राष्ट्रीय ध्रुवीय एवं समुद्री अनुसंधान केन्द्र
पृथ्वी प्रणाली विज्ञान संगठन
पृथ्वी विज्ञान मंत्रालय (भारत सरकार)
हेडलैण्ड सडा, वास्को-डा-गामा, गोवा-४०३ ८०४, भारत
NATIONAL CENTRE FOR POLAR AND OCEAN RESEARCH
Earth System Science Organisation
Ministry of Earth Sciences, (Goverment of India)
Headland Sada, Vasco-da-Gama, Goa - 403 804, INDIA

CERTIFICATE

This is to certify that Ms. **Lovely Anand** student of Goa University, Taleigao Plateau, Goa, has successfully completed a winter internship from **National Centre for Polar and Ocean Research** in the field of Arctic Ecology and Biogeochemistry from 01/12/2022 to 30/12/2022 under the guidance of **Dr. K.P. Krishnan** and under the supervision of **Dr. Anand Jain** and **Ms. Yuga Ghotge.**

During the period of her internship program with us, she had been exposed to various techniques and skills in the laboratory.

We wish her every success in her life and career.

05/04/2023

Supervisor

Archana Singh / अर्चना सिंह
Scientist / वैज्ञानिक
ESSO-National Centre for Antarctic and Ocean Research
ई.एस.एस.ओ.-राष्ट्रीय अंटार्कटिक एवं समुद्री अनुसंधान केन्द्र
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Archa Singh
05.04.2023

Authorized Signature
SIC, Arctic Ecology & Biogeochemistry
Arctic Operations

National center for polar and ocean research (NCPOR)

Internship report

1st December 2022 to 30th December 2022

Submitted by

Lovely Anand

M.Sc. Marine biotechnology (part 2)

School of Biological Sciences and Biotechnology

Goa University

Acknowledgement

First of all, I would like to express my sincere gratitude to **Department of biotechnology (DBT)- India, Dean of School of biological Sciences and Biotechnology and Marine Biotechnology programme Director** for providing me an opportunity to do my internship at **National centre for polar and ocean research**. For me, it was a unique experience to study about various research work that has been done by the institution and various practical, technical and experimental approach toward research. This internship period was a great chance of learning and professional development.

Now, I would like to express my deepest sense of gratitude to **Dr. K.P Krishnan** (scientist F – NCPOR) providing us an opportunity to work in the institution.

I also express my deepest thanks to **Dr. Anand Jain** (Project Scientist – III) and **Ms. Yuga Ghotge** ma'am (junior research fellow – NCPOR) for explaining both practical theory and practical work in details with lots of patience. I Thank them for their necessary advice and career guidance tips. It will definitely help me in choosing the best path.

content

About NCPOR

National Centre for Polar and Ocean Research (NCPOR) is India's premier R&D institution responsible for the country's research activities in the polar and Southern Ocean realms

- The NCPOR operates in different fields or tasks
- Storing ice core sample, from Antarctica and the Himalayas
- Operating the Himadri and Ind Arc research stations in Svalbard, Norway
- Managing the oceanic research vessel, ORV Sagar Kanya, the flagship of India's fleet of oceanographic study vessels. This ship has contributed significantly to India's study of the Arbian sea, the Bay of Bengal and the Indian Ocean.

On 9 October 2016, the National Centre for Antarctica and Ocean Research (NCAOR) established a high-altitude research station in Himalaya called Himansh. The station is situated above 13500 feet (>4000m) at a remote region in Spiti , Himachal Pradesh.

The NCPOR was established as an autonomous research and development institution of the ministry of Earth Sciences (formerly Department of ocean development). Government of India on the 25th may 1998.

Different Stations

Two research stations at antarctica namely Dakshin Gangotri (1984) Maitri (1989) and Bharati (2011) and artic namely Himadri (2008) – observations and studies are carried out in atmospheric biological, geographical and ecological sciences etc.

At antarctica scientific studies and investigation are undertaken on the continental part and contiguous shelf ice. At artic also, meteorological, biological, glaciological and past climate studies are undertaken in the vicinity of the station " Himadri".

