

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

Corey Lipchik, NSABP; Patrick Gavin, NSABP; Seong-Rim Kim MD, NSABP; Katherine Pogue-Geile PhD, NSABP

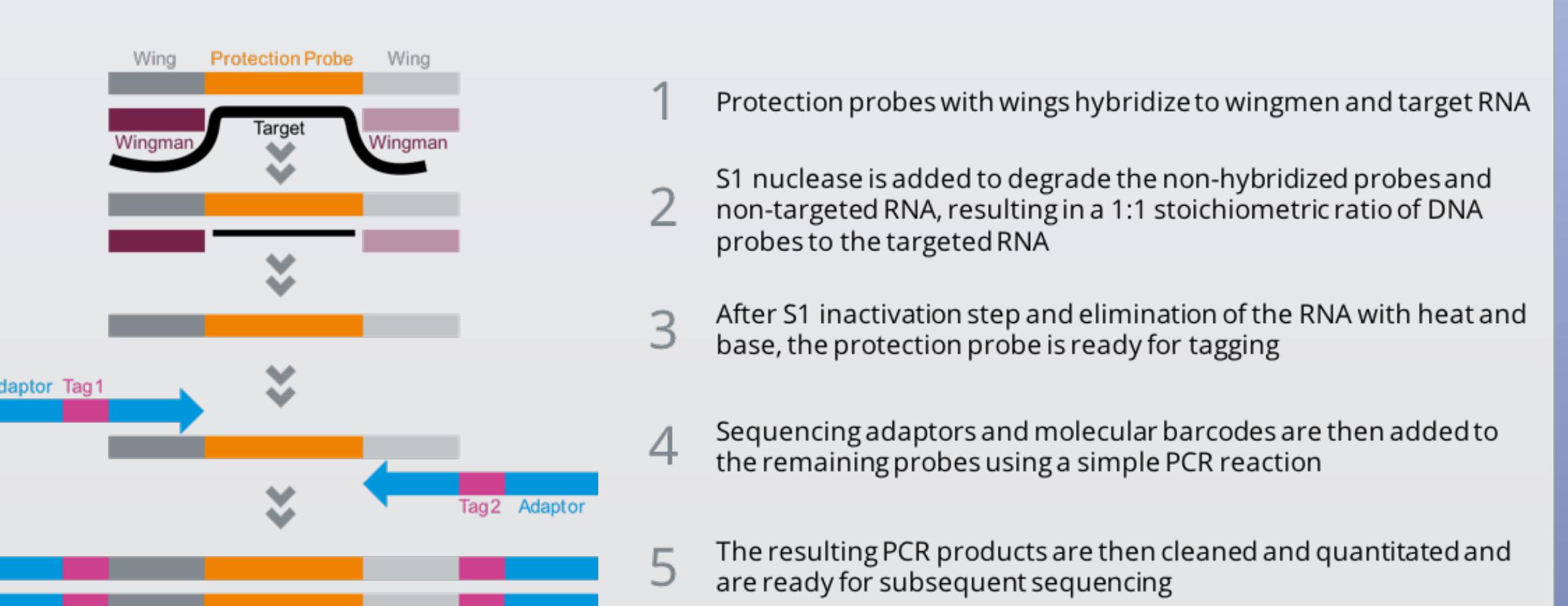


Introduction

- 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 ¼ the recommended input

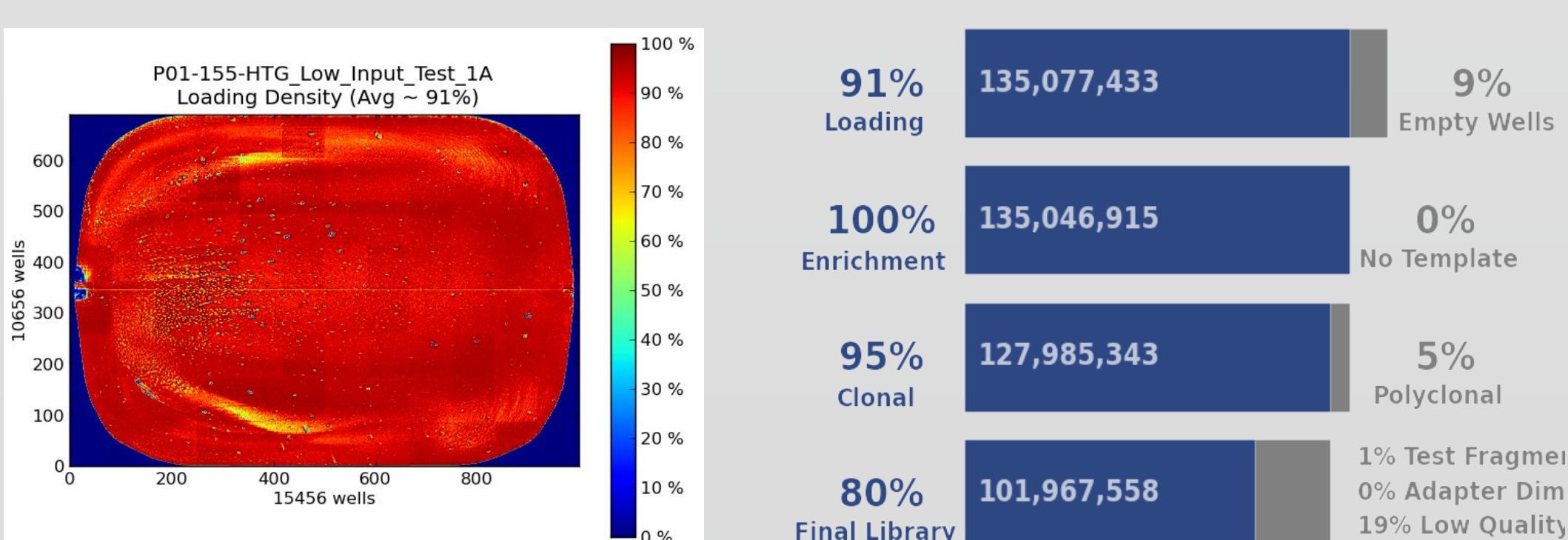
EdgeSeq Technology

- 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:



