# Expansion of Mouse Mammary Epithelial Stem Cells in Serum-Free Organoid Growth

David Rowbotham[1], Samantha Rothwell[1], Allen C. Eaves[1,2], Sharon A. Louis[1], and John Stingl[1]

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[1]STEMCELL Technologies Inc., Vancouver BC, Canada; [2]Terry Fox Laboratory, BC Cancer, Vancouver BC, Canada

## INTRODUCTION

Growing mammary epithelial cells as organoids in a three-dimensional (3D) cell culture environment represents a more physiological model system than conventional 2D adherent cell culture systems for studying many different aspects of mammary epithelial cell biology. We have developed MammoCult™ Organoid Growth Medium (Mouse; OGM), a serum- and phenol red-free medium for the long-term propagation of organoids from mouse mammary tissue. To initiate the cultures, mouse mammary glands are resected and enzymatically dissociated sequentially in Gentle Collagenase/Hyaluronidase, trypsin, and dispase/DNase I to generate a single-cell suspension, and 4.5 x $10^3$ of the liberated cells are then embedded in Corning® Matrigel® and maintained in MammoCult™ Organoid Growth Medium (Mouse). Alternatively, freshly dissociated mammary cells can be expanded in adherent culture in EpiCult™ Plus Medium prior to seeding into Matrigel®. Approximately 8.4 ± 0.6% (mean ± SEM; n = 10) of the freshly dissociated cells seeded in the Corning® Matrigel® will proliferate and generate organoids that are typically > 200 µm in diameter and have a highly branched morphology, although organoids with a cystic morphology are also observed. Immunostaining of these branched organoids reveals that they are composed of a polarized epithelium made up of keratin (K) 8-expressing luminal cells and K5/14-expressing basal cells (n = 7). Organoids upregulate transcripts for casein (*Csn2*), whey acidic protein (Wap), and lactoferrin (Ltf) upon stimulation with prolactin, hydrocortisone, and insulin (n = 4). These multilineage organoids can be established from multiple inbred mouse strains, including C57Bl6/J, FVB, BALB/c, and CD-1. Organoids can be passaged every 10 - 14 days as small fragments at a 1:3 to 1:4 split ratio, and can be maintained for a minimum of 10 passages while still maintaining multilineage potential and a normal karyotype. These results demonstrate that MammoCult™ Organoid Growth Medium (Mouse) efficiently generates and expands mammary epithelial cell-derived organoids and offers researchers a novel tool for in vitro mammary studies.

## METHODS

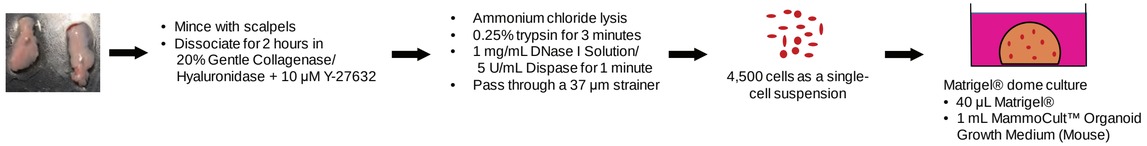



**FIGURE 1. Mammary Gland Dissociation and Seeding Protocol**

Protocol for dissociating mouse mammary glands to single cells and seeding into Matrigel® domes. Cells are seeded into Matrigel® domes at 5.5 x $10^3$ cells/40-µL dome. Once gelled, 1 mL of MammoCult™ OGM is added to the well. Medium is changed 3 times per week. When cultured this way, approximately 8.4 ± 0.6% (mean ± SEM; n = 10) of the seeded cells will proliferate and generate organoids > 200 µm in size.

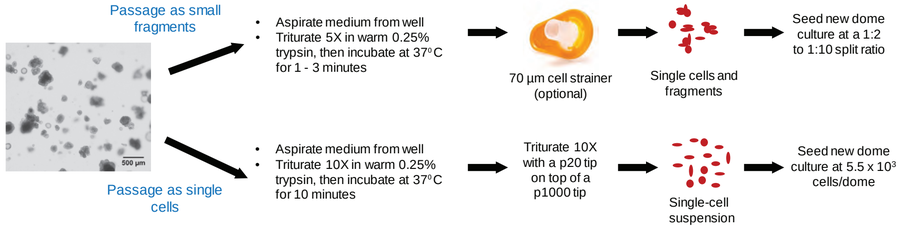



**FIGURE 2. Organoid Passaging Protocol**

Protocols for passaging of organoids either as small fragments (top) or as single cells (bottom). In both cases, organoids are ready for passaging again after 10 - 14 days in culture.

