Extraction-Free RNA Profiling of Formalin-Fixed Paraffin-Embedded Tissue

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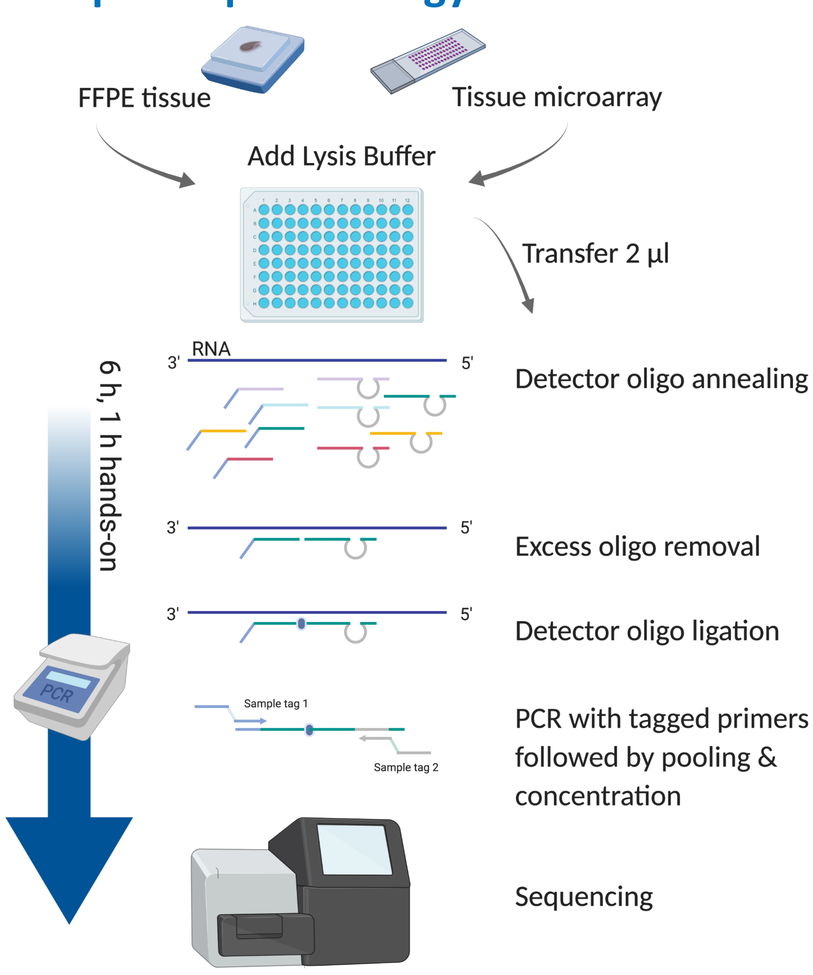
Abstract Clinical biomarker research using archived formalin-fixed paraffin-embedded (FFPE) tissue is often limited by sample access as well as quality and assay requirements for RNA input. TempO-Seq® is a targeted sequencing platform capable of measuring expression of focused gene panels up to whole transcriptomes of cultured cells, fresh, frozen or fixed tissue lysates without the typical requirements of extraction, reverse transcription or pre-amplification. The TempO-Seq assay is routinely used for whole transcriptome profiling in-vivo studies using ~5-10mm^2, 4µm sections from a variety of organs including thyroid gland, heart and pancreas. Further, successful profiling has also been achieved on multiple other more challenging sample types such as 27-year old archived FFPE tissue and <1mm^2 FFPE tissue input. Tissue microarray (TMA) is an efficient and ordered collection of fixed tissues, often cores from patient biopsies with diameters of 0.6-2mm, arranged in a grid thus fitting hundreds of samples in one block allow analysis across all samples in one experiment. A TMA block can then be cut into sections of ≥4µm thick and can thereafter be used for downstream analyses such FISH, IHC and H&E, empowering the level of details you can get from multiple patients in one experiment. However, using standard methods, gene expression analysis of FFPE samples is time-consuming, expensive and requires many sections of tissue (even with pre-amp) to obtain RNA yields appropriate for downstream analysis. TempO-Seq bypasses these limitations to provide higher precision measurements of gene expression from crude lysate. Furthermore, the TempO-Seq technology has previously been shown to generate exceptional quality data from small FFPE tissue input (~0.25mm^2, 5µm thick). Laser capture microdissection (LCM) is a microscopy-based technique developed to isolate focal areas from heterogenous histology-defined tissue. The Zeiss PALM MicroBeam uses a laser catapult to isolate the area of interest and transfer it into a sticky-cap or well of a 96-well plate without damaging the RNA. Here we use sections from breast tumour FFPE blocks or LCM catapulted tissue as input equivalent to TMAs for transcriptomic profiling using a 3600-plex panel, proving excellent reproducibility and the ability to identify significant treatment-specific differentially expressed genes.

