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SeqFISH Tissue Preservation V.1

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Protocol status: In development

We are still developing and optimizing this protocol

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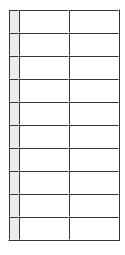
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Abstract

Tissue Preparation for segFISH



Materials





Fixation

- Clean surface with RNAzap. Make the following solutions.
 - 1L PBS 1x, RNAse-free
 - take 1 L RNAse-free water bottle
 - 2. use serologic pipet and draw up 100 mL water into another container
 - 3. add 100 mL of 10x RNase-free PBS
 - 4. mix thoroughly

50% sucrose

- tare 1 L RNase-free disposable container
- 2. take 500 g RNAse-free sucrose container
- add RNAse-free water to sucrose and transfer to 1 L container
- 4. continue to add RNAse-free water until mass to 1 kg
- mix thoroughly, may need agitation on platform at RT for some time
- 6. store at 4°C

NOTE: for fixation, minimum PFA to tissue volume is 20:1, ideal closer to 50:1

2 Prepare 4% PFA in 1x PBS, 120 mL total for 4 tissues 30 mL each in 50 mL conicals.

prepa re 3x conic als for 4 tissue s	
16% PFA	10 ml
10x PBS	4 ml
RNAs e free water	26 ml
total	40 ml



- 3 Cut freshly dissected tissue to small pieces for heart, try cutting slab, 2 cm x 1 cm x 0.4 cm thickness: 2 cm dimension from epicardium to endocardium.
- 4 Place each piece in a 50 ml tube (or smaller if you can cut to smaller pieces) in 4% PFA leave at RT for 3 hrs.
 - ** the sample should be completely covered.
- 5 Wash three times with equal volume (30mL) of 1x PBS to remove PFA leave a bit of extra liquid in the bottom between each wash.

Sucrose gradient

- 6 Place the tissue in 10% Sucrose at RT and wait for it to sink in 50 mL conical, add 10 mL of sucrose wait for sample to sink to bottom – minimum incubation time of 30 min remove supernatant for next bath.
- 7 Place the tissue in 20% Sucrose at RT and wait for it to sink.
- 8 Place the tissue in 30% sucrose in 4°C – ready for shipping. Make sure there is enough 30% sucrose solution in container e.g. if in 50 mL conicals, have 50 mL of 30% sucrose.