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Veksler and Baldin laboratory of High Energy Physics

FINAL REPORT ON THE START PROGRAMME

Molecular Identification of Sponges by PCR and Electrophoresis, and Experimental Determination of Distribution Coefficients

Supervisor:
Dr. Ayagoz Baimukhanova

Student:
Minyar Laouini, Tunisia,
Faculty of Sciences of Tunis,
University of Tunis El Manar

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Abstract

Tow independent experiments were conducted in this study to explore distinct applications of modern laboratory techniques. In the first experiment, the internal transcribed spacer 1 (ITS1) region was amplified by polymerase chain reaction (PCR) from ten sponge specimens collected form Lake Sevan. The desired fragment of 800–900 (bp) was obtained, along with non-specific products of 500–600 bp. Target bands were isolated using agarose gel electrophoresis, excised, and confirmed by re-electrophoresis. The purified DNA is to be sequenced, and comparison with reference databasesis expected to allow species identification and provide insights into sponge phylogeny.

In the second experiment, the distribution coefficient (Kd) was determined to describe of elements between immiscible phases, offering insights into ionic interactions. Together, these investigations highlight complementary aspects of biological and chemical analysis, emphasizing the importance of experimental approaches in advancing scientific understanding.

Keywords : PCR , ITS1 region , Sponge, Lake Sevan, DNA sequencing, Distribution coefficient, ICP-MS.

Introduction

Modern scientific research relies on experimental approaches that span multiple disciplines, from molecular biology to environmental chemistry, in order to understand both living systems and the behavior of elements in natural environments. Two such approaches, the Polymerase Chain Reaction (PCR) and the determination of distribution coefficients (Kd), illustrate the diversity and importance of laboratory methods in addressing fundamental scientific questions.

PCR, developed in the 1980s, enables exponential amplification of specific DNA sequences from minimal amounts of genetic material. It has become a cornerstone of molecular biology with wide applications in diagnostics, forensic investigations, and biodiversity studies [1] . In ecology, PCR is particularly valuable for organisms whose morphology is variable or difficult to interpret, such as freshwater sponges. Amplification of ribosomal regions like the internal transcribed spacer (ITS1) provides robust molecular markers that can be sequenced and compared to databases, allowing accurate identification and phylogenetic analysis [2]. In this study, PCR was applied to sponge specimens from Lake Sevan to support biodiversity assessment.

In environmental and radiochemical sciences, the distribution coefficient (Kd) is a key parameter describing how an element partitions between solid and liquid phases. It provides a quantitative measure of sorption efficiency and mobility, with high Kd values indicating strong retention and low values suggesting higher transport potential. Kd is essential for modeling contaminant migration, evaluating nuclear waste safety, and developing separation strategies[3] . It is most often determined by the batch method, in which a solid (e.g., resin, soil, or mineral) is equilibrated with solution and the concentrations are analyzed. Sensitive analytical techniques such as ICP-OES and ICP-MS are widely used, while radioactive tracer methods remain crucial for radionuclide studies . In this work, Kd values were determined for multiple elements on a Dowex 50×8 resin under different acid conditions to explore ion-exchange behavior relevant to radiochemistry.

The motivation for combining these two independent studies lies in the importance of understanding both molecular-level biological diversity and macroscopic geochemical processes. PCR-based identification of sponges illustrates the power of molecular tools in biodiversity research, while Kd measurements highlight the methodologies used to assess contaminant behavior in the environment. Together, these experiments provide valuable insight into how

theoretical knowledge is translated into laboratory practice and how such practice contributes to broader scientific and societal questions.

The objectives of the present work are therefore threefold:

- To conduct PCR amplification of selected DNA samples and analyze the products by gel electrophoresis.
- To prepare samples for sequencing and future phylogenetic analysis
- To perform Kd experiments and determine distribution coefficients under controlled conditions using standard analytical methods.
- To discuss the broader significance of these techniques, both in their respective fields and in terms of scientific practice.

