BD Rhapsody™ System

OMICS-One™ CRISPR and Targeted mRNA for poly-A sgRNA Capture

Library Preparation Protocol

23-25060(01)
2026-01

Regulatory information

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

History

Revision	Date	Change made
23-25060(01)	2026-01	Initial release.

Contents

Introduction

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

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

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

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

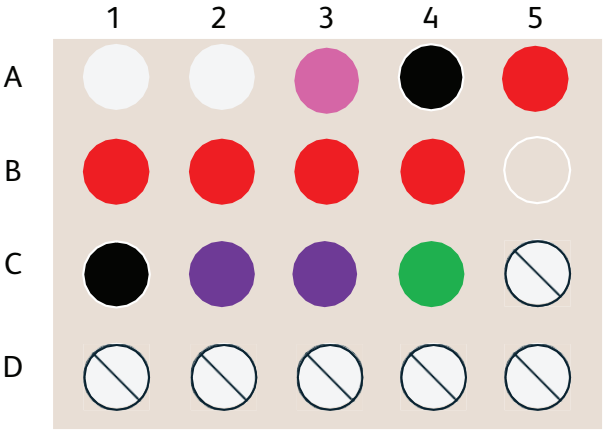
Symbols

The following symbols are used in this guide:

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

Protocol kits

Before you begin, ensure that you have the correct kits for this protocol. Matching cap colors indicate you have the correct kit, along with the catalog numbers found in the Required and recommended materials (page 7) section.



BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit

Cap color	Name	Part Number	Vial
	PCR Master Mix	91-1083	A1
	Universal Oligo	650000074	A2
	Elution Buffer	91-1084	A3
	Bead RT/PCR Enhancer	91-1082	A4
	Library Forward Primer	91-1085	A5
	Library Reverse Primer 1	650000080	B1
	Library Reverse Primer 2	650000091	B2
	Library Reverse Primer 3	650000092	B3
	Library Reverse Primer 4	650000093	B4
	Nuclease-Free Water	650000076	B5
	Bead Resuspension Buffer	650000066	C1
	Sample Tag PCR1 Primer Rhapsody	91-1088	C2
	Sample Tag PCR2 Primer Rhapsody	91-1089	C3
	AbSeq Primer	91-1086	C4

Workflows

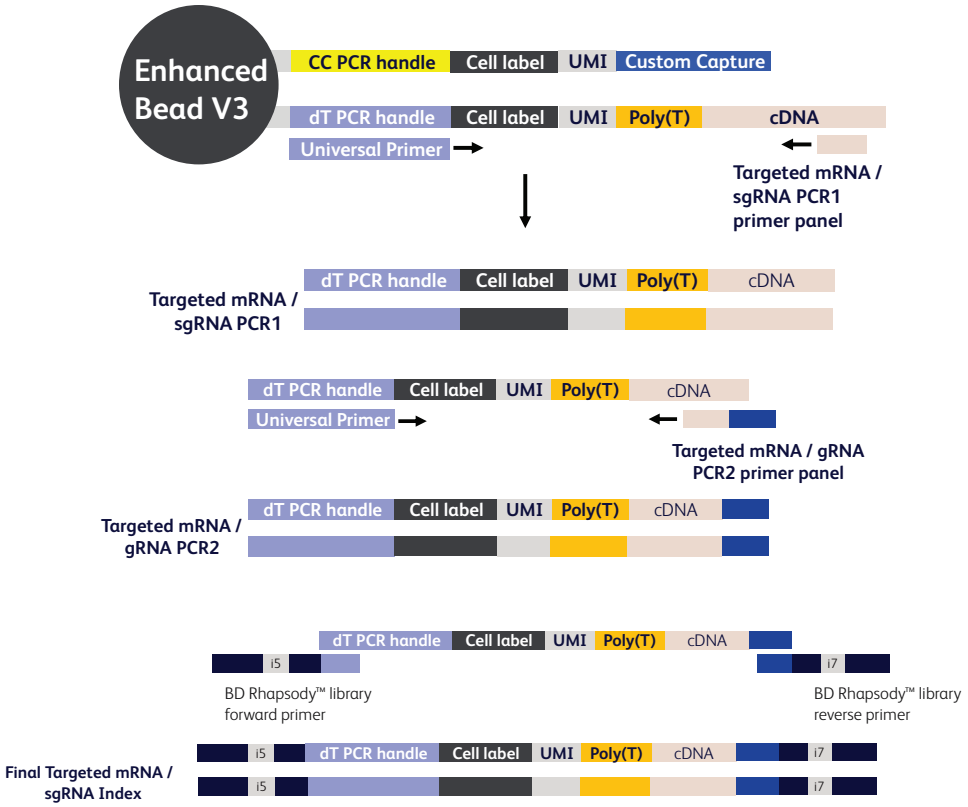
sgRNA and Targeted mRNA library amplification workflow

