Protocol for Preparing DNA for a Knockout/in Project by Electroporation

DNA Purification for Introduction into the ES cell Genome –

from **Manipulating the Mouse Embryo pg. 432**

It is critical to use high-quality DNA, free of contaminating chemicals. The DNA should be purified either on a Quigen column (e.g., Qiagen 12143) or by CsCL centrifugation. DNA is then linearized, extracted with phenol/chloroform/isoamyl alcohol (25:24:1) (e.g., Invitrogen Life Technologies, 15593-031), precipitated with ethanol, and dissolved in sterile PBS or water at a concentration of 1mg/ml. The completion of the restriction digestion to linearize the DNA should be assessed by agarose gel electrophoresis. When using Qiagen purification, it is important to follow the manufacturer's instruction precisely. In particular, do not grow bacterial colonies too densely in a very rich culture medium. This may result in carbohydrate copurifying with the DNA.

For the Transgenic Facility:

It is recommended to use 20ug of DNA per 10 million ES cells or 40ug per 20 million ES cells for electroporation. It is easiest to provide the DNA to us @ 1ug/ul and have it dissolved in sterile PBS. If 80ug @ 1ug/ul of DNA can be provided this would be best – if something were to go wrong with the electroporation it is convenient to have enough DNA on hand to repeat as soon as possible if necessary.