BD Rhapsody™ System

BD OMICS-One™ WTA Next and Sample Tag

Library Preparation Protocol

23-24993(01)
2025-10

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Regulatory information

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History

Revision	Date	Change made
23-24993(01)	2025-10	Initial release.

Contents

Introduction

This protocol provides instructions on creating single-cell whole transcriptome mRNA and Sample Tag libraries after cell capture on the BD Rhapsody™ HT Single-Cell Analysis System or the BD Rhapsody™ HT Xpress System for sequencing on various sequencers. For complete instrument procedures and safety information, 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*.

Before beginning this protocol, ensure that the cDNA of mRNA and Sample Tag targets is first encoded on BD Rhapsody™ Enhanced Cell Capture Beads, as described in the instrument user guides. At the same time, the barcode information from BD Rhapsody™ Enhanced Cell Capture Beads is also added to Ab-Oligos during reverse transcription, which enables amplification of Sample Tags in solution. To generate the Sample Tag sequencing libraries, the extended Sample Tags are first denatured from the BD Rhapsody™ Enhanced Cell Capture Beads, which are later amplified separately through a series of PCR steps. Meanwhile, the whole transcriptome amplification library is generated directly from the BD Rhapsody™ Enhanced Cell Capture Beads using a random priming approach, followed by an index polymerase chain reaction (PCR) step. The whole transcriptome mRNA and Sample Tag libraries can be combined together for sequencing on various next-generation sequencers.

This protocol is intended to provide a method to screen RNA expression of single cells using a 3' whole transcriptome analysis (WTA) approach using the BD OMICS-One™ WTA Next Amplification Kit for samples that have been labeled using Sample Tags from the BD® Single Cell Multiplexing Kit. The data set generated from this protocol can be used to generate a custom panel for subsequent 3' Targeted mRNA sequencing. Specifically, the protocol outlines how to generate whole transcriptome libraries for cell-capture inputs between 1,000 and 100,000 resting peripheral blood mononuclear cells (PBMCs) per sample for library generation. For cell-capture inputs between 1,000 to <5,000 cells per sample, there are sections in the protocol for additional cleanup. Cell inputs of 5,000 or more will give optimal performance. If sufficient cells are available, loading more cells and subsampling BD Rhapsody™ Enhanced Cell Capture Beads to reach the desired number of cells will improve data quality for lower cell inputs. For cell types other than resting PBMCs, protocol optimization might be required by the user.

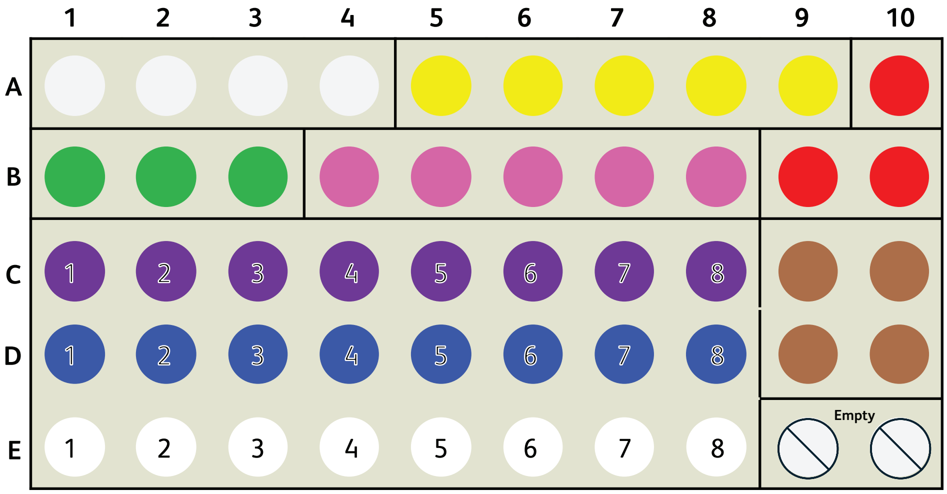
Symbols

The following symbols are used in this guide:

Symbol	Description
⚠	Important information for maintaining measurement accuracy or data integrity.
💡	Noteworthy information.
STOP	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 9) section.



BD OMICS-One™ WTA Next Amplification Kit

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

Part numbers for primers in rows C–E

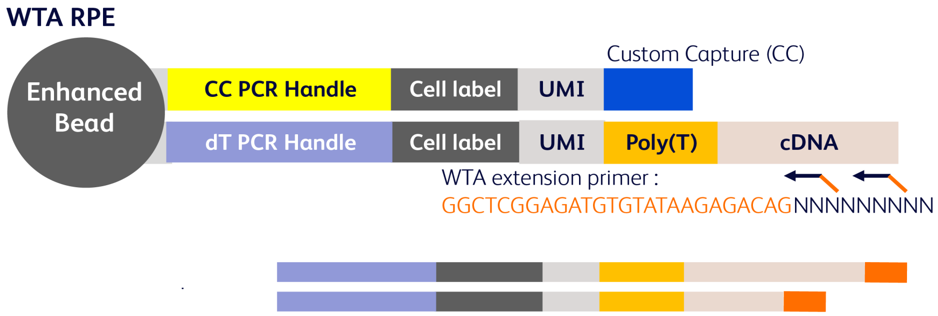
Name	Part Number
BD OMICS-One™ Library Forward Primer 1	51-9025472
BD OMICS-One™ Library Forward Primer 2	51-9025473
BD OMICS-One™ Library Forward Primer 3	51-9025474
BD OMICS-One™ Library Forward Primer 4	51-9025475
BD OMICS-One™ Library Forward Primer 5	51-9025476
BD OMICS-One™ Library Forward Primer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

Workflows

WTA library amplification workflow

1.1 Random priming and extension (RPE) (page 14): Random priming on the bead.

Denature off the RPE product.



1.2 RPE PCR (page 19): Amplify the RPE product.

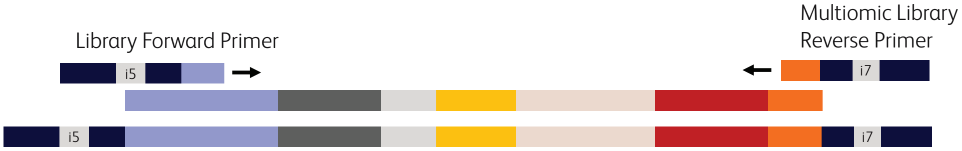
1.4 WTA Index PCR (page 25): Add sequencing adapters and indices.

Sample Tag library amplification workflow

2.1 Sample Tag PCR1 (page 35): Denatured products amplified together.

2.3 Sample Tag PCR2 (page 41): Sample Tag (ST) product further amplified in PCR2.

2.5 Sample Tag Index PCR (page 47): 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.
BD OMICS-One™ WTA Next Amplification Kit	BD Biosciences	572620
AMPure® XP beads for DNA Cleanup	Beckman Coulter	A63880
100% ethyl alcohol, molecular biology grade	Major supplier	–
Nuclease-free water	Major supplier	–

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

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

Libraries

- Sample Tag libraries can be sequenced separately or together with 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.