Part I : PCR Amplification of ITS1 Region from Lake Sevan Sponges

1.Polymerase Chain Reaction (PCR)

1.1Definition of Polymerase chain reaction (PCR) :

Polymerase chain reaction (PCR) is a molecular biology technique developed in 1983 by Kary Mullis. It is an in vitro method that allows specific amplification of a DNA sequence, producing millions or even billions of copies of a particular region from a very small initial quantity [4].

1.2Principle of PCR :

There are three major steps in a PCR, which are repeated for 30 or 40 cycles. This is done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time.

- **Denaturation:**
  The DNA sample is heated to approximately 95°C for about 30 seconds. This high temperature causes the two complementary strands of the double-stranded DNA to separate, resulting in single-stranded DNA templates.
- **Annealing :**
  The temperature is lowered to around 50–60°C. At this temperature, short DNA sequences called primers bind (anneal) specifically to their complementary sequences on the single-stranded DNA. These primers define the region of DNA to be amplified.
- **Extension :**
  The temperature is raised to approximately 72°C, the optimal temperature for the thermostable DNA polymerase enzyme (such as Taq polymerase). The enzyme synthesizes a new complementary DNA strand by adding nucleotides to the primers, extending the DNA sequence [5].

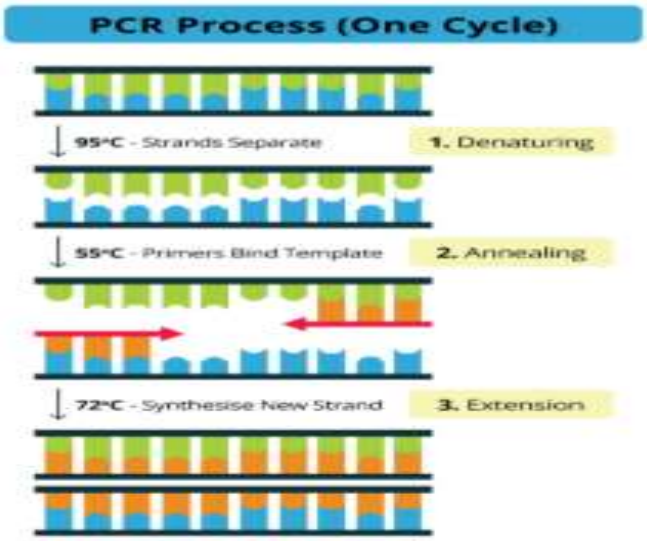

Figure 1 : PCR Process (One Cycle)[6].

2.Agarose Gel Electrophoresis

Agarose gel electrophoresis is a technique where DNA, RNA or proteins are separated in an electric field based on their size, using a gel formed from a polysaccharide (agarose, which can be derived from seaweed). During the electrophoresis process, as the gel is immersed in a buffer solution, negative electrodes are drawn towards the positive electrode, while smaller particles are able to move more rapidly through the gel in comparison to larger particles [7].

