ASUTOSH COLLEGE
(Estd. 1916)
92, S.P. Mukherjee Road
Kolkata – 700026

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Additional Information

METRIC NO: 2.3.1

STUDENT CENTRIC METHODS, SUCH AS EXPERIENTIAL LEARNING, PARTICIPATIVE LEARNING AND PROBLEM SOLVING METHODOLOGIES ARE USED FOR ENHANCING LEARNING EXPERIENCES

At Asutosh College, the focus is on student-centric methods to enhance learning experiences. Experiential learning allows students to engage in hands-on activities, making education more interactive and practical. Participative learning encourages students to actively participate in discussions and collaborative projects, fostering a sense of community and teamwork. Problem-solving methodologies challenge students to think critically and develop innovative solutions to real-world issues. These approaches collectively create a dynamic and engaging learning environment that prepares students for future challenges.

Examples of experiential learning, participative learning, and problem-solving initiatives by various departments at Asutosh College are provided here.

**Please click the above options to move quickly to the respective sections*

EXPERIENTIAL LEARNING

DEPARTMENT OF ZOOLOGY

REPORT ON MOU ACTIVITY BETWEEN ASUTOSH COLLEGE AND MAULANA AZAD COLLEGE

- **TITLE OF THE EVENT/PROGRAMME:** Faculty/Student Exchange Programme between Asutosh College and Maulana Azad College as part of their MOU activity. This was a 2-day event.

DAY 1

- **THEME OF THE EVENT/PROGRAMME:** Faculty/Student Exchange Programme
- **ACADEMIC SESSION:** 2023 – 2024
- **DATE:** 06.05.2024
- **VENUE:** Seminar Hall, Centenary Building, Asutosh College
- **OBJECTIVE:** To gain knowledge from experts in the field of UV, Visible and Fluorescence Spectroscopy and Flow Cytometry and its applications.
- **SPEAKER/RESOURCE PERSON:**
  - Speaker from Maulana Azad College: Dr. Samudra Prosad Banik, Assistant Professor and Head, Department of Microbiology Maulana Azad College
  - Speaker from Asutosh College: Dr. Satabdi Nandi, Assistant Professor, Department of Zoology, Asutosh College
- **TARGET AUDIENCE/PARTICIPANTS:**
  - Students of B.Sc Zoology Honours and Microbiology Honours (Semester VI) from Asutosh College and Maulana Azad College
  - Students of M.Sc Zoology (Semester II & IV) from Asutosh College and Maulana Azad College
- **ATTENDANCE SHEET:** https://drive.google.com/file/d/1BdwPni2lAmWJGhhF9mFAB4ziFHvpVJEc/view?usp=drive_link

- **BRIEF REPORT ABOUT THE EVENT:**

SPEAKER 1: The topic was "UV, Visible and Fluorescence Spectroscopy". Our first speaker spoke extensively about the principle, applications of Spectroscopy. It was a highly interactive session in which students participated and learnt the detailed mechanisms of spectroscopy. He discussed about the following

- ✓ Beer Lamberts Law and principles of scattering
- ✓ Importance of chromophore, its properties
- ✓ The different ranges for absorption by different proteins/amino acids
- ✓ The absorption spectral shift when proteins are dissolved in water and ethanol
- ✓ Hypochromatic and hyperchromatic shifts were discussed through graphical representations
- ✓ Florescence and Phosphorescence
- ✓ Intrinsic and extrinsic flurophores of biomolecules
- ✓ Labelling should not change the conformity of the protein - ideation
- ✓ Natural fluorescence observed Inosine and Wyosine, Application of ethidium bromide in staining nucleic acids
- ✓ The application of ethidium bromide as an intercalating agent when it reacts with DNA and shows fluorescence
- ✓ Static quenching – half life unaltered and dynamic quenching – half life changes
- ✓ Green fluorescence tag is used extensively in RDT, Localization of proteins can be visualized under the microscope

SPEAKER 2: The topic of her talk was "Flow cytometry Concepts and applications on Biomedical Research". The basic principle of FACS was discussed thoroughly with the students. She discussed the various aspects of flowcytometry

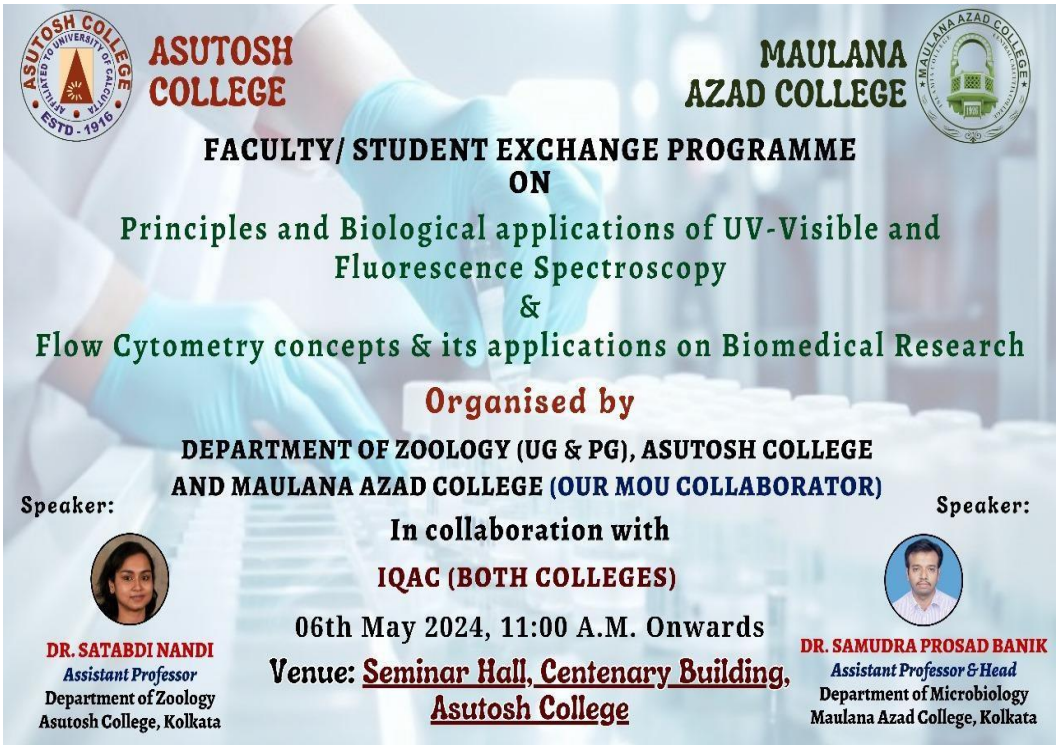
- ✓ The high throughput instrument has several applications, The basic scattering principle such as FSC/SSC and identify the sub populations of an organ
- ✓ The benefits and advantages of FACS are plenty, gives unbiased data
- ✓ The fluorescence activated cell sorter can simultaneously sort the data and sub populations can be identified
- ✓ The integral components of a FACS includes a fluidics, optics and electronics system
- ✓ The Graphical representation of FSC/SSC was explained using human blood sample

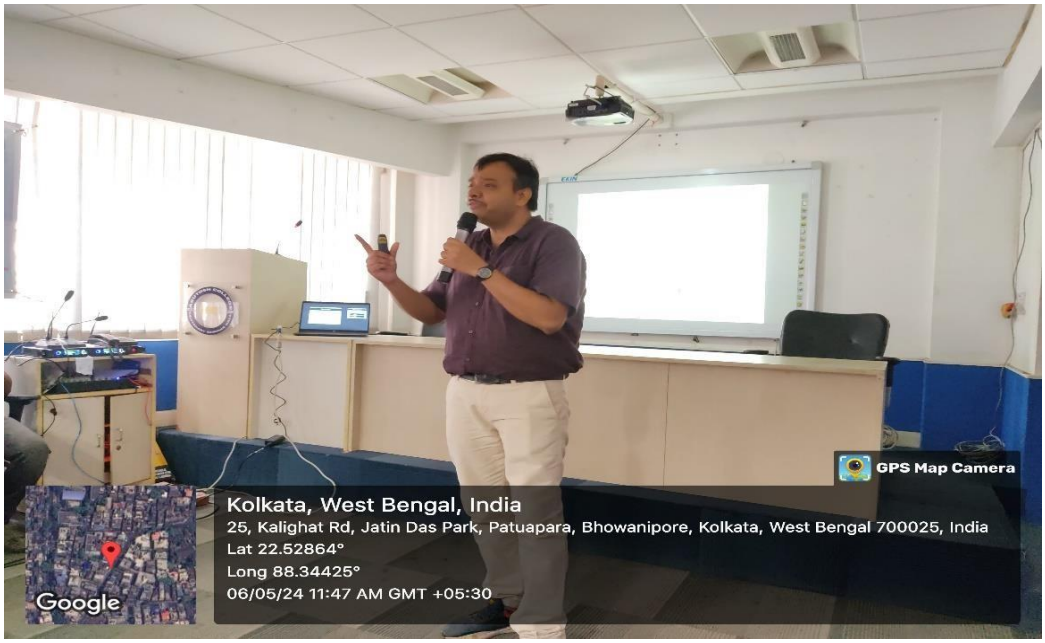
- ✓ The various fluorescence markers, unique fluorescence tags that are used to identify cells
- ✓ Fluorescence labelling with markers, is an unbiased technique, Green vs red fluorophore data is analysed using conjugated antibodies.
- ✓ The different applications of biomedical research were discussed at length, Cell counting, immunophenotyping, cell cycle analysis, imaging, biomarker discovery, apoptosis, cytokine was also discussed
- ✓ Bead based assays were done, Oncology and immuno-oncology was taken up as a case study for detection by FACS.
- ✓ Treatment of cancer is immensely benefitted by FACS, Imaging analysis can also be done.
- ✓ The disadvantages like single cells, live cells, tissue structure being lost, data analysis requires training etc. were also taken up for discussion.

At the end of both the talks, an active student interaction session was held to further enrich our students. The session concluded with an engaging question-and-answer session and thoughtful discussion among the faculty members.

- **EXPECTED OUTCOME:** To further enrich our students and encourage them to study and apply these high throughput instruments.

- **BANNER AND GEO-TAGGED PHOTOGRAPHS:**




GPS Map Camera
Kolkata, West Bengal, India
25, Kalighat Rd, Jatin Das Park, Patuapara, Bhowanipore, Kolkata, West Bengal 700025, India
Lat 22.52864°
Long 88.34425°
06/05/24 11:47 AM GMT +05:30
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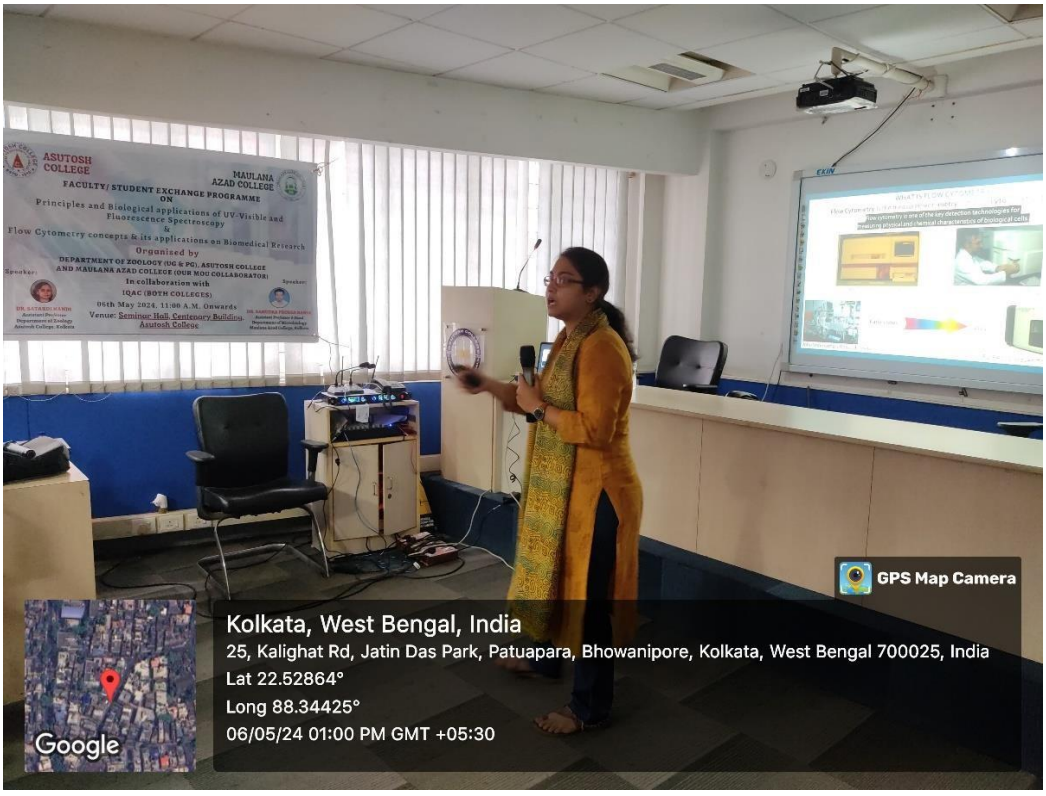

ASUTOSH COLLEGE
MAULANA AZAD COLLEGE
FACULTY/ STUDENT EXCHANGE PROGRAMME
ON
Principles and Biological applications of UV-Visible and Fluorescence Spectroscopy
&
Flow Cytometry concepts & its applications on Biomedical Research
Organised by
DEPARTMENT OF ZOOLOGY (UG & PG), ASUTOSH COLLEGE
AND MAULANA AZAD COLLEGE (OUR MOU COLLABORATOR)
In collaboration with
IQAC (BOTH COLLEGES)
05th May 2024, 11:00 A.M. Onwards
Venue: Seminar Hall, Centenary Building, Asutosh College
GPS Map Camera
Kolkata, West Bengal, India
25, Kalighat Rd, Jatin Das Park, Patuapara, Bhowanipore, Kolkata, West Bengal 700025, India
Lat 22.52864°
Long 88.34425°
06/05/24 01:00 PM GMT +05:30
Google

DAY 2

TITLE OF THE EVENT/PROGRAMME: Student Exchange Programme between Asutosh College and Maulana Azad College as part of their MOU activity

THEME OF THE EVENT/PROGRAMME: Experiential Learning for Post graduate students of Asutosh College and Maulana Azad College

ACADEMIC SESSION: 2023 – 2024

DATE: 07.05.2024

VENUE: Bose Institute Unified Campus, Central Instrumentation Facility, Sector V, Salt Lake

OBJECTIVE: To gain knowledge from experts in the field of Spectroscopy and Flow Cytometry, Gene Sequencing, Confocal Microscopy and other instruments and its applications.

ACCOMPANYING FACULTY MEMBERS:

Dr. Sriparna Datta Ray, Associate Professor in Zoology, Asutosh College Dr. Subhabrata Ghosh, Assistant Professor in Zoology, Asutosh College

TARGET AUDIENCE/PARTICIPANTS:

Students of M.Sc Zoology (Semester IV) from Asutosh College and Maulana Azad College

ATTENDANCE SHEET: Attached with this report as scanned copy

PROJECT REPORT ON ONE DAY VISIT TO BOSE INSTITUTE:

https://drive.google.com/file/d/1zPX3lWrZJUDR5-164w_xQQPJBTocu0vH/view?usp=sharing

बसु विज्ञान मंदिर
BOSE INSTITUTE
(विज्ञान एवं प्रौद्योगिकी विभाग, भारत सरकार के एक स्वायत्त संस्था)
(An Autonomous institute of Department of Science & Technology, Govt. of India)

रासायनिक विज्ञान विभाग / Department of Chemical Sciences
समन्वित शैक्षिक परिसर / Unified Academic Campus
ब्लॉक-ई एन, प्लॉट नं. ८०, सेक्टर-V, सॉल्ट लेक सिटी, कोलकाता 700 091
Block-EN, Plot No.-80, Sector-V, Salt Lake City, Kolkata-700 091

संदर्भ सं, / Ref. No. .....

दिनांक / Date : 7/5/24

To Whom It May Concern

This is to certify that the students of the PG department 4th semester and faculty members of Ashutosh College and Maulana Azad College visited Bose Institute, Unified Academic Campus, Salt Lake on the 7th of May,2024. The students visited the Central Instrument Facility and had the opportunity to observe the operation and applications of various sophisticated instruments available at the facility like FACS, Confocal Microscope, HPLC, DNA Sequencer, LCMS, MALDI TOF, and RT PCR. They also had the opportunity to engage in discussion with the technical staff regarding the specifications and principles of the instruments. The list of students and faculty members is attached herewith.
I wish them good luck in their future endeavour.

Prof. Jayanta Mukhopadhyay
Chairman, CIF

Dr. Jayanta Mukhopadhyay
CHAIRMAN
Central Instrument Facility
BOSE INSTITUTE
KOLKATA

बसु विज्ञान मंदिर
BOSE INSTITUTE
(विज्ञान एवं प्रौद्योगिकी विभाग, भारत सरकार के एक स्वायत्त संस्था)
(An Autonomous Institute of Department of Science & Technology, Govt. of India)

मुख्य कैंपस / **Main Campus :**
93/1, आचार्य प्रफुल्ल चंद्र रोड, कोलकाता - 700 009
93/1, Acharya Prafulla Chandra Road, Kolkata - 700 009
फोन/Phone: 2350-7073 (निदेशक/Director)
इपीबीएक्स/EPABX : 2350-6619/6702/2402/2403, 2303-0000/1111
फैक्स/Fax : 91-33-2350-6790

शतवार्षिकी भवन / **Centenary Building :**
पी-1/12, सी.आई.टी. स्कीम VII-एम, कोलकाता - 700 054
P-1 / 12, C.I.T. Scheme VII-M, Kolkata - 700 054
फोन/Phone: 2355-7434(निदेशक/Director), 2355-0595(रजिस्ट्रार/Registrar)
इपीबीएक्स/EPABX : 2355-9416/9213/9544, 2569-3271, फैक्स/Fax : 91-33-2355-3886

समन्वित शैक्षिक परिसर / **Unified Academic Campus :**
ब्लॉक-ईएन, प्लॉट नं-80, सेक्टर-V, सॉल्ट लेक सिटी, कोलकाता - 700 091
Block-EN, Plot No.-80, Sector-V, Salt Lake City, Kolkata - 700 091
फोन/Phone: 2569-3131(निदेशक/Director)
इपीबीएक्स/EPABX : 2569-3123/28, फैक्स/Fax : 91-33-2569-3127

संदर्भ सं. / Ref. No. _____ दिनांक / Date :

Name of the faculty members who accompanied students during the visit of Bose Institute on 07.05.2024

1. **Dr. Sriparna Datta Ray**
   Associate Professor
   Department of Zoology (UG & PG)
   Asutosh College
   Kolkata-700026

2. **Dr. Subhabrata Ghosh**
   Assistant Professor
   Department of Zoology (UG & PG)
   Asutosh College
   Kolkata-700026

Dr. Jayanta Mukhopadhyay
CHAIRMAN
Central Instrument Facility
BOSE INSTITUTE
KOLKATA

Name of the students who visited Bose Institute on 07.05.2024

Serial no	ASUTOSH COLLEGE	MAULANA AZAD COLLEGE
1	ABHIMANYU MAHANTA	ASHMITA DAS
2	SOURITRA GANGULY	ARNAB SARKAR
3	FAHIMA NAHID	NABANITA ROY
4	TANIA DEY	SHUBHAJIT MAJI
5	SUDATTA ROY	SURYENDU SAHA
6	SILPA HAZRA	PRAJNA DEBNATH
7	SNEHA TRIBADY	INDRANI ADHIKARI
8	RIYA GHOSH	MOHIMA MITRA
9	SAYAK MONDAL	PRIYA SAHA
10	SEEMRAN PAUL	TANIA SHAW
11	SHAHEENA RAHMAN	SHREYA GHOSH
12	SHANIA NASIM	SUDHRITI DAS
13	SHRIPARNA BAGH	SAYAK MONDAL
14	SOUMI SARDAR	SAYAN BHADRA
15	SOUMYAJIT DASGUPTA	SATYA RANJAN DAS
16	TAUSIF AMRUL KHILJI	
17	VEDANUSHA CHAKRABARTI	
18	RIMPA MONDAL	
19	PRIYANGANA MONDAL	
20	LAIBA ARIF	
21	KOYENA DAS	
22	ISHITA SAHA	
23	GOURAB BISWAS	
24	DIPANWITA MISHRA	
25	ARPITA MONDAL	
26	APRATIM GOSWAMI	
27	ANKIT PATRA	

Dr. Jayanta Mukhopadhyay
CHAIRMAN
Central Instrument Facility
BOSE INSTITUTE
KOLKATA

- **GEO-TAGGED PHOTOGRAPHS:**

PHOTOGRAPHS TAKEN DURING VISIT TO BOSE INSTITUTE ON 07.05.2024

Summer Internship (2024) under Curriculum and Credit Framework (CCF) of the University of Calcutta ZOOLOGY MAJOR

SEMESTER II

Name	**: MAHEK CHATTERJEE**
CU Roll Number	**: 233012-11-0271**
CU Registration Number	**: 012-1211-1112-23**

ASUTOSH COLLEGE

92, S.P. Mukherjee Road Kolkata-700026

Certificate of Completion

for

Summer Internship (2024) under Curriculum and Credit Framework (CCF) of the University of Calcutta

This is to certify that **MAHEK CHATTERJEE**, student of Zoology Major, Semester II, University Roll No 233012-11-0271 and Reg. No 012-1211-1112-23 of Asutosh College, successfully completed the 15day/60hours summer internship programme on Basic Molecular Biology Techniques (Level I) from 10/06/24 to 28/06/24 at Environmental Epigenomics Laboratory, Department of Environmental Science, Ballygunge Science College, University of Calcutta, Kolkata.

