# BD Rhapsody™ System

## BD OMICS-One™ WTA Next and BD® AbSeq

### Library Preparation Protocol

23-24992(01)
2025-10

### Regulatory information

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

### History

| Revision | Date | Change made |
|---|---|---|
| 23-24992(01) | 2025-10 | Initial release. |

# Contents

# Introduction

This protocol provides instructions on creating single-cell whole transcriptome mRNA and BD® AbSeq libraries after cell capture on the BD Rhapsody™ HT Single-Cell Analysis System or the BD Rhapsody™ HT Xpress System for sequencing on various sequencers. For complete instrument procedures and safety information, refer to the *BD Rhapsody™ HT Single-Cell Analysis System Instrument User Guide* or the *BD Rhapsody™ HT Xpress System Instrument User Guide for Scanner-Free Workflow*.

Before beginning this protocol, ensure that the cDNA of mRNA and BD® AbSeq 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 in solution. To generate the BD® AbSeq sequencing libraries, the extended Ab-Oligos are first denatured from the BD Rhapsody™ Enhanced Cell Capture Beads, which are later amplified separately through a series of PCR steps. Meanwhile, the whole transcriptome amplification library is generated directly from the BD Rhapsody™ Enhanced Cell Capture Beads using a random priming approach, followed by an index polymerase chain reaction (PCR) step. The whole transcriptome mRNA and BD® AbSeq 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. The data set generated from this protocol can be used to generate a custom panel for subsequent 3' Targeted mRNA sequencing. Specifically, the protocol outlines how to generate whole transcriptome libraries for cell-capture inputs between 1,000 and 100,000 resting peripheral blood mononuclear cells (PBMCs) per sample for library generation. For cell-capture inputs between 1,000 to <5,000 cells per sample, there are sections in the protocol for additional cleanup. Cell inputs of 5,000 or more will give optimal performance. If sufficient cells are available, loading more cells and subsampling BD Rhapsody™ Enhanced Cell Capture Beads to reach the desired number of cells will improve data quality for lower cell inputs. For cell types other than resting PBMCs, protocol optimization might be required by the user.

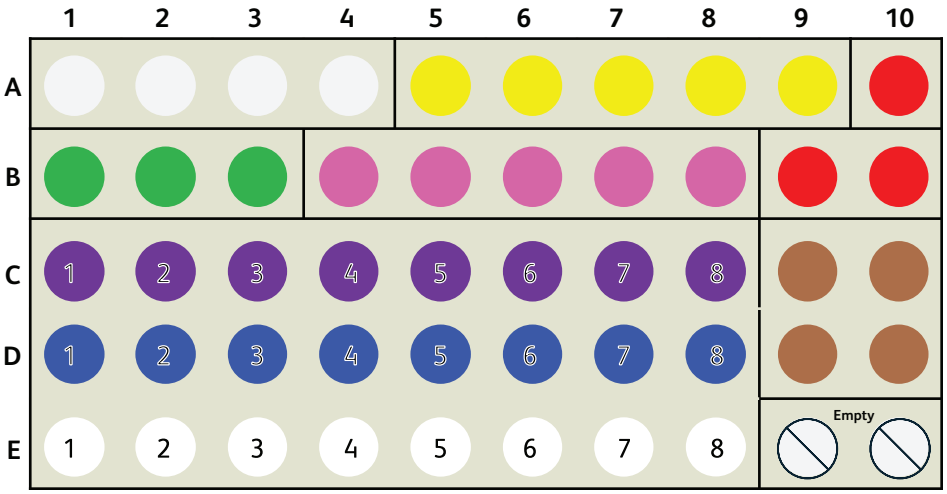
## Symbols

The following symbols are used in this guide:

| Symbol | Description |
|---|---|
|  | Important information for maintaining measurement accuracy or data integrity. |
|  | Noteworthy information. |
|   | 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 8) section.




**BD OMICS-One™ WTA Next Amplification Kit**

| Cap Color | Name | Part Number | Vial Placement |
|---|---|---|---|
| | BD OMICS-One™ Nuclease-Free Water | 51-9025552 | A1–A4 |
| | BD OMICS-One™ WTA Extension Buffer | 51-9025488 | A5 |
| | BD OMICS-One™ WTA Extension Primer | 51-9025467 | A6 |
| | BD OMICS-One™ dNTP Mixture | 51-9025491 | A7 |
| | BD OMICS-One™ Bead RT/PCR Enhancer | 51-9025495 | A8 |
| | BD OMICS-One™ WTA Extension Enzyme | 51-9025499 | A9 |
| | BD OMICS-One™ AbSeq Primer | 51-9025468 | A10 |
| | BD OMICS-One™ PCR Master Mix | 51-9025466 | B1 |
| | BD OMICS-One™ Universal Oligo | 51-9025553 | B2 |
| | BD OMICS-One™ WTA Amplification Primer | 51-9025469 | B3 |
| | BD OMICS-One™ Elution Buffer | 51-9025554 | B4–B8 |
| | BD OMICS-One™ Sample Tag PCR1 Primer | 51-9025470 | B9 |
| | BD OMICS-One™ Sample Tag PCR2 Primer | 51-9025471 | B10 |
| | BD OMICS-One™ Bead Resuspension Buffer | 51-9025555 | C9, C10, D9, D10 |
| | BD OMICS-One™ Library Forward Primer 1–8 | See Part numbers for primers in rows C–E (page 6) | C1–C8 |
| | BD OMICS-One™ WTA Library Reverse Primer 1–8 | | D1–D8 |
| | BD OMICS-One™ Multiomic Library Reverse Primer 1–8 | | E1–E8 |

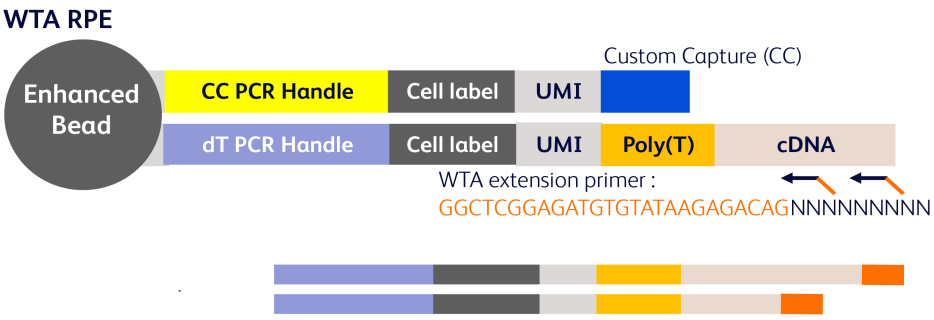
## Part numbers for primers in rows C–E

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

# Workflows

## WTA library amplification workflow

1.1 Random priming and extension (RPE) (page 13): Random priming on the bead.

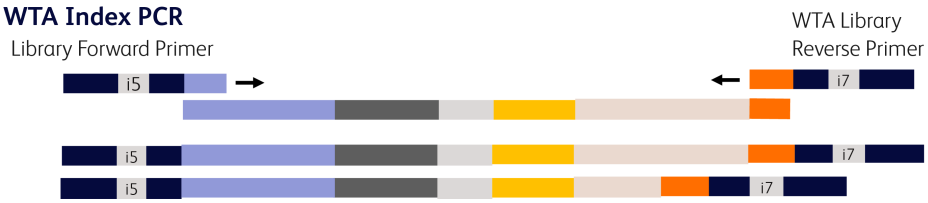



Denature off the RPE product.



