Systems and Solutions for Advanced Organoid Research

EMBO | EMBL Organoids Symposium - 21-24 October 2020

Alex Sim

President, AMSBIO

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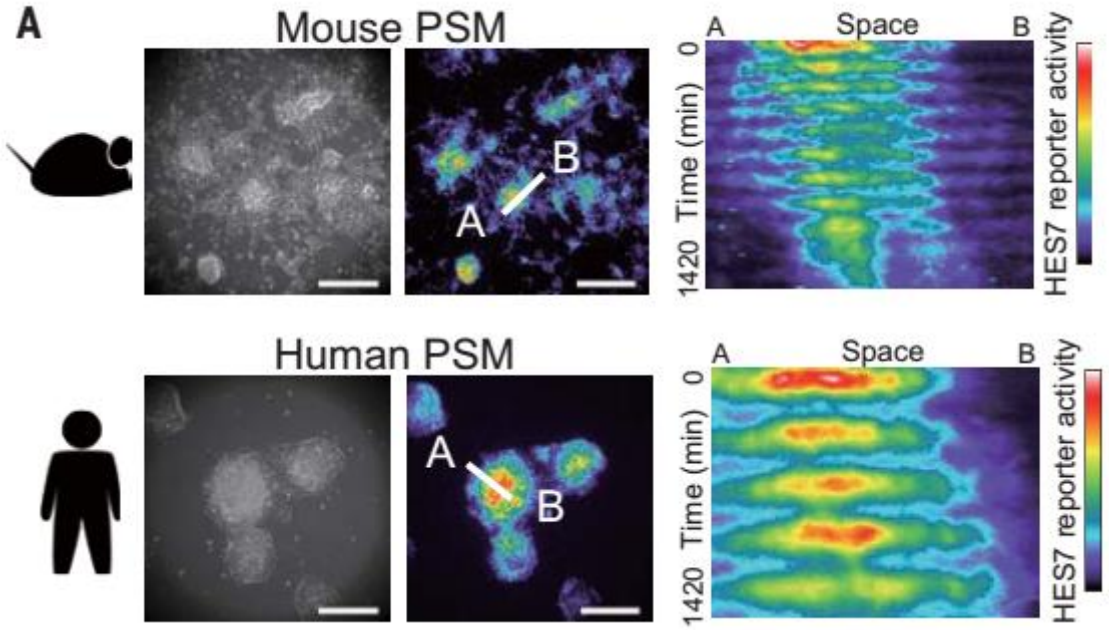
EMBO | EMBL Symposia

Stem Cell Culture



Images courtesy of Mikki Ebisuya (EMBL, Barcelona, Spain & RIKEN BDR, Kobe, Japan) and co-workers.

From Matsuda *et al*. (2020) Science 369.6510: 1450-1455 by permission.

StemFit®

Superior Feeder-Free Stem Cell Culture Medium

iMatrix-511

Recombinant Laminin 511 E8 Fragments

- Species-specific segmentation clocks modelled *in vitro* with presomitic mesoderm (PSM) cells
- Induced from mouse ESCs & human iPSCs cultured with iMatrix-511silk as ECM in StemFit® medium

