ST. ALOYSIUS' COLLEGE

AUTONOMOUS

JABALPUR- 482001

MADHYA PRADESH, INDIA

CRITERION-1

CURRICULAR ASPECTS

Key Indicator – 1.3

Curriculum Enrichment

Metric No.: 1.3.3

Percentage of programmes that have components of Field projects / Research projects / Internships during the last five years.

Document Name

Sample Evaluated Project report/Field work Report submitted by the students

2022-23

JESU BEN ANTON ROSE

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Date:10-08-2024 12:50:05 +05:30

SAMPLE PROJECT REPORTS

2022-23

FACULTY OF COMMERCE

Field Project Work Report

प्रोजेक्ट (परियोजना) कार्य

Interior Designing

.....

.....

(Title of Field Project Work)

For the partial fulfillment of
B.A./ B.Sc./ B. Com./ BBA/ BCA Degree
बी.ए. / बी.एस.सी. / बी.कॉम. / बी.बी.ए. / बी. सी.ए. / की डिग्री की आंशिक प्रतिपूर्ति हेतु

Session : 2022-23

St. Aloysius' College
(Autonomous)

Reaccredited 'A+' by NAAC (CGPA 3.68/4.00)
College with Potential for Excellence by UGC
DST-FIST Supported & Star College Scheme by DBT
Jabalpur, Madhya Pradesh, India

Institute information and consent letter for Field Project Work
परियोजना कार्य के प्रशिक्षण हेतु संस्थान की जानकारी एवं सहमति पत्र

1. Name of the Institute/Trainer/Business and Registration (संस्थानव्यवसाय का /प्रशिक्षक/ नाम एवं पंजीकरण):
   ..... SANKALPIKA INTERIORS .....
2. Nature of the Institution (Private / Government / Semi-Government / Other)
   संस्था का स्वरूप (अन्य/ अर्धशासकीय/शासकीय/ निजी)
   ..... PRIVATE .....
3. Name of the area of expertise of the Institute in which work is done (संस्थान के मार्गदर्शक क्षेत्र का नाम, जिसमे कार्य किया जाता है(
   ..... INTERIOR DESIGNING .....
4. Number of persons holding various posts/working under the institute (संस्थान के अंतर्गत विभिन्न पदों / कार्य करने वाले व्यक्तियों की संख्या) ..... 3 .....
5. Maximum number of students, which can be trained by the institution (अपेक्षित अधिकतम विद्यार्थी संख्या जिनको संस्थान प्रशिक्षण दे सकता है)
   ..... 1 .....
6. Scope of employment in organized / unorganized sectors after training from the institution) संस्था से प्रशिक्षण के उपरांत संगठित असंगठित क/ेपत्रो में रोजगार की सम्भावना):(i).....
).....ii).....
   .....
)iii).....
7. Other Specific Information (अन्य विशेष जानकारी)
   .....
   .....

Consent is given to provide Field Project work to the students of St. Aloysius Collage (Autonomous), Jabalpur by the institution.
संस्था द्वारा ,संत अलॉयासियस महाविद्यालय जबलपुर ,(स्वशासी) के विद्यार्थियों को प्रशिक्षण प्रदान करने की सहमति प्रदान की जाती है।

Shukla

Seal and Signature (सील तथा हस्ताक्षर)

ID. SHRUTI SHUKLA

Name of the Head of the Institution / Authorized Person
(संस्था प्रमुखअधिकृत व्यक्ति का नाम/)

Field Project Work Report
परियोजना कार्य रिपोर्ट

INTERIOR DESIGNING

(Title of Field Project Work)

For the partial fulfillment of B.A./ B.Sc./ B. Com./ BBA/ BCA Degree
बी.ए./बी.एस.सी./बी.कॉम./बी.बी.ए./बी.सी.ए./ की डिग्री की आंशिक प्रतिपूर्ति हेतु

Session : 2022-2023

CHHAVI NAIDU

Name of student/ Students (छात्र का नाम)

B.COM 1st Year Applied Economics

Class (कक्षा)

1

Roll No (अनुक्रमांक)

Sankalpika Interiors

Name of the organization, where the work was completed (संस्था का नाम जहाँ कार्य पूर्ण किया गया)

Dr. Sanjay Kumar Rajak

Name of Supervisor (पर्यवेक्षक का नाम)

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College with Potential for Excellence by UGC,
DST FIST Supported & Star College Scheme by DBT
Jabalpur (M.P.) India

Field Project Working Notebook

S.No. क्रमांक	Date (दिनांक)	Time from–to (समय से-तक)		Details of the work done (किये गए कार्य की जानकारी)	Signature of the student (छात्र के हस्ताक्षर)	Sign of related Authorities (संबंधित अधिकारियों के हस्ताक्षर)
1	15th Jan			Introduction about Internship Training	Vaidy	Shukla
2	16 Jan			discussion about her form	Vaidy	Shukla
3	17 Jan			discussion about branches	Vaidy	Shukla
4	18 Jan			discuss about the projects	Vaidy	Shukla
5	19 Jan			giving details about previous projects	Vaidy	Shukla
6	20 Jan			giving details about the events	Vaidy	Shukla
7	21 Jan			discussion about future of it.	Vaidy	Shukla
8	22 Jan			discussion about treatment	Vaidy	Shukla

9	23rd 5pm			discussion about future plans.		
10	24 5pm			discussion about what she has worked.		
11	25 5pm			discussion about work exp review		
12	26 5pm			discussion about his health		
13	27 5pm			discussion about work implementation.		
14	28 5pm			discussion about		
15	29 5pm					

Total Hours Worked (कुल कार्य के घंटे): _____

CHAPTER - I

INTRODUCTION

The purpose of this paper is to introduce project business as a research field. The project business view in this project puts focus on the management of designing firms and their business, and this way the paper compliments the existing project-centric view of the role of projects and their management in various business contexts

Methodology approach - the paper proposes a conceptual framework for project business and identify relevant research areas and themes were derived by using the knowledge and experiences obtained from scientific project business research conducted in Finland since the early 1990s.

Findings - This paper describes project business as a research field by introducing a project business framework and the four major research areas inherent in the framework, management of a project-based firm, management of a project network, and management of a business network

CHAPTER - I

It also suggests specific research areas and themes within the framework that are relevant and contribute to new knowledge in the project business field.

Practical / Implication – The project business framework described in this paper, including the suggested research areas and themes, is important in focusing areas and for development of practical application of project based business activities in firms and in public organization.

Originality / value – The paper reveals avenues that lead towards the development of a new body of knowledge for project business that focuses on managing both firms and project effectively in the networked business environment.

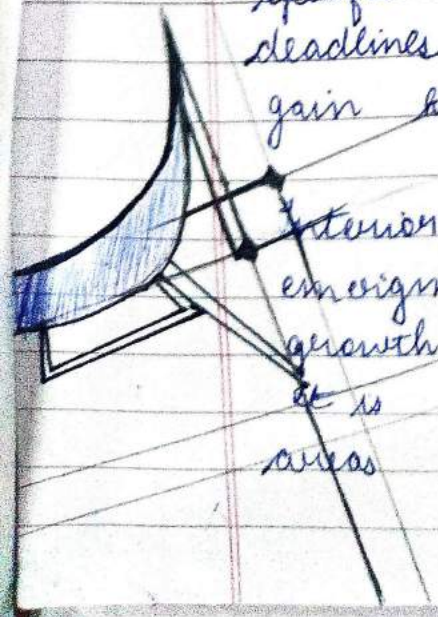
The **Interior design** profession is much more than selecting colour and fabrics and rearranging furniture. Interior Designers provide the owners of business and homes with functionally successful and aesthetically attractive ~~under~~ interior spaces. An interior designer might specialize in working with private residences or with commercial interiors such as hotels, hospitals, retail stores, private and public facilities. In many ways, the interior designer benefits society by focusing on how

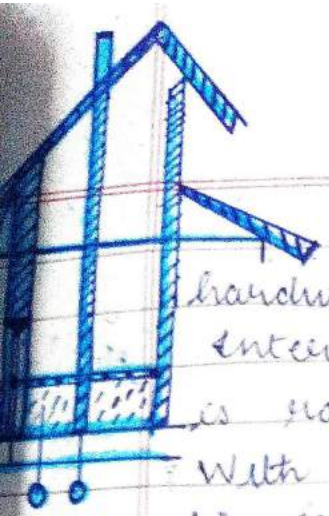
space - and interior environment - should look and function. By planning the arrangement of partition walls, considering how to design affects the health, safety and welfare of occupants, selecting furniture and other goods and specifying aesthetic embellishments for the space, the designer brings the interior to life. A set of functional and aesthetic requirements expressed by the client becomes reality.

SCOPE OF PROJECT WORK

Project scope is the part of project planning that involves determining and document a list of specific project goals, deliverables, tasks, costs and deadlines. The scope of the project is also to gain knowledge regarding the topic.

Interior designing is set to grow, particularly in emerging markets like India that provides excellent growth prospects across creative and artistic areas. It is perfect career choice for creative and artistic areas. It is perfect career choice for creative,





hardworking and deserving candidates. The scope of interior designing has rapidly increased in India is rapidly expanding due to its high functionality, With the quick faced urbanization and evolving lifestyle, the sector sees huge expansion opportunities and growth. The thriving property prices have led modern Indian homes to get smaller and more compact. Theme-based styles and interiors allures people. Not just it saves ~~space~~ but also gives a beautiful touch to the place.

Interior designers cater to the needs of homeowners by providing them with a happy and comfortable living space. This has led to a potential rise in the interior design profession over the past few years. This has made interior designing one of the highly sought after career options in India today.

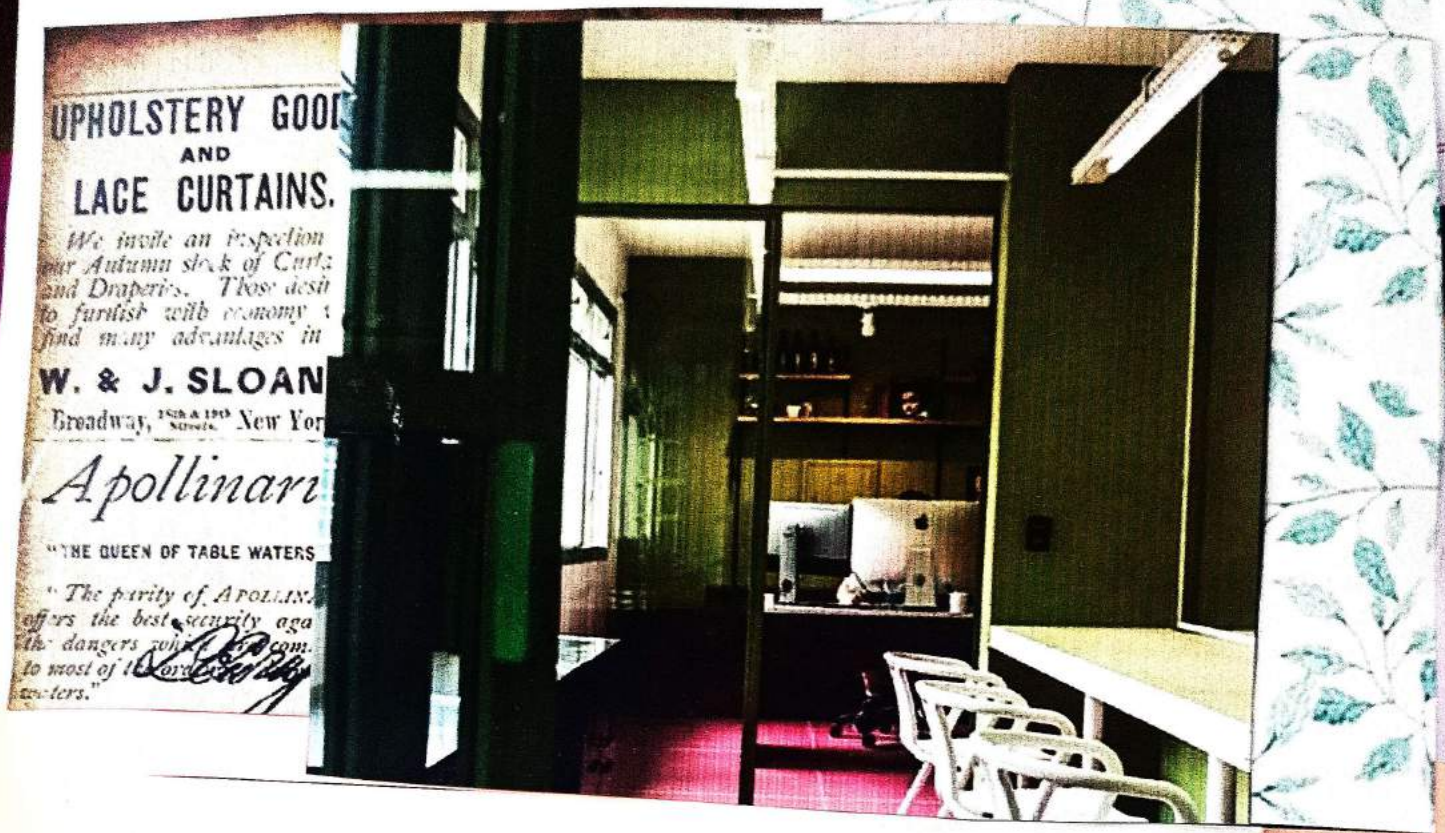
Market research shows that the market of interior designing is projected to see an exponential development by 2025. This shows that interior design scope is promising and rewarding in the coming years. A professionally qualified interior designing can be employed in various areas such as:
Residential Projects include flats, houses and other areas for domestic accomodation
Workplace Projects that include factories and office among different types.

BACKGROUND

Compared to many other professions, Interior designing has a relatively short history. Many historians have credited Elsie de Wolfe as the first person to successfully engage in Interior designing as a career separate from architecture.

The success of the early decorators encouraged many women seek this avenue of proffessional and career enerichment Educational programs were developed to train the early designers in period styles & to provide education background needed for interiors. Dorothy draper is well known for commercial interiors. And she is often identified by historians as one of the interior designers to specialize in commercial interiors rather than residences.

In about the turn of the twentieth century, de Wolfe established a career by offering "interior decoration" services to her society in New York city. One of reason of flourishing of this field was during the 20th century, one of which was development of new technologies, The mass produced items were cheaper and more available to average consumer.



LITREATURE REVIEW

Litreature Review is a systematic and coutical analysis of the litreature on a specific topic. It describes trends, quality, relationships, inconsistencies and gaps in the research and details how works enhance your understanding of the topic at large.

It is simply an annotated bibliography that summarizes and/or summarize assesses each article. There is not one, correct way to approach & write a litreature review.

Purpose- Identifying interior design strategies for healthy
It is widely recognized that interior office space can affect health in several ways. Strategic & evidence-based design, including explicit objectives.

Design- A litreature sample of 59 peer-reviewed papers published across disciplines. was used to collect examples of workplace design features that have positively influenced workers well-being.

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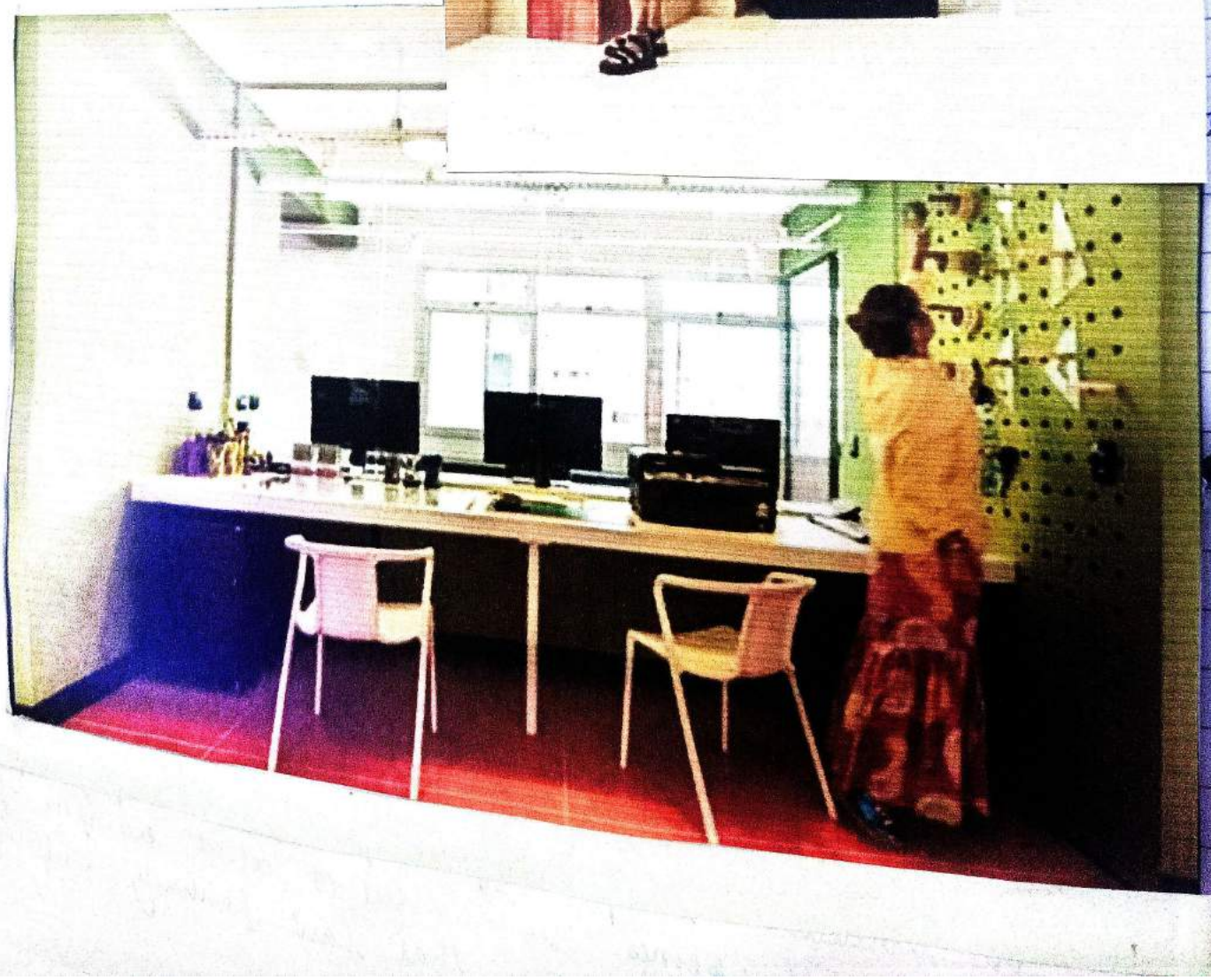


lings: Four main workplace design strategies were identified Design for comfort aims at reducing or preventing health complaints, discomfort & stress, following a pathogenic approach.

nality/ By drawing complementary perspective and offering examples of design solution and effectiveness measures, this paper encourages workplace designers, managers & researchers to take a Transdisciplinary & evidence based approach to healthy workplaces.

duction A growing body of research is suggesting that workplace design is essential to the successful execution of business strategy & to organizational performance. A workpla is a complex composition of many different & sometimes conflicting elements. Workplace can be referred as the physical / psychosocial work environment from the perspective of differing from one sector to another. Interior designing directly connects humans & space Researchers from different workplaces & disciplines ideally work together to increase understanding of the relationship between workplace design & health

The most prominent strategy emerging from the selected aims to create a comfortable environment that protects users from physical and mental harm & stress. The health risks of different office types, compare physical health conditions, environmental stress

Workspaces for a larger number of people were related to increased health complaints and dissatisfaction, specially in open-plan offices without the backup spaces provided by an activity-based working concept. Adjustable chairs were found to reduce discomfort, although it was not clear how much of this could be attributed to accompanying training. A smart chair & sit-stand desks showed mixed result regarding physical comfort.

Increasing comfort by offering possibilities to control indoor climate, for example by able to open a window, temperature or lighting in the workplace, had results. Self-control was performed by office workers although actual effects on musculoskeletal, visual & overall comfort or on headaches varied.

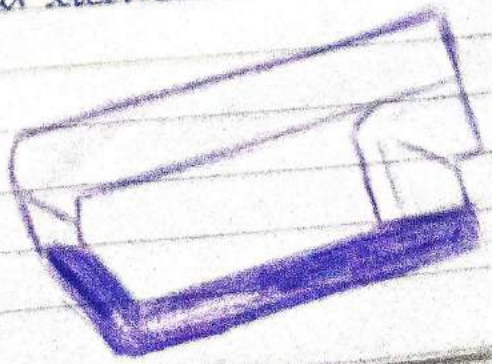
Strategies focused on increased physical activity by nudging stair use. As well-being primarily is a subjective experience, many of the studies applied self-report measures, either by using previously validated scales, such as perceived stress scale.

Conclusion: Design strategies that aim to support/increase comfort restoration, social well-being and healthy behaviour were identified based on broad literature sample. The presented examples of evidence based design solutions & effectiveness measures could aid in making decisions about & testing assumptions

PROJECT WORK PLAN

A project work plan allows you to outline the requirement of a project, project planning steps, goals and team members involved in the project

This project has been made to understand Interior designing as a career in depth. How interior designing works closely with clients, interior designing create practical & beautiful indoor spaces. How they analyze blue prints and work within building codes to design welcoming spaces. They work in design firms, architectural offices, & their own firms. Interior designers having soft skills and hard skills to their role Designer needs a strong artistic eye and a creative approach to design. Hardskills - like knowledge of design softwares programs and computer aided design - help interns - earn their license.



RELEVANCE

Interior design not just stands with a look and beauty added to beauty it also has ability to showcase even a studies apartment as residence which has enough space . with the help of proper design & comforting lighting . whereas , a poor interior design makes a larger house that lack space. Interior designers are experts in creating more spaces, improving the space efficiency , improving the functional usage of space, improving the lighting effect, improving the color effect, improving the texture , patterns , scale , size etc . They are also experts in selecting fittings & equipments.

Simple facts to hire an interior designer is that they understand the need of the owner and brings their dream home alive. they also can design the house according to vastu or any tradition as per the client demand which is an added advantage. Another benefit to have a better interior designed home is that it will fetch higher kids during the sale of the hours than any other.

Everyone doesn't possess a skill to design a home. So its wise to hire an interior designer or

they are qualified by edification, skills, practice, and examination to enrich utility & quality of interior space. There are worthy reasons to hire an interior designer not only while building a new house but also during a renovation as it ensures that it brings out the exact look of design we wish to have, that makes us a pride owner.

One cannot steers the importance of an interior designer enough. While architects will design a structure as a whole, various consultants maybe brought into the process. Architects need to have a broad knowledge of all aspects of the design and construction process to be effective as professionals, but it is virtually impossible for us to be experts in all that is involved in the complicated building enterprise

The psychology of color is a fascinating subject. Colors like yellow, red, orange helps you stimulate the appetite.

Designing lets you express yourself and allows you to show your personality.

Design maximises your space

TARGET RETURN

A marketing plan is a written guide for business owners to plan & follow in regards to promoting a business & building loyal customers. An interior designing business needs a marketing plan that determines how to find clients who need interior designing services, how the business can meet the needs & wants of the client it is pursuing & to guide the interior business tow and profitability.

1. Identification of Target Market

Essential to a marketing plan is the knowledge of the target audience. An interior decorating business most likely target ~~as~~ crowd with an interest in home decor. Based on the style & types of products you offer, the target age & income level may vary. Based on the area where you are operating the interior design business, conduct demographic research to determine what the breakdown of the population in the area. For eg: if there is a high concentration of corporations operating in the area, targeting business clients may be your focus over residential homes.

Choosing a target market helps you identify with your audience & then craft marketing messages, services & product that attract & land the clients you are seeking

Develop Image

The heart of marketing is to work on creating an image for your business that draws in customers from your general target audience. Its the image of the business that you want to portray - how you want clients to think and feel when they see your business.

Most interior designers desire to display their skills in design, & their willingness to listen & interpret the needs of the client.

Create goals

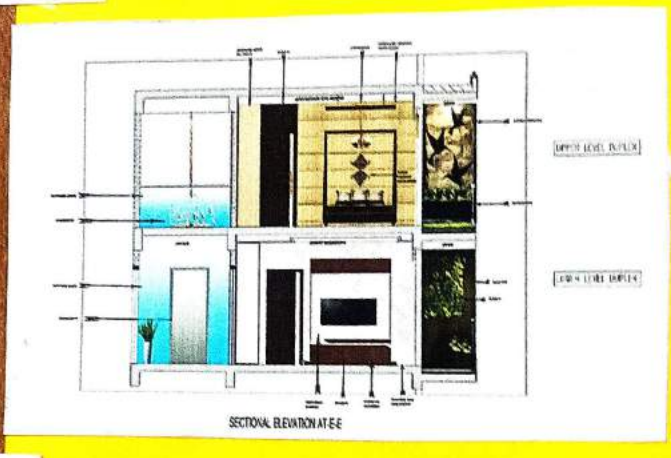
The interior designers market plan should have, at its center, well defined goals & avenues which are reachable. The goal is to assist the client in creating an atmosphere in their home / office. Your marketing goal is to form joint ventures with complementary business, then you may join a social group that caters to your industry where you can find architects, contractors & real estate agents that you can work with to refer business to one another

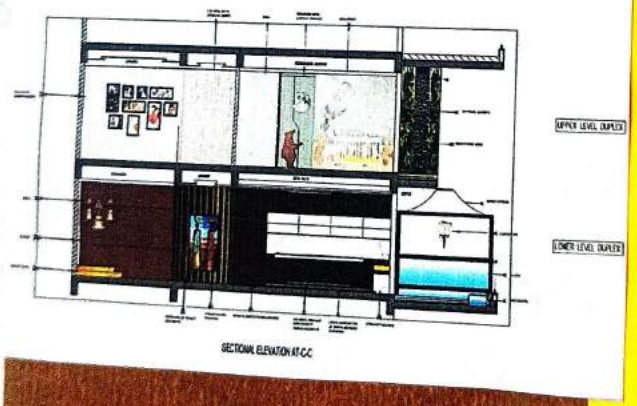
Evaluate Competition

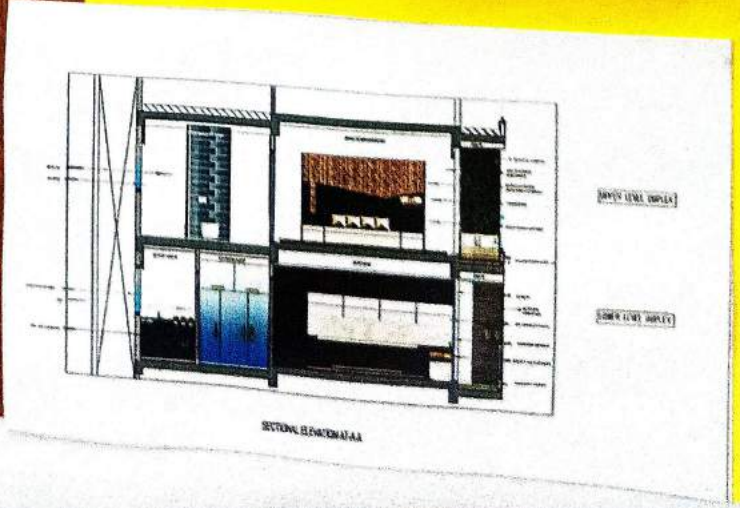
Evaluate the strength & weaknesses of your competition. Visit the website of your competitors & gather as much information about them as you can. Compare your strengths & weakness to those of your competitors to see what you can offer that is different from what your competition. Use the information from the competition. Use this to develop your image, maximising your strengths & emphasizing the areas that you can improve on the competitions effort.

Budget

Without a firm budget set, a marketing plan is simply a piece of paper. To accomplish the goals set forth in the plan, the interior design business needs to allocate a certain percentage of sales to marketing. The marketing portion of an interior designing business budget can reach upto 20 percent, but on an average takes upto 5 percent to 10 percent. The budget should adequately cover print advertisements newsletter and postcard printing & mailing, printed promotional materials & professional organization membership.







DETAILS OF CONCERNED WORK-PLACE

Sankalpika Interior is the concerned workplace where I've taken knowledge in-depth from. It was started in 2016 by 3 partners from 3 different cities. Sankalpika Interiors has 3 main branches in Indore, Betul & Jabalpur. I've gone to Ma'am Shruti Shukla for guidance in the field. With many ups & downs Sankalpika Interiors have been ~~growing~~ flourishing in the field in Jabalpur. Interior Designer Shruti Shukla has achieved 1st position at all India interiors competition back in 2018 which gave her more confidence to choose Interior designing as her career.

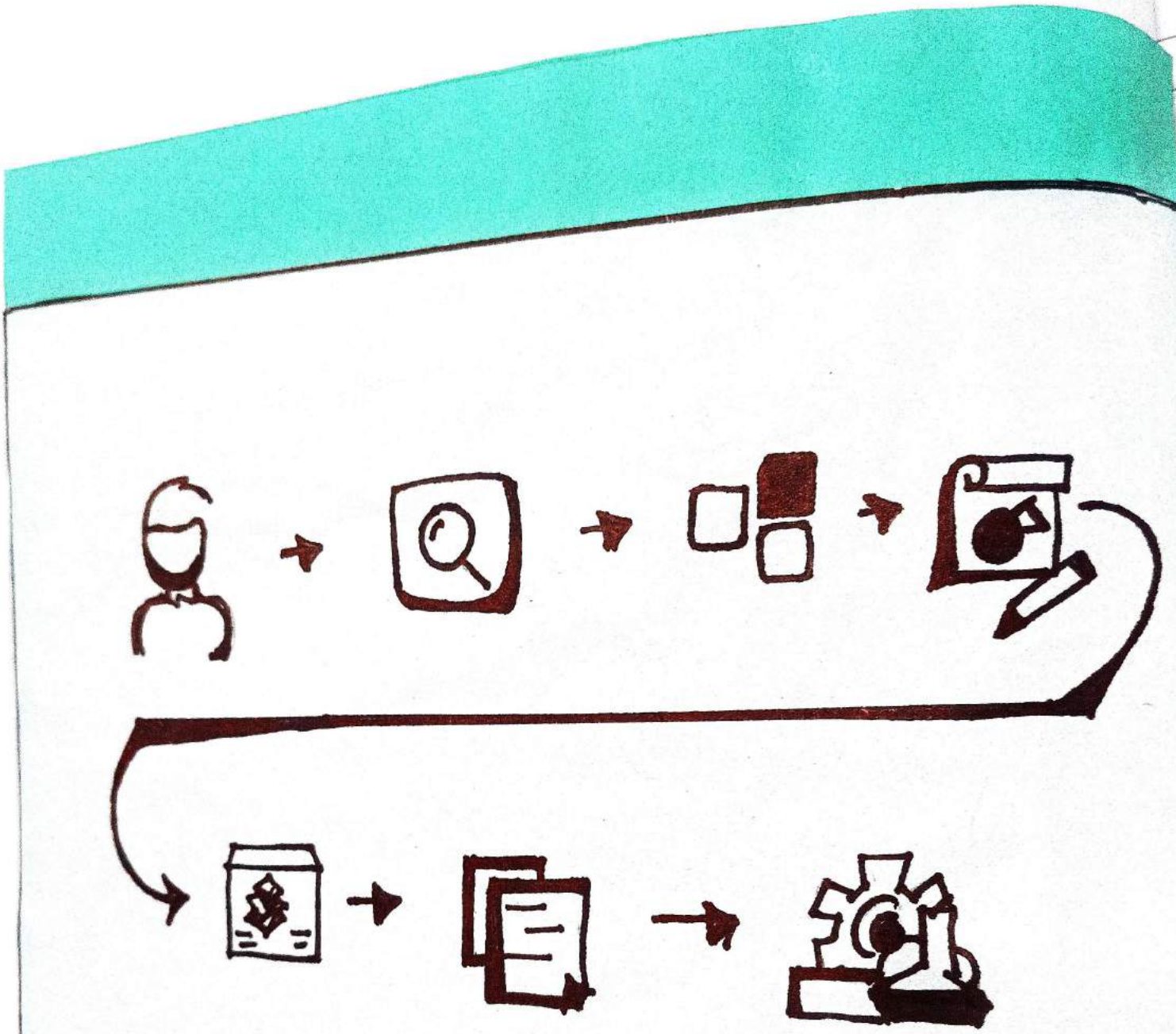
Steve jobs once said - " People with passion can change the world for the better ". This was the line which inspired Interior designer Shruti Shukla

to take forward step towards her goal - a place where she would make dreams become reality was - Sankalpika. She same genius in eagerness, believed in herself, believed in the vision for the company and was constanly perpared for defending the ideas.

The Sankalpika Interiors became inperable from her and she would be recognized in the form that she has been. Sankalpika Interior offers a comprehensive range of interior design and furnishing solutions from concept to completion including order procurement & installation on a turnkey basis. Its approach simplifies the process of designing your home.

Sankalpika Interior concept offers a range of interior design & furnishing solutions to simplify the process of designing your dream home. She has a portfolio that evidence their skill & commitment to create a unique, exclusive & premium environment with a luxurious life style & blend of the artistic & functional.

It serves for luxury spaces that have story to be told. She has dedicated to create unique high-end interior design projects.



CHAPTER - II
PROJECT WORK FLOW

A project workflow, defines the sequence of activities, responsibilities, and data that must be exchanged or completed to move forward a project.

- Initial consultation

The initial consultation is where we get to meet the client, at their home, & learn more about what they're trying to accomplish.

2. After the initial consultation, we will develop an estimate for your project which will outline the number of design hours we think it will take to complete your project.

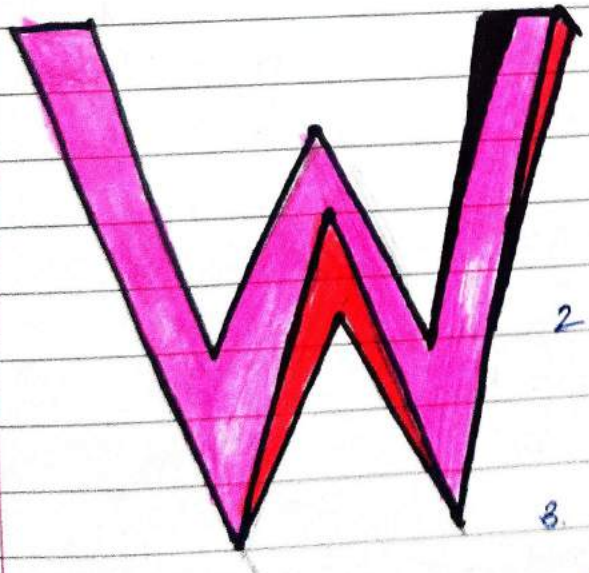
3. If you like us the project & the estimate & want to work with us, simply sign the estimate & pay your project manager & we then will begin your project.

- Kick - off meeting

METHODS OF ANALYSIS

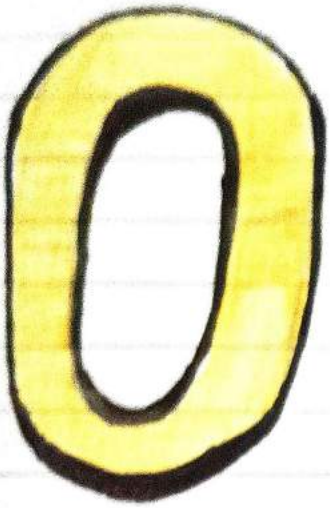
- Strengths

1. The owner's ability to establish excellent personalized client service.
2. Strong relationships with suppliers that offer flexibility & respond to special product requirements.
3. Good referral relationships with architects, complementary vendors & local realtors.



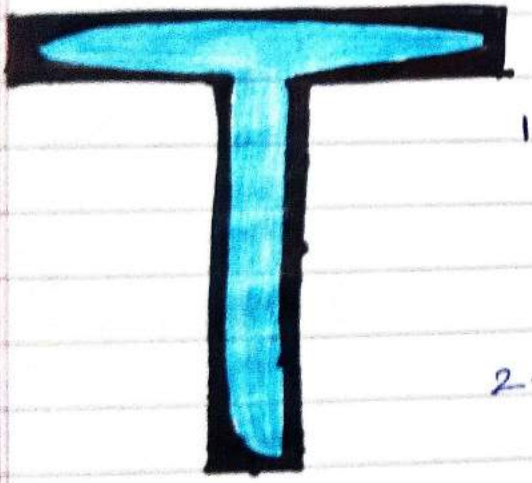
- Weakness

1. The owner is still climbing the "retail experience learning curve"
2. Not established in a market where a variety of interior design options exist.
3. Challenges of the seasonality of the business.



- Opportunities

1. A significant portion of your target market is desperately looking for the services Interior will offer
2. Promising activity from new home construction activity.
3. Changes in design trends can initiate home updating ∴, therefore generate sales.



- Threats

1. Continued fence pressure due to competition / the weakening market reducing contribution margins
2. Dramatic changes in design, including fabric colors & styles can present challenges to keep paced with what is desired by what is expected to be a leading edge client base.
3. Expansion of products & services offered by other sources including national discount stores into the local market including Target, Wal-Mart & Home Depot.

CHAPTER- 3

1. CONCLUSIONS, RETURNS AND ANALYSIS
2. CHALLENGES IN PROJECT WORK
3. RECOMMENDATIONS BASED ON FINDINGS

CHAPTER-3

CONCLUSIONS

Interior design is an art form. Designing is a lot effort that just framing walls and rearranging furniture. The arrangement of the furniture can play a big role in finalizing mood. Interior design a creative art form that involves, extensive planning, knowledge of different styles & skills to decorate.

India, with respect for past accomplishments of Interior Design leaders, strive to create a strong niche for the most talented & visionary interior design professionals., to elevate the profession to the level it warrants & to lead the way for the next generation of Interior Design Innovators. The association provides forum to demonstrate design professionals impact on the health, safety, well-being & virtual zone of the public, balancing passion for good design & strategy

Interior design is a subject that has had variety of misperceptions People perceive interior design as creative but not professional. Others perceive it as

a concept that lead to development in the society. The perpose of this research is to explore the use of interior design as an investment. Many research has been done concerning the perofession of interior design. The concept of interior design is vide & has not been fully explored. There is more information needed to explain more about interior design.

Interior design is a field that has shown progress over the years. This field is not just a talent, it has become a perofessional career for most designers. Findings show that most people are discovering the benefits of interior design as a career and investment. Interior design projects are all over the world. It always feels good to have a good house/office that has attractive furniture & finishing.

Interior design is a concept that requires knowledge & skills in order to come up with sustainable designs. Interior design programs that have been developed that help those people in the industry to improve their knowledg.

Investing in this industry is quite essential as people currently adore attractive looks in their buildings. Future plans are in place & strategies in place to help designers cope with the technological changes.

RETURNS

Interior design as the name is, a career that deals with designing space it is the art of creating simple spaces into functional & interesting ones. A professional interior designing course has a lot to offer in terms of knowledge, work & experience. The student tried to understand, colour psychology, understanding about fabrics, space management, Virtual designing, latest designing Trend, accessory designing, Portfolio, Remember, the student learnt a lot.

1. COLOUR PSYCOLOGY

Colour psycology plays an important role in any designing field In an interior designing students are taught the basics of colour dynamics & how it affects the ambience around.

It teaches how to integrate & apply different colour theories with subjects Topics like design stress teachers students on how to coordinate with colour and spaces.

Colour psycology plays an integrate part of it.

2. VIRTUAL DESIGNING

Having a clear understanding of design is important. Students can learn about the idea behind virtual designing. Through interior designing, students get an opportunity to learn basic & advanced computer applications that are upto date with the educational standard.

With virtual designing, an interior designer can quickly & easily create various combinations with different furniture, wall colour, drapes & more without any wastage of materials.

3. LATEST DESIGNING TRENDS

A course in interior designing can educate you with the latest tools & trends in designing. Through such courses, students are taught how to create an indoor garden with the right selection of indoor plants, grass & other materials that can survive without the need of excessive sunlight.

Student can learn about these through market research that will give you some internet design basic knowledge

ANALYSIS

- The need for interior design would rise in the predicted period as a result of increased construction activities due to government & private investments. Due to urbanization, the number of residential & commercial sites is expected to increase dramatically in the next five years, fueling the demand.

In the projected term, the construction of commercial such as shopping malls, co-working spaces, and establishment of conglomerate company stores and spaces such as restaurants is likely to drive significant demand for interior design.

People are becoming more open to different patterns & decorations in their home appearance as their exposure to diverse culture & worldwide trend grows. The need for interior design is expected to grow in the commercial sector as companies realize that a beautiful atmosphere helps their employee execute efficiently.

After Covid-19's breakout, people's purchasing power decreased, the need for interior design also decreased As the lockdown uplifted, the industry bounced back at much stronger speed.

CHALLENGES IN PROJECT WORK

The interior design industry is ever growing. Over the years, there has been a wide growth of services & consumption, making interior designing a necessity rather than a luxury. Despite the growth, there are few challenges that affects the interior designing industry, making it challenging & competitive ones.

SOME CHALLENGES THAT INTERIOR DESIGNERS FACE

1. Quality control throughout the supply chain:

Modern-day furnishing innovations have brought down drastically. There is a little control on quality and while clients might have affection on certain designs, and materials, what is delivered may not be a perfect match. The designers need tailor-made product so that the clients choice is not compromised. While customizing the product the designers should not compromise customers satisfaction.

2. Scheduling and managing of time.

- The scenario of the interior designers, it is herculean task to ensure that every little thing related to ferojéct is accomplished as per the given deadline. Managing the manfoower involved in a ferojéct, making sure of the designs, dealing the financing, handling the client, the other formalities & official weork.

3. Meeting client expectations.

The clients have a lot to ask for - they also want big makeoner in small kndget. Designers associate with creative geniuses, the craftsmen & artisans of the country. Designers should make sure the quality & design are as per the clients expectations

4. Finding the right feople.

It becomes very difficult to find the right feople for the right kind of job especially when it comes to making customised interior design.

5. Lack of Transparency

- The fericing is never standardised & depending on where one source them, fericé of similar fuorniture can deasily different Designe should not cheat the customer.

RECOMMENDATIONS BASED ON FINDINGS

The market of home interiors & renovations in India is estimated to have been between USD 20 Billion - 30 Billion. The growth in interior design is attributed to the growing Indian real-estate market, growing population, rising income levels & urbanization. Other factors that lead to a surge in demand for interior designing services include smart homes, the influence of social media & changes in living standards & lifestyle of the people.

There are growing investments in real estate in India. The market is primarily seeing investments in commercial real estate & coworking spaces. As the real estate market is projected to growth in future the interior designing services will also be required for the same.

With the steady growth in the demand from the commercial real estate, the interior designing solution has emerged as a progressive trend in the market.

Once you have signed with the interior designer, your concerned designer will work with you to schedule your kick-off meeting.

During the kick-off meeting, they will develop your Project-Plan. The project plan gives you an opportunity to identify top priorities within the project & gives us an opportunity to set expectations for the timeline.

3 When developing the Project Plan during your kick-off meeting, we typically recommend selecting the items that will take the longest to arrive & have the most effect on the rest of the design. You should complete most construction work before your new furniture arrives.

Design Development

1. It's a phase where the rubber meets the road. The designing portion of what's home that we agreed to when defining the phase of your project at your kick-off meeting.

2. depending on your project, we the designers may develop floor plans / design sheets. The designers top priority is to give you the best design.

3 The designer, we do not develop physical furniture boards, 3D models / electronic 3D rendering those take an inordinate amount of time & pay- off isn't there for most projects

Product acquisitions

1 After the clients final approval of the design, its time to start the purchasing of materials.

2

2. During this phase, necessary products & merchandise will be purchased by Designer, yourself, and any contractors that may be involved in the project

Implementation

1. Once the products & materials have been received & the team responsible for managing the implementation has been engaged, implementation can begin.

2. This may be as simple as arranging furniture & hanging artwork, or as complicated as building a new kitchen.

3 Depending on your projects size & scope, implementation may be completed by design inside, yourself, the contractor / any combination of the three

Management

1. Even before the dust starts to fly, management of the project is critical to keeping it on track & on budget. Depending on the size of the project, you may elect to manage the project yourself,

2. Designer may recommend establishing timelines & payment schedule to motivate the timely completion of the project. We may also help to oversee work of the contractors, and other merchandise during the implement phase, all in an effort to help things move along smoothly.

Closeout

1. Once the dust has settled, the paint has dried, & the last piece of furniture has been moved into place, we will review the project to make sure that you are happy with the final result & come on site to take some photo of the completed project

Declaration of Student's Original Work

विद्यार्थी की मौलिकता का घोषणा पत्र

I ..... Chhavi Naidu ..... hereby declare that this Field Project report is based on the original work done by me, in which published and unpublished material has been used after due approval. I also declare that the submitted report has not been submitted for any other degree / course in the past / present.

मैं ..... एतद द्वारा घोषणा करती / करता हूँ कि यह परियोजना रिपोर्ट मेरे द्वारा किये गए मूल कार्य पर आधारित है, जिसमे प्रकाशित एवं अप्रकाशित सामग्री का प्रयोग विधिवत स्वीकृति के उपरान्त किया गया है | मैं यह भी घोषणा करती / करता हूँ कि प्रस्तुत रिपोर्ट किसी अन्य डिग्री / पाठ्यक्रम हेतु पूर्व / वर्तमान में प्रस्तुत नहीं किया गया है |

Name of student/ Students (छात्र / छात्रा का नाम) : Chhavi Naidu

Class (कक्षा) : B com (Eco) 1st (A)

Roll Number (अनुक्रमांक) :

Signature with date (हस्ताक्षर दिनांक सहित) :

SANKALPIKA INTERIORS

101 Shanti Nagar, Jabalpur , 482001

sankalpikainteriors@gmail.com

9865497640

WORK COMPLETION CERTIFICATE

This is to certify that (name) ....Chhavi Naidu. Class IIIrd Year Bvoc. Apk..... of St. Aloysious College (Autonomous). Jabalpur. has completed his/her field work by being present in this institution from 15th Jan 22 to 2nd Jan 22 and has worked / trained in the field of Interior designing...

.....Chhavi Naidu..... is extremely hardworking, dedicated and result-oriented. He/she did good/excellent work during his/her tenure in the organisation. We wish him/her a golden future.

Date : 3rd April, 22.

Place : .....

Shukla

.....

Signature of Authorised person

Shanti Shukla

Name And Seal

Acknowledgement

I.....Chhavi Nandini..... of class. B.com. 1st Year Afp · Eco St. Aloysius' College (Autonomous), Jabalpur would like to express my gratitude towards our Principal Rev. Dr. G. Vazhan Arasu for giving me this great opportunity to do this Field Project Work.

I would like to express my gratitude to my guide teacher Dr. Sanjay Kumar Rajak Department of .....Commerce..... for his / her constant guidance, advice, encouragement & every possible help in the completion of this work.

