Dec 19, 2023

SOP: cell culture methods for non-model organisms

DOI
https://dx.doi.org/10.17504/protocols.io.n92ldm6q7l5b/v1

Ana Riesgo[1], María Conejero[1], C.DiNizo[2], Jonas Astrin[2], Cira Martínez[3], Tomàs arqués[4]

[1]Museo Nacional de Ciencias Naturales -CSIC; [2]Leibniz Institute for the Analysis of Biodiversity Change (LIB); [3]Universitat Pompeu Fabra; [4]Instituto Biología Evolutiva-CSIC

María Conejero
Museo Nacional de Ciencias Naturales

DOI: https://dx.doi.org/10.17504/protocols.io.n92ldm6q7l5b/v1

Document Citation: Ana Riesgo, María Conejero, C.DiNizo , Jonas Astrin, Cira Martínez, Tomàs arqués 2023. SOP: cell culture methods for non-model organisms. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldm6q7l5b/v1

Created: December 19, 2023

Last Modified: December 19, 2023

Document Integer ID: 92483

Keywords: cell culture, invertebrates, vertebrates for cell culture, sops for invertebrate, somatic cell, cell culture method, cell culture, invertebrate, vertebrate taxa, vertebrate taxa like amphibian, vertebrate, cell, organism, amphibian, sop

Funders Acknowledgements:
EU Horizon Europe Research and Innovation action
Grant ID: 101059492

Abstract

Acquisition of somatic cells from vertebrates for cell cultures is well stablished in the literature. However, some vertebrate taxa like amphibians prove to be challenging mostly because there is a higher chance of contamination in this group or because the cells do not survive for long periods preventing to be cryopreserved. For invertebrates, the challenge is even greater and so far, the protocols tested have not proven satisfactory and are still being developed. Therefore, the SOPs for invertebrates presented below are still in the testing phase.

The present SOP is in development and contains successful steps to be carried out to obtain cell cultures for non-model organisms from a range of taxa. All procedures should be done according to Animal Welfare regulations in your country, please make sure you know and abide to those regulations before starting the culture.
Once the tissue is obtained, all steps are carried out in safety cabinets. All materials must be cleaned with 70% ethanol before being inserted into the cabins in order to maintain the most sterile environment possible. Culture flasks are kept in cleaned incubator settled up with appropriate temperature required for each species with 5% CO_2 (for vertebrates only).

As each species requires a different culture medium and supplementation, these will be described below.

It is recommended that solutions be purchased in liquid form and prepared following sterile technique.

Tissue culture solutions used in this SOP

- Minimum Essential Medium (MEM), Schneider's Insect Medium, and Leibovitz L-15 supplemented with: 10 to 20%% Foetal Bovine Serum, 1% antibiotics-antimycotic.
- Biopsy media: Completed Alpha MEM (above) supplemented with additional antibiotics:1% antibiotic-antimycotic and
  gentamicin.
- Additional Solutions 1X HBSS: Hank's Balanced Salt Solution (1X).
- Tissue Dissociation Enzyme (i.e. collagenase): Liberase, Trypsin, Collagenase or dispase.

Materials needed

- Electric clippers (optional)
- Straight-edge razors
- 70% ethanol
- Gauze sponge
- Forceps and scalpel (stored in 70% ethanol)
- Biopsy vial with Biopsy media.

1. Cell cultures from tissue derived from amphibians *(based on Houck et al., 2017 – with modifications)*

Best results for amphibians were obtained from tongue, limb, skin, eye, kidney, and tadpole. The tissue is obtained from fresh material (be sure that you have the permission to handle animals/tissues, this is not covered in this SOP) and extracted after cleaning the area of excision with ethanol 70%. Put the tissue (approximately 1 to 3 cm) in a falcon tube containing 3 to 5 ml of biopsy media supplemented with antibiotics (100 ug/mL). The tissue has to be completely cover by the biopsy media. Next steps are performed on a safety sterile cabinet. For amphibians, the most used media are Minimal Essential Medium (MEM) or Leibovitz L-15 supplemented with 1% antibiotic-antimycotic and Foetal Bovine serum (FBS) in a concentration of 10 to 20%.

- Wash the tissue twice in Hanks' Balanced Salt Solution (HBSS) .
- Wash the tissue twice in washing solution (HBSS supplemented with antibiotics).
- Tap out the tissue into a petri dish with a few drops of washing solution and cut in small pieces.
- Use forceps to place (10 - 15) small pieces (as smaller as possible) in a T25 flask separated from each other.
- Space the fragments so that there is sufficient room for outgrowth, approximately 2–3 mm apart. Important: keep the tissues minimally wet.
- Leave the flask in a vertical position to let tissue adhere for 5 to 10 min (it is important to not let the tissue dry up but also important to let the tissue adhere for the cells to grow out).
- Add carefully a drop of media (1 to 3 ml media).
- Incubate in the appropriate temperature (20-30°C). It is recommended to culture within 5°C of the physiological optimum for the species.
- The next day, check cultures visually for contamination. Amphibian cultures have to be verified every day for the first two weeks, because of the high chance of contamination. Contamination may be removed by increasing the concentration of antibiotic / antifungal, but persistent contamination requires the flask to be discard.