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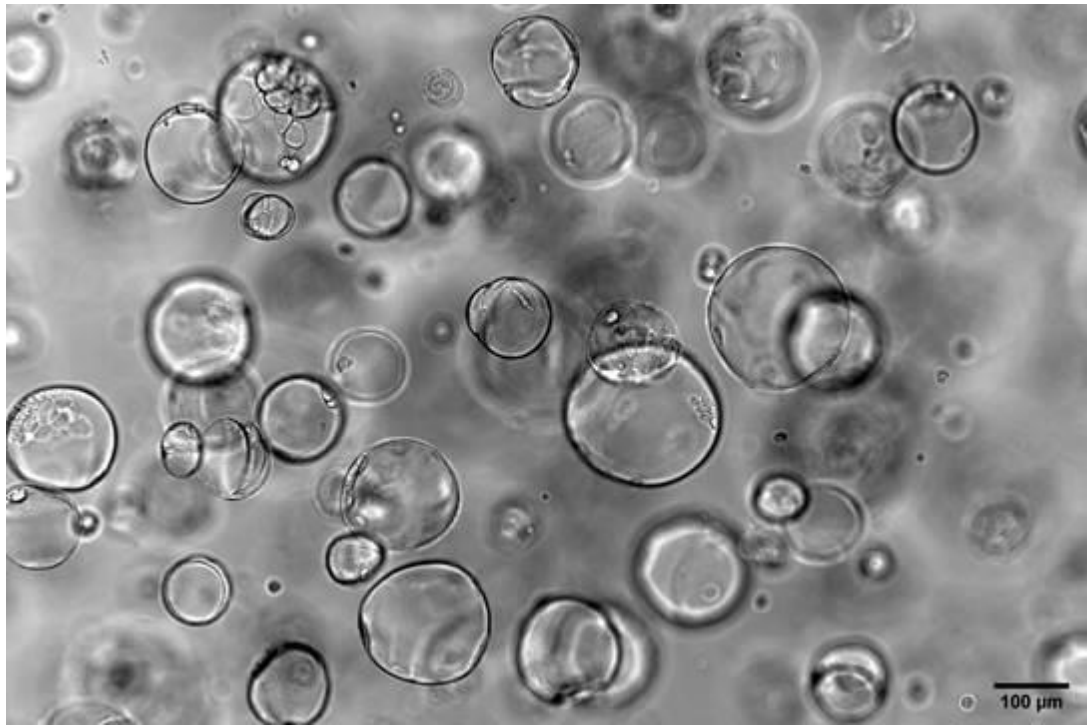
Stem Cell Culture



StemFit®

Superior Feeder-Free Stem Cell Culture Medium

iMatrix-511

Recombinant Laminin 511 E8 Fragments

Human intestinal colonies grown in LDTM gel supplemented with iMatrix laminin-511

Image courtesy of Saba Rezakhani and Matthias Lutolf, EPFL, Switzerland

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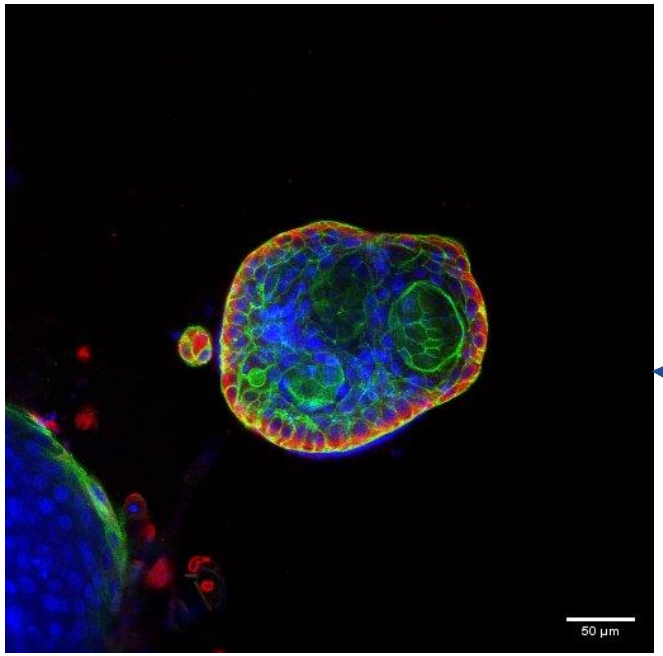
Cryopreservation of Organoids

Human Airway Organoid frozen in CELLBANKER® 1. Imaged post-thaw.



DAPI, KRT5, Phalloidin (blue/red/green).
Image courtesy of the Clevers Lab, Hubrecht Institute.

“On the day of the lockdown, me and some other researchers froze our organoids down for the first time in CELLBANKER®. The procedure was a lot easier and faster since I could simply transfer the organoids in CELLBANKER® to the freezer immediately… it genuinely helped the freezing when going into lockdown.”

- Jelte van der Vaart (Clevers Lab, Hubrecht Institute, Netherlands)

Presence of KRT5+ cells shows that basal cells survive freezing and the culture is still able to expand.

