BD Rhapsody™ System

OMICS-One™ CRISPR and WTA Next for poly-A sgRNA Capture

Library Preparation Protocol

23-25059(01)
2026-01

Regulatory information

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

History

Revision	Date	Change made
23-25059(01)	2026-01	Initial release.

Contents

Introduction

Single-cell CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) screening enables high-resolution functional genomics by linking thousands of targeted gene perturbations to transcriptomics and phenotypic responses within individual cells. This approach facilitates systemic interrogation of gene regulatory networks and identification of functional elements and elucidates mechanisms underlaying drug sensitivity and resistance. The BD Rhapsody™ System provides an integrated workflow for pooled CRISPR screens combined with whole-transcriptome or targeted scRNA-seq, enabling cost-efficient and scalable analysis of perturbation effects. Furthermore, incorporation of OMICS-One™ Protein Panels allows simultaneous quantification of cell surface proteins, delivering a multiomics perspective on gene perturbation responses. For large-scale studies, the BD Rhapsody™ HT Xpress System supports high-throughput single-cell CRISPR screening, ensuring robust and reproducible data generation.

This protocol provides detailed instructions for generating single-guide RNA (sgRNA) and whole transcriptome mRNA libraries following cell capture on the BD Rhapsody™ HT Single-Cell Analysis System or the BD Rhapsody™ HT Xpress System. It is intended for downstream sequencing on a variety of platforms. The resulting sgRNA and whole transcriptome mRNA libraries can be pooled and sequenced together, enabling integrated analysis of gene expression and perturbation identity from the same single-cell dataset. For complete instrument operation and safety guidelines, refer to the *BD Rhapsody™ HT Single-Cell Analysis System Instrument User Guide* or the *BD Rhapsody™ HT Xpress System Instrument User Guide for Scanner-Free Workflow*.

In this workflow, cDNA corresponding to both sgRNA and mRNA targets is synthesized directly on BD Rhapsody™ Enhanced Cell Capture Beads. sgRNA molecules are hybridized to barcoded beads via poly(A) capture, enabling single-cell resolution. Amplification of sgRNA sequences is performed using PCR primers compatible with BD Rhapsody™ library construction. sgRNAs are selectively amplified in parallel with whole transcriptome amplification targets using the BD Rhapsody™ Whole Transcriptome Analysis (WTA) Amplification Kit. Indexed libraries are then prepared for next generation sequencing. Downstream analysis, including sgRNA assignment per cell and transcriptome profiling, can be performed using the BD Rhapsody™ Sequence Analysis Pipeline and Cellismo™ Data Visualization Tool

sgRNAs should be designed to be captured with a poly(A)/dT oligo hybridization to enable efficient capture in single-cell 3′ transcriptome assays, such as those employed in CROP-Seq or Perturb-Seq workflows. The poly-A tail allows for sgRNA detection by hybridizing to oligo-dT primers on barcoded beads during reverse transcription, ensuring that sgRNA molecules are captured alongside mRNA for accurate cell-to-sgRNA mapping.

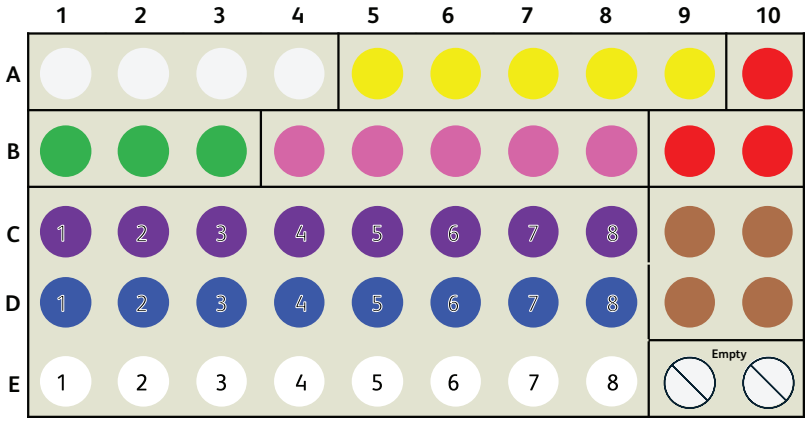
Symbols

The following symbols are used in this guide:

Symbol	Description
	Important information for maintaining measurement accuracy or data integrity.
	Noteworthy information.
	Procedural stopping point.

Protocol kits

Before you begin, ensure that you have the correct kits for this protocol. Matching cap colors indicate you have the correct kit, along with the catalog numbers found in the Required and recommended materials (page 10) section.



OMICS-One™ WTA Next Amplification Kit

Cap Color	Name	Part Number	Vial Placement
	OMICS-One™ Nuclease-Free Water	51-9025552	A1–A4
	OMICS-One™ WTA Extension Buffer	51-9025488	A5
	OMICS-One™ WTA Extension Primer	51-9025467	A6
	OMICS-One™-One dNTP Mixture	51-9025491	A7
	OMICS-One™-One Bead RT/PCR Enhancer	51-9025495	A8
	OMICS-One™ WTA Extension Enzyme	51-9025499	A9
	OMICS-One™ AbSeq Primer	51-9025468	A10
	OMICS-One™ PCR Master Mix	51-9025466	B1
	OMICS-One™ Universal Oligo	51-9025553	B2
	OMICS-One™ WTA Amplification Primer	51-9025469	B3
	OMICS-One™ Elution Buffer	51-9025554	B4–B8
	OMICS-One™ Sample Tag PCR1 Primer	51-9025470	B9
	OMICS-One™ Sample Tag PCR2 Primer	51-9025471	B10
	OMICS-One™ Bead Resuspension Buffer	51-9025555	C9, C10, D9, D10
	OMICS-One™ Library Forward Primer 1–8	See Part numbers for primers in rows C–E (page 7)	C1–C8
	OMICS-One™ WTA Library Reverse Primer 1–8		D1–D8
	OMICS-One™ Multiomic Library Reverse Primer 1–8		E1–E8

Part numbers for primers in rows C–E

Name	Part number
OMICS-One™ Library Forward Primer 1	51-9025472
OMICS-One™ Library Forward Primer 2	51-9025473
OMICS-One™ Library Forward Primer 3	51-9025474
OMICS-One™ Library Forward Primer 4	51-9025475
OMICS-One™ Library Forward Primer 5	51-9025476
OMICS-One™ Library Forward Primer 6	51-9025477
OMICS-One™ Library Forward Primer 7	51-9025478
OMICS-One™ Library Forward Primer 8	51-9025479
OMICS-One™ WTA Library Reverse Primer 1	51-9025480
OMICS-One™ WTA Library Reverse Primer 2	51-9025600
OMICS-One™ WTA Library Reverse Primer 3	51-9025482
OMICS-One™ WTA Library Reverse Primer 4	51-9025483
OMICS-One™ WTA Library Reverse Primer 5	51-9025484
OMICS-One™ WTA Library Reverse Primer 6	51-9025485
OMICS-One™ WTA Library Reverse Primer 7	51-9025486
OMICS-One™ WTA Library Reverse Primer 8	51-9025487
OMICS-One™ Multiomic Library Reverse Primer 1	51-9025489
OMICS-One™ Multiomic Library Reverse Primer 2	51-9025490
OMICS-One™ Multiomic Library Reverse Primer 3	51-9025492
OMICS-One™ Multiomic Library Reverse Primer 4	51-9025493
OMICS-One™ Multiomic Library Reverse Primer 5	51-9025494
OMICS-One™ Multiomic Library Reverse Primer 6	51-9025496
OMICS-One™ Multiomic Library Reverse Primer 7	51-9025497
OMICS-One™ Multiomic Library Reverse Primer 8	51-9025498

Workflows

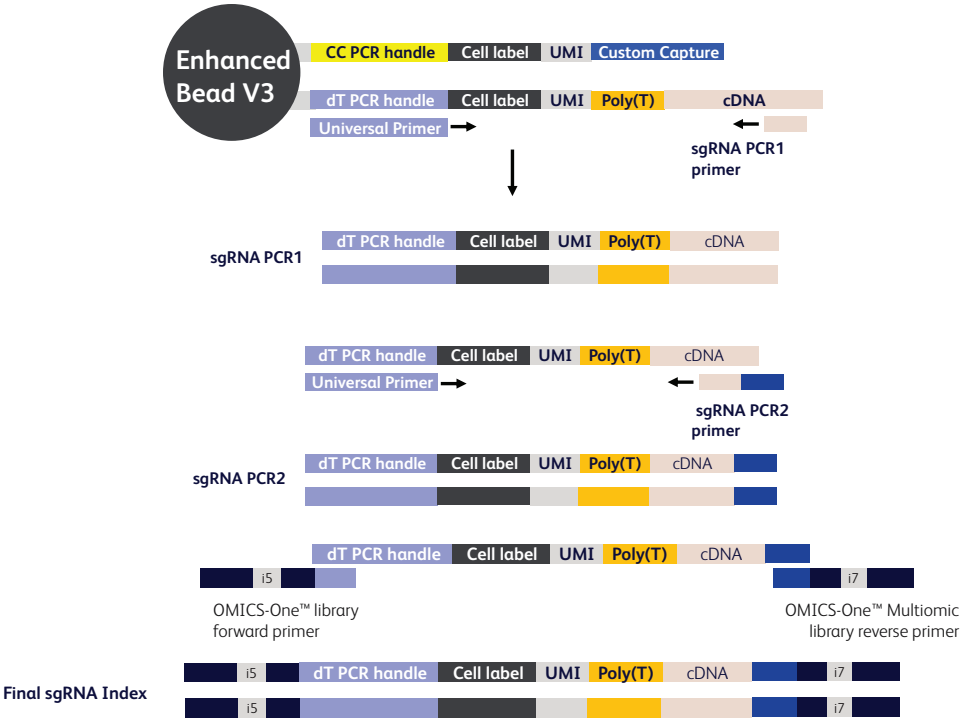
sgRNA library amplification workflow

1.1 sgRNA PCR1 (page 16): Universal primer and sgRNA PCR1 primer copy target region from bead.

Amplify in solution. Collect supernatant as sgRNA PCR1 product.

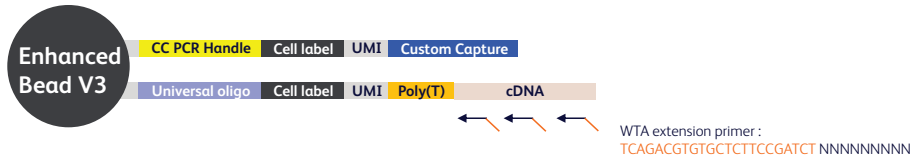
1.3 sgRNA PCR2 (page 22): Amplify using sgRNA PCR2 primer for nested PCR enrichment.

1.5 sgRNA Index PCR (page 28): Add adapters and indices.