1. sgRNA and Targeted mRNA PCR1 (page 13): Universal primer and Targeted mRNA/sgRNA PCR1 primer panel copy target region from bead.

Amplify in solution. Collect supernatant as Targeted mRNA/sgRNA PCR1 product.

3. sgRNA and Targeted mRNA PCR2 (page 19): Amplify using sgRNA PCR2 primer panel for nested PCR enrichment.

5. sgRNA and Targeted mRNA Index PCR (page 25): Add 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 Rhapsody™ Targeted mRNA and AbSeq Amplification Kit*	BD Biosciences	633774
AMPure® XP beads for DNA cleanup	Beckman Coulter	A63880
sgRNA PCR1 primer†	Major supplier	–
sgRNA PCR2 primer†	Major supplier	–
BD Rhapsody™ custom panel	BD Biosciences	Various
OMICS-One™ Dual Index Kit‡	BD Biosciences	571899
100% ethyl alcohol, molecular biology grade	Major supplier	–
TE Buffer [10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA]	Major supplier	–
Nuclease-free water	Major supplier	–

* If processing more than four libraries, two orders of this catalog number are required. This kit contains sufficient reagent volume to amplify both the Targeted and sgRNA libraries.

† Refer to the Appendix for recommended primer sequences and references.
Primer sequences can be adapted to your workflow. Customize based on target region, platform requirements, and PCR performance (e.g., Tm, GC content, avoiding dimers). Validate primers before large-scale use.
For designing and ordering primers for gene-specific and sgRNA amplification, contact your local BD single-cell technical support

‡ Recommended for unique dual indexing with high-throughput (more than eight) library preparation workflows.

Recommended consumables

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

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

Equipment

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

† Recommended for processing greater than six samples.

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

Best practices

Cell capture

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

Bead handling

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

  Never vortex the beads. Pipet-mix only.

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

  Do not freeze.

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

Libraries

- sgRNA libraries are sequenced together with Targeted mRNA libraries.

