

# A mouse mammary gland organoid protocol to mimic breast morphology in vitro

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## SCOPE OF THE METHOD

<b>The Method relates to</b>	Animal health
<b>The Method is situated in</b>	Basic Research
<b>Type of method</b>	In vitro - Ex vivo
<b>This method makes use of</b>	Animal derived cells / tissues / organs
<b>Species from which cells/tissues/organs are derived</b>	mouse
<b>Type of cells/tissues/organs</b>	mammary gland

## DESCRIPTION

### Method keywords

Mouse mammary gland organoids

primary material  
branched morphology  
ECM composition  
growth factor supplementation  
tumor initiation  
pubertal development

### **Scientific area keywords**

Developmental biology  
Oncology  
3D organoid models  
stem cell biology

### **Method description**

The protocol is aimed at developing primary mammary gland organoids that have a morphology similar to the one of the *in vivo* breast, which is organized as a complex network of interconnected branches. The organoids are derived from the mouse mammary gland by mechanical dissociation and enzymatic digestion of the tissue to obtain small mammary tissue fragments that spontaneously organize in sphere-shaped organoids. The sphere-shaped organoids are then transferred in mixed Basement Membrane Extract (BME) and collagen gels, supplemented with growth factors to induce an elongated and branched morphology. By combination of the right ECM stiffness (mixed collagen: Matrigel gels) and growth factor supplementation, we managed to obtain complex organoids, up to 1.2 mm in length and with branches up to the 5th level. This will allow to study how the branching process works in branched organs in our body with reduced use of animal models. Also, mutations can be introduced in the model, which can be used to study tumor initiation and progression.

### **Lab equipment**

## **Method status**

History of use

Published in peer reviewed journal

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

- Organoids are derived from primary material so they are not transformed,
- They show a complex morphology similar to the *in vivo* gland,
- Relatively fast procedures,
- No need of special equipment.

### **Challenges**

- Relatively low throughput,
- Technically challenging,
- Long culture time (15-20 days),
- Primary material cannot be expanded indefinitely so it still requires use of animals.

### **Modifications**

We are currently testing additional factors on the organoids to mimic *in vitro* the remodeling stages of the adult breast during pregnancy, lactation and involution upon weaning to have a model that can reduce the use of animals to study these developmental processes.

### **Future & Other applications**

The model can be used to study the impact of breast remodeling on tumor predisposition. The concept of modulating the matrix stiffness and providing a

growth factor alternation may apply to induce branching also in organoid models of other branched organs.

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### References

Caruso M, Huang S, Mourao L, Scheele CLGJ. A Mammary Organoid Model to Study Branching Morphogenesis. *Front Physiol.* 2022 Mar 16;13:826107. doi: 10.3389/fphys.2022.826107. PMID: 35399282; PMCID: PMC8988230.

Caruso M, Saberiseyadabad K., Mourao L, Scheele CLGJ. A guide towards 3D mammary and breast organotypic cultures. *Methods Mol Biol.* 2023, Springer Nature (submitted).

### Associated documents

[fphys-13-826107.pdf](#)

### Links

[A Mammary Organoid Model to Study Branching Morphogenesis.](#)

## PARTNERS AND COLLABORATIONS

### Organisation

**Name of the organisation** Oncology - KU Leuven

**Department** Oncology

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