JUMPCODE GENOMICS | Compatible PRODUCTS 10x GENOMICS

USER MANUAL v1.1

DepleteX® Single Cell RNA Boost Kit (Human) with compatible panels

KIT1037

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Contact Us

If you have any questions, contact Technical Support at support@jumpcodegenomics.com 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*, DepleteX® removes those reads *in-vitro*, redistributing sequencing reads to less abundant transcripts.

DepleteX technology leverages CRISPR/Cas9 technology with specifically designed guide RNAs to remove highly abundant transcripts. The DepleteX Single Cell RNA Boost Kit gives the user the ability to cut through the noise, with minimal impact on workflow, while maximizing the view of biology.

Contents for depletion were designed by surveying a collection of publicly available 10x Genomics® Chromium single cell datasets from 14 different tissues. CRISPR complexes of Cas9 endonuclease and guide RNA are applied to cDNA derived from single cell RNA content to deplete (1) highly expressed protein-coding ribosomal and mitochondrial genes, (2) genes ubiquitously expressed across different tissues (non-variable genes), and (3) conservatively captured intergenic regions. A list of the intervals targeted for depletion is provided on the Jumpcode Genomics website.

DepleteX Single Cell RNA Boost Kit (Human)

Description	
Assay Time	2 hours
Hands-On Time	45 minutes
Input	100-150 ng of cDNA • Generated from 10x Genomics Chromium 3’ single cell kit (v3.1), standard throughput • 3,000 - 10,000 target cell recovery per sample.
cDNA Fragment Size	500 – 1,500 bp
Method	CRISPR/Cas9 depletion
Designed to deplete	• Intergenic regions • Ribosomal genes • Mitochondrial genes • (Optional; included) Non-variable genes (NVG) • (Optional; not included) RNA Depletion Panels for Liver, Globin, Insulin, and *Ribo/Mito Genes. Targets for depletion can be downloaded from Jumpcode website.
Library Prep	• Chromium Next GEM Single Cell 3’ GEM, Library & Gel Bead Kit v3.1, 16 rxns PN-100012 • Chromium Next GEM Single Cell 3’ GEM, Library & Gel Bead Kit v3.1, 4 rxns PN-1000128
Sequencing Platform	Illumina® systems

*Ribo/Mito Gene panel is available for both human and mouse

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Workflow

DepleteX Single Cell RNA Boost is a simple 3-step protocol easily integrated into the 10x Genomics® Chromium Next GEM Single Cell 3' gene expression protocol. This workflow can also be adapted to any single cell or spatial method to deplete undesired fragments from cDNA prior to sequencing.

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 DepleteX protocol. After the depletion 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 - SPRIselect**

- 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. **DepleteX Single Cell RNA Boost Kit**

- Ribonucleoprotein complex formation
- CRISPR digestion
- Bead cleanup

C. Continue with Chromium Next GEM Single Cell 3' protocol to completion:

- Step 3.5: Sample Index PCR
- Step 3.6: Post Sample Index PCR Double Sided Size Selection – SPRIselect
- Continue with subsequent steps without modification until library preparation is complete

