JUMPCODE GENOMICS

USER MANUAL

KIT1018

CRISPRclean® Single Cell RNA Boost Kit

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Contact Us

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Find us at our website: jumpcodegenomics.com

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Product overview

Traditionally, single cell data processing incorporates certain filtering and normalization steps prior to canonical clustering and downstream interpretation. Instead of filtering those reads *in silico*, CRISPRclean removes those reads *in vitro*, redistributing 50% of sequencing reads to biologically relevant transcripts —allowing you to maximize gene and UMI sensitivity.

CRISPRclean leverages CRISPR depletion and a specifically designed guide RNA set to remove reads that are not incorporated in downstream analysis. The CRISPRclean Single Cell RNA Boost Kit gives you the ability to cut through the noise with minimal impact on your workflow, and maximum confidence on your results.

Content for depletion was designed by analyzing a cohort of publicly available 10x Genomics® Chromium single cell datasets from various sources. Roughly 30-50% of Chromium single cell reads align to the genome but not the transcriptome, and thus, are ignored during conventional data analyses. By tailoring CRISPR guides to deplete these genomic intervals, in addition to highly expressed protein-coding ribosomal and mitochondrial genes and non-variable genes that vary little in expression between different tissue types, one is able to redistribute ~50% of reads to the transcriptome. A list of the genomic intervals and genes targeted for depletion is provided on the Jumpcode Genomics website.

Description	
Assay time	2 hours
Hands-on time	45 min
Input	Uses one of four Chromium cDNA aliquots per prep
Method	Single cell 3’ gene expression libraries for 10x Genomics
Designed to deplete	• Unaligned reads • Ribosomal • Mitochondrial • Non-variable genes
Jumpcode validated	10x Genomics Chromium Next GEM Single Cell 3’ Reagent Kit v3.1

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Workflow

CRISPRclean Single Cell RNA Boost for 10x Genomics is a simple 3-step protocol easily integrated into 10x Genomics Chromium Next GEM Single Cell 3' gene expression protocol.

The user follows the Chromium Next GEM Single Cell 3' protocol v3.1, without modification, until the end of **Step 3.4 – Post Ligation Cleanup – SPRIselect**. Immediately after Step 3.4, the user switches to the CRISPRclean protocol. After the CRISPRclean protocol is complete, the user resumes the Chromium Next GEM Single Cell 3' protocol v3.1 at **Step 3.5 – Sample Index PCR** and follows the protocol until completion of the libraries.

Protocol Overview:

A. Perform library preparation up to and including Step 3.4 post-ligation cleanup
- **Step 1: GEM generation and barcoding**
- **Step 2: Post GEM-RT cleanup and cDNA amplification**
- **Step 3: Gene expression library construction**
  - At the end of **Step 3.4**, elute the sample in 16 µL of Nuclease-Free Water (instead of 30.5 µL of Buffer EB).

B. **CRISPRclean Single Cell RNA Boost Kit**
- Ribonucleoprotein complex formation
- CRISPR digestion
- Size selection

C. Continue with Chromium Next GEM Single Cell 3' protocol until completion of libraries
- **Step 3.5: Sample Index PCR**
- **Step 3.6: Post Sample Index PCR Double Sided Size Selection - SPRIselect**

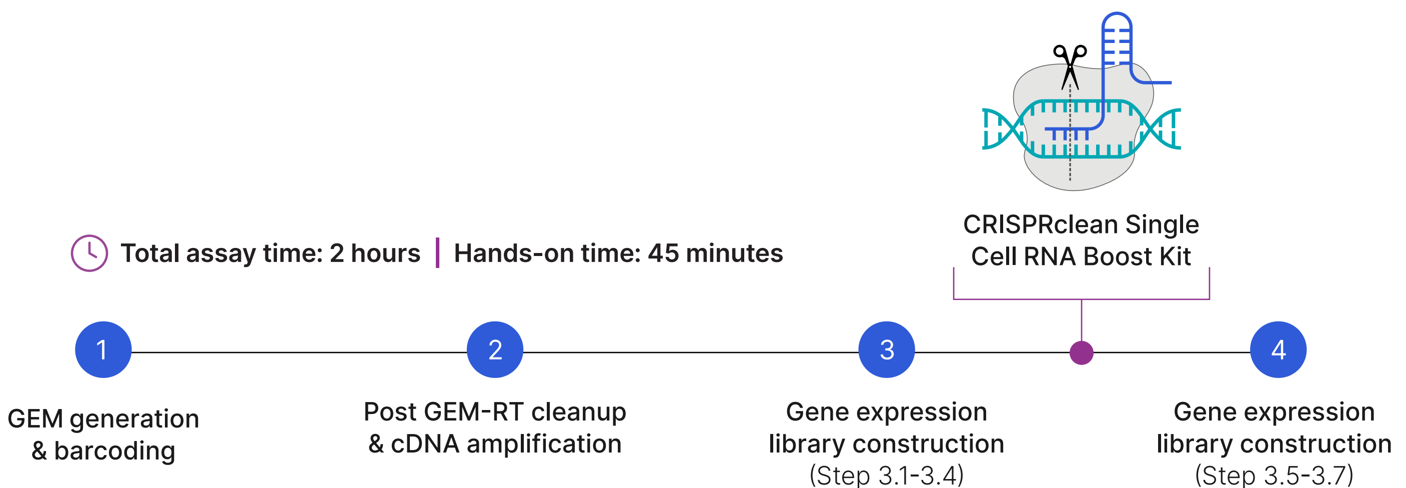

Figure 1: Schematic illustrating the position where CRISPRclean protocol fits into 10x Genomics Chromium Next GEM Single Cell 3' library preparation protocol.

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Kit contents, storage, and shelf life

The CRISPRclean Single Cell RNA Boost kit contains enough material to deplete 24 Chromium single cell 3′ gene expression libraries. The kit shelf life is 12 months from the date of manufacturing when stored according to manufacturer's recommendations. The kit contents and storage temperatures are indicated in the tables below.

ASY-1060: CRISPRclean Single Cell RNA Boost Kit

24 samples

Kit contents	Storage temperature
CRISPRclean Cas9	-20°C
CRISPRclean 10x Cas9 Buffer	-20°C
CRISPRclean RNase Inhibitor	-20°C
CRISPRclean Nuclease-free Water	*4°C

* **Note:** Store at 4°C after first use.

ASY-1061: CRISPRclean Single Cell RNA Boost Guide RNA

24 samples

Kit contents	Storage temperature
CRISPRclean Single Cell Guide RNA	-80°C

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Required materials provided by the user

Reagents

- cDNA-amplified and adapter-ligated product from Chromium Next GEM Single Cell 3' Reagent Kits protocol v3.1
- AMPure® XP beads (stored at 4°C)
- 80% Ethanol (freshly prepared and stored at room temperature)

Consumables and hardware

- 10, 20, 200, and 1000 µL pipettes
- RNase-free barrier pipette tips
- 0.2 mL, 0.5 mL and 1.5 mL nuclease-free microcentrifuge tubes (Eppendorf™ LoBind or similar)
- DNA analysis instrument, such as the Agilent 2100 Bioanalyzer® System in conjunction with the Bioanalyzer® RNA 6000 Nano or Pico Kit, and High Sensitivity DNA Kit or Qubit®
- Magnetic stand (depending on sample number, a plate version may be preferred)
- Microcentrifuge
- Thermal cycler
- Vortex instrument
- Heating block
- Ice

Warnings and precautions

General:

- Periodically, optimizations and revisions are made to the kit components and user manual. Therefore, it is important to follow the protocol included with or appropriate for the kit in question. This can be done by comparing the name and version number of the CRISPRclean product to the name and version number of the protocol. If you need further assistance in this regard, contact support@jumpcodegenomics.com.
- This protocol describes the reagents, best practices, workflow and method details for CRISPRclean depletion as it applies to the 10x Genomics Chromium Next GEM Single Cell 3' protocol v3.1. Please refer to the Chromium Next GEM Single Cell 3' protocol for warnings and precautions related to Chromium library preparation reagents.
- Use properly calibrated pipettes as library preparation is sensitive to pipetting error.
- Ensure that all pipette tips, microcentrifuge tubes, and other consumables are DNase- and RNase-free.
- Follow good laboratory practices throughout the protocol, including RNA-safe procedures when handling RNA.

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For specific reagents

- Do not remove Cas9 and RNase Inhibitor from -20°C until before use. Return to -20°C immediately after use.
- Store the Guide RNA at -80°C. Do not remove the reagent from -80°C until time of use. Return it to -80°C immediately after use.
- We recommend a maximum of 3 freeze-thaw cycles for the Guide RNA. The Guide RNA tube contains material for 24 samples. It is strongly recommended that multiple smaller aliquots of the Guide RNA be prepared when the reagent is first thawed in order to reduce the number of freeze-thaw cycles affecting the Guide RNA.
- Do not freeze AMPure® XP beads.
- Allow AMPure XP beads to come to room temperature for 30 minutes before use.
- Vortex AMPure XP beads immediately before use. Ensure that they are in a uniform suspension before use.

