Genetic identification of freshwater sponge lineages from Lake Sevan, Armenia

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Introduction

Freshwater sponges (Porifera) are important yet often undervalued components of aquatic ecosystems, contributing to water filtration and nutrient cycling. Lake Sevan, the largest lake in the Caucasus region, harbors unique biodiversity that remains poorly understood, particularly in regard to its sponge populations.

This practical course is designed to introduce students to basic molecular biology techniques by identifying sponge species collected from Lake Sevan through sequencing and phylogenetic comparison with other known freshwater sponge species.

Throughout this module, students will perform a complete molecular phylogenetic pipeline, including:

Polymerase chain reaction (PCR) – amplification of the ITS region from extracted sponge DNA (Fig.1).

Gel electrophoresis – verification of amplicon size (Fig.2).

Ligation – insertion of the ITS fragment into a plasmid vector.

Transformation – introduction of the recombinant plasmid into *E. coli* cells.

Blue-white screening – identification of colonies carrying the insert (Fig.3).

Plasmid DNA extraction – isolation of plasmid DNA from candidate positive clones.

Restriction enzyme digestion and gel electrophoresis – confirmation of insert length.

After the aforementioned steps, the plasmid will be submitted to a sequencing company and further analysis will be conducted using previously sequenced samples.

Phylogenetic analysis – interpretation of sequence data to infer evolutionary relationships.

For phylogenetic reconstruction, we will target the internal transcribed spacer (ITS) region of ribosomal DNA. The phylogenetic analysis will include multiple sequence alignment and tree reconstruction using the maximum likelihood method (Fig. 4), including searching the NCBI GenBank database and using Clustal Omega and MEGA software to construct the phylogenetic

tree.

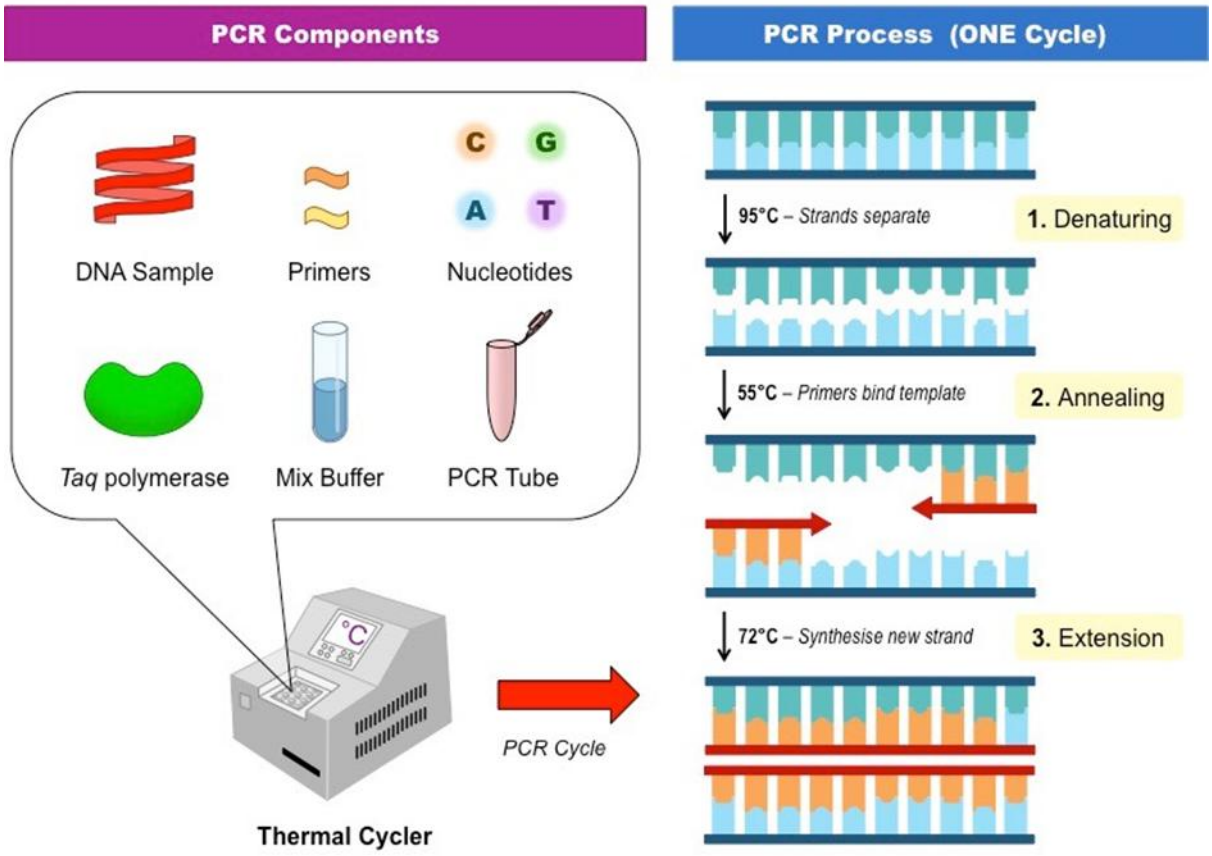

Figure 1. Overview of a Polymerase Chain Reaction cycle (https://old-ib.bioninja.com.au/standard-level/topic-3-genetics/35-genetic-modification-and/pcr.html).

