SOP: **Establishment and Propagation of Adult Mouse Fibroblast Cultures**
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Modified by: **S. Hansen, S. Girirajan, T. Canfield (UW)**

Adult mouse fibroblast cultures were established in the laboratory of Dr. Evan Eichler (University of Washington, Department of Genome Sciences) from mice that were less than 6 months old (ear punch or tail clip).

Materials List

1. Hank's Balanced Salt Solution (HBSS) (Invitrogen, Cat# 24020-125)
2. Collagenase (Type XI-S) (Sigma-Aldrich, Cat# C4785)
   Stock solution in HBSS is 2000U/ml
   Solid collagenase is added slowly to HBSS, sterile-filtered when dissolved, and stored at 4°C. Collagen digestion activity is usually supplied at 1500U/mg collagenase so for 50mg solid, add 37.5mL HBSS.
3. Trypsins:
   0.05% Trypsin-EDTA (Invitrogen, Cat# 25300-054=1X solution)
   0.25% Trypsin-EDTA (Invitrogen, Cat# 25200-056=1X solution)
4. Fibroblast Culture Media:
   Fetal Calf Serum (Invitrogen, Cat# 16000-044)
   Heat inactivate for 30 minutes at 56°C.
   10% Fetal Calf Serum
   1% MEM Nonessential Amino Acids (Invitrogen, Cat# 11140-050)
   1% Penicillin/Streptomycin (Invitrogen, Cat# 15140-122)
   DMEM Medium (Invitrogen, Cat# 11965-092)
5. 1.5mL microcentrifuge tubes
6. Petri dishes (6cm)
7. Tissue culture dishes (3cm)
8. Tissue culture flasks (T75)
9. Graduated pipets (1, 5, 10, 25, 50mL)
10. Conical centrifuge tubes (15, 50mL)
11. Eppendorf Refrigerated Centrifuge 5810R
12. Hemocytometer
13. Micropipet w/ P20 tips
14. Microscope

Primary Culture Procedure

1. Cut the sample (ear punch or tail clip) into a 1.5mL microcentrifuge tube containing 0.5mL HBSS.
2. Place the sample into a 6cm Petri dish and dice the tissue into small pieces using a razor blade; put back into the same microcentrifuge tube.
3. Add 0.5mL collagenase (final concentration after addition is 1000U/mL).
4. Incubate at 37°C for 25 minutes (30 minutes for older mouse tails).

5. Spin 5 minutes at 1000rpm in an Eppendorf 5810R centrifuge; carefully decant and discard supernatant.
6. Wash once with 1-3mL HBSS by mixing and centrifuging as above, discarding the supernatant.
7. Add 0.5mL 0.05% trypsin, mix thoroughly, and incubate at 37°C for 20 minutes.
8. Centrifuge and decant supernatant as above, resuspend pellet in 0.5mL fibroblast culture media.
9. Triturate (pipet up and down) to break up cell aggregates.
10. Plate suspension into a 3cm tissue culture dish, avoiding large pieces of tissue.
11. Add 2mL fibroblast culture medium (final culture volume about 2.5mL).
12. Incubate in a 37°C, 5% CO^2 humidified tissue culture incubator.

Sub-culture

1. Every 2-4 days, feed or split cultures 1:4 to 1:6.
2. For the initial passage from 3cm plates, for example, use 1mL 0.25% trypsin solution to detach, add 1mL culture media, and centrifuge 5 minutes at 1000rpm; resuspend in fibroblast culture media for replating.
3. Split similarly for expansion into T75 flasks for final harvest (to at least 10 million cells).