Signature of authorised signatory with official seal

Issued on: 16.08.24

Dr. Pritha Bhattacharjee
Asst. Professor
Department of Environmental Science
University of Calcutta, Kolkata - 700019

SUMMER INTERNSHIP

ON

BASIC MOLECULAR BIOLOGY TECHNIQUES

From 10th to 28th June 2024

ASUTOSH COLLEGE

92, S. P. MUKHERJEE ROAD KOLKATA -700026

Acknowledgements

I am grateful to Dr. Pritha Bhattacharjee, Assistant Professor, Environmental Epigenomics Laboratory, Department of Environmental Science, University of Calcutta for her continuous guidance as course coordinator.

I am grateful to our Principal, Dr. Manas Kabi for giving us this opportunity for the summer internship. Along with that, I am also thankful to Dr. Sriparna Datta Ray, Associate Professor, Department of Zoology, Asutosh College for helping us throughout this time and also to Dr. Tapan Kumar Roy, Head of the Department and all other faculties of our department.

I am also thankful to all the lab members of Environmental Epigenomics Laboratory, especially Dr. Tamalika Sanyal for her continuous support during the internship course.

Content

Course Content

Internship Program at Environmental Epigenomics Laboratory Department of Environmental Science, University of Calcutta Course name: Basic Molecular Biology Techniques Level-I (2 weeks program for UG Students)

Date	Topics for demonstration and hands on experiments	Mode	Marks
10/06/24	Basics of Molecular Biology -Orientation [Lecture & Quiz]	Offline	10
11/06/24	PCR- Principle, Methodology & Application [Lecture & Demonstration & Quiz]	Hybrid	10
12/06/24	*In silico* PCR- Primer Designing [Lecture & Demonstration & Assignment]	Online	20
13/06/24	Hands on by candidate, Trouble shooting	Offline	20
14/06/24	RFLP- Principle, Methodology & Application [Lecture & Demonstration & Quiz]	Offline	10
15/06/24	*In silico* identification of Restriction enzymes [Assignments]	Online	20
18/06/24	Hands on by candidate, Trouble shooting	Offline	20
19/06/24	Isolation of DNA [Lecture & Demonstration & Quiz]	Hybrid	10
20/06/24	Hands on by candidate	Offline	20
21/06/24	Quantification of DNA [Demonstration & Quiz]	Hybrid	10
24/06/24	Gel Electrophoresis [Demonstration & Quiz]	Offline	10
25/06/24	Hands on by candidate	Offline	20
26/06/24	Trouble shoot, Question-Answer Session, Interaction	Hybrid	10
27/06/24	Project report preparation & Remedial sessions	Hybrid	30
28/06/24	Power point presentation, Video documentation, certificate distribution and Submission of Report	Offline	30
	Total		250

INTRODUCTION

Molecular biology stands at the cutting edge of scientific exploration, where precision and innovation converge to drive groundbreaking discoveries. My recent summer internship at Environmental Epigenomics Laboratory in Department of Environmental Science, Ballygunge Science College, University of Calcutta, provided a profound experience within this rapidly evolving field. This 15-day summer training course offered a meticulously crafted blend of theoretical instruction and hands-on practice, truly supportive for future research initiation.

The internship commenced with an introductory overview of molecular biology, including its fundamental principles and diverse application fields. This initial phase established a critical understanding of the discipline, setting the stage for more specialized training. We began with foundational techniques essential to molecular biology, such as the precise handling of micropipettes—a fundamental skill in laboratory settings. As the program advanced, I was introduced to more sophisticated methodologies, including Polymerase Chain Reaction (PCR), DNA isolation, primer designing, use of restriction enzyme, gel electrophoresis and Restriction Fragment Length Polymorphism (RFLP) analysis. A significant aspect of the internship was the assessment process, designed to evaluate both our understanding of the techniques and our practical skills. Additionally, hands-on experience with state-of-the-art laboratory equipment, such as biosafety cabinets, -20°C freezers, cold centrifuges, PCR machine, RT-PCR, gel electrophoresis unit and GelDoc systems, provided invaluable insights into contemporary laboratory practices. This summer internship course ignited a profound passion for molecular biology and fostered a research-oriented mindset essential for future success in the field.

This project report represents my working experience in a day wise manner. All the assignments, photographic documentations and the format of final presentation are also given in this project report for complete understanding of the extensive coursework.

DAY 1: 10/06/24

Orientation programme: Introduction towards basics of molecular biology

On the very first day, our course coordinator Dr. Pritha Bhattacharjee Ma'am gave us a talk on the background of molecular biology and its importance in present perspective of analytical research in various fields like Zoology, Botany, Microbiology, Biochemistry etc. Molecular biology is the branch of biology that deals with the study of biological activity at molecular level. She explained the importance of molecular biology techniques like isolation of nucleic acids, PCR, Gel Electrophoresis, RFLP etc. that are present in the internship schedule. Next a short self-introductory session was arranged. We were introduced to the equipments present in the lab. We saw PCR machine, RT-PCR machine, and different types of table top centrifuge, Gel Electrophoresis apparatus, biosafety cabinet, -20°C freezer and many other basic instruments. We also learned about the primary safety guidelines about molecular biology lab and the use of PPE (Personal Protective Equipments) during the experiments.

Demonstration on the importance of PPE in Lab

Personal Protective Equipment, or PPE, is the clothing and equipment that forms the last line of defense between the researcher and harmful materials in the laboratory environment. Proper PPE and laboratory attire help to minimize the potential risk for skin exposure to hazardous chemicals, biological agents, and other hazardous materials. Basic PPE provided in the Molecular Biology laboratory includes: disposable gloves, lab coat, safety glasses, disposable face masks etc.

Demonstration and hands on training on micropipette handling

A demonstration was given on micropipette handling. Micropipettes are used to measure and deliver small volumes of liquid usually in the microliter range. We were informed that micropipettes are of great importance in laboratory work. Then we performed hands on practice with micropipette of different ranges. Gradually we learned about the proper handling and reagent measurement with the help of micropipette.

Remark

The first day orientation programme and research lab visit was a great experience for us. Please find the solved assignment attached with the project report.

DAY 2: 11/06/24

Demonstration on PCR methodology and applications

The day was started with a class on PCR. PCR (Polymerase Chain Reaction) is a scientific technique in molecular biology, which is used to amplify a single target DNA fragment for the generation of thousands to millions copies of similar DNA. We got to know the objectives and principles of PCR, the steps--- denaturation, annealing and extension, the reagents and the applications of PCR. Major applications of PCR –

- PCR is widely used in cloning DNA fragments of interest, in a technique known as PCR cloning and is important in recombinant DNA technology.
- It is important for medical diagnostics (pathogen identification) and forensic analysis.
- PCR is necessary for DNA sequencing and genotyping experiments like RFLP, RAPD, AFLP etc.
- PCR is also applicable in mutation detection, identification of specific allele, gene expression analysis, production of cDNA etc.

Procedure

A standard polymerase chain reaction (PCR) setup consists of four steps:

I. Addition of required reagents, i.e., mastermix (Green mastermix was used containing dNTPs, Taq DNA polymerase, Buffer, MgCl2, dye), template DNA and primers (forward and reverse) to PCR tubes.
II. Proper mixing and centrifugation (quick spin).
III. Set up of parameters in thermal cycler (temperatures for each step and no. of cycles).
IV. Evaluation of amplified DNA by agarose gel electrophoresis followed by ethidium bromide staining.

Remark

We observed each step very carefully and also listened the exact function of each reagent during the demonstration. Most unique of them was the Taq DNA polymerase, a thermostable enzyme derived from the thermophilic bacterium *Thermus aquaticus*. It is able to withstand repeated heating to 95°C (as is demanded by the PCR technique) without significant loss of activity.

DAY 3: 12/06/24

Demonstration on in silico primer designing for PCR reaction

The day was alloted for *in silico* work of PCR, i.e, primer designing demonstration. Primers are single stranded oligonucleotides that are complementary to the DNA segment of interest and are used for start of the reaction. We were informed about the different features of primer, which are

- Length of 18-24 bases.
- 40-60% G/C content.
- Start and end with 1-2 G/C pairs.
- Melting temperature (Tm) of 50-60°C.
- Primer pairs should have a Tm within 5°C of each other.

Procedure: Use of Primer3 software

We have learnt how to design a primer for a particular gene (also learned about the gene sequences from NCBI database). The demonstration was given on primer designing for *Brca2* gene. We had learnt the process of primer designing with the reference to SNP (Single nucleotide polymorphism) identification experiment and it is divided into two steps - identifying the SNP from NCBI database and designing the primer from Primer3 software. Then we had performed the hands on primer designing. We had designed the set of primers for *tp53* gene. The steps involved were as follows:

- Firstly, identified the SNP from NCBI database.
- Copied the fasta format of the gene in the word document.
- From the Primer3 software, designed the forward and reverse primer.
- Calculated the amplicon size.

Remark

We had a remarkable experience as we have learned about the primer designing. Also we got to know about the NCBS database. We were given assignments on the basics of PCR and primer designing. Please find the solved assignment attached with the project report.

DAY 4: 13/06/24

That day we performed the hands-on practice on PCR. The gene for that was *GAPDH*. We had to set a PCR reaction of 10 µl with the provided reagents like, template DNA -- 50ng/µl, 2x green master mix, forward and reverse primer both with a conc. of 10µmol/ µl and nuclease free water. We had done the calculation of the volumes of ingredients as ----

Template DNA 2 µl

Forward primer 1 µl

Reverse primer 1 µl

Green master mix 5 µl

Nuclease free water 1 µl

Then we had prepared the PCR mastermix by mixing the appropriate volumes of ingredients and put it into centrifuge. After centrifugation, PCR tubes were loaded into the thermal cycler for the amplification and adjusted the parameters as follows ---

Steps	Temperature	Time
Initial denaturation	95°C	5m
Denaturation	95°C	30S
Primer annealing	61°C	35S
Extension	72°C	40S
Final extension	72°C	7m
Storage	0°C	10m

30 cycles

After about one hour the process was completed and the PCR product was obtained. Then it was examined under gel electrophoresis.

Remarks

We had also learnt about the troubleshooting of PCR like how to identify primer dimers, how to reduce non-specific bands, how to optimize the primer annealing temperature, importance of positive and negative control reaction etc. This was indeed a great experience to perform hands on PCR reaction.

DAY 5: 14/06/24

Demonstration on RFLP methodology and applications

Today, we explored Restriction Fragment Length Polymorphism (RFLP), a key technique introduced by Alec Jeffreys in 1984 for detecting genetic variations. Our session combined theoretical insights with hands-on demonstrations, covering RFLP's principles, methodology, and applications.

At first, we were introduced towards various restriction enzymes and their cut sites to understand the basis of RFLP. The technique involved digestion of target DNA fragments with specific restriction enzymes and analyzing the resulting fragment lengths using agarose gel electrophoresis.

Procedure

The RFLP procedure was demonstrated by the instructors. Our practical work started with PCR amplification to generate DNA samples. Then, digestion mixture was prepared with the PCR product, restriction enzyme, buffer, and nuclease-free water. After incubating the mixture at 37°C for 1 hour and heating it to 65°C to inactivate the enzyme, we used gel electrophoresis to separate and visualize the DNA fragments. Observing the gel, we identified variations in DNA fragment patterns, such as differences in band numbers and sizes, which indicate genetic differences.

Importance and application

This process has essential application in identification of genetic diseases, DNA fingerprinting analysis and forensics, genetic mapping and detecting disease-causing mutations. We also discussed an example of sickle cell anemia detection using RFLP.

Remark

Today's experience clearly indicates significant role of RFLP analysis in genetic analysis and its continued relevance despite the advancement of newer sequencing technologies. Please find the solved assignment attached with the project report.

DAY 6: 15/06/24

Demonstration on in silico identification of restriction enzyme cut sites

Today's lab demonstration was focused on the *in silico* process of identifying restriction enzyme cut sites, a critical step in genetic analysis. We were introduced to the software WebCutter 2.0, a web-based tool, to facilitate this process.

Procedure

At first, webcutter 2.O site was opened in the desktop (it is a free site)

Next step, target DNA sequence was copied from the source and pasted in the provided space

Then, ―analyze sequence‖ was selected and clicked

Finally, result was observed on the screen which clearly indicates about your cut site details by different restriction enzymes within that fragment, no of cuts, position of cuts etc.

Importance and application

We were able to know specific information, such as the number of cuts made by the enzyme and their exact positions within the sequence. This information is essential for planning experiments and understanding how restriction enzymes interact with DNA. Also it indicates the nature of cuts (blunt or staggered) by the enzyme.

Remark

Today's session highlighted how *in silico* tools like WebCutter 2.0 can streamline the process of mapping restriction enzyme sites, aiding in precise genetic analysis and experimental planning.

DAY 7: 18/06/24

Hands on practice: In Silico Identification of Restriction Enzyme Cut Sites

Today's session provided a hands-on experience with the *in silico* process of identifying restriction enzyme cut sites, demonstrated on our previous classes. We used the WebCutter 2.0 tool to analyze DNA sequences and locate restriction enzyme cut sites.

We began by entering target DNA sequence into WebCutter 2.0. After submitting the sequence, we clicked on the ―Analyze‖ button to view the restriction enzyme cut sites. The tool provided details such as the number and positions of cuts within the sequence, essential for planning subsequent experiments.

Troubleshooting and problem solving during hands on experience

During the practical exercise, we encountered several troubleshooting scenarios. One common issue was the input of incorrect DNA sequences. We had to ensure that the sequence was accurately copied and free from errors, as even a single nucleotide change could affect the results. We also faced challenges with the tool's output. Occasionally, the results included unexpected cut sites or no cut sites at all. We resolved this by double-checking the DNA sequence and enzyme specifications, adjusting settings if necessary to align with the expected results. Overall, today's hands-on practice not only reinforced our understanding of RFLP but also provided valuable experience in troubleshooting common issues in the *in silico* identification of restriction enzyme cut sites.

Remark

This experience emphasized the importance of accuracy and careful analysis in genetic research and experimental planning.

Practice assignment was given at the completion of RFLP demonstration and hand on practice. Please find the solved assignment at the end of the report.

DAY 8: 19/06/24

Demonstration on DNA isolation protocol

DNA isolation is a key technique in molecular biology that separates DNA from other parts of a cell, such as proteins and RNA, so it can be studied. DNA isolation is important for many reasons, including genetic analysis and sequencing of genomes; forensic science ; medical tests and detecting bacteria and viruses etc.

Procedure

DNA Isolation can be performed in multiple ways. Lecture was delivered on this topic and two methods of DNA isolation were discussed.

- DNA isolation using conventional Phenol-Chloroform method
- DNA isolation from buccal smear following kit based protocol.

DNA isolation using Phenol-Chloroform method

In Phenol-Chloroform method, the basic principle is based on the principle of the liquid- liquid extraction of biomolecules. We started by lysing the cells to release the DNA. This is typically done using a lysis buffer, then added an equal volume of phenol: chloroform: isoamyl alcohol to the lysate followed by centrifuging the mixture at high speed (e.g., 16,000 × g) for about 5 minutes. This will separate the mixture into aqueous phase (containing DNA) and an organic phase (containing proteins) followed by transferring the upper aqueous phase to a new tube. Then, we added 2.5 volumes of cold 100% ethanol and 0.1 volume of sodium acetate to the aqueous phase followed by mixing it gently and incubating at -20°C for at least 30 minutes to precipitate the DNA. Then, the mixture was centrifuged again at high speed for about 10 minutes to pellet down the DNA followed by discarding the supernatant. The DNA pellet was washed with 70% ethanol to remove any remaining impurities. Centrifuged briefly and discarded the ethanol. Lastly, we air dried the pellet and re-suspended it in an appropriate buffer (TE buffer, pH 8).

Remark

Conventional phenol chloroform method is applicable for DNA isolation from various sources like animal and plant cells, tissues etc. It is most cost-effective but gives high yield of DNA.

DAY 9: 20/06/24

Hands on activity: DNA isolation from buccal smear

On that day, we had done the complete DNA isolation from our own buccal smear with the help of Buccal DNA isolation Kit (HiPurA® Buccal DNA Purification Kit, HiMedia). The protocol was given in the kit booklet, which was accurately followed.

Procedure

In this method, the stepwise protocol was as follows ---

I. To collect buccal cells, we scraped the inside of our mouth with a Buccal Collection Brush.
II. Then, dispensed 300 µl Cell Lysis Solution into a 1.5 ml microcentrifuge tube followed by removing the collection brush from its handle and placing the detached head in the tube.
III. Incubated for 5 min on ice followed by centrifuging for 3 min. Pipetted 300 µl isopropanol and 0.5 µl Glycogen Solution into microcentrifuge tube, and added the supernatant.
IV. Then, mixed by inverting gently and centrifuged for 5 min followed by discarding the supernatant, and then drained the tube by inverting on an absorbent paper, taking care that the pellet remains in the tube.
V. We then added 300 µl of 70% ethanol and inverted several times to wash the DNA pellet. Centrifuged for 1 min.
VI. Lastly, we carefully discarded the supernatant and drained the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube followed by air drying it for 5 min and mixed 50 µl of Nuclease free water and kept it for dissolving.

Remark

After complete dissolving, isolated DNA can be used in several experiments like PCR, RFLP analysis, sequencing etc. with proper quantification and purity check-up. Due to our short course duration, we were unable to perform any further experiments with our own DNA samples. But the isolation protocol was interesting and learned minute details from the experiment.

DAY 10: 21/06/24

Demonstration on DNA quantification

On the 10th day, we were introduced with the process of DNA quantification, DNA quantification is the process of determining the concentration and purity of DNA in a sample. Accurate quantification is essential for various downstream applications, such as PCR, sequencing, cloning, and other molecular biology experiments.

Principle: The spectrophotometric estimation of nucleic acid concentration is based on absorption by DNA, RNA and their constituents following a particular pattern. Quantification can be done based on the Beer-Lambert law. In a solution, DNA spectrophotometers can measure the levels of ultraviolet light absorbed by the bases. DNA and other nucleic acids absorb light at a peak wavelength of 260nm. The amount of light absorbed is proportional to the concentration of DNA in the sample.

Instrument Used: The NanoDrop spectrophotometer is widely used for measuring nucleic acids such as DNA, RNA, and oligonucleotides. Micro volume measurement technique uses tiny sample quantities (1-2 μL), reducing sample consumption and simplifying quantification. NanoDrop spectrophotometers are commonly employed in molecular biology labs because of their speed, precision, and ease of use.

Procedure: DNA Sample was loaded on the specific plate provided in NanoDrop machine. After setting up the software, it automatically determined the concentration of the sample at 260 nm absorbance. Result was displayed on the digital display monitor. Additionally, the A260/A280 and A260/A230 ratios were also observed to determine the DNA sample's purity.

Remark

Gaining experience in DNA quantification involves hands-on practice to prepare DNA samples, calibrate instruments, and interpret absorbance or fluorescence data to determine DNA concentration and purity. Troubleshooting is often part of the process, as one might encounter issues like sample contamination or equipment calibration errors. Through this experience, we will develop skills in precision, data analysis, and ensuring the accuracy of measurements for downstream applications like PCR and sequencing.

DAY 11: 24/06/24

Demonstration on Agarose Gel Electrophoresis

Today, we learned about gel electrophoresis. It is basically the method for separation of DNA molecules based on their molecular weights (sizes). The molecules to be separated are loaded on a gel matrix and an electrical field was generated which causes the movement of the DNA molecules through the gel pores. Movement can be affected by several factors such as electric field, charge of molecule and their size. Learning gel electrophoresis is an engaging and hands- on experience that involves understanding both the theory and practical aspects of the technique. Initially, we dive into the fundamental principles, such as how nucleic acids or proteins migrate through a gel matrix under an electric field based on their size and charge. Also, we learned about the details of the reagent preparation and their functions. We had learnt that there are primarily two types of gel electrophoresis:

1. **Agarose Gel Electrophoresis**: Used to separate DNA and RNA molecules by size, with smaller fragments moving faster than larger ones through the gel. The technique involves staining DNA with ethidium bromide, which fluoresces under UV light, and then viewing the gel under UV light to see the bands. The rate at which DNA moves through the gel is proportional to the voltage applied, but higher voltages can reduce the resolution of larger fragments.
2. **Polyacrylamide gel electrophoresis:** Used to analyze RNA fragments and separate proteins from mixtures. The gels are chemically cross-linked by polymerizing acrylamide with a cross-linking agent, such as bisacrylamide. Sodium dodecyl sulfate (SDS) can also be used to denature proteins into individual polypeptides. PAGE is also used tfor better resolution of smaller DNA fragments.

Procedure

1% agarose gel was made with 1 gm. of agarose powder dissolved in 100 ml of 1xTBE buffer and boiling. The solution was then poured into the casting tray with comb set-up. Next, the DNA samples were loaded mixed with the loading dye into the wells after the formation of proper gel matrix. Power source was adjusted and electrophoresis was done. Gel was stained in EtBr solution and the image was taken using the GelDoc system.

