An Inquiry Based Experiment on Natural Selection to Follow Up a Bacterial Transformation Kit Lab

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Once Transformed, Always Transformed?

Introduction & Objectives

Teaching about the process of transformation in bacteria has been made easier with the availability of kits that include all the background information, materials, and procedures necessary in one relatively small box. Both Carolina Biological and Edvotek market a variety of transformation kits. Lab equipment such as water baths, Bunsen burners, incubators, micro-tube holders, and beakers are also necessary, but most labs have these, or there are simple ways to improvise. For example, if a classroom does not have an incubator, cultures can be left to incubate at room temperature for a slightly longer period of time. These kits utilize well tested protocols so students can successfully transform bacteria. However, these kits can also be used to create an inquiry-based activity that ties into natural selection.

Materials & Plan

A good kit to use is one that utilizes a plasmid with an ampicillin resistance gene and a color marker gene such as β-galactosidase, which produces blue colonies (Edvotek pGAL kit or Carolina pBLU kit). From this particular kit, students are able to transform and grow bacteria that are resistant to the antibiotic, ampicillin, and which produce colorful blue colonies when grown on media with X-Gal.

Once students obtain these colonies, the lab experience does not have to end. This lab can be extended by asking students the following question. "Once bacteria are transformed, do all their descendants maintain and keep these new traits?" In other words, "Once transformed, will bacteria always be transformed?" The transformation kit lab takes on a new objective from this point. Students can design a controlled experiment to answer this question.

Extra sterile Luria broth, Luria broth agar media, ampicillin, X-Gal, and sterile petri dishes, like the ones used in the kit, are necessary for the students to design their experiment.

Pre-lab

The post-lab for the transformation can lead directly into the pre-lab for the extension.

For example, assuming the transformation lab was successful, the students should be able to explain properties plasmids must have to be useful in biotechnology:

- Why selection genes?
- Why color marker genes?
- Why restriction sites?

At some point, the teacher poses this question to the students. Are these transformed bacteria permanent? In other words, if these bacteria are allowed to continue to multiply

and grow, will all the future bacteria maintain their new traits or will they lose these traits over time? The discussion can include the following:

- If plasmids are useful to bacteria, why don't all bacteria have plasmids?
- What are the benefits and disadvantages of plasmids to a bacterium?
- What role does the environment play in shaping the traits of bacteria?
- Do bacteria with plasmids multiply at the same rate as bacteria without plasmids?

Eventually, the teacher asks the students to write a hypothesis to the original question: Will transformed bacteria remain so in future generations?

Lab Activity

Using the same kinds of materials from the transformation kit, the class can design a controlled experiment to test their hypotheses.

For example, students might use a sterile inoculating loop to streak transformed colonies onto three different plates: a plain Luria broth plate, a Luria broth plate with X-Gal, and a Luria broth plate with ampicillin and X-Gal. Incubate the plates overnight at 37˚C, or 48 hour if at room temperature and record results.

Another possible protocol might be to use a sterile inoculating loop to transfer transformed colonies to two different culture tubes of sterile Luria broth, one treated with ampicillin and one without. After incubating overnight (at 37˚C if possible), plate 250 μL of each culture on three different agar plates; Luria broth agar, Luria broth agar with ampicillin, and Luria broth agar with ampicillin and X-Gal. Incubate the plates overnight at 37˚C, or 48 hour if at room temperature.

Post Lab

The results students observe will depend on the design of their experiment, but it is highly likely that some of the new bacteria will no longer carry a plasmid if the laws of natural selection hold true. Transformed bacteria grown on media with ampicillin have a decided selective advantage over bacteria without the plasmid. But will transformed bacteria continue to replicate their plasmid if there is no selection pressure? Replicating the plasmid takes added resources and time, so there may be a time when a transformed bacterium is better off not replicating the plasmid, or even "throwing out" the plasmid during the replication process. Thus the transformation experiment has become an experiment on natural selection.