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# Xenium Prime (5K) data generation

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xiangwei[1]

[1]Washington University in St. Louis

Washington University i...

Xiangwei Fang
Washington University in St. Louis, School of Medicine





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**We use this protocol and it's working**

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## Abstract

Xenium Prime allows for in situ transcript detection of selected probe set of RNA targets (Up to 5000 + 100) revealing spatially resolved expression patterns at single-cell resolution.

## Tissue processing and QC

1 Determine suitable samples of interest by performing a test described in CG000578_Demonstrated_Protocol_XeniumInSituProtocolsFFPE_TissuePreparationGuide , sectioning guidelines described in Step 1 pgs. 18-25 and H&E staining in Appendix pgs. 38-45.

2 Evaluate section and staining quality, only choose samples or regions free of detachment or artifacts.

## Xenium slide preparation

3 Formalin-fixed paraffin-embedded (FFPE) blocks were sectioned at 5 µm and mounted onto Xenium slides following a modified version of the FFPE Tissue Preparation Guide (10x Genomics, CG000578, Rev D).

4 Slides were deparaffinized with sequential xylene and ethanol washes, followed by decrosslinking using the FFPE Tissue Enhancer and diluted Perm Enzyme B (10x Genomics, CG000580, Rev E).

5 Xenium Prime pre-designed and add-on custom priming oligos were hybridized to target RNAs in situ. After RNase treatment to release the RNA strand, a polishing step was performed, followed by overnight probe hybridization utilizing Xenium Prime pre-designed Panel (10x Genomics) of probes for 5001 genes and an additional custom panel of probes for extra 100 genes (10x Genomics, CG000760, Rev C).

6 Probes were subsequently ligated and underwent rolling circle amplification, generating multiple copies of the gene-specific barcode for each RNA target. Cell segmentation reagents were applied during a staining workflow to label nuclei, membranes, and cytoplasmic regions for automated morphology-based segmentation. The background was quenched using an auto-fluorescence mixture, and nuclei were counterstained with DAPI to aid in sample tracking and cell boundary segmentation (10x Genomics, CG000760, Rev C).

## Xenium analyzer

7 Xenium slides were then loaded onto the 10X Xenium Analyzer instrument (10x Genomics, 1000481) for imaging and initial data processing following manufacturer's instructions (10x Genomics, CG000584, Rev F). Fluorescently labeled oligos bind to the amplified DNA probes, and iterative rounds of probe hybridization, imaging, and stripping generated optical signatures for each barcode, which were decoded to gene identities.

## Post-Xenium H&E

8 The H&E staining of the post-Xenium slides was performed
after the run following the guidance provided (10X Genomics, CG000613, Rev B).