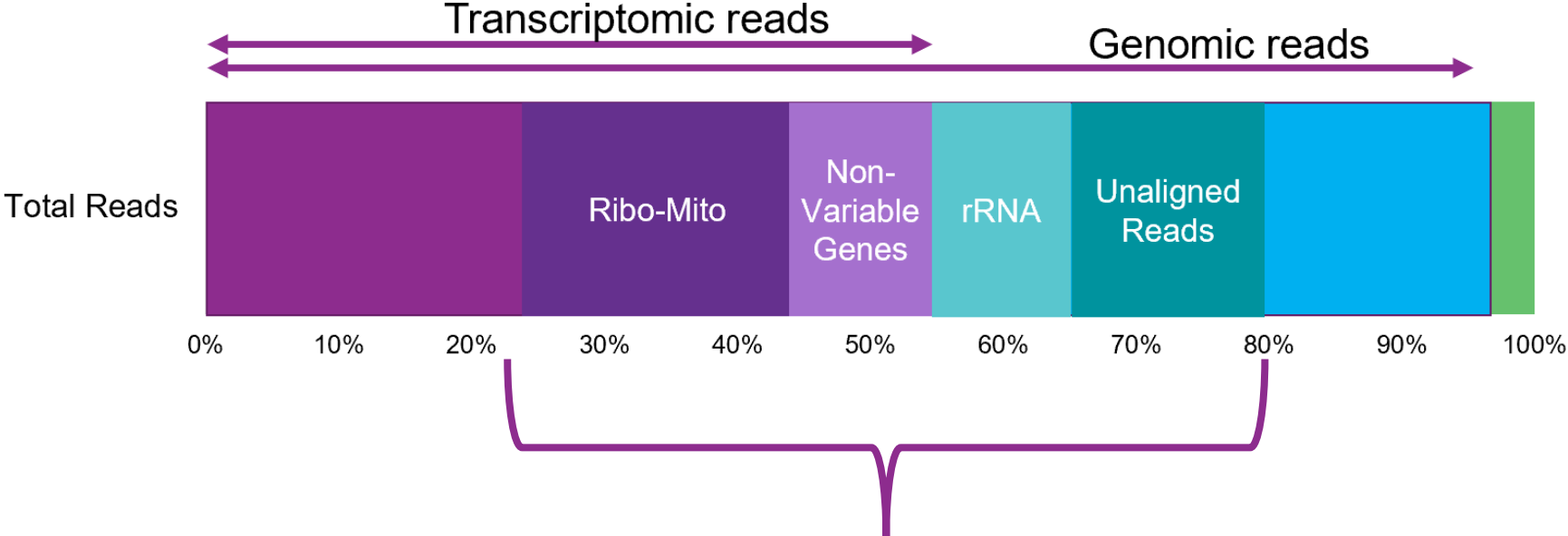


# Pre-flight Customer Information: Single Cell Sequencing with DepleteX™

To ensure customer success on their first single cell experiment using Jumpcode technology:

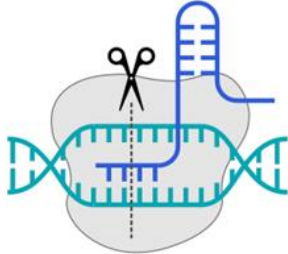
- Provide a high-level overview of the process
- Answer any customer questions including FAQ
- Provide point of contact for support in case any issues arise during the experiment or analysis

# What are we removing?

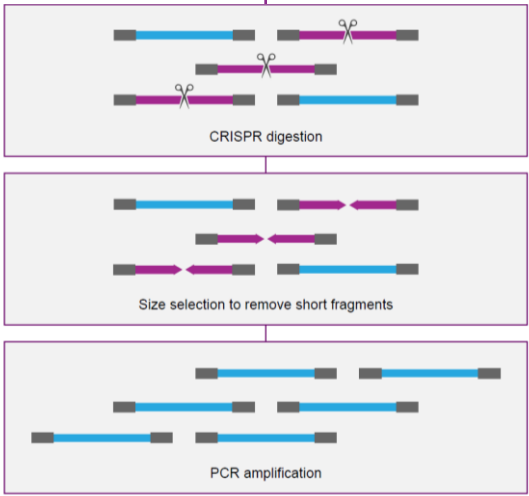


Typically uninformative for secondary analysis;  
Often removed informatically by Seurat / Scanpy

