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# OPEN ACCESS



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https://protocols.io/view/leeet-al-digestion-and-seedingadapted-from-annik-czfyx3pw

#### **MANUSCRIPT CITATION:**

https://www.sciencedirect.co m/science/article/pii/S009286 7418302976?via%3Dihub

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**Protocol status:** In development

We are still developing and optimizing this protocol.

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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  $\mu$ M 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

## 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

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

5 Preaheat Lee Organoid Medium to 37°C

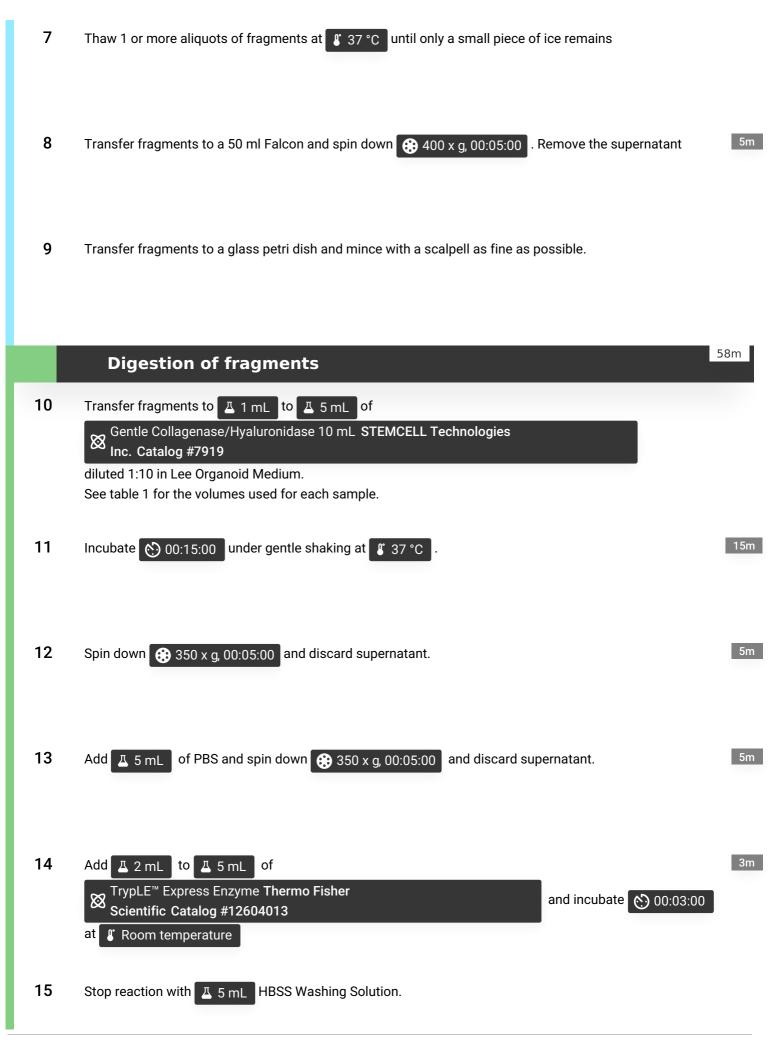
## Thaw fragments

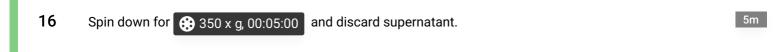
58m

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

Table 1: Sample Overview and Volumes for digestions





Resuspend in  $\mathbb{Z}_2$  mL of HBSS Washing Solution and pass through a  $-100 \, \mu \text{m}$  cell strainer. Wash with 5 ml of HBSS Washing solution.

## Seed cell clusters as organoids

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

Resuspend the cells in Δ 75 μL for each well of a 24-well plate or

Δ 250 μL for each well of a 6-well plate of 60%

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and carefully layer over the matrigel coating of the prepared wells. Solidify for 00:15:00 at

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

### Results

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