HTG EdgeSeq assays do not require processing steps such as reverse transcription, adenylation or ligation.

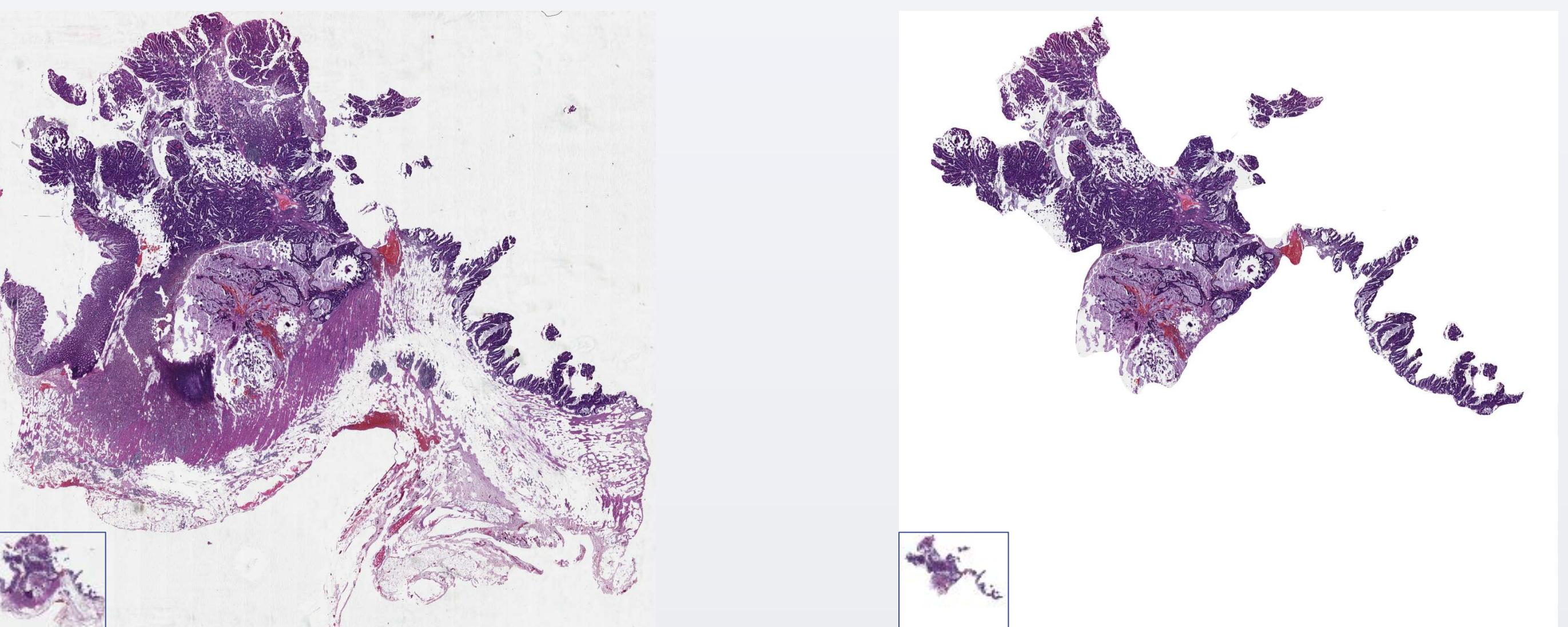
- Following PCR amplification and cleanup, sequencing libraries were templated on the Ion One Touch 2 / Ion Chef and sequenced on the Ion Proton
- Ion Torrent semiconductor sequencing technology meshes extremely well with EdgeSeq, as data loss to polyclonality can be nearly completely eliminated:



