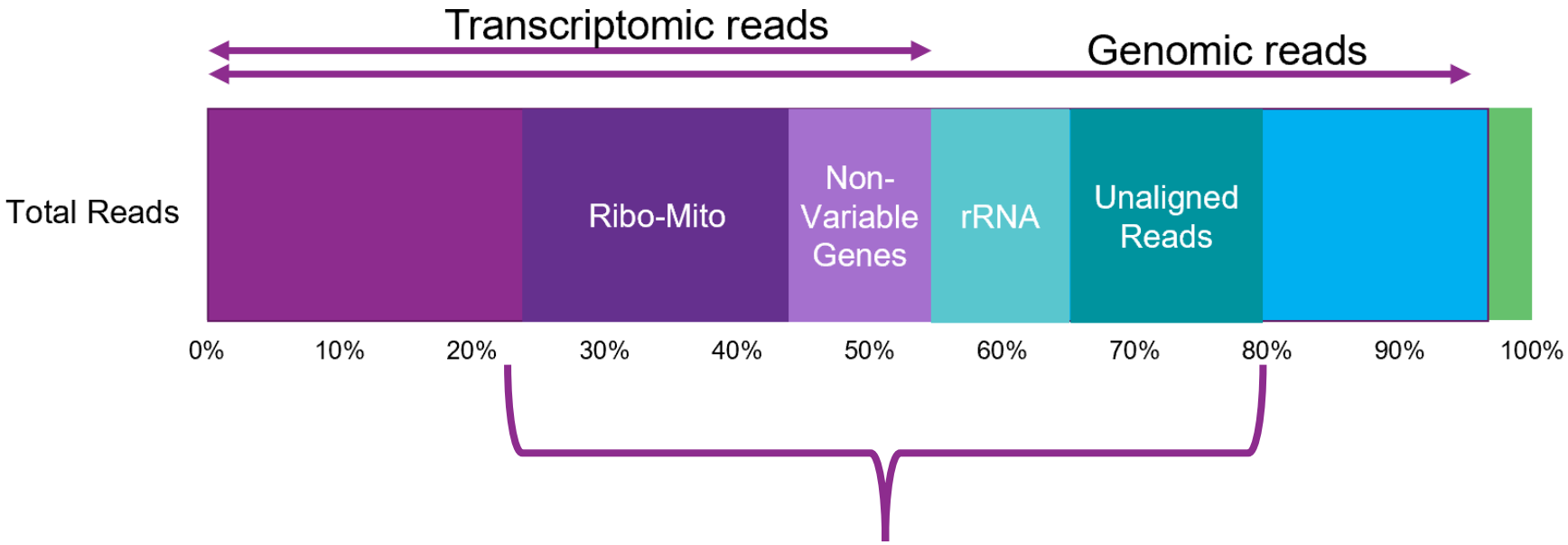
Pre-flight Customer Information: Single Cell Sequencing with MAS-Seq and Jumpcode Depletion

Goals

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

- Provide a high-level overview of the process
- Answer any customer questions, including FAQ
- Provide a 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

Workflow Overview

Single Cell RNA Boost for PacBio MAS-Seq for 10x Single Cell 3’ Kit

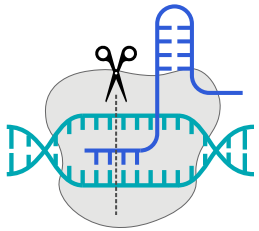
1. 10x Chromium Next GEM Single Cell 3’ Kit v3.1
2. **Single Cell RNA Boost Kit**
   - **Hands-on time: 45 min | Total time: 2.5 hrs**
   - A. Ribonucleoprotein Complex Formation
   - B. CRISPR Digestion
   - C. Proteinase K Treatment
   - D. Bead Cleanup
   - **Safe stopping point**
3. PacBio MAS-Seq library preparation
4. PacBio sequencing

Workflow Details

10X 3’ Single Cell Kit (to step 2.4, cDNA synthesis)

50-100ng cDNA **Control**

50-100ng cDNA **Depleted**



CRISPRclean Single Cell RNA Boost Kit

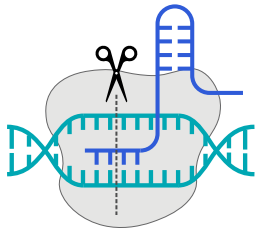
MAS-Seq Library Preparation with modification (Step 2.1)