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Cryopreservation of Organoids

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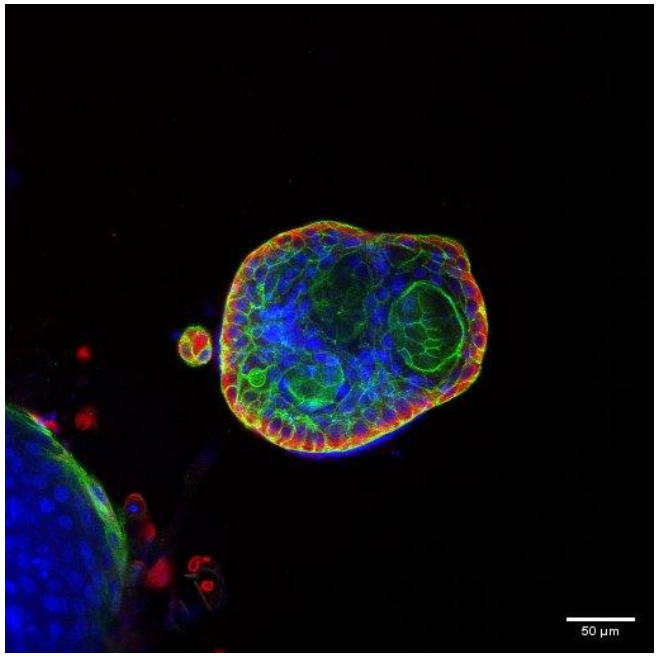

DAPI, KRT5, Phalloidin (blue/red/green).
Image courtesy of the Clevers Lab, Hubrecht Institute.

Published Citations for CELLBANKER® 2

Vallier Lab (Cambridge, UK) - Cryopreservation of cholangiocyte organoids
Tysoe *et al.* (2019) *Nature Protocols*, 14(6), 1884

Knoblich Lab (Vienna, Austria) - Archiving clones with desired genetic characteristics
Bagley *et al.* (2017) *Nature Methods*, 14, 743.

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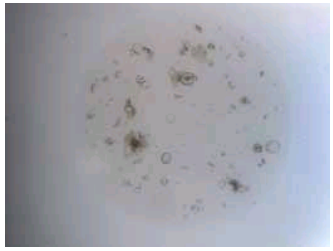
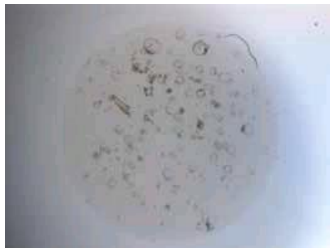
Cryopreservation of Organoids

> **Day 2 and Day 4 images clearly show that organoids recovered and grew well in these three CELLBANKER® Freezing Media.**

Meritxell Huch (Max Planck Institute of Molecular Cell Biology and Genetics, Germany)

	Day 2	Day 4	Organoid Growth Following Freezing
STEM-CELLBANKER® GMP			✓
CELLBANKER® 2			✓
CELLBANKER® 1			✓

Human liver organoids stored in CELLBANKER® series.
Images courtesy of Robert Arnés, Huch Lab, MPI-CBG, Germany

