Report of Internship at National Centre of Polar and Ocean Research [NCPOR]

1st December 2022 - 31st December 2022

Submitted by

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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 Mr. **Shubham Dilip Salve** 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 his internship program with us, he had been exposed to various techniques and skills in the laboratory.

We wish him every success in his 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 महासागरी अनुसंधान केन्द्र
Ministry of Earth Sciences (Govt. of India)
पृथ्वी विज्ञान मंत्रालय (भारत सरकार)
Headland Sada, Vasco-da-Gama, Goa - 403 804
हैडलैंड सडा, वास्को डा गामा, गोवा- 403 804

Archana Singh
05.04.2023
Authorized Signature
Scientist-in-Charge
Arctic Ecology & Biogeochemistry
Arctic Operations

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ACKNOWLEDGEMENT

I would like to thank Prof. Savita ma'am, Prof. Sanjeev C. Ghadi sir, Prof. Samantha ma’am, Prof. Meghnath sir, for providing this opportunity.

I would like to thank Dr. Krishnan K. P. Sir (Scientist E - NCPOR) for giving me the chance to work in the institute.

My special thanks to Dr. Anand. J(Project Scientist I - NCPOR) and Yuga ma’am (Junior research fellow - NCPOR) for explaining both theory and practical work in detail with lots of patience . I’m really grateful for your career guidance tips and motivation throughout the duration of Internship. It will definitely help me in choosing the best path and make better decisions in future.

ABOUT NCPOR

The National Centre for Polar and Ocean Research (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.

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 mandate of NCPOR is multi-dimensional: Leadership role in niche areas of scientific research in the domain of polar and ocean sciences.

With a mission mandate that is quite challenging, the Centre is designated as the nodal organization for the co-ordination and implementation of the Indian Antarctic Programme, including the maintenance of India’s permanent station in Antarctica.

NCPOR operates the Himadri and IndARC Arctic research stations in Svalbard, Norway and Himansh station in Spiti, Himachal Pradesh.

INFORMATION ABOUT THE INTERNSHIP

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

1st week

Analysis of the polar water samples collected from arctic region . Samples have been collected from different locations in the arctic (in and out stations).

Some samples were filtered to reduce the cell number hence reducing overcrowding of cells during the slide preparation .While other samples were used as unfiltered during slide preparation.

Sample filtration was done using a setup made of a column, a vaccum pump and a filter of pore size 0.2 micron. The water sample was passed through the column towards to filter and going into the tube below. The filtered sample goes into the receiving tube.

These filter papers are then stored in tubes with formalin in fridge at -20°C. Then later these filter papers with cells are subjected to DNA extraction later.

These filtered water samples thus obtained are then used for slide preparation to visualize the cells by fluorescence microscopy. DAPI was added to the tubes containing water samples to visualize cells as it is a fluorescent dye. The cells are visualized with oil at 10X objective lens.

Different types of cells were visualized shaped cocci and rods of varying sizes. The cell counts were done for these samples. Volume of cocci shaped cells were calculated by the formula of a sphere i.e. 4/3 πr^3 and the volume of rod shaped cells were calculated by the formula – $\pi r^2[L-2/3\ r]$

Then later the cell volumes were calculated for the cells visualized. Volumes for cocci and rods both were calculated. Up to 200 cell's volumes were obtained.

All the information thus obtained was put up in a tabular form for further analysis of samples.

2nd Week:-

Media preparation was done once filtered cell samples were obtained after performing the sample filtration and cell count.

Modified basal media was used of 4 different compositions , either of which didn't have yeast extract and peptone. MBM acted as a selective media so as to get the growth of desired microbes by limiting the nutrients and prevent growth of undesirable microbial species.

1. MBM + Sodium Alginate + Agar
2. MBM + Laminarin + Agar
3. MBM + Mannitol + Agar
4. MBM + Agar

Trace metals and vitamins were added to each of these 4 types of media and PO_4 was added to them media after autoclaving to increase the buffering capacity.

8 plates of each type of media were made for the spread plate technique and last 3 dilutions were used out of the 4 serial dilutions for plating the cultures.
The spread plate technique was performed and the plates were incubated for 2-3 weeks .

Meanwhile, the DNA extraction experiment was performed for the filtered samples which were stored at -20°C. The DNA extraction was performed using FastDNA™ Kit.