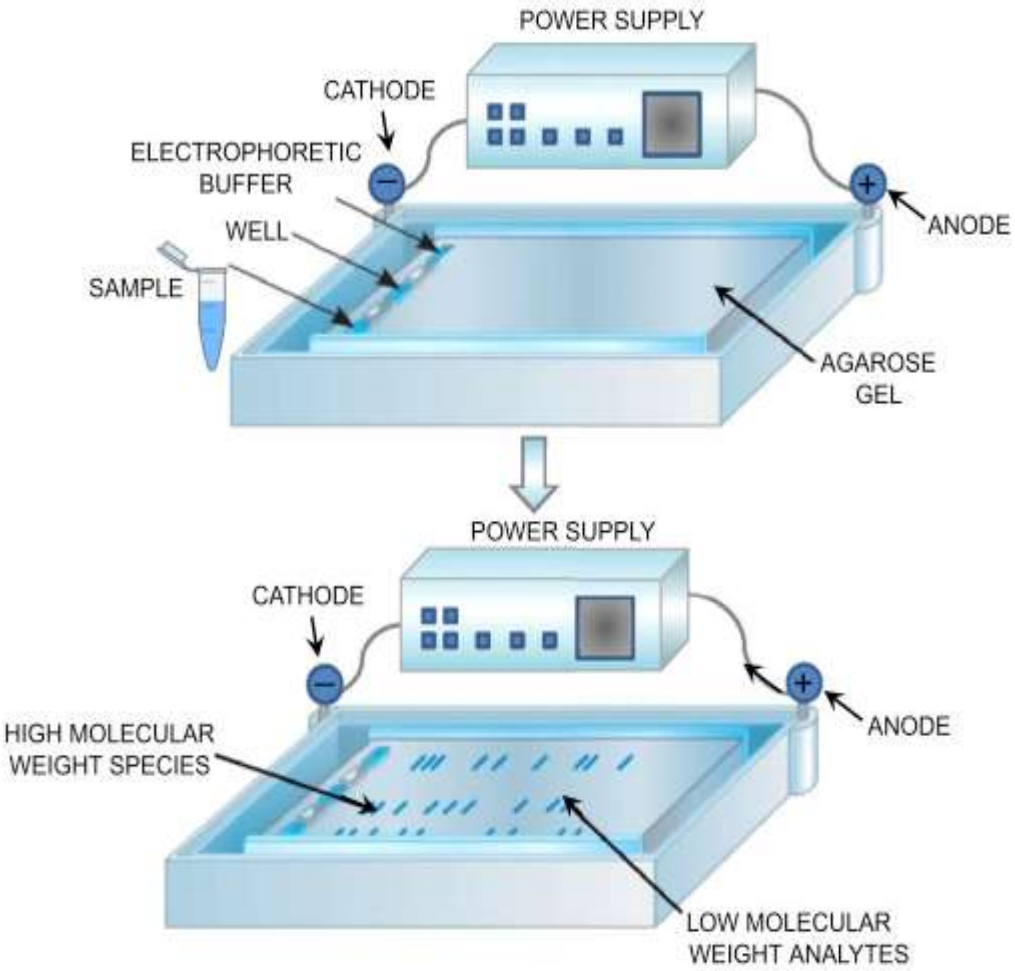

Figure 2 : Agarose electrophoresis system [8].

- After electrophoresis, DNA fragments are typically visualized using ultraviolet (UV) light. This is achieved by incorporating fluorescent dyes, such as ethidium bromide, which intercalate between the bases of the DNA double helix. When exposed to UV illumination, these dye–DNA complexes emit fluorescence, producing visible bands that correspond to DNA fragments of different sizes [9].

3.Materials and methods :

3.1 PCR Master Mix preparation:

To minimize pipetting errors and ensure uniformity across reactions, a PCR master mix was prepared. The composition of the reaction mixture (final volume of 20 µL per sample) was as follows :

- 10 µL of diluent
- 8 µL of primers
- 1 µL of nuclease-free water (H_2O)

For 10 reactions, the total reagent volumes were calculated. To account for potential pipetting 0losses and to guarantee sufficient volume, all components were multiplied by a factor of 1.1 (equivalent to 11 reactions). The resulting master mix was then aliquoted into individual PCR tubes.

3.2 PCR Solution Preparation and Amplification:

For each individual reaction, 1 µL of DNA template was added to the PCR tube containing the master mix. The total reaction volume was adjusted to 20 µL. The master mix was prepared in sufficient quantity for ten DNA samples.

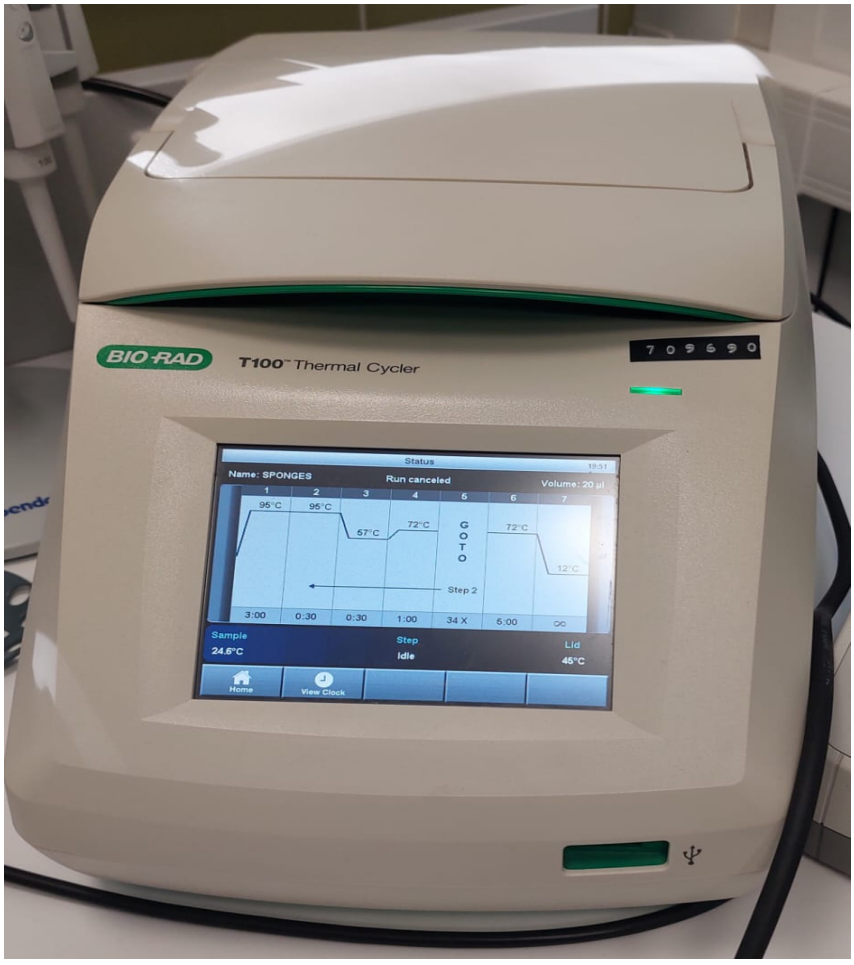

Figure 3 : PCR thermocycler used in the laboratory

3.3 Agorose Gel Preparation

For electrophoresis, a 1% agarose gel was prepared by dissolving 0.3 g of agarose in 30 ml of 1X TAE buffer, followed by heating in a microwave oven until complete dissolution. Ethidium bromide was added at a final concentration of 0.5 µg/ml for DNA staining.

The molten agarose was poured into a casting tray with a comb to form wells and left to solidify for ~30 minutes. Once solidified, the comb was carefully removed, and the gel was placed into the electrophoresis chamber containing 1X TAE buffer. DNA samples were mixed with 6X TriTrack DNA Loading Dye and loaded into the wells, alongside 2 µl of GeneRuler DNA Ladder Mix used as a size marker. Electrophoresis was performed at a voltage of 1–10 V/cm of gel length until proper separation was achieved.

