Picking and expanding ES Clones

- Note, typically, 100-500 clones are picked after electroporation. Rates of homologous recombination vary from less than 0.1% to over 10%.

- On the day prior to picking colonies, set up 24 well dishes with $G418^R$ feeders using 0.5 ml of medium per well. Note, the area of a 24 well plate is approximately that of a T75. Feeders do not distribute as evenly on 24 well plates as on larger dishes. To facilitate more even distribution, rock the 24 well plates each hour for the next 3 hours.

- Feed colonies on the 10 cm plates 1-3 hours before picking them. At the same time, change the medium in the 24 well plates using 1 ml ES medium per well (drugs are optional at this point).

- Add 10 µl HBSS to every other well in a 96 well plate.

- Wash colonies on 10 cm dish with HBSS.

- Under a dissecting or phase contrast microscope in a hood, pick colonies with a P20 Pipetman, picking up to 10 µl or so of HBSS with it and transfer it to a well in the 96 well plate.
  Use a fresh rack of tips, and take every other tip for each colony. It will be convenient later when using the 12 place pipetor to have a tip on every other position of the pipetor. By using every other tip when picking colonies now, you'll have a rack with tips conveniently placed for the 12 place pipetor).

- Pick 24 colonies this way.

- Add to the picked colonies 20 µl trypsin with the 12 place pipetor.

- Incubate at 37°C for 5 minutes.

- Add 100 µl ES media to trypsinized colonies with the 12 place pipetor.

- Pipette cells up and down several times to get single cell suspensions.

- Transfer trypsinized cells to the 24 well dishes with feeders. A tip at every other position of the 12 well pipetor allows you to transfer 6 clones at a time from the 96 well dish the 24 well dish.

- Grow cells on 24 well plates, feeding them daily with 0.5 ml ES medium.

- 5-10 days later, trypsinize cells for freezing, and expansion for DNA preps.