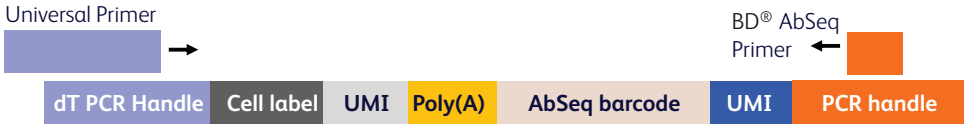
1.2 RPE PCR (page 18): Amplify the RPE product.


1.4 WTA Index PCR (page 24): Add sequencing adapters and indices.

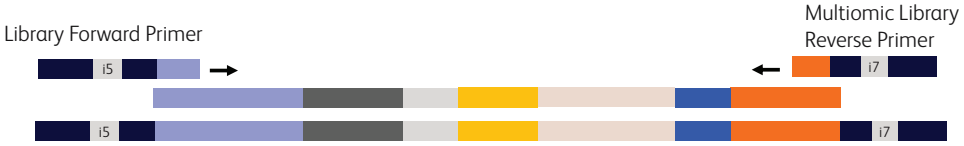



## BD® AbSeq library amplification workflow

2.1 BD® AbSeq PCR1 (page 34): Denatured products amplified together.




2.3 BD® AbSeq Index PCR (page 40): Add sequencing adapters and indices.



# Required and recommended materials

## Required reagents

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

| **Material** | Supplier | Catalog no. |
|---|---|---|
| BD OMICS-One™ WTA Next Amplification Kit | BD Biosciences | 572620 |
| AMPure® XP beads for DNA Cleanup | Beckman Coulter | A63880 |
| 100% ethyl alcohol, molecular biology grade | Major supplier | – |
| Nuclease-free water | Major supplier | – |

## Recommended consumables

| **Material** | Supplier | Part number/Catalog no. |
|---|---|---|
| Pipettes (P10, P20, P200, P1000) | Major supplier | – |
| Low-retention, filtered pipette tips | Major supplier | – |
| 0.2-mL PCR 8-strip tubes | Major supplier | – |
| Axygen® 96–Well PCR Microplates*<br>Or,<br>MicroAmp Optical 96–Reaction Plate* | Corning<br><br>Thermo Fisher Scientific | PCR96HSC<br><br>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<br>Or,<br>Agilent High Sensitivity D1000 ScreenTape<br>Agilent High Sensitivity D1000 Reagents<br>Or,<br>Agilent High Sensitivity D5000 ScreenTape<br>Agilent High Sensitivity D5000 Reagents | Agilent<br><br>Agilent<br>Agilent<br><br>Agilent<br>Agilent | 5067-4626<br><br>5067-5584<br>5067-5585<br><br>5067-5592<br>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<br>Or,<br>12-tube magnetic separation rack†<br>Or,<br>Invitrogen™ DynaMag™-2 magnet† | New England Biolabs<br><br>New England Biolabs<br><br>Thermo Fisher Scientific | S1506S<br><br>S1509S<br><br>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<br>Or,<br>Agilent® 4200 TapeStation System | Agilent Technologies<br><br>Agilent Technologies | G2940CA<br><br>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 libraries can be sequenced separately or together with WTA libraries.

**Master mix preparation**

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

**Supernatant handling**

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

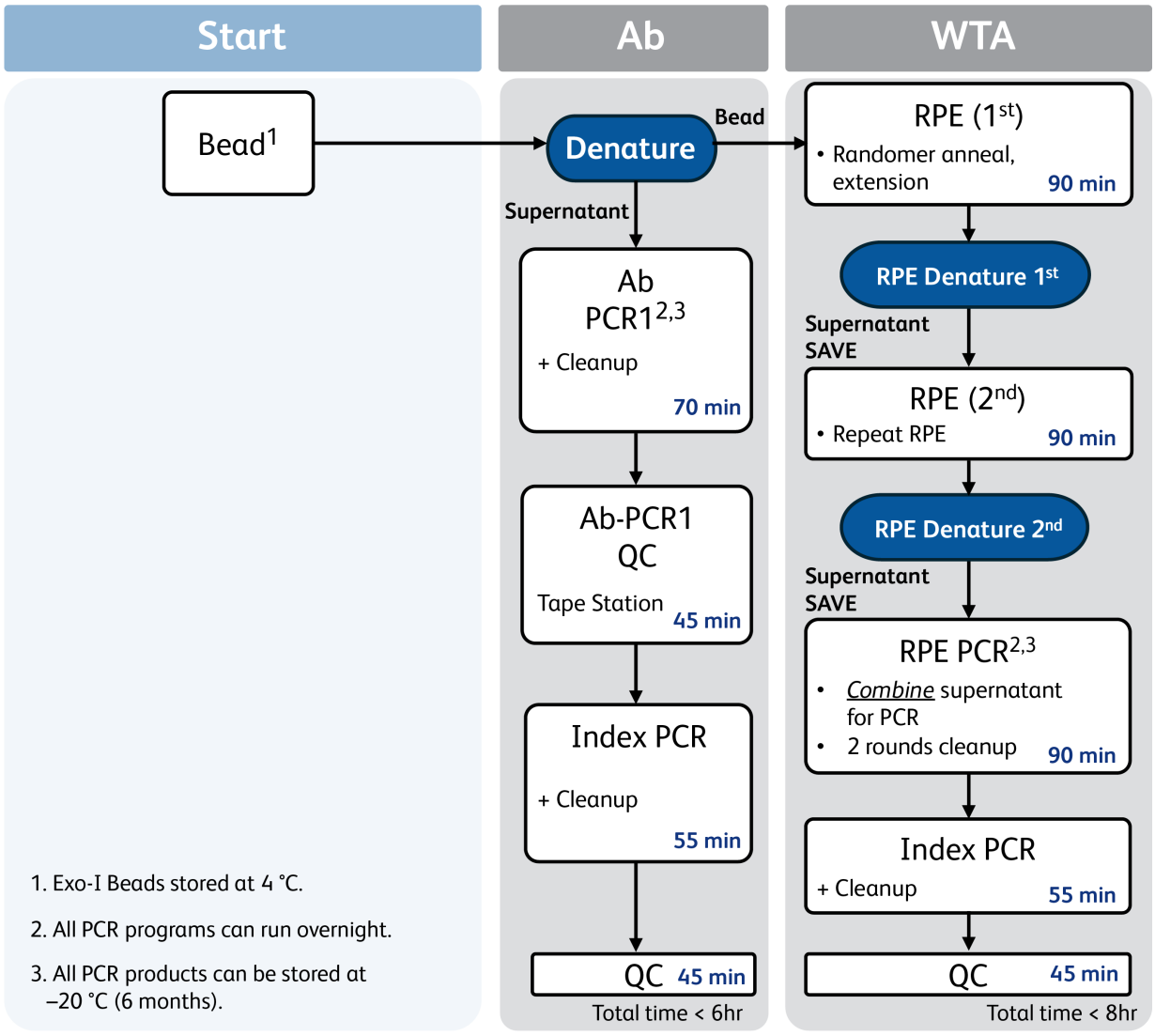
## Additional documentation

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

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)

