HOW-TO-DO-IT

Augmenting the Traditional Bacterial Transformation Lab

PAMELA A. MARSHALL

Using plasmid DNA to transform competent *E. coli* is a standard laboratory in many biology curriculum, including the AP Curriculum (Laboratory 6, Molecular Biology) and many general biology laboratory and genetics lab curricula (such as Mertens & Hammersmith, 2007; Vodopich & Moore, 2008). The standard transformation protocol of adding a plasmid coding for antibiotic resistance to competent cells is straightforward and elegantly demonstrates the concept of bacterial genetic exchange as well as illustrates the potential for transfer of antibiotic resistance to bacteria in the environment.

Although most students take for granted that nucleic acid is the genetic material, in the early part of the 20th century, there was an intense debate raging at that time about what constituted the genetic material. Many scientists believed that DNA was too simple to be the genetic material and instead proposed the protein was the genetic material. In 1944, researchers, including Oswald Avery, Colin MacLeod, and Maclyn McCarty, were interested in determining which biological molecule is the genetic material. The genetic material was also called the "transforming factor" because in previous experiments performed by Franklin Griffith it could transform bacteria of one genotype to another. In their experiment, Avery, MacLeod, and McCarty used enzymes to degrade one component of a bacterial extraction and then added the remainder of the biological material to bacteria to determine which component would change or transform the genotype of the starting bacteria (Avery et al., 1944; McCarty, 1985). Here I describe an extension of the general transformation lab in which the students mimic the hypothesis driven experiment of Avery, MacLeod, and McCarty to determine the "transforming factor."

Materials

Teachers need any type of transformation kit, such as:

- Bio-Rad's pGLO kit
- Carolina Biological Supply's pBLU Colony Transformation Kit
- those from Modern Biology (Producing a Strain of *E. coli* that Glows in the Dark; or *E. coli* Transformation Kit)
- Ward's Natural Science (Transformation of *E.coli* with Green and Blue Fluorescent Protein Lab Activity)
- their own homemade version (for examples see BIOTECH Project, The University of Arizona, 2002; Slish, 1999).

Additional reagents needed are amylase, DNase, RNase, protease, and Luria Bertani plates with the appropriate antibiotic.

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1. Amylase is available for free from human saliva (Jones & Cooper, 2005; Marini, 2005); appropriate cautions should be employed with human saliva. Bacterial amylase can be purchased (Carolina 202360) and has little to no DNAse activity.
2. DNase is available from many sources. The least expensive I have found is from Promega (1000 units for $43, M6101).
3. RNase A 5mg/ml (500 µl) is available from Carolina (211745).
4. Trypsin protease is available from Carolina (25 g, FA-89-7000) or Wards (5 g, 947 V 2400; or 10 g, 947 V 2401). Dissolve trypsin at a 1mg/ml concentration in double distilled water and freeze small aliquots for use.
5. Pre-poured Luria Bertani plates with ampicillin can be purchased from Ward's (88 V 0904) or Carolina (216601). Alternatively you can make your own (10 g tryptone, 5 g yeast extract, 10 g NaCl, in a total of 1 L of water. Autoclave to sterilize; cool to around 50 °C; add appropriate antibiotics and other supplements, such as X-Gal and IPTG, if needed; and then pour).

Reagent Companies

- Bio-Rad
  www.bio-rad.com
  800-424-6723
- Carolina Biological Supply
  www.carolina.com
  800-334-5551
- Modern Biology, Inc.
  www.modernbio.com
  800-733-6544
- Promega Corporation
  www.promega.com
  800-356-9526
- Ward's Natural Science
  www.wardsci.com
  800-962-2660

Methods

For this exercise, students treat their purified plasmid (transforming factor) with amylase, protease, DNase, or RNase, and then perform a transformation. Students follow a general transformation protocol (such as BIOTECH Project, The University of Arizona) but will first aliquot the

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"transforming factor" into five separate aliquots and treat each one with 1 μl of the appropriate enzyme or control. This enzymatic treatment is incubated at 37 °C for 30 minutes and then is used in a standard transformation lab protocol. DNAse is robust enough that it works in this procedure, even without the included buffer.

The following week students return to analyze their plates to determine what molecule is the genetic material that will transform bacteria. This activity can be done with any transformation lab exercise available and turns a simple transformation lab into an inquiry-based activity (National Research Council, 2003). The extension requires two additional steps to the traditional transformation protocol: to allow the students time to treat their DNA sample and then to have more plates available for the students to transform the appropriate number of aliquots of competent cells (usually six: no DNA negative control, positive control of plasma with no treatment, amylase, protease, DNase, and RNase treatment). This extension of the traditional transformation lab can demonstrate then to the students the results similar to what Oswald Avery, Maclyn McCarty, and Colin MacLeod saw, that DNA is in fact the transforming factor!

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BIO PAMELA A. MARSHALL is Assistant Professor, Division of Mathematical and Natural Sciences, Arizona State University at the West Campus, Phoenix, AZ 85069-7100; e-mail: Pamela.Marshall@ASU.edu.

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