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Preparation of Transgene DNA

The protocol below was communicated by Steven Vokes (previous member of the McMahon Lab). It has consistently yielded transgenic founder mice at a frequency of around 30%.

1. Digest 100 µg of DNA with the specific restriction endonucleases to separate the insert from the vector backbone

Following digestion, check small aliquot of DNA on analytical gel for complete digestion and good separation of fragments.

2. Separate fragments on a 0.8% agarose gel in TAE

Run preparative gel by taping teeth of gel comb yielding large well across the gel. Load up to 500 µl of digestion solution. Run at low voltage to obtain good separation of bands.

3. Excise the band which corresponds to the transgene

Check gel on low energy UV light protected with saran wrap and shield, excise band, place in sterile polypropylene tube

4. Place agarose piece into dialysis bag, 10,000 MW cutoff from Pierce, add around 1 ml of TAE and electro-elute for about 20 minutes

To extract DNA from gel, place agarose slice in dialysis tubing, seal on either end with clamps, place perpendicular in gel box, apply voltage to elute DNA from agarose. After complete elution, check agarose slice for remaining DNA under UV light. Reverse current for 1 min to dislocate DNA that stuck to dialysis tubing. Transfer to sterile polypropylene tube.

5. Clean up DNA by using 6 Qiagen PCR columns (each column will retain approximately 10 µg of DNA)

Clean up DNA over Qiagen PCR columns as per manufacturer's instructions.

6. Precipitate DNA in ethanol, re-dissolve in filtered injection buffer, consisting of 5 mM Tris-HCl pH 7.6, 0.1 mM EDTA (Buffer is commercially available by Millipore/Specialty Media)

After re-dissolving DNA in injection buffer, determine concentration using spectrophotometer, fluorometer, and run small sample on agarose gel to check integrity of transgene insert. Copy data on GMF Transgene DNA sample sheet. Submit to GMF for injection. Store at 4⁰ C.