SUBCLONING

Targeted ES cell clones, especially those obtained after selection for G418-resistance, are often subclonal. In this case the intensities of the endogenous and homologous recombinant bands on the Southern are not identical. Pure targeted ES cell clones can be obtained by subcloning:

- Prepare a 96-well plate (flat bottom) with a MEF feeder layer.
- Culture an ES cell line at a small scale (e.g. on a 4-well plate).
- Trypsinize the ES cell culture and count the number of cells.
- Add 50 cells to 20 ml of complete medium + β + LIF.
- Fill the 96-well plate with 200 μl per well.
- Grow individual colonies (15 - 20 will appear after 7 days).
- Wash the wells with PBS (100 μl) and trypsinize with 25 μl TVP(10x) (5 min 37 °C).
- Add 150 μl complete medium + β + LIF, resuspend and transfer the cells to a new 96-well plate with MEFs.
- Culture the cells overnight and refresh the medium (200 μl).
- Culture the cells to (semi)confluency (2-3 days).
- Expand the cell cultures to 4-well or 12-well surface.
- Store 1/3 in liquid N_2 and use the rest for DNA analysis.