



Element  
Biosciences

User Guide

# Element Adept<sup>TM</sup> Library Compatibility Workflow

Adept Standard Protocol

## FOR USE WITH

Element Adept Library Compatibility Kit v1.1, catalog # 830-00007

**ELEMENT BIOSCIENCES**

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# Table of Contents

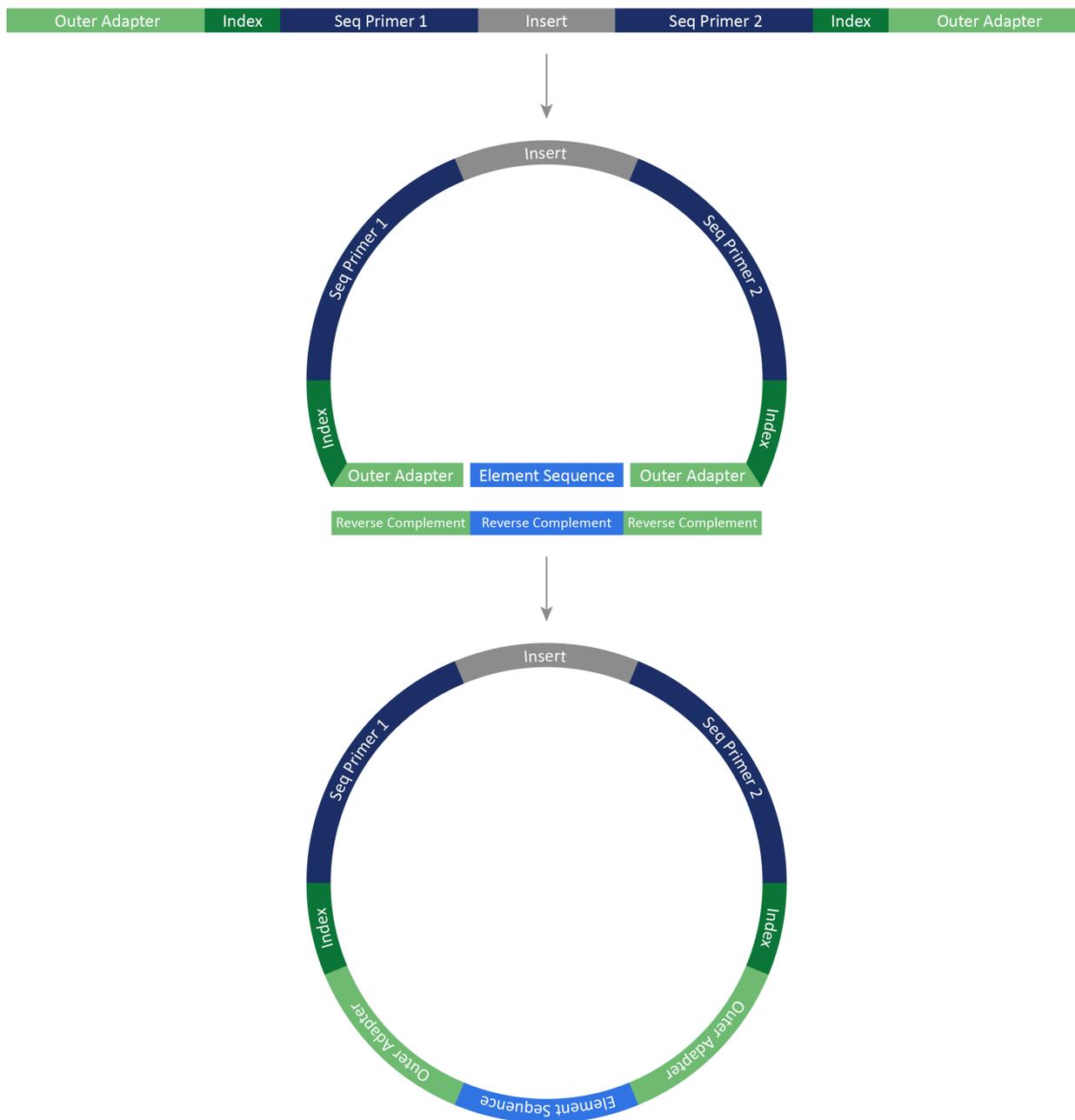
Introduction .....	4
Kit Contents and Storage .....	7
Input Requirements .....	10
Adept Standard Protocol .....	12
Technical Support .....	20
Document History .....	21

# Introduction

The Element Adept Library Compatibility Workflow adapts linear libraries prepared with third-party kits for sequencing on the Element AVITI™ System. The Adept Standard Protocol uses the Element Adept Library Compatibility Kit v1.1 to circularize up to 24 reactions. Each reaction supports input of one linear library or a pool of indexed linear libraries.

The protocol starts with denaturing a linear library into single strands. The library is then annealed to splint oligos, which introduce the Element surface primer binding sequences. A ligation reaction circularizes the library, followed by enzymatic digestion for cleanup.

**Figure 1:** Circularization of a linear library



# Sequencing Compatibility

Adept libraries are *only* compatible with Cloudbreak™ sequencing kits on the Element AVITI System. If you are using Cloudbreak Freestyle™ sequencing kits, converting your library with the Adept Workflow is not required. For more compatibility information, see [go.elembio.link/product-compatibility](https://go.elembio.link/product-compatibility).

## Supported Protocols

The Adept Workflow supports multiple kits and protocols. The compatibility kit generates a circular library and the PCR-plus kit generates a linear library that is circularized onboard the instrument. Thus, the AVITI System sequences a circular library regardless of whether you load a linear or circular library.

This guide documents the Adept Standard Protocol. To follow a different protocol, reference the applicable user guide.