1. The filter papers(having samples) were cut into pieces and transferred to fresh tubes.
2. To these tubes 978µL sodium phosphate butter was added then 122µL MT buffer after that.
3. Then these tubes were centrifuged at 14000 rpm for 5-10 minutes.
4. The supernatant thus obtained was transferred to fresh (2ml) microcentrifuge tubes and 250µL of PPS (Protein Precipitation Solution) was added to each of them respectively and mixed properly by inverting the tube 10 times.

5. These tubes were centrifuged at 14000 rpm for 5 minutes to pellet precipitate. Then the supernatant thus obtained was transferred to a clean (15ml) microcentrifuge tube.
6. Resuspended the binding matrix suspension and added 1mL to the supernatant in the 15mL tube.
7. They were placed on rotor or inverted by hand for 2 minutes to allow binding of DNA. The tube was placed in rack for 3 minutes to allow settling of the silica matrix.
8. Then 500µL of the mixture was discarded from the tubes.
9. Approximately 600µL of the mixture was transferred to the SPIN™ Filter and was then centrifuged at 14000 rm for 1 minute. The catch tube was emptied and the remaining mixture was added to the spin filter and centrifuged again the same way as before. The catch tube was emptied again later.
10. 500µL of SEWS-M solution was added and gently resuspended the pellet using the force of the liquid from the pipet tip.
11. It was then centrifuged at 14000 rpm for 1 minute. The catch tube was emptied and replaced.
12. Without the addition of any liquid they were centrifuged again at 14000 rpm for 2 minutes to dry the matrix of residual wash solution. The catch tube was discarded and replaced with a new clean catch tube.
13. Air dried the SPIN Filter for 5 minutes at room temperature.
14. Gently resuspended the binding matrix (above the SPIN filter) in 25µL DES (DNase/Pyrogen-free water) twice.
15. It was again centrifuged at 14000 rpm for 1 minute to bring eluted DNA into the clean catch tube. The SPIN filter was discarded.

The obtained DNA is now ready for the PCR analysis and other applications as per required. The DNA thus obtained is stored at -20°C for extended periods or 4°C until use.

3rd Week :-

DNA analysis by Agarose gel Electrophoresis

5µL of eluted DNA was loaded into each well on 1% agarose gel in 0.5X TBE electrophoresis buffer. Etbr loading dye, a 10X solution of bromophenol blue and sucrose solution, are used for this application.

PCR Amplification of the given sample

For ion studies library preparations

1. Nested PCR approach using (2TF/1492R)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 minutes denaturation

45 °C for 30 sec annealing

72 °C for 2 minutes extension

30 cycles

Step 2 : Checked on 1% agarose gel

Step 3 : Nested PCR using V3-V4 Primers

Reaction mixture : 50µL

Takara mastermix : 25µL

Forward primer : 1µL

Reverse primer : 1µL

Water : 17µL

Template : 1µL

PCR Conditions : 95 °C for 2 minutes denaturation

56 °C for 15 sec/72 °C for 30 sec

72 °C for 5 min extension

Step 4 :- Checked by % agarose gel electrophoresis

Step 5 : Extraction of band and purification

2% agarose gel in TBE buffer

100 bp marker DNA

2µL loading buffer

Sample

Apply 75V

Primer STAR 4S

Reaction mixture = 50µL

5X PrimeSTAR buffer= 10µL

dNTPs mixture = 4µL

primer1= 1µL

primer2= 1µL

Sample DNA = 1µL

PrimeSTARS DNA polymerase= 0.5µL

DNase free water =32.5µL

We used Promega PCR master mix kit

Protocol 50 reaction mixture

PCR Mastermix,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 mins denaturation

48 °C for 30 sec annealing

72 °C for 1-5 min extension

25-30 cycles required

Check on 2% agarose gel electrophoresis

Other instruments studied

The **Ion Chef System** provides automated library preparation, template preparation, and chip loading for users. In less than 15 minutes of up-front hands-on time and with the use of pre-packaged library preparation reagent kits, the Ion Chef System provides a convenient walk-away workflow resulting in equalized, pooled libraries ready for templating. The automated workflow supports Ion AmpliSeq one- and two-pool designs.

It is a Sangers sequencer.

The **Applied Biosystems 3500** and **3500xL Genetic Analyzers** are supplied as follows:

- Capillary electrophoresis instrument
- 8-capillary (3500 System) or 24-capillary (3500xL System) array and polymer
- DNA sequencing and/or fragment analysis reagents and consumables for system qualification
- Dell™ computer workstation with flat-screen monitor
- Integrated software for instrument control, data collection, quality control, and auto-analysis of sample files for basecalling and fragment sizing.

HPLC is a separation technique in which liquid is used to force the sample at high pressure through a column packed with stationary phase in order to identify and/or quantify the components.

