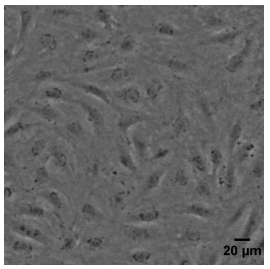
Dec 18, 2024

Primary cell culture of dorsal fin-derived fish fibroblasts in a CO2-independent environment



DOI
https://dx.doi.org/10.17504/protocols.io.rm7vzxd64gx1/v1

Catarina Santos[1], Mariana Moço[1], Gabriela Rodrigues[1], Vitor C Sousa[1], Joao Moreno[1,2]

[1]cE3c – Centre for Ecology, Evolution and Environmental Changes & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal;
[2]MARE – Centro de Ciências do Mar e do Ambiente (MARE) & ARNET—Aquatic Research Network, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

Joao M Moreno
cE3c – Centre for Ecology, Evolution and Environmental Chang...

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzxd64gx1/v1

Protocol Citation: Catarina Santos, Mariana Moço, Gabriela Rodrigues, Vitor C Sousa, Joao Moreno 2024. Primary cell culture of dorsal fin-derived fish fibroblasts in a CO2-independent environment. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzxd64gx1/v1

Protocol status: Working
We use this protocol and it's working

Created: January 25, 2024

Last Modified: December 18, 2024

Protocol Integer ID: 94126

Keywords: Cell culture, Fibroblasts, Fish cell culture, Primary cell culture, primary cell cultures from dorsal fin sample, primary cell culture of dorsal, derived fish fibroblast, freshwater cyprinid, obtaining primary cell culture, global freshwater fish assessment, primary cell culture, other freshwater fish species, cell culture, derived cell culture, dorsal fin sample, squalius genus, cell

Funders Acknowledgements:
Fundação para a Ciência e a Tecnologia
Grant ID: SFRH/BD/143199/2019
Fundação para a Ciência e a Tecnologia
Grant ID: PTDC/BIA-EVL/4345/2021
Human Frontier Science Program Young Research
Grant ID: RGY0081/2020

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

A strong limitation to conduct experimental studies on non-model freshwater fish is related to the conservation status of about 20% of the species that are currently classified at least as vulnerable by the IUCN Red List of Threatened Species (Global Freshwater Fish Assessment 2020). To address this limitation, fin-derived cell cultures have emerged as a promising non-lethal tool for various assays, enabling the assessment of factors such as pollutants, environmental changes, and more. In this study, we present a protocol for obtaining primary cell cultures from dorsal fin samples of freshwater cyprinids belonging to the *Squalius* genus. This protocol could potentially be applied and adapted for use with a broad range of other freshwater fish species.

Materials

- Sterilized dissection material (scissors, forceps, and bistoury)
- 1.5 mL microcentrifuge tubes
- 15 mL centrifuge tube
- Petri Ø12mm dishes
- 6-well tissue culture treated plates
- Pipette (variable volumes) and respective filter tips

Solutions and reagents

	Solution/Reagent	Brand	Catalog ID
	Chlorhexidine gluconate 1% (w/v)	Zoopan®	1000000832
	Sodium Hypochlorite solution 10% (w/v) technical grade	PanReac AppliChem®	211921.1211
	Ethanol, Absolute [100% (v/v)] (200 Proof), Molecular Biology Grade	Fisher BioReagents™	16606002
	Leibovitz's L-15 Medium	Gibco®	11415064
	Antibiotic-Antimycotic (100X) solution containing 10,000 units/mL of penicillin, 10,000 µg/mL of streptomycin, and 25 µg/mL of Amphotericin B	Gibco®	15240062
	Kanamycin Sulfate 10 mg/mL	Gibco®	15160047
	Nystatin, anti-fungal agent	Gibco®	15340029
	Donor Bovine Serum with Iron	Gibco®	10371029
	Phosphate buffered saline (PBS) 1X	Sigma-Aldrich®	P4417
	0.05% Trypsin - EDTA (1X) w: phenol	Gibco®	25300062

	Solution/Reagent	Brand	Catalog ID
	red		

Note: the PBS used in this protocol is sold in tablet form, so before used dissolve in distilled sterile water according to manufacturer's protocol and autoclave prior to use.

Before start

All materials should be sterilized in advance.

Tissue collection

2h 30m

1 Prepare decontamination solution:

1X Earle's Balanced Salt Solution (EBSS) or Hanks' Balanced Salt Solution (HBSS) supplemented with [M] 2 % (v/v) Antibiotic-Antimycotic (100X), [M] 50 µg/mL Kanamycin Sulfate and 1:200 Nystatin suspension

Note

Make sure you prepare enough decontamination solution for the washing protocol described in section 2.

2 After washing the area surrounding the dorsal fin (and fin included) with chlorhexidine gluconate [M] 1 % (w/v) , collect the dorsal fin of the fish using sterilized dissecting scissors and put it in 1.5 mL of decontamination media. Leave it at room temperature for at least 00:30:00 and up to 02:00:00 .