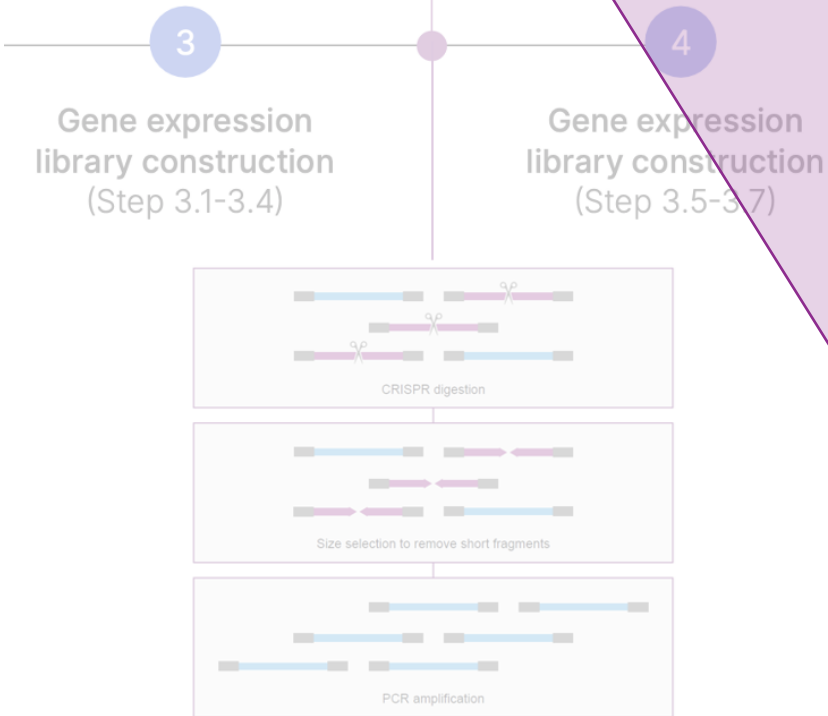
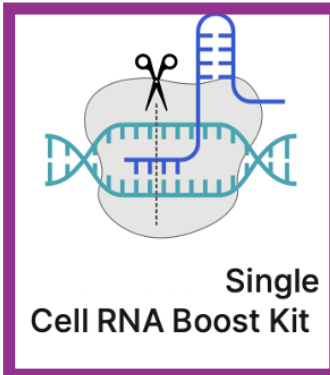
# Overview of DepleteX™ single cell workflow with 10x 3' Gene Expression



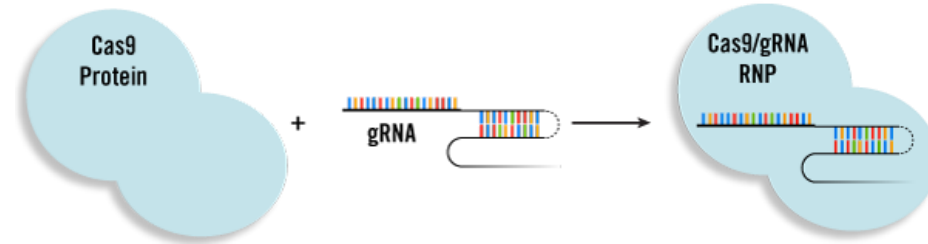
🕒 Total assay time: 2 hours | Hands-on time: 45 minutes



