



Aug 12, 2021

Bulk RNA sequencing (mRNA seq)

Klaus H. Kaestner Lab¹, Suzanne Shapira¹¹University of Pennsylvania Perelman School of Medicine

Suzanne Shapira: HIRN-HPAP correspondence: sshapira@penmedicine.upenn.edu

1 Works for me

 Sharedx.doi.org/10.17504/protocols.io.bwdcpa2w

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.

Date submitted: August 13, 2020

DOI

dx.doi.org/10.17504/protocols.io.bwdcpa2w

EXTERNAL LINK

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

PROTOCOL CITATION

Klaus H. Kaestner Lab, Suzanne Shapira 2021. Bulk RNA sequencing (mRNA seq). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bwdcpa2w>

KEYWORDS

null, mRNA seq, HPAP, HIRN

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CREATED

Jul 06, 2021

LAST MODIFIED

Aug 12, 2021

PROTOCOL INTEGER ID

51332

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)
 - $< 5 \times 10^6$ cells, **350 μ l**
 - $5 \times 10^6 - 1 \times 10^7$ cells, **600 μ l**
 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:

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).