Using Fresh Frozen Samples for RNA Analysis

Disclaimers

- Human fresh frozen tissues can carry pathogens and should be handled according to your institution's lab safety guidelines and IACUC.
- It is essential to avoid RNase contamination when processing fresh frozen tissues. Ensure all buffers are made with DEPC-treated water and all equipment is RNase-free.
- Please see the sample preparation guides for selecting and preparing fresh frozen blocks. NanoString has not optimized the assay for fresh frozen tissues and recommends empirical optimization be performed for your samples.

Protocol

- Unstained tissue sections should be 5 µm thick on SuperFrost Plus™ slides. Slides can be stored at -80°C after sectioning. Tissue sections should be placed in the center of the slide and be no larger than 36.2 mm long by 14.6 mm wide. If sections are larger than this size and/or placed off-center, it is likely that the tissue located outside the gray area will not be measured by the GeoMx DSP instrument.
- **Thaw frozen slides** in 10% NBF and allow to fix overnight (12–16 hours). After fixation, wash the slides 3 times in 1X PBS. Thorough fixation of tissues is required to maintain tissue integrity throughout the DSP process.
- Perform **target retrieval** (step 4 of the FFPE RNA protocol) for 15 minutes at 100°C. Timing and temperature may need to be empirically determined for different tissue types and samples.
- Perform **proteinase K digestion** (step 5 of the FFPE RNA protocol) for 15 minutes at room temperature (RT). Timing, temperature, and proteinase K concentration may need to be empirically determined for different tissue types and samples.
- Proceed to **post-fixation** (step 6 of the FFPE RNA protocol) from this point forward.