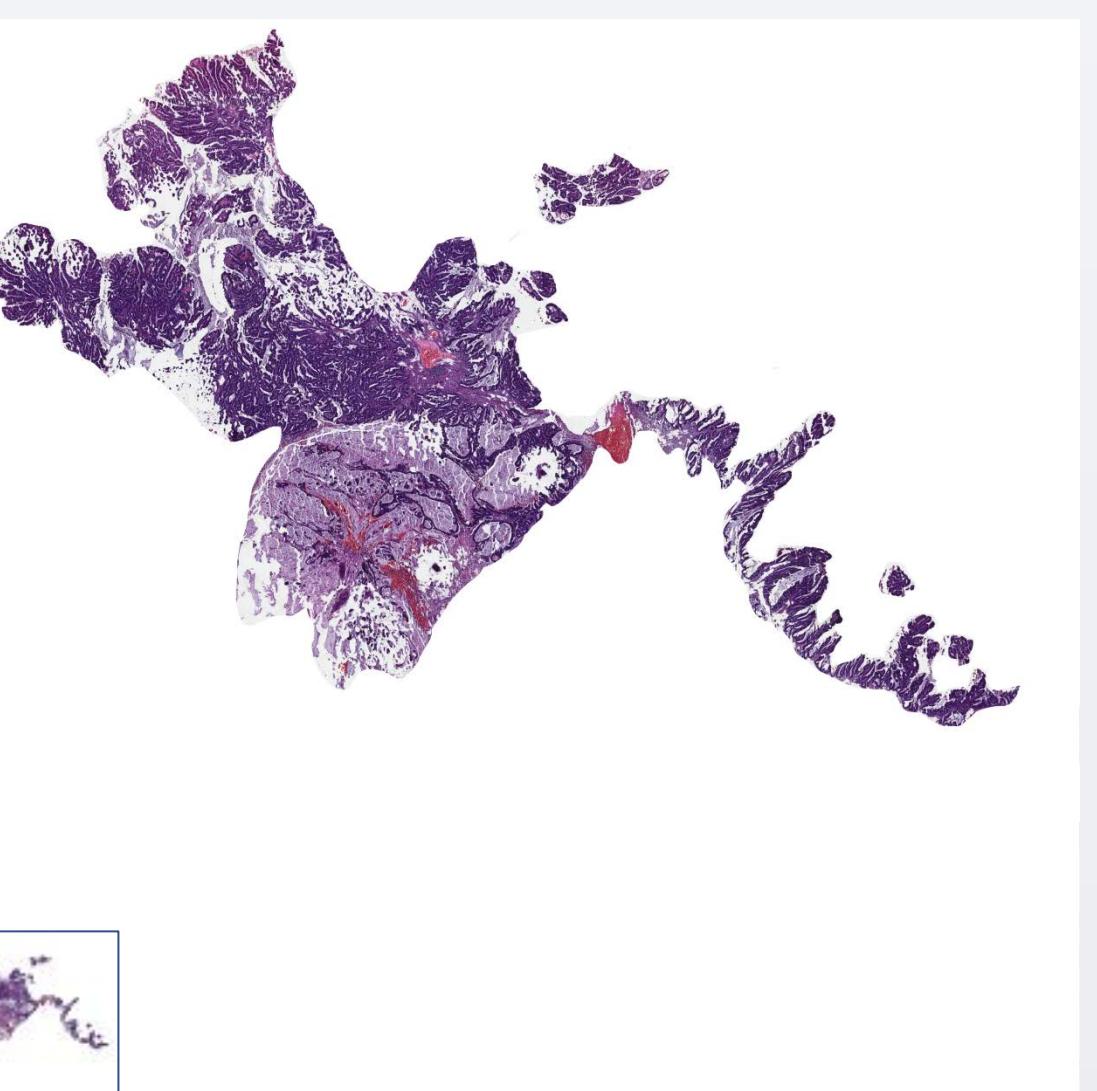
Tissue Treatment

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