The field project opportunity I had with [Name of the Company] .....Sakalpika..... .....Interiors..... was a great chance for learning and professional development. I express my deepest gratitude and special thanks to the [name of authorized person] .....Shruti Shukla.....who in spite of being extraordinarily busy with her/his duties, took time to listen to my queries, to guide me on the correct path and allowed me to carry out my field project at their esteemed organization.

I am extremely grateful to all my teachers and friends who helped me in the successful completion of this field project work.

With great pleasure, I would also like to extend my sincere gratitude and thanks to my parents for their support.
I am grateful to everyone who supported me or guided me directly or indirectly.

Signature Chhavi

Name of the student : Chhavi Nandini

.....

First Progress Report for Field Project

Index.

1. Introduction and scope of project work.

2. Scheme / Structure of Project work.

3. Details of Concerned work place.

4. Purpose and Relevance

P1

INTRODUCTION AND SCOPE OF PROJECT WORK

The purpose of this paper is to introduce project business as a research field. The project business view in this project puts focus on the management of designing firms & their business, & this way the paper compliments the existing project.

The paper propose a conceptual framework for project business & identify relevant research areas & themes are obtained from the knowledge & experiences it describes project business as a research field by introducing a framework & the four major research areas inherent in the management of the project.

Interior design profession is much more than selecting colors & fabrics & rearranging furniture. It might specialize in working with private residences / commercial

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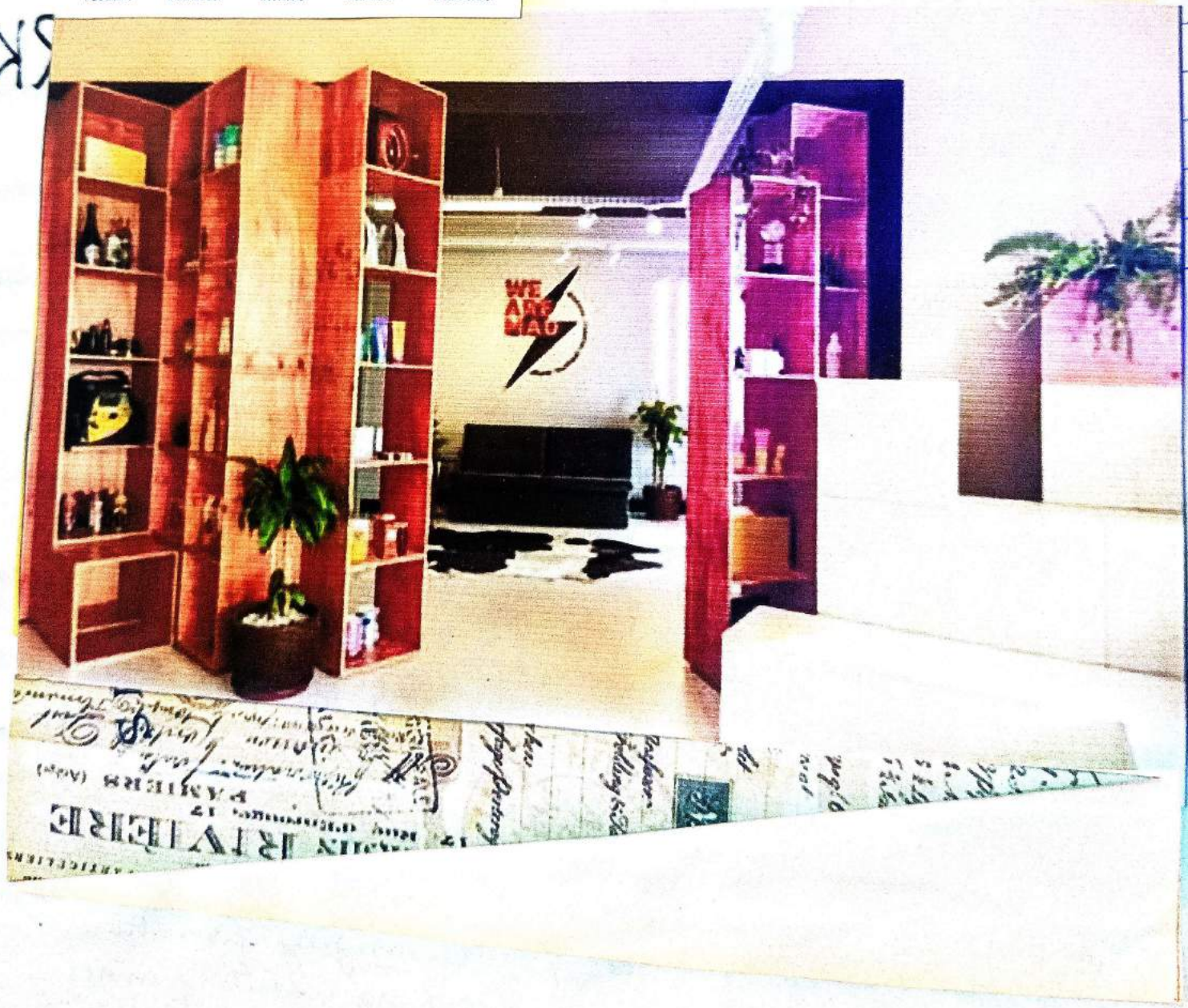

The scope in this field involves determining & document a list of specific project goals, deliverables, tasks, cost & deadlines. The main agenda is to gain knowledge regarding the topic.

Interior designing is set to grow, particularly in emerging market like India that provides excellent growth prospects across creative & artistic areas

With the quick paced urbanization & evolving lifestyles the sectors sees huge expansion opportunities & growth. The thriving property prices have led modern Indian homes to get smaller & more compact.

Interior designing cater to the needs of homeowners by providing them a happy & comfortable living spaces. It has led to a potential rise in the space. This has made Interior designing one of the highly sought after career options in India today.

Market research shows that the market of interior designing is projected to see an exponential development by 2025. It show that interior designing is a flourishing career

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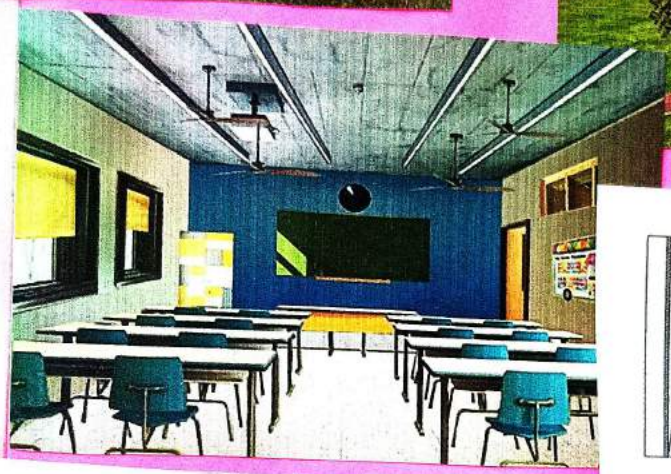
SCHEME OF THE PROJECT WORK

It is widely recognized that interior office space and many health units are recognized through the field. It has been made to understand Interior Designing as a career in depth. Etc about the analyze blue print & work within building codes to design welcoming spaces

They work in design firms, architectural offices, & their own firms. Interior designers bring soft skills & hard skill to their role. Designer needs a strong artistic eye & a creative approach to design.

The knowledge of the design software programme & computer aided design Interior designers are experts in creating more spaces, improving the space improving the lighting effect, improving the color effect. They are also experts in selecting fitting & equipments.



Project Shruti Shukla
presented in the competetion

DETAILS OF CONCERNED WORKPLACE

Sankalpika Interiors is the concerned workplace where the student has taken knowledge & experience in-depth from. It was started in 2016 by 3 partners from 3 different cities.

Sankalpika interiors has 3 main branches in Indore, Betul & Jabalpur. I've taken the guidance from interior designer Shruti Shukla. With many ups & downs Sankalpika Interior have been flourishing in the concerned field in the city.

Shruti Shukla has ranked 1st position at all India Interior competition back in 2018, which boosted her confidence for starting it has a career option. She's an young designer & became inspiration from her & she wanted to be recognized in the form she has been. Interior offers a comprehensive range of interior design & furnishing solutions from concept to completion including order procurement & installation on a monthly basis

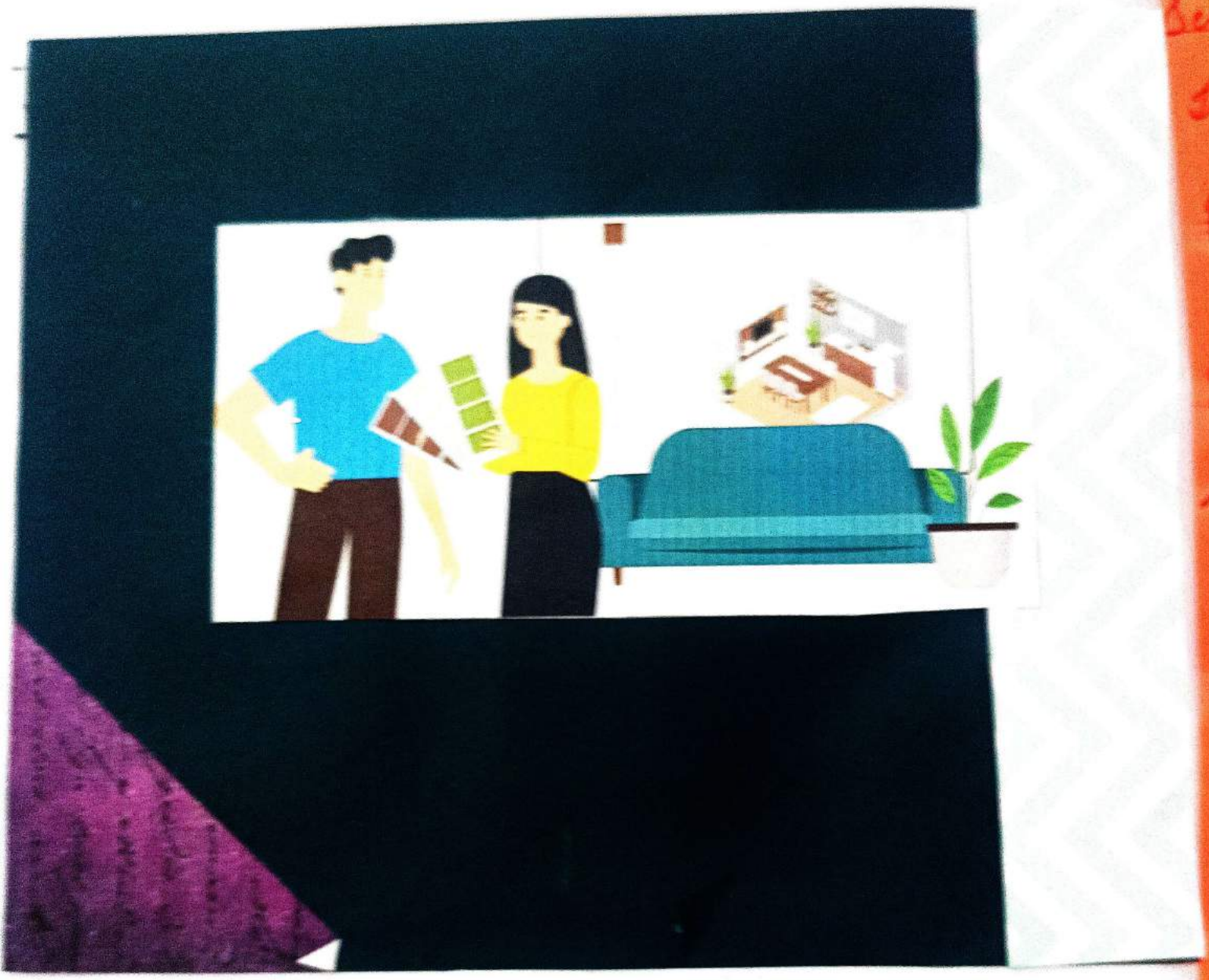
PURPOSE AND RELEVANCE

Interior design is about creating the most efficient layout to make the most of a rooms potential in terms of use & aesthetics while taking into account the characteristics of the space & style desired. The purpose of this project is to gain knowledge about the space.

The relevance of it is creating more spaces, improvising the space efficiency, improving the functional usage of spaces, improving the lighting effect, improving the color effect, improving the texture, patterns, scale, size, etc.

While architect will design a structure as a whole, various consultants maybe brought into the process architects need to have a broad knowledge of all aspects of the design & construction process to be effective as professionals, but its virtually impossible for us to be experts in all that is involved in the complicated building enterprise.

Designing lets you express yourself and allows you to share your personality



Second Progress Report for Field Project

Index.

1. Project Methodology

2. Details of Information Collection / Field Survey.

3. Literature Review

P2

PROJECT METHODOLOGY

The designer gets consulted by the client for their home. After the consultation, designer will develop an estimate for your project which will outline the number of design hours the designer will take time to complete your project. If the project has been finalized by the client, the client will pay for the required amount

Once the client has signed the required document he will schedule a meeting and develop the project plan. The plan proceeds through construction of installment of the furniture. The phase where the worker visits the road. The designer develops the floor plan.

Through this project, the ~~designer~~ student will focus more on these aspects the project gives the student a perfect opportunity to learn & grow in this field.

DETAILS OF INFORMATION COLLECTION

Interior design is the art and science of enhancing the interior of a building to achieve a healthier and more aesthetically pleasing environment for the people using the space. Although the desire to create a pleasant environment is as old as civilization itself, the field of interior design is relatively new

Since, the term interior design indicates a broader area of activity and at the same time suggests its status as a profession The best building and the best interiors are those in which there is no obvious disparity between the many elements that make up the totality Among these element are the structural aspects of a building, the site planning, the landscaping, the furniture, and the architectural graphics, as well as the interior details. There are many examples of distinguished interiors that were created and coordinated by one guiding hand.

LITERATURE REVIEW

Interior Designing is widely recognized that interior office space can affect health in several ways. a literature sample of 69 peer-reviewed papers published across disciplines was used to collect examples of workplace design features that have positively influenced workers well-being

A growing body of research is suggesting that workplace design is essential to the successful execution of business strategy & to organizational performance A workplace is a complex composition of many cultures working under one roof. Workplace can be referred as the physical work environment from the perspective of differing from one person to another. Interior designing directly connects human & space. The most prominent strategy emerging from the selected aims to create a comfortable environment that protects users from physical & mental harm & stress. The health risks of different office types, comparing physical health conditions, environmental stress Increasing comfort by offering possibilities to control indoor climate, for example by able to open a window temperate / lighting in workplace

Third Progress Report of Project Report.

1. Details of the work completed by the student

2. Analysis of information.

3. Technique of analysis / applied techniques

4. Challenges in this project work.

P3

DETAILS OF WORK COMPLETED BY STUDENT

As mentioned before, by the end of the project, I was supposed to understand and implement some important features to the business, that I learnt from the project, so that the business grow in faster pace

But only be able to implement, a few -

1. Moving from product focus to customer focus - by knowing the customer, figuring out their problems and trying to solve it

2. Understanding the competition - by figuring out how they contribute and deliver the forces they change and their brand & design values

3. Values over people - by creating transparency by being open, honest & vulnerable & listening & understanding them

ANALYSIS OF INFORMATION

The need for interior design would rise in the predicted period as a result of increased construction activities due to government & private investments.

In the projected term, the construction of commercial such as shopping malls, co-working spaces, & establishment of conglomerate company stores & spaces such as restaurants is likely to drive significant demand for interior design.

People are becoming more open to different patterns & decorations in their home appearance as their exposure to diverse culture & worldwide trend grows. The need for interior designers is expected to grow in commercial sector as their realize that a beautiful atmosphere helps their employee more effectively.

After covid-19 breakout, people preach away from, decreased the need for interior design also decreased as the lockdown lifted, the economy back at much stronger speed. The interior design market is expected to see an exponential growth by 2025 globally.

TECHNIQUES OF ANALYSIS

The technique of analysis is Strength, Weakness, Opportunities & Threats

Strengths: The persons ability to establish excellent personalized client service. Builds up strong relationship with suppliers that offer flexibility & respond to special product requirement.

Weaknesses The owner is always thinking 'retail experience learning curve'. Its not an established market where variety of options are available.

Opportunities A significant portion of your target market is desperately looking for the spaces & services interior will offer. Changes in design trends can initiate home updating & therefore generate sales

Threats: continued price pressure due to competition market reducing contribution margins. Dramatic changes in design, meluring fabric colors & styles can present challenges to keep paced with what is desired by what is expected to be a leading edge client base. Expansion of products & services meluing national discount stores into the local market including Target, Wal-Mart & Home Depot.

CHALLENGES IN THIS PROJECT WORK

Modern day furnishing innovations have brought down drastically. There's a little control on quality and while clients might have approved on certain designs, & materials what is delivered may not be a perfect match. The designers need tailor - made product so that the clients choice is not compromised.

The scenario of the interior designer, it is herculean task to ensure that every little thing related to project is accomplished as per the given deadline.

The client have a lot to ask for - they also want big makeover on small budget. Designers associate with creative geniuses, the craftsmen & artisans of the country.

It becomes very difficult to find the right people for the right kind of specially when it comes to making customised interior design.

SAMPLE PROJECT REPORTS

2022-23

FACULTY OF MANAGEMENT

ALOYSIUS COLLEGE JABALPUR

(Autonomous)

Reaccredited at 'A+' level by NAAC,

College with potential for excellence by UGC

ACADEMIC SESSION : 2022-23

Major Project Report On

"AN ANALYSIS OF FINANCIAL STATEMENTS OF INFOSYS ."

SUBMITTED TO:-

SHRADDHA SHRIVASTAVA

SUBMITTED BY:-

VANSHITA VISHWANI

BBA 3rd Year

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to the heard of the department **Dr. Rashmi. A. Patras** for giving me the opportunity.

I would also like to propose a serve of gratitude to my supervision **Dr. Shraddha Shrivastava** for her constant guidance and supervision I would like to appreciate her keen observation and dedication.

I am really thankful to my parents who stood beside me for every support.

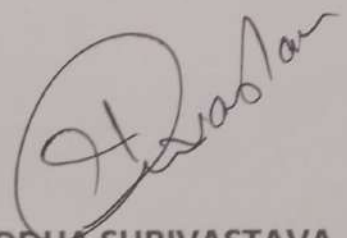
Date:

Vanshita Vishwani

B.B.A 3rd Year

CERTIFICATE

This is to certify that the Project Report entitled **"An Analysis of Financial Statements of Infosys ."** which has been submitted here for award of degree of Bachelors of business administration Jabalpur by **Vanshita Vishwani** of BBA 3rd year is done under our supervision on guide.



DR. SHRADDHA SHRIVASTAVA
(ASSISTANT PROFESSOR)
Dept. Of Management

DR. RASHMI PATRAS
(Head of the Department)

INTRODUCTION

Topic

Date : / /

ABOUT INFOSYS

Infosys Limited is an Indian multinational informational technology company that provides Bussiness Consulting, information technology and Outsourcing services. The Company was founded in Pune and is headquatered in Bangalore. Infosys is the second largest Indian IT Company after Tata Consultancy Services by 2020 revenue figures.

HISTORY

Infosys was founded by seven engineers in Pune, Maharastra, India Capital was $ 250. It was registered as Infosys Consultants Private Limited on 2 July 1961. In 1983 it relocated to Banglore Karnataka.

The Company charged its name to Infosys Technologies Private Limited in April 1992 and to Infosys Technologies Limited when it became a Public limited Company in June 1992. It was renamed Infosys Limited in June 2011

Infosys shares were listed on the Nasdaq Stock Exchange in 1999. It Became Became the first Indian Company to be listed on Nasdaq. The Share Price surged 8100 equivalent 30,000

Topic Date : / /

In 2012, Infosys announced a new office in Milwaukee, Wisconsin, to serve Harley Davidson Infosys hired 1200 United States employees in 2011 and expanded the workforce by 2000 employees in 2012 In April 2018. Infosys announced expansion in Indianapolis.

In July 14, Infosys started a Product subsidiary called EdgeVerve System, focusing on enterprise software Products for Business Operations, customer service, procurement and commerce network domains. In August 2015, assets from Finacle Global Banking Solutions were transferred from Infosys. thus Becoming Part of the Product company Edge Verve Systems Product Portfolio.

Products and Services

Infosys Provides Software development maintainance and Independent validation services to Companies in finance, insurance, manufacturing and other domains.

Its key Products are :-

1) Next Generation Intergrated AI Platform
2) Infosys Consulting - a global management Company Consulting service
3) Cloud Based enterprise transformation service
4) Digital Marketing
5) Panaya Cloud Suite

Topic Date : / /

Geographical Presence

Infosys has 82 Sales and Marketing offices and 123 development centres across the world as 31st Mar 18 with Major Presence in India, United States, China, Australia, Japan, Middle East and Europe

In 2019, 60%, 24% and 3% of its revenues were derived from Projects in North America Europe and India respectively. The remaining 13% of revenues were derived from rest of the World.

In 2022, Infosys Presence in Russia came under the Scrutiny. Infosys issued a clarification stating that they don't have active relationships with Russian firms. By November 2022, the only People working there were administrative staff helping with transfering the existing Contracts to the other Contractors.

Topic

Date : / /

ACQUISITIONS

Name of Acquired Company	Based In	Acquisition Cost	Business of Acquired Company
Expert Information System	Australia	US$23 million	IT Service Provider
Mc Camish System	U.S.A	US$38 million	Insurance Service.
Portland Group	Australia	AUD 37 Million	Strategic Sourcing
Lodestone	Switzerland	US$345 million	Management Consultancy
Panaya	Israel	US$200 million	Automation technology
Oddity	Germany	EUR 50 million	Digital Marketing
Simplus	USA-AU	TBD	Sales force Partner
Skava	USA	U$120 million	Digital Experience
Starter N.V	Netherland	EUR 127.5 million	Mortgage Services
Kaleidoscope	USA	US$42 million	Product Design Development

Topic

Date : / /

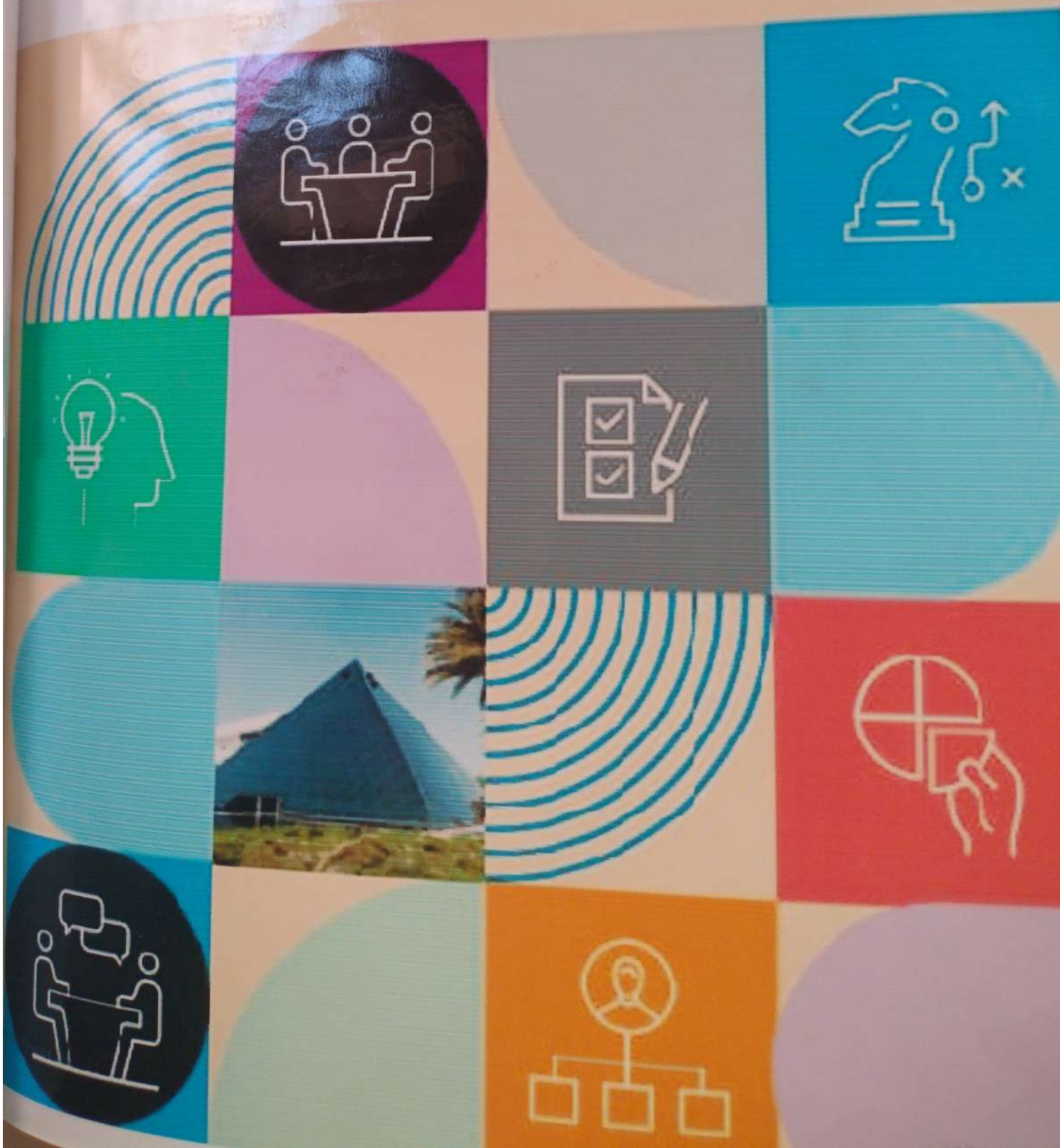
Listing and Shareholdings Pattern

In India, shares of Infosys are listed on BSE where it is Part of the BSE SENSEX and NSE where it is NIFTY 50 Constituent. Its shares are listed by way of American depository receipts at the New York Stock Exchange.

Over a period of time, the shareholding of its Promoters has gradually reduced, starting from June 1993 when its shares were first listed.

Shareholders	Shareholding
Promoters	13.95%
Foreign Institutions	31.64%
NB Banks Mutual Funds	15.44%
Others	12.18%
Retail	18.22%

DATA ANALYSIS



Topic Date : / /

Net Profit

Net Profit is the amount of money your Business earns after deducting all Operating, interest and tax expense over a given Period of time. If Value of Net Profit is Negative then it is called net loss.

FINANCIAL YEAR	AMOUNT (In Cr)
2017 - 18	16,155
2018 - 19	14,702
2019 - 20	15,543
2020 - 21	18,048
2021 - 22	21,235

Topic

Date : / /

Net Profit Before Tax (PBT)

Profit Before Tax is a measure that looks at a a Company's Profits before the Company has to Pay Corporate Income tax. Profit Before tax can be found on the Income Statement as Operating Profit minus interest.

FINANCIAL YEAR	AMOUNT
2017 - 18	19,908
2018 - 19	21,041
2019 - 20	22,007
2020 - 21	24,477
2021 - 22	28,495

Topic

Date : / /

GROSS TURNOVER

Turnover means the gross amount of revenue recognized in the Profit and loss account from the Sale, Supply, Or Distribution of goods or on account of services rendered or Both, by a Company during a financial year.

FINANCIAL YEAR	AMOUNT
2017 - 18	61,941
2018 - 19	73,107
2019 - 20	79,047
2020 - 21	1,00,472
2021 - 22	1,03,940

Topic

Date : / /

Current Ratio

This ratio compares a company's current assets to its current liabilities, testing weather its sustainably balances assets, financing and liabilities. Typically the current ratio is used as a general metric of financial health since it shows a company's ability to pay off short term debts.
Current ratio's of greater than 1.50 would indicate ample liquidity.

$$\text{Current Ratio} = \frac{\text{Current Assets}}{\text{Current Liabilities}}$$

YEAR	Current Assets	Current Liability	Current Ratio
2017-18	44,090	11,662	3.780
2018-19	46,233	15,430	2.996
2019-20	43,820	15,220	2.879
2020-21	48,282	17,622	2.739
2021-22	52,437	24,976	2.099

Topic

Date : / /

Net Profit Ratio

Net Profit Ratio, also referred to as the Net Profit Margin Ratio is a profitability ratio that measures the company's profits to the total amount of money brought into the Business.

$$\text{Net Profit Margin} = \frac{\text{Revenue} - \text{Cost}}{\text{Revenue}}$$

Topic Date : / /

Debt-Equity Ratio

The Debt-Equity ratio is a measure of the relative contribution of the creditors and shareholders or Owners in the capital employed in Business. Although it varies from Industry to Industry Debt to Equity ratio of 2·5 or 2 is generally considered good.

Debt to Equity =	Debt / Equity

YEAR	DEBT	EQUITY	RATIO
2017-18	713	63,502	0·0112
2018-19	789	62,711	0·0125
2019-20	3587	62,234	0·0576
2020-21	4786	71,531	0·06690
2021-22	5105	69,306	0·0736

Topic

Date : / /

Market Prospect Ratio

1. Price Earning Ratio

The Price to Earning Ratio is the ratio for valuing a company that measures its current share price relative to its earning per share (EPS). Also known as Price Multiple.

$$P.E. = \frac{\text{Stock Price}}{\text{Earnings Per Share}}$$

YEAR	Stock Price	EPS	Ratio
2017-18	565.90	35.68	15.860 times
2018-19	759.30	33.66	22.557 times
2019-20	636.25	36.34	17.50 times
2020-21	1362.55	42.37	32.158 times
2021-22	1903.55	50.27	37.86 times

RESEARCH METHODOLOGY

Topic

Date : / /

WHAT IS RESEARCH DESIGN?

Research Design is the frame work of research methods and techniques choosen by a researcher to Conduct a study. The design allows researchers to sharpen the research methods suitable for the subject matter and set up their studies for success.

Creating a research topic explain the type of research (experimental, survey research, correlational, semi-experimental, review) and its sub type (experimental design, research Problem, discriptive (case study).

There are three main types of designs for research.

- Data Collection.
- Measurement
- Data Analysis.

The Research Problem an Organisation face will determine the design not Vice Versa. The design phase of a study determines which tools to use and how they are used.

Topic

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- Surveys
- Questionnaires
- Test
- Databases
- Organisational Records.

This research Methodology is objective and is often quicker as researchers use software programs when analyzing data.

c) Mixed Method

This Contemporary research methodology combines quantative data and qualitative data approaches to provide additional prespectives, create a richer picture and present multiple findings. The Quantitative Methodology provides definitive facts and figures, while the Qualitative provides human aspect. This methodology can produce interesting results as it presents exact data while also being explanatory.

Topic Date : / /

OBJECTIVE OF THE STUDY

1 To Study the financial Report of Infosys

2 To Analyze the Various aspects of the financial Statement of Infosys.

3 To Interpret the result for the future decision.

Topic Date : / /

PROCESS OF RESEARCH DESIGN

The Research Design Process is a systematic and structured approach to conducting research. The Process is essential to ensure that study is Valid, realiable and Produces meaningful results.

1. Consider your aims and approaches.
2. Choose a type of Research Design.
3. Identify your population and Sampling Method.
4. Choose your Data Collection Methods.
5. Plan your Collection Procedures.
6. Decide on your Data Analysis strategies.

RESEARCH DESIGN ELEMENTS

Impactful research usually creates a minnimum bias in data and increases trust in the accuracy of Collected data. The essential elements are:

1 Accurate Purpose Statement
2 Techniques to be implemented for Collecting and Analysing research.
3. The method applied for analysing collected details
4 Type of research Methodology.
5. Probable Objections to research.
6 Timeline
7 Measurment of Analysis.

Topic Date : / /

TYPES OF RESEARCH METHODOLOGY

Here are different methodologies and their application:

a) Qualitative

Qualitative research involves collecting and analysing written or Spoken words and textual data. It may also focus body language or visual elements and help to create detailed description of a researchers observations. Researchers usually gather qualitative data through interviews observation and focus groups using a few carefully chosen participants.

This research methodology is subject and more time Consuming than using quantitive data. Researchers often use a qualitative methodology when the aims and Objectives of the research are explanatory.

b) Quantative

Researchers usually use a quantative methodology when the Objective of the research is to Confirm Something. It focuses on Collecting, testing and measuring numeric data. usually from a large sample of Participants. Popular methods used to gather are -

Topic

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WHAT IS RESEARCH METHODOLOGY ?

Research methodology is way of explaining how a researcher intends to carry out their research. It is a logical, systematic plan to resolve a research to ensure reliable, valid results that address their aims and objectives. It encompasses what data they're going to collect and where from, as well as how its being collected and analyzed.

IMPORTANCE OF RESEARCH METHODOLOGY

A Research Methodology gives research legitimacy and provides scientifically sounds findings. It also Provides a detailed plan that helps to keep researchers on track making the process smooth, effective and manageable. allows the reader to understand the approach and methods used to reach conclusions.

Having Sound research methodology provides following :

a) Other researchers who want to replicate the research have enough information to do so

b) Researchers who receive critisim can refer to the methodology and explain their approach.

Topic

Date : / /

Primary & Secondary Data

- **Primary Data.**
  Data that has been generated by the researcher himself / herself, Surveys interviews, experiments, specially designed to Understanding and Solving the research Problem at hand

- **Secondary Data**
  Using existing Data generated by large Government Institutions, healthcare facilities etc, as part of Organisational record keeping. The Data is then extracted from more Varied data files.

→ Secondary Data means data Collected
Data Collected by someone else earlier

→ It is a Past Data.
→ Quick and Easily Available.
→ Some Common Sources Government Publications, websites, books, journal articles, internal records etc.
→ Economical Method
→ Relatively less Accuracy and Realiability

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The data used in the analysis of financial statements of Infosys is considered as secondary data which means that it has been collected from sources other than original data source. In this case, Data has been obtained from realiable sources like Wikipedia. the company's website, and news articles of Economic Times. These sources are trusted and commonly used for financial performance.

Additionaly these sources are regularly updated ensuring that data used for analysis is current and relevant As such, the use of secondary data from realiable sources in analysis of Infosys financial statements and is considered essential for making informed investment decisions.

REVIEW OF LITERATURE

Topic

Date : / /

Infosys Recognized as One of the 2023 Worlds Most Ethical Companies for the Third Consective Year by Ethisphere

Recognized for demonstrating Business integrity through best in class ethics, compliance, and governance Practices

Bengaluru, India - Mar 13, 2023

Infosys, a Global leader in next Generation digital services and Consulting today announced that it has been recognized, by Ethisphere, a Global leader in defining and Advancing the standards of ethical business Practices, as one of the 2023 Worlds most Ethical Companies, for the third Consective year, for Demonstrating the high standards of Business integrity.

Infosys has been recognised among 135 honorees spanning 19 Countries and 46 industries. These Companies were evaluated based on Ethisphere Ethics Quotient.

"Ethics matter. Organisation that Commit to business integrity through robust Programs and Practices not only elevate standards and expectations for all, but also have better long term Performance" Said Ethisphere CEO

Topic

Date : / /

Nine Out of Ten Companies Lack the Culture and Organisational Structure to Unlock Digital Growth Infosys finds.

Infosys research reveals a blueprint for the 21st century enterprise focused on live Data, responsible risk taking and Product centricity

Bangalore, India Mar 7 2023

Only 7% of Companies have the correct Combination of Culture and Operating Structure to boost growth from digital technologies, according to new research from the Infosys Knowledge Institute, the thought leadership arm of Infosys.

Infosys is a Global leader in next generation digital Services and Consulting Over 3,00,000 of our People work to amplify human Potential and create the next Opportunity for the People Business and Communities. With Over 4 Decades of experience in managing the Systems we expertly steer Clients in more than 50 Countries, as they Navigate their digital tranformation.

Topic

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Infosys Foundation Collaborates with Several Social Organisations to Bolster Women Empowerment in India.

Grants totalling to INR 39.6 Crore to Propel women's education and employment in the country.

Bengaluru, India - March 8, 2023

Infosys Foundation, the Philantrophic and CSR arm of Infosys announced that it has signed Memorandum of Understanding in this financial year with Avanti Fellows, Nirmaan Organisation and Shrimad Rajchandra Atma Tattva Research Centre To Bolter Womens Empowerment in India These Strategic Collaborations are aimed at Providing Quality education and Employment Opportunities to Women from under privileged Communities across the country.

The Infosys foundations Strategic Collaboration for empowering women include :-

a) A Grant of INR 25.7 Crore to Avanti fellows to Provide free Engineering and medical test Preperation for 10,000 girl Students.

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b) A Grant of INR 4.9 Crore to Nirmaan Organisation to Create Purposeful livelihood for 5000 unemployed literate women from marginalised communities. This will help beneficiaries with Placements across Bangalore, Pune, Jaipur Gurugram

c) A Grant of INR 9 Crore to Shrimad Rajchandra Aatma Tatva Research Center for the construction of Hostle for under Privileged girl Students. Through this Collaboration Infosys Foundation aims to Provide Quality education to reduce school dropout rates among girl students

Infosys Foundation takes Pride in Working with all Sections of the Society with infinite care and working in areas that are traditionally overlooked by Society at large.

SAMPLE PROJECT REPORTS

2022-23

FACULTY OF SCIENCE

St Aloysius College Autonomous Jabalpur

PROJECT REPORT ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

PATRON
Rev. Dr. G. VazhanArasu
Principal

Department of Zoology
St. Aloysius' College (Autonomous), Jabalpur

PLACE OF WORK
Department of Zoology
St. Aloysius' College (Autonomous), Jabalpur

SUBMITTED BY
SONI YADAV
M.SC II SEMESTER

DEPARTMENT OF ZOOLOGY
Session 2022-23

DEPARTMENT OF ZOOLOGY

ST. ALOYSIUS COLLEGE, JABALPUR

DECLARATION

I **Soni Yadav** student of MSc IInd. Sem Zoology of St. Aloysius College (Autonomous), Jabalpur wish to state that, I have undergone project at Department of Zoology of St. Aloysius College (Autonomous), Jabalpur on the topic entitled: **"MENSTRUAL CYCLE-a biological indicator of health in girlsof rural area"** conducted during the month of January to march 2023 and this report has been prepared originally by me.

Soni

ST. ALOYSIUS COLLEGE, JABALPUR

ACKNOWLEDGEMENT

We respectfully thank **Dr. Fr. Vazhan Arasu**, Principal St. Aloysius College for giving us an opportunity to do the project work on "MENSTRUAL CYCE-a biological jindicator and providing us all support and guidance which made us complete this project on time. We are extremely grateful to him for providing such a nice support and guidance. We owe our profound gratitude to our project guide to head ,who took interest in our project work and guided us all along till the completion of our project work by providing all the necessary information for developing a good system. We would not forget to remember all the teachers of our Department of Zoology for their unlisted encouragement and more over for their timely support and guidance till the completion of our project work.

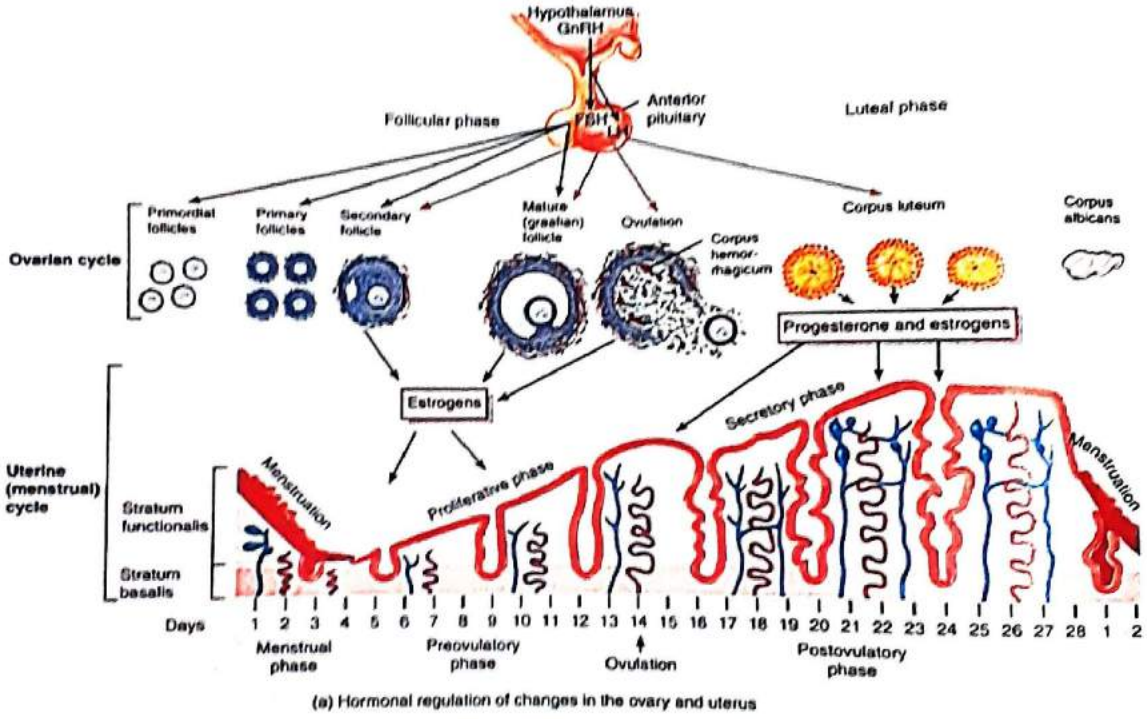
❖ INTRODUCTION-

Adolescence is the period of transition between puberty and adulthood. Menarche is one of the markers of puberty and therefore can be considered as an important event in the life of adolescent girls. Studies suggested that menarche tends to appear earlier in life as the sanitary, nutritional, and economic conditions of a society improve.For most females, it occurs between the age of 10 and 16 years; however, it shows a remarkable range of variation.

Menstruation is the shedding of the lining of the uterus (endometrium) accompanied by bleeding. Itoccurs in approximately monthly cycles throughout a woman's reproductive life, except during pregnancy. Menstruation starts during puberty (at menarche) and stops permanently at menopause. (Menopause is defined as 1 year after the last menstrual cycle.)
By definition, the menstrual cycle begins with the first day of bleeding, which is counted as day 1. The cycle ends just before the next menstrual period. Menstrual cycles normally range from about 24 to 38 days.
Only 10 to 15% of women have cycles that are exactly 28 days. Also, in at least 20% of women, cycles are irregular. That is, they are longer or shorter than the normal range. Usually, the cycles vary the most and the intervals between periods are longest in the years immediately after menstruation starts (menarche) and before menopause.
Normally, menstrual bleeding lasts 4 to 8 days. Blood loss during a cycle usually ranges from 1/5 to 2 ½ ounces. A sanitary pad or tampon, depending on the type, can hold up to an ounce of blood. Menstrual blood, unlike blood resulting from an injury, usually does not clot unless the bleeding is very heavy.
The menstrual cycle is regulated by hormones. Luteinizing hormone and follicle-stimulating hormone, which are produced by the pituitary gland, promote ovulation and stimulate the ovaries to produce estrogen and progesterone. Estrogen and progesterone stimulate the uterus and breasts to prepare for possible fertilization.
The menstrual cycle has three phases:

- Follicular (before release of the egg)
- Ovulatory (egg release)
- Luteal (after egg release)

The menstrual cycle begins with menstrual bleeding (menstruation), which marks the first day of the follicular phase. When the follicular phase begins, levels of estrogen and progesterone are low. As a result, the top layers of the thickened lining of the uterus (endometrium) break down and are shed, and menstrual bleeding occurs. About this time, the follicle-stimulating hormone level increases slightly, stimulating the development of several follicles in the ovaries. (Follicles are sacs filled with fluid.) Each follicle contains an egg. Later in this phase, as the follicle-stimulating hormone level decreases, usually only one follicle continues to develop. This follicle produces estrogen. Estrogen levels increase steadily.



(a) Hormonal regulation of changes in the ovary and uterus

The ovulatory phase begins with a surge in luteinizing hormone and follicle-stimulating hormone levels. Luteinizing hormone stimulates egg release (ovulation), which usually occurs 16 to 32 hours after the surge begins. The estrogen level decreases during the surge, and the progesterone level starts to increase.

During the luteal phase, luteinizing hormone and follicle-stimulating hormone levels decrease. The ruptured follicle closes after releasing the egg and forms a corpus luteum, which produces progesterone. During most of this phase, the estrogen

level is high. Progesterone and estrogen cause the lining of the uterus to thicken more, to prepare for possible fertilization.
If the egg is not fertilized, the corpus luteum degenerates and no longer produces progesterone, the estrogen level decreases, the top layers of the lining break down and are shed, and menstrual bleeding occurs (the start of a new menstrual cycle).
If the egg is fertilized, the corpus luteum continues to function during early pregnancy. It helps to maintain the pregnancy.

❖ OBJECTIVES –

- To understand the problems faced by girls during menstrual cycle.
- To find out which painkiller they use during menstrual cycle,
- To know the awareness among girls related to menstrual health
- Finding the solutions to improve menstrual health.

❖ MATERIAL & METHODS

- A cross sectional study was conducted on 100 menstruating girls of rural areas of KANPUR city of Uttarpradesh.
- The sample was collectedfrom the villages- chaudhripur,chittarpurwa,bithoor,neoraaz.
- Standarized self reporting questionnaires were used to obtain relevant data.The categorical data were analyzed using Bar graphs

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE-14

1. How old were you when you had your first period? Age (years) 10
2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☐24 - 30 days ☑30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes
5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes
6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☑1-3 days ☐more than 3 days
7. Do you experience the problem of bloating or gas during menstrual cycle? ☑No ☐Yes
8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑ 2or 3 days before menstrual cycle
☐ during the period of whole menstrual cycle
☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes
10. If "Yes" for above question then -

☐menstrual cramps/pain always hurts
☑menstrual cramps/pain hurts sometimes
☐menstrual cramps/pain doesn't hurt too much

11. From how many years you are experiencing menstrual cramps:

☐from the onset of menstrual cycle
☑from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days
13. Which painkiller do you take during menstrual pain/cramps?

☐Combiflam ☐Ibuprofean ☑Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days
15. Do you experience any side effects due to painkillers? ☑No ☐Yes
16. Do you Know about PCOD/PCOS? ☐No ☑Yes
17. Do you have the problem of PCOS or PCOD? ☑No ☐Yes
18. At what age you were diagnosed with PCOD/PCOS? _____
19. Do you use sanitary napkeen or cloth ? ☐No ☑Yes
20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE-20

1. How old were you when you had your first period? Age (years) 10
2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☐24 - 30 days ☑30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes
5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes
6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☑1-3 days ☐more than 3 days
7. Do you experience the problem of bloating or gas during menstrual cycle? ☑No ☐Yes
8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑2or 3 days before menstrual cycle
☐ during the period of whole menstrual cycle
☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes
10. If "Yes" for above question then -

☐menstrual cramps/pain always hurts
☑menstrual cramps/pain hurts sometimes
☐menstrual cramps/pain doesn't hurt too much

11. From how many years you are experiencing menstrual cramps:

☐from the onset of menstrual cycle
☑from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days
13. Which painkiller do you take during menstrual pain/cramps?