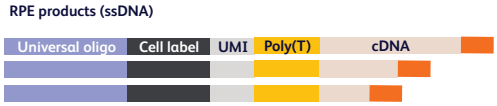


WTA library amplification workflow

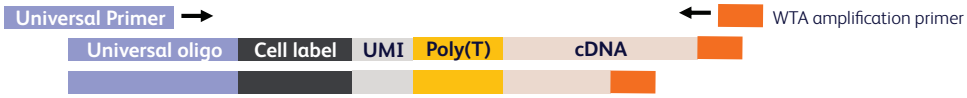
2.1 Random priming and extension (RPE) (page 36): Random priming on the bead.



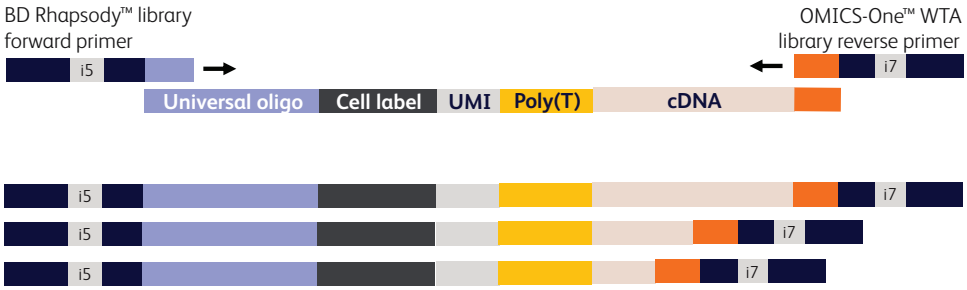
Denature off the RPE product.



2.2 RPE PCR (page 40): Amplify the RPE product.



2.4 WTA Index PCR (page 45): Add sequencing adapters and indices.



Required and recommended materials

Required reagents

Store the reagents at the storage temperature specified on the label.

Material	Supplier	Catalog no.
OMICS-One™ WTA Next Amplification Kit*	BD Biosciences	572620
AMPure® XP beads for DNA cleanup	Beckman Coulter	A63880
sgRNA PCR1 primer (10 µM)†	Major supplier	–
sgRNA PCR2 primer (10 µM)†	Major supplier	–
100% ethyl alcohol, molecular biology grade	Major supplier	–
TE Buffer [10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA]	Major supplier	–
Nuclease-free water	Major supplier	–

* This kit contains sufficient reagent volume to amplify both the WTA and sgRNA libraries.

† Refer to the Appendix for recommended primer sequences and references. Order primers at 25nmol scale, standard desalting. No additional purification required. Prepare a 10-µM primer working stock.
Primer sequences can be adapted to your workflow. Customize based on target region, platform requirements, and PCR performance (e.g., Tm, GC content, avoiding dimers). Validate primers before large-scale use.
For designing and ordering primers for sgRNA amplification, contact your local BD single-cell technical support

Recommended consumables

Material	Supplier	Part number/Catalog no.
Pipettes (P10, P20, P200, P1000)	Major supplier	–
Low-retention, filtered pipette tips	Major supplier	–
0.2-mL PCR 8-strip tubes	Major supplier	–
Axygen® 96–Well PCR Microplates* Or, MicroAmp Optical 96–Reaction Plate*	Corning Thermo Fisher Scientific	PCR96HSC N8010560
MicroAmp Clear Adhesive Film*	Thermo Fisher Scientific	4306311
15-mL conical tube	Major supplier	–
DNA LoBind® tubes, 1.5 mL	Eppendorf	0030108051
Qubit™ Assay Tubes	Thermo Fisher Scientific	Q32856
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851
Agilent High Sensitivity DNA Kit Or, Agilent High Sensitivity D1000 ScreenTape Agilent High Sensitivity D1000 Reagents Or, Agilent High Sensitivity D5000 ScreenTape Agilent High Sensitivity D5000 Reagents	Agilent Agilent Agilent Agilent Agilent	5067-4626 5067-5584 5067-5585 5067-5592 5067-5593

* Recommended for processing high-throughput (more than eight) library preparation workflows.

Equipment

Material	Supplier	Catalog no.
Microcentrifuge for 1.5–2.0-mL tubes	Major supplier	–
Microcentrifuge for 0.2-mL tubes	Major supplier	–
Vortexer	Major supplier	–
Digital timer	Major supplier	–
PCR thermal cycler	Major supplier	–
Eppendorf ThermoMixer® *	Eppendorf	5382000023
6-tube magnetic separation rack for 1.5-mL tubes Or, 12-tube magnetic separation rack† Or, Invitrogen™ DynaMag™-2 magnet†	New England Biolabs New England Biolabs Thermo Fisher Scientific	S1506S S1509S 12321D
Low-profile magnetic separation stand for 0.2 mL, 8-strip tubes	V&P Scientific, Inc.	VP772F4-1
Magnetic Stand–96‡	Thermo Fisher Scientific	AM10027
Qubit™ 3.0 Fluorometer	Thermo Fisher Scientific	Q33216
Agilent® 2100 Bioanalyzer Or, Agilent® 4200 TapeStation System	Agilent Technologies Agilent Technologies	G2940CA G2991AA
Heat block	Major supplier	–

* Two thermomixers are recommended. A heat block can be used for denaturation steps.

† Recommended for processing greater than six samples.

‡ Recommended for processing high-throughput (more than eight) library preparation workflows.

Best practices

Cell capture

- For best results, ensure that cells have high viability before proceeding with cell capture.

Bead handling

- When working with BD Rhapsody™ Enhanced Cell Capture Beads, use low-retention filtered tips and LoBind® tubes.

  Never vortex the beads. Pipet-mix only.

- Store BD Rhapsody™ Enhanced Cell Capture Beads at 4 °C.

  Do not freeze.

- Bring Agencourt® AMPure® XP magnetic beads to room temperature (15–25 °C) before use. See the *AMPure® XP User's Guide* for information.

Libraries

- sgRNA libraries can be sequenced together or separately from WTA libraries

Master mix preparation

- Thaw reagents (except for enzymes) at room temperature.
- Keep enzymes at –25 °C to –15 °C until ready for use.
- Return reagents to correct storage temperature as soon as possible after preparing the master mix.
- Use only nuclease-free water throughout the protocol.

Supernatant handling

- Read this protocol carefully before beginning each section. Note which steps require you to keep supernatant to avoid accidentally discarding required products.
- Remove supernatants without disturbing AMPure® XP magnetic beads.
- Make and use fresh 80% ethyl alcohol within 24 hours. Adjust the volume of 80% ethyl alcohol depending on the number of libraries.

Bead amplification

- To ensure uniform bead suspension, do not proceed to thermal cycling until each tube is gently mixed by pipette. Start the thermocycler program immediately after mixing.

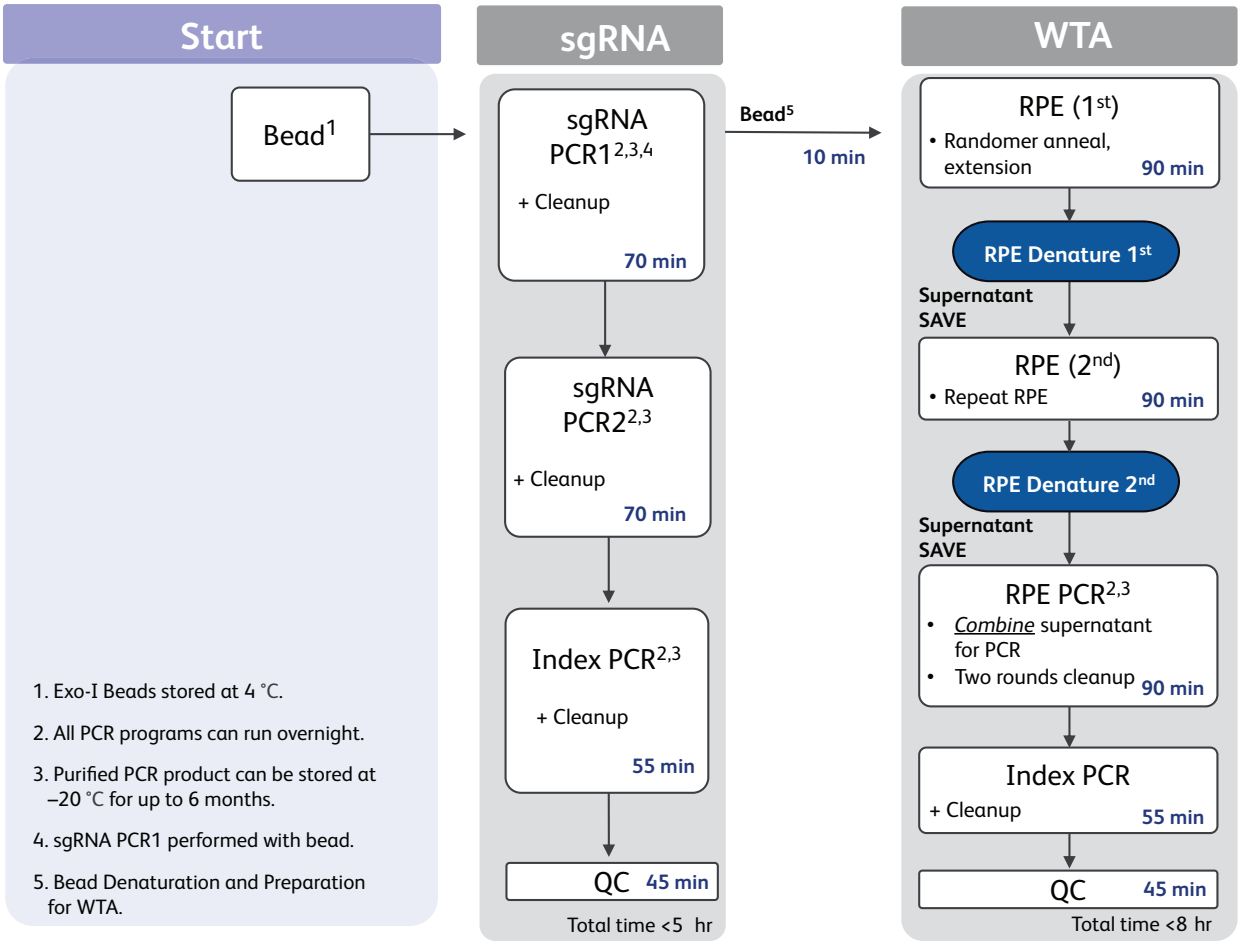
Additional documentation

- *BD Rhapsody™ HT Single-Cell Analysis System Extended-Lysis Single-Cell Capture and cDNA Synthesis Protocol* (doc ID 23-24984)
- *BD Rhapsody™ HT Xpress System Extended-Lysis Single-Cell Capture and cDNA Synthesis Protocol* (doc ID 23-24983)
- *BD Rhapsody™ Sequence Analysis Pipeline User's Guide* (doc ID 23-24580)

Safety information

For safety information, refer to the *BD Rhapsody™ HT Single-Cell Analysis System Instrument User Guide* (doc ID 23-24989) or the *BD Rhapsody™ HT Xpress System Instrument User Guide for Scanner-Free Workflow* (doc ID 23-24988).