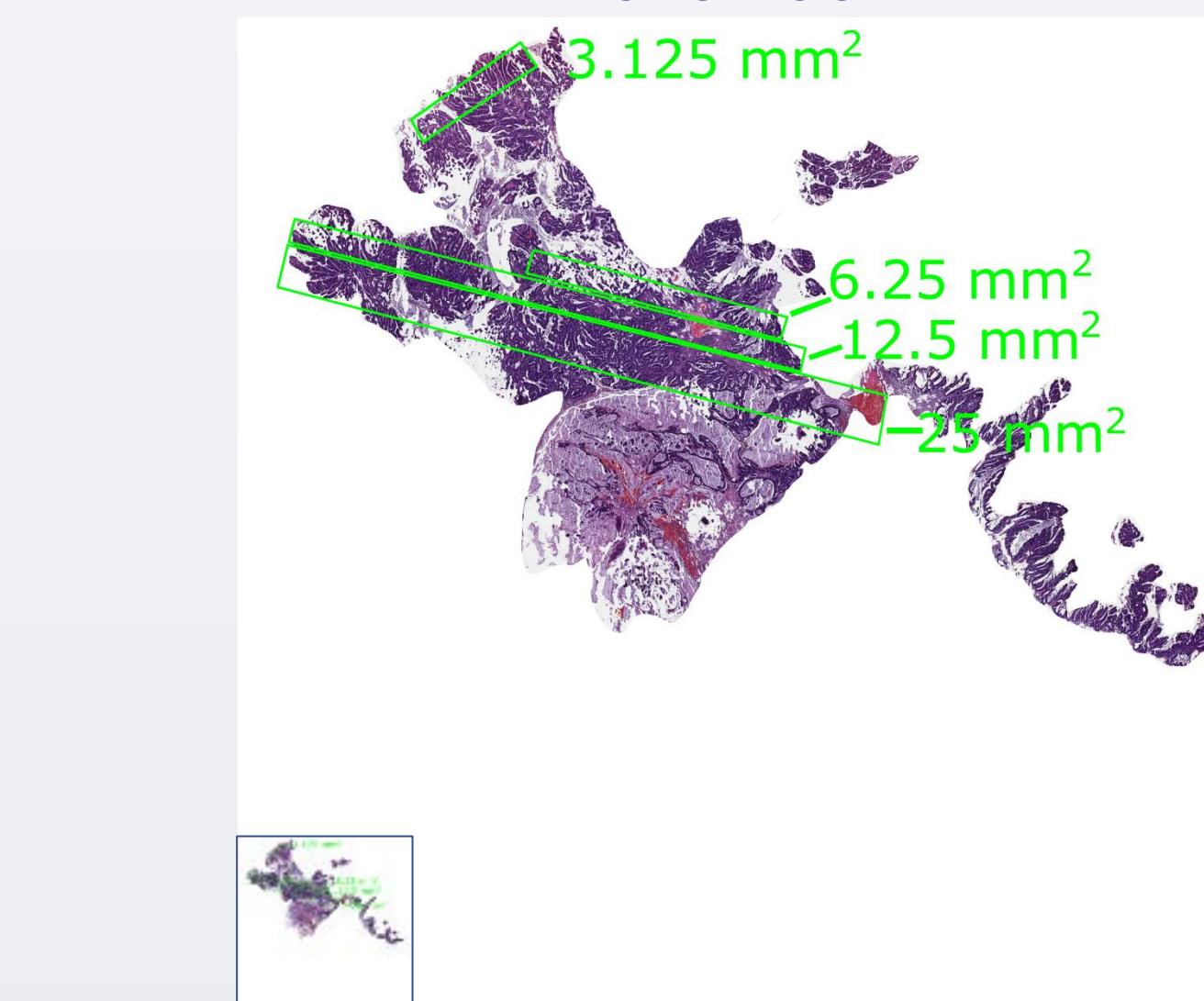
CRC-4 Full Section



