**Kartheek Gavini**

**Param Lab**

Leak Prevention Gel Protocol:

1. Set upper and lower plates on even surface
2. Make sure that both of the plates are even on bottom
3. Place the plates in cassette and make sure plates are even. Press the levers and close cassette
4. Place the plate cassette on the casting stand with a layer of parafilm on bottom
5. Make the desired stacking gel recipe w/ bromophenolblue (1:500 dilution) without adding the polymerization reagents (10% APS and TEMED)
6. Extract 500uL per gel in separate Eppendorf tubes
7. Add 2uL of 0.5% bromophenolblue in Eppendorf tubes
8. Vortex
9. Prepare everything near the vortex machine (Set 1000mL pipette to 450uL)
10. Add 10uL of 10% APS and 1uL of TEMED and quickly vortex **one Eppendorf tube at a time (Reaction will finish within a minute so be quick)**
11. Pipette 450uL of solution from Eppendorf into the plate by tilting the plate and pipetting slowly **(Reaction will finish within a minute so be quick)**
12. Rock the solution back and forth for a couple seconds after in the casting stand **(Reaction will finish within a minute so be quick)**
13. Wait two minutes for polymerization
14. Check the Eppendorf tube (which should have 50uL remaining) to see if polymerization is complete
15. Drain excess water with KimWipe by tilting the casting stand
16. Repeat Steps 10-15 per Well plate