2h 30m

Note

If you suspect severe contaminations use the following quarantine protocol: after collecting the tissue, put it in a 1.5 mL microtube with a mixture of 1X Earle's Balanced Salt Solution (EBSS) or Hanks' Balanced Salt Solution (HBSS) supplemented with 2% (v/v) Antibiotic-Antimycotic (100X), [M] 50 µg/mL Kanamycin Sulfate and 1:200 Nystatin suspension for 00:30:00 at room temperature. Next, transfer the tissue to a 1.5 mL microtube filled with 1.0 mL of culture medium supplemented with [M] 5 % (v/v) antibiotic/antimycotic ([M] 10000 mg/mL), 1:200 Nystatin suspension, and [M] 100 mg/mL Kanamycin sulfate for another 00:30:00 at room temperature.

Establishing primary cell culture

3 Prepare enough volume of complete L-15 media:

L-15 media supplemented with [M] 15 % (v/v) *Donor bovine serum with Iron and* [M] 1 % (v/v) *Antibiotic-Antimycotic (100X).*

Note

If you suspect strong contaminations add: [M] 50 µg/mL Kanamycin Sulfate (for bacterial contamination) and/or 1:200 Nystatin suspension (for fungal contamination). Note that both Kanamycin and Nystatin are toxic for the cells and should be avoided or their presence in the media should be minimal and as reduced as possible.

4 Dilute sodium hypochlorite solution to [M] 1 % (w/v) and absolute ethanol to [M] 65 % (v/v) .

5 Prepare six 1.5 mL tubes according to the following scheme:

- Tube 1: 1.0 mL [M] 65 % (v/v) ethanol
- Tube 2: 1.0 mL L-15 with [M] 2 % (v/v) antibiotic/antimycotic, 1:200 Nystatin suspension and [M] 50 µg/mL Kanamycin
- Tube 3: 1.0 mL [M] 1 % (v/v) sodium hypochlorite
- Tube 4: 1.0 mL L-15 with [M] 2 % (v/v) antibiotic/antimycotic, 1:200 Nystatin suspension and [M] 50 µg/mL Kanamycin
- Tube 5: 1.0 mL [M] 65 % (v/v) ethanol
- Tube 6: 1.0 mL L-15 with [M] 2 % (v/v) antibiotic/antimycotic, 1:200 Nystatin suspension and [M] 50 µg/mL Kanamycin

6 Use sterilized forceps to take the tissue out of the 1.5 mL microtube and proceed with the following washing protocol using the tubes prepared in the previous step:

- 2 to 5 seconds in the Tube 1.
- 5 to 8 seconds in the Tube 2.
- 5 seconds in the Tube 3.
- 5 seconds in Tube 4.

- 6 to 8 seconds in the Tube 5.

7 Transfer the tissue to tube 6 and leave it there for at least 45 seconds (ideally up to 2 – 5 min).

8 Transfer the tissue to a Ø12mm petri dish containing 1X PBS supplemented with [M] 2 % (v/v) antibiotic/antimycotic. Wash the tissue for around 00:00:30 . 30s

9 Transfer the tissue to a Ø12mm petri dish containing 750 µL trypsin-EDTA [M] 0.05 % (w/v) . Cut the tissue into tiny pieces with a sterile pair of tweezers or bistoury. 10m
Incubate the pieces of tissue in the trypsin for 00:10:00 at room temperature (shake occasionally).

Note: trypsin is toxic to cells and will cause cells to die off if incubated for longer periods.

10 Add 2.5 mL complete media (see step 3) to the petri dish.

Note

If you suspect severe contamination, supplement the complete media with 1:200 Nystatin suspension in case of fungus and/or [M] 50 µg/mL Kanamycin in case of bacteria.

11 Transfer the media (with remaining chunks of tissue) to a 6-well plate.

Note: plates with visible chunks of tissue seem to grow the most cells, but are also most prone to infection. It is possible to divide the previous suspension into more than one plate to plate each well with various amounts of visible tissue and assess the most manageable ratio of cell and bacterial growth.

12 After 18:00:00 to 24:00:00, all chunks of tissue that didn't adhere to the bottom of the plate must be removed, and the media must be changed. For that, all the media should be removed, and 3.0 mL fresh media should be added to each well. 1d 18h

Note: If you observe small debris in the media, wash the well with complete media (preferably) or 1X PBS, and then add 3.0 mL fresh complete media.

Maintenance and establishment of cell lines

13 Replace all of the media every 2-3 days, or whenever you notice a significant change in its color (towards a more yellow/orange or purple tone).

14 When cells reach around 80% confluency, trypsinize them and transfer all the cells to a different well. It is important that, in this step, no chunks of fin go into the new well.

Note: 150 µL of [M] 0.05 % (w/v) Trypsin - EDTA (1X) per well (6-well plate) is used for trypsinization.

15 After cells reach confluency in the new well, pass them into a new well or T25 flask to proliferate them, or use them for further experiments.

Protocol references

Rábová, M., R. Monteiro, M. J. Collares-Pereira, e P. Ráb. «Rapid Fibroblast Culture for Teleost Fish Karyotyping». In *Fish Cytogenetic Techniques: Ray-Fin Fishes and Chondrichthyans*. CRC Press, 2015. https://www.taylorfrancis.com/books/9781482211993