

TMCF Protocols: DNA Purification / ES Cell Targeting

This protocol is designed simply for the purification of DNA targeting vectors. If you need assistance in the design of an appropriate construct, please contact our office (Dr. Baolin Li, 496-3352)

1. Purify the DNA targeting vector using a CsCl gradient.
2. Linearize 50 µg of the purified DNA construct using **a unique restriction site present only in the vector or in the plasmid polylinker**. Do not digest the DNA such that the targeting vector itself is disrupted. It is not necessary to remove vector sequences for ES cell targeting.
3. Run a small sample of the digest on a gel to ensure complete digestion of the targeting vector. Incomplete digestion will result in reduced electroporation efficiency.
4. Ethanol precipitate the DNA using standard protocols and store at -70° C.

When submitting DNA for targeting, please prepare 3 tubes, each with ~50 µg of fully digested DNA, and give them to us as ethanol precipitates. We will spin down the DNA and prepare it for electroporation.