Transcriptomic dose response modeling in >21 year-old archival mouse liver tissue for risk assessment

Leah C. Wehmas, Charles E. Wood*, and Susan D. Hester
U.S. Environmental Protection Agency, Office of Research and Development, Center for Computational Toxicology and Exposure
*Currently, Boehringer Ingelheim, Ridgefield, CT
Leah Wehmas | wehmas.leah@epa.gov | 919-541-2931

Abstract

Gene expression data from short-term in vivo studies shows efficacy in quantifying dose-dependent effects that inform and precede toxicity outcomes of regulatory concern. With archival formalin-fixed paraffin-embedded (FFPE) tissue samples, this data can be obtained and related to pathological effects without the need for new animal studies. Advances in technology can help overcome the challenges of isolating and sequencing damaged nucleic acid from old FFPE tissue samples. This study investigated the efficacy of performing transcriptomic benchmark dose modeling (BMD_T, BMDExpress 2.0) on whole genome TempO-sequencing in >21 yr-old paired frozen (FR) and FFPE liver tissue harvested from male B6C3F1 mice exposed to 0, 1, 2, and 3.5 g/L dichloroacetic acid via drinking water for 6 d. for which total RNA-sequencing data existed. BMD_Ts calculated from preselected Cyp4a targets in FR total RNA sequencing data were 277-284 mg/kg-d, which was ~6.5-fold higher than the IRIS BMD for liver adenoma and carcinoma. BMD_T performed poorly on FFPE total RNA sequencing data due to a 97% loss in gene count signal; however, BMD_T analysis of whole transcriptome TempO-sequencing on the same FFPE tissue only suffered a 40% loss relative to paired FR. Furthermore, DESeq2 analysis of TempO-sequencing data identified 8865 and 16434 differentially expressed genes (DEGs) (FDR<0.05, ±2-fold change) across all doses for FR and FFPE datasets, respectively. Of those DEGs, 5355 were common and 1022 FR and 726 FFPE passed the Williams Trend Test (p-value <0.05) and had BMD_T values ≤ to the highest dose. The median BMD_T value for FFPE TempO-sequencing results was 335 mg/kg-d, 38% higher than paired FR and 16% higher than the FR total RNA sequencing BMD_T results. TempO-sequencing BMD_T for Cyp4a target genes in FFPE samples was quite consistent (166-325 mg/kg-d) but not as consistent as the FR (202-225 mg/kg-d). While median TempO-sequencing BMD_T values were still higher than the IRIS BMD, they were within an order of magnitude and calculated from a short in vivo exposure (6 d.) unlike cancer endpoints. These BMD_Ts may also be improved with molecular pathway level data, saving time and resources. Our results show very old FFPE tissue samples can be used to obtain quantitative gene expression data and with additional refinement, used to enhance traditional risk assessments. This abstract does not represent EPA policy.

Goals

- Evaluate TempO-sequencing (TempO-seq) approach compared to total RNA-sequencing (RNA-seq) in archival samples
- Apply benchmark dose modeling to gene expression data from old archival samples to estimate biologic potency

Design

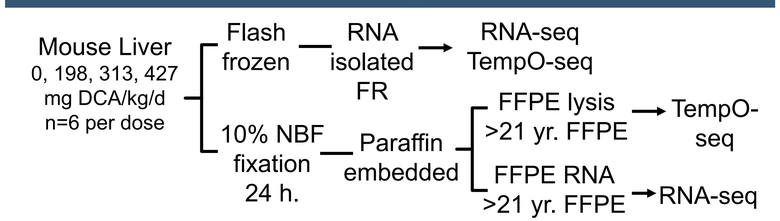


Figure 1. Liver from mice exposed to dichloroacetic acid (DCA) by drinking water was split and either flash frozen (FR) or fixed in 10% neutral buffered formalin (NBF) before being processed into paraffin-embedded blocks (FFPE). FR and FFPE samples age >21 yr. in freezer or block, respectively.

Methods

RNA-seq

- Purified mRNA and noncoding RNA (FR and FFPE)
- Ribo-Zero ribodepletion
- TruSeq Stranded Total RNA Libraries
- Paired-end 50 bp RNA seq
- Illumina Hi-seq 2500
- Target depth: 25 mil reads per sample

TempO-seq

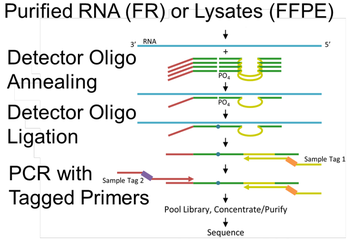


Figure 2. Depicts TempO-sequencing method as described in Yeakley 2017.https://doi.org/10.1371/journal.pone.0178302