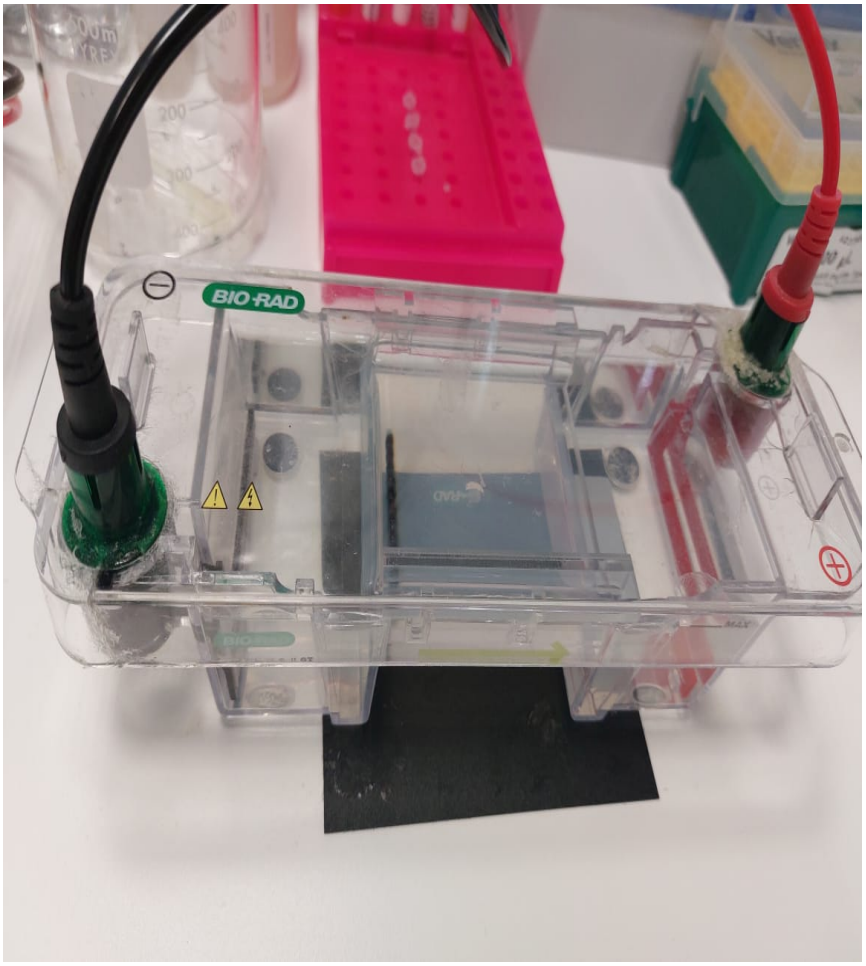

Figure 4 : Agarose Gel Electrophoresis chember with gel and power supply

3.4UV Visualization

At the end of electrophoresis, the gel was transferred onto a UV transilluminator.

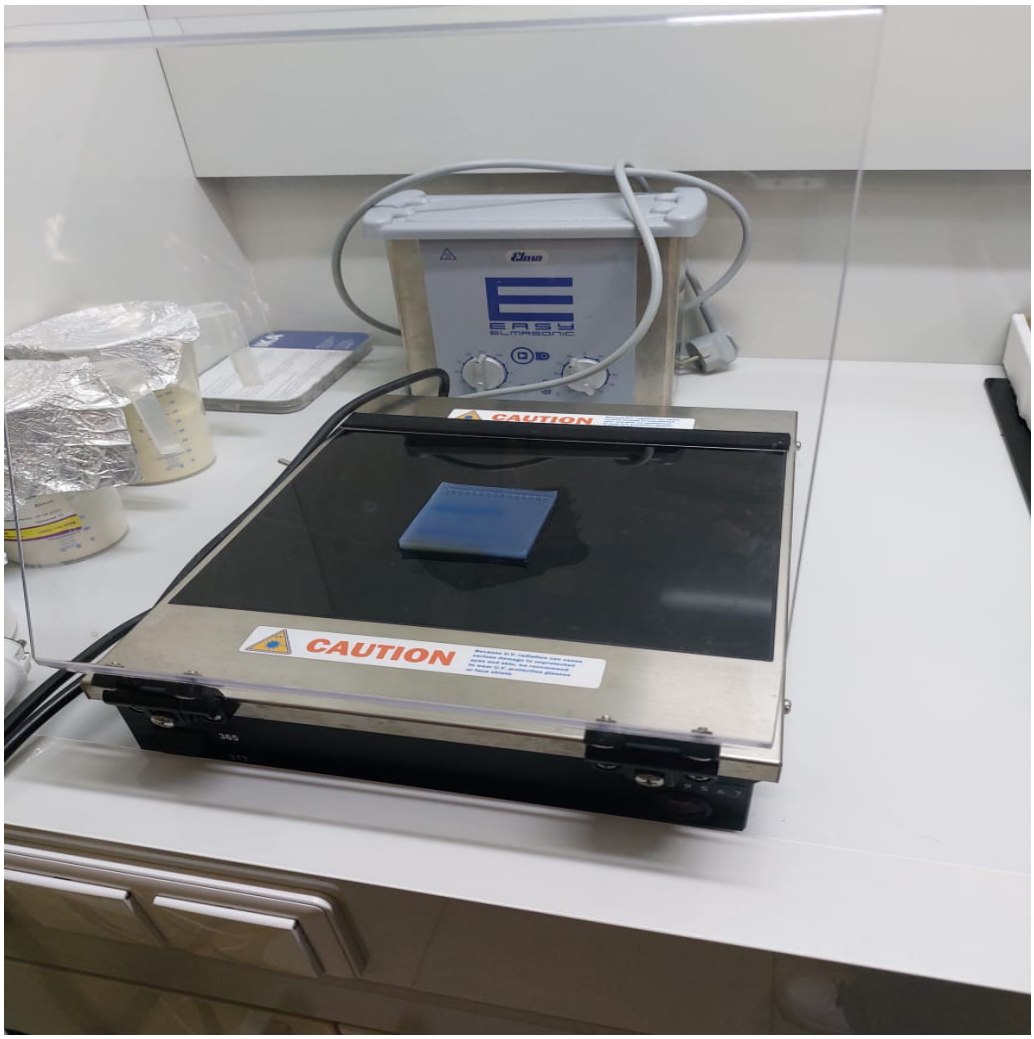

Figure 5 : UV transilluminator used in the laboratory

4.Results and Discussion :

4.1 PCR Product

The agarose gel electrophoresis of the amplified DNA revealed two distinct bands in the majority of sample lanes (Figure 6). The first band, migrating at approximately 800–900 base pairs, corresponded to the expected ITS1 region. However, a second, undesired band was also visible around 500–600 base pairs, indicating the presence of a non-specific amplification product. A molecular DNA ladder was included to provide accurate size estimation of the observed fragments.

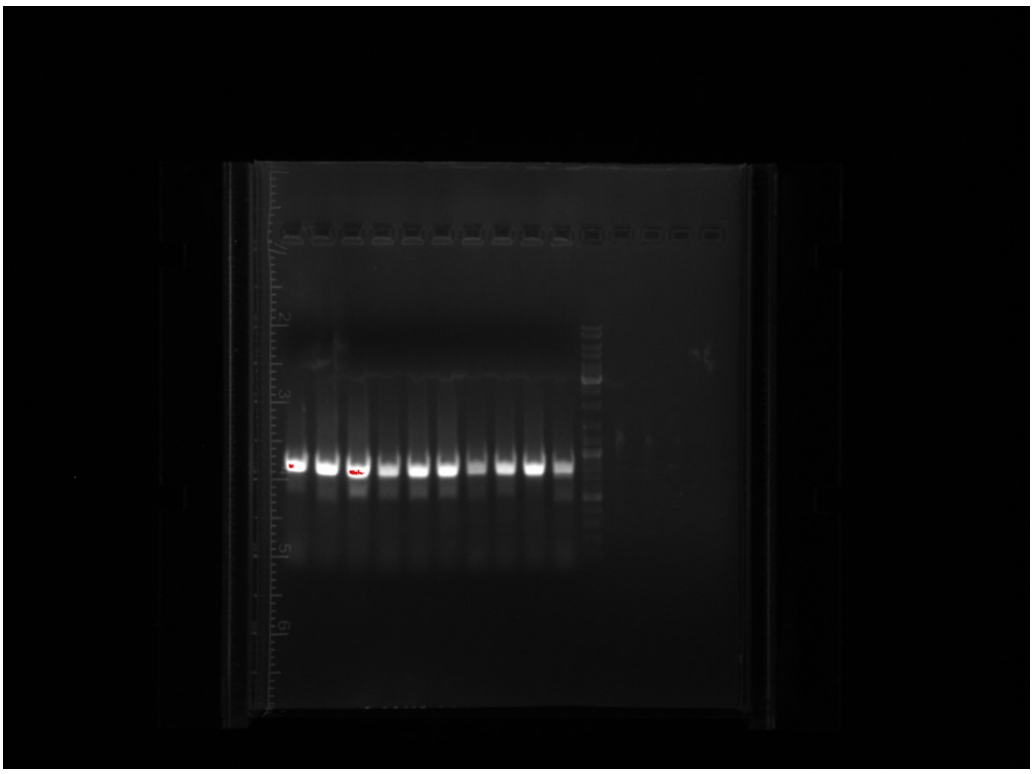

Figure 6 :Electrophoresis gel image of PCR products showing both the desired ITS1 fragment (~800–900 bp) and an additional non-specific fragment (~500–600 bp).