# The entire process can be inserted into any single cell application and only takes 45 minutes of hands-on time

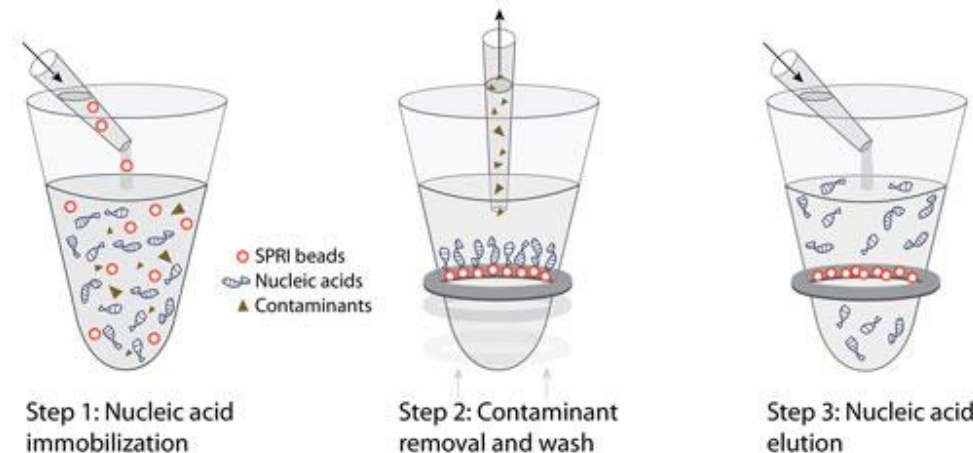


