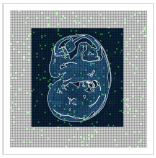


Feb 13, 2021

Creating sci-Space Grids for Spatial Barcoding

In 1 collection

Sanjay Srivatsan[1], Mary C Regier [1]

[1]University of Washington

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

Sanjay Srivatsan

SUBMIT TO PLOS ONE

ABSTRACT

Sci-Space is a technology for transferring spatially arrayed oligonucleotides onto tissue sections prior to single cell sequencing. The spatially arrayed oligonucleotides are arrayed on thin bed of dried agarose adhered to a glass slide. This protocol describes how to create an agarose backed microscope slide and print oligonucleotides onto these slides.

PROTOCOL CITATION

Sanjay Srivatsan, Mary C Regier 2021. Creating sci-Space Grids for Spatial Barcoding. **protocols.io** https://protocols.io/view/creating-sci-space-grids-for-spatial-barcoding-bm64k9gw

COLLECTIONS

Single Cell Combinatorial Indexing (SCI) - seqeuncing

CREATED

Oct 08, 2020

LAST MODIFIED

Feb 13, 2021

PROTOCOL INTEGER ID

42940

PARENT PROTOCOLS

Part of collection

Single Cell Combinatorial Indexing (SCI) - seqeuncing

Casting dried agarose slides

1 SeaPlaque

To prepare nuclease-free agarose, 3% w/v Agarose **Lonza Catalog #50101** (SeaPlaque, Lonza, Bend, OR) was added to deionized water containing 0.1% v/v Diethyl pyrocarbonate **Sigma Aldrich Catalog #D5758**, incubated 2 hr at room temperature, and autoclaved at 15 psi on liquid cycle for at least 15 min to inactivate the remaining diethyl pyrocarbonate.

2 The uniform thickness of the layer of agarose across the slide (Superfrost™ Plus and ColorFrost™ Plus Microscope Slides, Superfrost Plus, White **Thermo Fisher Catalog #4951PLUS4**) was patterned using spacers of two stacked 22 x 22 mm, number one thickness (0.15 ± 0.02 mm each) coverslips overhanging either end of the slide.

3 Molding of the agarose was performed by pipetting a 300 uL volume of heated agarose solution into the center of the slide and slowly placing a second slide onto the agarose solution avoiding the formation of bubbles. The molding slide was allowed to rest on the cover glass spacers.

4 After the agarose had gelled between the two slides (~30-60 min on ice) a razor blade was used to release the exposed edges of the agarose layer from the top, molding slide. 1h

5 The two slides were then carefully slid apart and the cover glass spacers were removed. The resulting thin layer of agarose gel was dried onto the bottom slide overnight in a biosafety cabinet. 1d

6 All agarose slides were UV-treated for 20-30 min prior to spotting to further protect against nuclease activity. 30m

Preparing Oligonucleotides

7 Hashing oligonucleotides (oligos) used as spot or sector oligos were designed with the following sequence structure: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[10bp-barcode sequence]-BAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3' (where B is C,G, or T)

7.1 The complete set of ordered hash oligos can be ordered from IDT using the following files -- **all_plates_for_ordering.xlsx**

7.2 Oligos were ordered from IDT at full yield and reconstituted in IDT Low TE pH 8.0 at a final concentration of 100uM.

8 Each well of a series of 18 full 384 well plates and 144 wells of another 384 well plate received a total volume of **20 µl** with a final concentration of **15 Micromolar (µM)** spot oligo and **2.5 Micromolar (µM)** sector oligo per well (Integrated DNA Technologies, Coralville, IA), and Glycerol **Sigma Aldrich Catalog #G789-3** **0.5 % (v/v)**, with or without SYBR Green SYBR™ Green I Nucleic Acid Gel Stain, 10,000X concentrate in DMSO **Thermo Fisher Catalog #S7585** (final concentration of 5X).

