Alkaline Lysis/Phenolic Extraction of DNA

Introduction:

In this lab you will isolate plasmid DNA from transformed bacteria. A single, well isolated colony of E. coli (which have been transformed with an ampicillin resistance containing plasmid) is placed in LB media containing ampicillin. As the bacteria grow overnight, they will replicate the plasmid at the same time they replicate their genome and it will become amplified. The next day, you will lyse the bacteria with an alkaline solution, and then extract the resultant solution to purify the plasmid.

In the alkaline lysis procedure, bacterial cells are exposed to NaOH and sodium dodecylsulfate (SDS - a strong detergent). This causes the cell walls and membranes to burst and the contents of the bacteria are spilled out. An acidic solution of sodium acetate is then added to neutralize the solution. At this point, most of the cell membrane material and the genomic DNA precipitate to form a phlegm-like mass. The cell contents (including plasmids) can be separated from this material by centrifugation. The resultant supernatant is then extracted to purify the plasmid DNA.

Extraction is an easy and quick way to purify DNA from a mixture of proteins, lipids and nucleic acids (e.g., a cell or bacterial lysate). The mixture is extracted with phenol or a 50/50 mixture of phenol and chloroform. The organic solvents have two effects: 1.) they dissolve hydrophobic molecules and 2.) they denature proteins (which makes them insoluble in water). As a result, cell membranes and cellular proteins are either dissolved in the phenol/$CHCl_3$ (which is then discarded) or trapped in the interface between the two phases. DNA and RNA remain in the aqueous phase, and are easily separated.

It is important to keep in mind the purpose of this procedure while performing it. The reason for this procedure is to separate the plasmid DNA from its associated proteins so that further manipulations can be done to it. Enzymes added to purified DNA *in vitro* can have unhindered access to it. These enzymes might be used for restriction mapping, ligation, sequencing, or other procedures to modify the DNA. A poorly purified plasmid preparation will only be partially accessible to the enzymes and this will cause many headaches in these later steps. For this reason, special care must be taken to ensure a pure DNA preparation.

Also, the procedure isolates both plasmid DNA and small pieces of cellular RNA. If you need to work with DNA, these RNA fragments can inhibit your reactions. For this reason the final pellet will be treated with DNAse-free RNAse, which is an enzyme that hydrolyses the RNA into nucleotides.

Although this is a quick, easy and cheap way to isolate DNA, there are other methods that give a more pure product. Very pure DNA can be made quickly by column chromatography over commercially available columns (e.g., Wizard® preps by Promega™) however these columns are expensive and this can become cost prohibitive if you are doing a large number of preps. Also, a high quantity of extremely pure DNA can be made by 24 hr. high speed (100,000 X g) centrifugation over a </>$CeCl_2$ gradient, however this procedure can take two to three days.

Procedure:

IMPORTANT!

The afternoon before performing the procedure, you must pick a single, well isolated colony from a plate with a sterile toothpick and put it in 20 ml of LB media with 50 mg/ml ampicillin in a 250 ml Erlenmeyer flask. Incubate overnight at 37^{o}C in rotating incubator (B 314A).

The DNA you will isolate in this lab will be used in four of the up-coming labs, so do your best to get the highest yield and purest DNA possible.

Put the tubes of 95% ethanol and 70% ethanol in your ice bucket.

1.) Put approximately 1.5 ml of an overnight culture of bacteria into each of four eppendorf tubes with a ten ml. pipette. Centrifuge in the microfuge at high speed for 5 min.

Do the following to all four tubes:

2.) Decant the supernatant into a waste beaker. Resuspend the pellet in 100 ul solution I (50 mM glucose, 25 mM Tris.Cl [pH 8.0], 10 mM EDTA [pH 8.0]). Resuspension is done by pipetting the solution up and down on the pellet until there are no more solid pieces. Be sure the pellet is totally resuspended before going on.

3.) Add 200 ul solution II (0.2 M NaOH, 1% SDS). Close the tube and invert several times to mix the solutions.

4.) Add 150 ul solution III (3 M sodium acetate). Close the tube and invert repeatedly to facilitate precipitation.

5.) Centrifuge at high speed for 5 min. Carefully remove the supernatants and put them into new eppendorf tubes. Discard the old tubes and pellets.

6.) Add 0.5 ml of phenol/chloroform and vortex for 30 seconds. Centrifuge at high speed for 3 min.

7.) Carefully remove the aqueous (upper) layer with a pipette and place it into a new eppendorf tube. Be careful to not take any of the interface (precipitated proteins) with the upper layer (it's better to have a low yield of clean DNA than a high yield of dirty DNA). Pour the used phenol into a waste container.

8.) Repeat steps 6 and 7 until there is no solid material apparent at the interface between the two phases.

9.) Add 0.5 ml of chloroform to the final aqueous phase. (Be careful when pipeting chloroform - rinse the inside of the tip with chloroform by pipeting up and down before transferring to the eppendorf tube. Otherwise chloroform [carcinogenic] will spill out of the tip and onto your bench.) Vortex briefly (5 s). This will remove any residual phenol from the aqueous phase. Spin this in the microfuge briefly (5 s).

10.) Carefully remove the aqueous phase from the tube and place it in a new tube. Add 2.5 volumes of ice cold 95% ethanol and mix well to precipitate the DNA. (At this point, if you have a small drop of liquid that separates out at the bottom of the tube, it is $CHCl_3$ and must be removed before centrifuging.) Centrifuge on high speed for 5 minutes.

11.) Remove the liquid with a pipet and carefully wash the pellet with 400 ul of ice cold 70% ethanol. Spin for 3 min.

12.) Remove the liquid; be careful not to disrupt the pellet (a small white or clear solid at the bottom of the tube), which will be very loose now. Keep an eye on the pellet so that you don't discard it with the waste ethanol. Wash the pellet with 400 ul of 95% ethanol. Spin briefly (5 s) and remove the liquid carefully. Put the tube back into the centrifuge and spin briefly again to cause the ethanol on the sides of the tube to move to the bottom. Remove this last bit of ethanol.

13.) Allow the pellet to air dry for 5-10 min. Resuspend the pellet in 20 ul of TE with RNAse (10 mM Tris.Cl [pH 7.5], 1 mM EDTA [pH 7.5], with 1 ug/ml RNAse).

14.) Store in the freezer at -20^{o}C for later use.

If you have questions please send me a message at my Email address