Protocol	Kit	User Guide
Adept Standard	Element Adept Library Compatibility Kit v1.1	Current user guide
Adept Rapid PCR-Free	Element Adept Library Compatibility Kit v1.1	<i>Element Adept Library Compatibility Workflow User Guide for the PCR-Free Protocol (MA-00033)</i>
Adept Rapid PCR-Plus	Adept Rapid PCR-Plus Kit	<i>Element Adept Library Compatibility Workflow User Guide for the PCR-Plus Protocol (MA-00040)</i>

## Library Compatibility

The Adept Standard Protocol supports libraries prepared with the library prep and index kits listed at [go.elembio.link/compatible](https://go.elembio.link/compatible). If your kit is not supported, follow the Adept PCR-Plus Protocol.

The following factors make a library unsupported:

- Truncated ends
- End-blocked libraries
- Additional bases at the end, such as a polymerase-generated adenine (A) overhang

## Quantification Method

Quantifying the final library ensures the appropriate input for sequencing. By detecting only circular library, quantitative PCR (qPCR) ensures consistent and accurate quantification of libraries prepared with the Element Adept Library Compatibility Kit v1.1.

Qubit is an alternative quantification method that requires testing and modifications. For more information, see the *Accurate Quantification of Circular Libraries for Sequencing on the Element AVITI System Technical Note (LT-00009)*.



### NOTE

Linear and circular Adept libraries have different recommended loading concentrations.

## Low-Diversity Amplicon Library

When preparing a low-diversity amplicon library, such as 16S, for sequencing with a 2 x 300 kit, meet the following requirements:

- An insert size of > 200 bp
- High plexity of  $\geq 64$  unique dual indexed (UDI) libraries
- A 1–5% spike-in of PhiX Control Library

## Custom Primers

The AVITI System accepts custom primers for any Adept library. However, custom primers require special consideration and planning. To make sure a run with custom primers meets specifications, contact Element Technical Support early in experiment planning. Technical support can also help determine whether your library requires custom primers.

## Workflow Summary

The following figure summarizes the protocol, which takes 75 minutes, including 25 minutes of hands-on time. All durations are approximate and depend on lab-specific factors.

**Figure 2:** Adept Standard Protocol

	Procedure	Duration	Kit Reagents	User-Supplied Reagents
1	Anneal splint oligos	15 minutes	Adept Annealing Mix 2, Elution Buffer	None
2	Circularize library	25 minutes	Ligation Buffer, Ligation Enzyme 1, Ligation Enzyme 2	None
3	Digest linear DNA	17 minutes	Digestion Enzyme 1, Digestion Enzyme 2	None
4	Clean up circular library	20 minutes	Elution Buffer	Freshly prepared 80% ethanol, sample purification beads
5	Quantify library		qPCR Primer Mix, qPCR Standard 2	SYBR Green PCR Master Mix; Tris-HCl 10 mM with 0.05% Tween-20, pH 8.0

## Safety Data Sheets

When using the Element Adept Library Compatibility Kit v1.1 and other reagents, always wear personal protective equipment (PPE): a lab coat, powder-free disposable gloves, and protective goggles. Review the safety data sheets (SDS) for chemical properties. The SDS inform safety, disposal, and hazards for your region and are available at [elementbiosciences.com/resources](https://www.elementbiosciences.com/resources).

# Kit Contents and Storage

The Element Adept Library Compatibility Kit v1.1 is packaged in one box and shipped on dry ice. When you receive your kit, promptly store reagents at the proper temperature. Reference reagent labels for fill volumes.

In addition to the kit, the protocol requires the user-supplied materials listed in the following sections. The protocol specifies processing libraries in a plate, but you can substitute tubes.

Reagent	Quantity	Cap Color	Storage Temperature
Adept Annealing Mix 2	1	Green	-25°C to -15°C
Digestion Enzyme 1	1	Clear	-25°C to -15°C
Digestion Enzyme 2	1	Clear	-25°C to -15°C
Elution Buffer	1	Clear	-25°C to -15°C
Ligation Buffer	2	Clear	-25°C to -15°C
Ligation Enzyme 1	1	Clear	-25°C to -15°C
Ligation Enzyme 2	1	Clear	-25°C to -15°C
qPCR Primer Mix 2	1	Clear	-25°C to -15°C
qPCR Standard 2	1	Clear	-25°C to -15°C

## User-Supplied Consumables

Supplier	Consumable	Catalog #
General lab supplier	96-well PCR plates	Not applicable
	96-well qPCR plates	Not applicable
	Absolute ethanol	Not applicable
	Filtered pipette tips	Not applicable
	Nuclease-free water	Not applicable
	Sample purification beads	Not applicable <sup>1</sup>
Agilent	High Sensitivity D5000 Reagents	Part # 5067-5593 <sup>2</sup>
	High Sensitivity D5000 ScreenTape	Part # 5067-5592 <sup>2</sup>

Supplier	Consumable	Catalog #
Bio-Rad	Microseal 'B' Film, adhesive	Catalog # MSB1001 <sup>2</sup>
	Microseal 'C' Film, optical	Catalog # MSC1001 <sup>2</sup>
Eppendorf	DNA LoBind Tubes, 1.5 ml	Catalog # 022431021
Teknova	10 mM Tris-HCl with 0.05% Tween-20, pH 8.0	SKU # T1485 <sup>2</sup>
Thermo Fisher Scientific	96-well 0.8 ml deepwell storage plates	Catalog # AB0765 <sup>2,3</sup>
	Either kit: <ul style="list-style-type: none"> <li>• Qubit dsDNA BR Assay Kit</li> <li>• Qubit dsDNA HS Assay Kit</li> </ul>	The corresponding catalog #: <ul style="list-style-type: none"> <li>• Q33216</li> <li>• