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# Best Practice Guidelines for Spatial Transcriptome Sequencing

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Spatial Transcriptome Sequencing combines gene expression with its tissue morphology. By overlaying transcription profiles with microscopic tissue images from FFPE tissue sections, biological architecture can be disentangled, tissue heterogeneity unraveled, and different cell populations can be distinguished based on expression profiles. The most important factor to ensure good quality results for Spatial Transcriptome Sequencing is the sample material. In the following guideline, we outline the best practices for preparing FFPE samples for Spatial Transcriptome Sequencing.

## Best Practice: Tissue Preparation & Fixation

The following aspects need to be considered when handling tissue and preparing FFPE samples for spatial gene expression:

The tissue needs to be handled gently prior to formalin fixation and paraffin embedding to avoid mechanical stress. Mechanical stress can, for example, be introduced by ischemia, coagulative necrosis caused by electrocautery, or hemorrhages from surgical trauma.

To preserve tissue quality, the tissue should be processed immediately after resection. Delayed tissue fixation may promote autolysis, leading to tissue degradation and, consequently, negatively impacting the results. If fixation is not possible immediately after resection, tissues can be kept in an isotonic solution; however, this should be avoided for more than four hours. Thus, an isotonic solution is not suitable for long-term storage.

The tissue should be stored in a moist and cool environment to prevent it from drying and degrading.

To maximize the RNA quality in the sample, a few fixation aspects should be kept in mind:

Depending on the organism, tissue type, and potential disease status, the fixation time of the tissue might differ. While under-fixation may lead to continued activity of some enzymes, potentially resulting in degradation of nucleic acids, proteins, and lipids, over-fixation can cause the loss of the structural integrity of certain molecular features and exacerbate sectioning of the tissue. Both under- and over-fixation can lead to tissue artifacts.

The tissue thickness significantly affects the fixation process. Most standard procedures ensure the proper fixation of tissues of 5 – 10 mm thickness. If these measures are not met, the freshly collected tissue should be reduced with a scalpel.

For optimal results, the fixation should be performed at 4°C. As indicated above, the fixation time at this temperature might differ depending on the tissue type.

To prevent degradation and reduction of RNA quality, the following embedding aspects should be considered:

The tissue needs to be dehydrated after the fixation to remove the remaining water in the tissue to avoid poor preservation of the tissue structure and damage during sectioning. An ethanol series is used for the dehydration. After the dehydration, the remaining ethanol needs to be cleared using a solvent to facilitate the following wax infiltration.

After the dehydration and clearing, wax is added to infiltrate the tissue. Poor wax infiltration can lead to gaps or holes in the final block, causing difficulties during sectioning. High-quality wax and regular checks of wax temperatures are important.

In the final step, the tissue is embedded in paraffin. Note the orientation of the tissue during the embedding step to ensure proper spatiality. To maintain the integrity of the FFPE block, avoid exposure to high temperatures or humidity, and store it appropriately.

## Best Practice: Quality Assessment

After the fixation and embedding process, RNA quality can be assessed by isolating RNA from an additional FFPE tissue section and calculating its DV200 value. For our Spatial Transcriptome Sequencing products, the DV200 value should be > 30%. Low DV200 values do not necessarily result in poor data, but high values are more likely to correlate with higher sensitivity. Furthermore, the total yield of the isolated RNA might give an indication of the future performance of the FPPE material for Spatial Transcriptome Sequencing.

Additionally, if you have extra sections available, you can optionally assess the tissue morphology based on DAPI staining or H&E staining. DAPI staining should result in punctate nuclei that are clearly defined and don't look washed out. With H&E staining, processing artifacts that have a negative impact on the assays, like necrosis, large cracks, and hemorrhaging, can be detected.

## Best Practices: Preparation and Shipment of FFPE Slides

If the RNA quality is sufficient (DV200 > 30%) and no processing artifacts are present, you can ship the samples to CeGaT. If you choose to provide slides, please refer to our Slide-Guide for Spatial Transcriptome Sequencing, which provides additional information on approved slides and allowable placement areas for the tissue. Additionally, please consider the following aspects:

- Always prepare fresh FFPE slides. If the slides have already been prepared, they should not be older than 6 months.
- Dry the slides at 42°C for 3 hours and store them in a desiccator at room temperature for at least 12 hours before shipping.
- After drying, the slides can be shipped in a slide mailer with desiccant pouches. Please ensure that the slide mailer generates a tight seal.
- If drastic temperature changes are anticipated during transportation, please include ice packs.

Thus, properly preparing your sample can significantly influence the result of your Spatial Transcriptome Sequencing project. Figure 1 summarizes the key aspects you need to consider for good quality results. For further information on best practice for preparing FFPE samples for Spatial Transcriptome Sequencing projects, please also have a look at the dedicated article provided by 10x Genomics.

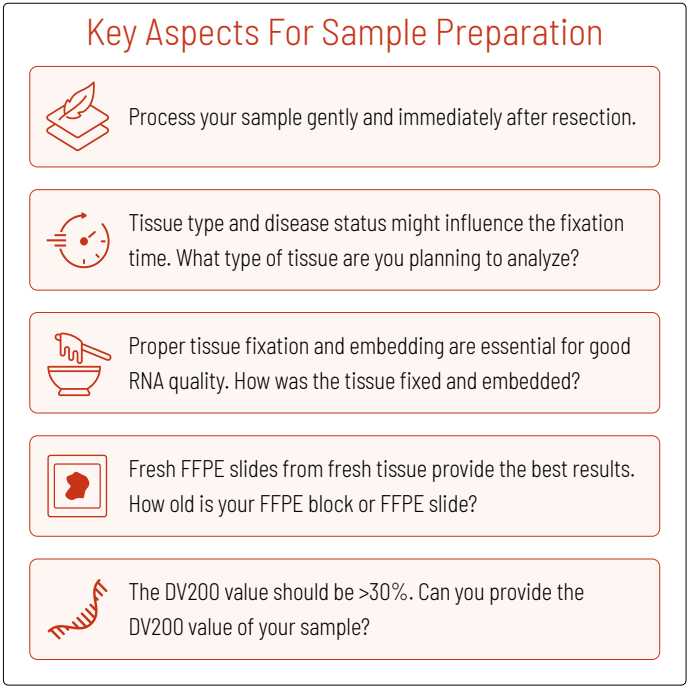


**Figure 1 | Key aspects for sample preparation.**

# About Us

CeGaT was founded in 2009 in Tübingen, Germany. Our scientists are specialized in next-generation sequencing (NGS) for genetic diagnostics, and we also provide a variety of sequencing services for research purposes and pharma solutions. Our sequencing service portfolio is complemented by analyses suited for microbiome, immunology, and translational oncology studies.

Our dedicated project management team of scientists and bio-informaticians works closely with you to develop the best strategy to realize your project. Depending on its scope, we select the most suitable library preparation and conditions on our sequencing platforms.

**We would be pleased to provide you with our excellent service. Contact us today to start planning your next project.**

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2026/02