Rhapsody™ System

Mouse TCR/BCR Next and BD OMICS-One™ WTA Next

Library Preparation Protocol

23-24999(02)
2026-09

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Regulatory information

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History

Revision	Date	Change made
23-24999(01)	2025-10	Initial release.
23-24999(02)	2026-09	Updated with corrections for customer-impacted issues.

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Introduction

This protocol enables high throughput single-cell transcriptome alongside TCR and BCR profiling of individual cells captured on the BD Rhapsody™ system, providing instructions for amplifying Illumina-compatible single-cell barcoded mRNA, TCR, and BCR libraries.

cDNA is encoded on BD Rhapsody™ Enhanced Cell Capture Beads using both the 3' and 5' ends of transcripts as templates. Whole transcriptome library is generated directly from the beads using a random priming approach, followed by an index polymerase chain reaction (PCR) step. TCR and BCR libraries are amplified from cDNA on bead using a two-step nested PCR, followed by additional random priming to capture complementarity determining regions (CDR) 1–3 and framework regions (FR) 1–4.

Symbols

The following symbols are used in this guide:

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

Protocol kits

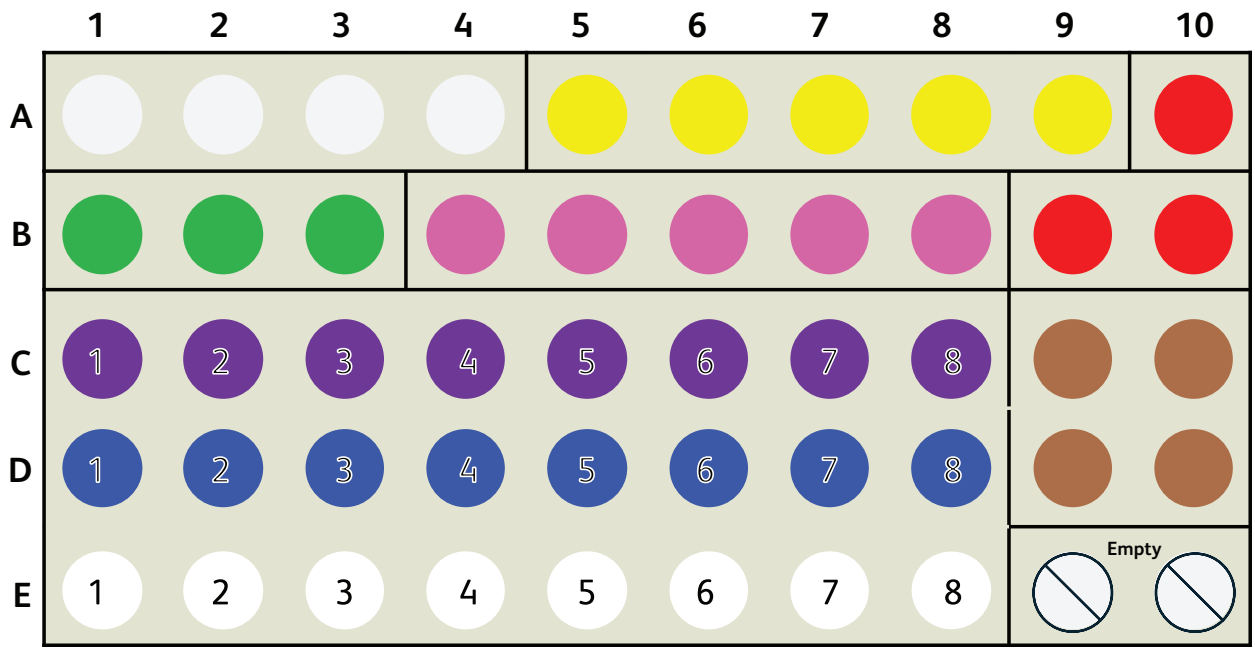
Before you begin, ensure that you have the correct kits for this protocol. Matching cap colors indicate you have the correct kit, along with the catalog numbers found in the Required and recommended materials (page 14) section.

BD Rhapsody™ cDNA Kit

Cap Color	Name	Quantity
🟠	RT buffer	1
🟠	RT 0.1M DTT	1
🟠	Reverse transcriptase	1
🟠	dNTP	1
🟠	RNase inhibitor	1
⚫	Bead RT/PCR enhancer	1
🟡	10X Exonuclease I buffer	1
🟡	Exonuclease I	1
⚪	Nuclease-free water	2
⚫	Bead Resuspension buffer	1

BD Rhapsody™ Enhanced Cartridge Reagent Kit v3 (4 °C)

Cap Color	Name	Quantity
🟤	BD Rhapsody™ HT Enhanced Cell Capture Beads v3	4
○	Sample buffer	1
○	Cartridge wash buffer 1	1
○	Cartridge wash buffer 2	1
○	Lysis buffer	4
○	Bead wash buffer	1
○	Waste collection container	4
○	1M DTT	1



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 8)	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

BD Rhapsody™ Mouse TCR/BCR Next Amplification Kit

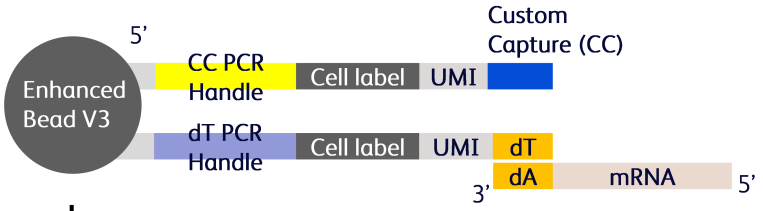
Cap Color	Name	Quantity
○	TCR/BCR extension primers	1
○	TCR/BCR extension buffer	1
○	TCR/BCR extension enzyme	1
●	10 mM dNTP	2
○	Nuclease-free water	2
●	Bead RT/PCR enhancer	1
●	TSO Next	1
●	TCR N1 primer - Mouse	1
●	TCR N2 primer - Mouse	1
●	BCR N1 primer - Mouse	1
●	BCR N2 primer - Mouse	1
○	PCR master mix	1
●	TCR/BCR universal oligo N1	1
●	TCR/BCR universal oligo N2	1
●	Elution buffer	2
●	1M $MgCl_2$	1
●	Hybridization buffer	4

Workflows

On bead workflow

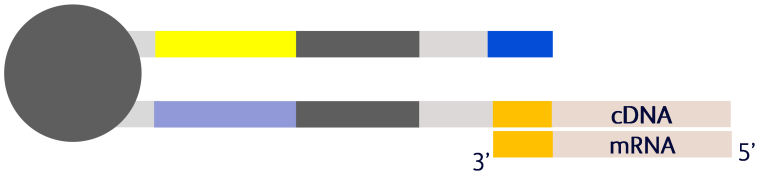
Cell lysis in microwell

mRNA is captured by dT



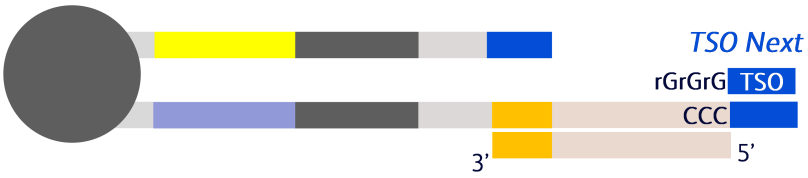
Reverse transcription

Complementary DNA (cDNA) is synthesized from captured mRNA.



Template-switching oligo priming

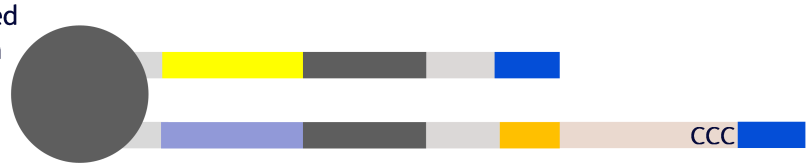
TSO is added to 3' of the cDNA on the bead, to allow for bead self-hybridization



Denaturation

Supernatant:
The mRNA template is denatured off the bead. Discard the supernatant.

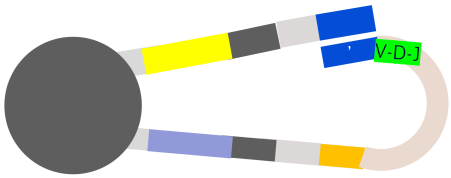
Bead: Single-stranded DNA is generated on beads to prepare for self-hybridization.



Self-hybridization

Resuspend the beads in pre-warmed hybridization buffer, then gradually cool down to allow the cDNA self-hybridized on bead by CC.