Areas under study

Polar Science and Cryosphere

Polar science and cryosphere studies at NCPOR are aimed at a holistic understanding at the polar region.

Geoscience

The geoscientific programs at NCPOR seek to carry out a systematic geoscientific survey of the ocean realm around india.

- Exploring the origin of Indian ocean
- Evolution of the Himalaya and the origin of the monsoon.
- Exploration for the mineral resources

The mandate of NCPOR is currently confined to the exploration of gas hydrate occurrences with the EEZ as well as multi-metal hydrothermal mineralization in the Indian ridge area

Hydrothermal vents

At antarctica scientific studies and investigation are undertaken on the continental part and contiguous shelf ice area.

About the internship

This internship report contains the details of my winter internship at NCPOR. I worked in the Polar Biology laboratory.

Aim

Polar biology basically deals with the sample that they got from the arctic regions, and tries to find out how various environmental factor affects the vegetation of the arctic region.

They are trying to find out how carbon exchange (carbon cycle) takes place within different vegetation in arctic and how it affects the environmental condition and role of bacterial communities in carbon cycling of coastal arctic ecosystem and factor influencing bacterial community.

1st week

Cell counting and cell volume determination of the given sample by using fluorescent microscopy

Sample collected from the different stations of the arctic regions and prepared a slide for cell counting and compared the unfiltered sample and filtered sample of the sample from same domain to determine the cell growth, cell size and cell volume of the given sample.

Main motive of doing cell counting and cell volume estimation is to determine the biomass of the marine bacterioplankton present in a given area. It is desirable to know size distribution more accurately for studies of selective grazing by bacterioplankton contribution to optical properties of seawater.

Cell volume and cell counting of the 12 different samples (filtered and unfiltered) were determined by using fluorescent microscopy.

2nd week

Slide preparation of the sample for cell counting by using fluorescent microscopy.

Steps

- Filtration of sample
- Samples were collected from different stations.
- 2ml sample was required for the filtration.
- Sample was stained with DAPI stain and incubate at 4°c for 20 min.
- Filtered paper was washed by PBS to avoid any contamination.
- Sample was fixed by using 2% formaldehyde
- filtered through a black-stained 0.2um pore size nucleopore filter (25 diameter) (black polycarbonate filtered paper)
- transferred on the wet filtered to a slide, covered with paraffin oil and a coverslip, viewed under 1000X (oil immersion) on a epifluorescence microscope.
- Cells were photographed for measurements. Bacterioplanktons size were categorized into spheres (volume = $4/3\pi r^3$) and rods (volume = πr^2[L-2/3r].

Result

The abundance of cell was calculated for the sample collected from 10 different stations in arctic (HN-OUT, HN-MDS, HF-OUT, HF-MDS, KN-OUT, KN-MDS, NA-OUT, NA-MDS, BD-OUT, BD-MDS)

Abundance of cell was found to be more in unfiltered sample than filtered one.

Conclusion

With this we can conclude that the unfiltered sample has larger cell biomass than the filtered one , the main reason for this result might

be the presence of another organic molecule along with the bacterioplankton.

3rd week

Preparation of media and plates for culturing the sample from arctic.

We prepared MBM (modified basal media) for culturing samples

For the preparation of MBM media we used basic salt composition of sea water to this we added trace metal and vitamins.

4 different kinds of media were prepared to check in which media the growth is more

MBM + sodium alginate + agar

MBM + Laminarin + agar

MBM + mannitol + agar

MBM + Agar

Small serial dilutions were made and first 3 dilutions were taken for the spread plating i.e., 10^2, 10^3, 10^4, For each media.

Media dilutions : 15 ml in each plate.

Composition of media

Composition	Gram/l
NaCl	25
KCl	0.75
CaCl2	0.2
MgSO4	7
NH4Cl	0.5
Yeast Extract	2.0
Peptone	0.3
K2HPO4 – 10%	7ml
KH2PO4 – 10%	3ml
Trace metal solution	1ml
Vitamin solution	1ml

Distilled water	1000ml

PH 7.0

Added K2HPO4 and KH2PO4 after autoclaving. Adjust pH 7 using 1N/0.1 NaOH.

