**Passaging and Seeding Mouse Embryonic Fibroblasts (MEFs) for Experiments**

1. Passage cells when confluency exceeds 60%
2. Remove media
3. Wash cells with 5mL prewarmed PBS without Calcium and magnesium.
4. Aspirate and discard PBS
5. Add 3-5mL room temp TrypLE Express (enough to ensure complete coverage of cells).
6. Incubate cells at 37°C until they have detached
	1. check at 3-5min intervals under an inverted microscope, gently tap flask to dislodge cells if needed
7. Add 5-10mL (or at least 1:1 ratio of media : TrypLE) of pre-warmed complete media to flask
	1. pipette up and down to dislodge cells as needed.
8. Transfer cell suspension to conical tube
9. Spin down at 1300-1800rpm (300xg) for 3-5 mins
	1. *During this time label one microcentrifuge tube for each cell line being used and add 20μL of Trypan blue*
10. Check for cell pellet then aspirate and discard supernatant
	1. avoid disturbing the cell pellet
11. Resuspend cells in appropriate volume of prewarmed complete media
12. Take 20μL of resuspended cell mix (gently pipetting up and down 10 times) and add to 20μL of trypan blue
	1. pipette up and down 10 times to mix cells with trypan blue
13. Count the cells using a Countess 3 (Thermofisher Scientific)
	1. Clean the glass hemocytometer slide and add 10μL of cell / trypan blue mix to chamber A and B
	2. Avoid bubbles and dirt
14. Perform cells count ensuring working in same units e.g. x105 cells per mL or x106 cells per mL
A=\_\_\_\_\_\_\_\_ B=\_\_\_\_\_\_\_\_ Average A & B =\_\_\_\_\_\_\_\_\_\_\_\_\_
15. Once counts are performed mix cell suspension again as cells will have settled to the bottom of the tube by the time counts are done
Add \_\_\_\_\_\_\_\_\_\_\_\_\_\_ μL/mL of to \_\_\_\_\_\_\_\_\_\_\_ μL/mL of media
16. Seed cells in appropriate volumes
17. Record the following:
18. Cell type, strain and genotype\*
19. Number of wells
20. Density per well\*
21. Passage\*
22. Time and Date of Seeding\*
23. Temperature (if not at standard room temp)
24. If left over cell are needed, place in an appropriate volume of media and transfer to a fresh flask for future experiments
25. Incubate plate and flasks overnight (O/N) in 37°C incubator