Preparation of Transgene DNA for Microinjection

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1. Purify plasmid DNA by QIAGEN plasmid Maxi Kit (do not over load the column!), or QIAprep Mini prep kit, #27106 (if you do not need the DNA for other purpose), or by CsCl centrifugation.

2. Digest DNA with appropriate enzyme(s). Digest 60-120 µg of DNA with enzyme(s) in a total volume of 150-300ul to separate the insert from the vector backbone. Following digestion, check a small aliquot of DNA on analytical agarose gel for complete digestion and good separation of fragments.

3. Separate the transgene fragment from the vector. Separate fragments on an agarose gel in TAE. Run preparative gel by taping teeth of gel comb yielding large well across the gel (e.g. load up to 300ul of digestion solution). Run at a voltage to obtain good separation of bands (e.g. 0.8% agarose gel, 80-90 V for 3-8kb bands; or, 20-30V, o/n, for larger bands).

4. Excise the transgene band. Check gel on low energy UV light, excise the band corresponding to the transgene, place it in sterile polypropylene tube. Minimize DNA exposure to UV light to prevent photochemical damage (~ 1 minute).

5. Extract DNA from gel slice. One of the kits can be used, and DNA can be eluted with injection buffer (Millipore/Specialty Media, MR-095-10F), consisting 5mM Tris-HCl, pH 7.4, 0.1mM EDTA, 0.2uM filtered. (a) QIAEX II Gel Extraction Kit: Cat.# 20021, in a total volume of 80-100ul, or (b) Clontech NucleoSpin Gel and PCR Clean-Up, Cat# 740609.50, or (c) QIAGEN MinElute Gel Extraction Kit; Cat# 28604, or (d) QIAGEN Qiaquick Gel Extraction Kit: Cat.# 28704, or (e) GeneClean Turbo Kit, Cat#1102-000, or (f) using Dialysis Tubing (see details in the following "Note").

6. Filter the DNA prep again through a 0.2uM Syringe Filter (PALL Co. Cat# 4602, Acrodisc Syringe Filters, Supor Membrane, 0.2uM, 13mm, sterile), or (Whatman, Cat# 6809-1122, Anotop 10 Syringe Filter, 0.2uM, 10mm, sterile). This step is highly recommended. Prewash the filter with ~0.5ml Injection Buffer before filter the DNA prep.

- If the DNA fragment is larger than 15Kb, please spin the prep at 14k, 20min, 4C in a micro-centrifuge, then take the supernatant; repeat this step 3 times to remove any particles.

7. Determine DNA concentration. Use UV spectrophotometer, and run 50 – 100ng on agarose TAE gel, along with High DNA Mass Ladder (Invitrogen Cat# 10496-016) to check DNA quality and concentration. Store DNA prep at 4C.

- *You need a concentration of around 50ng/ul, and a total volume of around 50ul for the injection process.*
- *If the DNA concentration is too high, dilute an aliquot of your DNA, and re-check the diluted DNA prep on a gel, along with High DNA Mass Ladder, to give you a more accurate concentration for the injection process.* ***An accurate concentration of DNA is very important!!***

8. Record data on GMF Transgene DNA Sample Sheet. Label your tube: name of construct, DNA concentration, size of the fragment, date, & GMF Job#. Submit DNA prep to GMF for injection after fill out the GMF Service Request Form.

Note:

(f) Method of using dialysis tubing (Pierce #68100; 10,000 MW cutoff):

a. Place gel slice in dialysis tubing, add 1ml TAE buffer, remove bubbles, and seal both ends with clamps. (This assembling process can take place in ~300ml 1 x TAE buffer in a large glass container).
b. Orientate the gel slice so that DNA has the shortest distance to exit. Apply voltage, 80-90V for 20-30min, to elute DNA from gel slice.
c. Check the gel slice under UV light to make sure the elution is completed. Rub the dialysis tubing to dislocate DNA that stuck to the tubing. Transfer elute from the dialysis tubing to a 15ml sterile polypropylene tube.
d. Clean up DNA by DNA columns. Using 4-6 Qiagen PCR columns (Cat#:28104; each column will retain approximately 10µg DNA, for size 10Kb or less) to clean up DNA as per manufacturer's instructions. Combine all elutes into an Eppendorf tube.
e. Precipitate DNA:
    i. Precipitate DNA with 0.1 Vol 5M NH4Acetate (pH 5.5) and 2.5 Vol cold 100% EtOH (e.g. 300ul DNA + 30ul NH4Acetate + 750ul EtOH); mix well, and place sample at -80C for overnight.
    ii. Spin to pellet the DNA, wash the DNA pellet 1-3 times with 70% EtOH, and spin again. Remove 70% EtOH and air dry the DNA pellet.
f. Resuspend the DNA pellet in 50-100ul injection buffer (Millipore/Specialty Media, MR-095-10F), consisting 5mM Tris-HCl, pH 7.4, 0.1mM EDTA, 0.2uM filtered. (The injection buffer is not TE buffer.)