UCSD Transgenic Mouse and
Gene Targeting Core
9500 Gilman Drive, MC 0687
La Jolla, CA 92093-0687
(858) 534-3178

DNA Isolation from ES cells in 96 well plates

After the clones from your transfection have been picked and split, you will receive duplicate 96-well plates that have been grown to 100% confluence. The media will have been removed and the plates will have been frozen at –20° C. You should prepare DNA from one of the plates first and store the duplicate covered with parafilm at -20° C as a backup.

Required Reagents and Materials:

1. Lysis Buffer	For 100 ml:	
	10 mM Tris-HCl pH 7.5	0.5 ml of 2 M stock
	10 mM EDTA:	2 ml of 0.5 M stock
	10 mM NaCl:	0.2 ml of 5 M stock
	0.5% Sarcosyl	w/v: 0.5 g / 100 ml
	1 mg/ml proteinase K	100 mg proteinase K (add fresh each time; stored in –80° freezer)
2. Precipitation Buffer	For 100 ml:	
	150 mM NaCl	3 ml of 5 M stock
	100% EtOH	97 ml 100% ETOH
		(This solution remains a slurry)
3. Wash Buffer	For 200 ml:	
	70% EtOH	140 ml 100% EtOH
		60 ml MQ water
4. Resuspension Buffer:	For 25 ml:	
	10 mM Tris-HCl pH 8.0	0.125 ml of 2 M stock
	10 mM EDTA	0.5 ml of 0.5 M stock

5. A 60° C incubator

Method:

1. Add 50 µl of Lysis Buffer to each well.
2. Dampen some paper towels and place in the bottom of a tupperware container.
3. Place the plates on the paper towels and seal the lid of the tupperware container.
4. Incubate the plates (with lids on) in this sealed humid container in a 60° C incubator for 2-3 hours.
5. Add 150 µl of Precipitation Buffer to each well; leave at RT for 30 min to allow the DNA to precipitate.
6. Decant the supernatant—be extremely cautious with this step because it is easy to lose DNA pellets if you don't invert the plate very slowly. In addition, don't let the paper towels touch the wells, otherwise the DNA will be sucked onto the paper towels! As an alternative which is safer, you can transfer the contents of each well into individual eppendorf tubes and continue the prep from there.
7. Wash the precipitated DNA with 150 µl of Wash Buffer 3 times. Decant.
8. Let the pellets dry by leaving the plate on the bench (with the lid off or the eppy tubes open).
9. Resuspend the DNA with 30 µl of Resuspension Buffer. Incubate o/n in the 37° C incubator (with the lid on or the tubes closed to prevent evaporation).

** The expected yield of DNA from each plate well is 10 µg. The DNA from one plate will be sufficient to perform 1 southern blot (10 µg per gel well) or several PCR reactions. With the duplicate plates we provide, you will have enough DNA to analyze both the 5' and 3' ends of your clones.**

10. If you need to run a PCR reaction, you may use 1-2 µl of DNA from step 9. If you need to perform a Southern blot, continue to step 11.

11. Make up 10 µl per sample of digest cocktail containing:
    -restriction enzyme
    -restriction enzyme buffer
    -BSA (if required by enzyme)
    -spermidine (to a final concentration of 1 mM; stored in –80° C freezer)

12. Add that 10 µl to the 30 µl DNA from step 9 for a final volume of 40 µl.
13. Digest o/n @ 37° C (with the lid on or the tubes closed).
14. Electrophorese the digested DNA on an agarose gel. Do not exceed 80V.