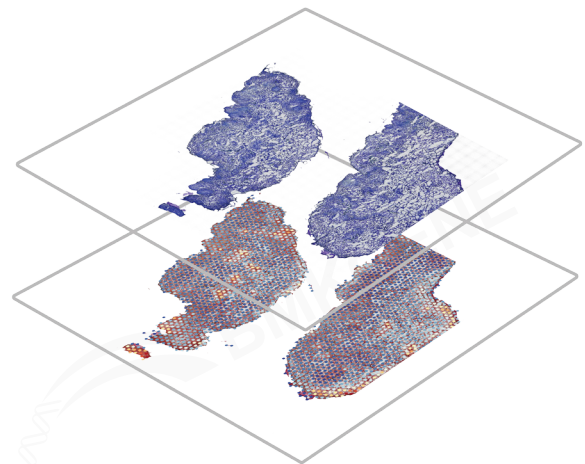


# Sample Preparation Guidance for Spatial Transcriptomics

(For Plant Samples)



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# Sample Preparation Guidance for Spatial Transcriptomics

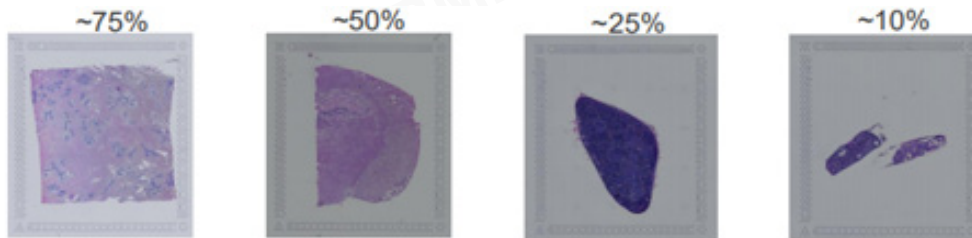
## (For Plant Samples)



### 1. Sample Requirements

For 10x Spatial Transcriptomics, tissue samples should not exceed 6.5 x 6.5 mm<sup>2</sup> in length and width, while for BMKMANU S3000 Spatial Transcriptomics, tissue samples should not exceed 6.8 x 6.8 mm<sup>2</sup> in length and width. The thickness should be greater than 1.5 mm.

For tissues with a tissue section coverage of less than 25%, we recommend embedding multiple samples into one OCT embedding block with minimal tissue spacing, but no overlapping. Multiple tissues should be kept on the same horizontal level to fully utilize the capture area.



#### Note 1 - Plant Tissue:

- If sample quantity permits, it is recommended to prepare at least 3-5 embedding blocks for each plant sample.
- Given the thin target structures in plant tissues, we recommend implementing the Express Workflow, a single embedding block undergoes RNA quality control (slice quality control standards outlined in section 3), structural confirmation, slice selection, and for applying both the permeabilization and expression chips in a single sectioning session to avoid structural loss from repeated slicing. The remaining two or more blocks are reserved as backups.
- If the sample is extremely thin, the Express Workflow is not applicable, for RNA quality control (slice quality control standards outlined in section 3), structure confirmation and slice selection, and for applying both the permeabilization and expression chips, one embedding block should be prepared for each of these steps. This will help avoid project delays in case of abnormal sample conditions or other unforeseen circumstances requiring resampling.



## 2. Sample Preparation and Transportation

### 2.1 Pre-sampling Preparation

- (1) All instruments and environments should be sterilized and disinfected, and all instruments (such as scissors, forceps, etc.) should be pre-cooled on ice.
- (2) Place a layer of ice on a medical tray, cover it with aluminum foil, and then lay several layers of sterile gauze. Place the individual on the sterile gauze for sampling, and keep the entire sampling process at low temperature to delay nucleic acid degradation.
- (3) Use the designated brand of OCT (Sakura-4583 or Leica-FSC 22) for embedding, and pre-cool the OCT on ice for 30 minutes before use.

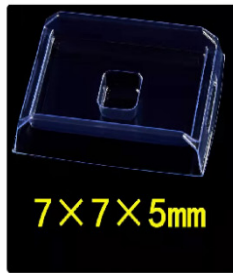
### 2.2 Sampling (Plant Sample)

- (1) For samples cultured in the medium: Take out the tissue in a sterile clean bench, remove the medium adhering to the tissue surface, and wash the tissue surface clean with ultrapure water. Remove the fresh target tissue from the living plant and place it in a culture dish. Use forceps and scissors pre-cooled at 4°C to remove as much of the tissue structure outside the target area as possible. If there are cavities inside the tissue, trim the tissue to expose the cavities as much as possible. Then cut the tissue into small pieces of  $< 6.5 \times 6.5 \text{ mm}^2$  (10x Genomics) /  $6.8 \times 6.8 \text{ mm}^2$  (BMKMANU S3000).