2. Cell cultures from arthropod material ***(based on Lynn, 2001 and Mandriolli et al., 2015 – with modifications)***

Rearing and animal harcesting is not covered by this SOP, please make sure you act according to your national/local regulations. The tissue is obtained fresh and extracted after cleaning or submerging the dead animal in ethanol 70%. Preferred tissues for arthropods are embryos, larvae, ovaries, and imaginal discs. Depending on the size, entire specimen can be used. Put the tissue (or the entire animal) in a falcon tube containing 3 to 5 ml of biopsy media supplemented with antibiotics (100 ug/mL). The tissue has to be completely cover by the biopsy media. Next steps are performed on a safety sterile cabinet. For arthropods, the most used media are Schneider's Insect Medium or Leibovitz L-15 supplemented with 1% antibiotic-antimycotic and Foetal Bovine serum (FBS) in a concentration of 10%,

- Wash the tissue twice in HBSS .
- Wash the tissue twice in washing solution (HBSS supplemented with antibiotics).
- Put the animal in a petri dish with a few drops of washing solution and cut the body in half using forceps (in case of whole specimens). Shake the abdomen with a pair of sterile forceps to release immunocytes.
- Remove the body fragments using forceps. Transfer the material to a culture flask.
- Add 1ml culture medium to the flask.
- Incubate at 24-28°C.
- Check daily for contamination and change 50% or 100% of the media when necessary.

3. Obtention of cell culture from crustaceans and molluscs *(based on Toullec 1999, Yoshino et al. 2013, and Potts et al., 2020 – with modifications)*

Animal collection, handling and euthanasia are not covered in this SOP and must be in accordance with local regulations. The tissue is obtained fresh and extracted after cleaning or submerging the dead animal in ethanol 70%. Put the tissue (or the entire animal) in a falcon tube containing biopsy media supplemented with antibiotics. Next steps are performed on a safety sterile cabinet. For crustaceans and molluscs, the most used

medium is Leibovitz L-15 supplemented with 1% antibiotic-antimycotic and Foetal Bovine serum (FBS) in a concentration of 10%, For marine species, osmolarity should be adapted using artificial sea water or adding inorganic ions previously sterilized. Artificial sea water without calcium and magnesium (CMFSS) can be prepared by dissolving salts in deionized SuperQ water: 25.5 g/L NaCl, 0.8 g/L Na2SO4, 2.86 g/L HEPES (Sigma, United States). The solution has to be autoclaved.

- Wash the tissue twice in HBSS
- Wash the tissue twice in washing solution (HBSS supplemented with antibiotics)
- Put the animal or tissue in a petri dish with a few drops of washing solution and cut in small pieces.
- Use forceps to place the pieces in a T12.5 or T25 flask separated from each other
- Add culture medium sufficient to cover the tissues.
- Add 1ml culture medium to the flask
- Incubate at 25-28°C.
- Check daily for contamination and change 50% or 100% of the media when necessary.
- Alternatively, dissociation techniques (mechanical or enzymatic) can be performed in order to obtain cells. Mechanical dissociation can be achieved with magnetic stirring or by repeated aspiration using a pipette or syringe. Enzymatic treatment can be made using Trypsin, Collagenase or dispase.

Protocol references

1. Houck, M. L.; Lear, T. L.; Charter, S. J. (2017). Animal cytogenetics. The AGT Cytogenetics Laboratory Manual, 1055-1102.
2. Lynn, D. E. (2001). Novel techniques to establish new insect cell lines. In Vitro Cellular & Developmental Biology-Animal, 37, 319-321.
3. Mandrioli, M.; Monti, M.; Tedeschi, R. (2015). A practical guide to insect cell cultures: establishment and maintenance of primary cell cultures. *Halteres*, 6, 132-141.
4. Potts, R. W.; Gutierrez, A. P.; Cortés-Araya, Y.; Houston, R. D.; Bean, T. P. (2020). Developments in marine invertebrate primary culture reveal novel cell morphologies in the model bivalve Crassostrea gigas. *PeerJ*, 8, e9180.
5. Toullec, J. Y. (1999). Crustacean primary cell culture: a technical approach. Methods in cell science, 21, 193-198.
6. Yoshino; T. P.; Bickham, U.; Bayne, C. J. (2013). Molluscan cells in culture: primary cell cultures and cell lines. *Canadian*
journal of zoology, 91(6), 391-404.