9 To achieve a predetermined spotted oligo array layout, each well of the 384-well plates received a specified combination 12h

of spot oligo, array oligo, and +/- SYBR green taking into account the pattern in which the spotting pins covered the positions of the grid array and the order in which the plates were spotted from.

Spotting oligonucleotide arrays

10 A QArray2 microarray scanner (Genetix, New Milton, Hampshire, GB) was set up with 16 spotting pins arrayed in a 4x4 layout and the slide bed was filled with cast and dried agarose slides with a subset designated for blotting the pins prior to spotting the arrayed transfer slides.

11 The spotting height was adjusted to ensure consistent contact of the spotting pins with the transfer slides' agarose coating. 15m

12 The spotting layout was set to 21x21 spots/pin for the 16 pins giving 16 adjacent sectors (4x4) comprised of 441 unique spots in each sector and a total of 7056 spots with unique combinations of spot and sector oligos. Each spot oligo occured 4 or 6 times across the array and never more than once in a sector. The layout of the source plates was optimized to maintain separation between the each spot oligo as they occured in multiple sectors to avoid ambiguous hashing. 1d

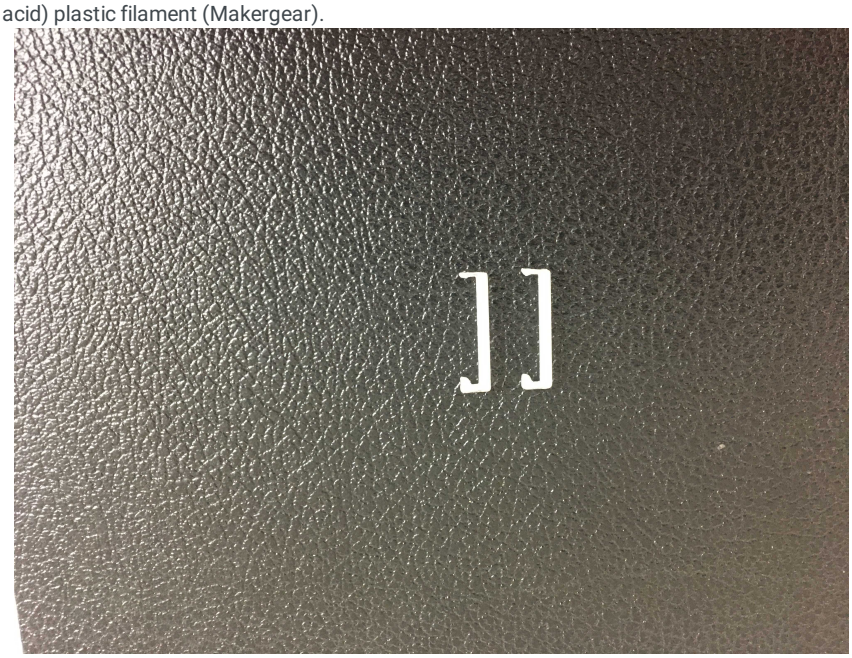
Transfer clip design and fabrication

13 Clips that securely held the tissue section slide and oligo transfer slide together during transfer were designed in SolidWorks v24 (Dassault Systèmes SolidWorks Corp., Waltham, MA). These clips featured a bottom support that spanned the width of the stacked slides and side supports capped with teeth that overhung the top surface of the stacked slides.

14 For a stack of one Superfrost Plus microscope slide (Thermofisher) with agarose coating and one Zyagen tissue section slide, internal dimensions of 22.4 mm x 2.6 mm and a tapered overhang of 0.75 mm allowed slides to snap into the clips with a snug fit preventing sliding of one slide across the other. Clip dimensions should be optimized for the specific slides and coatings used for sections and oligo transfer arrays.

slide_clip _superfrost+Tanner_slides.STL

15 The clips were 3D printed on a Makergear M2 (Makergear, Beachwood, OH) printer using consumer grade poly(lactic acid) plastic filament (Makergear).



Final result