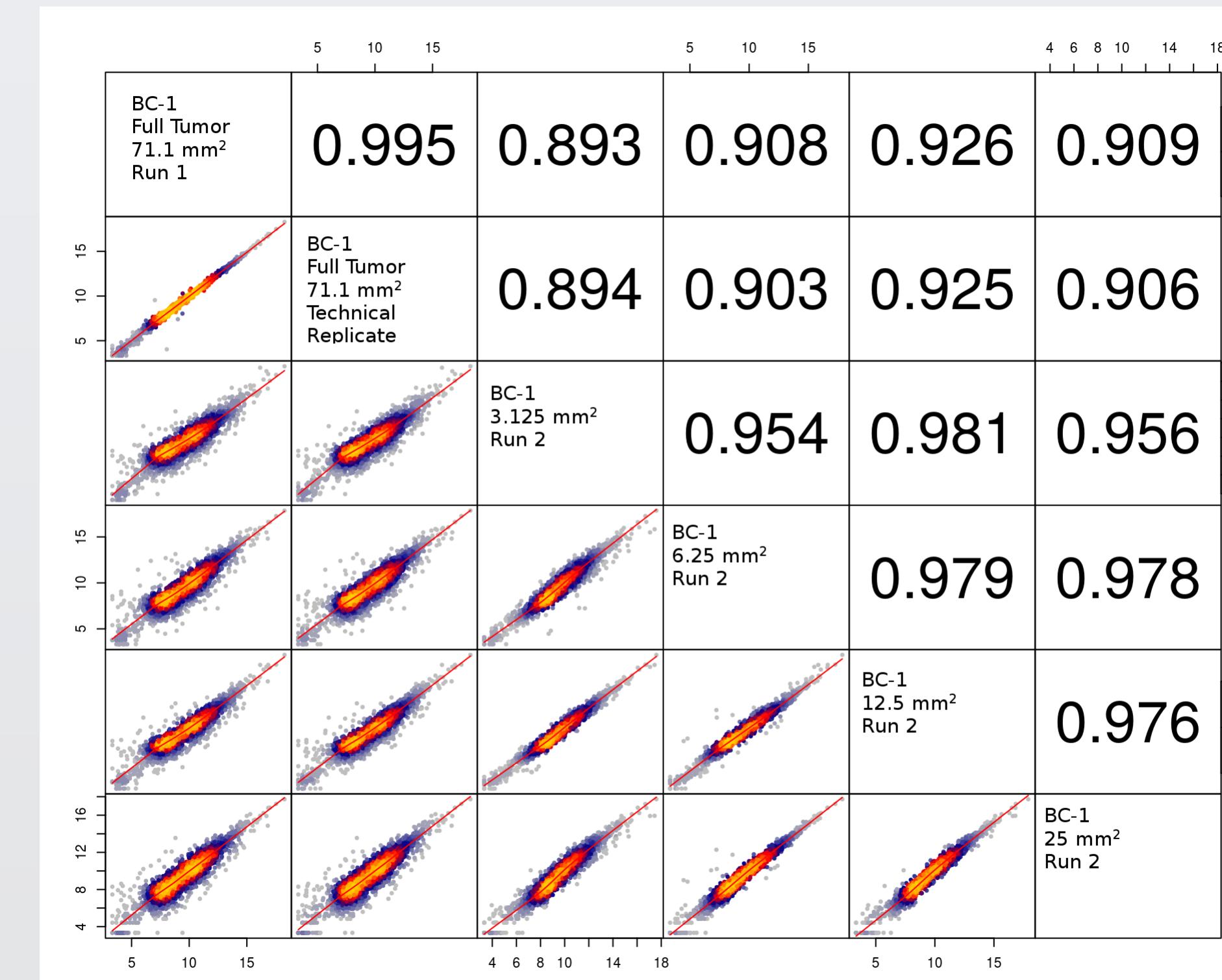
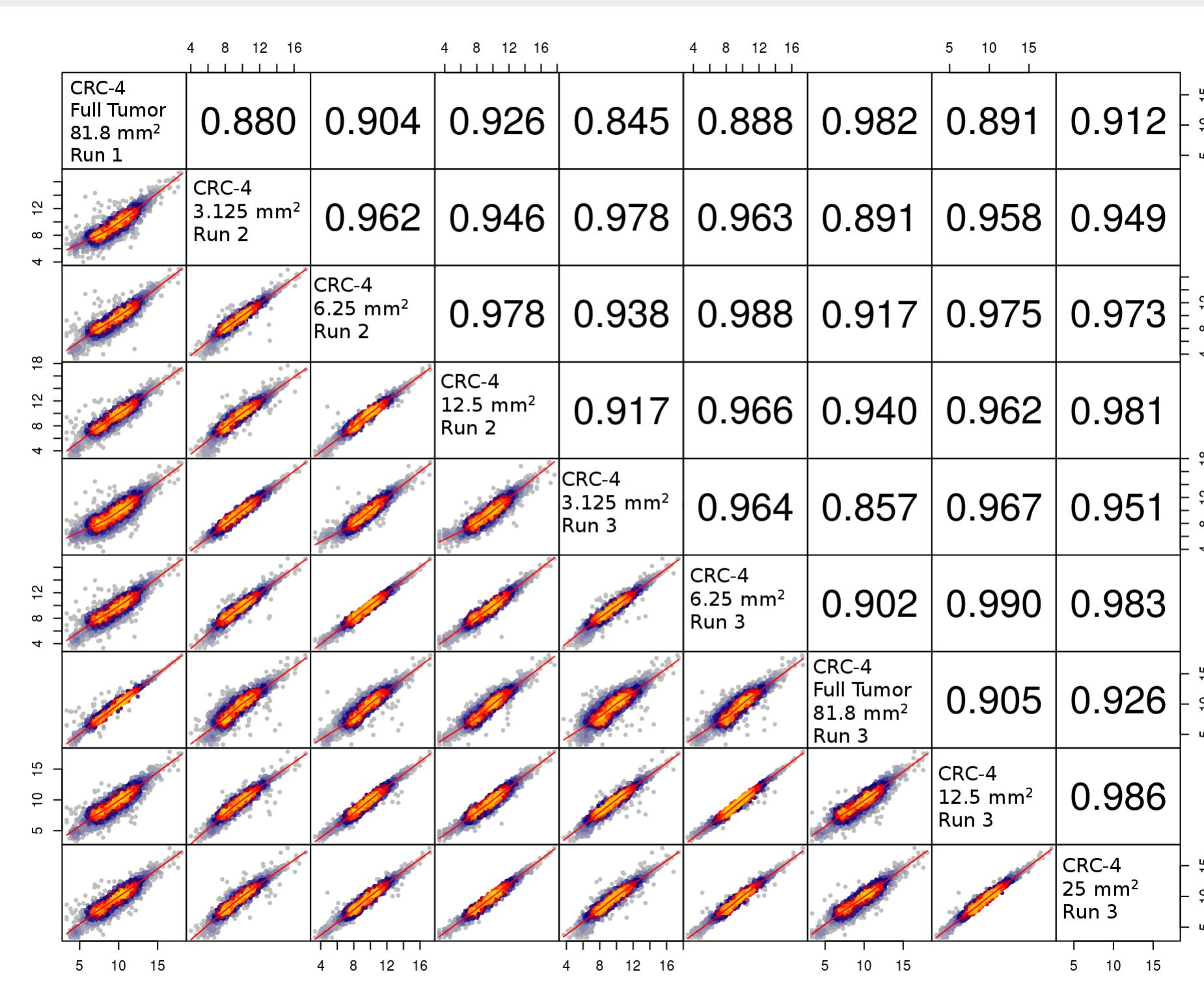
CRC-4 Tumor



