

## Promega Miniprep Kit Method for P.69 and GFP Plasmid DNA

Kay Finn

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### Preparation of Cell Lysate

1. Streak a fresh LB/amp plate (100 ug/ml amp for P69; 50 ug/ml amp for GFP) 37°C
2. 5pm: start 25 ml overnight culture of LB/amp (50ul 50 mg/ml stock for P69, 25ul for GFP) plus one colony from fresh plate, in 125 ml sterile flasks, shake in 37°C
3. 8am: put 2 x 1.5 ml (3ml total) from overnight culture in sterile 2 x 1.5 ml centrifuge tubes.
4. Spin 10K RPM in room temp microcentrifuge for 2 minutes. Pour off supernatant and dry tubes upside down on paper towel.
5. Resuspend cell pellet in 100 ul Cell Resuspension Solution. Combine the two tubes into one tube.
6. Add 200 ul Cell Lysis Solution, mix by inverting the tubes 4 times. If cell suspension does not clear immediately, wait for 3-5 minutes for complete lysis.
7. Add 400 ul Neutralization Solution and mix by inverting tubes 4 times. Incubate 10 minutes at room temp.
8. Centrifuge the lysate at 10K RPM in room temp microcentrifuge for 5 minutes. If pellet has not formed, centrifuge for an additional 15 minutes. **SAVE SUPERNATANT!! ...THIS IS THE CELL LYSATE FOR THE NEXT STEP!**

### Plasmid Purification Using the Vacuum Manifold

1. Attach one syringe barrel to one minicolumn from kit. Place on manifold. Close stopcock.
2. Pipet 1 ml of resuspended resin into barrel. If crystals or aggregates are present, heat solution to 37 °C for 10 minutes and cool before use.
3. Carefully transfer the supernatant (cell lysate) to barrel.
4. Open stopcock and apply vacuum to pull mix into barrel of minicolumn. Stop vacuum when everything is in minicolumn.
5. Add 2ml of 40% Isopropyl Alcohol/4.2M GuHCl solution and apply vacuum to get all thru column; wait 30 sec after all thru column, then stop vacuum. This will be slower than the first vacuum step.
6. Add 2ml of Column Wash Solution to the barrel. Apply vacuum until 30 sec after all solution thru the column. Stop vacuum. **DO NOT DRY LONGER THAN THE RECOMMENDED 30 SEC TIME!**
7. Remove barrel and cut off luer top part of minicolumn. Transfer minicolumn to 1.5 ml microcentrifuge tube, spin at 10K RPM for 2 minutes to remove any column wash solution.
8. Transfer minicolumn to a fresh 1.5 ml tube, add 50 ul of 70°C sterile H<sub>2</sub>O. Wait 1 minute. Centrifuge at 10 K RPM for 20 seconds to elute the DNA.
9. Remove the column. Add 5ul of 10X TE buffer to the eluted 50ul of DNA. Store at 4 °C.

### 40% IPA/4.2M GuHCl

(BL21(DE3)pLysS is an EndA+ strain; this is a method for this type of strain.)

Make a 7M GuHCl solution

(66.9 g Guanidine Hydrochloride + 50ml Sterile H<sub>2</sub>O, Mix, bring up to 100 ml vol after all is dissolved. Filter sterilize.)

In 50 ml Falcon tube: add 20 ml of Isopropyl Alcohol + 30 ml of above 7M GuHCl. Mix, RT storage.