Master mix preparation

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

Supernatant handling

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

Bead amplification

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

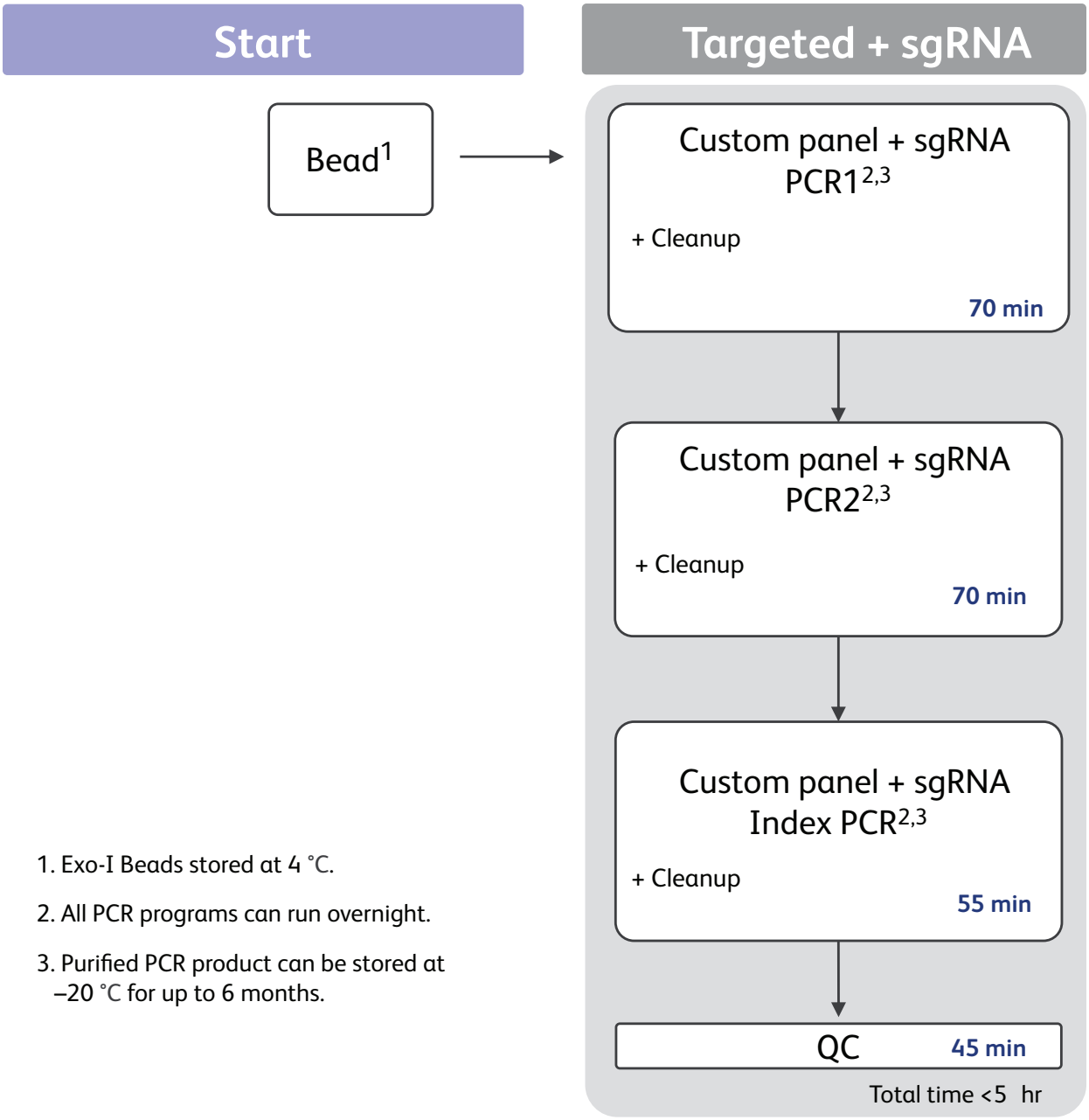
Additional documentation

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

Safety information

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

Time considerations



1. Exo-I Beads stored at 4 °C.
2. All PCR programs can run overnight.
3. Purified PCR product can be stored at –20 °C for up to 6 months.

Procedure

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

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

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

sgRNA and Targeted mRNA library amplification

This section comprises the following tasks:

1. sgRNA and Targeted mRNA PCR1

Before using BD Rhapsody™ 10X PCR1 custom primers or BD Rhapsody™ 10X PCR1 supplement primers, dilute 1 part of the 10X PCR primer stock to 9 parts of TE buffer to prepare a 1X primer solution.

Summary:

- Prepare sgRNA and Targeted mRNA PCR1 mix
- Amplify using sgRNA and Targeted mRNA PCR1 program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
◯	Universal oligo	650000074	Fully thaw and mix reagents prior to use. Centrifuge briefly. Keep on ice until ready.	–20 °C
	PCR1 Targeted mRNA primer panel			
	sgRNA PCR1 primer			
◯	Nuclease-free water	650000076		
Leave in freezer until ready to use:				
◯	PCR master mix	91-1083	Centrifuge briefly before adding to mix.	–20 °C
●	Bead RT/PCR enhancer	91-1082		
Obtain:				
Exonuclease I-treated cell capture beads			Centrifuge briefly and keep on ice until ready.	4 °C
Ice bucket				
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Thermocycler sgRNA and Targeted mRNA PCR1 program				

Procedure steps:

This section describes how to amplify sgRNA and Targeted mRNA products through PCR.

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

Gene-specific custom panels can be ordered from BD Biosciences, and sgRNA primers may be incorporated as a supplemental panel for targeted amplification. For assistance with designing primers for sgRNA amplification, contact your local BD single cell representative.

sgRNA and Targeted mRNA 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	100.0	120.0	480.0	960.0
○	Universal oligo	20.0	24.0	96.0	192.0
●	Bead RT/PCR enhancer	12.0	14.4	57.6	115.2
	PCR1 Targeted mRNA primer panel* †	40.0	48.0	192.0	384.0
	sgRNA PCR1 primer* †‡	10.0	12.0	48.0	96.0
○	Nuclease-free water	18.0	21.6	86.4	172.8
	Total	**200.0**	**240.0**	**960.0**	**1920.0**

* Order from BD Biosciences or oligo vendor.

