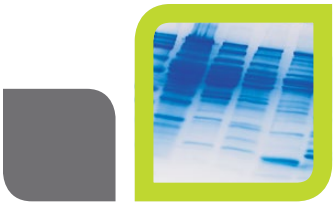
Comparative Proteomics Kit 1: Protein Profiler Module



Mastering Inquiry Can Be Easy with Bio-Rad

The Protein Profiler Module is inherently inquiry based since students must select the fish species to be analyzed. In addition, Appendix B of the curriculum manual provides instructions for conducting BLAST searches on the amino acid sequences from the fish species used. Use the following 20 questions for additional student-based inquiry about the processes contained in this kit. Whenever possible let your students develop protocols and choose the variables to test.

Level 1 questions are simple to adapt and do not add extra days to the running of this laboratory. An example of how to organize and execute a Level 1 question is given below.

Level 2 questions may add a few days onto the lab and may require some additional materials to answer.

Level 3 questions are for students seeking a real challenge and will require additional days, techniques, and materials to answer.

EXAMPLE

Level 1, Question #1: What is the optimal temperature for denaturing your protein? Would freezing work?

Have students decide which temperatures they want to test, including the one recommended in the manual (95°C). They can then perform the lab as written, with one or multiple groups incubating all their fish samples at one of the desired temperatures. For example, groups 1 and 2 incubate for 5 min at 50°C, groups 3 and 4 incubate for 5 min at 75°C, groups 5 and 6 incubate for 5 min at 95°C, and groups 7 and 8 leave theirs at room temperature. Alternatively, each group can incubate one fish sample at several different temperatures. In this case, students won't be able to compare the proteins among the different fish — only the effect of heating on their fish sample.

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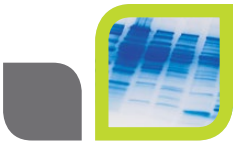

Level 1

1. What is the optimal temperature for denaturing your protein? Would freezing work?
2. Is there an optimal time to incubate your sample at 95°C?
3. What happens if you mash up your fish sample before adding it to the Laemmli buffer?
4. Can you tell the difference between proteins in crab and “krab” (artificial crab often used in California sushi rolls)?
5. Can you see differences between proteins of wild-caught and farmed fish, or between the same species caught in different regions of a river or lake?
6. Can you tell the difference between cooked fish samples and raw ones?
7. What happens to your protein bands if you load more or less of your sample?
8. Is there a difference between using Laemmli buffer with and without dithiothreitol (DTT)?
9. What happens if you use a higher or lower concentration of Tris/glycine/SDS (TGS) running buffer?
10. What is the optimal staining time for the gels?

Level 2

11. What happens if you increase the sodium dodecyl sulfate (SDS) in the Laemmli buffer?
12. What do the samples look like when you use a different percentage or gradient gel?
13. What do the proteins in muscle tissue from different animals look like?
14. Can you use another reducing agent instead of DTT? What is its optimal concentration?
15. Can you make your own Laemmli buffer? How does it compare to the buffer in the kit?

Level 3

16. How can you conclusively identify the myosin light chain proteins (other than by comparing your sample to the protein standard)?
17. Can you see a difference in the proteins expressed in cardiac, skeletal, and smooth muscle?
18. If you extract proteins from different organs of the same animal (for example, chicken liver, heart, and muscle), what does your gel look like?
19. Choose a different protein that is common to your samples (for example, ß-hemoglobin, DNA polymerase, or adenosine triphosphate [ATP] synthase). Construct a cladogram based on the amino acid differences and compare it to the cladogram based on the protein sizes. Do your cladograms match up?
20. Can you use PCR to amplify the gene for actin or myosin?

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