DAY 12: 25/06/24

Hands on activity: Agarose Gel Electrophoresis and gel imaging

We had performed complete gel electrophoresis experiment on that day. All the steps were as follows ---

Principle:

Agarose gel electrophoresis is an electrophoretic method using agarose, a polysaccharide derived from seaweed, as the supporting medium. It has the dual functions of ―molecular sieve‖ and ―electrophoresis‖ and is now widely used for nucleic acid detection and analysis. When an electric current is applied across this gel, charged molecules (such as DNA or RNA fragments) migrate through the agarose matrix at a rate determined by their size. Usually, different concentrations of agarose gel can separate 200 bp to 50 kb DNA fragments.

Instruments used: Gel Electrophoresis Apparatus: Horizontal gel apparatus consists of Gel Casting Tray, Comb, Electrophoresis Chamber, Power Supply unit, Micropipette for loading, Staining tray, GelDoc machine for imaging.

Materials required: Electrophoresis Buffer: TBE buffer, Agarose, Loading dye, Staining solution (Ethidium bromide).

Procedure

1) 1gm of agarose powder was dissolved in 100 ml 1X TBE (Tris, Boric acid, EDTA) buffer solution to prepare 1% agarose gel.

2) The glass conical flask was heated until it becomes a liquid and poured into tray with comb inserted to create wells where samples will be loaded.

3) Power supply was switched on and the DNA migration was tracked following the dye front.

Result

After running gel electrophoresis, the agarose gel was visualized in GelDoc imaging system. EtBr was used as the fluorescent staining dye.

Remark

A hand on experience on gel electrophoresis was very important in the context of DNA analysis. We have also learned the reagent preparation and imaging part from this experiment. Please find the solved assignment at the end of the project report.

DAY 13: 26/06/24

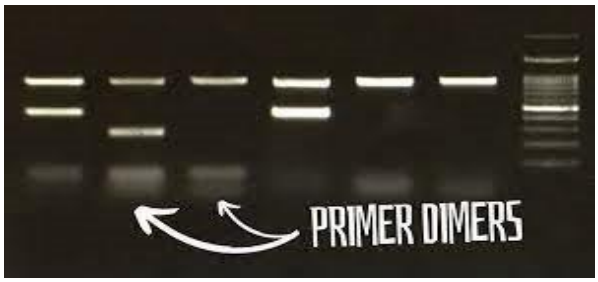
Troubleshoot, question – answer, interactions

Today was all about troubleshooting and refining our techniques in PCR and gel electrophoresis.

1. **PCR Troubleshooting:**

We learned that during PCR, common issues include primer dimer formation, improper primer binding, and unwanted amplifications. We discussed the problems with the demonstrators and they suggested us the following ---

- Primer Dimer Formation: To reduce the primer concentration for avoiding non-specific interactions.
- Improper Primer Binding: Increase the reaction temperature to improve binding specificity.
- Unwanted Amplifications: Ensure the number of PCR cycles is between 30 and 40 to avoid over-amplification. Also check the concentration of MgCl2 in reaction buffer.



2. Gel Electrophoresis Troubleshooting:

For gel electrophoresis, precision is crucial:

- Sample Loading: Be meticulous when loading samples into the gel to avoid errors.
- Choosing the right percentage and type of Gel: Decide between PAGE and agarose gels based on your sample size to ensure optimal resolution. Also keep in mind about the pore size depending on percentage of gel.

DAY 14: 27/06/24

Discussion on Project report preparation

Our course coordinator Dr. Pritha Bhattacharjee and all other demonstrators (Research Scholars) guided us on how to make project reports for our internship. We were advised to make day to day reports which included every detail of the experiments, in-silico work and hands-on experiments, which were conducted and keep it updated as it would help us in the long run. Assignments were given on a regular basis and we were asked to solve it and maintain a record of it which would be evaluated by our professors later. The research scholars also helped us in the making of our PowerPoint presentation which we were preparing for the last day of our summer internship by guiding us highlight the important points, solved every doubt that came in our mind and gave us important tips on how to display a presentation with confidence which indeed was very fruitful.

Other than that we were also introduced to other professors in the Department of Environmental Science. Head of the Department, Prof. Aniruddha Mukhopadhyay was encouraged us for our future research related activity and gave us valuable suggestions about academic as well as cultural aspects of the department and University of Calcutta.

Figure: Group photograph was taken in the Environmental Epigenomics Laboratory.

DAY 15: 28/06/24

This was the last day of our training. We presented our 15-day experience in a nut shell with the help of power point presentation in front of all the lab members and other trainees from various institutions.

Power point presentation on the last day of the course work

Photograph with our course coordinator, other lab members and trainee friends.

Conclusion

In these 15 days of training, we have learnt several basic techniques of Molecular Biology and its application in various sectors. It has been a great experience for all of us. We have absorbed a lot of knowledge that shall be validated for the rest of our lives. We learnt: DNA Isolation techniques and how to Quantify DNA by NanoDrop Spectrophotometer. Primer designing for a desirable gene before PCR with the help of Primer3 website. PCR (Polymerase Chain Reaction), the technology to amplify DNA with the help of primers and Taq polymerase. We operate the thermal cycler machine by ourselves. Also got to know about RFLP (Restriction Fragment Length Polymorphism) procedure. We learned to determine the cut site of Restriction enzymes in a particular sequence by *in silico* mechanism. We had done Gel Electrophoresis to check the DNA amplification after PCR and also the interpretation of gel images.

In conclusion, the summer internship on basic techniques of molecular biology has provided a comprehensive understanding of essential methods such as gel electrophoresis, DNA quantification, PCR, and DNA isolation. These basic techniques form the backbone of modern molecular biology research, allowing for the precise analysis, manipulation, and quantification of nucleic acids and proteins. Mastery of these methods not only enhances laboratory proficiency but also facilitates the advancement of genetic research, diagnostics, and biotechnology applications. The hands-on experience gained reinforces the theoretical knowledge and prepares for further exploration in the field.

SIGNATURE & SEAL OF COURSE COORDINATOR:

Pritha Bhattacharjee

Dr. Pritha Bhattacharjee
16.8.2024
Asst. Professor
Department of Environmental Science
University of Calcutta, Kolkata - 700019

SIGNATURE & SEAL OF THE HEAD OF THE DEPARTMENT:

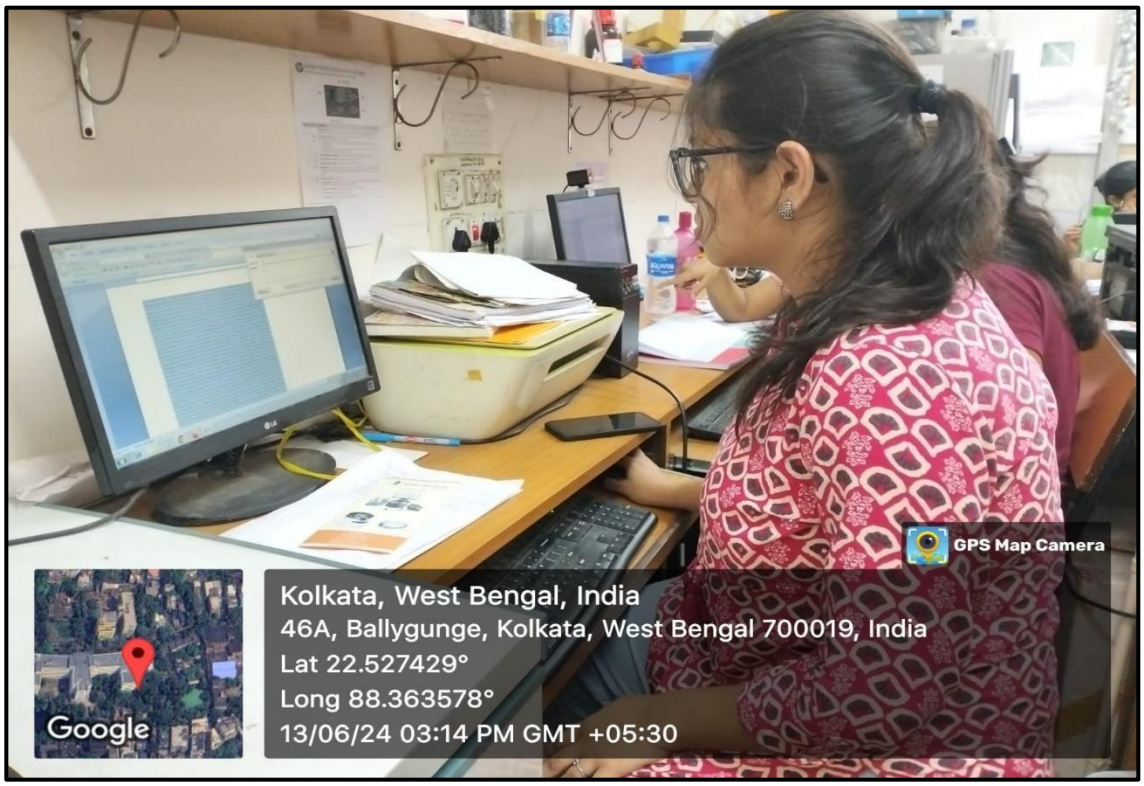
17/8/2024

Tapan Kumar Roy

PHOTOGRAPHIC DOCUMENTATION

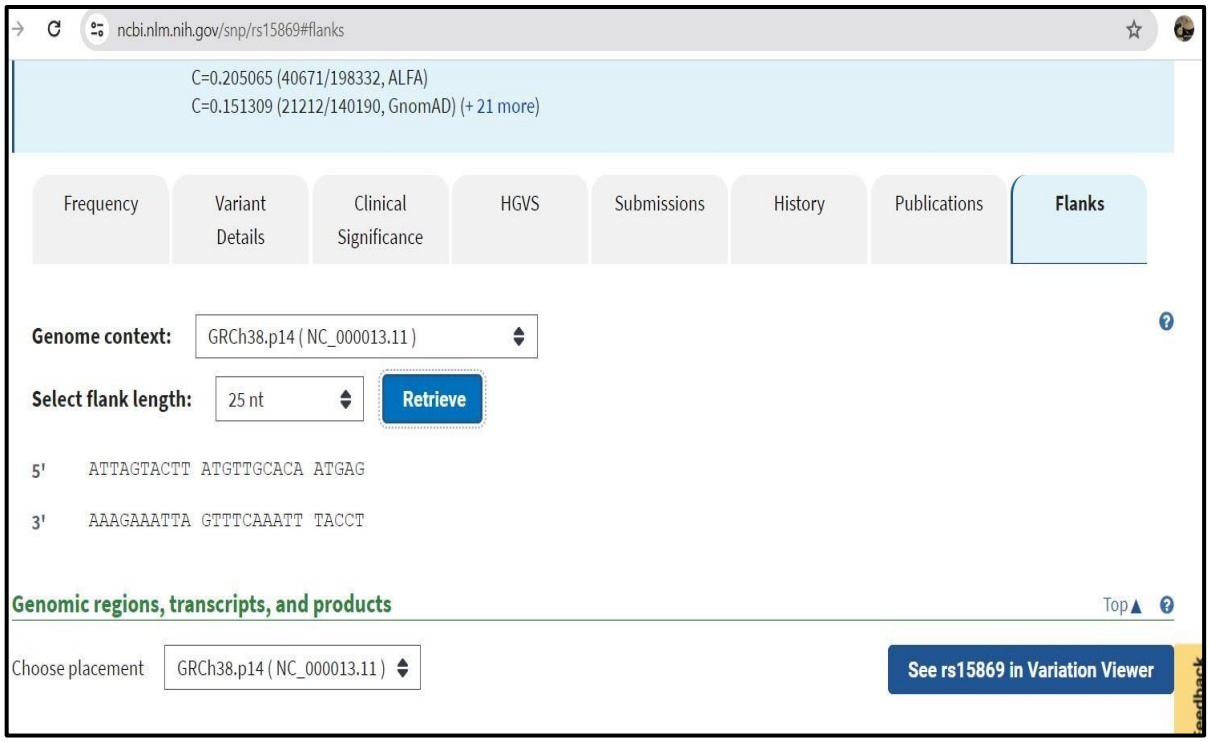
PRACTISING MICROPIPETTING

PCR MACHINE

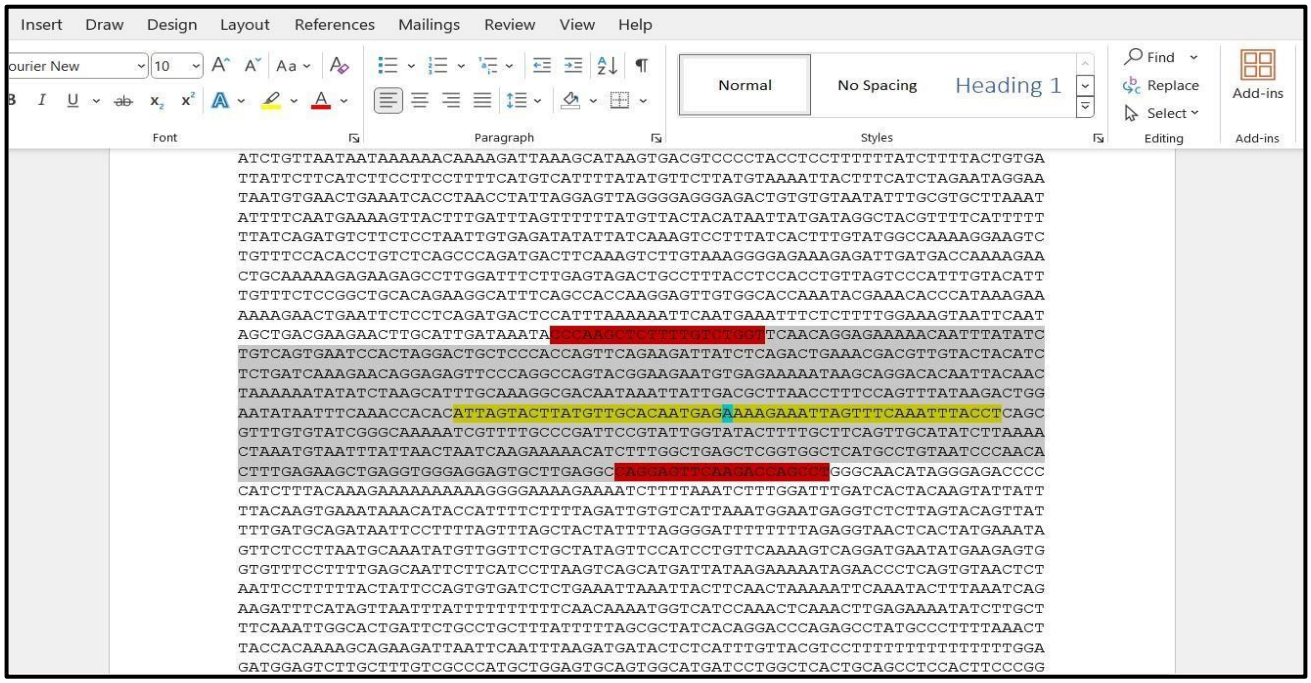


PRIMER DESIGNING (HANDS-ON)

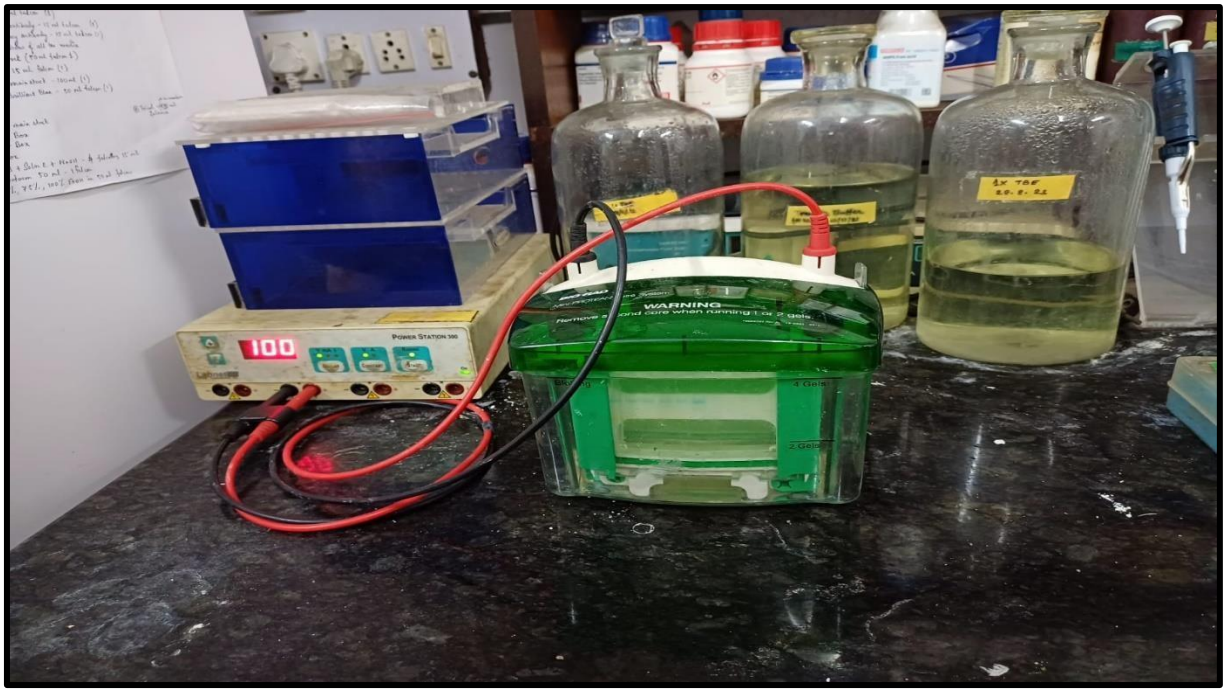
SELECTION OF THE GENE DURING PRIMER DESIGNING



RETRIEVING FLANKING LENGTH

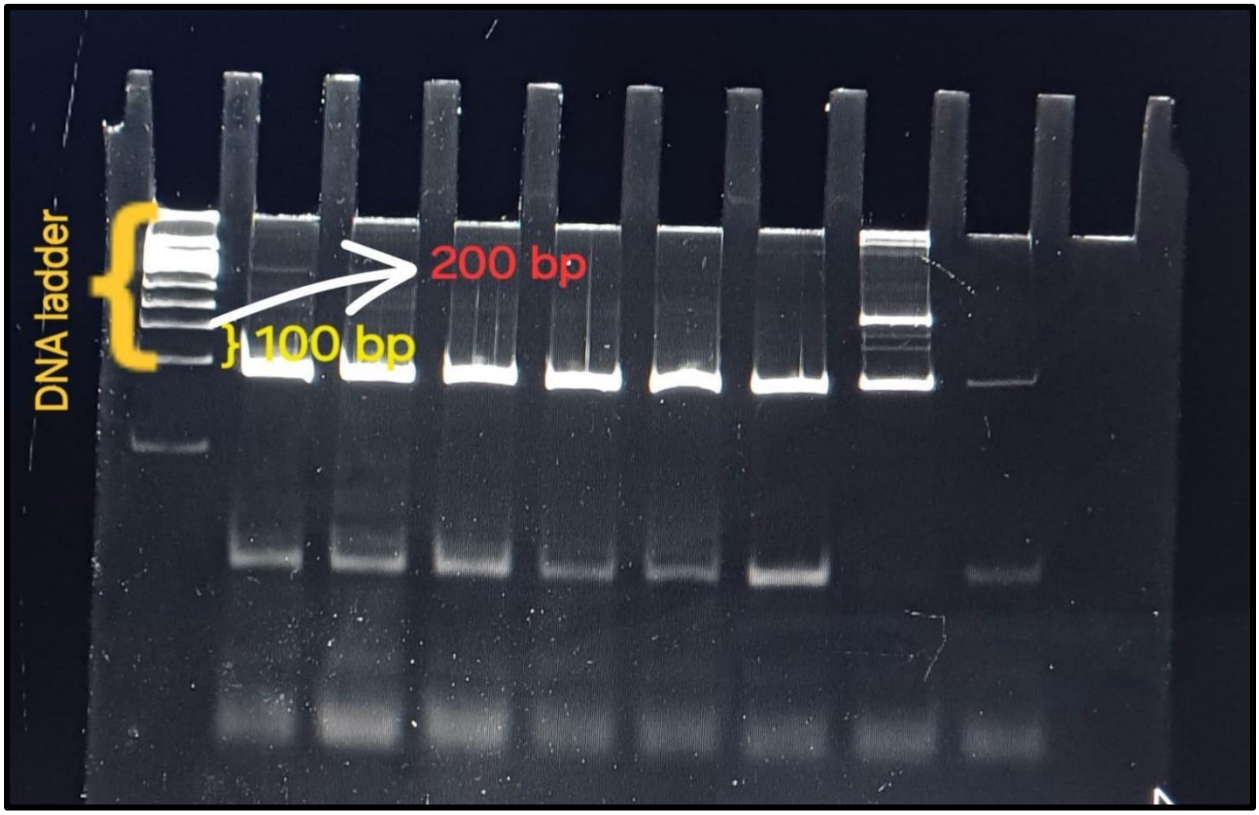


REQUIRED DNA TEMPLATE & THE SNP SITE ALONG WITH PRIMERS

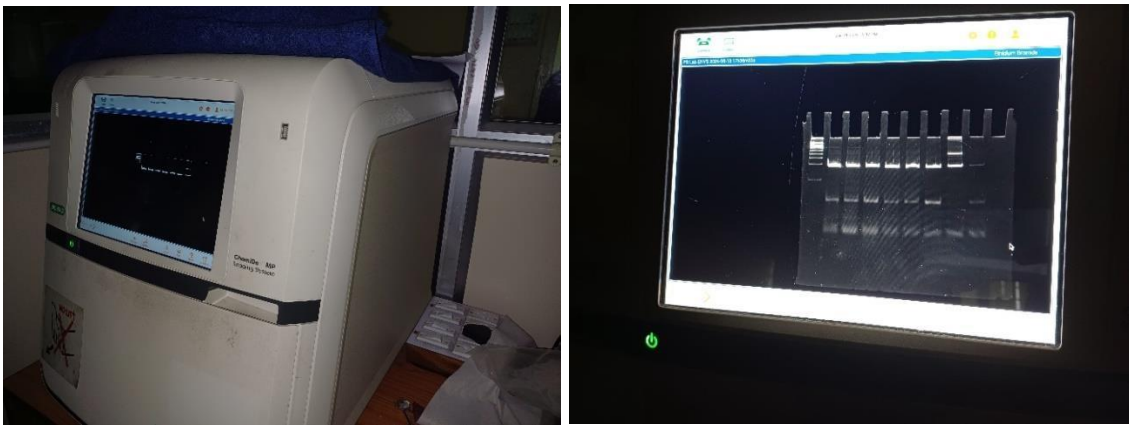


GEL ELECTROPHORESIS SYSTEM

PERFORMING GEL ELECTROPHORESIS



VISUALIZATION OF THE PCR PRODUCTS IN AGAROSE GEL



GEL DOC SYSTEM AND IMAGE OF THE DNA BANDS

Power Point Presentation on 15-day Internship Programme

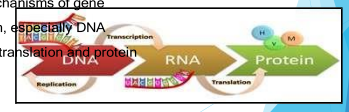
BASIC MOLECULAR BIOLOGY TECHNIQUES

NAME : MAHEK CHATTERJEE

COLLEGE: ASUTOSH COLLEGE

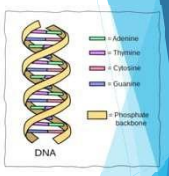
MOLECULAR BIOLOGY

Molecular biology is the study of the structure and function of molecules and macromolecular systems associated with biological processes, especially the molecular basis of inheritance and protein synthesis. It includes mechanisms of gene expression and regulation, especially DNA replication, transcription, translation and protein synthesis.



DNA

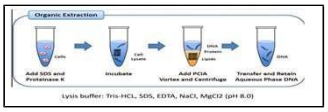
Deoxyribonucleic acid (abbreviated DNA) is the molecule that carries genetic information for the development and functioning of an organism. DNA is made of two linked strands that wind around each other to resemble a twisted ladder — a shape known as a double helix. Each strand has a backbone made of alternating sugar (deoxyribose) and phosphate groups. Attached to each sugar is one of four bases: adenine (A), cytosine (C), guanine (G) or thymine (T). The two strands are connected by Hydrogen bonds between the bases: adenine bonds with thymine by two H bonds, and cytosine bonds with guanine by three H bonds. The sequence of the bases along DNA's backbone encodes biological information, such as the instructions for making a protein or RNA molecule.



DNA ISOLATION

Using Phenol-chloroform method

- Water is a polar solvent and phenol is a non-polar solvent. On the other hand, DNA is a polar molecule with a net negative charge on its backbone and protein is non-polar. Since, the polar molecule can only dissolve in polar solutions hence DNA can dissolve in water but not in phenol.
- When we mix phenol with the cell suspension, the protein portion of the cell gets denatured and when we centrifuge it, the denatured protein settles into the bottom of the tube along with the phenol as protein has a higher density.

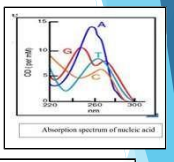


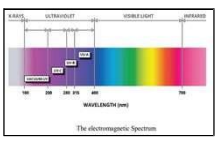
Using Buccal smear

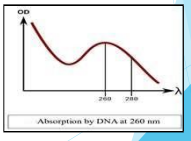
- To collect buccal cells, scrape the inside of the mouth 10 times with a Buccal Collection Brush.
- Dispense 300 pl Cell Lysis Solution into a 1.5 ml microcentrifuge tube..
- Incubate for 5 min on ice. Centrifuge for 3 min at 13,000-16,000 x g.Pipet isoprpanol into a 1.5 ml microcentrifuge tube and add the supernatant.
- Centrifuge for 5 min at 13,000-16,000 x g. Carefully discard the supernatant
- Add 300 pl of 70% ethanol. Centrifuge for 1 min at 13,000-16,000 x g.
- Carefully discard the supernatant Allow to air dry for 5 min.
- Add 100 pl DNA Hydration Solution and vortex for 5 s at medium speed to mix. Incubate at 65°C for 1 hour to dissolve the DNA. Incubate at room temperature overnight with gentle shaking. Transfer the samples to storage tube

DNA QUANTIFICATION

The spectrophotometric estimation of nucleic acid concentration is based on absorption by DNA, RNA and their constituents following a particular pattern is known as DNA Quantification. UV-vis spectroscopy is a widely adopted method for DNA quantification due to its non-destructive nature, rapid analysis, and simplicity.

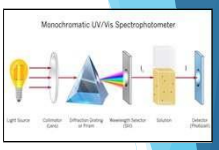






Instrumentation of UV-Vis Spectroscopy:

A UV-Vis spectrophotometer is a type of analytical tool that measures a sample's transmittance or absorbance in the



NanoDrop Spectrophotometer :

When UV light travels through a sample containing nucleic acids, the molecules absorb it at exact wavelengths determined by their chemical structure. NanoDrop can quantify the concentration of nucleic acids in a sample by measuring its absorbance at

Applications of DNA Quantification

- Polymerase Chain Reaction

PRIMER DESIGNING

1) Using any browser, search for NCBI.

2Choose a gene of your choice and click on rs number.

3) Click on the option Flank and then upon retrieving, we will get the 5' and 3' sequences.

4) Copy the whole DNA sequence from FASTA and paste it on a newly created word document

5))First, copy the 5' DNA sequence and then in the navigation menu in the word document, paste it to find it in the whole sequence. Upon finding it, highlight that portion. Similarly , follow the same steps for the 3' DNA sequence.

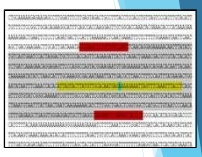
6) Upon highlighting both the sequence, we will find that the 'A' gene is in the middle of the two sequences. Highlight 'A' to make it more prominent (A is the

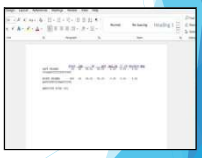
8) Then for the 3' DNA sequence, copy it and paste in Reverse complement box to reverse the sequence. Then, copying the newly formed sequence search it in the navigation menu and find. If it happens otherwise, follow the same steps to find the suitable one.

9) Always the record the length, G/C content and Melting temperature of the selected 5' and 3' DNA sequence by creating another word document.

10) The starting region of the Forward primer and the ending region of the Reverse primer is the required DNA template.

11) The starting region of the Forward primer and the ending





PCR

Principle

It is a technique for obtaining large amounts of a specific DNA sequence from a DNA sample. This amplification is based on the replication of a double-stranded DNA template. It is broken down into three phases: a denaturation phase, a hybridization phase with primers, and an elongation phase. The products of each synthesis step serve as a template for the following steps, thus exponential amplification is achieved.

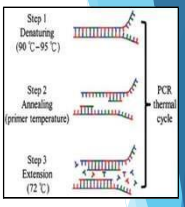
Applications of PCR

- Detection of genetic diseases

Steps of PCR

1) Denaturation : High temperature incubation is used to "melt" double-stranded DNA into single strands and loosen secondary structure in single-stranded DNA. (95 Oc)

2) ANNEALING : During annealing, complementary sequences have an opportunity to hybridize, so an appropriate temperature is used that is based on the calculated melting temperature (Tm) of the primers.



PCR Mix :

2) Primers (Forward and reverse primers)
3) Taq polymerase
4) dNTP
5)Divalent cation
6) Buffer
7) Green master mix
8)Nuclease free water

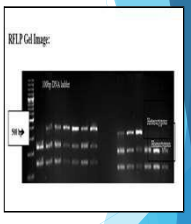
RFLP

Principle

Restriction Fragment Length Polymorphism (RFLP) is a difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples with specific restriction endonucleases. This technique was invented in 1984 by the English scientist Alec Jeffreys during research into hereditary diseases.

Applications of RFLP :

- To determine genetic diseases.
- To determine and confirm the source of DNA such as in paternity test of at crime site.



PROCEDURE

- Identify the restriction endonuclease enzyme through WebCutter V2.0. Proceed with the PCR amplification using the pre-designed forward and reverse primers as per protocol.
- Prepare a mix using the PCR product, 1unit of enzyme/µg of DNA, Restriction Buffer and Nuclease free water.
- Incubate the reaction mix at 37ºC for 1 hour followed by an enzyme inactivation step at 65ºC for 15 minutes. Identify the fragmentation pattern through PAGE/agarose gel electrophoresis.

Enzyme Finding

GEL ELECTROPHORESIS

Gel electrophoresis is a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size. In gel electrophoresis, the molecules to be separated are pushed by an electrical field through a gel that contains small pores. The molecules travel through the pores in the gel at a speed that is inversely related to their lengths. This means that a small DNA molecule will travel a greater distance through the gel than will a larger DNA molecule.

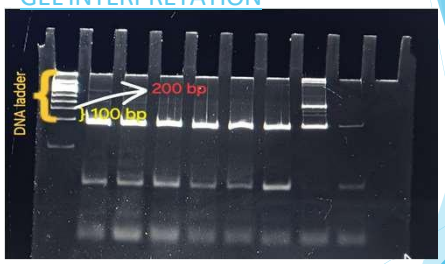
Types of Gel Electrophoresis:

- **Agarose Gel Electrophoresis**: Agarose gel electrophoresis is a method to separate DNA or RNA molecules by size. This is achieved by moving negatively charged DNA through an agarose matrix with an electric field. Shorter molecules move faster and migrate faster than the long ones. Agarose is isolated from the seaweed genera *Gelidium* and *Gracilaria*.
- **PAGE (Polyacrylamide Gel Electrophoresis):** The basic principle of PAGE is to separate biological molecules by passing them through the pores of a polyacrylamide gel using an electric current. Polyacrylamide gel (PAG) is a synthetic gel prepared from pure acrylamide and bis-acrylamide.

Applications of Agarose Gel Electrophoresis

- Genetic fingerprinting : Combined with PCR, agarose gel electrophoresis can be a powerful technique for identifying individuals based on their genetic code.
- Diagnostics : Electrophoresis can be used in a range of diagnostic tests, primarily in the screening of genetic disorders but also to identify abnormal proteins.

GEL INTERPRETATION



SUMMARY

- Molecular Biology : It is the field of biology that studies the composition, structure and interactions of cellular molecules.
- DNA : It is the genetic information inside the cells of the body that helps make people who they are.
- DNA isolation: It describes the general procedure as lysing cells to release DNA, precipitating proteins and cell membranes, and isolating DNA using alcohol precipitation.
- DNA quantification: Quantification refers to determining the quality and concentration of extracted DNA in a biological sample.
- Primer designing: It involves selecting sequences that not only hybridize perfectly with the target DNA but also resist forming dimers that can hinder the PCR reaction
- PCR: It is a very sensitive technique that allows rapid amplification of a specific segment of DNA which allows detection and identification of gene sequences using visual techniques based on size and charge.
- RFLP: It is useful in finding where a specific gene for a disease lies on a chromosome.
- Gel Electrophoresis: It I used to separate mixtures of biomacromolecules such as DNA, RNA and protein. This technique separates by molecular size and / or charge .

THANK YOU !!

I want to thank Asutosh College wholeheartedly for giving me this golden opportunity to work on this Summer Internship in a prestigious college like Ballygunge Science College. This internship turned out to be quite fun and interesting because of Dr. Pritha Bhattacharjee , research scholars and our professors Dr. Tamalika Sanyal, Dr. Tapan Kumar Ray, Dr. Sriparna Dutta Ray, Smt. Lopamudra Mukherjee, Dr.

A.R. Md. Mustafizur Rahaman, Dr. Deep Chandan Chakraborty, Dr. Subhabrata Ghosh and Dr. Satabdi Nandi who guided us in this journey. It was really a fruitful experience for me.

Solved Assignments Given During Our Course Work

SUMMER INTERNSHIP ASSIGNMENTS

ASSIGNMENT 1 (Date: 10/06/2024)

I) What is molecular biology?

Ans: Molecular biology is a branch of biology that deals in the study of biological processes and activities at the molecular level, specially the interactions between biomolecules like DNA, RNA, and proteins. It helps in understanding and visualizing the structure of these molecules and how various biological processes occurs inside our cells.

- Molecular biology focuses into the mechanisms of replication, transcription, and translation, which are the basic processes governing the flow of genetic information within cells.
- It also explores how alterations in molecular processes can lead to mutations and how these insights can be of utmost importance for medical advancements, like the development of targeted therapies and various diagnostic tools.
- Techniques such as PCR (polymerase chain reaction), DNA sequencing, and gene editing technologies are core part of molecular biology research, which enables scientists to analyse DNA and other biomolecules.

This field has a wide spectrum of applications across medicine, biotechnology, agriculture, and environmental science.

II) State the applications of molecular biology in present day research.

Ans: Some of the applications of molecular biology in present day research are as follows :

- Medicine: Molecular biology techniques are used for diagnosing genetic disorders, cancer etc. They play a crucial role in discovery of various drugs, personalized medicine, and gene therapy.
- Biotechnology: Molecular biology explores a ton of biotechnological advances such as the production of recombinant proteins, genetically modified organisms (GMOs), and synthetic biology applications.
- Forensics: Molecular biology helps in the production of various DNA analysis

techniques which are essential in forensic investigations for identifying individuals, determining paternity, and solving criminal case.

- Evolutionary biology: Molecular techniques like DNA sequencing and phylogenetics help in understanding evolutionary relationships among species, tracing histories of evolution, and studying genetic diversity.

III) What is micropipette? Describe its importance in laboratory work.

Ans: A micropipette is an instrument for precision which is used in laboratories to accurately measure and transfer small volumes of liquid. It mainly consists of a piston-driven system with disposable tips that come in various volume ranges, from microliters to millilitres.

Importance of a micropipette in laboratory work:

- Precision: Micropipettes provide precise measurement of liquid volumes, often down to the microliter (μL) range. This accuracy is important for ensuring reliability in experiments, especially in fields like molecular biology, biochemistry, and microbiology.
- Minimization of contamination: Micropipettes with disposable tips help minimize the risk of cross-contamination between samples. While conducting an experiment, the tips should not come in contact with any substance otherwise there will be a probability of the micropipette getting contaminated.
- Versatility: Micropipettes are versatile tools that can be used in a wide range of applications, which includes DNA sequencing, PCR (polymerase chain reaction), ELISA (enzyme-linked immunosorbent assay), cell culture, and protein assays.

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ASSIGNMENT – 2 (Date: 12/06/2024)

Q1) What is PCR? Tabulate the reagents used for a PCR reaction with their function.

Ans: The Polymerase chain reaction is a scientific technique in molecular biology to amplify a single or a few copies of a piece oof DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

The reagents used in PCR with their functions are:

i) Template DNA: Template DNA containing genomic DNA or cDNA sample from the patient can be used in single or double stranded form.

ii) Buffer: To maintain the pH of the solution.

iii) DNA polymerase: A thermostable DNA polymerase which can withstand the denaturation temperature (94 – 95 oC), is essential to catalase the template dependent synthesis of DNA.

iv) Primers: During annealing step, PCR primers hybridize to a specific location on the single stranded DNA template via complementary base pairing.

v) Divalent cations: Free divalent cations are needed for the activity of thermostable polymerases.

vi) Deoxy nucleoside triphosphatases (dNTP): Using dNTP during extension phase provides single bases ready to go into DNA and double it, like building blocks.

Q2) State the applications of PCR.

Ans: Some of the applications of PCR are:

i) Detecting pathogens using genome specific primer pairs in clinical samples.

ii) Identification of genetic mutations like deletions, insertions and point mutations.

iii) Sub cloning DNA targets using PCR.

iv) Generation of libraries from small amounts of mRNA and hence establish cDNA libraries.

v) In forensic science, using restriction fragment length polymorphism (RFLP) analysis, DNA specific to particular individuals can be obtained.

Q3) What are the important features of the PCR primers?

Ans: The important features of the PCR primers are:

i) It should be specific to the DNA region that is to be amplified.

ii) The melting temperature for both the primers should be in a similar range.

iii) Melting temperature should be between 50-60 ˚C.

iv) G/C content should be between 40-60%.

Q4) What type of DNA polymerase is used in PCR?

Ans: Taq DNA polymerase is the most commonly used enzyme in standard end-point PCR. It is isolated from the eubacterium Thermus aquaticus.

Q5) Who invented PCR? Give a short biography of the inventor.

Ans: Polymerase chain reaction was developed in 1984 by the American biochemist, Kary Mullis. Mullis received the Nobel prize and the Japan prize for developing PCR in 1993.

Kary Banks Mullis, Nobel Prize winning chemist, was born on December 28, 1944, in Lenoir, North Carolina. After receiving a doctorate in biochemistry from the University of California, Berkeley, in 1973, Mullis held research posts at various universities. In 1979 he joined Cetus Corp., a California biotechnology firm, where he carried out his prizewinning research. From 1986 to 1988 he was director of molecular biology for Xytronyx, Inc., in San Diego, California; thereafter he worked as a freelance consultant. During his seven years in Cetus Corp., he conducted research on oligonucleotide synthesis and invented the polymerase chain reaction. Dr. Mullis received a Nobel Prize in chemistry in 1993, for his invention of the polymerase chain reaction (PCR). Dr. Mullis was awarded the Japan Prize in 1993 for the PCR invention. It is one of international science‟s most prestigious awards. His many publications include “The Cosmological Significance of Time Reversal” (Nature), “The Unusual Origin of the Polymerase Chain Reaction” (Scientific American), “Primer-directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase” (Science), and “Specific Synthesis of DNA In Vitro via a Polymerase Catalysed Chain Reaction” (Methods in Enzymology).Dr. Mullis has written an autobiographical book titled Dancing Naked in the Mind Field published by Pantheon Books in 1998. Dr. Mullis serves on the board of scientific advisors of several companies, provides expert advice in legal matters involving DNA, and is a frequent lecturer at college campuses, corporations and academic meetings around the world.

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4) https://www.karymullis.com/biography.shtml

ASSIGNMENT -3 (Date :14/06/2024)

Q1) What is the importance of bioinformatics in present day molecular biology research?

Ans: Importance of bioinformatics in present day molecular biology research are:

i) DNA microarrays: It is used to measure the levels of gene expression in cells, in various diseases to detect SNPs.

ii) Comparative genomics: The complete genomes of different organisms can be compared and studied easily

iii) Structural genomics: This involves the prediction of the 3D structure and functions of the proteins.

iv) Medical informatics: This involves the management of biomedical and medical data with respect to biomolecules and assays.

Q2) What is NCBI database?

Ans: The National center for Biotechnology Information (NCBI), a center within the National Library of Medicine at the National Institutes of Health, was created in 1988 to develop information systems for molecular biology. NCBI provides facilities for submitting and downloading data, analysis and visualization software, educational events and materials about NCBI products, and software and services to support an expanding developer community.

Q3) What is SNP? State the basic difference between SNP and mutation.

Ans: Single Nucleotide Polymorphism (SNP) is a variation in a genetic sequence that affects only one of the basic building blocks – Adenine (A), Guanine(G), Thymine (T), or Cytosine (C) – in a segment of a DNA molecule and that occurs in more than 1% of a population.

SNP:

i) SNP represents a change in a single nucleotide of a genome and is a type of mutation.

ii) The SNP variation is present in at least 1% of the population.

iii) For instance, in the sequence ATAGC, if G is replaced by C, the sequence becomes ATACC. This alteration in a single nucleotide is referred to as SNP.

Mutation:

i) A mutation is a change in DNA base pairs due to insertion, deletion, duplication or substitution of base pairs.