The electrophoresis profile demonstrates that while the primers successfully amplified the ITS1 region, they also showed a degree of non-specificity, producing an additional fragment. This outcome is relatively common in PCR experiments, especially when working with complex DNA templates such as sponge samples. The presence of two bands highlights the need for purification to ensure that downstream analyses are based only on the correct DNA fragment. To address this issue, the desired band at ~800–900 bp was carefully excised from the gel, and the DNA was purified using the Cleanup Mini Kit (Evrogen). This step was crucial to eliminate the shorter, non-specific fragment and to prepare high-quality DNA for subsequent analysis.

4.2 Desired Fragment

Following purification of the desired bands, a second round of PCR was performed to validate that only the ITS1 fragment was retained. The resulting agarose gel image displayed a single, sharp band corresponding to ~800–900 base pairs across all lanes (Figure 7). Importantly, the non-specific 500–600 bp fragment observed in the initial amplification was no longer present, confirming the effectiveness of the purification procedure.

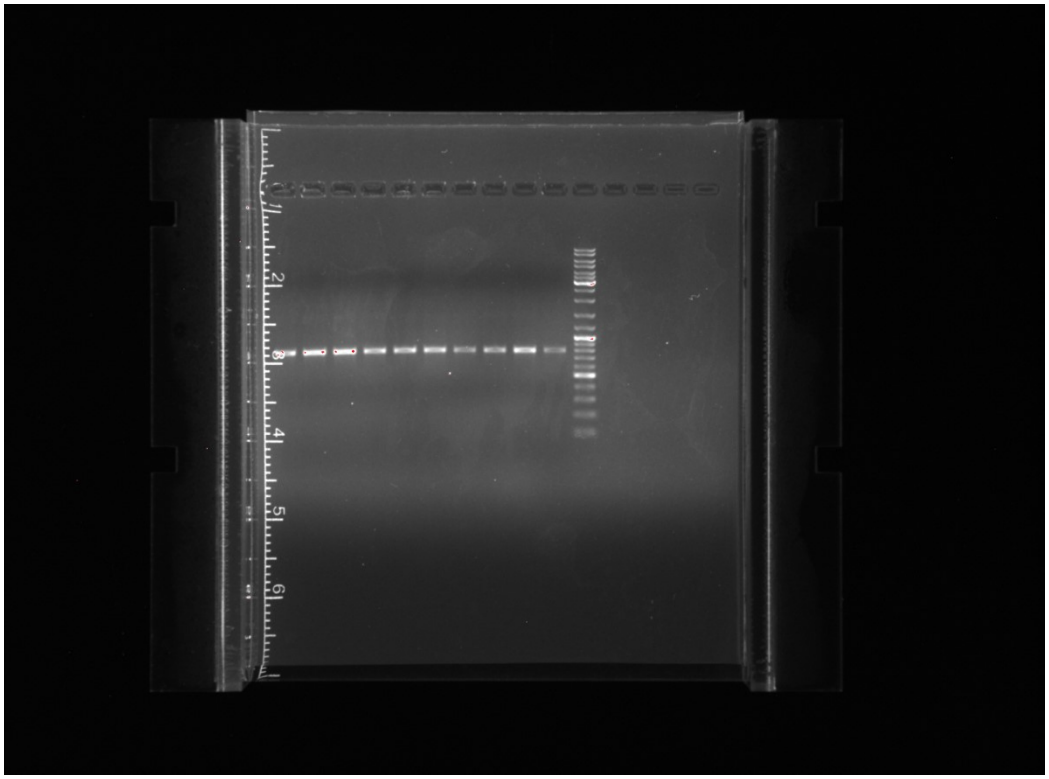

Figure 7 : Electrophoresis gel image depicting purified ITS1 DNA fragments after gel extraction and re-amplification.

