DNA PURIFICATION FOR MAKING TRANSGENIC MICE

REAGENTS REQUIRED

QIAGEN Maxi Prep Kit (Used HiSpeed Plasmid Maxi Kit – Cat. No. 12663)
QIAGEN Gel Extraction Kit (Used QIAquick Gel Extraction Kit – Cat. No. 28704)
TLE: 10mM Tris pH 7.4, 0.1mM EDTA, filter sterilized using 0.2μm filter
Restriction enzyme to linearize the plasmid

1 Maxi prep the plasmid containing the transgene to be injected

2 Digest 100μg of DNA overnight (16hours)
Make sure to use a restriction enzyme that will remove as much of the plasmid backbone as possible.

3 Clean the fragment using QIAGEN Gel Extraction Kit.
It is important to spread out the digested DNA as much as possible to avoid overloading. To do this, I taped together 19 wells of a 20 well comb and loaded the digested DNA across this large "single" well of 2, 0.8% gels.

All of the transgene DNA was cut out in a single band and the DNA from the 2 gels was dissolved together in buffer QG. The agarose/buffer QG/isopropanol mixture was then distributed equally over 10 gel extraction columns.

DNA was eluted from these columns using 80μL of TLE as follows. 40μL of TLE was used to elute the DNA from column 1 and this eluted DNA was then transferred to column 2 etc. up to column 5. The same technique was used for columns 6-10. The two final 40μL portions of eluted DNA were then combined.

Do not reprecipitate the DNA using ethanol. Do not use phenol chloroform to clean the fragment. Trace amounts of these chemicals are toxic to the embryo.

4 Quantify the DNA.
The facility requires 10-20μg of DNA with a minimum concentration of 100ng/μL.

5 Confirm Pure Transgene
Run a portion of your purified transgene next to a known quantity of uncut plasmid and cut plasmid. The facility requires a photo of the gel with these controls to confirm pure transgene.

M. Lamarche, 2011