Submission ID #: 68619
Scriptwriter Name: Pallavi Sharma
Project Page Link: https://review.jove.com/files_upload.php?src=20928843

Title: Comprehensive Spatial Profiling of Species-Agnostic Transcriptomes *via* Stereo-seq

Authors and Affiliations:

Anna K. Casasent[1,*], Danielle L. Stolley[1,*], Basant T. Gamal[2,*], Arnold Dahay[2], Samuel Mok[2], Lidia Rocha[3], Vincent Li[3], Arizona T. Nguyen[3], Boyu Zhang[4], Clay T. Brasuell[4], Erika J. Thompson[4], Thomas Huynh[3], Jared K. Burks[1], Sammy Ferri-Borgogno[2]

[1]Department of Hematopoietic Biology and Malignancies, The University of Texas MD Anderson Cancer Center
[2]Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center
[3]Department of Veterinary Medicine & Surgery, University of Texas MD Anderson Cancer Center
[4]Department of Genetics, The University of Texas MD Anderson Cancer Center

*These authors contributed equally

Corresponding Authors:
Jared K. Burks jburks@mdanderson.org
Sammy Ferri-Borgogno sferri@mdanderson.org

Email Addresses for All Authors:

Anna K. Casasent akcasasent@mdanderson.org
Danielle L. Stolley dstolley@mdanderson.org
Basant T. Gamal btgamal@mdanderson.org
Arnold Dahay amdahay@mdanderson.org
Samuel Mok scmok@mdanderson.org
Lidia Rocha lrocha1@mdanderson.org
Vincent Li vli1@mdanderson.org

Arizona T. Nguyen ATNguyen6@mdanderson.org
Boyu Zhang BZhang6@mdanderson.org
Clay T. Brasuell ctbrasuell@mdanderson.org
Erika J. Thompson ejthomps@mdanderson.org
Thomas Huynh thuynh@mdanderson.org
Jared K. Burks jburks@mdanderson.org
Sammy Ferri-Borgogno sferri@mdanderson.org

Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes , all done**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: 10/17/2025

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 34
Number of Shots: 55

Introduction

~~**REQUIRED:** What is the scope of your research? What questions are you trying to answer?~~

1.1. **Sammy Ferri-Borgogno:** Our research focuses on characterizing the ovarian tumor microenvironment in detail to identify predictive and therapeutic biomarkers. For example, finding markers for chemo response or novel molecular therapeutic targets for second-line treatments.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What are the current experimental challenges?~~

1.2. **Anna K. Casasent:** Sample handling, assessing quality and the length of the protocol remain challenging, while on the analysis side sparse data, genome mapping when examining repeated elements can complicate analysis.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What research gap are you addressing with your protocol?~~

1.3. **Sammy Ferri-Borgogno:** The adaptations of the protocol address issues we had in full transcriptomic spatial profiling of fatty or fragile tissues by improving tissue adhesion, RNA accessibility and handling for Stereo-seq on these difficult tissue types.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.3.1.*

~~What advantage does your protocol offer compared to other techniques?~~

1.4. **Anna K. Casasent:** Stereo-seq currently provides higher resolution than other spatial transcriptomics methods, with a resolution of 500 nm compared to 2 microns. It also provides unbiased capture needed for the study of microbiome, viral, and non-polyadenylated transcript.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.3.1*

Ethics Title Card

This research has been approved by the Institutional Review Board (IRB) at The University of Texas MD Anderson Cancer Center, Department of Gynecologic Oncology and Reproductive Medicine

Protocol

2. Stereo-seq Random Oligonucleotide Capture Chip (N-chip) Preparation

Demonstrators: Basant T. Gamal and Arnold Dahay

2.1. After removing the Stereo-sequencing N-chip from its packaging, record the chip identification number **[1]**. Using 100 percent ethanol, clean the edges of both the Stereo-sequencing N-chip and the glass slide without touching the chip surface **[2-TXT]**.

2.1.1. WIDE: Talent shows the chip identification number.

2.1.2. Talent cleaning the edges of the Stereo-seq N-chip and glass slide with 100 percent ethanol, taking care not to touch the chip surface. **TXT: Allow the chip to air dry completely**

2.2. Using a micropipette, carefully apply 10 microliters of ultraviolet-curable ceramic resin around the edge of the Stereo-sequencing N-chip **[1]**. Use a sealed foam swab to remove excess resin, leaving only a thin line around the chip edge **[2]**. Place the slide into the ultraviolet curing device, ensuring the chip surface is not touched **[3]**.

2.2.1. Talent applying 10 microliters of resin along the edge of the chip using a micropipette.

2.2.2. Talent using a sealed foam swab to remove excess resin, leaving a thin border around the chip.

2.2.3. Talent placing the prepared slide into the ultraviolet curing device.

2.3. Now, place the resin-applied chip on top of an opaque mask on the 405-nanometer ultraviolet light source to illuminate the back of the slide and cure for 5 minutes **[1]**.

2.3.1. Talent positioning the resin-applied chip onto an opaque mask and placing it under the 405-nanometer ultraviolet light source.

2.4. After curing, use sealed foam swabs soaked in 100% molecular grade ethanol, then 70% ethanol, and finally nuclease-free water to clean around the resin-applied edge **[1-TXT]**. Submerge the chip three times in fresh nuclease-free water using a 50-milliliter conical tube **[2].**

2.4.1. Talent cleaning the chip edge with a swab soaked in 100 percent molecular grade ethanol. **TXT: If white specks detach, re-clean edge; Avoid touching chip surface**

2.4.2. Talent dipping the chip into a 50 milliliter conical tube filled with fresh nuclease-

free water.

2.5. Using compressed air, dry the chip with the full force of air at an angle of approximately 60 to 80 degrees, moving diagonally across the surface from about 5 centimeters away **[1-TXT]**.

2.5.1. Talent drying the chip with compressed air, holding the nozzle at a diagonal angle and distance specified. **TXT: If the chip looks cloudy, rewash with fresh NFW and dry**

2.6. Next, apply 150 microliters of poly-L-lysine solution evenly across the entire chip surface and incubate the chip for 10 minutes at room temperature **[1]**.

2.6.1. Talent pipetting 150 microliters of poly-L-lysine solution onto the chip, covering the surface and keeping it aside.

2.7. Then, remove the poly-L-lysine solution from the chip **[1]**. Wash the chip twice with nuclease-free water for 10 to 15 seconds each time **[2]**.

2.7.1. Talent aspirating the poly-L-lysine solution off the chip.

2.7.2. Talent washing the chip twice with nuclease-free water.

2.8. After the second wash, carefully dry the edges of the slide without touching the chip surface **[1]**. Firmly dry the chip using compressed air as demonstrated earlier **[2]**.

2.8.1. Talent carefully drying only the edges of the slide with a lint-free tissue, avoiding contact with the chip surface.

2.8.2. Talent firmly drying the chip with compressed air.

2.9. While sectioning the FPPE *(F-P-P-E)* tissue, do not touch the chip directly, and only cover 80 percent of the chip with tissue **[1-TXT]**.

2.9.1. Close-up of tissue sections being mounted to cover only 80 percent of the chip surface without touching it. **TXT: Use new blades & NFW for baths/ice**

3. Nuclear Imaging and ssDNA Staining of the FPPE Tissue Sections

Demonstrators: Sammy Ferri-Borgogno and Danielle L. Stolley

3.1. To prepare 2 microliters of single-stranded DNA staining solution in SSC buffer, dilute the Qubit single-stranded DNA reagent with 5 times SSC buffer **[1-TXT]**.

3.1.1. Talent pipetting Qubit single-stranded DNA reagent and mixing it with 5 times

SSC buffer to prepare the staining solution. **TXT: Use 1:1 dilution/1-2chips**

3.2. Create a master mix of staining solution **[1]** using a 1 to 20 dilution of ribonuclease inhibitor, a 1 to 200 dilution of the single-stranded DNA solution, and the remaining volume with 5 times SSC buffer **[2]**.

3.2.1. Shot of all the required reagents.

3.2.2. Talent adding the reagents into a tube and mixing them.

3.3. Add 150 microliters of staining solution to each chip **[1-TXT]**. Then, gently remove the staining solution from the corner of each chip using a pipette **[2]**.

3.3.1. Talent adding 150 microliters of staining solution. **TXT: Incubate in dark at RT for 5 min**

3.3.2. Talent carefully aspirating the staining solution from the corner of the chip with a pipette.

3.4. After washing the chip twice with 0.1 times SSC buffer for 10 to 15 seconds, carefully dry the edges of the slide **[1]**.

3.4.1. Talent carefully drying the slide edges with a lint-free tissue, avoiding the chip surface.

3.5. Add approximately 3 to 5 microliters of glycerol onto the slide to mount it **[1]**. Drop a coverslip from about 1 centimeter above the slide, keeping it level and ensuring it does not touch the chip surface **[2-TXT]**.

3.5.1. Talent pipetting 3 to 5 microliters of glycerol onto the slide.

3.5.2. Talent dropping a coverslip from 1 centimeter height onto the slide. **TXT: If the coverslip slides off, remove it and remount**

3.6. Capture fluorescent images of single-stranded DNA-stained tissue using a widefield microscope, ensuring 10 percent overlap between image tiles **[1]**.

3.6.1. SCREEN: stomics_screencaptureCONFOCAL_edit_30s.mp4 00:00-00:20

3.7. Stitch the acquired images in image processing software such as ImageJ by first utilizing theoretical overlap to generate approximate tile positions, then applying pixel-matching computational overlap to fine-tune the tiling **[1]**.

3.7.1. SCREEN: SCREEN_JOVE_ImageJ_28sec.mp4 00:00-end

3.8. Process the stitched images in StereoMap *(Stereo-Map)* software and confirm that image quality control passes before continuing **[1]**.

3.8.1. SCREEN: SCREEN_Jove_Image_QC_sec27. Mp4 00:08-00:26

3.9. Submerge the Stereo-sequencing slide in 30 milliliters of 0.1 times SSC buffer until the coverslip detaches **[1]**.

3.9.1. Talent placing the Stereo-seq slide into a container with 30 milliliters of 0.1 times saline-sodium citrate buffer.

4. Decrosslinking Process of the Stained FPPE Sections

Demonstrators: Sammy Ferri-Borgogno and Anna K. Casasent

4.1. Ensure the FFPE decrosslinking reagent is at room temperature and inspect it for particles **[1]**.

4.1.1. Talent inspecting the FFPE decrosslinking reagent vial at room temperature.

4.2. Turn on the thermocycler and set it to 30 degrees Celsius for equilibration, 95 degrees Celsius for a 30-minute incubation, and infinite hold at 4 degrees Celsius **[3]**.

4.2.1. Thermocycler display showing the temperature settings.

4.3. Assemble the cassette gasket **[1]** and place the Stereo-sequencing chip slide into the cassette **[2]**.

4.3.1. Talent assembling the cassette gasket on the bench.

4.3.2. Talent inserting the Stereo-seq chip slide into the assembled cassette.

4.4. Then, add FFPE decrosslinking reagent into the well of the Stereo-sequencing slide cassette **[1]**, apply sealing tape to the cassette, and confirm it is tightly sealed **[2]**.

4.4.1. Talent pipetting decrosslinking reagent into the well of the cassette.

4.4.2. Talent applying sealing tape over the cassette and pressing firmly to ensure it is tightly sealed.

4.5. Start the 30-minute incubation at 95 degrees Celsius **[1]**. After 10 minutes, place 25 milliliters of methanol in a 50-milliliter container in a minus 20 degrees Celsius freezer, preparing about 1 milliliter per slide for later use **[2]**.

4.5.1. Talent placing the chip in thermocycler.

4.5.2. Talent placing a 50-milliliter container with 25 milliliters of methanol into a minus 20 degrees Celsius freezer.

4.6. After removing the chip from the thermocycler, transfer the slide cassette to the bench and peel off the sealing tape **[1]**.

4.6.1. Talent placing the Stereo-seq slide cassette onto the bench and peeling off the sealing tape from the cassette.

.

4.7. Using a pipette, remove and discard the FFPE decrosslinking reagent from the cassette **[1]**. Once the reagent has been completely removed, detach the cassette and gasket, and discard them **[2]**.

4.7.1. Talent aspirating and discarding the FFPE decrosslinking reagent with a pipette.

4.7.2. Talent detaching and discarding the used cassette and gasket. **TXT: Equilibrate the Stereo-seq slide to RT**

4.8. Under a sterile fume hood, dry the edge of the slide and apply a new silicone chamber if needed **[1]**. Add 500 microliters of chilled methanol from the minus 20 degrees Celsius freezer, ensuring the entire section is submerged **[2]**.

4.8.1. Talent drying the edges of the slide and attaching a new silicone chamber inside a sterile fume hood.

4.8.2. Talent pipetting 500 microliters of chilled methanol from the minus 20 degrees Celsius freezer into the chamber, fully submerging the section.

4.9. Prewarm PR solution on a 37 degrees Celsius dry block for 10 minutes before use **[1]**.

4.9.1. Talent placing a tube of PR solution on a 37 degrees Celsius dry block.

4.10. After fixation, in a fume hood, wipe off any excess methanol from the slide and wait for the remaining methanol on the chip to evaporate **[1]**. Assemble a new cassette and gasket **[2]**. Once evaporation is complete, place the Stereo-sequencing chip slide into the cassette **[3]**.

4.10.1. Talent wiping the edges of the slide.

4.10.2. Talent assembling a new cassette and gasket.

4.10.3. Talent placing the Stereo-seq chip slide into the newly assembled cassette.

4.11. Add 200 microliters of permeabilization reagent to the chip **[1]**. Apply sealing tape to the Stereo-sequencing slide cassette and ensure it is tightly sealed **[2]**.

4.11.1. Talent pipetting 200 microliters of permeabilization reagent into the cassette well.

4.11.2. Talent applying sealing tape to the cassette and pressing firmly to confirm it is tightly sealed.

5. Collection of the cDNA Release Mix

Demonstrator: Sammy Ferri-Borgogno

5.1. Preheat nuclease-free water to 37 degrees Celsius **[1]**.

5.1.1. Talent placing tubes of nuclease-free water into a 37 degrees Celsius dry block to preheat.

5.2. Remove the seal from the chip **[1]**. Pipette vigorously in each corner and in the center of the chip over the tissue without scraping or touching it **[2]**. Collect all the complementary DNA release mix from the chip and transfer it into a 1.5 or 2.0 milliliter centrifuge tube **[3-TXT]**.

5.2.1. Talent peeling the seal from the Stereo-seq chip.

5.2.2. Talent pipetting vigorously over the tissue in each corner and the center of the chip without touching the tissue.

5.2.3. Talent transferring the complementary DNA release mix into a centrifuge tube. **TXT: Record the chip identification number on the tube**

5.3. Add 350 microliters of preheated nuclease-free water directly onto the chip surface **[1]**. Pipette vigorously with a smaller pipette, angling the tip to apply shear force without touching the tissue or scraping the chip **[2]**.

5.3.1. Talent pipetting 350 microliters of preheated nuclease-free water onto the chip.

5.3.2. Talent vigorously pipetting with a P200 pipette at an angle, avoiding contact with the tissue.

5.4. Combine the wash with the 400 microliters of complementary DNA release mix collected earlier, ensuring that only material from a single chip is combined **[1]**.

5.4.1. Talent pipetting the 350 microliter wash into the tube containing the previously collected complementary DNA release mix from the same chip.

5.5. Place 100 microliters of nuclease-free water on the chip **[1]**. Seal the Stereo-seq chip slide and store it in the refrigerator until the end of the protocol **[2]**.

5.5.1. Talent pipetting 100 microliters of nuclease-free water onto the chip.

5.5.2. Talent sealing the Stereo-seq chip slide and placing it into the refrigerator.

Results

6. Results

6.1. Single-cell segmentation using the SAW pipeline was achieved on FFPE sections **[1],** enabling mapping to non-polyadenylated RNAs such as tRNA TRDMT1 *(T-R-D-M-T-One)* **[2].**

6.1.1. LAB MEDIA: Figure 4.

6.1.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the overlay showing TRDMT1-positive cells marked in color against the black and white background.*

6.2. Interactive spatial visualization showed putative cell types using the proprietary software StereoMap **[1],** with clusters displayed in various colors across a zoomed-in region of the tissue **[2].**

6.2.1. LAB MEDIA: Figure 4B.

6.2.2. LAB MEDIA: Figure 4B. *Video editor: Zoom in on the red, green, and purple colored cells in the top-left, center, and bottom-right zones of the tissue region*.

