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Establishment of adherent fibroblasts from adult *P. vitticeps* lung tissue

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We use this protocol and it's working

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Abstract

This protocol describes the primary culture of lung fibroblasts from *Pogona vitticeps* and their application in lentivirus transfection efficiency assays. The protocol has been optimized for *Pogona vitticeps* but can be adapted for other species. The cultured fibroblasts provide a stable and reproducible system for studying gene delivery efficiency, making them a valuable tool for downstream molecular applications. The method ensures high cell viability and proliferation capacity, supporting long-term culture and experimental manipulation. Additionally, the fibroblasts maintain their characteristic morphology and gene expression profiles, allowing for accurate assessment of transfection efficiency and viral vector performance in reptilian cells.

Materials

Cell culture consumables

100 x Anti-Anti (Gibco, Cat: 15240062)

pH 7.2 PBS without calcium, magnesium,

phenol red (Thermofisher, Cat: 20012027)

Fetal bovine serum (Gibco, Cat: A3382001)

RPM1 1640 with GlutaMAX and HEPES (Thermofisher, Cat: 72400047)

Collagenase/Dispase (Roche) (Merck, Cat: 10269638001)

Trypsin 0.25% EDTA (Thermofisher, Cat: 25200056)

Other equipment/consumables

Stainless steel scissors

Stainless steel forceps/tweezers

70% ethanol

Sterile 1.5 mL microcentrifuge tubes

T24 culture flasks

Shaking water bath

Sterile 0.22µM filter

31°C, 5% CO₂ incubator

Refrigerated centrifuge



Before start

This protocol has been developed and optimized using *Pogona vitticeps* as a model system. Before starting, ensure all dissection equipment is autoclaved, and make sure multiple are available so they can be changed frequently during the dissection.

Washing solution

Prepare sterile tubes with PBS with 1% Anti-Anti.

Digestion working solution

Prepare working solution of collagenase/dispase (1mg/mL) by diluting with PBS (must be calcium and magnesium free) and filter through a sterile 0.22µM filter.

Culture Medium

Prepare cell culture media using RPMI 1640 supplemented with 15% FBS and 1% Anti-Anti. Prewarm complete culture media in a 37°C water bath for at least 15 minutes.

Note: The addition of 15% FBS supports optimal growth of primary cells.

Cryopreservation solution

FBS and DMSO in a 9:1 ratio, preparing 500 µL per tube.

Dissection

- 1 Thoroughly clean the abdominal surface with 70% ethanol. Make a long vertical incision along the abdomen taking care not to puncture the stomach or intestines. Ensure that new scissors and tweezers are used after cutting open the abdominal skin.
Note: Ensure that the skin remains away from the exposed body cavity as it is a major source of bacterial contamination. Horizontal cuts to the skin flaps can help to lay it open.
- 2 With clean instruments, remove a piece of lung tissue approximately 3 cm × 1 cm in size. Try to complete the dissection as quickly as possible to reduce the risk of contamination in the cell cultures.
- 3 Immediately place the tissue into a sterile tube pre-prepared with washing solution.

Washing

- 4 Using clean scissors finely mince the lung tissue in the tube. Centrifuge for 5 minutes at 1000 rpm. Replace the Washing solution. Repeat this process three times. This step aims to remove blood from the tissue as much as possible.



Digestion

- 5 Add 1 mL of Digestion working solution to the tissue and mix thoroughly. Incubate the mixture overnight at 37°C with shaking 500 rpm.

Culture

- 6 After digestion, remove the centrifuge tube from the water bath and dry off any moisture. Centrifuge at 3000 rpm for 5 minutes and discard the supernatant, being careful not to disturb the cell pellet. Add approximately 300 µL of culture medium to the tissue, then transfer the mixture to a 25 cm² culture flask.
- 7 Incubate statically overnight at 31°C. The next day, carefully add 2 mL of culture medium along the inner wall of the flask and continue static incubation.
Note:  31 °C is the most suitable temperature for pogona primary cells.



Maintenance

- 8 Monitor regularly for contamination. Refresh culture medium every 3 days.
- 9 When cells reach 80%-90% confluency, passage the cells. Avoid passaging the cells more than 10 times.
Note: if over 10 times, the shape of the cells becomes longer.

Cryopreservation

- 10 Prepare the fresh cryopreservation solution. For 12 tubes, prepare 6 mL of cryopreservation solution, which includes 5.4 mL of FBS and 0.6 mL of DMSO.
- 11 Pre-treatment for Cell Freezing - Passage
24-48 hours before freezing, passage the cells to ensure they are in the active division phase. At this time, add 1.5 mL of trypsin to a 25 cm² culture flask and incubate in a 37°C incubator for 3 minutes. Observe the cells becoming round, then gently tap to detach them. Add 6.5 mL of culture medium, gently pipette against the flask wall to mix thoroughly, and transfer half (4 mL) to a new clean culture flask for incubation. Continue incubate 3 days.
- 12 Trypsin Digestion
Discard the supernatant, add 2 mL of trypsin, and wait until the cells are round and detached. Then, add 1 mL of culture medium to terminate digestion, pipette against the flask wall, and transfer the cell suspension to a 10 mL centrifuge tube. Centrifuge at 3000 rpm for 5 minutes and discard the supernatant, leaving the cell pellet.
- 13 Gradient Cryopreservation
Add 500 µL of the cryopreservation solution to the cell pellet and transfer to a 2 mL cryopreservation tube. Place the 12 cryopreservation tubes in a cooler cell box at -80°C overnight, then transfer to liquid nitrogen for long-term storage the next day.

Recovery

- 14 Thawing the Cells
Remove the frozen vial from liquid nitrogen or -80°C freezer.
Note: Hold the vial in your hand and gently swirl to accelerate thawing (~1 minute).
- 15 Diluting the Cells
Immediately after thawing, gently transfer the entire contents of the vial (500 µL) into a sterile 12 mL conical tube. Slowly add 10 mL of prewarmed culture media dropwise while gently swirling the tube to mix. This gradual dilution helps reduce osmotic shock.



- 16 Centrifugation
Centrifuge the tube at 1400 rpm (approximately 300 x g) for 3 minutes at 22°C. Carefully aspirate and discard the supernatant without disturbing the cell pellet.
- 17 Resuspending the Cells
Gently tap the tube against the palm of your hand to loosen the pellet. Add 4 mL of fresh prewarmed culture media. Pipette up and down gently 3-5 times to ensure a uniform suspension.
- 18 Seeding the Cells
Transfer the cell suspension into a T25 culture flask or an appropriate vessel. Gently rock the flask to evenly distribute the cells. Place the flask in a 31°C, 5% CO₂ incubator and allow the cells to recover.
- 19 Monitoring the Cells
After 24 hours, check cell attachment under a microscope. Replace the media after 48 hours to remove dead cells and debris. Continue routine culture maintenance according to standard protocols.

Protocol references

Ezaz, T., O'Meally, D., Quinn, A.E., Sarre, S.D., Georges, A. and Marshall Graves, J.A., 2008. A simple non-invasive protocol to establish primary cell lines from tail and toe explants for cytogenetic studies in Australian dragon lizards (Squamata: Agamidae). *Cytotechnology*, 58, pp.135-139.

Clark, H.F., Cohen, M.M. and Karzon, D.T., 1970. Characterization of reptilian cell lines established at incubation temperatures of 23 to 36. *Proceedings of the Society for Experimental Biology and Medicine*, 133(3), pp.1039-1047.

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