The purified DNA profile clearly demonstrates that the cleanup procedure successfully removed the undesired products. The single, well-defined band in the gel reflects both the specificity and the reliability of the second PCR amplification. The absence of smearing or secondary bands indicates that the DNA fragments were intact and of sufficient purity for downstream applications. By eliminating the ~500–600 bp fragment, the procedure ensured that only the correct ITS1 region was retained, paving the way for sequencing and phylogenetic analysis. These results emphasize the importance of combining gel extraction with re-amplification in order to achieve both accuracy and reproducibility in molecular studies.

Part II : Distribution Coefficient

1.Distribution Coefficient (Kd)

1.1Definition of the Distribution Coefficient (Kd)

The distribution coefficient, usually denoted as Kd, is a parameter that describes the equilibrium distribution of a solute between two phases: a solid phase

(such as an ion-exchange resin) and a liquid phase (the solution in contact with the resin). It is mathematically defined as:

$$K_d = \frac{C_{Solid}}{C_{Solution}}$$

Where:

- C_{Solid} = concentration of the elements in solid phase per (mg/g) of dry DOWEX
- $C_{Solution}$ = concentration of elements per (mg/mL) in the mobile phase

The value of Kd is expressed in units of mL/g[10] .

High Kd values indicate strong sorption of the analyte onto the resin, while low values imply weak sorption and predominance in the liquid phase. This simple parameter is, therefore, a powerful tool for characterizing the sorption behavior of elements in ion-exchange systems [11].

1.2Determination

In practice, Kd is usually determined by the batch method, where a known mass of solid sorbent (m) is equilibrated with a solution of volume (V) containing the target element at an initial concentration . After equilibrium, the remaining concentration in solution is measured. The Kd can then be calculated as:

$$K_d = \frac{(C_0 - C) \times V}{C \times m}$$

- C_0 = concentration in control solution (mg/L)
- C = concentration in equilibrium solution (mg/L)
- V = volume of solution (mL)
- m= mass of dry resin (g)

This equation reflects the ratio of the amount retained on the solid phase to the concentration in the solution. Analytical methods such as ICP-OES or ICP-MS are commonly used to measure and with high precision [12].

1.3Application

Kd values are critical for evaluating the migration and retention of elements in natural and engineered systems. They are used in environmental studies to model contaminant transport in soils and sediments, in nuclear waste management to predict radionuclide mobility, and in radiochemistry for optimizing separation methods, ion-exchange chromatography, and the design of radionuclide generators for medical applications[13].

2.Materials and methods :

2.1Materials

- **Resin:**
  Dowex 50×8 cation-exchange resin, 100–200 mesh, H^+ form.
- **Solutions:**
  Multi-element calibration standard ICP-MS-68B-250 Solution A (48 elements, 100 mg/L in 4% HNO_3, High Purity Standards, USA).
  Nitric and phosphoric acids for adjusting acidity.
- **Equipment:**
  15 mL polypropylene tubes.
  ICP-OES instrument for elemental analysis.

2.2Preparation of Samples

Polypropylene tubes (15 mL) were filled with 300 mg of Dowex 50×8 resin in H^+ form. Each tube received 5 mL of a solution containing 1.25 mL of the multi-element calibration standard and the appropriate acid mixture to achieve the desired acidity. Control samples, prepared identically but without resin, were included to determine baseline concentrations.

2.3Experimental Procedure

The experiments were performed at eight different acid concentrations to assess the influence of acidity on sorption. Tubes were kept at room temperature for two weeks to allow equilibrium to be established between the resin and the solution. After equilibration, the liquid phase was carefully separated from the resin for further analysis.

Conclusion

This work combined molecular and chemical approaches to demonstrate the value of interdisciplinary laboratory research. The ITS1 region of sponge DNA from Lake Sevan was successfully amplified by PCR, and agarose gel electrophoresis confirmed recovery of the target fragment, providing material suitable for sequencing and phylogenetic analysis. In parallel, distribution coefficients (Kd) of multiple elements on Dowex 50×8 resin were determined under different acid conditions using the batch method and ICP-OES, yielding insight into sorption efficiency and ion-exchange selectivity.

Together, these studies highlight how PCR contributes to biodiversity assessment while Kd determination informs radiochemical separations and environmental safety, underscoring the importance of integrating biological and chemical techniques in modern research.

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