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MINISOUTHERN ANALYSIS OF ES CELL CLONES

Initial cloning

1. Wash plate with selected colonies twice with PBS.

2. Dispense 25 μl of trypsine in 96-well plate using a multichannel pipette.

3. Pick individual colonies into each well of 96-well plate (about 100~150 clones/hour).

4. Add 25 μl of ES medium per well with multichannel pipette. Pipette up and down to disaggregate ES cells.

5. Transfer 25 μl cells to a 96-well feeders plate (with 100 μl ES medium) and allow growing about 3 days for frozen storage. Add 100 μl ES medium to rest of the cells and culture about 5 days to confluence for mini-Southern analysis. To generate duplicate plate, passage and split cells after 3 day culture. This procedure can usually result in more homogenous DNA yield from each well.

Mini-Southern analysis

6. Wash cells with PBS twice and add 50 μl of lysis buffer. Incubate overnight at 60°C in a humid chamber.

7. Add NaCl/EtOH mixture [1.5 μl of 5 M NaCl to 100-μl cold absolute ethanol (stored at –20°C). The salt will precipitate. Mix well and dispense 100 μl to each well with a multichannel pipette]. Leave the plate on the bench for about 30 min. (usually DNA precipitation can be seen immediately).

8. Cover the plate with paper towel, flip over and discard the solution. Wash with 150 μl 70% EtOH twice (try not disturb the DNA).

9. Invert plate on paper towel for a few minutes (about the time to prepare restriction cocktail). Do not let the DNA dry completely.

10. Add 30 μl of restriction cocktail to each well with a multichannel pipette. (A typical mixture will contain 1 X restriction buffer for the enzyme to be used, 1 mM spermidine, 100 μg/ml BSA (optional), and 10-15 units of enzyme). Incubate at 37°C overnight in a humid chamber.

11. Add 6 μl of 6 X loading buffer and load an agarose gel. Run gel at 80-100 V for about 3 hours. The rest is the same as normal Southern procedure.

Lysis Buffer: 10 mM Tris pH 7.5, 10 mM EDTA, 10 mM NaCl, 0.5% sarcosyl. Add Proteinase K before use to 1 mg/ml

	250 ml
1 M Tris.HCl, pH7.5	2.5 ml
0.5M EDTA, pH8.0	5 ml
5M NaCl	0.5 ml
Sarcosyl	1.25 g