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Stereo-seq for FF-OCT and FFPE

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We use this protocol and it's working

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Abstract

Stereo-seq protocol for both FF-OCT and FFPE samples

FF-OCT

1 FF-OCT samples were sectioned into 10 µm sections using a cryostat (Leica, CM1950) and mounted onto 1 cm x 1 cm Stereo-seq T chips (Complete Genomics, 211ST114).

2 Mounted chips were processed through the Version 1.2 fresh-frozen Stereo-Seq workflow using kit components (Complete Genomics, 111KT114) and according to the manufacturer's protocol.

2.1 Briefly, chips were dried at 37°C for 4 minutes, followed by methanol fixation for 30 minutes at -20°C, Qubit ssDNA staining (Invitrogen, Q10212), and FITC imaging (Zeiss, Axioscan Z1).

2.2 Tissues were then permeabilized for 24 minutes, followed by reverse transcription for 3 hours at 42°C, tissue removal, and cDNA collection.

2.3 cDNA were then purified (Vazyme, N411-01) and PCR amplified using kit components.

3 Library preparation was performed using a commercial kit (Complete Genomics, 111KL114) according to manufacturer's directions.

4 DNA Nanoballs (DNBs) were prepared using the OneStep DNB kit (MGI, 1000020563) and sequenced on a DNBSeq-T7 Sequencer (Complete Genomics, 900-000698-00) using the PE100 sequencing set (Complete Genomics 940-001889-00, with custom primer set 940-000037-00).

4.1 Sequencing was done using 50 cycles for Read 1 (dark cycles 26-40), 100 cycles for Read 2, and 10 cycles of index read.

5 Primary analysis was performed using STOmics' Standard Analysis Workflow pipeline (SAW, Version 7), aligning to the human reference genome Hg38.

FFPE

6 FFPE samples were sectioned into 5 µm sections using a microtome (Dakewe, MT1) and mounted onto Stereo-seq N chips (STOmics, 210CN114-EA).

7 The chips were processed through the Stereo-Seq FFPE early-access workflow using the kit components (STOmics, 211KN114-EA) according to the manufacturer's protocol.

7.1 Briefly, mounted chips were dried at 42°C for 3 hours, 37°C for 16 hours, and 60°C for 1 hour.

7.2 Tissue was cleared in Sub-X (Leica, 3803670) twice for 20 minutes each, followed by rehydration with serial incubations in 100% ethanol, 96% ethanol, 90% ethanol, 80% ethanol, 70% ethanol, and nuclease-free water.

7.3 Decrosslinking was performed for 30 minutes at 85°C, followed by methanol fixation for 20 minutes at -20°C.

7.4 Staining, imaging, and permeabilization (30 minutes) were performed in the same way as for FF-OCT samples.

7.5 Following permeabilization, chips were processed through dimerization with kit reagents for 1 hour at room temperature.

8 Subsequent reverse transcription, tissue removal, cDNA collection, cDNA amplification, library preparation, and DNB preparation were conducted according to the manufacturer's protocol.

9 Sequencing was performed on a DNBSeq-T7 Sequencer using custom MDA primer 5′ CTGCTGACGTACTGAGAGGC 3′ and read 2 primer 5′ GAGACGTTCTCGACTCAGCAGAGGG 3′.

9.1 The sequencing protocol included 91 cycles of Read 1 (dark cycles 26-85), 100 cycles of Read 2 (dark cycles 1-25), and 10 cycles of index read.

10 Primary analysis was performed using STOmics' Standard Analysis Workflow pipeline (SAW FFPE EA, with cell segmentation added from SAW V7 modified by expansion to 10 pixels).