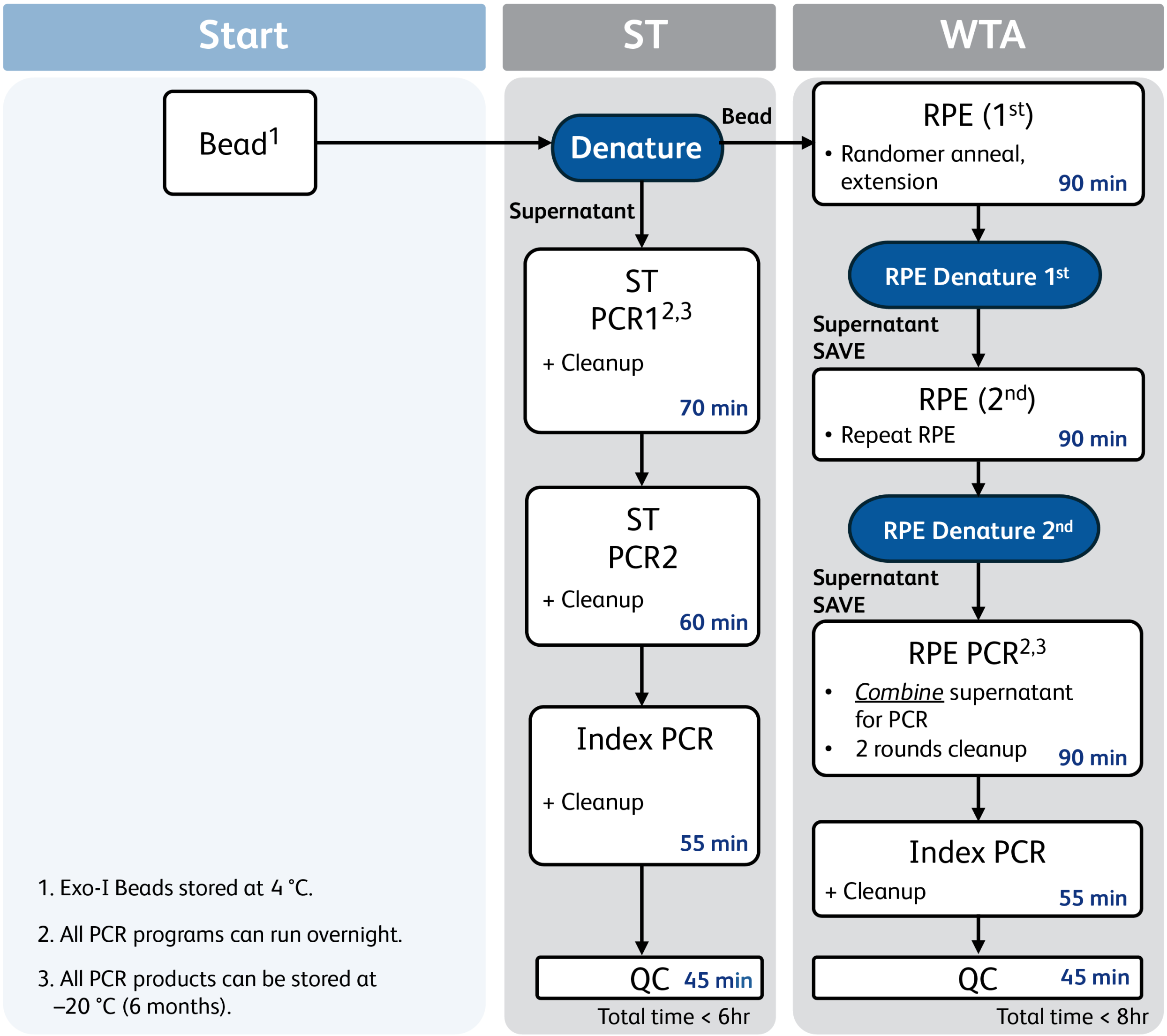
Additional documentation

- *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™ System Single-Cell Labeling with BD® Single-Cell Multiplexing Kits Protocol* (doc ID 23-21340)
- *BD Rhapsody™ System Single-Cell Labeling with BD® Flex Single-Cell Multiplexing Kits Protocol* (doc ID 23-24311)
- *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



1. Exo-I Beads stored at 4 °C.
2. All PCR programs can run overnight.
3. All PCR products can be stored at –20 °C (6 months).

Procedure

Continue this procedure after staining the antibodies as described in one of the following protocols:

- *BD Rhapsody™ System Single-Cell Labeling with BD® Single-Cell Multiplexing Kits Protocol* (doc ID 23-21340)
- *BD Rhapsody™ System Single-Cell Labeling with BD® Flex Single-Cell Multiplexing Kits Protocol* (doc ID 23-24311)

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 whole transcriptome amplification library generation of cell inputs between 1,000 to 100,000 single cells, specifically resting PBMCs. For cell inputs between 1,000 and 5,000 single cells, follow the extra steps outlined in the additional cleanup section.

Ensure that the intended total cell load is between 1,000 and 100,000 single cells for this protocol. Cell load below or above this recommendation might not be suitable for the current protocol configuration. Then proceed as described in the following procedure.

1. WTA library amplification

This procedure comprises the following tasks:

- 1.1 Random priming and extension (RPE) (page 14)
- 1.2 RPE PCR (page 19)
- 1.3 RPE PCR cleanup and quantification (page 22)
- 1.4 WTA Index PCR (page 25)
- 1.5 WTA Index PCR cleanup and quality check (page 28)
- (Optional) 1.6 Additional WTA Index PCR cleanup (page 32)

1.1 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	Equilibrate to room temperature 30 minutes before setting up RPE. Centrifuge briefly.	–20 °C
🟡	WTA extension primer	51-9025467		
🟡	dNTP mixture	51-9025491		
⚪	Nuclease-free water	51-9025552		
🟣	Elution buffer	51-9025554		
Place on ice:				
🟡	Bead RT/PCR enhancer	51-9025495	Centrifuge briefly before adding to mix.	–20 °C
Leave in freezer until ready to use:				
🟡	WTA extension enzyme	51-9025499	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Exonuclease I-treated cell capture beads			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.

Perform this procedure in the pre-amplification workspace. 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 21 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 primer	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 Exonuclease I-treated BD Rhapsody™ Enhanced Cell Capture Beads, and then complete one of the following actions.
   - If you are using a subsample of the beads, proceed to step 6.
   - If you are using the entire sample of beads, skip to step 7.
6. (Optional) To subsample the Exonuclease I-treated BD Rhapsody™ Enhanced Cell Capture Beads:
   a. Based on the expected number of viable cells captured on the beads in the final bead resuspension volume, determine the volume of beads to subsample for sequencing.
   b. Completely resuspend the beads by pipet-mixing, then pipet the calculated volume of the bead suspension into a new 1.5-mL tube. Bring the total volume up to 200 µL using bead resuspension buffer.

      The remaining Exonuclease I-treated beads can be stored in bead resuspension buffer at 4 °C for up to 1 year.
7. Place the tube with beads on a magnet until the supernatant is clear **(<2 minutes)**.
8. Remove and discard the supernatant.
9. Remove the tube from the magnet.
10. Add **75 µL** of elution buffer to the tube.

11. To denature Sample Tag 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. Briefly centrifuge the tube.
    d. Place the tube on a magnet until the supernatant is clear (**<2 minutes**).

    Save supernatant at this step. Do not discard!

    e. Transfer **75 µL** of the supernatant (Sample Tag product) to a new 1.5-mL tube.

    To minimize Sample Tag contamination, ensure that all the supernatant is removed from the tube.

    f. Keep the tube with Sample Tag product on **ice** or at **4 ˚C** for up to 24 hours until ready to proceed to 2.1 Sample Tag PCR1 (page 35).
12. Remove the tube from the magnet.
13. Pipet **200 µL** of elution buffer to the tube.
14. Pipet-mix 10 times to resuspend the beads.
15. Briefly centrifuge the tube.
16. Place the tube on a magnet until the supernatant is clear **(<2 minutes)**.
17. Remove and discard the supernatant.
18. Remove the tube from the magnet.
19. Add **87 µL** Random primer mix to the tube.
20. 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.

21. 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—If you are using one thermomixer, skip the 37 ˚C incubation step.

    c. Thermomixer at 1,200 rpm and at **25 ˚C** for **5 minutes**.
22. Briefly centrifuge the tube.
23. Leave at **room temperature** until ready to use.

24. 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**

25. Add **13 µL** of the Extension enzyme mix to the tube with beads from step 23 (total volume of 100 µL).
26. Place on **ice** until ready to use.

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

27. 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.

28. Place the tube of Extension enzyme mix with beads in the programmed thermomixer (see step 27).
29. Remove the tube after the program is complete.
30. Place the tube on a magnet until the supernatant is clear **(<2 minutes)**.
31. Remove and discard the supernatant.
32. Remove the tube from the magnet.
33. Pipet **200 µL** of elution buffer into the tube.
34. Pipet-mix 10 times until the beads are fully resuspended.
35. Place the tube on a magnet until the supernatant is clear **(<2 minutes)**.
36. Remove and discard the supernatant.
37. Remove the tube from the magnet.
38. Add **80 µL** of elution buffer to the tube.

39. 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.
40. Place the tube containing the RPE product on ice.
41. Repeat steps 19–40 to perform a second RPE.

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

42. Combine the two RPE products for each sample, for a total volume of **160 µL** (80 µL from the first RPE + 80 µL from the second RPE).

1.2 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	Equilibrate to room temperature 30 minutes before setting up RPE PCR. Centrifuge briefly.	–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.

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**

1. Pipet-mix the RPE PCR mix.
2. Place on ice until ready to use.
3. Add **84 µL** of the RPE PCR mix to the tube with the **160 µL** of RPE product.
4. Pipet-mix 10 times to create the RPE PCR reaction mix.
5. Split the mix into **four** 0.2-mL PCR tubes with **60 µL** mix per tube.
6. Transfer any residual mix to one of the tubes.

Bring the tubes to the post-amplification workspace.

7. 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 - 30,000 cells: 8 cycles 30,000 - 70,000 cells: 7 cycles 70,000 - 100,000 cells: 6 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.

For subsampled beads with <5,000 cells, use 10 cycles.

Two additional cycles are recommended for PBMC nuclei.

STOP The PCR can run overnight.

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

1.3 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
Qubit dsDNA HS Assay Kit			Manufacturer's recommendations	
AMPure® XP magnetic beads				
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:

This section describes how to perform a single-sided AMPure® cleanup to remove unwanted products from the RPE PCR products. The final product is purified double-stranded DNA (~200–2,000 bp).

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 1.4 WTA Index PCR (page 25).

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

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

1.4 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	Equilibrate to room temperature 30 minutes before setting up WTA Index PCR. 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.

The same indices can be used for all library types for each lane (WTA and Sample Tag, for example). The libraries will be demultiplexed using the BD Rhapsody™ Sequence Analysis Pipeline. If you prefer to index each library separately, you can use combinatorial dual indexing for more index combinations.

⚠ 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.

💡 In the pre-amplification workplace, in a new 1.5-mL tube, pipet the following components:

WTA 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	2.5	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**	**48.0**	**168.0**	**336.0**

1. Pipet-mix the WTA Index PCR mix.
2. Pipet **35 µL** of WTA Index PCR mix into a separate 0.2-mL PCR tube for each sample.
3. Add **2.5 µL** of forward primer and **2.5 µL** of reverse primer to each sample.
4. Place on ice until ready to use.

   💡 Bring the tubes to the post-amplification workspace.

5. Dilute an aliquot of the purified RPE PCR product from step 46 of 1.3 RPE PCR cleanup and quantification (page 22) 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".

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

8. Run the following PCR program.

WTA Index PCR program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	RPE PCR concentration* 0.05 ng/µL: 12 cycles 0.1 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.

STOP The PCR can run overnight.

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

1.5 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.

