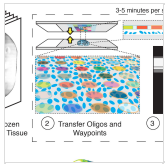

Feb 13, 2021

Spatial Transfer of Oligonucleotides and Imaging

In 1 collection

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2 *Works for me* This protocol is published without a DOI.

Sanjay Srivatsan

SUBMIT TO PLOS ONE

PROTOCOL CITATION

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COLLECTIONS

Single Cell Combinatorial Indexing (SCI) - seqeuncing

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PARENT PROTOCOLS

Part of collection
Single Cell Combinatorial Indexing (SCI) - seqeuncing

DISCLAIMER:

Prep work

1 Prepare the following reagents prior to beginning the protocol

1. Nuclei Buffer (NB)
2. Indexed Cold Lysis Buffer (CLB)
3. 5% PFA solution
4. Nuclei Buffer + SuperaseIn + BSA (NSB)

1.1 Nuclei Buffer (NB):
10mM Tris/HCl pH 7.4
Trizma hydrochloride solution **Sigma Aldrich Catalog #T2194-100ML**
Sodium chloride solution **Sigma**
10mM NaCl **Aldrich Catalog #S6546-1L**
3mM $MgCl_2$
Magnesium Chloride (1M Solution) **Invitrogen - Thermo Fisher Catalog #AM9530G**
Solvent: Nuclease Free water
Nuclease-free water or water filtered using a Milli-Q filtering system **Ambion Catalog #AM9932**

Prepare enough NSB for **0.5 mL** mL per section and an additional 4mL to account for overage and washing nuclei after pooling

1.2

Indexed Cold Lysis Buffer Solution (CLB):

Prepare **500 µl** of cold lysis buffer solution for every slide used.
If you are preparing solution for more than one slide prepare the pool in a 15mL conical.

1uM DAPI (final concentration)
IGEPAL® CA-630 **Sigma**
0.25% IGEPAL v/v **Aldrich Catalog #I8896** -- Make a 10% solution as a working stock
Solvent: Nuclei Buffer

Mix well after preparation, keep cold and out of light as much as possible.

1.3 For each slide that you are planning to index, aliquot 495uL of the Cold Lysis Buffer into a separate 1.5mL tube.
To each tube add 5uL of a 10uM slide-specific indexing oligo

Note: Make sure that the slide oligo used is unique from any sequence found in the sci-Space grid.

1.4 Wide-bore p1000 pipette tips. Cut the tip of p1000s or
ART™ Barrier Specialty Pipette Tips, 1000, wide bore **Thermo Fisher Catalog #2079G**

1.5 5% PFA solution:

Make enough solution for 1mL per section.

☒10X PBS **Thermo**

1.25x PBS **Fisher Catalog #QVC0508** d

5% PFA

☒Paraformaldehyde 32% (methanol free) **Electron Microscopy Sciences Catalog #15714**

Solvent: Nuclease Free water

☒Nuclease-free water or water filtered using a Milli-Q filtering system **Ambion Catalog #AM9932**

Mix well after preparation. The remaining PFA can aliquoted and stored at -20C for future use. Make sure to handle concentrated PFA solution in a fume hood.

