### Introduction

- The overwhelming majority of archival samples of cancer tissue are formalin-fixed, paraffin-embedded (FFPE).
- Many FFPE samples, especially pre-treatment biopsy specimens, are too small to reliably extract sufficient RNA for gene expression analysis.
- HTG Molecular recommends 12.5 mm² of tissue from one 5 µm FFPE section with no RNA extraction necessary for gene expression data from their EdgeSeq nuclease-protection assay.
- EdgeSeq Oncology Biomarker Panel covers 2,560 genes.
- This pilot study examined different amounts of tissue input from 3 colorectal and 2 breast tumors down to 3.1 mm², representing ⅛ the recommended input.

### EdgeSeq Technology

- FFPE samples were scraped into tubes and lysed with proteinase K, followed by a 90°C incubation.
- Lysates were transferred to the Edge instrument, where the nuclease-protection assay was performed overnight.

### Tissue Treatment

- Small biopsies were approximated by macrodissecting small amounts of tissue from tumor samples ranging in age from 5-12 years old.

### Intra-sample Reproducibility

- How Gold Are The Standards?
  
  Typically, 50-70% correlation is not great, but that’s how well data from the two NanoString codesets and TaqMan correlate with each other. This level of inter-platform concordance is common.

### Conclusions

- The HTG EdgeSeq Oncology Biomarker Panel provides a solid platform to obtain gene expression data from FFPE samples far too small for reliable RNA extraction.
- Reproducibility was robust, with technical replicates correlating >0.98, and small areas of the tumor correlating very well with each other, and reasonably well with NanoString and TaqMan data from extracted RNA.
- The lower limit for tissue input for this assay was not reached in this experiment, but most FFPE biopsy samples of interest will not be smaller than 3.125 mm².

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**FFPE Tissue and the HTG Oncology Biomarker Panel: a Low-Input, Extraction-Free Gene Expression Pilot Study**

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