

Promega DNA MidiPrep Kit for P69 and GFP

Clark Lab

Kay Finn

last updated: July 17, 2003

Cell Growth and Lysis Process

Start with overnight culture of 10 ml-100 ml in LB with appropriate antibiotic.

Pellet cells by centrifugation at 10Kxg for 10 min at 4°C.

- 10Kxg = 7000 RPM for JLA10.5 rotor, or 9000 RPM for JA20

- if have 100 ml or more, use GSA centrifuge tubes in JLA10.5 rotor (BIG) with adaptors

- if have <100 ml, use SS34 tubes in JA20 rotor (SMALL)

Pour off supernatant. Dry tube upside down on paper towel to remove excess liquid.

Can freeze here at -20°C for up to several weeks.

Completely resuspend cell pellet in 3 ml of Cell Resuspension Solution. VIP step!

Add 3 ml of Cell Lysis Solution, and mix by inverting tube 4 times. DO NOT VORTEX.

Cell suspension should clear immediately. [cultures of >50 ml need 3-5 additional minutes for lysis step; do not go longer, as this may result in ssDNA]

Add 6 ml of Neutralization Solution, mix by inverting tube 4x, incubate at RT for 10 min.

Centrifuge at 14Kxg for 15 min at 4°C in JA20 rotor (=11K RPM; use SS34 tubes). If tight pellet has not formed in 15 min, centrifuge another 15 minutes.

Decant supernatant into new tube. **SAVE SUPERNATANT.** Avoid any pellet.

Plasmid DNA Purification Process

Add 10 ml of resuspended Wizard Midiprep Resin to saved DNA supernatant. Swirl to mix.

Immediately load onto column in place on vacuum manifold. Pull solution through column, stop vacuum.

Add 15 ml of 40% IPA/4.2 M GuHCl reagent. Pull through column. All will not fit at one time, so add most and add the remaining after some run through. This will be slower.

Add 15 ml of Column Wash Solution and pull through column.

Repeat with 15 more ml of Column Wash Reagent and pull through column, this may take 30 minutes. Add **30 sec dry time ONLY** at end.

Transfer the end of the column tip [cut off with razer blade or cleaned up box cutter] to microfuge tube. Centrifuge at 10K RPM for 2 minutes to remove any additional Wash Solution.

Transfer column tip to new microfuge tube.

Add 300 ul of preheated [65-70°C] nuclease free H₂O to the column. Wait 1 minute. Elute DNA by spinning at 10K RPM for 20 seconds. Remove and discard column.

DNA solution may contain unseen fines from the resin. Spin at 10K RPM for 5 minutes to pellet fines, transfer the supernatant to a fresh microfuge tube and ethanol precipitate the prep.

DNA stable at 4°C in TE buffer or at -20°C in nuclease free H₂O.