

Biology Lab 3: Small Scale Plasmid DNA Purification (Minipreps)

Plasmids are small, circular pieces of DNA (about 3-5 kilobases in length on average) that can 'carry' a cloned gene or piece of DNA. They can be inserted into *E. coli* bacteria using a technique called transformation. Once inside the bacterial cell, the plasmid DNA will be replicated along with the bacteria's own genomic DNA when the cell prepares to divide. In our first lab, you were able to witness bacterial growth in a liquid culture. The *E. coli* used for that lab did not have plasmid DNA in it. In today's lab, the same strain of *E. coli* has been transformed with a plasmid carrying a gene called *Spatzle* (this gene is involved in determining the fate of cells in *Drosophila* development). The reason for putting plasmids into *E. coli* is so that you can have the bacteria amplify the DNA as they grow in a nutrient broth. The technique we will use today involves harvesting the plasmid DNA from the *E. coli* without isolating the bacteria's genomic DNA.

Plasmid purification procedures selectively enrich plasmid DNA over genomic DNA, which is present in the cell in much greater quantities. The two most widely used techniques are the alkaline lysis (Birnboim and Doly 1979) and rapid boiling (Holmes and Quigley 1981) procedures. Both protocols involve a precipitation of cell debris and protein and take advantage of physical properties of plasmids to separate them from the chromosomal DNA. Either of these procedures can be done in 1 to 2 hours, allowing rapid screening of many different plasmids. The plasmid DNA produced in these protocols is relatively clean and can be used in restriction digestions, transformations, and even DNA sequencing. In today's lab, we will employ the alkaline lysis method.

In the alkaline lysis procedure, cells from the overnight culture are pelleted rapidly in a microcentrifuge and the pellet is resuspended in a buffered medium. Then the cells are lysed with a solution of SDS (Sodium dodecyl sulfate – a detergent that will denature proteins) and NaOH (sodium hydroxide – a base). In addition to lysing the cells, these components serve several other valuable functions. The SDS and the NaOH solubilize and denature cellular constituents, and the elevated pH will begin to degrade RNA. The alkaline conditions also result in DNA denaturation (strand separation). Because the chromosomal DNA is very large, it is broken by shear forces when the cells lyse, while the small plasmids remain intact. Thus, when denatured, the plasmids remain linked to their complementary strand much as two links of a chain are linked together. When the lysate is neutralized with potassium acetate, the hydrogen bonds reform. Because the complementary plasmid DNA strands are held in close proximity to each other by the linked strands, they will immediately find their complementary strand and base pair properly to form an intact double-stranded plasmid. However, the chromosomal DNA will not be near its complementary strand. When the solution is neutralized, the DNA will base pair, but not necessarily to its complementary strand. The result will be a rather large, interlinked mass of DNA strands. The elevated salt concentration resulting from the potassium acetate addition will cause the SDS and protein to form a flocculent precipitate. The precipitated SDS and protein trap the chromosomal DNA fragments and are removed by centrifugation. The plasmids (and RNA) in the supernatant are then precipitated with alcohol and dissolved in a Tris/EDTA (TE) buffer.

Prior to starting:

• *Get ice and all other reagents together. Everything will be on the cart or on the benchtop area. Nancy will tell you what you will need.*

Protocol

1. Fill a 2.0 mL Eppendorf tube with the overnight culture. Label your tube with a Sharpie and place it in the centrifuge. Spin at 12,000 x g speed for 1 minute. This should pellet the *E. coli* at the bottom of the tube.
 2. Gently remove the tubes and look for the bacteria to be pelleted at the bottom of the tube. The supernatant is the TSB media that they were grown in. Remove the supernatant (SN) by pouring off into the sink and using a pipetman, carefully remove any remainder/trace of TSB and discard.
 3. Briefly (~1 min) respin to remove any remaining traces of SN, leaving the bacterial pellet as dry as possible. Be very careful not to suck up the pellet!
 4. To resuspend the pellet add 100µl of ice-cold Miniprep Solution I. Vortex/WhirlyMix the samples to get the bacteria in suspension.
 5. Incubate the bacteria for 5 minutes at room temperature in the tube rack.
 6. To lyse the cells, add 200µl of Miniprep Solution II. Mix by inversion. DO NOT VORTEX. Incubate on ice for 5 minutes.
 7. Add 150µl of ice-cold Miniprep Solution III to neutralize the lysate. Mix by inversion or gentle vortexing for 10 seconds. Incubate for 5 minutes on ice.
 8. Centrifuge at maximum speed for 5 minutes.
 9. Transfer the supernatant to a fresh tube, avoiding the white precipitate.
 10. Add 1000µL of 95% ethanol. Mix and allow to precipitate for 5 minutes on ice. The DNA is not soluble in ethanol and will begin to precipitate out of solution.
 13. Centrifuge at 12,000 x g for 5 minutes and look for a pellet at the bottom of the tube. Nancy can help you see this.
 13. Remove the supernatant and dissolve the pellet in 50µL of sterile TE buffer.
- Nancy will demonstrate how to quantify the amount of DNA you isolated using a spectrophotometer.