BD Rhapsody™ System BD OMICS-One™ WTA Next, BD® AbSeq, and Sample Tag Library Preparation Protocol

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Regulatory information

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History

Revision	Date	Change made
23-24994(01)	2025-10	Initial release.

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Introduction

This protocol provides instructions on creating single-cell whole transcriptome mRNA, BD[®] AbSeq, 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, BD® AbSeq, 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 the Ab-Oligos and Sample Tags in solution. To generate the BD[®] AbSeq and Sample Tag sequencing libraries, the extended Ab-Oligos and 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, BD[®] AbSeq, 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 BD[®] AbSeq Ab-Oligo reagents, as well as 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.

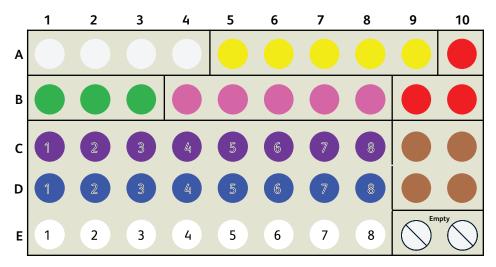
Symbols

The following symbols are used in this guide:

Symbol	Description
\wedge	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 10) section.



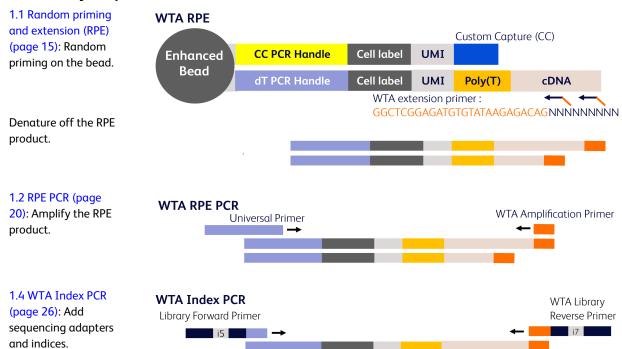
	BD OMICS-One™ WTA Next Amplificat	tion Kit	
Cap Color	Name	Part Number	Vial Placemen
	BD OMICS-One™ Nuclease-Free Water	51-9025552	A1-A4
	BD OMICS-One™ WTA Extension Buffer	51-9025488	A5
	BD OMICS-One™ WTA Extension Primer	51-9025467	A6
	BD OMICS-One™ dNTP Mixture	51-9025491	A7
	BD OMICS-One™ Bead RT/PCR 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	В2
	BD OMICS-One™ WTA Amplification Primer	51-9025469	В3
	BD OMICS-One™ Elution Buffer	51-9025554	B4-B8
	BD OMICS-One™ Sample Tag PCR1 Primer	51-9025470	В9
	BD OMICS-One™ Sample Tag PCR2 Primer	51-9025471	B10
	BD OMICS-One™ Bead Resuspension Buffer	51-9025555	C9, C10, D9, D1
	BD OMICS-One™ Library Forward Primer 1–8	See Part numbers	C1–C8
	BD OMICS-One™ WTA Library Reverse Primer 1–8	for primers in rows	D1-D8
\bigcirc	BD OMICS-One™ Multiomic Library Reverse Primer 1–8	C–E (page 7)	E1–E8

Part numbers for primers in rows C–E

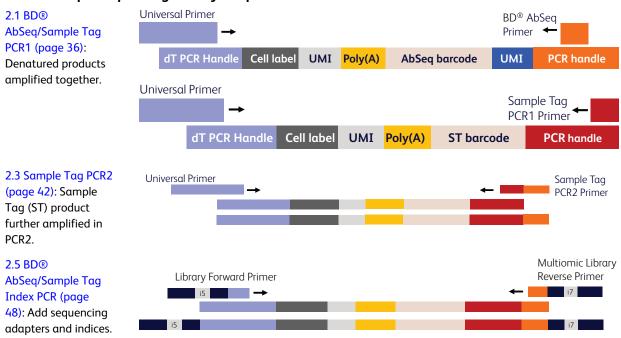
Name	Part Number
BD OMICS-One™ Library Forward Primer 1	51-9025472
BD OMICS-One™ Library Forward Primer 2	51-9025473
BD OMICS-One™ Library Forward Primer 3	51-9025474
BD OMICS-One™ Library Forward Primer 4	51-9025475
BD OMICS-One™ Library Forward Primer 5	51-9025476
BD OMICS-One™ Library Forward Primer 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



BD® AbSeq/Sample Tag library amplification workflow





BD® AbSeq and Sample Tag Index PCR are done separately.

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*	Corning	PCR96HSC
Or, MicroAmp Optical 96–Reaction Plate*	Thermo Fisher Scientific	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	5067-4626
Agilent High Sensitivity D1000 ScreenTape Agilent High Sensitivity D1000 Reagents Or,	Agilent Agilent	5067-5584 5067-5585
Agilent High Sensitivity D5000 ScreenTape Agilent High Sensitivity D5000 Reagents	Agilent Agilent	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	New England Biolabs	S1506S
Or, 12-tube magnetic separation rack [†]	New England Biolabs	S1509S
Or, Invitrogen™ DynαMag™-2 magnet [†]	Thermo Fisher Scientific	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	Agilent Technologies	G2940CA
Or, Agilent [®] 4200 TapeStation System	Agilent Technologies	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

• BD® AbSeq and Sample Tag 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.