☐Combiflam ☐Ibuprofean ☑Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days
15. Do you experience any side effects due to painkillers? ☑No ☐Yes
16. Do you Know about PCOD/PCOS? ☐No ☑Yes
17. Do you have the problem of PCOS or PCOD? ☑No ☐Yes
18. At what age you were diagnosed with PCOD/PCOS? _____
19. Do you use sanitary napkeen or cloth ? ☐No ☑Yes
20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME- — AGE-23

1. How old were you when you had your first period? Age (years) 10

2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☐24 - 30 days ☑ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☑1-3 days ☐more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☑No ☐Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑2or 3 days before menstrual cycle

☐ during the period of whole menstrual cycle

☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes

10.If "Yes" for above question then -

☐menstrual cramps/pain always hurts

☑menstrual cramps/pain hurts sometimes

☐menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☐from the onset of menstrual cycle

☑from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☐Combiflam ☐Ibuprofean ☑Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days

15. Do you experience any side effects due to painkillers? ☑No ☐Yes

16.Do you Know about PCOD/PCOS? ☐No ☑Yes

17. Do you have the problem of PCOS or PCOD? ☑No ☐Yes

18. At what age you were diagnosed with PCOD/PCOS? _____

19.Do you use sanitary napkeen or cloth ? ☐No ☑Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE-22

1. How old were you when you had your first period? Age (years) 10
2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☐24 - 30 days ☑30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes
5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes
6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☑1-3 days ☐more than 3 days
7. Do you experience the problem of bloating or gas during menstrual cycle? ☑No ☐Yes
8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑2or 3 days before menstrual cycle
☐ during the period of whole menstrual cycle
☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes
10. If "Yes" for above question then -

☐menstrual cramps/pain always hurts
☑menstrual cramps/pain hurts sometimes
☐menstrual cramps/pain doesn't hurt too much

11. From how many years you are experiencing menstrual cramps:

☐from the onset of menstrual cycle
☑from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days
13. Which painkiller do you take during menstrual pain/cramps?

☐Combiflam ☐Ibuprofean ☑Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days
15. Do you experience any side effects due to painkillers? ☑No ☐Yes
16. Do you Know about PCOD/PCOS? ☐No ☑Yes
17. Do you have the problem of PCOS or PCOD? ☑No ☐Yes
18. At what age you were diagnosed with PCOD/PCOS? _____
19. Do you use sanitary napkeen or cloth ? ☐No ☑Yes
20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON
"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE-21

1. How old were you when you had your first period? Age (years) 10
2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☐24 - 30 days ☑30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes
5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes
6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☑1-3 days ☐more than 3 days
7. Do you experience the problem of bloating or gas during menstrual cycle? ☑No ☐Yes
8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑2or 3 days before menstrual cycle
☐ during the period of whole menstrual cycle
☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes
10. If "Yes" for above question then -

☐menstrual cramps/pain always hurts
☑menstrual cramps/pain hurts sometimes
☐menstrual cramps/pain doesn't hurt too much

11. From how many years you are experiencing menstrual cramps:

☐from the onset of menstrual cycle
☑from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days
13. Which painkiller do you take during menstrual pain/cramps?

☐Combiflam ☐Ibuprofean ☑Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days
15. Do you experience any side effects due to painkillers? ☑No ☐Yes
16. Do you Know about PCOD/PCOS? ☐No ☑Yes
17. Do you have the problem of PCOS or PCOD? ☑No ☐Yes
18. At what age you were diagnosed with PCOD/PCOS? _____
19. Do you use sanitary napkeen or cloth ? ☐No ☑Yes
20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 21

1. How old were you when you had your first period? Age (years) 10

2. Do you have regular periods? (Tick one box only)

☑ Yes ☐ No, they have never been regular ☐ No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐ Less than 24 days ☐ 24 - 30 days ☑ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐ No ☑ Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐ No ☑ Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☑ 1-3 days ☐ more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☑ No ☐ Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑ 2or 3 days before menstrual cycle

☐ during the period of whole menstrual cycle

☐ after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐ No ☑ Yes

10.If "Yes" for above question then -

☐ menstrual cramps/pain always hurts

☑ menstrual cramps/pain hurts sometimes

☐ menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☐ from the onset of menstrual cycle

☑ from last few years

12. For how many days the menstrual cramps/pain persist? ☑ 1-3 days ☐ more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☐ Combiflam ☐ Ibuprofean ☑ Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑ 1-3 days ☐ more than 3 days

15. Do you experience any side effects due to painkillers? ☑ No ☐ Yes

16.Do you Know about PCOD/PCOS? ☐ No ☑ Yes

17. Do you have the problem of PCOS or PCOD? ☑ No ☐ Yes

18. At what age you were diagnosed with PCOD/PCOS? _____

19.Do you use sanitary napkeen or cloth ? ☐ No ☑ Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐ No ☑ Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME: AGE: 20

1. How old were you when you had your first period? Age (years) 12

2. Do you have regular periods? (Tick one box only)

☐Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☐No ☑Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑ 2or 3 days before menstrual cycle

☐ during the period of whole menstrual cycle

☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes

10.If "Yes" for above question then -

☐menstrual cramps/pain always hurts

☑menstrual cramps/pain hurts sometimes

☐menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☑from the onset of menstrual cycle

☐from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☑Combiflam ☐Ibuprofean ☐Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days

15. Do you experience any side effects due to painkillers? ☑No ☐Yes

16.Do you Know about PCOD/PCOS? ☑No ☐Yes

17. Do you have the problem of PCOS or PCOD? ☐No ☐Yes

18. At what age you were diagnosed with PCOD/PCOS? _____

19.Do you use sanitary napkeen or cloth ? ☐No ☑Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 21

1. How old were you when you had your first period? Age (years) 12
2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes
5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes
6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days
7. Do you experience the problem of bloating or gas during menstrual cycle? ☐No ☑Yes
8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑2or 3 days before menstrual cycle
☐ during the period of whole menstrual cycle
☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes
10. If "Yes" for above question then -

☐menstrual cramps/pain always hurts
☑menstrual cramps/pain hurts sometimes
☐menstrual cramps/pain doesn't hurt too much

11. From how many years you are experiencing menstrual cramps:

☑from the onset of menstrual cycle
☐from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days
13. Which painkiller do you take during menstrual pain/cramps?

☑Combiflam ☐Ibuprofean ☐Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days
15. Do you experience any side effects due to painkillers? ☑No ☐Yes
16. Do you Know about PCOD/PCOS? ☑No ☐Yes
17. Do you have the problem of PCOS or PCOD? ☐No ☐Yes
18. At what age you were diagnosed with PCOD/PCOS? _____
19. Do you use sanitary napkeen or cloth ? ☐No ☑Yes
20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 24

1. How old were you when you had your first period? Age (years) 12

2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☐No ☑Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑2or 3 days before menstrual cycle

☐ during the period of whole menstrual cycle

☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes

10.If "Yes" for above question then -

☐menstrual cramps/pain always hurts

☑menstrual cramps/pain hurts sometimes

☐menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☑from the onset of menstrual cycle

☐from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☑Combiflam ☐Ibuprofean ☐Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days

15. Do you experience any side effects due to painkillers? ☑No ☐Yes

16.Do you Know about PCOD/PCOS? ☑No ☐Yes

17. Do you have the problem of PCOS or PCOD? ☐No ☐Yes

18. At what age you were diagnosed with PCOD/PCOS? _____

19.Do you use sanitary napkeen or cloth ? ☐No ☑Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE-18

1. How old were you when you had your first period? Age (years) 12
2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes
5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes
6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days
7. Do you experience the problem of bloating or gas during menstrual cycle? ☐No ☑Yes
8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑ 2or 3 days before menstrual cycle
☐ during the period of whole menstrual cycle
☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes
10. If "Yes" for above question then -

☐menstrual cramps/pain always hurts
☑menstrual cramps/pain hurts sometimes
☐menstrual cramps/pain doesn't hurt too much

11. From how many years you are experiencing menstrual cramps:

☑from the onset of menstrual cycle
☐from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days
13. Which painkiller do you take during menstrual pain/cramps?

☑Combiflam ☐Ibuprofean ☐Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days
15. Do you experience any side effects due to painkillers? ☑No ☐Yes
16. Do you Know about PCOD/PCOS? ☑No ☐Yes
17. Do you have the problem of PCOS or PCOD? ☐No ☐Yes
18. At what age you were diagnosed with PCOD/PCOS? _____
19. Do you use sanitary napkeen or cloth ? ☐No ☑Yes
20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 21

1. How old were you when you had your first period? Age (years) 10

2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☑No ☑Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑ 2or 3 days before menstrual cycle

☐ during the period of whole menstrual cycle

☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☑No ☐Yes

10.If "Yes" for above question then -

☐menstrual cramps/pain always hurts

☐menstrual cramps/pain hurts sometimes

☑menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☒from the onset of menstrual cycle

☒from last few years

12. For how many days the menstrual cramps/pain persist? ☒1-3 days ☐more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☐Combiflam ☐Ibuprofean ☐Meftal Any other painkiller —

14. For How may days you take painkiller? ☐1-3 days ☐more than 3 days

15. Do you experience any side effects due to painkillers? ☐No ☐Yes

16.Do you Know about PCOD/PCOS? ☐No ☑Yes

17. Do you have the problem of PCOS or PCOD? ☑No ☐Yes

18. At what age you were diagnosed with PCOD/PCOS? —

19.Do you use sanitary napkeen ~~or cloth~~ ? ☐No ☑Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 27

1. How old were you when you had your first period? Age (years) 29
2. Do you have regular periods? (Tick one box only)

☐Yes ☐No, they have never been regular ☑No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☑Less than 24 days ☐24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☑No ☐Yes
5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☑No ☐Yes
6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days
7. Do you experience the problem of bloating or gas during menstrual cycle? ☐No ☑Yes
8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☐ 2or 3 days before menstrual cycle
☑during the period of whole menstrual cycle
☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes
10. If "Yes" for above question then -

☐menstrual cramps/pain always hurts
☑menstrual cramps/pain hurts sometimes
☐menstrual cramps/pain doesn't hurt too much

11. From how many years you are experiencing menstrual cramps:

☐from the onset of menstrual cycle
☑from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days
13. Which painkiller do you take during menstrual pain/cramps?

☐Combiflam ☐Ibuprofean ☑Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days
15. Do you experience any side effects due to painkillers? ☑No ☐Yes
16. Do you Know about PCOD/PCOS? ☐No ☑Yes
17. Do you have the problem of PCOS or PCOD? ☐No ☑Yes
18. At what age you were diagnosed with PCOD/PCOS? 21
19. Do you use sanitary napkeen ~~or cloth~~ ? ☐No ☑Yes
20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 26

1. How old were you when you had your first period? Age (years) 10

2. Do you have regular periods? (Tick one box only)

☐Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☐24 - 30 days ☑ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☑No ☐Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☑No ☐Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☑1-3 days ☐more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☐No ☑Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☐ 2or 3 days before menstrual cycle

☑during the period of whole menstrual cycle

☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☑No ☐Yes

10.If "Yes" for above question then -

☐menstrual cramps/pain always hurts

☐menstrual cramps/pain hurts sometimes

☑menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☐from the onset of menstrual cycle

☐from last few years

12. For how many days the menstrual cramps/pain persist? ☒1-3 days ☒more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☐Combiflam ☐Ibuprofean ☐Meftal Any other painkiller_____

14. For How may days you take painkiller? ☐1-3 days ☒more than 3 days

15. Do you experience any side effects due to painkillers? ☒No ☐Yes

16.Do you Know about PCOD/PCOS? ☐No ☑Yes

17. Do you have the problem of PCOS or PCOD? ☐No ☑Yes

18. At what age you were diagnosed with PCOD/PCOS? 23

19.Do you use sanitary napkeen ~~or cloth~~ ? ☐No ☑Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 22

1. How old were you when you had your first period? Age (years) 12

2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☐No ☑Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑2or 3 days before menstrual cycle

☐ during the period of whole menstrual cycle

☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes

10.If "Yes" for above question then -

☐menstrual cramps/pain always hurts

☑menstrual cramps/pain hurts sometimes

☐menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☑from the onset of menstrual cycle

☐from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☑Combiflam ☐Ibuprofean ☐Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days

15. Do you experience any side effects due to painkillers? ☑No ☐Yes

16.Do you Know about PCOD/PCOS? ☑No ☐Yes

17. Do you have the problem of PCOS or PCOD? ☐No ☐Yes

18. At what age you were diagnosed with PCOD/PCOS? _____

19.Do you use sanitary napkeen or cloth ? ☐No ☑Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 26

1. How old were you when you had your first period? Age (years) 12

2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☐No ☑Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑2or 3 days before menstrual cycle

☐ during the period of whole menstrual cycle

☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes

10.If "Yes" for above question then -

☐menstrual cramps/pain always hurts

☑menstrual cramps/pain hurts sometimes

☐menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☑from the onset of menstrual cycle

☐from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☑Combiflam ☐Ibuprofean ☐Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days

15. Do you experience any side effects due to painkillers? ☑No ☐Yes

16.Do you Know about PCOD/PCOS? ☑No ☐Yes

17. Do you have the problem of PCOS or PCOD? ☐No ☐Yes

18. At what age you were diagnosed with PCOD/PCOS? _____

19.Do you use sanitary napkeen or cloth ? ☐No ☑Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE-24

1. How old were you when you had your first period? Age (years) 10

2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☐24 - 30 days ☑30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☑1-3 days ☐more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☐No ☑Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑ 2or 3 days before menstrual cycle

☐ during the period of whole menstrual cycle

☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes

10.If "Yes" for above question then -

☐menstrual cramps/pain always hurts

☑menstrual cramps/pain hurts sometimes

☐menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☐from the onset of menstrual cycle

☑from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☐Combiflam ☐Ibuprofean ☑Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days

15. Do you experience any side effects due to painkillers? ☑No ☐Yes

16.Do you Know about PCOD/PCOS? ☐No ☑Yes

17. Do you have the problem of PCOS or PCOD? ☑No ☐Yes

18. At what age you were diagnosed with PCOD/PCOS? _____

19.Do you use sanitary napkeen or cloth ? ☐No ☑Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 19

1. How old were you when you had your first period? Age (years) 17

2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☐No ☑Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

☑2or 3 days before menstrual cycle

☐during the period of whole menstrual cycle

☐after end of menstrual cycle

9. Do you experience the menstrual cramps/pain? ☐No ☑Yes

10.If "Yes" for above question then -

☐menstrual cramps/pain always hurts

☑menstrual cramps/pain hurts sometimes

☐menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☑from the onset of menstrual cycle

☐from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☑Combiflam ☐Ibuprofean ☐Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days

15. Do you experience any side effects due to painkillers? ☑No ☐Yes

16.Do you Know about PCOD/PCOS? ☑No ☐Yes

17. Do you have the problem of PCOS or PCOD? ☐No ☐Yes

18. At what age you were diagnosed with PCOD/PCOS? _____

19.Do you use sanitary napkeen or cloth ? ☐No ☑Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 21

1. How old were you when you had your first period? Age (years) 11

2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months the next)?

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☐No ☑Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes

6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days

7. Do you experience the problem of bloating or gas during menstrual cycle? ☑No ☐Yes

8. If YES (for above question then)then ,you experience problem of gas or bloating during:

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9. Do you experience the menstrual cramps/pain? ☐No ☑Yes

10.If "Yes" for above question then -

☒menstrual cramps/pain always hurts

☒menstrual cramps/pain hurts sometimes

☒menstrual cramps/pain doesn't hurt too much

11.From how many years you are experiencing menstrual cramps:

☐from the onset of menstrual cycle

☑from last few years

12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days

13. Which painkiller do you take during menstrual pain/cramps?

☑Combiflam ☐Ibuprofean ☐Meftal Any other painkiller_____

14. For How may days you take painkiller? ☑1-3 days ☐more than 3 days

15. Do you experience any side effects due to painkillers? ☐No ☑Yes

16.Do you Know about PCOD/PCOS? ☐No ☑Yes

17. Do you have the problem of PCOS or PCOD? ☑No ☐Yes

18. At what age you were diagnosed with PCOD/PCOS? _____

19.Do you use sanitary napkeen or cloth ? ☐No ☑Yes

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☑No ☐Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 16

1. How old were you when you had your first period? Age (years) 9

2. Do you have regular periods? (Tick one box only)

☐Yes ☐No, they have never been regular ☑No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

4. Do you experience the symptoms of Anger or irritability the days before or around your menstrual periods? ☑No ☐Yes

5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☑No ☐Yes

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☑No ☐Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE- 22

1. How old were you when you had your first period? Age (years) 11

2. Do you have regular periods? (Tick one box only)

☐Yes ☐No, they have never been regular ☑No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

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20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☐No ☑Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE-17

1. How old were you when you had your first period? Age (years) 11
2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

☐Less than 24 days ☑24 - 30 days ☐ 30 &More than 35 days

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5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes
6. For how many days you experience mood swings,anger or irritability ot anxiety or depression before and after menstrual cycle? ☐1-3 days ☑more than 3 days
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☐menstrual cramps/pain doesn't hurt too much

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12. For how many days the menstrual cramps/pain persist? ☑1-3 days ☐more than 3 days
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20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☑No ☐Yes

SURVEY BASED ON

"MENSTRUAL CYCLE-a biological indicator of health in girls of rural area"

NAME-_____ AGE-15

1. How old were you when you had your first period? Age (years) 10

2. Do you have regular periods? (Tick one box only)

☑Yes ☐No, they have never been regular ☐No, they have been irregular for a few months

3. What is the usual interval between your periods? (from the first day of one period to the first day of the next)? (Tick one box only)

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5. Do you usually experience Anxiety or depression, the days before or around your menstrual periods? ☐No ☑Yes

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19.Do you use sanitary napkeen or cloth ?

20. Do you experience itching or other symptoms in intimate area, during or after the menstrual cycle?

☑No ☐Yes

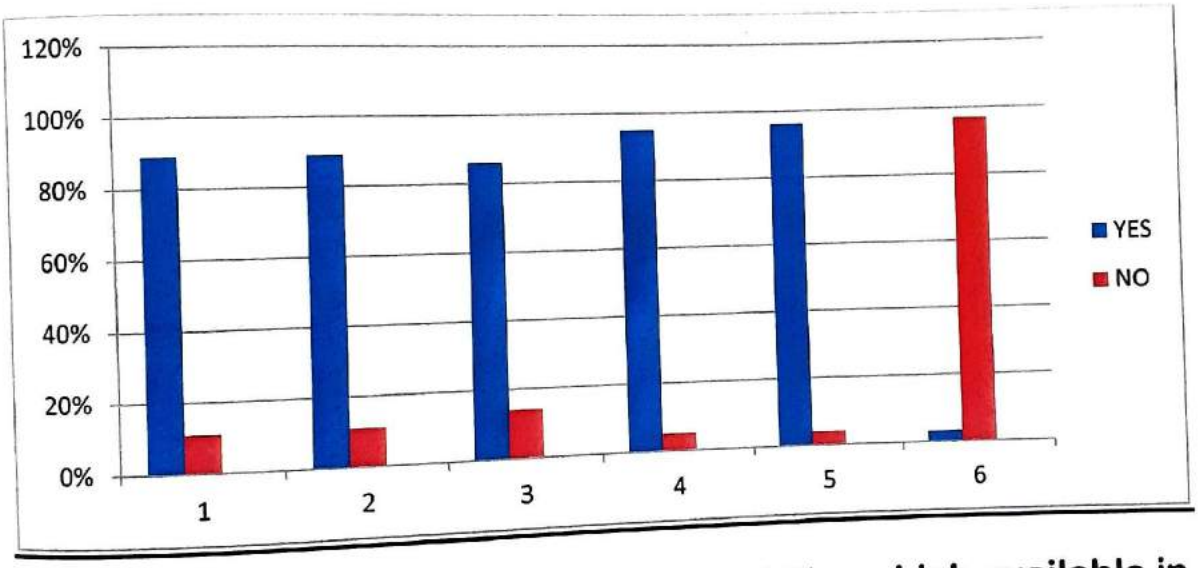
❖ OBSERVATION & RESULT

A total of 100 valid responses were successfully obtained. The majority of respondent were females above age 20 years. Mean age of menarche was 10.

The responses of the survey report are as follows: Ques:)

i. Symptoms of anger or irritability
ii. Symptoms of anxiety or depression
iii. Problem of bloating and gas during menstrual cycle
iv. Do they experience menstrual cramps
v. Do they take painkiller
vi. Do they know about PCOD/PCOS

THE RESPONSES (YES OR NO) ARE AS FOLLOWS IN THE FORM OF GRAPH



Most of the people take the normal painkiller which available in their nearby shop. Here Combiflam is the most common painkiller followed by ibuprofean and meftal. Around 80% girls face the problem of menstrual pain and stomach related issues like bloating etc. Around 10% of girls in rural areas use cloth instead of sanitary napkeen. Almost all the girls encounter the problem of menstrual pain, itching and irritation in intimate areas.

CONCLUSION

Menstruation and menstrual health issues which is one of the major areas of concern in reproductive health affects a large number of women throughout their reproductive life from adolescence. The present study was conducted to explore the menstrual characteristics among the unmarried adolescents across different age groups (early and late adolescence) and to find out association with menstrual pattern.

Dysmenorrhea and menstrual irregularity are more prevalent among adolescent females. Common menstrual symptoms are tiredness, mood swings,anxiety,irritability,anger. It appears that occurrence of dysmenorrhea is increasing in the population; such sufferings would affect the productivity among females.

Therefore, it can be stated that a comprehensive school education program , awareness campaign on menarche and menstrual problems,regular and free medical checkups in rural areas may help girls to cope better and seek proper medical assistance.

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www.mayoclinic.org
www.clevelandclinic.org
www.betterhealth.vic.gov

STRAINS FROM BIOFILMS COLLECTED FROM THE HISTORI
SITE -CHOSATH YOGINI MANDIR"

A

Dissertation

SUBMITTED FOR DEGREE OF

MASTER OF SCIENCE

IN

MICROBIOLOGY

UNDER THE GUIDANCE OF

PROF. SURENDRA SINGH

RANI DURGAVATI VISHWAVIDYALAYA, JABALPUR (M.P.)

EPARTMENT OF POST GRADUATE STUDIES AND RESEARCH
BIOLOGICAL SCIENCE

CO-GUIDANCE OF

DR. (MRS.) SONALI NIGAM

ST. ALOYSIUS' COLLEGE (AUTONOMOUS)

JABALPUR

SUBMITTED BY

Miss. ANKITA MARAVI

M.Sc. IV SEM (MICROBIOLOGY)

2022-23

My Beloved Family And Respected Teachers

ST. ALOYSIUS' COLLEGE
(AUTONOMOUS), JABALPUR (M.P.)
Reaccredited 'A+' Grade by NAAC (CGPA 3.68/4.00)
College with Potential for Excellence (CPE) by UGC
DST-FIST Supported

CERTIFICATE

This is to certify that the Dissertation work embodied in this thesis entitled **REENING, SELECTION AND ISOLATION OF CYNOBACTERIAL STRAINS FROM BIOFILMS LECTED FROM THE HISTORICAL MOUNMENTS - Chosath Yogini Mandir"** is submitted miss. **Ankita Maravi** for partial fulfillment of the degree of Master Of Science In robiology 2022-23. The Dissetation has been duly completed under the supervision and co-lance of **Dr. (Mrs.) Sonali Nigam** HOD Department of Botany And Microbiology **ST. YSIUS COLLEGE (AUTONOMOUS) JABALPUR (M.P.)**

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CERTIFICATE

This is to certify that the dissertation work embodied in this thesis entitled **"Screening, Selection And Isolation Of Cynobacterial Strains From Biofilms Collected From The Historical Mounments - Chosath Yogini Mandir"** has been submitted by miss. **Ankita Maravi** from the partial fulfillment of the degree of Master Of Science In Microbiology 2022 from **St. Aloysius College (Autonomous) Jabalpur (M.P.)** Under the supervision of **Dr. (Mrs.)Sonali Nigam** (HOD) Department of Botany And Microbiology st. Aloysius collage (autonomous), jabalpur (m.p.). This work is original and no part of this theises has been submitted for the award of anyother diploma or degree to any other university or organization

Date :

Place : Jabalpur (M.P.)

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Reference no. .....

Date-.....

CERTIFICATE

This Is To Certify That The Dissertation Entitled **"Screening, Selection And Isolation Of Cynobacterial Strains From Biofilms Collected From The Historical Mounments - Chosath Yogini Mandir"** Has Been Submitted By **ANKITA MARAVI** A Student Of M. Sc. Microbiology St. Aloysius' College (Autonomous) Jabalpur (M.P.) For The Academic Year 2023. He Has Duly Completed This Dissertation Under My Supervision And Guidance. This Dissertation Is Being Submitted For The Degree Of "Master Of Science" In Microbiology At The Department Of Post Graduate Studies And Research In Biological Sciences, Rani Durgavati Vishwavidyalaya, Jabalpur, (M. P).

26/6/2

DATE:

Place:

Prof. Dr. Surendra Singh
(Supervisor)

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My Sincere thanks to Principal of ***Dr. Fr. G Vazhan Arasu St. Aloysius College (Autonomous) Jabalpur (M.P.)****& co-guidance of* ***Dr.(Mrs.) Sonali Nigam*** *HOD Department of Botany And Microbiology* ***St. Aloysius College (Autonomous) Jabalpur (M.P.)*** *Permitting me to carry out my dissetation work from RDVV JABALPUR.*

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I feel immense pleasure and privilege to express my deep sense of gratitude for this constant encouragement and guidance during my dissertation, to ***Prof. S.N. Bagchi,*** *Head Of The Department Of Biological Science,* ***Prof. Divya Bagchi Prof. S.S. Sandhu*** *Rani Durgavati Vishwavidyalaya, Jabalpur, (M.P.) for providing the facilities and encouragement. I sincerely thank* ***Dr. Sunil Chaudhary,*** *of algal Biotechnology Laboratory, Department Of Post Graduate Studies And Research In Biological Sciences, Rani Durgavati Vishwavidyalaya, Jabalpur, for his advice and constant support.*

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The completion of my work would not have been possible without the contribution of my lab-mates. lastly and most importantly, I owe all my thanks to my beloved parents, and sisters for their unconditional love and support throughout my life.

DATE:-

PLACE: JABALPUR

Ankita

MISS. Ankita Maravi

ABBREVIATIONS

% .....Percentage

H.....Hour Centimetre

G .....Gram

H.....Hour

IAA.....Indole acetic acid

Kg.....Kilogram

M.P.....Madhya Pradesh

Mg.....Miligram

N2.....Nitrogen

S.No.....Serial number

CONTENTS

CHAPTER-1
INTRODUCTION

INTRODUCTION

Cyanobacteria successfully colonize almost all illuminated environment including some of the most hostile for life (Stal 2007) . The presence and deteriorating action of microorganisms on monuments and stone work of art have received considerable attention in the last few year . Stone monuments statues and historic building are exposed to the effects of physical chemical and biological deteriorating factors . This review will foucs on the damages caused by micro-organisms . Due to their photo autotrophic nature these micro-organisms develop easily on stone surface , giving rise to coloured patinas and incrustation (Tomaselli et al. 2000) . For Cyanobacteria ,Infact the colonization of stone is closely correlated with porosity. Roughness ,hygroscopicity and capillary water obsorption , which strongly influence water availability for micro – organisms (Urzi&Ralini 1998; prieto&silva 2005) . Physico - Chemical characteristics of the material favour the establishment of the material favour the establishment of photo synthetic communities at depths that also depend on external environmental factors, especially light , Which influences the total biomass of the community(Saiz Jimenez 1995).

The mechanical fracturing and disintegration of natural stone substrates by lithobionts and corrosion through the metabolic processes of micro -organisms the the greatest biodeterioration threats to the conservation of stone based cultural heritage , How everundersired bio-logical colonisation of stone materials can also lead to unwanted aesthetic changes such as unsightly discoloration that cover art details (Warscheid and braam , 2000 charola et al ,2011; Dias et , al 2020 ;faverolango and viles 2020).

The open air condition of exposure of building and monuments induce a large influence of climatic factors on the bio weathering by changing of sunlight radiation , temperatures and rainfall which vary with respect to their geographical location and ongoing climate change. Underground cultural heritage like caves in the natural state , generally have a weak connection with the external atmosphere (Sanchez –Moral et al , 2021) and are considered to be extreme environment for microbial growth , due to the low nutrient availability simon et al, 2007) . In such evironmentschemolithoautotrophic organisms can thrive on stone surface as a result of stable conditions contant air and solute water temperature high moistureand

solute rich groundwater (Bastian and Alabouvette , 2009). How ever , underground caves now represent appealing tourist attraction (Cigna and fort 2013) and the introduction of lighting equipment can rapidly and significantly alter previously stable environmental conditions by increasing ambient temperature and CO2 concentration and decreasing relative humidity (Mulec el al 2012 , BaqnedanoEstenaz et al , 2019 ;Caneva et al 2020) Furthermore intensified human activity also bring organic matter and new strains of micro –organisms from outside which gradually adapt to subterranean environments(Mulec 2014 Marques et al 2016).One reason for this is that inereasedgeotourism can foster the perfect combination of microbes the typically thrive on the surface along with the need for well –lit and illuminated areas , meanings that phototrophic oganisms (Bastian et al ,2010 Albertano 2012 Perez 2018).

After studying it was found that In other case it has been shown that pigmentation changes in response to environmental factors including light intensity, light quality, nutrient availability, temperature and the age of cells (Bartonlini et al 2004) . That have thick shealths with intense colour being the expression of different ecology stages and environmental adaptations, Cyanobacteria and chlorophyta colonize a wide varicty of substrata and that this is related primarily to physical characteristics of the surface microclimate and environmental conditionsand secondary to the lithotype. Colour and aesthetical and physic chemical damages. The bio colonization is dependent of the material and on the environment .The response is more intense in winter as temperature islower and relative humidity (RH) higher. Cyanobacteria are less sensitive to this seasonal effect as they are more resistant to desiccation than green algae . A considerable number of green algae (Chlorophyta) having adapted to life on land . The Chlorophyta constitute the most common group of algae colonizing stone cultural heritage (Ortega – Calvo et al 1993).

Microbial geochemical cycles have been going on since life on earth began. This activity results in chemical and mineral changes of the different types of rocks and minerals within the biosphere and involves essentially the solubilisation processes of major mineral element from silicates ,carbonates , phosphates ,oxides etc.

While the weathering of minerals is natural environments results in the destruction of rocks, thus contributing to soil formation process, it has a deleterious effect when these rocks are part of the historical buildings and monuments.

cyanobacteria) and to chemoorganotrophic and phototrophic eukaryotic microorganisms such fungi and algae. The most important factors are, however, ecological or geophysical equilibria between rock exposure and the biological surroundings. Microbial mats and microbial films, often called Biofilms, grow practically on and in all stones exposed for some time to the atmosphere. Through their growth, they can changethe chemical and mineralogical composition of the original rock. They change the stability, permeabilit1ryand colour of the stone as well as the density. Microbial mats can create crusts inside and outsidethe original stone material. Lithobionts(epiliths and endoliths) also have an important impact on building stone andalter the stone stability. The colour change is usually caused by oxidation pro some rare cases, bleaching orreduction were also observed. Usually, the micro flora to nearthe surface.

Biological alterations differ according to the ecological peculiarities such as the substrate, the nature of the microorganisms or organisms involved, and the characteristics of environment where the artwork is located (micro- and macro-environment, atmospheric pollution). Frequently, these alterations may by clearly recognized and correlated to the presence of living organisms. In other cases it may be impossible to recognize a biological regent involve with the naked eye and only the results of analyses will identify the deterioration The weathering of building stone is a natural process in which physical, chemical and biological agents are involved. Weathering mobilizations and anions that are needed by all life forms. But, deterioration(weathering) of rock surfaces, of building stones and prehistoric/historic monuments has accelerated recently, and it has now become important to understand the reasons for the actual weathering processes.

THIS IS CONSEQUENT WITH THEIR WAY OF COLONATION OF BUILDING STONE SURFACES

EPILITHIC CYANOBACTERIA

The cyanobacteria, because of their ability to perform oxygenic photosynthesis, areconsidered as phototrophic bacteria cultural studies are somewhat uselessfor ecological purpose as opposed to the traditional botanical classification using collected field.All the reports on historic buildings with one exception use the botanical classification when dealing taxonomically with the cyanobacteria.

Cyanobacteria often develop abundantly on damp, porous, and particularly calcareous rocks. All the epilithic Cyanobacteria cited with rare exceptions, arecommon in soils and corticolous (tree trunk) habitats. The genera cited include unicellularas well as filamentous forms. The unicellular forms are either ensheathed*(Aphanothece,Chroococcus, Gloecoapsa, Gomphosphaeria, Mixosarcina)* or lack a visible sheath (*Synechococcus*). The filamentous ones also include ensheathed (*Lyngbya, Microcoleus, Phormidium, Schizotrix*) and unsheathed forms *(Oscillatoria);* they may have false or true branching *(Stigonema*), and heterocysts (*Calothrix, Nostoc, Scytonema*). Extensive taxonomy of the cyanobacteria present and found that they dominate over diatoms and green algae. It was observed that most of the cyanobacteria present possessed visible gelatinous sheaths. This also happened with the cyanobacteria from other monuments.The sheath in terrestrial cyanobacteria acts as a reservoir of water, where it is bound throughstrong molecular forces ,sheath production by the cyanobacteria has been consideredof importance for stability of the substrate, acting as a cementing agent and increasing and increasing is physical properties.

ENDOLITHIC CYANOBACTERIA

Very few studies on historic buildings report the presence of endolithic cyanobacteria. (Saiz-Jimenez et al. (1990a)). Found a cryptoendolithic microbial community developed as a green layer 1 mm below the surface of a limestone from the Basilica of Tongeren, Belgium dominated by unicellular cyanobacteria . Their presence was confirmed by microscopic techniques as well as by chemical characterization of theCyanobacteria and Algae on historic Buildingsorganic matter present in the green layer. The importance has been stressed of the use of chemical signatures (biomarkers) in obtaining valid measurements of the biomass and metabolic activities of microbial ecosystems . Analytical pyrolysis has been used for the chemical characterization of the endolithic communities. Specific cyanobacterial biomarkers, such as 7-methylheptadecane, were identified. These communities resembled those found in semi-desert and cold temperate biomes. An endolithic filamentous cyanobacterium (*Phormidium sp.)* was also found growing under black sulphated crusts developed on limestones in the Cathedral of Seville, Spain. The endolithic active borer Hyellafontana was found in marble statues in Rome, and it appeared that this cyanobacterium had a role in the decay of the stone.

CHAPTER-2
REVIEW OF LITERATURE

Cyanobacteria and algae are commonly found on building in humid places growing on cornices, in holes and crevices ore beneath crusts, where water is retained and evaporation is slow due to protection against winds or direct sunshine . Furthermore, growth may also be due, for example, to leaking or badly sited roof guttering, inadequate drainage of flat areas, frequently forming streaks that follow the areas of dampness. Their growth is rarely uniform, frequently forming streaks that follow the areas of dampness. Their presence is more apparent on the north faces of building than the south because the latter dry out more readily.

Only water and a minimal supply of mineral salts are required for colonization. It is the duration of the period of wetness that is mineral that is crucial, rather than the frequency of wetting in predisposing a surface to colonization. Inoculation is more rapid where there is adjacent or over changing vegetation form which cyan bacteria and algae can be brought by wind and rain and development is further accelerated if bird droppings agricultural fertilizer and pollution-derived nitrogen oxides introduce additional nitrogen and phosphorous Rough or porous surfaces facilitate attachment of air-borne propagules and the accumulation of nutrients. Although cyanobacteria and algae are phototrophic organisms, deriving their energy for growth form light, they can also be found is very poorly illuminated of nutrients. Although cyanobacteria and algae are phototrophic organisms, deriving their energy for growth from light, they can also be found in very poorly illuminated places inside building .

The presence of cyan bacteria and algae on and in a stone can be studied using different techniques. They can be investigated in situ with optical and electron microscopy, which supply basic information about these microscopic organisms and their surrounding micro-environment. Scanning electron microscopy has been proved to be very useful in the study of lithobionticmicroorganisms . Differential interference contrast optics and epifluorescence can also be used for in situ observation . Enrichment culture techniques (with mineral culture media that favour the growth of cyanobacteria and algae) organisms using standard microbiological techniques, may give additional information about relevant taxonomic characters absent in the direct microscopic observations of the field samples.

A quantitative approach consists of the estimation of cyanobacteria / algae biomass, which has been made with viable count methods, including plate counts and most probable number methods.These obsolete methods have some disadvantages, such as eventual selectivity of dormant microorganisms and differential propagule production by the species of the community. The biomass of lithobiontic cyanobacteria and algae can also be estimated through chlorophyll extraction technique. Although the chlorophyll content can change with the physiological state of the cells, this method has been suggested to be the most reliable method for quantifying soil algae(Tino et al.). Applied the ATP-bioluminescence method measuring the ATP levels of the whole microbial community, to several samples of a stone monument that contained microbial community to several with a green patina.They suggested the suitability of this method for detecting and quantifying the presence of microorganisms in a sample of stone.

MICROBIAL POPULATIONS ON STONE :-

The microbial colonization of stone is considered to start with phototrophic organisms which build up avisible biofilms of enriched organisms biomass on the stone surface.The growth and metabolic activity of these algae, cynobacteria and liches as well as mosses and higher plants is regulated by parameters such as light and moisture.

Phototropic microorganisms may grow on the stone surface or may penetrate some millimetres into the rock pore system. It used to be believed that phototrophic micro organisms caused only asetheric damage to stone surfaces associated with their pigment and did not have any direct effect on the deterioration of such stones.

The accumulation of photosynthetic biomass provides an excellent organic nutrient base for subsequent heterotrophic microbiota and their biodeterioration activities.

THE ROLE OF CYANOBACTERIA IN THE DEGRADATION HISTORICAL MONUMENTS:-

Cyanobacteria colonize a wide varity of terrestrial habitats, including rocks, hot and cold desert crusts , as well as modern and ancient building. The role of cyanobacteria in the deterioration of surface of historic building has been the subject of several recent studies.

The increase in local ph level in phototropic bio-films throws some doubts on the acid degration suggested to be produced by algae and cyanobacteria even though (Van der Oost et al) , showed that Cyanoacteria other carries out mixed acid fermentation and could , therefore, induce acid degradation of stone. However, there is little evidence that cyanobacteria produced acid in situ and Waterbury, suggest that it is the associates heterotrophic bacteria that lead to acid decay of calcareous materials in the presence of cyanobacteria, as also show by Friedmann and weed. All of which contribute to their protection against desiccation and intense solar radiation. Due to their phototrophic nature and many being nitrogen fixers, cyanobacteria colonize easily on exposed surfaces that in due course lead to formation of patinas and incrustations causing aesthetic damage.

Cyanobacteria being important from evolutionary and ecological point of view, the mode of their diversity analysis and taxonomy are changing with recent information and techniques. Very little information is available on identification and phylogeny of cyano- bacteria species from Indian environments following molecular approach (Sahu and Adhikary, 2012; Keshari and Adhikary, 2013). Organisms occurring in desiccated state on sub-Baterial surfaces are difficult to identify following available monographs as seldom the morphological features of the species can be seen even after prolong wetting of the natural material.

DETERIORATION MECHANISMS

Apart from the unaesthetic appearance, evident in most of the reports on cyanobacteria and algae on historic buildings, there are references in the literature that point to direct decay mechanisms. In fact, it has been reported that epilithic cyanobacteria may play a role in rock surface weathering in nature through various effects upon the carbonate dissolution system. The presence of a layer of microorganism on the rock surface will lead to the development of a distinctive microenvironment, where respiration and photosynthesis will affect partial pressures of CO2 over the diurnal cycle, and acids may be produced as metabolic by products. Furthermore, substrate utilization by developing microbial communities increases proportionally with thickness, up to a critical point where nutrient diffusion through the biofilm becomes limiting, leading to

anoxic and reducing conditions within the biofilm and close to the substratum surface.

Cyanobacteria and Algae on Historic Buildings obtain nutrients. Some algal over growths, produce organic acids which corrode rock surface.Epilithic cyanobacteria may also act upon rock weathering in nature by influencing precipitation and deposition of calcium carbonate. Some species of the genera *Rivularia, Schizothrix, Geitleria, Plectonema and Microcoleus* are capable of directly precipitating calcium carbonate on Species without this capability may still encourage precipitation of calcium carbonate by providing nuclei for the crystallization of dissolved calcium carbonate, or by trapping particulatecarbonate material. These precipitating roles have been observed in intertidalcyanobacterial mats and on tufa deposits, but may also operate on terrestrial surfaces where epilithic growths are thick enough.

Cyanobacteria and algae may also have a role in the mechanical biodegradation of the stone , exerting considerable force through repeated shrinking and relaxation when they are going through cycles of drying and moistening . This is mainly attributed to the mucilage formed by the sheaths of the cyanobacteria and algae, which adheres them to the substrate and suffers deep changes in volume due to its water retention properties. The sheath allows the establishment of a self-contained aquatic environment within which the cells can attain adequate irradiation and gaseous exchange. In addition, the presence of this water reservoir with a high water potential ensures that the cells remain essentially aquatic, thus permitting the cyanobacteria and algae to overcome drought periods. Through sheath contraction and expansion, they can loosen rock grains, constituting a possible factor in the gradual destruction of the rocks. The formation of crusts induced by cyanobacterial and algal growth results in a longer moisture retention at the surface of the stone, increasing the mechanical damage produced by the freezing and thawing of water present in the pores ofthe stone.

ERADICATION AND CONTROL

Methods for the eradication and control of cyanobacteria and algae on constructional materials and painted surfaces have been extensively reviewed and the effects of biocides on soil.

Chausath Yogini Temples . The group of 81 is a mark of royalty, im
that the temple was founded by a king . The large Temple is on a hilltro
the river Narmada in Bhedaghat. Geographical coordinates of Chausatn
temple $23^0$07'48 N" $79^0$48'04'E/23.129872^{0}N 79.801244^{0}E.

Figuer 2.Coverd by dry Cyanobacterial biofilms

CHAPTER-3
MATERIAL REQUIRE & METHOD

MATERIAL AND METHOD:-

Sampling sites :-District Jabalpur is situated in the Mahakoshal region of Madhya Pradesh, India. and lies between geographical coordinates of Chausath Yogini Temple 23°07'48'N, 79°48'04"E/ 23.129872°N, 79.801244°E. Cyanobacterial culture was established in a BG11 medium on Petridishes. Culture were successfully transferred to semisolid BG11 agar medium and incubation at 25+_ 2'c and 75 W/m2 light for further taxonomic enumeration.

MASS CALTIVATION OF CYANOBACTERIA CULTURE :-

The mass cultivation of cyanobacteria has been done by Algal Biotechnology Laboratory, Department of postgraduate studies and Research in Biological Science, Rani Durgavati University, Jabalpur BG11 Agar medium at 30 -35C temp under 2500/Lux light 12-12 hours dark and light keep it at one week in culture room.

GROWTH MEDIUM :-

1979 Rippka1988 without combined based medium BG11 medium is used for successfully for cyanobacteria

TABLE :- COMOSITION FOR BG11 MEDIUM :-

COMPOSITION OF THE MEDIUM COMPONENT	CONCERTRATION
K2HPO4	0.04 G/L
MGSO4	0.075 G/L
CACL2 .H2O	0.036 G/L
CITRIC ACID	0.006 G/L
FERRIC AMMONIUM CITRATE	0.006G/L
EDTA	0.01 G/L
NACO3	0.02 G/L

COMPOSITION OF THE MEDIUM COMPONENT

CONCENTRATION

H3BO3	0.04 G/L
ZNSO4.7H2O	0.075 G/L
MNCL2.4H2O	0.036 G/L
NAMOCU.5H2O	0.006 G/L
CUSO4.5H2O	0.006G/L
EDTA	0.01G/L
CO(NO3)2.H2O	0.02G/L

The experiment of microorganisms (cyanobacteria) contamination and cyanobacteria growth performed under indoor environmental condition.

METHODS

one ml of sample was added to agar plates made with (25 ml of sterilized BG-11 media (Rippka at al. 1979) in petri dishes & simultaneously one ml of sample was inoculated in 50 ml of sterilized BG11 media in flask. after inoculation sample were incubated for 45 days atr 2500 Lux light intensity for 12 hours and 12 hour of dark interval at temperature 25±2. After 12 days of incubation cyanobacteria colonies appeared on the agar plates and broth media in flasks. Isolated species further spread on to fresh agar plates. After the development, colonies appearing in agar plates were examined microscopically and transferred to agar slants. This process was repeated until axenic cultures were obtained.

MICROSCOPIC ANALYSIS

cynobacteria species were observed under microscope for morphometric analysies, were prepared. Texonamically important data such as trichome shape, filaments colour, akinetes and heterocyst shape, size, position were recorded.

DETECTION AND IDENTIFICATION TECHNIQUE :-

Sample were incubated ,under standard., low light condition ,on solid media for algae and cyanobacteria (BG11) Adhesive tape and point flake sample were examined directly , with low power binocular and high power optical microscopes ,

CHAPTER-4
RESULT AND DISCUSSION

RESULT AND DISCUSSION

Cyanobacteria biodiversity was higerduring study period and dominated by *AphanocapsaChroocous, Phormidium, Chloraphyta, Microcoleus, Aphnnothece, Nostocspecies*,algae, liches, mosses on the surface ,wall and pillars.

The pH is one of major characteristics which determine the growth of cyanobacteria. Organisms were identified to genus level, where possible, based on their morphological characteristics.

DISCUSSION

The majority of the results suggest that green algae and cyanobacteria can colonize a wide variety of substrata and this is primarily related to the physical. Cyanobacteria and green algae on monuments characteristics of the stone surface (porosity, roughness and permeability) and secondarily to the nature of the substratum, Most cyanobacteria and chlorophyta did not show a clear relationship with the nature of the substratum, suggesting that environmental variables and site-specific characteristics (eg exposure to light, special architectural features) together with secondary, tertiary and/or extrinsic stone bioreceptivity have a stronger influence on community development than the substratum itself. In this complex amalgam of factors, it is often difficult to determine the influence of each factor alone: the evaluation of their combined effects, their synergy and dynamics is complex, and probably all factors are relevant. In order to ascertain a correlation between stone substratum and organisms, we need more detailed data about lithotype properties, and the microclimatic and environmental conditions of the monuments studied. Cyanobacteria and green algae play an important role in the deterioration of monuments and other stone works of art. being responsible for aesthetic, biogeophysical and biogeo- chemical damage. Future work should focus on ecological and physiological studies of specific species of these micr organisms in order to gain a better understanding of their role in stone colonization and biodeterioration processes. Moreover, an interdisciplinary team wicking on the same 'case study is necessary in order to simultaneously investigate all the factors involved in the biodeterioration process such as mineralogical-petrographic, physico-chem- ical and climatic (and microclimatic) parameters.

