THE NORTHWESTERN UNIVERSITY
TRANSGENIC AND TARGETED MUTAGENSIS LABORATORY

Isolation of DNA from 12 Well Plates

Materials & Solutions:

Lysis Buffer	10 mM Tris pH 7.5
	10 mM EDTA pH 8.0
	0.5% Sarcosyl (*do not use SDS*)
	1mg/ml Proteinase K *(added just before use*)
	Store at room temperature
Isopropanol	
TE (1x)	10 mM Tris
	1 mM EDTA, pH 8.0

ES cells densely growing on a 12 well flat bottom plate
Tape seals and roller for 12 well plate (Whatman UniSeal, thickness 0.5mm, cat #7704-001)

Note: The cells of interest should be very dense prior to beginning this procedure.

Day 1

1. Add the Proteinase-K dry powder to the Lysis Buffer to a final concentration of 1mg/ml.
2. Add 0.5ml of Lysis Buffer containing Proteinase-K to the wells.
3. To prevent evaporation, tape lid down using tape seals (roll across plate to form a seal, watch for bubbles around edge that may cause wells to dry out. It is VERY important that the wells do not dry out, so take care when sealing).
4. Incubate overnight at 55^{o}C.

Day 2

5. Transfer the solution from one well into an eppendorf tube and add equal volume of isopropanol. Invert the tube a couple of times to mix and look for the precipitated DNA (it will look like a ball of thread).
    a. If the precipitate forms, repeat this process with the remaining wells.
    b. If the DNA has not become soluble, then the precipitate will not form. If no precipitate is seen, add 1μl of fresh proteinase-K to the remaining wells, incubate at 55^{0}C for 1-2 hr, and repeat step 5.
6. Spool the DNA onto pulled glass pipet and allow to dry BRIEFLY.
7. Transfer DNA to an eppendorf containing 50-100μl of sterile TE buffer. Be careful not to transfer too much liquid with the DNA.
8. Place the DNA into a 37^{0}C waterbath for 1-2 hr. This will help resuspend the DNA. Alternatively, place at 4^{0}C overnight. In either case the DNA should be gently tap-mixed and kept at 4^{0}C overnight.

Day 3

9. Gently tap-mix eppendorf tubes again. The DNA should be viscous. This indicates that you have high molecular weight DNA (which is good). DNA is ready for analysis.