Time considerations



Procedure

Perform the experiment on the BD Rhapsody™ Single-Cell Analysis system using either of the following guides for cell capture, reverse transcription, and Exonuclease treatment:

- *BD Rhapsody™ HT Single-Cell Analysis Extended-Lysis System Single-Cell Capture and cDNA Synthesis Protocol* (doc ID 23-24984)
- *BD Rhapsody™ HT Xpress System Extended-Lysis Single-Cell Capture and cDNA Synthesis Protocol* (doc ID 23-24983)

This protocol is intended for the Targeted mRNA amplification library generation of cell inputs of up to 100,000 single cells captured per lane.

1. sgRNA library amplification

This section comprises the following tasks:

1.1 sgRNA PCR1

Summary:

- Prepare sgRNA PCR1 mix
- Amplify using sgRNA PCR1 program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Universal oligo	51-9025553	Fully thaw and mix reagents prior to use. Centrifuge briefly. Keep on ice until ready.	–20 °C
	sgRNA PCR1 primer (10µM)*			
○	Nuclease-free water	51-9025552		
Leave in freezer until ready to use:				
●	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
●	Bead RT/PCR enhancer	91-1082		
Obtain:				
Exonuclease I-treated cell capture beads			Centrifuge briefly and keep on ice until ready.	4 °C
Ice bucket				
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Thermocycler sgRNAPCR1 program				

* Order from BD Biosciences or oligo vendor. If ordered from BD Biosciences, ensure sgRNA is diluted to 1X with TE Buffer before use.

Procedure steps:

This section describes how to amplify sgRNA products through PCR.

In the pre-amplification workspace, in a new 1.5-mL tube, pipet the following components.

sgRNA PCR1 mix

Cap	Component	1 library (µL)	1 library with 20% overage (µL)	4 libraries with 20% overage (µL)	8 libraries with 20% overage (µL)
	PCR master mix	50.0	60.0	240.0	480.0
	Universal oligo	2.0	2.4	9.6	19.2
	Bead RT/PCR enhancer	12.0	14.4	57.6	115.2
	sgRNA PCR1 primer (10 µM)*	8.0	9.6	38.4	76.8
	Nuclease-free water	128.0	153.6	614.4	1,228.8
	Total	**200.0**	**240.0**	**960.0**	**1920.0**

* Order from BD Biosciences or oligo vendor. Ensure sgRNA primer is diluted with TE Buffer to 1X before use if ordered from BD Biosciences.

1. Pipet-mix the sgRNA PCR1 mix.
2. Place on **ice** until ready to use.
3. Briefly spin the tube with the bead suspension. Place the tube of beads in Bead Resuspension Buffer on a 1.5-mL magnet for ≤1 minute. Discard the supernatant.
4. Remove the tube from the magnet and resuspend the beads in **200 µL** of sgRNA PCR1 reaction mix. Do not vortex.
5. Ensuring that the beads are fully resuspended, pipet **50 µL** of sgRNA PCR1 reaction mix with beads into each of four 0.2-mL PCR tubes. Transfer any residual mix to one of the tubes.

Bring the tubes to the post-amplification workspace.

6. Run the following PCR program:

sgRNA and PCR1 program

Step	Cycles	Temperature	Time
Hot start	1	98 °C*	45 seconds
Denaturation	12†	98 °C	15 seconds
Annealing		60 °C‡	1 minute
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

* To avoid beads settling due to prolonged incubation time on the thermal cycler before the denaturation step, it is critical to pause the instrument at 98 °C before loading the samples. Different thermal cyclers might have different pause time settings. In certain brands of thermal cyclers, however, we have observed a step-skipping error with the pause/unpause functions. To ensure that the full 45-second denaturation is not skipped, verify that the pause/unpause functions are working correctly on your thermal cycler. To avoid the step-skipping problem, a 1-minute 98 °C pause step can be added immediately before the 45-second 98 °C denaturation step.

† The cycle number depends on the number of cells, the amount of input cDNA, the number of amplified genes, and the expression levels of these genes. As a start, 12 cycles is recommended but might require optimization.

‡ The annealing temperature depends on the primer melting temperature (Tm), primer length, GC content, and the complexity of the template. As a starting point, an annealing temperature about 3–5 °C below the primer Tm is recommended, but this might require optimization. Gradient PCR can be used to determine the optimal temperature for specificity and yield.

7. Ramp the heated lid and heat block of the post-amplification thermal cycler to ≥95 °C by starting the thermal cycler program and then pausing it.

Do not proceed to thermal cycling until each tube is gently mixed by pipette to ensure uniform bead suspension.

8. For each 0.2-mL PCR tube, gently pipet-mix, immediately place the tubes in thermal cycler, and unpause the thermal cycler program.

The PCR can be run overnight.

9. When the sgRNA PCR1 program is complete, briefly centrifuge the tubes.

1.2 sgRNA PCR1 cleanup and quantification

Summary:

- sgRNA PCR1 cleanup

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	51-9025554	Centrifuge briefly.	–20 °C
	Bead Resuspension Buffer	51-9025555		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit dsDNA HS Assay Kit				
Obtain:				
sgRNA PCR1 product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

This section describes how to perform a single-sided AMPure® cleanup to remove primer dimers from the sgRNA PCR1 products. The final product is purified double-stranded DNA.

Perform the purification in the post-amplification workspace.

1. Bring AMPure® XP magnetic beads to room temperature.
2. Make fresh 80% ethyl alcohol for use within **24 hours**.

   Adjust the volume depending on the number of samples—one sample requires 1 mL of 80% ethyl alcohol.
3. Vortex the AMPure® XP magnetic beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with the sgRNA PCR1 product.
5. Pipet-mix and combine the **four** tubes of **50-µL** PCR1 product into a new 1.5-mL tube.
6. Place the 1.5-mL tube on a magnet for 2 minutes, and carefully pipet the supernatant (sgRNA PCR1 products) into a new 1.5-mL LoBind® tube without disturbing the beads.

   Save supernatant at this step. Do not discard!

   To maintain data integrity, remove tube with the cell capture beads from the magnet, and pipet 200 µL cold Bead Resuspension Buffer into tube. Pipet-mix. Do not vortex. Store beads at 2–8 °C in post-amplification workspace.

   Important: These beads will be used for WTA amplification. Do not throw away!
7. Pipet-mix the supernatant 10 times.

   The volume must be exactly **200 µL**. If the volume is less than 200 µL, use nuclease-free water to achieve the final volume.
8. Pipet **300 µL** AMPure® XP beads (1.5x) into the tube containing the PCR1 products.
9. Pipet-mix 10 times.
10. Briefly centrifuge the tube.

    Avoid getting AMPure® beads on the lid of the tube. Residual beads and PCR mix buffer can negatively impact downstream results.
11. Incubate at room temperature for **5 minutes**.
12. Place the tube on a magnet until the supernatant is clear **(<5 minutes)**.
13. Remove and discard the supernatant.
14. Keeping the tube on the magnet, gently pipet **500 µL** of fresh 80% ethyl alcohol into the tube.
15. Incubate for **30 seconds**.

16. Remove and discard the supernatant without disturbing the beads.
17. Repeat steps 14–16 once for a total of **two ethyl alcohol washes**.
18. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
19. Air-dry the beads at room temperature until the beads no longer look glossy **(~3 minutes)**.

    Do not overdry the AMPure® beads after the ethanol washes. Overdried beads appear cracked.

20. Remove the tube from the magnet.
21. Pipet **50 µL** of elution buffer into the tube.
22. Pipet-mix 10 times until the beads are fully resuspended.
23. Incubate at room temperature for **2 minutes**.
24. Briefly centrifuge the tube.
25. Place the tube on a magnet until the supernatant is clear (**~30 seconds**).
26. Pipet the eluate **(~50 µL)** into a new 1.5-mL tube.
27. Estimate the concentration by quantifying 2 µL of the sgRNA PCR1 product with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay Kit. Follow the manufacturer's instructions.

    If the yield is too low to measure using the Qubit™ Fluorometer, use the maximum volume for PCR2 and adjust the volume of nuclease-free water accordingly.

28. Dilute an aliquot of the PCR2 products with nuclease-free water to **1.0 ng/µL**.

    The purified sgRNA and PCR1 product is ready for 1.3 sgRNA PCR2 (page 22).

STOP The sgRNA PCR1 libraries can be stored at –20 °C for up to 6 months.

1.3 sgRNA PCR2

Summary:

- Prepare sgRNA PCR2 mix
- Amplify using sgRNA PCR2 program

Preparation list

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟢	Universal oligo	51-9025553	Fully thaw and mix reagents prior to use. Centrifuge briefly. Keep on ice until ready.	–20 °C
	sgRNA PCR2 primer (10 µM)			
⚪	Nuclease-free water	51-9025552		
Leave in freezer until ready to use:				
🟢	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Purified sgRNA PCR1 product (diluted with nuclease-free water to **1.0 ng/µL**)				4 °C
Ice bucket				
1.5-mL LoBind® tube				
0.2-mL PCR tubes				
Set up:				
Thermocycler with with sgRNA PCR2 program				

Procedure steps

This section describes how to amplify sgRNA PCR1 products through PCR. The PCR primers include partial sequencing adapters that enable the additions of full-length sequencing indices during index PCR.

In the pre-amplification workspace, pipet reagents into a new 1.5-mL tube placed on ice. Use component mix volumes from a column in the following table according to your needs:

sgRNA PCR2 mix

Cap	Component	1 library (µL)	1 library with 20% overage (µL)	4 libraries with 20% overage (µL)	8 libraries with 20% overage (µL)
●	PCR master mix	12.5	15.0	60.0	120.0
●	1:10 dilution* of universal oligo	5.0	6.0	–	–
●	Universal oligo	–	–	2.4	4.8
	sgRNA PCR2 primer (10 µM)†	2.0	2.4	9.6	19.2
○	Nuclease-free water	20.5	24.6	120.0	240.0
	Total	**40.0**	**48.0**	**192.0**	**384.0**

* If preparing sgRNA PCR2 mix for one sample, dilute univeral oligo 1:10 for pipetting accuracy.

† Order from BD Biosciences or oligo vendor. Ensure sgRNA primer is diluted with TE Buffer to 1X before use if ordered from BD Biosciences.

If the PCR1 reaction produces low DNA concentration, increase the template input to achieve a total input amount of approximately 10 ng while maintaining the final reaction volume at 50 µL. Adjust the water volume accordingly to keep the total reaction volume constant.

1. Pipet-mix the the sgRNA PCR2 mix.
2. Place on **ice** until ready to use.
3. Pipet **40 µL** of sgRNAPCR2 mix into one 0.2-mL PCR tube for each sample.

   Bring the tubes to the post-amplification workspace.

4. Add **10 µL** of **diluted** sgRNAPCR1 product.
5. Pipet-mix 10 times.

6. Run the following PCR program:

sgRNA PCR2 program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	10*	98 °C	15 seconds
Annealing		60 °C†	1 minute
Extension		72 °C	1 minute
Final extension	1	72 °C	5 minutes
Hold	1	4 °C	∞

* As a start, 10 cycles is recommended but might require optimization.