CRC-4 Biopsy Approximation

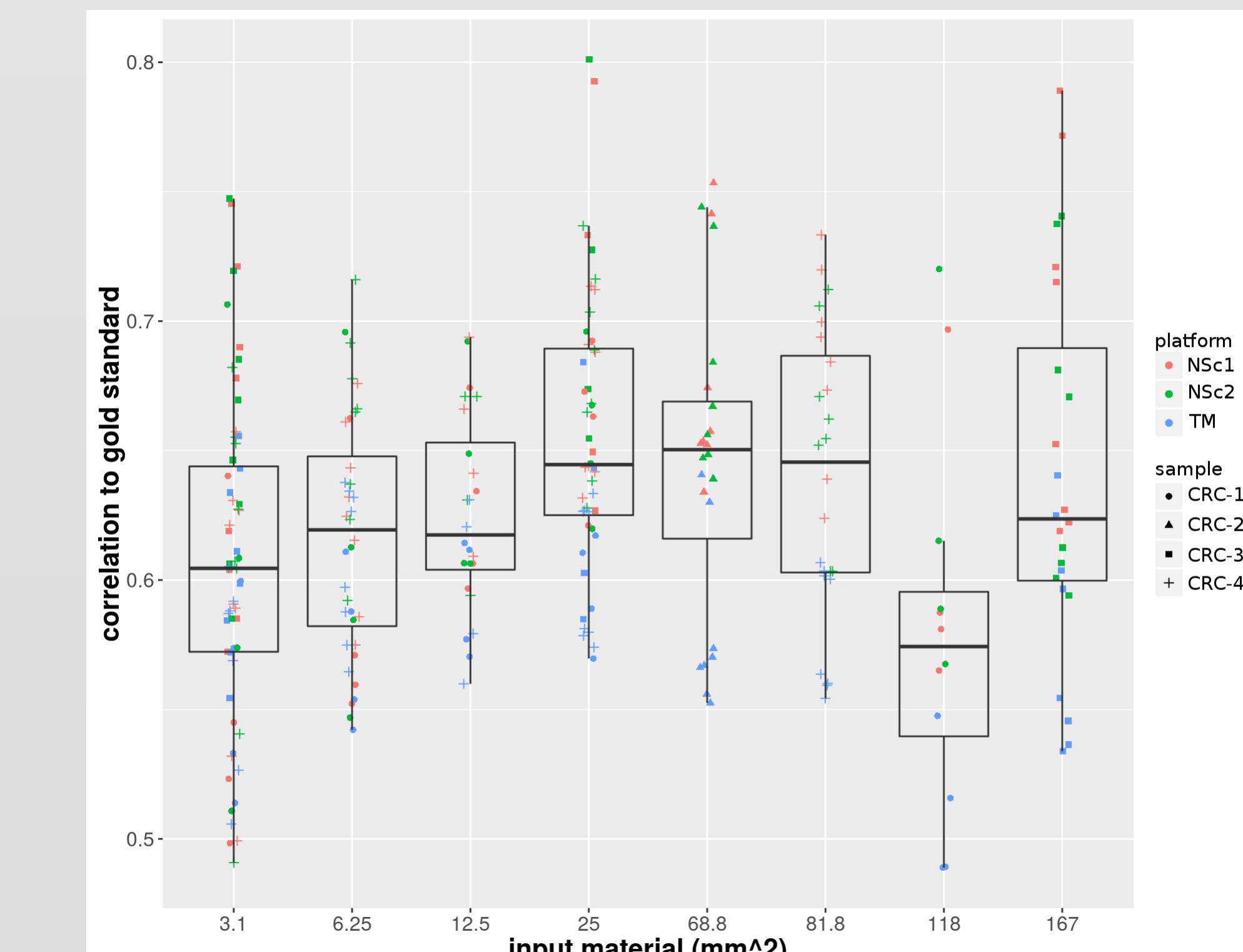
