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RNA Sequencing

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Dahlia Rohm[1,2], Joshua Black[1,2], Sean R. McCutcheon[1,2], Alejandro Barrera[1,2], Shante Berry[1,2], Daniel Morone[1,2], Xander Nuttle[3,4], Celine de Esch[3,4], Derek Tai[3,4], Michael Talkowski[3,4], Nahid Iglesias[1], Charles A. Gersbach[1,2]

[1]Duke University; [2]Center for Advanced Genomic Technologies; [3]Massachusetts General Hospital; [4]Broad Institute of Massachusetts Institute of Technology and Harvard University

Gersbach Lab

Andrea R Daniel
Duke University

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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 Dahlia Rohm and colleagues in the Gersbach lab at Duke University.

Abstract

This protocols describes methods for RNA sequencing in human induced pluripotent stem cells.

Materials

Norgen Total RNA Purification Plus Micro kit (Norgen, 48500); Qubit RNA Broad Range quantification assay (Thermo, Q10211); Agilent TapeStation (Agilent TapeStation 4200) RNA ScreenTape (Agilent, 5067-5576); Genewiz (Azenta Life Sciences); Trimmomatic v0.32; STAR aligner; featureCounts; DESeq2.

Cell preparation and RNA preparation

1 Harvest cells, protocol defined by user. Pellet ~100,000 cells (between 1000 and 500,000) and proceed to RNA isolation immediately or flash-freeze in liquid nitrogen and store pellets at -80°C until processing.

0.2 Isolate total RNA using Norgen Total RNA Purification Plus Micro kit (Norgen, 48500).

RNA sequencing

0.3 Quantify RNA with the Qubit RNA Broad Range quantification assay (Thermo, Q10211) and optionally validate RNA integrity with Agilent TapeStation (Agilent TapeStation 4200) RNA ScreenTape (Agilent, 5067-5576) or a similar automated electrophoresis system.

0.4 Submit total RNA to Genewiz (Azenta Life Sciences) for total RNA sequencing for 2×150bp paired-end short read sequencing. Genewiz verifies quality of samples and libraries and performed DNAse treatment as necessary.

0.5 Subject resulting reads to adapter trimming using Trimmomatic v0.32, align to GRCh37 or GRCh38 with the STAR aligner, and retrieve gene counts using featureCounts.

0.6 Perform differential expression analysis using DESeq2. Determine genes with significant differential expression using a threshold of FDR<0.01 and absolute value of Log2(FC)>1.