## Culture Splitting Quick Guide

### Get everything ready

* New flask, labeled:
  + cell type
  + transfected gene
  + date
  + passage #
  + your initials
* Coverslip-bottom dish, also labeled
* Tube of DMEM, labeled with your initials
* Warm PBS, labeled with your initials
* Trypsin (shared)
* Pipetters and tips
* 5 mL serological pipets
* pipet gun
* waste beaker
* spray bottle of ethanol

### Protocol – Keep everything sterile!

1. Spray all surfaces. Spray everything that goes into the hood.
2. Spray your gloves
3. Label new flask to receive cells
4. 2.5 mL DMEM into flask, 2 mL into dish, move to incubator
5. Take flask of cells out of incubator, label with your initials
6. Use 5 mL serological pipet or a transfer pipet to remove medium
7. Put ~ 1 mL warm PBS into flask (sterile transfer pipet or Pasteur pipet)
8. rock to rinse, remove PBS
9. Add ~0.5 mL trypsin (KEEP STERILE), rock to coat cells, put in incubator
10. Incubate 3 minutes
11. Check that cells are in suspension; incubate longer if needed
12. Add 2 mL cold DMEM (KEEP STERILE) to stop the reaction.
13. Transfer 0.7 mL to new flask; 0.2 mL to dish
14. Done!