Low-cost toxicogenomic profiling of Human iPSC derived minibrain reveals key adverse outcome pathways

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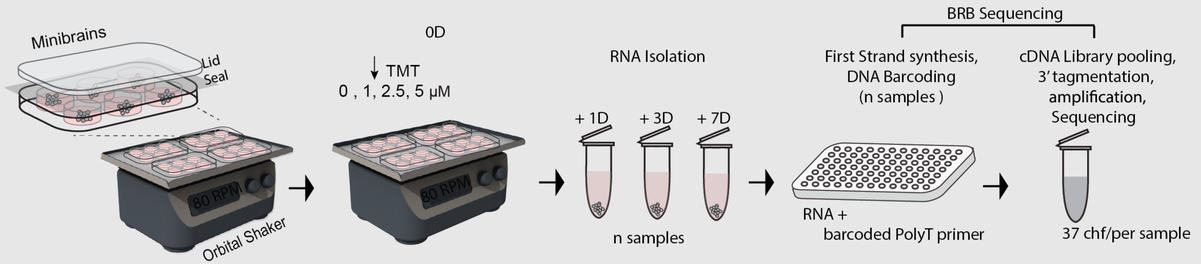
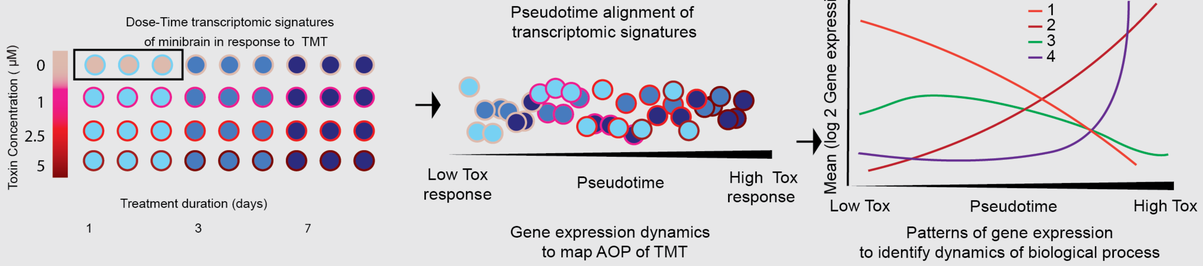
Genome-wide transcriptomic interrogation of organoid models are gaining popularity in characterising drug, toxicity responses and neurodevelopmental disorders. In this study, we have adopted a novel low-cost transcriptomic pipeline to study neurotoxicity associated adverse outcome pathway. We utilise a 3D minibrain spheroid model developed in the lab called minibrain, an RNA sequencing library preparation methodology called BRBSeq and low depth sequence profiling for toxicogenomic profiling. Using this pipeline, we were able to uncover the molecular changes and differentiation dynamics involved in minibrain development, illustrating the ability to identify key cellular dynamic using our pipeline. We compare the transcriptomic changes associated with the dose-time neurotoxic response of minibrain to trimethyltin chloride both using high and low depth sequence profiling.

Study Design & Methods

A. ultra low cost transcriptomic sequencing of minibrains upon TMT treatment



B. Illustration of the transctriptomic mapping



Design and Novel Pipeline for toxicogenomic screening of TMT Neurotoxicity: A, Illustrates multiplexed generation of minibrains maintained in an orbital shaker [1] that is exposed to different dose and time of TMT treatment. Treated Minibrains are then collected for ultralow cost RNA sequencing (BRB Sequencing) [2] . B. Illustrates the distinct transcriptomic signatures obtained using BRB sequencing across dose-time exposure of TMT. Down stream analysis was performed to create low toxicity to high toxicity axis which was used to map gene expression dynamics of TMT Toxicity.

