Bulk RNA sequencing (mRNA seq)

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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)

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 10^6 cells, 350ul
        - 5 x 10^6 – 1 x 10^7 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.

II. Links to kits used in post-processing

1. All recent samples (using 750pg input) were processed using Takara Pico kit with: https://www.takarabio.com/products/cdna-synthesis-and-library-construction/next-gen-sequencing-kits/total-rna-seq-kits/pico-input-strand-specific-total-rna-seq-for-illumina

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