Recombinant DNA Kit

Student Worksheet

Day 1 of the experiment:

1. After your teacher gives some background, you will divide into teams and will be asked to develop and explain a hypothesis to the following question:

> *Given 2 strains of bacteria, strain* A^rN^r *(r: resistant to the antibiotics ampicillin and naladixic acid), and strain (resistant to the antibiotic streptomycin), which strain will grow on each of the following media? (Assume the two strains are kept separate while growing so no conjugation occurs.): 1) medium containing no antibiotic 2) medium containing streptomycin and ampicillin 3) medium containing ampicillin 4) medium containing streptomycin 5) medium containing naladixic acid.*

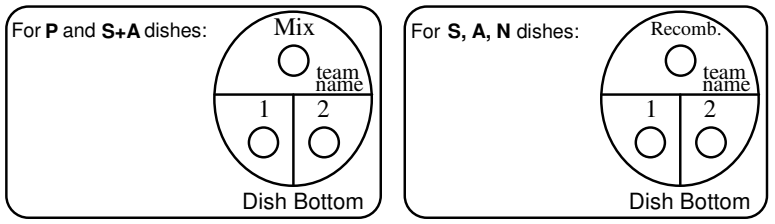
Describe and explain your hypothesis:

Fill out Table 1 below based on your hypothesis using a plus (+) for growth and a minus (-) for no growth:

Table 1

Media containing:	strain A^rN^r	strain S^r
no antibiotic		
ampicillin		
streptomycin		
naladixic acid		

Drawing 1



2. Wash your work area with 10% bleach. Wear lab coats and gloves if available. Do not get bleach on your clothes.
3. Your team will receive the following: 5 Petri dishes labeled P (plain, no antibiotic), S+A (streptomycin + ampicillin), A (ampicillin), S (streptomycin), and N (naladixic acid). For the first part of the experiment, you will also receive 2 sterile swabs.
4. Think of a short name for your team. Use a permanent marker or wax pencil to draw lines, circles, and your team name on the bottoms of all your petri dishes, as shown in drawing 1.
5. When your team is called, go to the first culture station (*E. coli* 1) with all 5 of your petri dishes and open one sterile swab. Do not let the cotton end touch anything other than the culture broth, the inside of the bottle, or the inside of the petri dish.
6. Dip the swab into the culture bottle marked *E. coli* 1. Wring the swab out by pressing it against the inside of the culture bottle.
7. Be sure to follow the next instructions in order:
   a. Gently rub the swab in the circle in the area marked "1" of each petri dish. **Do not press hard.**
   b. Do not rewet swab. On the P and S+A petri dishes **only**, gently rub the swab in the circle in the area marked "mix". It is important that this "mix" section is inoculated last. When finished, place swab in 10% bleach beaker.
8. Take your petri dishes to the second culture station (*E. coli* 2). Open your second swab.
9. Dip the swab into the culture bottle marked *E. coli* 2. Wring the swab out by pressing it against the inside of the culture bottle.
   a. Gently rub the swab in the circle in the area marked "2" of each petri dish. **Do not press hard.**
   b. Do not re-wet swab. On the P and S+A petri dishes **only**, gently rub the swab in the circle in the area marked "mix". You want to rub over the same area that you rubbed with the *E. coli* 1 culture so that both 1 & 2 are mixed together.. It is important that this "mix" section is inoculated last. When finished, place swab in 10% bleach beaker.
10. Your teacher will either collect the plates or tell you where to place them for incubation.
11. Wash your work area with 10% bleach, and then wash your hands with soap.

Day 2 of the experiment:

12. Examine your petri dishes for growth in the inoculated areas. Record your results on table 2 using a plus (+) indicating growth or a minus (-) indicating no growth. Copy your results onto the table that your teacher will place on the board. Do your results correspond with other teams results?

Table 2

	E. coli 1	E. coli 2	Mix	Recomb.
Plain				
Streptomycin + Ampicillin				
Streptomycin				
Ampicillin				
Naladixic Acid				

13. Your teacher will draw a picture on the board depicting the two strains of *E. coli*, A^rN^r and S^r. Match the drawings to the 2 *E. coli* cultures that you used in this experiment and explain your answers based on what you have discovered thus far:

***E. coli* 1=_____** because

***E. coli* 2=_____** because

14. Since only one of the strains can be a DNA donor, is there any way to tell which of the two strains was the DNA donor and which was the recipient? Is there any way to determine whether the DNA that was transferred was chromosomal DNA or plasmid DNA? Explain both of your answers.

15. Your teacher will provide each team with a new swab. Open the swab and roll the swab in some of the bacterial growth from the "Recomb." section of the S+A dish. Do not "load" the swab with a gob of bacteria. A little will go a long way.
16. Gently rub the swab in the circle in the "Recomb." sections of the S, A, and N petri dishes. Dispose of the swab in the 10% bleach disposal container.
17. Your teacher will either collect the plates or tell you where to place them for incubation.
18. Wash your work area with 10% bleach, and then wash your hands with soap.

Day 3 of the experiment:

19. Examine the results in the "Mix" sections of your petri dishes and record the results on Table 2.
20. Answer the following study questions:
    a. Which strain(s) of *E. coli* (1, 2 and/or recombinant) had antibiotic resistance to 2 antibiotics?

    b. Why did the mix of *E. coli* 1 and *E. coli* 2 grow on the S+A dish when neither strain grew alone?

    c. Which strain was the DNA donor? How do you know?

    d. What type of DNA was transferred, chromosomal or plasmid? How do you know?

    e. A patient has a blood infection which has been determined to be *E. coli*. When a sample of the patient's blood is spread onto plain nutrient agar, the entire dish becomes covered with the bacterium after the dish is incubated. Another sample is placed on a new dish of nutrient agar, but before it is incubated, three antibiotic disks are placed on separate areas of the dish. One of the disks contains penicillin, the second contains ampicillin, and the third contains neomycin. After the dish has incubated overnight, there is a 15 cm bare area (devoid of bacterial growth) around the ampicillin, a 6 cm bare area around the ampicillin, and the third disk has no bare area around it. There is bacterial growth everywhere except in the bare areas. If you were this patient's doctor, how would you treat the infection? Why?