February 2026

Supplementary Protocol

Convert and normalize QIAseq® Multimodal Panel library for sequencing on Element Biosciences AVITI™ System

QIAseq Multimodal Panels (except QIAseq Beads, QIAseq Bead Binding Buffer, and Nuclease-free Water) are shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer.

QIAseq Beads, QIAseq Bead Binding Buffer, and Nuclease-free Water are shipped in a separate box on cold packs. Upon receipt, QIAseq Beads, QIAseq Bead Binding Buffer, an Nuclease-free Water should be stored at 2–8°C.

QIAseq Multimodal Index Kits are shipped on dry ice and should be stored at –30°C to –15°C upon arrival. Under these conditions, the components are stable until the expiry date indicated on the label.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- LoBind® tubes for storage of generated libraries (Eppendorf®, cat. no. 0030108094, 0030108116, or 0030108132) or equivalent
- 96-well PCR plates or PCR tubes/strips
- Foil seal for 96-well plates, heat-resistant
- 100% ethanol (ACS grade)
- Nuclease-free water
- Pipette tips and pipettes
- Vortexer
- Microcentrifuge and/or plate centrifuge for 96-well plates
- Thermal cycler
- Magnetic stand (e.g., DynaMag™ 2 Magnet or DynaMag™ 96 Side Skirted Magnet, Thermo Fisher, cat. no. 12027)
- **Optional**: QIAGEN QIAxcel®, Agilent® Bioanalyzer®, or similar method to assess the quality of DNA library

Notes before starting

- Ensure QIAseq Multimodal Panel libraries are in the correct size without primer dimers, and the library yields are consistent with concentrations well above 15–20 nmol/L in a minimal volume of 15 µL.
- The recommended number of libraries in the sequencing pool is 6 or higher.
- Use the following kit for normalization:

QIAseq Universal Normalizer Kit **Catalog no.** **Number of reactions**	**(24)** **180613** **24**	**(96)** **180615** **96**
QIAseq Normalizer Reagent Kit		
Normalizer Reagent	1 × 135 µL	1 × 580 µL
Normalizer Wash Buffer	1 × 14 mL	4 × 14 mL
Normalizer Elution Buffer	1 × 1.9 mL	2 × 1.9 mL
RNase-free Water	1 × 1.9 mL	1 × 1.9 mL
QIAseq Normalizer Primer Kit		
Normalizer Primer Mix	1 × 40 µL	1 × 165 µL
QIAseq Normalizer PCR Kit		
HiFi PCR Master Mix	2 × 300 µL	2 × 1.5 mL
RNase-free Water	1 × 1.9 mL	2 × 1.9 mL
QIAseq Beads		
QIAseq Beads	1 × 5 mL	1 × 10 mL

- The QIAseq Universal Normalizer Kit is shipped in 4 boxes at different temperatures. When stored correctly, the kit is good until the expiration date printed on the outer package
    - Store the QIAseq Normalizer Reagent Kit and QIAseq Beads at 2–8°C upon arrival (do not freeze).
    - Store the QIAseq Normalizer Primer Kit and QIAseq Normalizer PCR Kit at –30°C to –15°C upon arrival.

Protocol: (Tube Format) Workflow A – Library modification for use with QIAseq Universal Normalizer Kits

This protocol describes the modification and normalization of QIAseq Multimodal Panel library. Libraries can be sequenced on the Element Biosciences AVITI instrument using Cloudbreak Freestyle™ flowcells.

Important points before starting

- Thaw QIAseq HiFi PCR Master Mix and the Normalizer Primer Mix on ice. Once reagents are thawed, mix them thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use.
- Prepare fresh 80% ethanol
- Equilibrate QIAseq Beads to room temperature (15–25°C). Directly before use, vortex the beads thoroughly to homogenize.

Procedure

1. Set up a thermal cycler with a heated lid according to Table 1.

Table 1. Thermal cycling parameters

Time	Temperature (°C)	Number of cycles
2 min	98	1
20 s	98	4 cycles*
30 s	60	
30 s	72	
2 min	72	1
∞	4	Hold

*If library volume is <23.5 µL, adjust the cycle number accordingly.

2. Prepare the library modification reaction mix on ice according to Table 2. Mix the components in a PCR tube/strip or 96-well plate.

Table 2. Reaction mix for Normalizer modification PCR

Component	Volume/reaction (µL)
HiFi PCR Master Mix, 2x	25
Normalizer Primer Mix	1.5
Finalized Illumina library*	≤23.5
RNase-free Water	Variable
Total reaction volume	**50**

*If available, always use 23.5 µL of finalized QIAseq Multimodal Panel library. Otherwise use all available volume and fill with RNase-free Water.