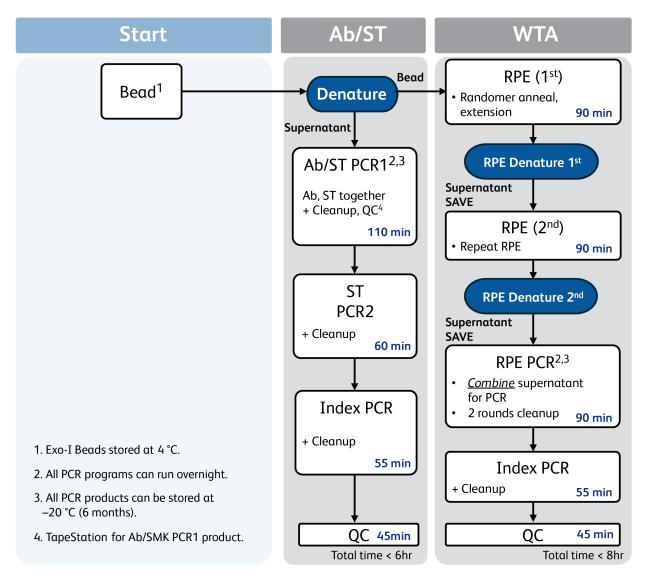
Additional documentation

- 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[®] AbSeq Ab-Oligos (1 plex to 40 plex) Protocol (doc ID 23-24262)
- BD Rhapsody[™] System Single-Cell Labeling with BD[®] AbSeq Ab-Oligos (41 plex to 100 plex) Protocol (doc ID 23-22314)
- BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos for Intracellular CITE-seq Protocol (doc ID 23-24464)
- 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 and BD[®] AbSeq Ab-Oligos Protocol (doc ID 23-24312)
- 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

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

- BD Rhapsody[™] System Single-Cell Labeling with BD[®] AbSeq Ab-Oligos (1 plex to 40 plex) Protocol (doc ID 23-24262)
- BD Rhapsody[™] System Single-Cell Labeling with BD[®] AbSeq Ab-Oligos (41 plex to 100 plex) Protocol (doc ID 23-22314)
- BD Rhapsody™ System Single-Cell Labeling with BD® AbSeq Ab-Oligos for Intracellular CITE-seq Protocol (doc ID 23-24464)
- 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 and BD® AbSeq Ab-Oligos Protocol (doc ID 23-24312)

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 15)
- 1.2 RPE PCR (page 20)
- 1.3 RPE PCR cleanup and quantification (page 23)
- 1.4 WTA Index PCR (page 26)
- 1.5 WTA Index PCR cleanup and quality check (page 29)
- (Optional) 1.6 Additional WTA Index PCR cleanup (page 33)

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	
Equili	brate to room temperature	: :			
	WTA extension buffer	51-9025488			
	WTA extension primer	51-9025467	Facilibrate to some town overture 20 minutes		
	dNTP mixture	51-9025491	Equilibrate to room temperature 30 minutes before setting up RPE.		
\bigcirc	Nuclease-free water	51-9025552	Centrifuge briefly.		
	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 reαdy to us	se:			
	WTA extension enzyme	51-9025499	Centrifuge briefly before adding to mix.	–20 °C	
Obtai	n:				
Exonuclease I-treated cell capture beads Centrifuge briefly and keep on ice until ready. 4 °C		4 °C			
Ice bu	Ice bucket				
1.5-m	L DNA LoBind [®] tubes				
1.5-m	L tube magnetic rack				
Set up	p:				
Heat I	block at 95 °C				
Therm	nomixer at 25 °C				
Therm	nomixer at 37 °C (Optional)				
Progra	ammed thermomixer with RI	PE 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 $95\,^{\circ}$ 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

Сар	Component	1 librαry (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
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 $4 \,^{\circ}$ 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** μ L of elution buffer to the tube.

- 11. To denature BD[®] AbSeq/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** μ L of the supernatant (BD[®] AbSeq/Sample Tag product) to a new 1.5-mL tube.



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

- f. Keep the tube with BD[®] AbSeq/Sample Tag product on ice or at 4 °C for up to 24 hours until ready to proceed to 2.1 BD® AbSeq/Sample Tag PCR1 (page 36).
- 12. Remove the tube from the magnet.
- 13. Pipet **200** μ L 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** μ L 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

Сар	Component	1 librαry (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 μ L of the Extension enzyme mix to the tube with beads from step 23 (total volume of 100 μ L).
- 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** μ L 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:
 - 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 μ L 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 μ L (80 μ L 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			
Equili	Equilibrate to room temperature:						
	Universal oligo	51-9025553	Equilibrate to room temperature 30 minutes	−20 °C			
	WTA amplification primer	51-9025469	before setting up RPE PCR. Centrifuge briefly.				
Leave	Leave in freezer until ready to use:						
	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C			
Obtai	n:						
RPE p	roduct			4 °C			
Ice bu	ıcket						
0.2-m	0.2-mL PCR tubes						
Set u	Set up:						
Therm	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

Сар	Component	1 librαry (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** μ L of the RPE PCR mix to the tube with the **160** μ L 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** μ L 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*	98 °C	15 seconds
Annealing	1,000–20,000 cells: 9 cycles 20,000 - 30,000 cells: 8 cycles	60 °C	30 seconds
Extension	30,000 - 70,000 cells: 7 cycles 70,000 - 100,000 cells: 6 cycles	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.



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			
Equilibra	Equilibrate to room temperature:						
	Elution buffer	51-9025554	Centrifuge briefly.	−20 °C			
Qubit ds[DNA HS Assay Kit		NA for the most of the control				
AMPure [®]	XP magnetic beads		Manufacturer's recommendations				
Obtain:							
RPE PCR	product			4 °C			
1.5-mL D	NA LoBind [®] tubes						
0.2-mL P0	CR tubes						
1.5-mL tu	1.5-mL tube magnetic rack						
Set up:	Set up:						
Prepare f	Prepare fresh 80% ethyl alcohol						

Procedure steps:

This section describes how to perform a single-sided AMPure $^{(8)}$ cleanup to remove unwanted products from the RPE PCR products. The final product is purified double-stranded DNA (\sim 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 $^{\textcircled{R}}$ 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-\muL** 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.
- Place the tube on a magnet until the supernatant is clear (~30 seconds).
- Pipet the eluate (~40 μ L) into a new 1.5-mL tube. 26.
- 27. Add 60 μ L of nuclease-free water to the eluate for a final volume of 100 μ L.