† Ensure custom and supplement panels are diluted to 1X before use if ordered from BD Biosciences.

‡ If necessary, add the sgRNA primer at 8× excess relative to each single primer in the Targeted mRNA primer panel. This ratio ensures strong amplification of gRNA sequences alongside targeted gene transcripts. Adjust the volumes of other components as needed but keep the total reaction volume at 200 µL.

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

Bring the tubes to the post-amplification workspace.

6. Run the following PCR program:

sgRNA and Targeted mRNA PCR1 program

Step	Cycles	Temperature	Time
Hot start	1	95 °C*	3 minutes
Denaturation	12†	95 °C	30 seconds
Annealing		60 °C‡	3 minutes
Extension		72 °C	1 minute
Final extension	1	72 °C	5 minutes
Hold	1	4 °C	∞

* To avoid beads settling due to prolonged incubation time on the thermal cycler before the denaturation step, it is critical to pause the instrument at 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.

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

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

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

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

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

The PCR can be run overnight.

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

2. sgRNA and Targeted mRNA PCR1 cleanup and quantification

Summary:

- sgRNA and Targeted mRNA PCR1 cleanup

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
	Elution buffer	91-1084	Centrifuge briefly.	–20 °C
	Bead Resuspension Buffer	650000066		
AMPure® XP magnetic beads			Manufacturer's recommendations	
Qubit dsDNA HS Assay Kit				
Obtain:				
sgRNA and Targeted mRNA PCR1 product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
1.5-mL tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

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

Perform the purification in the post-amplification workspace.

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

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

   Save supernatant at this step. Do not discard!

   To maintain data integrity, remove tube with the cell capture beads from the magnet, and pipet 200 µL cold Bead Resuspension Buffer into tube. Pipet-mix. Do not vortex. Store beads at 2–8 °C in post-amplification workspace.
7. Pipet-mix the supernatant 10 times.

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

    Avoid getting AMPure® beads on the lid of the tube. Residual beads and PCR mix buffer can negatively impact downstream results.
11. Incubate at room temperature for **5 minutes**.
12. Place the tube on a magnet until the supernatant is clear **(<5 minutes)**.
13. Remove and discard the supernatant.
14. Keeping the tube on the magnet, gently pipet **500 µL** of fresh 80% ethyl alcohol into the tube.
15. Incubate for **30 seconds**.
16. Remove and discard the supernatant without disturbing the beads.
17. Repeat steps 14–16 once for a total of **two ethyl alcohol washes**.

18. Keeping the tube on the magnet, use a P20 pipette to remove and discard any residual supernatant from the tube.
19. Air-dry the beads at room temperature until the beads no longer look glossy **(~3 minutes)**.

    Do not overdry the AMPure® beads after the ethanol washes. Overdried beads appear cracked.
20. Remove the tube from the magnet.
21. Pipet **50 µL** of elution buffer into the tube.
22. Pipet-mix 10 times until the beads are fully resuspended.
23. Incubate at room temperature for **2 minutes**.
24. Briefly centrifuge the tube.
25. Place the tube on a magnet until the supernatant is clear (**~30 seconds**).
26. Pipet the eluate **(~50 µL)** into a new 1.5-mL tube.
27. Estimate the concentration by quantifying 2 µL of the sgRNA PCR1 product with a Qubit™ Fluorometer using the Qubit™ dsDNA HS Assay Kit. Follow the manufacturer's instructions.

    If the yield is too low to measure using the Qubit™ Fluorometer, use the maximum volume for PCR2 and adjust the volume of nuclease-free water accordingly.
28. Dilute an aliquot of the PCR2 products with nuclease-free water to **1.0 ng/µL**.

    The purified sgRNA and Targeted mRNA PCR1 product is ready for 3. sgRNA and Targeted mRNA PCR2 (page 19).

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

3. sgRNA and Targeted mRNA PCR2

Before using BD Rhapsody™ 10X PCR2 custom primers or BD Rhapsody™ 10X PCR2 supplement primers, dilute 1 part of the 10X PCR primer stock to 9 parts of TE buffer to prepare a 1X primer solution.

