

## 96 Well DNA Prep.

### ES Cell lysis buffer:

	<u>for 100 mls</u>	<u>final concentration</u>
5 M NaCl	2 ml	100 mM
1 M Tris-HCl pH 7.5	5 ml	50 mM
0.5 M EDTA	1 ml	10 mM
20% SDS	2.5 ml	0.5%
Sterile H <sub>2</sub> O	89.5 ml	

\* 1.0 mg/ml proteinase K added before use

### Procedure:

- For cells growing in 96 well dishes without feeders.

\* Make sure that cells are dense to the point of the medium being a yellow color.

- Rinse wells 2x's with 100 µl PBS
- Place in - 80° C freezer for at least 1 hour
- Add 50 µl lysis buffer w/PK per well
- Parafilm dish and enclose in a container lined with wet paper towels
- Place at 60° C overnight
- Spin moisture down from lid (1000 rpm for 5 min)
- Add 50 µl isopropanol - tap plate to mix sample. Let sit for 30-45 min (longer is better) and should see a stringy white DNA ppt. Tap the plate if you don't.
- Spin the plate to stick the DNA firmly to the bottom (1500 rpm for 5 min)
- Gently invert and drain liquid onto paper towels - blot
- Wash with COLD 70% ethanol (fill wells with a squirt bottle)
- Spin plate to make sure DNA is attached (not absolutely necessary) - 1500 rpm
- Gently invert and drain liquid onto paper towels - blot
- Repeat ethanol wash 3x's
- Air dry DNA for 10-20 min or longer. It's very important that wells are dry - otherwise samples won't load.
- Add DNA digestion mixture directly to well and digest overnight at 37° C (use 4 µl of enzyme per well - total volume of 40 µl/well)