



## **Bulk RNA sequencing (mRNA seq)**

Kaestner lab

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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)
    - $< 5 \times 10^6$  cells, 350ul
    - $5 \times 10^6 - 1 \times 10^7$  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 [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](#)