## RESULTS

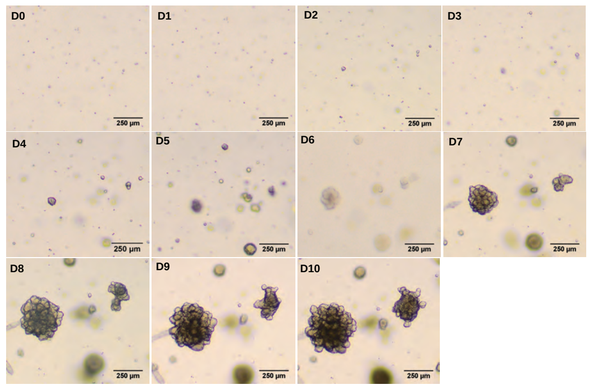



**FIGURE 3. Mammary Organoid Growth From Single-Cell Suspensions, Day 0 - 10**

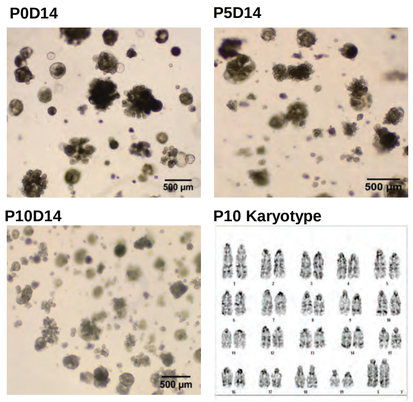



**FIGURE 4. Organoids Can Be Maintained For a Minimum of 10 Passages With a Normal Karyotype**

Data obtained from 2 pooled independent cultures.

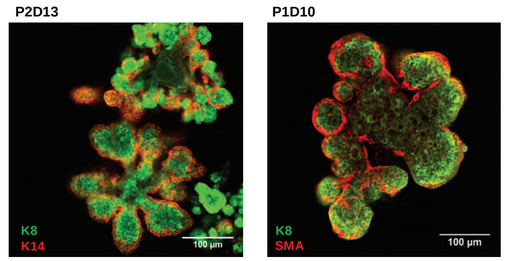



**FIGURE 5. Mammary Organoids Are Multilineage**

Mammary organoids are composed of K8-expressing luminal cells and K14- and smooth muscle actin (SMA)-expressing basal cells. The left panel shows organoids at passage 2 day 13, and the right panel shows organoids at passage 1 day 10.

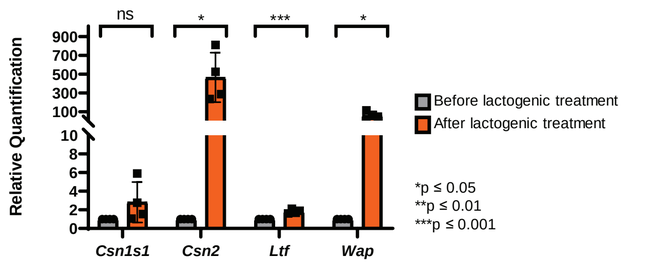



**FIGURE 6. Organoids Can Undergo Lactogenic Differentiation**

Pre-established mammary organoids (passage 1 day 10), when treated with 5 µg/mL prolactin, 5 µg/mL insulin, and 100 ng/mL hydrocortisone for 4 days, upregulate expression of transcripts for casein 2 (*Csn2*), lactoferrin (*Ltf*), and whey acidic protein (*Wap*). Transcripts for casein alpha s1 (*Csn1s1*) were not significantly upregulated. Data in lactogenic hormone treatment was normalized to non-treated cells from the same mouse (n = 4), and significance was determined using paired t-tests.

