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## Bulk RNA-seq

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**We use this protocol and it's working**

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## Disclaimer

This protocol was adapted from the work of Lexi Bounds in the Gersbach lab at Duke University.

## Abstract

This protocol describes methods for bulk RNA-seq using WTC11 cells and Neural Progenitor Cells (NPCs).

## Materials

High Sensitivity RNA ScreenTape Analysis (Agilent #5067)

## Bulk RNA-seq

- 1 Total RNA is purified from WTC11 iPSCs and NPCs using the Qiagen RNeasy kit according to the manufacturer's protocol.
- 2 RNA quality is verified to have RIN score > 8 for all samples using High Sensitivity RNA ScreenTape Analysis (Agilent #5067).
- 3 RNA-sequencing libraries are prepared by Genewiz and sequenced on an Illumina HiSeq with 2×150bp configuration.
- 4 Perform RNA-sequencing in duplicate for each cell type.

## Bulk RNA-seq analysis

- 5 The pipeline for processing and analysis of the bulk RNA-seq data can be found here: [https://github.com/Duke-GCB/GGR-cwl/blob/master/v1.0/RNA-seq\\_pipeline/pipeline-pe-unstranded-with-sjdb.cwl](https://github.com/Duke-GCB/GGR-cwl/blob/master/v1.0/RNA-seq_pipeline/pipeline-pe-unstranded-with-sjdb.cwl).
- 6 Quality control of FASTQ files is performed using FASTQC (Ref. 1). Adapter reads were then trimmed using Trimmomatic (Ref.2) and mapped using the STAR aligner (Ref. 3).
- 7 Quantification is performed using RSEM (Ref. 4).
- 8 For comparisons of basal gene expression levels, a mean transcripts per million (TPM) value is calculated using two biological replicates.



## Protocol references

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