VDJ region is flipped and captured by bead CC strand

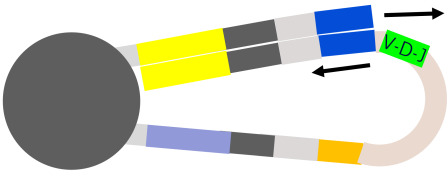


Extension

Copy cell label, UMI, TCR/BCR universal oligo

Exo I

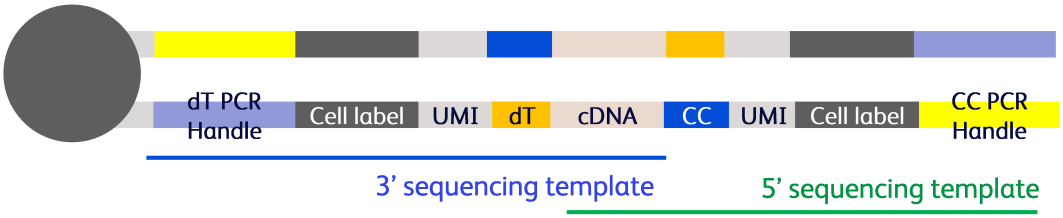
Remove unused oligo capture sequences



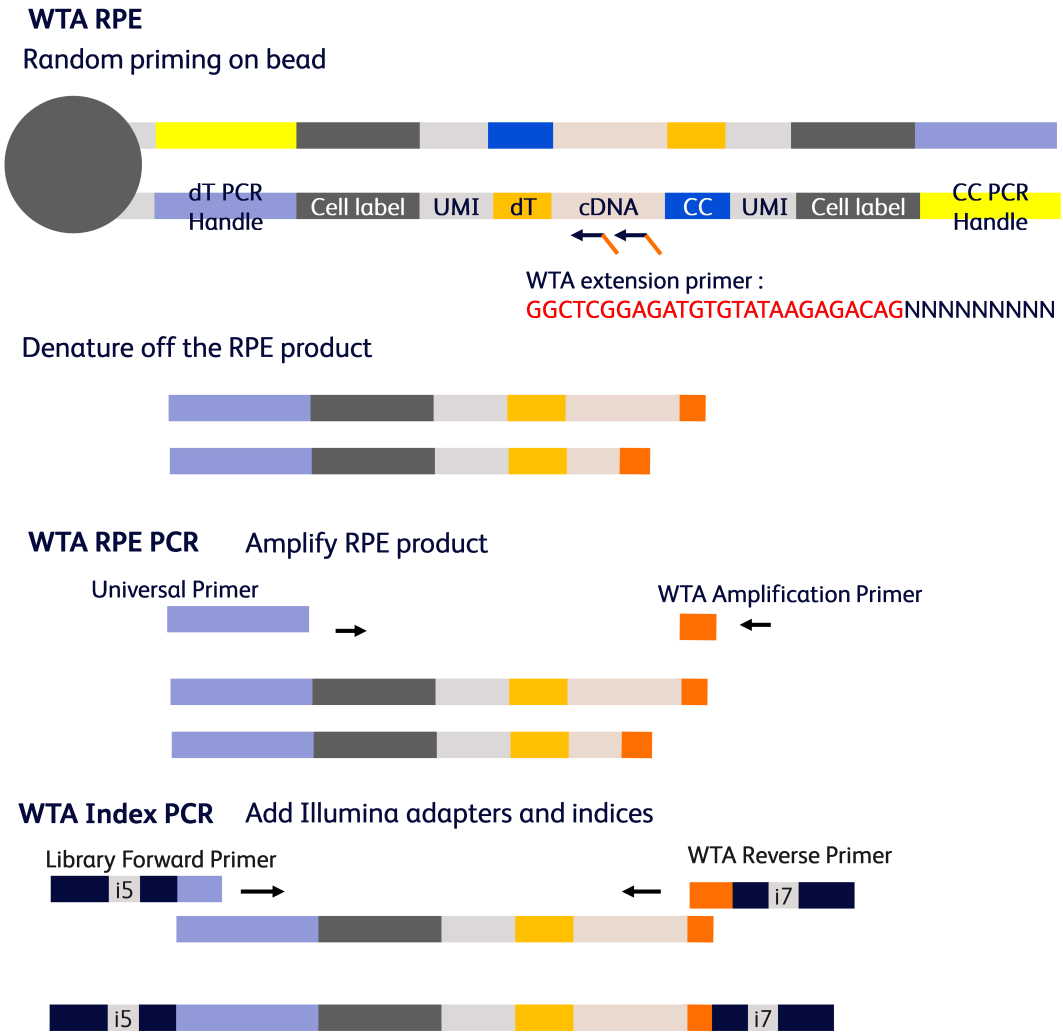
Final bead layout

3' sequencing template (blue): mRNA library

5' sequencing template (green): TCR/BCR library



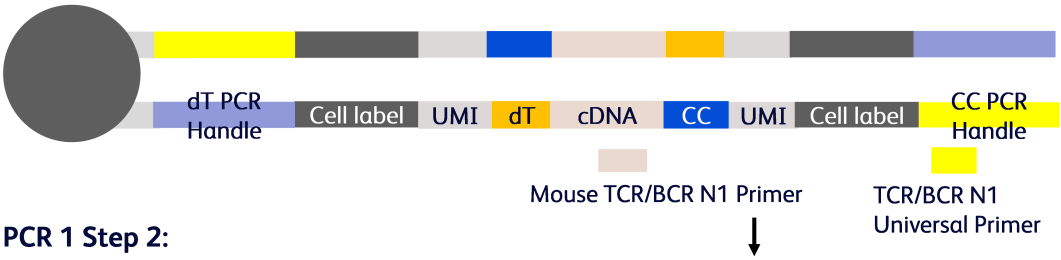
WTA library amplification workflow



TCR/BCR library amplification workflow

PCR 1 Step 1:

TCR/BCR universal primer and Mouse TCR/BCR N1 primer copy target region from bead



PCR 1 Step 2:

Amplify in solution (collect supernatant as TCR/BCR PCR1 product)

PCR 2:

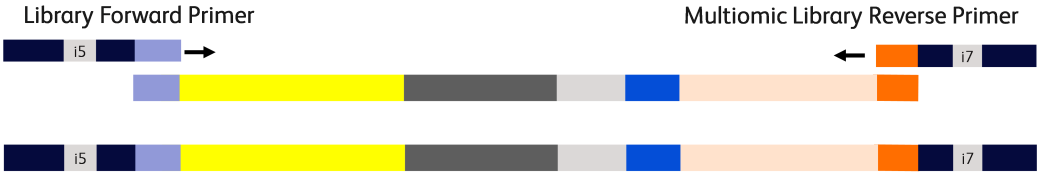
TCR/BCR universal N2 primer adds sequencing handle; Mouse TCR N2 or BCR N2 primer for nested PCR enrichment

RPE for fragmentation:

Random priming is used for fragmentation for sequencing, full-length VDJ is assembled bioinformatically. TCR and BCR RPE are done separately

Index PCR:

Add Illumina adapters and indices. TCR and BCR Index 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 Rhapsody™ cDNA Kit[a]	BD Biosciences	633773
BD Rhapsody™ Enhanced Cartridge Reagent Kit V3[a,b]	BD Biosciences	667052
BD OMICS-One™ WTA Next Amplification Kit	BD Biosciences	572620
BD Rhapsody™ Mouse TCR/BCR Next Amplification Kit[a]	BD Biosciences	667059
AMPure® XP magnetic beads	Beckman Coulter	A63880
100% ethyl alcohol, molecular biology grade	Major supplier	–
Nuclease-free water	Major supplier	–

a. For processing more than four libraries, two orders of this catalog number are required.

b. The Enhanced Cartridge Reagent Kit V3 must be used before beginning this protocol.

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[a] Or, MicroAmp™ Optical 96–Reaction Plate[a]	Corning Thermo Fisher Scientific	PCR96HSC N8010560
MicroAmp™ Clear Adhesive Film[a]	Thermo Fisher Scientific	4306311
15-mL conical tube	Major supplier	–
DNA LoBind® Tubes, 1.5 mL	Eppendorf	0030108051
DNA LoBind® Tubes, 5.0 mL	Eppendorf	0030108310
Qubit™ Assay Tubes	Thermo Fisher Scientific	Q32856
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851
Agilent High Sensitivity DNA Kit Or, Agilent High Sensitivity D1000 ScreenTape Agilent High Sensitivity D1000 Reagents Or, Agilent High Sensitivity D5000 ScreenTape Agilent High Sensitivity D5000 Reagents	Agilent Agilent Agilent Agilent Agilent	5067-4626 5067-5584 5067-5585 5067-5592 5067-5593

a. Recommended for processing high throughput 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	–
Eppendorf ThermoMixer® C	Eppendorf	5382000023
6-tube magnetic separation rack for 1.5-mL tubes Or, 12-tube magnetic separation rack[a] Or, Invitrogen™ DynaMag™-2 magnet[a]	New England Biolabs New England Biolabs Thermo Fisher Scientific	S1506S S1509S 12321D
Low-profile magnetic separation stand for 0.2 mL, 8-strip tubes	V&P Scientific, Inc.	VP772F4-1
Magnetic Stand–96[b]	Thermo Fisher Scientific	AM10027
Qubit™ 3.0 Fluorometer or similar	Thermo Fisher Scientific	Q33216
Agilent® 2100 Bioanalyzer Or, Agilent® 4200 TapeStation System	Agilent Technologies Agilent Technologies	G2940CA G2991AA
Heat block	Major supplier	–

a. Recommended for processing greater than six samples.

b. Recommended for processing high throughput library preparation workflows.

Best practices

The BD Rhapsody™ Enhanced Cartridge Reagent Kit V3 (Catalog no. 667052) must be used for this protocol. The BD Rhapsody™ Mouse TCR/BCR Next Amplification Kit (Catalog no. 667059) is not compatible with the BD Rhapsody™ Enhanced Cartridge Reagent Kit (Catalog no. 664887).

Cell capture

- Ensure that the intended total cell load is 7,500–20,000. Cell loads outside this recommended range might require protocol optimization and might yield suboptimal results.
- For best results, ensure that cells have high viability before proceeding with cell capture.

Bead handling

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

  Never vortex the beads. Pipet-mix only.

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

  Do not freeze.

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

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.

Denaturation and self-hybridization

- Remove supernatant promptly after 95 °C denaturation step (≤30 seconds after placing on magnet).
- Ensure that hybridization buffer is preheated at 80 °C for at least 20 minutes before resuspending beads in step 9 of 1.2 Denaturation and Self-hybridization (page 23).

  Using cold or room temperature hybridization buffer might negatively impact self-hybridization efficiency.

Supernatant handling

- Read the 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 beads.
- Make and use fresh 80% ethyl alcohol within 24 hours. Adjust the volume of 80% ethyl alcohol depending on the number of libraries.

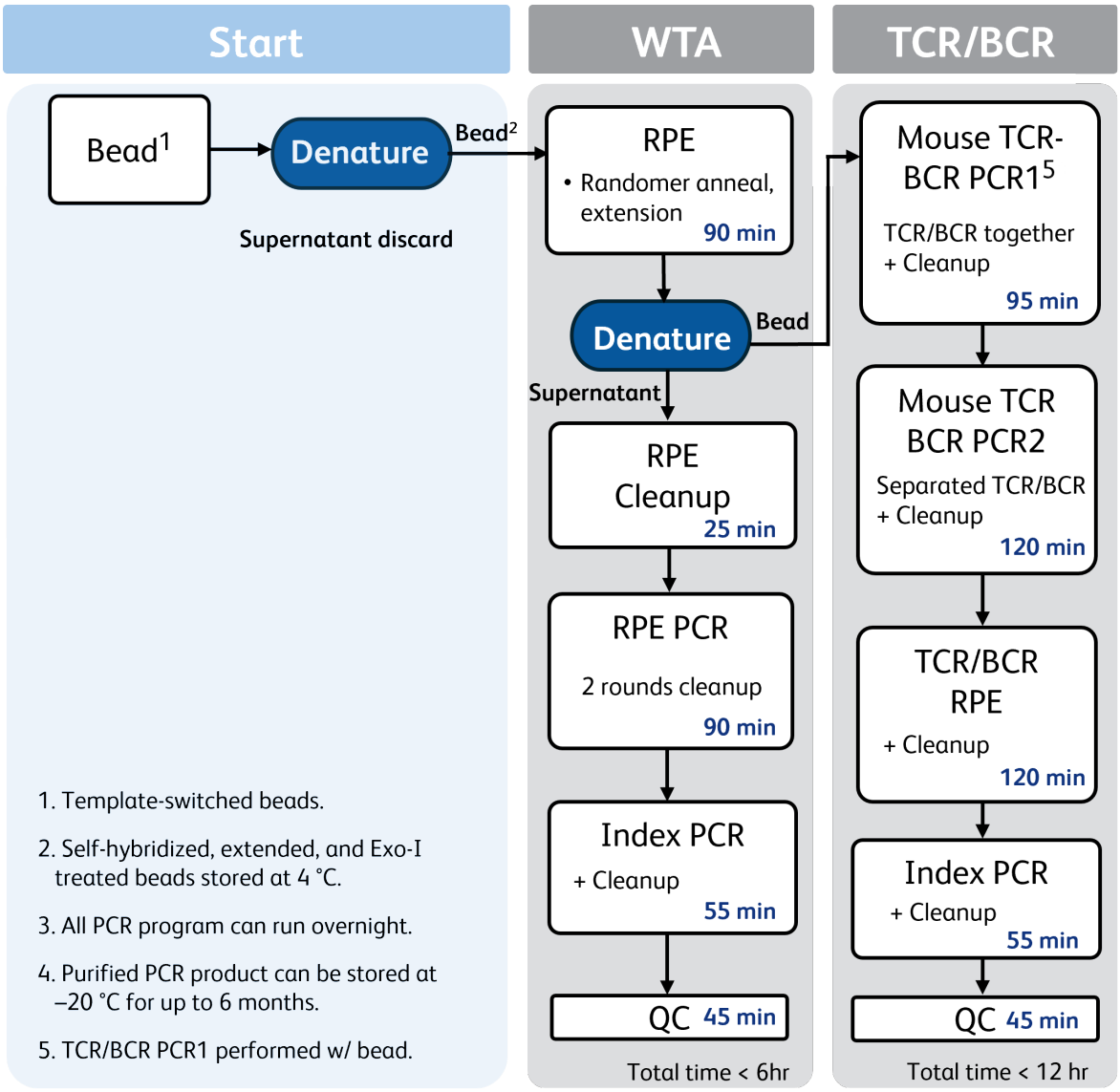
Bead amplification

- Do not proceed to thermal cycling until each tube is gently mixed by pipette to ensure uniform bead suspension. Start the thermocycler program immediately after mixing.
- Save beads after the first amplification step (WTA Random Priming and Extension (RPE) (page 29)). They must be used again for the second bead amplification step (TCR/BCR PCR1 (page 47)).

Safety information

For safety information, refer to the *BD Rhapsody™ HT Single-Cell Analysis System Extended-Lysis Single-Cell Capture and cDNA Synthesis Protocol* (doc ID: 23-24984) or *BD Rhapsody™ HT Xpress System Extended-Lysis Single-Cell Capture and cDNA Synthesis Protocol* (doc ID: 23-24983).

Time considerations



Procedure

Perform the experiment on the BD Rhapsody™ Single-Cell Analysis system following either the:

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

  STOP

  After the "Washing BD Rhapsody™ Enhanced Cell Capture Beads" section and follow this protocol from Reverse transcription, template switching, and Exonuclease I treatment (page 20) and subsequent steps.

  or

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

  STOP

  After the "Washing BD Rhapsody™ Enhanced Cell Capture Beads" section and follow this protocol from Reverse transcription, template switching, and Exonuclease I treatment (page 20) and subsequent steps.

The BD Rhapsody™ Enhanced Cartridge Reagent Kit V3 (Catalog no. 667052) must be used for this protocol.

Ensure that the intended total cell load is between 7,500–20,000 single cells for this protocol. Cell load below or above this recommended range might not be suitable for current protocol configuration. Then proceed as described in the following procedure.

1. Reverse transcription, template switching, and Exonuclease I treatment

1.1 cDNA Synthesis and Template Switching

Summary:

- Prepare cDNA mixture
- cDNA synthesis
- Add Template Switch Oligo (TSO Next)

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟠	RT buffer	650000067	Equilibrate to room temperature 30 minutes before setting up cDNA synthesis. Centrifuge briefly.	–20 °C
🟠	dNTP	650000077		
🟠	0.1 M DTT	650000068		
⚪	Nuclease-free water	650000076		
⚫	1M $MgCl_2$	91-1198		
Place on ice:				
⚫	Bead RT/PCR enhancer	91-1082	Centrifuge briefly before adding to mix.	–20 °C
🟡	TSO Next	91-1295		
Leave in freezer until ready to use:				
🟠	RNase inhibitor	650000078	Centrifuge briefly before adding to mix.	–20 °C
🟠	Reverse transcriptase	700026321		
Obtain:				
Washed enhanced cell capture beads			Centrifuge briefly and keep on ice until ready.	4 °C
Ice bucket				
1.5-mL tube magnetic rack				
1.5-mL DNA LoBind® tubes				
Set up:				
Thermomixer at 42 °C				

Procedure steps:

This section should be performed in the pre-amplification workspace.

1. Set a thermomixer to 42 °C.
2. If performing self-hybridization on the same day, set a second thermomixer to:
   - 1,200 rpm and at 80 °C for 3 minutes.
   - 1,200 rpm and at 25 °C for 1 minute.
   - 1,200 rpm and at 25 °C infinite (optional).

   The thermomixer set to 80 °C will be used as a heat block to warm the hybridization buffer, and then used with programmed cooling during 1.2 Denaturation and Self-hybridization (page 23).

   Confirm "Time Mode" on the thermomixer is set to "Temperature Control" to ensure that the 25 °C temperature is reached before the 1 minute at 25 °C begins. This will ensure gradual cooldown from 80 °C to 25 °C.

3. In a new 1.5-mL LoBind® tube, pipet the following reagents.

cDNA/template switching mix

Cap	Component	1 library (µL)	1 library with 20% overage (µL)	4 libraries with 20% overage (µL)	8 libraries with 20% overage (µL)
🟠	RT buffer	40.0	48.0	192.0	384.0
🟠	dNTP	20.0	24.0	96.0	192.0
🟠	RT 0.1 M DTT	10.0	12.0	48.0	96.0
⚫	Bead RT/PCR enhancer	12.0	14.4	57.6	115.2
🟠	RNase inhibitor	10.0	12.0	48.0	96.0
🟠	Reverse transcriptase	10.0	12.0	48.0	96.0
⚪	Nuclease-free water	98.0	117.6	470.4	940.8
	Total	**200.0**	**240.0**	**960.0**	**1920.0**

4. Gently vortex mix, briefly centrifuge, and place back on ice.
5. Place the tube of washed BD Rhapsody™ Enhanced Cell Capture Beads on a magnet for **≥2 minutes**.
6. Discard the supernatant.
7. Remove the tube from the magnet and pipet **200 µL** of cDNA mix into the beads. Pipet-mix.

Keep the prepared cDNA mix with beads on ice until the suspension is transferred in the next step.

8. Transfer the bead suspension to a new 1.5-mL LoBind® tube.
9. Incubate the bead suspension on the thermomixer at 1,200 rpm and **42 °C** for **30 minutes**.

Shaking is critical for this incubation.

10. While the bead suspension is still incubating at 1,200 rpm and 42 °C, pipet the following reagents in a new 1.5-mL LoBind® tube.

Prepare the TSO mix approximately **within 2 minutes** before the 30 minute incubation at 42 °C is finished.

Use immediately.

TSO mix

Cap	Component	1 library (µL)	1 library with 20% overage (µL)	4 libraries with 20% overage (µL)	8 libraries with 20% overage (µL)
	TSO Next	6.0	7.2	28.8	57.6
	1M $MgCl_2$	2.0	2.4	9.6	19.2
	Total	**8.0**	**9.6**	**38.4**	**76.8**

11. Gently vortex mix, briefly centrifuge, and keep on ice.
12. Add **8 µL** of TSO mix to the reaction, gently pipet-mix, and incubate on the thermomixer for another **30 minutes** at 1,200 rpm and **42 °C**.

If you are performing self-hybridization on the same day, complete steps 3 and 4 from 1.2 Denaturation and Self-hybridization (page 23) now.

13. Place the bead suspension on the 1.5-mL tube magnet until the solution is clear (**≤1 minute**). Discard the supernatant.
14. Remove the tube from the magnet and pipet **200 µL** of elution buffer into the tube. Pipet-mix. Place on ice.

OPTIONAL

STOP

BD Rhapsody™ Enhanced Cell Capture Beads can be stored up to 7 days at 2–8 °C after template switching.

15. If using the *BD Rhapsody™ HT Single-Cell Analysis System Instrument User Guide*, view the BD Rhapsody™ Scanner image analysis to see if the analysis metrics passed.

1.2 Denaturation and Self-hybridization

Summary:

- Denature mRNA
- Add hybridization buffer to hybridize TSO onto bead

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Hybridization buffer	91-1199	Equilibrate to room temperature 30 minutes before setting up hybridization. Centrifuge briefly.	–20 °C
	Elution buffer	91-1084		
Obtain:				
Enhanced cell capture beads after cDNA Synthesis and Template Switching				
Ice bucket				
1.5-mL tube magnetic rack				
1.5-mL DNA LoBind® tubes				
Set up:				
Heat block at 95 °C				
Heat block at 80 °C (Optional)				
Thermomixer with self-hybridization program				

Procedure steps:

1. Set a heat block to 95 °C.
2. Program a thermomixer with the self-hybridization program for gradual cooldown from 80 °C to 25 °C.
   a. 1,200 rpm and at 80 °C for 3 minutes.
   b. 1,200 rpm and at 25 °C for 1 minute.
   c. 1,200 rpm and at 25 °C infinite (optional).

   If you performed cDNA synthesis on the same day, this is the same thermomixer from 1.1 cDNA Synthesis and Template Switching (page 20), and the thermomixer is already programmed.

   Confirm "Time Mode" on the thermomixer is set to "Temperature Control" to ensure that the 25 °C temperature is reached before the 1 minute at 25 °C (step 2b) begins. This will ensure gradual cooldown from 80 °C to 25 °C.

3. Prepare hybridization buffer for self-hybridization.
4. Aliquot **1.2 mL** hybridization buffer into a new 1.5-mL LoBind® tube and place the tube in the pre-heated 80 °C thermomixer (from step 2a) without shaking.
5. Keep the tube of hybridization buffer in the 80 °C thermomixer until ready to use, at least **20 minutes** before resuspending beads in step 9 of this section.
6. To denature, incubate the tube in the following order:
   a. Pipet-mix to resuspend the beads.
   b. Incubate the tube at **95 °C** in a heat block for **5 minutes**.
   c. Immediately after the completion of the 95 °C incubation, slightly open the lid of the tube to release air pressure within the tube.
7. Immediately place the tube on the magnet for **≤30 seconds** until clear.
8. Discard the supernatant.
9. Resuspend the beads in **1.0 mL** of pre-heated 80 °C hybridization buffer, and immediately place in the pre-programed thermomixer from step 2a. Start the program.

   Incubation will take approximately 25 minutes.

10. After the hybridization step, place tube on **ice** for at least **1 minute** while TCR/BCR extension mix is being prepared.

1.3 TCR/BCR Extension

Summary:

- Prepare extension enzyme mix
- Extend TSO to copy cell label from bead

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
○	TCR/BCR extension buffer	91-1206	Equilibrate to room temperature 30 minutes before setting up extension. Centrifuge briefly.	–20 °C
●	dNTP	650000077		
○	Nuclease-free water	650000076		
Leave in freezer until ready to use:				
○	TCR/BCR extension enzyme	91-1207	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Enhanced cell capture beads after self-hybridization			Centrifuge briefly and keep on ice until ready.	4 °C
Ice bucket				
1.5-mL tube magnetic rack				
1.5-mL DNA LoBind® tubes				
Set up:				
Thermomixer at 37 °C				

Procedure steps:

1. Set a thermomixer to 37 °C.
2. Ensure all reagents other than the TCR/BCR extension enzyme are at room temperature.
3. In a new 1.5-mL LoBind® tube, pipet the following reagents.

TCR/BCR extension mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
○	TCR/BCR extension buffer	20	24	96	192
●	dNTP	20	24	96	192
○	TCR/BCR extension enzyme	10	12	48	96
○	Nuclease-free water	150	180	720	1440
	Total	**200**	**240**	**960**	**1920**

4. Gently vortex mix, briefly centrifuge, and keep at room temperature.
5. Briefly spin the tube with the bead suspension.
6. Place the tube of BD Rhapsody™ Enhanced Cell Capture Beads on a magnet for **≤2 minutes**. Discard the supernatant.
7. Remove the tubes from the magnet and resuspend using **200 µL** of TCR/BCR extension mix. Pipet-mix.
8. Incubate the bead suspension on a thermomixer at 1,200 rpm and **37 °C** for **30 minutes**.
9. Briefly spin the tube with the beads suspension and place the tube on **ice**.

1.4 Exonuclease I Treatment

Summary:

- Prepare exonuclease I enzyme mix
- Treat beads with exonuclease I
- Heat inactivation

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟡	10X exonuclease I buffer	650000071	Equilibrate to room temperature 30 minutes before setting up Exo-I treatment. Centrifuge briefly.	–20 °C
⚪	Nuclease-free water	650000076		
⚫	Bead resuspension buffer	650000066		
Leave in freezer until ready to use:				
🟡	Exonuclease I	650000072	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Enhanced cell capture beads after TCR/BCR extension			Centrifuge briefly and keep on ice until ready.	4 °C
Ice bucket				
1.5-mL tube magnetic rack				
1.5-mL DNA LoBind® tubes				
Set up:				
Thermomixer at 37 °C				
Heat block at 80 °C				

Procedure steps:

1. Set one thermomixer to 37 °C and a heat block to 80 °C.
2. In a new 1.5-mL LoBind® tube, pipet the following reagents.

Exonuclease I mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
	10X exonuclease I buffer	20	24	96	192
	Exonuclease I	10	12	48	96
	Nuclease-free water	170	204	816	1632
	Total	**200**	**240**	**960**	**1920**

3. Gently vortex-mix, briefly centrifuge, and keep at room temperature.
4. Place the tube of BD Rhapsody™ Enhanced Cell Capture Beads with TCR/BCR extension mix on a 1.5-mL tube magnet for **≤1 minute**.
5. Discard the supernatant.
6. Remove the tube from the magnet and pipet **200 µL** exonuclease I mix into the tube. Pipet-mix.
7. Incubate the bead suspension on thermomixer at 1,200 rpm and **37 °C** for **30 minutes**.
8. Incubate the bead suspension in the heat block at **80 °C** for **20 minutes**.
9. Place the tube on ice for **~1 minute**.
10. Briefly spin the tube with the bead suspension.
11. Place the tube on the magnet for **≤1 minute** until clear. Discard the supernatant.
12. Remove the tube from the magnet and pipet **200 µL** of cold bead resuspension buffer into the tube. Pipet-mix.

STOP Exonuclease I-treated beads can be stored in bead resuspension buffer at 4 °C for up to 1 year.

13. Proceed to library preparation.

2. WTA library amplification

2.1 WTA Random Priming and Extension (RPE)

Summary:

- Prepare random priming mix and extension enzyme mix
- Anneal random primers
- Extend random primers
- Denature RPE products

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🟡	WTA extension buffer	51-9025488	Equilibrate to room temperature 30 minutes before setting up RPE. Centrifuge briefly.	–20 °C
🟡	WTA extension primer	51-9025467		
🟡	dNTP mixture	51-9025491		
⚪	Nuclease-free water	51-9025552		
🟣	Elution buffer	51-9025554		
Place on ice:				
🟡	Bead RT/PCR enhancer	51-9025495	Centrifuge briefly before adding to mix.	–20 °C
Leave in freezer until ready to use:				
🟡	WTA extension enzyme	51-9025499	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Exonuclease I-treated enhanced cell capture beads			Centrifuge briefly and keep on ice until ready.	4 °C
Ice bucket				
1.5-mL tube magnetic rack				
1.5-mL DNA LoBind® tubes				
Set up:				
Heat block at 95 °C				
Thermomixer at 37 °C (Optional)				
Thermomixer at 25 °C				
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 11b.

2. In a new 1.5-mL LoBind® 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**	**1670.4**

3. Pipet-mix the random primer mix and keep at room temperature.
4. 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 the next step.
   - If you are using the entire sample of beads, skip to step 6.
5. (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 LoBind® tube.

   The remaining Exonuclease I-treated beads can be stored in bead resuspension buffer at 4 °C for up to 1 year.

   Subsample is only optional for WTA + TCR/BCR.

6. Place the tube on a magnet until the supernatant is clear (**<2 minutes**).
7. Remove and discard the supernatant.
8. Remove the tube from the magnet.

9. Add **174 µL** of random primer mix into the tube.
10. Pipet-mix 10 times to resuspend the beads.
11. Incubate the tube in the following order:
    a. 95 °C in a heat block (no shaking) for 5 minutes.
    b. Thermomixer at 1,200 rpm and at 37 °C for 5 minutes.

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

    c. Thermomixer at 1,200 rpm and at 25 °C for 5 minutes.
12. Briefly centrifuge the tube.
13. Place at room temperature until ready to use.
14. In a new 1.5-mL LoBind® tube, pipet the following reagents.

Extension enzyme mix

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

15. Pipet **26 µL** of the extension enzyme mix into the sample tube containing the beads (for a total volume of 200 µL) and keep on **ice** until ready.
16. 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.

17. Place the tube of extension enzyme mix with BD Rhapsody™ Enhanced Cell Capture Beads in the programmed thermomixer. The program takes 45 minutes.
18. Remove the tube after the program is complete.
19. Place the tube on a magnet until the supernatant is clear (**<2 minutes**).

20. Remove and discard the supernatant.
21. Remove the tube from the magnet.
22. Pipet **200 µL** of elution buffer into the tube.
23. Pipet-mix 10 times until the beads are fully resuspended.
24. Place the tube on a magnet until the supernatant is clear (**<2 minutes**).
25. Remove and discard the supernatant.
26. Remove the tube from the magnet.
27. Pipet **80 µL** of elution buffer into the tube.
28. To denature the random priming products off the beads.
    a. Pipet-mix 10 times to resuspend the beads.
    b. Incubate the tube 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 LoBind® tube.
29. Place the tube containing the RPE product on ice. The total volume of RPE product will be 80 µL. Proceed to WTA RPE cleanup (page 33).
30. Pipet **200 µL** of cold bead resuspension buffer to the tube with leftover beads. Gently resuspend the beads by pipet-mixing only. Do not vortex.
31. Store the beads on ice or at 4 °C in the pre-amplification workspace until needed.

    These beads will be used for TCR/BCR library amplification (page 47). DO NOT THROW AWAY!

2.2 WTA RPE cleanup

Summary:

- RPE cleanup

Preparation list:

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

Procedure steps:

This section describes how to perform a single-sided AMPure® cleanup, which removes primer dimers and other small molecular weight byproducts. The final product is purified single-stranded DNA.

Perform the RPE purification in the pre-amplification workspace.

1. 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 of 80% ethyl alcohol.

2. Bring AMPure® XP beads to room temperature. Vortex the AMPure® XP beads at high speed for **1 minute** until the beads are fully resuspended.
3. Pipet **128 µL** of AMPure® XP beads into the tube containing the **80 µL** of RPE product supernatant (**1.6x**). Pipet-mix at least 10 times, then briefly centrifuge.
4. Incubate at room temperature for **5 minutes**.
5. Place the tube on the magnet for **3 minutes**. Discard the supernatant.
6. Keeping the tube on the magnet, gently add **250 µL** of fresh 80% ethyl alcohol into the tube and incubate for **30 seconds**. Discard the supernatant.
7. Repeat step 6 for a total of **two ethyl alcohol washes.**
8. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
9. Air-dry the beads at room temperature for **5 minutes** or until the beads no longer look glossy.
10. Remove the tube from the magnet and resuspend the bead pellet in **80 µL** of elution buffer.
11. Incubate the sample at room temperature for **2 minutes**. Briefly centrifuge the tube to collect the contents at the bottom.
12. Place the tube on the magnet until the solution is clear, usually **~30 seconds**.
13. Pipet the eluate (**~80 µL**) to a new PCR tube. This is the purified RPE product.
14. Keep on **ice** until ready to proceed with WTA RPE PCR (page 35).

2.3 WTA RPE PCR

Summary:

- Prepare RPE PCR mix
- Amplify using RPE PCR program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Universal oligo	51-9025553	Equilibrate to room temperature 30 minutes before setting up RPE PCR. Centrifuge briefly.	–20 °C
●	WTA amplification primer	51-9025469		
Leave in freezer until ready to use:				
●	PCR master mix	51-9025466	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
RPE product				
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, so that there are multiple copies of each random-primed molecule.

Perform this section in the pre-amplification workspace.

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

RPE PCR mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
●	PCR master mix	30.0	36.0	144.0	288.0
●	Universal oligo	6.0	7.2	28.8	57.6
●	WTA amplification primer	6.0	7.2	28.8	57.6
	Total	**42.0**	**50.4**	**201.6**	**403.2**

2. Pipet-mix the RPE PCR mix.
3. Place on ice until ready to use.
4. Add **42 µL** of the RPE PCR mix to the tube with the **80 µL** of RPE product.
5. Pipet-mix 10 times to create the RPE PCR reaction mix.
6. Split the RPE PCR reaction mix into two 0.2-mL PCR tubes with **61 µL** mix per tube.
7. Transfer any residual mix to one of the tubes.

Bring the tubes to the post-amplification workspace.

8. Run the following PCR program.

RPE PCR program

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 seconds
Denaturation	Recommended PCR cycles for resting peripheral blood mononuclear cells (PBMCs)* 7,500 – 20,000 cells: 10 cycles	98 °C	15 seconds
Annealing		60 °C	30 seconds
Extension		72 °C	1 minute
Final extension	1	72 °C	2 minutes
Hold	1	4 °C	∞

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

STOP The PCR can run overnight.

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

2.4 WTA RPE PCR cleanup and quantification

Summary:

- RPE PCR cleanup (2 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
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit dsDNA HS Assay Kit				
Obtain:				
RPE PCR product				
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

Perform the purification in the post-amplification workspace.

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

   Adjust the volume of 80% ethyl alcohol depending on the number of samples. One sample requires 1 mL 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 **two** tubes of **61 µL** RPE PCR into a new 1.5-mL LoBind® tube.
6. Pipet-mix 10 times.
7. Transfer exactly **110 µL** RPE PCR product to a new 1.5-mL LoBind® tube.
8. Pipet **88 µL** of AMPure (**0.8x**) 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 AMPure® XP 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 **200 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 0.2-mL PCR strip tube.
27. Add **60 µL** of water to the eluate for a final volume of **100 µL**.

    The volume must be exactly 100 µL. Adjust the water volume if needed.

28. Pipet **80 µL** of AMPure® XP beads (**0.8x**) 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 **200 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 PCR strip tube.

    The purified RPE PCR product is ready for Section 2.5: WTA index PCR (page 41).

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

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

2.5 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 8 samples.

The same indices can be used for all library types for each lane/experiment (WTA, TCR, and BCR, 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.

Note: Though the index sequences can be the same, the index primers for WTA libraries are different than those of the multiomic libraries (SMK, AbSeq, VDJ etc.).

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

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

WTA index PCR mix

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

2. Pipet-mix the WTA index PCR mix.
3. Pipet **35 µL** into separate 0.2-mL PCR tubes for each sample.
4. Add **2.5 µL** of forward primer and **2.5 µL** of reverse primer to each sample.

   If index primers have already been added to the PCR mix, i.e. for 1 library, they do not need to be added again.

5. Place on ice until ready to use.
6. Dilute an aliquot of the purified RPE PCR product from step 46 of WTA RPE PCR cleanup and quantification (page 38) with water 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.

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

9. Run the following PCR program.

WTA index PCR program

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

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

STOP The PCR can run overnight.

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

2.6 WTA index PCR cleanup and quality check

Summary:

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

Preparation list:

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

Procedure steps:

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

Perform the purification in the post-amplification workspace.

1. Bring AMPure® XP beads to room temperature.
2. Make fresh 80% ethyl alcohol and use within 24 hours. Adjust the volume of 80% ethyl alcohol 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. Add **60 µL** of 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 **65 µL** of AMPure® XP beads (**0.65x**) into the 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 steps 12–14 once for a total of **two ethyl alcohol washes**.
16. Keeping the tube on the magnet, use a P20 pipette to remove and discard 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 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 LoBind® tube. The purified eluate is the final sequencing library.

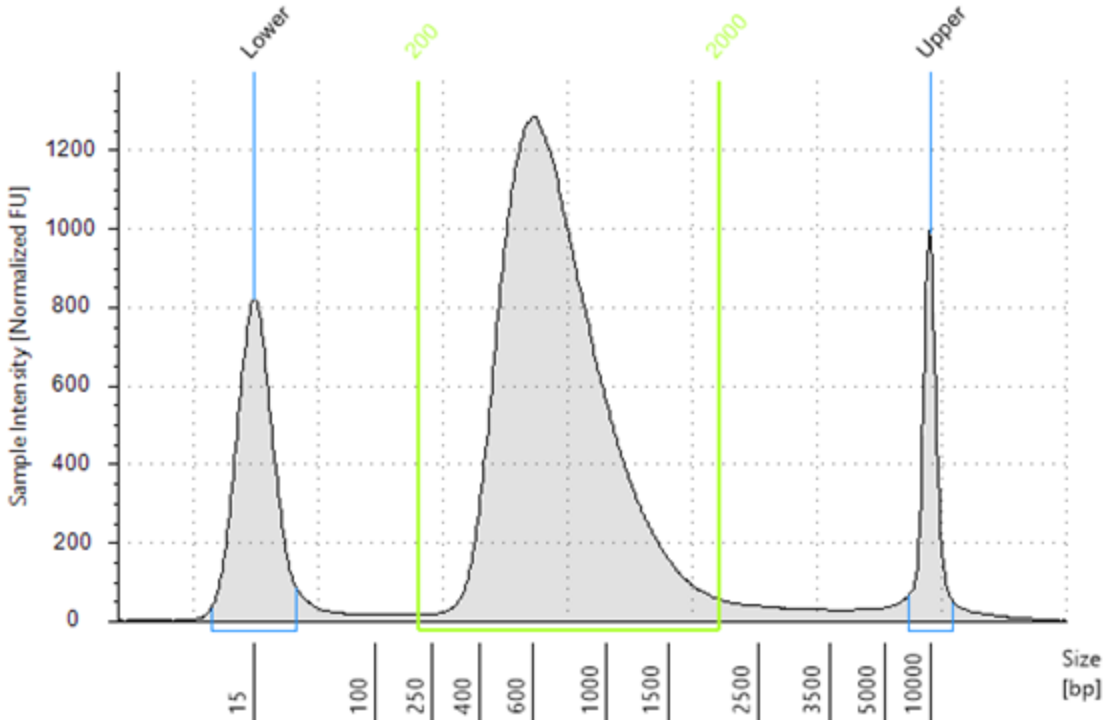
STOP Purified PCR product can be stored at –20 °C for up to 6 months.

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:
    - Agilent 2100 BioAnalyzer using the Agilent High Sensitivity DNA Kit
    - 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



3. TCR/BCR library amplification

3.1 TCR/BCR PCR1

Summary:

- Prepare TCR/BCR PCR1 mix
- Amplify using TCR/BCR PCR1 program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Thaw then place on ice until ready for use:				
○	PCR master mix	91-1083	Thaw then place on ice until ready for use. Centrifuge briefly. Keep on ice until ready.	–20 °C
Equilibrate to room temperature:				
●	TCR/BCR universal oligo N1	91-1204	Equilibrate to room temperature 30 minutes before setting up TCR/BCR PCR1. Centrifuge briefly. Keep on ice until ready.	–20 °C
●	TCR N1 primer - Mouse	91-1212		
●	BCR N1 primer - Mouse	91-1214		
○	Nuclease-free water	650000076		
Place on ice:				
●	Bead RT/PCR enhancer	91-1082	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Enhanced cell capture beads after WTA RPE Denaturation				4 °C
Ice bucket				
0.2-mL PCR tubes				
Set up:				
Thermocycler with TCR/BCR PCR1 program				

Procedure steps:

1. Obtain beads from step 30 of 2.1 WTA Random Priming and Extension (RPE) (page 29).
2. In the pre-amplification workspace, pipet the following reagents into a new 1.5-mL LoBind® tube.

TCR/BCR PCR1 mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
○	PCR master mix	100.0	120.0	480.0	960.0
●	TCR/BCR universal oligo N1	10.0	12.0	48.0	96.0
●	Bead RT/PCR enhancer	12.0	14.4	57.6	115.2
●	Mouse TCR N1 primer[a]	2.4	2.9	11.5	23.0
●	Mouse BCR N1 primer[a]	2.4	2.9	11.5	23.0
○	Nuclease-free water	73.2	87.8	351.4	702.8
	Total	**200.0**	**240.0**	**960.0**	**1920.0**

a. If only doing TCR or BCR amplification, replace N1 primer volume with water. For example, if only doing TCR amplification, replace BCR N1 primer with water.

3. Gently vortex mix, briefly centrifuge, and place back on ice.
4. Briefly spin the tube with the bead suspension.
5. Place the tube of beads on a magnet for **≤1 minute**.
6. Discard the supernatant.
7. Remove the tube from the magnet and resuspend the beads in **200 µL** of TCR/BCR PCR1 mix to create the TCR/BCR PCR1 reaction mix.
8. Do not vortex.
9. Ensuring that the beads are fully resuspended, pipet **50 µL** of TCR/BCR PCR1 reaction mix with beads into each of four 0.2-mL PCR tubes.
10. Transfer any residual mix to one of the tubes.

    Bring the TCR/BCR PCR1 reaction mix to the post-amplification workspace.

11. Run the following PCR program on the thermal cycler.

TCR/BCR PCR1 program

Step	Cycles	Temperature	Time
Hot start	1	95 °C[a]	3 minutes
Denaturation	Recommended PCR cycles[b]	95 °C	30 seconds
Annealing	7,500 – 10,000 cells: 11 cycles	60 °C	1 minute
Extension	20,000 cells: 10 cycles	72 °C	1 minute
Final extension	1	72 °C	5 minutes
Hold	1	4 °C	∞

a. To avoid beads settling due to prolonged incubation time on the thermal cycler before the denaturation step, it is critical to pause the instrument at 95 °C before loading the samples. Different thermal cyclers might have different pause time settings. In certain brands of thermal cyclers, however, we have observed a step-skipping error with the pause/unpause functions. To ensure that the full 3-minute denaturation is not skipped, verify that the pause/unpause functions are working correctly on your thermal cycler. To avoid the step-skipping problem, a 1-minute 95 °C pause step can be added immediately before the 3-minute 95 °C denaturation step.

b. Recommended PCR cycles might need to be optimized for different cell types and cell number.

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

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

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

    STOP The PCR can run overnight, but proceed with purification within 24 hours after PCR.

14. After PCR, briefly centrifuge the tubes.
15. Put the tubes on a magnet for **>30 seconds**.
16. For each sample, collect and combine the supernatant from the four 0.2-mL PCR tubes into one new 1.5-mL LoBind® tube without disturbing the beads.
17. Discard the beads.

3.2 TCR/BCR PCR1 cleanup

Summary:

- TCR/BCR PCR1 cleanup

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	91-1084	Centrifuge briefly.	–20 °C
	Nuclease-free water	650000076		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Obtain:				
TCR/BCR 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® XP beads cleanup to remove primer dimers from the TCR/BCR PCR1 products. The final product is purified double-stranded DNA.

Perform the purification in the post-amplification workspace.

1. 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.

2. Bring the AMPure® XP beads to room temperature. Vortex at a high speed for **1 minute** until the beads are fully resuspended.
3. To **200 µL** of TCR/BCR PCR1 products, pipet **140 µL** AMPure® XP bead (**0.7x**) (from step 17 in TCR/BCR PCR1 (page 47)).
4. Pipet-mix 10 times. Incubate at room temperature for **5 minutes**.
5. Place the 1.5-mL LoBind® tube on the magnet for **5 minutes**.
6. Discard the supernatant.
7. Keeping the tube on the magnet, gently add **500 µL** of fresh 80% ethyl alcohol into the tube and incubate for **30 seconds**.
8. Discard the supernatant.
9. Repeat steps 7–8 once for a total of **two ethyl alcohol washes**.
10. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
11. Air-dry the beads at room temperature for **5 minutes**.
12. Remove the tube from the magnet and resuspend the bead pellet in **50 µL** of elution buffer.
13. Vigorously pipet-mix until the beads are uniformly dispersed. Small clumps do not affect performance.
14. Incubate at room temperature for **2 minutes** and briefly centrifuge.
15. Place the tube on the magnet until the solution is clear, usually **~30 seconds**.
16. Pipet the eluate (**~50 µL**) into a new 1.5-mL LoBind® tube (purified TCR/BCR PCR1 products).

STOP

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

3.3 TCR/BCR PCR2

Summary:

- Prepare TCR/BCR PCR2 mix
- Amplify using TCR/BCR PCR2 program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Thaw then place on ice until ready for use:				
○	PCR master mix	91-1083	Thaw then place on ice until ready for use. Centrifuge briefly. Keep on ice until ready.	–20 °C
Equilibrate to room temperature:				
●	TCR/BCR universal oligo N2	91-1205	Equilibrate to room temperature 30 minutes before setting up PCR2. Centrifuge briefly. Keep on ice until ready.	–20 °C
● ●	TCR N2 primer - Mouse **OR** BCR N2 primer - Mouse	91-1213 **OR** 91-1215		
○	Nuclease-free water	650000076		
Obtain:				
Purified TCR/BCR PCR1 product				
Ice bucket				
0.2-mL PCR tubes				
Set up:				
Thermocycler with TCR/BCR PCR2 program				

Procedure steps:

This section describes how to amplify TCR/BCR products through PCR.

1. In the pre-amplification workspace, pipet reagents into a new 1.5-mL LoBind® tube on ice.

TCR and BCR PCR2 mixes

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
○	PCR master mix	25.0	30.0	120.0	240.0
●	TCR/BCR universal oligo N2	2.0	2.4	9.6	19.2
● ●	Mouse TCR N2 primer **OR** Mouse BCR N2 primer[a]	6.0	7.2	28.8	57.6
○	Nuclease-free water	12.0	14.4	57.6	115.2
	Total	**45.0**	**54.0**	**216.0**	**432.0**

a. PCR2 mixes for TCR and BCR are made separately.

2. Gently vortex mix, briefly centrifuge, and place back on ice.
3. Pipet **45 µL** of PCR2 Mix into one 0.2-mL PCR tube for each library.

Bring the TCR PCR2 mix and the BCR PCR2 mix to the post-amplification workspace.

4. Pipet **5.0 µL** of PCR1 products into **45 µL** of PCR2 mix for each library to create the TCR PCR2 Reaction Mix and BCR PCR2 reaction mix, respectively. Total volume of reaction will be **50 µL** for PCR2.
5. Gently vortex and briefly centrifuge.
6. Run the following PCR program on the thermal cycler.

TCR/BCR PCR2 program

Step	Cycles	Temperature	Time	
Phase I:	1	95 °C	3 minutes	Press **Option** > **Auto Delta Starting cycle** > "2" Delta > "1 degree" > Done The temperature decreases by 1 °C each cycle, from 70 °C to 55 °C.
	15	95 °C	30 seconds	
		70–55 °C	1 minute	
		72 °C	1 minute	
Phase II:	8	95 °C	30 seconds	
		55 °C	1 minute	
		72 °C	1 minute	
	1	72 °C	5 minutes	
	1	4 °C	∞	

STOP The PCR can run overnight.

3.4 TCR/BCR PCR2 cleanup

Summary:

- TCR/BCR PCR2 cleanup
- Quality check using Qubit Fluorometer

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
●	Elution buffer	91-1084	Centrifuge briefly.	–20 °C
○	Nuclease-free water	650000076		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit dsDNA HS Assay Kit				
Obtain:				
TCR/BCR PCR2 product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
0.2-mL PCR tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

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

Perform PCR2 purification in the post-amplification workspace.

1. 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 of 80% ethyl alcohol.

2. Bring AMPure® XP beads to room temperature and vortex at high speed for **1 minute** until beads are fully resuspended.
3. To **50 µL** PCR2 products, pipet **35 µL** of AMPure® XP beads (**0.7x**).
4. Pipet-mix 10 times and incubate at room temperature for **5 minutes**.
5. Place the tube on the magnet for **3 minutes**. Discard the supernatant.
6. Keeping the tube on the magnet, gently add **200 µL** of fresh 80% ethyl alcohol into the tube and incubate for **30 seconds**.
7. Discard the supernatant.
8. Repeat steps 6–7 once for a total of **two ethyl alcohol washes**.
9. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
10. Air-dry the beads at room temperature for **3 minutes**.
11. Remove the tube from the magnet and resuspend the bead pellet in **50 µL** of elution buffer. Pipet-mix until the beads are fully resuspended.
12. Incubate at room temperature for **2 minutes** and briefly centrifuge.
13. Place the tube on the magnet until the solution is clear, usually **~30 seconds**.
14. Pipet the eluate (**~50 µL**) into a new 1.5-mL LoBind® tube.

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

15. Estimate the concentration by quantifying 2 µL of the TCR/BCR PCR2 library with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay Kit. Follow the manufacturer's instructions.

3.5 TCR/BCR RPE

Summary:

- Prepare random priming mix and extension enzyme mix
- Anneal random primers
- Extend random primers

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
○	TCR/BCR extension buffer	91-1206	Equilibrate to room temperature 30 minutes before setting up RPE. Centrifuge briefly.	–20 °C
○	TCR/BCR extension primers	91-1208		
●	dNTP	650000077		
○	Nuclease-free water	650000076		
●	Elution buffer	91-1084		
Leave in freezer until ready to use:				
○	TCR/BCR extension enzyme	91-1207	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Purified TCR/BCR PCR2 product				
Ice bucket				
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
Set up:				
Thermocycler with TCR/BCR Denaturation and random priming program				
Thermocycler with TCR/BCR random primer extension program				

Procedure steps:

1. Dilute an aliquot of the TCR and BCR PCR2 products with water to **1.0 ng/µL**.

   If PCR2 concentration is <1 ng/µL, increase the volume of PCR2 product needed to ensure 5 ng total concentration and decrease the volume of water in the random primer mix accordingly.

2. In pre-amplification workspace, pipet the following reagents into a new 1.5 mL LoBind® tube:

   Random primer mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
◯	TCR/BCR extension buffer	5.0	6.0	24.0	48.0
◯	TCR/BCR extension primers	2.5	3.0	12.0	24.0
◯	Nuclease-free water	Up to 34.0	Up to 40.8	Up to 163.2	Up to 326.4
	Total	**41.5**	**49.8**	**199.2**	**398.4**

3. Pipet-mix the random primer mix and keep at room temperature.
4. Pipet **41.5 µL** of random primer mix into one 0.2-mL PCR tube for each library.

   Bring the TCR RPE mix and the BCR RPE mix to the post-amplification workspace.

5. Add **5 µL** of 1.0 ng/µL purified TCR or BCR PCR2 products into each 0.2-mL PCR tube containing random primer mix.
6. Total volume of reaction will be **46.5 µL** for random priming.
7. Perform denaturation and random priming on thermocycler using the following program:

   Program

Temperature	Time	Cycles
95 °C	5 minutes	1
37 °C	5 minutes	
25 °C	15 minutes	

8. Briefly centrifuge the tube and keep at room temperature.
9. In pre-amplification workspace, pipet the following reagents into a new 1.5 mL LoBind® tube:

Primer extension enzyme mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
●	dNTP	2.0	2.4	9.6	19.2
○	TCR/BCR Extension Enzyme	1.5	1.8	7.2	14.4
	Total	**3.5**	**4.2**	**16.8**	**33.6**

10. Gently vortex mix, centrifuge, and place on ice.
11. Add **3.5 µL** primer extension enzyme mix to the random priming reaction tube to bring total volume up to **50 µL**. Run the following protocol on a thermocycler for extension:

Program

Temperature	Time	Cycles
25 °C	10 minutes	1
37 °C	15 minutes	
45 °C	10 minutes	
55 °C	10 minutes	

12. When the PCR program is complete, briefly centrifuge the tubes.

3.6 TCR/BCR RPE cleanup

Summary:

- TCR/BCR RPE cleanup

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	91-1084	Centrifuge briefly.	–20 °C
	Nuclease-free water	650000076		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Obtain:				
TCR/BCR RPE 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:

Perform purification in the post-amplification workspace.

1. 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 of 80% ethyl alcohol.