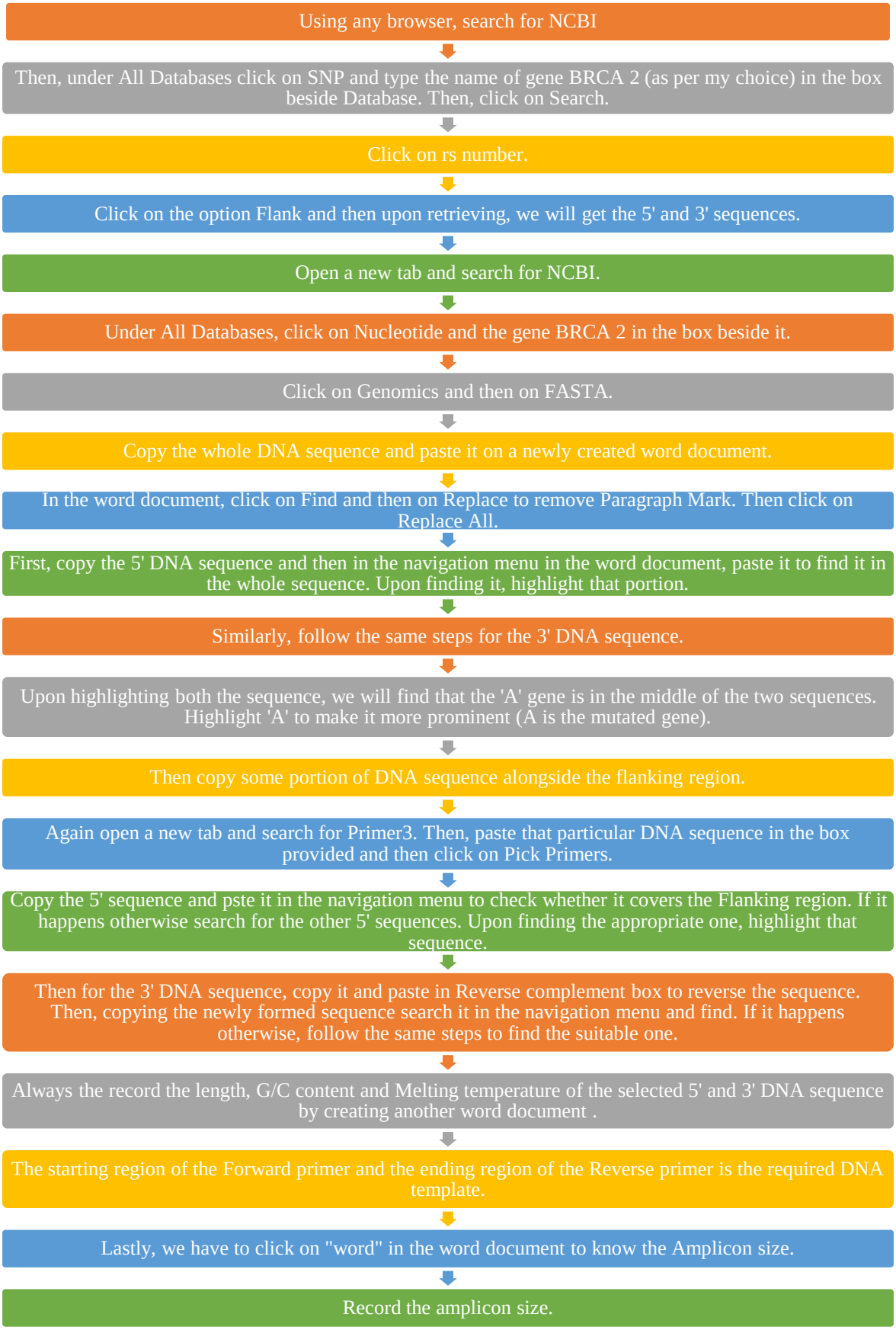
ii) The frequency of the mutation is found in less than 1% of the population.

iii) There are various types of mutations, including missense mutation, silent mutation, and nonsense mutation.

Q4) Name one primer designing software. Elaborate the process of primer designing for identification of a particular SNP associated with any gene of your choice.

Ans: Primer3 is a primer designing software.

The steps to design a primer are



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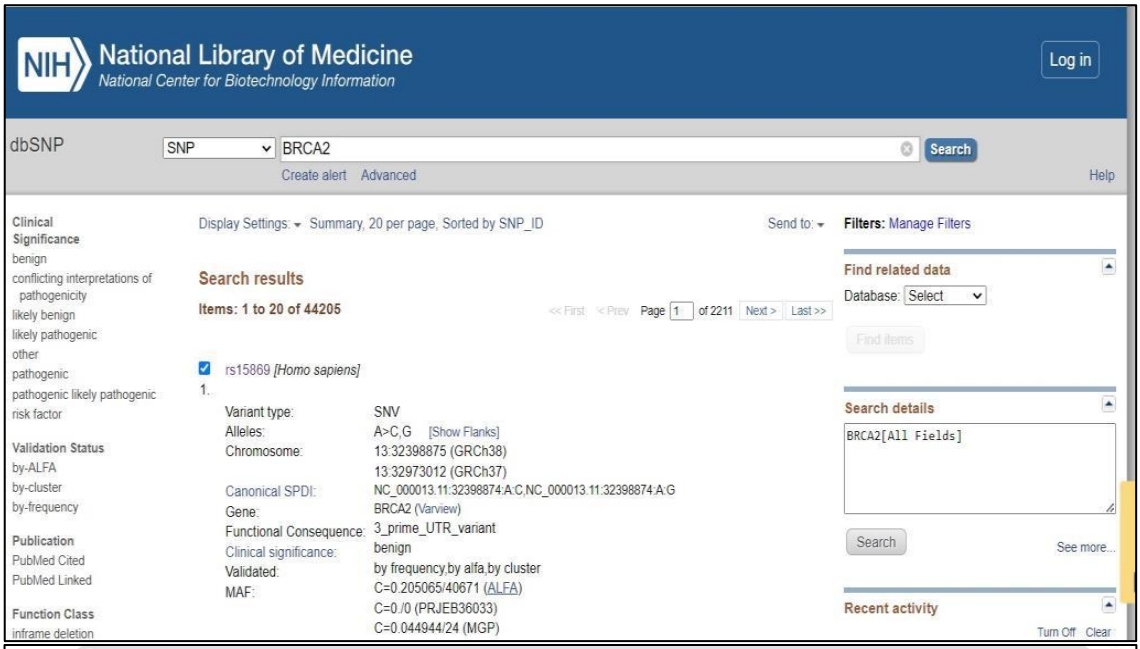
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National Center for Biotechnology Information
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SNP
BRCA2
Search
Create alert
Advanced
Help
Clinical Significance
benign
conflicting interpretations of pathogenicity
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likely pathogenic
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pathogenic likely pathogenic
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<< First < Prev Page 1 of 2211 Next > Last >>
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Alleles: A>C,G [Show Flanks]
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Validated: by frequency,by alfa,by cluster
MAF: C=0.205065/40671 (ALFA)
C=0./0 (PRJEB36033)
C=0.044944/24 (MGP)
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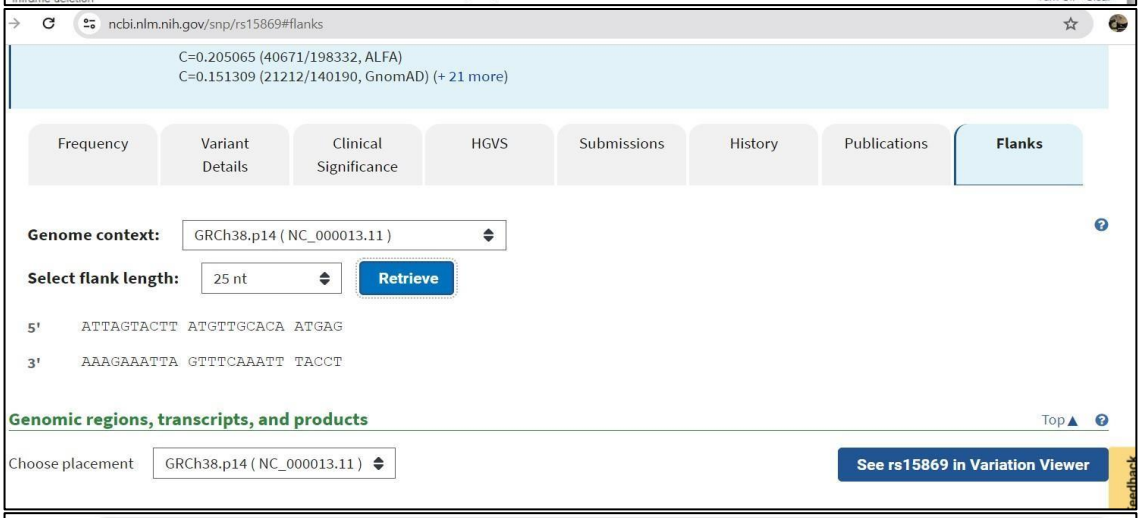
2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Frequency
Variant Details
Clinical Significance
HGVS
Submissions
History
Publications
Flanks
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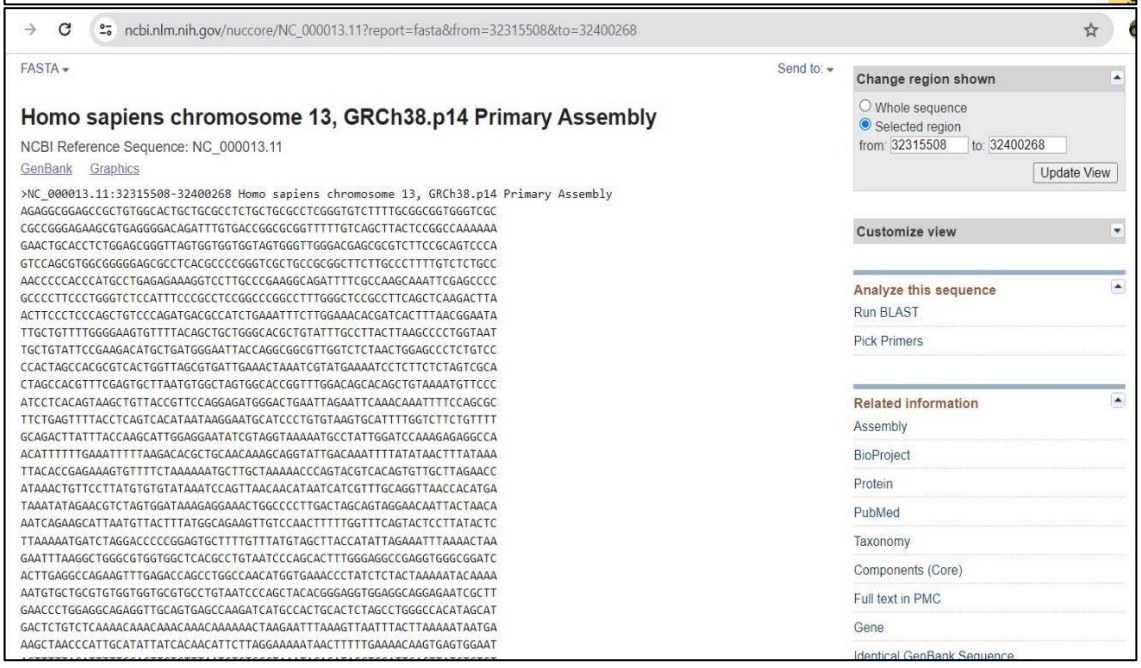
3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Change region shown
Whole sequence
Selected region
from: 32315508 to: 32400268
Update View
Customize view
Analyze this sequence
Run BLAST
Pick Primers
Related information
Assembly
BioProject
Protein
PubMed
Taxonomy
Components (Core)
Full text in PMC
Gene
Identical GenBank Sequence

4

Primer3web version 4.1.0 - Pick primers from a DNA sequence.

disclaimer | code | cautions

Select the Task for primer selection: generic

Template masking before primer design (available species)

Select species: Example: Mus musculus

Primer failure rate cutoff: < 0.1

Nucleotides to mask in 5' direction: 1

Nucleotides to mask in 3' direction: 0

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library): NONE

```
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AAGTATTATT
```

Pick left primer, or use left primer below	Pick hybridization probe (internal oligo), or use oligo below	Pick right primer, or use right primer below (5' to 3' on opposite strand)

Pick Primers | Download Settings | Reset Form

Sequence Id: A string to identify your output.

Targets: E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [and]: e.g. ...ATCT[CCCC]TCAT.. means that primers must flank the central CCCC.

Overlap Junction List: E.g. 27 requires one primer to overlap the junction between positions 27 and 28. Or mark the source sequence with -: e.g. ...ATCTAC-TGTCAT.. means that primers must overlap the junction between the C and T.

5

Primer3 Output

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PRIMER PICKING RESULTS FOR

Template masking not selected
No mispriming library specified
Using 1-based sequence positions
OLIGO            start  len      tm      gc%  any_th  3'_th hairpin seq
LEFT PRIMER        370   20   59.00   55.00    0.00   0.00    0.00 CTCAGCGTTTGTGTATCGGG
RIGHT PRIMER       547   20   59.02   55.00    0.00   0.00    0.00 CTCCCACCTCAGCTTCTCAA
SEQUENCE SIZE: 675
INCLUDED REGION SIZE: 675

PRODUCT SIZE: 178, PAIR ANY_TH COMPL: 0.00, PAIR 3'_TH COMPL: 0.00

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   61 AAAACAATTTATATCTGTCAGTGAATCCACTAGGACTGCTCCCACCAGTTCAGAAGATTA

  121 TCTCAGACTGAAACGACGTTGTACTACATCTCTGATCAAAGAACAGGAGAGTTCCCAGGC

  181 CAGTACGGAAGAATGTGAGAAAAATAAGCAGGACACAATTACAACTAAAAAATATATCTA

  241 AGCATTTGCAAAGGCGACAATAAATTATTGACGCTTAACCTTTCCAGTTTATAAGACTGG

  301 AATATAATTTCAAACCACACATTAGTACTTATGTTGCACAATGAGAAAAGAAATTAGTTT

  361 CAAATTTACCTCAGCGTTTGTGTATCGGGCAAAAATCGTTTTGCCCGATTCCGTATTGGT
```

6

Reverse Complement

Reverse Complement converts a DNA sequence into its reverse, complement, or reverse-complement counterpart. You may want to work with the reverse-complement of a sequence if it contains an ORF on the reverse strand.

Paste the raw or FASTA sequence into the text area below.

```
AGGCTGGTCTTGAACTCCTG
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SUBMIT | CLEAR

- Convert the DNA sequence into its reverse-complement counterpart.

[home]

7

about:blank

The Sequence Manipulation Suite: Reverse Complement

Results for 20 residue sequence starting "AGGCTGGTCT".

CAGGAGTTCAAGACCAGCCT

8

Insert Draw Design Layout References Mailings Review View Help

Font Paragraph Styles Editing Add-ins

Normal No Spacing Heading 1

Find Replace Select

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Design Layout References Mailings Review View Help

Font Paragraph Styles Editing

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Find Repla Select

	start	len	tm	gc%	any_th	3' th	hairpin	seq
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RIGHT PRIMER	580	20	59.31	55.00	0.00	0.00	0.00	AGGCTGGTCTTGAACTCCTG

AMPLICON SIZE: 551

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2) Schuler., Epstein., Okawa. and Kans,J.A. (1996) Entrez: molecular biology database and retrieval system. Methods Enzymol.,266, 141–162.
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4) https://testbook.com/key-differences/difference-between-snp-and-mutation

ASSIGNMENT 4 (Date :17.06.2024)

Q1) What is the principle of electrophoresis?

Ans: The fundamental principle of electrophoresis is the existence of charge separation between the surface of a particle and the fluid immediately surrounding it. An applied electric field acts on the resulting charge density, causing the particle to migrate and the fluid around the particle to flow.

Q2) What is agarose gel electrophoresis? Mention its application.

Ans: Agarose gel electrophoresis separates DNA fragments based on size, ranging from 100 bp to 25 kb. Agarose, from seaweed like Gelidium and Gracilaria, forms a network of pores crucial for molecular sieving. DNA, negatively charged due to its phosphate backbone, migrates towards the anode under an electric field. Factors like DNA size, agarose concentration, voltage, and dye presence affect migration rate. Post- separation, DNA bands are visualized with ethidium bromide under UV light, aiding size determination. This method revolutionized DNA analysis, offering precise separation over previous techniques like sucrose density gradient centrifugation.

Applications of Agarose Gel Electrophoresis:

- Estimation of the size of DNA molecules
- Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting
- Separation of restricted genomic DNA prior to Southern analysis, or of RNA prior to Northern analysis.
- The agarose gel electrophoresis is widely employed to estimate the size of DNA fragments after digesting with restriction enzymes, e.g. in restriction mapping of cloned DNA.

- Agarose gel electrophoresis is commonly used to resolve circular DNA with different supercoiling topology, and to resolve fragments that differ due to DNA synthesis.
- In addition to providing an excellent medium for fragment size analyses, agarose gels allow purification of DNA fragments. Since purification of DNA fragments size separated in an agarose gel is necessary for a number molecular techniques such as cloning, it is vital to be able to purify fragments of interest from the gel.

Q3) State the composition and function of the following reagents-

a. 1%Agarose solution

Ans: Composition: 1gm agarose in 100ml of buffer solution

Function: 1% agarose means small amount of agarose mixed with buffer so that the pores in gel will be large sizes for running large sized DNA sample through it.

b. TBE Buffer

Ans: Composition: we basically used 10X TBE buffer which contains Tris base, Boric acid, EDTA

Function: TBE (Tris-Borate-EDTA) buffer is a crucial component in gel electrophoresis for DNA and RNA separation.

1. Buffering pH: TBE buffer maintains a stable pH, typically around 8.3, which is optimal for the stability and migration of nucleic acids (DNA and RNA) during electrophoresis.
2. Ion Conductivity: The ions in TBE buffer (Tris, boric acid, and EDTA) facilitate the conduction of electrical current through the gel.
3. Prevention of Enzymatic Activity: EDTA (Ethylenediaminetetraacetic acid) chelates divalent cations such as Mg^{2+}, which are cofactors for many nucleases.
4. Maintaining Low Conductivity: TBE buffer is designed to have low electrical conductivity, which minimizes heat generation during electrophoresis and helps maintain the stability of the gel and the integrity of separated DNA or RNA fragments.

c. Loading dye

Ans: Composition:

- Bromophenol Blue
- Xylene Cyanol FF
- Other Components: Besides dyes, 6X DNA Loading Dye typically contains glycerol or another viscous agent and EDTA.

Function:

1. 6X DNA Loading Dye contains bromophenol blue and xylene cyanol FF dyes that facilitate the visualization and tracking of DNA migration during gel electrophoresis.
2. This ensures precise loading of samples and enables researchers to monitor DNA movement within the gel, which is essential for accurately analysing DNA fragment sizes and patterns.
3. Glycerol ensures that DNA samples form a stable layer at the bottom of the wells
4. EDTA prevents enzymatic degradation by chelating divalent metal ions necessary for nuclease activity.

d. EtBr solution

Ans: Function: Ethidium Bromide (EtBr) is added to running buffer during the separation of DNA fragments by agarose gel electrophoresis. It is used because upon binding of the molecule to the DNA and illumination with a UV light source, the DNA banding pattern can be visualized.

e. DNA ladder

Ans: Composition:

- DNA Fragments: The ladder contains multiple DNA fragments of varying lengths, typically ranging from 50 base pairs (bp) to several thousand base pairs.
- Loading Dye: Often, a loading dye is added to the DNA ladder to help visualize the DNA fragments during electrophoresis and to ensure they migrate properly through the gel
- Buffer Solution: The DNA fragments are suspended in a buffer solution that provides stability and optimal conditions for their preservation and migration through the gel matrix during electrophoresis.
- Preservatives and Stabilizers: To ensure long-term stability and usability, DNA ladders may contain preservatives or stabilizers.
- Purification Agents: DNA ladders are typically highly purified to remove any contaminants or unwanted DNA fragments that could interfere with accurate size determination of unknown samples.

Functions:

1. **Size Estimation**: The primary function of a DNA ladder is to provide a reference for estimating the size of DNA fragments in a sample. By comparing the migration of sample DNA fragments to the known sizes in the ladder, scientists can determine the approximate length of the sample fragments.

2. **Quality Control**: DNA ladders help verify the success of DNA extraction, PCR amplification, or restriction enzyme digestion. If the expected bands appear in the ladder, it indicates that the experimental procedures were performed correctly.

3. **Quantification**: Some DNA ladders are designed to provide not only size markers but also approximate quantities of DNA. This can help in estimating the concentration of DNA in a sample.

Q4) Describe different parts of an agarose gel electrophoresis unit with proper diagram.

Ans: **Electrophoresis Chamber and Power Supply**: This is the main apparatus where the gel is placed and the electric current is applied. The power supply provides the necessary voltage to drive the electrophoresis process.

Gel Casting Tray: This tray is used to cast the agarose gel. It is usually made of UV-transparent plastic to allow for visualization of the gel under UV light.