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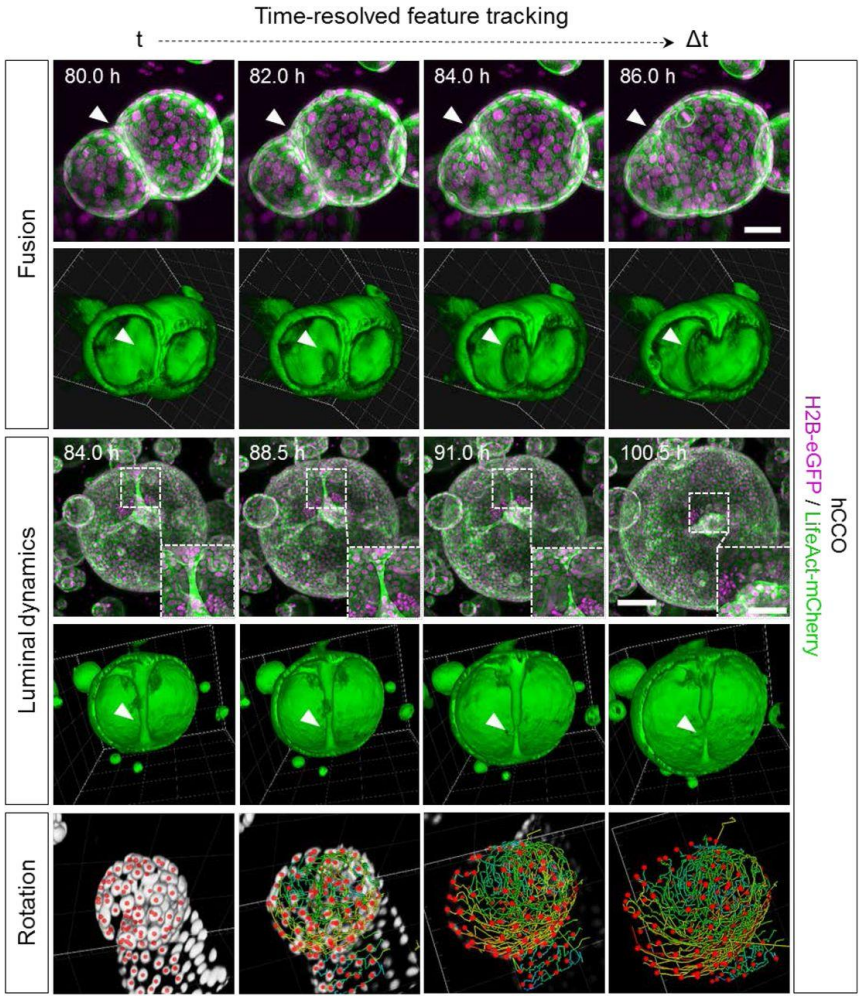
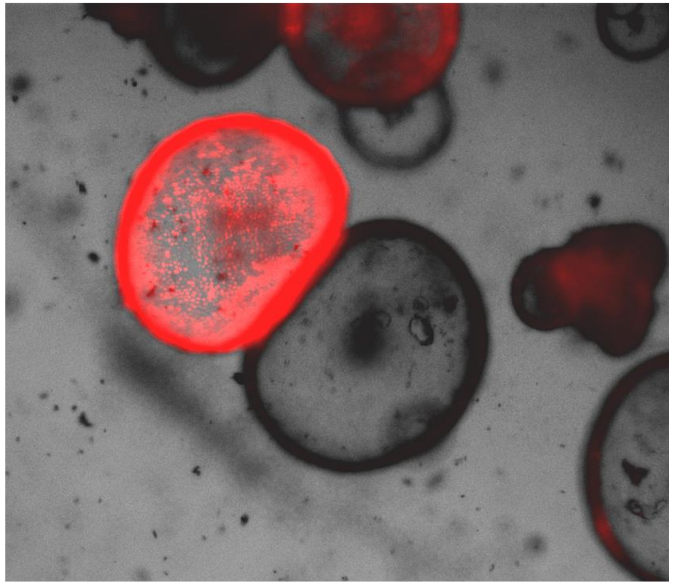
Lentivirus for Organoid Research



Cellular dynamics of liver organoid development was tracked with AMSBIO lentivirus expressing H2B-EGFP (nuclei) & LifeAct-mCherry (F-actin cytoskeleton).

Hof, Lotta, *et al.* (2020) Long-term live imaging of epithelial organoids and corresponding multiscale analysis reveal high heterogeneity and identifies core regulatory principles. *bioRxiv.*



Cholangiocarcinoma-nuclear red organoids, generated with AMSBIO lentivirus (LVP360-R) for nuclear RFP

Image courtesy of Robert Arnés, Huch Lab, MPI-CBG, Germany

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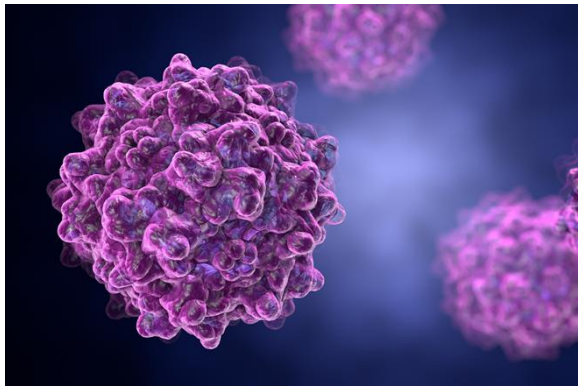
Lentivirus & AAV for Organoid Research