CHAPTER-5
CONCLUSION

CONCLUSION:-

Cyanobacteria are probably the most important colonizes of stone buildings since they are not dependent on any organisms source of carbon and very resistant to environmental changes. They can be directly responsible for ability to grow endolithically, but can also act as an organism food material for the growth of otherbiodeterioration, such as fungi. It is impossible to prevent the growth of micro organisms on surface exposed to the open air and regular cleaning with substance that do not damming the stone is recommended. Many potential treatments to prevent or retard growth have been tested over the year, but the discussion of the word require another review.

CHAPTER-6
FUTURE PROSPECTS

FUTURE PROSPECT

Growing concern for the presentation of culture heritage has led to a greater interest in the biological attack on these building . The importance of cyanobacteria as deteroigens is emphasized and the traditional and more modern molecular method used to detect these micro organisms are discussed . The development of molecular techniques for the rapid identification of cyanobacteriawith out need for culture and isolation is fundamental if our knowledge of these communities in biofilms on the surface of historical buildings is the extended.

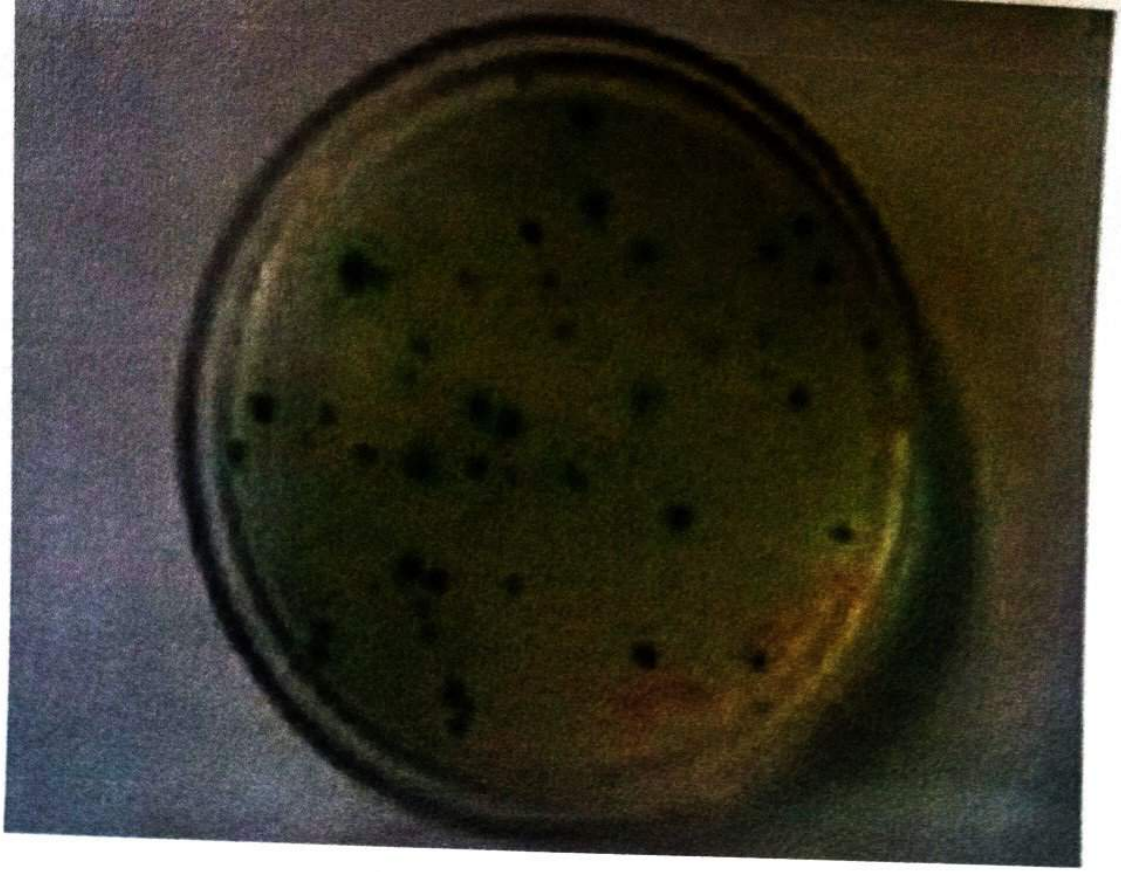
In general , these is no direct evidence for a chemical role of cyanobacteria and algae in stone decay. In some causes the results of some authors, as well as the inferred data from citations to works of an auther in different publication, are contradictory. It is clear that future work to resolve the question of stone deterioration by Cynobacteria and algae must be focused on eliminating the ambiguities of previous studies. Such studies should be made at the ecological / physiological level and laboratory experiments should provide valuable information on metabolic behaviour of phototrophic organisms and interactions between microbial extracellular and intracellular compounds and inorganic materies.

Furthermore, the suggested role of organic acid in biodeterioration mechanisms should be proved. The searching of models for understanding the impact of microbial surface – associated consortia or biofilms on stone is highly desirable. There are very few studies on interactions of different groups of organisms in a single ecosystem eg. bacteria – algae, fungi –algae , mosses- algae etc., Which could be of importance since organisms can act in a sinergistic way in the deterioration of stone.

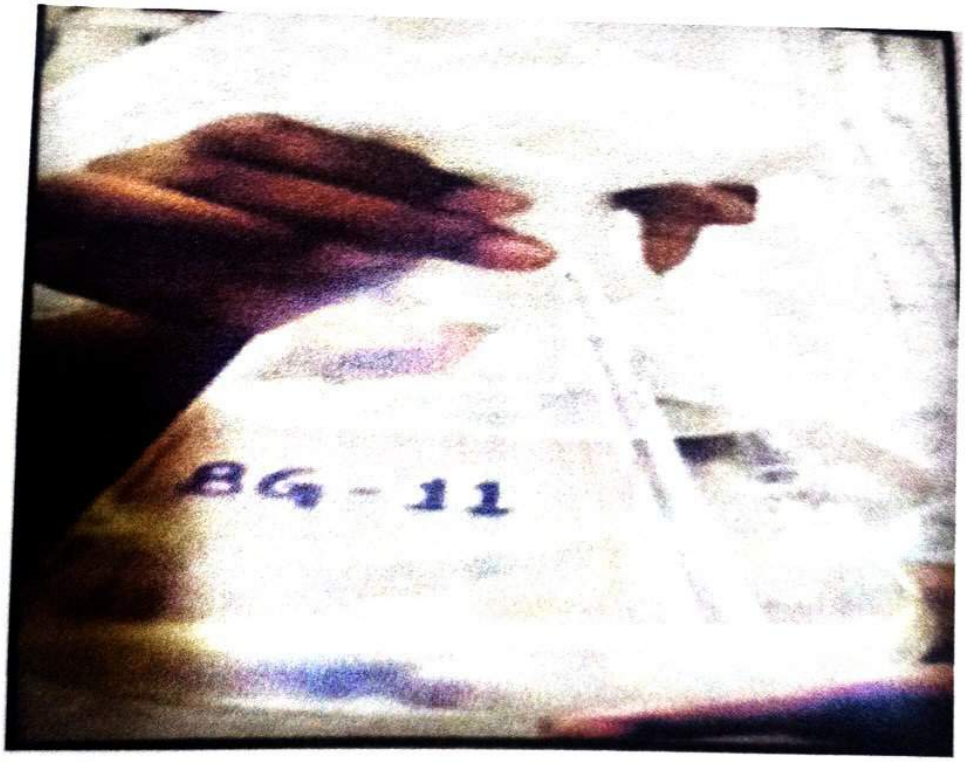
CHAPTER-7
PHOTOGRAPHS

The formatin of black crusts during the dry season.

Cynobacterial biofilms on Chausath Yogini tempal:



Growth of Cynobacteria



BG-11 medium

Studies on the role of guanine quadruplex motifs on DnaA and DnaB protein functions in *Deinococcus radiodurans*

A

DISSERTATION REPORT

Submitted in the partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

SUBMITTED BY

NEHA GHOSH

To

THE DEPARTMENT OF BIOTECHNOLOGY

ST. ALOYSIUS' COLLEGE (AUTONOMOUS), JABALPUR

Under the supervision of

DR. SWATHI KOTA

MRS. SHRUTI MISHRA

MOLECULAR BIOLOGY DIVISION

BHABHA ATOMIC RESEARCH CENTRE

2023

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14 June 2023

CERTIFICATE

To,
Whomsoever it may concern,

Ms.Neha Ghosh, M.Sc. Biotechnology student of St. Aloysius' college (Autonomous), Jabalpur, Madhya Pradesh, underwent project work in our division for five months from 2nd January 2023 to 30th May 2023. During this period, she worked on the project "**Studies on guanine quadruplex motifs role on the DnaA and DnaB proteins functions in *Deinococcus radiodurans*.**" In this work she purified recombinant deinococcal DnaA and DnaB proteins and studied the role of guanine quadruplex (G4) motifs on their functions. She did PCR amplification of "*ori*" sequence of chromosome 1 and induced G4 structures in the PCR purified fragment. She compared the DNA binding activity, ATPase activity and oligomerization pattern of Dna A and Dna B proteins in presence of double strand form and G4 structure induced form of "*ori*" sequence. She also used the overlap PCR technique to generate "RGG" motif site-directed and deleted alleles of *mutS*, an important gene involved in mismatch repair pathway in *Deinococcus radiodurans*.

During this period, she has acquired an experience in molecular biology techniques like PCR, plasmid DNA isolation, bacterial transformation, protein purification, electrophoretic mobility shift assay, ATPase activity estimation and dynamic light scattering techniques

In addition to her excellent scientific aptitude, Ms. Neha is sincere and honest to her duties. I personally found her very helpful and co-ordeal to other colleagues in the laboratory.

I strongly feel that Ms. Neha will be an asset to any laboratory / organization she joins in her future endeavors.

I wish her "All the Best".

Sincerely Yours
K. Swathi 14/06/2023
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NAAC REACCREDITED A+ (CGPA-3.68/4.00)

This is to certify that **Neha Ghosh** has completed her dissertation work entitled "**Studies on the role of Guanine quadruplex motifs on *DnaA* and *DnaB* protein functions in *D. radiodurans***" from **Bhabha Atomic Research Centre** under **Dr. Swathi Kota** towards her partial fulfilment for the award of degree of Masters of Science in Biotechnology. The matter embodied in this thesis is not submitted by her for the award of any other degree or diploma for this or any other university.

I wish her success for her future endeavours.

5/6/23

Date:

Place:

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Statement by the candidate

The work entered in this report is the work done by me entitled **"Studies on the role of guanine quadruplex motifs on *DnaA* and *DnaB* protein functions in *D. Radiodurans*"** submitted to the Department of Biotechnology, St. Aloysius' College (Autonomous), Jabalpur is carried out as a partial fulfilment of Masters of Science (M.Sc) Biotechnology IV semester, under the guidance of **Dr. Swathi Kota** at **Molecular Biology Division, Bhabha Atomic Research Centre**, Mumbai. No part of this work has ever been submitted for any other degree of this or any other university. Further, no part of this work would be presented or published in whatsoever form in any symposium proceedings / journals, without proper consent from the Institute where this work was carried out.

Neha Ghosh

Neha Ghosh
(SIGNATURE OF THE CANDIDATE)

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It is certified that the above statements made by the candidate are correct to the best of my knowledge.

K. Swathi 14/06/2023

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Declaration

I hereby declare that the dissertation thesis entitled "**Studies on the role of guanine quadruplex motifs on DnaA and DnaB protein functions in *Deinococcus radiodurans***" submitted for partial fulfilment of the degree of Master of Science in Biotechnology from the **Bhabha Atomic Research Centre** is an authentic record of my work, carried under the guidance of **Dr. Swathi Kota, Scientific Officer-G, Molecular Biology Division, Bhabha Atomic Research Centre.**

It is also declared that no part of this thesis has been submitted elsewhere for any other degree, diploma, fellowship, or similar title.

Neha Ghosh.

Date: 23/06/2023

Place: JABALPUR

Neha Ghosh

M.Sc. Biotechnnology (IV Sem)

St. Aloysius' (Autonomous) College

Jabalpur, M.P.

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I want to sincerely express my gratitude to everyone who supported and mentored me throughout my training. I got a fantastic opportunity to learn and live in a scientific environment during my internship with B.A.R.C., which aided in the development of my professional and laboratory abilities.

I would especially like to thank my mentor and guide, **Dr. Swathi Kota** (SO/G) B.A.R.C., Mumbai, for her guidance and words of wisdom. I sincerely appreciate your advice, which helped me stay motivated and upbeat despite the experimental problems. It was a great pleasure to work under her direction.

I am extremely grateful to my co-guide, **Mrs. Shruti Mishra** (SO/D), for helping me with the project and providing me with essential guidance and suggestions. I was able to finish my thesis thanks to her timely insights and suggestions, as well as her compassion and excitement.

My sincere gratitude to **Ms. Himani Tewari** for the assistance, direction, and maintenance of a positive atmosphere in the lab. Her encouragement and moral backing have always been my greatest sources of drive.

For their invaluable advice and support throughout the coursework, I would like to extend my sincere gratitude to **Dr. Laxmikant Pandey** (H.O.D., Department of Biotechnology), **Mr. Enosh Phillips**, and the other faculty members at the Department of Biotechnology, St. Aloysius' (Autonomous) College, Jabalpur.

In addition, I would like to express my sincere gratitude to **Mr. Abhijeet Garg** for motivating me to leave my comfort zone and travel all of this distance in order to receive training from the institute of my dreams.

Last but not least, without my parents' unwavering support through all the highs and lows of my trip, I would never have made it this far in my life. I am and will always be grateful to them. And **Shruti Ghosh**, my sweetest little sister, for being the stress buster of my life!

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
APS	Ammonium persulphate
ATP	Adenosine triphosphate
DLS	Dynamic Light Scattering
DSB	Double Strand Breaks
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EMSA	Electrophoretic Mobility Shift Assay
IMAC	Immobilized Metal Affinity Chromatography
IPTG	Isopropyl β-D-1-thiogalactopyranoside
LB	Luria Bertani
MMR	Mismatch repair
Ni-NTA	Nickel-nitrilotriacetic acid
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate Buffered saline
PDS	Pyridostatin
PEG	Polyethylene glycol
PMSF	Phenylmethylsulfonyl fluoride
PPIs	Protein-Protein Interactions
PRB	Protein running buffer
SDS	Sodium dodecyl Sulphate
SSB	Single Strand Breaks
TAE	Tris acetate EDTA

ABSTRACT

Guanine quadruplex (G4) structures are formed in guanine-rich nucleic acid sequences and are known to regulate a variety of cellular functions in higher eukaryotes. Extremophile Deinococcus radiodurans has a multipartite genome made up of two chromosomes and two plasmids. Bioinformatics analysis showed that the *Ori* region on chromosome I contains putative G4 motifs. Here, it was investigated how this bacterium's replication was regulated by G4-forming motifs. PCR amplification of the *Ori* region was carried out to perform the in vitro experiments with replication initiation proteins DnaA and DnaB. Results from the electrophoretic mobility shift assay, ATPase activity, and dynamic light scattering experiments suggested that DnaA has a stronger affinity for G4 motifs and aggregates more readily in its presence as compared to double-stranded DNA. Similar preliminary research with DnaB indicates that, compared to double- or single-stranded DNA, G4 structures are not significantly preferred.

Simultaneously, the overlap PCR technique was used to generate site-directed and deleted mutant alleles of *mutS* in *Deinococcus radiodurans*. PCR-amplified fragments were digested with appropriate restriction enzymes, and attempts were made to clone the mutant alleles into the *Escherichia coli* expression vector pET-28a (+).

CHAPTER-1

INTRODUCTION

Introduction

The double helix structure of the B form of DNA is stabilised by the Watson-Crick base pairing, making it the DNA type that has been studied the most. Over the past few decades, more research has been done on non-canonical DNA and RNA structures and how they affect biological processes. The genomes of many species contain sequences that can build alternate secondary structures that could block activities associated with genome functioning. Examples of these alternate secondary structures include triple helices, i-motifs, guanine quadruplexes (G4s), R-loops, and others. They are produced through base pairing, a far less powerful interaction than Watson-Crick base pairing. The G4 structure has drawn the most interest among these non-canonical nucleic acid structures. Guanine bases can form planar Hoogsteen-base-paired rings (also known as G quartets) in G-rich sequences. These G quartets are stabilised by cations and frequently stack to create thermodynamically stable G4 secondary structures. Guanine quadruplex (G4) structures are prevalent in both DNA and RNA molecules of organisms with GC-rich genomes. These structures can achieve parallel, antiparallel, or mixed symmetry depending on the directionality of the strand's folding.

The human genome contains approximately 700,000 motifs with 1–12 nucleotide loops and over 350,000 potential G4-forming sequences (PQS) with 1–7 nucleotide loops, according to bioinformatics research. These G4 motifs were discovered to be particularly abundant in promoters, first introns, and telomeres, three regulatory areas. Replication, transcription, RNA processing, DNA recombination, and telomere preservation are just a few of the key biological activities that G4 structures have been linked to as having a role in maintaining genome integrity. Depending on where G4 motifs are located in the genome, these roles change. Chromatin status and cell type both affect how dynamic the G4 structure is in vivo. G4BPs are also involved in the direct or indirect regulation of G4 structure development and unwinding across the entire genome and transcriptome, which has an impact on a number of

biological processes. By interacting with proteins, G4s carry out many biological tasks. Important physiological functions like telomere preservation, DNA replication, gene transcription, and mRNA processing are regulated by G4-binding proteins.

The bacterial chromosome's (*ori*C) discrete *ori*gin of replication is a region with AT-rich conserved DNA motifs and a variable number of 9-mer repeats of nonpalindromic sequences known as DnaA boxes. DnaA, a replication initiator protein, recognises these boxes, which trigger the construction of the replication initiation complex at *ori*C. Numerous bacteria that have a single circular chromosome with a restricted number of copies each have inheritable mechanisms for replication initiation. It has been demonstrated that the DnaA-ATP oligomer binds to DnaA boxes at *ori*C in *Escherichia coli* and unwinds the nearby AT-rich region. The prepriming complex is then formed as a result of the recruitment of a hexameric complex of the replicative helicase DnaB and its loader DnaC (DnaB6-DnaC6) to the unwinding region in *ori*C. This gives primase a place to bind and activate numerous processes necessary for the replication complex, which includes DNA polymerase III holoenzyme, to advance. While the DNA polymerase complex extends the primer's 3'- end, the DnaB hexameric ring translocates in both directions to unwind the parental duplex DNA. The first round of *ori*C-mediated DNA replication must wait until the *ori*C of freshly replicated daughter chromosomes is fully methylated in *E. coli* because the beginning of DNA replication at the *ori*C site is strictly regulated. Therefore, it is anticipated that there will be fewer than two copies of the main chromosome in each cell under typical growth conditions. Recently, bacteria with a multipartite genome system in multiple copies have been found.

Deinococcus radiodurans contains a multipartite genome system made up of a megaplasmid (177,466 bp) and a plasmid (45,704 bp) in addition to two chromosomes (Chr I (2,648,638 bp) and Chr II (412,348 bp). It's interesting to note that each genomic component exists in

numerous copies in each cell. The putative DnaA and DnaB are encoded by Chr I in *D. radiodurans*.

When it comes to all known species, *Deinococcus radiodurans* is unmatched in its ability to withstand oxidative stress, which affects all cellular macromolecules. Reactive oxygen species (ROS), which can develop as a result of metabolic processes or as a result of exposure to physical and chemical factors such as desiccation, ionising radiation, UV radiation, mitomycin C (MMC), or hydrogen peroxide, cause oxidative stress. All ROS-generating compounds are very resistant to *D. radiodurans*. Proteins, lipids, nucleic acids, and carbohydrates are damaged by ROS produced by desiccation and ionising radiation, which also causes double-strand DNA breaks (DSBs) in the bacterial genome that may be fatal. *D. radiodurans* can endure high doses of ionising radiation that can fragment its genome into up to 2,000 DSBs per multigenomic cell without suffering significant protein loss. The robustness of this bacteria is a result of potent anti-oxidative stress defence mechanisms that shield proteins from oxidative damage and a DNA repair process that successfully assembles DNA fragments in an effective and exact manner. It is possible for DNA repair proteins and other proteins to maintain their catalytic activity and respond quickly to oxidative stress thanks to their antioxidation protection.

Genomic stability is mostly maintained by the biological system known as DNA mismatch repair (MMR), which has undergone extensive evolutionary conservation. MMR is particularly specific for base-base mismatches and insertion/deletion mispairs that are produced during DNA replication and recombination. If left untreated, DNA damage has the potential to lead to mutations in somatic or germline cells, which can change the cellular phenotype and result in dysfunction and illness. Cells have several mechanisms to repair DNA damage and, hence, stop mutations in order to avoid such harmful effects and protect the integrity of the genome. The vital route known as DNA mismatch repair (MMR), for

example, is one such system. DNA mismatch repair (MMR), depends on the enzyme *MutS*. Mismatches are recognised by *MutS*, which then activates the MMR's downstream responses. Virtually all species, including viruses, archaea, bacteria, and eukaryotes, have *MutS* orthologues. A growing body of genomic data has shown that all three domains of life contain proteins that are somewhat similar to *MutS*. Bacterial *MutS*2 is one of the *MutS* paralogues that has been relatively thoroughly characterised. While knocking out *mutS*2 in *Helicobacter pylori* was found to increase the frequency of homologous recombination, disruption of *mutS*2 in *Bacillus subtilis* and *Deinococcus radiodurans* had no effect on either strain's phenotype. This finding points to *MutS*2's potential involvement as an inhibitor of homologous recombination.

Mutagenesis is a crucial laboratory technique in molecular biology where DNA mutations are purposefully produced to produce libraries of mutant genes, proteins, bacterial strains, or other genetically modified animals. The function of a genetic locus, process, or product can be thoroughly explored by altering the many components of a gene, as well as its regulatory components and gene products. The mutation may result in mutant proteins with intriguing characteristics, improved functions, or novel functions that could be useful comMercially. Additionally, mutant strains that are useful or enable the study of the molecular underpinnings of a certain cell function may be generated.

In terms of genomics, a deletion is a sort of mutation that happens when one or more nucleotides are lost from a section of DNA. Any number of nucleotides can be lost during a deletion, ranging from one nucleotide to the loss of an entire section of a chromosome.

Utilising the molecular biology technique of site-directed mutagenesis, one can specifically and purposefully alter the DNA sequence of a gene and its gene products. It is also known as oligonucleotide-directed mutagenesis or site-specific mutagenesis, and it is employed for protein engineering as well as research into the structure and biological function of DNA, RNA, and protein molecules.

CHAPTER-2

AIMS & OBJECTIVES

AIM

To check the role of guanine quadruplexes in DNA replication initiation in *Deinococcus radiodurans.*

OBJECTIVES

1. To purify the DnaA and DnaB proteins from recombinant strains of *E. coli.*

2. To study the interaction of DnaA and DnaB proteins with G4 DNA and double-stranded DNA.

3. To construct the recombinant plasmids for deletion and site directed mutagenesis for *mutS* gene of *Deinococcus radiodurans.*

CHAPTER-3

LITERATURE REVIEW

3.1 GUANINE QUADRUPLEXES

3.1.1 Guanine quadruplexes- the most conserved nucleic acid structure

The Watson-Crick base pairing stabilises the double helix structure B type of DNA, which is the form of DNA that has been researched the most. Research into the non-canonical structures of nucleic acids (DNA and RNA) and their functions in biological processes has increased during the past few decades. Many organisms have sequences in their genomes that are capable of creating alternate secondary structures that could obstruct activities related to genome functions (Mirkin & Mirkin, 2007). These alternative secondary structures include R-loops, guanine quadruplexes (G4s), i-motifs, triple helices, and more. They are created through base pairing, a far less energy-intensive process than Watson-Crick. The G4 structure is among the noncanonical nucleic acid structures that have been investigated the most in-depth of these. Guanine bases can form planar Hoogsteen-base-paired rings (also known as G quartets) in G-rich sequences. These G quartets are stabilised by cations and frequently stack to create thermodynamically stable G4 secondary structures (Gellert, Lipsett, & Davies, 1962) (Williamson, Raghuraman, & Cech, 1989). According to bioinformatics research, the human genome contains over 350,000 PQS (possible G4-forming sequences) with 1–7 nucleotide loops and over 700,000 motifs with 1–12 nucleotide loops (Maizels & Gray, 2013).

3.1.2 Formation of Guanine Quadruplex

The versatile and durable secondary nucleic acid structure known as the G-quadruplex (G4) is most frequently produced in guanine-rich areas of single-stranded DNA or RNA (Hänsel-Hertsch, Spiegel, Marsico, Tannahill, & Balasubramanian, 2018; Jochen Spiegel, Santosh Adhikari, & Shankar Balasubramanian, 2020; Varshney, Spiegel, Zyner, Tannahill, & Balasubramanian, 2020). The G-quartet, a square planar arrangement of four guanines bound

together by Hoogsteen hydrogen bonding, is the fundamental structural component of the G4 (Tarsounas & Tijsterman, 2013). In general, stacks of 2-4 G-quartets and monovalent cations like Na+ and K+ in the central channel of the G4 helix promote the creation of stable G4 structures. The same (intramolecular) or different (intermolecular) G-rich strands could be used to build G4s. The number of stacked G-quartets, the direction and polarity of the nucleic acid strands, and the glycosidic conformation of the guanine bases in quartets all influence the possible topologies that G4 structures might fold into(Daniela Rhodes & Hans J Lipps, 2015; Tarsounas & Tijsterman, 2013; Varshney et al., 2020). For instance, RNA G4 is more likely to form a parallel structure and has higher thermal stability than DNA G4, which can form a parallel, anti-parallel, or mixed structure(Ding et al., 2020; Fay, Lyons, & Ivanov, 2017).

3.1.3 Guanine Quadruplex in double-stranded DNA

Genomic DNA normally exists in a double-stranded condition, with the exception of the telomeric ends of chromosomes. Therefore, G4 functioning may be related to procedures like replication or transcription in which the DNA strands separate since the existence of a complementary strand inhibits G4 development. Other elements, like DNA-binding proteins or supercoiling, might also contribute to local double helix unwinding or instability, which would favour G4 development. Additionally, it has been demonstrated that certain solution circumstances, such as molecular crowding brought on by the presence of polyethylene glycol (PEG), cause the equilibrium to shift in favour of the G4 DNA structure(Miyoshi, Fujimoto, & Sugimoto, 2013; Miyoshi, Nakao, & Sugimoto, 2002; Zheng, Chen, Hao, & Tan, 2010). Recently, pyridostatin (PDS), a highly selective G-quadruplex ligand (Rodriguez et al., 2008), and its derivatives were effectively used in a number of experiments, including the targeting and localization of G4 structures across the entire genome (Chambers et al.,

2015) as well as the visualisation of G4 structures in living cells (Di Antonio et al., 2020) and ex vivo (Biffi, Tannahill, McCafferty, & Balasubramanian, 2013).

3.1.4 The significance of G4 motifs

All organisms' genomes contain sequences called potential G4 (pG4) that have the ability to generate G4 structures(Huppert & Balasubramanian, 2005; Daniela Rhodes & Hans J Lipps, 2015). Due to their predominance in functional sections of the genome and high level of conservation across species, pG4s do not just appear randomly across the genome(Daniela Rhodes & Hans J Lipps, 2015). Significantly, it was discovered that pG4s are highly abundant in regulatory areas including telomeres, gene promoters, and the boundary between introns and exons. Furthermore, pG4 motifs have been found to colocalize with the region-specific 5′ -untranslated region (UTR) of the transcribed mRNAs in 3000 human genes and are present in 90% of human DNA replication *ori*gins (Bugaut & Balasubramanian, 2012; Daniela Rhodes & Hans J Lipps, 2015). As can be seen, G4 structures are crucial for DNA replication, transcription, translation, and epigenetic modification and are intimately correlated with genome activities(Mendoza, Bourdoncle, Boulé, Brosh Jr, & Mergny, 2016).

3.1.5 G-quadruplex-binding proteins

Protein involvement is required for the development of G4 structures and the accomplishment of their biological roles. The term "G-quadruplex-binding proteins" (G4BPs) often refers to proteins that can specifically bind to G4s. First, G4BPs can be divided into two major categ*ori*es based on their regulatory mechanisms and functional connections to G4s, namely G4-folding proteins that influence G4 structures and G4-recruited proteins, which are functional proteins that G4 recruits (Figure 1). Second, based on how G4s are distributed across the genome, G4BPs can also be separated into two groups: DNA G4BPs and RNA G4BPs (Figure 2). To be more precise, there are two ways in which G4-folding proteins

affect the G4s structurally. These proteins have the capacity to unfold G4s while simultaneously encouraging pG4 to establish a stable quadruplex structure(Mendoza et al., 2016; Sauer & Paeschke, 2017; Z.-Y. Sun, Wang, Cheng, Su, & Ou, 2019). Additionally, the recruitment of G4BPs frequently occurs concurrently with the accomplishment of the biological tasks of G4 structures (Václav Brázda, Hároníková, Liao, & Fojta, 2014). A variety of helicases and DNA repair proteins recruited to the G4 formation sites unwind G4 structures and remove DNA lesions to ensure effective DNA replication and maintain genome integrity (Mendoza et al., 2016; Pavlova, Kubareva, Monakhova, Zvereva, & Dolinnaya, 2021; Sauer & Paeschke, 2017). Epigenetic modulators regulate the methylation of DNA and histones through their interaction with G4s (Mao et al., 2018; Oyoshi & Masuzawa, 2020). Transcription factors bind to G4s at the promoter sites to aid or suppress gene transcription(Kim, 2019; Spiegel et al., 2021). Telomere-binding proteins can bind to G4s and unfold these structures at telomeres to maintain the length and integrity of telomeres(Takahama, Kino, Arai, Kurokawa, & Oyoshi, 2011; Takahama et al., 2013). Additionally, G4BPs play a role in a variety of biological functions, including mRNA maturation, post-transcriptional processing, and translational control (Amato et al., 2019).

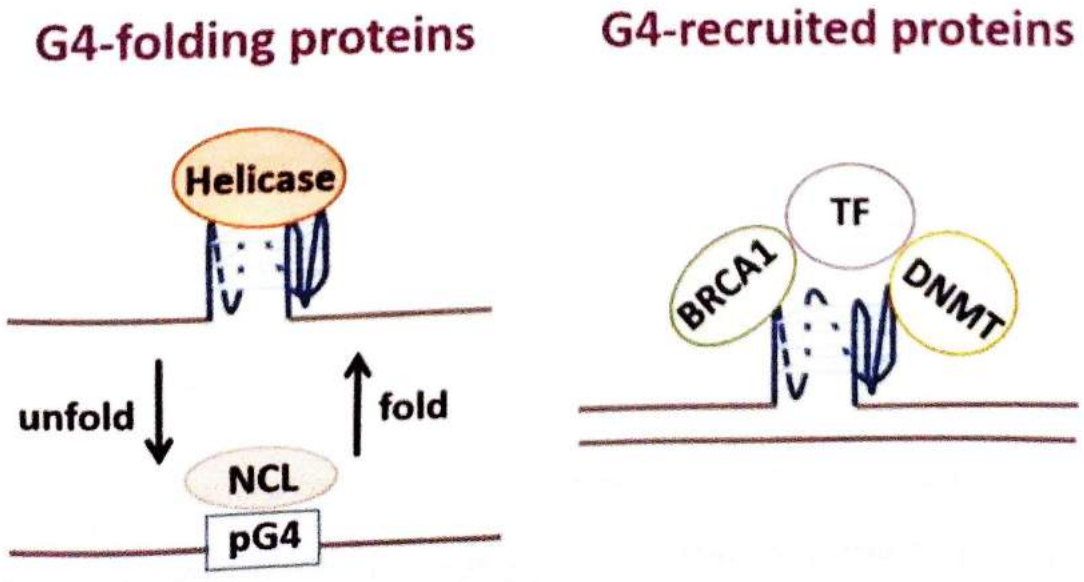


Figure 1 : Classification of proteins that bind G-quadruplexes. G4BPs fall into two categories: those that fold or unfold G4s, such as nucleolin (NCL) and helicases, and those that G4s can recruit, such as transcription factors (TF), DNA repair proteins (BRAC1), and chromatin remodelling proteins (DNA methyltransferase, or DNMT).

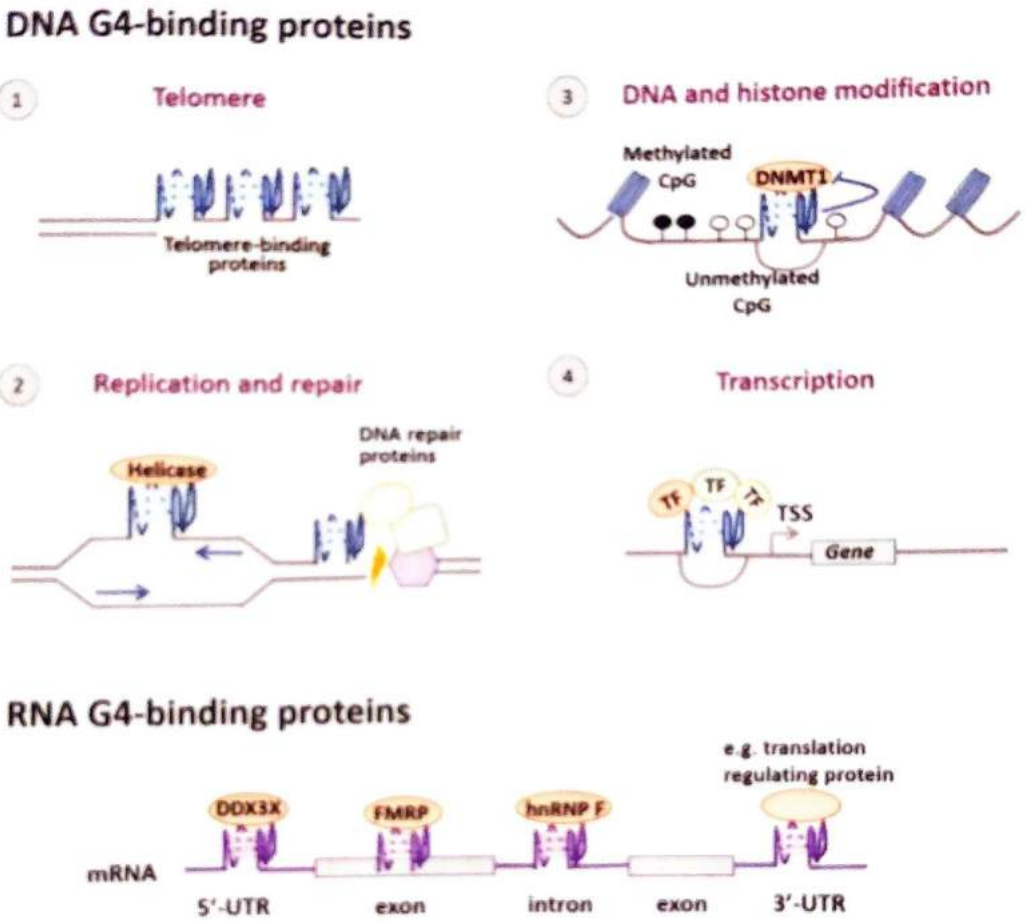


Figure 2 : Location and biological purposes of proteins that bind G-quadruplexes. G4BPs can be divided into two groups based on where they are found: DNA G4BPs and RNA G4BPs. G4BPs carry out a variety of biological tasks in cells. (1) At telomeres, telomere-binding proteins form a ternary complex with the G4 structures of telomeric DNA. (2) Replication machinery encounters G4s during replication, which helicases must eliminate to ensure successful replication. In the meantime, DNA double-strand breaks (DSBs) might be repaired at G4 locations by DNA repair proteins. (3) In terms of epigenetic regulation, DNA methyltransferase 1 (DNMT1) becomes inactive when it binds G4s, which causes

hypomethylation at CpG islands. (4) Multiple transcription factors have the ability to bind G4s in the promoter regions and initiate transcription of genes. RNA G4BPs affect translational regulation as well as mRNA maturation, including mRNA export and splicing.

3.1.5.1 DNA G-Quadruplex-Binding Proteins

Below is a discussion of recent findings regarding the role of DNA G4BPs in the regulation of cellular essential processes.

Telomeric G-Quadruplex-Binding Proteins : Telomeres, which make up the ends of eukaryotic chromosomes and play a key role in preserving the stability and integrity of the genome, are nucleoprotein complexes. They have the capacity to create G4 structures and are composed of identical TTAGGG short repeat sequences with a guanine-rich single-stranded 3′ overhang (Václav Brázda et al., 2014; Izumi & Funa, 2019). Several proteins, including TRF1, TRF2 (Telomere Repeat Binding Factor 1 and 2), POT1 (Protection of Telomeres protein 1), and TPP1 (TIN2 Interacting Protein), bind to double-stranded telomeric DNA and control how the G4 complexes fold and unwind(Baumann & Cech, 2001; Václav Brázda et al., 2014; Chaires et al., 2020; F. Wang et al., 2007). To preserve telomere integrity and guarantee telomere replication, the RecQ family helicases WRN (Werner syndrome ATP-dependent helicase) and BLM (Bloom syndrome protein) are attracted to the telomeres and unfold the G4 structures (Budhathoki et al., 2014; Wu et al., 2018).

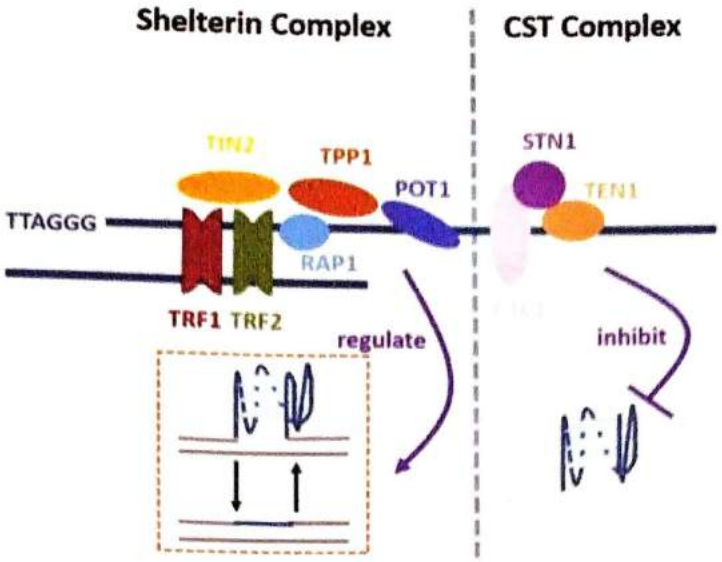


Figure 3 : Schematic diagram of the telomere-associated protein complexes shelterin and CST. In order to maintain telomeres, shelterin and CST are essential. Shelterin's TPP1-POT1 subunit controls how G4 complexes fold and unwind. G4 structure creation could be prevented and resolved using CST.

A protein complex in mammalian cells known as CST (CTC1-STN1-TEN1) is essential for effective telomere replication and for maintaining telomere length (Bhattacharjee, Wang, Diao, & Price, 2017; Miyake et al., 2009; Surovtseva et al., 2009; Zhang et al., 2019). CST is a complex of single-stranded DNA-binding proteins that aids in the resolution of replication issues throughout the genome (Bhattacharjee et al., 2017; Zhang et al., 2019). CST's ability to attach to the G4s and unfold them, improving the efficiency of double-stranded telomeric DNA replication, was demonstrated through experiments (Bhattacharjee et al., 2017). hnRNP A1 and hnRNP A2/B1 (heterogenous nuclear ribonucleoproteins) are two additional telomere-binding proteins that regulate telomere length by forming macromolecular complexes with telomere-maintaining elements (González, Guo, Hurley, & Sun, 2009; Hudson, Ding, Le, Lewis, & Graves, 2014; Krüger et al., 2010).

G-Quadruplex-Binding Proteins Involved in Replication : The G4 structure affects DNA replication in two different ways. It encourages the start of DNA replication and might stop

the leading- and lagging-strand polymerases from becoming uncoupled. However, it can impede the replication fork's development and affect DNA synthesis, which might result in genomic alterations and deletions (Sauer & Paeschke, 2017; Valton & Prioleau, 2016). A 5′ - 3′ DNA helicase known as FANCJ (Fanconi anaemia complementation group J) is involved in a number of biological activities, including the repair of DNA damage, G4 resolution, homologous recombination, and maintenance of genome stability (Z.-Y. Sun et al., 2019). For effective DNA replication, FANCJ can unfold and break down G4 structures, but its lack will prevent replication at G4s and eventually result in DNA damage (Castillo Bosch et al., 2014). Recent research indicates that Mms1(ubiquitin ligase complex protein) is not only a DNA G4-binding protein but also helps Pif1 bind to a specific G4 structure on the lagging strand (Schwindt & Paeschke, 2018). Pif1 from yeast is able to bind and unfold G4 structures to facilitate DNA replication(Sauer & Paeschke, 2017).

G-Quadruplex-Binding Proteins Involved in Transcription : When found at the first intron downstream of the transcription start site, G4s play a crucial function in the regulation of gene expression because they inhibit RNA polymerase and decrease transcription (Kim, 2019). Endogenous G4s in promoters have been demonstrated to be important binding sites for numerous transcription factors, including SP1, MAZ, and PARP-1 (Cogoi, Paramasivam, Membrino, Yokoyama, & Xodo, 2010; Kim, 2019; D. Rhodes & H. J. Lipps, 2015; J. Spiegel, S. Adhikari, & S. Balasubramanian, 2020). The nuclease hypersensitivity element III1 (NHE III1), which is located upstream of the P1 promoter of the proto-oncogene c-MYC, is where the first documented G4 on the promoter is produced (Simonsson, Pecinka, & Kubista, 1998; Z.-Y. Sun et al., 2019). Numerous genes, including the pro-oncogenes VEGF (D. Sun, Guo, Rusche, & Hurley, 2005), KRAS (Cogoi & Xodo, 2006), BCL-2 (Dexheimer, Sun, & Hurley, 2006), and c-KIT (Rankin et al., 2005), the human platelet-derived growth factor receptor PDGFR-β (Qin et al., 2010), and the human telomerase reverse transcriptase

hTERT (Palumbo, Ebbinghaus, & Hurley, 2009), have been shown to produce G4 structures in the promoter regions. The multifunctional phosphoprotein known as nucleolin (NCL) is found mostly in the nucleolus and has a role in the synthesis of ribosomes, chromatin remodelling, transcriptional control, G4 binding, and apoptosis (González et al., 2009). A member of the NM23 family of nucleoside diphosphate kinases (NDPK), NM23-H2 is a protein with several different roles, such as kinase activity, promoter binding, transcriptional control, and DNA repair (Thakur et al., 2009). p53 is a tumour suppressor protein with roles in apoptosis, DNA repair, cell cycle regulation, and ageing. Through a variety of mechanisms, p53 can prevent the expression of genes that control the cell cycle and promote growth. In p53-mediated transcriptional regulation, the interaction between p53 and G4 structures in the promoter regions of p53 target genes may be crucial (Petr et al., 2016).

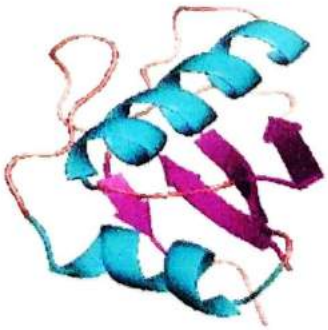
Other DNA G-Quadruplex-Binding Proteins : The dynamic structuring of the endogenous human G4 DNA landscape is influenced by a number of critical variables, including chromatin relaxation, CTCF, hypomethylation, and remodelling (Guilbaud et al., 2017; Martin J Law et al., 2010; Tikhonova et al., 2021). Since CpG islands in the human genome are hypomethylated, ATR-X colocalizes with CpG islands (CGI) that have the potential to generate G4 structures (Martin J Law et al., 2010; M. J. Law et al., 2010; Y. Wang et al., 2019). According to studies, DNMT1 (DNA methyltransferase 1) interacts with these G4 sites, and the development of G4 prevents DNMT1 from methylating particular CpG islands and inhibiting local methylation (Mao et al., 2018). G4s interact with CTCF in vitro and colocalize with CTCF (CCCTC-binding factor) binding sites in CpG islands. Typically, hypomethylated CpG islands are where CTCF is recruited, and G4 can help CTCF attach to genomic DNA by attracting chromatin proteins (Hou et al., 2019).

A thorough investigation found that a protein's amino acid sequence, which includes domains and motifs, affects how it functions. These domains are the fundamental components that make up a protein's intrinsic features, such as its ability to interact with nucleic acid sequences, and they also affect the pathways to which it belongs (Thandapani, O'Connor, Bailey, & Richard, 2013). The G4 recognition of proteins is a multi-step process where the crucial domain interacts with nearby disordered areas to recognise the G4 structures. According to assessments of existing G4BPs, the known or projected binding sites in G4BPs share specific domains or patterns (Figure 4). According to several studies, these domains are enriched in G4BPs, which helps them interact with G4s (Kharel, Becker, Tsvetkov, & Ivanov, 2020; McRae, Booy, Padilla-Meier, & McKenna, 2017). A greater understanding of their traits will advance our understanding of the G4-protein interaction's binding mechanisms while also supplying precise structural targets for future drug development.

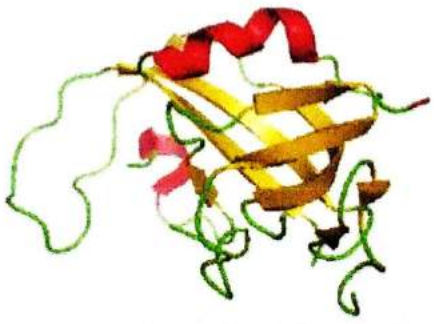
RGG/RG motif

NCL	RGGGRGGFGGRGGGRGGRGGFGGRGRGGFGG
hnRNP U	RGGGHRGRGGFNMRGGNFRGGAPGNRGG
CIRBP	RGGSAGGRGFFRGGRGRGRGFSRGG

RRM domain



OB-fold domain



CIRBP all include RGG/RG motifs. The Protein Data Bank's structural code 2KRR (NCL) is where the RRM domain structure is found. One four-stranded antiparallel sheet and two helices arranged against the sheet make up the sandwich structure that is the RRM domain. Protein Data Bank's structural code 5W2L (CTC1) is where the OB-fold domain structure is found. A barrel known as the OB-fold domain is made up of five antiparallel -sheets.