Summary:

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

Preparation list:

| Item | | BD Part Number | Preparation and Handling | Storage |
|---|---|---|---|---|
| **Equilibrate to room temperature:** | | | | |
| 🟡 | WTA extension buffer | 51-9025488 | Equilibrate to room temperature 30 minutes before setting up RPE.<br>Centrifuge briefly. | –20 °C |
| 🟡 | WTA extension primer | 51-9025467 | | |
| 🟡 | dNTP mixture | 51-9025491 | | |
| ⚪ | Nuclease-free water | 51-9025552 | | |
| 🟣 | Elution buffer | 51-9025554 | | |
| **Place on ice:** | | | | |
| 🟡 | Bead RT/PCR enhancer | 51-9025495 | Centrifuge briefly before adding to mix. | –20 °C |
| **Leave in freezer until ready to use:** | | | | |
| 🟡 | WTA extension enzyme | 51-9025499 | Centrifuge briefly before adding to mix. | –20 °C |
| **Obtain:** | | | | |
| Exonuclease I-treated cell capture beads | | | Centrifuge briefly and keep on ice until ready. | 4 °C |
| Ice bucket | | | | |
| 1.5-mL DNA LoBind® tubes | | | | |
| 1.5-mL tube magnetic rack | | | | |
| **Set up:** | | | | |
| Heat block at 95 °C | | | | |
| Thermomixer at 25 °C | | | | |
| Thermomixer at 37 °C (Optional) | | | | |
| Programmed thermomixer with RPE program | | | | |

Procedure steps:

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

Perform this procedure in the pre-amplification workspace. We recommend using a separate heat block for the 95 °C incubations.

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

   If you are using one thermomixer, skip the 37 °C incubation in Step 21 b.

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

**Random primer mix**

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

3. Pipet-mix the Random primer mix.
4. Leave at **room temperature** until ready to use.
5. Briefly centrifuge the tube of Exonuclease I-treated BD Rhapsody™ Enhanced Cell Capture Beads, and then complete one of the following actions.
   - If you are using a subsample of the beads, proceed to step 6.
   - If you are using the entire sample of beads, skip to step 7.
6. (Optional) To subsample the Exonuclease I-treated BD Rhapsody™ Enhanced Cell Capture Beads:
   a. Based on the expected number of viable cells captured on the beads in the final bead resuspension volume, determine the volume of beads to subsample for sequencing.
   b. Completely resuspend the beads by pipet-mixing, then pipet the calculated volume of the bead suspension into a new 1.5-mL tube. Bring the total volume up to 200 µL using bead resuspension buffer.

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

11. To denature BD® AbSeq products off the beads:
    a. Pipet-mix 10 times to resuspend the beads.
    b. Incubate the sample at **95 ˚C** in a heat block for **5 minutes (no shaking)**.
    c. Briefly centrifuge the tube.
    d. Place the tube on a magnet until the supernatant is clear (**<2 minutes**).

    **Save supernatant at this step. Do not discard!**

    e. Transfer **75 µL** of the supernatant (BD® AbSeq product) to a new 1.5-mL tube.

    To minimize BD® AbSeq contamination, ensure that all the supernatant is removed from the tube.

    f. Keep the tube with BD® AbSeq product on **ice** or at **4 ˚C** for up to 24 hours until ready to proceed to 2.1 BD® AbSeq PCR1 (page 34).
12. Remove the tube from the magnet.
13. Pipet **200 µL** of elution buffer to the tube.
14. Pipet-mix 10 times to resuspend the beads.
15. Briefly centrifuge the tube.
16. Place the tube on a magnet until the supernatant is clear **(<2 minutes)**.
17. Remove and discard the supernatant.
18. Remove the tube from the magnet.
19. Add **87 µL** Random primer mix to the tube.
20. Pipet-mix 10 times until the beads are fully resuspended.

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

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

    Optional—If you are using one thermomixer, skip the 37 °C incubation step.

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

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

**Extension enzyme mix**

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

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

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

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

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

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

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

    **Save supernatant at this step. Do not discard!**

    g. Transfer **80 µL** of the supernatant (RPE product) to a new 1.5-mL tube.
40. Place the tube containing the RPE product on ice.
41. Repeat steps 19–40 to perform a second RPE.

    If working with multiple samples, ensure that the supernatants are combined correctly.
42. Combine the two RPE products for each sample, for a total volume of **160 µL** (80 µL from the first RPE + 80 µL from the second RPE).

## 1.2 RPE PCR

Summary:

- Prepare RPE PCR mix
- Amplify using RPE PCR program

Preparation list:

| Item | | BD Part Number | Preparation and Handling | Storage |
|---|---|---|---|---|
| **Equilibrate to room temperature:** | | | | |
| ● | Universal oligo | 51-9025553 | Equilibrate to room temperature 30 minutes before setting up RPE PCR.<br>Centrifuge briefly. | –20 °C |
| ● | WTA amplification primer | 51-9025469 | | |
| **Leave in freezer until ready to use:** | | | | |
| ● | PCR master mix | 51-9025466 | Centrifuge briefly before adding to mix. | –20 °C |
| **Obtain:** | | | | |
| RPE product | | | | 4 °C |
| Ice bucket | | | | |
| 0.2-mL PCR tubes | | | | |
| **Set up:** | | | | |
| Thermocycler with RPE PCR program | | | | |

Procedure steps:

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

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

**RPE PCR mix**

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

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

Bring the tubes to the post-amplification workspace.

7. Run the following PCR program.

**RPE PCR program**

| Step | Cycles | Temperature | Time |
|---|---|---|---|
| Hot start | 1 | 98 °C | 45 seconds |
| Denaturation | Recommended number cycles for resting PBMCs*<br><br>1,000–20,000 cells: 9 cycles<br>20,000 - 30,000 cells: 8 cycles<br>30,000 - 70,000 cells: 7 cycles<br>70,000 - 100,000 cells: 6 cycles | 98 °C | 15 seconds |
| Annealing | | 60 °C | 30 seconds |
| Extension | | 72 °C | 1 minute |
| Final extension | 1 | 72 °C | 2 minutes |
| Hold | 1 | 4 °C | ∞ |

* Recommended PCR cycles might require optimization for different cell and sample types.

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

Two additional cycles are recommended for PBMC nuclei.


The PCR can run overnight.

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

## 1.3 RPE PCR cleanup and quantification

Summary:

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

Preparation list:

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

Procedure steps:

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

Perform the purification in the post-amplification workspace.

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

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

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

    Do not overdry the AMPure® XP beads after the ethyl alcohol washes. Overdried beads appear cracked.
20. Remove the tube from the magnet.
21. Pipet **40 µL** of elution buffer into the tube.
22. Pipet-mix 10 times until the beads are fully resuspended.