† The annealing temperature depends on the primer melting temperature (Tm), primer length, GC content, and the complexity of the template. As a starting point, an annealing temperature about 3–5 °C below the primer Tm is recommended, but this might require optimization. Gradient PCR can be used to determine the optimal temperature for specificity and yield.

The PCR can run overnight.

7. When the sgRNA PCR2 program is complete, briefly centrifuge the tubes.

1.4 sgRNA PCR2 cleanup and quantification

Summary:

- sgRNA PCR2 cleanup
- Quantify using Qubit Flurometer

Preparation List:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	51-9025554	Centrifuge briefly.	–20 °C
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit dsDNA HS Assay Kit				
Obtain:				
sgRNA PCR2 product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

This section describes how to perform a single-sided AMPure® cleanup to remove primer dimers from the sgRNA PCR2 products. The final product is purified double-stranded DNA.

Perform the purification in the post-amplification workspace.

1. Bring AMPure® XP magnetic beads to room temperature.
2. Make fresh 80% ethyl alcohol and use within 24 hours.

   Adjust the volume depending on the number of samples—one sample requires 0.5 mL of 80% ethyl alcohol.
3. Vortex the AMPure® XP beads until they are fully resuspended.
4. Briefly centrifuge the sgRNA PCR2 products.

   The final volume must be exactly **50 µL**. If the volume is less than 50 µL, use nuclease-free water to achieve the final volume.
5. Pipet **75 µL** of AMPure® (1.5x) XP beads into the tube.
6. Pipet-mix 10 times.
7. Briefly centrifuge the tube.

   Avoid getting AMPure® XP beads on the lid of the tube. Residue of AMPure® XP beads and PCR mix buffer can negatively impact downstream results.
8. Incubate at room temperature for **5 minutes**.
9. Place the tube on a magnet until the supernatant is clear (**<5 minutes**).
10. Remove and discard the supernatant.
11. Keeping the tube on the magnet, gently pipet **200 µL** of fresh 80% ethyl alcohol into the tube.
12. Incubate for **30 seconds**.
13. Remove and discard the supernatant without disturbing the beads.
14. Repeat steps 11–13 once for a total of **two ethyl alcohol washes**.
15. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
16. Air-dry the beads at room temperature until the beads no longer look glossy **(~2 minutes)**.

    Do not overdry the AMPure® beads after the ethanol washes. Overdried beads appear cracked.
17. Remove the tube from the magnet.
18. Pipet **30 µL** of elution buffer into the tube.
19. Pipet-mix 10 times until the beads are fully resuspended.
20. Incubate at room temperature for **2 minutes**.

21. Briefly centrifuge the tube.
22. Place the tube on a magnet until the supernatant is clear (**~30 seconds**).
23. Pipet the eluate **(~30 µL)** into a new 1.5-mL tube.
24. Quantify the PCR2 products with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay.
25. Dilute an aliquot of the PCR2 products with nuclease-free water to **1.0 ng/µL**.

    The sgRNA PCR2 product is ready for 1.5 sgRNA Index PCR (page 28).

The sgRNA PCR2 libraries can be stored at Store at –20 °C for up to 6 months.

1.5 sgRNA Index PCR

Summary:

- Prepare sgRNA Index PCR mix
- Amplify using sgRNA Index PCR program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Forward primer 1–8	Various	Fully thaw and mix reagents prior to use. Centrifuge briefly. Keep on ice until ready.	–20 °C
○	Multiomic reverse primer 1–8	Various		
○	Nuclease-free water	51-9025552		
Leave in freezer until ready to use:				
●	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Purified sgRNA PCR2 product (diluted with nuclease-free water to **1.0 ng/µL**)				4 °C
Ice bucket				
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
Set up:				
Thermocycler with sgRNA Index PCR program				

Procedure steps:

This section describes how to generate sgRNA libraries compatible with various sequencing platforms, by adding full-length sequencing adapters and indices through PCR.

Add the sgRNA PCR2 product in the post-amplification workspace.

In the pre-amplification workspace, in a new 1.5-mL tube, pipet the following components.

sgRNA Index PCR mix

Cap	Component	1 library (µL)	1 library with 20% overage (µL)	4 libraries with 20% overage (µL)	8 libraries with 20% overage (µL)
	PCR master mix	12.5	15.0	60.0	120.0
	Forward primer 1–8	2.0	2.4	N/A	N/A
	Multiomic reverse primer 1–8[†]	2.0	2.4	N/A	N/A
	Nuclease-free water	23.5	28.2	112.8	225.6
	Total	**40.0**	**48.0**	**172.8**	**345.6**

[†] For more than one library, use different library reverse primers for each library. For recommendations about how to index libraries, contact your local Field Application Specialist or go to scomix@bd.com.

If the PCR2 reaction produces low DNA concentration, increase the template input to achieve a total input amount of approximately 10 ng while maintaining the final reaction volume at 50 µL. Adjust the water volume accordingly to keep the total reaction volume constant.

1. Pipet-mix the sgRNA Index PCR mix.
2. For multiple samples, pipet **36 µL** of Index PCR mix into a separate 0.2-mL PCR tube for each sample.
3. Add **2 µL** of forward primer and **2 µL** of multiomic reverse primer to each sample.
4. Place on **ice** until ready to use.

Bring the sgRNA Index PCR mix to the post-amplification workspace.

When performing dual indexing with multiple samples, ensure that the appropriate combinations of forward primer and reverse primer are used. Accurate primer assignment is essential to maintain sample identity during multiplexed sequencing.

5. Add **10 µL** of diluted sgRNA PCR2 product.
6. Pipet-mix 10 times.

7. Run the following PCR program:

sgRNA Index PCR program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	8*	98 °C	20 seconds
Annealing		60 °C	30 seconds
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

* As a start, 8 cycles is recommended but might require optimization.

The PCR can run overnight.

8. When the sgRNA Index PCR program is complete, briefly centrifuge the tubes.

1.6 sgRNA Index PCR cleanup and quality check

Summary:

- sgRNA Index PCR cleanup
- Quality check using a Qubit Fluorometer and BioAnalyzer/TapeStation

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	51-9025554	Centrifuge briefly.	–20 °C
	Nuclease-free water	51-9025552		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit dsDNA HS Assay Kit				
Agilent BioAnalyzer High Sensitivity Kit **OR** Agilent TapeStation ScreenTape and Reagents				
Obtain:				
sgRNA Index PCR product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
0.2-mL PCR tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

This section describes how to perform a single-sided AMPure® cleanup to remove primer dimers from the sgRNA Index PCR products. The final product is purified double-stranded DNA with full-length adapter sequences.

Perform the purification in the post-amplification workspace.

1. Bring AMPure® XP magnetic beads to room temperature.
2. Make fresh 80% ethyl alcohol for use within 24 hours.

   Adjust the volume depending on the number of samples—one sample requires 0.5 mL 80% ethyl alcohol.
3. Vortex the AMPure® XP magnetic beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with sgRNA Index PCR product.

   The volume must be exactly **50 µL**. If the volume is less than 50 µL, use nuclease-free water to achieve the final volume.
5. Pipet **50 µL** of AMPure® beads (1.0x) into the tube.
6. Pipet-mix 10 times.
7. Briefly centrifuge the tube.
8. Incubate at room temperature for **5 minutes**.
9. Place the tube on a magnet until the supernatant is clear (**<5 minutes**).
10. Remove and discard the supernatant.
11. Keeping the tube on the magnet, gently pipet **200 µL** of fresh 80% ethyl alcohol into the tube.
12. Incubate for **30 seconds**.
13. Remove and discard the supernatant without disturbing the beads.
14. Repeat steps 11–13 once for a total of **two ethyl alcohol washes**.
15. Keeping the tube on the magnet, use a P20 pipette to remove and discard the residual supernatant from the tube.
16. Air-dry the beads at room temperature until the beads no longer look glossy (**~2 minutes**).

    Do not overdry the AMPure® XP beads after the ethyl alcohol washes. Overdried beads appear cracked.
17. Remove the tube from the magnet.
18. Pipet **30 µL** of elution buffer into the tube.
19. Pipet-mix 10 times until the beads are fully suspended.
20. Incubate at room temperature for **2 minutes**.
21. Briefly centrifuge the tube.

22. Place the tube on the magnet until the solution is clear (**~30 seconds**).
23. Pipet the eluate (**~30 µL**) into a new 1.5-mL tube.

    The purified eluate is the final sequencing library.

    The Index PCR libraries can be stored at –20 °C for up to 6 months until sequencing.

24. Quantify and perform quality control of the sgRNA Index PCR product with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay and one of the following systems:
    - Agilent 2100 BioAnalyzer using the Agilent High Sensitivity DNA Kit
    - Agilent 4200 TapeStation system using the Agilent High Sensitivity D1000 or D5000 ScreenTape assay

    The sgRNA library should typically fall within the range of ~250–350 bp. Actual size might vary depending on the insert length and adapter design. Exact size might vary due to instrument or sample purification efficiency.

1.7 Bead denaturation and preparation for WTA

Procedure steps:

This section outlines the preparation of BD Rhapsody™ cell capture beads following targeted amplification for whole-transcriptome amplification.

Perform in the post-amplification workspace.

1. Set a heat block to 95 °C.
2. Pipet-mix the beads in **200 µL** bead resuspension buffer. Do not vortex—see Section 1.2 Step 6 (page 20).
3. Immediately incubate the sample at 95 °C in a heat block for **5 minutes** (without shaking).
4. Briefly centrifuge the tube.
5. Place the tube on a magnet until the supernatant is clear (**<1 minute**).
6. Remove the supernatant without disturbing the beads.

Ensure that all the supernatant is removed from the tube.

7. Remove the tube from the magnet.
8. Pipet **200 µL** of cold bead resuspension buffer to the tube with beads. Store at 4 °C.
9. Keep the tube with beads on ice or at 4 °C until ready to proceed to 2. WTA library amplification (page 35).

2. WTA library amplification

This procedure comprises the following tasks:

- 2.1 Random priming and extension (RPE) (page 36)
- 2.2 RPE PCR (page 40)
- 2.3 RPE PCR cleanup and quantification (page 42)
- 2.4 WTA Index PCR (page 45)
- 2.5 WTA Index PCR cleanup and quality check (page 48)
- (Optional) 2.6 Additional WTA Index PCR cleanup (page 52)

2.1 Random priming and extension (RPE)

Summary:

- Prepare random primer mix and extension enzyme mix
- Anneal random primers
- Extend random primers
- Denature RPE products
- Repeat RPE (2× total)

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟡	WTA extension buffer	51-9025488	Fully thaw and mix reagents prior to use. Centrifuge briefly. Keep on ice until ready.	–20 °C
🟡	WTA extension primer	51-9025467		
🟡	dNTP mixture	51-9025491		
⚪	Nuclease-free water	51-9025552		
🟣	Elution buffer	51-9025554		
Place on ice:				
🟤	Bead Resuspension Buffer	51-9025555	Centrifuge briefly before adding to mix.	–20 °C
🟡	Bead RT/PCR enhancer	51-9025495		
Leave in freezer until ready to use:				
🟡	WTA extension enzyme	51-9025499	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Beads prepared for WTA after sgRNA amplification			Centrifuge briefly and keep on ice until ready.	4 °C
Ice bucket				
1.5-mL DNA LoBind® tubes				
1.5-mL tube magnetic rack				
Set up:				
Heat block at 95 °C				
Thermomixer at 25 °C				
Thermomixer at 37 °C (Optional)				
Programmed thermomixer with RPE program				

Procedure steps:

This section describes how to generate random priming products. First, random primers are hybridized to the cDNA on the BD Rhapsody™ Enhanced Cell Capture Beads, followed by extension with an enzyme.

