Ligation in situ Hybridization (LISH): A Multiplexed Gene Expression Platform for Analysis of FFPE RNA

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Clinical tissues are prepared for histological analysis and long-term storage via formalin fixation and paraffin embedding (FFPE). The FFPE process results in fragmentation and chemical modification of RNA, rendering it less suitable for analysis by techniques that rely on reverse transcription (RT) such as RT-qPCR and RNA-Seq. Multiplexed gene expression measurements of the tumor-immune microenvironment promises to provide improved patient stratification for appropriate immunotherapeutic intervention. We have developed a broadly applicable, probe-based technique called "Ligation *in situ* Hybridization" (LISH), which locally converts FFPE RNA sequences into amplifiable DNA for quantification by sequencing or other means. LISH utilizes the T4 RNA Ligase 2 to efficiently join adjacently hybridized chimeric RNA-DNA probe pairs. We demonstrate several unique advantages of LISH-based assays using patient-derived FFPE tissue. These include >100-plex capability, compatibility with common histochemical stains, and suitability for analysis of decade-old materials and exceedingly small microdissected tissue fragments. High throughput DNA sequencing modalities, including single molecule sequencing, can be used to analyze ligation products from complex panels of LISH probes ("LISH-seq"), which can be amplified efficiently and with negligible bias.

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Conflict of Interest: HBL and JJC are listed as co-inventors on a related patent application currently pending review at the USPTO.