



Transgenic and Gene Targeting Core
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TRANSGENIC MICE: METHOD FOR PREPARING DNA FOR MICROINJECTION

(Courtesy of Rosenfeld lab members, UCSD)

THIS METHOD STRONGLY RECOMMENDED BY THE CORE

1. Grow your plasmid in a bacterial strain like XL-1 Blue (Stratagene) that has no endoA activity and does not produce a lot of carbohydrates. Grow a 250-500 ml culture from a fresh transformation.
2. Make a Qiagen-500 DNA preparation according to the manufacturer's instructions. Resuspend DNA in 500 ul TE.
3. Further purify the DNA obtained from the Qiagen preparation over a CsCl gradient, or **use the Promega Midi prep kit called "Pure yield Plasmic Midiprep." Catalog #A2492 instead of the CsCL gradient.** Add 50 ul EtBr (10mg/ml) to DNA sample. Add sample to 4.5 ml CsCl solution (0.9 mg/ml). Place sample in sealing tube, heat seal, and spin in ultracentrifuge at 78K for 3-4 hours. Remove band, add 1 volume of TE, and extract 4-5 times with H₂O saturated butanol, to remove ALL EtBr (this is critical). Precipitate DNA and resuspend in 400 ul TE.
4. Digest 100 ug DNA O/N with the appropriate restriction enzyme in a volume of 200 ul or more.
5. Separate fragment from vector backbone by gel electrophoresis. Run on a 0.8% gel with NO EtBr (critical). Make a wide well (50-75 mm) and run gel for 2-3 hours at 80-120V. Run a marker lane beside the experimental lane. Cut out the appropriate band from the gel, representing your fragment. This is done by cutting off the marker lane and staining it with EtBr and then lining it up with the unstained gel over a light box.
6. **THE CORE HAS AN ELUTRAP DEVICE THAT CAN BE LOANED OUT TO INVESTIGATORS. PLEASE EMAIL TG@AD.UCSD.EDU TO RESERVE IT.** Electroelute the DNA fragment from the gel using for example, the Elutrap apparatus from Schleicher & Schuell, cat # 46170, following the manufacturer's instructions. Don't turn up the voltage too high or you may decrease your yield. Remember to reverse the leads 20 seconds at the end to pull the DNA off the membrane. This is critical. Recover the DNA in an 800ul volume (you may have more). From this step on, prepare all solutions with tissue grade water, endotoxin tested. (eg. Gibco cat#15230-147)

7. Take the eluate with the DNA (if you have more than 800ul, just split it) and equilibrate each 800 ul eluate with 50 ul MOPS pH 7.0 (1 M stock) + 150 ul NaCl (5 M stock).

Bind this equilibrated DNA to a Qiagen-20 column, pre-equilibrated according to the manufacturer's instructions. Allow to flow through by gravity and use a different column for each 800ul of equilibrated eluate.

Wash the bound DNA 3X with buffer QC, a total of 3 ml pass-through.

Finally elute the DNA with 800 ul buffer QF.

8. Precipitate the DNA in an eppendorf tube by adding 560 ul isopropanol to each eluate. Put at -80 for 20 minutes, then spin in the cold, 15 min, max speed.
9. Wash pellet with 70% EtOH, spin again 10 min max speed..
10. Do not overdry the pellets. Combine the DNA pellets (if you have more than one, you may not) by taking them up in 40 ul filtered 1X injection buffer (1X inj. Buffer = 7.5 mM Tris pH 7.4 + 0.15 mM EDTA).
11. Let DNA pellet fully dissolve then spin at full speed to get rid of all undissolved particles.
12. Dialyze the supernatant against 1X inj. Buffer on a Millipore filter catalog # VSWP 02500 (0.025 micron pore size). Prerinse a 100 mm petri dish with ~30 ml minjection buffer. Pour off and fill dish near to top. Float membrane on surface of buffer and add your sample to the center of the membrane. Dialyze o/n.
13. Remove membrane from the petri dish and place on a piece of SaranWrap. Remove sample - about half the volume or more will have evaporated. Add an additional 30-50 ul of injection buffer to the membrane to remove any DNA left on the surface. Respin DNA at 14-15,000 rpm for 10-15 min and place in new tube. You should still have about 30 ug left from the 100 ug you started with.
14. Prepare the DNA solution for injection as follows:
Dilute to a final concentration of 4 ug/ml corresponding to an OD reading of 0.080. It is always better to OD the DNA rather than estimate the concentration on a gel, especially for larger fragments. Divide the prep in half to determine this concentration and use the other half for injection. In addition you should run a mini gel to ensure that the DNA is not degraded, and confirm the concentration using GIBCO/BRL High DNA Mass Ladder Cat.# 10496-016. Bring a photo of this gel to the transgenic Core when you deliver your DNA,
15. Finally, the DNA is injected at a concentration of 1.8 ug/ml (that dilution is made in the transgenic facility. Be sure your DNA is free of any particulates before bringing it over, by spinning the DNA for 2 min at max speed in the microfuge Take off the supernatants. The Core needs at least 500ng of purified DNA.