Summary:

- Prepare sgRNA and Targeted mRNA PCR2 mix
- Amplify using sgRNA and Targeted mRNA PCR2 program

Preparation list

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
◯	Universal oligo	650000074	Fully thaw and mix reagents prior to use. Centrifuge briefly. Keep on ice until ready.	–20 °C
	PCR2 Targeted mRNA primer panel			
	sgRNA PCR2 primer			
◯	Nuclease-free water	650000076		
Leave in freezer until ready to use:				
◯	PCR master mix	91-1083	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Purified sgRNA and Targeted mRNA PCR1 product (diluted with nuclease-free water to **1.0 ng/µL**)				4 °C
Ice bucket				
1.5-mL LoBind® tube				
0.2-mL PCR tubes				
Set up:				
Thermocycler with with sgRNA and Targeted mRNA PCR2 program				

Procedure steps

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

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

sgRNA and Targeted mRNA PCR2 mix

Cap	Component	1 library (µL)	1 library with 20% overage (µL)	4 libraries with 20% overage (µL)	8 libraries with 20% overage (µL)
◯	PCR master mix	25.0	30.0	120.0	240.0
◯	Universal oligo	2.0	2.4	9.6	19.2
	PCR2 Targeted mRNA primer panel* †	10.0	12.0	48.0	96.0
	sgRNA PCR2 primer * † ‡	2.5	3.0	12.0	24.0
◯	Nuclease-free water	0.5	0.6	2.4	4.8
	Total	**40.0**	**48.0**	**192.0**	**384.0**

* Order from BD Biosciences or oligo vendor.

† Ensure custom and supplement panels are diluted to 1X before use if ordered from BD Biosciences.

‡ If necessary, add the sgRNA primer at 8× excess relative to each single primer in the targeted mRNA primer panel. This ratio ensures strong amplification of gRNA sequences alongside targeted gene transcripts. Adjust the volumes of other components as needed but keep the total reaction volume at 50 µL.

If the PCR1 reaction produces low DNA concentration, increase the template input to achieve a total input amount of approximately 10 ng while maintaining the final reaction volume at 50 µL. Adjust the water volume accordingly to keep the total reaction volume constant. If you need to increase the reaction volume to 100 µL, make sure to adjust all other components proportionally to maintain the correct concentrations.

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

   Bring the tubes to the post-amplification workspace.

4. Add **10 µL** of **diluted** sgRNA and Targeted mRNA PCR1 product.
5. Pipet-mix 10 times.

6. Run the following PCR program:

sgRNA and Targeted mRNA PCR2 program

Step	Cycles	Temperature	Time
Hot start	1	95 °C	3 minutes
Denaturation	10*	95 °C	30 seconds
Annealing		60 °C†	3 minutes
Extension		72 °C	1 minute
Final extension	1	72 °C	5 minutes
Hold	1	4 °C	∞

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

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

The PCR can run overnight.

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

4. sgRNA and Targeted mRNA PCR2 cleanup and quantification

Summary:

- sgRNA and Targeted mRNA PCR2 cleanup
- Quantify using Qubit Flurometer

Preparation List:

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

Procedure steps:

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

Perform the purification in the post-amplification workspace.

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

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

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

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

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

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

    The sgRNA PCR2 product is ready for 5. sgRNA and Targeted mRNA Index PCR (page 25).

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

5. sgRNA and Targeted mRNA Index PCR

Summary:

- Prepare sgRNA and Targeted mRNA Index PCR mix
- Amplify using sgRNA Index PCR program

Preparation list:

Item		BD Part Number	Preparation and Handling	Storage
Equilibrate to room temperature:				
🔴	Forward primer 1	91-1085	Fully thaw and mix reagents prior to use. Centrifuge briefly. Keep on ice until ready.	–20 °C
🔴	Reverse primer 1–4	Various		
⚪	Nuclease-free water	650000076		
Leave in freezer until ready to use:				
⚪	PCR master mix	91-1083	Centrifuge briefly before adding to mix.	–20 °C
Obtain:				
Purified sgRNA and targeted mRNA PCR2 product (diluted with nuclease-free water to **1.0 ng/µL**)				4 °C
Ice bucket				
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
Set up:				
Thermocycler with sgRNA and Targeted mRNA Index PCR program				

Procedure steps:

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

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

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

sgRNA and Targeted mRNA 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	25.0	30.0	120.0	240.0
●	Library forward primer	2.0	2.4	9.6	19.2
●	Library reverse primer 1–4[†]	2.0	2.4	N/A	N/A
○	Nuclease-free water	11.0	13.2	52.8	105.6
	Total	**40.0**	**48.0**	**182.4**	**384.0**

[†] For more than one library, use different library reverse primers for each library. For recommendations about how to index libraries, contact your local Field Application Specialist or go to scomix@bd.com.
For more than four libraries, use the OMICS-One™ Dual Index Kit.