Quantification and PacBio Sequencing

MAS-Seq Depleted cDNA Input Material	Recommended MAS-Seq TSO PCR Cycles	Target Yield:
50 – 75 ng	4-5*	150ng – 1,000ng
25 – 49 ng	5-6*	
15 – 24 ng	6-7*	

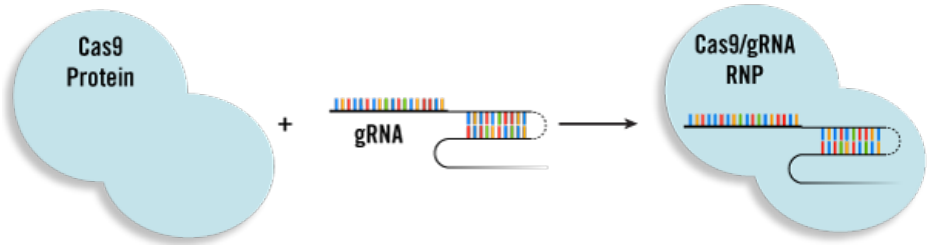
*Normalization of depleted cDNA input material to PB MAS-Seq 15ng or 60-75ng is not recommended

The entire process can be inserted into any single cell application



CRISPRclean Single Cell RNA Boost Kit

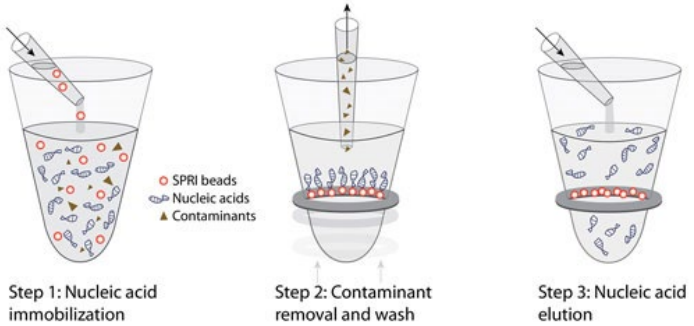
1. **Make RNP**: combine guides with Cas9 *(15 min)*