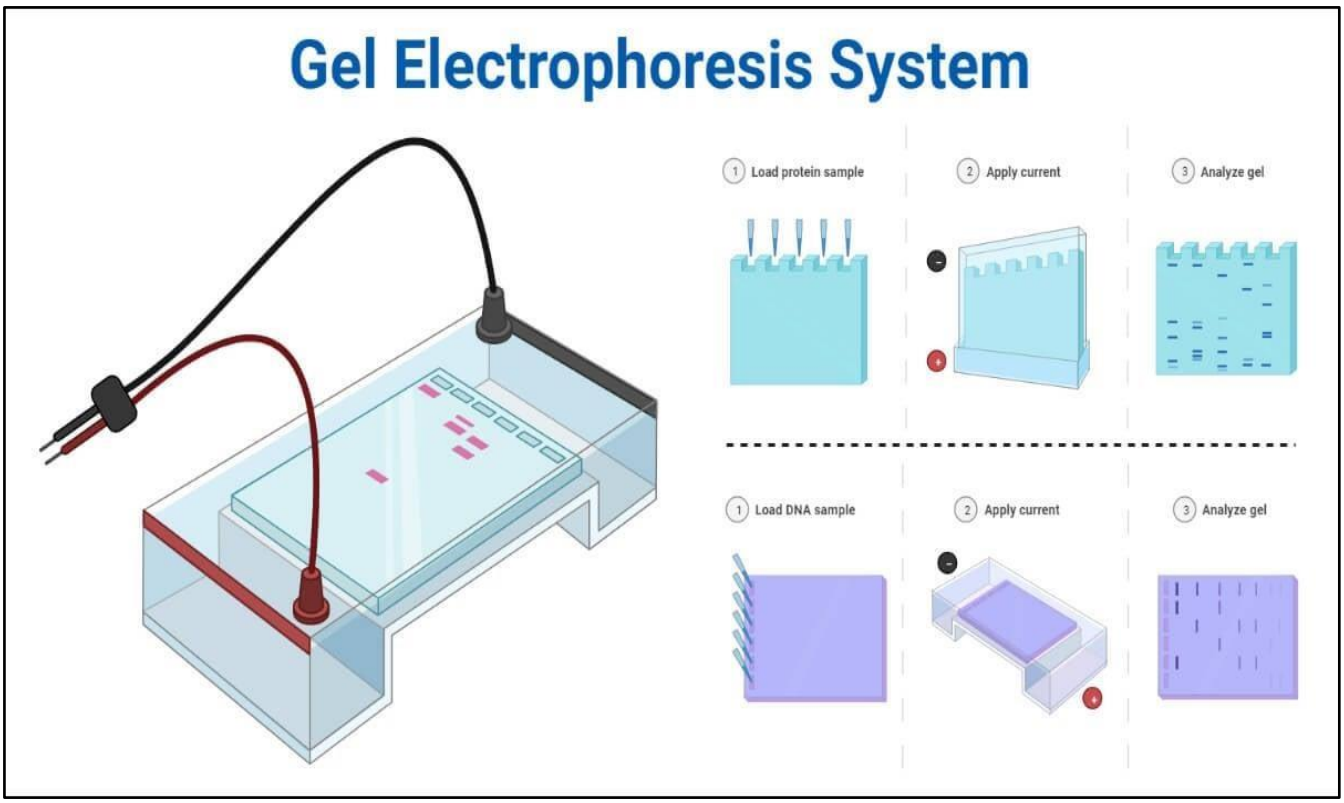
Sample Combs: These are placed in the gel casting tray before the gel solidifies to create wells where the samples will be loaded.

Agarose Gel: The gel itself is made from agarose, a polysaccharide extracted from seaweed. It forms a matrix with pores through which the DNA fragments migrate.

Buffer Solution: The gel is submerged in a buffer solution (commonly TAE or TBE) that conducts the electric current and maintains a stable pH during electrophoresis.

Loading Buffer: This is added to the DNA samples before loading them into the wells. It contains a dense substance like glycerol to help the samples sink into the wells and tracking dyes to monitor the progress of the electrophoresis.

Staining Dye: After electrophoresis, the gel is stained with a dye such as ethidium bromide or SYBR Green, which binds to DNA and fluoresces under UV light, allowing visualization of the DNA bands.



Q5) Elaborate the steps of agarose gel electrophoresis with the help of a flow chart.

Ans:

Prepare the Agarose Gel Solution

- Dissolve agarose powder in electrophoresis buffer (e.g., TAE & TBE Buffer)
- Heat the solution until the agarose is completely dissolved.
- Allow the solution to cool to about 50°C.

Cast the Gel

- Pour the cooled agarose solution into a gel casting tray with a comb in place to create wells.
- Allow the gel to solidify at room temperature.

Set Up the Electrophoresis Chamber

- Place the solidified gel in the electrophoresis chamber.
- Fill the chamber with electrophoresis buffer until the gel is submerged.

Prepare DNA Samples

- Mix DNA samples with loading dye.
- Load the DNA samples and a DNA ladder (size marker) into the wells of the gel.

Run the Gel

- Connect the electrophoresis chamber to a power supply.
- Apply an electric current to the gel. DNA fragments will migrate towards the positive electrode.

Stain the Gel

- After electrophoresis, stain the gel with a DNA-binding dye (e.g., ethidium bromide or SYBR Safe).
- Visualize the DNA bands under UV light or a blue light transilluminator.

Analyse the Results

- Compare the DNA bands to the DNA ladder to determine the size of the fragments.

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4. https://www.thermofisher.com/order/catalog/product/R0611

ASSIGNMENT 5 (Date :19/06/2024)

Q1) What is restriction endonuclease (RE)? Give example.

Ans: A restriction enzyme, also known as a restriction endonuclease, is an enzyme that cleaves DNA at specific recognition nucleotide sequences called restriction sites. This enzymatic action involves making two incisions, each through one of the sugar-phosphate backbones of the DNA double helix.

In bacterial cells, restriction enzymes play a crucial role by cleaving foreign DNA, thereby protecting the cell from invading organisms. These enzymes can be extracted from bacterial cells and are invaluable tools in genetic engineering and recombinant DNA technology. They are used in the laboratory to precisely manipulate DNA fragments, including those containing genes, facilitating the creation of genetically modified organisms and the study of gene function.

Eg: EcoRI

Q2) What is the source of RE?

Ans: Restriction endonucleases, also known as restriction enzymes, are naturally found in bacterial cells. These enzymes serve as a defence mechanism for bacteria, protecting them from viral infections by cutting the viral DNA into fragments. This process is known as restriction digestion.

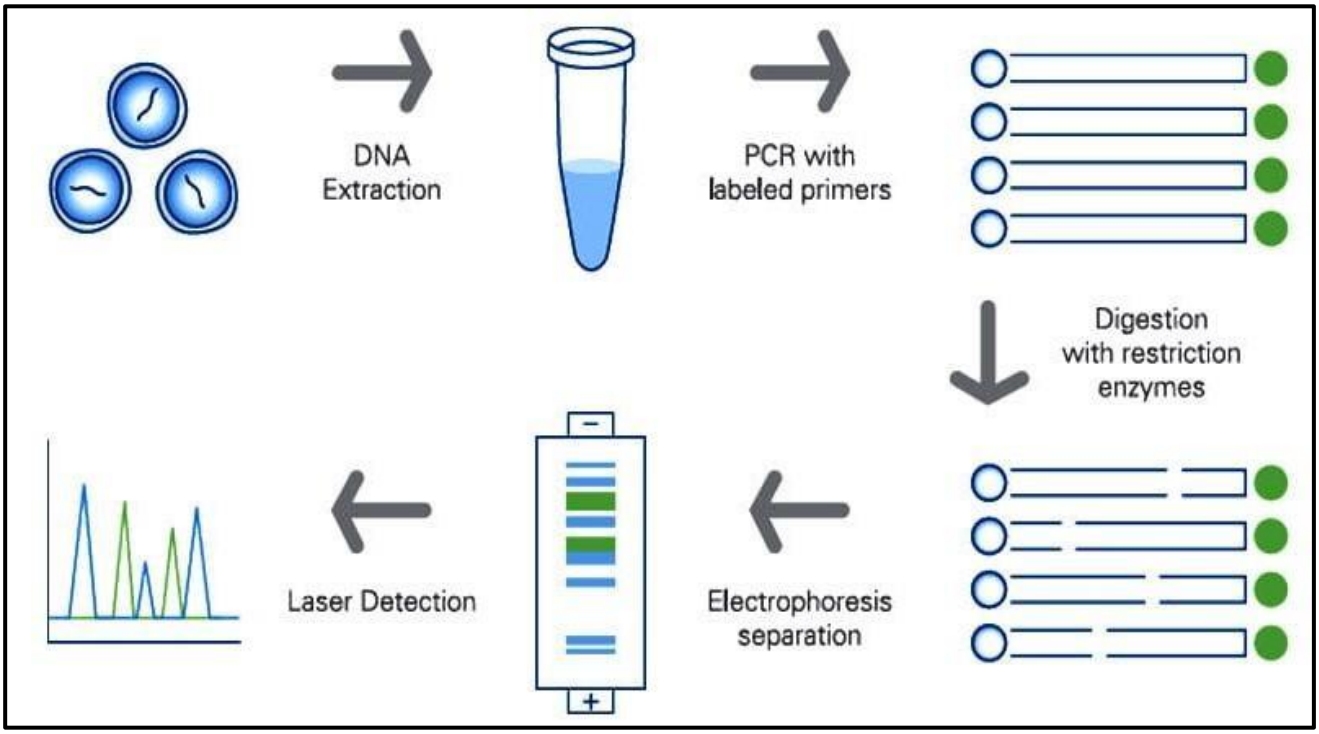
Q3) Mention the application of RE in molecular biology.

Ans: Applications of RE in molecular biology are:

1. **Gene Cloning**: REs are used to cut DNA at specific sequences, allowing scientists to insert genes into plasmids or other vectors for cloning.
2. **DNA Mapping**: By cutting DNA with different REs, researchers can create restriction maps that show the locations of specific sequences within a DNA molecule.
3. **Genetic Engineering**: REs enable the precise cutting and pasting of DNA fragments, which is essential for creating genetically modified organisms (GMOs).
4. **Molecular Diagnostics**: REs are used in techniques like Restriction Fragment Length Polymorphism (RFLP) analysis to detect genetic variations and mutations.

Q4) What is RFLP? Explain with diagram.

Ans: Restriction Fragment Length Polymorphism (RFLP) is a molecular technique that detects variations in DNA sequences by fragmenting DNA with restriction enzymes. Fragments of different lengths are separated using agarose gel electrophoresis and transferred to a membrane for analysis via Southern blotting with a labelled DNA probe. RFLP markers are co-dominant and locus-specific, used historically for genome mapping, genetic variation studies, and applications in genotyping, forensics, and disease diagnostics. While largely replaced by DNA sequencing technologies, RFLP remains foundational in molecular biology and genetics.



Q5) Discuss the experimental methodology involving PCR-RFLP for the identification of three different individuals – normal healthy, with sickle cell anaemia (affected), carrier of sickle cell anaemia. (Mention the genetic change in terms of nucleotide, then in genetic code, then the RE site, RFLP, DNA banding pattern).

Ans: SICKEL CELL ANAEMIA:

Sickle cell disease is a group of inherited red blood cell disorders that impact haemoglobin, the protein responsible for transporting oxygen throughout the body. Normally, red blood cells are disc-shaped and flexible, allowing them to move easily through blood vessels. However, in sickle cell disease, a genetic mutation causes red blood cells to assume a crescent or "sickle" shape. These abnormal cells are rigid and can obstruct blood flow, leading to complications by impeding oxygen delivery to tissues and organs.

CAUSE OF SICKEL CELL ANAEMIA

Sickel cell anaemia occur when point mutation occur in the DNA sequence. basically „A" replaced by „T" (GAG → GTG) in a particular part of DNA sequence and for that valine will produce instead of glutamic acid.

Genetic Change

1. **Nucleotide Level:**

    - Normal Healthy (βAβA): The normal beta-globin gene has the sequence GAG at codon 6.
    - Sickle Cell Anaemia (βSβS): The mutation involves a single nucleotide change from adenine (A) to thymine (T), changing the sequence from GAG to GTG.
    - Carrier (βAβS): One allele is normal (GAG) and the other is mutated (GTG).

2. **Genetic Code:**

    - Normal Healthy: GAG codes for the amino acid glutamic acid
    - Sickle Cell Anaemia: GTG codes for the amino acid valine.
    - Carrier: Both glutamic acid and valine are present due to the heterozygous nature.

Restriction Enzyme (RE) Site

- The mutation affects the recognition site for the restriction enzyme **MstII**.
- **Normal Healthy**: The sequence GAG is recognized and cut by **MstII**.
- **Sickle Cell Anaemia**: The sequence GTG is not recognized by **MstII**, so it remains uncut.
- **Carrier:** Both cut and uncut fragments are present

RFLP and DNA Banding Pattern

1. PCR Amplification: Amplify the region of the beta-globin gene containing the mutation using specific primers.

2. Restriction Digestion: Digest the PCR products with MstII enzyme.

3. Gel Electrophoresis: Separate the digested products on an agarose gel to visualize the banding pattern.

DNA Banding Pattern

- **Normal Healthy (βAβA):**

  MstII cuts the PCR product into two fragments.

  Banding Pattern: Two bands.

- Sickle Cell Anaemia (βSβS):

  MstII does not cut the PCR product.

  Banding Pattern: One band (undigested).

- **Carrier (βAβS):**

  MstII cuts one allele but not the other.

  Banding Pattern: Three bands (one undigested and two digested).

Q6) Discuss the in silico method for the identification of RE cut site in any DNA sequence for RFLP experiment designing. Ans: We can identify the restriction enzyme cut side by following some steps:

Open webcutter2.O site

Then put the DNA sequence

Then click on analyse sequence.

Then, the RE cut site details like no of cut, position of cut etc. will be displayed.

Untitled sequence

8 base pairs

Graphic map | Table by enzyme name

BseRI
tgaggagt base pairs
actcctca 1 to 8

Table by Enzyme Name

Enzyme name	No. cuts	Positions of sites	Recognition sequence
BseRI	1	7	gaggag

The following endonucleases were selected but don't cut this sequence:

AatI, AatII, Acc113I, Acc16I, Acc65I, AccB1I, AccB7I, AccBSI, AccI, AccIII,
AclNI, AcsI, AcyI, AfeI, AflII, AflIII, AgeI, AhdI, Alw21I, Alw44I, AlwNI,
Ama87I, AocI, Aor51HI, ApaI, ApaLI, ApoI, AscI, AseI, AsnI, Asp700I, Asp718I,
AspEI, AspHI, AspI, AtsI, AvaI, AviII, AvrII, BalI, BamHI, BanI, BanII,
BanIII, BbeI, BbiII, BbrPI, BbsI, BbuI, Bbv12I, Bbv16II, BcgI, BclI, BcoI,
BfrI, BglI, BglII, BlnI, BlpI, BpiI, BpmI, Bpu1102I, Bpu14I, BpuAI, Bsa29I,
BsaAI, BsaBI, BsaHI, BsaI, BsaMI, BsaOI, BsaWI, BscI, Bse118I, Bse21I,
Bse8I, BseAI, BseCI, BsePI, BsgI, Bsh1285I, Bsh1365I, BshNI, BsiEI, BsiHKAI,

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2. https://emedicine.medscape.com/article/205926-overview?form=fpf
3. https://www.sigmaaldrich.com/IN/en/technical-documents/technical-article/genomics/sequencing/restriction-enzymes
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PARTICIPATIVE LEARNING

DEPARTMENT OF CHEMISTRY

Participative learning is a parallel process of conventional classroom learning which encompasses various departmental activities like alumni lecture series, students' seminar, poster presentation and learning through LMS. Details of the above-mentioned aspect of learning are summarized below:

Students' Seminar

Date 23.4.2024	**Semester 6**	**Session 2023-2024**
Name of the Mentee	**Topic of your presentation**	**Name of the mentor**
Subrata Ghosh	Niosomes	Arpita Roy
Priyabrata Naskar	Polymeric Micelles	Arpita Roy
Sanchita Giri	Photoactivable metal complexes: From theory to applications in biotechnology and medicine.	Ipsita Bhattacharya
Aritra Mondal	Smart polymer	Ipsita Bhattacharya
Uttaran Talukdar	Photochemical Pinacolone Rearrangement	Keya Ghosh
Biplab Mahato	Aldol Condensation	Keya Ghosh
Sultana Ruhi	DFT	Manas Kumar Biswas
Sreyasi Panda	Analysis of various bands in IR Spectroscopy	Manas Kumar Biswas
Sreeja Maitra	Liebeskind-Srogl Cross Coupling	Paramita Das
Priyanshu Nandi	Effect of curcumin in seed germination	Srijita Basumullick
Shayan Paul	Cross Coupling Grignard	Keya Ghosh
Koyel Saha	Use of chromatography in pharmaceutical industry and medicine	Ipshita Bhattacharya
Debdutta Halder	Vertically aligned carbon nanotubes prospects in biomedical engineering	Ipsita Bhattacharya
Md Sahil	Polyacetic Acid/ Biodegradable Polymers	Madhusudan Banerjee
Tamal Pakrasi	Photocatalytic Nitrogen Fixation	Monoj Kumar Barman
Ayon debnath	Photo catalytic Water splitting	Monoj Kumar Barman
Sharmi Das	Synthesis of colloidal nanocrystals by using quantum dot	Niladri Sekhar Karan
Soumi Pharikal	Grignard Reaction	Paramita Das
Sritama Maiti	Curcumin as Anti-cancer agent	Srijita Basu Mallick
Dipankar Dutta	In-vivo bioimaging with Quantum Dots	Niladri Sekhar Karan