For samples cultivated in soil: Extract the tissue completely from the potting soil, remove the adhering soil and debris. Cleanse the tissue surface thoroughly with ultrapure water. Remove the fresh target tissue from the living plant and place it in a culture dish. Use tweezers and scissors pre-cooled at 4°C to remove as much of the tissue structure outside the target area as possible. If there are cavities inside the tissue, trim the tissue to expose the cavities as much as possible. Then cut the tissue into small pieces of  $< 6.5 \times 6.5 \text{ mm}^2$  (10x Genomics) /  $6.8 \times 6.8 \text{ mm}^2$  (BMKMANU S3000).

- (2) Embed the target tissue according to specific embedding method based on its type.

# Sample Preparation Guidance for Spatial Transcriptomics (For Plant Samples)



OCT embedding medium (Sakura-4583)

Minimum size of embedding cassette

Isopentane purity: 95% (CAS: 78-78-4) (If not obtainable, dry ice pellets can be used as a substitute.)

Note: Reagents for tissue freezing and embedding are not provided by BMKGENE. Customers need to prepare them.

## 2.3 Freezing and Embedding of Plant Tissue

Different embedding methods are recommended for different tissue types:

Tissue Type	Recommended Protocol
Young bud, flower bud, seed, stem, root (Tissue with cavities)	Protocol 1: Vacuum Infiltration & Dry Ice Freezing OCT Embedding
Lateral root, root tips, leaf, callus (Tissue without cavities or unsuitable for vacuum processing)	Protocol 2: Direct Dry Ice Freezing OCT Embedding

Note: The recommendations provided are applicable to most sample types. However, tissues from different species, growth stages, or physiological states may exhibit unique characteristics. Perform trial sectioning to evaluate the structural integrity of the slices and select the most suitable method for specific samples is recommended. For further guidance, please contact technical support.



# Sample Preparation Guidance for Spatial Transcriptomics (For Plant Samples)



## Protocol 1: Vacuum Infiltration & Dry Ice Freezing OCT Embedding (no fixation required)

- (1) Please refer to the sampling method for tissue excision. For tissues with numerous cavities (e.g., flower buds, shoot tips), partially cut open the tissue and remove as much non-target tissue as possible.
- (2) Immerse the plant tissue in 1 mL of 0.05% Tween 20 at room temperature for 15 minutes.
- (3) Pre-cool the 75% OCT embedding agent solution on ice (7.5 ml OCT + 2.5 ml sterilized water, invert and mix), add 1ml of 75% OCT to a 1.5ml centrifuge tube, infiltrate the plant tissue in it, and place a flat bottom adsorption column without the adsorption film in the centrifuge tube to prevent the tissue from floating on the surface of the OCT during vacuum infiltration. Open all tube caps, place them in a vacuum concentrator (pay attention to balancing), and perform vacuum infiltration at room temperature for 10 minutes. Adjust the vacuum concentrator to V-AQ to promote the OCT solution to fully wrap and enter the tissue voids and ensure the integrity of the OCT-filled embedding tissue. See the following figure:



OCT liquid surface  
Bottom surface of the flat adsorption column



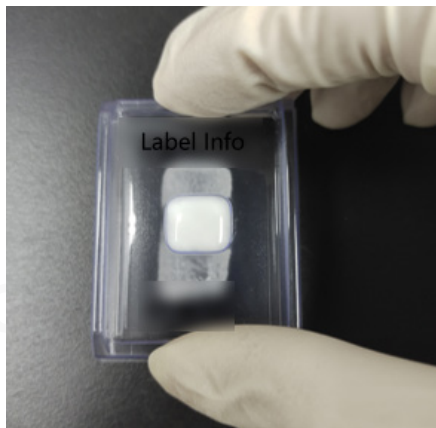
Vacuum Concentrator



## Sample Preparation Guidance for Spatial Transcriptomics (For Plant Samples)



- (4) Place the embedding cassette on ice, label it (including the direction of tissue placement and sample name, etc.), which can be recorded by taking a photo, and add an appropriate amount of OCT embedding agent to the bottom of the embedding cassette (about one-fourth depth), to prevent the tissue from settling directly on the bottom of the embedding cassette.
- (5) Gently grasp the tissue with forceps on ice, wipe off the surface OCT, and slowly place it into the pre-cooled embedding cassette containing OCT. Add pre-cooled OCT until the tissue is completely enveloped, and adjust the position of the tissue in OCT to ensure that the target plane is consistent with the cutting plane. If multiple tissues are embedded together, they must be arranged neatly in the middle of the embedding cassette to avoid overlapping. During this process, avoid generating bubbles. If bubbles are present, gently remove them with a needle or a 200  $\mu$ l pipette tip.
- (6) Gently press the embedding block containing tissue and OCT onto dry ice pellets (as shown on the left in the figure below) to ensure that it is level (otherwise, it will affect the orientation of the tissue). At the same time, it can ensure that the contact area between the dry ice pellets and the embedding block is maximized to achieve rapid cooling. Continue pressing until the embedding block is completely frozen and turns white (as shown on the right in the figure below).



Dry ice pellet quick freezing of embedding block



Embedding block after OCT freezing.

- (7) Wrap the OCT-embedded tissue blocks and embedding cassettes in aluminum foil, and store them directly in a sealed container at  $-80^{\circ}\text{C}$ . Properly label the samples and transport them using dry ice.