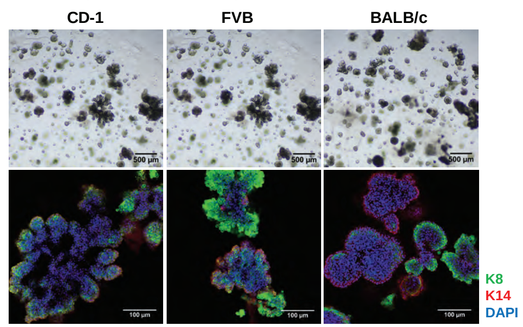



**FIGURE 7. Multiple Inbred Mouse Strains Can Generate Organoids**

Images showing passage 2 day 9 organoids generated from CD-1, FVB and BALB/c mouse strains.

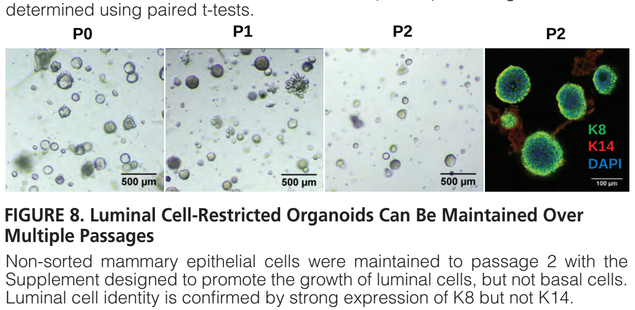



**FIGURE 8. Luminal Cell-Restricted Organoids Can Be Maintained Over Multiple Passages**

Non-sorted mammary epithelial cells were maintained to passage 2 with the Supplement designed to promote the growth of luminal cells, but not basal cells. Luminal cell identity is confirmed by strong expression of K8 but not K14.

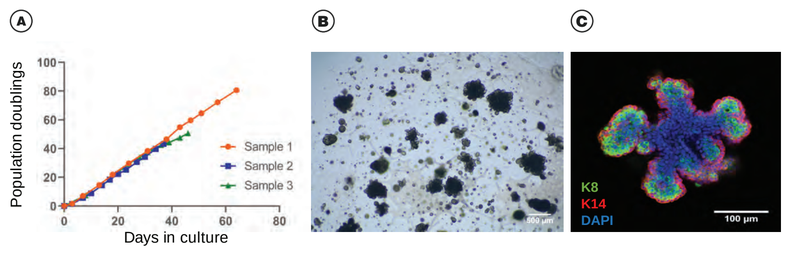



**FIGURE 9. Mammary Organoid-Forming Cells Can Be Expanded In EpiCult™ Plus Medium Prior to Seeding Into an Organoid Assay**

**(A)** Mouse mammary epithelial cells can be expanded for > 40 population doublings in adherent culture in EpiCult™ Plus Medium.
**(B)** Mammary epithelial cells expanded for approximately 12 population doublings in EpiCult™ Plus Medium can form organoids when seeded into MammoCult™ OGM.
**(C)** Organoids are multilineage and are composed of K8-expressing luminal cells and K14-expressing basal cells.

## Summary

- MammoCult™ Organoid Growth Medium (Mouse) consists of a phenol red-, serum-, estrogen-, and progesterone-free defined medium formulation for efficient and reproducible generation of mouse mammary organoids within 10 - 14 days of culture.
- These organoids can be maintained for a minimum of 10 passages while maintaining a stable karyotype.
- Mouse mammary organoids generated with MammoCult™ Organoid Growth Medium (Mouse) are composed of K8-expressing luminal cells and K14- and SMA-expressing basal cells. These organoids can also undergo lactogenic differentiation when exposed to lactogenic hormones.
- MammoCult™ Organoid Growth Medium (Mouse) promotes the growth of mammary organoids from multiple mouse strains.
- MammoCult™ Organoid Growth Medium (Mouse) is supplied in a modular format, and can be used to promote the growth of multilineage organoids or luminal cell-restricted organoids, depending on the combination of supplements used.
- Mouse mammary organoid-forming cells can be expanded in EpiCult™ Plus Medium prior to seeding into the organoid assay.

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