**1. Make RNP:** combine guides with Cas9 (15 min)

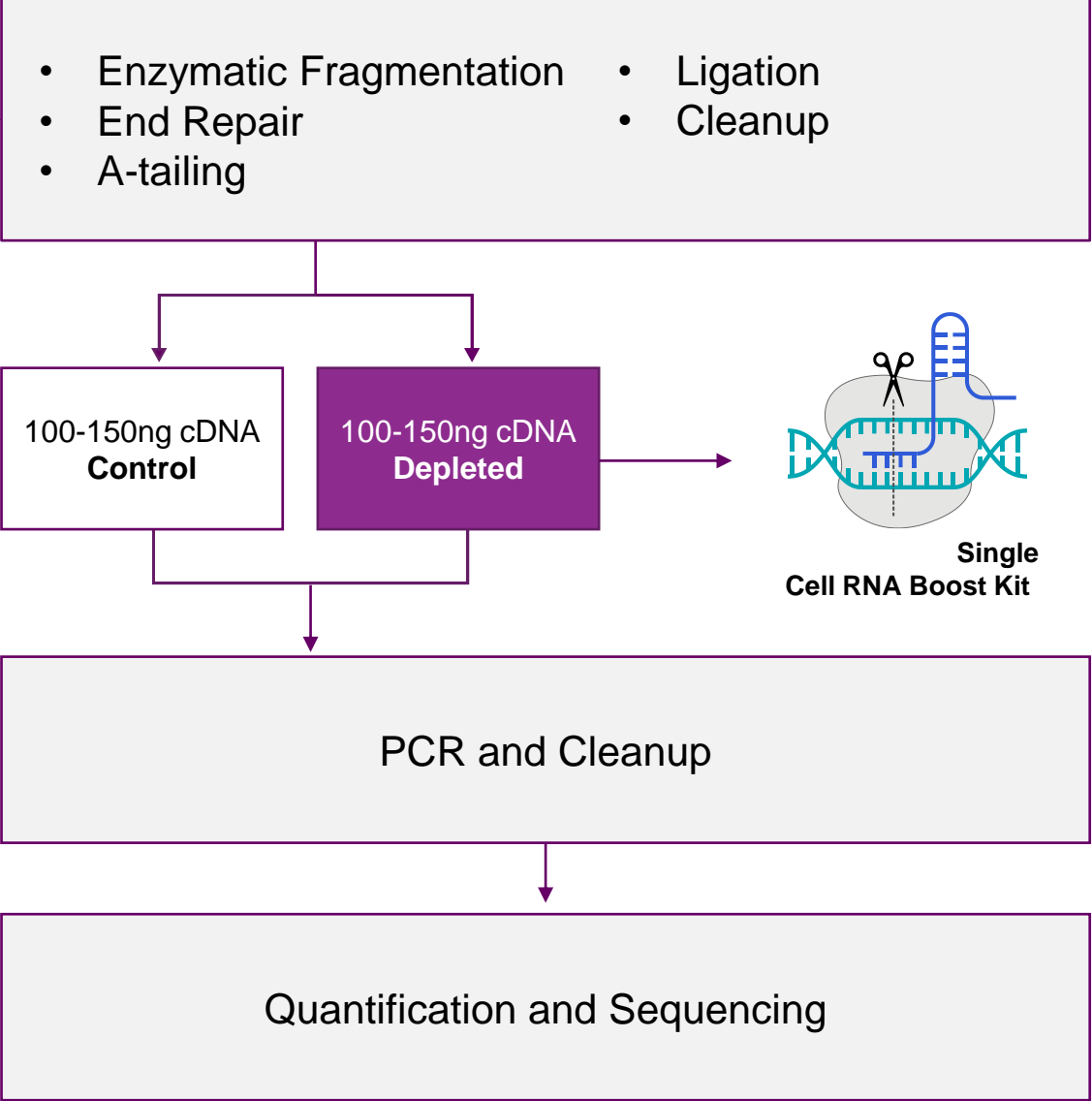
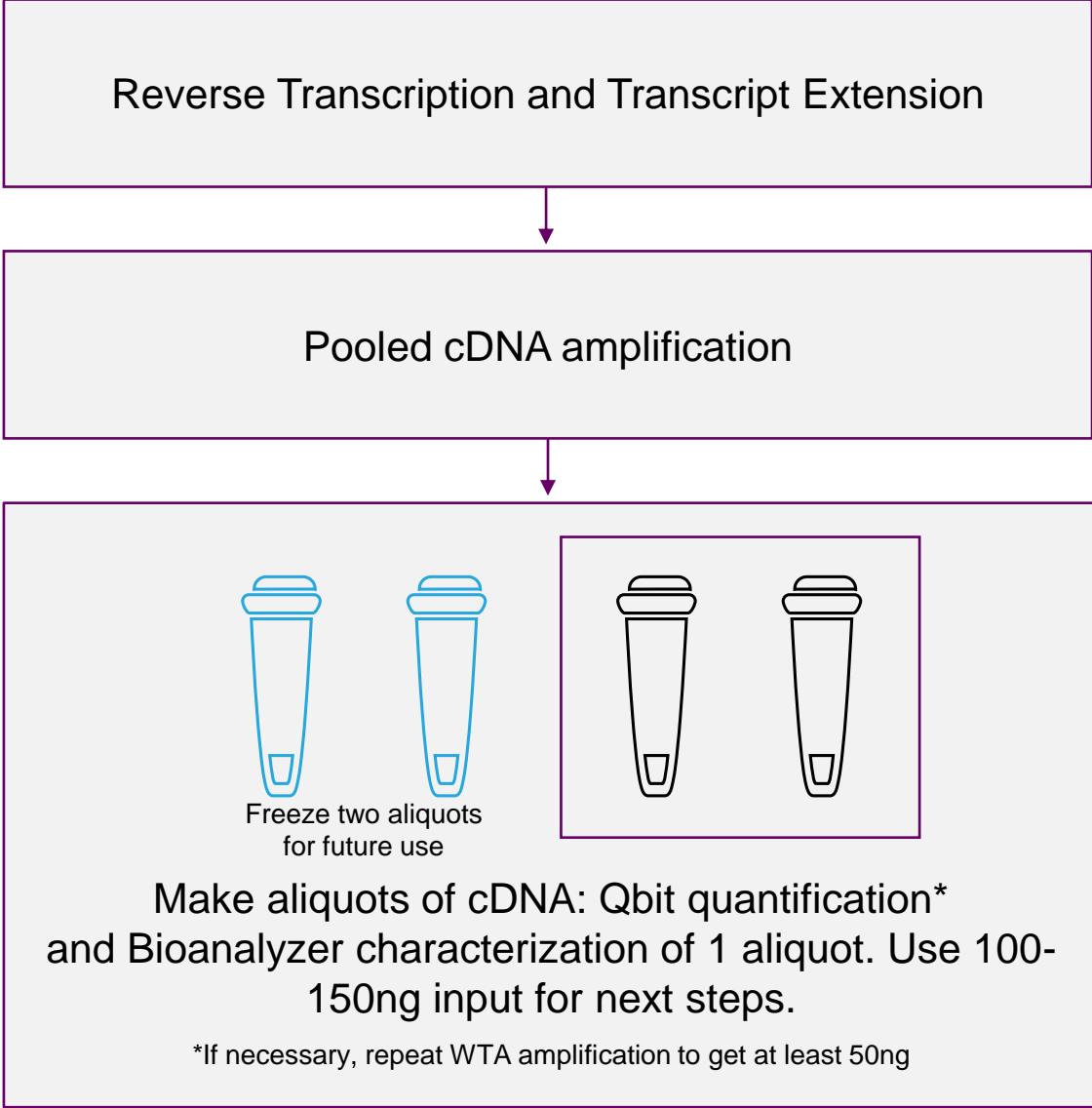


