THE NORTHWESTERN UNIVERSITY
TRANSGENIC AND TARGETED MUTAGENSIS LABORATORY

Isolation of DNA from 96 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
NaCl/Ethanol	1.5ml 5 M NaCl
	100ml (100%) Ethanol
	Store at –20°C
70% Ethanol	70ml (100%) Ethanol
	30ml H_2O
TE (1x)	10 mM Tris
	1 mM EDTA, pH 8.0

ES cells densely growing on a 96 well flat bottom plate
Tape Seals for 96 well plate (Whatman UniSeal, thickness 0.5mm, cat #7704-001)
Multichannel pipetor

Day 1

Note: The cells of interest should be very dense prior to beginning this procedure (i.e. ES cells seeded at $1x10^5$ cells/well, grown a minimum of 5 days before harvesting).

1. Add the Proteinase-K dry powder to the Lysis Buffer to a final concentration of 1mg/ml.
2. Add 50μl of Lysis Buffer containing Proteinase-K to the empty 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).
4. Incubate overnight at 55°C.

Day 2

5. Spin down plate for 3-5 minutes at 3200 rpm to collect condensation (optional).
6. Add 100 μl/well NaCl/Ethanol (@ -20°C). The salt precipitates, so keep the mixture well mixed. Incubate at -20°C for at least 30 minutes until precipitated DNA is visible as long threads under tissue culture microscope.
7. Quickly invert plate over sink to dump out liquid then blot on paper towel.
8. Rinse 3 times with 100μl 70% ethanol. With each rinse, quickly invert plate over sink then blot on paper towel.
9. Air dry 15-20 minutes.
10. For PCR screening, add 40μl of TE/well. Incubate at 65°C for 1 hour to allow DNA to resuspend. DNA can also be incubated at 4°C overnight.
    For Southern screening, DNA can be resuspended in 1x restriction buffer + 100μg/ml BSA + 1 mM spermidine. Resuspend the DNA by pipetting up and down 20-30 times. To prevent evaporation, place adhesive seal on plate and cover with plastic lid taped down. Incubate at a temperature appropriate for the enzyme.
11. Spin plate down 3-5 minutes at 3200 rpm to collect condensation.
12. Resuspend the DNA by pipetting up and down a least 20-30 times before use.
13. DNA can be quantitated with a Fluorometer.

Note: Average yield for dense culture of ES cells is ~4.5 μg/well (range = 1 to 7.2 μg/well). DNA is suitable for use in PCR or Southern Blots. For PCR use 1-3μl of sample. Use all of the DNA sample for Southern Blot Analysis. Store DNA plates at –20^{o}C.