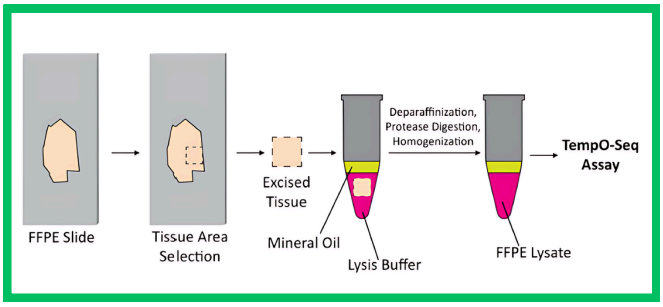
# Extraction Free TempO-Seq from FFPE Tissue

## Introduction

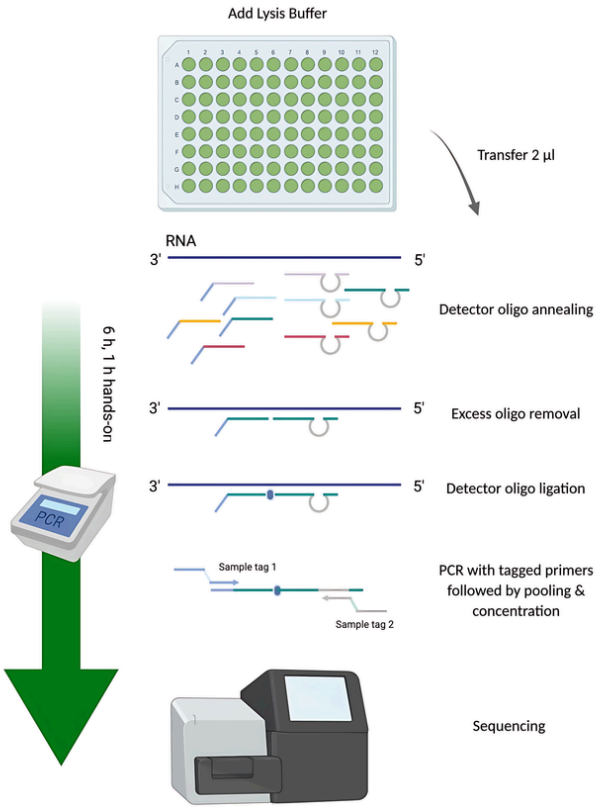
BioSpyder has adapted the TempO-Seq™ technolog to profile gene expression in formalin-fixed paraffin-embedded (FFPE) tissue. This novel approach works by directly lysing tissue scraped from FFPE slides without the need to extract RNA or convert it to cDNA. Kits can purchased, or sample testing services. Just as for crude cell lysates, TempO-Seq FFPE Assay is capable of targeted whole transcriptome, S1500+ Surrogate, gene panel expression profiling from human, mouse, or rat while maintaining the sensitivity of purified RNA and cell lysate input. The TempO-Seq FFPE assay can also be customized to recognize custom mRNA gene panels, panels of alternatively spliced isoforms amd DNA variants from all other mammalian species as well as any species bacteria and viruses. The FFPE assay has a high degree of reproducibility and produces gene expression profiles that correlate strongly with samples prepared fresh ($R^2$ = .97) nor to RNAseq of RNA extracted from FFPE. The assay can handle input areas of a wide range, and maintains sensitivity for input as small as 2mm² scraped from a 5 µm thick section. Further, the assay remains robust for samples that have undergone extended fixation times and samples that have been archived for more than 30 years or are highly degraded. A thorough explanation of the assay and its capabilities are detailed in Trejo et al. (2019) PLoS ONE 14(2): e0212031.

## Lysate Preparation




The TempO-Seq FFPE Assay was recently compared favorably to other methods of analyzing transcriptomic profiles from clinical FFPE tissues in an independent study by investigators at the University of Edinburgh: Turnbull et al. (2020) BMC Bioinformatics 21(1), 1-10. Of note, TempO-Seq was the only approach which did not require prior RNA extraction.

## Assay Workflow




## TempO-Seq FFPE Assay

The TempO-Seq assay for FFPE samples uses standard TempO-Seq chemistry. The protocol, without modification, involves scraping a sample from an FFPE section and placing it in a lysis buffer. After overlaying with mineral oil, the sample is heated to dissolve the paraffin, separating it from the lysate. Protease is then added, and the mixture is incubated at 37°C for 30 minutes, followed by homogenization. The lysates can then be either frozen or used immediately. A 2 µL aliquot of the processed lysate is added to a microplate with annealing buffer and Detector Oligos (DOs) for gene measurement. The mixture is heated from 70°C to 45°C, then incubated overnight at 45°C to ensure DOs fully anneal to their target RNAs. This hybridization is efficient despite RNA fragmentation, as DOs anneal to short RNA sequences. A nuclease mix is added to degrade unbound and incorrectly bound DOs. Then, a ligase mixture is added to form full-length probes from correctly bound DOs. After inactivating the enzymes at 80°C for 15 minutes, the probes are amplified via PCR. The PCR primers index individual samples, allowing multiplexing of many samples in a single sequencing library.

BioSpyder™
BIOLOGY TO BARCODES™