**2. Incubate** with cDNA at 37°C - 1 hour incubation

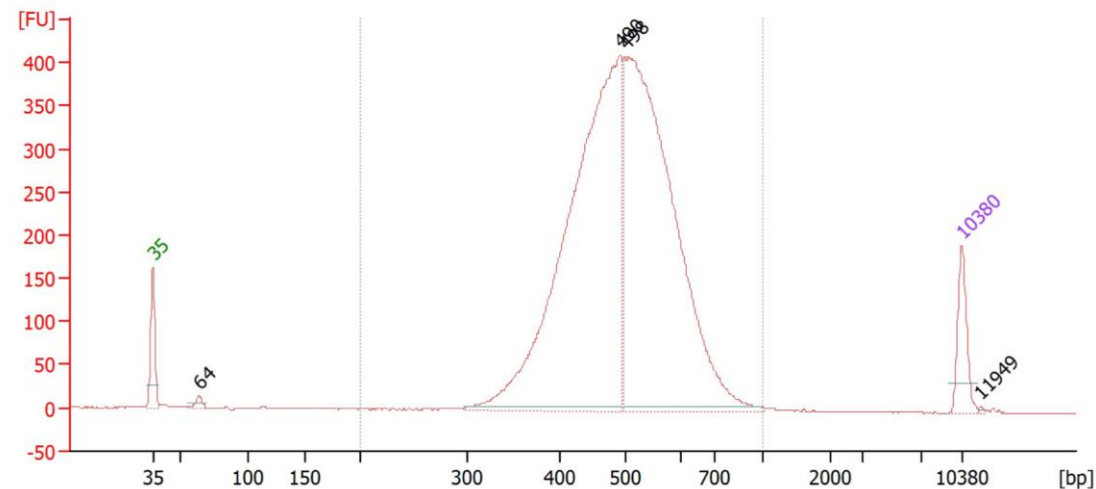
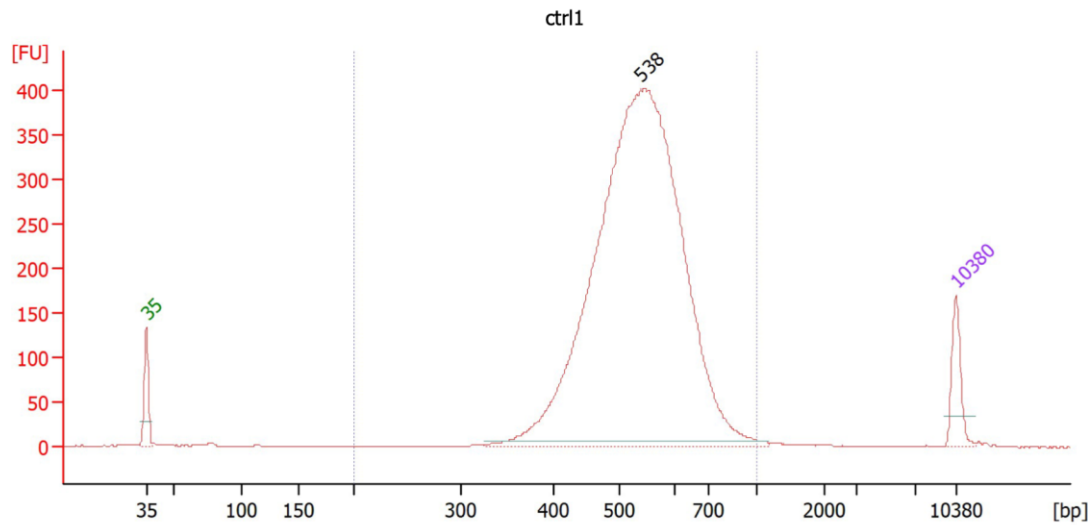
**3. Bead clean** to remove cut fragments (30 min)