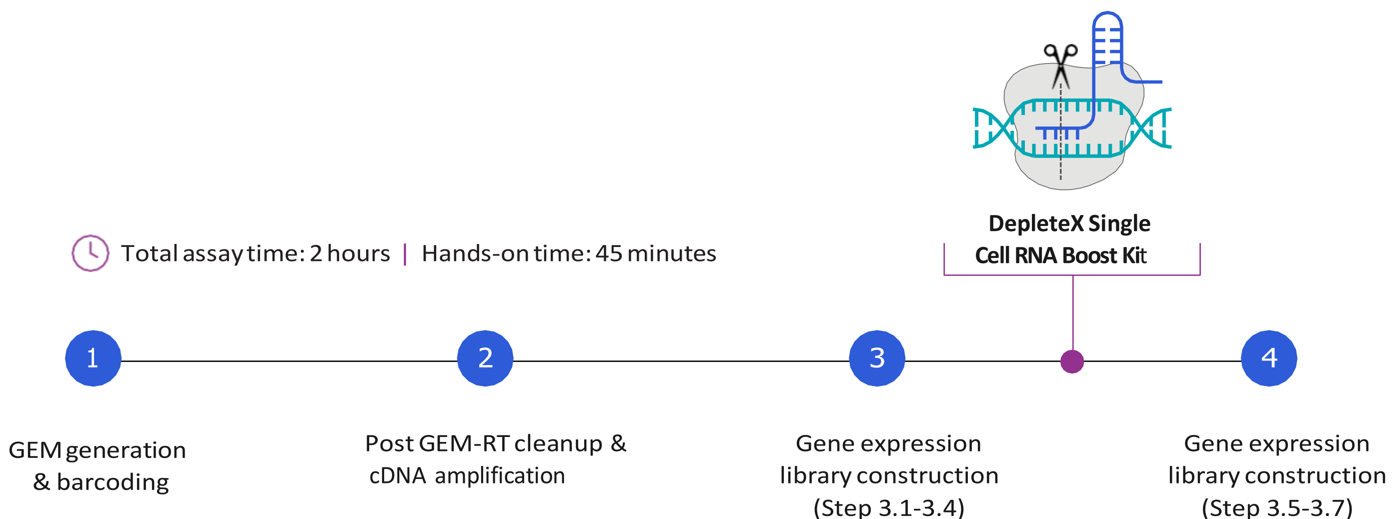

Figure 1: Schematic illustrating the point in the protocol where the DepleteX protocol fits into the 10x Genomics Chromium Next GEM Single Cell 3' library preparation protocol.

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Kit contents and storage

DepleteX Single Cell RNA Boost Kit (Human) contains sufficient material to deplete 24 10x Genomics® Chromium single cell 3' gene expression libraries. Kit contents and storage temperatures are indicated in the tables below.

KIT1037: ASY1060: Single Cell RNA Boost Kit (24 Samples)

Storage at -20°C

Kit contents	Part number	Quantity Per Box
Nuclease-Free Water	REA1023	2 tubes
10x Cas9 Buffer	REA1001	1 tube
RNase Inhibitor	REA1007	1 tube
Cas9	REA1000	1 tube

KIT1037: ASY1061-003: Single Cell RNA Boost Guide RNA (24 Samples)

Storage at -80°C

Kit contents	Part number	Quantity Per Bag
Human Single Cell Boost Guide RNA	REA1025-003	1 tube

KIT1037: ASY1080-001: Single Cell RNA Boost NVG Guide RNA (24 Samples)

Storage at -80°C

Kit contents	Part number	Quantity Per Bag
Human Single Cell Boost NVG Guide RNA	REA1120-001	1 tube

Compatible Jumpcode Guide RNA Panels for use in this protocol (sold separately):

Storage at -80°C

Kit	Kit Contents	Part Number	Quantity Per Bag
KIT1022: ASY1065	Guide RNA (Liver) (24 Samples)	REA1048	1
KIT1024: ASY1071	Guide RNA (Globin) (24 Samples)	REA1053	1
KIT1025: ASY1072	Guide RNA (Insulin) (24 Samples)	REA1054	1
KIT1027: ASY1068	Guide RNA (Ribo/Mito Genes) (24 Samples)	REA1051	1
KIT1036: ASY1067	Guide RNA (Mouse Ribo/Mito Genes) (24 Samples)	REA1050	1

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Required materials and equipment provided by the user

