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A Sequential Approach to Teaching Transformation, Gene Splicing, and PCR in a Biotechnology Unit

Introduction

When teaching a unit on biotechnology, hands-on labs are essential for students to understand the many techniques used to manipulate DNA. Because these techniques are so new to students, they are often intimidated when faced with common biotechnology skills such as micropipetting, loading gels, or spreading agar plates. Labs done only once do not give students confidence with these skills, nor do they help them understand how the different processes are connected. To aid in building students' confidence with biotech skills, and understanding important techniques and processes, I propose teaching a sequence of three transformation lab activities, using three different kits over a three week period.

Materials

Edvotech kit #221 – Transformation of *E. coli* with pGAL

Carolina Biological kit #211162A – E-Z Gene Splicer DNA Recombination and Transformation

Peyer Laboratories – Cloning a Fluorescent Gene

37^{o} Incubator

42^{o} water bath

ice

Refrigerator/freezer

Microwave oven or hot plate

Procedure

Beginning with the Edvotek kit, students get their first introduction to bacterial transformation. The kit comes with LyphoCells^{TM} *E. coli* which make the teacher prep very easy. Students learn about the "heat shock" method for bacterial transformation, as well as basic principles of plasmid selection and marker genes. The success rate of transformation is high so students will see blue colonies of transformed bacteria when plated on LB agar plates with ampicillin and X-Gal.

The follow-up to the above lab is the kit from Carolina Biological. In this lab, students must ligate genes from two different plasmid digests in order to make a recombinant plasmid containing genes for both ampicillin and kanamycin resistance. In this part of the lab, students learn about the importance of restriction enzymes, "sticky ends," and ligase for cutting and pasting DNA. Then using a similar heat shock method, students transform E. coli bacteria with the recombinant plasmid. Only

the bacteria transformed with the recombinant plasmid will grow on LB agar plates with ampicillin and kanamycin.

The final lab of the sequence is the kit from Peyer Laboratories. In this lab, students amplify the Green Fluorescent Protein gene(GFP) by the Polymerase Chain Reaction (PCR) using a thermocycler that is leased from the company. At this point, students learn about the role of primers and Taq polymerase in the PCR sequence. After many copies of the gene are made, students ligate the gene to a vector carrying an ampicillin resistance gene to make a recombinant plasmid. The recombinant plasmid carries two genes, one for ampicillin resistance and one for GFP. Finally, *E. coli* and the recombinant plasmid are heat shocked to induce transformation. Transformed bacteria will glow when exposed to a long wave UV lamp.

Comments

Except for the lab items list in materials section, each kit comes with all the necessary supplies. If you have micropipettes, these can be used in place of the ones that come in the Edvotek and Carolina kits. The Peyer kit is different in that company leases a thermocycler for PCR, a long wave UV lamp to spotlight the bacteria transformed with the GFP gene, and micropipettes.

In terms of teacher preparation, the Edvotek LyphoCells™ come ready to go, which saves a lot of time. Carolina sends an E. coli slant culture which means the teacher needs extra time to prepare streak plates to obtain fresh bacteria colonies for the transformation. Peyer sends a ready prepared streak plate, which saves some time.

All the kits come with extensive instructor manuals to compliment the student manuals. The Peyer student manual is exceptional in the details of each process, and could be used in place of a textbook chapter on transformation, gene splicing, and PCR.

Finally, by using all three kits, students work with three different plasmids so there is variation in the final transformed bacteria phenotype they observe. The sequence of adding a new process with each kit should help students grasp how PCR, gene splicing, and transformation are connected, while at the same time, they should become more confident and adept with the lab techniques.