Plate pouring and spread plating was done and kept it in incubator for 15 days at low temperature for growth.

Instrumentation demonstration

Fluorescent microscopy

Principle: Fluorescence microscopy is a light microscope that works on the principle of fluorescence. A substance is said to be fluorescent when it absorbs the energy of invisible shorter wavelength radiation (such as UV light) and emits longer wavelength radiation of visible light (such as green or red light). This phenomenon, also called fluorescence, is widely used in clinical and diagnostic settings to detect microorganisms, antibodies, and many other substances rapidly.

Commonly used fluorescent dyes are; DAPI (49,6-diamidino-2-phenylindole), acridine orange, auramine-rhodamine, Alexa Fluors, or DyLight 488.

Most cellular components are colorless and cannot be clearly distinguished under a microscope. The basic premise of fluorescence microscopy is to stain the components with dyes.

The emission light can then be filtered from the excitation light to reveal the location of the fluorophores.

- Fluorescence microscopy uses a much higher intensity light to illuminate the sample. This light excites fluorescence species in the sample, which then emits light of a longer wavelength.
- The image produced is based on the second light source or the emission wavelength of the fluorescent species rather than from the light originally used to illuminate, and excite, the sample.

HPLC

Principle: High-performance liquid chromatography or commonly known as HPLC, is an analytical technique used to separate, identify or quantify each component in a mixture.
The mixture is separated using the basic principle of column **chromatography** and then identified and quantified by spectroscopy.
HPLC is thus basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres.

Ion exchange chromatography

Principle: Ion exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to ion exchangers.

The principle of separation is thus by reversible exchange of ions between the target ions present in the sample solution to the ions present on ion exchangers.

Qubit fluorometer

Principle : The Qubit fluorometer is a DNA quantification device **based on the fluorescence intensity of fluorescent dye binding to double-stranded DNA (dsDNA)**. Qubit is generally considered useful for checking DNA quality before next-generation sequencing because it measures intact dsDNA.

Spectrophotometer

Principle: The spectrophotometer technique is to measure light intensity as a function of wavelength. It does this by diffracting the light beam into a spectrum of wavelengths, detecting the intensities with a charge-coupled device, and displaying the results as a graph on the detector and then on the display device.

Genetic analyzer

Principle: Genetic analyzers are automated systems capable of sequencing DNA or analyzing fragments for a variety of applications. In capillary electrophoresis-based systems, **DNA fragments bound to probes migrate through a polymer and the fluorescence emissions are measured**.

Gene sequencer/ sanger sequencer

Principle: This method is based on the principle that **single-stranded DNA molecules that differ in length by just a single nucleotide can be separated from one another using polyacrylamide gel**

electrophoresis, described earlier. One dideoxynucleotide, either ddG, ddA, ddC, or ddT.

4th week

DNA extraction, PCR amplification and gel elution

DNA extraction of the sample by using fastDNA SPIN Kit for Soil.

Protocol

- Filter paper containing the sample was cut with the help of septic scissors and forceps and added to the lysing matrix E tube.
- 978µl Sodium Phosphate buffer was added to the sample in lysing matrix E tube.
- 122µl MT buffer was added
- Vortexed the sample for 5 min
- Centrifuged the sample at 14000rpm for 5-10 min to pellet debris
- Transfered supernatant to a clean 2.0 ml microcentrifuge tube. And 250µl PPS (protein precipitation solution) and mixed by inverting the tubes 10 times.
- Sample was centrifuged at 14000 rpm for 5 min to pellet precipitate. Transfer supernatant to a clean 15 ml microcentrifuge tube.
- The binding matrix suspension was resuspended and 1.0 to the supernatant was added in the 15ml tube.
- Placed on rotator for 2 min to allow binding of DNA. Tube was placed on a rack for 3 min to allow settling of silica matrix.
- 10. Removed and discarded 500µl of supernatant being careful to avoid settled binding matrix.