1.6 Nuclei Buffer + SuperaseIN + BSA (NSB) solution:

1% SuperaseIN

☒SUPERase• In™ RNase Inhibitor (20 U/μL) **Thermo Fisher Catalog #AM2696**

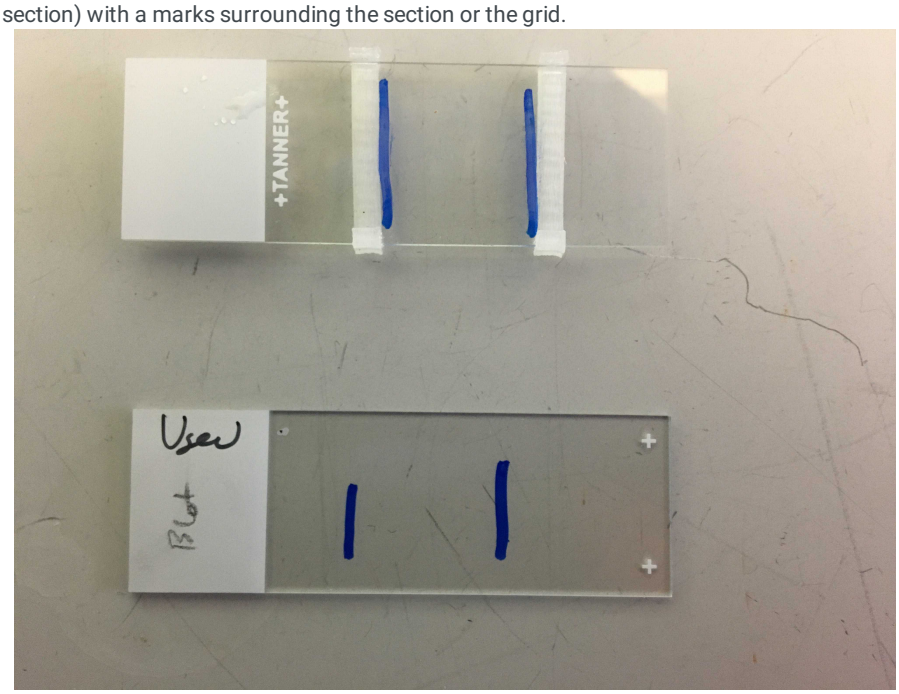
☒BSA 20

1% v/v BSA mg/ml **NEB Catalog #B9000S**

Solvent: Nuclei Buffer

Spatial Transfer of Oligonucleotide

2 A single mouse embryo section (stored at -80C) and a single sci-Space grid (stored at -20C) were removed. The back of each was wiped to remove moisture. Outline on the back of each slide (side that does not contain the oligo grid or section) with a marks surrounding the section or the grid.



embryo section (top) and sci-Space grid (below) with sharpie marking the boundaries of the embryo and spatial oligo grid. These marks are made on the back of each slide

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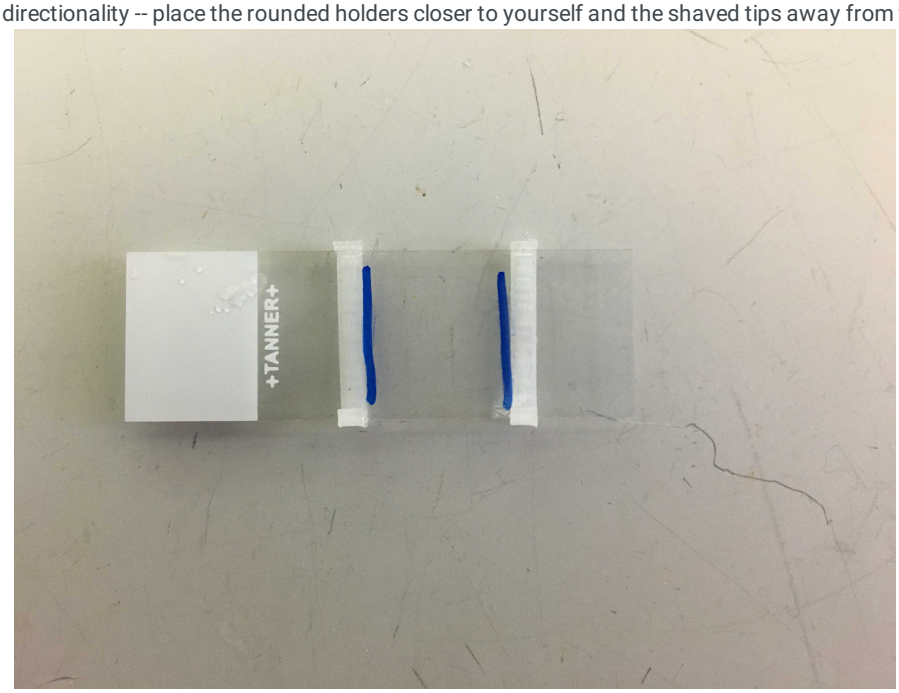
This is to help place the grid directly over the embryo.