A typical **HPLC system** consists of a pump (consistent flow rate is key), an injector or autosampler, and at least one detector. Also, HPLC systems often use a column oven to adjust and control the temperature of the separation step.

A variety of detector types are available, including UV-VIS, refractive index (RI), photo diode array, and fluorescence. Biochemistry, biology, and the pharmaceutical industry all make use of the high resolution and fast separation possible with an HPLC system.

The **Ion GeneStudio S5 System** is a semiconductor-based next-generation sequencing (NGS) system that enables simple targeted sequencing workflows. The Ion GeneStudio S5 System is optimized to provide affordability for labs focused on smaller panels and lower sequencing throughput requirements.

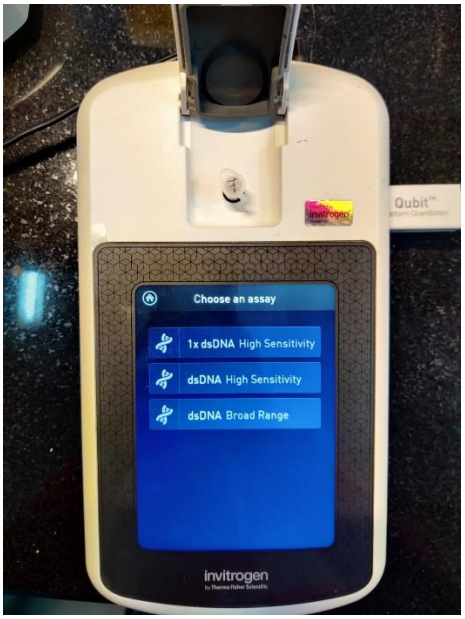
With cartridge-based reagents, the system is simple to use and offers scalability and flexibility. It uses Ion 5 Series chips to support a broad range of high-throughput sequencing for clinical research and research applications from microbial genomes and gene panels to exomes and transcriptomes.

Key features:

• Select the chip (Ion 510–540 chip) that fits particular application and throughput needs to achieve from 2–80 million reads per run

• Reduce setup time and complexity with cartridge-based plug-and-play reagents

• Complete run and analysis in hours.



Qubit Fluorometers detect fluorescent dyes specifically bound to the target molecule. With optimized Qubit Assays, they can distinguish dsDNA from ssDNA or intact from degraded RNA, even in extremely small amounts or in the presence of contaminants. A simple, intuitive interface and onboard calculators streamline the process. Results appear within seconds and can be exported to a computer or downstream device.

Qubit provides RNA assays to detect both large, intact RNA molecules (such as rRNA or large mRNA) and small, intact RNA molecules (such as microRNA and siRNA). These assays are highly selective for their RNA type, even in the presence of DNA or the other RNA type, or of common contaminants. In this sensitivity test, both the Qubit RNA and microRNA assays measured quite close to the actual concentrations of their target RNAs.

Qubit Fluorometers are orders of magnitude more sensitive than UV absorbance, an alternative method that can quantify nucleic acids due to their absorption of ultraviolet light at 260 nm.

Savant™ SpeedVac™ Integrated Vacuum Concentrator Systems and Kits

Dry and concentrate aqueous or non-aggressive samples with speed and efficiency with Thermo Scientific™ Savant SPD1030 and SPD2030 Integrated SpeedVac™ Systems and kits. Fully integrated for fast installation, programming. Ideal for drying DNA/RNA precipitates, PCR samples, proteins, enzyme, HPLC fractions in plates or tubes in water/ethanol or isopropanol and other organic solvents. SpeedVac SPD1030 System is designed for medium capacity (i.e.,120 x 1.5 mL tubes, other formats). SpeedVac SPD2030 System allows higher capacity (i.e.,200 x 1.5 mL tubes, other formats).

The **Eppendorf BioSpectrometers** are small, very compact spectrophotometers that allow users to conduct measurements in the UV/ Vis and fluorescence range. Absorbance spectra can be recorded, and individual wavelength measurements can be conducted, in a spectral range of 200 nm to 830 nm.

Conclusion

From my internship at NCPOR, I gained hands on experience in Molecular biology and Microbiological techniques.

I gained practical exposure to various instruments and techniques used in research laboratory.

I got a clear idea of how to do a high quality research with proper knowledge and skills.

I got a better understanding of how the scientific research work progresses and how effective it is.

I gained a lot of interest in research after completing the internship.

Overall, I found the winter internship experience to be positive, and I'm sure I will be able to use the skills I learned in my career in future.