- Binding matrix was gently resuspended in the remaining amount of supernatant. Approximately 600µl of the mixture was transferred to a SPIN filter and centrifuged at 14000rpm for 1 min. the catch tube was emptied and the remaining mixture was added to the SPIN filter and centrifuged as before. The catch tube was emptied again.
- 500µl prepared SEWS-M was added and gently resuspended the pellet using the force of the liquid from the pipet tip
- Centrifuged at 14000rpm for 1 min. Emptied the catch tube and replaced.
- Centrifuged a second time at 14000rpm for 2 min without addition of any liquid, to dry the matrix of residual wash solution. Discarded the catch tube with a new clean catch tube.
- Air dried the SPIN filter for 5 min at room temperature.
- Gently resuspend Binding Matric in 25 and 25µl of DES (DNase/pyrogen-free water).
- Centrifuge at 14000rpm for 1 min to bring eluted DNA into the clean catch tube. Discard the spin filter. DNA is now ready for PCR and other downstream applications. Store at -20°C for extended periods or 4°C until use.

DNA Analysis by Agarose Gel Electrophoresis

Load 5µl of the eluted DNA per well on a 1% agarose gel in 0.5* TBE electrophoresis buffer.

Etbr loading dye, a 10X solution of bromophenol blue and sucrose solution, is supplied for this application.

PCR Amplification of the given sample

For ion studies library preparations

1. Nested PCR approach using (2TF/1492 R)pro V3 – V4

Step 1: NORMAL PCR

Reaction Mixture = Final volume (50µl)