3. Transfer the PCR tube/strip or plate to the thermal cycler and start the program.
4. Once PCR is complete, transfer the whole reaction to a new 1.5 mL Eppendorf tube and add 50 µL of homogenized QIAseq Beads to each reaction.
5. Mix by thoroughly pipetting up and down or vortex. Then, incubate the mixture for 5 min at room temperature.
6. If required, pulse spin the tubes to collect all liquid at the bottom.
7. Pellet the beads on a magnetic stand for 5 min or until the solution is clear.
8. Carefully discard the supernatant without disturbing the pellet.
9. Wash the beads by adding 200 µL fresh 80% ethanol to each pellet. Pellet the beads on the magnetic stand and carefully discard the supernatant.
10. Repeat step 9 for a total of 2 ethanol washes.
11. Remove as much excess ethanol as possible.

12. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Remove from the magnetic stand.

13. Elute modified libraries by resuspending in 32.5 µL of RNase-free Water or 10 mM Tris·Cl, pH 8.0. Pellet the beads on the magnetic stand and carefully transfer 30 µL of the supernatant into a new tube.

    Note: If required, the elution volume can be reduced to 20 µL to increase the library concentration. The QIAseq Normalizer procedure will require 15 µL of modified library.

14. The collected supernatant contains the modified library and can be used for normalization. Proceed to "Protocol: (Tube format) QIAseq Library Normalization". Alternatively, modified libraries can be stored for later use at –20°C.

Protocol: (Tube format) QIAseq Library Normalization

This protocol describes the normalization of previously modified QIAseq Multimodal Panel libraries. Only use libraries that have been modified or amplified with the Normalizer Primer Mix. Using non-modified libraries will not normalize to the expected concentration. QIAseq Normalization will yield double-stranded libraries at a concentration of approximately 4 nmol/L. Normalized libraries can be pooled for sequencing at equal volumes without further quantification.

Things to do before starting

- Modify libraries to be normalized following "Protocol: (Tube Format) Workflow A – Library modification for use with QIAseq Universal Normalizer Kits". Do not use non-modified libraries.
- For normalization, use libraries that consistently reach concentrations of at least 15–20 nmol/L or more.
- Thoroughly mix the Normalizer Reagent by vortexing for 60 s. Make sure the pellet of beads is completely dissolved and the solution is well homogenized.
- Homogenize the bottle(s) of Normalizer Wash Buffer by vortexing and preheat the whole reagent bottle(s) to 55°C in a water bath.

  Alternatively, use a heating block for 1.5/2.0 mL tubes or Falcon tubes. Fill the homogenized wash buffer into compatible receptacles to preheat. Prepare 450 µL wash buffer (400 µL are required) per normalization reaction.
- Preheat a heating block for 1.5 mL tubes to 55°C.
- It is not required to equilibrate the Normalizer Reagent and Normalizer Elution Buffer to room temperature before use.

Procedure

1. Homogenize the bottle(s) of Normalizer Wash Buffer by vortexing and preheat the whole reagent bottle(s) to 55°C in a water bath. Alternatively, use a heating block for 1.5/2.0 mL tubes or Falcon tubes. Fill the homogenized wash buffer into compatible receptacles to preheat. Prepare 450 µL wash buffer (400 µL are required) per normalization reaction. Leave the wash buffer at 55°C until use.
2. Vortex the Normalizer Reagent for 60 s to mix thoroughly. Make sure the pellet of beads is completely dissolved and the solution is well homogenized.
3. Directly before use, pulse spin the Normalizer Reagent at low force to collect all liquid at the bottom. Then use a 200 µL pipette to homogenize the reagent. Be sure to disperse a sediment of beads that may have formed during pulse spin.
4. For every library to be normalized, pipet 5 µL of homogenized Normalization Reagent to a 1.5 mL tube. Leave the tubes at room temperature.
5. Add 15 µL of the modified library to be normalized to the 1.5 mL tube containing Normalization Reagent. Mix well by pipetting or vortexing.
6. Incubate for 10 min at room temperature.
7. Add 200 µL pre-warmed Normalizer Wash Buffer (55°C) to each tube.
8. Pellet the beads on a magnetic stand for 2–7 min, until the solution is clear.

   Note: Ensure all beads have been pelletized.
9. Carefully discard the supernatant without disturbing the pellet.
10. With the tube on the magnet, add 200 µL pre-warmed Normalizer Wash Buffer (55°C) to each pellet.

