MCMV production:

D0: Plate 3T3s

1. Harvest 3T3s by washing quickly with 1XPBS followed by trypsin
2. Remove trypsin right away and incubate dishes @ 37ºC for 3-5min
3. Resuspend cells in DMEM, and spin down cells @ 1200rpm for 5min
4. Resuspend cells in smaller volume, and count cells
5. Plate 3X10^6 cells/dish/20ml of DMEM in large 15cm TC dishes
6. Incubate o.n. until they are 70-80% confluent

D1: Infection of 3T3s

1. Remove all the media, and add 10ml of fresh DMEM
2. Quick thaw virus in 37°C water bath, 3min
3. Vortex virus stock briefly to mix virus that has settled on the bottom of the tube
4. Infect cells @ MOI 0.2-0.3 by adding appropriate volume of virus stock directly into culture media in the dishes

   NOTE: use a little bit higher titer if virus has been freeze-thawed. You can still re-use virus that has been re-frozen, just the titer may be a little lower
5. Incubate dishes for 2hrs @ 37°C
6. Add 10ml of fresh DMEM directly to 10ml of media + virus (no washing necessary)
7. Incubate dishes for 3-4days @ 37°C until most of the cells have rounded up and floated in the media. Monitor daily.

D4: Harvest supe (D3 p.i.)

1. Collect all supe in 50ml conical tubes
2. Spin @ 1200rpm for 5min to spin out all cells
3. Take all supe and transfer into ultra-centrifuge tubes with caps (~35ml/tube)

   NOTE: Take note of the volume of supe to know how much volume to resuspend virus pellet in after centrifugation
4. Balance tubes
5. Ultracentrifuge supe for 2hrs @ 15,000rpm @ 4°C in JA-20 rotor to pellet virus
6. Gently pipette off supe, and resuspend pellet in DMEM, 1/10 of the original supe volume
7. Aliquot in cryotubes, and freeze @ -80°C