VERSION 1

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External link:
<https://hpap.pmacs.upenn.edu/explore/workflow/islet-molecular-phenotyping-studies?protocol=6>

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Protocol status: Working
 We use this protocol and it's working

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🌐 Bulk RNA sequencing (mRNA seq) V.1

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ABSTRACT

Bulk RNA sequencing (RNA-seq) is a quantitative method used to interrogate the transcriptome of a biological sample at a given point in time. This protocol describes a method for generating bulk RNA-seq libraries from human islet material. This method also allows for isolation of genomic DNA, which can be used for additional assays such as whole genome bisulphite sequencing.

Note

Date submitted: August 13, 2020

Steps in pre-processing

- 1
 1. 250,000 to 500,000 cells for use in [Qiagen DNA/RNA AllPrep kit](#); for >500,000 cells, use DNA/RNA Universal AllPrep kit; for <500,000 cells, use Qiagen DNA/RNA AllPrep Micro kit.
 - a. Centrifuge cells, then carefully remove all supernatant by aspiration.
 - b. Loosen pellet by flicking and add RLT Plus buffer (prepared with Beta-mercaptoethanol)
 - < 5 x 10⁶ cells, 350 µL
 - 5 x 10⁶– 1 x 10⁷ cells, 600 µL
 2. Pipet the lysate directly into a QIAshredder spin column and centrifuge for 2 min at maximum speed.
 3. Continue with AllPrep protocol, or snap freeze and store at -80 °C for future use.

Links to kits used in post-processing

- 2
 1. All recent samples (using 750pg input) were processed using Takara Pico kit with: [https://www.takarabio.com/learning-centers/next-generation-sequencing/technical-notes/rna-seq/stranded-libraries-from-picogram-input-total-rna-\(v2\)](https://www.takarabio.com/learning-centers/next-generation-sequencing/technical-notes/rna-seq/stranded-libraries-from-picogram-input-total-rna-(v2))
 2. All samples prior to 2020 (used 100ng input) were processed using Illumina TruSeq Stranded Total RNA Library prep Gold: <https://www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/truseq-stranded-total-rna.html>

Note

Note:

The cells go through the All Prep kit to get RNA and then utilized for bulk RNA-seq processing. From this preparation, we also acquire DNA which is used for Whole Genome Bisulphide Sequencing (WGBS).