Analysis

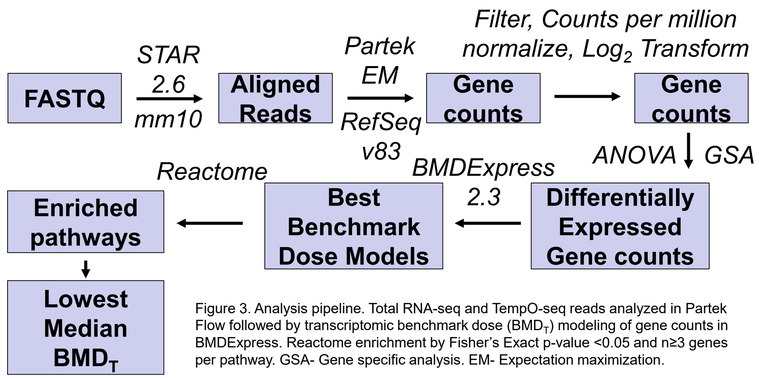
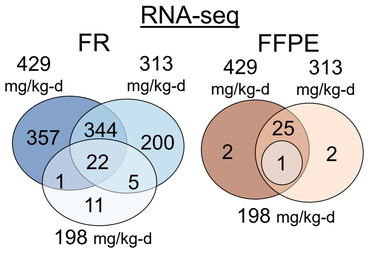


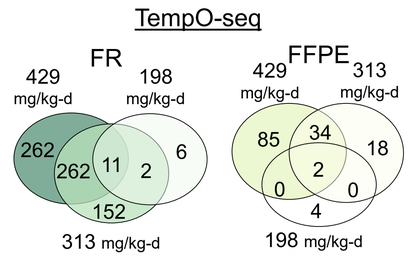
Figure 3. Analysis pipeline. Total RNA-seq and TempO-seq reads analyzed in Partek Flow followed by transcriptomic benchmark dose (BMD_T) modeling of gene counts in BMDExpress. Reactome enrichment by Fisher's Exact p-value <0.05 and n≥3 genes per pathway. GSA- Gene specific analysis. EM- Expectation maximization.

1. TempO-seq maintains better gene detection in paired FFPE samples

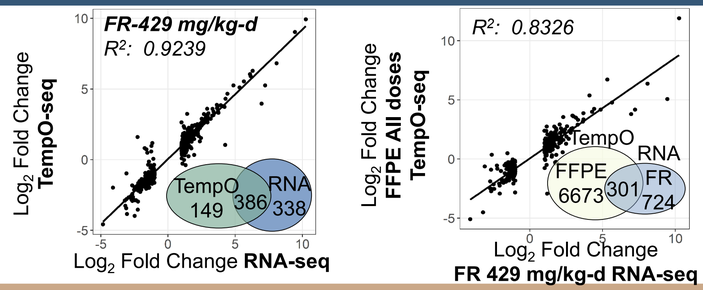
RNA-seq detects 21-fold fewer significant genes in FFPE vs. FR



TempO-seq detects 5-fold fewer significant genes in FFPE vs. FR

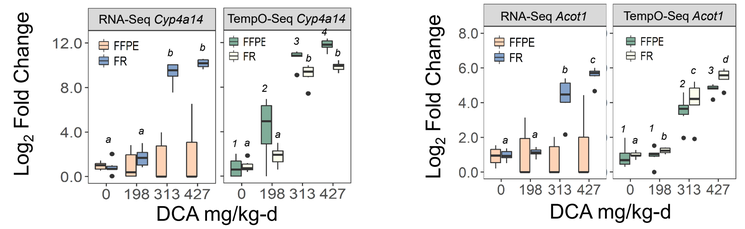


2. TempO-seq FFPE genes concordant (R^2>0.76) with FR RNA-seq



3. Select biomarker genes

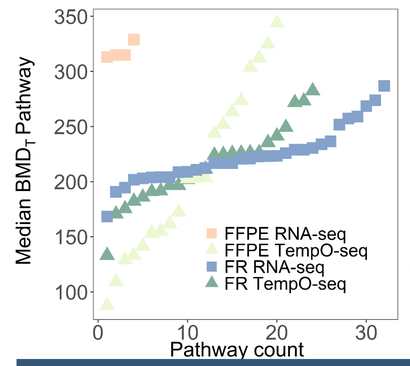
RNA-seq shows 8-10 fold difference in marker gene counts in FFPE vs. FR ; TempO-seq shows 1-3 fold difference



a, b, c, and d: FR significant difference (p-value <0.05) between dose groups. 1, 2, 3, and 4: FFPE significant difference (p-value <0.05) between dose groups.

4. TempO-seq BMD_T for 6-day FFPE samples within 2.1-fold of 2-year BMD_A estimates

BMD_T highest for FFPE RNA-seq at 313 mg/kg-d whereas FR and FFPE TempO-seq are 130 mg/kg-d



	Lowest Median BMD (mg/kg-d)	Median BMDL (mg/kg-d)	BMD_T:BMD_A*
FR TempO-seq	133.2	100.7	3.1
FFPE TempO-seq	87.7	55.1	2.1
FFPE RNA-seq	313.1	202.0	7.3
FR RNA-seq	168.4	127.8	4.0

*BMD_A: IRIS BMD for mouse liver adenoma and carcinoma = 42.6 mg/kg-d

Conclusions

- In frozen tissues, TempO-seq provided highly concordant results to total RNA-seq, based on genes detected and fold-change responses.
- TempO-seq outperformed RNA-seq in older FFPE samples based on enhanced detection of target gene responses to chemical treatment.
- Similar estimates for FFPE TempO-seq BMD_T at 6 days and apical effects at 2 years indicate this method could be used to estimate biological potency of chemicals for rapid safety assessments.
- IMPACT-Mining aged archival samples can provide a useful resource for linking early stage molecular toxicity pathways with later adverse effects without the need for new studies, thereby achieving CSS goals.