

# TempO-Seq Gene Expression Platform

Profile expression using panels from dozens of genes to whole transcriptome using a sensitive high-performance high-throughput assay without extraction, cDNA synthesis, or complicated bioinformatics.

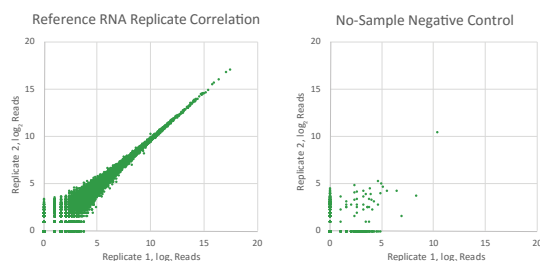
## A POWERFUL NEW EXPRESSION PLATFORM

BioSpyder Technologies has developed a novel product for gene expression profiling called TempO-Seq, a tool designed to monitor dozens to thousands of genes at once quickly and efficiently. TempO-Seq can analyze expression in samples with thousands of cells or from single cells without pre-amplification, maximizing utilization of precious or limited samples. Sample barcoding, together with sequencing only targeted regions of each gene, enables pooling up to 6,144 samples in one sequencing run (or lane). Assay content is flexible and customizable delivering unprecedented accuracy and sensitivity with simplified data analysis that eliminates the need for time-consuming bioinformatics. Needing no specialized hardware, TempO-Seq assays deliver an easy-to-implement solution for profiling expression of any gene set in any species.

## APPLICATION FLEXIBILITY

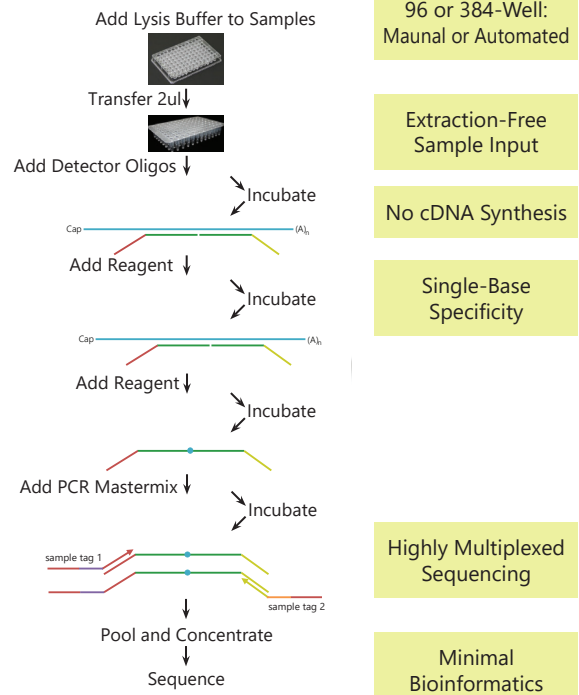
TempO-Seq is a highly flexible gene expression platform, with standard or custom designs for virtually any content, from any input source. Standard assay panels include whole transcriptome, surrogate transcriptome capturing most of the information in the transcriptome and comprehensive pathway coverage with 80-90% fewer probes, application-focused panels (e.g., predictive toxicology, oncology), and custom panel designs (either as a subset of validated probes from other panels, or probes designed to order). The extraction-free protocol does not require (although does support) purified RNA samples, saving cost and time by performing expression analysis immediately from cell lysates, and making use of either FFPE or frozen samples with equivalent results. The assay

**FIGURE 2: HIGH REPRODUCIBILITY WITH MINIMAL BACKGROUND**



Whole transcriptome assay replicates are highly reproducible, while no-sample control background is exceptionally low.

**FIGURE 1: TEMPO-SEQ ASSAY**



Highly precise assay prepares multiplexed libraries that reduce sequencing costs and overcome accuracy and informatics limitations of RNA-Seq.

exhibits sensitivity down to and below the single-cell level, for limited samples or single-cell application. While standard panels target individual gene expression levels, custom panels can be designed to assay differential splicing/isoforms, fusion gene events, expressed SNPs, and other mutations.

## EFFICIENCY FOR HIGH THROUGHPUT

In addition to not requiring RNA isolation, the assay also does not require any special equipment aside from a thermocycler. The capture-free, add-only assay simply requires manual or automated reagent additions and short incubations in a thermocycler. Manual users can easily process 198 samples daily, with <1 hour hands on time (however, the whole transcriptome assay requires an overnight incubation).