11. Switch the tube position on the magnet to wash the beads, and wait until all beads have pelletized. Alternatively, rotate the tube by 180° to force the beads to the opposite side of the tube.

12. Carefully discard the supernatant without disturbing the pellet. Remove as much remaining liquid as possible, then proceed to the next step immediately.

    Note: Drying the beads is not required.

13. Add 26 µL Normalizer Elution Buffer to each pellet and mix well by vortexing. Make sure the pellet is completely dissolved.

14. Tap the tube on the benchtop to collect the liquid at the bottom. If there is liquid remaining in the lid, pulse spin the tube at low force. Do not spin to form a compact bead pellet.

15. Incubate for 5 min at 55°C in a heating block.

16. Pulse spin to collect all liquid at the bottom, then pellet the beads on a magnetic stand for 2 min and wait until the solution is clear.

17. Carefully transfer 25 µL of the supernatant to a new tube.

    Important: Do not discard the supernatant. The supernatant contains the ready-to-sequence library.

18. Proceed to "Guidelines: Pooling and sequencing of normalized libraries on Element AVITI". Alternatively, the normalized libraries can be stored at –20°C for up to 3 months.

Guidelines: Pooling and sequencing of normalized libraries on Element AVITI

The following guidelines describe the pooling and sequencing of normalized libraries for Element AVITI. Libraries normalized using QIAseq Universal Normalizer Kits are double-stranded and have a concentration of approximately 4 nmol/L. Quantification of the library pool prior to sequencing is optional.

Pooling

- If normalized libraries were frozen, thaw them completely, mix by pulse vortexing, and spin down to avoid localized concentrations.
- For a homogeneous distribution of sequence reads, combine equal volumes of normalized libraries into a pool. For example, combine 5 µL of each normalized library. The pool will have an overall concentration of approximately 4 nmol/L.
- For joint sequencing, only combine libraries that have different indexes to allow complete demultiplexing of sequence data.

Sequencing with Element Biosciences AVITI and CloudBreak FS kits

1. Thaw cartridge for 2.5 h at room temperature, or 26 h at 4°C.
2. Prepare 0.2 N NaOH.
3. Prepare 0.2 M Tris-HCL buffer, pH 7.0.
4. Dilute library pool to 1 nM with low TE buffer.
5. For denaturation: 12.6 µL 1 nM pool + 12.6 µL 0.2 N NaOH, mix by vortexing.
6. Spin down and incubate at room temperature for 5 min.
7. Add 12.6 µL 0.2 M Tris-HCL buffer, pH 7.0.
8. Vortex, quick spin, and add 1362.2 µL library loading buffer.

9. Place on ice.
10. Set loading concentration at 9 pM.
11. Refer to the *Element Biosciences AVITI User Manual* for more information to plan the run.

Planned run

Populate the following fields according to their respective actions indicated in the table below.

Field	Action
Title	Enter **Run Name**
Browse	Import **manifest**
Library type	Select **Third party**
Sequencing Kit	Select **Kit type**
Library Structure	Select **Linear**
Low-diversity High-multiplex option	Select **No**
Library pools	Enter **1**
Cycles	Select **10 index 1**, **10 index 2**, **150 Read 1**, and **150 Read 2**

Initiate Sequencing Run

1. Select **New run**, select **Open Nest**, insert used flow cell, then select **Close Nest**.
2. Select Side: **Side A** / **Both** / **Side B**
3. Select **Cloudbreak** as chemistry type.
4. Select **Manual Run** as run type.
5. Input **Run Name**.
6. Inspect and mix Reagents.

7. Invert cartridge 10 times to mix reagents.
8. Replace R1 and R2 tubes in reagent cartridge with fresh tubes from the Custom Primer kit 820-00025.
9. Add custom primers as follows:
    a. 32.4 µL of 100 µM Custom Read 1 A primer to R1.
    b. 19 µL of 100 µM Custom Read 2 primer to R2.
10. Add libraries to the cartridge.
11. Load reagents and buffers.
12. Confirm reagent preparation.
13. Empty waste and prime reagents.
14. Select **Next** to automatically start priming.
15. Equilibrate flow cell to room temperature (priming takes approximately 15 min).
16. After priming, select **Next** for Load Flow Cell Screen.
17. Load flow cell, select **Close Nest**, then select **Next**.
18. Select **Run**.

Document Revision History

Date	Changes
02/2026	Initial release

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