The volume must be exactly 100 μ L.

- 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**.
- Place the tube on a magnet until the supernatant is clear (<5 minutes).
- Remove and discard the supernatant.
- Keeping the tube on the magnet, gently pipet 500 μ L 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.
- 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.
- Pipet-mix 10 times until the beads are fully resuspended. 42.
- 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 μ L) into a new 1.5-mL tube.

The purified RPE PCR product is ready for 1.4 WTA Index PCR (page 26).

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.

1.4 WTA Index PCR

Summary:

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

Preparation list:

Item B		BD Part Number	Preparation and Handling	Storage			
Equili	Equilibrate to room temperature:						
	Forward primer 1–8	Various	Equilibrate to room temperature 30 minutes				
	WTA reverse primer 1–8	Various	before setting up WTA Index PCR.	−20 °C			
\bigcirc	Nuclease-free water	51-9025552	Centrifuge briefly. Keep on ice until ready.				
Leave	Leave in freezer until ready to use:						
	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C			
Obtai	n:						
Purifie	ed RPE PCR product			4 °C			
Ice bu	ucket						
1.5-m	L DNA LoBind [®] tubes						
0.2-m	0.2-mL PCR tubes						
Set up	Set up:						
Therm	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, BD[®] AbSeq 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

Сар	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 \muL** of forward primer and **2.5 \muL** 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 23) to $0.5 \text{ ng/}\mu\text{L}$.



If RPE PCR product concentration is <0.5 $ng/\mu L$, 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

WITH INDEX I CR program					
Step	Cycles	Temperature	Time		
Hot start	1	98 °C	45 seconds		
Denaturation	RPE PCR concentration*	98 ℃	15 seconds		
Annealing	0.05 ng/μL: 12 cycles 0.1 ng/μL: 11 cycles	60 °C	30 seconds		
Extension	0.2 ng/μL: 10 cycles 0.5 ng/μL: 8 cycles	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.

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		BD Part Number	Preparation and Handling	Storage			
Equili	Equilibrate to room temperature:						
	Elution buffer	51-9025554		20.00			
	Nuclease-free water	51-9025552	Centrifuge briefly.	–20 °C			
AMPu	re [®] XP magnetic beads			•			
Qubit	dsDNA HS Assay Kit		M				
Agilent BioAnalyzer High Sensitivity Kit OR Agilent TapeStation ScreenTape and Reagents		-	Manufacturer's recommendations				
Obtai	n:						
WTA :	Index PCR product			4 °C			
1.5-m	L DNA LoBind [®] tubes						
0.2-m	L PCR tubes						
0.2-m	0.2-mL PCR tube magnetic rack						
Set u	Set up:						
Prepa	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 $^{\textcircled{R}}$ XP beads until the beads are fully resuspended.
- 4. Add **60 \muL** of nuclease-free water to **50 \muL** of the WTA Index PCR product.
- 5. Transfer 100 μ L of WTA Index PCR product into a new 0.2-mL PCR tube.



The volume must be exactly 100 μ L.

- 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 μ L 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 (\sim 30 μ L) into a new 1.5-mL tube.

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



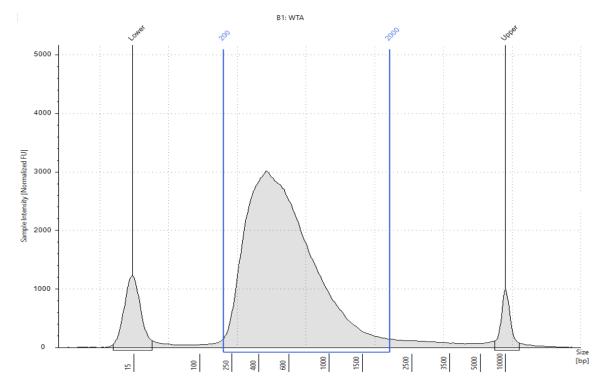
The Index PCR libraries can be stored at -20 °C for up to 6 months until sequencing.

- 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.

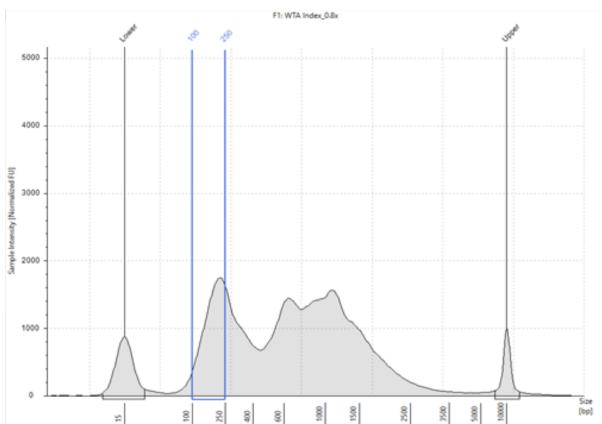
The TapeStation trace should show a peak from ~200 to 2,000 bp. Refer to the representative traces in the following figures.

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 33) 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 31) in 1.5 WTA Index PCR cleanup and quality check (page 29), bring up the total volume to $100 \mu L$ with nuclease-free water.
- 2. Pipet-mix 10 times.
- 3. Briefly centrifuge the tube.



The volume must be exactly 100 μ L.

- 4. Pipet **80 \muL** of AMPure[®] XP beads (0.8x) into the tube containing 100 μ L of sample.
- 5. Pipet-mix 10 times.
- 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 μ L of fresh 80% ethyl alcohol into the tube.
- 11. Incubate for 30 seconds.
- 12. Remove and discard the supernatant without disturbing the beads.
- 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 μ L 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 μ L) into a new 1.5-mL tube.