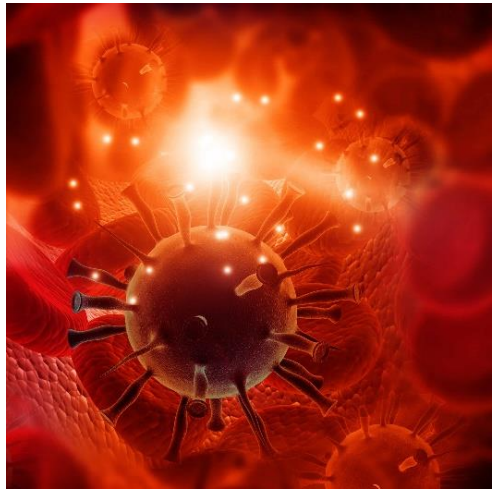
AAV

- ORF, shRNA, miRNA expression
- Wide range of promoters available
- High titer AAV
- Custom service
- Wide range of reporter virus available off the shelf
- Wide range of AAV serotypes available
- GMP service



Lentivirus

- 3rd generation lentivirus, replication incompetent
- shRNA, ORF, miRNA expression
- High titer lentivirus available
- Reprograming lentivirus sets
- Ready made particles for fluorescent proteins, luciferase expression
- Reporter lentivirus for different pathways (Notch, Wnt, Hypoxia…)
- Organelle labelling lentivirus
- Custom service available
- Stable cell line service available



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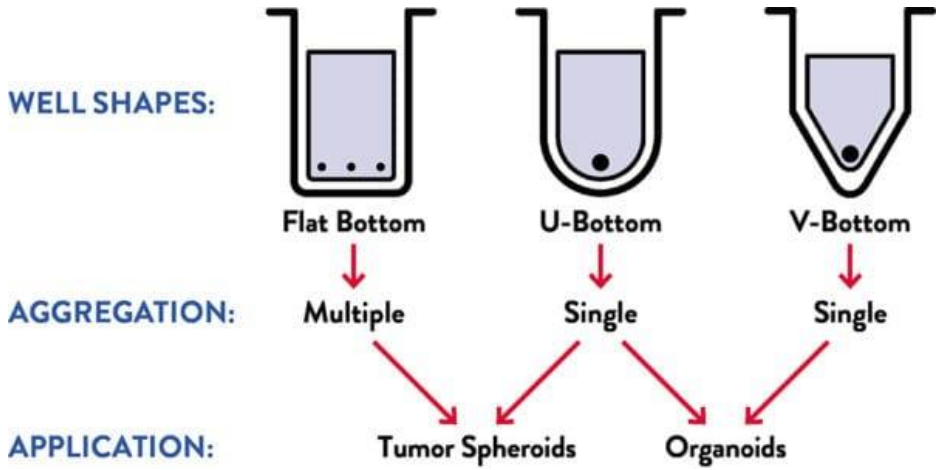
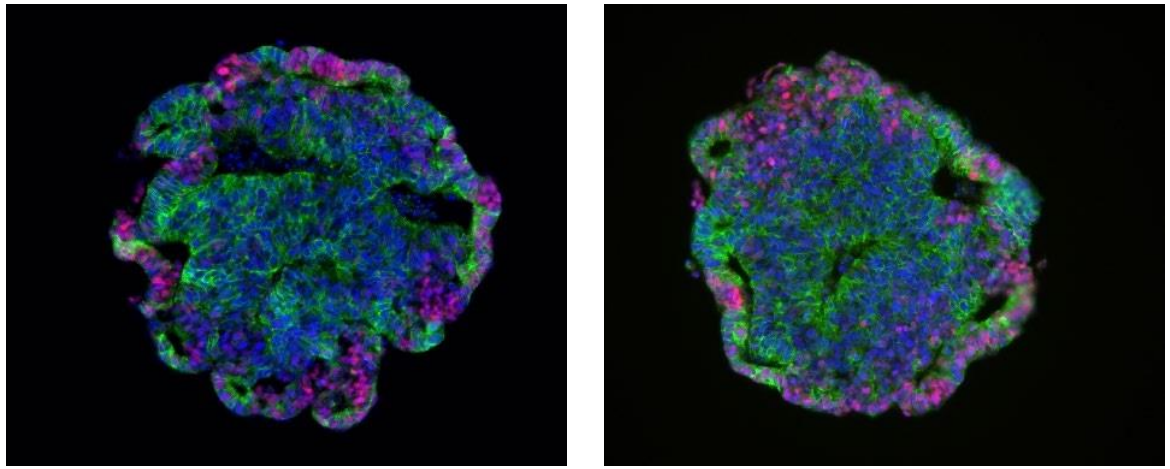
Lipidure-Coat Plates: Superior Low-Attachment Cell Culture

