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Bulk RNA sequencing (mRNA seq) V.2

Klaus H. Kaestner Lab[1], Suzanne Shapira[1]

[1]University of Pennsylvania Perelman School of Medicine

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Human Islet Research Network

Lili Liang

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.

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EXTERNAL LINK

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

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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 10^6cells, **350 µl**
- 5 x 10^6– 1 x 10^7cells, **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 Total RNA-Seq Kit v2

2. All samples prior to 2020 (used 100ng input) were processed using Illumina TruSeq Stranded Total RNA Library prep Gold

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).

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