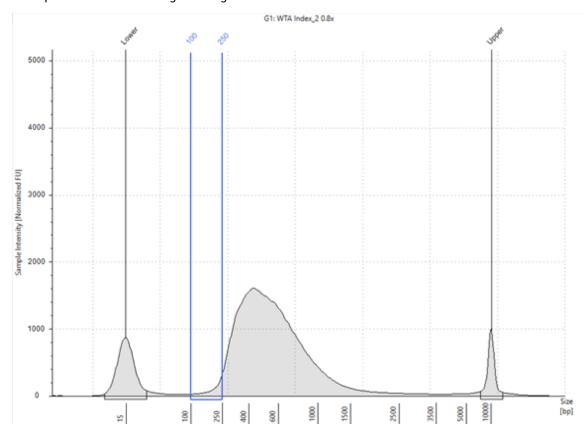
The purified eluate is the **final sequencing library**.

23. Repeat step 25 (page 31) in 1.5 WTA Index PCR cleanup and quality check (page 29) to perform a quality check of the final library.



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. BD® AbSeq/Sample Tag library amplification

This section comprises the following tasks:

- 2.1 BD® AbSeq/Sample Tag PCR1 (page 36)
- 2.2 BD® AbSeq/Sample Tag PCR1 cleanup and quantification (page 39)
- 2.3 Sample Tag PCR2 (page 42)
- 2.4 Sample Tag PCR2 cleanup and quantification (page 45)
- 2.5 BD® AbSeq/Sample Tag Index PCR (page 48)
- 2.6 BD® AbSeq/Sample Tag Index PCR cleanup and quality check (page 51)

2.1 BD® AbSeq/Sample Tag PCR1

Summary:

- ullet Prepare BD[®] AbSeq/Sample Tag PCR1 mix
- Amplify using BD^{\circledR} AbSeq/Sample Tag PCR1 program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage			
Equili	Equilibrate to room temperature:						
	Universal oligo	51-9025553					
	BD [®] AbSeq primer	51-9025468	Equilibrate to room temperature 30 minutes before setting up BD [®] AbSeq/Sample Tag PCR1. Centrifuge briefly.	–20 °C			
	Sample Tag PCR1 primer	51-9025470		-20 C			
\bigcirc	Nuclease-free water	51-9025552					
Leave	Leave in freezer until ready to use:						
	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C			
Obtai	n:						
Dena	tured BD [®] AbSeq/Sample Tag product			4°C			
Ice bu	ıcket						
0.2-m	L PCR tubes						
1.5-mL tube magnetic rack							
Set up:							
Thern	Thermocycler BD® AbSeq/Sample Tag PCR1 program						

Procedure steps:

This section describes how to amplify BD® AbSeq/Sample Tag products through PCR.



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

BD® AbSeq/Sample Tag PCR1 mix

Сар	Component	1 librαry (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
	BD [®] AbSeq primer	10.0	12.0	48.0	96.0
	Nuclease-free water	68.5	82.2	328.8	657.6
	Total	132.0	158.4	633.6	1,267.2

- 1. Pipet-mix the BD^{\circledR} AbSeq/Sample Tag PCR1 mix.
- 2. Place on ice until ready to use.
- 3. In a new 1.5-mL tube, add 132 μ L of the mix with 68 μ L of BD[®] AbSeq/Sample Tag product.
- 4. Pipet-mix to create the BD® AbSeq/Sample Tag PCR1 reaction mix.
- 5. Split the reaction mix into **four** 0.2-mL PCR tubes with **50** μ L 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:

BD® AbSeq/Sample Tag PCR 1 program

Step	Cycles	Temperature	Time
Hot start	1	98 ℃	45 seconds
Denaturation	1,000 cells: 14 cycles 2,500 cells: 13 cycles	98 ℃	15 seconds
Annealing	5,000 cells: 12 cycles 10,000 cells: 11 cycles 20,000 cells: 10 cycles	60 °C	30 seconds
Extension	40,000 cells: 9 cycles >80,000 cells: 8 cycles*	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.



The PCR can be run overnight.

8. When the BD[®] AbSeq/Sample Tag PCR1 program is complete, briefly centrifuge the tubes.

2.2 BD^{\circledR} AbSeq/Sample Tag PCR1 cleanup and quantification

Summary:

- BD® AbSeq/Sample Tag PCR1 cleanup
- Quantification using BioAnalyzer/TapeStation

Preparation list:

Item BD Part Numb		BD Part Number	Preparation and Handling	Storage
Equili	brate to room temperature:			
	Elution buffer	51-9025554	Centrifuge briefly.	−20 °C
AMPu	re [®] XP magnetic beads			
Agilent BioAnalyzer High Sensitivity Kit OR Agilent TapeStation ScreenTape and Reagents			Manufacturer's recommendations	
Obtai	Obtain:			
BD® AbSeq/Sample Tag PCR1 product				4 °C
1.5-m	L DNA LoBind [®] tubes			
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Prepa	Prepare fresh 80% ethyl alcohol			

Procedure steps:

This section describes how to perform a single-sided AMPure[®] cleanup to remove primer dimers from the BD[®] AbSeq/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 $^{\textcircled{R}}$ XP beads until the beads are fully resuspended.
- 4. Briefly centrifuge the tubes with the ${\sf BD}^{\it \&}$ AbSeq/Sample Tag PCR1 product.
- 5. Combine the **four** tubes of **50-\muL** PCR1 product into a new 1.5-mL tube.
- 6. Pipet-mix 10 times.



The volume must be exactly **200 \muL**. If the volume is less than 200 μ L, use nuclease-free water to achieve the final volume.