Tokara master mix= 25µl

Forward primer= 1µl

Reverse primer= 1µl

Water DNase/RNase free= 16µl

Template= 2µl

PCR conditions : 95°C for 2 mins denaturation

45°C for 30 sec annealing

72°C for 2 min extension

30 cycles

Step 2: Check on 1% agarose gel

Step 3: Nested PCR using pro V3-V4 primers

Reaction Mixture= 50µl

Takara master Mix = 25µl

Forward Primer= 1µl

Reverse Primer = 1µl

Water= 17µl

Template= 1µl

PCR conditions : 95°C for 2 min denaturation

56°C for 15 sec/ 72°C for 30sec annealing

72°C for 5 min extension

Step 4: check on 2% Agarose Gel Electrophoresis

Step 5: Extraction of band and purification

2% agarose gel in TBE buffer

100bp marker DNA

2µl loading buffer

Sample

Apply 75V

<u>Primer STAR 4S</u>

Reaction Mixture = 50µl

5X PrimeSTAR Buffer = 10µl

dNTPs mixture= 4µl

primer 1 = 1µl

primer2= 1µl

sample DNA= 1µl

primeSTARS DNA polymerase = 0.5µl

DNase free water= 32.5µl

We used Promega PCR master mix kit

Protocol for 50µl reaction mixture

PCR master Mix, 20X = 25µl

Upstream primer, 10µM= 0.5-5.0µl

Downstream primer, 10µM= 0.5-5.0µl

DNA Template= 1-5µl

Nuclease free water= 23µl

PCR conditions

95°C for 2 min denaturation

48°C for 30sec annealing

72°C for 1 – 5min extension

25- 30 cycle required

Check on 2% agarose electrophoresis

Gel Extraction

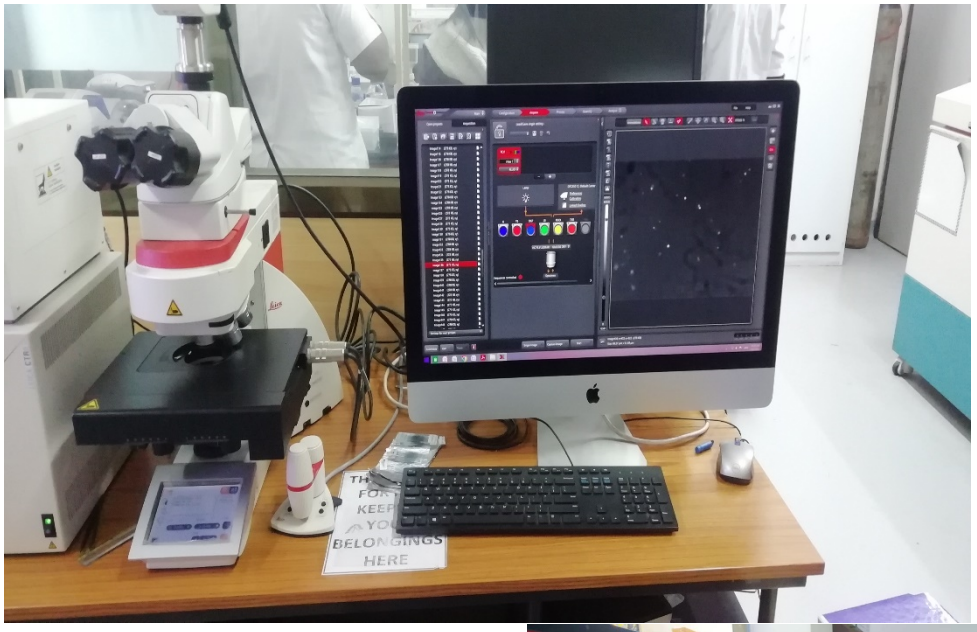
Protocol

- Desired PCR band was cut from the gel and put it into 2ml centrifuge tube .
- 10µl MBS along with gel slice
- Vortexed and incubate at 56° until slice is dissolved
- Transfered the dissolved sample into 2ml collection tube.
- 700µl MSW was added and centrifuged it for1 min at 15000rpm.
- Discarded the filtrate and used collection tube.
- 500µl MWS and centrifuged again for 1 min at 15000rmp.
- Repeated the step again at 15000rpm for 5 min.