Kit contents	Part number	Quantity
Plastics	0.5 mL, 1.5 mL DNA LoBind Tubes	Eppendorf (Cat# 022431021)
	0.2 mL thin wall PCR tubes	General Lab Supplier
	Low-Retention Filtered Sterile Tips (10 µl, 20 µl, 200 µl and 1000 µl)	General Lab Supplier
Reagents	cDNA-amplified and adapter-ligated product from Chromium Next GEM Single Cell 3' Reagent Kits protocol v3.1	10X Genomics
	AMPure XP Beads	Beckman Coulter (Cat# A63881)
	Absolute Ethanol, 200 Proof	General Lab Supplier
	Qubit dsDNA HS Assay Kit	ThermoFisher Scientific (Cat# Q32854)
Equipment	Single Channel Pipettes (10 µl, 20 µl, 200 µl, and 1000 µl)	General Lab Supplier
	Multichannel Pipettes (10 µl, 20 µl, and 200 µl)	General Lab Supplier
	Vortex Mixer	General Lab Supplier
	Microcentrifuge	General Lab Supplier
	PCR Magnetic Rack or Stand for use with tubes	General Lab Supplier
	Ice Bucket	General Lab Supplier
	PCR Thermal Cycler	General Lab Supplier
	Qubit Fluorometer	ThermoFisher Scientific (Cat# Q33238)
	Automated electrophoresis such as TapeStation	General Lab Supplier
	DNA analysis instrument, such as the Agilent 2100 Bioanalyzer® System	General Lab Supplier

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Best Practices

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 Jumpcode 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 DepleteX 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.

Reagent Handling

- 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 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.
- Use magnetic stands appropriate for PCR tubes.

Revision log

Version	Date	Description
V1.0	September 2023	Launch Release
V1.1	April 2024	Edited for clarity and format. Appendix added to provide information on compatible guide panels.

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Input Material

The DepleteX Single Cell RNA Boost Kit is optimized for 100 - 150 ng of single-cell 3’ cDNA of input material. Efficient depletion and high-quality single cell data have been generated with single-cell cDNA generated from 3,000 - 10,000 individual cells per sample using the 10x Genomics® Chromium Next GEM Single Cell 3’ Kit (v3.1) standard throughput workflow. Lower amounts of starting material may result in higher duplication rates, reduced library complexity and additional changes to sequencing data quality.

Accurate cDNA quality assessment is recommended to maximize depletion efficiency and the efficiency of downstream steps. Validate input cDNA using a fluorometric based method, such as a Qubit Fluorometer. Evaluate quality using an automated electrophoresis method, such as the Agilent Bioanalyzer System with a High Sensitivity DNA reagent kit.

Guide Panels

This protocol contains instructions for the use of single guide RNA panels or combinations of two guide RNA panels for depletion.

Instructions for the following guide RNA panels are provided in the protocol:

- KIT1022 RNA Depletion Panel (Liver)
- KIT1024 RNA Depletion Panel (Globin)
- KIT1025 RNA Depletion Panel (Insulin)
- KIT1027 RNA Depletion Panel (Ribo/Mito Genes)
- KIT1036 RNA Depletion Panel (Mouse Ribo/Mito Genes)

If using two guide RNA panels together, follow the instructions in the Appendix:

- KIT1037 Single Cell RNA Boost Kit in combination with KIT1037 RNA Depletion Panel (NVG)
- KIT1037 Single Cell RNA Boost Kit in combination with KIT1024 RNA Depletion Panel (Globin)
- KIT1037 Single Cell RNA Boost Kit in combination with KIT1025 RNA Depletion Panel (Insulin)
- KIT1037 Single Cell RNA Boost Kit in combination with KIT1022 RNA Depletion Panel (Liver)

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Protocol

Before starting the DepleteX Single Cell RNA 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 Nuclease-Free Water.
3. Transfer **15 µL** of the sample to a new 0.2 mL or 0.5 mL nuclease-free microcentrifuge tube.
4. For depletion with single guide panels, proceed to **Step A: Single-Guide Panel Depletion** of Single Cell 3' Chromium cDNA. For combinations of guides, refer to Appendix A.

Step A: Single-Guide Panel Depletion of Single Cell 3' Chromium cDNA

☐ Hands-on time: ~30 minutes | Total time: ~2 hours

Reagent Preparation

Item	Storage	Handling
Adapter-ligated cDNA library from Step 3.4 of the Chromium Next GEM Single Cell 3' protocol v3.1	-20°C	If frozen, thaw on ice. Finger-tap mix and spin down. Keep on ice. Return to freezer immediately after use.
Cas9		
RNase Inhibitor		
Single-Guide Panel for Single-Cell 3' Depletion	-80°C	
10X Cas9 Buffer	-20°C	Thaw at room temperature. Vortex briefly and spin down. Keep on ice.
Nuclease-Free Water		
AMPure XP Beads	2°C to 8° C	Bring to room temperature. Vortex and invert mix.
80% Ethanol	Room temperature	Prepare fresh.

A.1: Ribonucleoprotein (RNP) Complex Formation

1. At room temperature, combine the following reagents in the order listed below in a 0.2 mL PCR tube:

	RNP Complex Formation Reaction[1]		
	Single Cell RNA Boost	Globin or Insulin[2]	Liver or Ribo/Mito Genes[2,3]
Nuclease-Free water	1.0 µL	4.3 µL	2.7 µL
10X Cas9 Buffer	1.0 µL	1.0 µL	1.0 µL
RNase Inhibitor	1.0 µL	1.0 µL	1.0 µL
Cas9	2.3 µL	0.9 µL	1.5 µL
Guide RNA	3.5 µL	1.6 µL	2.6 µL
Total Volume	8.8 µL	8.8 µL	8.8 µL

[1]Note: This table is for single guide panels. For combinations of guide panels, refer to Appendix A.
[2]Note: These guide panels are sold separately.
[3]Note: The Mouse Ribo/Mito Guide Panel can be substituted here using the same reagent volumes.

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2. Mix gently by flicking the tube or pipetting up and down slowly. Collect the contents at the bottom of the tube by briefly spinning the tube in a microcentrifuge.
3. Incubate the reaction at room temperature for 10 minutes. This constitutes the "Ribonucleoprotein (RNP) Complex."
4. To the tube containing the RNP Complex, add the following reagents in the order listed below at room temperature:

CRISPR Digestion Reaction	Volume
RNP Complex Reaction (Step A.1.3)	8.8 µL
Adapter-ligated cDNA library from Step 3.4 of the Chromium Next GEM Single Cell 3' protocol v3.1	15 µL
10X Cas9 Buffer	1.5 µL
Total Volume	~25 µL

5. Mix gently by flicking the tube or pipetting up and down slowly. Collect the contents at the bottom of the tube by briefly spinning the tube in a microcentrifuge.
6. Place the tube in a thermal cycler with a heated lid set to ≥45°C. Run the following program:

Temperature	Cycle Time
42°C	1 hour
4°C	HOLD

7. Proceed immediately to the next step (Bead Cleanup).

A.2: Bead Cleanup

1. Add 25 µL of Nuclease-Free Water to the reaction.
2. Add 30 µL (0.6X) of resuspended Ampure XP Beads to the reaction. Mix by pipetting a few times. Briefly spin the tube in a microcentrifuge to collect all liquid at the bottom of the tube.
3. Incubate the sample at room temperature for 10 minutes.
4. Place the tube on a magnet stand until the solution is clear (3-5 minutes). Discard the supernatant without disturbing the beads.
5. Add 200 µL of freshly prepared 80% ethanol to the tube while still on the magnetic stand. Wait 30 seconds, then remove and discard the ethanol. Do not remove the tube from the magnetic stand while performing the ethanol wash.
6. Repeat the previous wash step.
7. Briefly spin the tube, place back on the magnet, and remove any traces of ethanol. Do not allow the beads to air dry to optimize elution yield.
8. Remove the tube from the magnet and immediately add 31 µL of Nuclease-free Water to the beads. Slowly pipette mix several times to resuspend. Briefly spin the sample in a microcentrifuge to collect all liquid at the bottom of the tube.
9. Incubate the sample at room temperature for 5 minutes.
10. Place the tube on the magnet. Allow the solution to clear and transfer 30 µL of the supernatant containing the eluted DNA to a new 0.2 mL tube. This constitutes the "depleted single-cell cDNA."
11. 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.

SAFE STOPPING POINT: If stopping after Step A2.10, store the "depleted single-cell cDNA" sample at -25°C to -15°C.

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Next Steps

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 DepleteX 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.

Follow the Chromium Next GEM Single Cell 3' protocol *without modification* after Step 3.6.

