

Ex vivo culture of gastric and intestinal stem cells as organoids

Commonly used acronym: Gut organoids

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SCOPE OF THE METHOD

The Method relates to	Animal health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vitro - Ex vivo
This method makes use of	Animal derived cells / tissues / organs
Species from which cells/tissues/organs are derived	Mouse
Type of cells/tissues/organs	Gastric and intestinal stem cells

DESCRIPTION

Method keywords

intestine

development
cellular differentiation
gut organoids
epithelium
adult stem cells
foetal progenitors

Scientific area keywords

intestinal stem cells
gastric stem cells
intestinal development
Organoid biobank
organoid preservation
gut regeneration
cellular differentiation

Method description

The tri-dimensional (3D) culture protocols allow isolation and culture of gastric epithelial antral and intestinal stem cells to efficiently generate organoids that recapitulate the mature pyloric and intestinal epithelium *in vitro* and foetal epithelial progenitors of these tissues growing as immature undifferentiated spheroids. The *ex vivo* culture approach is suitable to study gastric and intestinal function in homeostasis as well as in disease and developmental aspects of the gut.

Lab equipment

- Biological safety cabinet,
- Cell culture incubator (37°C, 5% CO₂),
- Inverted bright field microscope Binocular,
- Cold light source,

- Ultra-low temperature upright Freezer (for biobank storage),
- FACS for single cell isolation.

Method status

History of use

Internally validated

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

The organoid technology offers the possibility to grow indefinitely foetal progenitors and adult stem cells in culture. The cryo-preserved organoid samples (as a biobank) can be regrown upon thawing, keeping their original properties. This substantially reduces the use of individual animals (pre and post-natally). For adult-derived organoid cultures, all epithelial cell lineages are spontaneously differentiated from adult stem cells, in a proportion similar to that observed in the tissue of origin *in vivo*. Cellular imaging, genomic, transcriptomic and proteomic studies can be easily performed on organoids as well as Drug screening assays and gene editing. A similar protocol is reported for generation of human biobanks from biopsies.

Challenges

This 3R compliant method requires initial use of animals as starting material to generate the biobank. Moreover, this method only allows to investigate epithelium function and does not reproduce the complex *in vivo* environment of a tissue (which includes stromal mesenchymal, immune, nervous cells and systemic derived-factors). Therefore, this organoid technology cannot totally avoid animal use in research.

Modifications

Epithelial-derived organoids can be co-cultured with additional cell types to recomplexify the *in vivo* environment.

Future & Other applications

Patient-derived organoids for personalized medicine.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Mustata, R. C., Vasile, G., Fernandez-Vallone, V., Strollo, S., Lefort, A., Libert, F., Monteyne, D., Perez-Morga, D., Vassart, G. and Garcia, M. I. (2013). Identification of Lgr5-independent spheroid-generating progenitors of the mouse fetal intestinal epithelium. *Cell Rep* 5(2): 421-432.

Fernandez Vallone, V., Leprovots, M., Strollo, S., Vasile, G., Lefort, A., Libert, F., Vassart, G. and Garcia, M. I. (2016). Trop2 marks transient gastric fetal epithelium and adult regenerating cells after epithelial damage. *Development* 143(9): 1452-1463.

Mustata R., Van Loy T., Lefort A, Libert F., Strollo S., Vassart G., Garcia MI. Lgr4 is required for Paneth cell differentiation and maintenance of intestinal stem cells *ex vivo*. (2011). *EMBO Reports*. 12:558-564.

Fernandez Vallone, V., Leprovost, M., Ribatallada Soriano, D., Gerbier, R., Lefort, A., Libert, F., Vassart, G., & Garcia, M.-I. (2020). Lgr5 controls extracellular matrix production by stem cells in the developing intestine. (2020). *EMBO Reports* e49224, 1-14.

Alia Hadeji, Morgane Leprovots, Max Thulliez, Orianne Bastin, Anne Lefort, Frédérick Libert, Antoine Nonclercq, Alain Delchambre, Francois Reniers, Jacques Deviere, MI Garcia. (2022). Cold Atmospheric Plasma Differentially Affects Cell Renewal and Differentiation of Stem Cells and Apc-Deficient-Derived Tumor Cells in Intestinal Organoids. *Cell Death Discovery*. *Cell Death Discov.* 8, 66.

Sato, T., Vries, R. G., Snippert, H. J., van de Wetering, M., Barker, N., Stange, D. E., van Es, J. H., Abo, A., Kujala, P., Peters, P. J. and Clevers, H. (2009). Single Lgr5 stem cells

build cryptvillus structures in vitro without a mesenchymal niche. Nature 459(7244): 262-265.

Barker, N., Huch, M., Kujala, P., van de Wetering, M., Snippert, H. J., van Es, J. H., Sato, T., Stange, D. E., Begthel, H., van den Born, M., Danenberg, E., van den Brink, S., Korving, J., Abo, A., Peters, P. J., Wright, N., Poulsom, R. and Clevers, H. (2010). Lgr5+ve stem cells drive selfrenewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell 6(1): 25-36.

Associated documents

Links

[Ex vivo Culture of Fetal Mouse Gastric Epithelial Progenitors](#)

[Ex vivo Culture of Adult Mouse Antral Glands](#)

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