DNA preparation for microinjection
(transgenic mouse production)

1. Purify plasmid DNA containing insert for microinjection by CsCl banding or Qiagen prep column

2. Excise insert from plasmid DNA (use 50 ug of a 3-4kb plasmid containing a 1kb insert) by enzyme digestion

NOTE: it is important to remove all bacterial sequences, and ideally the restriction sites should immediately flank the promoter and poly A sequences

3. Separate insert from plasmid DNA on a preparative 0.9% agarose gel containing ethidium bromide in 1X TAE buffer

NOTE: it is important to begin with a clean electrophoresis chamber and unused buffer

4. Visualize band representing insert DNA on a transilluminator, and cut out gel piece using a new razor blade

NOTE: try to minimize exposure of gel slice to UV light

5. Prepare dialysis tubing (Spectrapor, 12-14K cutoff) by boiling in water for 5 min and rinsing three times in cold water, and seal one end with a clamp or knot

6. Place gel slice in tubing, and enough 0.5X TAE to cover slice; then seal top end of tubing with a clamp or knot

7. Place sealed tubing in clean dry horizontal electrophoresis chamber, orient in horizontal fashion, and secure to floor of chamber with tape

8. Fill chamber with enough 0.5X TAE to cover tubing and run at 40 mA for 25 min

9. Reverse orientation (or rotate tubing 180°), and run at 40 mA for 30 sec

10. Carefully remove solution within tubing using a long pipet tip, and transfer to eppendorf tube on ice

NOTE: using transilluminator, confirm that DNA has migrated out of gel slice and been removed from tubing

11. Add 3M sodium acetate (0.1 volumes) and cold ethanol (2.5 volumes), mix well, allow DNA to precipitate for 30 min at –80°C or overnight at –20°C

12. Microfuge for 15 min at 4°C to recover precipitated DNA, wash once with 70% ethanol, then let air dry on bench top

13. Resuspend DNA in 2 mL Loading buffer (200 mM NaCl, 20 mM Tris, 1 mM EDTA, pH 7.4)
14. Equilibrate Elutip-d column (Schleicher & Schuell) with 3 mL Loading buffer, then apply DNA solution and slowly push through column; collect flow-through and re-run through column

15. Wash column with 3 mL Loading buffer, then elute DNA with 0.4 mL Elution buffer (1 M NaCl, 20 mM Tris, 1 mM EDTA, pH 7.4); elute with an additional 0.1 mL Elution buffer to increase yield

16. Add eluted DNA to eppendorf tube containing 1 mL cold ethanol, then precipitate, wash and dry DNA as above

17. Resuspend purified DNA in 30 uL Injection buffer (10 mM Tris, 1x10^{-4} M EDTA, pH 7.4)

18. Fill 3 wells of a 6-well plate each with 5 mL Injection buffer, and float a 0.05 um / 13 mm filter (VMWP 01300, Millipore) in each – shiny side facing up

19. Carefully add DNA to center of first floating filter by pipet. The solution should round up and remain as a drop on the filter. After 20 min, carefully recover solution by pipet and transfer to second filter. Finally, after another 20 min, transfer solution to third filter. 

NOTE: Expect to lose as much as 1/2 of the sample by the end of this process

20. Estimate DNA concentration by running a small amount on agarose gel

21. Store at –20°C until ready for microinjection