23. Incubate at room temperature for **2 minutes**.
24. Briefly centrifuge the tube.
25. Place the tube on a magnet until the supernatant is clear **(~30 seconds)**.
26. Pipet the eluate **(~40 µL)** into a new 1.5-mL tube.
27. Add **60 µL** of nuclease-free water to the eluate for a final volume of **100 µL**.

    The volume must be exactly 100 µL.

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

    The purified RPE PCR product is ready for 1.4 WTA Index PCR (page 24).

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 | | BD Part Number | Preparation and Handling | Storage |
|---|---|---|---|---|
| **Equilibrate to room temperature:** | | | | |
|  | Forward primer 1–8 | Various | Equilibrate to room temperature 30 minutes before setting up WTA Index PCR. Centrifuge briefly. Keep on ice until ready. | –20 °C |
|  | WTA reverse primer 1–8 | Various | | |
|  | Nuclease-free water | 51-9025552 | | |
| **Leave in freezer until ready to use:** | | | | |
|  | PCR master mix | 51-9025466 | Centrifuge briefly before adding to mix. | –20 °C |
| **Obtain:** | | | | |
| Purified RPE PCR product | | | | 4 °C |
| Ice bucket | | | | |
| 1.5-mL DNA LoBind® tubes | | | | |
| 0.2-mL PCR tubes | | | | |
| **Set up:** | | | | |
| Thermocycler with WTA Index PCR program | | | | |

Procedure steps:

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

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

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

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

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

**WTA Index PCR mix**

| Cap | Component | 1 library (µL) | 1 library with 20% overage (µL) | 4 libraries with 20% overage (µL) | 8 libraries with 20% overage (µL) |
|---|---|---|---|---|---|
| | PCR master mix | 12.5 | 15.0 | 60.0 | 120.0 |
| | Forward primer 1–8 | 2.5 | 3.0 | N/A | N/A |
| | WTA reverse primer 1–8 | 2.5 | 3.0 | N/A | N/A |
| | Nuclease-free water | 22.5 | 27.0 | 108.0 | 216.0 |
| | **Total** | **40.0** | **48.0** | **168.0** | **336.0** |

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

Bring the tubes to the post-amplification workspace.

5. Dilute an aliquot of the purified RPE PCR product from step 46 of 1.3 RPE PCR cleanup and quantification (page 21) to **0.5 ng/µL**.

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

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

8. Run the following PCR program.

**WTA Index PCR program**

| Step | Cycles | Temperature | Time |
|---|---|---|---|
| Hot start | 1 | 98 °C | 45 seconds |
| Denaturation | RPE PCR concentration*<br>0.05 ng/µL: 12 cycles<br>0.1 ng/µL: 11 cycles<br>0.2 ng/µL: 10 cycles<br>0.5 ng/µL: 8 cycles | 98 °C | 15 seconds |
| Annealing | | 60 °C | 30 seconds |
| Extension | | 72 °C | 1 minute |
| Final extension | 1 | 72 °C | 2 minutes |
| Hold | 1 | 4 °C | ∞ |

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


The PCR can run overnight.

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

## 1.5 WTA Index PCR cleanup and quality check

Summary:

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

Preparation list:

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

Procedure steps:

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

Perform the purification in the post-amplification workspace.

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

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

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

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

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

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

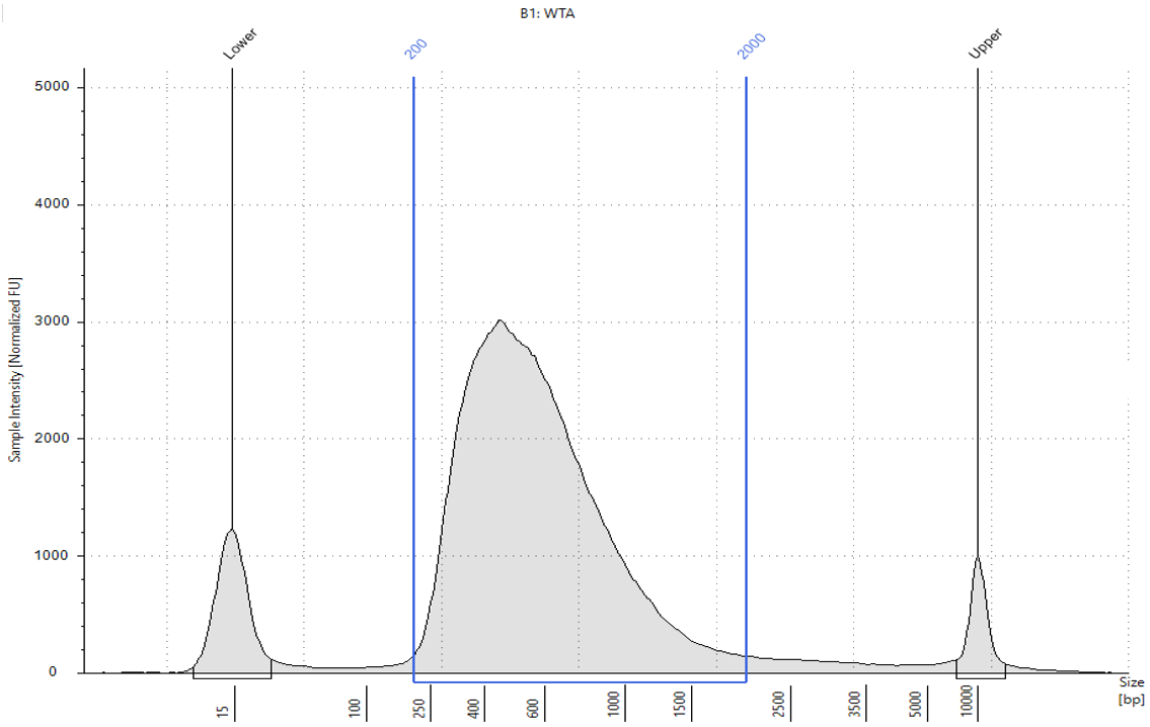
25. Quantify and perform quality control of the WTA Index PCR product with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay and one of the following systems:

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

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

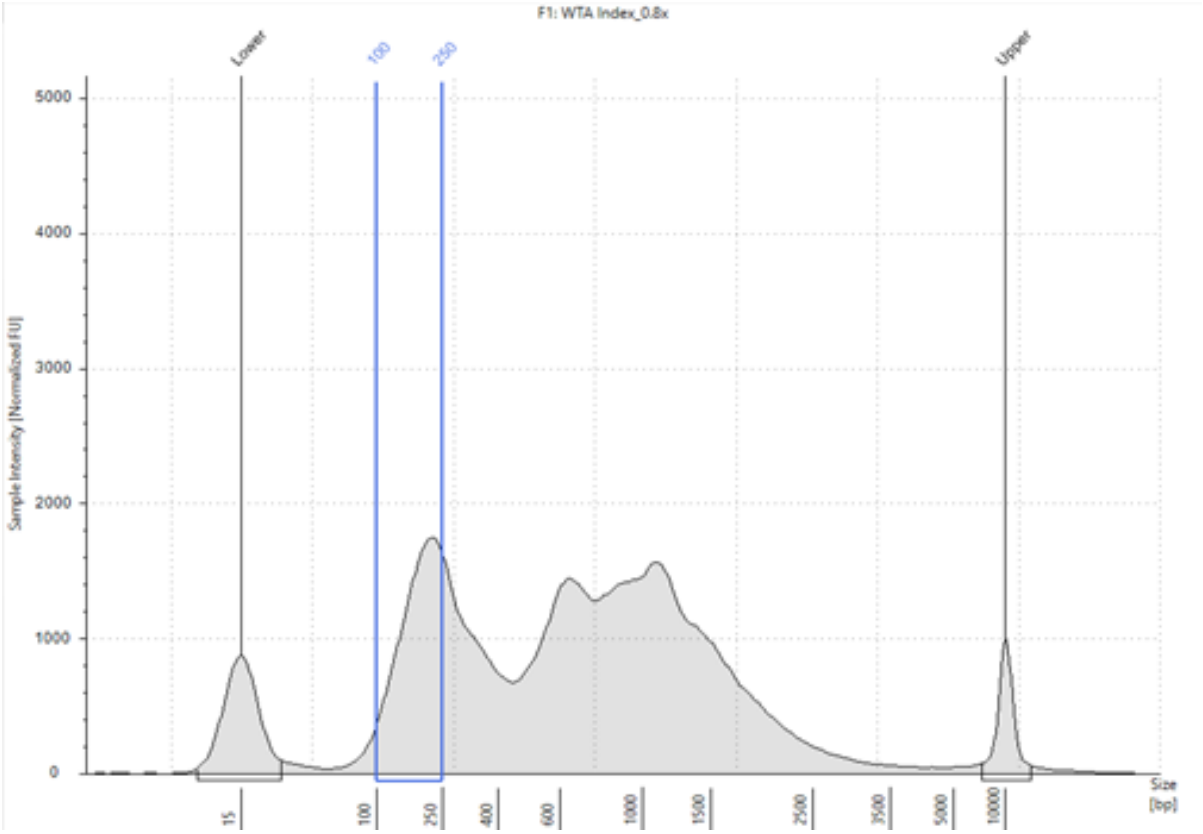
    The TapeStation trace should show a peak from ~200 to 2,000 bp. Refer to the representative traces in the following figures.

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



If smaller products (<250 bp) are observed (such as the peaks shown in Figure 2), we recommend a second round of AMPure® XP bead purification. See 1.6 Additional WTA Index PCR cleanup (page 31) 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 29) in 1.5 WTA Index PCR cleanup and quality check (page 27), bring up the total volume to **100 µL** with nuclease-free water.
2. Pipet-mix 10 times.
3. Briefly centrifuge the tube.

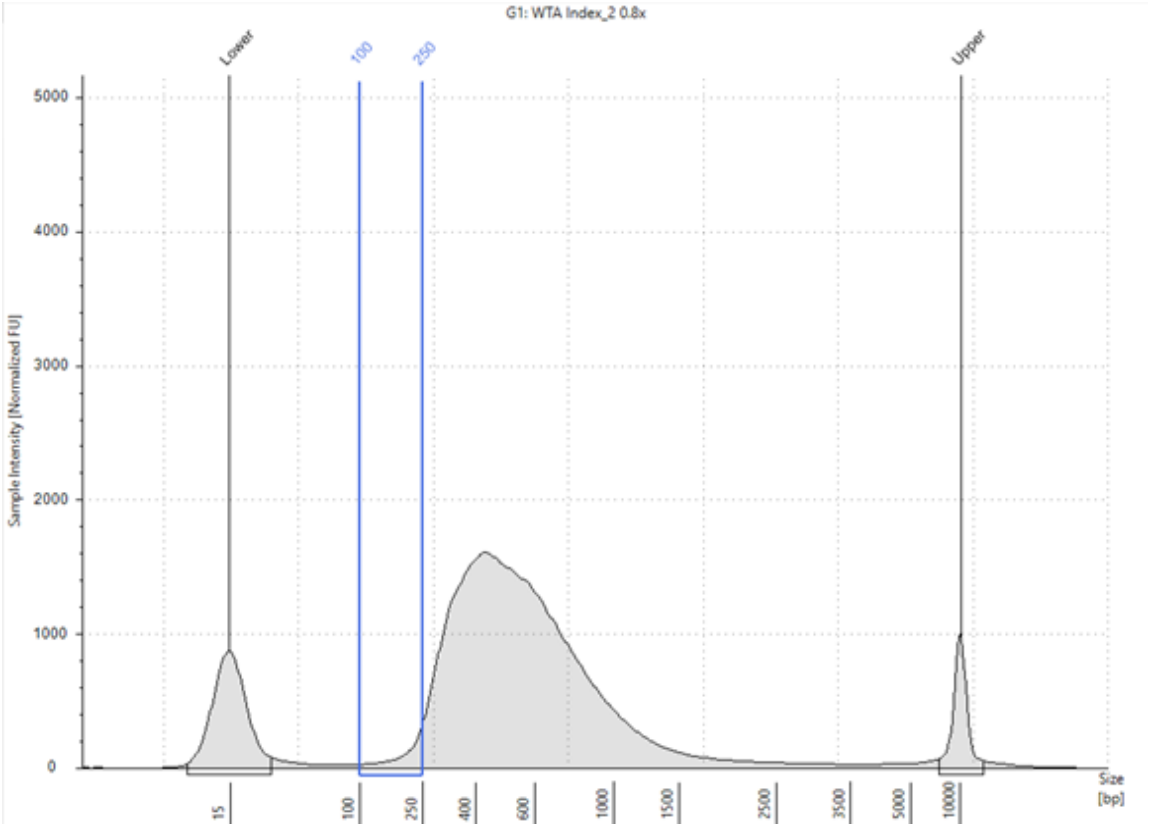
   The volume must be exactly 100 µL.

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

    The purified eluate is the **final sequencing library**.
23. Repeat step 25 (page 29) in 1.5 WTA Index PCR cleanup and quality check (page 27) to perform a quality check of the final library.

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

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



# 2. BD® AbSeq library amplification

This section comprises the following tasks:

## 2.1 BD® AbSeq PCR1

Summary:

- Prepare BD® AbSeq PCR1 mix
- Amplify using BD® AbSeq PCR1 program

Preparation list:

| Item | | BD Part Number | Preparation and Handling | Storage |
|---|---|---|---|---|
| **Equilibrate to room temperature:** | | | | |
| 🟢 | Universal oligo | 51-9025553 | Equilibrate to room temperature 30 minutes before setting up BD® AbSeq PCR1. Centrifuge briefly. | –20 °C |
| 🔴 | BD® AbSeq primer | 51-9025468 | | |
| ⚪ | Nuclease-free water | 51-9025552 | | |
| **Leave in freezer until ready to use:** | | | | |
| 🟢 | PCR master mix | 51-9025466 | Centrifuge briefly before adding to mix. | –20 °C |
| **Obtain:** | | | | |
| Denatured BD® AbSeq product | | | | 4 °C |
| Ice bucket | | | | |
| 0.2-mL PCR tubes | | | | |
| 1.5-mL tube magnetic rack | | | | |
| **Set up:** | | | | |
| Thermocycler BD® AbSeq PCR1 program | | | | |

Procedure steps:

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

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

**BD® AbSeq PCR1 mix**

| Cap | Component | 1 library (µL) | 1 library with 20% overage (µL) | 4 libraries with 20% overage (µL) | 8 libraries with 20% overage (µL) |
|---|---|---|---|---|---|
| ● | PCR master mix | 50.0 | 60.0 | 240.0 | 480.0 |
| ● | Universal oligo | 2.5 | 3.0 | 12.0 | 24.0 |
| ● | BD® AbSeq Primer | 10.0 | 12.0 | 48.0 | 96.0 |
| ○ | Nuclease-free water | 69.5 | 83.4 | 333.6 | 667.2 |
| | **Total** | **132.0** | **158.4** | **633.6** | **1,267.2** |

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

Bring the tubes to the post-amplification workspace.

