mPSM protocol V.1

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[1]EMBL

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Protocol to perform 2D ex vivo segmentation assay (Lauschke et al. 2013).

Takehito Tomita 2022. mPSM protocol. **protocols.io**
https://protocols.io/view/mpsm-protocol-b349qqz6

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1 Coat dishes/plates with fibronectin.

1.1 Dilute fibronectin (Sigma, Cat.No: F1141) with PBS to 50ug/mL (20 fold dilution).

1.2 Fill each well of 8-well plate (NuncTMLab-TekTMII Chambered Coverglass, Cat.No: 155409PK) with ~160µl of fibronectin solution and incubate for 1 hour in 37°C.

1.3 Remove all fibronectin solution from the well(s) and dry for 1 hour at room temperature (~25°C).

2 Prepare dissection medium and culture medium.

2.1 Mix the following ingredients in a 50 mL falcon tube:

- **0.4 g BSA**
- **400 µL glutamine**
- **35.2 µL 45% glucose**
- **40 mL DMEM-F12**

Vortex well.

2.2 Separate **10 mL** from the mix in step 2.1 and filter through a 0.22µm filter (Millex-GV, Cat.No: SLGV033RS) into a fresh 15 mL falcon tube. Add **100 µL Penicillin/Streptomycin (Gibco, Cat.No: 15140-122)** to achieve
100U/mL. This mix is used as culture medium. Equilibrate in 37°C 5% CO2 until use.

2.3 Add **510 µL HEPES** to the remaining **30 mL** mix in step 2.1. This mix is used as dissection medium. It can be kept at the bench until use.

3 Wash the dried wells with ~200µL culture medium per well. Apply fresh culture medium (160uL ~ 300µL per well) and equilibrate in 37°C 5% CO2 until use.

4 Extract mouse embryos and collect tails in dissection medium.

5 Cut the tail bud using a scalpel in dissection medium. Be careful not to flick the tail buds as this damages the tissue.

6 Wash the tail buds once in a dish containing culture medium. Quickly move the tail buds to the fibronectin coated wells. Align the tissue orientation using forceps to place the cut side downward.

7 Incubate in 37°C 5% CO2 for 1hr to let the tissue attach.

8 Place the well into the metal box to fit into the microscope. Add a strip of wet paper towel inside the metal box to prevent drying. Carefully move the box into the microscope, as tissue can detach quite easily.

9 Set up imaging.