Purification of plasmid DNA

Plasmids are small double-stranded DNA pieces of DNA that exist outside the chromosome of the cell in which they are located. Almost all plasmids are circular. They contain a small number of genes, which can include genes that make cells resistant to antibiotics. Plasmids are used extensively in cloning DNA. You insert some foreign DNA into the plasmid DNA, and then infect cells with the DNA in a process called transformation.

There are several ways to purify plasmids. These methods are often called "minipreps" because you are purifying DNA from a small volume of cells. Minipreps involve lysing the cells and purifying the DNA via centrifugation and/or membrane binding. The method we will do uses a silica-gel membrane to bind the DNA, which has been developed by the company Qiagen. You will purify the plasmid DNA and analyze it.

We lyse the cells using a modified alkaline lysis procedure. This technique was invented by Birnboim and Doly. Other types of minipreps may use a different procedure for lysing the cells. In general, in the alkaline lysis method, the cells are brought to a high pH to not only lyse the cells, but also to denature the DNA. The DNA solution is then neutralized. Since plasmid DNA is circular and supercoiled, when the pH is brought back down to neutral, the plasmid DNA snaps back to being double-stranded. By contrast, genomic DNA is so large that it is broken into linear pieces no matter what we do. The linear DNA denatures in alkali and forms precipitates when the pH is lowered. The precipitate is removed via centrifugation. The supernatant is then applied to the silica-gel membrane to further purify the plasmid DNA. Under the right conditions double-strand plasmid DNA sticks to the membrane while single-strand DNA and RNA do not. The membrane does not require the use of organic solvents and lets us easily wash the DNA to remove contaminants. We then elute the DNA from the membrane by using a low-ionic strength buffer. The plasmid DNA that we obtain is very pure, and can be restricted or used in DNA sequencing.

Materials

DNA purification

1.5 ml cultures of plasmid infected E. coli, grown overnight in TB (Terrific broth)
TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
Buffer P1
Buffer P2
Buffer N3
Buffer PB
Buffer PE
Buffer EB: 10 mM Tris-HCl, pH 8.5
QIAprep spin columns.
Microfuge tubes (1.7 ml)
Collection tubes: 2 ml capless microfuge tubes

Methods

We will be purifying DNA from plasmid infected *E. coli*. It is very important that you label the tubes correctly. It is also very important to use the right solutions and to pipet correctly.

Notes:

1) This procedure is designed for 1.5 ml of cells, and works with up to 10 A_{600} units of culture.
2) All centrifugation steps are at the *maximum* speed of the microfuge (10,000 rpm or more).

Plasmid DNA purification

0) Grow 1.5 ml cultures of plasmid infected *E. coli* overnight in TB (Terrific broth) containing 50-100 μg/ml of ampicilin. If you are doing this for a class, this step will probably be done by your instructor. Otherwise, you use a loop to pick colonies off of streak plates or 96-well plates. For the procedure to be successful, the culture should look *very cloudy* before proceeding to the next step.
1) Label 1.7 ml microfuge tubes with the numbers of your samples.
2) Vortex the overnight cultures to resuspend all of the cells, and then transfer each overnight culture to a separate microfuge tube.
3) Centrifuge the samples for 60 seconds to pellet the cells.
4) Remove as much of the supernatant as possible without disturbing the cell pellet.
5) Resuspend the pellet completely in 250 μl of Buffer P1 by vigorously vortexing the samples. No clumps should be visible in the tube.
6) Add 250 μl of Buffer P2 and mix the sample by gently inverting the tube 4 to 6 times. *Do not vortex or shake the sample vigorously.* The bacterial suspension should begin to clear. You have lysed the bacterial cells in this step. **Warning:** Do not stop here for more than five minutes, as the high pH hurts your DNA!
7) Add 350 μl of Buffer N3 and mix by gently inverting the tube 4 to 6 times. *Do not shake vigorously, as it might break the genomic DNA.*
8) Centrifuge at *maximum speed* for 10 minutes at room temperature to pellet the cell debris. You should see a white precipitate in the tube after the centrifugation.
9) While your samples are centrifuging, for *each* sample, label a QIAprep spin column (which is in a 2-ml collection tube).
10) Apply the supernatants from step 8 to the columns.
11) Centrifuge for 30 to 60 seconds. Discard the flow-through in the collection tube.
12) Wash the QIAprep spin column by adding 0.5 ml of Buffer PB, centrifuging for 30 to 60 seconds, and discarding the flow-through in the collection tube.
13) Wash the column by adding 0.75 ml of Buffer PE. Centrifuge 30 to 60 seconds and discard the flow-through. Then centrifuge the tubes again for 60 seconds to remove all the moisture.
14) Place the columns in clean 1.5 ml centrifuge tubes that are labeled with the sample number. To elute the DNA, add 50 μl of Buffer EB to the center of each column. Let the samples stand for **2 or more** minutes, and then centrifuge for 1 minute. The sample in the centrifuge tube (bottom) is your plasmid DNA. Discard the column and save the sample in the microfuge tube by placing it in the freezer (-20˚C).

Important note: SAVE THE SAMPLE IN THE FREEZER. The tubes **must** be labeled with the number of the sample.

References

Birnboim, H.C., and Doly, J. *A rapid alkaline extraction procedure for screening recombinant plasmid DNA*. **Nucleic Acids Research 7**, 1513-1523 (1979).

Griffiths, et.al. **Modern Genetic Analysis, W. H. Freeman & Co., NY. Chapter 10 (1999).**

Hartwell, L, Hood, L, Goldberg, M, Reynolds, A, Silver, L, and Veres, R. **Genetics, From Genes to Genomes**. McGraw Hill, San Francisco. p. 280-283.

QIAprep Miniprep Handbook. January, 1999.

Raven, P.H. and Johnson G.B. (2002). **Biology, Sixth Edition**. McGraw Hill, San Francisco. P. 381, 389-397, 402-403,

Weaver, RF. **Molecular Biology, 2nd edition**. McGraw Hill, San Francisco. p 93-94 (2002).