I. PLASMID ISOLATION

Background

Most methods available for plasmid isolation have the following steps in common:

1. Growth and Harvesting of Plasmid-Containing Bacterial Cells - The cells from an antibiotic-resistant colony are usually grown overnight in a liquid culture to stationary phase and then harvested as a pellet by centrifugation.

2. Preparation of the Cleared Lysate - These steps involve the lysis (breaking open) of the bacteria and the separation of plasmid DNA from most other components in the lysate including chromosomal DNA, protein and RNA.

3. Final Purification of the Plasmid DNA – These steps involve the final purification of the plasmid DNA using a silica matrix.

The procedure described below incorporates these steps and yields high-quality plasmid DNA that is suitable for transformation, restriction nuclease digestion, and DNA ligation.

Procedure

A. Harvesting the Bacteria

1. Transfer about 1.5 ml of your three overnight cultures to three labeled 1.5 ml microcentrifuge tubes and centrifuge at maximal speed for 1-2 minutes to pellet the bacteria. Discard the supernatant, fill the tubes again with the appropriate cultures and centrifuge for 1-2 minutes.

3. Carefully remove and discard the supernatant fluid. Invert the tubes and allow the cell pellets to dry for a minute.

B. Preparing the Plasmid DNA

1. Add 250 ul of Solution I to each tube and suspend the pellets in the solution by vigorous shaking or by using a vortex mixer. This solution contains RNAse, which destroys RNA. Complete re-suspension (no visible clumps) is important in order to have high plasmid yield.

2. Incubate the tubes at room temperature for 2 minutes.

3. Add 250ul of Solution II, cap the tubes and mix by inversion several times until the contents are mixed thoroughly. The solution should become clear and viscous within 1-2 minutes. Solution II contains the detergent sodium dodecyl sulfate (SDS) and sodium hydroxide, which dissolve the bacterial membranes and causes cell lysis. The sodium hydroxide also denatures the DNA. Upon neutralization in the step below, the plasmid DNA strands renature because of their proximity while the bacterial DNA does not.

4. Add 350 ul of Solution III to each tube, cap the tubes and shake until the contents are mixed thoroughly. A white flocculent precipitate should form. The acetic acid in Solution III will neutralize the solution. The Guanidium Chloride in Solution III and the SDS from the previous step produce a tangled network of long strands of bacterial chromosomal DNA and cell debris, and this matrix can be separated from the smaller plasmid DNA by centrifugation.

5. Centrifuge the tube for 10 minutes at maximal speed. After centrifugation, plasmid DNA and small amounts of cellular proteins are in the supernatant, and bacterial chromosomal DNA and most of the proteins are in the pellet. This supernatant is known as a "cleared lysate". A common procedure that is used to remove the proteins, SDS and salts that are present in the cleared lysate involves the use silica, which binds to the DNA but not to the contaminants. The silica is packed into the bottom of a column that you will use, and the lysate is added to the column over the silica. The column is then washed by centrifugation to remove the contaminants and the plasmid DNA is eluted by using a low salt buffer.

6. Obtain three blue columns, and three collections tubes, label each, and insert the columns into the collection tubes. Add 100 ul of Equilibration Buffer to the columns and centrifuge for 1 minute at maximal speed.

7. Carefully pour the three cleared lysates from step 5 into the columns making sure that the pellets are not dislodged. Centrifuge the columns with collecting tubes for 1 minute at maximal speed.

8. Discard the liquid in the collection tubes, place the columns back into the collection tubes and add 500 ul of HB Buffer to the columns. Centrifuge the columns/collecting tubes for 1 minute.