2. Bring AMPure® XP beads to room temperature and vortex at high speed for **1 minute** until beads are fully resuspended.
3. To the **50 µL** of TCR and BCR RPE products, add **90 µL** AMPure® XP beads (**1.8x**).
4. Pipet-mix 10 times and incubate at room temperature for **5 minutes**.
5. Place the tube on the magnet for **3 minutes**.
6. Discard the supernatant.
7. Keeping the tube on the magnet, gently add **200 µL** of fresh 80% ethyl alcohol into the tube and incubate for **30 seconds**.
8. Discard the supernatant.
9. Repeat steps 7–8 once for a total of **two ethyl alcohol washes**.
10. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
11. Air-dry the beads at room temperature for **3 minutes**.
12. Remove tubes from the magnet and add **50 µL** of elution buffer.
13. Incubate at room temperature for **2 minutes** and briefly centrifuge.
14. Place the tube on the magnet until the solution is clear, usually **≤30 seconds**.
15. Pipet the entire eluate (**~50 µL**) into a new 1.5-mL LoBind® tube separately (purified TCR and BCR RPE products).

3.7 TCR/BCR index PCR

Summary:

- Prepare TCR/BCR index mix
- Amplify using TCR/BCR index program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Thaw then place on ice until ready for use:				
○	PCR master mix	91-1083	Thaw then place on ice until ready for use. Centrifuge briefly. Keep on ice until ready.	–20 °C
Equilibrate to room temperature:				
●	Forward primer 1–8	Various	Equilibrate to room temperature 30 minutes before setting up TCR/BCR Index PCR. Centrifuge briefly. Keep on ice until ready.	–20 °C
○	Multiomic reverse primer 1–8	Various		
○	Nuclease-free water	650000076		
Obtain:				
Purified TCR/BCR RPE product				
Ice bucket				
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
Set up:				
Thermocycler with TCR/BCR index PCR program				

Procedure steps:

This section describes how to generate TCR/BCR libraries compatible with the Illumina sequencing platform, by adding full-length Illumina sequencing adapters and indices through PCR.

1. In the pre-amplification workspace, pipet reagents into a new 1.5-mL LoBind® tube on ice.

TCR/BCR index PCR mix

Cap	Component	For 1 library (µL)	For 1 library with 20% overage (µL)	For 4 libraries with 20% overage (µL)	For 8 libraries with 20% overage (µL)
○	PCR master mix	25.0	30.0	120.0	240.0
●	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
	Total	**29.0**	**34.8**	**120.0**	**240.0**

2. Gently vortex mix, briefly centrifuge, and place back on ice.

Bring the TCR/BCR index PCR mix to post-amplification workspace.

3. For multiple samples, pipet **25 µL** of PCR master mix into separate 0.2-mL PCR tubes to each sample.
4. Add **2 µL** of forward primer and **2 µL** of multiomic reverse primer to each sample.

If index primers have already been added to the PCR mix, i.e. for 1 library, they do not need to be added again.

5. Add **21 µL** of TCR/BCR RPE purified products into **29 µL** of TCR/BCR index PCR mix. Total volume of reaction will be **50 µL** for index PCR.
6. Gently vortex, and briefly centrifuge.
7. Run the following PCR program on the thermal cycler.

TCR/BCR index PCR program

Step	Cycles	Temperature	Time
Hot start	1	95 °C	3 minutes
Denaturation	10	95 °C	30 seconds
Annealing		60 °C	30 seconds
Extension		72 °C	30 seconds
Final extension	1	72 °C	1 minute
Hold	1	4 °C	∞

STOP The PCR can run overnight.

3.8 TCR/BCR index PCR cleanup and quality check

Summary:

- TCR/BCR 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	91-1084	Centrifuge briefly.	–20 °C
○	Nuclease-free water	650000076		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit dsDNA HS Assay Kit				
Agilent BioAnalyzer High Sensitivity Kit **OR** Agilent TapeStation ScreenTape and Reagents				
Obtain:				
TCR/BCR index PCR product				4 °C
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 to remove primer dimers from the TCR/BCR index PCR products. The final product is purified double-stranded DNA with full-length Illumina adapter sequences.

Perform index PCR purification in the post-amplification workspace.

1. 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 of 80% ethyl alcohol.

2. Bring AMPure® XP beads to room temperature and vortex at high speed for **1 minute** until the beads are fully resuspended.
3. Briefly centrifuge all the index PCR products.
4. Transfer **40 µL** of the TCR and/or BCR index PCR products to a new 0.2-mL PCR tubes.
5. Pipet **26 µL** of AMPure® XP beads (**0.65x**).
6. Pipet-mix 10 times and incubate at room temperature for **5 minutes**.
7. Place the tube on the strip tube magnet for **3 minutes**.
8. Discard the supernatant.
9. Keeping the tube on the magnet, gently add **200 µL** of fresh 80% ethyl alcohol into the tube and incubate for **30 seconds**.
10. Discard the supernatant.
11. Repeat steps 9–10 for a total of **two ethyl alcohol washes**.
12. Keeping the tube on the magnet, use a P20 pipette to remove and discard the residual supernatant from the tube.
13. Air-dry the beads at room temperature for **1 minute.**
14. Remove the tube from the magnet and resuspend the bead pellet in **50 µL** of elution buffer. Pipet-mix until the beads are fully resuspended.
15. Incubate at room temperature for **2 minutes**, and briefly centrifuge.
16. Place the tube on the magnet until the solution is clear, usually **~30 seconds**.
17. Pipet the entire eluate (**~50 µL**) into a new 1.5-mL LoBind® tube (final sequencing libraries).
18. Perform quality control before freezing samples.
    a. Estimate the concentration by quantifying 2 µL of the final sequencing library with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Kit to obtain an approximate concentration of PCR products to dilute for quantification on an Agilent 2100 Bioanalyzer. Follow the manufacturer's instructions. The expected concentration of the libraries is >1.5 ng/µL.

b. Measure the average fragment size of the TCR/BCR library within the size range of 200–1,000 bp by using the Agilent Bioanalyzer with the High Sensitivity Kit for 50–7,000 bp, 5–1,000 pg/µL. Follow the manufacturer's instructions.

Figure 2 Sample TapeStation high-sensitivity D5000 trace - Mouse TCR index PCR product

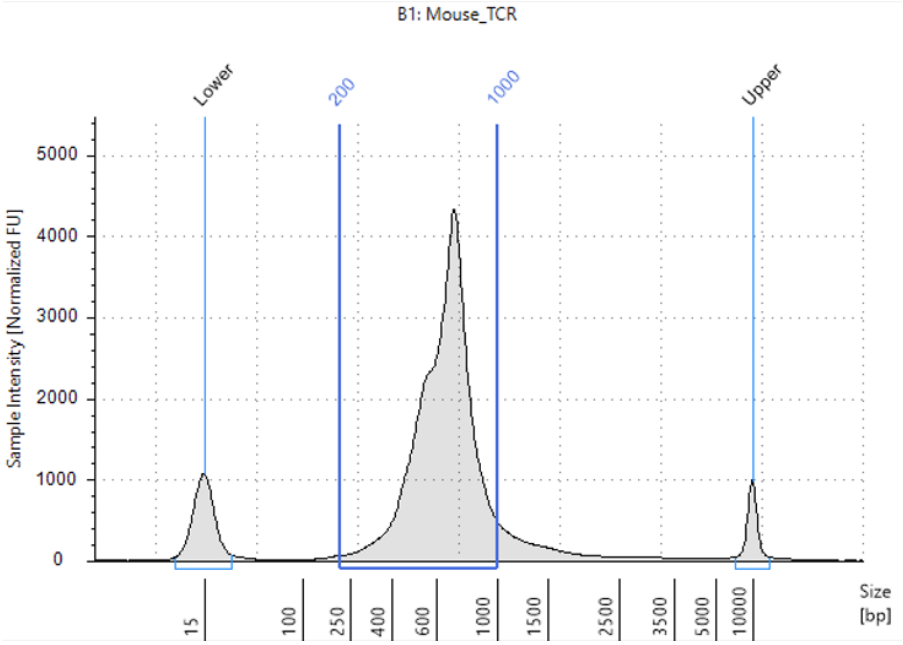
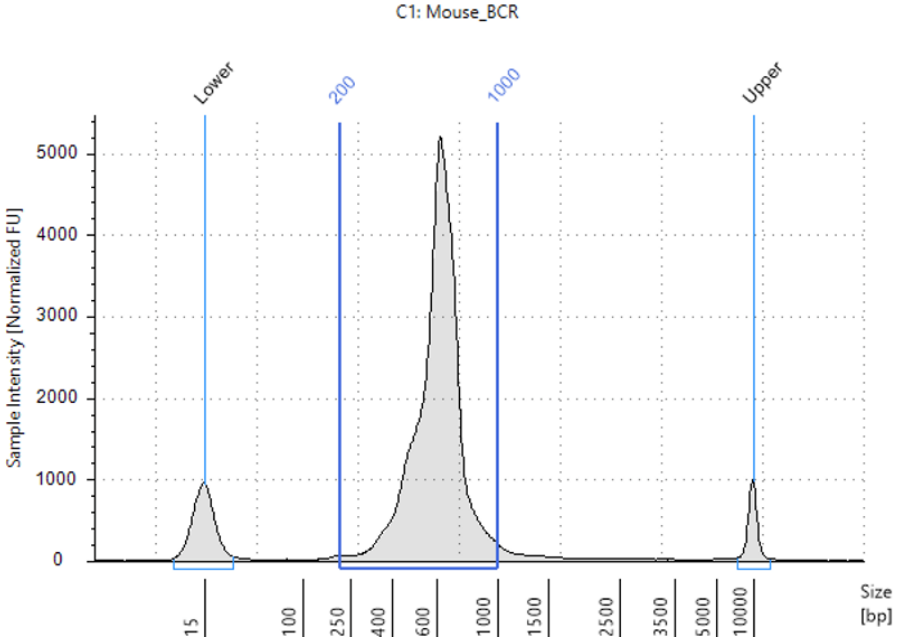


Figure 3 Sample TapeStation high-sensitivity D5000 trace - Mouse BCR index PCR product



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

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 mRNA, TCR, and BCR libraries.

Read requirements for libraries

Library	Read requirement for data analysis
WTA mRNA	10,000–100,000 reads/cell
TCR	~5,000 reads/T cell
BCR	~5,000 reads/B cell

Required parameters

Parameter	Requirement
Platform	Illumina and Element*
Paired-end reads	Minimum: Read 1: 65 cycles; Read 2: 150 cycles Recommended: Read 1: 150 cycles; Read 2: 150 cycles Index 1 (i7): 8 cycles; Index 2 (i5): 8 cycles
PhiX	Required (3%)
Analysis	Refer to the *BD® Single-Cell Multiomics Bioinformatics Handbook*

* To review Index sequences, see the Appendix (page 74).

