Preparation of Mouse Embryonic Fibroblasts

1. Order a timed pregnant CF-1 mouse ($35 dollars from Charles River Laboratories strain #023)

2. On embryonic day 13.5, sacrifice the pregnant female and sterilize with 70% alcohol. Dissect out the uterus of the pregnant female using sterile tools and place it in a 50 mL conical containing PBS and Pen/Strep.

3. From this step forward, all steps should be performed using strict sterile conditions in a tissue culture biosafety cabinet. Transfer the uterus into a p100 dish containing 10 mL of PBS. Dissect out the embryos from the uterus and remove any extra uterine or placental tissue, leaving just the embryos.

4. Transfer the embryos into a new p100 dish containing 2mL of 0.05% trypsin per 3 embryos. Finally mince the embryos using a sterile razor blade.

5. Incubate the dish in a 37°C incubator for 5 minutes.

6. Add an equal volume of complete media (DMEM; 10% FBS; 1% P/S; +L-Glut) to neutralize the trypsin.

7. Aliquot the equivalent of 3 embryos per T75 flask and bring the volume up to 15 mL of media.

8. The following day, aspirate out the media and re-feed.

9. The following day (depending on how the cells look), aspirate off the media and wash the flasks. If there appears to be a confluent layer of MEFs pass 1:4 and continue culture accordingly.

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