- Differentiate embryoid bodies to organoids in 96-well format
- U-bottom or V-bottom well shapes available



Inner Ear Organoids

Day 6 Aggregates, showing ruffling, indicating formation of vesicles: DAPI (nuclear) - blue, ECAD (epithelial) - green and TFAP2A (sequence-specific DNA-binding factor) – purple.



Images courtesy of the Hashino Lab, Indiana University School of Medicine.

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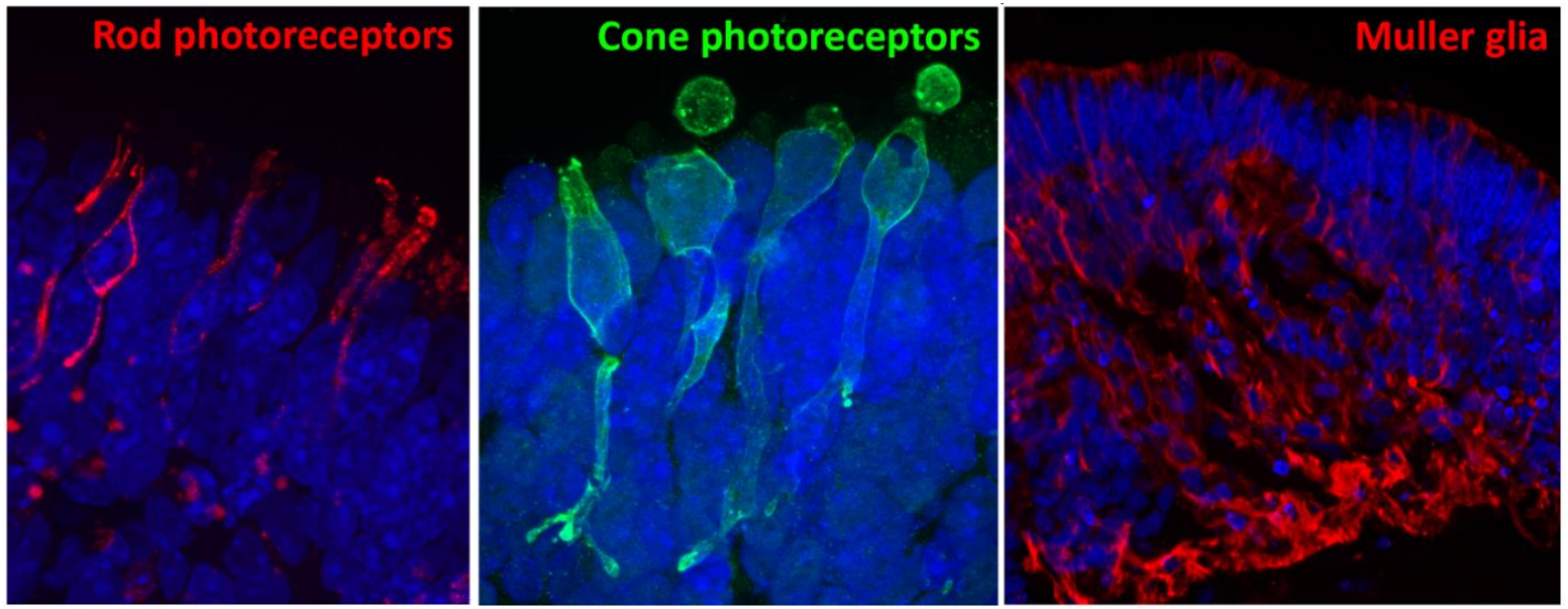
Lipidure-Coat Plates: Superior Low-Attachment Cell Culture