Sequencer throughput is maximized, since reads are focused on counting targeted regions rather than entire genes, and are

not wasted on unimportant genes. Thus, for the same or better data quality, approximately 10-times more whole transcriptome samples can be run than with RNA-Seq, and focused panels can enable thousands of samples per lane (Table 1).

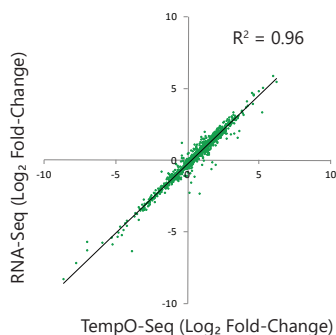
Matching the ease of high throughput processing, the assay obviates the need for complicated or time consuming bioinformatics. The probe design pipeline ensures there is no need for isoform deconvolution or multi-mapping genes. Since the alignment space is reduced at least 30-fold, alignment time is faster than 3 minutes per sample on a standard computer. This rapidly generates a flat file table of genes with counts for each sample, for use in virtually any pipeline for differential expression or pathway analysis.

**PERFORMANCE**

More critically, TempO-Seq assays pair this efficiency with outstanding data quality. Experimental samples run in triplicate regularly demonstrate high reproducibility (correlation R<sup>2</sup> val-

**FIGURE 3: EQUIVALENT PERFORMANCE TO RNA-SEQ WITH 90% FEWER READS**

**2285-Plex Rat Surrogate Transcriptome Cross-Platform Comparison**



Despite requiring only 1/10 (or fewer) of the reads, TempO-Seq yields equivalent fold-difference results to RNA-Seq. This RNA-Seq data were as published as part of the SEQC Toxicogenomics Study, and the same RNA was run using a Rat 2,284 gene TempO-Seq panel.

**TABLE 1: HIGH THROUGHPUT WITH DUAL INDEXING**

EXAMPLE ASSAY PANEL	25M READS/RUN	400M READS/RUN	3B READS/RUN
Whole Transcriptome (250 avg counts/gene, 50% expressed)	9	152	1,142
Surrogate Transcriptome 2700 (500 avg counts/gene, 80% expressed)	23	370	2,777
Focused 1000-Gene Panel (500 avg counts/gene, 80% expressed)	125	2,000	15,000
Screening 200-gene Panel (500 avg counts/gene, 90% expressed)	555	6,144*	49,152*

ues greater than 0.99). The assay has the sensitivity to generate high quality results even when sample input is limited—down to and below the quantity contained in a single cell (10 pg)—combined with near absence of background in no-sample controls (Fig 2). Single-base specificity is 99.8%, ensuring confident gene counts even in highly-multiplexed panels and enabling detection of coding variants. The assay is also robust to degraded samples or FFPE treatment; comparison of matched fixed and frozen inputs result in essentially no loss in sensitivity, and no impact of RNA degradation due to fixation.

In sum, these performance characteristics and high correlation to RNA-Seq results (Fig 3, R<sup>2</sup> = 0.96) combined with the efficiency to run tens to thousands-fold more samples with the same sequencing expense make it an ideal replacement method for nearly all expression array and RNA-Seq experiments.

**FOR MORE INFORMATION**

For detailed product specifications, technical publications, and ordering information, view [www.biospyder.com](http://www.biospyder.com) or contact [info@biospyder.com](mailto:info@biospyder.com).

**ORDERING INFORMATION**

Panel	Samples per Kit			
	24	48	96	384 (4x96)
Human Whole Transcriptome	KT-02-024	KT-02-048	KT-02-096	KT-02-384
Human Surrogate + Tox	KT-01-024	KT-01-048	KT-01-096	KT-01-384
Rat Whole Transcriptome	KT-05-024	KT-05-048	KT-05-096	KT-05-384
Rat Surrogate + Tox	KT-04-024	KT-04-048	KT-04-096	KT-04-384
Mouse Whole Transcriptome	KT-06-024	KT-06-048	KT-06-096	KT-06-384
Mouse Surrogate + Tox	KT-03-024	KT-03-048	KT-03-096	KT-03-384
Custom Panels	Available: Contact <a href="mailto:support@biospyder.com">support@biospyder.com</a> for information			
Human Pan Cancer	Available Soon			