3.2 RGG DOMAIN

The RGG (Arginine-Glycine-Glycine) domain, also known as the RGG/RG motif or the GAR (glycine-arginine-rich) domain, is made up of repetitive sequences rich in RGG or RG and exhibits remarkable evolutionary conservation (Kharel et al., 2020; Thandapani et al., 2013). More than 1000 human proteins have been shown to contain RGG/RG motifs, which are known to have an impact on transcription, mRNA translation, DNA damage signalling pathways, precursor mRNA splicing, and apoptosis (Thandapani et al., 2013). In a recent study, 77 human G4-binding proteins were examined to determine their amino acid makeup. The study showed a considerable enrichment of glycine and arginine as well as high abundance in RR, GR, and RG in G4BPs when compared with a random subset of the human proteome and a well-defined group of nucleic acid binding proteins. The presence of a conserved RG-rich motif, a defining feature of G4BPs, was investigated through research (V. Brázda et al., 2018).

The RGG domain has been demonstrated to mediate G4-protein interactions and is typically present in G4BPs. For instance, the RGG domain is present in hnRNP U (Izumi & Funa, 2019). For the recognition of the c-MYC NHE III1 sequence and the encouragement of G4 development, nucleolin's C-terminal region, which is made up of the RNA-binding domains (RBD) 3 and 4, as well as the RGG domain, is crucial. Additionally, the GAR domain, which

has been shown to be essential for NRAS rG4-DDX3X interaction, is present in more than half of the newly discovered NRAS rG4BPs (Herdy et al., 2018; Huang et al., 2018).

In the RGG domain, the small residue gaps between RGG repeats usually include aromatic amino acids. The tiny segment RGG motif in the RGG domain significantly increases the G4 binding affinity, according to research on the binding mechanisms of this domain. According to Huang et al., the internal configuration of RGG repeats and gap amino acids is more important for G4-protein interactions than the length and quantity of RGG repeats. The seven RGG repeats in the peptide 12's structure were shown to be effective DNA G4 binders in experiments. On the basis of the aforementioned findings, they learned that the RGG peptide 12 found in the cold-inducible RNA-binding protein (CIRBP) could bind G4s both in vitro and in vivo and that this peptide is crucial for CIRBP's recognition of G4s. The team's investigation of G4-binding RGG motifs led to the discovery of a new G4-binding protein and a wealth of fresh information on the interaction between the RGG peptide and G4s (Huang et al., 2018).

3.3 ***DEINOCOCCUS RADIODURANS***

3.3.1 Scientific Classification and Background

Kingdom: **Bacteria**

Phylum: **Deinococcus-Thermus**

Class: **Deinococci**

Order: **Deinococcales**

Family: **Deinococcaaceae**

Genus: ***Deinococcus***

Species: ***radiodurans***

Gram-positive bacteria called *Deinococcus radiodurans*, also known as a polyextremophile, can withstand extreme cold, dehydration, hoovering, acid, and radiation. It was initially discovered in a can of beef that had received a significant dose of radiation at the Oregon Agricultural Experiment Station (Anderson, 1956). Living up to its Latin name, "strange little berry that withstands radiation," it has been shown to withstand radiation doses up to 1,000 times higher than those that would normally kill a human. It is a spherical, 1.5–3.5 mm in diameter, non-spore-forming, non-pathogenic bacteria. It is a mesophile with a thermal radiation of over 39 degrees and thrives between 30 and 37°C (Makarova et al., 2001).

D. radiodurans demonstrates exceptional resilience to desiccation, radiation, and other factors that might damage DNA(Lange, Wackett, Minton, & Daly, 1998). This bacteria has effective methods for repairing damaged DNA and guards against oxidative damage to its other biomolecules. It is a proteolytic-living organotrophic bacteria. The following minimal conditions are needed for *D. radiodurans* to develop productively: a carbon source, nicotinic acid, sulphur, nitrogen, and manganese (Mn) sources (Minton, 1994; Moseley & Evans, 1983; P. Wang & Schellhorn, 1995).

3.3.2 Cell Structure

It exists in tetrads, and its genetic material is packed in toroidal form. Under regulated growth conditions, cells of dimer, tetramer, and even multimer morphologies can be formed. Bacteria can be easily grown at temperatures between 30°C and 37 °C, with a doubling period between 1.5 and 3 hours. It can tolerate about 200 double strands and 3000 single strand breaks per genome and demonstrates an excellent biphasic DNA strand break repair process (Carroll, Daly, & Minton, 1996). The cell envelope consists of six layers: the plasma membrane, peptidoglycan layer, compartmentalised layer, electron-transparent zone, outer membrane, and a hexagonally packed intermediate layer. Some *Deinococcus* bacteria also

have a thick layer of carbohydrates. During cell division, the septum is only formed by the cytoplasmic membrane and the peptidoglycan layer. The additional layers are thought of as a sheath because they form on the surface of daughter cells when they separate and surround clusters of cells. The mucopeptide that makes up the holey layer contains four major amino acids (glutamic acid, alanine, glycine, and L-ornithine), glucosamine, and muramic acid. Carotenoids, lipids, proteins, and polysaccharides are found in the layer that is hexagonally packed. Heptose is absent from the polysaccharide, which also contains residues of rhamnose and mannose as well as galactose and glucose. Phosphoglycolipids, which are thought to be exclusive to *D. radiodurans*, account for 43% of the membrane lipids and have alkyl amines as structural constituents. There aren't any prevalent bacterial phospholipids like phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, or phosphatidylinositol (Work & Griffiths, 1968).

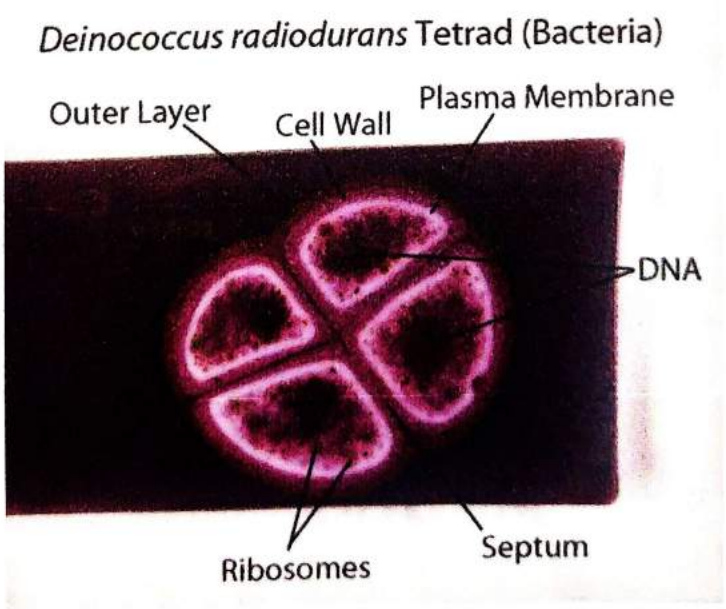


Figure 5 : Tetrads of *D. radiodurans*

3.3.3 Genome structure

It contains a multipartite genome with a total length of 3,284,156 base pairs. This genome is made up of two chromosomes (2,648,638 and 412,348 base pairs each), a mega plasmid (177,466 base pairs), and a small plasmid (45,704 base pairs). There are 3,195 genes in it (White et al., 1999). The bulk of the proteins required for this bacterium's normal growth are encoded on chromosome I, which serves as the primary chromosome. In contrast, other genome replication units encode proteins that are mostly responsible for this bacterium's secondary phenotypes (Maurya, Chaudhary, Pandey, & Misra, 2021).

3.3.4 Manganese content

Presence of manganese in *Deinococcus radiodurans* helps in efficient growth and resistance against irradiation. Different concentrations of Manganese display the capacity of these cells to survive irradiation. When *D. radiodurans* cultures are starved of Mn(II), their resistance to ionizing radiation decreases (Daly et al.,2004). The numbers of DNA double-strand breaks formed are the same for a specific dose of ionizing radiation both in the presence or absence of Mn(II), so Mn(II) does not prevent DNA damage. Instead, cellular damage that results from exposure to high radiation doses is better tolerated if Mn(II) is present.

3.3.5 DNA Replication in *D. radiodurans*

The bacterial chromosome's *ori*gin of replication (*oriC*) is a distinct locus that includes AT-rich conserved DNA motifs and a variable number of 9-mer repeats of nonpalindromic sequences known as DnaA boxes. DnaA, a protein that initiates replication, recognises these boxes, and *oriC* then assembles the replication initiation complex (Messer, 2002; Mott & Berger, 2007). It has been demonstrated that the DnaA-ATP oligomer binds to DnaA boxes at *oriC* in *Escherichia coli* and unwinds adjacent AT-rich regions. The prepriming complex is then formed as a result of the recruitment of a hexameric complex of the replicative helicase

DnaB and its loader *DnaC* (DnaB6-*DnaC*6) to the unwinding region in *oriC* (Chodavarapu & Kaguni, 2016; Skarstad & Katayama, 2013). This gives primase a place to bind and activate numerous processes necessary for the replication complex, which includes DNA polymerase III holoenzyme, to advance. While the DNA polymerase complex extends the primer's 3' end, the DnaB hexameric ring translocates in both directions to unwind the parental duplex DNA (McHenry, 2011; Yao & O'Donnell, 2010).

The multipartite genome system of *Deinococcus radiodurans* consists of two chromosomes—Chr I (2,648,638 bp) and Chr II (412,348 bp)—as well as a megaplasmid (177,466 bp) and a plasmid (45,704 bp) (White et al., 1999). It's interesting to note that each genomic component exists in numerous copies in each cell (Hansen, 1978). *D. radiodurans*' chromosome I carries the DnaA and DnaB genes, while chromosome II carries the *PprA* gene, which has been studied for a variety of roles (Adachi et al., 2014; Kota, Charaka, Ringgaard, Waldor, & Misra, 2014; Kota et al., 2016; Narumi et al., 2004). Recent research on *PprA*'s expanded structure raised the notion that it might function as a protein scaffold (Adachi et al., 2019). In *D. radiodurans*, the functional characterization of the chromosome replication initiation proteins DnaA and DnaB was published, and it was shown that *PprA* is crucial for the control of DNA replication. Chromosome I and Chromosome II had more copies in the *PprA* mutant, which was balanced by the in-trans production of *PprA* from the wild type. *PprA* overexpression had an interestingly neutral effect on the number of copies of the various genome components in wild-type cells. *PprA* overexpression had an interestingly neutral effect on the number of copies of the various genome components in wild-type cells. DnaA was identified as an *oriCI*-sensitive ATPase and a sequence-specific *ori*gin of replication (*oriCI*) binding protein. In addition to having a higher affinity for single-stranded DNA (ssDNA) than double-stranded DNA (dsDNA), DnaB was discovered to be an ATP-dependent 5' →3' dsDNA helicase. It's interesting to note that *PprA* suppressed both

homotypic and heterotypic interactions between these proteins by interacting with DnaA with a higher affinity than DnaB. Furthermore, DnaA's ATPase activity was downregulated by *PprA*, but DnaB's ATPase and helicase activities were unaffected. These findings imply that in *D. radiodurans*, DnaA and DnaB perform the essential tasks required for the start of replication at *oriCI*. Further evidence for *PprA*'s role in regulating chromosomal replication in this bacterium came from its interference with the physicochemical properties of these replication proteins as well as from an increase in the copy numbers of both primary and secondary chromosomes in its absence (Maurya et al., 2021).

DnaA

Bacteria were the first organisms among which the replicon model was established. In Escherichia coli, the initiator protein DnaA selectively binds to the 250-bp AT-rich replicator sequence *ori*C, causing local ATP-dependent DNA unwinding and replicative DNA helicase (DnaB) loading (Figure 6). After that, DNA polymerases and other replication fork components are assembled, and replication is then started (Chesnokov & Akhmetova, 2021).

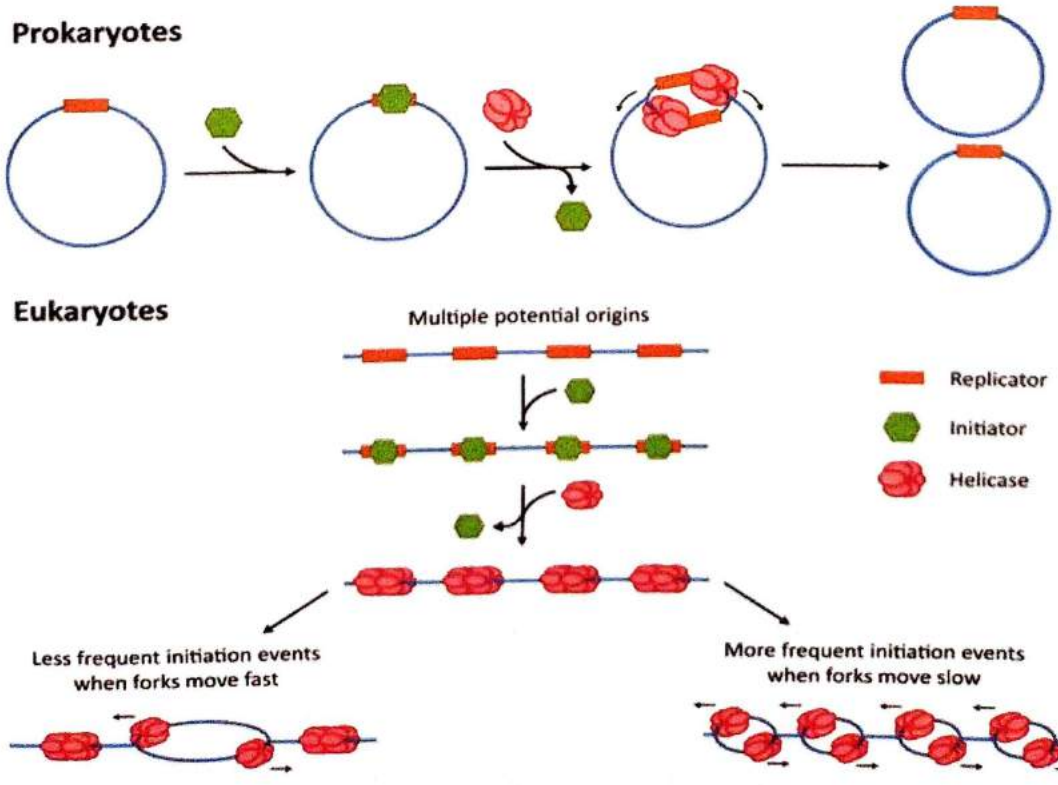


Figure 6 : Origin of replication in prokaryotes and eukaryotes. One origin starts one replication fork on each bacterial chromosome. On eukaryotic chromosomes, there are numerous probable *ori*gins. The rate at which replication forks advance is correlated with the activation of these *ori*gins; if forks advance slowly, more *ori*gins are activated.

Structure :

The DnaA protein is made up of an amino-terminal domain I that is involved in helicase recruitment and has specific residues that are necessary for oligomerization at *ori*C, a variable and possibly inconsequential domain II, a core domain III that contains the nucleotide binding site and an oligomerization motif, and a carboxy-terminal DNA binding domain (Boeneman et al., 2009).

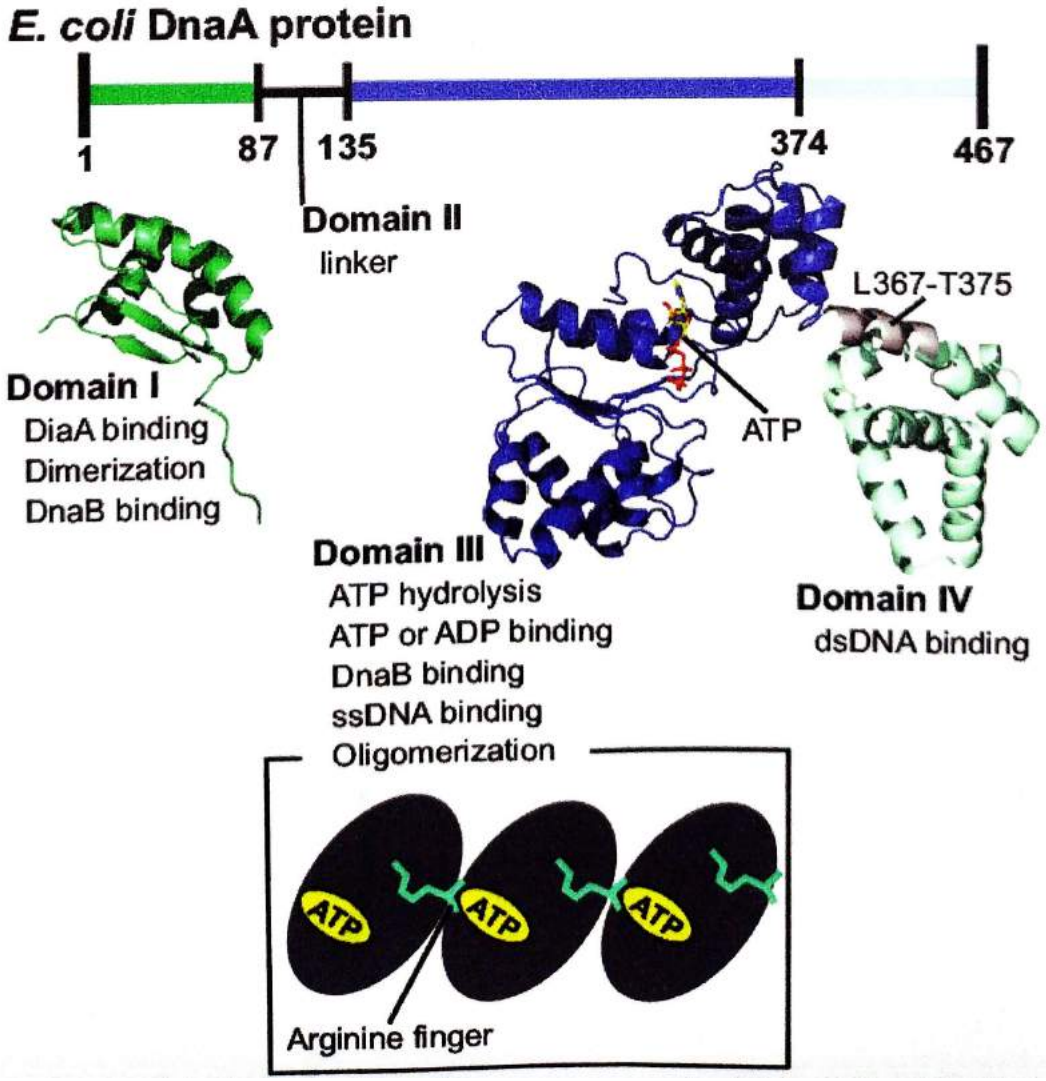


Figure 7 : The DnaA protein's domain structure. (Top) The structures and functions of the four domains that make up DnaA are listed below. Domains III–IV are homology models, while domain I is an NMR structure from Protein Data Bank 2E0G. (Bottom) The homooligomerization of DnaA domain III proceeds from head to tail. One molecule interacts with the arginine finger side of another molecule through its ATP-bound side.

3.3.6 DNA Damage Resistance and DNA Repair Mechanisms:

D. radiodurans can repair about 200 DSBs or 190 cross-links per genome copy without losing viability, and it is 30 times and 1,000 times more radiation resistant than E. coli and humans, respectively. It is extremely resistant to numerous DNA-damaging chemicals that cause various types of DNA damage. Double-strand breaks (DSBs), single-strand breaks (SSBs), and base damage are produced by ionising radiation and desiccation; different pyrimidine dimers are created by UV radiation; and severe base and nucleotide damage is produced by hydrogen peroxide, methyl methane sulfonate (MMS), N-methyl-N′ -nitro-N-nitrosoguanidine, nitrous acid, and hydroxylamine.

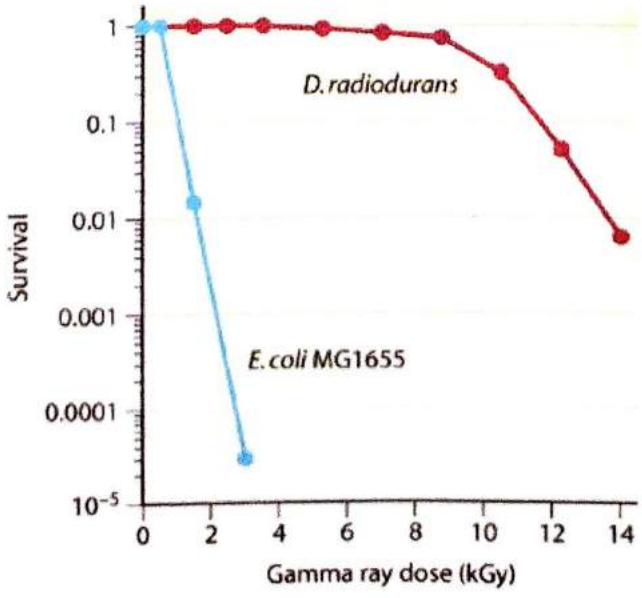


Figure 8 : Extreme resistance of *D. radiodurans* to gamma rays

Diverse DNA repair mechanisms, including direct damage reversal, base and nucleotide excision repair, mismatch repair, and recombinational repair, are used by D. radiodurans to withstand DNA damage. Ionising radiation damages the DNA by causing double-strand breaks into several pieces, but it also causes at least ten times as many SSBs and significantly more base damage sites. The most serious type of DNA damage, DSBs, is repaired sequentially by two mechanisms called "extended synthesis-dependent strand annealing" (ESDSA) and homologous recombination by crossovers (Makarova et al., 2001).

Double Strand Break (DSBs) Repair by Homologous Recombination

DNA double-strand breaks (DSBs) can result in significant genomic rearrangements and pose a serious danger to genome integrity and cell survival. Homologous recombination (HR) is the main DSB repair mechanism in bacteria. DSBs are accurately repaired by HR in a step-by-step manner using data from an undamaged homologous template. DSB recognition, DNA end processing, RecA loading, strand invasion and branch migration, and Holliday junction resolution are the five main phases of HR. It has been discovered that in D. radiodurans, an extended synthesis-dependent strand annealing (ESDSA) mechanism occurs before HR, enabling quick reconstruction of a complete genome post-ionising radiation.

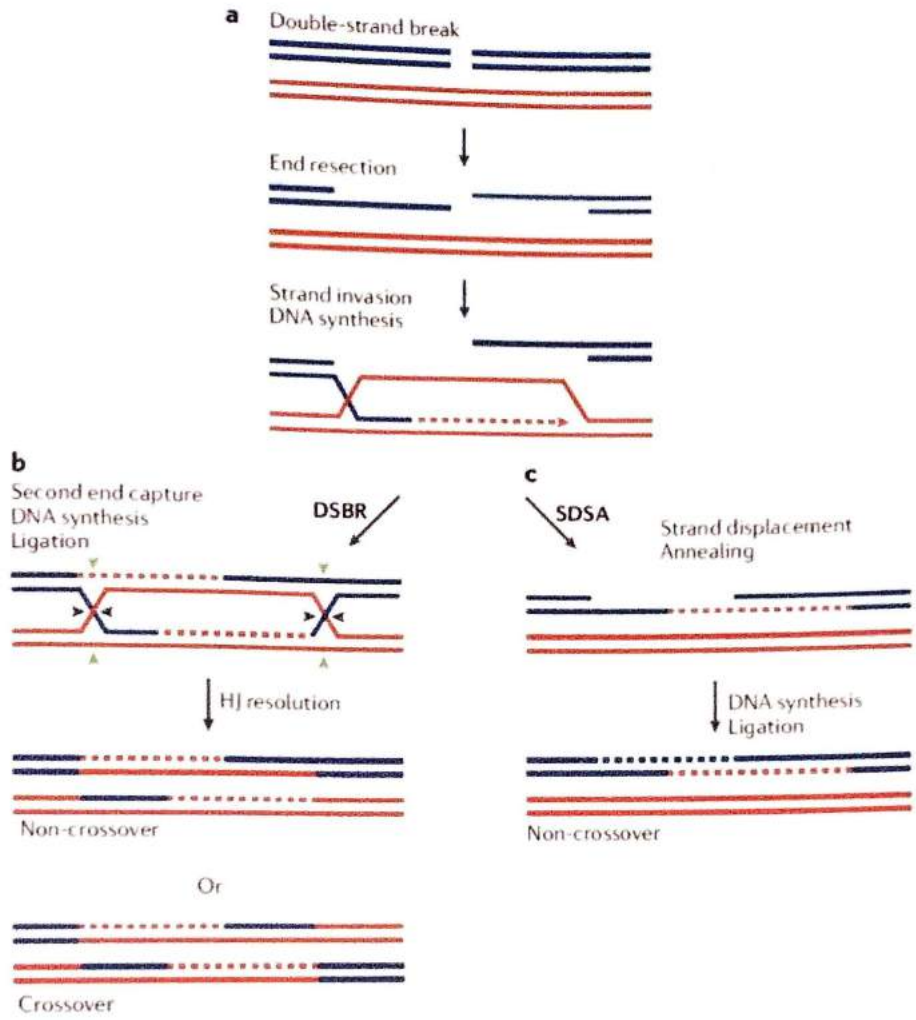


Figure 9 : Multiple homologous recombination (HR)-mediated mechanisms, including double-strand break repair (DSBR) and synthesis-dependent strand annealing (SDSA), are capable of repairing double-strand breaks (DSBs). **(a)** Repair is started in both pathways by resecting a DSB to create 3' single-stranded DNA (ssDNA) overhangs. These 3' ssDNA overhangs cause strand invasion into a homologous sequence, which is followed by DNA synthesis at the invasive end. **(b)** The second DSB end can be caught to create an intermediate with two Holliday junctions (HJs) after strand invasion and synthesis. After gap-repair DNA synthesis and ligation, the structure is resolved at the HJs in a non-crossover (black arrowheads at both HJs) or crossover mode (green arrowheads at one HJ and black arrowheads at the other HJ). **(c)** By strand displacement, annealing the stretched single-strand end to the ssDNA on the other break end, gap-filling DNA synthesis, and ligation, the reaction can also progress to SDSA. SDSA always offers non-crossover repair products.

3.4 PROTEIN PURIFICATION

For the purpose of characterization of the function, structure, and interactions of the target protein, protein purification is essential. In the process of purification, the protein and non-protein components of the mixture may be separated out, and then the desired protein can be separated from all other proteins. In most separation processes, differences in protein size, physico-chemical characteristics, binding affinity, and biological activity are taken advantage of. Either preparative or analytical methods are used to purify proteins. The goal of preparative purifications is to create a sizable amount of purified proteins for later usage. The analytical purification procedure yields a relatively small quantity of a protein for a range of analytical or research applications, such as identification, quantification, and examinations of the protein's structure, post-translational changes, and function.

3.4.1 Affinity Chromatography

Affinity Chromatography is a separation technique based upon molecular conformation, which frequently utilizes application specific resins. These resins have ligands attached to their surfaces which are specific for the compounds to be separated. This relies on the reversible interactions between the protein and the affinity ligand coupled to the chromatographic matrix. The proteins are expressed with a specific affinity tag such as a sequence of 6 to 8 histidines into the N- or C-terminal of the protein and purified using immobilized metal- affinity chromatography (IMAC). The polyhistidine binds strongly to divalent metal ions such as nickel and cobalt. The protein can be passed through a column containing immobilized nickel ions, which binds the polyhistidine tag. All untagged proteins pass through the column. The protein can be eluted with increasing concentrations of imidazole, which competes with the polyhistidine tag for binding to the column.

CHAPTER-4

MATERIALS

4.1 Bacterial strains used

Deinococcus radiodurans R1

This wild type strain of *Deinococcus radiodurans R1* was used to isolate gene of interest.

Nova Blue

Nova Blue cells are used for routine molecular cloning applications. It is a K-12 derived strain and offers high transformation efficiency, aids stability of the plasmid, along with blue/white screening with appropriate plasmids. It also has *recA* and *endA* mutations resulting in higher yields of plasmid with better quality.

DH5α

DH5α cells are used for molecular cloning applications. They are defined by three mutations: *recA1, endA1*, which help plasmid insertion, and *lacZM15*, which enables blue white screening. The recA1 mutation is a single-point mutation that replaces glycine 160 of the *recA* polypeptide with an aspartic acid residue in order to disable the activity of the recombinases and inactivate homologous recombination. The *endA1* mutation inactivates an intracellular endonuclease to prevent it from degrading the inserted plasmid.

BL21 (DE3)

E. coli BL21 (DE3) is the ideal choice for routine protein expression and is one of the most widely used systems for expression of recombinant proteins. It harbors a lambda DE3 prophage, that is derived from bacteriophage λ that carries the gene for T7 RNA polymerase under the control of *lacUV5* promoter. This allows the expression of T7 RNA polymerase upon induction with IPTG.

SHuffle

SHuffle strains have been engineered from two different E. coli strains; E. coli B (C3029) and E. coli K-12 (C3026) to catalyze the formation and permit the maintenance of stable disulfide bonds within the cytoplasm of E. coli. SHuffle strains are capable of oxidising cysteines in proteins to create disulfide bonds. These strains have proved extremely helpful in creating novel disulfide-bonded proteins that previously couldn't be generated in traditional *E. coli* production strains.

4.2 Plasmid

pET series of expression plasmids are one of the widely used systems for expression of recombinant proteins in *E. coli.* pET-28a (+)(+) is the most popular expression plasmid in which the target genes are cloned under the control of strong bacteriophage T7 promoter and adjacent *lac* operator to suppress uninduced expression. The size of this plasmid is 5.3 kb. The gene of interest has to be inserted downstream of this region. The pET-28a (+) vector contains an N-terminal His Tag/thrombin/T7 tag configuration including an optional C-terminal His Tag sequence. This allows for easy purification of proteins on the Ni^{2+} affinity chromatography. It contains gene for kanamycin resistance that facilitates screening of plasmids with the desired gene of interest.

4.3 Plastic and glass ware

Disposable polypropylene micro centrifuge tubes (0.2ml, 0.5 ml, 1.5 ml and 2 ml) and micropipette tips were from Tarsons, India or Axygen, USA. Polypropylene SS34 tubes were from Tarsons, India. All the glasswares were from Corning, USA or Borosil, India. All the plastic wares and glasswares were sterilized by autoclaving. Glasswares were also sterilized by baking at higher temperature for 3 hrs.

4.4 Chemicals and Media

All the dehydrated components of the media were from Difco laboratories, USA. Other chemicals were from Sigma Aldrich, USA.

4.5 Composition of Stock Solutions.

Table1. Stock Solutions, its concentration and Storage

Chemical	Concentration		Storage conditions
	Stock	Final/Working	
$CaCl_2$	1.0M	100mM	4°C
Ethidium Bromide	5mg/ml	0.5µg/ml	RT
Sodium Chloride	5.0N	As required	RT
Sodium Acetate	3.0M (pH 5.0)	As required	RT
TrisHCl (pH 8.8)	1.5M	As required	RT
TrisHCl (pH 8.0)	1.0M	As required	RT
TrisHCl (pH 7.6)	1.0M	As required	RT
TrisHCl (pH 6.8)	1.0M	As required	RT
Sodium Hydroxide	10N	As required	RT
Potassium Chloride	2.5 M	20mM	RT
EDTA	5 mg/ml	0.5 µg/ml	RT
Glycerol	100%	As required	RT
Imidazole	8M	As required	4°C
Lysozyme	100mg/ml	1mg/ml	-20°C
PMSF	100MM	1mM	4°C
Triton X-100	10%	0.2%	RT
Acrylamide	30%	As required	4°C
APS	10%	As required	4°C
TEMED	Stock (Sigma)	As required	4°C
SDS	10%	As required	RT
IPTG	1.0M	As required	4°C
NP40	10%	0.3%	RT
Sodium Lauroyl Sarosinate	10%	0.3%	RT
Pyridostatin (PDS)			-20°C
CHAPS	10%	1%	RT
Nickel Chloride	0.5 M	As required	RT

4.6 Composition of commonly used reagents

1M TrisHCl

121.1g of Tris base was dissolved in 800ml of Distilled water and the pH was adjusted using conc. HCl. The solution was then cooled to RT and it was adjusted to the required pH and checked with the help of pH meter. The final volume was made to 1 litre using Autoclaved distilled water, then autoclaved and stored at RT.

0.5M EDTA (pH 8.0)

186.1 gm of $C_{10}H_{14}N_2O_8*2Na*2H_2O$ (Ethylenedinitrilotetraacetic acid disodium salt dehydrate) was dissolved in 800ml DDW and the pH was adjusted to 8.0 using 10N NaOH or using Sodium hydroxyl pellets. The final volume was made up to 1 litre using DDW, the solution was autoclaved and stored at RT.

5N NaCl

292.2g of NaCl was dissolved in 800ml of distilled water. The solution was stirred on a magnetic stirrer to facilitate complete dissolution of the salt. The volume was made up to 1 litre using DDW and the solution was filtered using Whatman Filter paper, autoclaved and stored at RT.

10N NaOH

200g of NaOH was dissolved in 400ml of DDW for a total volume of 500ml. The final volume was made up to 500ml using autoclaved Distilled water and stored.

1M Calcium Chl*or*ide

111g of Calcium Chl*or*ide was dissolved in 900ml of DDW. The mixture was kept on magnetic stirrer to ensure uniform mixing and distribution. The solution volume was made up to 1 litre, autoclaved and stored at 4°C. The stock solution

was diluted further to make working solution of 100mM using autoclaved distilled water.

8M imidazole

54.46 gm Imidazole was dissolved in 100mL sterile water and stored in an amber colored bottle.

Lysozyme

Lysozyme was prepared at a concentration of 100mg/ml in 10mM TrisHCl (pH 8.0) and freshly used.

100mM PMSF

0.87 gm of PMSF was dissolved in 50mL of Isopropanol and kept for through mixing, and stored at 20°C for further use.

1M IPTG

Dissolve 2.38 gm of IPTG in around 8mL of 15 mL falcon tube and make the volume to 10 mL using distilled water. The solution was sterilized with 0.22-micron filter paper and aliquots of 1mL were distributed in 1.5 mL microfuge tubes and stored at -20°C.

Acrylamide Stock (30 %)

29 gm of Acrylamide and I gm of N, N-Methylene bis-acrylamide was dissolved in 70 ml double distilled water at 37°C. The solution was kept at magnetic stirrer for 30 mins to ensure proper mixing. The solution was filtered through whatman filter paper and stored at 4°C.

2X Laemelli Cracking buffer/2x SDS gel loading dye

460 gm of SDS, 7.6 mL of EDTA, 2 mL of Glycerol, 20 mg of sodium azide, 1 mL of β-mercaptoethanol in 2.5 ml of 0.5M TrisHCl, pH 6.8, 150 µl of 100mM

PMSF, and 0.5% of Bromophenol blue were mixed and the volume was made upto 10mL using Distilled water.

10X Tris Glycine buffer (SDS Tank buffer/Protein running buffer)

To, 800 ml of autoclaved distilled water 30 gm of Trizma base, 144 gm of Glycine and 10 gm of SDS was added. The pH was adjusted to 8.3 and the volume was adjusted to 1 L with D/W.

TE Buffer

10mM Tris-HCl (pH 8.0) and 1mM EDTA (pH 8.0) were diluted from their respective stock solutions. Small aliquots of this solution were distributed, autoclaved and stored at room temperature.

10X PBS

1.37M (80g) Sodium Chl*ori*de in 800 ml of distilled water, 18mM (2.4g) Mono potassium Phosphate, 100mM di-Sodium Hydrogen Phosphate Dehydrate, 27mM (2g) Potassium Chl*ori*de the solution was autoclaved and stored at RT. The buffer is diluted to 1X with sterile autoclaved distilled water before use.

5X TBE

54 g of Tris base, 27.5 g of b*oriC* acid and 20 mL 0.5M EDTA (pH 8.0) is dissolved in distilled water and the volume is made upto 1 litre. The buffer is diluted to 0.5X with sterile autoclaved water before use.

2.5 M KCl

Dissolve 46.25 g of KCl in 250 mL of distilled water. The solution is autoclaved before use.

De-stain solution I

500 ml of Methanol was mixed with 100 ml of Glacial Acetic Acid, and the volume was made to 1 litre with distilled water.

Coomassie Brilliant Blue Stain

Dissolve 0.25 gm of CBB R250 in around 90 mL of methanol: H2O (1:1, v/v) and 10 ml of glacial acetic acid. Mix the solution thoroughly and filter with the help of Whatman filter paper no.1 to remove any particulate matter and store at RT.

4.7 Preparation of Antibiotic Stock solutions

Preparation of stock solution of antibiotic is mentioned in Table. All the antibiotics were prepared either using sterile diluents or filter sterilized. Small aliquots were distributed in vials and stored at -20°C.

Table2. Stock solution of Antibiotics

Antibiotic	Stock solution (mg/ml)	Working concentration (µg/ml)	
		For *E. coli*	For *D. radiodurans*
Kanamycin	50	25	8
Ampicillin	100	100	-
Spectinomycin	100	70	75
Chloramphenicol	34	20	5

4.8 Composition of Media

Composition of Media used is mentioned in Table. The final volume was made up using Stage -1 Distilled water. The media was sterilized by autoclaving at 121°C at 15 p.s.i for 20 minutes and stored at RT unless specifically mentioned. For Agar the final concentration of 1.7% was used. The media was allowed to cool down before adding appropriate antibiotics and poured. All the plates with or without antibiotics were sealed using parafilm and stored at 4°C until further use.

Table3. Luria Bertani Broth and Luria Bertani Agar composition

Components	Luria Broth (1L)	Luria Bertani Agar (200ml)
Bactotryptone	10gm	2gm
Bacto yeast extract	5gm	1g.
NaCl	10gm	2gm
Bacto Agar	-	3.4gm (1.7%)
Stage 1 Distilled water	1L	200ml

Table4. Tryptone Yeast Glucose (TYG) broth and Tryptone Yeast Glucose Agar composition

Components	Tryptone Yeast Glucose Broth (1L)	TryptoneYeast Glucose Agar (200ml)
Bactotryptone	10gm (1%)	2gm
Bacto yeast extract	5gm (0.5%)	1g.
Glucose	1gm (0.1%)	0.2gm
Bacto Agar	-	3.4gm (1.7%)
Distilled water	1L	200ml

4.9 Kits used for the experiments

Table5. Purification kits

Sr.no	Purpose	Kits used
1	Plasmid extraction	QIA prep Spin Miniprep Kit- QIAGEN
2	Gel extraction	QIA quick Gel extraction Kit-QIAGEN
3	PCR Purification	QIA quick PCR Purification Kit-QIAGEN

4.10 Agarose Gel electrophoresis reagents and buffer composition

Reagents

Agarose used was from Sigma Aldrich for qualitative and quantitative work.

Buffers used in Agarose Gel electrophoresis

50X TAE

242g of Trizma Base was dissolved in around ,800ml.of Distilled water, and 57.1 ml of 100% Glacial Acetic Acid and 100ml of 0.5M EDTA were added. The volume of the solution was makeup using autoclaved distilled water to 1L, and autoclaved and stored at RT. Before use, the buffer was diluted to 1X with autoclaved distilled water.

6X DNA loading buffer /dye

To prepare 10 ml of 6X DNA loading dye, weigh out 25 mg Bromophenol blue. Transfer it to a 15-mL screw-capped tube. Add 10 ml of 40% Sucrose solution

4.11 Enzymes used along with other molecular biology reagents

PCR reagents

2X Phusion master mix along with 100% DMSO were provided by New England Bio labs.

Restriction digestion Components

Restriction endonucleases (E*cori*, NdeI, NcoI, BamHI) along with 10X CutSmart buffer were from New England Bio labs.

Ligation components

The enzyme T4 DNA ligase 100 Units and 10X ligation buffer with ATP were provided by Roche.

4.12 Plasmid extraction (Chloroform-isoamyl alcohol)

Resuspension buffer (50mM glucose, 25mM TrisHCl pH 8.0, 10mM EDTA)

0.18g of Glucose is dissolved in 80mL of MilliQwater, in this 2.5mL 1M TrisHCl pH 8.0, along with 2mL 0.5M EDTA is added. The volume of the

solution was makeup using MilliQ grade water to 100ml. The solution was autoclaved and RNaseA (100ug/ml) was added and stored at 4°C.

Lysis buffer (1% SDS and 0.2N NaOH)

0.2ml of 10N NaOH with 1ml of 10% SDS is added and the solution volume is adjusted to 10ml by autoclaved distilled water. The solution was stored at RT. Note: This solution is to be prepared freshly while use.

Neutralization Buffer (5M Potassium Acetate)

29.4 gm of Potassium Acetate was dissolved in 50ml of autoclaved distilled water and around 11.5ml glacial acetic acid was added to get the (pH 4.8). The volume of the solution was makeup to 100mL using Autoclaved distilled water and stored at 4°C.

4.13 Composition of SDS-PAGE (For 2 gels)

Table6. Components of SDS-PAGE gel

Components	Resolving gel (10 mL)	Stacking gel (5 mL)
Autoclaved distilled water	2.6 mL	3.4 Ml
Tris Buffer	2.8 mL pH (8.8)	630 µLpH (6.8)
30% Acrylamide	3.4 mL	630 µL
10% SDS	100 µL	50 µL
10% APS	100 µL	50 µL
TEMED	10 µL	5 µL

4.14 Protein Purification using IMAC

Lysis Buffer – 1

20mM Tris-cl pH 7.6, 500mM NaCl, 1mM PMSF, 10% Glycerol

Lysis Buffer -2

20mM Tris-cl pH 7.6, 500mM NaCl, 10mM Imidazole, 1mM PMSF, 5MM, 0.1% Triton-X 100, 0.1% NP-40, Protease inhibitor, 10% Glycerol and Lysozyme 3mg/ml

Wash Buffer

20mM Tris-cl pH 7.6, 300mM NaCl, 80mM Imidazole, 1mM PMSF, 10% Glycerol

Elution Buffer

20mM Tris-cl pH 7.6, 300mM NaCl, 1mM PMSF, 10% Glycerol. 12 ml of buffer used with different concentration of Imidazole – 100mM, 300mM, 500mM

Pellet Solubilization Buffer

50mM Tris-cl pH 7.6, 300mM NaCl, 1mM PMSF, 0.5% Sodium Lauroyl Sarosinate

Dialysis Buffer

20mM Tris-cl pH 7.6, 150mM NaCl, 1mM PMSF, 1mM EDTA, 1mM $MgCl_2$, Protease inhibitor cocktail, 10% or 50% glycerol.

Equilibration Buffer

20mM Tris (pH 7.6), 300mM NaCl, 1mM PMSF, 10% Glycerol.

4.15 Western Blotting

10X Western Transfer Buffer

For 10X Transfer buffer-25 mM Tris, 192 mM Glycine

For 1X Transfer Buffer (100 ml)-10 ml 10X Transfer Buffer; 20 ml methanol, and the volume is made up with autoclaved distilled water.

Blocking buffer:

20 mM Tris (pH 7.6); 800mM NaCl, 3% skim milk powder was dissolved in autoclaved distilled water.

Washing buffer-TBS 20:

20 mM Tris (pH 7.6), 800mM-1M NaCl, 0.1% Tween-20, was added in autoclaved distilled water.

Alkaline Phosphatase buffer (Detection Buffer):

100mM Tris (pH 8.8), 100 mM NaCl, 50mM $MnCl_2$ was added in 50 ml of autoclaved distilled water.

BCIP/NBT

5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium–Color Development Substrate

4.16 Antibody used

Table7. Antibody and their characteristics

Antibody	Purpose	Characteristics
Anti His Antibody	WB/Co-IP	Antibody against 6X His Tag
Mouse Anti-T18 Primary Ab	WB/Co-IP	Primary antibody recognizes an epitope on the target antigen. It is produced by a host organism that is of a different species than the specimen.
Rabbit Anti-Mouse Secondary Ab	WB/Co-IP	Secondary antibodies bind to the heavy chains of primary antibodies, so that they don't interfere with the primary antibody binding to the antigen. This secondary antibody is made in a species that is different than both those of the primary antibody or the specimen.

4.17 Composition of 15% Native PAGE gel (For 2 gels)

Table8. Components of Native-PAGE gel

Components	Resolving gel (20 mL)
Autoclaved distilled water	5.9 Ml
5X TBE	2 Ml
30% Acrylamide	9.8 mL
2.5M KCl	160 µL
10% APS	150 µL
100% Glycerol	2 mL
TEMED	10 µL

4.18 Electrophoretic Mobility Shift Assay (EMSA)

EMSA Running Buffer

0.5X TBE, 20mM KCl in required amount of autoclaved water.

10X Protein Buffer

500mM Tris-cl pH 7.6, 250mM KCl, 100mM NaCl, 1mM $MgCl_2$, 1mM DTT, 10% Glycerol, the volume was made upto 1mL.

CHAPTER-5

METHODOLOGY

5.1 Growth of bacterial Culture

For *E. coli* strains (NB/DH5α/BL21)

1. Bacterial culture was streaked on LB agar plate from glycerol stock.
2. The plate was incubated overnight at 37°C.
3. A single colony from the plate was further inoculated into 5ml LB broth with or without antibiotic and incubated overnight at 37°C, 150 rpm.

For *Deinococcus radiodurans* R1

- Bacterial culture was taken from glycerol stock and streaked on TYG agar plates without any antibiotic.
- The plate was incubated overnight at 32°C.
- A single isolated colony was inoculated into sterile 5ml TYG broth and incubated at 32°C overnight at 150 rpm.

5.2 Isolation of plasmid DNA (QIAprep Spin Miniprep Kit-QIAGEN)

Alkaline lysis method was used to isolate plasmid DNA from the cells. In this method the cells are lysed under alkaline conditions and makes use of three buffers namely Buffer P1, Buffer P2 and N3 buffer. Buffer P1 is a Resuspension buffer with Tris buffer, EDTA, RNase A and Glucose. It helps resuspending bacterial cell pellet. The buffer P2 is a lysis buffer with NaOH and SDS used for lysis of cells. The SDS in the buffer solubilizes the phospholipids and proteins associated with the cell membrane. It creates pores inside the cell, and leads to release of all the cellular content outside the cell. NaOH denatures the chromosomal DNA irreversibly whereas the Plasmid DNA being smaller in size renatures back. The two strands of plasmid DNA are topologically constrained, even though denatured they remain together. Lysis reaction is allowed to proceed not more than 5 min,

during which plasmid DNA is released without release of chromosomal DNA. The N3 Buffer is the neutralization Buffer that contains potassium Acetate, hence in the presence of high salt conditions cellular debris such as proteins, ssDNA, and other components of the cell settle at the bottom, whereas plasmid DNA renatures and remains in the solution.

