PROTOCOL- **Purification of DNA for ES Cell Transfection**

Please provide **500 ug of linearized plasmid DNA** as an ethanol precipitate under 70% ethanol wash for each transfection request.

1. Purify plasmid DNA using ion exchange (i.e. Qiagen or NucleoSpin), silica gel (Sigma, NucleoSpin), other resin-based (Promega) system or CsCl gradient.
2. Quantify the isolated DNA by absorbance. The A_{260}:A_{280} ratio should be $\geq$1.7
3. Digest DNA in an appropriate volume of compatible buffer to linearize the construct. Linearization should not interrupt the sequence of interest. Complete digestion is required; please provide gel documentation with the plasmid DNA prep to confirm complete digestion. Indicate the injection fragment on gel documentation of the plasmid DNA digest.
4. Ethanol precipitate the digested DNA and leave the linearized DNA pellet in 70% ethanol wash.
5. The DNA precipitate will be stored at -20°C in the Transgenic/Knockout Core until final preparation for transfection.
6. The Transgenic/Knockout Core will resuspend the DNA ppt in embryo tested water to give a final concentration of 50-75 ug/25 uL based on the original DNA concentration provided with the DNA at the time of delivery to the Transgenic/Knockout Core Facility.
7. Each DNA aliquot in embryo tested water will be used for a single transfection experiment. Generally 2 transfection experiments are performed side-by-side to generate sufficient clones for 2 or 3 96-well plates after selection. Additional transfection experiments will be performed at a later date if too few clones are generated from the original transfection selections.