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Molecular Cartography for FF-OCT (Resolve Biosciences)

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We use this protocol and it's working

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Abstract

Molecular Cartography protocol for FF-OCT (Resolve Biosciences), used for intestine tissue.

1 FF-OCT samples were sectioned into 10 µm sections with a Cryostat (Leica, CM1850UV), placed within the imageable area on the Resolve observation slides, and stored at -80˚C until use.

2 Upon retrieval from the freezer, the slide was heated at 37˚C for 5 minutes. Sticky wells were assembled using an aligner and adaptor.

3 The sections were fixed with MF1 at 4˚C for 30 minutes, followed by washes with PBS and ethanol.

4 They were subsequently rehydrated in a sequential series of isopropanol, 95% ethanol, and 70% ethanol.

5 DST1 was applied to stain the sections at room temperature for 5 minutes, followed by washes with 50% ethanol and Wash Buffer 1.

6 To prepare for probe hybridization, the sections were incubated in Priming Solution at 37˚C for 30 minutes.

7 A custom probe mix of 100 genes was then added to each section and incubated at 37˚C on a thermal cycler for 16–24 hours.

8 Excess probe mix was removed by three washes with Wash Buffer 2, and the sections were stored in Wash Buffer 1.

9 For imaging, the slides were loaded onto the Molecular Cartography Workflow, following the manual (Workflow Setup v2.2, Resolve Biosciences).

10 The sample preparator unit, tray handler, imager, and PC were set up according to the manual.

11 The imager captured a brightfield overview scan, where regions of interest (ROIs) were selected for each section. ROIs were scanned to detect signals from target transcripts.