Protocol for Colony Picking

1. Thaw trypsin, warm media (M10G+LIF)
2. Remove the plate from incubator.
3. Remove the media from the plate.
4. Add 7ml of PBS to the plate.
5. Add 25µl trypsin (2x+glu trypsin) to 'U' bottom 96 well plate.
6. Pick up the colony with 20µl pipette set to aspirate 10µl of PBS with the colony. Cut round the colony with the pipette tip and aspirate in 10µl volume. Add the colony and PBS to the trypsinised well.
7. Incubate the plate in an Incubator at 37º for 10 minutes.
8. Add 165µl of media to the plate.
9. Triturate 4-5 times to disperse the colony
10. Transfer the cells to the flat bottomed gelatinised 96 well plate.
11. Use microscope to check the cells are properly dispersed. Mix further if required.
12. Incubate the 96 well plates overnight.
13. The following morning, change the media to M10G+Lif+G418 (100µg/ml).