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

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

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

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

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

7. Run the following PCR program:

Index PCR conditions for Targeted mRNA

Step	Cycles	Temperature	Time
Hot start	1	95 °C	3 minutes
Denaturation	8*	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	∞

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

The PCR can run overnight.

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

6. sgRNA and Targeted mRNA Index PCR cleanup and quality check

Summary:

- sgRNA and Targeted mRNA 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		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:				
sgRNA and Targeted mRNA Index PCR product				4 °C
1.5-mL DNA LoBind® tubes				
0.2-mL PCR tubes				
0.2-mL PCR tube magnetic rack				
Set up:				
Prepare fresh 80% ethyl alcohol				

Procedure steps:

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

Perform the purification in the post-amplification workspace.

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

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

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

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

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

    The purified eluate is the final sequencing library.

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

24. Quantify and perform quality control of the sgRNA and Targeted mRNA 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 sgRNAand Targeted mRNA libraries should typically fall within the range of ~250–600 bp. Actual size might vary depending on the insert length and adapter design. Exact size might vary due to instrument or sample purification efficiency.

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 sgRNA and Targeted mRNA libraries.

Sequencing depth of the Targeted mRNA libraries can vary depending on whether the sample contains high- or low-content RNA cells.

Read requirements for libraries

For resting PBMCs, we recommend:

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

* 2,000 reads/cell can be sufficient for cell-type clustering and classification. For deeply saturated sequencing (RSEC depth >6), use 20,000 reads/cell.

Required parameters

Parameter	Requirement
Platform	Illumina and Element*
Paired-end reads	Recommend Read 1: 51 cycles; Read 2: 83 cycles; Index 1: 8 cycles; Index 2: 8 cycles
PhiX	1% recommended
Analysis	See the *BD® Single-Cell Multiomics Bioinformatics Handbook*†

* To review Index sequences, see the Appendix (page 35).

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

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

Sequencing recommendations

- For a NextSeq High or Mid Output run and MiniSeq High or Mid Output run, load the flow cell at a concentration between 1.5 and 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.

Sequencing analysis pipeline

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

Troubleshooting

Library preparation

Observation	Possible causes	Recommended solutions
PCR2 product yield too low.	PCR1 and PCR2 primers might have been swapped by mistake.	Ensure the correct primers are used for each step.
	cDNA synthesis might have failed due to incomplete washing of lysis buffer.	Avoid leaving behind lysis buffer or bubbles after removing lysis buffer from the tube during bead wash after retrieval from the cartridge. Use new tubes for each wash step, as described in the protocol.
	cDNA synthesis might have failed due to thermomixer not shaking during reverse transcription.	Samples need to be on the thermomixer in shake mode. Where applicable, ensure that a SmartBlock™ Thermoblock is installed on the thermomixer for 1.5-mL tubes so that the reaction can proceed at the designated temperature.
	BD Rhapsody™ Enhanced Cell Capture Beads not fully resuspended immediately before PCR1.	Gently pipet-mix BD Rhapsody™ Enhanced Cell Capture Beads in PCR1 reaction mix immediately before starting PCR1 thermal cycling to ensure uniform bead suspension.
	Thermal cycler mis-programming.	Ensure that the correct thermal cycling program is used.
	Too few PCR1 cycles.	Optimize the number of PCR cycles for the specific sample type.
	Incorrect volume of AMPure® XP magnetic beads used during PCR2 cleanup.	Use the specified volume of AMPure® XP beads.
	Incorrect solution or incorrect concentration of 80% ethyl alcohol used for washing AMPure® XP magnetic beads, resulting in premature elution of PCR products from beads.	Use 80% ethyl alcohol for washing AMPure® XP beads.