TempO-Seq Technology



FFPE tissue preparations

Prior to processing invaluable patient TMA material, a feasibility study of equivalent low input material was performed using breast tumour sections (Amsbio, Product # T2235086). This was lysed following the TempO-Seq FFPE protocol using practical excisable areas (2mm^2, 5mm^2 and 10mm^2) and the protocol recommended lysis buffer volume or excess volume to mimic the lower TMA tissue input tissue:volume concentration. Two lysis buffer volumes (80µL and 120µL) were used to evaluate the performance and variability low tissue input can produce. Data not shown for 5mm^2 and 10mm^2 tissue inputs lysed in 80µL and 120µL.

LCM tissue preparations

SpheroMatrices® (patent pending) microTMA technology from MicroMatrices aligns 3D tissue spheroids on a 2D geometric grid to facilitate simultaneous sectioning of hundreds of individual spheroids for subsequent histological staining. Rat liver microtissues, control or compound treated, were organised in a microTMA and embedded in paraffin. 6µm thickness sections mounted on PALM-membrane slides, were catapulted into sticky-cap tubes via LCM. One or four ~200µm diameter sections were catapulted into a sticky-cap and lysed using the TempO-Seq FFPE protocol.

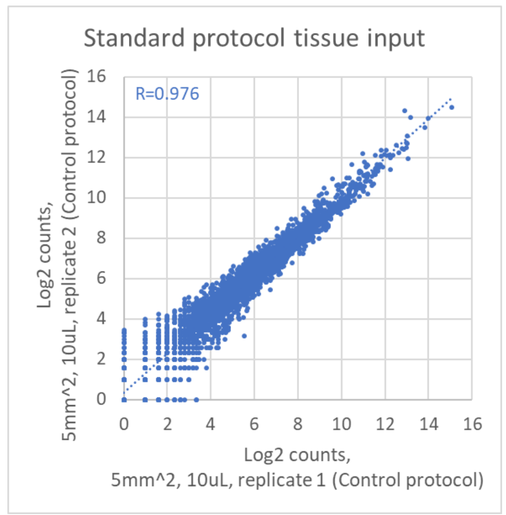
The table below shows concentration range across breast tumour or LCM input and lysis volumes tested.

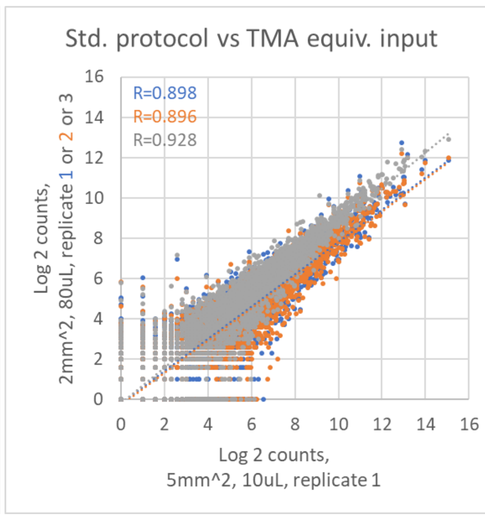
Sample type	Tissue input	Tissue input	Lysate volume	Concentration
Breast tumour	2mm^2, 5µm thick	0.01mm^3	120µL	0.000083mm^3/µL
Breast tumour	2mm^2, 5µm thick (TMA: 0.6µm diameter, 7µm thick)	0.01mm^3	80µL (TMA equivalent)	0.000125mm^3/µL
Breast tumour	5mm^2, 5µm thick	0.025mm^3	10µL (Standard protocol)	0.002500mm^3/µL
LCM spheroid	0.0314mm^2, 6µm thick (one, 200µm, diameter)	0.000188mm^3	10µL	0.000019mm^3/µL
LCM spheroid	0.126mm^2, 6µm thick (four, 200µm, diameter)	0.000756mm^3	10µL	0.000076mm^3/µL