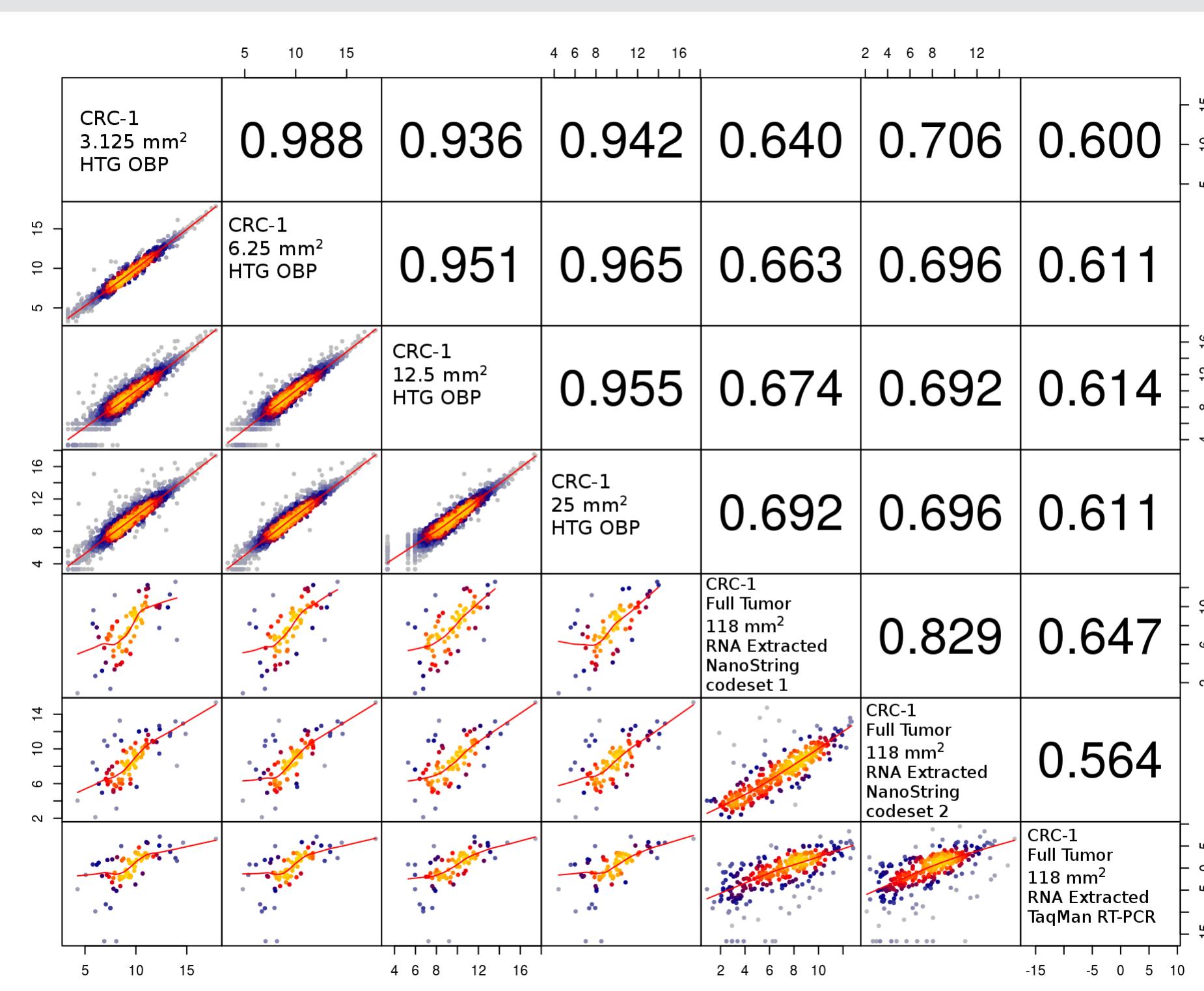