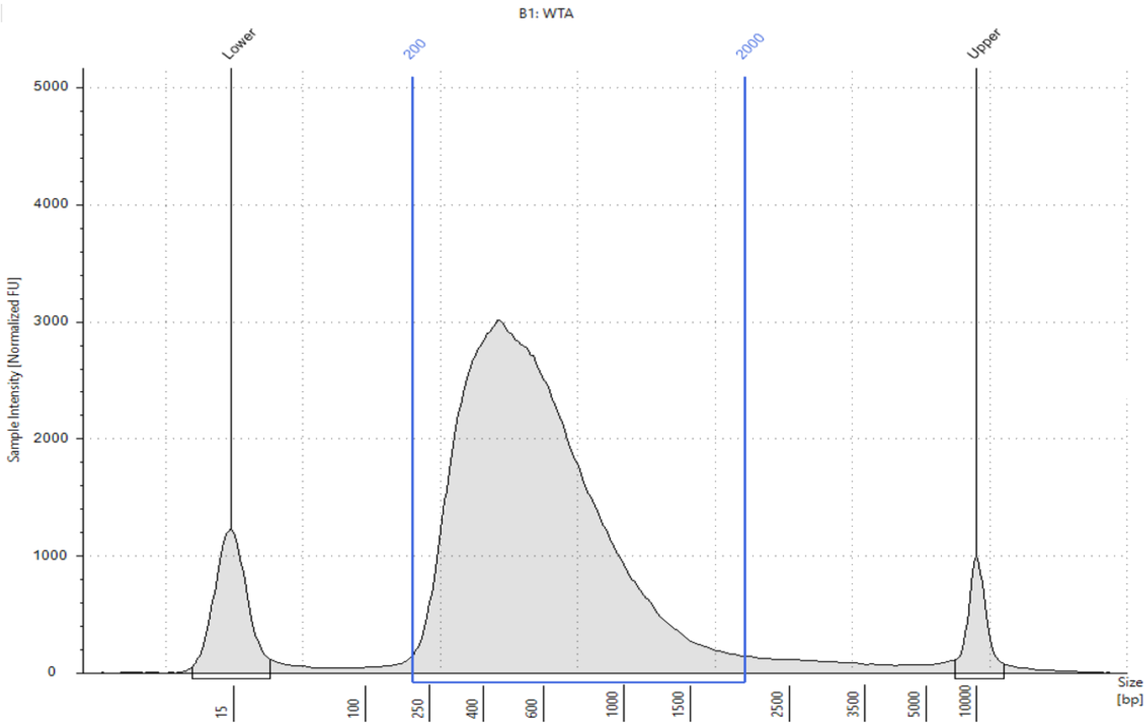
25. Quantify and perform quality control of the WTA Index PCR product with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay and one of the following systems:

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

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

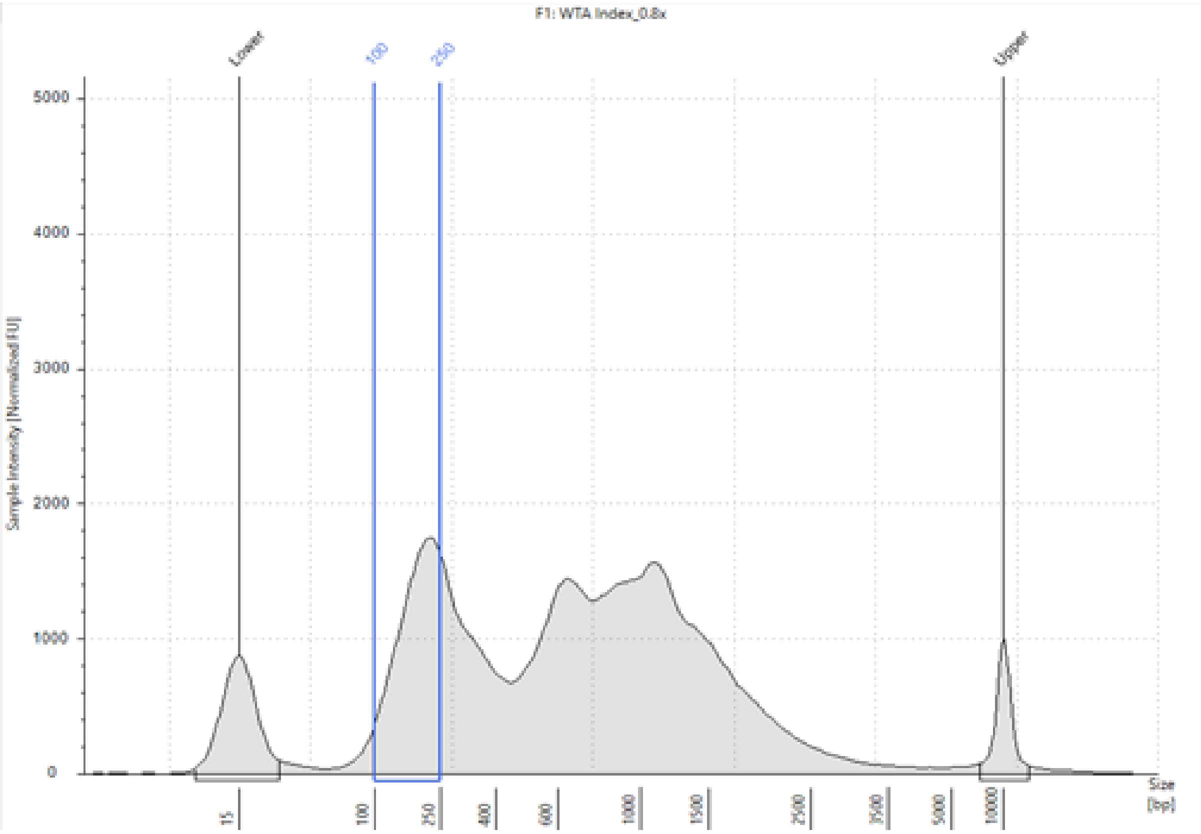
    The TapeStation trace should show a peak from ~200 to 2,000 bp. Refer to the representative traces in the following figures.

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 1.6 Additional WTA Index PCR cleanup (page 32) 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



1.6 Additional WTA Index PCR cleanup

Perform the purification in the post-amplification workplace.

1. To the eluate from step 24 (page 30) in 1.5 WTA Index PCR cleanup and quality check (page 28), 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.

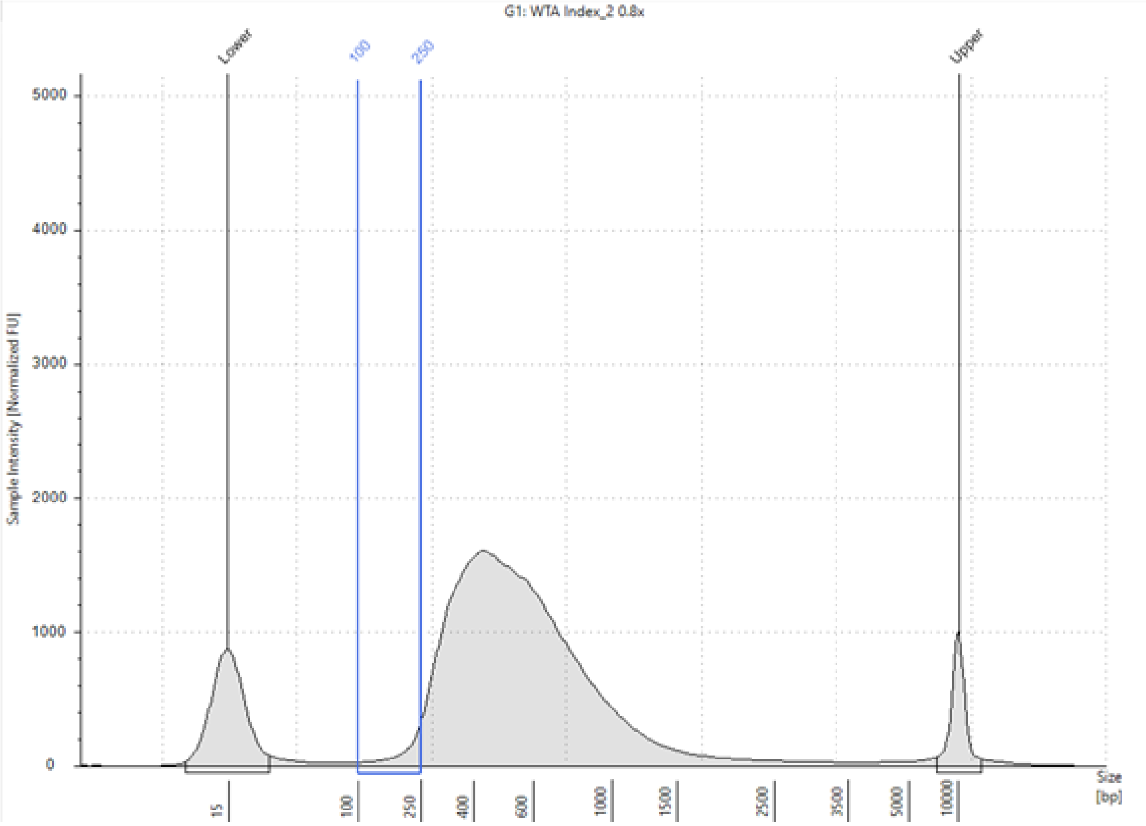
4. Pipet **80 µL** of AMPure® XP beads (0.8x) into the tube containing 100 µL of sample.
5. Pipet-mix 10 times.
6. Briefly centrifuge the tube.
7. Incubate at room temperature for **5 minutes**.
8. Place the tube on a magnet until the supernatant is clear **(<5 minutes)**.
9. Remove and discard the supernatant.
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 30) in 1.5 WTA Index PCR cleanup and quality check (page 28) 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



2. Sample Tag library amplification

This section comprises the following tasks:

2.1 Sample Tag PCR1

Summary:

- Prepare Sample Tag PCR1 mix
- Amplify using Sample Tag PCR1 program

Preparation list:

Item		**BD Part Number**	**Preparation and Handling**	**Storage**
Equilibrate to room temperature:				
🟢	Universal oligo	51-9025553	Equilibrate to room temperature 30 minutes before setting up Sample Tag PCR1. Centrifuge briefly.	–20 °C
🔴	Sample Tag PCR1 primer	51-9025470		
⚪	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:				
Denatured Sample Tag product				4 °C
Ice bucket				
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Thermocycler Sample Tag PCR1 program				

Procedure steps:

This section describes how to amplify Sample Tag products through PCR.

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

Sample Tag 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	2.5	3.0	12.0	24.0
🔴	Sample Tag PCR1 primer	1.0	1.2	4.8	9.6
⚪	Nuclease-free water	78.5	94.2	376.8	753.6
	Total	**132.0**	**158.4**	**633.6**	**1,267.2**

1. Pipet-mix the Sample Tag PCR1 mix.
2. Place on **ice** until ready to use.
3. In a new 1.5-mL tube, add **132 µL** of the mix with **68 µL** of Sample Tag product.
4. Pipet-mix to create the Sample Tag PCR1 reaction mix.
5. Split the reaction mix into **four** 0.2-mL PCR tubes with **50 µL** mix per tube.
6. Transfer any residual mix to one of the tubes.

Bring the tubes to the post-amplification workspace.

7. Run the following PCR program:

Sample Tag PCR 1 program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	1,000 cells: 14 cycles 2,500 cells: 13 cycles 5,000 cells: 12 cycles 10,000 cells: 11 cycles 20,000 cells: 10 cycles 40,000 cells: 9 cycles >80,000 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 need to be optimized for different cell types.

STOP The PCR can be run overnight.

8. When the Sample Tag PCR1 program is complete, briefly centrifuge the tubes.

2.2 Sample Tag PCR1 cleanup

Summary:

- Sample Tag PCR1 cleanup

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	
Obtain:			
Sample Tag 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 Sample Tag PCR1 products. The final product is purified double-stranded DNA.

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 1 mL of 80% ethyl alcohol.

3. Vortex the AMPure® XP beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with the Sample Tag PCR1 product.
5. Combine the **four** tubes of **50-µL** PCR1 product into a new 1.5-mL tube.
6. Pipet-mix 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.

7. Pipet **280 µL** AMPure® XP beads (1.4x) into the tube.
8. Pipet-mix 10 times.
9. 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.

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

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

19. Remove the tube from the magnet.
20. Pipet **30 µL** of elution buffer into the tube.

21. Pipet-mix 10 times until the beads are fully resuspended.
22. Incubate at room temperature for **2 minutes**.
23. Briefly centrifuge the tube.
24. Place the tube on a magnet until the supernatant is clear (**~30 seconds**).
25. Pipet the eluate **(30 µL)** into a new 1.5-mL tube.

    The purified Sample Tag PCR1 product is ready for 2.3 Sample Tag PCR2 (page 41).

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

2.3 Sample Tag PCR2

Summary:

- Prepare Sample Tag PCR2 mix
- Amplify using Sample Tag PCR2 program

Preparation list

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟢	Universal oligo	51-9025553	Equilibrate to room temperature 30 minutes before setting up Sample Tag PCR2. Centrifuge briefly.	–20 °C
🔴	Sample Tag PCR2 primer	51-9025471		
⚪	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 Sample Tag PCR1 product				4 °C
Ice bucket				
1.5-mL LoBind® tube				
0.2-mL PCR tubes				
Set up:				
Thermocycler with Sample Tag PCR2 program				

Procedure steps

This section describes how to amplify Sample Tag 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, in a new 1.5-mL tube, pipet the following components:

Sample Tag 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
🔴	Sample Tag PCR2 primer	3.0	3.6	14.4	28.8
⚪	Nuclease-free water	24.5	29.4	139.2	278.4
	Total	**45.0**	**54.0**	**216.0**	**432.0**

* If preparing Sample Tag PCR2 mix for one sample, dilute universal oligo 1:10 for pipetting accuracy.

1. Pipet-mix the Sample Tag PCR2 mix.
2. Place on **ice** until ready to use.
3. Pipet **45 µL** of Sample Tag PCR2 mix into a separate 0.2-mL PCR tube for each sample.

   Bring the tubes to the post-amplification workspace.

4. Add **5 µL** of **undiluted** Sample Tag PCR1 product.
5. Pipet-mix 10 times.

6. Run the following PCR program:

Sample Tag PCR2 program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	10*	98 °C	15 seconds
Annealing		66 °C	30 seconds
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

* Recommended PCR cycles might need to be optimized for different cell types.

STOP The PCR can run overnight.

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

2.4 Sample Tag PCR2 cleanup and quantification

Summary:

- Sample Tag 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:			
Sample Tag PCR2 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 Sample Tag PCR2 products. The final product is purified double-stranded DNA.

Perform the purification in the post-amplification workspace.

1. Bring AMPure® XP 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 tubes with Sample Tag PCR2 product.

   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 **60 µL** of AMPure® XP beads (1.2x) 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® 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 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.

    The Sample Tag PCR2 product is ready for 2.5 Sample Tag Index PCR (page 47).

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 **0.1–1.1 ng/µL**.

STOP The Sample Tag PCR2 libraries can be stored at –20 °C for up to 6 months.

2.5 Sample Tag Index PCR

Summary:

- Prepare Sample Tag Index PCR mix
- Amplify using Sample Tag Index PCR program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟣	Forward primer 1–8	Various	Equilibrate to room temperature 30 minutes before setting up Sample Tag Index PCR. 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 Sample Tag PCR2 product				4 °C
Ice bucket				
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
Set up:				
Thermocycler with Sample Tag Index PCR program				

Procedure steps:

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

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

Sample Tag 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	30.5	36.6	146.4	292.8
	Total	**47.0**	**56.4**	**206.4**	**412.8**

1. Pipet-mix the Sample Tag Index PCR mix.
2. For multiple samples, pipet **43 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 Sample Tag Index PCR mix to the post-amplification workspace.

5. Dilute an aliquot of Sample Tag PCR2 product from 2.4 Sample Tag PCR2 cleanup and quantification (page 44) with nuclease-free water to **0.1–1.1 ng/µL**.

   Add **3 µL** of the diluted Sample Tag PCR2 to Sample Tag Index PCR mix.

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

6. Pipet-mix 10 times.

7. Run the following PCR program:

Sample Tag Index PCR program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	Index PCR concentration* 0.5–1.1 ng/µL: 6 cycles 0.25-0.5 ng/µL: 7 cycles 0.1-0.25 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 PCR cycles might need to be optimized for different cell types.

STOP The PCR can run overnight.

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

2.6 Sample Tag Index PCR cleanup and quality check

Summary:

- Sample Tag 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:			
Sample Tag 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 Sample Tag 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 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 beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with Sample Tag 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 **40 µL** of AMPure® XP beads (0.8x) 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 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.

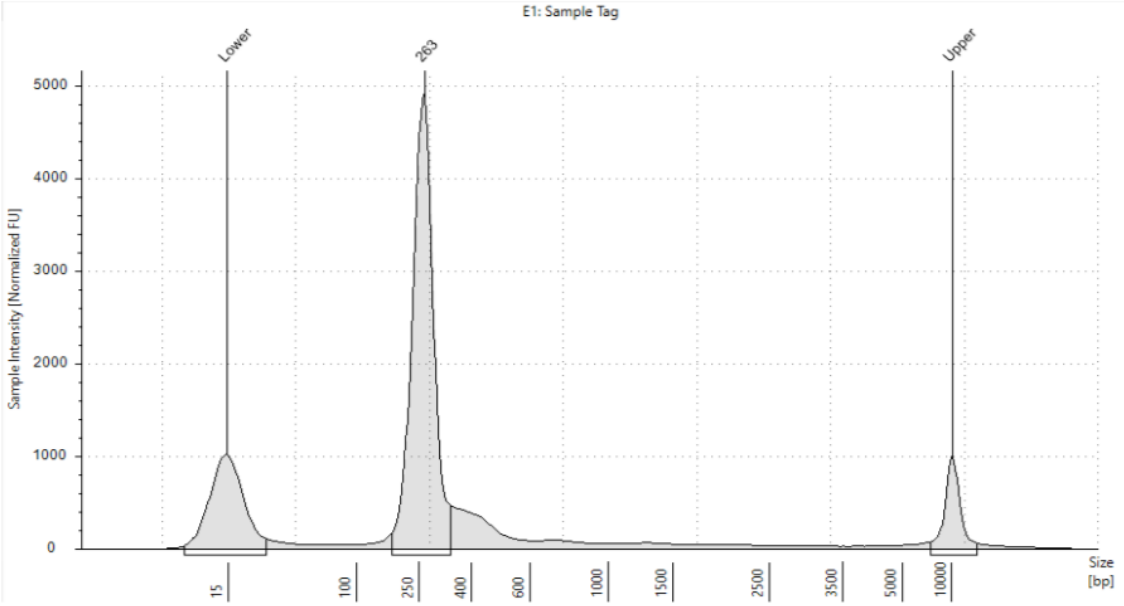
    The purified eluate is the final sequencing library.