6.3. Whole-tissue expression of the non-polyadenylated RNA TRDMT1 was visualized using stereopy **[1].**

6.3.1. LAB MEDIA: Figure 4D.

Pronunciation Guide:

1. Microenvironment
   Pronunciation link: https://www.merriam-webster.com/dictionary/microenvironment
   IPA: /ˌmaɪ.kroʊ.ɪnˈvaɪ.rən.mənt/
   Phonetic Spelling: my-kroh-in-vy-ruhn-muhnt
2. Biomarker
   Pronunciation link: https://www.merriam-webster.com/dictionary/biomarker
   IPA: /ˈbaɪ.oʊˌmɑːr.kər/
   Phonetic Spelling: by-oh-mar-ker
3. Transcriptomic
   Pronunciation link: No confirmed link found
   IPA: /ˌtrænˌskrɪpˈtoʊ.mɪk/
   Phonetic Spelling: tran-skrip-toh-mik
4. Microbiome
   Pronunciation link: https://www.merriam-webster.com/dictionary/microbiome
   IPA: /ˌmaɪ.kroʊˈbaɪ.oʊm/
   Phonetic Spelling: my-kroh-by-ohm
5. Polyadenylated
   Pronunciation link: No confirmed link found
   IPA: /ˌpɑː.li.əˈdɛn.əˌleɪ.tɪd/
   Phonetic Spelling: pah-lee-uh-den-uh-lay-tid
6. Oligonucleotide
   Pronunciation link: https://www.merriam-webster.com/dictionary/oligonucleotide
   IPA: /ˌoʊ.lɪ.goʊˈnuː.kli.əˌtaɪd/
   Phonetic Spelling: oh-lih-goh-noo-klee-uh-tyd
7. Ultraviolet
   Pronunciation link: https://www.merriam-webster.com/dictionary/ultraviolet
   IPA: /ˌʌl.trəˈvaɪ.lət/
   Phonetic Spelling: uhl-truh-vy-luht
8. Nanometer
   Pronunciation link: https://www.merriam-webster.com/dictionary/nanometer
   IPA: /ˈnæn.əˌmiː.tər/
   Phonetic Spelling: nan-uh-mee-ter
9. Nuclease
   Pronunciation link: https://www.merriam-webster.com/dictionary/nuclease
   IPA: /ˈnuː.kliˌeɪs/
   Phonetic Spelling: noo-klee-ace
10. Glycerol
    Pronunciation link: https://www.merriam-webster.com/dictionary/glycerol
    IPA: /ˈglɪs.əˌrɔːl/
    Phonetic Spelling: glis-uh-rawl

11. Fluorescent
    Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescent
    IPA: /flɔːˈrɛs.ənt, flʊəˈrɛs.ənt/
    Phonetic Spelling: flor-eh-sent / floo-eh-sent
12. Decrosslinking
    Pronunciation link: No confirmed link found
    IPA: /ˌdiːˈkrɔːs.lɪŋ.kɪŋ/
    Phonetic Spelling: dee-krawss-ling-king
13. Thermocycler
    Pronunciation link: No confirmed link found
    IPA: /ˈθɜːr.moʊˌsaɪ.klər/
    Phonetic Spelling: ther-moh-sy-klur
14. Methanol
    Pronunciation link: https://www.merriam-webster.com/dictionary/methanol
    IPA: /ˈmɛθ.əˌnɔːl/
    Phonetic Spelling: meth-uh-nawl
15. Permeabilization
    Pronunciation link: No confirmed link found
    IPA: /ˌpɜːr.miː.ə.bɪ.ləˈzeɪ.ʃən/
    Phonetic Spelling: per-mee-uh-bil-uh-zay-shun
16. Complementary (biological sense)
    Pronunciation link: https://www.merriam-webster.com/dictionary/complementary
    IPA: /ˌkɑːm.pləˈmɛn.təˌri/
    Phonetic Spelling: com-pluh-men-tuh-ree
17. Segmentation
    Pronunciation link: https://www.merriam-webster.com/dictionary/segmentation
    IPA: /ˌsɛg.mɛnˈteɪ.ʃən/
    Phonetic Spelling: seg-men-tay-shun
18. Proprietary
    Pronunciation link: https://www.merriam-webster.com/dictionary/proprietary
    IPA: /prəˈpraɪ.əˌtɛr.i/
    Phonetic Spelling: pruh-pry-uh-tair-ee