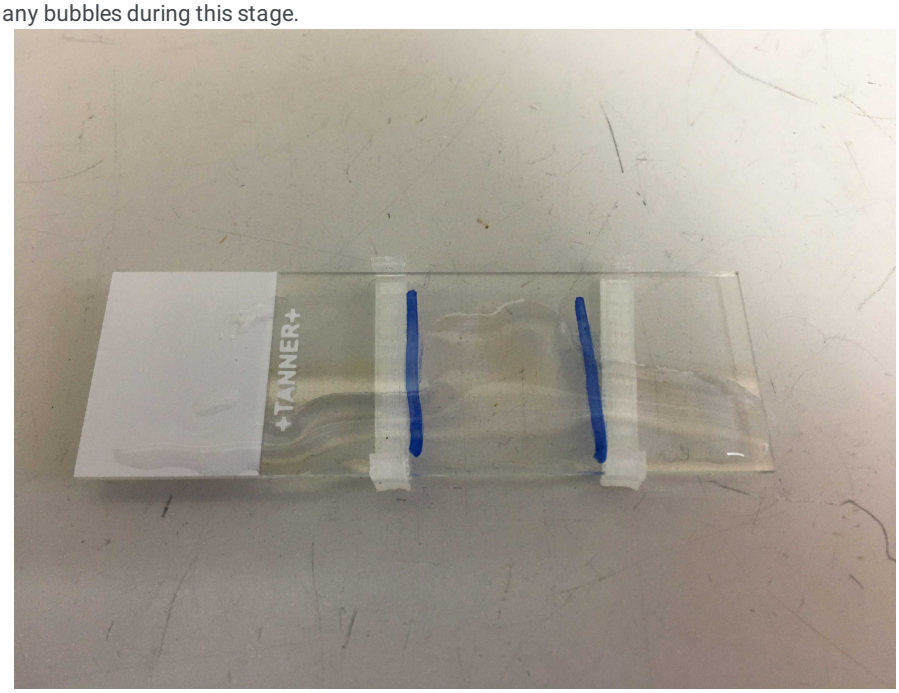
2.1 At the moment the sci-Space protocol has been optimized for OCT-embedded fresh-frozen sections. In the sci-Space study these sections were purchased from zyagen -- https://zyagen.com

3 Place the embryo section on two transfer clips with the transfer clips surrounding the section. The clips have a directionality -- place the rounded holders closer to yourself and the shaved tips away from you.



Note: The transfer clips are not crucial, but they are very helpful in securing the stacked slides during transfer.

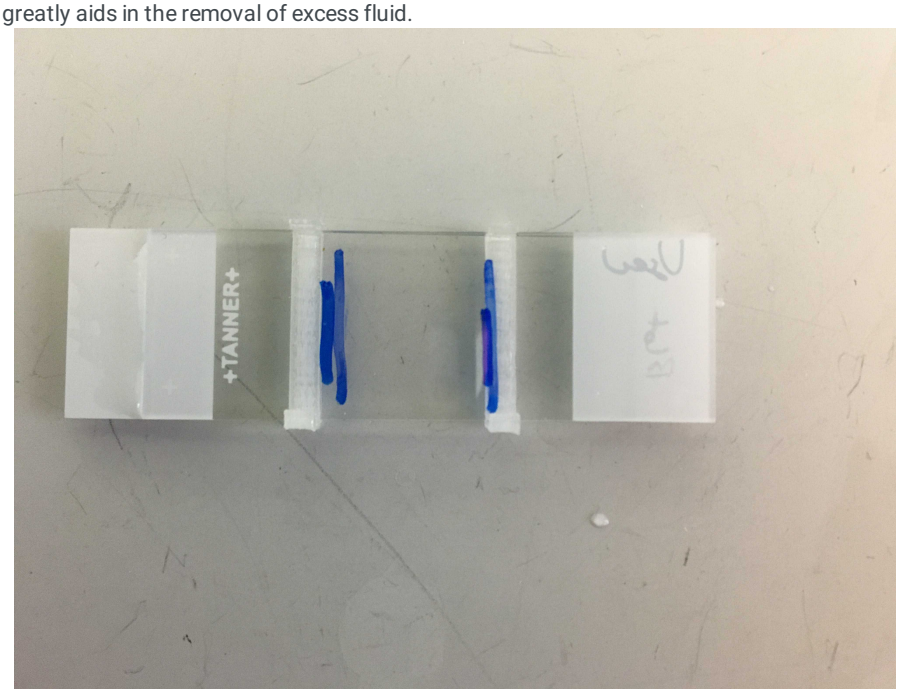
4 Using a wide-bore tip draw up 500uL of the indexed CLB solution and start by gently pipetting onto the embryo. As the embryo becomes covered in solution, move the pipette to cover the bottom of the slide. It's important not to introduce any bubbles during this stage.



