



Genome Modification Facility Protocols **Harvard University – MCB Department / HSCI**

Preparation of Transgene DNA

The protocol below was communicated by Steven Vokes from the McMahon Lab; it has consistently yielded transgenic founder mice at a frequency of > 30%.

1. Digest 100 μg of DNA with the specific restriction endonucleases to separate the insert from the vector backbone.
2. Separate fragments on a 0.8% agarose gel in TAE.
3. Excise the band which corresponds to the transgene.
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
5. Clean up DNA by using 6 Qiagen PCR columns (each column will retain approximately 10 μg of DNA).
6. Precipitate DNA in ethanol, resuspend 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).

Isolation of Transgene DNA using the S&S EluTrap Apparatus

Materials:

- Elutrap apparatus
- large gel box
- BT1 and BT2 membranes (S & S)
- gel slices

Brief Description: * Nucleic acids in agarose gel slice are removed out of solid substrate by electromotive forces and trapped between semi-permeable and non-permeable membranes. The time it takes to elute depends on the size of the fragment. The eluate can then be concentrated by ethanol precipitation.

Procedure. (wear gloves!!)

- 1) Apparatus assembly (must be done rapidly to keep membranes moist). Add 1 X TBE buffer to gel box with elution apparatus (but not the traps you will be loading). Buffer can go as high as halfway up circular cutouts.
- 2) Place one BT1 membrane (NONpermeable, trapezoidal shaped, translucent membrane) just across from arrow on trap top, between 2 U-shaped inserts. The highest

comer should be closest to the arrow. This will be farthest from your gel slice [closest to the electrode].

Next, place one BT2 membrane (SEMIpermeable, white, rectangular membrane) between U-shaped inserts closer to middle of apparatus (i.e., closer to where you put the gel slice). Tighten up inserts, make sure membranes are upright. Place in chamber with buffer.

3) Open chambers that you will be loading and close the ones not in use (forces current to flow through the chambers to be used only). Make sure elutrap tray is pushed as far to the (+) end (right side) as possible.

4) Put gel slice in appropriate trough (LABEL trough!) close to the SEMIpermeable membrane. Check that buffer just covers agarose slice and that there is buffer in the eluate space (i.e., the space between the membranes). Make sure electrodes are hooked up correctly (current flow negative to positive).

Run at 100-200 Volts for appropriate time (2 - 5 hours). (No more than 100 V if overnight). Monitor with long wave UV light. Reverse leads and run at 200 Volts for ~20 seconds to remove any DNA which may be attached to the BT-1 membrane.

Purification of electroeluted fragment:

Remove eluate from trap with a sterile Pasteur pipette and place into clean, labeled microfuge tube.

* Extract sample with an equal volume of butanol; discard top phase. One extraction should be sufficient unless there is a large amount of ethidium bromide in sample.

Ethanol precipitate:

- add 1/10 volume of 3M NaOAc
- add 2 volumes of 95% EtOH; mix well by inverting tube
- precipitate at -20° overnight (for at least 2 hrs)
- centrifuge @ 4° for 30 min; discard supernatant
- wash pellet with 70% EtOH; centrifuge for 10 min
- dry pellet and resuspend pellet in Microinjection buffer* to a concentration of at least 50 ng/ul

* Microinjection Buffer:

- 5 mM Tris-Cl (pH 7.5)
- .1 mM EDTA (pH 7.5)
- dH₂O to appropriate volume
- Filter through a .2µm syringe filter and store at 4°C

Before injection, dilute transgene to 2 ng/ul in Microinjection buffer and filter through a Millipore Ultrafree MC centrifugal filter device.