Revision log

Version	Date	Description
V1.0	December 2021	Early access release
V2.0	March 2022	Full launch release

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Protocol

Before starting CRISPRclean Single Cell Boost Kit protocol

1. Follow the Chromium Next GEM Single Cell 3' protocol v3.1 up to and including Step 3.4.
2. At the end of **Step 3.4: Post Ligation Cleanup – SPRIselect**, elute the library in 16 µL of CRISPRclean Nuclease-Free Water, instead of 30.5 µL of Buffer EB.
3. Transfer 15 µL of the sample to a new 0.2 mL or 0.5 mL nuclease-free microcentrifuge tube.
4. Proceed to the **Step A: Ribonucleoprotein complex formation**.

Step A: Ribonucleoprotein complex formation

Materials provided

- CRISPRclean 10X Cas9 Buffer
- CRISPRclean RNase Inhibitor
- CRISPRclean Guide RNA
- CRISPRclean Cas9

Required materials provided by the user

- Nuclease-free microcentrifuge tubes

1. Combine the following reagents in the order listed below in a 0.2 mL or 0.5 mL nuclease-free microcentrifuge tube.

Component	Volume
CRISPRclean 10X Cas9 Buffer	1.0 µL
CRISPRclean RNase Inhibitor	1.0 µL
CRISPRclean Guide RNA	3.9 µL
CRISPRclean Cas9	2.3 µL
Total Volume	**8.2 µL**

2. Mix the contents gently by pipetting up and down. Centrifuge briefly to collect the contents at the bottom of the tube.
3. Leave the tube on the laboratory bench for 10 minutes at room temperature. This is the ribonucleoprotein complex (RNP).
4. Proceed immediately to **Step B: CRISPR digestion**.

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Step B: CRISPR digestion

Materials provided

- CRISPRclean 10X Cas9 Buffer
- Ribonucleoprotein complex (RNP) from Step A

Required materials provided by the user

- Adapter-ligated cDNA library from Step 3.4 of the Chromium Next GEM Single Cell 3' Reagent Kits protocol v3.1
- Nuclease-free microcentrifuge tubes
- Thermal cycler or heating block
- AMPure XP beads (stored at room temperature)
- Ice

1. Combine the following reagents in a 0.5 mL nuclease-free microcentrifuge tube:

Component	Volume
CRISPRclean 10X Cas9 Buffer	1.5 µL
Adapter-ligated cDNA library from Step 3.4 of the Chromium Next GEM Single Cell 3' protocol	15 µL
Ribonucleoprotein complex (RNP) from Step A	8.2 µL
Total Volume	**~25 µL**

2. Mix the CRISPR digestion reaction gently by pipetting up and down. Centrifuge briefly to collect the contents at the bottom of the tube. Incubate the tube at 37°C for 60 minutes.

> **Note:** During this incubation period, remove the AMPure XP beads from the refrigerator and place them on the laboratory bench to bring them to room temperature. A minimum of 30 minutes at room temperature is recommended before use.

3. After the incubation is complete, transfer the tube to ice for ~2 minutes.

4. Briefly centrifuge the tube to collect the contents at the bottom of the tube. Transfer the tube to the laboratory bench. Immediately proceed to **Step C: Size Selection**.

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Step C: Size Selection

Hands-on time: 15 min | Total time: 45 min

Materials provided

- CRISPRclean Nuclease-Free Water

Required materials provided by the user

- CRISPR-digested product from Step B
- AMPure XP beads (brought to room temperature prior to use)
- 80% Ethanol, freshly prepared (room temperature)
- Nuclease-free microcentrifuge tubes
- Magnetic stand

1. Add 25.3 µL of Nuclease-Free Water to the CRISPR digestion reaction.
2. Mix gently by pipetting up and down several times. Place the tube on the laboratory bench at room temperature.
3. Add 30 µL of well-resuspended, room-temperature AMPure XP beads, pipette up and down several times to mix and incubate the tube at room temperature for 10 minutes. Mix the sample halfway through the incubation by pipetting up and down several times.
4. Place the tube on the magnetic stand. Allow the solution to clear (3–5 minutes). Remove and discard the supernatant without disturbing the beads.
5. Add 200 µL of freshly prepared 80% ethanol to the tube.
6. After 30 seconds, remove and discard the ethanol. Leave the tube on the magnetic stand during this step.
7. Repeat the wash step (previous two steps) with 200 µL of 80% ethanol. Remove as much ethanol as possible after the second wash.
8. Open the cap of the tube while it is on the magnetic stand and allow the beads to air dry for 5 minutes.
9. Add 31 µL of CRISPRclean Nuclease-Free Water to the beads.
10. Remove the tube from the magnetic stand. Mix the contents by pipetting up and down several times to fully resuspend the beads in the liquid.
11. Incubate the tube at room temperature for 10 minutes. Mix the sample halfway through the incubation (5 minutes) by pipetting up and down several times.
12. Place the tube on the magnetic stand. Allow the solution to clear.
13. Transfer 30 µL of the supernatant to a new microcentrifuge tube. Place the tube on ice. This tube contains the CRISPRclean-depleted Chromium library.

