SEP 01, 2023

OPEN ACCESS

Protocol Citation: Annika Fendler 2023. Lee et al. Digestion and Seeding (adapted from. Annika). **protocols.io** https://protocols.io/view/lee-et-al-digestion-and-seeding-adapted-from-annik-czfyx3pw

MANUSCRIPT CITATION: https://www.sciencedirect.com/science/article/pii/S0092867418302976?via%3Dihub

Protocol status: In development
We are still developing and optimizing this protocol.

Created: Sep 01, 2023

Last Modified: Sep 01, 2023

PROTOCOL integer ID: 87256

Lee et al. Digestion and Seeding (adapted from. Annika)

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ABSTRACT

This Protocol is adapted from Lee et a. (2018) (https://www.sciencedirect.com/science/article/pii/S0092867418302976?via%3Dihub)

The authors have established organoids from clusters of bladder cancer cells after TURB. They use a medium with CS-FCS and EGF and culture the organoids as a sandwich in matrigel, The effiency was around 70%, genomics were comparable to the parental tumour but phenotypically the organoids tended to evolve into basal cells even when arising from a tumour with a luminal phenotype.

MATERIALS

Lee Organoid Medium:
Hepatocyte media with 10 ng/ml EGF, 5% CS-FBS, 10 µM Y-27632 (STEMCELL Technologies), and 1X Glutamax (GIBCO)), supplemented with 1x Zellshield.

HBSS Washing Solution:

HBSS with 10 mM HEPES, Without Phenol Red 500 mL **STEMCELL Technologies Inc. Catalog #37150**

supplemented with 5% CS-FBS, 10 µM of the ROCK inhibitor Y-27632 and 1x Zellshield.

PROTOCOL MATERIALS

Gentle Collagenase/Hyaluronidase 10 mL **STEMCELL Technologies Inc. Catalog #7919**

Step 10

TrypLE™ Express Enzyme **Thermo Fisher Scientific Catalog #12604013**

Step 14

Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix **Corning Catalog #356231**

In 4 steps

HBSS with 10 mM HEPES, Without Phenol Red 500 mL **STEMCELL Technologies Inc. Catalog #37150**

Materials

Keywords: Orgnaoids,
bladder, matrigel, clusters

Prepare medium and enzyme mixes

1 Heat the shaking incubator to 37°C

2 Prepare and adequate volume of Lee Organoid Medium and HBSS Washing Solution.

3 Thaw

Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix **Corning Catalog #356231**

o/n

4 Coat an adequate number of wells of a 24-well or 6-well plate with 60%

Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix **Corning Catalog #356231**

(diluted in organoid medium).

Add 75 µl or 250 µl of 60% Matrigel to each well. Keep the plate on ice and try to spread the

Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix **Corning Catalog #356231**

evenly with a pipette tip. Solidify for 15 min at 37°C

5 Preaheat Lee Organoid Medium to 37°C

Thaw fragments

58m

6

	Sample ID	Number of Aliquots	Number of fragments	Volume Collagenase/Hyaluronidase (ul)	Volume Medium (ml)

Table 1: Sample Overview and Volumes for digestions

7 Thaw 1 or more aliquots of fragments at 37 °C until only a small piece of ice remains

8 Transfer fragments to a 50 ml Falcon and spin down 400 x g, 00:05:00 . Remove the supernatant 5m

9 Transfer fragments to a glass petri dish and mince with a scalpell as fine as possible.

Digestion of fragments 58m

10 Transfer fragments to 1 mL to 5 mL of
Gentle Collagenase/Hyaluronidase 10 mL **STEMCELL Technologies Inc. Catalog #7919**
diluted 1:10 in Lee Organoid Medium.
See table 1 for the volumes used for each sample.

11 Incubate 00:15:00 under gentle shaking at 37 °C . 15m

12 Spin down 350 x g, 00:05:00 and discard supernatant. 5m

13 Add 5 mL of PBS and spin down 350 x g, 00:05:00 and discard supernatant. 5m

14 Add 2 mL to 5 mL of 3m
TrypLE™ Express Enzyme **Thermo Fisher Scientific Catalog #12604013**
and incubate 00:03:00
at Room temperature

15 Stop reaction with 5 mL HBSS Washing Solution.

16 Spin down for 350 x g, 00:05:00 and discard supernatant. 5m

17 Resuspend in 2 mL of HBSS Washing Solution and pass through a 100 µm cell strainer. Wash with 5 ml of HBSS Washing solution.

Seed cell clusters as organoids

18 Spin down for 350 x g, 00:05:00 and carefully discard as much supernatant as possible. 5m

19 Resuspend the cells in 75 µL for each well of a 24-well plate or 250 µL for each well of a 6-well plate of 60% Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix **Corning Catalog #356231** and carefully layer over the matrigel coating of the prepared wells. Solidify for 00:15:00 at 37 °C in incubator . 15m

20 Add 250 µL for each well of a 24-well plate or 1.5 mL for each well of a 6-well plate of Lee Organoid Medium per well.

Results

21 Add text and pictures here to describe how organoids are growing. Please also reference the next protocol that you are using to continue culturing