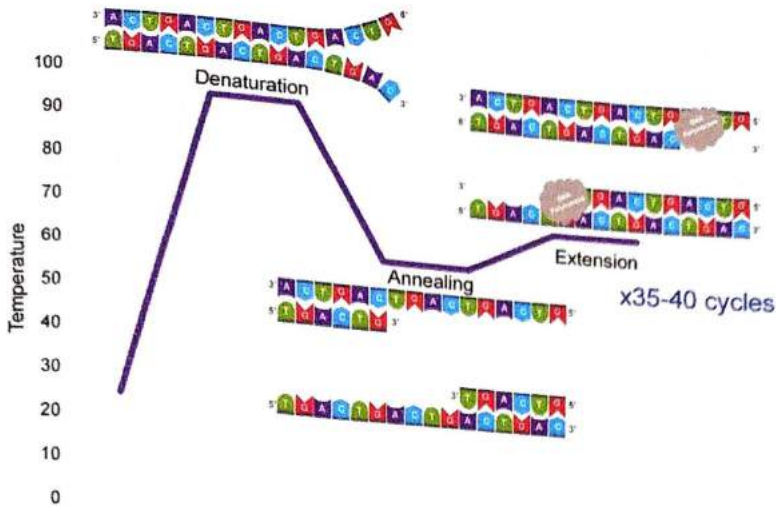
Protocol

- Pellet down 1-5ml of Overnight grown bacterial culture at 7000 rpm for 1 min in a tube. Resuspend the pellet in 250-300µl of Resuspension buffer P1 and vortex properly to ensure uniform distribution of cells without clumps.
- Add 250-300µl of Lysis buffer P2 and mixed by inverting the tubes 4-5X and allow the reaction to proceed for not more than 5 mins.
- Add 350-400µl of Neutralization Buffer N3 and mix thoroughly by gently inverting the tubes 4-5X incubate for 5 mins on ice and spin at 12,000 rpm for 10mins to obtain pellet.
- The supernatant obtained was loaded on QIAprep 2.0 Spin column by carefully pipetting the supernatant and the pellet was discarded.
- The supernatant was centrifuged at 8,000 rpm for 30sec. The flow through obtained was again put on the same column and centrifuged to get better yields. After centrifugation at same speed, the flow through was discarded.
- The column was washed with 300µl of Wash Buffer PE and centrifuged again at 8000 rpm for 30sec. The flow through was discarded again. Repeat this step twice.
- Empty spin of around 1:30 mins at 10,000 rpm was done to remove traces of alcohol.

- Meanwhile, (Stage2) MilliQ water is filtered using 0.2micron filter and is used for elution.
- Transfer the spin column to 1.5ml micro centrifuge tube and add 50µl of MilliQ water, incubate for 5 mins and spin at 10,000 rpm for 1:30 min.
- The purity of the plasmid was checked on Agarose Gel electrophoresis and concentration of the plasmid was estimated by Nanodrop.
- The plasmid was stored at -20°C until further use.

5.3 Amplification of inserts by PCR

Polymerase chain reaction is a technique most comMonly employed in molecular biology laborat*orie*s to amplify the desired segment of gene using polymerases. A template DNA is used in the reaction, on to which specifically designed primers anneal to the region which has to be amplified at specific temperature conditions. The primers for the gene can be comMercially synthesized. In order to determine the optimum annealing temperature of the primers to the template, gradient PCR was performed for successful amplification of inserts or segment of gene. Phusion Rich GC buffer master mix is used. It is a 2X master mix that contains deoxynucleotides, reaction buffer, and $MgCl_2$. The final concentration used is 1X. For gradient PCR, 100µl reaction mixture was prepared, and 20µl aliquots were made in five 0.2ml sterile PCR tubes as per the reaction given in Table. All reaction tubes were kept at different temperatures and amplification was carried out, and once optimization of annealing temperature was done, a larger volume reaction is prepared. Aliquots of the mixture were distributed in four 0.2 ml sterile PCR tubes and amplified at respective temperature that was optimized. Amplified PCR products were checked on 1% Agarose Gel.



https://iastate.pressbooks.pub/genagbiotech/chapter/pcr-and-gel-electrophoresis/

Figure 10 : Process of Polymerase Chain Reaction

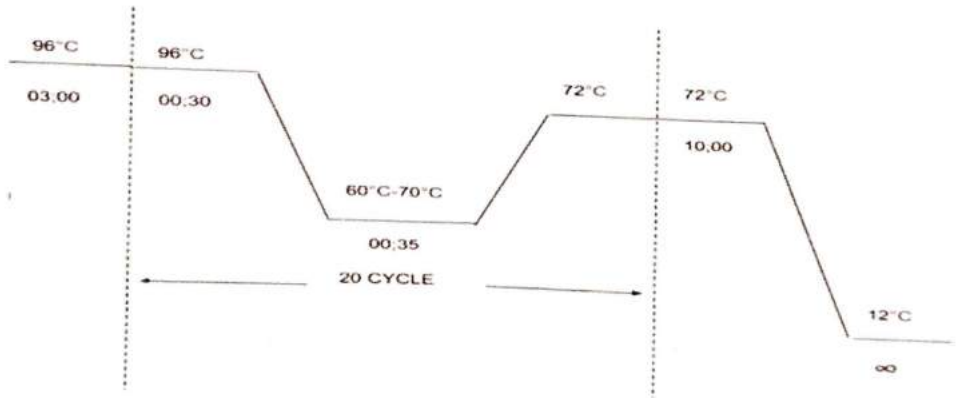


Figure 11 : Cycles in Polymerase chain reaction

Table9. Gene annealing temperature and extension time

GENE	Annealing Temperature	Extension time
RGG del *mutS* up	64.4 °C	1 min
RGG del *mutS* down	62.4 °C	1 min
mutS	64 °C	2 min 30 sec
Ori Chromosome I	64 °C	1 min

Table10. Reaction mixture for Gradient PCR

Components	Volume (µl)
Template DNA	1
Primers (Forward and Reverse)	4
2X Phusion master mix	50
10% DMSO	10
MilliQ water	35
Total	100

Table11. Reaction mixture for amplification at optimized Temperature conditions

Components	Volume (µl)
Template	2
Primers (Forward and Reverse)	8
2X Phusion master mix	100
10% DMSO	20
MilliQ water	70
Total	200

For visualization of DNA

0.5g of Agarose powder (Sigma Aldrich) was added in 50ml of 1X TAE buffer and heated for 1 minute 30 seconds to thoroughly dissolve the agarose. After allowing the solution to cool, 0.5µg/ml of Ethidium Bromide was added after the temperature reached 50°C. The gel was put into a Bio-Rad gel casting equipment and allowed to polymerize at room temperature. The PCR-amplified material was combined in a 5:1 ratio with 6X gel loading dye and loaded into the wells. The gel was run at 90 volts until the tracking dye reached 3/4 down the gel. Under short-wavelength UV, DNA bands were visualized on a gel documentation system (SYNGENE). If a single band is observed, PCR purification is done, and if multiple bands are observed, gel extraction is carried out.

5.4 Generation of mutants

5.4.1 Generation of mutants using Deletion

To create a specific point mutation, insertion, or deletion inside a specific DNA sequence of interest, overlap extension polymerase chain reaction (PCR) mutagenesis can be used. Compared to other mutagenesis techniques, it requires very little preparation and doesn't require the use of restriction enzymes. Overlap extension PCR mutagenesis is a more adaptable technique than its predecessors. With this technique, it is practically possible to insert or delete DNA at any point along a given DNA sequence, regardless of size. An insertion fragment and two flanking fragments must first be prepared by PCR in order to produce an insertion mutation. The insertion fragment is recombined with two flanking fragments produced from the original template during the secondary PCR.

5.4.2 Generation of mutants using Site-Directed Mutagenesis (SDM)

The method of site-specific nucleotide sequence alterations is known as site-directed mutagenesis. It has evolved into a crucial tool for determining the significance of specific residues in protein engineering. The nucleotide sequence can be altered by replacing a nucleotide with a different nucleotide, which can alter the protein's overall stability, solubility, and function. In rare cases, SDM can lead to the production of new gene products in addition to enabling structural and functional studies of the gene. Many methods for introducing mutations have been devised. One method is to use PCR, which amplifies the nucleotide sequence using pairs of primers. These primers anneal in the opposite direction at either end of the sequence, amplifying the target mutation exponentially in the target sequence.

5.4 Generation of mutants

5.4.1 Generation of mutants using Deletion

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Principle of Overlap Extension PCR.

In mutagenesis by overlap extension PCR, altered genes with mutations are produced from cloned DNA using PCR. With just a few steps, this technique may quickly insert precise mutations from a cloned gene into the sequence. In a PCR procedure, two PCR fragments with overlapping regions are combined, eliminating the need for DNA ligases and restriction endonucleases.

Mutagenesis by using overlap extension PCR involved generating fragments of DNA, by incorporating the primers in two rounds of PCR reactions. The Overlapping Extension PCR's mechanism is shown in Figure 11. Separate PCRs using mutagenic primers are used to create the fragments with specific mutations. Here, each reaction uses a flanking primer (Primer a and Primer d) that binds to either end of the target sequence and a mutagenic primer (Primer b and Primer c) that binds to the mutation site containing a mismatched nucleotide. Since mutagenic primers incorporate into and become a part of the PCR result, the WT sequence devoid of any mutation cannot be amplified. The two internal primers with overlapping areas can be fused together in the subsequent round of PCR using two flanking primers that hybridise to each end of the sequence. Due to this overlap, one strand of the fragment can serve as a primer for the other, and when this process is extended, a full-length fragment with the desired mutation is produced. Short fragment overlap happens less frequently and is prevented by using flanking primers a and d.

Here we report the generation of a mutants using SDM of *MutS* gene. One conserved site was identified based on the MSA data. The conserved amino acid Arginine is replaced by serine.

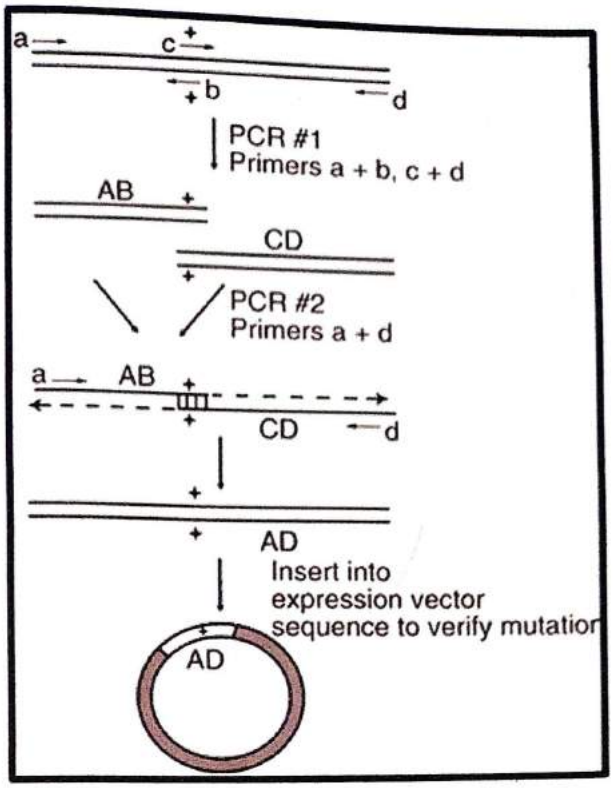

Figure 12 : Site directed mutagenesis by overlap extension PCR

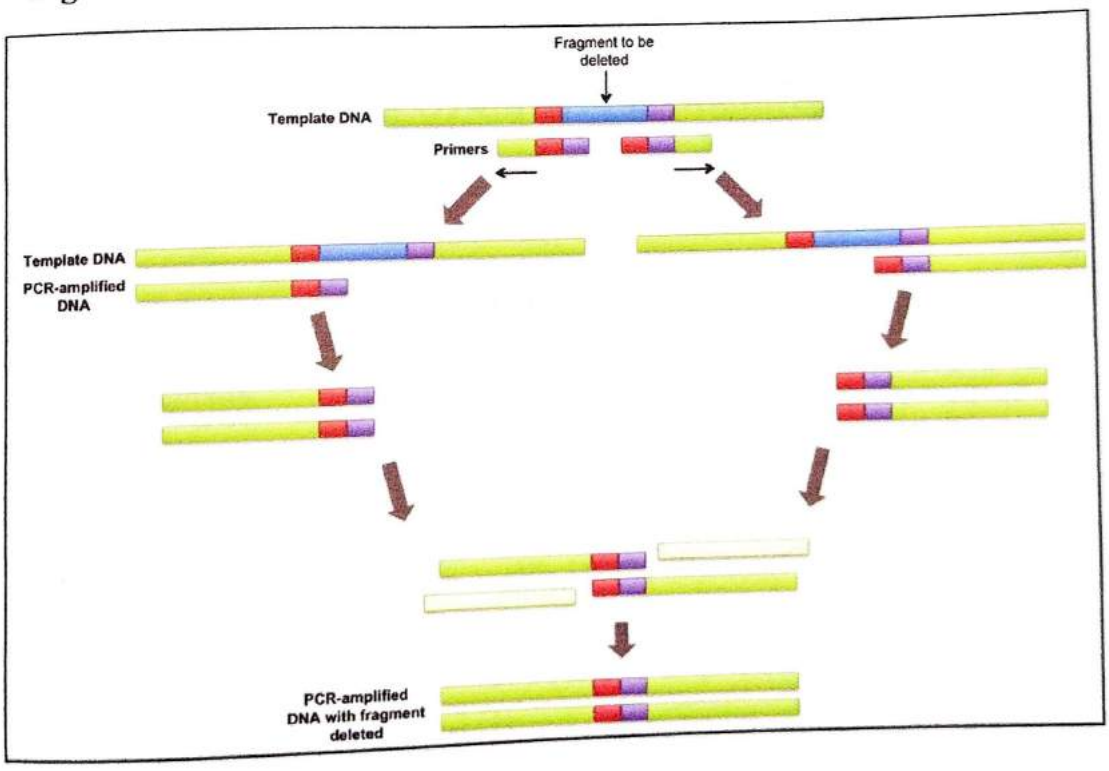

Figure 13 : Deletion by overlap extension PCR

5.5 PCR Purification using High Pure Roche PCR purification Kit

- To the total volume of PCR product Roche PCR Binding Buffer is added in 1:5 ratios.
- Incubate for 2-3 mins and add 1/3rd volume of Isopropanol in it.

- Load the above product to the PCR spin columns and spin for 30sec 8000 rpm. The flow through obtained was again put on the same column and centrifuged to get better yields. After centrifugation at same speed, the flow through was discarded.
- The column was washed with 350µl of Roche Wash Buffer for 30 sec at 800 rpm.
- Empty spin of around 1:30 mins at 10,000 rpm was done to remove traces of alcohol
- Meanwhile, (Stage2) MilliQ water is filtered using 0.2µ filter and is used for elution.
- Transfer the spin column to 1.5ml micro centrifuge tube and add 50µl of MilliQ water, incubate for 5 mins and spin at 10,000 rpm for 1:30 min.
- The purity of the product is checked on Agarose Gel electrophoresis and concentration of the was estimated by Nanodrop.
- The product was stored at -20°C until further use

5.6 PCR Gel extraction using QIAprep Spin Miniprep Kit-QIAGEN

After amplification of inserts, the PCR-amplified insert was gel purified to remove PCR components or if multiple bands were observed that might interfere with further experiments.

- The PCR-amplified product was combined in a 5:1 ratio with 6X gel loading purple dye and loaded into the wells of 1% Agarose gel kept in a clean and sterile Bio-Rad electrophoresis unit containing 1X TAE buffer.
- After electrophoresis is completed, the DNA is visualized by handset UV lamp at long wavelength and the DNA is cut with a sterile blade.
- Take the cut piece of the gel in a 2 ml Eppendorf tube and add QG Gel Solubilization Buffer in 1:3 ratios. Heat the tube at 55° C until all the gel melts. Vortex at appropriate intervals.
- Add 1/3rd volume of Isopropanol in it.

- Load the melted gel solution and spin at 8000 rpm for 30sec pass 600µl of solution through column. Spin for 30 sec 8000 rpm. The flow through obtained was again put on the same column and centrifuged to get better yields. After centrifugation at same speed, the flow through was discarded.
- The column was washed with 300µl of Wash Buffer PE and centrifuged again at 8000 rpm for 30sec. The flow through was discarded again. Repeat this step twice.
- Empty spin of around 1:30 mins at 10,000 rpm was done to remove traces of alcohol.
- Meanwhile, (Stage2) MilliQ water is filtered using 0.2µ filter and is used for elution.
- Transfer the spin column to 1.5ml micro centrifuge tube and add 50µl of MilliQ water, incubate for 5 mins and spin at 10,000 rpm for 1:30 min.
- The purity of the product is checked on Agarose Gel electrophoresis and concentration of the was estimated by Nanodrop.
- The product was stored at -20°C until further use.

5.7 Restriction Digestion

- Restriction digestion is a process in which restriction enzymes are used to create specific cuts in the DNA. All the restriction enzymes have specific recognition sites, and they cleave the DNA only at that specific position.
- By using two different restriction endonucleases to cleave the vector and insert, it is ensured that the insert will ligate in a proper *ori*entation in the vector; this is referred to as directional cloning. The pET-28a (+) and pNOS plasmids isolated along with PCR-amplified and gel-purified inserts were digested with the respective enzymes.
- It is important to check the efficiency of enzymes. Hence, a small-scale single digestion reaction is also prepared along with the large-scale double digestion. Vortexing of samples are important to ensure uniform distribution of enzymes in the

solution. The samples were kept at the respective incubation temperature for 3–4 hours or overnight. The digested samples were checked on Agarose gel to confirm the activity of enzymes.

- Small quantity of digested product was checked on Agarose gel to confirm the digestion, and the remaining volume of the digested product was put on 72°C (10 min) for inactivation of restriction enzymes.

Table12. Restriction enzymes used for plasmids and inserts

DNA	ENZYMES and Incubation temp.
pET-28a (+)(+)	NdeI, BamHI (both at 37 °C)
RGG del mutS	NdeI, BamHI (both at 37 °C)
RGG sd mutS	NdeI, BamHI (both at 37 °C)

Table13. Reaction for RE digestion of plasmid and inserts

Components		Enzyme Single digestion 1	Enzyme Single digestion 2	Double digestion plasmid	Double digestion insert
MilliQ		11 µl	11 µl	48 µl	45 µl
10X Cut smart buffer		1.5 µl	1.5 µl	7.5 µl	7.5 µl
Enzyme	1	0.5 µl	-	1 µl	1 µl
	2	-	0.5 µl	1 µl	1 µl
Plasmid		2 µl	2 µl	20 µl	30 µl
Total Volume		15 µl	15 µl	75 µl	75 µl

Purification of double digested plasmid

After inserts and plasmids have been successfully digested with their respective restriction enzymes, the double digested plasmid is gel filtered to remove undigested plasmid. As a result, following confirmation of restriction digestion, the inserts were maintained at 72 °C for heat inactivation to inactivate enzymes. The double-digested

plasmid was put on a low-melting agarose gel and run at 50V for purification. Following electrophoresis, the plasmid-containing gel fragments were visualized using a portable UV light at a long wavelength, and the gel was excised. The QIAquick gel extraction kit was then used to purify the plasmid DNA for the gel.

5.8 Ethanol precipitation by Sodium acetate

A precipitation process with a greater molar ratio of insert to vector was put up to speed up the reaction. For sticky end ligation, a 1:3 ratios are typically used. The minimum concentration of vector required for a successful ligation reaction should be greater than 100 ng, and the insert volume is then adjusted accordingly. 10% Sodium Acetate is added to the reaction. Sodium acetate offers high ion concentration and ensures proper pH for DNA precipitation.

Table14. Reaction for DNA Precipitation

Vector	40 µl
Insert	25 µl
Na Acetate	10 µl
MQ	2.5 µl
Total	**75 µl**

- To the total volume of 75 µl add 2.5 times the volume of absolute alcohol and incubate at -20°C for overnight precipitation.
- Next day, the mixture was centrifuged at 12,000 rpm for 10 minutes. The supernatant was discarded, and approximately 200–300 µl of 70% alcohol was used to wash the

pellet. The mixture was centrifuged at 12000 rpm for 10 minutes, and the supernatant was discarded.

- Dry spin the DNA sample in a vacuum concentrator at 45 °C for 30 minutes to remove alcohol. The pellet obtained was then resuspended in a small amount of MilliQ water or ligation mixture, was directly added to it.

5.9 Ligation reaction

- The ligation reaction was setup according to table below.
- After setting up the reaction, the mixture was kept at 16 °C for 4-5 hrs or overnight.

Table15. Ligation mixture reaction

Ligation Mixture	**Volume**
T4 DNA 10X Buffer	1.5µl
ATP	1.5µl
T4 DNA Ligase enzyme	1.5µl
MQ	11.5 µl
Total Volume	**16 µl**

5.10 Preparation of competent cell and transformation in bacteria

Competent cells are bacterial cells that can take up foreign DNA from their environment through a process known as transformation. If the cell walls of *E. coli* are changed, the cells are more likely to absorb the DNA. Calcium chl*ori*de and heat shock therapy can make the cells competent. Rapidly developing cells can be

rendered more competent than cells at other phases of development. Following transformation, the cells may express the acquired genetic information. The technique is comMonly used to introduce recombinant plasmid DNA into competent bacterial cells. Competent cells have modified cell walls that allow DNA to pass through readily. To become competent, certain cells must be subjected to chemical or electrical treatments. Calcium ion treatment is effective. Calcium chl*ori*de allows binding of the plasmid DNA to the lipopolysaccharide (LPS) of the cell, by forming a divalent cation coordination bonds formed between the plasmid DNA and the LPS. Negatively charged DNA and LPS interact, and heat shock treatment allows the plasmid DNA to enter the bacterial cell.

Protocol

- The overnight grown primary culture of NB/DH5α strain was diluted to 100-fold in fresh LB medium.
- The culture was allowed to grow at 37 °C until the $O.D_{600}$ reaches up to 0.3-0.4 and the culture was then transferred on ice for about 30-45 min to stop the cells from further entering the stationary phase.
- The culture was transferred to pre-chilled SS34 tubes and centrifuge at 6,000 rpm for 10 min at 4^0C.
- The pellet obtained was gently resuspended in half volume of 100mM CaCl2 of initial volume of the culture in sterile conditions and the suspension was kept on ice for around 45 mins.
- The suspension was then centrifuged at 4000 rpm for 10 min at 4°C. After the spin is completed, an eye shape pellet is observed and competent cells were gently suspended in 1mL of 100mM CaCl2. The competent cells were

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- The suspension was then centrifuged at 4000 rpm for 10 min at 4°C. After the spin is completed, an eye shape pellet is observed and competent cells were gently suspended in 1mL of 100mM CaCl2. The competent cells were

then kept on ice for around 1 hour before they can be used for transformation.

- The competent cells can be stored in 20% Glycerol for one month at -70^{0}C without losing the competence of cells.
- Competent cells were then aliquoted in 1.5 ml pre-chilled eppendorfs tubes and specific amount of DNA is added depending on the concentration of DNA.
- After adding the DNA, then it was gently mixed by tapping.
- For positive control, uncut plasmid was used and added in the competent cells, for negative control, only competent cells without any DNA was used.
- The mixture was incubated on ice for 45 min to facilitate binding of DNA.
- Heat shock at 42°C for 90 secs is given to allow entry of plasmid inside the cells, followed by cold shock by incubating in ice for 5-10 min.
- The transformation mixture was further diluted by adding1mL of fresh LB medium. The mixture was incubated at 37°C for 60 min.
- After incubation, tubes containing LB broth and the competent cells were centrifuged at 8000rpm for 1 min.
- After carefully discarding the supernatant, the pellet was resuspended in the remaining supernatant and approximately 100-200µL of transformation mixture was plated using spread plate technique on LB agar plates with the appropriate antibiotics.
- The plates were allowed to dry and incubated at 37°C overnight.
- The positive control and negative control are kept to determine the efficiency of transformation.

- Next day random colonies were selected and streaked on LB agar plates containing appropriate antibiotic and incubated at 37^0C.

5.11 Plasmid Screening using Chloroform-isoamyl alcohol plasmid extraction method after transformation

- The screening process of the transformants is carried out in two steps:
  1. Plasmid shift
  2. RE Digestion

The basic principle of chloroform isoamyl alcohol is the solubility of DNA in imMiscible solutions. It is a liquid-liquid plasmid isolation method involving chloroform and isoamyl alcohol.

- In 1.5mL of Microfuge tube, add around 200uL of Soln1. With the help of autoclaved tips, scrape the patched colonies from LB agar plates and vortex ensure complete Resuspension of the cells in the solution.
- After 5 min of incubation, add 200uL of lysis solution (0.2N NaOH, 1% SDS) was added and gently mixed by inverting the tubes 4-5 times and kept for 5 min.
- After 5 min, when the tubes were opened, thread like structures were visible indicating complete lysis of the cells, then300 µl pre-chilled Soln 3 (potassium acetate 5M Solution) was added and mixed thoroughly by inverting the tubes 4-5 times. The mixture was incubated on ice for 5 min and then spin at 12,000 rpm for 15 mins.
- The supernatant was then transferred to another 1.5mL tubes and 600µL of chloroform isoamyl alcohol prepared in the ratio of 24:1.
- Mix by gently inverting the tubes, and centrifuge at 12000 rpm for 10 min.

- After centrifugation, the mixture was separated in two phases, the upper aqueous phase and the lower organic phase. The aqueous phase was separated by carefully pipetting and transferred into 1.5mL microfuge tube.
- Isopropanol was added about $1/3^{rd}$ volume to aqueous layer and incubated at 30 min at RT.
- The tubes were centrifuged at 12,000 rpm for 10mins and the supernatant was discarded. The pellet obtained was washed with 70% ethanol and centrifuged again same speed.
- Supernatant was discarded and the pellet obtained were kept for dry spin in a vaccum concentrator for 30-40 min at 45^0C to remove excess alcohol.
- The pellet obtained was resuspended in approx. 30µL of MilliQ water.
- All the plasmids were checked on Agarose gel electrophoresis with a plasmid that was used for transformation as the control plasmid.
- Positive plasmids can be identified by the increase in molecular weight of the plasmid because of ligation of insert in the vector, an upper shift in the gel can be observed.
- Positive plasmids are selected and stored at -20^0C for further use.

5.12 Screening of transformants

- After plasmid isolation of clones, the plasmids are loaded on the agarose gel to check for the shift.
- If insert and vector are ligated properly the plasmid size is increased than the normal plasmid and it shows a slower migration on the gel.

5.13 Restriction digestion of clones

- Plasmids which were selected to be positive based on the shift observed on the gel, were subjected to restriction digestion with the respective enzymes use during cloning.
- Plasmids which have been cloned successfully, show the release of insert whose size can be confirmed with the DNA ladder.
- Positive clone(s) selected from this were streaked on LB agar plates and broth with appropriate antibiotics and stored and maintained for further experiments.
- Positive clones were also stored in 20% Glycerol at -70^{0}C for further use.

5.14 Transformation of Positive clones in BL21 (DE3) for expression of protein

In order to express recombinant proteins produced by the desired gene of interest cloned in a particular vector, the plasmids have to be transformed in *E.coli* strain BL21 (DE3) which is one of the most comMonly used expression system for recombinant protein expression. Plasmid with the desired gene of interest was isolated from NB/DH5α cells and transformed in *E. coli* BL21 in a similar manner as compared to transformation in NB/DH5α cells.

Protocol for transformation in BL21 (DE3)

- The overnight grown primary culture of BL21 (DE3) strain was diluted to 100-fold in fresh LB medium.
- The culture was allowed to grow at 37 °C until the $O.D_{600}$ reaches up to 0.3-0.4 and the culture was then transferred on ice for about 30-45 min to stop the cells from further entering the stationary phase.

  The culture was transferred to pre-chilled SS34 tubes and centrifuges at 6,000 rpm for 5 min at 4^{0}C.

- The pellet obtained was gently suspended in half the volume of 100mM CaCl2 of the initial volume of the culture in sterile conditions and the suspension was kept on ice for around 2 hours.
- The suspension was then centrifuged at 4000 rpm for 10 min. After the spin is completed, an eye shape pellet is observed and competent cells were gently suspended in 1ml of 100mM CaCl2. The competent cells were then kept on ice for around 1 hour before they can be used for transformation.
- The competent cells can be stored in 20% Glycerol for one month at -20^{0}C without losing the competence of cells.
- Then 100µl of competent cells were aliquot in 1.5ml pre-chilled tubes and plasmid DNA isolated from NB/DH5α cells around 5 µl was added.
- After adding the DNA, the ligated mixture was mixed by tapping.
- The mixture was incubated on ice for 30-45 min to facilitate binding of DNA.
- Heat shock at 42^{0}C was given for 90 secs to allow entry of plasmid inside the cells, followed by cold shock by incubating in ice for 5-10 min.
- The transformation mixture was further diluted by adding1mL of fresh LB medium. The mixture was incubated at 37^{0}C for 60 min.
- After incubation, tubes containing LB broth and the competent cells were centrifuged at 8000rpm for 1 min.
- After carefully discarding the supernatant, the pellet was resuspended in the remaining supernatant and approximately 100-200µl of transformation mixture was spread on LB agar plates containing appropriate antibiotics.
- The plates were allowed to dry and incubated at 37^{0}C overnight.
- Random colonies were selected and streaked on grids on LB agar plates containing appropriate antibiotic and incubated at 37^{0}C.

5.15 Small scale Induction of proteins

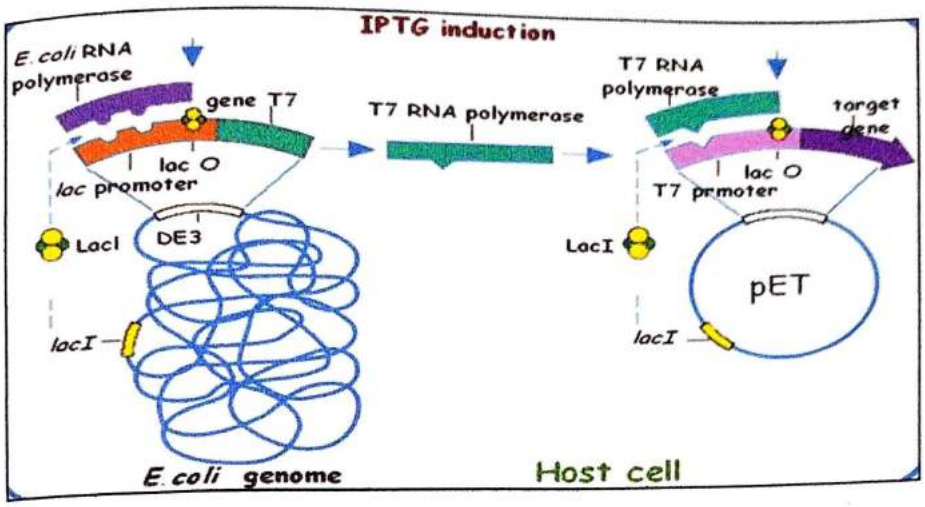

Figure 14 : IPTG induced expression of recombinant proteins (https://www.rrnursingschool.biz/restriction-enzyme/images/8170_146_157-iptg-induction-polymerase.jpg)

Small scale induction is used to check the production of desired proteins. IPTG (iso-propyl-D-1-Thiogalctopyranoside) is most comMonly used to check induction of proteins. When IPTG is present in the medium, lac repressor binds to the operator region of the operon, blocking the *E. coli* RNA polymerase from further transcribing and translating the T7 gene. The T& bacteriophage system is used for the expression of recombinant proteins because it recognizes only bacteriophage promoter such as T7 promoter, not present in *E. coli*, and T7 RNA polymerase is highly processive enzyme and can transcribe genes that cannot efficiently transcribe by *E. coli* RNA polymerase. Optimal expression of proteins can be achieved only if the growth conditions are controlled. The culture conditions play an important role in expression of recombinant proteins, and it is optimized before proceeding with large scale induction.

- Clones are inoculated in 2ml of LB broth containing respective antibiotic and incubate until the $O.D_{600}$ reaches up to 0.3-0.4.
- Then induced with 0.5mM IPTG after taking uninduced aliquot and incubate for 3hrs at 37°C.
- Spin the culture medium at 8000rpm for 30 secs to harvest the pellet from the culture.
- Re-suspend the pellet in TE buffer and add equal volume of 2X Laemlli buffer to it.
- Heat the sample at 95°C for 15 minutes. Short spin and load on 10% PAGE gel.

5.16 SDS-PAGE gel

Polyacrylamide gel provides a matrix for electrophoretic separation of polypeptides. The use of sodium dodecyl sulfate (SDS) and polyacrylamide gel in SDS-PAGE eliminates the influence of structure and charge, allowing proteins to be separated solely based on polypeptide chain length. SDS is a detergent with a strong protein-denaturing effect and binds to the protein backbone at a constant molar ratio. In the presence of SDS and a reducing agent, proteins unfold into linear chains with negative charge proportional to the polypeptide chain length. In denaturing SDS-PAGE therefore, migration is determined by the molecular weight.

Table16. Composition of 10 % resolving polyacrylamide gel and 5% stacking gel

Acrylamide 30%	6.7 ml	Acrylamide 30%	830µl
1.5 M Tris-HCl pH 8.8	5.6 ml	1.5 M Tris-HCl pH 6.8	630µ
10% SDS	200µl	10% SDS	50µl
10%APS	200µl	10%APS	50µl
TEMED	15µl	TEMED	12µl
Water	5.3 ml	Water	3.4 ml
Total Volume	20 ml	Total Volume	5 ml

- Glass plates were cleaned with liquid soap, rinsed with water and wiped with 70% ethanol. Gaps between the plate and stand were sealed with agar and SDS-PAGE mixture of resolving gel was poured, followed by addition of isopropanol.

- After 20-30 min, the isopropanol was removed and washed with water. Stacking mixture was poured and comb was fitted in the gel.
- Samples were prepared by heating equal volume of sample and 2X SDS gel loading dye at 95°C.
- The denatured samples were centrifuged at 12,000 rpm for 10 min and clear supernatant was loaded in the gel.
- The electrophoresis tank was filled with electrophoresis buffer (10% SDS-Tris-Glycine, pH 8.8) and gel was run at 90 volts. The gel was then processed as needed.
- After a full run, gel was removed and stained with Coomassie Blue for 30 minutes, kept on a rocker for better staining.
- On completion of the staining, the stain was poured off and destained with destaining solution-I , kept on rocker for 10–15 minutes.
- The samples are separated out as blue bands on a transparent gel.
- If western blotting is planned, then gel should be used without staining.

5.17 Large Scale Protein induction

- The overnight-grown primary culture of BL21 (DE3)/ SHuffle pET clones was diluted to a 1:500 ratio in fresh LB medium containing the respective antibiotic and incubated at 37 °C until the O.D.600 reached up to 0.3–0.4 (exponential phase).
- 0.5 mM IPTG was added to the medium, and further incubation was carried out at appropriate growth conditions.
- After incubation, the media is decanted into buckets of a swinging bucket centrifuge, balanced properly, and centrifuged at 4000 rpm for 20 minutes at 4 °C.

- Decant the supernatant and resuspend the pellet in a small amount of initial media in the bucket.
- Transfer this to a 50-ml sterile flask and centrifuge at 4 °C for 5 minutes at 6000 rpm.
- Decant the supernatant and resuspend the pellet in Lysis Buffer 1.
- Keep overnight at -80 °C.
- The next day, the falcon is thawed at 37 °C, and Lysis Buffer 2 is added to it. Incubate at 37 °C for 1 hour.
- Add DnaAse and incubate for 30 minutes at 37 °C.
- Further, incubate the sample at 7°C on a rocker for 1 hr.
- Proceed with sonication. Sonication of cells is the third class of physical disruption comMonly used to break open cells. The method uses pulsed, high-frequency sound waves to agitate and lyse cells, bacteria, spores, and finely diced tissue. Sonication is done for 10 minutes at 32 amplitude, 10 seconds on and 10 seconds off. The tube is surrounded by ice to avoid heating the sample.
- After sonication, the sample is centrifuged at 4 °C for 6000 rpm for 10 minutes.
- Meanwhile, column preparation is done for affinity chromatography. After the columns are ready, the supernatant is loaded onto the column and incubated overnight at 7 °C on shaker conditions to increase binding efficacy.
- The next day, the columns are clamped on stands, and the flow through is allowed to elute out. The flow through is also collected to check on SDS-PAGE.
- The column is washed using wash buffer 2 times the amount of supernatant loaded onto the column.
- Further elution is carried out using elution buffer containing different concentrations of imidazole in it. The eluted sample is collected in different fractions, which are then

checked on SDS-PAGE gel and further processed for concentration using an appropriate size protein filter column followed by dialysis.

5.18 Pellet Solubilizaton

Inclusion bodies, highly aggregated proteins, are produced when numerous recombinant proteins are expressed at high levels in Escherichia coli. Normally, inclusion bodies form in the cytoplasm; however, if a secretory vector is utilised, they can form in the periplasm. By using low-speed centrifugation, inclusion bodies can be extracted from cell lysates.

- To solubilize the pellet, add 50MM Tris, 300MM NaCl, 0.5% Sodium Lauroyl sarcosinate 1.5MM PMSF and make up the volume to 20Ml.
- Keep the tubes overnight on a rocker at 4°C.
- The next day, load the contents into affinity chromatography columns.

5.19 Protein Purification

A. **Column preparation** - Ni-NTA Agarose is an affinity chromatography matrix for purifying recombinant proteins carrying a His tag. Histidine residues in the His tag bind to the vacant positions in the coordination sphere of the imMobilized nickel ions with high specificity and affinity.

- Rinse the column with Stage 1 water and let the whole thing pass through it fully. Repeat this step twice.
- Wash twice with 70% ethanol.

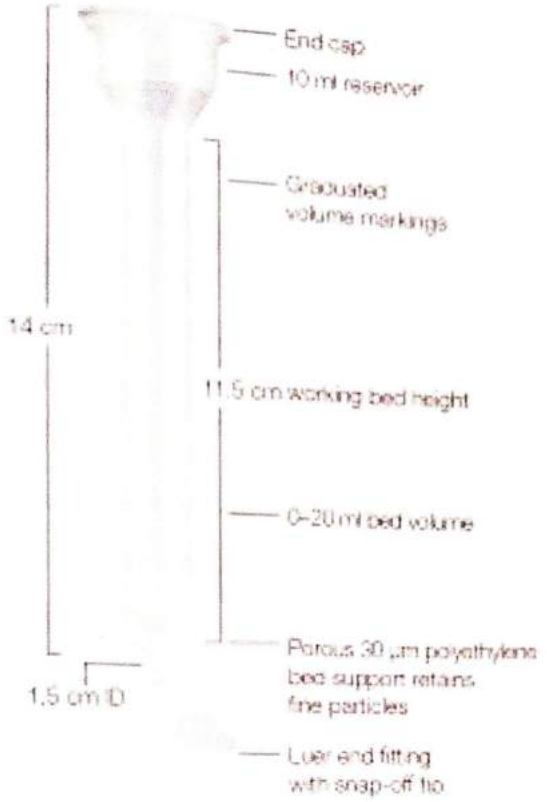

Figure 15 : Protein columns used for purification and protein concentration

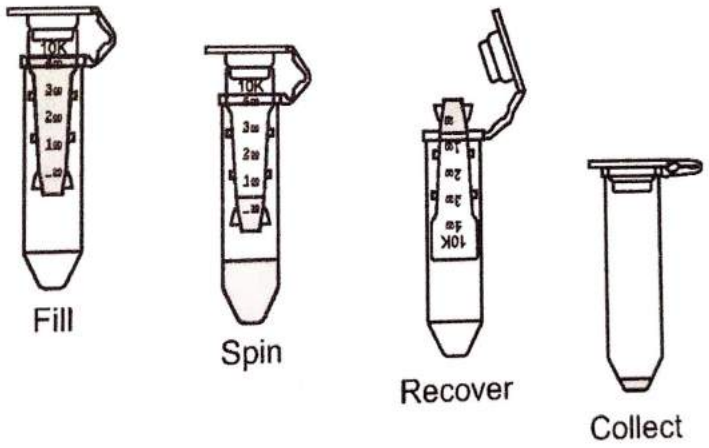

Figure 16 : Centrifugal filters used to concentrate protein

- Rinse again with Stage 1 water.
- In a tub, add Stage 1 water until the columns are fully dipped in it. Give warm water sterilization in a microwave for 10 minutes.
- Rinse with Stage 1 water once and dd chelating sepharose (1–2 ml) (Sephadex G25 Beads)
- Wash twice with Stage 1 water.
- To it, add 1 ml of O.5M Nickel Chloride solution.

- Wash twice with Stage 1 water.Equilibrate the column with the Equilibration Buffer, and load the cell free extract into the column.

B. Concentration of protein using appropriate size centrifugal filters

- Wash concentration columns/filters using Stage II distilled water four times and spin once to remove water.
- Load the pooled eluted fractions of protein into the column.
- Spin at 5500 rpm for 15 minutes at 4°C.
- Repeat the process (steps 2 and 3) till the volume reaches 500 µl.

C. Dialysis

Dialysis is a separation process that uses selective and passive diffusion via a semi-permeable membrane to remove undesirable chemicals from macromolecules in solution. Tiny molecules and buffer salts move readily through the membrane, lowering the concentration of those molecules in the sample. Sample molecules bigger than the membrane pores are retained on the sample side of the membrane, whereas tiny molecules and buffer salts are retained on the sample side of the membrane. A dialysis membrane is a semi-permeable film (often a sheet of regenerated cellulose) with varying pore sizes.

- The dialysis membrane, clips, and magnetic stirrer are washed in a boiling water bath containing 10mM for 15 minutes to sterilize and remove sodium azide salts
- The dialysis membrane is clipped from one side, then protein sample is inserted into the dialysis membrane and clipped from the other side, leaving no air space in between.
- Overnight dialysis is done for a better resultS at 4 °C.
- Next day a small amount of the recovered sample is checked on SDS-PAGE Gel
- The sample is stored at -20 °C until further use.

5.20 Western Blot

After running the SDS PAGE and well resolved protein mixture along with the color protein standard marker it is transferred to a membrane. The transfer is done using an electric field oriented perpendicular to the surface of the gel, causing proteins to move out of the gel and onto the membrane. The membrane is placed between the gel surface and the positive electrode in a sandwich. The sandwich includes a fibre pad (sponge) at each end, and filter papers to protect the gel and blotting membrane. Here two things are very important:

(1) The close contact of gel and membrane to ensure a clear image

(2) The placement of the membrane between the gel and the positive electrode. The membrane must be placed as such, so that the negatively charged proteins can migrate from the gel to the membrane. This type of transfer is called electrophoretic transfer, and can be done in semi-dry or wet conditions. Wet conditions are usually more reliable as it is less likely to dry out the gel, and is preferred for larger proteins.

- Dismantle the SDS PAGE gel from the protein running apparatus, and transfer it to 1X western transfer buffer.
- Simultaneously wet the sponge pads using 1X western transfer buffer by keeping it on the apparatus.
- Cut the PVDF membrane of required size and to charge the PVDF membrane dip in methanol.
- All the layers are arranged in a sandwich form as shown below.

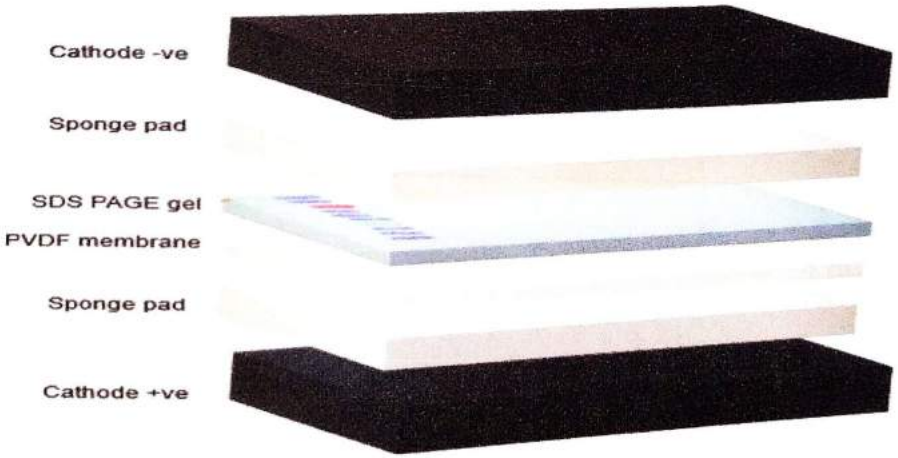

Figure 17 : Western blot arrangement.

- Ensure there are no air bubbles between the gel and PVDF membrane, and squeeze out extra liquid
- Relocate the sandwich to the transfer apparatus, Add transfer buffer to the apparatus, and ensure that the sandwich is covered with the buffer. Place electrodes on top of the sandwich, ensuring that the PVDF membrane is between the gel and a positive electrode.
- Transfer is done for 45 minutes
- After run is complete place the membrane in blocking buffer and keep on rocker for 1 hr and the gel is kept for staining.
- Add Primary Ab of Mouse Anti T18 and keep on rocker for overnight.
- Wash the membrane with TBS-20 thrice each for 15 mins.

(Note: All washing and antibody incubation steps should be done on a shaker at room temperature to ensure even agitation).

- Single wash with TBS solution (TBS solution is same as TBS-20 excluding Tween-20)
- Add blocking buffer and secondary antibody of Rabbit Anti-mouse Ab.
- Wash the membrane with TBS-20 for 15 minutes. Washing is done thrice for 15 mins each.
- Add alkaline phosphate buffer (Detection buffer) into membrane and keep it on shaker.
- Develop the blot by adding BCIP/NBT substrate. (As it is light sensitive should be used in dim light or lights off.)

5.21. Electrophoretic Mobility Shift Assay (EMSA)

Table17. EMSA buffer composition

0.5X TBE Buffer
20 mM KCl
Stage I water

1. The purified DnaA protein was centrifuged at 22,000 X g for 15 min at 4°C to remove any aggregate.
2. 0.5µM, 1.0µM and 1.5µM 2µM and 2.5µM of protein was mixed with dsDNA and G4 DNA and 1 mM of ATP containing 10X Buffer was kept at 37°C for incubation for 20 mins. DNA without protein was also taken.
3. After incubation, the samples were loaded on 0.7% Agarose gel (without SDS) and run at 50V.
4. After run, the gel was stained in EtBr for 10 mins and destained . using Destaining solution I, the gel was visualized under UV.

5.22. ATPase Assay

The goal of the malachite green assay is to quantify the concentrations of inorganic phosphate in a solution. Malachite green molybdate interacts with inorganic phosphate in an acidic environment to generate a green complex, which is the basis for this colorimetric technique. When measured using a spectrophotometer at 620 nm, the quantity of green molybdophosphoric acid complexes is directly associated with the amount of free inorganic phosphate present in the reaction. The standard curve that was previously established in the

lab was used to measure the concentrations of released phosphate and determine the ATPase activity.

- Different concentrations of DnaA was preincubated with 10X buffer and assay buffer before the reaction was initiated by 1mM ATP.
- The reaction mixtures were incubated for 20 minutes at 37°C and terminated using 200µL freshly prepared malachite green reagent.
- Absorbance at 620nm was measured relative to a buffer control and normalized with protein control (without ATP).
- The graph was plotted concentration v/s absorbance.

5.23. Dynamic light scattering

This technique is used for measuring the size and size distribution of proteins.

