pGLO[™] Bacterial Transformation Kit



Mastering Inquiry Can Be Easy with Bio-Rad

Use the following 20 questions for student-based inquiry about the processes contained in the pGLO Bacterial Transformation 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 #4: How does transformation temperature affect transformation success?

Have two student groups run the experiment using the protocol temperature of 42°C. These groups will act as the control for the experiment. Have two student groups run the experiment at a temperature above 42°C and two student groups run it at a temperature below 42°C, and have the last pair of student groups not perform the heat shock at all (0°C). Multiple student groups running the experiment at the same temperature will provide confirmation of results. Have students compare their transformation efficiencies and make a class graph of temperature vs. transformation efficiency.

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Level

- 1. How does plasmid concentration affect transformation success?
- 2. How does bacterial concentration affect transformation success?
- 3. Can a salt other than CaCl₂ be used for transformation?
- 4. How does transformation temperature affect transformation success?
- 5. How does CaCl₂ concentration affect transformation success?
- 6. Is the 10 min ice incubation prior to heat shock necessary?
- 7. How does time at 42°C affect transformation success?
- 8. Is the ice incubation after heat shock transformation necessary?
- 9. Does the amount of LB broth added after transformation affect success?
- 10. Is 37°C the ideal temperature to grow the transformed bacteria?

Level 2

- 11. How does arabinose concentration affect green fluorescent protein (GFP) concentration?
- 12. What concentration of antibiotic is needed to kill bacteria that do not receive a plasmid?
- 13. How can the plasmid map be verified?
- 14. Can bacteria other than Escherichia coli be transformed using the same protocol?
- 15. Does growing transformed E. coli when GFP is being produced affect its growth rate?

Level

- 16. How fast do E. coli strains lose their plasmids if they aren't under selective pressure to keep them?
- 17. Can you isolate plasmid from your transformed bacteria and transform new bacteria?
- 18. What happens if a section of *bla* is removed from the plasmid?
- 19. What happens if all of *bla* is removed from the plasmid?
- 20. What happens to GFP production if *araC* is removed from the plasmid?

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