7. Run the following PCR program:

**BD® AbSeq PCR 1 program**

| Step | Cycles | Temperature | Time |
|---|---|---|---|
| Hot start | 1 | 98 °C | 45 seconds |
| Denaturation | 1,000 cells: 14 cycles<br>2,500 cells: 13 cycles<br>5,000 cells: 12 cycles<br>10,000 cells: 11 cycles<br>20,000 cells: 10 cycles<br>40,000 cells: 9 cycles<br>>80,000 cells: 8 cycles* | 98 °C | 15 seconds |
| Annealing | | 60 °C | 30 seconds |
| Extension | | 72 °C | 1 minute |
| Final extension | 1 | 72 °C | 2 minutes |
| Hold | 1 | 4 °C | ∞ |

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


The PCR can be run overnight.

8. When the BD® AbSeq PCR1 program is complete, briefly centrifuge the tubes.

## 2.2 BD® AbSeq PCR1 cleanup and quantification

Summary:

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

Preparation list:

| Item | | BD Part Number | Preparation and Handling | Storage |
|---|---|---|---|---|
| **Equilibrate to room temperature:** | | | | |
| ● | Elution buffer | 51-9025554 | Centrifuge briefly. | –20 °C |
| AMPure® XP magnetic beads | | | Manufacturer's recommendations | |
| Agilent BioAnalyzer High Sensitivity Kit<br>OR<br>Agilent TapeStation ScreenTape and Reagents | | | | |
| **Obtain:** | | | | |
| BD® AbSeq PCR1 product | | | | 4 °C |
| 1.5-mL DNA LoBind® tubes | | | | |
| 0.2-mL PCR tubes | | | | |
| 1.5-mL tube magnetic rack | | | | |
| **Set up:** | | | | |
| Prepare fresh 80% ethyl alcohol | | | | |

Procedure steps:

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

Perform the purification in the post-amplification workspace.

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

   Adjust the volume depending on the number of samples—one sample requires 1 mL of 80% ethyl alcohol.
3. Vortex the AMPure® XP beads until the beads are fully resuspended.
4. Briefly centrifuge the tubes with the BD® AbSeq PCR1 product.
5. Combine the **four** tubes of **50-µL** PCR1 product into a new 1.5-mL tube.
6. Pipet-mix 10 times.

   The volume must be exactly **200 µL**. If the volume is less than 200 µL, use nuclease-free water to achieve the final volume.
7. Pipet **280 µL** AMPure® XP beads (1.4x) into the tube.
8. Pipet-mix 10 times.
9. Briefly centrifuge the tube.

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

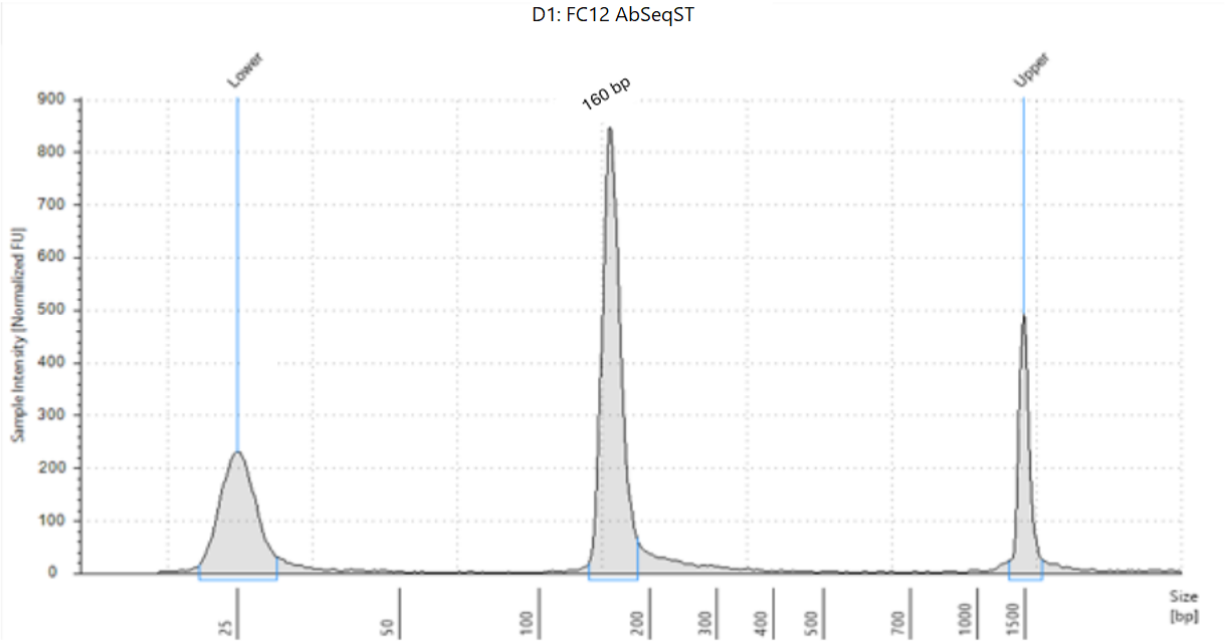
    Do not overdry the AMPure® beads after the ethyl alcohol washes. Overdried beads appear cracked.
19. Remove the tube from the magnet.
20. Pipet **30 µL** of elution buffer into the tube.

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

    The purified BD® AbSeq 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), dilute BD® AbSeq PCR1 product to **0.1–1.1 ng/ µL**, ready for 2.3 BD® AbSeq Index PCR (page 40).

