G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Conservation of Genetic Information

Teacher's Guidebook

(Cat. # BE-409)

MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for 24 students (six groups of four students).

- 1 vial Human Protein
- 1 vial Bovine Protein
- 1 vial Sheep Protein
- 2 vials Trypsin Porcine
- 1 vial Protease-T Buffer
- 1 vial PAGE: Sample Loading Buffer (2X)
- 60 1.5ml Tubes
- 1 bottle 500ml LabSafe GelBlue

SPECIAL HANDLING INSTRUCTIONS

- Store Trypsin at 4°C.
- All protein samples can be stored at room temperature for 4 weeks. Store at 4°C for long-term storage.
- All other reagents can be stored at room temperature.

ADDITIONAL EQUIPMENT REQUIRED

- Protein Electrophoresis Equipment, SDS PAGE gels and electrophoresis running buffer.

TIME REQUIRED

- 3-4 hours

OBJECTIVES

- Learn manipulation of protein samples.
- Learn the concept of protein sequence.
- Learn time course dependent experiments.
- Use of protein electrophoresis for problem solving tasks.
- Identification of sample by protein fingerprinting.

BACKGROUND

Although the protein make up of an organism is unique to that individual species and as a result protein fingerprinting can be used to compare and contrast different organisms, many proteins are highly conserved. Proteins that perform identical biochemical functions are often highly conserved between different species. The Conservation of Genetic Information kit is an advanced protein analysis lab activity designed to teach delicate manipulation of protein samples and the use of a powerful and highly sensitive protein electrophoresis method.

In this lab activity students learn to perform carefully controlled experiments to generate protein fragments using a protease enzyme and then analyze the protein fragments by electrophoresis. By analysis of protein fragmentation patterns, i.e. protein fingerprints, students learn about protein sequence, structure, and their variation or conservation. Students are challenged into considering why the distribution of proteins vary throughout the animal kingdom and use protein fingerprinting to understand similarities of proteins between varying species.

This kit is provided with a set of 3 functionally identical protein samples selected from throughout the animal kingdom and unknown samples. Protein samples are treated with the protease supplied with kit. The protease digestion results in highly specific protein fragments. These protein fragments are separated using electrophoresis which results in a protein specific protein-fragmentation map or protein fingerprint. After generating fingerprints by electrophoresis students examine the protein fingerprint of each sample to compare the similarity or differences between the species and attempt to determine the origin of the unknown sample.

TEACHER'S PRE EXPERIMENT SET UP

Acrylamide/Bis-acrylamide is toxic. Always wear gloves and protective clothing when handling the chemicals.

1. Prepare one 8-12% polyacrylamide gel or use premade gel for each student group. G-Biosciences Protein Electrophoresis Kit is recommended for making your own gel.

2. Before you open the vials centrifuge all protein samples and Trypsin vials for 5 minutes to bring down all pellets to the bottom of the tubes.

3. Add 150µl Protease-T Buffer to each vial of human, bovine and sheep protein. Soak the protein samples for 5 minutes with periodical vortexing to dissolve them completely.

4. Add 100µl Protease-T Buffer to each Trypsin Protease vial just before the class activity starts. Vortex the tubes to dissolve the Trypsin Protease completely.

5. Prepare your own electrophoresis running buffer (25mM Tris base, 192mM Glycine and 0.1% SDS).

6. OPTIONAL: Protein markers may be provided to each group. Provide 10µl protein standard maker for each group and instruct students to load the protein markers in the first lane.

7. Aliquot reagents for each student group according to the next section.

MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components will be shared by the whole class and should be kept on a communal table.

- 1 8-12% Polyacrylamide gel
- 50ml LabSafe GelBlue
- 20µl Human Protein
- 20µl Bovine Protein
- 20µl Sheep Protein
- 25µl Protease Solution
- 50µl Protease-T Buffer
- 150µl Sample Loading Buffer (2X)
- 7 1.5ml Tubes
- 1X Electrophoresis running buffer (shared with whole class)

PROCEDURE

SDS-Page gels contain Acrylamide/Bis-acrylamide that is toxic. Always wear gloves and protective clothing when handling the gel.

1. Label 7 tubes 1-7. Tubes 1-3 are for human, bovine and sheep protein control, tubes 4-6 are the experimental tubes. Tube 7 is for a Trypsin control.

2. Aliquot 10µl protein solution into tubes 1-7 according to the table below.

Tube 1	Human
Tube 2	Bovine
Tube 3	Sheep
Tube 4	Human
Tube 5	Bovine
Tube 6	Sheep

3. Add 5µl Protease to tubes 4-6 and mix. Incubate tubes 4-6 for 1 hour at 37°C.

4. Add 5µl Protease-T Buffer to tubes 1-3 and 10µl to tube 7.

5. At the end of the incubation, add 15µl 2X Sample Loading Buffer to all the tubes (1-7). Tighten the caps and boil for 5 minutes.

6. Vortex the tubes briefly and centrifuge for 1 minute.

7. For running the samples, assembly the gel according your teacher's instructions.

8. Load 15µl of each sample in separate lanes.

9. Run the gel run 30mA/gel until the blue dye front is half centimeter from the gel bottom.

10. Disassembly the gel carefully. Wash the gel twice in distilled water, five minutes each wash.

11. Add 50ml LabSafe GelBlue to cover the gel. Gently shake the gel for 60 minutes at room temperature.

12. Decant the LabSafe GelBlue and rinse the gel with distilled water. The gel can be stored in water. Longer destaining in water will give a clearer view.

RESULTS, ANALYSIS & ASSESSMENT

Observe the protein bands on the gel carefully. Compare the protein band pattern for the human, bovine and sheep samples and discuss similarities and differences. Describe your results and conclusions below:

Q. Why do some proteins have distinctive protein fingerprints?

A Protein fingerprints are determined by the primary structure of the protein molecule, i.e. the sequence of their amino acids. Proteases have the unique ability to cut proteins at specific sites creating distinctive protein fragments termed protein fingerprints.

Q. Why are protein fingerprints different between species, even with a protein that has the same function?

A Protein fingerprints are determined by the primary structure of the protein molecule, i.e. the sequence of their amino acids. As species evolve and adapt to changes in their environment there protein make up changes. A particular mutation in a protein of one species will not necessarily occur in a different species, resulting in a different protein fingerprint.

Q. Why are some proteins highly conserved between species?

A. Proteins that successfully perform a specific function are often conserved between species as they have evolved to be efficient as possible, therefore further evolution is not required, hence their similarity.

Q. How you can alter the protein fingerprint of a protein?

A. Amino acid sequences of proteins cannot be changed, but if you change the Protease used to digest the protein, the protein fingerprints will change, because each protease cuts proteins at specific sites).

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Q. Why are protein fingerprints different between species, even with a protein that has the same function?

Q. Why are some proteins highly conserved between species?

Q. How you can alter the protein fingerprint of a protein?

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