Colony PCR

The purpose of this colony PCR is to determine if the cloning worked, by checking to see if the insert is present, and estimating its size.

Homework

a) Use the map of the plasmid given to determine how big the PCR product will be if the insert goes into the plasmid.

b) What would the size of the PCR product be if the insert does not go into the plasmid?

1. On the bottom of each petri dish, label 8 colonies to be tested. The colonies should be white.

   Blue colonies are producing the enzyme β-galactosidase, which breaks down X-gal in the media into a blue compound. These colonies are able to produce this enzyme while white colonies are not. β-galactosidase synthesis is interrupted in while colonies because we have inserted DNA right into the middle of its gene, lacZ. This is called blue/white screening, and means that white colonies should have an insert, while blue colonies do not.

2. Label the bottom of a fresh petri dish with 8 squares to transfer colonies into.

3. For each colony, put 15 μl of water in 0.2 ml strip tubes. Label them with the colony number. Prepare 5ml cultures of LB broth for each colony and add 10ul of Ampicillin (25mg/ml).

4. Take a 20 μl pipet tip and touch a colony **very lightly** and dip the tip a couple of times into the 15 μl of water, then with the **same tip**, streak onto the fresh media in the appropriate square and put into LB broth. Repeat for each colony.

5. Heat the strip tubes in the PCR machine for 5 minutes at 95°C. Place tubes on ice immediately afterwards.

6. Thaw PCR reagents. Fill out PCR sheet, with 10 μl total volume and 1 μl of colony DNA for each reaction. Use primers M13F and M13R, these bind to the plasmid DNA on either side of the insert and amplify across it. This will help to determine what size the insert is.

7. Make a master mix according to the calculations above, with all reagents except for the DNA. Dispense the master mix into strip tubes (9 μl each) and then add the DNA prepared in step 4 (1 μl each). Label tubes and put in PCR machine.

Materials Necessary

Plates from cloning
LB Broth
PCR reagents
8-strip or single 0.2 ml PCR tubes
Sterilized DI H_2O
10 and 20 μL pipette tips
M13 F and M13 R primers

Equipment Necessary

10 μL micropipette
20 μL micropipette
PCR Thermocycler