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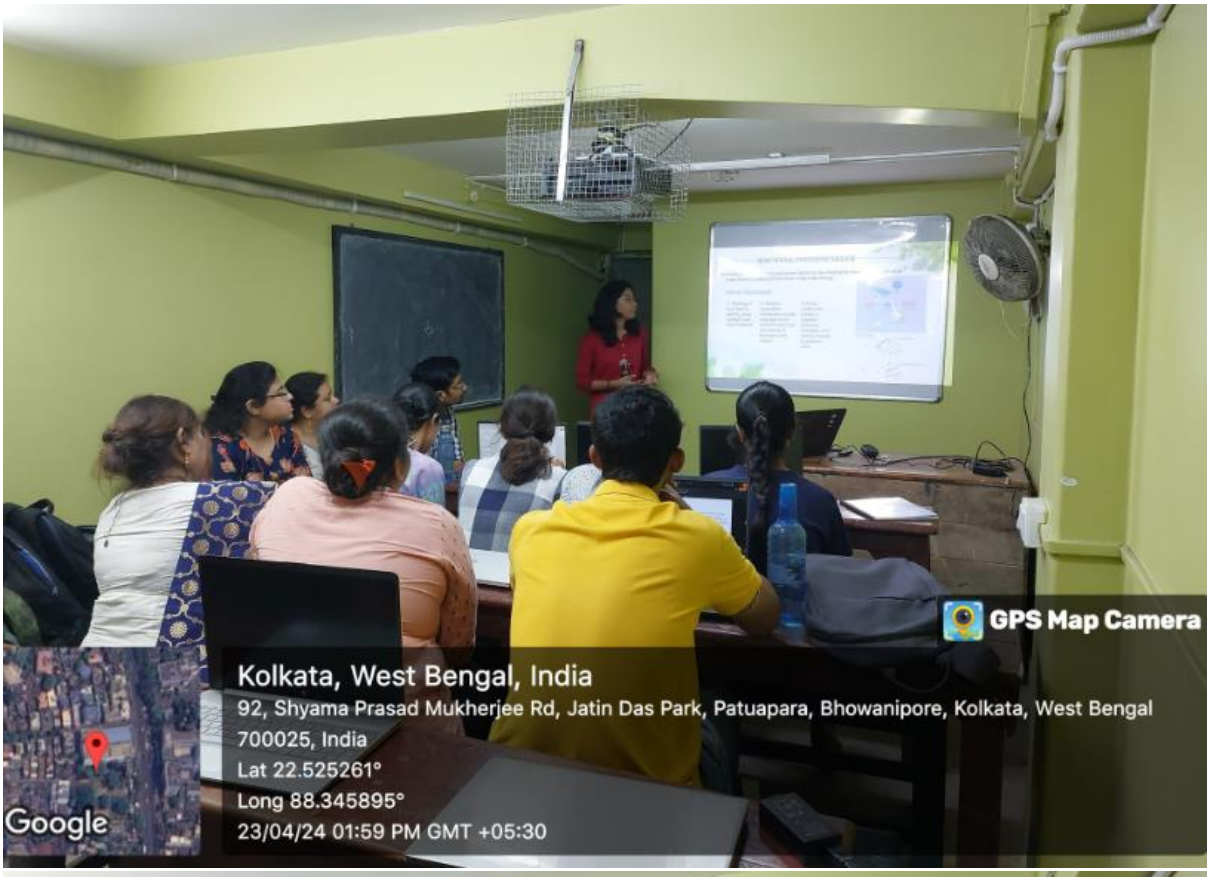
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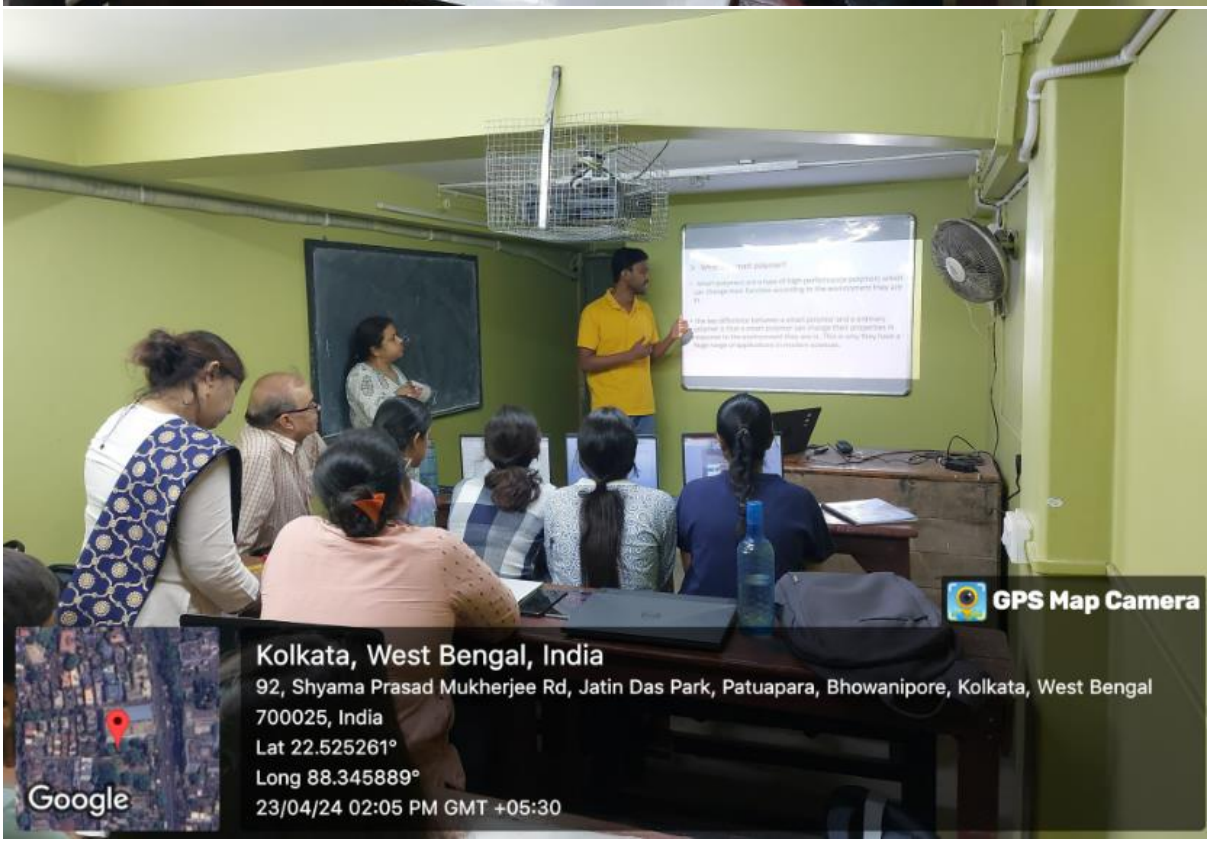
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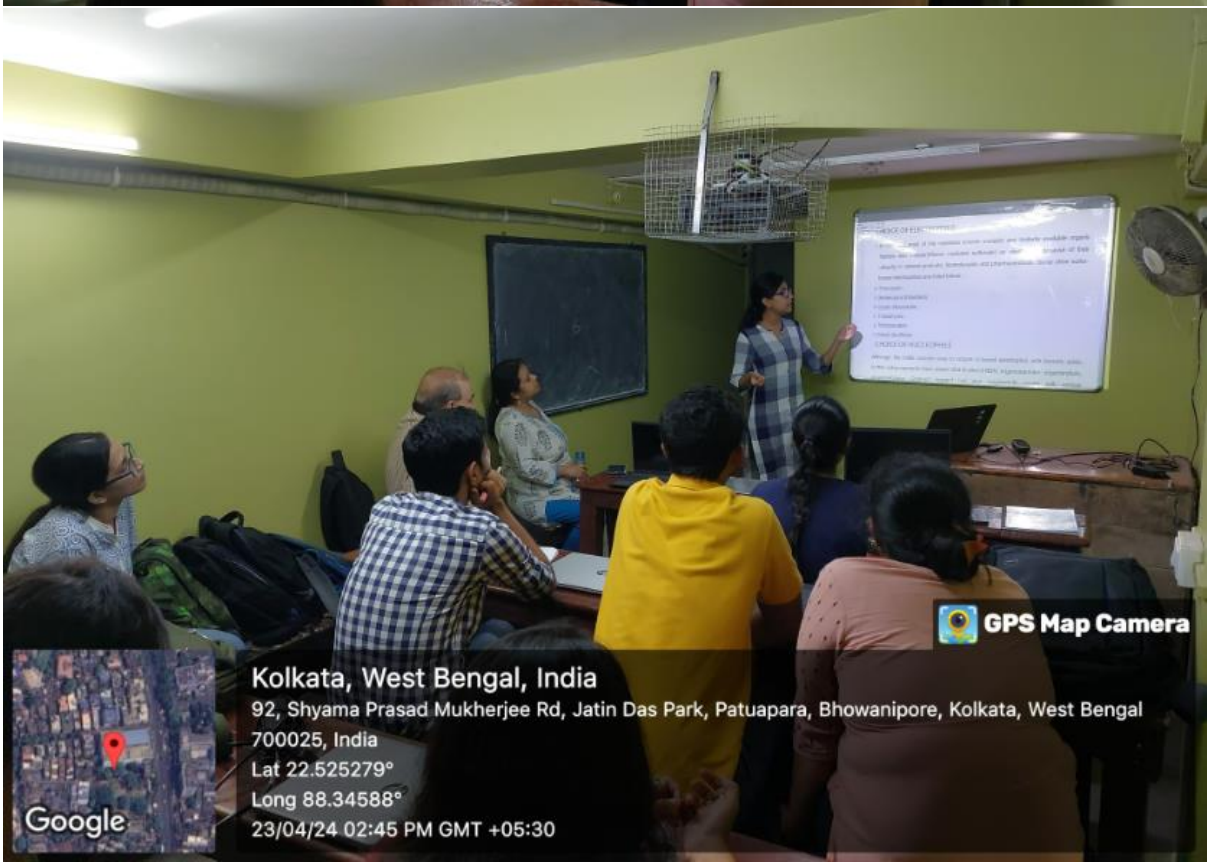
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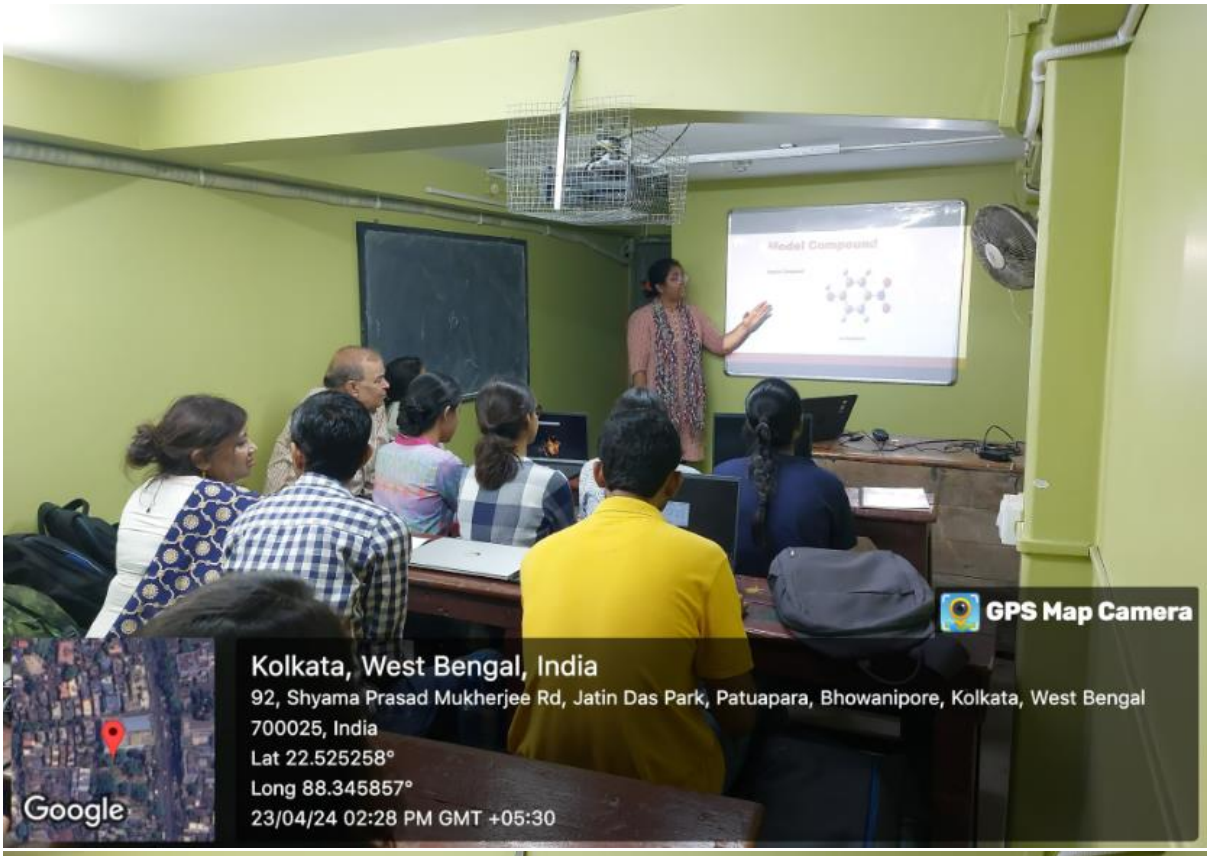
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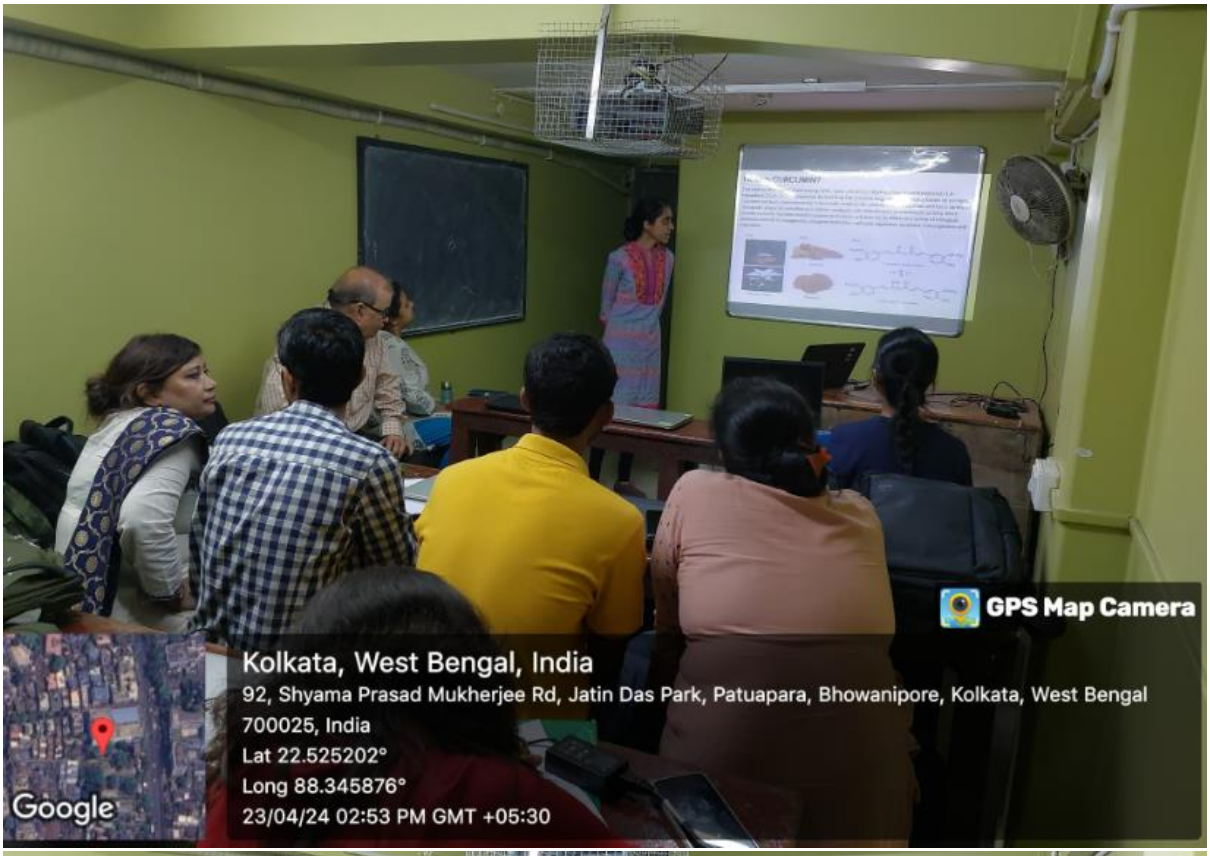
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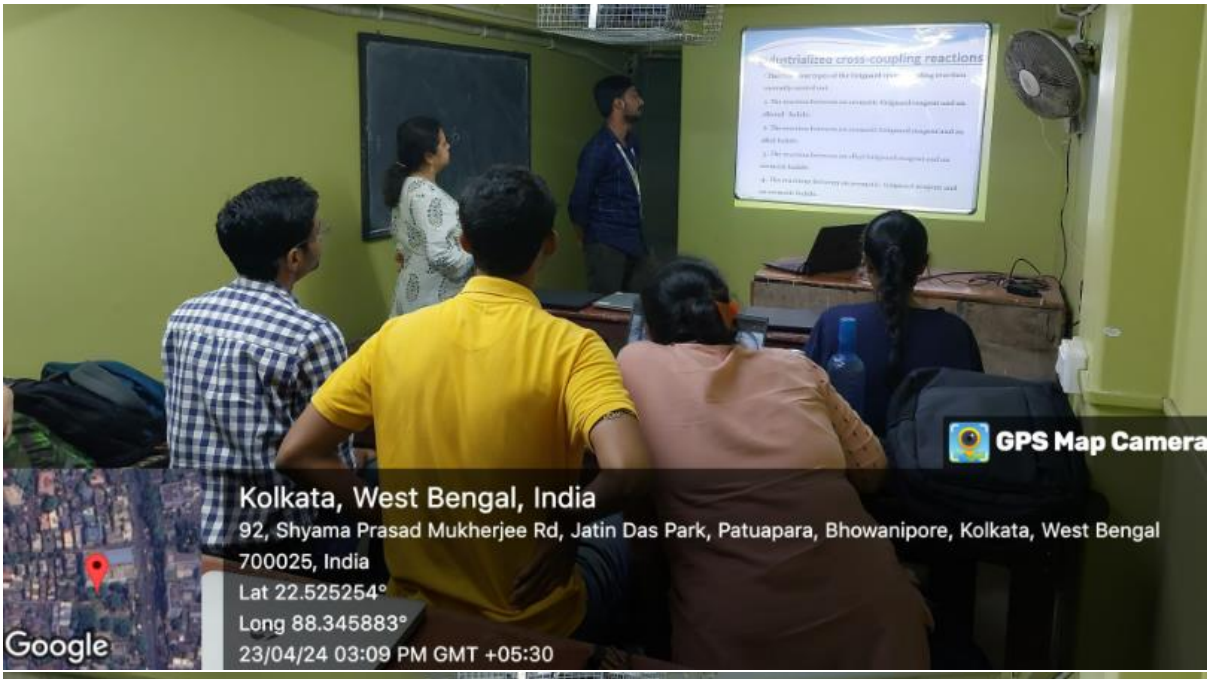
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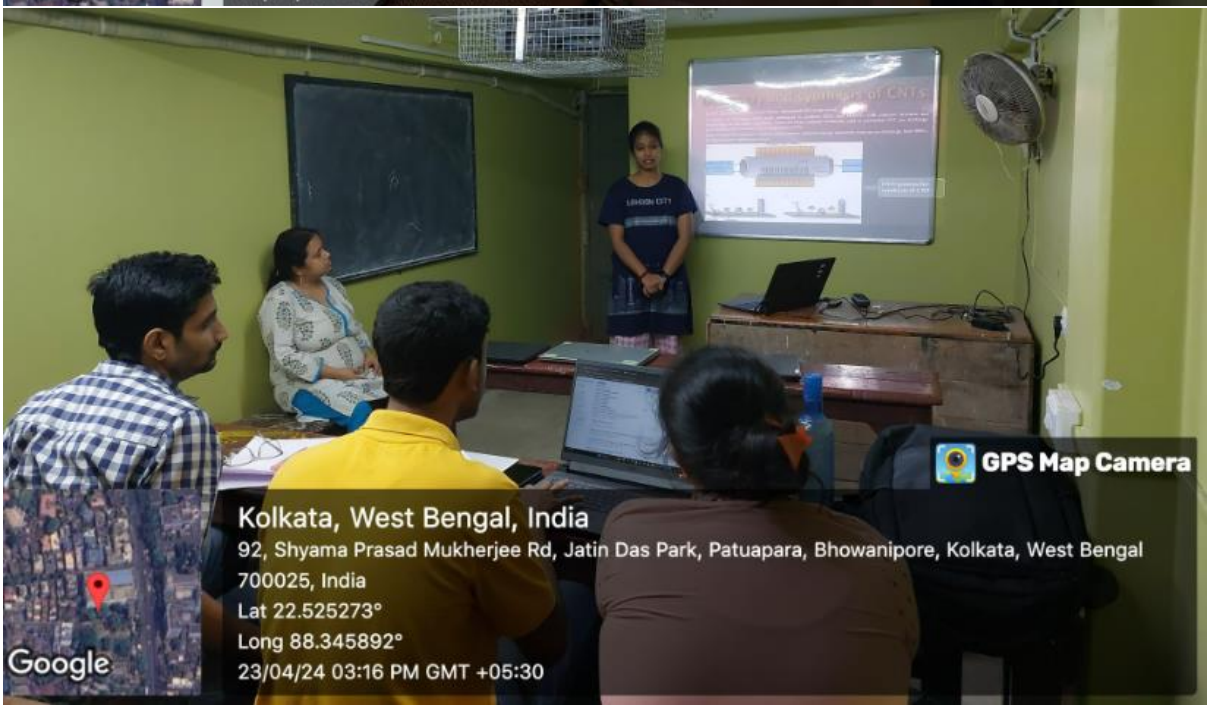
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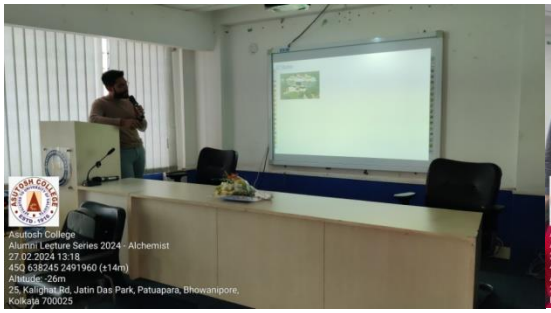
Poster Presentation

Alumni Lecture Series

- TITLE OF EVENT/ PROGRAMME: Alumni Lecture Series 2023-2024
- THEME OF THE EVENT/ PROGRAMME: Journey of the molecular world using theory and computation
- DATE: 27thFebruary,2024
- VENUE: Centenary Building, Asutosh College.
- COLLABORATOR: IQAC, Asutosh College.
- OBJECTIVE/ PURPOSE: This particular Alumni Lecture series: Alchemist was kept open to all candidates with the larger aim of knowledge transfer to the willing candidates (students and faculties of Asutosh College and others). The sole purpose of conducting this Alumni Lecture series in the Department of Chemistry is to provide the participants, to interact with their pass-out senior.
- SPEAKER/S / RESOURCE PERSON/S: Dr. Sutirtha Narayan Chowdhury, R&D Engineer, Intel Corporation, USA.
- TARGET AUDIENCE/ PARTICIPANTS: UG and PG Students and faculties of Asutosh College as well as other colleges and Universities
- ATTENDANCE SHEET: Total Number of Participants – 53
- BRIEF REPORT ABOUT THE EVENT/ PROGRAMME: The event started with the welcome address from Dr.Keya Ghosh, Head of the Department of Chemistry, Asutosh College. Then Prof. Manas Kabi, Principal, Asutosh College was felicitated from the department. Then with a brief introduction of the speaker, the lecture started. All the students interacted with the speaker and actively participated in the lecture. The speaker answered the queries of the participants satisfactorily and with a vote of thanks from the Chemistry Department the lecture ended.
- EXPECTED OUTCOME: Students were introduced with the knowledge in the modern trends of chemical science.
- GEO-TAGGED PHOTOGRAPHS:



- ATTENDANCE SHEET

Day - I

27.2.24

ALCHEMIST : ALUMNI LECTURE SERIES

-1

Speaker :- Subirtha Chowdhury, R&D Engineer, Intel Corp., USA

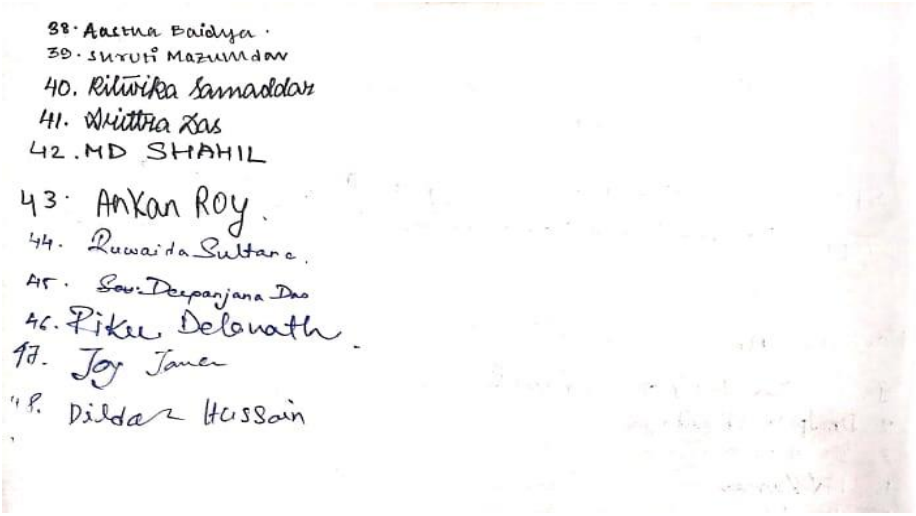
Topic - Journey to the Molecular World, with Theory and computation

Faculty Members :-

1. Dr. Keya Ghosh.
2. Dr. Ipsita Bhattacharya
3. Dr. Arpita Roy.
4. N Karan
5. Dr. Manas Kumar Biswas

Students :—

1) Ankush Das
2. Soumitro Moitra.
3. Srinjan Majumder.
4. Ritam Mandal.
5. Chayan Samanta.
6. Pratyusha Das
7. Shayeni Banerjee
8. Poulomi Basak
9. Shreabanti Nath
10. Subarna Ghosh
11. Shiksha Mishra
12. Agnik kumar Halder.
13. Nihar Mandal
14. Abhra Pal
15. Sritama Maiti
16. Sanjukta Patra
17. Debadutta Halder.
18. Uttaran Talukdar.
19. Sanchita Giri.
20. Tamal Pakrasi
21. Shayan Paul
22. Priyanshu Nandi
23. Ayon Debnath
24. Anitra Mondal.
25. Sultana Ruhi
26. [redacted]
26. Sk. Sahib Rahaman.
27. Md Sayed Anwar
28. Aditya kumar
29. Somashree Dinda
30. Srijnik chakraborty
31. Devatma Chatterjee
32. Priya Roy.
33. Pratayee Manna.
34. Rishi Das.
35. Farhad Khan
36. Rohan Mondal.
37. Maniur Ali Khan

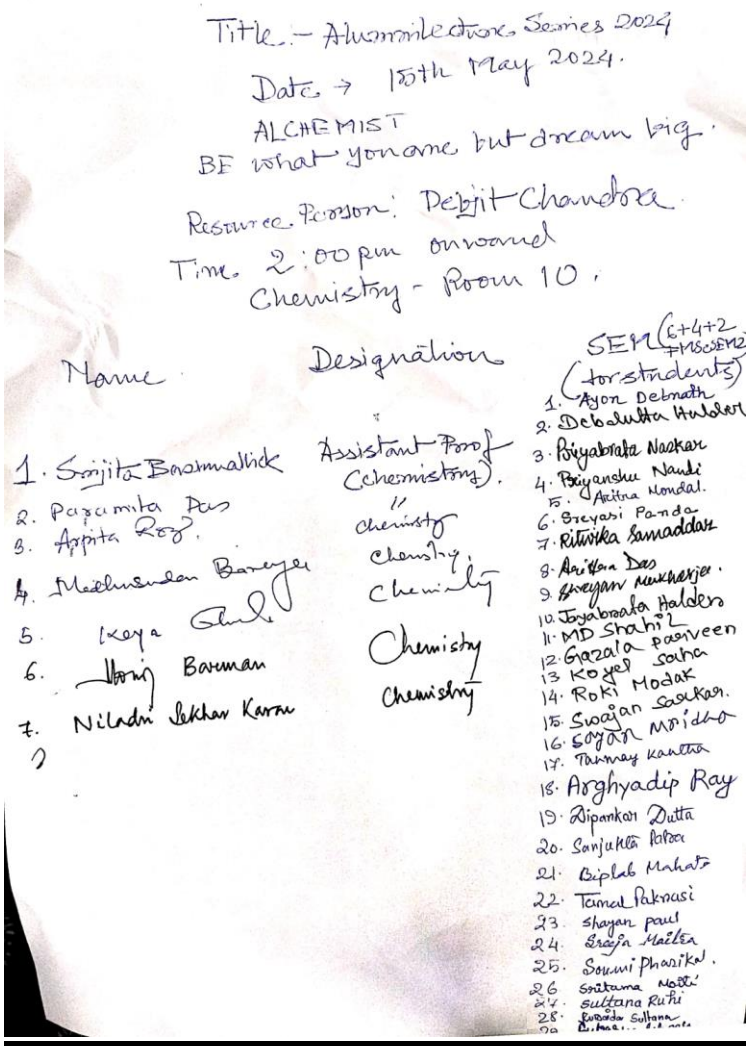

38. Aastha Baidya.
39. Shruti Mazumdar
40. Rituvika Samaddar
41. Shiktha Das
42. MD SHAHIL
43. Ankan Roy.
44. Ruwaida Sultana.
45. Sou. Deepanjana Das
46. Rikki Debnath
47. Joy James
48. Dildar Hussain

- TITLE OF EVENT/ PROGRAMME: Alumni Lecture Series 2023-2024
- THEME OF THE EVENT/ PROGRAMME: Be what you are but dream big
- DATE: 15th May,2024
- VENUE: Main Building, Room number 10, Asutosh College.
- COLLABORATOR: IQAC, Asutosh College.
- OBJECTIVE/ PURPOSE: This particular Alumni Lecture series: Alchemist was kept open to all candidates with the larger aim of knowledge transfer to the willing candidates (students and faculties of Asutosh College and others). The sole purpose of conducting this Alumni Lecture series in the Department of Chemistry is to provide the participants, to interact with their pass-out senior.
- SPEAKER/S / RESOURCE PERSON/S: Speaker: Mr. Debjit Chandra, Global Technical Services Manager – Dorf Ketal.
- TARGET AUDIENCE/ PARTICIPANTS: UG and PG Students and faculties of Asutosh College as well as other colleges and Universities
- ATTENDANCE SHEET: Total Number of Participants – 37
- BRIEF REPORT ABOUT THE EVENT/ PROGRAMME: The event started with the welcome address from Dr.Keya Ghosh, Head of the Department of Chemistry, Asutosh College. Then Dr.Manas Kabi, Principal, Asutosh College was felicitated from the department. Then with a brief introduction of the speaker, the lecture started. All the students interacted with the speaker and actively participated in the lecture. The speaker answered the queries of the participants satisfactorily and with a vote of thanks from the ChemistryDepartment the lecture ended.

- EXPECTED OUTCOME: Students were introduced with the knowledge in the modern trends of chemical science.

- GEO-TAGGED PHOTOGRAPHS:

- ATTENDANCE SHEET:


Title :- Alumni Lecture Series 2024
Date → 15th May 2024.
ALCHEMIST
BE what you are but dream big.
Resource Person: Debjit Chandra.
Time. 2:00 pm onwards
Chemistry - Room 10.

Name — Designation

1. Sujita Basumallick — Assistant Prof (Chemistry).
2. Paramita Das — "
3. Arpita Roy — Chemistry
4. Madhusudan Banerjee — Chemistry
5. Kavya — Chemistry
6. Hiranmoy Barman — Chemistry
7. Niladri Sekhar Karan — Chemistry

SEM (6+4+2 + MSc SEM 2 for students)

1. Ayon Debnath
2. Debalutta Halder
3. Priyabrata Naskar
4. Priyanshu Nandi
5. Aditra Mondal.
6. Sreyasi Panda
7. Ritwika Samaddar
8. Aaikan Das
9. Shreyan Mukherjee.
10. Jayabrata Halder
11. MD Shahil
12. Ghazala Parveen
13. Koyel Saha
14. Roki Modak
15. Swajan Sarkar.
16. Sayan Mondal
17. Tanmay Kantha
18. Arghyadip Ray
19. Dipankar Dutta
20. Sanjukta Patra
21. Biplab Mahato
22. Tamal Pakrasi
23. Shayan Paul
24. Sraija Maitra
25. Soumi Pharikal.
26. Sritama Maiti
27. Sultana Ruthi
28. Farzada Sultana

DEPARTMENT OF SANSKRIT

ACTIVITIES (SEMINARS / WEBINARS / WORKSHOPS / EXTENSION LECTURES / KNOWLEDGE TRANSFER ACTIVITY/ALUMNI TALK)

TITLE OF EVENT/ PROGRAMME (SEMINAR/ WEBINAR/ WORKSHOP/ EXTENSION LECTURE/ EXTENSION ACTIVITY/ KNOWLEDGE TRANSFER ACTIVITY/ ANY OTHER ACTIVITY): **KNOWLEDGE TRANSFER ACTIVITY**

THEME OF THE EVENT/ PROGRAMME: **Yoga Manabiyo Kartyabyabodhar Pradhan Dhap (Faculty Exchange Programme)**

DATE: 11.10.23

VENUE: **ABN SEAL COLLEGE, COOCH BEHAR**

COLLABORATOR/S (IF ANY): **ABN SEAL COLLEGE, COOCH BEHAR**

OBJECTIVE/ PURPOSE: To Impart Knowledge to Students.

SPEAKER/S / RESOURCE PERSON/S: **Dr. Somnath Das**

TARGET AUDIENCE/ PARTICIPANTS: UG & PG Students **ABN SEAL COLLEGE, COOCH BEHAR**

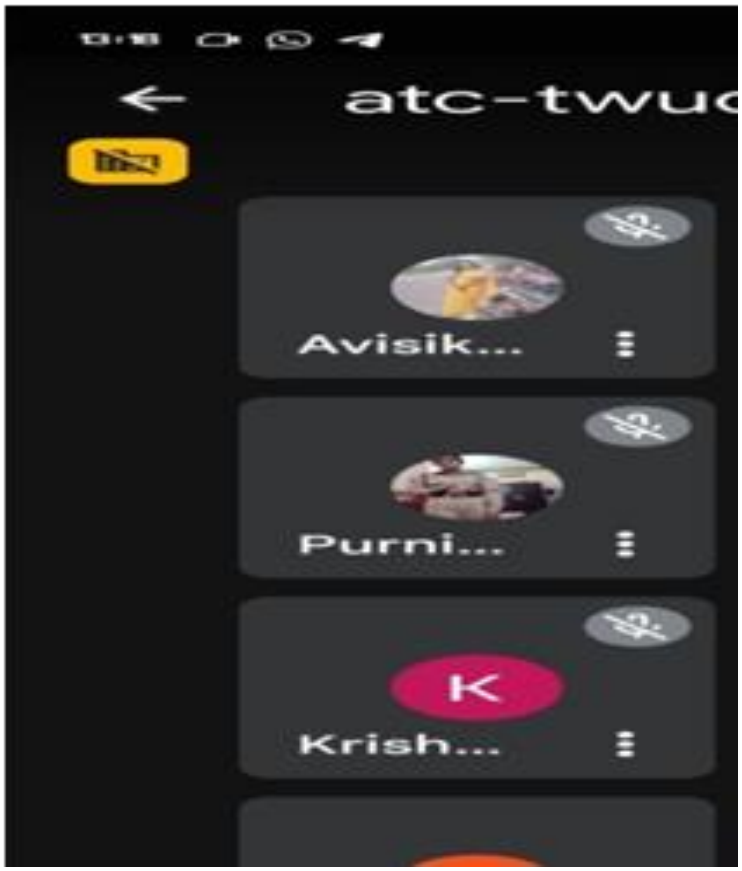
ATTENDANCE SHEET (If available): Attached

BRIEF REPORT ABOUT THE EVENT/ PROGRAMME: Expressing the fact that yoga is the Main means of human sense of duty. Informing the fact that the role of Yoga is Important in Different Stages of Life, thereby Gaining People's Knowledge about the Value of Yoga.

EXPECTED OUTCOME: To make aware about psychosocial Development, to determine the relationship of yoga with body.

GEO-TAGGED PHOTOGRAPHS:


GPS Map Camera
Cooch Behar, West Bengal, India
8CCX+8QV, Cooch Behar, West Bengal 736101, India
Lat 26. 320905°
Long 89. 449415°
11/10/23 02:11 PM GMT +05:30
Google


atc-twu
Avisik...
Purni...
K
Krish...

ACTIVITIES (SEMINARS / WEBINARS / WORKSHOPS / EXTENSION LECTURES / KNOWLEDGE TRANSFER ACTIVITY / ALUMNI TALK)

TITLE OF EVENT / PROGRAMME (SEMINAR / WEBINAR / WORKSHOP / EXTENSION LECTURE / EXTENSION ACTIVITY / KNOWLEDGE TRANSFER ACTIVITY / ANY OTHER ACTIVITY): **KNOWLEDGE TRANSFER ACTIVITY**

THEME OF THE EVENT/ PROGRAMME: **DURGA PUJA IN VEDIC AND PURANIC AGE**

(Faculty Exchange Programme)

DATE: 17.10.23

VENUE: **KANDRA RADHAKANTA KUNDU MOHAVIDYALAYA**

COLLABORATOR/S (IF ANY): **KANDRA RADHA KANTA KUNDU MAHAVIDYALAYA**

OBJECTIVE/ PURPOSE: To Impart Knowledge to Students.

RESOURCE PERSON**: Dr. Somnath Das**

TARGET AUDIENCE/ PARTICIPANTS: UG All Semeesters Students **(KANDRA RADHA KANTA KUNDU MAHAVIDYALAYA**

ATTENDANCE SHEET (If available): Attached

BRIEF REPORT ABOUT THE EVENT/ PROGRAMME: Since the vedic and Puranic era, durga puja distributes auspiciousness inn the human soul. That is why it is said that durga puja is one of the manifestations of the madness of mankind.

EXPECTED OUTCOME: To Provide information to People about the Practice of Durga Puja in Vedic and Puranic Times.

GEO-TAGGED PHOTOGRAPHS:

PROBLEM SOLVING METHODOLOGIES

DEPARTMENT OF GEOGRAPHY

- **TITLE OF EVENT/ PROGRAMME:** Problem solving methodologies
- **THEME OF THE EVENT/ PROGRAMME:** Breaking Barriers: Effective Problem-Solving Approaches
- **ACADEMIC SESSION:** 2023-24
- **DATE:** NA
- **VENUE: Asutosh College**
- **OBJECTIVE/ PURPOSE:** To encourage creative and innovative solutions and promote efficiency and effectiveness
- **SPEAKER/S / RESOURCE PERSON/S:** Departmental faculty members
- **TARGET AUDIENCE/ PARTICIPANTS:** Both UG and PG students
- **ATTENDANCE SHEET:** NA
- **BRIEF REPORT ABOUT THE EVENT/ PROGRAMME:**

  Problem-solving is a key educational objective that equips students with the ability to apply theoretical knowledge in real-world scenarios. In geography, where students engage with both physical and human environments, problem-solving techniques are integral to understanding complex systems. Skill Enhancement Courses (SECs) and Class Tests through Learning Management Systems (LMS) like Google Classroom and Moodle offer structured methodologies to enhance these skills. This report explores the use of SECs and LMS-based assessments for undergraduate and postgraduate students in the Department of Geography at Asutosh College, emphasizing both theory and practical components.

  Problem-solving in geography involves the following steps: Identifying the Problem, Analysing Data, Formulating Solutions: Evaluating Outcomes. This process can be enhanced by incorporating Skill Enhancement Courses that develop specific abilities like data analysis, GIS applications, and research techniques, along with class tests through LMS platforms that assess understanding at every stage. Skill Enhancement Courses are an integral part of the curriculum, designed to impart practical skills that go beyond theoretical knowledge. SECs are particularly effective for geography students because they help bridge the gap between theory and practical application.

Learning Management Systems such as Google Classroom or Moodle provide a centralized platform for managing course content, assignments, and assessments. By utilizing these platforms, instructors can enhance problem-solving methodology through structured assessments that test both theoretical understanding and practical applications. Class tests, administered through LMS platforms, provide timely feedback on students' understanding and their ability to apply knowledge to problem-solving scenarios. These tests can be designed in several ways to ensure that students develop problem-solving skills effectively.

Problem-solving methodology is crucial for the holistic development of geography students, preparing them for both academic challenges and real-world applications. Skill Enhancement Courses (SECs) and assessments through Learning Management Systems (LMS) provide structured avenues for developing these skills. By incorporating SECs like GIS and cartographic techniques, along with LMS-based class tests and assignments, the Department of Geography at Asutosh College can foster an environment where students not only understand theoretical concepts but also excel in practical problem-solving. This approach ensures that students are well-prepared for careers in research, planning, and environmental management.

- **EXPECTED OUTCOME:** Clear and precise problem identification and stronger organizational resilience

GEO-TAGGED PHOTOGRAPHS: NA

CLASS TEST REPORT THROUGH LMS:

https://drive.google.com/file/d/1DDPTp91ITXSDlOApisyhM3HCdo8gWjqt/view?usp=sharing

CERTIFICATE COURSE REPORT:

https://drive.google.com/file/d/19DGi9i36OdraDRk6NYeZktWpb0HW1n_o/view?usp=sharing

DEPARTMENT OF BENGALI

Department uses LMS facilities for problem solving. One example is cited here.

আশুতোষ কলেজ

বাংলা বিভাগ

ক্লাস টেস্ট–১

(পদ্ধতি: অনলাইন LMS)

সেমেস্টার: ৫ (সাম্মানিক স্নাতক)

পত্র: সি সি ১১

মডিউল: ১

বিষয়: কাব্য-কবিতার রূপভেদ (মহাকাব্য ও গীতিকাব্য)

তারিখ: ১২.১০.২০২৩

সময়: দুপুর ১২.০০টা—দুপুর ১.০০টা

পূর্ণমান: ১০

পূর্ণ বাক্যে উত্তর লেখো। (৫×২=১০)

১. মহাকাব্য বিষয়ে পাশ্চাত্যের কোন বিশিষ্ট মনীষী প্রথম আলোচনা করেছিলেন? তাঁর বইটির নাম লেখো। (১+১=২)

২. মহাকাব্য কয় প্রকার ও কী কী? (২)

৩. গীতিকাব্যের দু'টি বৈশিষ্ট্য লেখো। (২)

৪. গীতিকাব্যের সঙ্গে কথাসাহিত্য ও নাটকের কোন কোন সংরূপের ভাবগত সাদৃশ্য লক্ষ করা যায়? (২)

৫. 'ভোরের পাখি' কাকে বলা হয়? তাঁর লেখা একটি গীতিকাব্যের নাম লেখো। (১+১=২)

Assignment Result List

Subject: BNGHCC

Paper: CC11

Semester: 5

Full marks: 10, Qualifying marks: 4 Date: 12-10-2024

Sr No	Univ. Reg. No	Univ. Roll No	Student ID	Student Name	Marks Obtained
1	012-1111-0009-21	212012-21-0003	0593	Sourav Samanta	10
2	012-1211-0012-21	212012-11-0007	1177	Asmita Roy	10
3	012-1111-0017-21	212012-21-0006	1183	Anushko Ghosal	10
4	012-1112-0060-21		0606	Saurav Mondal	10
5	012-1211-0040-21		2458	Shreya Roy	10
6	012-1211-0034-21		2279	Shireen Hossain	10
7	012-1112-0066-21	212012-21-0123	1106	Manas Halder	10
8	012-1211-0039-21		2456	Bidisha Mukherjee	10
9	012- 1211-0004-21		0540	Shreyashi Sarkar	10
10	012-1212-0078-21		2087	Oliva Roy	9
11	012-1111-0008-21		0752	Sanway Chakraborty	10
12	012-1211-0047-21		2777	Amiya Saha	9
13	012-1115-0050-21	212012-21-0187	1097	Rohit Mahato	10
14	012-1212-0067-21		1132	Riya Halder	10
15	012-1211-0016-21	212012-11-0010	1095	Tiyasa Debnath	10
16	012-1211-0024-21	212012-11-0015	1565	Piyasha Purkait	10
17	012-1211-1964-21		1771	Triparna Sarkar	10
18	012-1214-0053-21	212012-11-0313	1779	Shalini Malakar	10
19	012-1212-0062-21	212012-11-0238	0890	Urmila Malick	10
20	012-1212-0071-21	212012-11-0245	1512	Shreya Sarkar	9
21	012-1111-1963-21	212012-21-0210	1185	Sudipta Ghosh	9
22	012-1212-0064-21		0906	Rishita Das	10

23	012-1212-0056-21		0051	Puja Halder	9
24	012-1111-0001-21		0199	Aritra Chatterjee	10
25	012-1213-0080-21		0259	Sabita Mardi	7
26	012-1211-0021-21		1376	Rayeshree Ghosh	9
27	012-1212-0063-21		0907	Baisakhi Mandal	10
28	012-1111-0048-21		2792	Deep Adhikary	10
29	012-1212-0055-21	212012-11-0233	0367	Arpita Saha	9
30	012-1111-1697-21	212012-21-0200	2124	Yasdin Islam Mondal	9
31	012-1211-0018-21		2056	Sunandita Paul	10
32	012-1111-0011-21		1043	Souvik Bhattacharyya	10
33	012-1212-0068-21		1276	Trisha Bairagi	9
34	012-1211-0014-21		1212	Sayoni Roy	9
35	012-1211-0046-21		2772	Moumita Maity	8
36	012-1212-0058-21	212012-11-0236	0678	Bithi Naskar	10
37	012-1111-0019-21	212012-21-0007	1322	Piyush Mondal	10
38	012-1211-0022-21		1475	Arpita Bakshi	10
39	012-1111-0015-21		1204	Arpan Kar	10
40	012-1214-0051-21	212012-11-0312	0602	Shipra Saha	10
41	012-1211-0020-21	212012-11-0012	1444	Kashmira Khatun	8
42	012-1211-0013-21		1159	Anushka Talukder	10
43	012-1212-0076-21	212012-11-0249	1782	Debjany Roy	10
44	012-1211-0045-21		2642	Ashmita Das	8
45	012-1212-0072-21	212012-11-0246	1749	Swathi Naskar	10
46	012-1112-0079-21	212012-21-0125	2638	Shuvo Mondal	8