We recommend using a separate heat block for the 95 °C incubations.

1. Set a heat block to 95 °C and set two thermomixers to 37 °C and 25 °C, respectively.

   If you are using one thermomixer, skip the 37 °C incubation in Step 11 b.

2. In a new 1.5-mL tube, pipet the following reagents.

Random primer mix

Cap	Component	1 library (µL)	1 library with 20% overage (µL)	4 libraries with 20% overage (µL)	8 libraries with 20% overage (µL)
	WTA Extension Buffer	20.0	24.0	96.0	192.0
	WTA Extension Primers	40.0	48.0	192.0	384.0
	Nuclease-free water	114.0	136.8	547.2	1,094.4
	Total	**174.0**	**208.8**	**835.2**	**1,670.4**

3. Pipet-mix the Random primer mix.
4. Leave at room temperature until ready to use.
5. Briefly centrifuge the tube of beads prepared after sgRNA library amplification.
6. Place the tube with beads on a magnet until the supernatant is clear **(<2 minutes)**.
7. Remove and discard the supernatant.
8. Remove the tube from the magnet.
9. Add **87 µL** of random primer mix to the tube.
10. Pipet-mix 10 times until the beads are fully resuspended.

    Save the remaining volume of Random primer mix at **room temperature** for a second RPE.

11. Incubate the tube in the following order:
    a. 95 °C in a heat block (no shaking) for **5 minutes**.
    b. Thermomixer at 1,200 rpm and at **37 °C** for **5 minutes**.

       Optional: Skip this step if using only one thermomixer.

    c. Thermomixer at 1,200 rpm and at **25 °C** for **5 minutes**.

12. Briefly centrifuge the tube.
13. Leave at **room temperature** until ready to use.
14. In a new 1.5-mL tube, pipet the following reagents.

Extension Enzyme mix

Cap	Component	1 library (µL)	1 library with 20% overage (µL)	4 libraries with 20% overage (µL)	8 libraries with 20% overage (µL)
	dNTP mixture	8.0	9.6	38.4	76.8
	Bead RT/PCR enhancer	12.0	14.4	57.6	115.2
	WTA Extension Enzyme	6.0	7.2	28.8	57.6
	Total	**26.0**	**31.2**	**124.8**	**249.6**

15. Add **13 µL** of the Extension Enzyme mix to the tube with beads from step 13 (total volume of 100 µL).
16. Place on ice until ready to use.

Save the remaining volume of extension enzyme mix on **ice** for a second RPE.

17. Program the thermomixer.
    a. 1,200 rpm and at 25 °C for 10 minutes.
    b. 1,200 rpm and at 37 °C for 15 minutes.
    c. 1,200 rpm and at 45 °C for 10 minutes.
    d. 1,200 rpm and at 55 °C for 10 minutes.

Confirm “Time Mode” is set to “Time Control” before the program begins.

18. Place the tube of extension enzyme mix with beads in the programmed thermomixer (see step 27).
19. Remove the tube after the program is complete.
20. Place the tube on a magnet until the supernatant is clear **(<2 minutes)**.
21. Remove and discard the supernatant.
22. Remove the tube from the magnet.
23. Pipet **200 µL** of elution buffer into the tube.
24. Pipet-mix 10 times until the beads are fully resuspended.
25. Place the tube on a magnet until the supernatant is clear **(<2 minutes)**.
26. Remove and discard the supernatant.
27. Remove the tube from the magnet.
28. Add **80 µL** of elution buffer to the tube.

29. To denature the random priming products off the beads:
    a. Pipet-mix 10 times to resuspend the beads.
    b. Incubate the sample at **95 °C** in a heat block for **5 minutes (no shaking)**.
    c. Slightly open the lid of the tube to release air pressure within the tube.
    d. Place the tube on **ice** for **1 minute**.
    e. Briefly centrifuge the tube.
    f. Place the tube on a magnet until the supernatant is clear **(<2 minutes)**.

    Save supernatant at this step. Do not discard!

    g. Transfer **80 µL** of the supernatant (RPE product) to a new 1.5-mL tube.
30. Place the tube containing the RPE product on ice.
31. Repeat steps 9–30 to perform a second RPE.

If working with multiple samples, ensure that the supernatants are combined correctly.

32. Combine the two RPE products for each sample, for a total volume of **160 µL** (80 µL from the first RPE plus 80 µL from the second RPE).
33. Pipet **200 µL** of Bead Resuspension Buffer to the tube with leftover beads. Gently resuspend the beads by pipet-mixing only. Do not vortex.
34. Incubate the beads in Bead Resuspension Buffer at 95 °C for **5 minutes**, followed by shaking incubation at 1,200 rpm for 10 seconds at room temperature.
35. Place the tube on a magnet until the supernatant is clear **(<2 minutes)**.
36. Remove and discard the supernatant.
37. Pipet **200 µL** of Bead Resuspension Buffer to the tube with beads. Gently resuspend the beads by pipet-mixing only. Do not vortex.
38. Store the beads on ice or at 4 °C in the post-amplification workspace.

2.2 RPE PCR

Summary:

- Prepare RPE PCR mix
- Amplify using RPE PCR program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Universal oligo	51-9025553	Fully thaw and mix reagents prior to use. Centrifuge briefly. Keep on ice until ready.	–20 °C
●	WTA amplification primer	51-9025469		
Leave in freezer until ready to use:				
●	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
RPE product				4 °C
Ice bucket				
0.2-mL PCR tubes				
Set up:				
Thermocycler with RPE PCR program				

Procedure steps:

This section describes how to generate more RPE product through PCR amplification, resulting in multiple copies of each random-primed molecule.

1. In the pre-amplification workspace, in a new 1.5-mL tube, pipet the following components.

RPE PCR mix

Cap	Component	1 library (µL)	1 library with 20% overage (µL)	4 libraries with 20% overage (µL)	8 libraries with 20% overage (µL)
	PCR master mix	60.0	72.0	288.0	576.0
	Universal oligo	12.0	14.4	57.6	115.2
	WTA amplification primer	12.0	14.4	57.6	115.2
	Total	**84.0**	**100.8**	**403.2**	**806.4**

2. Pipet-mix the RPE PCR mix.
3. Place on ice until ready to use.
4. Add **84 µL** of the RPE PCR mix to the tube with the **160 µL** of RPE product.
5. Pipet-mix 10 times to create the RPE PCR reaction mix.
6. Split the mix into **four** 0.2-mL PCR tubes with **60 µL** mix per tube.
7. Transfer any residual mix to one of the tubes.
8. Bring the tubes to the post-amplification workspace.
9. Run the following PCR program:

RPE PCR program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	Recommended number cycles for resting PBMCs* 1,000–20,000 cells: 9 cycles >20,000 cells: 8 cycles	98 °C	15 seconds
Annealing		60 °C	30 seconds
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

* Recommended PCR cycles might require optimization for different cell and sample types. Two additional cycles are recommended for PBMC nuclei.

The PCR can run overnight.

10. When the RPE PCR reaction is complete, briefly centrifuge the tubes.

2.3 RPE PCR cleanup and quantification

Summary:

- RPE PCR cleanup (two rounds)
- Quantify using Qubit Fluorometer

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	51-9025554	Centrifuge briefly.	–20 °C
Obtain:				
RPE PCR product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

Perform the purification in the post-amplification workspace.

1. Bring AMPure® XP beads to room temperature.
2. Make fresh 80% ethyl alcohol for use within 24 hours.

   Adjust the volume depending on the number of samples. One sample requires 2 mL of 80% ethyl alcohol.
3. Vortex the AMPure® XP beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with the RPE PCR product.
5. Combine the **four** tubes of **60-µL** RPE PCR product into a new 1.5-mL tube.
6. Pipet-mix 10 times.
7. Transfer exactly **220 µL** RPE PCR product to a new 1.5-mL tube.
8. Pipet **264 µL** of AMPure® XP beads (1.2x) into the tube.
9. Pipet-mix 10 times.
10. Briefly centrifuge the tube.

    Avoid getting AMPure® XP beads on the lid of the tube. Residual beads and PCR mix buffer can negatively impact downstream results.
11. Incubate at room temperature for **5 minutes**.
12. Place the tube on a magnet until the supernatant is clear **(<5 minutes)**.
13. Remove and discard the supernatant.
14. Keeping the tube on the magnet, gently pipet **500 µL** of fresh 80% ethyl alcohol into the tube.
15. Incubate for **30 seconds**.
16. Remove and discard the supernatant without disturbing the beads.
17. Repeat steps 14–16 once for a total of **two ethyl alcohol washes**.
18. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
19. Air-dry the beads at room temperature until the beads no longer look glossy **(~3 minutes)**.

    Do not overdry the AMPure® XP beads after the ethyl alcohol washes. Overdried beads appear cracked.
20. Remove the tube from the magnet
21. Pipet **40 µL** of elution buffer into the tube.
22. Pipet-mix 10 times until the beads are fully resuspended.
23. Incubate at room temperature for **2 minutes**.
24. Briefly centrifuge the tube.

25. Place the tube on a magnet until the supernatant is clear **(~30 seconds)**.
26. Pipet the eluate **(~40 µL)** into a new 1.5-mL tube.
27. Add **60 µL** of nuclease-free water to the eluate for a final volume of **100 µL**.

    The volume must be exactly 100 µL.

28. Pipet **120 µL** of AMPure® XP beads (1.2x) into the tube.
29. Pipet-mix 10 times.
30. Briefly centrifuge the tube.
31. Incubate at room temperature for **5 minutes**.
32. Place the tube on a magnet until the supernatant is clear **(<5 minutes)**.
33. Remove and discard the supernatant.
34. Keeping the tube on the magnet, gently pipet **500 µL** of fresh 80% ethyl alcohol into the tube.
35. Incubate for **30 seconds**.
36. Remove and discard the supernatant without disturbing the beads.
37. Repeat steps 34–36 for a total of **two ethyl alcohol washes**.
38. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
39. Air-dry the beads at room temperature until the beads no longer look glossy **(~3 minutes)**.
40. Remove the tube from the magnet.
41. Pipet **30 µL** of elution buffer into the tube.
42. Pipet-mix 10 times until the beads are fully resuspended.
43. Incubate at room temperature for **2 minutes**.
44. Briefly centrifuge the tube.
45. Place the tube on a magnet until the supernatant is clear **(~30 seconds)**.
46. Pipet the eluate **(~30 µL)** into a new 1.5-mL tube.