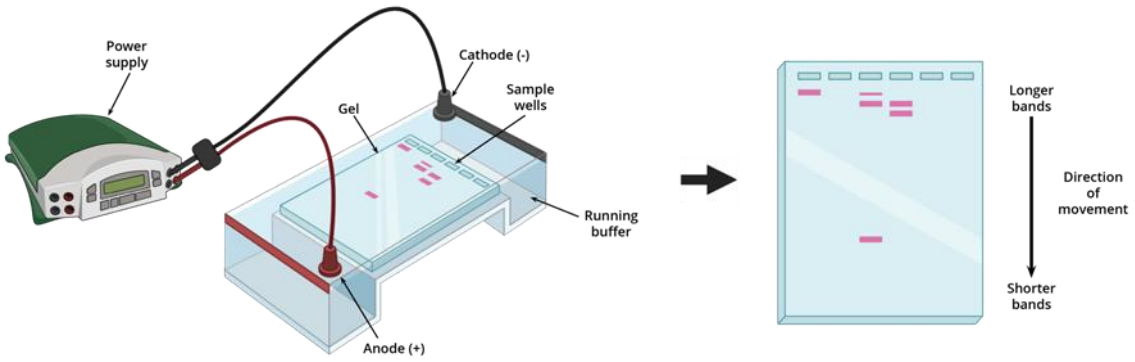

Figure 2. An illustration of gel electrophoresis workflow (https://www.stratech.co.uk/aat-bioquest/gel-electrophoresis/).

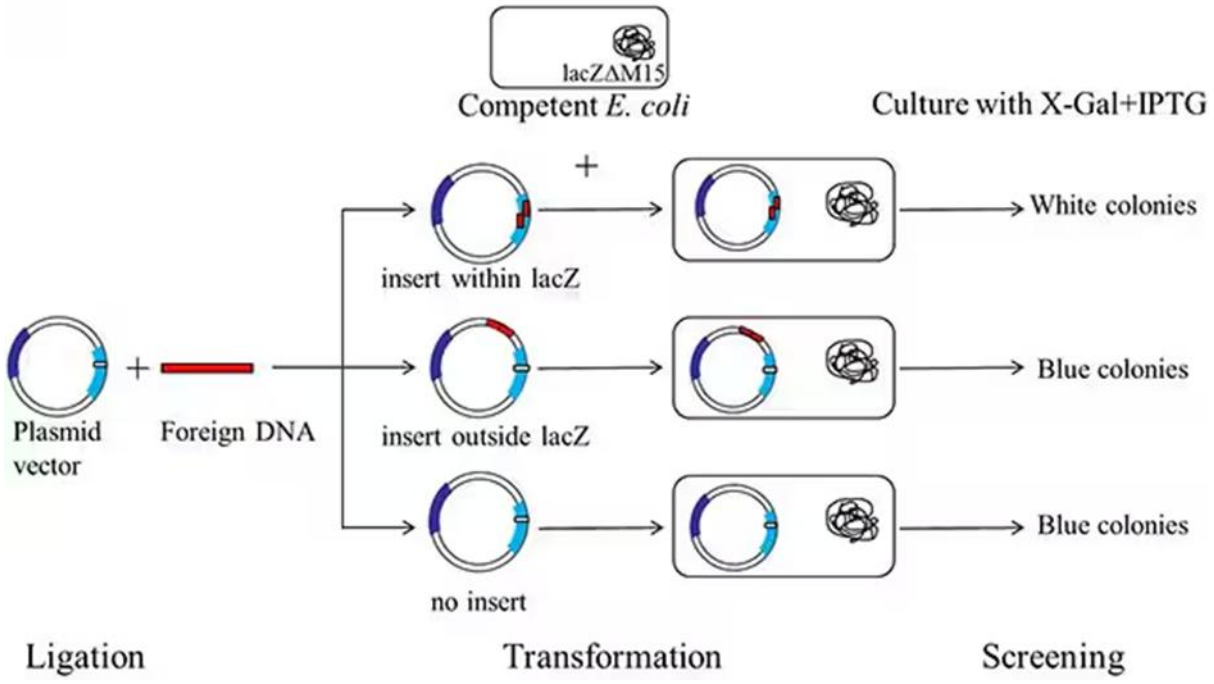

Figure 3. A schematic representation of a typical blue-white screening procedure (https://www.sigmaaldrich.com/RU/en/technical-documents/technical-article/genomics/cloning-and-expression/blue-white-screening?srsltid=AfmBOoqRJgE0RGeO_PMK2EBNZX5dNrjO6Yl4zAGrHGFBARulyotT9Izy).