    STOP 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 Sample Tag 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 Sample Tag library should show a peak of ~276 bp. Exact size may vary due to instrument or sample purification efficiency. Select the region size between 200 and 500 bp.

Figure 4 Representative TapeStation High Sensitivity D5000 trace—Sample Tag Index PCR product



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 Sample Tag libraries.

Read requirements for libraries

Library type	Read requirement for data analysis
BD Rhapsody™ WTA	20,000–100,000 reads/cell
Sample Tag	600 reads/cell

Required parameters

Parameter	Requirement
Platform	Illumina and Element*
Paired-end reads	Recommend Read 1: 51 cycles; Read 2: 71 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).

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 Sample Tag library to pool for sequencing, use the sequencing calculator available by contacting your local Field Application Specialist (FAS) or 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 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 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.	Sample Tag 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 Sample Tag), perform a second round of AMPure® purification according to 1.6 Additional WTA Index PCR cleanup (page 32).

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.
Yield of Sample Tag library too low after Index PCR (<1 ng/µL).	Sample Tag labeling incubation time too short.	Ensure that the cells were labeled with Sample Tags correctly and that the correct incubation time was used.
	PCR1 and PCR2 primers swapped.	Ensure that correct primer is used for each step.
	Only one primer (library forward or library reverse primer) added to Index PCR mix.	Ensure that both the library forward primer and library reverse primer are added to the Index PCR mix, and repeat Index PCR.
	Too few Index PCR cycles.	Increase the number of Index PCR cycles.
Expected size of Sample Tag products is too short (<276 bp).	Upper and lower markers on the Agilent Bioanalyzer or Agilent TapeStation are incorrectly called.	Ensure that the markers are correct. Follow the manufacturer's instructions.
	Inefficient Sample Tag labeling.	Ensure that the cells were labeled with Sample Tags correctly and that the correct incubation time was used.
	Sample Tags were not amplified in PCR steps due to incorrect primers used.	Perform PCR2 again. See 2.3 Sample Tag PCR2 (page 41). Analyze products using the Agilent Bioanalyzer or the Agilent TapeStation and look for a ~182 bp peak that corresponds to Sample Tag PCR2 products.

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.

Observation	Possible causes	Recommended solutions
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.
High proportion of undetermined Sample Tag calls in sequencing results.	Insufficient sequencing of the Sample Tag Library.	1. Set pooled samples of the same cell type to 120 reads/cell. 2. Set pooled samples of different cell types to 600 reads/cell. 3. Repeat sequencing of Sample Tag library. If issue persists, contact your local Field Application Specialist (FAS) or scomix@bd.com.
	Insufficient washes after labeling cells with Sample Tags.	Follow the washing steps in the Sample Tag staining protocol.
	BD Rhapsody™ cartridge overloaded with cells.	Follow the cell loading steps in the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide*.

Appendix

Oligonucleotides in BD 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	**Use**	**Part/Catalog No.**	**Sequence (5' – 3')**
BD OMICS-One™ Universal Oligo	Forward primer for WTA RPE PCR, Sample Tag PCR1 and PCR2, and BD® AbSeq PCR1	51-9025553	ACA CGA CGC TCT TCC GAT CT
BD OMICS-One™ AbSeq Primer	Reverse primer for BD® AbSeq PCR1	51-9025468	CAG ACG TGT GCT CTT CCG ATC T
BD OMICS-One™ WTA Extension Primer	Random primer for WTA RPE	51-9025467	GGC TCG GAG ATG TGT ATA AGA GAC AG NNNNNNNNN
BD OMICS-One™ WTA Amplification Primer	Reverse primer for WTA RPE PCR	51-9025469	GGC TCG GAG ATG TGT ATA AGA GAC AG

Oligonucleotide	Use	Part/Catalog No.	Sequence (5' – 3')
BD OMICS-One™ Library Forward Primer 1	Forward primer for WTA, Sample Tag, and BD® AbSeq Index PCR	51-9025472	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TATAGCCT ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
BD OMICS-One™ Library Forward Primer 2		51-9025473	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC ATAGAGGC ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
BD OMICS-One™ Library Forward Primer 3		51-9025474	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC CCTATCCT ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
BD OMICS-One™ Library Forward Primer 4		51-9025475	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC GGCTCTGA ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
BD OMICS-One™ Library Forward Primer 5		51-9025476	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC AGGCGAAG ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
BD OMICS-One™Library Forward Primer 6		51-9025477	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TAATCTTA ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
BD OMICS-One™ Library Forward Primer 7		51-9025478	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC CAGGACGT ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
BD OMICS-One™ Library Forward Primer 8		51-9025479	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC GTACTGAC ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T

Oligonucleotide	Use	Part/Catalog No.	Sequence (5' – 3')
BD 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
BD OMICS-One™ WTA Library Reverse Primer 2		51-9025600	CAA GCA GAA GAC GGC ATA CGA GAT AGGCTCCG GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
BD OMICS-One™ WTA Library Reverse Primer 3		51-9025482	CAA GCA GAA GAC GGC ATA CGA GAT GCAGCGTA GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
BD OMICS-One™ WTA Library Reverse Primer 4		51-9025483	CAA GCA GAA GAC GGC ATA CGA GAT CTGCGCAT GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
BD OMICS-One™ WTA Library Reverse Primer 5		51-9025484	CAA GCA GAA GAC GGC ATA CGA GAT GAGCGCTA GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
BD OMICS-One™ WTA Library Reverse Primer 6		51-9025485	CAA GCA GAA GAC GGC ATA CGA GAT CGCTCAGT GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
BD OMICS-One™ WTA Library Reverse Primer 7		51-9025486	CAA GCA GAA GAC GGC ATA CGA GAT GTCTTAGG GTCTCGTGGGCTCGGAGATGTGTATAAGA*G
BD OMICS-One™ WTA Library Reverse Primer 8		51-9025487	CAA GCA GAA GAC GGC ATA CGA GAT ACTGATCG GTCTCGTGGGCTCGGAGATGTGTATAAGA*G

Oligonucleotide	Use	Part/Catalog No.	Sequence (5' – 3')
BD OMICS-One™ Multiomic Library Reverse Primer 1	Reverse primer for Sample Tag and BD® AbSeq Index PCR	51-9025489	CAA GCA GAA GAC GGC ATA CGA GAT TACTACGC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 2		51-9025490	CAA GCA GAA GAC GGC ATA CGA GAT AGGCTCCG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 3		51-9025492	CAA GCA GAA GAC GGC ATA CGA GAT GCAGCGTA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 4		51-9025493	CAA GCA GAA GAC GGC ATA CGA GAT CTGCGCAT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 5		51-9025494	CAA GCA GAA GAC GGC ATA CGA GAT GAGCGCTA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 6		51-9025496	CAA GCA GAA GAC GGC ATA CGA GAT CGCTCAGT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 7		51-9025497	CAA GCA GAA GAC GGC ATA CGA GAT GTCTTAGG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD 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
BD OMICS-One™ Library Forward Primer 1	TATAGCCT	AGGCTATA
BD OMICS-One™ Library Forward Primer 2	ATAGAGGC	GCCTCTAT
BD OMICS-One™ Library Forward Primer 3	CCTATCCT	AGGATAGG
BD OMICS-One™ Library Forward Primer 4	GGCTCTGA	TCAGAGCC
BD OMICS-One™ Library Forward Primer 5	AGGCGAAG	CTTCGCCT
BD OMICS-One™ Library Forward Primer 6	TAATCTTA	TAAGATTA
BD OMICS-One™ Library Forward Primer 7	CAGGACGT	ACGTCCTG
BD OMICS-One™ Library Forward Primer 8	GTACTGAC	GTCAGTAC

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

Sample Tag sequences

Human Sample Tag sequences

Each Human Sample Tag is a human universal antibody conjugated with a unique oligonucleotide sequence to allow for sample identification. Each Sample Tag has common 5' and 3' ends, with the Sample Tag sequence between them:

- GTTGTCAAGATGCTACCGTTCAGAG**(Sample Tag sequence)**AAAAAAAAAAAAAAAAAAAAAAAAA

Sample Tag	Sample Tag sequence
Sample Tag 1–Human	ATTCAAGGGCAGCCGCGTCACGATTGGATACGACTGTTGGACCGG
Sample Tag 2–Human	TGGATGGGATAAGTGCGTGATGGACCGAAGGGACCTCGTGGCCGG
Sample Tag 3–Human	CGGCTCGTGCTGCGTCGTCTCAAGTCCAGAAACTCCGTGTATCCT
Sample Tag 4–Human	ATTGGGAGGCTTTCGTACCGCTGCCGCCACCAGGTGATACCCGCT
Sample Tag 5–Human	CTCCCTGGTGTTCAATACCCGATGTGGTGGGCAGAATGTGGCTGG
Sample Tag 6–Human	TTACCCGCAGGAAGACGTATACCCCTCGTGCCAGGCGACCAATGC
Sample Tag 7–Human	TGTCTACGTCGGACCGCAAGAAGTGAGTCAGAGGCTGCACGCTGT
Sample Tag 8–Human	CCCCACCAGGTTGCTTTGTCGGACGAGCCCGCACAGCGCTAGGAT
Sample Tag 9–Human	GTGATCCGCGCAGGCACACATACCGACTCAGATGGGTTGTCCAGG
Sample Tag 10–Human	GCAGCCGGCGTCGTACGAGGCACAGCGGAGACTAGATGAGGCCCC
Sample Tag 11–Human	CGCGTCCAATTTCCGAAGCCCCGCCCTAGGAGTTCCCCTGCGTGC
Sample Tag 12–Human	GCCCATTCATTGCACCCGCCAGTGATCGACCCTAGTGGAGCTAAG