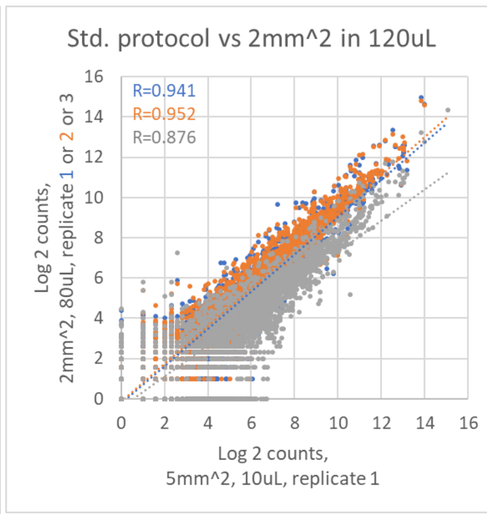
Assay performance

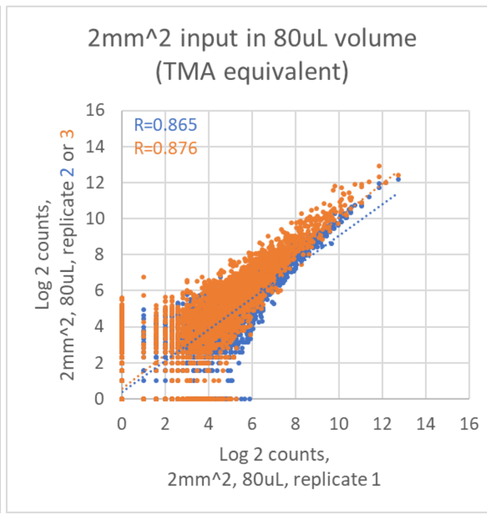
TempO-Seq and the Human/Rat surrogate panel provides excellent data from either FFPE section or LCM microTMAs, as a performance control the Rat Surrogate panel was used for analysis of purified RNA at 100ng and 1ng input, while the Human Surrogate panel was used for analysis using the standard FFPE protocol recommended input showing excellent reproducibility across the wide range of tissue input, R>0.875. For breast tissue and LCM spheroids, low input show minimal variability, which is slightly higher in the low expressing genes. Correlation coefficient (R) as a measure of reproducibility are >0.8 between technical as well as biological replicates. Differential gene expression analysis comparing untreated and treated LCM sections significantly identified compound specific genes.