1. Dynamic light scattering was measured using a Malvern Panalytical, Zetasizer Nano range instrument.
2. Protein was centrifuged at 12000rpm for 30 min at 4°C.
3. 5µM protein was incubated in the absence and presence of 1MM ATP for 10 min. Light scattering at 90° angle was measured at 37°.
4. To the same reaction, 0.5mM dsDNA of *Ori* Chromosome I was added and incubated for 10 min. Light scattering at 90° angle was again measured at 37°C. The data obtained as kilo counts per second was analyzed using in-built software (SZ-100) and plotted.
5. The same process for G4 DNA.

CHAPTER-6
RESULTS AND DISCUSSIONS

6.1 To check the role of guanine quadruplexes in DNA replication initiation in *Deinococcus radiodurans*.

6.1.1 Protein overexpression and optimization of protein purification of DnaA and DnaB protein

The recombinant plasmids containing DnaA and DnaB_encoding sequences were previously transformed to SHuffle and BL21 (DE3) in the laboratory. To confirm the induction of the proteins we did small-scale induction as described in materials and methods. Both uninduced and induced samples of C-terminal truncated DnaA and DnaB with histidine tag were processed and checked on SDS-PAGE for protein induction as shown in Figure

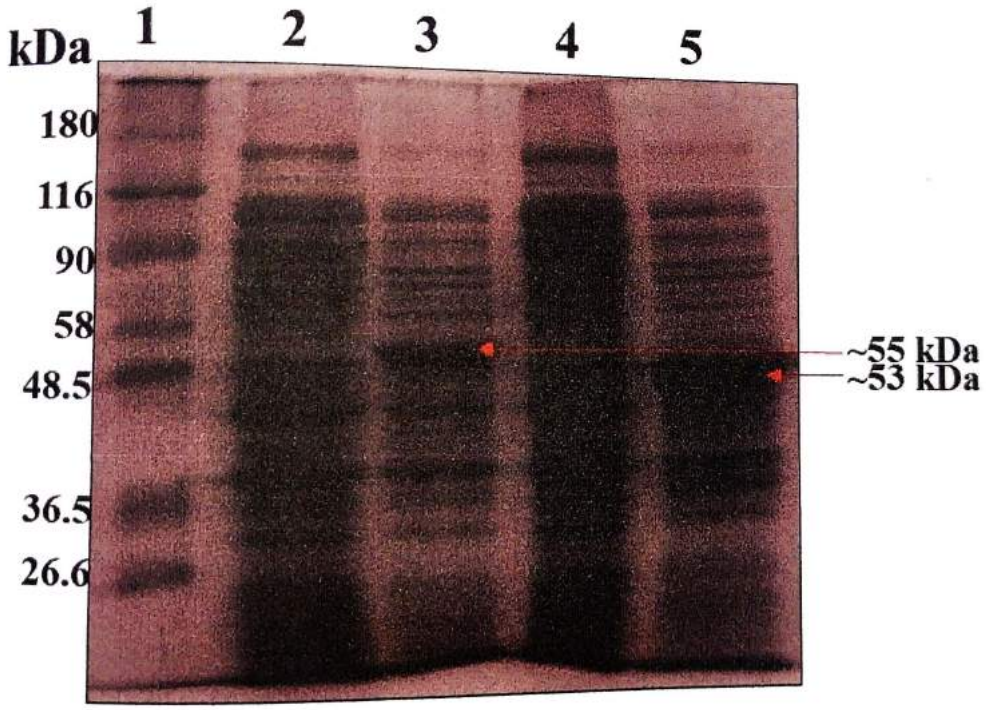

Figure 18 : Small scale induction of DnaA and DnaB proteins. The uninduced and induced samples were checked by SDS PAGE, and the size of proteins was compared with a molecular weight marker (Lane 1). Lane 2 and Lane 4 show uninduced samples, while Lane 3 and Lane 5 show induced samples of DnaA and DnaB, respectively.

Lane 3 and Lane 5 in the SDS PAGE, confirms the successful induction of our desired protein, hence we proceeded with large scale induction.

6.1.2 Large scale induction and purification

After confirming the over expression of proteins from the recombinant strains, large scale induction was carried out as mentioned in the methods. The proteins were purified by treating the cell free extract with lysis buffer followed by sonication and loading onto the column for binding with Ni-NTA overnight. Following a column wash, the bound protein was eluted using elution buffers containing imidazole at concentrations of 100 mM, 300 mM, and 500 mM. Then, as shown in the figure, two samples from each concentration of the eluted fractions, along with the uninduced and induced samples, were examined on the SDS-PAGE gel.

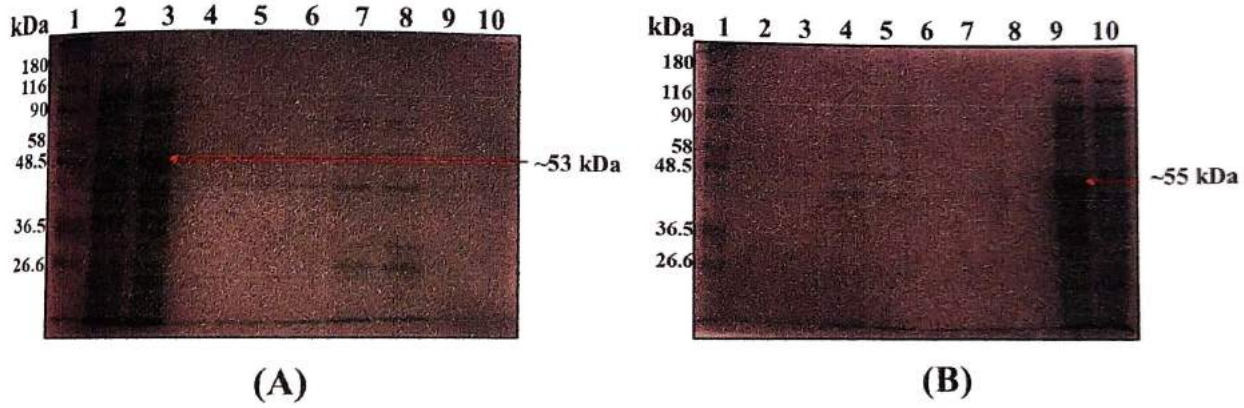

Figure 19 : SDS PAGE of eluted (A) DnaA and (B) DnaB protein fractions from Ni-NTA bound cell-free extract. **(A)** Lane 1- SDS7B2 Protein Marker, Lane 2- uninduced, Lane 3- induced, Lane 4- washout, Lane 5 and 6- 100mM conc., Lane 7 and 8- 300mM conc., Lane 9 and 10- 500mM conc. **(B)** Lane 1- SDS7B2 protein marker, Lane 2 and 3- 500mM conc., Lane 4 and 5- 300mM conc., Lane 6 and 7- 100mM conc., Lane 8- washout, Lane 9- induced, Lane 10- uninduced.

As the fractions do not show a detectable amount of protein, it was concluded that the proteins must have been entrapped in the inclusion bodies. For the recovery of proteins, the cell pellet was further processed.

6.1.3 Recovery of protein from inclusion bodies

As previously mentioned, large-scale recovery of bioactive proteins presents significant difficulty due to inclusion body formation in bacterial hosts. Inclusion bodies are frequently formed when recombinant protein molecules are produced at high levels in *Escherichia coli*. The intended protein is frequently expressed at a high translational rate when high temperatures, high inducer concentrations, and strong promoter systems are used during protein production. The bacterial protein quality control mechanism is exhausted as a result, and the misfolded and partially folded protein molecules clump together to form inclusion bodies. Inclusion bodies are also influenced by altered bacterial cytosolic conditions, a deficiency in eukaryotic chaperones, and post-translational machinery.

Isolation and solubilization of inclusion bodies are the first steps in the process of removing insoluble protein from inclusion bodies. The inclusion bodies will be found in the pellet following centrifugation and cell lysis. A subsequent centrifugation step is necessary to eliminate any leftover aggregates after the inclusion bodies have been solubilized in a buffer. The fraction of the inclusion body that has been solubilized can then be used for further purification and refolding. Figure 20 shows the eluted protein fractions from the inclusion bodies.

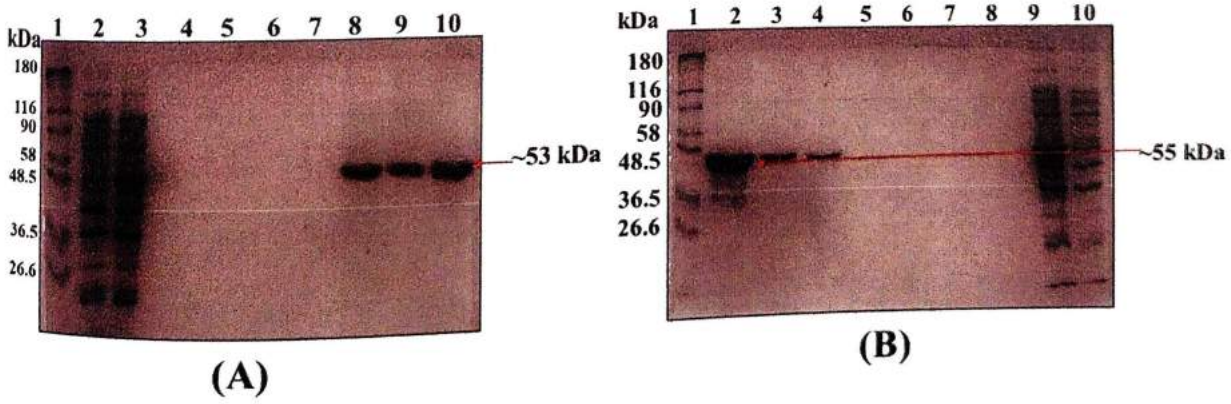

Figure 20 : SDS PAGE of eluted DnaA (A) and DnaB (B) protein fractions isolated from the cell pellet. **(A)** Lane 1- SDS7B2 Protein Marker, Lane 2- uninduced, Lane 3- induced, Lane 4- washout, Lane 5 and 6- 100mM conc., Lane 7 and 8- 300mM conc., Lane 9 and 10- 500mM conc. **(B)** Lane 1- SDS7B2 protein marker, Lane 2 and 3- 500mM conc., Lane 4 and

5- 300mM conc., Lane 6 and 7- 100mM conc., Lane 8- washout, Lane 9- induced, Lane 10- uninduced.

In the eluted fractions of the 300 mM and 500 mM imidazole concentrations, we observe single bands of our desired protein with very minimal contamination. As described in the protocols, these fractions were pooled and concentrated to a volume of 2 mL. These concentrated samples were dialyzed in a buffer containing 50% glycerol for further purification. The dialyzed protein was examined by SDS PAGE and then placed in storage at -20°C.

6.1.4 PCR Amplification of *Ori* Chromosome I , II and MP of *Deinococcus radiodurans*

Previously it was shown that DnaA binds specifically to *Ori* sequences of *D. radiodurans*. The PCR reaction was performed using gene specific forward and reverse primers targeting the *Ori* Chromosome I, II and Mega plasmid respectively in *D. radiodurans*. The reaction mixture consisted of BRIT GC rich buffer 2.5X, which contains Taq DNA polymerase. This buffer is specifically designed for GC-rich templates, such as *D. radiodurans*, which has a high GC content of 69.7% in its large genome. PCR optimization was conducted by varying the temperature within the range of 58°C to 68°C for the *Ori* Chromosome I, II and Mega plasmid of *D. radiodurans*. The extension time for each PCR cycle was set at 1 minute.

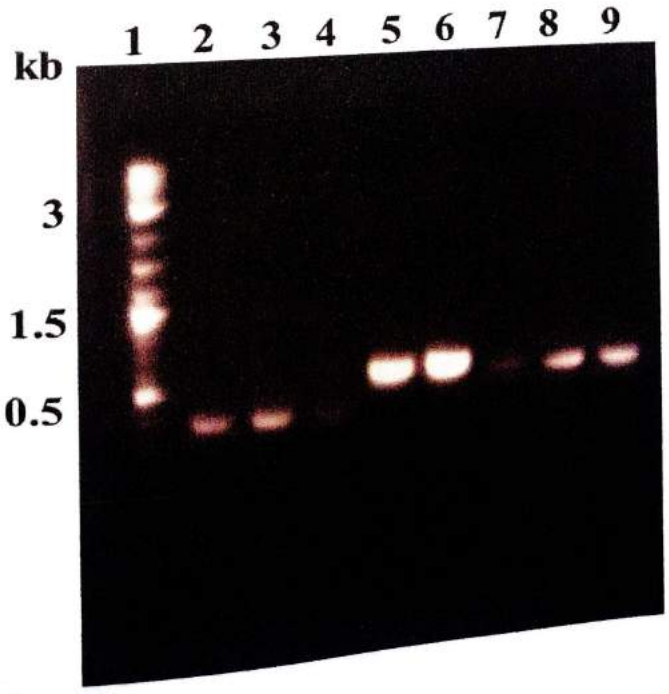

Figure 21 : Gradient PCR for *Ori* Chromosome I , II and MP. Lane 1 represents 2 log DNA ladder, Lane 2-4 represents *Ori* Chromosome II in temperatures 64.4°C, 62°C and 60°C respectively. Lane 5-6 represents *Ori* Chromosome I in temperatures 64.4°C and 62°C. Lane 7-9 represents Mega Plasmid in temperatures 64.4°C, 62°C and 60°C respectively An intense and sharp band observed at 64.4°C and 62°C of size ~ 0.5 kb.

To assess the PCR product, a small volume was used for agarose gel analysis. The gel was prepared with a 1% agarose concentration and subjected to electrophoresis at 90 V. A 2-log DNA ladder was employed as a molecular weight marker. During gel electrophoresis, strong bands were detected between temperatures of 64.4°C and 62°C. To remove potential interference from other PCR components that could affect further experiments, the PCR product purification using the QIA Quick PCR Purification Kit from QIAGEN was done. The purified product was stored in -20°C until further use.

6.1.5 Large scale PCR amplification and purification of *OriCI*

A large-scale PCR reaction was performed for *Ori* Chromosome I using gene-specific primers. The amplification was carried out at a temperature of 62°C with an extension time of 1 minute. Once the PCR run was completed, the PCR product was checked, purified to remove unwanted components, and stored at -20 °C for further use.

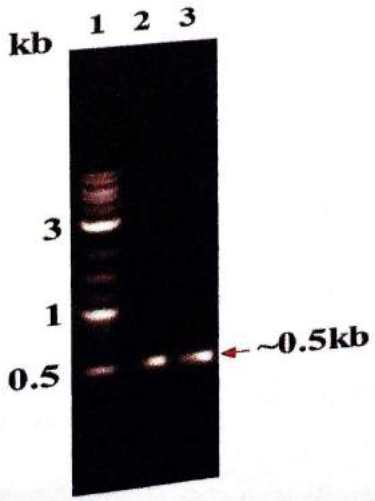

Figure 22 : Purified PCR product of *Ori* Chromosome I (Lane 2 & 3). Sharp intense bands observed of size ~ 0.5 kb. Lane 1 represents 2 log DNA ladder.

6.1.6 Guanine quadruplex structure formation in Chromosome I *Ori* sequence

Bioinformatics analysis using QGRS software has shown presence of putative guanine quadruplex forming motifs in the *ori* region of chromosome 1 of *D. radiodurans* R1 (as shown in the table below).

G4 DNA structures were shown to be formed under specific solution conditions, such as molecular crowding brought on by the presence of polyethylene glycol, the presence of certain DNA-binding proteins, single-strand DNA, etc. Further, previous studies have used either pyridostatin (PDS), a highly selective G-quadruplex ligand, or polyethylene glycol (PEG) to induce G4 structures in double-stranded DNA. The former was used by us in our experiments for inducing G4 structures in the PCR-amplified *ori* sequence.

PUTATIVE G4 SEQUENCES IN *ORI* CHROMOSOME I

The putative G4 sequence was perceived using *Ori* finder 3 and QGRS application.

Position	Length	QGRS	G-score
87	21	GGAAGGCCCAAGGTCACCTGG	17
205	30	GGACAAAAGTTTTCCACAGAGGGGGTGTGG	4
273	30	GGGGGGTTATCCACAGGGCATTTTTAGGGG	33

Reverse compliment:

Position	Length	QGRS	G-score
399	30	GGCCAGGTGACCTTGGGCCTTCCACAAAGG	13

100 mM KCl, 10 µM PDS, and 20 mM Tris were used for G4 induction. The reaction mixture was heated to 95 degrees for 15 minutes, after which it was let to gradually cool at room temperature overnight. The shift between dsDNA and G4 DNA was examined in a 15% native PAGE gel the next day. Retarded DNA migration in polyacrylamide gel electrophoresis makes it simple to track these significant structural changes in DNA.

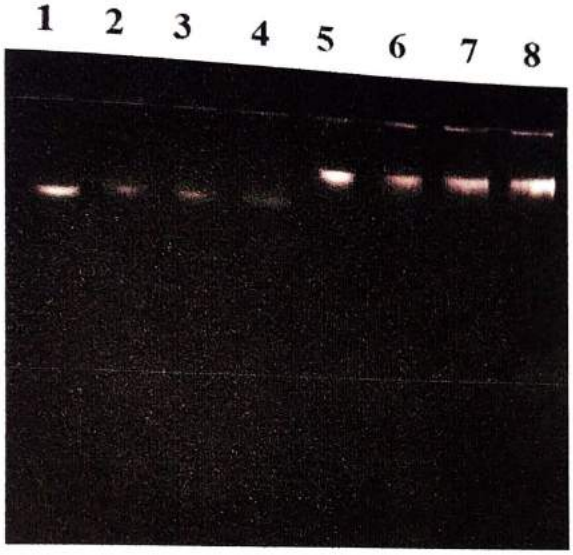

Figure 23 : Confirmation of the formation of G4 structures. In 15% native PAGE, the shift between dsDNA (Lanes 1-4) and G4 DNA (Lanes 5-8) strongly suggests that molecular crowding brought on by the presence of PDS in the solution produced G-quadruplex formation inside double-stranded DNA.

Previously it was reported in the lab that, DnaA binds to *oriCI*, which has 13 repeats of the DnaA-boxes. An electrophoretic mobility shift assay (EMSA) was carried out to ascertain the binding affinity of G4 DNA with the DnaA protein and compare it to that of the dsDNA of *oriCI*.

6.1.7 Electrophoretic mobility shift assay (EMSA)

The G4 DNA binding activity with DnaA was monitored using the electrophoretic mobility shift assay (EMSA). A shift in the DNA band on the agarose gel indicates that the protein has a stronger affinity for G4 DNA than dsDNA does. The nucleoprotein complex (NPC) size

steadily increased as protein concentration increased with G4 DNA as compared to the protein control (shown as slower mobility).

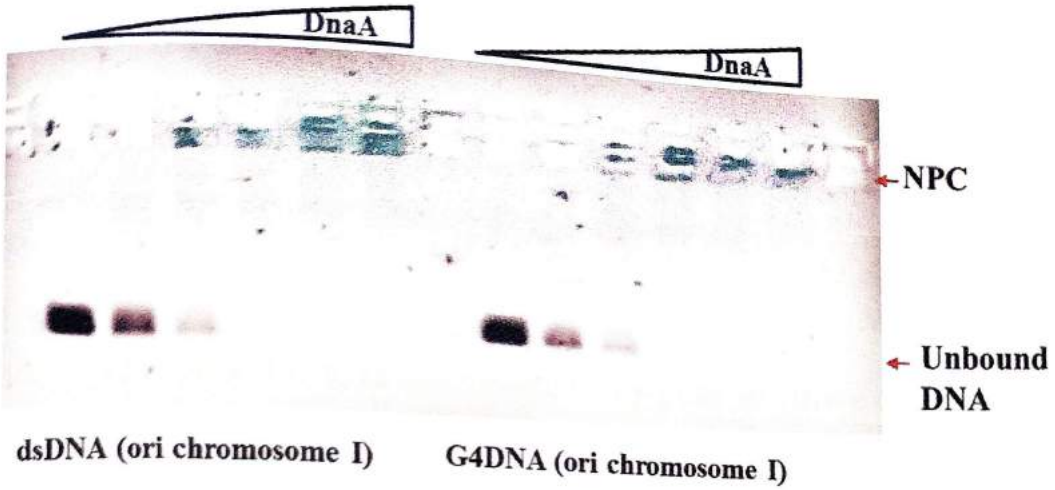

Figure 24 : DNA binding assay for the interaction of the DnaA protein with the G4 and dsDna of *oriCI*. The linear dsDNA and G4 DNA were incubated with 1X buffer and DnaA protein in increasing concentrations (0, 0.5, 1.0, 1.5, 2.0, and 2.5 μM). The amount of nucleoprotein complexes formed in each case was analysed on a 0.7% agarose gel made in 0.5X TBE buffer and 20 mM KCl.

The electrophoretic mobility shift assay (EMSA) was used to evaluate the DNA binding activity of DnaB protein interaction with double stranded DNA or single stranded DNA and their corresponding G4 structure forms. For this, the ssDNA, dsDNA, and their corresponding G4 DNA were incubated with 1X protein buffer and increasing concentrations of DnaB protein for 20 minutes at 37°C. A 0.7% agarose gel was prepared in a buffer containing 0.5X and 20 mM KCl, for the investigation of DNA-protein binding.

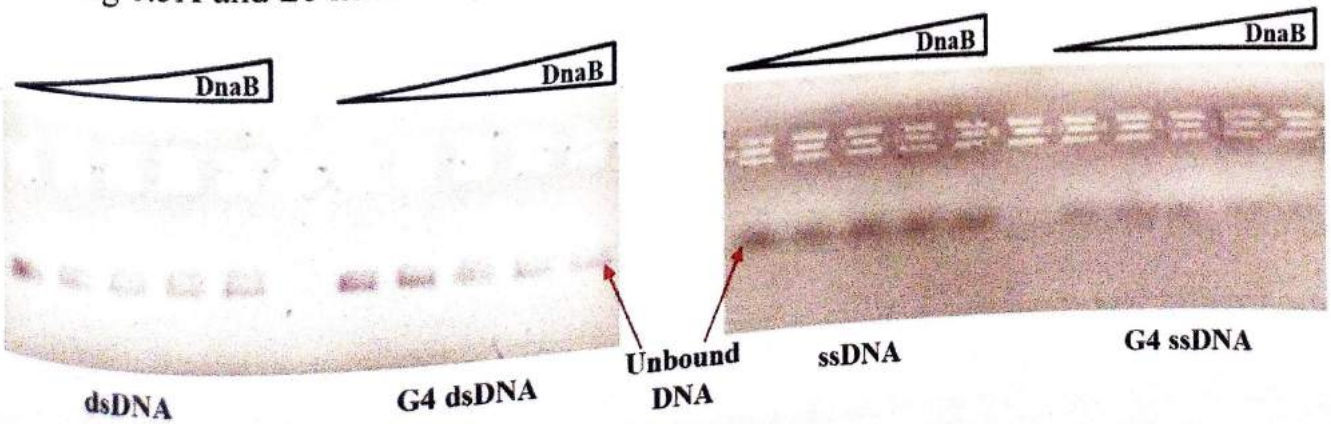

Figure 25 : **EMSA with DnaB protein.** An electrophoretic mobility shift assay was used to test the DnaB protein's affinity for dsDNA, G4dsDNA, ssDNA, and G4ssDNA amid increasing protein concentrations.

The preliminary results in Figure 25 indicate that DnaB has no preferential binding to G4 structures compared to the other forms of DNA, which needs to be checked further.

DnaA exhibits ATPase activity, as demonstrated by prior in vitro experiments of the purified recombinant proteins in the laboratory. The activity of DnaA's ATPase was induced by *ori*CI. A minimum amount of DnaA binding at the *ori*C site is necessary for the *ori*C-mediated initiation of DNA replication. It was discovered that DnaA interacts with the *ori*CI region and undergoes both homotypic and heterotypic oligomerization. It is well known that DnaA uses energy from the ATP hydrolysis process to melt the AT-rich area in *oriC*, which is required for the start of replication.

An ATPase assay was then carried out to investigate how the presence of dsDNA and G4 DNA affects the ATPase activity of the DnaA protein.

6.1.8 ATPase Assay

The goal of the malachite green assay is to quantify the concentrations of inorganic phosphate in a solution. Malachite green molybdate interacts with inorganic phosphate in an acidic environment to generate a green complex, which is the basis for this col*ori*metric technique. When measured using a spectrophotometer at 620 nm, the quantity of green molybdophosph*oriC* acid complexes is directly associated with the amount of free inorganic phosphate present in the reaction. The standard curve previously established in the lab was used to measure the concentrations of released phosphate and determine the ATPase activity.

According to the procedures described in the methods section, the ATPase assay of the DnaA protein was carried out in triplicates in the presence of dsDNA and G4 DNA at three distinct concentrations (1.0, 2.0, and 3.0 µM). At 620 nm, the absorbance was measured. The standard curve that was previously constructed in the lab was used to compute the number of micromoles of inorganic phosphate released per minute per microliter of the reaction mixture. The acquired data was then presented as a bar graph versus the DnaA protein concentration (µM).

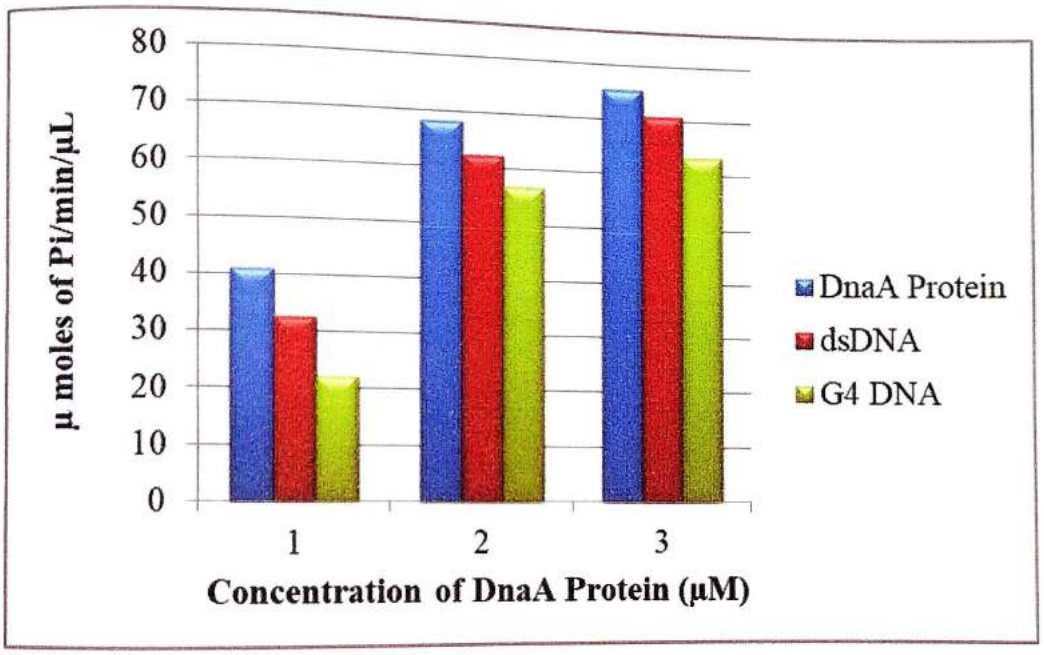

Figure 26 : ATPase Assay. The graph displays the DnaA protein's ATPase activity at different concentrations as a control and how it changes when dsDNA and G4 DNA are added to the reaction mixture.

We can infer from the aforementioned graph (Figure 26) that the presence of G4 DNA in the reaction mixture marginally decreases the DnaA protein's ATPase activity relative to that of the dsDNA, which needs to be confirmed further.

6.1.9 Dynamic Light Scattering

Dynamic light scattering (DLS) was used to examine whether DnaA forms higher-order nucleoprotein complexes with dsDNA and G4 DNA in response to ATP. To get rid of the

aggregates, DnaA protein was centrifuged at 12,000 rpm for 30 min at 4 °C. Centrifugation was followed by the incubation of 2.5 M of the purified DnaA with dsDNA and G4 DNA of *oriC*I in the presence of 1 mM ATP, as explained in the Methodology section. Using Origin software, the light scattering intensity (measured in kilocounts per second, or kcps) was plotted versus time.

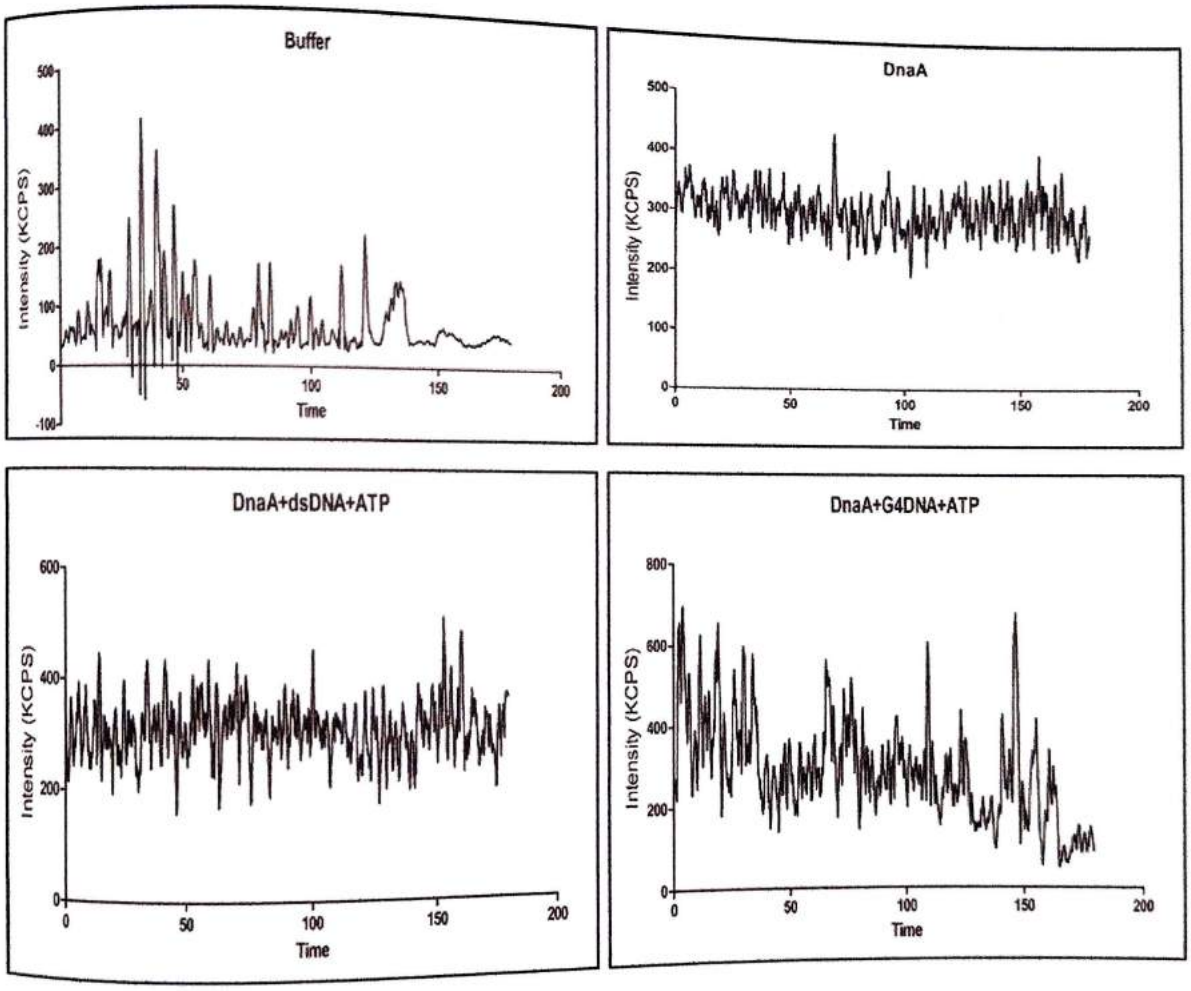

Figure 27 : Dynamic Light Scattering data of DnaA, in the presence of dsDNA, G4DNA and ATP.

The graphs (Figure 27) reveal that, in comparison to dsDNA, the DnaA protein exhibits larger intensity fluctuations and an increase in hydrodynamic radius in the presence of G4DNA, indicating that G4DNA promotes the development of large-size aggregates.

6.2 Construction of mutants for *mutS* gene

The extremely radiodurable strain of *Deinococcus radiodurans* has a mismatch repair system (MMR), which has previously been characterised. The MMR system is observed to be active in this organism, where it helps to ensure the accuracy of DNA replication and recombination. *MutS*1 and MutL, two essential proteins that make up a conserved core involved in mismatch recognition, are essential for the system's function.

Previous studies showed that MutS from *Escherichia coli* was active on G4 structures and the RGG motif in the protein plays an important role in structure specific binding, hence we sought to check whether MutS from *Deinococcus radiodurans* is involved in G4 metabolism in this bacterium. For this, two distinct types of mutants of *mutS* from *Deinococcus radiodurans* were planned, to check their functions towards G4 structures compared to wild type allele:

1. Deletion of the RGG sequence to determine if it aids in guanine quadruplex binding.
2. Site-directed mutagenesis, where serine was substituted for arginine in the RGG sequence.

6.2.1 PCR Amplification of the RGG del *mutS* up and down fragments

A gradient temperature range of 58°C to 68°C was used for the hypothesised del *mutS* coding sequence amplification. The genomic DNA of *D. radiodurans* was employed as a template, combined with primers that are specific to the given sequence. The Phusion GC Rich Buffer 2X, which is designed especially for GC-rich templates like *D. radiodurans*, was utilised. *D. radiodurans* has a large genome with a high GC content of 69.7%. To check for amplification and to measure the size of the amplified product, a tiny portion of the PCR reaction was run on a 1% agarose gel with a DNA marker.

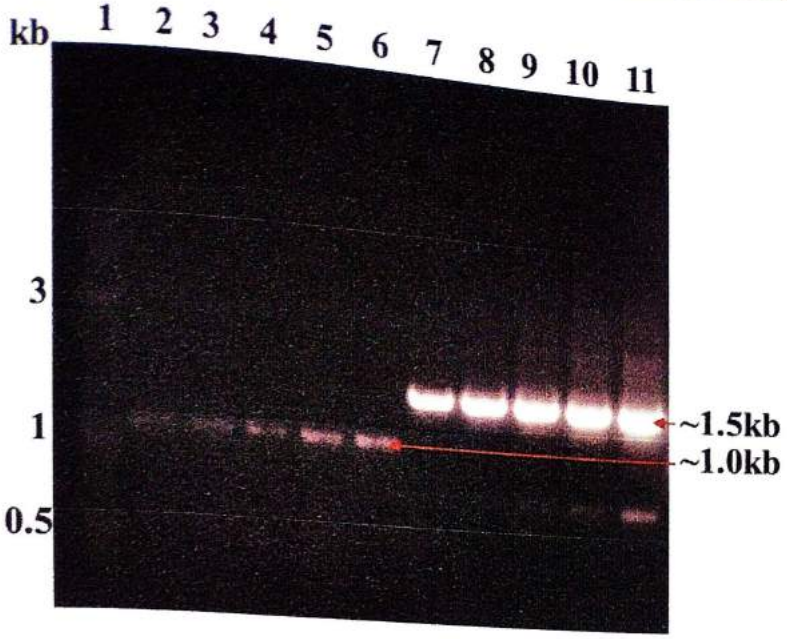

Figure 28 : displays the **results of gradient PCR for del *mutS* up and down fragments in the temperature range of 58 °C to 68 °C.** The temperature range of 58°C–68°C was used for the del *mutS* up fragment (lanes 1–5) and del *mutS* down fragment (lanes 6–11). The PCR-amplified product was examined in a 1% agarose gel corresponding to the following temperatures: 67°C, 64°C, 62°C, 60°C, and 58°C. At 58 °C, a distinct, intense band of size 1.5 kb (the del *mutS* down fragment) and 1 kb (the del *mutS* up fragment) was seen.

Both fragments were amplified in significant quantities using PCR at 58°C, and the amplified products were gel-purified and kept at -20°C for later use.

6.2.2 Overlapping PCR to obtain full length gene with desired mutation

All of the PCR-amplified fragments containing the appropriate mutations were then used as templates for overlapping PCR. Two primers, one of which serves as a flanking primer and binds to each end of the nucleotide sequence, are used to overlap the fragments. A gradient PCR of a 50-l reaction mixture was performed. After identifying the ideal annealing temperature, a high-volume reaction was prepared, and the PCR parameters were set using table 18. After amplification, 4 l of the sample was loaded onto the gel, and a ladder was used to confirm the size of the fragments. For simplicity of nomenclature, the fragments were given the numbers 1 and 2.

Table18. Optimization of temperature conditions for Overlapping extension PCR

Steps	PCR (30 cycles)	
	Temperature	Time
Initial Denaturation	95 °C	5 min
Denaturation	95 °C	30 sec
Annealing	58 °C	40 sec
Extension	72 °C	2 min 30 sec

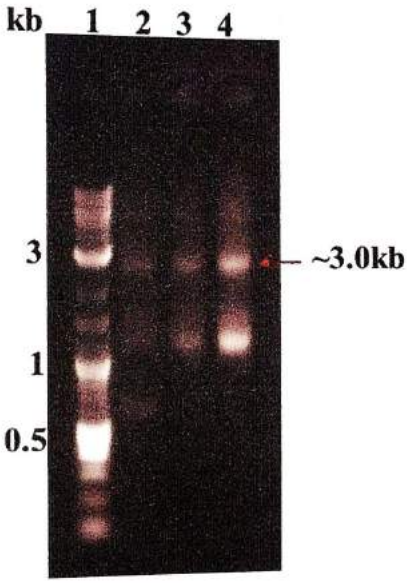

Figure 29 : shows the outcomes of the **Overlapping Extension PCR used to create *mutS* mutants with the RGG motif removed**. The utilised ladder is in lane 1, and the PCR products are identified as lanes 2, 3, and 4 in various tubes. The full-length RGG del *mutS* mutant is represented by the fragments in lanes 2–4. It is significant to note that the PCR product was produced at an optimal annealing temperature of 58°C.

The amplified product was gel purified and stored at -20°C for later use.

6.2.3 Amplification of the up and down fragments of site-directed *mutS* allele by PCR

The site-directed mutagenesis *mutS* coding sequence amplification was conducted at a gradient temperature range of 58°C to 68°C. As a template, the genomic DNA of *D. radiodurans* was used along with primers that are specific to the given sequence. It was done

using the Phusion GC Rich Buffer 2X, which has been designed for GC-rich templates like *D. radiodurans*. A small amount of the PCR reaction was tested with a DNA marker on a 1% agarose gel to determine whether amplification had occurred and to calculate the size of the amplified product.

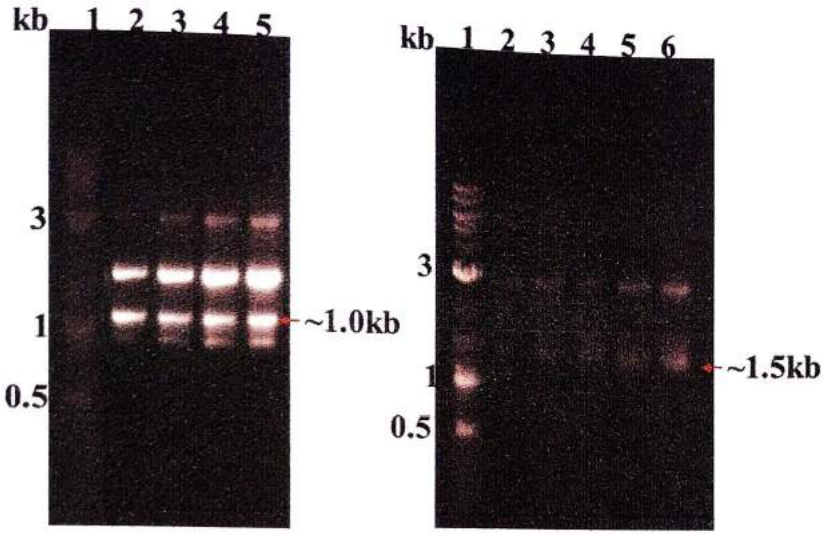

Figure 30 : displays the **SD *mutS* up- and down-fragment gradient PCR results at the 58–68 °C temperature range.** For (A) the SD *mutS* up fragment (lanes 2–5) and (b) the SD *mutS* down fragment (lanes 2–6), temperatures between 58°C and 68°C were used. The DNA marker is denoted by Lane1 in both (A) and (B). A 1% agarose gel was used to evaluate the PCR-amplified product at 67°C, 64°C, 62°C, and 60°C for the SD *mutS* up fragment (A), and 67°C, 64°C, 62°C, 60°C, and 58°C for the SD *mutS* down fragment (B). The SD *mutS* down fragment, measuring 1.5 kb, and the SD *mutS* up fragment, measuring 1 kb, were both clearly visible at 58 °C.

Both segments were amplified significantly by PCR at 58°C; the amplified products were then gel-purified and stored for future use at -20°C.

6.2.4 Overlapping PCR for Site Directed *mutS* to obtain full length gene

Overlapping PCR was performed using all of the PCR-amplified fragments with the necessary mutations as templates. To overlap the fragments, two primers are used, one of

which acts as a flanking primer and binds to both ends of the nucleotide sequence. A gradient PCR was run on a 50 µl reaction mixture. The optimal annealing temperature was determined, a high-volume reaction was made, and the PCR parameters were established using table 19. 4 µl of the sample was placed onto the gel after amplification, and a ladder was used to confirm the size of the pieces. The numerals 3 and 4 were assigned to the fragments for ease of designation.

Table19. Optimization of temperature conditions for Overlapping extension PCR

Steps	PCR (30 cycles)	
	Temperature	**Time**
Initial Denaturation	95 °C	5 min
Denaturation	95 °C	30 sec
Annealing	58 °C	40 sec
Extension	72 °C	2 min 30 sec

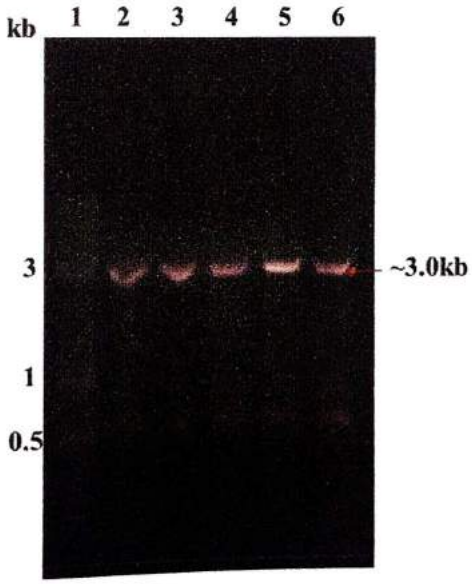

Figure 31 : demonstrates the results of the **Overlapping Extension PCR used to produce *mutS* mutants with serine in place of the amino acid arginine in the RGG motif in *D. radiodurans***. The used ladder is located in lane 1, while the PCR products are located in lanes 2–6 that correspond to the various temperatures (67°C, 64°C, 62°C, 60°C, and 58°C). The fragments in lanes 2–6 represent the complete site-directed mutagenesis *mutS* mutant.

As deduced from the gel image, we understand that the intense band of SD *mutS* was observed at 58°C. A large scale of overlapping PCR was then deployed to amplify the SD *mutS*. The amplified product was gel-purified and stored for later use at -20 °C.

6.2.5 Isolation of the pET-28a (+) plasmid, digestion of the vector, and insert

The pET-28a (+) plasmid was extracted using the QIAprep Spin Miniprep kit from QIAGEN following the successful amplification of both inserts. The concentration of the plasmid yield, which was evaluated by nanodrop and visualisation on Agarose gel electrophoresis, was found to be 90 ng/L. The plasmid was then exposed to restriction digestion using the proper enzymes, followed by gel purification to get rid of buffers and enzyme activity. Two restriction endonucleases were used, and the restriction enzyme sites were inserted into the primers used to amplify the genes to insert the gene of interest in a certain *ori*entation. The enzymes BamHI and NdeI were used to digest the mutants, and plasmid single- and double-digests were used to assess the enzymes' effectiveness.

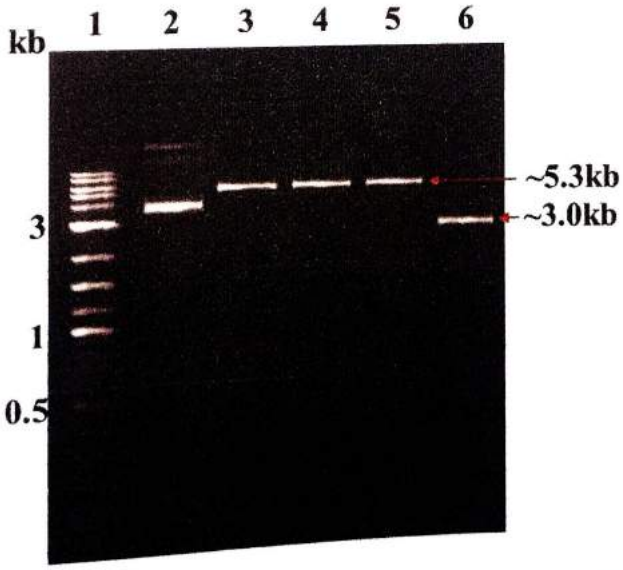

Figure 32 : Digestion of Vector and Insert. The restriction enzymes BamHI and NdeI were used to double digest the vectors and Insert (RGG del *mutS*). To test the efficiency of the utilised restriction enzymes, a single digestion of the vector with BamHI and NdeI was also performed. The DNA marker is displayed in lane 1, the undigested vector is displayed

in lane 2, the vector is double digested in lane 3, the single digested vector using BamHI and NdeI is displayed in lane 4 and 5, respectively, and the double digested insert (RGG del *mutS*) is displayed in lane 6.

6.2.6 Ethanol Precipitation, Ligation of vector and gene and Transformation into competent cells.

The double digested vector and inserts were both precipitated in ethanol in an Eppendorf tube before the ligation procedure was started. The vector and insert amounts were added to establish a 1:3 molar ratio for ligation. The reaction mixture was made as described in the methods section, and it was carried out overnight at 16°C. The recombinant vector was ligated and then transformed into *E. coli* (Nova blue) cells. The transformed *E. coli* (Nova blue) cells were then plated on LB agar plates with 25 µg/ml kanamycin and incubated at 37°C overnight. To assess the effectiveness of competent cells, plating of positive and negative controls was also done. A few colonies were seen on the positive control plate, which only contained intact pET-28a (+) vector, while none were seen on the negative control plate, demonstrating the competency of the cells. On the LB plates containing the ligated mixture, a moderate transformation of 20–30 colonies was attained.

6.2.7 Screening of Transformants

On a new LB plate with 25 µg/mL kanamycin, transformed colonies were streaked and incubated at 37 °C overnight. As described in the methods section, the plasmids were extracted the following day using the miniprep technique. Plasmid shift/band shift, release of the insert on double plasmid digestion, and colony PCR are the three methods used to identify positive clones.

