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Fibroblast culture from rodent/lagomorph ear

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the procedure for isolating and culturing either primary fibroblasts from the ear pinnae or fetal fibroblasts from the ear buds and head skin of rodents and lagomorphs, including *Acomys*, *Mus*, *Rattus*, and *Oryctolagus*, used in the Seifert Lab.

Note that any protocol involving animals should be approved by your Institutional Animal Care and Use Committee (IACUC) before use.

Protocol materials

⊗ IsoThesia (Isoflurane) Solution **Henry Schein Animal Health Catalog #029405**

⊗ 4 mm biopsy punch **Sklar Catalog #96-1146**

⊗ 1.5 mL Eppendorf tubes

⊗ FBS (HyClone)

⊗ Antibiotic/Antimycotic (100X) **Thermo Fisher Scientific Catalog #15240062**

⊗ DMEM and Hams F-12 50/50 **Fisher Scientific Catalog #MT10090CV**

⊗ Tissue culture dish (10 cm) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z707651**

⊗ Falcon® 70 µm cell strainer **Corning Catalog #352350**



1 Anaesthetize/euthanize animal appropriately

Note

For *Acomys* and *Mus*, we anaesthetize with [M] 3 % (v/v)

 IsoThesia (Isoflurane) Solution **Henry Schein Animal Health Catalog #029405**

at  1 μ L oxygen flow rate

Primary fibroblasts

2 Follow the following procedure for primary fibroblasts from adult animals

2.1 Harvest tissue from the center of the left and right ear pinna with a

 4 mm biopsy punch **Sklar Catalog #96-1146**

2.2 Place tissue from both ears for each animal into a single 1.5 mL Eppendorf tubes

2.3 Enzymatically dissociate the tissue first with a with 1:1 trypsin (0.25%)-dispase mix for 1 hour at 37°C. Dice up tissue with a razor and transfer tissue into a new eppendorf tube with 1X HBSS. Spin at 2000 rpm for 7 minutes. After that take out the HBSS and add collagenase IV (2.5 mg/ml) and incubate at 37°C for 1 hour.

2.4 Remove the enzyme, add 1X HBSS and mechanically dissociate cells by filtering through a  Falcon® 70 μ m cell strainer **Corning Catalog #352350** , with the gentle nudge of a syringe (3-10ml) plunger.

2.5 Pellet the suspension by spinning at 2000g x 7 minutes at 26°C.

2.6 Resuspend pellet in

 DMEM and Hams F-12 50/50 **Fisher Scientific Catalog #MT10090CV**

supplemented with 10%  FBS (HyClone) and 1%

 Antibiotic/Antimycotic (100X) **Thermo Fisher Scientific Catalog #15240062**



2.7 Culture at 37 °C under 3% O₂ and 5% CO₂ in



Tissue culture dish (10 cm) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z707651**

2.8 Passage at ~80% confluency. Count cells using a hemocytometer and seed 300,000-500,000 cells in a fresh



Tissue culture dish (10 cm) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z707651**

Embryonic fibroblasts

3 Follow the following procedure for fetal fibroblasts from embryos from timed-pregnant females after euthanasia

3.1 Dissect the ear buds and head skin from the embryo

3.2 Place tissue into 1.5 mL Eppendorf tubes

3.3 Enzymatically dissociate cells with 0.25% trypsin for 00:10:00 at 37 °C

3.4 Neutralize the enzyme in complete growth medium containing serum

3.5 Mechanically dissociate the tissue using a 20 gauge needle to obtain a single-cell suspension

3.6 Culture cells in



DMEM and Hams F-12 50/50 **Fisher Scientific Catalog #MT10090CV**

supplemented with 10% FBS (HyClone) and 1%



Antibiotic/Antimycotic (100X) **Thermo Fisher Scientific Catalog #15240062** at



37 °C under 3% O₂ and 5% CO₂ in



Tissue culture dish (10 cm) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z707651**