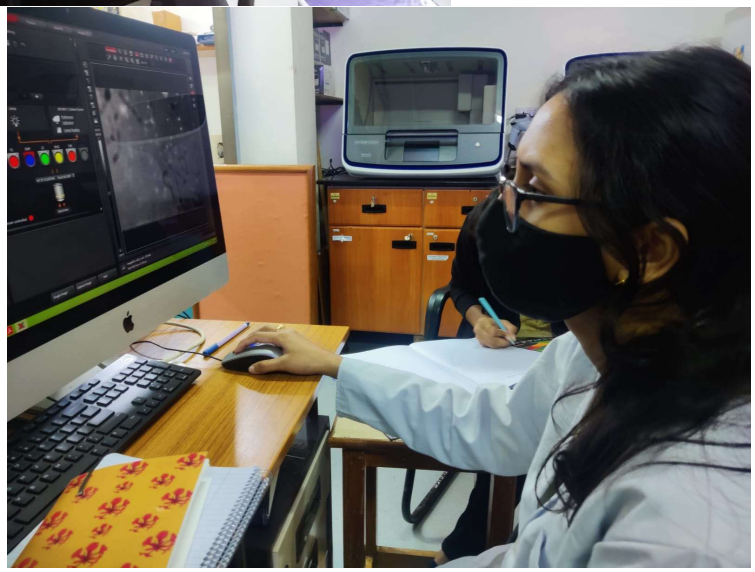
cryobiology lab

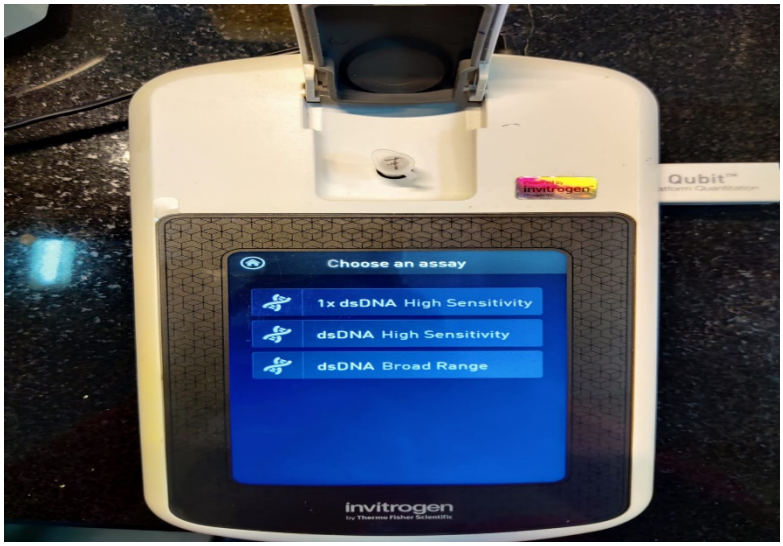
HPLC (high performance liquid chromatography)

