Plating subclones for electrophysiological testing

1. Choose 12 wells on the 96-well plate that contain one colony. You will need 3.2 ml selecting media (F12, P/S, FBS, Blasticidin and Zeocin) per colony picked.

2. Remove media from selected wells, rinse once with 200ul PBS, then add 50 ul trypsin. Watch to see when cells detach.

3. While cells are trypsinizing, For each colony, put 1 ml media in a well of a 12-well plate, and put 2 ml media in a 35 mm tissue culture treated dish. Mark well and dish with name of a well a clone is to be picked from.

4. Once they detach, add 200uL of selecting media. Triturate 5x to suspend cells. Remove 200ul suspension. Plate one drop of cell suspension in appropriately marked 35mm tissue culture dish. Place rest of cells in appropriate well of 12 well plate.

5. Add 200 ul media to picked wells of 96-well plate

6. Place cells incubator

*Cell Media: Ham's F12 from Gibco, +10% FBS, + 1% 100X Penicillin/Streptomycin