Screening of the clones by Plasmid shift

The migratory shift of the plasmids was evaluated using a 1% agarose gel. When compared to a plain (uncut) plasmid, the vector after ligation exhibits a size shift as the size of the insert is added up. The ligation of the insert with the vector was effective, as shown by the shift, proving that cloning had taken place. Further confirmation will be done with the restriction analysis.

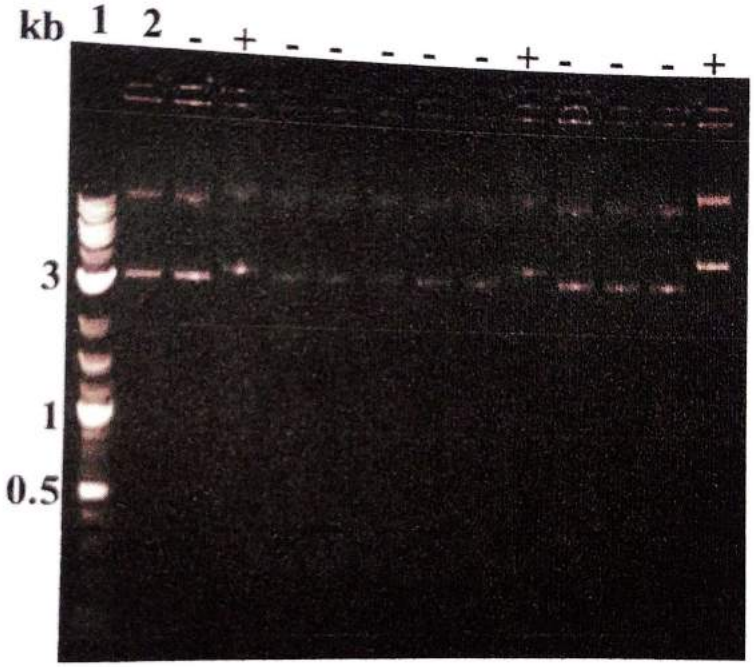

Figure 33 : Screening of transformants for plasmid shift. Following the isolation of the plasmid from each colony, a tiny volume was loaded onto a 1% agarose gel with a DNA marker and an uncut PET-28a (+) vector in order to look for any size differences and compare shifts. The DNA ladder is shown in lane 1, and the uncut PET-28a (+) vector is shown in lane 2. When compared to the uncut PET-28a (+) vector, the wells with a "+" showed a considerable change in the plasmid size.

CHAPTER- 7
CONCLUSIONS

CONCLUSION

This report tested whether guanine quadruplex (G4) structures play any regulatory role in replication initiation in an radioresistant multipartite genome containing organism *Deinococcus radiodurans*. Replication initiation proteins of *D. radiodurans*, recombinant DnaA and DnaB were purified to near homogeneity from *E. coli* host. PCR amplification of *ori* sequence of chromosome I was carried out and through bioinformatics analysis putative G4 motifs were identified in *ori* sequence. EMSA experiments with G4 DNA and non G4 forms suggested that DnaA is having high affinity for G4 structures while DnaB does not have any preference for G4 structures. Further results from dynamic light scattering suggests that DnaA forms higher molecular weight oligomers in presence of G4 strucutures.

The findings of the ATPase activity assays of DnaA protein revealed that protein's ATPase activity was higher in the presence of dsDNA than it was in the presence of G4 DNA, indicating that the reduced availability of free DnaA protein in the reaction mixture as a result of enhanced DnaA protein binding to G4 DNA.

Furthermore, two mutant alleles for the mismatch DNA repair protein MutS were created using overlapping PCR technique. One was obtained by deleting the RGG sequence from the *mutS* encoding sequence (RGG del *mutS*), and the other by substituting serine for arginine in the RGG sequence of *mutS* (SD *mutS*). Both research efforts sought to identify the function of the RGG sequence found in the MutS protein in the DNA mismatch repair process. Three RGG del *mutS*-positive clones were obtained from the screening of transformants which needs to be confirmed further through restriction analysis.

CHAPTER- 8
FUTURE PROSPECTS

- Use of a more sensitive technique like radioactive EMSA to check the affinity of DnaA and DnaB proteins towards G4 structures
- In vitro characterization of putative G4 motifs identified in the *ori* region of chromosome I in *Deinococcus radiodurans*
- It is planned to confirm the cloning of the *mutS* alleles by restriction analysis. The confirmed recombinant plasmids will be expressed in *E. coli* hosts, and proteins will be purified to understand the function of the RGG region.

CHAPTER – 9

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SAMPLE PROJECT REPORTS

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SESSION : 2022-23

PROJECT ON

REVIEW ON DHAVALESWARAPU RATNA HASANTI "ECO-WOMANISM IN ALICE WALKER'S COLOR PURPLE"

SUBMITTED TO
Ms. Neha Marawar
Department of English

SUBMITTED BY
Deepa Joseph Wilson
M.A. 2nd Year
Admission No. 49843

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Certificate

It is certified that Deepa Joseph Wilson has completed the project on the Topic "Review on Dhavaleswarapu Ratna Hasanti 'Ecowomanism in Alice Walker's Colour Purple'" During this procedure his work and conduct were satisfactory.

Date : 11th Nov, 2022

Signature :

Place : Jabalpur

Name : Deepa Joseph Wilson

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First and foremost, praises and thanks to God the almighty, for his blessings throughout my dissertation work to complite it successfully

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Declaration

I Deepa Joseph Wilson student of MA III Semester (English) hereby declares that the dissertation work presented in this report is my own work and has been carried out under the supervision of

This work is done by me and it is authentic. It has not been previously submitted to any other place.

Date - 11th Nov, 2022 Signature of the Student - D. J

Place - Jabalpur

Topic _____ Date _____

What is a Project?

A project is a unique endeavour to which either one person or a group of people work in order to solve a problem.

This word 'project' is an integral part of our life i.e both personally and professionally we face problems on each step we take, and we also set some goals to fulfill our various needs. A project, therefore, is a unique venture undertaken by people either individually or they collaborate to achieve some required goals in life.

General Definition of a Project -

According to Max Wideman - "A project is a process or undertaking that encompasses an entire set of activities having a definable starting point and well-defined objectives the delivery of which, signal the completion of the project."

Topic

Date

Types of Project

Human life is a Collection of goals. Personally and Professionally we make goals in our lives and put our efforts to achieve them. Projects are of different kinds:

1) Personal or Professional Projects – Marriage, building a house and planning a family will come under personal projects. Whereas, developing a design, or achieving a significant manufacturing Status will be professional.

2) Personal and Social Projects – Taking a loan from a bank to complete the education will come under personal projects. But the projects undertaken by the government for the sake of the people will come under social project.

3.) Individual and Group Projects – A Project can be taken by a single person or a large group of people. It entirely depends upon the magnitude of the project and its scope. For Example: preparing for exam is an individual project but working to increase factory manufacturing capacity is a group project.

Topic Characteristics of a Project 5

Every projects involves some activity. But every activity cannot be called a project. A project has some characteristics.

1) Definite Time Duration - Every project has to begin at some time and has to meet a deadline. In other words every projects has a definite time. An activity cannot be called a project if people are walking on it for a long time

2) Uniqueness - Projects are not conducted to achieve regular goals. They are conducted to get a result which is uncommon in daily life.

3) Resource Factor - Project could be big or small, low-budget or mega-budget, But every project involves the use of resources, such as: Capital, Knowledge and human resources.

4) Risk Factor - One of the most important Characteristic of a project that is ever free from the risk of failure. People involve in a project prepare themselves mentally that they may not get success.

Topic

Importance of a Project

Date

Projects are important because they are a group of actions performed to achieve goals, often for a collective good.

They achieve goals - We often make goals which cannot be achieved by the actions of daily life. They required special attention, more time and large efforts.

Team and Time Management - Conducting projects are important in a Company, because it brings chance for the employees to show their talents for the leader to learn how to manage time and a team.

Personal and Social Guard - Projects are important both from personal as well from social aspect. Personally, they are helpful for achieving goals and to fulfill the needs. From the social aspect, projects are important because they improve the quality of living in a society.

What is Literature Review

A literature review is a piece of academic writing demonstrating knowledge and understanding of the academic literature on a specific topic Have a in content. A literature review also includes a critical evaluation of the material. The review should enumerate, describe, summarize, objectively evaluate and clarify this previous research. The literature review acknowledge the work of previous researchers, and in so doing, assures the reader that your work has been well conceived. It is assumed that by mentioning a previous work in the field of study, that the author has read, evaluated and assimilated.

Topic Date 8

Types Of Review

There are some common types of Reviews

1.) General Review - That provides a review of the most important and critical aspects of the current knowledge of the topic. This general review forms the introduction of a thesis or dissertation and must be defined by research Objective.

2.) Theoretical Review - Which examines how theory shapes or frames research.

3.) Methodological Review - Where the research methods and design are described. These methodological reviews outline the strengths and weaknesses of the methods used and provide for future direction

4.) Historical Review - Which focus on examining research throughout a period of time, often starting with the first time an issue, concept, theory, phenomena emerged in the literature, then tracing its evolution within the scholarship of a discipline.

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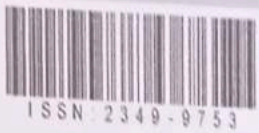

ECOWOMANISM IN ALICE WALKER'S *THE COLOR PURPLE*

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ABSTRACT

Women and nature are indispensible parts of the works of Alice Walker and she has empathetically written about them. Through her novels, Walker has brought into focus the exploitation of women and the environment. Her Pulitzer Prize winning novel *The Color Purple (1982)* is a manifestation of ecowomanism. Walker in her novels has given equal importance to men, women, animals and inanimate nature to promote an ecocentric world view through womanism. In the novel, *The Color Purple* she has linked environmental issues to questions of gender and race. This paper traces the ecowomanist consciousness of the main female characters in the novel. The ecowomanism that goes beyond ecofeminism, employed in the novel, asserts that both nature and women are to be handled with care. Walker shows how an ecological perspective when linked with womanism is a harbinger of true self-reliance and happiness for women. Shug, Celie, Sofia and Squeak enrich their lives with ecowomanist awareness, overcome tribulations, become truly self-reliant and triumphant by the end of the novel. Walker through the novel asks women to develop and put forth their ecowomanist awareness in action, by promoting eco-friendly ways of life.

Keywords: *Ecocrticism, Ecowomanism, Ecofeminism, Ecology, Ecosphere*

Women and nature are indispensible parts of the works of Alice Walker and she has empathetically written about them. Through her novels, Walker has brought into focus the exploitation of women and the environment. Her Pulitzer Prize winning novel *The Color Purple* (1982) is a manifestation of ecowomanism. Definitions of the words ecology and ecosystem, throw light on the interconnectivity amidst the flora and fauna on planet earth. The Oxford Advanced Learner's Dictionary defines 'Ecology' as "the relation of plants and living creatures to each other and to their environment" ("Ecology," Def. 485). It defines 'Ecosystem' as "all plants and living creatures in a particular area considered in relation to their physical environment" ("Ecosystem,"Def.486). Both the definitions show the connectivity between the animate and inanimate world. Perchance, human beings have dominated the ecosystem and have modified it for their benefit. This has created an imbalance in ecology which needs to be corrected for posterity on the planet earth. Moreover, the relationship between man and the

non-human part of the environment has been a lopsided one, with man dominating the rest, even women. Walker in her novels has given equal importance to men, women, animals and inanimate nature to promote an ecocentric world view through womanism. In the novel, *The Color Purple* she has linked environmental issues to questions of gender and race.

Ecocrticism talks about environmental degeneration, pollution, global warming, climate changes, and species extinction. It stresses on the need to usher in environmental awareness through proper representation in literature. Ecocritics like Cheryl Glotfelty, Lawrence Buell and Greg Garrard, through their works like *The Ecocrticism Reader: Landmarks in Literary Ecology* (1996), *The Environmental Imagination: Thoreau, Nature Writing and the Formation of American Culture* (1995) and *Ecocrticism* (2004) have talked about the ecological crisis faced by modern man, and have suggested ecological solutions like returning to nature and interdisciplinary study of environmental degradation. They haven't focussed on the link between oppression of women and degradation of the environment, which is important for the emancipation of both women and the environment. The term 'Ecofeminism' first appeared in 1974 in Francoise d'Eaubonne's *Le Fe'minisme ou la mort*, in which she talked about the direct link between the oppression of nature and the oppression of women. It is to be observed that: "Sexism and exploitation of the environment are parallel forms of domination" (Warren i). As adroitly pointed out by Spretnak "Ecofeminism will address not only the interlinked dynamics in patriarchal culture of terror of nature and the terror of elemental power of the female, but also the ways of the mesmerizing conditioning that keeps women and men so cut off from our grounding in the natural world, so alienated from our larger sense of self" (6). Ecofeminists have depicted how women are tied to nature and how feminist issues cannot be separated from ecological issues. They haven't linked issues of racism to environmental issues along with that of gender, which ecowomanism does.

Alice Walker coined the term 'Womanism' in her collection of essays titled *In Search of Our Mothers' Garden's: Womanist Prose* (1983) to speak about and against the oppression faced by women of colour. In it, she has said that, a womanist is "A black feminist or feminist of color. . . . Appreciates and prefers women's culture, women's emotional flexibility (values tears as natural counterbalance of laughter), and women's strength. . . . " She has further stated that a womanist " Loves music. Loves dance. Loves the moon. Loves the Spirit. Loves love and food and roundness. Loves struggle. Loves the Folk. Loves herself. Regardless" (Walker In Search xi-xii). From the aforesaid definitions, it is clear that love for nature is an integral part of womanism. Walker through her literary oeuvre has showcased her concern for black women and the environment. As hinted by ecofeminists Spretnak and others, Walker advocates 'ecological wisdom' as a route to the prevention of environmental degradation. She has surpassed the concept of ecofeminism bringing into focus the questions of race along with gender, by proposing an un-anthropocentric attitude towards nature, to solve the problems created by the nexus of race and gender. As put forth by Smith the term Ecofeminist "expresses the perception that the degradation of the Earth is of a piece with the subordinating and bullying of women, racial minorities, the poor and the marginalized, the term 'ecowomanist' expresses the burden of this perception on a woman of color" (476). Moreove ecowomanism is based on the authentic experien of African American women.

Walker as a committed ecowomanist inci her readers through a composed, reticent investigation and study of the threats shoved on nature by human beings in their specious ways. She suggests that the indemnities of environmental pollution, deforestation, and global warming need to be corrected by developing true ecowomanist consciousness. She upholds the view that, this alone can topple patriarchal hierarchy and preserve the interests of women and the environment. The works of Alice Walker are effectual pieces of protest, that reverberate with environmental issues like nurture nature, deforestation, nuclear disarmament and

preservation of nature centered culture and many other issues concerned with the upliftment of women. The novel *The Color Purple* brims with ecowomanist consciousness. This paper traces the ecowomanist consciousness of the main female characters in the novel. The ecowomanism that goes beyond ecofeminism employed in the novel, asserts that both nature and women are to be handled with care.

Black women much before their import to America have been children nurtured by nature within a matriarchal culture that protected their interests. After their import to America, they have been denied a true understanding of nature along with their own true nature. Their being and interests have been sabotaged to nurture and protect the interests of patriarchal culture instituted and propagated by the white man. Submerged and subjugated under a white patriarchal system, nature alone remained their source of happiness and solace. On account of their closeness to nature, they were labelled and castigated as pagans, heathens and witches. Moreover, patriarchal religiosity in the name of Christianity disengaged them from an ecocentric world view and coerced them to an anthropocentric world view. Walker through her works has sincerely tried to usher in an ecocentric worldview to protect the interests of nature and human beings as pointed out by Capra. Capra in *The Web of Life: A New Synthesis of Mind and Matter* (1996) has clearly elucidated the "complex interweaving of living and non-living systems within a single web" (309). Walker like Capra endorses the preservation of the ecosystem for the better survival of women of colour and mankind.

Walker in her work *Living by the Word: Selected Writings 1973-1987* (1988) has shown how racism and sexism are connected to environmental issues by stating that " some of us have become used to the thinking that woman is the nigger of the world, that a person of color is the nigger of the world, that a poor person is the nigger of the world. But in truth, Earth itself has become the nigger of the world. . ." (147). Walker in *The Color Purple*, the most famous of all her novels, has put forth the aforesaid perspective. It brings into focus the metamorphosis in the life of Celie the protagonist of the novel, after she develops an ecowomanist consciousness, and establishes a true communion with nature. Walker shows how an ecological perspective when linked with womanism is a harbinger of true self-reliance and happiness for women. Though gender issues amidst racism pervade the novel, it is the ecological perspective that gives the women characters the duress to reclaim their selves. Walker in the preface to the tenth edition of *The Color Purple* has rightly said that purple is the "color that is always a surprise but is everywhere in nature". As implicated in the aforesaid statement, the novel truly showcases the links between race, gender and nature.

Walker in the novel explores the connections between women and nature through spiritual amendments. She has said that "In day-today life, I worship the Earth as God-- representing everything-- and nature as its spirit" (Walker Anything 9). She argues that an anthropocentric, patriarchal Christian view that the universe is only for man, will be very taxing for the ecosystem and she recommends paganism as a way of promoting an ecocentric worldview. As pointed out by Pamela Smith, Walker asks for the adoption of "pan-religious and pantheist sensibilities" (7), as they alone can save the ecosphere. Celie in *The Color Purple* realizes this perspective through the enlightenment of Shug. She makes Celie realize that she is not an insignificant part of creation. Real redemption happens for Celie when she "discovers that she is part of 'the creation,' that she fits into the natural order of the world, . . ." (Barker 61-62). This spiritual rebirth of Celie is far away from patriarchal religiosity and is an outcome of her developing ecowomanist perspective.

Walker through the novel drives home the point that the critique of patriarchy alone can lead to the preservation of the interests of women and the earth. She showcases that patriarchal forces erase rituals and culture that embrace female sexuality and pleasure. Walker in the novel advocates that a free female sexuality alone can give humans a respite from their burdened sexuality. Free sexuality is part of animate nature and Walker argues against it, being tabooed. Regarding this, Lovalerie King has astutely stated that: "Walker's womanist is in touch with her

own fluid sexuality, which she shares at her discretion and pleasure with women and/or men. The womanist embraces and openly expresses her sexuality in relationships with others" (138). The lesbian relationship that Celie has with Shug can be viewed as an attack "on male hegemony, especially the violent abuse of black women by black men, . . . as a revolutionary leap forward into a new social order based on sexual egalitarianism" (Bell 263). Shug embraces her bisexuality with grace and so does Celie later on. Sofia discovers her sexuality with Buster. Squeak discovers it after overcoming the trauma of rape. The solacing sisterhood offered by the women around her, helps her open up, and overthrow unwanted male domination. Walker opines that an unencumbered sexuality can bestow wholeness on the lives of women and help them nurture humanity and nature, and she has promoted the same through the novel.

Walker in her work *Anything We Love Can Be Saved: A Writer's Activism* (1997) has disclosed her urge to take a walk amidst nature and see its beauty. She has said that this brings to her mind many blacks who she has known "are flexible like the grass and sheltering like the trees" (111). The solacing quality of nature has been given importance in the text *The Color Purple*. Celie imagines herself as a tree, while facing domestic violence under Albert. Celie tells Harpo, her stepson: "I say to myself, Celie, you a tree" (Walker *Color* 22). Imagining herself as a tree, gives her the strength to combat oppression, offer resistance and make life a happy ride. Shug shelters the other women characters in the novel, like a tree, under her ecowomanist wisdom and makes them self-reliant. Furthermore, Celie as a self-reliant entrepreneur gets firmly rooted like a tree and offers employment to many women. Walker advocates that human suffering and devastation of nature, can be surmounted when the toxicity in the mind, body and the earth are removed completely. Moreover, the text is profusely laden with vivid descriptions of nature.

Reclamation of the body and spirit are very important for a woman to redeem herself and nurture nature and Walker promotes this concept through the novel. Celie discovers herself with the help of Shug, and in the process reclaims both her body and spirit, by saving it from domestic abuse, nurturing it, and discovering her sexuality. Sofia Butler, wife of Harpo finds a new home and boyfriend to overthrow the nonchalance, and male chauvinism of Harpo. Squeak overcomes the domination and domestic violence of Harpo and reclaims her voice. Celie and Shug revitalize themselves with ecowomanist awareness and become whole and are ready to face life with renewed strength. Walker through the novel showcases that nature is the greatest revitalizer of life.

Preservation of matriarchal culture alone can outcast patriarchal domination, and protect the interests of women and the environment. Walker clearly showcases this in the novel. Celie is introduced to soothing blues songs by Shug and Squeak. They introduce her to the possibility of finding one's voice and being, and highlighting them for the positive catalysis of the self. Walker through *The Color Purple* endorses the idea of Sherley Ann Williams, who has talked about the solacing quality of blues songs as they express the pain and triumph of the black community. She has said: "pain plays a large part in Black music is evident in the lyrics of the blues, . . . Yet, there is the beautiful lyricism . . . which also expresses triumph and transcendence, the sly humor and laughing confidence, the will to make it on through, to work it on out . . . " (144). The women characters in the aforesaid novel realize this and revitalize themselves with blues songs, to realize their selves and lessen the burden of racism, sexism and classism by establishing an emotional connection with the black community on the whole. With their help, they come close to nature and their true nature.

Walker asks women to stand against patriarchal power by dismantling the hierarchies established by organized religion and cultural practices that place women and mother earth below the interests of man and his monopoly. Walker believes that "all of creation is of the same substance and therefore deserving ... same respect ... We are connected to them [animals] at least as intimately as we are connected to trees" (Walker "*The Universe*

Responds" 307-308). Having said so, she has spoken against the anthropocentric view of man's supremacy over the rest of nature. Walker endorses the view that connectivity between things in this world is cyclic. Walker has said that a womanist "Loves music. Loves dance. Loves the moon. Loves the Spirit. Loves love and food and roundness. Loves struggle. Loves the Folk. Loves herself. Regardless" (Walker, *In Search* xii). This definition implicates the ecological perspective of womanism. The ecological womanism or ecowomanism employed in the novels gives equal importance to both animate and inanimate aspects of nature. The healing circles that the women characters in the novel establish with their sisterhood, are evocative of the aforesaid statement. Eleanor Jane, the mayor's daughter in *The Color Purple* realizes this and comes in unison with Celie, Shug, Sofia and Squeak, keeping aside her hierarchy. The reversal of gender roles showcased at the end of the novel between Celie and Albert, and Sofia and Harpo hint at the dismantled hierarchies in the novel. The dismantled hierarchies aid in establishing better relationships between men and women, blacks and whites in the novel, and help the women characters preserve their interests including nature.

Walker has talked of a pantheistic perspective of God in an interview with John O'Brien. She has said that "Certainly I don't believe there's a God beyond nature. The world is God, man is God, So is a leaf or a snake . . . " (75). The aforesaid quote reflects the ecological perspective of her thinking and her womanist ideology. She speaks against the thought of Eve being labelled as a temptress. She defies the thought pattern that God is a 'he' and is traditionally found in the "white folks' white bible" (Walker *Color* 202). Shug elucidates to Celie, to overlook the hierarchy established by white Christianity by explaining to her that "God is inside you and inside everybody else You come into the world with God. But only them that search for it inside find it. And sometimes it just manifest itself even if you are not looking, or don't know what you are looking for" (Walker *Color* 202). Departure from Christianity to pantheism, first followed by Shug and later on by Celie can be best summarized in Shug's words as " My first step from the old white man was trees. Then air. Then birds. Then other people" (Walker *Color* 203). Walker having made Shug say so, in the novel, asks women to be authoritative tools of change for themselves, planet earth and future generations. This initiates a positive change in Celie's conception of God. Celie realizes how racism has been deviously connected to Christianity by whites and patriarchy. Commenting on this aspect of the novel, Rashmi Gaur has fittingly recorded that "Celie's changing attitude to life records a shift in her attitude towards God too. . . . At a later stage when she is put on a path of recovering her identity she is also able to understand the full extents of gender-based and racial connotation of Christian patriarchy" (173). Nettie and Samuel though Christian missionaries dwell deep on the act of "not being tied to what God looks like" (Walker *Color* 218). This helps them overthrow white monopoly of Christianity. The ecowomanist thread in the text encourages unity between God, humans and nature especially between God, women and nature. This realization empowers the women characters in the novel.

Resurrection of nature, its worship and preservation are of utmost importance to ecowomanism and Walker has talked about them in *The Color Purple*. Celie finds solace amidst the clamour of plantation life, nurturing nature on the farm. Shug makes her realize that "it pisses God off if you walk by the color purple in a field somewhere and don't notice it" (Walker *Color* 167). Later on, Celie becomes a self-reliant entrepreneur and builds a home in Memphis for herself amidst nature wit many artefacts of nature, symbolic of h ecowomanist awareness. Celie and Shug impart th awareness to the other women characters in th novel and accentuate their ecowomanist awareness. Nettie's stay in Olinka speaks about how, nature worship and preservation be it as simple as the worship of the roof leaf can act as a source of strength to fight oppression. Moreover, Walker has cleverly linked issues of environmental pollution, deforestation, and global warming with Nettie's stay in Olinka. She has showcased how destruction of greenery leads to the aforesaid problems, one after the other, even in a thickly vegetated country like Olinka. As pointed out by Bush "Through Netties

story, the theme of women's exploitation by men is set in the larger context of the exploitative relationship between races and nations" (1039). The act of making a spiritual union with nature helps Nettie in healing herself after having seen the ecological disaster the white man has made out of Olinka.

The Ecowomanist transformation in Celie is established by the fact that Celie addresses the last letter to everything that matters to the ecosphere. She starts it writing "Dear God, Dear Stars, dear trees, dear sky, dear peoples. Dear everything . . . " (Walker *Color* 292). With her ecowomanist metamorphosis, Celie gets all that she wants, and deserves in life. Walker, the writer par excellence, environmental activist, lover and "worshipper of nature" (Walker *In Search* xi), through the novel endorses the view that close contact with nature is a manifestation of the female spirit and it helps women in realizing their true potential. Shug, Celie, Sofia, and Squeak enrich their lives with ecowomanist awareness, overcome tribulations and become truly self-reliant and triumphant by the end of the novel.

The women characters in the novel overcome all the hindrances that have hindered their mental and spiritual evolution earlier. Ecological womanism needs hard-headed persuasion and application and Walker recommends this through the novel. Through the novel, she drives home the point that the subjugation of women and the conflagration that men have made of mother earth needs immediate amendments. Walker through the novel asks women to develop and put forth their ecowomanist awareness in action, through their activism, and by promoting eco-friendly ways of life.

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Topic Date

PREFACE

is like a bridge between theoretical and practical working. With the willingness I joined this particular diss

This dissertation and review paper deals with the study of some famous research paper one of them being Dhavaleswarapu Ratna Hasanti "Ecowomanism in Alice Walker's Color Purple", the paper fully focused on how nature and environment are wholly interconnected to the Woman.

The Review begans with the summary of the novel Color Purple and then reviews how Hasanti has beautifully and appropriately evaluated, analysed the nature and Women. How Nature is dependent on the Women.

I have tried to use some example from within the novel, that will help more to understand the quality work Hasanti has tried and had done it.

Topic Date

About The Author

Poet, essayist and novelist Alice Walker was born in 1944, in Eatonton, Georgia. The author of numerous books, she is one of the Country's best-selling writers of literary fiction. According to Renu Taiwa. Walker is a feminist and vocal advocate for human rights, and she has earned critical and popular acclaim as a major American novelist and intellectual.

Upon the release of The Color Purple, Critics sensed that Walker had created something special. "The Color Purple. . . . Could be the kind of popular and literary event that transforms an intense reputation into a national one," according to Gloria Steinem of Ms. Walker "has succeeded". The Color Purple is an American novel of permanent importance, that rare sort of book which amounts to 'a diversion in the field of dread"

An excellent student, Walker was awarded a scholarship to Spelman College in 1961. The Civil rights movement attracted her, and she became an activist.

Some reviewers criticize Walker's fiction for portraying an overly negative view of Black men

The idea of reformation, this sense of hope even in dispair, is at the core of Walker's vision. In spite of the brutal effects of sexism and racism suffered by the characters of her short stories and novels.

In 1982, Walker received the Pulitzer Prize for literature for her third novel, The Color Purple. Following this great achievement. She published a collection of essay, In search of Our Mothers Garden, in 1983 and In 1984 released a collection of poems.

Topic Date

WRITING STYLE

Alice Walker is an American activist, poet, short story writer and novelist. She is best known for her novel 'The Colour Purple'. Alice Walker is known to creati work that sounds poetic and rhythmic is nature.

Walker's writing style is known to ~~be~~ hold the consistent theme of African-American oppression and can be seen in many forms in her work. Despite the often saddening and serious themes of her work, Walker has a gift at making her work sound graceful and elegant. She uses many tactics found in poetic work, and incorporates them into her writing, such as the use of sentences varying in length and rhythm.

Walker's writing reflects her roots in Georgia, where Black Vernacular was prominent and the stain of slavery and oppression were still present. When she was eight, ~~Walker~~ coined the term "Womanist" to describe her philosophical stance on the issue of gender. As a Womanist she sees herself as someone who appreciates women's culture and feminity. Her work often reflects this stance as well as the universality of human experience. Walker's central character are almost always

Black women. She speaks the female experience more powerfully for being able to pursue it across boundaries of race and class. Walker also likes to break up long flowing sentences with short and powerful sentences. This makes it so she is able to add a sentence that is important and powerful to the reader since it's been embedded in the middle of long flowing sentences.

She has made a lasting impact on American literature, and helped people better understand the struggles African American women face. Walker strives to inspire people with her writing.

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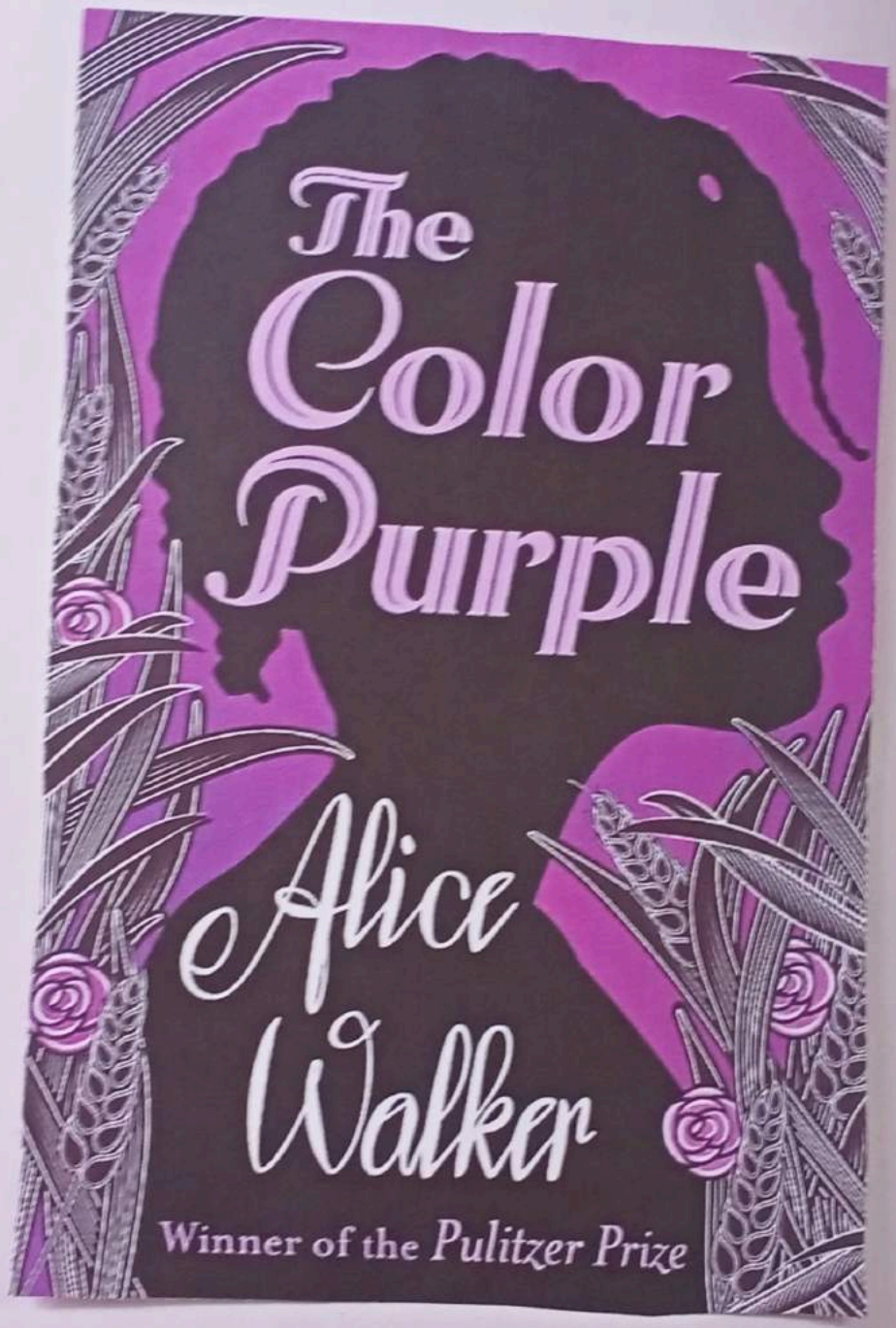

BOOK COVER

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Summary Of Color Purple

The Color Purple is a 1982 epistolary novel by American author Alice Walker which won the 1983 Pulitzer Prize for Fiction.

Through the novel Alice Walker traces the gradual liberation of Celie, a poor, Black Woman who must overcome abuse and separation from her beloved sister Nettie. Set in the South and an unnamed African Country during the 1930 to 1940s, the novel is a study in the way in which Black women use their faith, relationship, and creativity to survive racial and sexual oppression.

At fourteen, Celie begins writing to God about her life after Alphonso, the man she believes to be her father, threatens her if she tells anyone that he is raping her. Celie's only love in Nettie, her clever little sister. Celie eventually marries an older man, Albert after her father declines to let the man marry Nettie. Albert beats her, Verbally abuses her. On the other hand he is infatuated with the blues singer Shug Avery, a woman he has loved his entire life but cannot marry.

Celie's household is thrown into upheaval when Albert brings an ailing Shug home for Celie to nurse to health

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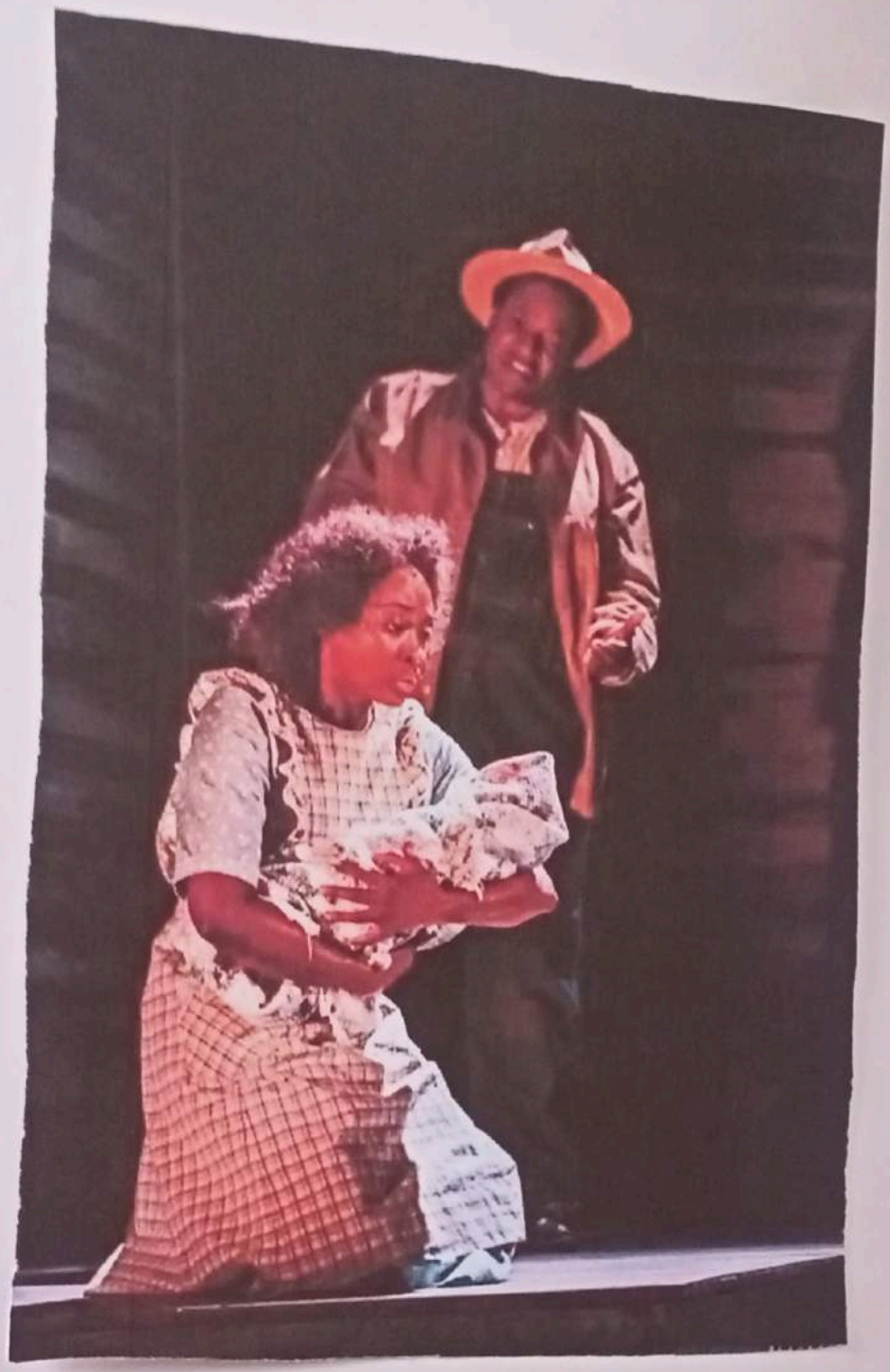
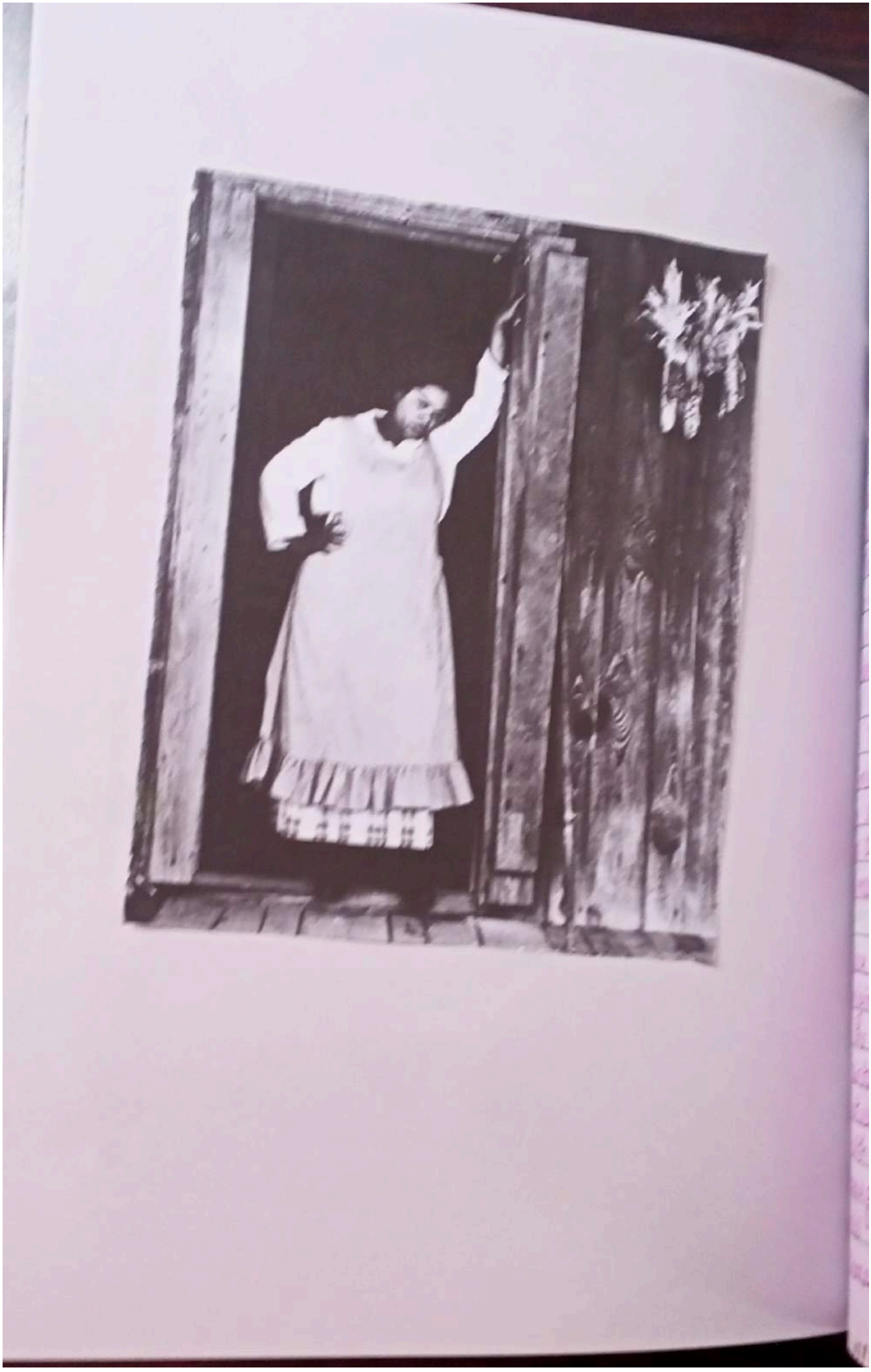


Celie manages to heal Shug with good food and leaves and the two women strike up an unlikely friendship that puzzles their family. Celie stands by as Shug heals and resumes her affairs with Albert. Celie learns to love Shug, who teaches Celie about her own pleasure and uncovers that Albert has been hiding letters from Nettie for years. Celie reads years and years of letter from her sister. Celie's life changes dramatically over the years as well. She lives with Shug in Memphis for a time after Albert's betrayal in hiding the letters becomes too much to bear. She starts her own company and becomes Shug's lover and companion.

When Alphonso dies, Celie and Nettie inherit a store, a fine house, and land. Celie experiences heartbreak however when Shug leaves Celie for a younger man. Celie also receives word that the Ship carrying Nettie and the children sank, which eventually turns out to be false.

Shug after few months turns up, ready to take up life with Celie again. Nettie, Olivia and Adam at last make it to Georgia. The family reunites and Celie is content.



Date 23

Review on Dhavaleswarapu Ratna Hasanti "Ecowomanism in Alice Walker's Color Purple"

According to Melanie L. Harris Ecowomanism is a reflective and contemplative study of ecowisdom that is theorized, constructed and practiced by women of African descent. Ecowomanism also involves lifting up positive examples of African and African American women's contribution to earth justice.

The word "Womanist" was coined by Alice Walker in her short story, Coming Apart. And Womanism is a social theory based on the history and everyday experience of Black women. Since Walker's initial use the term has evolved to envelop a spectrum of varied perspectives on the issues facing Black women. And hence Eco-Womanism helps to restore the balance between people and the environmental and reconcile human life with the spiritual dimension.

In her paper Ratna Hasanti has very beautifully combined and coordinated nature with women. In the novel Color Purple, Walker herself has maintained a proper proportionate relationship between men, women especially Women along with the eco-system. Ratna Hasanti through her paper has very well told us how the Women mainly the Afro-American Women were directly connected and

Topic

associated with nature and there are too many examples to second that in the novel Color Purple that most of the things related to farming were done by women and Men enjoyed the final results.

Women were subjected to exploitation and discrimination, especially Black women were subjected to double exploitation that is of being a women and that too black. Through Ratna Hasanti's paper it is very clear of the idea why Alice Walker kept the title "The Color Purple", one because of the beautiful purple lavender flowers these women used to grow and the other is the title of the novel is similar to the colour of these women when they are beaten black-blue by their male oppressors.

Alice Walker has remarked that a womanist- "loves music. Loves dance. Loves the moon. Love the spirit Loves love and food and roundness. Loves struggle. Loves the Folk. Loves herself. Regardless". These lines herself tells and indicates Women's love for nature. Nature is a very important and integral part of Womanism. Man has always been given the authority of everything even the nature but Hasanti has through her research paper has

Topic _____ Date _____

given the correct authority and position to women. Even in the novel there are many instances where we see that even after getting herself into beating formed shape and everything Celie returns back to her children and most important the field and starts working as if nothing has happened. Nature has always been the major supporting system for the women for it has accepted her as it is without any interdoubt unlike how the society has treated her. As Hasanti rightly exclaims in her paper that "Subjugated and Submerged under a white patriarchal system, nature alone remained their source of happiness and solace."

Hasanti by reviewing the works of Alice walker has greatly exclaimed that Alice walker's female protagonists has always been likened like the grass and sheltering like the trees. This is very apt because in the novel itself Celie was shown always as a token of love even her husbands step children and Shug herself found her as a source of shelter in Celie. Celie was everyone's comfort and Celie found her comfort in the womb of nature.

Resurrection of nature, it's worship and preservation are of utmost importance to Eco-Womanism

Topic _____ Date _____

Tayanti through her research paper has greatly reviewed how the protagonist Celie has been raised above and came out as a wonder among all the discrimination and exploitation done to her by men. In the story itself she finds solace amidst the plantation life, nurturing nature on the farm. Celie becomes a self-reliant entrepreneur and builds a home for herself.

It is truly remarkable to read how Tayanti in her research paper had connected the threads of womanism to the ecology and environment and woman are interlinked to and with each other. She not only gave correct and detailed reviews about eco womanism but also gave proper justice to the term 'Womanism' which was coined by Walker herself.

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Conclusion

The research paper by Hasanti has identified feminism and that too, the Eco-Womanism as a major theme that stands out in the book 'The Color Purple'. Using this subject matter, the author has been in a position to describe the pain, issues, and challenges many young women in the targeted society have to go through and their strategies to get rid of them.

The purpose of such aspects is to promote the social equality, economic gains, and political positions of all sexes or gender groups. This kind of potrayal is essential since it becomes a powerful gubline that many women in the novel consider to become free and successful.

The description also becomes a new tool for encouraging many people and their ideologies to take the issue of feminism to the next level. and promote gender equality.

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Critical Comment

From the detailed review and analysis, it is evident that Walker focuses on the concept of Womanism and environment in her book and Ratna Hasanti has made a wonderful attempt to present the unique challenges and problems many women experience in their respective families or communities. She also goes further to offer unique examples and guidlines using the cases of two characters, such as Celie and Sofia.

These developments will continue to guide more women and feminists to engage in additional initiatives and movements.

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