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ORGANOID SAMPLE EMBEDDING AND ORGANOID LAMINATION ON Stereo-seq CHIPS

Community Developed Protocol

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Contributor Profile

Dr. Zhang Xue, recipient of "Chongqing New Phoenix Talent" and "New Phoenix Talent" awards, leads the Spatial Pathology Tool Platform at Southwest BGI Life Sciences Research Institute. With 12 SCI publications, 2 granted patents, and 10 pending applications (2024), research focuses on spatial biochemical technology innovation and clinical adaptation. Expertise in constructing spatial technology workflows drives integration of advanced omics into research and clinical applications.

The founding team of Synorg Biotechnology (Synorg), from Tsinghua University, combines exceptional medical and engineering technologies to enhance small-scale organoid sample preparation, focusing on engineering, standardization, large-scale production, and application.

Synorg's high-throughput 3D printing equipment facilitates standardized organoid modeling, successfully creating various human organoids. Their OrgFab® technology involves preparing living-cell microspheres and 3D bioprinting, using microfluidic droplets to encapsulate primary cells for accurate, rapid, high-throughput in vitro models of cancers, including pancreatic, lung, kidney, cervical, liver, thyroid, gastric, breast, skin, and rectal cancers. They have reduced modeling time for larger tumor organoids (over 500 microns) from 4-6 weeks to one week, achieving "7-days modeling & 2-days screening" for individualized medication suggestions within 9 days. These advancements are expected to boost the chemotherapy clinical benefit rate from below 40% to over 80%.

Synorg received the Gold Prize at the 2022 Geneva Exhibition of Innovation. Their organoid modeling system consistently aligns with clinical feedback, allowing precise identification of potential drugs based on clinical ranges or sequencing data. Engineered organoids from microfluidic 3D printing show greater fidelity than traditional non-engineered organoids at the molecular level.

STOmics Products

Stereo-seq Transcriptomics Solution **https://en.stomics.tech/products/stereo-seq-transcriptomics-solution/list.html**

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Protocol 1

Required Materials

Consumables	Disposable plastic base mold, CITOTEST, 7 x 7 x 5 mm
	100 µL wide-bore pipette tips
	10 µL standard pipette tips
	Plastic petri dish (bottom diameter: 35mm, lid diameter: 38mm)
Reagents	Tissue-Tek® O.C.T. Compound, 4583
	Eosin staining solution (Baso, BA-4099) or an alternative Eosin Y Solution (Alcoholic).
	1X PBS

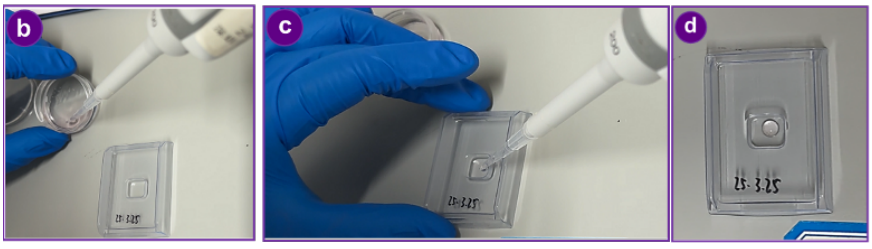
Organoid Embedding in OCT

Organoid Transfer and Washing

1. Use a 100µL wide-bore pipette tip to transfer the organoid spheres to a 35-mm disposable petri dish.
2. Add sufficient 1X PBS to fully submerge the organoid spheres.
3. Aspirate and discard the PBS.
4. Repeat the washing process once with fresh 1X PBS.

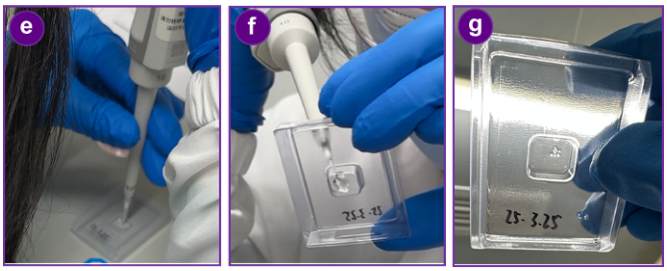
Organoid Transfer to Embedding Base Mold

1. Use a 100µL wide-bore pipette tip to slowly transfer the organoids to the center of a pre-labeled disposable embedding base mold.



Residual PBS Removal and Organoid Aggregation

1. Use a standard 10 μL pipette tip to aspirate all residual 1X PBS from the base mold.
2. Gently guide any unaggregated organoids to the center of the base mold using the pipette tip.



Organoid Staining and Rinsing

1. Add 20 μL of alcohol-based eosin staining solution (Baso BA-4099) to fully immerse the organoid spheres.
2. Stain for 30 seconds, then aspirate and discard the staining solution.
3. Wash the organoids twice with 1X PBS: Add PBS, allow organoids to settle naturally, and aspirate the supernatant.
4. After the final wash, use a 10 μL pipette tip to remove residual PBS and gently aggregate any displaced organoids to the center of the base mold.

OCT Embedding and Freezing

1. Fill the base mold with OCT compound to ensure complete immersion of the organoid spheres.
2. Place the base mold on dry ice for flash-freezing to complete the embedding process.
3. Pre-label a self-sealing bag, transfer the embedded base mold into it, and store at -80°C.

Critical Notes

- When removing supernatants, avoid aspirating organoids. After liquid addition, allow the organoids to settle naturally. To prevent structural damage, do not perform vigorous pipetting or centrifugation.
- Ensure all steps are performed under sterile conditions to maintain organoid viability.

Cryosectioning was performed according to the instructions outlined in the User Manual:

STUM-SP001 Stereo-seq Sample Preparation, Sectioning and Mounting Guide for Fresh Frozen Samples on Stereo-seq ChipSlides.

Protocol 2

Laminated Organoid Preparation for Stereo-seq

Organoids were recovered from culture by discarding the medium, washing with DPBS, and immersing in an organoid recovery solution containing RNase Inhibitor (2 U/μL) for 15 min at 4 °C to digest residual Matrigel. After removing the recovery solution and washing with DPBS, the organoids were dissociated by incubation in dissociation enzyme for 2 min at room temperature. The enzyme was diluted and quenched by adding DPBS, and the organoids were washed again with DPBS. The organoids were then fixed in cold methanol for 30 s before arraying onto a Stereo-seq sequencing chip. Using a cut pipette tip, up to 4 organoids were arrayed onto each 1 cm × 1 cm area of the chip, and excess liquid was removed by aspiration and gentle air drying.

The chip was placed onto a glass slide in the groove of a compression device. Another glass slide was positioned vertically above the chip, and a weight of 1,000 g was placed on top. The mechanical knob of the compression device was slowly turned to raise the slide-chip platform until it contacted the top slide, causing it to separate from the support. After 5 min at room temperature, the weight and top slide were carefully removed.

Reference: **https://doi.org/10.1073/pnas.2408939121**

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