9/10/19 Meeting with Allie

About: protocol for influenza reverse genetics

1. Transfect cells:
   1. Transfect in 6-well plates, 1 well should yield enough virus for experiments
      1. Good titer: >105 TCID50/uL \* 2x103 uL (2 mL per well)
      2. Bad titer: ~103 TCID50/uL \* 2x103 uL
      3. Need 103 TCID50 over 50uL for injection, so should have plenty from one well
   2. Transfect a mix of 293T and MDCK-SIAT1-TMPRSS2 cells
      1. 293T cells transfect better, but MDCK cells get infected better
   3. Make transfection master mix in media without FBS (but add it dropwise to cells in D10 media with FBS)
   4. Transfect 250ng each plasmid:
      1. All 8 segments plus TMPRSS2 plasmid (needed because 293T does not express TMPRSS2, which cleaves HA- so you don’t have to add trypsin)
      2. Do transfection control with PB1-GFP plasmid plus all 8 segments (should only see green if all RNPs and PB1-GFP are transfected)
      3. No HA control with PB1-GFP: 293Ts should be green but not MDCKs (can’t infect)
      4. No HA (without PB1-GFP) as TCID50 control: should see 0 infectious particles
   5. Change media to IGM 20-24 hours post-transfection
      1. Can wash with PBS first (to get rid of FBS), but this can wash away 293T cells. Not necessary
   6. Harvest virus 54-72 hours post-transfection
      1. Harvest into 2mL tubes (1.8mL max per tube)
      2. Centrifuge and take supernatant
      3. Make enough aliquots for each planned use of the virus, to avoid freeze/thaw
2. pHW2000 plasmid is bidirectional
   1. pol2 transcribes mRNA in one direction
   2. pol1 transcribes vRNA (has no tails) in the other direction
3. Making plasmids:
   1. Miniprep ok (don’t need to use endotoxin free maxi)
   2. Allie uses NEB Q5 mutagenesis kit
4. Safety:
   1. Dilute bleach doesn’t stay active for long. Replace this in the waste container often
   2. Don’t fill containers with all the way (prevent virus from touching lid)
   3. Store virus in screw top cryovials at -80 so snap top doesn’t break
   4. Use lids on centrifuge to prevent spilling virus