# Process Workflow



# Bioanalyzer Traces



- Traces of the control vs depleted condition for libraries treated with CRISPRclean
- The depleted condition is around 50bp shorter in size in this example, however, it is normal for the size shift to increase or decrease.

Can I use the CRISPRclean depletion on mouse samples?

- Yes, but the depletion rate is lower since the guides were designed for human samples. We sell mouse-specific ribo-mito depletion guides.

Does the CRISPRclean depletion use all of the cDNA from my 10X prep?

- No, only 1 tube of the available cDNA from a given 10X prep is used in general. The customer is free to customize this as needed to hit the 100-150ng input recommendation.

Is this kit compatible with the 5' Chromium assay? What about Visium spatial?

- Yes, it is compatible with both kits. Our depletion panel is designed to tile across the full length of transcripts.

What is the sequencing depth recommendation for the depleted condition?

- In general, single cell users use between 25K – 50K reads/cell depending on their application. For the depleted condition, users can choose to sequence 12.5K – 25K reads/cell to see the same information or sequence at the same depth to see more information.

Can I use SPRI beads instead of AMP beads?

- Yes, we have tested this in-house and they both work with our assay.



Due to depletion, lower yield on the depleted product is expected

- Please load the depleted sample and control sample at the same loading concentration.

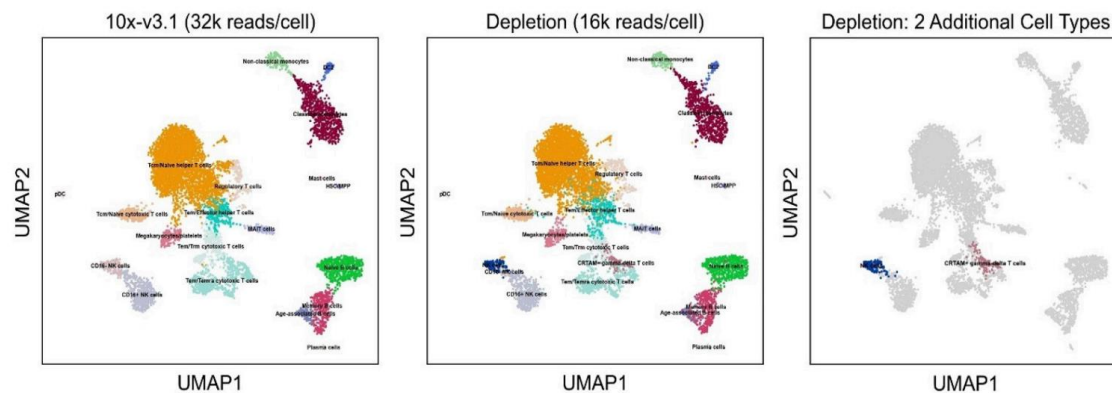
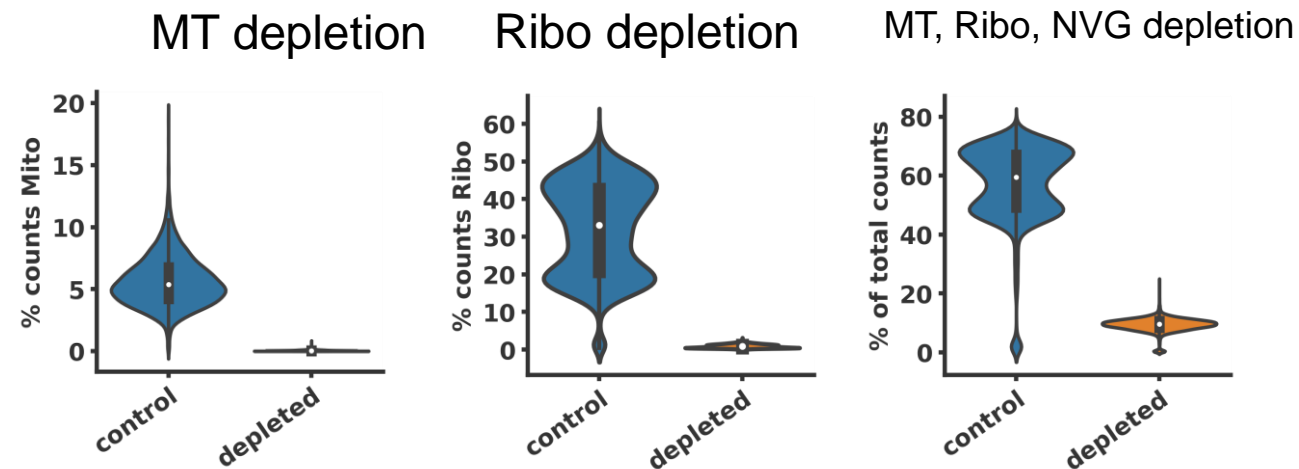
qPCR is recommended as the quantification method for highest accuracy in controlling cluster density on the sequencer