- 7. Pipet **280 \muL** 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.

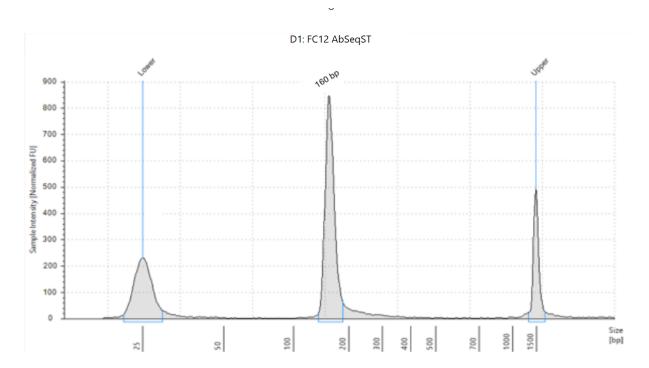
- 21. Pipet-mix 10 times until the beads are fully resuspended.
- 22. Incubate at room temperature for 2 minutes.
- Briefly centrifuge the tube.
- 24. Place the tube on a magnet until the supernatant is clear (~30 seconds).
- 25. Pipet the eluate (30 μ L) into a new 1.5-mL tube.

The purified BD® AbSeq/Sample Tag PCR1 product is ready for the next step.

- 26. Quantify the PCR1 products using 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
- 27. Based on the yield of the largest peak (~160 bp)
 - a. Dilute BD[®] AbSeq/Sample Tag product to **0.1–1.1 ng/ μL**, for 2.5 BD® AbSeq/Sample Tag Index PCR (page 48).
 - b. Use **5** µL of undiluted BD[®] AbSeq/Sample Tag PCR1 product for **2.3** Sample Tag PCR2 (page 42).

The $BD^{\textcircled{8}}$ AbSeq/Sample Tag PCR1 libraries can be stored at -20 °C for up to 6 months.

Figure 4 Representative TapeStation High Sensitivity D1000 Trace—BD® AbSeq PCR1



2.3 Sample Tag PCR2

Summary:

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

Preparation list

Item BD Part Number		BD Part Number	Preparation and Handling	Storage	
Equili	Equilibrate to room temperature:				
	Universal oligo	51-9025553	Equilibrate to room temperature 30 minutes before		
	Sample Tag PCR2 primer	51-9025471	setting up Sample Tag PCR2.	−20 °C	
\bigcirc	Nuclease-free water	51-9025552	Centrifuge briefly.		
Leave	Leave in freezer until ready to use:				
	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	−20 °C	
Obtai	n:				
Purifie	ed Sample Tag PCR1 produc	t		4 °C	
Ice bu	icket				
1.5-m	1.5-mL LoBind [®] tube				
0.2-m	0.2-mL PCR tubes				
Set up	Set up:				
Thern	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

Сар	Component	1 librαry (μL)	1 library with 20% overage (μL)	4 libraries with 20% overage (μL)	8 librαries 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 $45 \mu L$ 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 $5 \mu L$ 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		98 °C	15 seconds
Annealing	10*	66 °C	30 seconds
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4°C	∞

 $^{^{\}ast}\,$ Recommended PCR cycles might need to be optimized for different cell types.



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	1	BD Part Number	Preparation and Handling	Storage		
Equil	Equilibrate to room temperature:					
	Elution buffer	51-9025554	Centrifuge briefly.	−20 °C		
AMP	ure [®] XP magnetic beads		Manufacturer's recommendations			
Qubit dsDNA HS Assay Kit			manuracturer's recommendations			
Obto	Obtain:					
Sample Tag PCR2 product 4 °C			4 ℃			
1.5-n	nL DNA LoBind [®] tubes					
0.2-n	nL PCR tubes					
0.2-n	0.2-mL PCR tube magnetic rack					
Set u	Set up:					
Prepo	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 \muL**. If the volume is less than 50 μ L, use nuclease-free water to achieve the final volume.

- 5. Pipet **60** μ L 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.

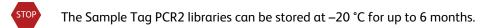
- 8. Incubate at room temperature for 5 minutes.
- 9. Place the tube on a magnet until the supernatant is clear (<5 minutes).
- 10. Remove and discard the supernatant.
- 11. Keeping the tube on the magnet, gently pipet 200 µL of fresh 80% ethyl alcohol into the tube.
- 12. Incubate for 30 seconds.
- 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 μ L 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 μ L) into a new 1.5-mL tube. The Sample Tag PCR2 product is ready for 2.5 BD® AbSeq/Sample Tag Index PCR (page 48).
- 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 $0.1-1.1 \text{ ng/}\mu\text{L}$.



2.5 BD® AbSeq/Sample Tag Index PCR

Summary:

- Amplify using BD[®] AbSeq/Sample Tag Index PCR program

Preparation list:

Item BD		BD Part Number	Preparation and Handling	Storage	
Equili	Equilibrate to room temperature:				
	Forward primer 1–8	Various	Equilibrate to room temperature 30 minutes		
\bigcirc	Multiomic reverse primer 1–8	Various	before setting up BD [®] AbSeq/Sample Tag Index PCR.	−20 °C	
\bigcirc	Nuclease-free water	51-9025552	Centrifuge briefly. Keep on ice until ready.		
Leave	Leave in freezer until ready to use:				
	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C	
Obtai	n:				
Purifie	ed BD® AbSeq PCR1/Sample Tag I	PCR2 product		4 °C	
Ice bu	ucket				
1.5-m	1.5-mL DNA LoBind [®] tubes				
0.2-m	0.2-mL PCR tubes				
Set u	Set up:				
Thern	Thermocycler with BD [®] AbSeq/Sample Tag Index PCR program				

Procedure steps:

This section describes how to generate BD® AbSeq/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.