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

3. **Thermolabile Proteinase K Treatment**

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



Single-Cell 10x cDNA Bioanalyzer Electropherograms

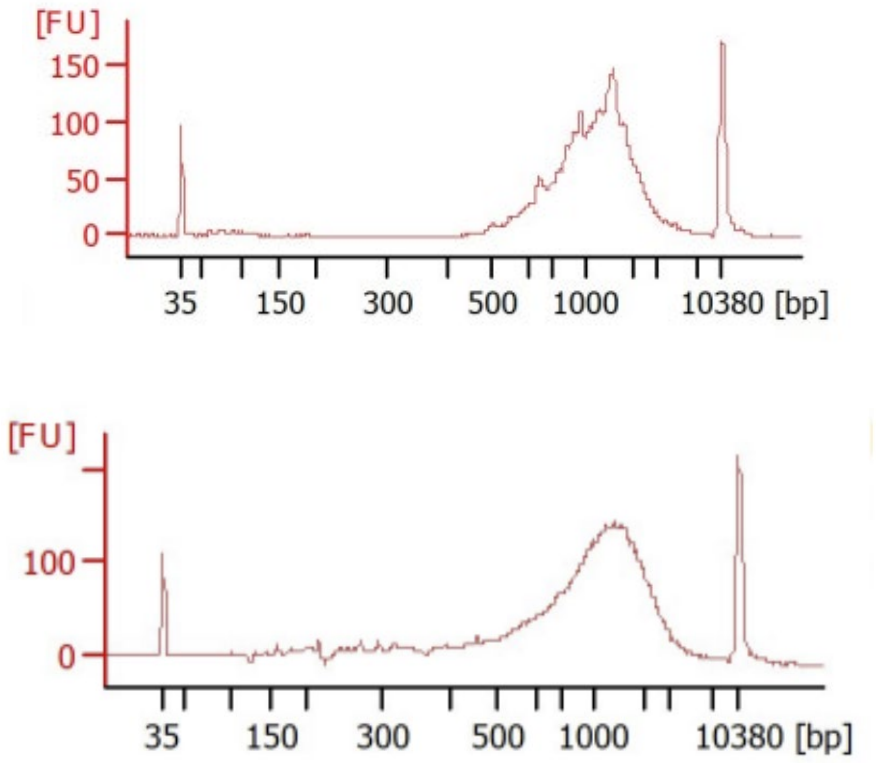
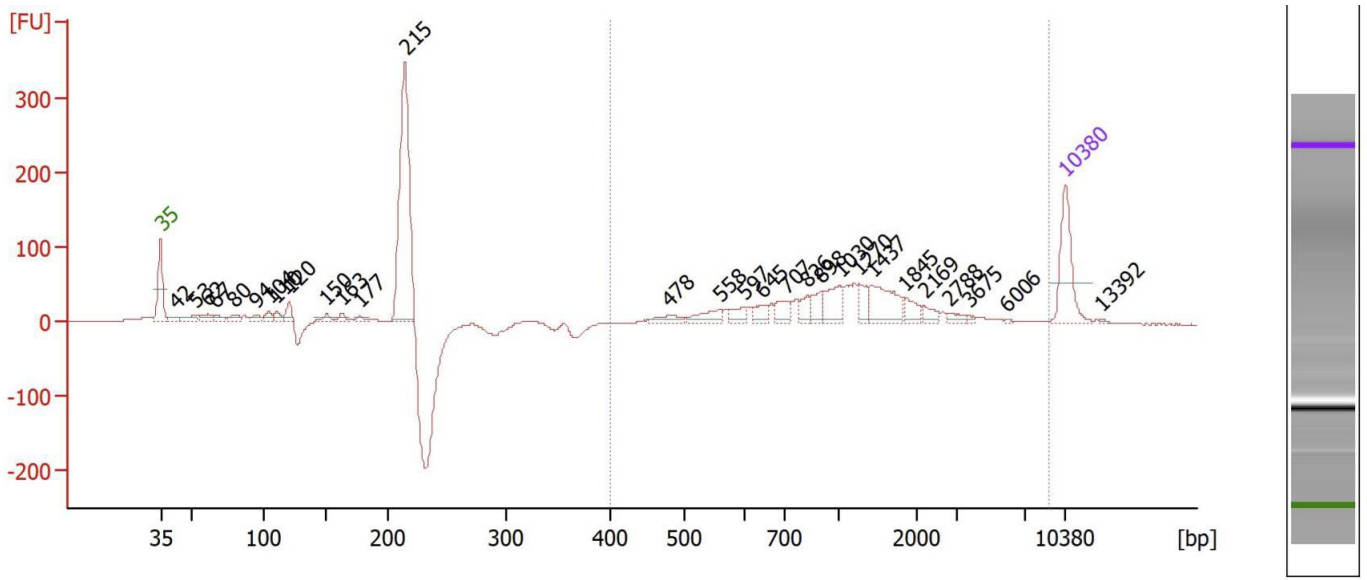


Figure 1. Example of an expected cDNA size distribution using 50-100 ng of PBMC single-cell cDNA for CRISPR-Cas9 depletion. An aliquot of the depleted cDNA was loaded on the Agilent Bioanalyzer using the Agilent High Sensitivity DNA Kit.

- Single-cell amplified cDNA was generated from PBMC (3,000-10,000 target) cells using the 10x Chromium 3' single cell v3.1 kit.
  - Samples were evaluated at 1 ng/uL using the Bioanalyzer DNA HS system.
- Expected size distribution 500 – 1,500 bp remains consistent between untreated and depleted samples.
  - Untreated Condition (Top)
  - **CRISPRclean Depleted Condition (Bottom)**

