THE NORTHWESTERN UNIVERSITY
TRANSGENIC AND TARGETED MUTAGENSIS LABORATORY

Preparation of DNA for Microinjection

In choosing a method from isolating microinjection DNA, it is important to remember that one of the most critical factors affecting integration efficiency is the purity of the DNA. High transgenesis rates can be achieved with ultrapure DNA. It is also essential that microinjection DNA be absolutely free of contaminants that are toxic to embryos. These include trace amount of phenol, ethanol or enzymes.

Plasmid DNA preparation:

Cesium Chloride gradient centrifuge preparation yields the purest plasmid DNA. Other methods that are routinely used include: NucleoSpinTM Extract Kit (available from Clontech, Cat# K3051-1); NucleoBond AX 500 Tip (Clontech, Cat# 4003-1); and Qiagen Endo Free Plasmid Kit (Qiagen, Cat# 12362).

Preparation of microinjection quality DNA:

The facility requires a total of 20μg of linear, purified transgenic DNA in microinjection buffer (supplied by the facility) at a concentration in excess of 40μg/ml for microinjections. Start with enough plasmid to yield this amount, assuming that 50% will be lost during purification.

The transgenic construct must have a promoter, a start codon, cDNA/genomic DNA/reporter gene, a stop codon with a poly A region. There should be little (100bp) or no vector present in the excised fragment as vector sequences may negatively affect expression**.**

Digest vector from the construct

1. Digest plasmid with the appropriate enzymes.
2. Electrophoresis in 0.7% agarose gel (regular mol biol grade agarose): electrophorese well into gel for good seperation of bands.
3. Excise band from band is excised and purified with a Qiagen kit (QIAquick Cat#28706).
4. Elute DNA 50^{0}C to ensure maximum recovery of DNA. The elution step is done twice; that is, following the first 50μl elution a second 50μl of EB buffer is applied to the column.
5. Check 2.5μl of the sample on a gel to assess recovery.
6. Precipitate eluted DNA overnight at -20^{0}C by addition of 10μl of 3M NaAcetate pH 5.2 and 225μl of ethanol. Note, precipitation of large-molecular-weight DNA molecules is more efficient with ammonium acetate, compared to sodium acetate.
7. Centrifuge DNA precipitate at 4^{0}C for 15 min.
8. Wash pellet with cold 70% EtOH, spin and remove EtOH
9. Wash pellet with cold 95% EtOH, spin and remove EtOH
10. Dry pellet for ten mins.

Elutip column purification (Schleicher and Schuell Cat# 10462615):

11. Dissolve pellet in elutip binding buffer and applied to an equilibrated elutip column.
12. Follow elutip protocol strictly adhering to the published procedure.
13. Precipitate final eluate and wash pellet as above (#6-10).
14. Resuspend pellet in small amount of microinjection buffer supplied by the TTML.
15. Run sample on gel to check quality and quantity. Submit photo of gel to TTML.
16. Store at -20^{0}C

Additional steps, which may improve DNA quality:

Isopropanol precipitation:

Purified DNA can be precipitated a second time followed by several 70% ethanol washes and extensive dialysis.

Standard phenol/chloroform extraction:

a. Phenol step: extract with 2 volumes phenol
b. Phenol/chloroform step: extract with 1 volume phenol, mix; then add 1 volume chloroform containing 5% isoamyl alcohol
c. Chloroform step: extraction 2X with chloroform containing 5% isoamyl alcohol
d. Precipitation step: ethanol precipitation as described above and wash several times with 70% ethanol followed by extensive dialysis if desired.

Dialysis for 16–48 hours against a large volume of TAE buffer removes traces of salts, CsCl and organic solvents (phenol/chloroform). The buffer should be changed several times during the dialysis.