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14. Proceed to the beginning of **Step 3.5: Sample Index PCR** of the Chromium Next GEM Single Cell 3' protocol v3.1. **Before proceeding, please read the following notes regarding Steps 3.5 and 3.6**.

> **Step 3.5: Sample Index PCR:** Refer to recommendations in the Chromium Next GEM Single Cell 3' protocol to determine the ideal number of PCR cycles. No adjustment in PCR cycles is necessary to compensate for CRISPRclean depletion.

> **Step 3.6: Post Sample Index PCR Double Sided Size Selection – SPRIselect:** Elute the library in 20 µL of Buffer EB, instead of 35.5 µL of Buffer EB, to increase the concentration of the DNA library.

Library validation

It is recommended that the user assess library yield using a dsDNA-specific fluorescence-based method (such as a Qubit fluorometer) and library fragment profile on an Agilent Bioanalyzer 2100 or equivalent instrument before sequencing. CRISPRclean-depleted libraries have a similar fragment profile to those of standard Chromium single cell libraries with a typical shift of 20-50 bp to the left of the electropherogram (i.e., the average fragment size is 20-50 bp smaller than that of a standard Chromium library).

It is also recommended that qPCR quantification be performed to ensure optimal cluster density on an Illumina sequencing instrument.

Once the library has been quantitated, it is ready for cluster generation on an Illumina instrument. Please follow standard Illumina protocols for the loading of the library and for cluster generation on the instrument. If the library needs to be stored before sequencing, please store it at -20°C.

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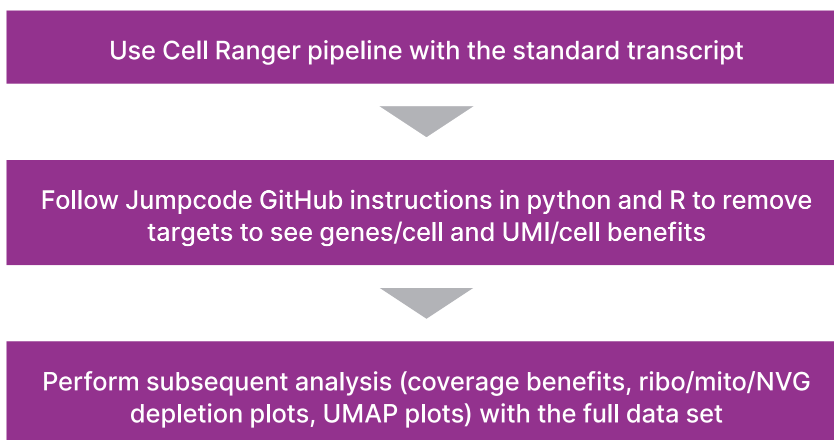
Bioinformatics guidelines

10x Genomics Chromium single cell data generated with CRISPRclean Single Cell RNA Boost Kit can be analyzed using the Cell Ranger pipeline and other commonly used single cell data analysis tools.

How to compare CRISPRclean-depleted data to the control data

The standard data analysis workflow must be modified to observe the impact of CRISPRclean depletion on metrics that are typically used to assess single cell library quality, such as numbers of genes per cell and numbers of UMIs per cell. Jumpcode has developed a workflow in python and R for users to see the UMI/cell and genes/cell benefits. Additionally, guidelines are provided to remove dead cells from downstream analysis. UMAP visualization is also available in the GitHub site.



Warning: If data from CRISPRclean-depleted libraries is aligned to the comprehensive standard human Cell Ranger Index to perform a comparative analysis between standard Chromium data and equivalent CRISPRclean-derived data, no significant improvement in the number of UMIs and genes would be observed with the latter. This is the case because the genes targeted for CRISPRclean depletion typically constitute ~35% of all Chromium sequencing reads and a significant proportion of unique reads in the sequencing data. When molecules derived from these genes are removed from the library, so are a significant proportion of UMIs in the library.

How to compare CRISPRclean-depleted data to the control data

https://github.com/JumpcodeGenomics/Single-Cell-RNA-Boost

Example outputs:

- Ribo, Mito, and total depletion including NVG content
- Genes detected and UMI benefit
- UMAP plots

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