**Lab: Cloning (DNA Ligation) & Transformation**

AP Biology
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In this lab, you will be making recombinant plasmid DNA by ligating fragments of DNA with T4 DNA Ligase. You will then be transforming these plasmids into E.coli cells using antibiotic resistant agar plates to isolate the colonies.

## Day 1: DNA Ligation

In this step, you will be preparing three controls and one sample to be used in the rest of this experiment. Follow the procedure below:

Control 1:
1. Add 35 µl of pre-cut plasmid DNA (purified pUC 19 pre-cut with only EcoRI) in a microcentrifuge tube.
2. Add 5µl of 10x ligase buffer.
3. Add 10µl of sterile water.
4. Mix the contents and spin the tube to bring the contest down.
5. Incubate the tube at 14°C overnight.

Control 2:
1. Add 35µL of Pre-cut plasmid DNA (purified pUC 19 pre-cut with only EcoRI) in a microcentrifuge tube.
2. Add 5µl of 10x ligase buffer.
3. Add 8µl of sterile water.
4. Add 2µl of ligase enzyme.
5. Mix the contents and spin the tube to bring the contest down.
6. Incubate the tube at 14°C overnight.

Control 3:
1. Add 35µl of pre-cut plasmid DNA (purified pUC 19 pre-cut with two enzymes: PstI and EcoRI) in a microcentrifuge tube.
2. Add 5µl 10x ligase buffer.
3. Add 8µl sterile water.
4. Add 2µl ligase enzyme.
5. Mix the contents and spin the tube to bring the contest down.
6. Incubate the tube at 14°C overnight.

Sample:
1. Add 35µl of pre-cut plasmid DNA (purified pUC 19 pre-cut with PstI and EcoRI) in a microcentrifuge tube.
2. Add 5µl of 10x ligase buffer

3. Add 5µl of DNA insert.
4. Add 3µl of sterile water.
5. Add 2µl of ligase enzyme.
6. Mix the contents and spin the tube to bring the contest down.
7. Incubate the tube at 14°C overnight.

**Questions:**

1. Why is it important to make three control solutions in this experiment? How are they different from one another? What results do you expect from each and why?

**Day 2: Innoculation of bacteria and plate preparation**

1. Innoculate 20µl of E.Coli bacterial cells with 15ml sterile (autoclaved) bacterial media in a 50ml polypropylene tube overnight at room temperature (constant shaking).
2. Add 110 ml of distilled water into a clean 500ml beaker containing 4.1g powdered LB agar media. Mix the contents thoroughly and bring the solution to a boil for 7 min (shaking often).
3. Allow the solution to cool to 45°C.
4. Add 20µl X-Gal, 20µl IPTG solution, and 100µl antibiotic ampicillin solution.
5. Mix the contents, swirling gently to remove any bubbles.
6. Pour ~25ml of bacterial agar media onto each bacterial plate. Once the media is solidified, invert the plates, and store in the refrigerator.

**Day 3: Transformation**

1. Transfer 1.5ml of the grown bacterial culture into each of 4 microcentrifuge tubes. Pellet the bacterial cells by spinning the tubes for ~15sec.
2. Discard the supernatant (solution on top)using a pipet and place tubes in the ice bucket.
3. Resuspend the bacterial pellet (tubes still in ice!) with 200µl ice cold transformation solution buffer (TS buffer). Bacterial cells will become competent in about 20min.
4. Add 10µl of each of the plasmid DNA into each of the microcentrifuge tubes containing 200µl of competent cells. Keep the tubes on ice ~30 min.
5. Heat shock the bacterial cells by incubating the tubes at 42°C for approximately 1min. After heat shock, keep the tubes on ice for about 5min.
6. Using a pipet, transfer the contents into X-gal/ITPG bacterial plates containing ampicillan. Spread or streak the contents gently onto the plates using sterile loops. Let the solution dry over the plates (15-20min).
7. Incubate UPSIDE DOWN overnight at 37°C.

**Day 4: Identification and quantification of colonies**

1. Record the color and the quantity of colonies found on each plate.

**Questions:**

1. Did you obtain the results that you had predicted after making the control and sample plasmids? Explain why each plate yielded the number and color of colonies that it did.

2. How might you use gel electrophoresis to identify the fragments generated by addition of restriction endonuclease in this experiment? Please include a diagram with your explanation.