    The purified RPE PCR product is ready for 2.4 WTA Index PCR (page 45).

47. Quantify the RPE PCR products with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay.

    The RPE PCR libraries can be stored at –20 °C for up to 6 months or 4 °C for up to 6 weeks.

2.4 WTA Index PCR

Summary:

- Prepare WTA Index PCR mix
- Amplify using WTA Index PCR program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Forward primer 1–8	Various	Fully thaw and mix reagents prior to use. Centrifuge briefly. Keep on ice until ready.	–20 °C
●	WTA reverse primer 1–8	Various		
○	Nuclease-free water	51-9025552		
Leave in freezer until ready to use:				
●	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Purified RPE PCR product				4 °C
Ice bucket				
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
Set up:				
Thermocycler with WTA Index PCR program				

Procedure steps:

This section describes how to generate mRNA libraries compatible with various sequencing platforms, by adding full-length sequencing adapters and indices through PCR.

We provide reagents for unique dual-indexing, with different library forward primers and reverse primers for up to eight samples.

Consult sequencing platform guidelines for low-plex pooling to ensure the indices chosen meet the color balancing guidelines for the sequencing instrument that will be used.

1. In a new 1.5-mL tube, pipet the following components:

WTA Index PCR mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
	PCR master mix	12.5	15.0	60.0	120.0
	Forward Primer 1–8	2.5	3.0	N/A	N/A
	WTA reverse primer 1–8	2.5	3.0	N/A	N/A
	Nuclease-free water	22.5	27.0	108.0	216.0
	Total	**40.0**	**42.0**	**168.0**	**336.0**

2. Pipet-mix the WTA Index PCR mix.
3. Pipet **35 µL** of WTA Index PCR mix into a separate 0.2-mL PCR tube for each sample.
4. Add **2.5 µL** of forward primer and **2.5 µL** of reverse primer to each sample.
5. Place on ice until ready to use.
6. Dilute an aliquot of the purified RPE PCR product from step 46 of 2.3 RPE PCR cleanup and quantification (page 42) to **0.5 ng/µL**.

If RPE PCR product concentration is <0.5 ng/µL, adjust the number of Index PCR cycles as outlined in the table "WTA Index PCR program".

7. Add **10 µL** of diluted RPE product to **40 µL** Index PCR mix.
8. Pipet-mix 10 times.

9. Run the following PCR program.

WTA Index PCR program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	RPE PCR conc.* <0.2 ng/µL: 11 cycles 0.2 ng/µL: 10 cycles 0.5 ng/µL: 8 cycles	98 °C	15 seconds
Annealing		60 °C	30 seconds
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

* Recommended number of PCR cycles might require optimization for different cell types.

The PCR can run overnight.

10. When the WTA Index PCR program is complete, briefly centrifuge the tubes.

2.5 WTA Index PCR cleanup and quality check

Summary:

- WTA Index PCR cleanup
- Quality check using Qubit Fluorometer and BioAnalyzer/TapeStation

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
Elution buffer		51-9025554	Centrifuge briefly.	–20 °C
Nuclease-free water		51-9025552		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit dsDNA HS Assay Kit				
Agilent BioAnalyzer High Sensitivity Kit **OR** Agilent TapeStation ScreenTape and Reagents				
Obtain:				
WTA Index PCR product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
0.2-mL PCR tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

This section describes how to perform a single-sided AMPure® XP beads cleanup for sequencing. The final product is purified double-stranded DNA with full-length adapter sequences.

Perform the purification in the post-amplification workspace.

1. Bring AMPure® XP beads to room temperature.
2. Make fresh 80% (v/v) ethyl alcohol for use within 24 hours,

   Adjust the volume depending on the number of samples. One sample requires 0.5 mL of 80% ethyl alcohol.
3. Vortex the AMPure® XP beads until the beads are fully resuspended.
4. Add **60 µL** of nuclease-free water to **50 µL** of the WTA Index PCR product.
5. Transfer **100 µL** of WTA Index PCR product into a new 0.2-mL PCR tube.

   The volume must be exactly 100 µL.
6. Pipet **80 µL** of AMPure® XP beads (0.8x) to the 0.2-mL PCR tube.
7. Pipet-mix 10 times.
8. Briefly centrifuge the tube.
9. Incubate at room temperature for **5 minutes**.
10. Place the tube on a magnet until the supernatant is clear **(<5 minutes)**.
11. Remove and discard the supernatant.
12. Keeping the tube on the magnet, gently pipet **200 µL** of fresh 80% ethyl alcohol into the tube.
13. Incubate for **30 seconds**.
14. Remove and discard the supernatant without disturbing the beads.
15. Repeat step 12–14 for a total of **two ethyl alcohol washes**.
16. Keeping the tube on the magnet, use a P20 pipette to remove any residual supernatant from the tube.
17. Air-dry the beads at room temperature until the beads no longer look glossy **(~2 minutes)**.
18. Remove the tube from the magnet.
19. Pipet **30 µL** of elution buffer into the tube.
20. Pipet-mix 10 times until the beads are fully resuspended.
21. Incubate the sample at room temperature for **2 minutes**.
22. Briefly centrifuge the tube.
23. Place the tube on the magnet until the solution is clear **(~30 seconds)**.

24. Pipet the eluate **(~30 µL)** into a new 1.5-mL tube.

    The purified eluate is the **final sequencing library**.

    STOP The Index PCR libraries can be stored at –20 °C for up to 6 months until sequencing.

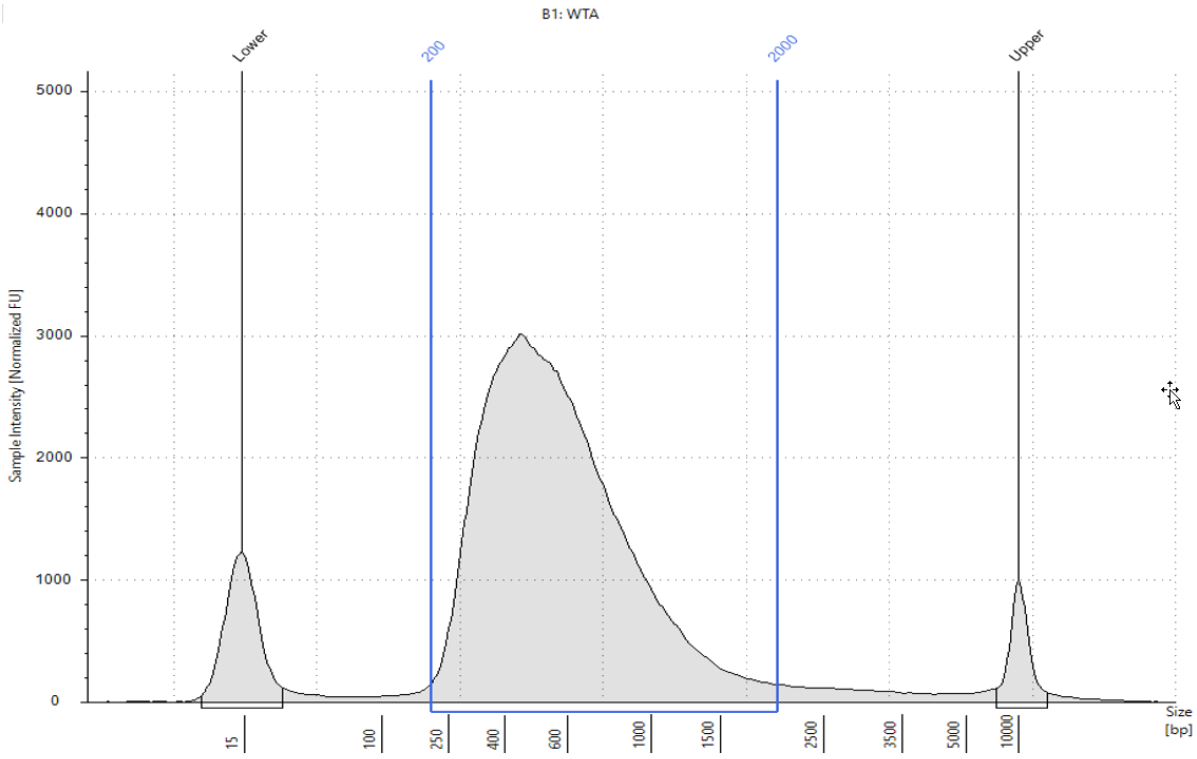
25. Quantify and perform quality control of the WTA Index PCR product with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay and one of the following systems:

    - The Agilent 2100 BioAnalyzer using the Agilent High Sensitivity DNA Kit
    - The Agilent 4200 TapeStation system using the Agilent High Sensitivity D1000 or D5000 ScreenTape assay

    The expected concentration from the Qubit™ Fluorometer is **>1 ng/µL**.

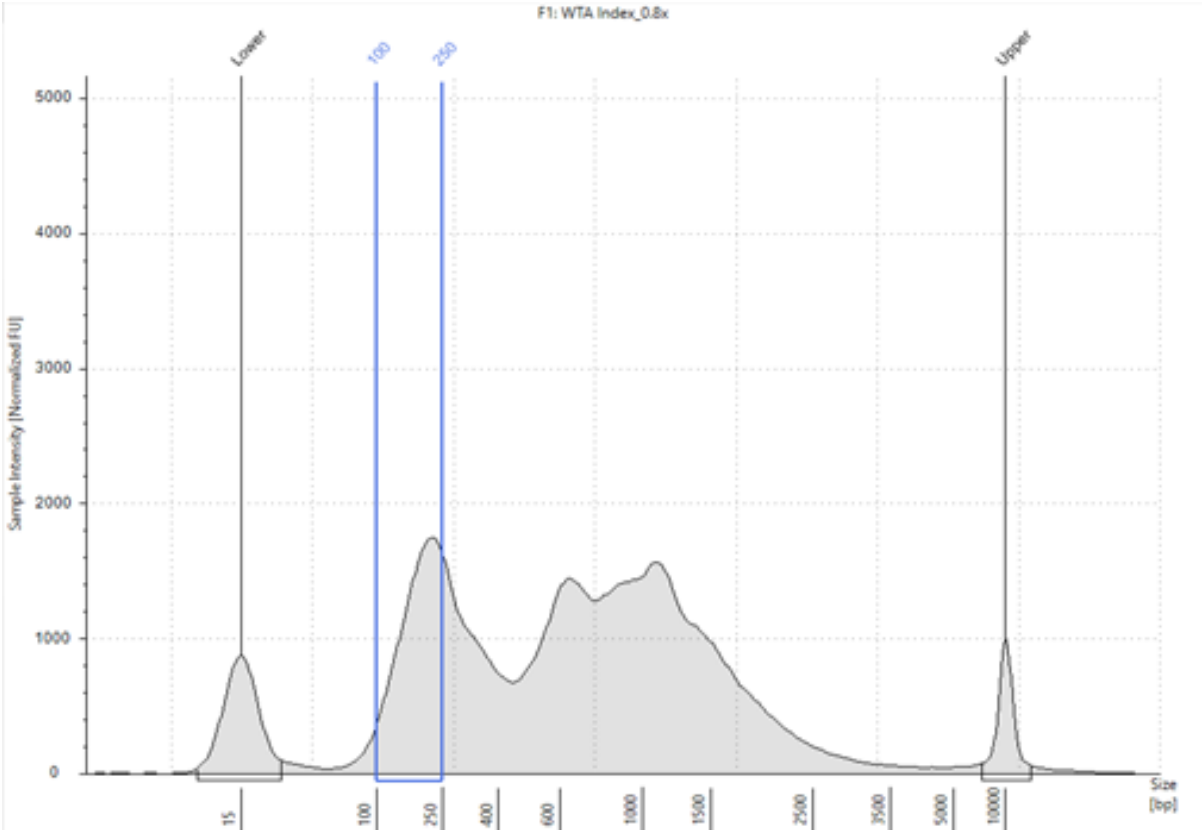
    The TapeStation trace should show a peak from ~200 to 2,000 bp. Refer to the representative traces in the following figures.

