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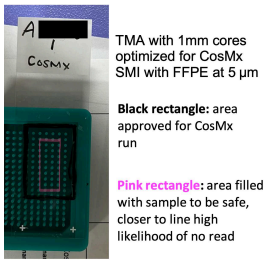
# FFPE Tissue Microarray protocol

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**We use this protocol and it's working**

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## Abstract

Protocol generated to as a step by step walk through of how to go from FFPE blocks to a tissue microarray (TMA) in preparation for NanoString CosMx SMI.

## Materials

| Item | Cat # | Vendor |
|---|---|---|
| **No charge slides** *do not add a sticker label either hand write or print directly on* | 9035 | Tissue-Tek‰ SmartWrite‰ Frosted Slides |
| **T-sue Microarray 1mm Mold** | 69132-07 | T-Sue Microarray Mold Kit, 1mm, 170 Cores : Amazon |
| **Biopsy punch** *need 1 per sample* *Tansey lab optimized with 1mm and were able to fit 40 samples per slide for CosMx* | MT3331AAP25 | MILTEX Sterile Disposable Biopsy Punch with Plunger, 1mm diameter, 25/box. MFID: 33-31AA-P/25: HOSPEQ |
| **Cassette** *if possible use smaller cassettes, generally green as opposed to the white, to decrease the depth of the paraffin* | 18000-004 | VWR‰ Premium Tissue Cassettes: Standard Cassettes |
| **Liquid paraffin/embedder** | 3801360 | Paraplast X-Tra or Blue-ribbon paraffin from Leica Biosystems |

## Troubleshooting

## Block generation

1 Put the T-sue mold in oven for 30 minutes at 70 °C

2 Once warm, bring tissue mold to embedder and dispense liquid paraffin into mold until the top of the core is fully submerged. Remove any bubbles with heated forceps

3 Place a cassette flat side down on top of mold, pick up mold with gloved hands (caution will be hot) and dispense liquid paraffin directly over the cassette until paraffin fills ½ of the depth of the cassette.

4 Put mold with cassette on top on cooling plate for 1 hour

5 After mold has cooled, carefully peel off the T-sue green mold from the cassette

6 Trim any excess wax around the edge of the cassette

## Extracting and placing tissue into the block:

7 Retrieve sample blocks and place on horizontal surface. Hover the punch needle perpendicular to the preferred sampling area, then slowly insert the punch needle into the block

8 Retrieve the recipient block that was made, slowly push the core punched from the sample block into desired hole. Repeat steps 7-8 for all samples.

9 Once all samples have been loaded, make sure the top surface area is completely flat, if not carefully trim off excess tissue

10 Place the recipient block containing samples face down on a glass slide and incubate at 37 °C for at least 24 and up to 48 hours.

## Preparing the block for sectioning: **if block is for CosMx do NOT cut until two weeks or less before experiment per NanoString manual**

11 Remove block from incubator and remove slide by placing on a warm plate glass slide down. Be very careful to remove block from glass slide as fast as possible. Allow block to cool.

12 TMA is now ready for sectioning. For 5um sections, allow block to rehydrate by placing face down on
paper towel on top of ice with 1/3 volume water added.

12.1 Make sure to orient filled tissue area to centered approved imaging area if preparing for NanoString CosMx.

13 After 4 hours of rehydration, fix block to microtome and section the block