Mouse Immune Sample Tag sequences

Each Mouse Immune Sample Tag is an Anti-Mouse CD45, Clone 30-F11 antibody conjugated with a unique oligonucleotide sequence to allow for sample identification. Each Sample Tag has common 5' and 3' ends, with the Sample Tag sequence between them:

- GTTGTCAAGATGCTACCGTTCAGAG**(Sample Tag sequence)**AAAAAAAAAAAAAAAAAAAAAAAAA

Sample Tag	Sample Tag sequence
Sample Tag 1–Mouse Immune	AAGAGTCGACTGCCATGTCCCCTCCGCGGGTCCGTGCCCCCCAAG
Sample Tag 2–Mouse Immune	ACCGATTAGGTGCGAGGCGCTATAGTCGTACGTCGTTGCCGTGCC
Sample Tag 3–Mouse Immune	AGGAGGCCCCGCGTGAGAGTGATCAATCCAGGATACATTCCCGTC
Sample Tag 4–Mouse Immune	TTAACCGAGGCGTGAGTTTGGAGCGTACCGGCTTTGCGCAGGGCT
Sample Tag 5–Mouse Immune	GGCAAGGTGTCACATTGGGCTACCGCGGGAGGTCGACCAGATCCT
Sample Tag 6–Mouse Immune	GCGGGCACAGCGGCTAGGGTGTTCCGGGTGGACCATGGTTCAGGC
Sample Tag 7–Mouse Immune	ACCGGAGGCGTGTGTACGTGCGTTTCGAATTCCTGTAAGCCCACC
Sample Tag 8–Mouse Immune	TCGCTGCCGTGCTTCATTGTCGCCGTTCTAACCTCCGATGTCTCG
Sample Tag 9–Mouse Immune	GCCTACCCGCTATGCTCGTCGGCTGGTTAGAGTTTACTGCACGCC
Sample Tag 10–Mouse Immune	TCCCATTCGAATCACGAGGCCGGGTGCGTTCTCCTATGCAATCCC
Sample Tag 11–Mouse Immune	GGTTGGCTCAGAGGCCCCAGGCTGCGGACGTCGTCGGACTCGCGT
Sample Tag 12–Mouse Immune	CTGGGTGCCTGGTCGGGTTACGTCGGCCCTCGGGTCGCGAAGGTC

BD® Flex SMK sequences

Each Flex Sample Tag is an anti-PE antibody conjugated with a unique oligonucleotide sequence to allow for sample identification. Each Sample Tag has common 5' and 3' ends, with the Sample Tag sequence between them:

- GTTGTCAAGATGCTACCGTTCAGAG**(Sample Tag sequence)**AAAAAAAAAAAAAAAAAAAAAAAAA

BD® Flex Single-Cell Multiplexing Kit A (Catalog No. 633849)

⚠ Not for use together with Hu SMK Tags 1–6 (Catalog No. 633781) in the same experiment.

Sample Tag	Sample Tag sequence	Notes
Sample Tag 1–Flex	ATTCAAGGGCAGCCGCGTCACGATTGGATACGACTGTTGGACCGG	Barcode sequence is the same as human SMK Sample Tag 1
Sample Tag 2–Flex	TGGATGGGATAAGTGCGTGATGGACCGAAGGGACCTCGTGGCCGG	Barcode sequence is the same as human SMK Sample Tag 2
Sample Tag 3–Flex	CGGCTCGTGCTGCGTCGTCTCAAGTCCAGAAACTCCGTGTATCCT	Barcode sequence is the same as human SMK Sample Tag 3
Sample Tag 4–Flex	ATTGGGAGGCTTTCGTACCGCTGCCGCCACCAGGTGATACCCGCT	Barcode sequence is the same as human SMK Sample Tag 4
Sample Tag 5–Flex	CTCCCTGGTGTTCAATACCCGATGTGGTGGGCAGAATGTGGCTGG	Barcode sequence is the same as human SMK Sample Tag 5
Sample Tag 6–Flex	TTACCCGCAGGAAGACGTATACCCCTCGTGCCAGGCGACCAATGC	Barcode sequence is the same as human SMK Sample Tag 6

BD® Flex Single-Cell Multiplexing Kit B (Catalog No. 633850)

Not for use together with Hu SMK Tags 7–12 (Catalog No. 633781) in the same experiment.

Sample Tag	Sample Tag sequence	Notes
Sample Tag 7–Flex	TGTCTACGTCGGACCGCAAGAAGTGAGTCAGAGGCTGCACGCTGT	Barcode sequence is the same as human SMK Sample Tag 7
Sample Tag 8–Flex	CCCCACCAGGTTGCTTTGTCGGACGAGCCCGCACAGCGCTAGGAT	Barcode sequence is the same as human SMK Sample Tag 8
Sample Tag 9–Flex	GTGATCCGCGCAGGCACACATACCGACTCAGATGGGTTGTCCAGG	Barcode sequence is the same as human SMK Sample Tag 9
Sample Tag 10–Flex	GCAGCCGGCGTCGTACGAGGCACAGCGGAGACTAGATGAGGCCCC	Barcode sequence is the same as human SMK Sample Tag 10
Sample Tag 11–Flex	CGCGTCCAATTTCCGAAGCCCCGCCCTAGGAGTTCCCCTGCGTGC	Barcode sequence is the same as human SMK Sample Tag 11
Sample Tag 12–Flex	GCCCATTCATTGCACCCGCCAGTGATCGACCCTAGTGGAGCTAAG	Barcode sequence is the same as human SMK Sample Tag 12

BD® Flex Single-Cell Multiplexing Kit C (Catalog No. 633851)

⚠ Not for use together with Ms SMK Tags 1–6 (Catalog No. 633793) in the same experiment.

Sample Tag	Sample Tag sequence	Notes
Sample Tag 13–Flex	AAGAGTCGACTGCCATGTCCCCTCCGCGGGTCCGTGCCCCCCAAG	Barcode sequence is the same as mouse SMK Sample Tag 1
Sample Tag 14–Flex	ACCGATTAGGTGCGAGGCGCTATAGTCGTACGTCGTTGCCGTGCC	Barcode sequence is the same as mouse SMK Sample Tag 2
Sample Tag 15–Flex	AGGAGGCCCCGCGTGAGAGTGATCAATCCAGGATACATTCCCGTC	Barcode sequence is the same as mouse SMK Sample Tag 3
Sample Tag 16–Flex	TTAACCGAGGCGTGAGTTTGGAGCGTACCGGCTTTGCGCAGGGCT	Barcode sequence is the same as mouse SMK Sample Tag 4
Sample Tag 17–Flex	GGCAAGGTGTCACATTGGGCTACCGCGGGAGGTCGACCAGATCCT	Barcode sequence is the same as mouse SMK Sample Tag 5
Sample Tag 18–Flex	GCGGGCACAGCGGCTAGGGTGTTCCGGGTGGACCATGGTTCAGGC	Barcode sequence is the same as mouse SMK Sample Tag 6

BD® Flex Single-Cell Multiplexing Kit D (Catalog No. 633852)

Not for use together with Ms SMK Tags 7–12 (Catalog No. 633793) in the same experiment.

Sample Tag	**Sample Tag sequence**	**Notes**
Sample Tag 19–Flex	ACCGGAGGCGTGTGTACGTGCGTTTCGAATTCCTGTAAGCCCACC	Barcode sequence is the same as mouse SMK Sample Tag 7
Sample Tag 20–Flex	TCGCTGCCGTGCTTCATTGTCGCCGTTCTAACCTCCGATGTCTCG	Barcode sequence is the same as mouse SMK Sample Tag 8
Sample Tag 21–Flex	GCCTACCCGCTATGCTCGTCGGCTGGTTAGAGTTTACTGCACGCC	Barcode sequence is the same as mouse SMK Sample Tag 9
Sample Tag 22–Flex	TCCCATTCGAATCACGAGGCCGGGTGCGTTCTCCTATGCAATCCC	Barcode sequence is the same as mouse SMK Sample Tag 10
Sample Tag 23–Flex	GGTTGGCTCAGAGGCCCCAGGCTGCGGACGTCGTCGGACTCGCGT	Barcode sequence is the same as mouse SMK Sample Tag 11
Sample Tag 24–Flex	CTGGGTGCCTGGTCGGGTTACGTCGGCCCTCGGGTCGCGAAGGTC	Barcode sequence is the same as mouse SMK Sample Tag 12

BD OMICS-One™ WTA Next and Sample Tag Library Preparation Quick Reference Guide

This guide is designed as a quick reference for users who are already familiar with the long-form BD OMICS-One™ WTA Next and Sample Tag Library Preparation protocol but do not need detailed instructions.

Introduction

This quick reference assumes that users are experts in the use of AMPure® XP Magnetic Beads (AMPure), the preparation of 80% ethyl alcohol (ethanol), and knowledgeable about the proper technique for WTA library preparation and common pitfalls. Users who are not familiar with these steps should review them in the long-form protocol.