    Figure 1 Representative TapeStation High-Sensitivity D5000 trace–WTA Index PCR product



If smaller products (<250 bp) are observed (such as the peaks shown in Figure 2), we recommend a second round of AMPure® XP bead purification. See 2.6 Additional WTA Index PCR cleanup (page 52) for more information.

Figure 2 Representative TapeStation High-Sensitivity D5000 trace–WTA Index PCR product with an observable noise peak in the smaller fragment region



2.6 Additional WTA Index PCR cleanup

Perform the purification in the post-amplification workplace.

1. To the eluate from step 24 (page 50) in 2.5 WTA Index PCR cleanup and quality check (page 48), bring up the total volume to **100 µL** with nuclease-free water.
2. Pipet-mix 10 times.
3. Briefly centrifuge the tube.

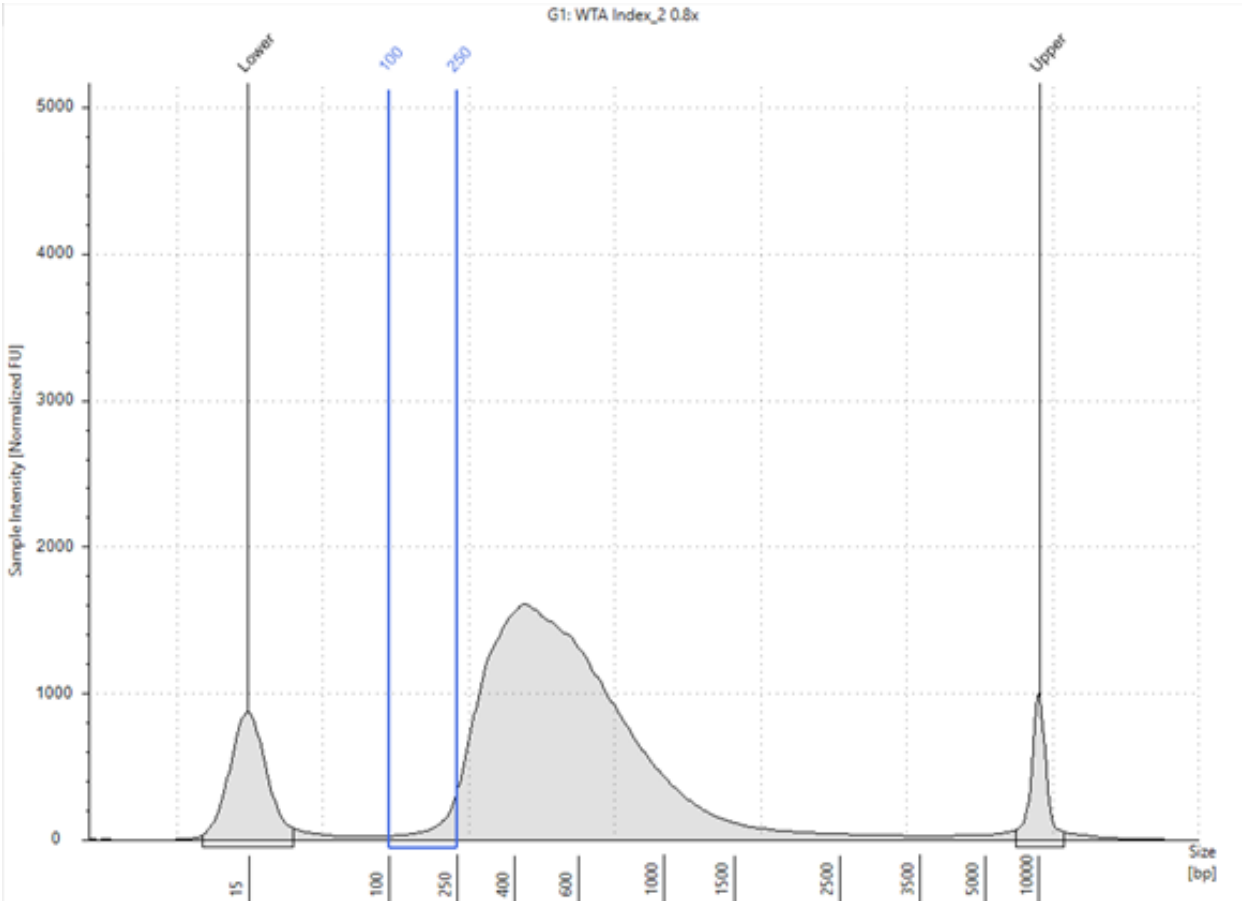
   The volume must be exactly 100 µL. If the volume is less than 100 µL, use nuclease-free water to achieve the final volume.
4. Pipet **80 µL** of AMPure® XP beads (0.8x) into the tube containing 100 µL of sample.
5. Pipet-mix 10 times.
6. Briefly centrifuge the tube.
7. Incubate at room temperature for **5 minutes**.
8. Place the tube on a magnet until the supernatant is clear **(<5 minutes)**.
9. Remove and discard the supernatant.
10. Keeping the tube on the magnet, gently pipet **200 µL** of fresh 80% ethyl alcohol into the tube.
11. Incubate for **30 seconds**.
12. Remove and discard the supernatant without disturbing the beads.
13. Repeat steps 10–12 once for a total of **two ethyl alcohol washes**.
14. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
15. Air-dry the beads at room temperature until the beads no longer look glossy **(~2 minutes)**.
16. Remove the tube from the magnet.
17. Pipet **30 µL** of elution buffer into the tube.
18. Pipet-mix 10 times until the beads are fully resuspended.
19. Incubate at room temperature for **2 minutes**.
20. Briefly centrifuge the tube.
21. Place the tube on a magnet until the supernatant is clear **(~30 seconds)**.
22. Pipet the eluate **(~30 µL)** into a new 1.5-mL tube.

    The purified eluate is the **final sequencing library**.

23. Repeat step 25 (page 50) in 2.5 WTA Index PCR cleanup and quality check (page 48) to perform a quality check of the final library.

STOP The Index PCR libraries can be stored at –20 °C for up to 6 months until sequencing.

Figure 3 Representative TapeStation High Sensitivity D5000 trace–WTA Index PCR product after removal of noise peak in the smaller fragment region



Sequencing

The sequencing depth for each library is dependent on application. For cell-type clustering, shallow sequencing is sufficient. However, for in-depth analysis such as comparison across multiple samples, deep sequencing is advised. We recommend meeting the requirement for recursive substitution error correction (RSEC) sequencing depth of ≥6 to reach the threshold of sequencing saturation where most molecules of the library have been recovered, approximately 80%. The RSEC sequencing depth and sequencing saturation are both reported by the analysis pipeline. The actual sequencing reads/cell required to achieve this depth can vary as it is dependent on the gene expression levels, number of cells, and sequencing run quality. The following reads/cell are recommended for WTA and sgRNA libraries.

Read requirements for libraries

Gene panel	Read requirement for data analysis
BD Rhapsody™ WTA	20,000–100,000 reads/cell
sgRNA	2,000–5,000 reads/cell

Required parameters

Parameter	Requirement
Platform	Illumina and Element*
Paired-end reads	Recommended for WTA only sequencing: Read 1: 51 cycles; Read 2: 71 cycles; Index 1: 8 cycles; Index 2: 8 cycles Recommended for sgRNA together with WTA sequencing: Read 1: 51 cycles; Read 2: 83 cycles; Index 1: 8 cycles; Index 2: 8 cycles
PhiX	1% recommended
Analysis	See the *BD® Single-Cell Multiomics Bioinformatics Handbook*†

* To review Index sequences, see the Appendix (page 58).

† Downstream analysis, including sgRNA assignment per cell and transcriptome profiling, can be performed using the BD Rhapsody™ Sequence Analysis Pipeline and Cellismo™ Data Visualization Tool.

Ensure that the instrument uses the most updated version of firmware (for Illumina and Element).

Sequencing recommendations

- For a NextSeq High or Mid Output run and MiniSeq High or Mid Output run, load the flow cell at a concentration of 1.5–1.8 pM with 1% PhiX for a sequencing run.
- For NovaSeq:

Sequencing platform	Cycles	Recommended loading concentration
NovaSeq 6000 S Prime (Single Lane)	2×50, 2×100, 2×150, 2×250*	180–250 pM (XP workflow)
NovaSeq 6000 S Prime (Single Flow Cell)	2×50, 2×100, 2×150, 2×250*	350–650 pM (standard workflow)
NovaSeq 6000 S1 (Single Lane)	2×50, 2×100, 2×150*	180–250 pM (XP workflow)
NovaSeq 6000 S1 (Single Flow Cell)	2×50, 2×100, 2×150*	350–650 pM (standard workflow)
NovaSeq 6000 S2 (Single Flow Cell)	2×50, 2×100, 2×150*	350–650 pM (standard workflow)
NovaSeq 6000 S4 (Single Lane)	2×100, 2×150	180–250 pM (XP workflow)
NovaSeq 6000 S4 (Single Flow Cell)	2×100, 2×150	350–650 pM (standard workflow)
NovaSeq X 10B	2×100, 2×150	Contact local Field Application Specialist (FAS)

* NovaSeq 100 cycle kit (v1.0 or v1.5) can be used. The 100-cycle kit contains enough reagents for up to 130 cycles.

- For other sequencing platforms (e.g. Element AVITI System), follow the manufacturer's sequencing recommendations. Loading concentration might need to be titrated to optimize yield.

Sequencing depth can vary depending on whether the sample contains high- or low-content RNA cells. For resting PBMCs, we recommend:

- 10,000 reads per cell for shallow sequencing. Genes per cell and UMI per cell detected is generally lower, but this can be useful for cell type identification.
- 20,000–50,000 reads per cell for moderate sequencing.
- 100,000 reads per cell for highly saturated deep sequencing to identify the majority of UMIs in the library.

To determine the ratio of BD Rhapsody™ WTA library to sgRNA library to pool, contact your local Field Application Specialist (FAS) or go to scomix@bd.com.

Sequencing analysis pipeline

Contact customer support at scomix@bd.com for access to the latest whole transcriptome sequencing analysis pipeline.