STOP The BD® AbSeq 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 BD® AbSeq Index PCR

Summary:

- Prepare BD® AbSeq Index PCR mix
- Amplify using BD® AbSeq Index PCR program

Preparation list:

| Item | | BD Part Number | Preparation and Handling | Storage |
|---|---|---|---|---|
| **Equilibrate to room temperature:** | | | | |
| ● | Forward primer 1–8 | Various | Equilibrate to room temperature 30 minutes before setting up BD® AbSeq Index PCR. Centrifuge briefly. Keep on ice until ready. | –20 °C |
| ○ | Multiomic reverse primer 1–8 | Various | | |
| ○ | Nuclease-free water | 51-9025552 | | |
| **Leave in freezer until ready to use:** | | | | |
| ● | PCR master mix | 51-9025466 | Centrifuge briefly before adding to mix. | –20 °C |
| **Obtain:** | | | | |
| Purified BD® AbSeq PCR1 product | | | | 4 °C |
| Ice bucket | | | | |
| 1.5-mL DNA LoBind® tubes | | | | |
| 0.2-mL PCR tubes | | | | |
| **Set up:** | | | | |
| Thermocycler with BD® AbSeq Index PCR program | | | | |

Procedure steps:

This section describes how to generate BD® AbSeq 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 Index PCR mix**

| Cap | Component | 1 library (µL) | 1 library with 20% overage (µL) | 4 libraries with 20% overage (µL) | 8 libraries with 20% overage (µL) |
|---|---|---|---|---|---|
| | PCR master mix | 12.5 | 15.0 | 60.0 | 120.0 |
| | Forward primer 1–8 | 2.0 | 2.4 | N/A | N/A |
| | Multiomic reverse primer 1–8 | 2.0 | 2.4 | N/A | N/A |
| | Nuclease-free water | 30.5 | 36.6 | 146.4 | 292.8 |
| | **Total** | **47.0** | **56.4** | **206.4** | **412.8** |

1. Pipet-mix the BD® AbSeq Index PCR mix.
2. For multiple samples, pipet **43 µL** of Index PCR mix into a separate 0.2-mL PCR tube for each sample.
3. Add **2 µL** of forward primer and **2 µL** of multiomic reverse primer to each sample.
4. Place on **ice** until ready to use.

   Bring the BD® AbSeq Index PCR mix to the post-amplification workspace.

5. Dilute an aliquot of BD® AbSeq PCR1 product, from 2.2 BD® AbSeq PCR1 cleanup and quantification (page 37) with nuclease-free water to **0.1–1.1 ng/µL**.

   Add **3 µL** of the diluted BD® AbSeq PCR1 product to BD® AbSeq Index PCR mix.

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

6. Pipet-mix 10 times.

7. Run the following PCR program:

**BD® AbSeq Index PCR program**

| Step | Cycles | Temperature | Time |
|---|---|---|---|
| Hot start | 1 | 98 °C | 45 seconds |
| Denaturation | Index PCR concentration*<br>0.5–1.1 ng/µL: 6 cycles<br>0.25-0.5 ng/µL: 7 cycles<br>0.1-0.25 ng/µL: 8 cycles | 98 °C | 15 seconds |
| Annealing | | 60 °C | 30 seconds |
| Extension | | 72 °C | 1 minute |
| Final extension | 1 | 72 °C | 2 minutes |
| Hold | 1 | 4 °C | ∞ |

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


The PCR can run overnight.

8. When the BD® AbSeq Index PCR program is complete, briefly centrifuge the tubes.

## 2.4 BD® AbSeq Index PCR cleanup and quality check

Summary:

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

Preparation list:

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

Procedure steps:

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

   The volume must be exactly **50 µL**. If the volume is less than 50 µL, use nuclease-free water to achieve the final volume.
5. Pipet **40 µL** of AMPure® XP beads (0.8x) into the tube.
6. Pipet-mix 10 times.
7. Briefly centrifuge the tube.
8. Incubate at room temperature for **5 minutes**.
9. Place the tube on a magnet until the supernatant is clear (**<5 minutes**).
10. Remove and discard the supernatant.
11. Keeping the tube on the magnet, gently pipet **200 µL** of fresh 80% ethyl alcohol into the tube.
12. Incubate for **30 seconds**.
13. Remove and discard the supernatant without disturbing the beads.
14. Repeat steps 11–13 for a total of **two ethyl alcohol washes**.
15. Keeping the tube on the magnet, use a P20 pipette to remove and discard the residual supernatant from the tube.
16. Air-dry the beads at room temperature until the beads no longer look glossy (**~2 minutes**).

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

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

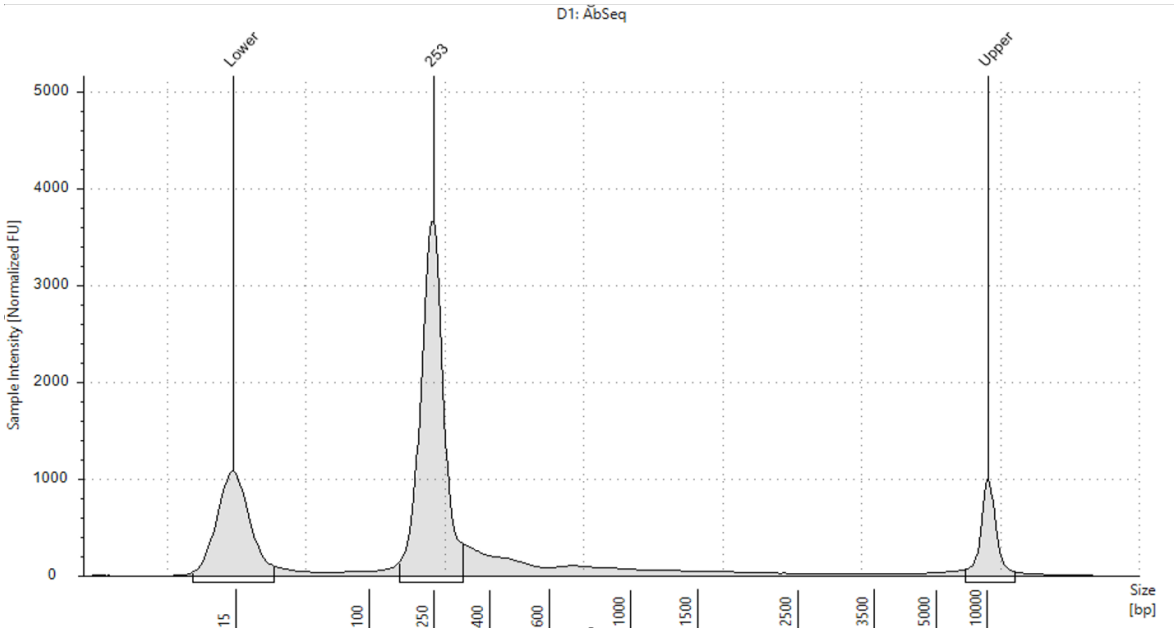
    The purified eluate is the final sequencing library.

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

24. Quantify and perform quality control of the BD® AbSeq 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® AbSeq library should show a peak of ~264 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



# Sequencing

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

## 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 50).

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

## Sequencing recommendations

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

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

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

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

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

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

Sequencing amount for BD® AbSeq libraries:

- The amount of sequencing needed for BD® AbSeq libraries will vary depending on application, BD® AbSeq panel plexy, and cell type. We have observed that using 40,000 sequencing reads per cell for 40-plex BD® AbSeq libraries prepared from resting PBMCs achieves an RSEC sequencing depth of ~2.