BD® AbSeq/Sample Tag Index PCR mix

Сар	Component	1 librαry (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 BD® AbSeq/Sample Tag Index PCR mix.
- 2. For multiple samples, pipet 43 μ L of Index PCR mix into a separate 0.2-mL PCR tube for each sample.
- 3. Add $2 \mu L$ of forward primer and $2 \mu L$ of multiomic reverse primer to each sample.
- 4. Place on ice until ready to use.



Bring the BD[®] AbSeq/Sample Tag Index PCR mix to the post-amplification workspace.

5. Dilute an aliquot of BD® AbSeq/Sample Tag PCR1 product, from 2.2 BD® AbSeq/Sample Tag PCR1 cleanup and quantification (page 39) or Sample Tag PCR2 product from 2.4 Sample Tag PCR2 cleanup and quantification (page 45) with nuclease-free water to **0.1–1.1 ng/μL**.

Add $3 \mu L$ of the appropriate template:

- BD[®] AbSeq PCR1 product for BD[®]AbSeq Index PCR
- Sample Tag PCR2 product for Sample Tag Index PCR



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:

BD® AbSeq/Sample Tag Index PCR program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	Index PCR concentration*	98 °C	15 seconds
Annealing	0.5–1.1 ng/μL: 6 cycles 0.25-0.5 ng/μL: 7 cycles	60 °C	30 seconds
Extension	0.1-0.25 ng/μL: 8 cycles	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.



The PCR can run overnight.

8. When the BD^{\circledR} AbSeq/Sample Tag Index PCR program is complete, briefly centrifuge the tubes.

2.6 BD® AbSeq/Sample Tag Index PCR cleanup and quality check

Summary:

- BD® AbSeq/Sample Tag Index PCR cleanup
- Quality check using a Qubit Fluorometer and BioAnalyzer/TapeStation

Preparation list:

Item BD Part Number		BD Part Number	Preparation and Handling	Storage	
Equili	brate to room temperat	ure:			
	Elution buffer	51-9025554			
\bigcirc	Nuclease-free water	51-9025552	Centrifuge briefly.	_20 °C	
AMPu	ıre [®] XP magnetic beads				
Qubit	dsDNA HS Assay Kit		Manufacture's account data		
Agilent BioAnalyzer High Sensitivity Kit OR Agilent TapeStation ScreenTape and Reagents		-	Manufacturer's recommendations		
Obtai	n:				
BD [®] AbSeq/Sample Tag Index PCR product				4 °C	
1.5-m	L DNA LoBind [®] tubes				
0.2-m	0.2-mL PCR tubes				
0.2-m	0.2-mL PCR tube magnetic rack				
Set up:					
Prepa	re fresh 80% ethyl alcoho	ol			

Procedure steps:

This section describes how to perform a single-sided AMPure[®] cleanup to remove primer dimers from the BD[®] AbSeq/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 BD® AbSeq/Sample Tag Index PCR product.



The volume must be exactly **50 \muL**. If the volume is less than 50 μ L, 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 μ L 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 $30 \mu L$ 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 ($\sim 30~\mu L$) 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 BD[®] AbSeq/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 BD^{\otimes} AbSeq library should show a peak of ~264 bp. 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 5 Representative TapeStation High Sensitivity D5000 trace—BD® AbSeq Index PCR product

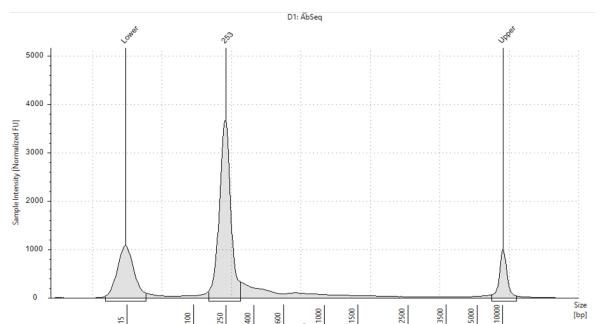
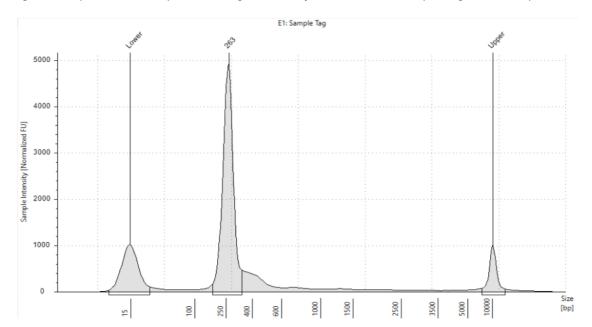


Figure 6 Representative TapeStation High Sensitivity D5000 trace—Sample Tag Index PCR product



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 , Sample Tag, and BD® AbSeq libraries.

Read requirements for libraries

Library type	Read requirement for data analysis
BD Rhapsody™ WTA	20,000–100,000 reads/cell
BD [®] AbSeq	300 reads/cell/AbSeq
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 61).



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)	
NovαSeq 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)	
NovαSeq 6000 S1 (Single Flow Cell)	2×50, 2×100, 2×150*	350–650 pM (standard workflow)	
NovαSeq 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)	
NovαSeq 6000 S4 (Single Flow Cell)	2×100, 2×150	350–650 pM (standard workflow)	
NovαSeq 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 138 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 BD[®] AbSeq 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. BD® AbSeq/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 BD [®] AbSeq/Sample Tag), perform a second round of AMPure [®] purification according to 1.6 Additional WTA Index PCR cleanup (page 33).	