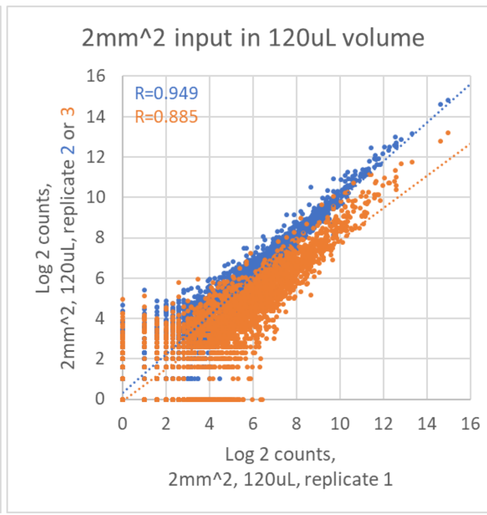
FFPE section input



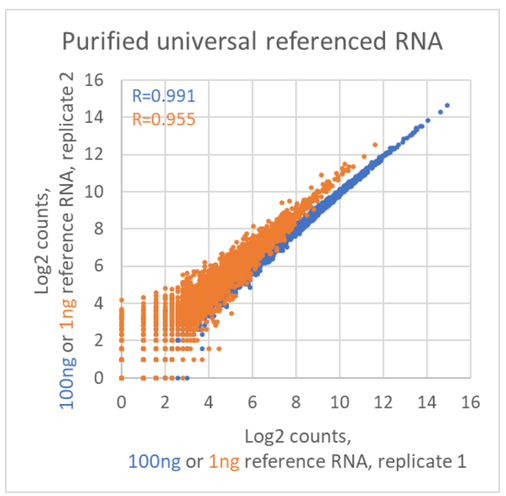


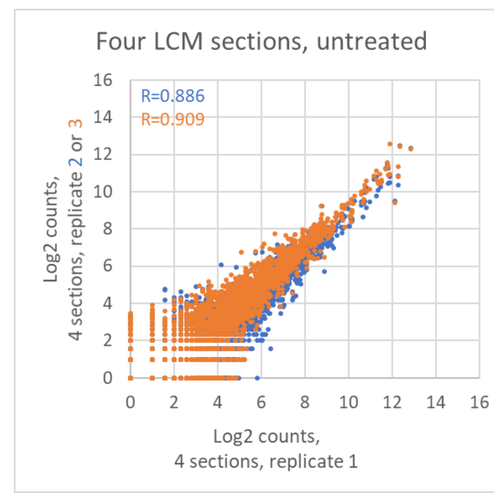


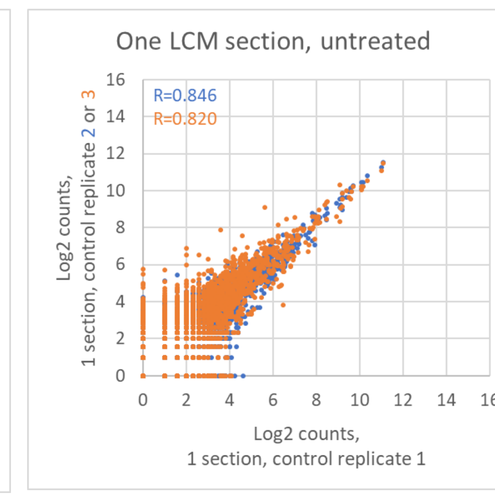


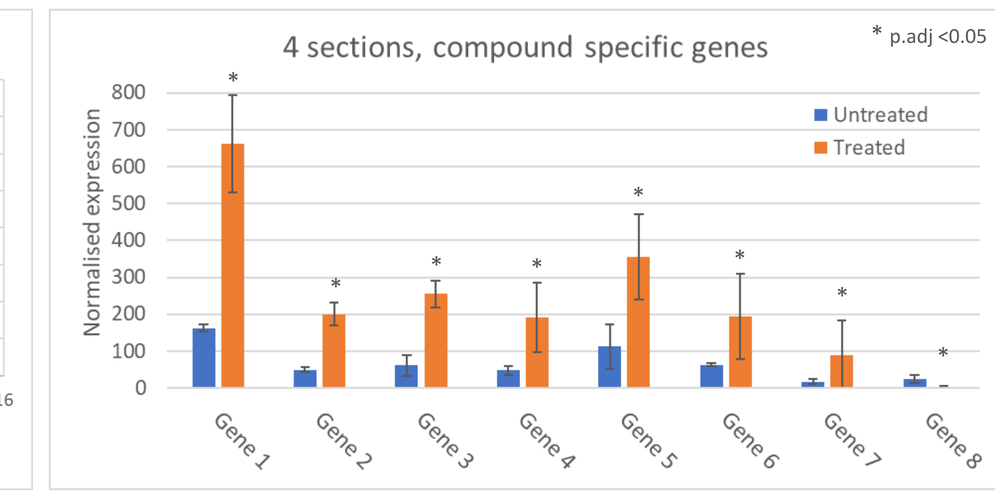


LCM spheroid input









Conclusion and Additional Comments

Combining the archival of clinical specimens such as FFPE and TMAs and the TempO-Seq technology enable excellent opportunities for clinical biomarker research, both retrospectively and prospectively as part of clinical trial with no special requirement for biopsy storage or preparation. Where very small, focused areas of FFPE sections are of interest LCM can easily be applied, however, care needs to be taken when selection LCM technology and mounting membrane. Several observations have showed interference with the TempO-Seq assay and non-PEN LCM membranes, the AutoLPC function of the PALM Zeiss LCM as well as other LCM technologies.

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