



Transgenic and Gene Targeting Core
9500 Gilman Drive, MC 0687
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Preparing Plasmid DNA for Electroporation

PLEASE DO NOT USE QUIAGEN COLUMNS FOR ANY PURIFICATION STEPS.

1. Isolate plasmid DNA by CsCl density gradient purification. (Maniatis, et al. Molecular Cloning, pages 93 and 94) OR USE Promega Midi prep kit, called "Pure Yield Plasmid Midi Prep." (Catalog #A2492)
2. Linearize 500 mg of purified plasmid DNA (**the core needs 200 g DNA**) with appropriate restriction enzyme/s in a total volume of 500 ml (1 unit enzyme/ mg DNA). After incubating for approximately 2 hr, analyze 1 ml of the reaction by agarose-gel electrophoresis to ensure the DNA is completely linearized.
Please present a photo of this gel to the Core.
3. Add equal amounts of phenol (phenol means phenol equilibrated with buffer and containing 0.1% hydroxyquinoline and 0.2% beta mercaptoethanol) to the reaction tube.
4. Mix the contents of the tube until an emulsion forms (the organic and aqueous phases may be mixed by vortexing when isolating small DNAs i.e. <10 kb, or by gentle shaking when isolating DNAs of moderate size i.e. 10-30 kb).
5. Centrifuge the tube for 15 seconds in an eppendorf centrifuge at room temperature. If the organic and aqueous phases are not well separated, centrifuge again for a longer time or at a higher speed.
6. Use a pipette to transfer 80-90% of the upper, aqueous phase to a fresh tube. Leave and discard at least 10% of the aqueous phase with the interface and the lower organic phase.
Note: We believe this routine phenol-chloroform step of DNA purification is one of the most crucial steps in the overall success of your targeting project. Please take note of every detail outlined. It is of the utmost importance to keep away from the interface and organic phase of the phenol-chloroform extractions AT ALL TIMES. We suggest leaving at least 10% of the aqueous phase above the interface each time you transfer the remaining aqueous phase to the next step.
7. Add an equal volume of a 1:1 mixture of phenol and chloroform (chloroform means a 24:1 v/v mixture of Chloroform and isoamyl alcohol) to the aqueous phase. Repeat steps 4, 5, and 6.
8. Add an equal volume of chloroform to the aqueous phase and repeat steps 4, 5, and 6.

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9. Centrifuge the aqueous phase from the last chloroform extraction **alone** (with no more additions of phenol or chloroform) for 15 seconds in an Eppendorf centrifuge at room temperature.
10. **After the third centrifugation, take 90% of the aqueous phase and precipitate it with sodium acetate (see below)** Save the remaining 10% till later (see # 14).
11. For sodium acetate precipitation, estimate the volume of the DNA solution, and add sodium acetate to a final concentration of 0.25 M. Mix well. Add exactly 2 volumes of ice-cold ethanol. Mix well and chill to -20°C for 30-60 minutes. (If volume is too large, split between two tubes).

Centrifuge at 0°C for 10 minutes at 1200g.

12. Wash the DNA pellet with ice-cold 70% ethanol. (Add 70% ethanol until the tube is 2/3 full. Vortex briefly, and centrifuge at 0°C for 10 minutes at 1200g.) After the 70% ethanol wash, the pellet does not adhere tightly to the wall of the tube, so care must be taken when pipetting off the supernatant. Repeat this procedure 2 times.
13. Add a third wash of 70% ethanol to pellet, but this time do not centrifuge. Bring your pellet under 70% ethanol to the Core.
14. With the remaining 10% aqueous phase left in #10, remove 80-90% to a fresh tube and precipitate with Na acetate, as described above (#11). Wash 3 times with 70% EtOH
15. After the third wash, invert tube on an absorbent surface to allow as much of the supernatant to drain away as possible. Use drawn out capillary pipettes to remove any drops of fluid that adhere to the walls of the tube.
16. The DNA pellet can now be dissolved in TE to approximately 1 mg/ml. Use 3-4 ug of DNA in your dilution for your spectrophotometer reading to check the O.D. 260 value to determine DNA concentration and the O.D. 260/280 value to check DNA purity. Provide this information to the Core.

To summarize, you will bring to the Core:

- A photo of your digested DNA.
- At least 200ug of your linearized, purified DNA as a pellet under 70% ethanol.
- A spectrophotometric reading of your DNA indicating concentration and purity.