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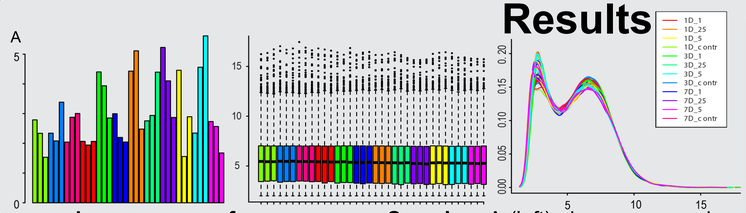
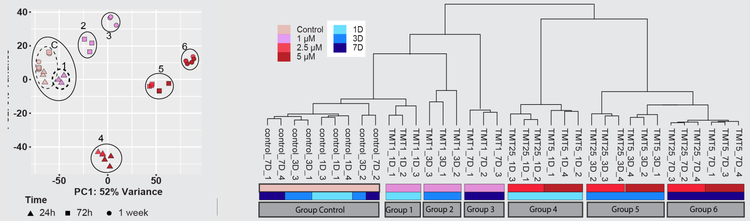
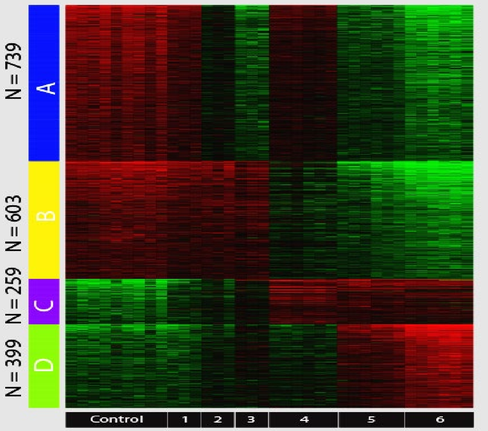
Results



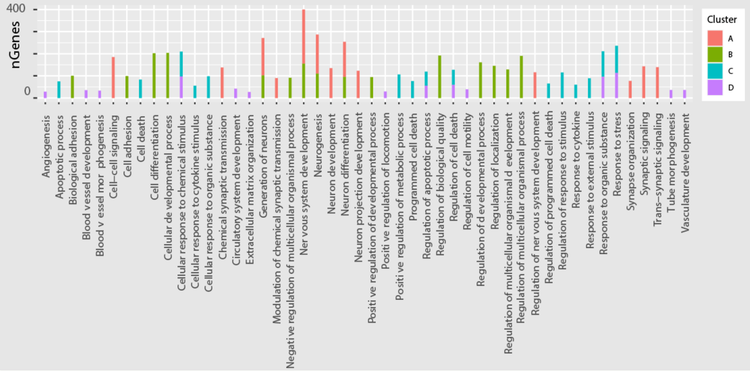
Sequencing coverage of genes across Samples: A (left) shows sequencing depth across multiple samples treated with distinct dose and time, (Middle and right) Upon filtering genes with similar expression values are represented across all samples.



Progressive grouping of Transcriptomic signatures using Principal component analysis and hierarchical clustering highly correlates with dose-time exposure of TMT Neurotoxicity (left, right).



Clustering of top 2000 most variable genes reveal 4 clusters, cluster A and cluster D genes are downregulated and upregulated based on time of treatment respectively. Cluster B and cluster C genes are downregulated and upregulated based on the group identity.



Cluster A and B represent genes upregulated temporally and groupwise order respectively. Cluster C and D are genes downregulated groupwise and temporally respectively.

Gene ontologies related to cluster A ,B overlap on certain gene ontologies and represent genes related to development, synapse and neuronal function related ontologies. Cluster C and D represent genes related to angiogenesis, response to stress, cytokine signaling, organic substance response and cell death.

The distinct gene expression changes and molecular candidates identified with our pipeline provide insight into the key events involved in the adverse outcome pathways of trimethyltin chloride associated neurotoxicity. We identify key processes such as endoplasmic reticulum stress, dysregulation of synaptic molecules and downregulation of neuron-morphology associated molecules to chronic but not acute exposure to trimethyltin chloride. We further validate these results functionally to confirm the validity of the low cost toxicogenomic profiling pipeline.

[1] Govindan S, Batti L, Osterop SF, Stoppini L and Roux A (2021) Mass Generation, Neuron Labeling, and 3D Imaging of Minibrains. *Front. Bioeng. Biotechnol.* 8:582650. :
[2]Alpern, D., Gardeux, V., Russeil, J. *et al.* (2019) BRB-seq: ultra-affordable high-throughput transcriptomics enabled by bulk RNA barcoding and sequencing. *Genome Biol* 20, 71.