To maintain brevity in this guide, longer names and terms are shortened as indicated in the following key:

Abbreviation	Full-length description
Amp.	Amplification
Analyzer	Refers to either the Agilent® 2100 Bioanalyzer System or Agilent® 4200 TapeStation® System. Used for all quality checking steps.
Beads, Cell Capture Beads	BD Rhapsody™ Enhanced Cell Capture Beads
Fluorometer	Invitrogen™ Qubit™ Fluorometer. Used for all quantification steps.
Fwd.	Forward
Fwd. Index Primer	Library Forward Primer 1–8
Magnet	The action of putting the sample tube on a magnet, then waiting for the solution to clear. Typically takes <2 minutes.
Purification	Purification using the AMPure reagent following the standard workflow in the long-form protocol. Includes: Adding the indicated volume of AMPure and pipet-mixing the sample (~10x), incubating for 5 minutes, putting the sample with AMPure on a magnet, removing and discarding the supernatant, washing twice with sufficient 80% ethanol to cover the AMPure beads, drying the AMPure beads, then eluting into room-temperature elution buffer.
Rev.	Reverse
Rev. Index Primer	Library Reverse Primer 1–8
RPE	Random Priming and Extension
Spin-down	Briefly centrifuge <1 min to collect liquid at bottom of tube.
ST	Sample Tag
Wash	Includes: Putting the sample tube on the magnet, adding the indicated volume of buffer, removing the buffer while the sample is still on the magnet, and then discarding the buffer.
Water	Nuclease-free water
WTA Amp. Primer	WTA Amplification Primer

Introduction to purification with AMPure® XP magnetic beads (AMPure)

The BD OMICS-One™ WTA Next protocol uses AMPure® XP magnetic beads (AMPure) for size-selection and purification throughout the workflow. This page provides general instructions for using AMPure, eliminating the need to repeat instructions with every purification.

- All PCR purifications will follow the same mixing, washing, and incubating steps.
- The volumes of PCR, AMPure, and room temperature elution buffer differ for each purification.
- Specific PCR purification volumes will be provided in tables contained within the guide.

NOTE: Correct volumes are **critical** for accurate size selection.

Volumes in these instructions are color-coded to match the volumes in Table 1 Example PCR purification.

Users who need more guidance should see the long-form protocol for detailed instructions.

Getting started

1. Bring AMPure to room temperature approximately 15 minutes before starting.
2. Determine the amount of ethanol required for purification using the following table.

Ethanol required per wash (two washes per purification)

Volume PCR + AMPure	Tube needed	Vol. Ethanol per wash (mL)
>220 µL	1.5-mL	0.5
<220 µL	0.2-mL (PCR)	0.2

3. Prepare 80% ethanol according to the following table.

Component	1 mL	2 mL	5 mL	10 mL
Water	0.2	0.4	1	2
100% ethanol	0.8	1.6	4	8

Purifying the sample with AMPure (example)

1. Pipet 220 µL PCR product into a new 1.5-mL tube. See row 1 of Table 1 Example PCR purification.
2. Vortex AMPure until beads are completely resuspended.
3. Add 264 µL AMPure to the 220 µL sample.
4. Pipet-mix ~10 times.
   - Do NOT mix by vortexing.
   - Avoid getting AMPure buffer on the tube cap.
5. Incubate **5 min at room temperature**.
6. Magnet for **3 min**.
7. Discard supernatant.
8. Wash AMPure pellet as follows:
   a. Add **0.5 mL** 80% ethanol.
   b. Discard **0.5 mL** ethanol.
   c. Repeat steps a and b for a total of **two ethanol washes**.
9. Remove the tube containing the AMPure pellet from the magnet.
10. Spin-down tube with pellet.
11. Magnet tube containing the pellet.
12. Remove all residual ethanol with a P20 pipet.
13. Air-dry pellet.
    Do not let the pellet over-dry and crack.
14. Remove tube from the magnet.
15. Resuspend pellet in 40 µL room temperature elution buffer.
16. Incubate 2 min at room temperature.
17. Magnet until solution is clear (about 30 sec).
18. Collect supernatant into a new PCR tube.
19. Add 60 µL water to the purified PCR product.
20. Mix.
21. Repeat purification steps 1–19 using the corresponding volumes in row 2 of Table 1 Example PCR purification.

Table 1 Example PCR purification

Purification	Water Vol. µL	PCR Vol. µL	AMPure Vol. µL	Elution Vol. µL
PCR product	0	220	264	40
1x purified PCR	60	100	120	30

BD OMICS-One™ WTA Next and Sample Tag library preparation

Preparing the thermomixers

Preheat the following instruments:

- 95 °C heat block
- 37 °C thermomixer
- 25 °C thermomixer

Preparing the reagent mixes

1. Prepare the **Random Primer mix** using the following table. Keep the mixture at room temperature.

Cap	Component	Sample count with % overage (volumes in µL)								
		1+0%	1+20%	2+20%	3+20%	4+20%	5+20%	6+20%	7+20%	8+20%
🟡	WTA Extension Buffer	20.0	24.0	48.0	72.0	96.0	120.0	144.0	168.0	192.0
🟡	WTA Extension Primer	40.0	48.0	96.0	144.0	192.0	240.0	288.0	336.0	384.0
⚪	Water	114.0	136.8	273.6	410.4	547.2	684.0	820.8	957.6	1094.4
	Total	**174.0**	**208.8**	**417.6**	**626.4**	**835.2**	**1044.0**	**1252.8**	**1461.6**	**1670.4**

2. Prepare the **Extension Enzyme mix** using the following table. Keep the mixture on ice.

Cap	Component	Sample count with % overage (volumes in µL)								
		1+0%	1+20%	2+20%	3+20%	4+20%	5+20%	6+20%	7+20%	8+20%
🟡	dNTP	8.0	9.6	19.2	28.8	38.4	48.0	57.6	67.2	76.8
🟡	Bead Enhancer	12.0	14.4	28.8	43.2	57.6	72.0	86.4	100.8	115.2
🟡	WTA Extension Enzyme	6.0	7.2	14.4	21.6	28.8	36.0	43.2	50.4	57.6
	Total	**26.0**	**31.2**	**62.4**	**93.6**	**124.8**	**156.0**	**187.2**	**218.4**	**249.6**

Denaturing the Sample Tag (ST) product

1. Label a new 1.5-mL tube as *ST product.*
2. Spin-down cell capture beads.
3. Magnet beads and discard supernatant.
4. Resuspend beads in **75 µL room temperature elution buffer.**
5. Incubate beads in a **95 °C heat block for 5 minutes (no shaking).**
6. Magnet beads and **collect supernatant** containing ST product into the labeled 1.5-mL tube.
7. Store **ST product** on ice until the ST PCR 1 section. See "Amplifying the Sample Tags (ST)" on page QRG-7.
8. Wash beads with **200 µL elution buffer.**

Generating the RPE product

1. Add **87 µL of Random Primer mix** (keep at room temperature).
2. Pipet-mix to resuspend beads (~10 times).
3. Incubate the sample as follows:
    a. **95 °C heat block (no shaking) for 5 minutes.**
    b. **37 °C thermomixer at 1,200 rpm for 5 minutes.**
    c. **25 °C thermomixer at 1,200 rpm for 5 minutes.**
4. Add **13 µL of the Extension Enzyme mix** to the tube containing beads.
5. Incubate the tube in the thermomixer programmed with the following **RPE program.**

RPE program (all steps at 1,200 rpm)

Step	Temperature	Time	Mode
1	25 °C	10 min	TIME
2	37 °C	15 min	
3	45 °C	10 min	
4	55 °C	10 min	

6. If this is the first round of RPE, label a new 1.5-mL tube as *RPE product.*
7. After the RPE program completes, spin-down the sample.
8. Magnet beads.
9. Discard supernatant.
10. Wash beads in **200 µL room temperature elution buffer.**
11. Denature the product as follows:
    a. Resuspend beads in **80 µL room temperature elution buffer.**
    b. Incubate sample at **95 °C for 5 min.**
    c. Incubate sample **1 min on ice.**
    d. Magnet sample.
    e. Transfer **80 µL** supernatant containing denatured **RPE product** to previously labeled 1.5-mL tube.
    f. Store RPE product on ice. You will need the RPE product for step 2 on page QRG-5.
12. Repeat steps 1–11 **of this section** for a total of **two rounds** of RPE and **160 µL RPE product**.
13. Continue to the "WTA RPE PCR" section on the next page.

⚠ DO NOT STOP.

Amplifying the RPE product

1. Prepare the **RPE PCR mix** using the following table.

Cap	Component	Sample count with % overage (volumes in µL)								
		1+0%	1+20%	2+20%	3+20%	4+20%	5+20%	6+20%	7+20%	8+20%
🟢	PCR Master Mix	60.0	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0
🟢	Universal Oligo	12.0	14.4	28.8	43.2	57.6	72.0	86.4	100.8	115.2
🟢	WTA Amp. Primer	12.0	14.4	28.8	43.2	57.6	72.0	86.4	100.8	115.2
	Total	**84.0**	**100.8**	**201.6**	**302.4**	**403.2**	**504.0**	**604.8**	**705.6**	**806.4**

2. Combine **84 µL RPE PCR mix** with **160 µL RPE product.**
3. Mix well.
4. Split all the PCR mix with product into **four PCR tubes** containing ~60 µL each.
5. Amplify using the **RPE PCR program** table.

RPE PCR program

Step	Cycles	Temp	Time
Hot start	1	98 °C	45 sec
Denaturation	1k–20k cells: 9 cycles 20k–30k cells: 8 cycles 30k–70k cells: 7 cycles 70k–100k cells: 6 cycles	98 °C	15 sec
Annealing		60 °C	30 sec
Extension		72 °C	1 min
Extension	1	72 °C	2 min
Hold	1	4 °C	∞

STOP **Safe stopping point.**
PCR can run overnight.

6. After PCR, spin-down sample.
7. Combine into a 1.5-mL tube.
8. Purify the RPE PCR product **twice** according to the following **RPE PCR purification** table.