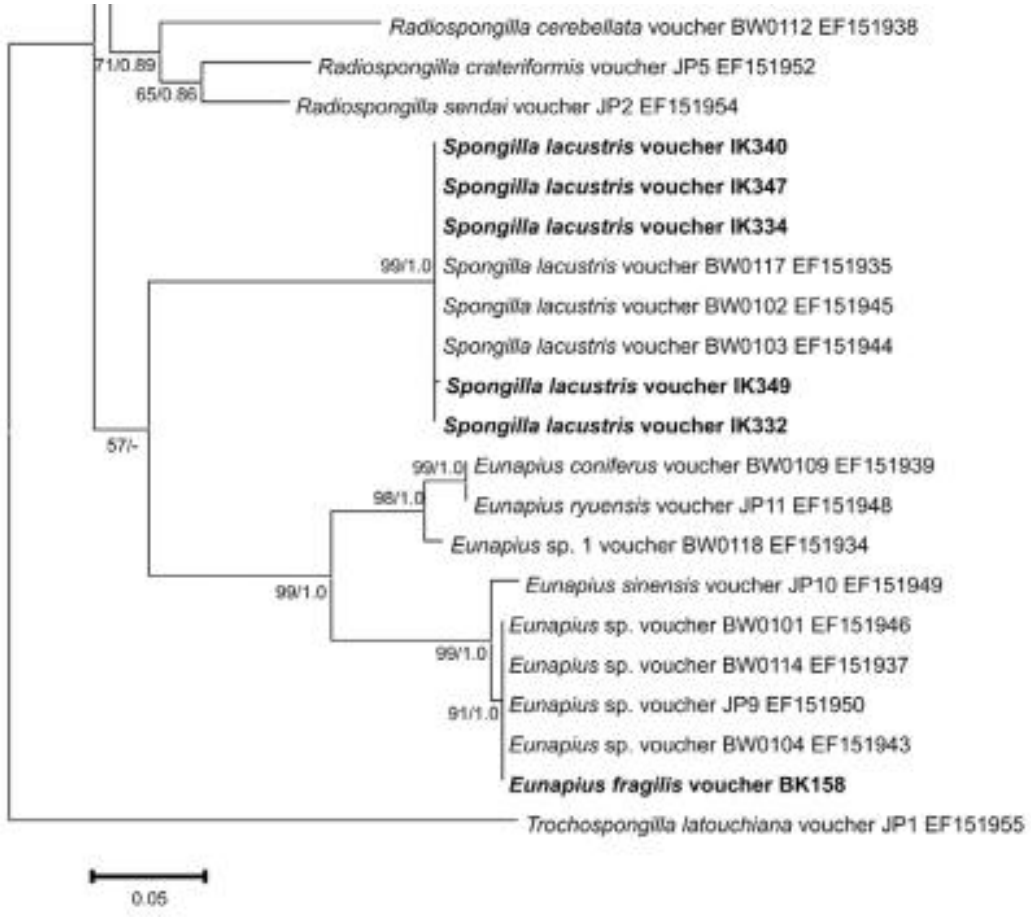

Figure 4. The example of Phylogenetic tree (from Itskovich V. et al., 2017.)

Upon completion of this practical session, students will not only be introduced to the basic techniques of molecular biology but will also gain a practical understanding of how these tools can be applied in various fields such as medical diagnostics, DNA analysis, mutation detection, agricultural genotyping, environmental metabarcoding, vaccine development and population genetics.

In the course of the work, the students get accustomed with the following **molecular biology techniques**:

- Plasmid DNA extraction
- Polymerase Chain Reaction
- Agarose gel preparation, DNA gel electrophoresis, analysis of PCR-fragments
- PCR-fragment extraction from the agarose gel
- TA Cloning
- Heat-shock transformation of bacterial cells
- Blue-white colony screening
- Phylogenetic analysis

Number of participants:

Four participants can work on the project at the same time.

Requirements for students:

- **accuracy and attentiveness!!!**
- biological background
- basic knowledge of DNA structure, mechanisms of DNA replication

If you have your own lab coat, please bring it with you.

Recommended literature:

1. https://asm.org/asm/media/protocol-images/polymerase-chain-reaction-protocol.pdf
2. https://en.wikipedia.org/wiki/Blue%E2%80%93white_screen
3. https://www.thermofisher.com/ru/ru/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/na-electrophoresis-education/na-electrophoresis-workflow.html
4. https://www.thermofisher.com/ru/ru/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/molecular-cloning/transformation/bacterial-transformation-workflow.html
5. Itskovich V. et al., 2017. Intraspecific and interspecific sequence variability in the ITS region of the rDNA of freshwater sponges of Lake Baikal and East Siberia. *Inland Waters*