Bulk RNA sequencing (mRNA seq)

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I. Steps in pre-processing

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, 350ul
    - 5 x 106 – 1 x 107 cells, 600ul

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 for future use.

4. The whole RNA sample is cleaned using the RNeasy MinElute Qiagen kit (Cat. No. 74204), the optional DNA digestion step is performed following kit instructions.

5. RNA quality is assessed by Tape station or bioanalyzer.

II. Links to kits used in post-processing

1. Some early HPAP samples (using 750pg input) were processed using Takara Pico kit with Total RNA-Seq Kit v2.

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

3. From HPAP-059 onward 100ng input of clean RNA is used to the QIAseq FastSelect Qiagen kit (Cat. No. 334387) combined with the NEBNext Ultra II Directional RNA library kit (Cat. No. 334387).

Note:

1. The cells go through the AllPrep kit to get RNA and then utilized for Bulk RNA-seq processing. From this preparation, we also acquire DNA that is used for Whole Genome Bisulphide Sequencing (WGBS).
2. The library kit was used to process each RNA sample, can be found in the metadata information.