Purification	Water vol. µL	PCR vol. µL	AMPure vol. µL	Elution vol. µL
RPE PCR product	0	220	264	40
1x purified RPE PCR	60	100*	120	30

*100 µL = 40 µL elution 1 + 60 µL water

STOP **Safe stopping point.**
Purified PCR product can be stored at –20 °C for up to 6 months.

Quantifying the RPE PCR product

1. Quantify purified RPE PCR product with the fluorometer.
2. Dilute purified RPE PCR product to **0.2–0.5 ng/µL**.
3. Continue to the “WTA Index PCR” section.

Indexing the WTA libraries

1. Prepare the **WTA Index PCR mix** using the following table.

Cap	Component	Sample count with % overage (volumes in µL)								
		1+0%	1+20%	2+20%	3+20%	4+20%	5+20%	6+20%	7+20%	8+20%
🟢	PCR Master Mix	12.5	15.0	30.0	45.0	60.0	75.0	90.0	105.0	120.0
🟣	Fwd. Index Primer	2.5	3.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A
🔵	WTA Rev. Index Primer	2.5	3.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A
⚪	Water	22.5	27.0	54.0	81.0	108.0	135.0	162.0	189.0	216.0
	Total	**40.0**	**48.0**	**84.0**	**126.0**	**168.0**	**210.0**	**252.0**	**294.0**	**336.0**

2. Combine **40 µL WTA Index PCR mix** with **10 µL** *diluted* and *purified* **RPE PCR product.** See "Amplifying the RPE product" on page QRG-5.
3. Mix well.
4. Amplify using the **WTA Index PCR program** table.

STOP **Safe stopping point.**
PCR can run overnight.

WTA Index PCR program

Step	Cycles	Temp	Time
Hot start	1	98 °C	45 sec
Denaturation	0.05 ng/µL: 12 cycles 0.1 ng/µL: 11 cycles 0.2 ng/µL: 10 cycles 0.5 ng/µL: 8 cycles	98 °C	15 sec
Annealing		60 °C	30 sec
Extension		72 °C	1 min
Extension	1	72 °C	2 min
Hold	1	4 °C	∞

5. After PCR, spin-down sample.
6. Purify according to the following **WTA Index PCR purification** table.

Purification	Water Vol. µL	PCR Vol. µL	AMPure Vol. µL	Elution Vol. µL
WTA Index PCR	60	100	80	30

STOP **Safe stopping point.**
Purified PCR product can be stored at –20 °C for up to 6 months.

Quantifying the indexed WTA libraries

1. Quantify purified WTA Index PCR with the fluorometer.
2. Quality check purified WTA Index PCR product using an analyzer.
3. Determine the average fragment size from 200–2000 bp.
4. If a noise peak is present, repeat the 0.8X WTA Index PCR purification.

Amplifying the Sample Tags (ST)

1. Prepare the **ST PCR 1 mix** using the following table.

Cap	Component	Sample count with % overage (volumes in µL)								
		1+0%	1+20%	2+20%	3+20%	4+20%	5+20%	6+20%	7+20%	8+20%
🟢	PCR Master Mix	50.0	60.0	120.0	180.0	240.0	300.0	360.0	420.0	480.0
🟢	Universal Oligo	2.5	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0
🔴	ST PCR1 Primer	1.0	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6
⚪	Water	78.5	94.2	188.4	282.6	376.8	471.0	565.2	659.4	753.6
	Total	**132.0**	**158.4**	**316.8**	**475.2**	**633.6**	**792.0**	**950.4**	**1108.8**	**1267.2**

2. Combine **68 µL ST product** (see “Denaturing the Sample Tag (ST) product” on page QRG-4) with **132 µL ST PCR 1 mix.**
3. Mix well.
4. Split the ST PCR mix-containing product across **four PCR tubes** containing 50 µL each.
5. Amplify using the following table.

Step	Cycles	Temp	Time
Hot start	1	98 °C	45 sec
Denaturation	1,000 cells: 14 cycles 2,500 cells: 13 cycles 5,000 cells: 12 cycles 10,000 cells: 11 cycles 20,000 cells: 10 cycles 40,000 cells: 9 cycles >80,000 cells: 8 cycles	98 °C	15 sec
Annealing		60 °C	30 sec
Extension		72 °C	1 min
Extension	1	72 °C	2 min
Hold	1	4 °C	∞

STOP **Safe stopping point.**
PCR can run overnight.

6. After PCR, spin-down sample.
7. Combine into a 1.5-mL tube.
8. Purify according to the following **ST PCR 1 purification** table.

Purification	PCR Vol. µL	AMPure Vol. µL	Elution Vol. µL
ST PCR 1	200	280	30

STOP **Safe stopping point.**
Purified PCR product can be stored at –20 °C for up to 6 months.

Generating the ST PCR 2 product

1. Prepare the **ST PCR 2 mix** using the following table.

Cap	Component	Sample count with % overage (volumes in µL)								
		1+0%	1+20%	2+20%	3+20%	4+20%	5+20%	6+20%	7+20%	8+20%
🟢	PCR Master Mix	12.5	15.0	30.0	45.0	60.0	75.0	90.0	105.0	120.0
🟢	1:10 Dilution* of Universal Oligo	5.0	6.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A
🟢	Universal Oligo	N/A	N/A	1.2	1.8	2.4	3.0	3.6	4.2	4.8
🔴	ST PCR 2 Primer	3.0	3.6	7.2	10.8	14.4	18.0	21.6	25.2	28.8
⚪	Water	24.5	29.4	69.6	104.4	139.2	174.0	208.8	243.6	278.4
	Total	**45.0**	**54.0**	**108.0**	**162.0**	**216.0**	**270.0**	**324.0**	**378.0**	**432.0**

*If preparing ST PCR 2 mix for 1 sample, dilute the Universal Oligo 1:10 for pipetting accuracy.

2. Combine **5 µL** *purified* and *undiluted* **ST PCR 1 product** with **45 µL ST PCR 2 mix.**
3. Mix well.
4. Amplify using the following table.

Step	Cycles	Temp	Time
Hot start	1	98 °C	45 sec
Denaturation	10	98 °C	15 sec
Annealing		**66 °C**	30 sec
Extension		72 °C	1 min
Extension	1	72 °C	2 min
Hold	1	4 °C	∞

STOP **Safe stopping point.**
PCR can run overnight.

5. After PCR, spin-down sample.
6. Purify according to the following **ST PCR 2 purification** table.

Purification	PCR Vol. µL	AMPure Vol. µL	Elution Vol. µL
ST PCR 2	50	60	30

STOP **Safe stopping point.**
Purified PCR product can be stored at –20 °C for up to 6 months.

Quantifying the ST PCR 2 product

1. Quantify purified ST PCR 2 product with the fluorometer.
2. Dilute ST PCR 2 product to **0.1–1.1 ng/µL** and continue to the “Sample Tag (ST) Index PCR” section.

Indexing the ST libraries

1. Prepare the **ST Index PCR mix** using the following table.

Cap	Component	Sample count with % overage (volumes in µL)								
		1+0%	1+20%	2+20%	3+20%	4+20%	5+20%	6+20%	7+20%	8+20%
🟢	PCR Master Mix	12.5	15.0	30.0	45.0	60.0	75.0	90.0	105.0	120.0
🟣	Fwd. Index Primer	2.0	2.4	N/A	N/A	N/A	N/A	N/A	N/A	N/A
○	Multiomic Rev. Index Primer	2.0	2.4	N/A	N/A	N/A	N/A	N/A	N/A	N/A
⚪	Water	30.5	36.6	73.2	109.8	146.4	183.0	219.6	256.2	292.8
	Total	**47.0**	**56.4**	**103.2**	**154.8**	**206.4**	**258.0**	**309.6**	**361.2**	**412.8**

2. Combine **47 µL ST Index PCR mix** with **3 µL** *diluted* and *purified* **ST PCR 2 product.** See "Generating the ST PCR 2 product" on page QRG-8.
3. Mix well.
4. Amplify using the following table.

Step	Cycles	Temp	Time
Hot start	1	98 °C	45 sec
Denaturation	0.1–0.25 ng/µL: 8 cycles 0.25–0.5 ng/µL: 7 cycles 0.5–1.1 ng/µL: 6 cycles	98 °C	15 sec
Annealing		60 °C	30 sec
Extension		72 °C	1 min
Extension	1	72 °C	2 min
Hold	1	4 °C	∞

STOP **Safe stopping point.**
PCR can run overnight.

5. After PCR, spin-down sample.
6. Purify according to the following **ST Index PCR purification** table.

Purification	PCR Vol. µL	AMPure Vol. µL	Elution Vol. µL
ST Index PCR	50	40	30

STOP **Safe stopping point.**
Purified PCR product can be stored at –20 °C for up to 6 months.

Quantifying the indexed ST libraries

1. Quantify purified ST Index PCR product with the fluorometer.
2. Quality check purified ST Index PCR product using an analyzer.
3. Determine average fragment size from 200–500 bp.
4. Refer to sequencing guidelines in the long-form protocol.

BD OMICS-One™ WTA Next and Sample Tag Quick Reference Guide

The following note sheet is provided for user convenience.

Experiment ____________ User ____________ Date ____________ Kit Lot ____________ Expiry ____________

Use the area below to record sample information:

Sample Description	RPE PCR Quant. ng/µL	WTA Fwd. Index	WTA Rev. Index	WTA Index PCR Quant. ng/µL	ST PCR 2 Quant. ng/µL	ST Fwd. Index	ST Rev. Index	ST Index PCR Quant. ng/µL	Notes
1									
2									
3									
4									
5									
6									
7									
8									

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