Pooling libraries for sequencing

The efficiency of sequencing on Illumina instruments is influenced by many conditions, library size being one of them. The TCR and BCR libraries are ~200–300 bp larger than the WTA mRNA library which will cause them to produce less sequencing data if pooled in a 1:1 ratio with the other libraries. To overcome the difference in sequencing efficiency, more DNA of the TCR and BCR libraries needs to be included in the pool than would be expected when calculating ratios based on read depth. The following tables show examples of different pooling strategies and the expected sequencing outcome, with correction for the size of the TCR and BCR libraries. Validation data indicates a 5X volume correction factor is needed for sequencing TCR and BCR libraries with WTA mRNA libraries.

Example of pooling with correction

In this example, a total of 5,000 enriched T cells were processed. These calculations are using a correction factor of 5 for the TCR library to overcome the differences in sequencing efficiency. The amount of data

needing to be generated (Column D) is based on the cell number (Column B) and expected number of reads per cell (Column C). Based on this example, 150 million reads are needed to achieve the appropriate read depths. Changing the pooling ratios by correcting for the lower TCR sequencing efficiency will help ensure the correct amount of data is generated for each library. This modified pooling scheme, however, does not change the total amount of data needing to be generated, which is 150 million reads.

Pooling for WTA mRNA and TCR libraries

A	B	C	D	E	F	G	H	I	J
Library type	**Number of cells**	**Expected reads/cell**	**Reads needed**	**Pooling ratio before correction**	**Correction**	**Reads needed for pooling**	**Pooling ratio with correction**	**Sequencing results**	**Sequencing results (reads/cell)**
WTA mRNA	5,000	25,000	125,000,000	83%	N/A	125,000,000	50%	125,000,000	25,000
TCR	5,000	5,000	25,000,000	17%	5[a]	125,000,000	50%	25,000,000	5,000
Total			**150,000,000[b]**	**100%**	–	**250,000,000[c]**	**100%**	**150,000,000**	–

a. The 5X correction factor is a recommended starting point and some fine tuning might be required to achieve the optimal library balance.

b. Total amount of data to be requested from the sequencing facility plus 3% PhiX.

c. Read total only for pooling purposes.

After sequencing, the total amount of data generated (Column I) as well as the reads/cell for each library (Column J) are as expected (Columns D and C, respectively). The correction for library pooling did not change the amount of data generated (150 million reads) but helped ensure the data was spread out appropriately to each library.

Example of pooling with a mixed population

The following table shows the pooling logic for a mixed population of cells.

Pooling for WTA mRNA, TCR, and BCR libraries

A	B	C	D	E	F	G	H	I	J
Library type	**Number of cells**	**Expected reads/cell**	**Reads needed**	**Pooling ratio before correction**	**Correction**	**Reads needed for pooling**	**Pooling ratio with correction**	**Sequencing results**	**Sequencing results (reads/cell)**
WTA mRNA	10,000	25,000	250,000,000	88%	N/A	250,000,000	59%	250,000,000	25,000
TCR	4,000	5,000	20,000,000	7%	5[a]	100,000,000	23%	20,000,000	5,000
BCR	3,000	5,000	15,000,000	5%	5[a]	75,000,000	18%	15,000,000	5,000
Total			**285,000,000[b]**	**100%**	–	**425,000,000[c]**	**100%**	**285,000,000**	–

a. The 5X correction factor is a recommended starting point and some fine tuning might be required to achieve the optimal library balance.

b. Total amount of data to be requested from the sequencing facility plus 3% PhiX.

c. Read total only for pooling purposes.

Additional considerations

- The 5X volume correction factor for TCR/BCR libraries is a recommended starting place for pooling these libraries. This might need to be adjusted to accommodate different types of flow cells (for example patterned vs non-patterned) or different sequencing platforms.
- It can be easier to achieve the desired sequencing depth when sequencing multiple TCR or BCR libraries alone since all the libraries are the same size. Pooling will not require a correction and will only be dependent on the number of cells and the reads/cell. This scheme, however, would require 10–15% PhiX, rather than the 3% PhiX when sequencing with the WTA mRNA library.
- Sequencing runs with only WTA libraries require Read 1 of 51 cycles and Read 2 of 71 cycles .
- The same indices can be used for all library types for each lane/experiment (WTA, TCR, and BCR, 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.

  Note: Though the index sequences can be the same, the index primers for WTA libraries are different than those of the multiomic libraries (SMK, AbSeq, VDJ etc.).

For additional support with pooling and sequencing, reach out to your local Field Application Specialist (FAS) or scomix@bd.com.

Sequencing recommendations

WTA and TCR/BCR libraries (with or without BD® AbSeq/Sample Tag).

For a NextSeq High or Mid Output run and MiniSeq High or Mid Output run, load the flow cell at a concentration between 1.4–1.8 pM with 3% PhiX for a sequencing run.

If using less than 10,000 reads/cell for the WTA library, increase PhiX percentage to 5–10% to account for lower library diversity.

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 may need to be titrated to optimize yield.

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 indexing PCR.	Input DNA not high enough or cycle number too low.	Repeat indexing PCR with higher cycle number. Alternatively, if RPE-PCR product was diluted before adding to indexing PCR, repeat indexing PCR with less or no dilution.
Lower number of reads/cell than expected from mRNA.	264 bp or ~160 bp products taking reads from mRNA library.	If noise peak is seen in the 264 bp or ~160 bp range, perform a second round of AMPure purification according to WTA index PCR cleanup and quality check (page 44).

TCR/BCR metrics

Observation	Possible causes	Recommended solutions
Low yield of TCR/BCR PCR2, unexpected profile of TCR/BCR Index products, or low TCR/BCR pairing efficiency.	Incorrect components were used.	Ensure BD Rhapsody™ Enhanced Cartridge Reagent Kit V3 (PN 667052) and TSO Next (PN 91-1295) are used for all TCR/BCR Next assays.
	Incorrect handling during earlier protocol steps (cDNA synthesis, template switching, denaturation, self-hybridization, and TCR/BCR extension).	Carefully follow all protocol steps in Section 1, especially noting warnings for thermomixer settings, reagent storage temperatures, and incubation timing.
	Low viability cells or other challenging samples.	Optimization might be required. 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.

Observation	Possible causes	Recommended solutions
Failed cluster generation or other sequencing challenges with low-plex pooling.	Sequencing instrument-specific color balance guidelines were not met. For example, the NovaSeq X platform requires signal in the green channel for every cycle. Low % reads in the green channel in a given cycle might not be sufficient.	Consult indexing and pooling guidelines for your sequencing platform. Be sure to consider the final pooling ratio—some libraries might make up a small fraction of the final pool.

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 TCR/BCR, 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 TCR/BCR, Sample Tag and BD® AbSeq Index PCR	51-9025489	CAA GCA GAA GAC GGC ATA CGA GAT TACTACGC GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 2		51-9025490	CAA GCA GAA GAC GGC ATA CGA GAT AGGCTCCG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 3		51-9025492	CAA GCA GAA GAC GGC ATA CGA GAT GCAGCGTA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 4		51-9025493	CAA GCA GAA GAC GGC ATA CGA GAT CTGCGCAT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 5		51-9025494	CAA GCA GAA GAC GGC ATA CGA GAT GAGCGCTA GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 6		51-9025496	CAA GCA GAA GAC GGC ATA CGA GAT CGCTCAGT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 7		51-9025497	CAA GCA GAA GAC GGC ATA CGA GAT GTCTTAGG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD OMICS-One™ Multiomic Library Reverse Primer 8		51-9025498	CAA GCA GAA GAC GGC ATA CGA GAT ACTGATCG GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T

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

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

Mouse T cell PCR1 primers

Primer name	Primer sequence (5'–3')
Ms_TRAC_N1	TTTTCGGCACATTGATTTGGGAG
Ms_TRBC_N1	CTCAGGCAGTAGCTATAATTGCT
Ms_TRDC_N1	CAATCTTCTTGGATGATCTGAGACT
Ms_TRGC1-TRGC2_N1	GGAAAGAACTTTTCAAGGAGACAAAGG

Mouse T cell PCR2 primers

Primer name	Primer sequence (5'–3')
Ms_TRAC_N2	AGGTTCTGGGTTCTGGATGT
Ms_TRBC_N2	CAATCTCTGCTTTTGATGGCTC
Ms_TRDC_N2	GTAGAAATCTTTCACCAGACAAGC
Ms_TRGC1-TRGC2_N2	TTGGGGGAAATGTCTGCA
Ms_TRGC4_N2	ATAGTAGGCTTGGGAGAAAAGTCTGA

Mouse B cell PCR1 primers

Primer name	Primer sequence (5'–3')
Ms_IGHA_N1	AACTGGCTGCTCATGGTGTA
Ms_IGHD_N1	AAGTGTGGTTGAGGTTCAGTTCTG
Ms_IGHE_N1	GAAGTTCACAGTGCTCATGTTC
Ms_IGHG1_N1	CAGAGTGTAGAGGTCAGACT
Ms_IGHG2A-IGHG2C_N1	TCGAGGTTACAGTCACTGAG
Ms_IGHG2B_N1	GATCCAGAGTTCCAAGTCACAG
Ms_IGHG3_N1	TACGTTGCAGATGACAGTCT
Ms_IGHM_N1	TGGATGACTTCAGTGTTGTTCTG
Ms_IGKC_N1	TGTAGGTGCTGTCTTTGCTG
Ms_IGLC1_N1	CTGTAACTGCTATGCCTTTCCC
Ms_IGLC2-IGLC3_N1	TTGGTGGGATTTGAAGTGTCC

Mouse B cell PCR2 primers

Primer name	Primer sequence (5'–3')
Ms_IGHA_N2	TGTCAGTGGGTAGATGGTGG
Ms_IGHD_N2	CTGACTTCCAATTACTAAACAGCC
Ms_IGHE_N2	TAGAGCTGAGGGTTCCTGATAG
Ms_IGHG1_N2	CAGTGGATAGACAGATGGGGGT
Ms_IGHG2A-IGHG2C_N2	ATGGGGCTGTTGTTTTGG
Ms_IGHG2B_N2	GTGGATAGACTGATGGGGGTGTT
Ms_IGHG3_N2	AGGGAAGTAGCCTTTGACAAG
Ms_IGHM_N2	GACATTTGGGAAGGACTGACTC
Ms_IGKC_N2	AGATGTTAACTGCTCACTGGATG
Ms_IGLC1_N2	GTTAGTCTCGAGCTCTTCAGA
Ms_IGLC2-IGLC3_N2	CAGTGTGGCTTTGTTTTCCT

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