Assay FAQs:
Is Proteinase K Treatment Required after depletion? **Yes!**

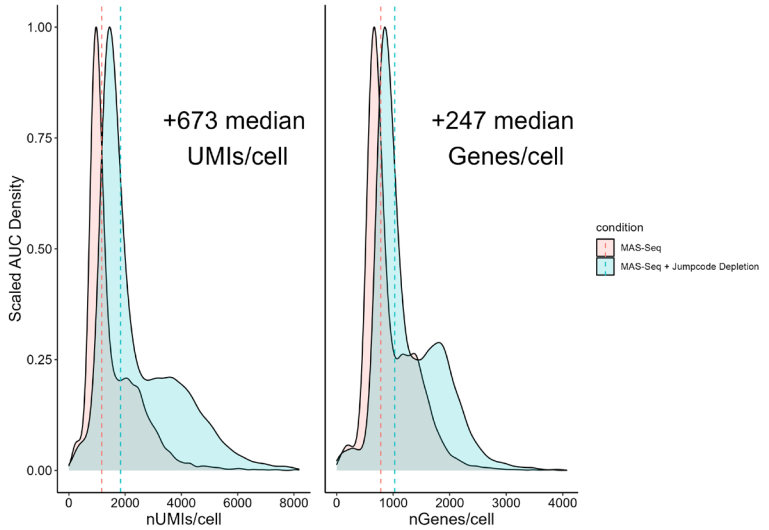
BA trace without Proteinase K treatment

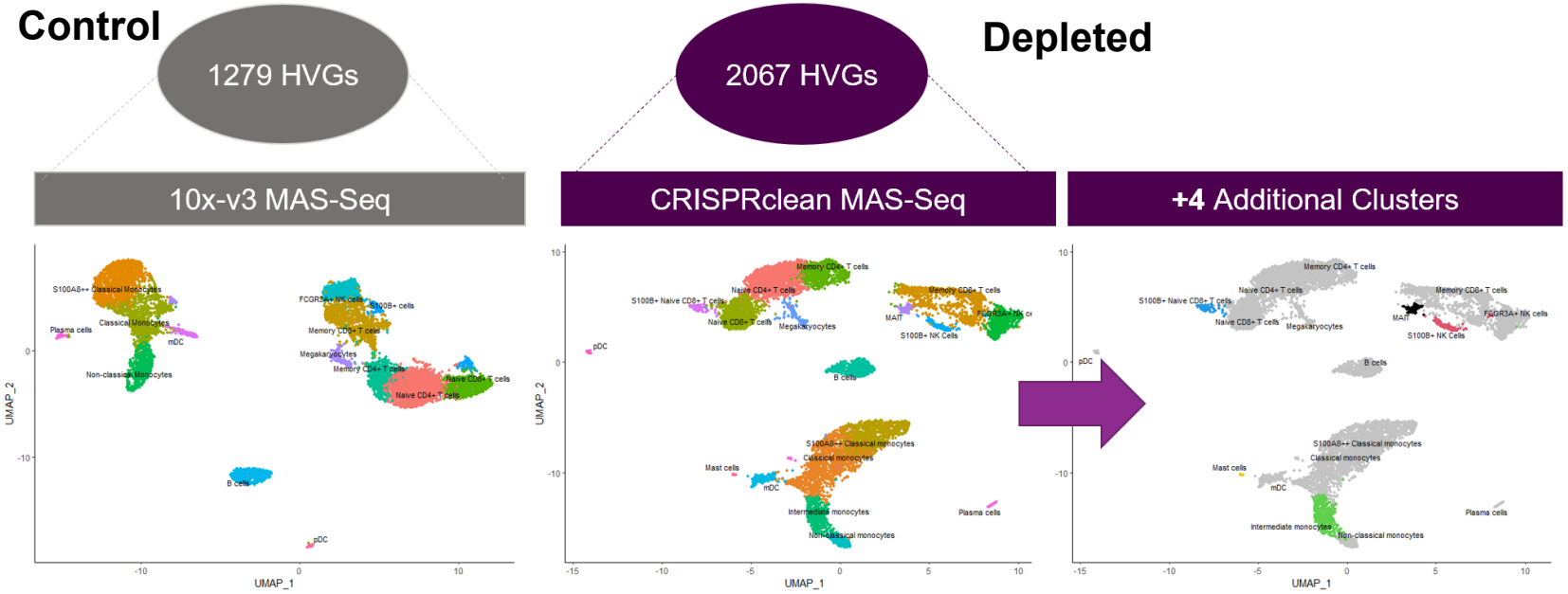


- Artifact peak at ~215bp indicates the presence of Cas9 protein attached to cDNA.
- Thermolabile Proteinase K treatment and heat inactivation @ 65C of both enzymes will allow these components to be washed away during Pronex bead cleanup.

Data Analysis

- PacBio data analysis is recommended to compare the control to the depleted sample.
- 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
  - Genes detected and UMI benefit
  - UMAP plots





Feedback Request

- We are interested in your feedback!

Additional Questions or Concerns?

Please contact me directly: smita.p@jumpcodegenomics.com
and/or support@jumpcodegenomics.com