Intra-sample Reproducibility



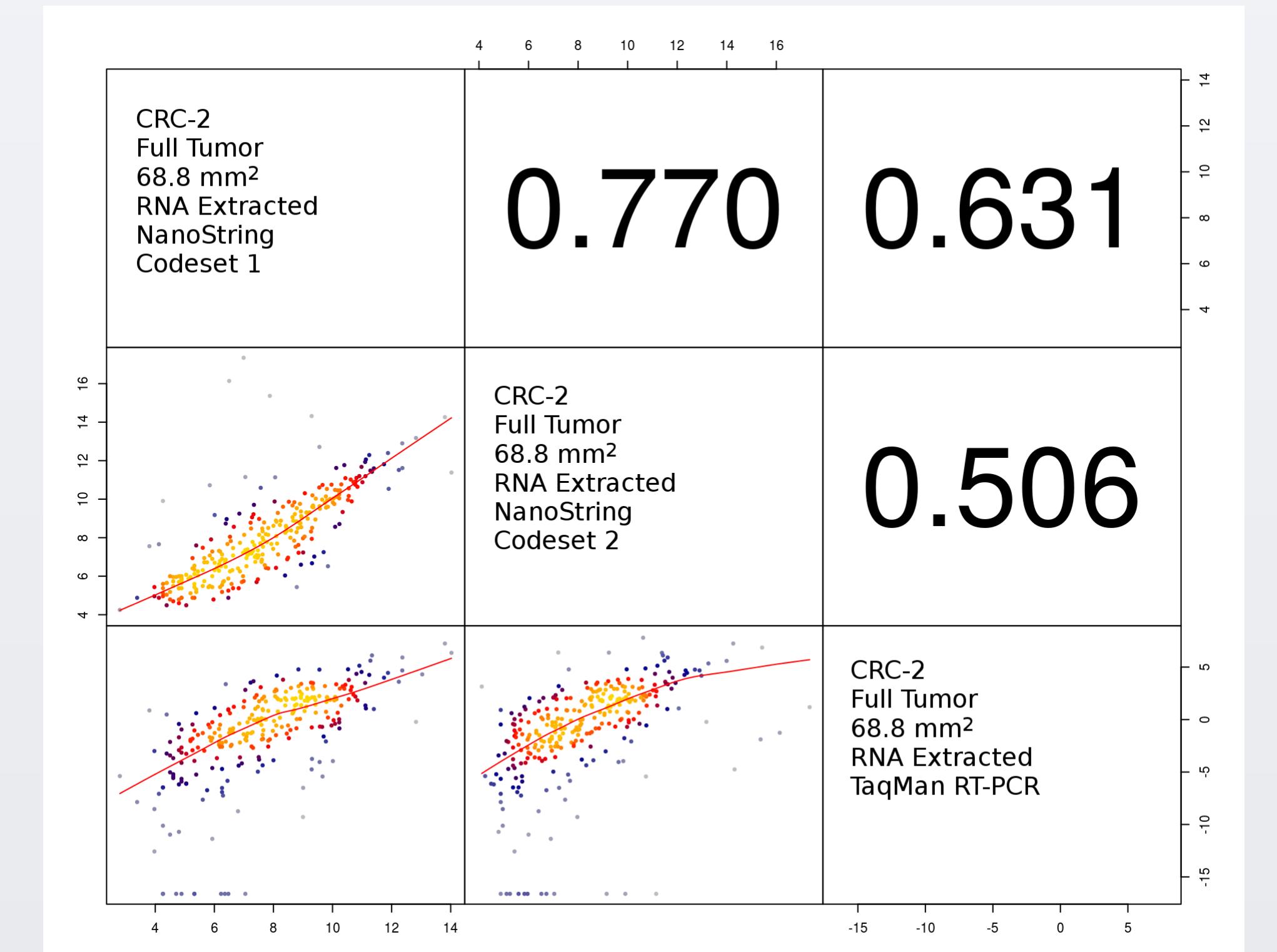
Colorectal Tumor

Comparison to Gold Standards NanoString & TaqMan



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

Thanks!

The authors would like to sincerely thank :

Terry Oeler, Histologist, NSABP
Melanie Finnigan, Lab Manager, NSABP
Cassiee Russel, Data Manager, NSABP
Karen Ross, Data Manager, NSABP
Fouad Janat, Field Applications Scientist, HTG Molecular
David Karolenko, Dir. Business Development, HTG Molecular