Observation	Possible causes	Recommended solutions
Concentration of final mRNA sequencing library too low.	Issue with PCR2 product yield or quality.	1. Determine the product size range: a. Load 1 µL of purified PCR2 product at 1 ng/µL in a High Sensitivity DNA Chip on the Agilent Bioanalyzer. b. Follow the manufacturer's instructions. 2. Confirm that the Targeted mRNA PCR2 products show an average size range of 350–600 bp. • If the products pass quality control, see 5. sgRNA and Targeted mRNA Index PCR (page 25) to prepare final libraries. Repeat the index PCR. • If the products do not pass quality control, contact your local Field Application Specialist (FAS) or scomix@bd.com.
	Thermal cycler misprogramming.	Ensure that the correct thermal cycling program is used.
Final sequencing product size too large.	Over-amplification during index PCR or input amount of PCR2 products too high.	Repeat the index PCR with a lower input of PCR2 products.
	Upper and lower markers on the Agilent Bioanalyzer or TapeStation are incorrectly called.	Ensure that markers are correct. Follow manufacturer's instructions.
	Incorrect volume of Agencourt® AMPure® XP magnetic beads used.	Use volume specified in protocol.

Sequencing

Observation	Possible causes	Recommended solutions
Over-clustering on the Illumina 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.

Appendix

References for sgRNA Amplification

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

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

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

Oligonucleotides in BD Rhapsody™ Targeted mRNA and BD® AbSeq Amplification Kit

The following table lists the sequences of all oligonucleotides included in the BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit (Cat No. 633774).

Oligonucleotide		Part/Catalog No.	Sequence (5' – 3')
BD Rhapsody™ Universal Oligo	Forward primer for WTA RPE PCR, Sample Tag PCR1 and PCR2, and BD® AbSeq PCR1	650000074	ACACGACGCTCTTCCGATCT
BD Rhapsody™ Sample Tag PCR1 Primer	Reverse primer for Sample Tag PCR1	91-1088	GTTGTCAAGATGCTACCGTT
BD Rhapsody™ Sample Tag PCR2 Primer	Reverse primer for Sample Tag PCR2	91-1089	CAGACGTGTGCTCTTCCGATCTGTTGTCAAGATGCTACCGTT
BD Rhapsody™ Library Forward Primer	Forward primer for WTA, Sample Tag, and BD® AbSeq Index PCR	91-1085	AATGATACGGCGACCACCGAGATCTACACTATAGCCT ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
BD Rhapsody™ Library Reverse Primer 1	WTA, Sample Tag, and BD® AbSeq Index PCR	650000080	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD Rhapsody™ Library Reverse Primer 2		650000091	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD Rhapsody™ Library Reverse Primer 3		650000092	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD Rhapsody™ Library Reverse Primer 4		650000093	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC*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 Rhapsody™ Library Forward Primer 1	TATAGCCT	AGGCTATA

Reverse Index name	i7 bases for sample sheet
BD Rhapsody™ Library Reverse Primer 1 (N709)	GCTACGCT
BD Rhapsody™ Library Reverse Primer 2 (N710)	CGAGGCTG
BD Rhapsody™ Library Reverse Primer 3 (N711)	AAGAGGCA
BD Rhapsody™ Library Reverse Primer 4 (N712)	GTAGAGGA

OMICS-One™ Dual Index Kit sequences

Forward index name	i5 bases for sample sheet NovaSeq, MiSeq, HiSeq 2000/2500	i5 bases for sample sheet iSeq, MiniSeq, NexSeq, HiSeq 3000/4000
Dual index forward primer 1	TATAGCCT	AGGCTATA
Dual index forward primer 2	ATAGAGGC	GCCTCTAT
Dual index forward primer 3	CCTATCCT	AGGATAGG
Dual index forward primer 4	GGCTCTGA	TCAGAGCC
Dual index forward primer 5	AGGCGAAG	CTTCGCCT
Dual index forward primer 6	TAATCTTA	TAAGATTA
Dual index forward primer 7	CAGGACGT	ACGTCCTG
Dual index forward primer 8	GTACTGAC	GTCAGTAC

Reverse index name	i7 bases for sample sheet
Dual index reverse primer 1	ATTACTCG
Dual index reverse primer 2	TCCGGAGA
Dual index reverse primer 3	CGCTCATT
Dual index reverse primer 4	GAGATTCC
Dual index reverse primer 5	ATTCAGAA
Dual index reverse primer 6	GAATTCGT
Dual index reverse primer 7	CTGAAGCT
Dual index reverse primer 8	TAATGCGC

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