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.	
BD [®] AbSeq PCR1 product size too low.	BD® AbSeq primer not added to PCR1 or too few PCR1 cycles. Incorrect volumes of AMPure® XP beads used during single-sided selection.	Contact your local Field Application Specialist (FAS) or scomix@bd.com.	
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.	
Yield of BD® AbSeq library too low	Too few Index PCR cycles.	Increase the number of cycles for Index PCR.	
after Index PCR, but yield of BD [®] AbSeq/Sample Tag PCR1 products is sufficient.	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.	

Observation	Possible causes	Recommended solutions	
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 42). Analyze products using the Agilent Bioanalyzer or the Agilent TapeStation and look for a ~182 bp peak that corresponds to Sample Tag PCR2 products. ~160 bp peak might be present that corresponds to BD® AbSeq products. If the ~182 bp peak is observed, proceed to index PCR. If the ~182 bp peak is not observed, contact your local Field Application Specialist (FAS) or scomix@bd.com.	

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.

Observation	Possible causes	Recommended solutions
High proportion of undetermined Sample Tag calls in sequencing results.	Insufficient sequencing of the Sample Tag Library.	 Set pooled samples of the same cell type to 120 reads/cell. 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 $^{\text{\tiny{M}}}$ 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 NNNNNNNN
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	51-9025489	CAA GCA GAA GAC GGC ATA CGA GAT TACTACGC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 2	PCR	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	i5 bases for sample sheet
	NovaSeq, MiSeq, HiSeq 2000/2500	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	ССТАТССТ	AGGATAGG
BD OMICS-One™ Library Forward Primer 4	GGCTCTGA	TCAGAGCC
BD OMICS-One™ Library Forward Primer 5	AGGCGAAG	СТТССССТ
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:

Sample Tag	Sample Tag sequence
Sample Tag 1–Human	ATTCAAGGGCAGCCGCGTCACGATTGGATACGACTGTTGGACCGG
Sample Tag 2–Human	TGGATGGGATAAGTGCGTGATGGACCGAAGGGACCTCGTGGCCGG
Sample Tag 3–Human	CGGCTCGTGCTGCGTCCTCAAGTCCAGAAACTCCGTGTATCCT
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:

Sample Tag	Sample Tag sequence
Sample Tag 1–Mouse Immune	AAGAGTCGACTGCCATGTCCCCTCCGCGGGTCCGTGCCCCCAAG
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	GCCTACCCGCTATGCTCGGCTGGTTAGAGTTTACTGCACGCC
Sample Tag 10–Mouse Immune	TCCCATTCGAATCACGAGGCCGGGTGCGTTCTCCTATGCAATCCC
Sample Tag 11–Mouse Immune	GGTTGGCTCAGAGGCCCCAGGCTGCGGACGTCGTCGGACTCGCGT
Sample Tag 12–Mouse Immune	CTGGGTGCCTGGGTTACGTCGGCCCTCGGGTCGCGAAGGTC

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:

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	CGGCTCGTGCGTCGTCTCAAGTCCAGAAACTCCGTGTATCCT	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	GCAGCCGGCGTCGTACGAGGCCACAGCGGAGACTAGATGAGGCCCC	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	AAGAGTCGACTGCCATGTCCCCTCCGCGGGTCCGTGCCCCCAAG	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	CTGGGTGCCTGGGTCGGGTTACGTCGGCCCTCGGGTCGCGAAGGTC	Barcode sequence is the same as mouse SMK Sample Tag 12

BD® AbSeq Ab-Oligo common motifs

The antibody in each BD[®] AbSeq Ab-Oligo is conjugated to an oligonucleotide that contains an antibody clone-specific barcode (ABC) flanked by a poly-A tail on the 3' end and a common PCR handle (PCR primer binding site) followed by a unique molecular identifier on the 5' end, with the AbSeq Ab-Oligo sequence (5'-3') between them:

 CAGACGTGTGCTCTTCCGATCTVNNVNNNNVNNV(Barcode sequence [ABC])AAAAAAAAAAAAAAAAAAAAAAAAA

The poly-A tail of the oligonucleotide allows the ABC to be captured by the BD Rhapsody™ System or other oligo-dT-based capture systems. The 5' PCR handle allows for efficient sequencing library generation for various sequencing platforms.

Viewing antibody barcode sequences

To view the ABC (barcode sequence) for an antibody:

- 1. Go to the BD Biosciences website for BD® AbSeq assays.
- 2. Click **View Products**. The page displays products pertaining to BD^{\otimes} AbSeq assays.
- 3. Click a specific product to view product details, and scroll down to the Barcode Sequence field.

BD OMICS-One™ WTA Next, BD® AbSeq, 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, BD® AbSeq, 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	BD Rhapsody™ Enhanced Cell Capture Beads
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
Rev.	AMPure beads, then eluting into room-temperature elution buffer. 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)

- 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.
- 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.
- Air-dry pellet.
 Do not let the pellet over-dry and crack.
- 14. Remove tube from the magnet.
- 15. Resuspend pellet in $40 \mu L$ 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, BD® AbSeq, 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.

Сар	Component	Sample count with % overage (volumes in μL)								
Сар	Component	1+0%	1+20%	2+20%	3+20%	4+20%	5+20%	6+20%	7+20%	8+20%
0	WTA Extension Buffer	20.0	24.0	48.0	72.0	96.0	120.0	144.0	168.0	192.0
0	WTA Extension Primer	40.0	48.0	96.0	144.0	192.0	240.0	288.0	336.0	384.0
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.

Сар	Component	Sample count with % overage (volumes in μL)								
	Component	1+0%	1+20%	2+20%	3+20%	4+20%	5+20%	6+20%	7+20%	8+20%
0	dNTP	8.0	9.6	19.2	28.8	38.4	48.0	57.6	67.2	76.8
0	Bead Enhancer	12.0	14.4	28.8	43.2	57.6	72.0	86.4	100.8	115.2
0	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 BD® AbSeq/ST product

- 1. Label a new 1.5-mL tube as BD® AbSeq/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 BD® AbSeq/ST product into labeled 1.5-mL tube.
- 7. Store BD® AbSeq/ST product on ice until BD® AbSeq/ST PCR 1. See "Amplifying the BD® AbSeq and Sample Tag (ST) product" on page QRG-7.
- 8. Wash beads with $200 \mu L$ elution buffer.