High complexity libraries going into depletion give the best results

- If low input into depletion, risk is that the benefit of depletion will not be observed
- Additional PCR is recommended prior to depletion, if necessary, rather than post depletion

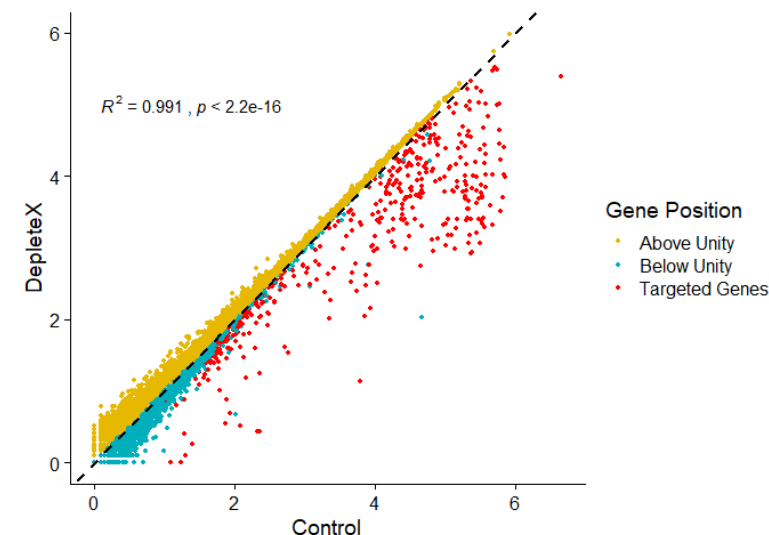
# Data Analysis

- Cell Ranger is recommended to compare the control to the depleted sample to see the benefit of depletion in sequencing saturation metrics.
- Secondary analysis benefits, including a boost in UMI/cell and genes/cell, can be found using our tutorial in [R](#)
- Example outputs:
  - Ribo, Mito, and total depletion including NVG content
  - UMAP plots



**Figure 2:** UMAP plots for PBMC samples. 10X v3.1 (32k reads/cell) annotated cell types (left), Depletion (16k reads/cell) annotated cell types (middle), and highlighted additional cell types with depletion (right). PBMCs were annotated using the CellTypist python package here: <https://doi.org/10.1126/science.ab15197>

R-Squared shows only Targeted Genes are being depleted



# Feedback Request

- We are interested in your feedback!
- .csv files for the control and depleted samples from Cell Ranger
  - If not using Cell Ranger, please provide the following metrics for control and depleted samples
    - Sequencing saturation

# Additional Questions or Concerns?



Please contact me directly: [smita.p@jumpcodegenomics.com](mailto:smita.p@jumpcodegenomics.com)  
and/or [support@jumpcodegenomics.com](mailto:support@jumpcodegenomics.com)