Ion exchange-chromatography



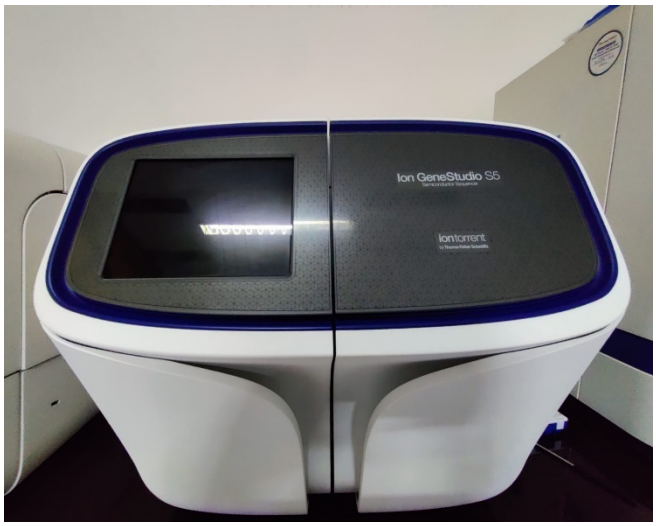
Fluorescent Microscopy

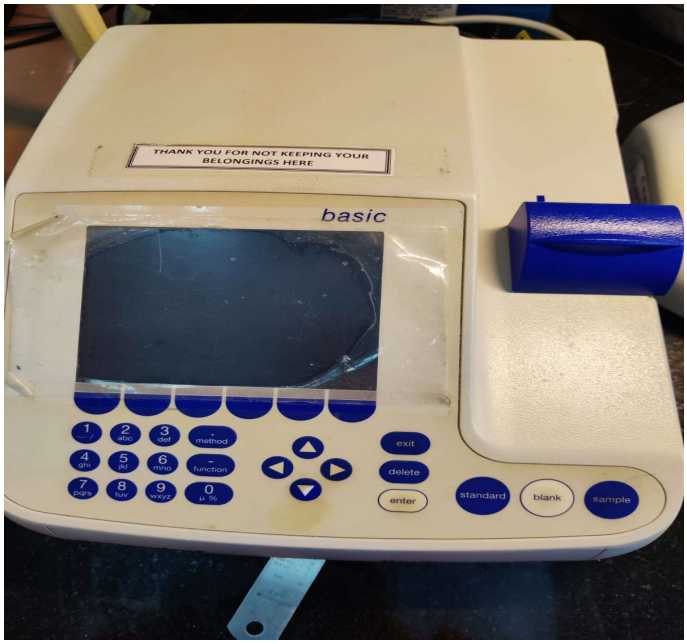




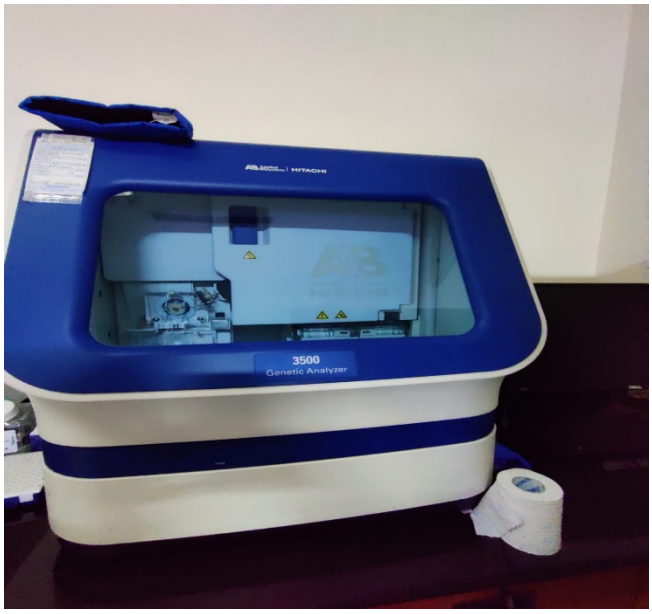
Qubit Fluorometer

Gene sequencer / sanger sequencer



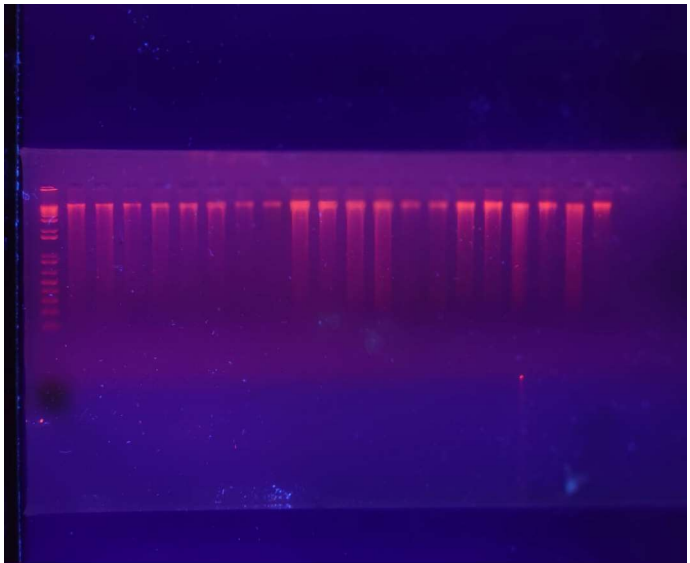


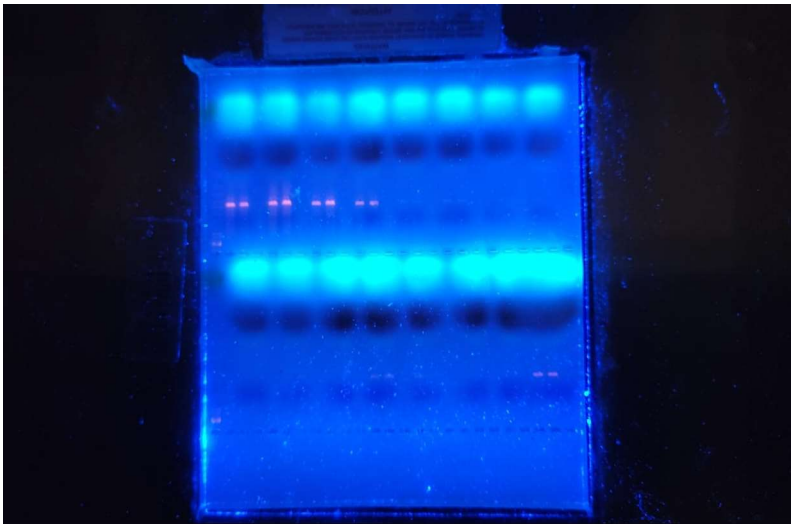
Spectrophotometer



Genetic Analyzer

DNA isolation from the given sample using fast DNA SPIN kit





PCR of the sample

Conclusion

The scientific experience I encountered during the internship at NCPOR allowed me to develop hand on experience in molecular and microbiological techniques.

I gained practical exposure to various instruments used in research laboratory. Learnt about how actually scientific research world work and how effective it is.

Overall, I fount the winter internship experience to be positive, and I’m sure I will be able to use the skills that I learnt in my career later.