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. Depleted libraries have a similar fragment profile to those of standard Chromium single cell libraries with a typical shift of ~50 base pairs to the left of the electropherogram (i.e., the average fragment size is 50 base pairs 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 -20ºC.

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Appendix: Dual-Guide Panel Depletion of Single Cell 3' cDNA

This appendix is for users who would like to perform Single Cell RNA Boost (KIT1037) depletion in combination with the NVG Guide RNA included as part of KIT1037.

Additionally, this section details the use of the Single Cell RNA Boost (KIT1037) with compatible panels that are sold separately, including the Globin RNA Depletion Panel (KIT1024), Insulin RNA Depletion Panel (KIT1025), or Liver RNA Depletion Panel (KIT1022).

We do not recommend combining more than 2 guide panels per depletion.

Note: KIT1022, KIT1024 and KIT1025 are sold separately.

☐ Hands-on time: ~30 minutes | Total time: ~2 hours

Reagent Preparation

Item	Storage	Handling
Adapter-ligated cDNA library from Step 3.4 of the Chromium Next GEM Single Cell 3' protocol v3.1	-20°C	If frozen, thaw on ice. Finger-tap mix and spin down. Keep on ice. Return to freezer immediately after use.
Cas9	-20°C	If frozen, thaw on ice. Finger-tap mix and spin down. Keep on ice. Return to freezer immediately after use.
RNase Inhibitor	-20°C	If frozen, thaw on ice. Finger-tap mix and spin down. Keep on ice. Return to freezer immediately after use.
Guide Panels for Single-Cell 3' Depletion	-80°C	If frozen, thaw on ice. Finger-tap mix and spin down. Keep on ice. Return to freezer immediately after use.
10X Cas9 Buffer	-20°C	Thaw at room temperature. Vortex briefly and spin down. Keep on ice.
Nuclease-Free Water	-20°C	Thaw at room temperature. Vortex briefly and spin down. Keep on ice.
AMPure XP Beads	2°C to 8° C	Bring to room temperature. Vortex and invert mix.
80% Ethanol	Room temperature	Prepare fresh.

* Program thermal cycler(s) prior to beginning the protocol for the first time.

App.1: Ribonucleoprotein (RNP) Complex Formation

1. At room temperature, combine the following reagents in the order listed below in a 0.2 mL PCR tube:

	RNP Complex Formation Reaction		
	A. Single Cell RNA Boost + **NVG**	B. Single Cell RNA Boost + **Globin OR Insulin**	B. Single Cell RNA Boost + **Liver**
Nuclease-free water	N/A	2.7 µL	1.0 µL
10X Cas9 Buffer	1.0 µL	1.0 µL	1.0 µL
RNase Inhibitor	1.0 µL	1.0 µL	1.0 µL
Cas9	2.3 µL	3.2 µL	3.8 µL
Guide RNA - Single Cell Boost	3.5 µL	3.5 µL	3.5 µL
Guide RNA (KIT1037, KIT1024, KIT1025, KIT1022)	1.0 µL	1.6 µL	2.7 µL
Total Volume	8.8 µL	13 µL	13 µL

2. Mix gently by flicking the tube or pipetting up and down slowly. Collect the contents at the bottom of the tube by briefly spinning the tube in a microcentrifuge.

3. Incubate the reaction at room temperature for 10 minutes. This constitutes the "Ribonucleoprotein (RNP) Complex."

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4. At room temperature, combine the following reagents in the order listed below to the RNP complex:

CRISPR Digestion Reaction	A. Single Cell RNA Boost + NVG	B. Single Cell RNA Boost + Globin OR Insulin OR Liver
RNP Complex Reaction (Previous Step)	8.8 µL	13 µL
Single-Cell 3’ cDNA (100-150 ng)	15 µL	15 µL
10X Cas9 Buffer	1.5 µL	2 µL
Total Volume	~25 µL	30µL

5. Mix gently by flicking the tube or pipetting up and down slowly. Collect the contents at the bottom of the tube by briefly spinning the tube in a microcentrifuge.
6. Place the tube in a thermal cycler with a heated lid set to ≥45°C. Run the following program:

