

mPSM protocol V.1

Takehito Tomita¹

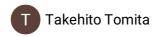
¹EMBL





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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 ~200 μ L culture medium per well. Apply fresh culture medium (160 μ L ~ 300 μ L 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.
- 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.