

# In-Class Cloning Experiment Protocol

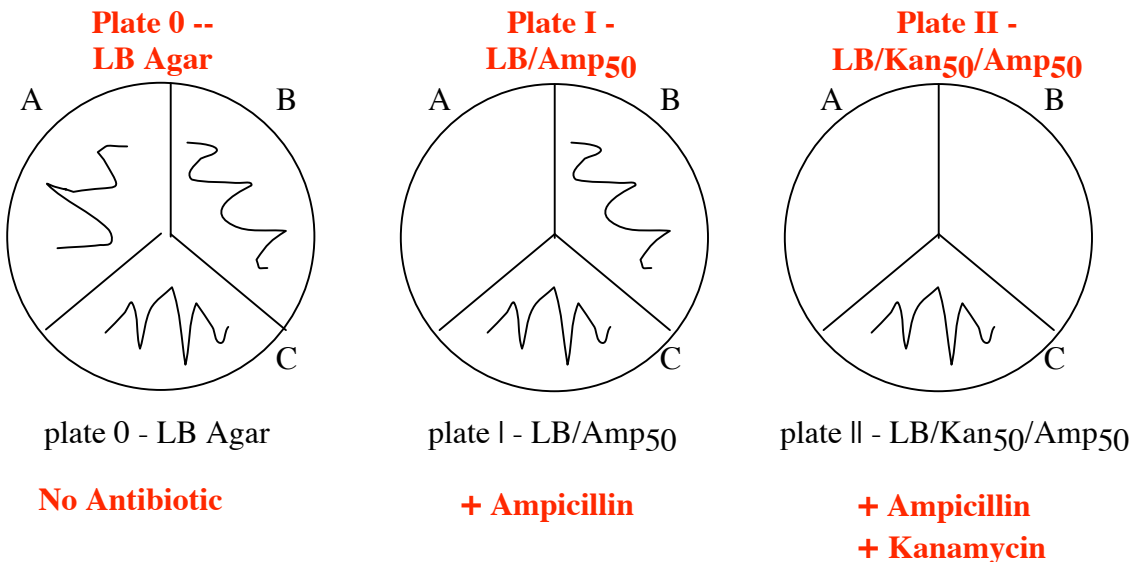
## HC70A: Genetic Engineering in Medicine, Agriculture, and Law

### **Purpose:**

The purpose of the in-class cloning experiment is to give the students a "hands-on" appreciation of cloning technology, and how science is done. This protocol will outline the steps taken to prepare the cloning experiment for a class of 50 students using the cells and materials available.

### **Experimental Design:**

Each student will receive 3 agar plates (wrapped in foil), 3 sterile toothpicks (wrapped in foil), and 3 cell suspensions in 1.5mL microcentrifuge tubes (on microcentrifuge rack – 2 students per rack). The plates are labeled to indicate their antibiotic selection: the unlabeled plate contains agar with no selective antibiotic, the plate with one tick mark contains one selectable antibiotic (Amp), and the plate labeled with two tick marks contains dual selectable markers (Kan & Amp). The students divide each plate into three equal sections, and label them A, B, and C (on the bottom of the plates). The students will also write their name on the bottom of the plates. They then streak cells from each of the labeled suspensions onto the appropriate sectors of each of the plates. *The "A" cells contain no antibiotic resistance genes, and are expected to only grow on the unlabeled plates (which contain no selection).* *The "B" cells contain a ampicillin resistance gene, and are expected to grow on the plates with no selection and ampicillin selection.* *The "C" cells contain antibiotic resistance genes for both kanamycin and ampicillin, and are thus expected to thrive on all three plates.* After incubation of these plates for one day at 37°C, the plates are returned to the students. The students are expected to write a brief report explaining their findings, and providing a hypothesis that explains the differential growth on the three plates.



## **Materials:**

1. 3 plates w/ LB Agar (base) media per student (100 x 15 mm plates)
2. 3 sterile toothpicks wrapped in tinfoil per student
3. 3 microfuge tubes labeled A, B, and C per student
4. *E. coli* cells without plasmid (DH5 $\alpha$ )
5. *E. coli* cells transformed with pBluescriptII (Amp-resistant)
6. *E. coli* cells transformed with pCR2.1 (Kan-resistant/Amp-resistant)
7. 50 mg/mL kanamycin stock (stored in 500  $\mu$ l aliquots)
8. 100 mg/mL Ampicillin stock (stored in 500  $\mu$ l aliquots)
9. One black sharpie pen per student
10. Three strips of parafilm

## **Protocol:**

### **1. Calculate the number of students in the class**

50 students + 2 TAs + 1 test set = 53 =  $\sim$  55 sets

(Note: Make 6 large plates (150 x 15 mm) for demonstration purposes)

### **2. Prepare enough LB Agar media for number of sets calculated above**

(55 sets) x (3 plates/set) x (25 ml/plate) = 4125 ml LB Agar media =  $\sim$  4.5 L of LB Agar media

### **3. Separate prepared media into 3 equal aliquots**

4.5 Liters LB Agar media / 3 = 1.5 Liter of LB Agar in TWO 2-Liter flasks (750 mL per flask)

## **Preparing plates:**

LB agar plates (1.5 L):

- a. Weigh out 37.5 g of LB Broth powder (Difco – catalog no. 244620)
- b. Add LB broth to 2-Liter beaker containing 1-Liter of distilled water.
- c. Stir to dissolve the LB powder.
- d. Transfer the LB medium to a 2-Liter graduated cylinder.
- e. Bring the final volume to 1.5 L.
- f. Transfer 750 mL of LB medium into a 2-Liter flasks containing 10.5 g of Bacto-Agar (Difco – catalog no. 214010)
- g. Autoclaved at 121 $^{\circ}$ C for 15 min.
- h. Cool the liquid LB medium to 55-60 $^{\circ}$ C.
  - i. For no antibiotic plates, go to steps i and j.
  - ii. For the Amp-resistant plates, add 0.75 mL of 100 mg/mL Ampicillin stock to 750 mL LB medium. Swirl to mix, then proceed to steps i and j.
  - iii. For the Kan-resistant/Amp-resistant plates, add 1.5 mL of 50 mg/mL Kanamycin stock and 0.75 mL of 100 mg/mL Ampicillin stock to 750 mL LB medium. Swirl to mix, then proceed to steps i and j.
- i. Pour the plates in the laminar flow hood.
- j. Allow LB agar to solidify ( $\sim$ 30 min.)
- k. Store the plates in the cold room (4 $^{\circ}$ C)

## **Preparing cultures:**

50 mL cultures:

- a. Streak of *E. coli* cells from glycerol stock on appropriate selection medium plates.
- b. Incubate the plates at 37°C for 12-16 hours.
- c. Inoculate 50mL of LB liquid medium in 250 mL Erlenmeyer flask.
  - i. For no antibiotic strains, go to steps d and e.
  - ii. For the Amp-resistant strains, add 25  $\mu$ L of 100 mg/mL Ampicillin stock to 50 mL LB medium. Swirl to mix, then proceed to steps d and e.
  - iii. For the Kan-resistant/Amp-resistant plates, add 50  $\mu$ L of 50 mg/mL Kanamycin stock and 25  $\mu$ L of 100 mg/mL Ampicillin stock to 50 mL LB medium. Swirl to mix, then proceed to steps d and e.
- d. Incubate at 37°C with 200 rpm shaking for 16 hours.
- e. Store the cultures in the cold room (4°C)
- f. Aliquot 500  $\mu$ l of cultures to labeled 1.5 mL microcentrifuge tubes.