Temperature	Cycle Time
42°C	1 hour
4°C	HOLD

7. Proceed immediately to the next step (Bead Cleanup).

App.3: Bead Cleanup

1. Bring reaction volume up to 50 µL with Nuclease-Free Water.
2. Add 30 µL (0.6X) of resuspended AMPure XP Beads to the reaction. Mix by pipetting 10 times. Briefly spin the sample in a microcentrifuge to collect all liquid at the bottom of the tube.
3. Incubate the sample at room temperature for 10 minutes.
4. Place the sample tube on a magnet stand until the solution is clear (3-5 minutes). Discard the supernatant without disturbing the beads.
5. Add 200 µL of freshly prepared 80% ethanol to the tube while still on the magnetic stand. Wait 30 seconds, then remove and discard the ethanol. Do not remove the tube from the magnetic stand while performing the ethanol wash.
6. Repeat the previous wash step.
7. Briefly spin the tube, return to the magnet and remove any traces of ethanol. For optimum elution yield, do not allow the beads to air dry.
8. Remove the tube from the magnet and immediately add 31 µL Nuclease-free Water to the beads. Slowly pipette mix several times to resuspend. Briefly the sample in a microcentrifuge to collect all liquid at the bottom of the tube.
9. Incubate the sample at room temperature for 5 minutes.
10. Place the tube on the magnet. Allow the solution to clear and transfer 30 µL the supernatant containing the eluted DNA to a new 0.2 mL tube. This constitutes the “depleted single-cell cDNA” sample.
11. 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.

SAFE STOPPING POINT: If stopping after Step App.3.10, store the sample at -25°C to -15°C.

Proceed with Next Steps (see page 10)

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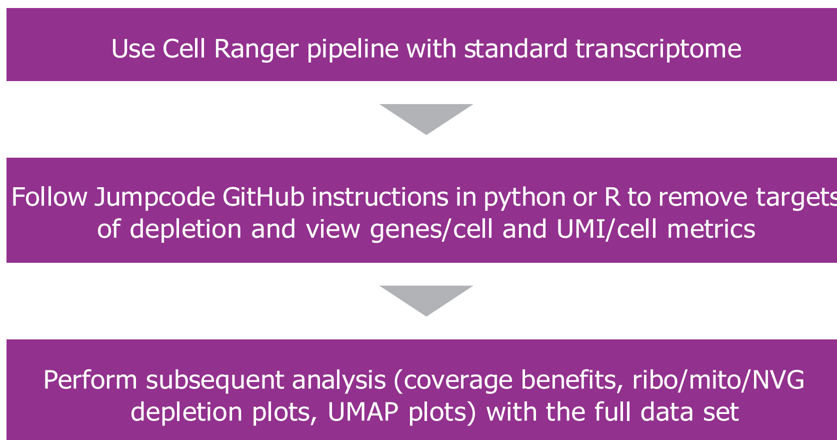
Bioinformatics guidelines

10x Genomics Chromium single cell data generated with the DepleteX 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 DepleteX-depleted data to control data

The standard data analysis workflow must be modified to observe the impact of DepleteX 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. Additionally, guidelines are provided to remove dead cells from downstream analysis. UMAP visualization is also available on the GitHub site.



Warning: If data from DepleteX-depleted libraries is aligned to the comprehensive standard human Cell Ranger Index to perform a comparative analysis between standard Chromium data and equivalent DepleteX-derived data, no significant improvement in the number of UMIs and genes would be observed with the latter. This is because the genes targeted for 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.

Access multiple options to support your analysis needs on our Quick Start page:

https://www.jumpcodegenomics.com/resources/quick-start/crisprclean-single-cell

- Video walkthrough with our public dataset files.
- Down sampling guidelines for Cell Ranger
- Step-by-step guide in Python or R on how to analyze data and visualize benefits of depletion
- Generate graphs such as the following:
  - Ribo, Mito and total target depletion (including NVG content)
  - Gene and UMI metrics
  - UMAP plots

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