EMSC (Ears Mesenchymal Stem Cells) Isolation and Culture

Prepare:

-HBSS (Invitrogen #14025-092)
   Add 100 µl Primocin (Invivogen #ant-pm-1) per 50 ml HBSS
   Filter with 0.22 µm
-Collagenase I (Worthington Biomedical Corporation #LS004196)
   2mg/ml Collagenase in sterile HBSS
   Filter with 0.22 µm
-Red Blood Cell Lysing Buffer (Sigma #R7757)
-Autoclave all needed tools

Isolate Cells:

1) Sacrifice mice and submerge collected ears only into 6 well plates with 70% ethanol

2) Collect external ears and put in sterile HBSS + antibiotics

Perform remaining steps under the hood:

3) Wash ears with sterile HBSS + antibiotics in the 6 well plates. 3 ml per well

4) Using sterile scissors and razor blades, cut ears into tiny pieces in collagenase solution. It will be easier to cut if you use a small amount of collagenase to cut the ears and then add the minced ears to the remaining collagenase.

5) Digest for 1 hour at 37°C in a water bath. Shake every 15 minutes.

6) Filter through 70 µm cell strainer to remove extra unwanted pieces. (BD Biosciences #352350)

7) Centrifuge at 1350 rpm for 8-9 minutes

8) Discard supernatant

9) Resuspend the pellet in red blood cell lysing buffer following Sigma’s instructions. Be gentle! Add 1 ml of buffer, pipette to mix, let sit for 1 minute, add 10 ml cell culture medium and pipette it again to mix.

10) Centrifuge at 1350 rpm for 8-9 minutes
11) Discard supernatant

12) Resuspend the pellet in culture medium (EMSC + Primocin) and seed

   Seed cells from 6 animals/12 ears in one 100mm dish

13) Change medium every 2-3 days.

**Split Cells:**

1) Remove medium from wells

2) Wash subconfluent primary cultures with PBS (Invitrogen #10010-023)

3) Remove PBS

4) Add 0.25% Trypsin (Invitrogen #25300-054)

   2-3 ml to a 100mm dish

5) Place in incubator for about 3 minutes until cells detach

6) Add culture medium to neutralize the trypsin

   6-8 ml to a 100mm dish

7) Collect and centrifuge at 1350 rpm for 8-9 minutes

8) Discard supernatant

9) Resuspend the pellet in culture medium and seed

   Cells are typically diluted 1:5 or 1:6 at each passage

**Culture Medium:**

DMEM/F12 (Invitrogen #11330-032) + 15% FBS (Invitrogen #10082-147) + 100 μl Primocin (Invivogen #ant-pm-1) per 50ml Media