Images courtesy of Dr. Valeria Chichagova (Newcells Biotech) and Prof. Lako (Newcastle University, UK)

> **“The Lipidure-coated plates provided by AMSBIO were extremely useful for generating with ease large numbers of homogeneous retinal organoids which responded to light and contained all the key retinal cell types.”**

Prof. Majlinda Lako (Newcastle University, UK)

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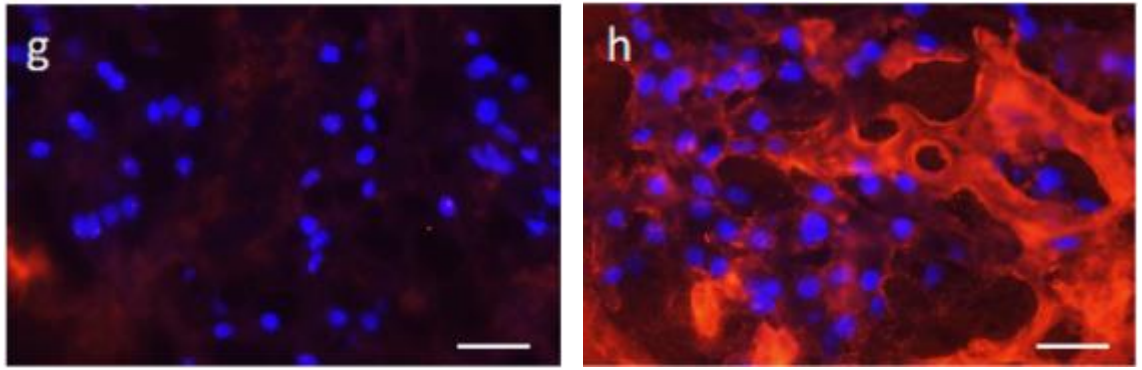
Expression of Heparan Sulfate by Lung Bud Organoids

IHC of Lung Bud Organoids for Heparan Sulfate (red) using 10E4 antibody (AMSBIO). Nuclei in blue (DAPI). Section digested with Heparinase III (AMSBIO) to remove glycans included as negative staining control (left). Scale bar 50 µm.



Negative Staining Control HS Staining Red

Images courtesy of Helena Meyer-Berg & Prof. Deborah Gill (University of Oxford, UK)

> **"The investigation of viral entry receptors in lung organoids, such as heparan sulphate stained with AMSBIO α-heparan sulphate antibody (clone F58-10E4), allowed us to compare our model to adult human lung and discuss viral entry mechanisms."**
>
> Helena Meyer-Berg (University of Oxford, UK)

Meyer-Berg, H., Zhou Yang, L., de Lucas, M. P., Zambrano, A., Hyde, S. C., & Gill, D. R. (2020). Identification of AAV serotypes for lung gene therapy in human embryonic stem cell derived lung organoids. Stem Cell Res. Ther. *in press*.

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ScreenIn3D – Drug Screening Service

Microfluidic Technology

↓

Complex *in-vitro Models*
and/or
Patient Biopsy

↓

Micro-Tumors

↓

In-vitro Drug Efficacy

Tangible Results

- **20-fold increase** in screening capabilities using **human biopsy tissue** (USP)
- **Quantifiable** tests of drug efficacy (IC50)
- Screening of human biopsy tissue in 1-3 weeks **(feasible clinical times)**
- Continuous **label-fee readouts** of spheroid condition in addition to standard readouts
- Spheroid/organoid culture using **perfusion** and **shear stress-free** conditions
- **Quicker results** than with costly animal models