Troubleshooting

Library preparation

Observation	Possible causes	Recommended solutions
PCR2 product yield too low.	PCR1 and PCR2 primers might have been swapped by mistake.	Ensure the correct primers are used for each step.
	cDNA synthesis might have failed due to incomplete washing of lysis buffer.	Avoid leaving behind lysis buffer or bubbles after removing lysis buffer from the tube during bead wash after retrieval from the cartridge. Use new tubes for each wash step, as described in the protocol.
	cDNA synthesis might have failed due to thermomixer not shaking during reverse transcription.	Samples need to be on the thermomixer in shake mode. Where applicable, ensure that a SmartBlock™ Thermoblock is installed on the thermomixer for 1.5-mL tubes so that the reaction can proceed at the designated temperature.
	Thermal cycler mis-programming.	Ensure that the correct thermal cycling program is used.
	Too few PCR1 cycles.	Optimize the number of PCR cycles for the specific sample type.
	Incorrect volume of AMPure® XP magnetic beads used during PCR2 cleanup.	Use the specified volume of AMPure® XP beads.
	Incorrect solution or incorrect concentration of 80% ethyl alcohol used for washing AMPure® XP magnetic beads, resulting in premature elution of PCR products from beads.	Use 80% ethyl alcohol for washing AMPure® XP beads.
Low yield of RPE-PCR.	Cell number lower than expected.	Repeat PCR using the RPE PCR product for additional cycles. Alternatively, increase Index PCR cycles.
Index PCR BioAnalyzer trace of WTA library has 264 bp peak.	sgRNA library contamination in mRNA library.	If peak takes up high percentage of sequencing reads (manifests as lower reads/cell than expected for WTA library, alongside higher reads/cell than expected for sgRNA), perform a second round of AMPure® purification according to 2.6 Additional WTA Index PCR cleanup (page 52).

Observation	Possible causes	Recommended solutions
Low yield of Index PCR.	Input DNA not high enough or cycle number too low.	Repeat Index PCR with higher cycle number. Alternatively, if RPE PCR product was diluted before adding to Index PCR, repeat Index PCR with less or no dilution.

Sequencing

Observation	Possible causes	Recommended solutions
Over-clustering on the flow cell due to under-estimation of the library.	Inaccurate measurement of the library concentration.	Quantify library according to instructions in protocol.
Low sequencing quality.	Insufficient PhiX.	Use the recommended concentration of PhiX with the library to be sequenced.
	Suboptimal cluster density, or library denaturation, or both.	See troubleshooting in sequencing platform documentation.
One or more libraries are not correctly demultiplexed.	Failure to correctly detect one or more index sequences during sequencing.	Try demultiplexing with a single index. For Illumina sequencers, adjust the mismatch threshold from the default of 1 to allow 2 mismatches.
Failed cluster generation or other sequencing challenges with low-plex pooling.	Sequencing instrument-specific color balance guidelines were not met. For example, the NovaSeq X platform requires signal in the green channel for every cycle. Low % reads in the green channel in a given cycle might not be sufficient.	Consult indexing and pooling guidelines for your sequencing platform. Be sure to consider the final pooling ratio—some libraries might make up a small fraction of the final pool.

Appendix

References for sgRNA Amplification

Renz PF, Ghoshdastider U, Baghai Sain S, Valdivia-Francia F, Khandekar A, Ormiston M, et al. (2024). In vivo single-cell CRISPR uncovers distinct TNF programmes in tumour evolution. *Nature*, 632, 419–428. doi: 10.1038/s41586-024-07663-y

Oligonucleotide	Use	Sequence (5' – 3')
sgRNA PCR1 primer	Reverse primer for sgRNA PCR1	TCTTGTGGAAAGGACGA
sgRNA PCR2 primer	Reverse primer for sgRNA PCR2	CAGACGTGTGCTCTTCCGATCTCTTGTGGAAAGGACGAAACA*C*C*G
		[Sequencing_primer_partial] TCTTGTGGAAAGGACGAAACA*C*C*G

Refer to *Enabling High-Throughput CRISPR Screening with the BD Rhapsody™ System* (doc ID 166243) for more detailed information.

Oligonucleotides in OMICS-One™ WTA Next Amplification Kit

The following table lists the sequences of all oligonucleotides included in the BD® OMICS-One WTA Next Amplification Kit (Catalog No. 572620).

Oligonucleotide		Part/Catalog No.	Sequence (5' – 3')
OMICS-One™ Universal Oligo	Forward primer for WTA RPE PCR, sgRNA PCR1 and PCR2, and BD® AbSeq PCR1	51-9025553	ACA CGA CGC TCT TCC GAT CT
OMICS-One™ AbSeq Primer	Reverse primer for BD® AbSeq PCR1	51-9025468	CAG ACG TGT GCT CTT CCG ATC T
OMICS-One™ WTA Extension Primer	Random primers for WTA RPE	51-9025467	GGC TCG GAG ATG TGT ATA AGA GAC AG NNNNNNNNN
OMICS-One™ WTA Amplification Primer	Reverse primer for WTA RPE PCR	51-9025469	GGC TCG GAG ATG TGT ATA AGA GAC AG
OMICS-One™ Library Forward Primer 1	Forward primer for WTA, sgRNA, and BD® AbSeq Index PCR	51-9025472	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TATAGCCT ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
OMICS-One™ Library Forward Primer 2		51-9025473	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC ATAGAGGC ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
OMICS-One™ Library Forward Primer 3		51-9025474	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC CCTATCCT ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
OMICS-One™ Library Forward Primer 4		51-9025475	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC GGCTCTGA ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
OMICS-One™ Library Forward Primer 5		51-9025476	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC AGGCGAAG ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
OMICS-One™ Library Forward Primer 6		51-9025477	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TAATCTTA ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
OMICS-One™ Library Forward Primer 7		51-9025478	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC CAGGACGT ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
OMICS-One™ Library Forward Primer 8		51-9025479	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC GTACTGAC ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T

Oligonucleotide		Part/Catalog No.	Sequence (5' – 3')
OMICS-One™ WTA Library Reverse Primer 1	Reverse primer for WTA Index PCR	51-9025480	CAA GCA GAA GAC GGC ATA CGA GAT TACTACGC GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
OMICS-One™ WTA Library Reverse Primer 2		51-9025600	CAA GCA GAA GAC GGC ATA CGA GAT AGGCTCCG GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
OMICS-One™ WTA Library Reverse Primer 3		51-9025482	CAA GCA GAA GAC GGC ATA CGA GAT GCAGCGTA GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
OMICS-One™ WTA Library Reverse Primer 4		51-9025483	CAA GCA GAA GAC GGC ATA CGA GAT CTGCGCAT GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
OMICS-One™ WTA Library Reverse Primer 5		51-9025484	CAA GCA GAA GAC GGC ATA CGA GAT GAGCGCTA GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
OMICS-One™ WTA Library Reverse Primer 6		51-9025485	CAA GCA GAA GAC GGC ATA CGA GAT CGCTCAGT GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
OMICS-One™ WTA Library Reverse Primer 7		51-9025486	CAA GCA GAA GAC GGC ATA CGA GAT GTCTTAGG GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
OMICS-One™ WTA Library Reverse Primer 8		51-9025486	CAA GCA GAA GAC GGC ATA CGA GAT ACTGATCG GTCTCGTGGGCTCGGAGATGTGTATAAGA*G

Oligonucleotide		Part/Catalog No.	Sequence (5' – 3')
OMICS-One™ Multiomic Library Reverse Primer 1	Reverse primer for sgRNA and BD® AbSeq Index PCR	51-9025489	CAA GCA GAA GAC GGC ATA CGA GAT TACTACGC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
OMICS-One™ Multiomic Library Reverse Primer 2		51-9025490	CAA GCA GAA GAC GGC ATA CGA GAT AGGCTCCG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
OMICS-One™ Multiomic Library Reverse Primer 3		51-9025492	CAA GCA GAA GAC GGC ATA CGA GAT GCAGCGTA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
OMICS-One™ Multiomic Library Reverse Primer 4		51-9025493	CAA GCA GAA GAC GGC ATA CGA GAT CTGCGCAT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
OMICS-One™ Multiomic Library Reverse Primer 5		51-9025494	CAA GCA GAA GAC GGC ATA CGA GAT GAGCGCTA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
OMICS-One™ Multiomic Library Reverse Primer 6		51-9025496	CAA GCA GAA GAC GGC ATA CGA GAT CGCTCAGT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
OMICS-One™ Multiomic Library Reverse Primer 7		51-9025497	CAA GCA GAA GAC GGC ATA CGA GAT GTCTTAGG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
OMICS-One™ Multiomic Library Reverse Primer 8		51-9025498	CAA GCA GAA GAC GGC ATA CGA GAT ACTGATCG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T

Forward Index name	i5 bases for sample sheet NovaSeq, MiSeq, HiSeq 2000/2500	i5 bases for sample sheet iSeq, MiniSeq, NextSeq, HiSeq 3000/4000
OMICS-One™ Library Forward Primer 1	TATAGCCT	AGGCTATA
OMICS-One™ Library Forward Primer 2	ATAGAGGC	GCCTCTAT
OMICS-One™ Library Forward Primer 3	CCTATCCT	AGGATAGG
OMICS-One™ Library Forward Primer 4	GGCTCTGA	TCAGAGCC
OMICS-One™ Library Forward Primer 5	AGGCGAAG	CTTCGCCT
OMICS-One™ Library Forward Primer 6	TAATCTTA	TAAGATTA
OMICS-One™ Library Forward Primer 7	CAGGACGT	ACGTCCTG
OMICS-One™ Library Forward Primer 8	GTACTGAC	GTCAGTAC

Reverse Index name	Bases in adapter	i7 bases for sample sheet
OMICS-One™ WTA Library Reverse Primer 1 OMICS-One™ Multiomic Library Reverse Primer 1	TACTACGC	GCGTAGTA
OMICS-One™ WTA Library Reverse Primer 2 OMICS-One™ Multiomic Library Reverse Primer 2	AGGCTCCG	CGGAGCCT
OMICS-One™ WTA Library Reverse Primer 3 OMICS-One™ Multiomic Library Reverse Primer 3	GCAGCGTA	TACGCTGC
OMICS-One™ WTA Library Reverse Primer 4 OMICS-One™ Multiomic Library Reverse Primer 4	CTGCGCAT	ATGCGCAG
OMICS-One™ WTA Library Reverse Primer 5 OMICS-One™ Multiomic Library Reverse Primer 5	GAGCGCTA	TAGCGCTC
OMICS-One™ WTA Library Reverse Primer 6 OMICS-One™ Multiomic Library Reverse Primer 6	CGCTCAGT	ACTGAGCG
OMICS-One™ WTA Library Reverse Primer 7 OMICS-One™ Multiomic Library Reverse Primer 7	GTCTTAGG	CCTAAGAC
OMICS-One™ WTA Library Reverse Primer 8 OMICS-One™ Multiomic Library Reverse Primer 8	ACTGATCG	CGATCAGT

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