Generating the RPE product

- 1. Add $87 \mu L$ 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 $13~\mu L$ 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 ℃	10 min	
2	37 <i>°</i> ℃	15 min	- - TIMF
3	45 °C	10 min	- I IIVIE
4	55 ℃	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.



Amplifying the RPE product

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

Сар	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.



- 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.

RPE PCR program

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

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} μ L = 40 μ L elution 1 + 60 μ L water



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.

Can	Component	Sample count with % overage (volumes in µL)								
Сар	Component	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						
	WTA Rev. Index Primer	2.5	3.0	N/A						
0	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

- 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.



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

WTA	Index	PCR	program

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

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



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 BD® AbSeq and Sample Tag (ST) product

1. Prepare the BD® AbSeq/ST PCR 1 mix using the following table.

Can	Component			Sample	count with	n % overa	ge (volum	es in μL)		
Сар	cup component	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
	BD® AbSeq Primer	10.0	12.0	24.0	36.0	48.0	60.0	72.0	84.0	96.0
	ST PCR1 Primer	1.0	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6
0	Water	68.5	82.2	164.4	246.6	328.8	411.0	493.2	575.4	657.6
	Total	132.0	158.4	316.8	475.2	633.6	792.0	950.4	1108.8	1267.2

- 2. Combine **68 µL BD® AbSeq/ST product** (see "Denaturing the BD® AbSeq/ST product" on page QRG-4) with **132 µL BD® AbSeq/ST PCR 1 mix.**
- 3. Mix well.
- 4. Split BD® AbSeq/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 ℃	45 sec
Donaturation	1,000 cells: 14 cycles	98 ℃	15 sec
Denaturation	2,500 cells: 13 cycles	98 °C	15 sec
	5,000 cells: 12 cycles		
Annealing	10,000 cells: 11 cycles	60 °C	30 sec
	20,000 cells: 10 cycles		
Extension	40,000 cells: 9 cycles	72 °C	1 min
	>80,000 cells: 8 cycles		
Extension	1	72 °C	2 min
Hold	1	4 ℃	8



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

Purification	PCR Vol. μL	AMPure Vol. μL	Elution Vol. μL
BD® AbSeq/ ST PCR 1	200	280	30



Safe stopping point.

Purified PCR product can be stored at -20 °C for up to 6 months.

Quantifying the BD® AbSeq/ST PCR 1 product

- 1. Quality check purified BD® AbSeq/ST PCR 1 using an analyzer.
- Dilute BD® AbSeq/ST PCR 1 product to 0.1–1.1 ng/μL for BD® AbSeq Index PCR.
- 3. Use 5 μ L undiluted BD® AbSeq/ST PCR 1 product for ST PCR 2.
- 4. Continue to the "Sample Tag (ST) PCR 2" section.

Generating the Sample Tag PCR 2 product

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

Can	Component	Sample count with % overage (volumes in μL)									
Сар	Component	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							
	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	
0	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 BD® AbSeq/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 ℃	45 sec		
Denaturation		98 ℃	15 sec		
Annealing	10	66 °C	30 sec		
Extension		72 °C	1 min		
Extension	1	72 °C	2 min		
Hold	1	4 ℃	∞		

- 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



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 "BD® AbSeq/Sample Tag (ST) Index PCR" section.

Indexing the BD® AbSeq or ST libraries

1. Prepare the BD® AbSeq/ST Index PCR mix using the following table.

Сар	Component	Sample count with % overage (volumes in μL)									
Сар	Component	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							
0	Multiomic Rev. Index Primer	2.0	2.4	N/A							
0	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 BD® AbSeq/ST Index PCR mix with 3 μL of one of the following *diluted* and *purified* product.
 - BD® AbSeq/ST PCR 1 product (see "Amplifying the BD® AbSeq and Sample Tag (ST) product" on page QRG-7).
 - ST PCR 2 product (see "Generating the Sample Tag PCR 2 product" on page QRG-8).
- 3. Mix well.
- 4. Amplify using the following table.

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



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

Purification	PCR Vol.	AMPure Vol.	Elution Vol.		
	μL	μL	μL		
BD® AbSeq/ST Index PCR	50	40	30		



Safe stopping point.

Purified PCR product can be stored at -20 °C for up to 6 months.

Quantifying the indexed BD® AbSeq and ST libraries

- 1. Quantify purified BD® AbSeq and ST Index PCR product with the fluorometer.
- 2. Quality check purified BD® AbSeq and 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, BD® AbSeq, and Sample Tag Quick Reference Guide

Date The following note sheet is provided for user convenience.

Experiment User

Use the area below to record sample information:

				Sample	Description	1	2	က	4	2	9	7	8
		RPE	PCR	Quant.	ng/µL								
			WTA	Fwd.	Index								
			WTA	Rev.	Index								
	WTA	Index	PCR	Quant.	ng/µL								
BD _®	AbSeq/	ST PCR	_	Quality	bp (avg)								
		BD ®	AbSeq	Fwd.	Index								
		BD⊚	AbSeq	Rev.	Index								
BD®	AbSeq	Index	PCR	Quant.	ng/ µL								
		ST PCR	7	Quant.	ng/µL								
				ST Fwd.	Index								
			ST	Rev.	Index								
	ST	Index	PCR	Quant.	ng/µL								

Contact Information

Becton, Dickinson and Company BD Biosciences 155 North McCarthy Boulevard Milpitas, California 95035 USA

bdbiosciences.com scomix@bd.com