To determine the ratio of BD Rhapsody™ WTA library to BD® AbSeq 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 |
|---|---|---|
| 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 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), perform a second round of AMPure® purification according to 1.6 Additional WTA Index PCR cleanup (page 31). |
| 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 BD® AbSeq library too low after Index PCR, but yield of BD® AbSeq PCR1 products is sufficient. | Too few Index PCR cycles. | Increase the number of cycles for Index PCR. |
| | 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. |

## Sequencing

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

# Appendix

## Oligonucleotides in BD OMICS-One™ WTA Next Amplification Kit

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

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

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

| Oligonucleotide | Use | Part/Catalog No. | Sequence (5' – 3') |
|---|---|---|---|
| BD OMICS-One™ WTA Library Reverse Primer 1 | Reverse primer for WTA Index PCR | 51-9025480 | CAA GCA GAA GAC GGC ATA CGA GAT TACTACGC GTCTCGTGGGCTCGGAGATGTGTATAAGA*G |
| BD OMICS-One™ WTA Library Reverse Primer 2 | | 51-9025600 | CAA GCA GAA GAC GGC ATA CGA GAT AGGCTCCG GTCTCGTGGGCTCGGAGATGTGTATAAGA*G |
| BD OMICS-One™ WTA Library Reverse Primer 3 | | 51-9025482 | CAA GCA GAA GAC GGC ATA CGA GAT GCAGCGTA GTCTCGTGGGCTCGGAGATGTGTATAAGA*G |
| BD OMICS-One™ WTA Library Reverse Primer 4 | | 51-9025483 | CAA GCA GAA GAC GGC ATA CGA GAT CTGCGCAT GTCTCGTGGGCTCGGAGATGTGTATAAGA*G |
| BD OMICS-One™ WTA Library Reverse Primer 5 | | 51-9025484 | CAA GCA GAA GAC GGC ATA CGA GAT GAGCGCTA GTCTCGTGGGCTCGGAGATGTGTATAAGA*G |
| BD OMICS-One™ WTA Library Reverse Primer 6 | | 51-9025485 | CAA GCA GAA GAC GGC ATA CGA GAT CGCTCAGT GTCTCGTGGGCTCGGAGATGTGTATAAGA*G |
| BD OMICS-One™ WTA Library Reverse Primer 7 | | 51-9025486 | CAA GCA GAA GAC GGC ATA CGA GAT GTCTTAGG GTCTCGTGGGCTCGGAGATGTGTATAAGA*G |
| BD OMICS-One™ WTA Library Reverse Primer 8 | | 51-9025487 | CAA GCA GAA GAC GGC ATA CGA GAT ACTGATCG GTCTCGTGGGCTCGGAGATGTGTATAAGA*G |

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

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

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

## 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])**AAAAAAAAAAAAAAAAAAAAAAAA

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® AbSeq assays.
3. Click a specific product to view product details, and scroll down to the **Barcode Sequence** field.

# BD OMICS-One™ WTA Next and BD® AbSeq Library Preparation Quick Reference Guide

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

## Introduction

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

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

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

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

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

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

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

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

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

### Getting started

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

**Ethanol required per wash** (Two washes per purification)

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

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

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

### Purifying the sample with AMPure (example)

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

**Table 1 Example PCR purification**

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

## BD OMICS-One™ WTA Next and BD® AbSeq library preparation

### Preparing the thermomixers

Preheat the following instruments:

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

### Preparing the reagent mixes

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

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

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

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

## Denaturing the BD® AbSeq product

1. Label a new 1.5-mL tube as *BD® AbSeq 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 product into labeled 1.5-mL tube.
7. Store **BD® AbSeq product** on ice until BD® AbSeq PCR 1. See "Amplifying the BD® AbSeq product" on page QRG-7.
8. Wash beads with **200 µL elution buffer.**

## Generating the RPE product

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

**RPE program** (all steps at 1,200 rpm)

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

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

DO NOT STOP.

## Amplifying the RPE product

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

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

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

**RPE PCR program**

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

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

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

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

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

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

## Quantifying the RPE PCR product

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

## Indexing the WTA libraries

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

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

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

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

**WTA Index PCR program**

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

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

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

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

## Quantifying the indexed WTA libraries

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

## Amplifying the BD® AbSeq product

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

| Cap | Component | Sample count with % overage (volumes in µL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1+0% | 1+20% | 2+20% | 3+20% | 4+20% | 5+20% | 6+20% | 7+20% | 8+20% |
| | PCR Master Mix | 50.0 | 60.0 | 120.0 | 180.0 | 240.0 | 300.0 | 360.0 | 420.0 | 480.0 |
| | Universal Oligo | 2.5 | 3.0 | 6.0 | 9.0 | 12.0 | 15.0 | 18.0 | 21.0 | 24.0 |
| | BD® AbSeq Primer | 10.0 | 12.0 | 24.0 | 36.0 | 48.0 | 60.0 | 72.0 | 84.0 | 96.0 |
| | Water | 69.5 | 83.4 | 166.8 | 250.2 | 333.6 | 417.0 | 500.4 | 583.8 | 667.2 |
| | **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 product** (see "Denaturing the BD® AbSeq product" on page QRG-4) with **132 µL BD® AbSeq PCR 1 mix.**
3. Mix well.
4. Split BD® AbSeq PCR mix-containing product across **four PCR tubes** containing 50 µL each.
5. Amplify using the following table.

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

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

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

| Purification | PCR Vol. µL | AMPure Vol. µL | Elution Vol. µL |
|---|---|---|---|
| BD® AbSeq PCR 1 | 200 | 280 | 30 |

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

## Quantifying the BD® AbSeq PCR 1 product

1. Quality check purified BD® AbSeq PCR 1 using an analyzer.
2. Dilute BD® AbSeq PCR 1 product to **0.1–1.1 ng/µL** for BD® AbSeq Index PCR.

## Indexing the BD® AbSeq libraries

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

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

2. Combine **47 µL BD® AbSeq Index PCR mix** with **3 µL** *diluted* and *purified* **BD® AbSeq PCR 1** product. See "Amplifying the BD® AbSeq product" on page QRG-7.
3. Mix well.
4. Amplify using the following **BD® AbSeq Index PCR program.**

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

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

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

| Purification | PCR Vol. µL | AMPure Vol. µL | Elution Vol. µL |
|---|---|---|---|
| BD® AbSeq Index PCR | 50 | 40 | 30 |

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

## Quantifying the indexed BD® AbSeq libraries

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

## BD OMICS-One™ WTA Next and BD® AbSeq Quick Reference Guide

The following note sheet is provided for user convenience.

Experiment _____ User _____ Date _____ Kit Lot _____ Expiry _____

Use the area below to record sample information:

| **Sample Description** | **RPE PCR Quant.** ng/µL | **WTA Fwd. Index** | **WTA Rev. Index** | **WTA Index PCR Quant.** ng/µL | **BD® AbSeq PCR 1 Quality** bp (avg) | **BD® AbSeq Fwd. Index** | **BD® AbSeq Rev. Index** | **BD® AbSeq Index PCR Quant.** ng/ µL | **Notes** |
|---|---|---|---|---|---|---|---|---|---|
| **1** | | | | | | | | | |
| **2** | | | | | | | | | |
| **3** | | | | | | | | | |
| **4** | | | | | | | | | |
| **5** | | | | | | | | | |
| **6** | | | | | | | | | |
| **7** | | | | | | | | | |
| **8** | | | | | | | | | |

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