Isolation of Embryonic Fibroblasts from Livestock Species

This protocol describes the isolation of embryonic fibroblasts from livestock species. It has been tested on pigs and sheep to generated porcine and ovine embryonic fibroblast primary cell cultures successfully.

The protocol is designed for collection of a skin sample from late stage fetus in a farm facility setting and the processing in a tissue culture lab. The protocol can be adapted to work with earlier stage whole embryos if the embryos are eviscerated before starting.

Embryonic Fibroblast Isolation

1. Warm growth media (20ml), trypsin (7ml), PBS+P/S (15ml) and outgrowth media (30ml) (volumes per sample)
2. Prepare growth media 20 ml per 50ml falcon, one for each sample, and take to farm facility at room temperature.
3. **At farm facility:** Harvest skin sample (about 3cm^2) and transfer into 15ml of growth media in a 50ml falcon – keep at room temperature (process within 5-6 hours)
4. In sterile flow hood remove skin sample from growth media.
5. Wash x3 using a total of 15ml PBS+P/S
   5.1. By pipetting 5ml PBS+P/S into 3 sterile petri dishes
   5.2. add the skin sample and swirl gently
   5.3. Continue until 3 washes have been carried out
6. In final petri dish macerate the skin sample and transfer to a 15ml falcon
7. Add 5ml trypsin and incubate at 37°C for 5 minutes.
8. Vortex for 30 secs to 1 minute
9. Incubate at 37’C for a further 5 minutes
10. Remove 3ml of the trypsin/cell mix (avoiding any large clumps) and pass the mix through a 100uM cell strainer (yellow) into a 50ml Falcon tube.
11. Once the suspension has passed through the strainer, rinse the strainer with 6ml of fibroblast outgrowth medium.
12. Transfer the cell suspension into a 15ml falcon tube
13. Centrifuge for 3 minutes at 200 x g
14. Resuspend cell pellet in 9ml (1ml then +8ml) outgrowth medium

15. Split the 9ml cell suspension between 3 T75 flasks (i.e. 3ml suspension in each flask)
16. To the remaining 2ml of trypsin + embryo, add another 2ml trypsin and continue from Step 6 onwards.
17. Incubate flasks in outgrowth medium for 5-7 days (keeping the cells in the outgrowth medium helps the fibroblasts to outgrow keratinocytes and gives the antibiotics/antifungal time to kill of anything that might be in your embryos). Don't move or disturb the flasks for the first 3 days.
18. When the flasks are 80-90% confluent, passage them 1:2 into standard fibroblast growth medium or freeze down.

Fibroblast outgrowth medium		**500ml**
DMEM, high glucose, glutamine, pyruvate		382.5ml
FBS	20%	100ml
MEM NEAA	1x	5mls (100x)
Pen-strep	1%	5ml
Fungizone	2.5ug/ml	5ml (250ug/ml)
Gentamicin	50ug/ml	2.5ml (10mg/ml)

Fibroblast growth medium		**500ml**
DMEM, high glucose, glutamine, pyruvate		440ml
FBS	10%	50ml
MEM NEAA	1x	5ml
Pen-strep	1%	5ml

PBS+P/S		**500ml**
PBS		498.5ml
P/S	X1	1.5ml