# Sample Preparation Guidance for Spatial Transcriptomics (For Plant Samples)



## Protocol 2: Direct Dry Ice Freezing OCT Embedding (without vacuum infiltration)

- (1) Please refer to the sampling method for tissue excision.
- (2) Immerse the plant tissue in 1 mL of 0.05% Tween 20 at room temperature for 15 minutes.
- (3) Place the embedding cassette (frozen section OCT embedding cassette, 7x7x5 mm, actual size is 10x10x5 mm) on ice, label it (including the direction of tissue placement and sample name, etc.)(can be recorded by taking a photo), and add an appropriate amount of pre-cooled OCT embedding agent to the bottom of the embedding cassette (about one-fourth depth), to prevent the tissue from settling directly on the bottom of the embedding cassette.
- (4) Gently grasp the tissue with forceps on ice, and slowly place it into the pre-cooled embedding cassette containing OCT. Add pre-cooled OCT until the tissue is completely enveloped.
- (5) Adjust the position of the tissue in OCT to ensure that the target plane is consistent with the cutting plane, and place the tissue in the middle of embedding cassette. If multiple tissues are embedded together, they must be arranged neatly in the middle of the embedding cassette to avoid overlapping. During this process, avoid generating bubbles. If bubbles are present, gently remove them with a needle or a 200 µl pipette tip.
- (6) Gently press the embedding block containing tissue and OCT onto dry ice pellets (as shown on the left in the figure below) to ensure that it is level (otherwise, it will affect the orientation of the tissue). At the same time, it can ensure that the contact area between the dry ice pellets and the embedding block is maximized to achieve rapid cooling. Continue pressing until the embedding block is completely frozen and turns white (as shown on the right in the figure below).



Dry ice pellet quick freezing of embedding block



Embedding block after OCT freezing.



# Sample Preparation Guidance for Spatial Transcriptomics

## (For Plant Samples)

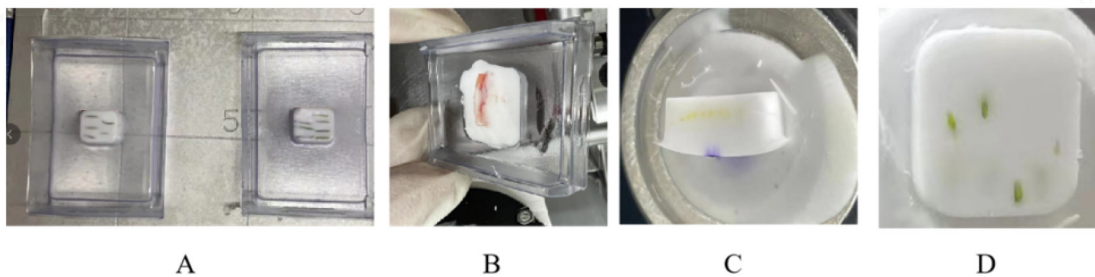


- (7) Wrap the OCT-embedded tissue blocks or embedding cassettes in tin foil, and store them directly in a sealed container at  $-80^{\circ}\text{C}$ . Properly label the samples and transport them using dry ice.

### 2.4 Sample Transportation

After embedding, wrap the tissue in aluminum foil, seal it in a sealed bag, and place it in a sample box for long-term storage at liquid nitrogen or  $-80^{\circ}\text{C}$  freezer. Alternatively, place it on dry ice for frozen transportation. For dry ice shipment, it is recommended to use approximately 5 kg per day.

#### Examples of Non-standard Sample Submission:



- A. The tissue is placed near the bottom during embedding, and the tissue is not wrapped in OCT, resulting in RNA degradation and failed quality inspection.
- B. The tissue is exposed to air, resulting in sample degradation. The tissue is not supported by OCT on both sides, which may damage the sample structure during sectioning.
- C. The tissue is too small to meet the minimum requirement of 25% coverage for expression analysis.
- D. Multiple samples are embedded in the same block, but not in the same plane, and each sample tissue is too small and has limited coverage area, which may result in lower effective data.





### 3. Section Quality Control Standards

**Tissue morphology inspection:** Perform Toluidine Blue staining on the sections to observe whether the target area is obtained and whether the integrity of tissue morphology is within an acceptable range (e.g. whether there are large cracks, obvious bubbles, wrinkles, etc.).

**RNA integrity inspection:** Take 10-15 sections and use Agilent 2100 (or equivalent) to inspect RNA integrity. It is required that RNA RIN  $\geq 6$ , which indicates that the sample integrity meets the experimental requirements and can proceed to subsequent experiments. The specific section quality inspection quantity is as follows:

- (1) 10  $\mu\text{m}$  section thickness, tissue area accounts for 1/5-1/4 of the embedding block surface area, and the quality inspection quantity is  $\sim 20$  sections (left figure).
- (2) 10  $\mu\text{m}$  section thickness, tissue area accounts for  $>1/3$  of the embedding block surface area, and the quality inspection quantity is  $\sim 15$  sections (right figure).

