Transformation

Honors Biology Time: 90 min

What is Recombinant DNA?

Learning Objectives

Students will use paper to model recombinant plasmids in order to understand how plasmid DNA can be used in synthetic biology. Students will add an insert of interest (for example, an insulin producing gene from a mammal) into a bacterial vector. They will review membrane properties and learn how plasmid DNA can enter bacteria through horizontal transfer. They will discuss how scientists select for bacteria that took up the plasmid. Finally, students will examine the costs and benefits of using plasmid DNA instead of chromosomal integration.



Materials

Transformation handout to make inserts and backbones: https://www.teachengi neering.org/content/uoh_/activitie s/uoh_genetic/uoh_genetic_lesson O1_activity1_dna_cutouts.pdf Tape

Procedure



Scissors

Approximate Cost: Free



Cut out the plasmid backbone and the insulin insert using scissors, which represent a restriction enzyme. Use tape to make the plasmid DNA sequence into a ring. Cut both the backbone and the insert at their restriction sites.

Recognition Site 5' >3'

Recognition Site 5' →3'

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Match up the sticky ends of the insulin insert with the plasmid and use tape, or DNA ligase, to hold it together.

Background

This activity is a representation of how restriction enzymes can be used to create recombinant DNA. Restriction enzymes are also called "molecular scissors" because they cut DNA at specific base pair sequences, called restriction sites. The nucleotides are cut in a staggered manner that leaves an overhang which is called a sticky end. Because restriction enzymes cut at the same recognition site for different DNA sequences, sticky ends will match up and can be bound together using another enzyme called ligase. This new plasmid is recombinant DNA.

The next step is transformation. Transformation is the process by which scientists can introduce recombinant DNA into a bacterial cell. Because bacteria have no nuclear envelope (they are prokaryotes), plasmids transformed into the bacteria will be translated/transcribed in the same way as the chromosomal DNA. The main difference is that while there is only one chromosome, there may be many plasmids! During transformation, some bacteria may pick up only one plasmid, while others may pick up two or three or even a hundred! This can cause some difficulties when using bacteria as vectors for biological devices from which a scientist is trying to take measurements. In the case of insulin producing bacteria, though, this just means some bacteria will make more insulin than others.

Critical Thinking Questions

In nature, why do you think bacteria take up plasmids? How might this be beneficial?

Can you think of any ways in which a synthetic biologist could encourage bacteria to take up plasmids?

Adapted from: