Picking ES cell colonies and transferring them to 96-well plates

About 7~8 days after drug selection is started, colonies are big enough to be picked with naked eyes. Once you know your general targeting efficiency, you may adjust the numbers to pick. We pick 48 colonies per construct.

Materials:

- round-bottom 96 well plate
- flat-bottom gelatin-coated 96 well plate

Methods:

1. Aliqot 20ul of trypsin per well to a 96-well round bottom plate.
2. Prepare a ES cell plate by rinsing with 10ml of PBS and add 10ml of GMEM to cover the plate.
3. Place the plate under the microscope to select undifferentiated healthy colonies.
4. Pick colonies using a P-20 pipette set to 10ul. Carefully pick a colony without breaking up the colony into single cells, and transfer it into a 96-well plate with trypsin. Pipette up and down a couple of times to disperse the colony.
5. Once all 48 colonies have been picked into the wells, incubate the plate in a 37C incubator for 7-10 minutes.
6. Add 170ul of media to each well. Pipette up and down ~10-15 times using the multichannel pipette to break up the colonies.
7. Transfer the cell suspension to a 96-well gelatinized plate. Change media everyday until ready to be split again.