

## Picking ES Cell Colonies and Transferring Them to 96-Well Plates

This is done after the ES cell colonies have been growing for 8-21 days. It is up to you how many colonies you wish to pick from your plate, but most people do one or two 96-well plates of colonies per electroporation.

1. Add 50  $\mu$ l of trypsin per well to a 96-well, round bottom plate.
2. Prepare your plate of ES cell colonies by washing it with PBS. Cover the bottom of the plate with PBS (about 7ml).
3. There's a special tool which will swivel the microscope eyepiece to make picking more comfortable. Adjust it to a good position for you. Using the microscope, pick colonies from the plate using a P-20 set to 4 $\mu$ l. P-10 tips may be best for picking, but standard "yellow" tips are fine, too. Transfer each colony into a well of the 96-well plate. If possible, try to alternate between small and large colonies (alternating rows is a good way to do this), in order to get a good range of events. An experienced picker can do two plates in about an hour. To keep your place on the plate, place a small piece of tape with a mark on it underneath the plate.
4. It is not necessary to do an incubation at 37°C after picking the colonies, although if your colonies are really huge, a 10' incubation would not do any harm.
5. Add 50  $\mu$ l of M-15 to each well. Pipette up and down 10-12 times using the multi-channel pipet, to break up the colonies.
6. Transfer the cell suspension to a 96-well feeder plate, preferably one which has been freshly fed with about 100 $\mu$ l M-15/well. Return plates to the TC 37°C incubator.
7. If you want to isolate DNA from your cells, wait until the plate is confluent, and split it 1:2 between a feeder plate (to freeze or continue growing) and a gelatinized plate (for isolating DNA). These will re-reach confluence within a few days, and can be further processed at that time (see protocols for freezing plates and making DNA from plates).