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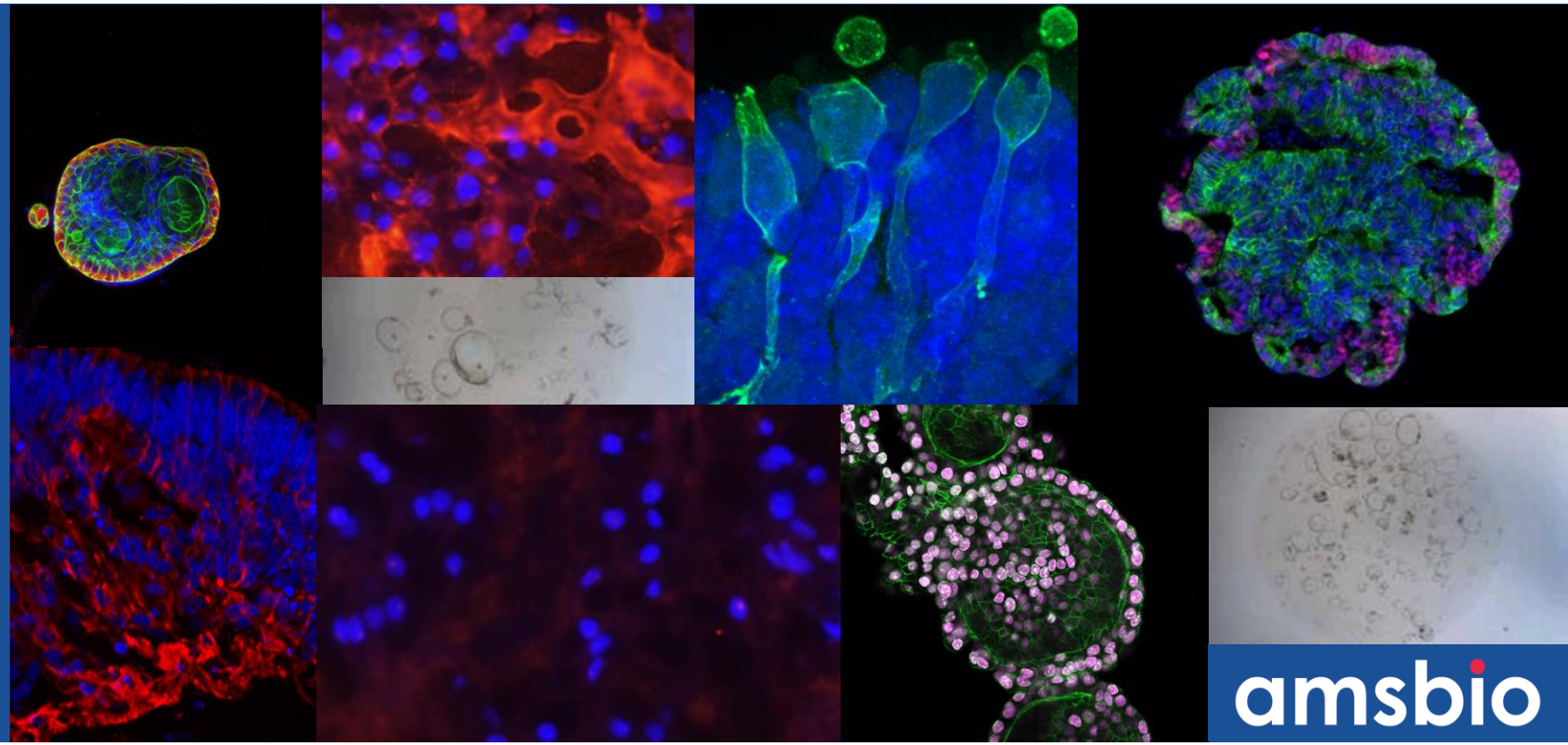
Systems and Solutions for Advanced Organoid Research

- StemFit® Stem Cell Media
- iMatrix-511 Recombinant Laminin
- Lentivirus and AAV Solutions
- Lipidure-Coat Plates
- CELLBANKER® Freezing Media
- Heparan Sulfate Antibodies
- ScreenIn3D Services



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