Embryo section is wet with indexed permeabilization buffer. Buffer is first applied to embryo and then applied to the bottom of the slide. This aids in overlaying the sci-Space slide without bubbles.

Use the sci-Space grid and align the marks made during step2. Carefully snap the grid onto the tissue section by

5 lowering the bottom half of the sci-Space grid onto the section and then slowly pushing until the two slides are completely flush. At this stage it is also important that bubbles do not form. Press the stacked transfer with the thumbs and apply gentle pressure to evacuate any excess fluid between the two slide. Use of a napkin or KimWipe at this stage greatly aids in the removal of excess fluid.



Slides are placed together with the sharpie marks aligned.

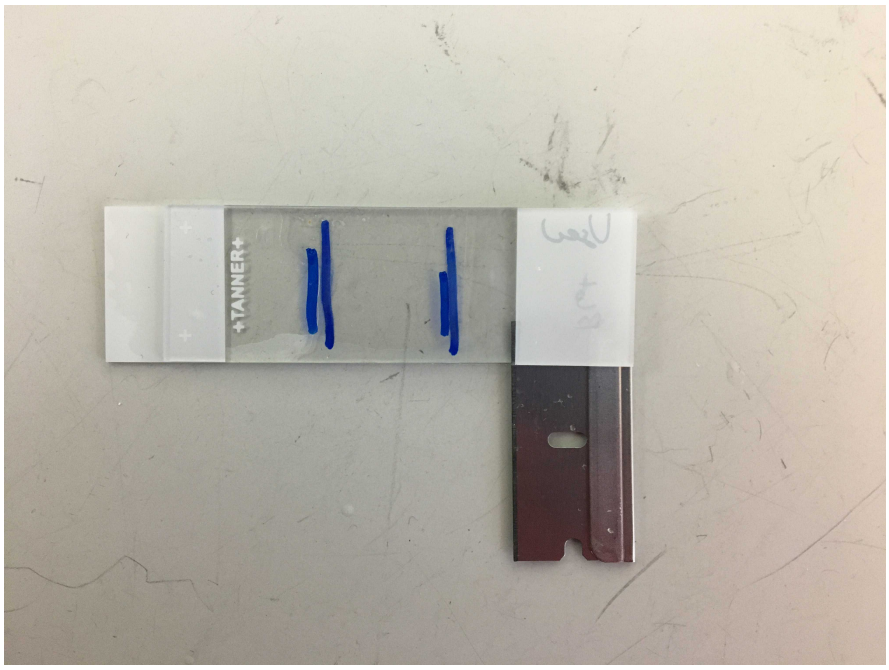
6 Video of this process --

Imaging

7 Place the stacked transfer onto the microscope for imaging. We used a Zeiss Axio Observer microscope with a 2.5x fluorescent objective. At this stage using the microscope, the boundaries of the image were marked by the microscope and tiled images were captured on two fluorescent channels (DAPI and FITC).

Cell recovery and fixation

8 After imaging insert a razor blade between the two slides in the stack and lift to separate them. Place the used sci-Space grid aside and add 100uL of 5% PFA solution to the embryo. Allow the solution to sit for 15seconds before using a cell scraper Cell scraper **Corning Catalog #3011** to scrape the solution into a 15mL conical tube. Use an additional 900uL of the 5% PFA solution to recover all the material on the slide. Mix the entire 1mL solution with a few strokes of pipetting.



Razor blade placed between the two slides to crack them apart

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The same cell scraper can be used for multiple slides provided that it is cleaned with some ethanol.

9 Sonicate the solution for 10seconds using the bioruptor sonicator and then place on ice for 15 minutes.

10 Prepare all slides in this manner using a different indexed CLB solution for each slide. We normally prepare slide 5 or 6 at a time. This means that the slides prepared first are sitting in fixation solution for the longest. We have not seen a negative impact on data quality as a result of this. Make sure to let the last slide prepared sit on ice for at least 15 minutes.

11 Using a swinging bucket rotor in a chilled centrifuge, spin cells down at 800g for 10 minutes.

12 Carefully remove all supernatant from all tubes and resuspend each pellets in 250 uL of NSB (Nuclei Buffer + SuperaseIN + BSA). Combine the pellets and centrifuge again at 800g for 6 minutes.

13 Resuspend pellet in 1mL of NSB and flash freeze. Nuclei can be stored at -80C for at least one year.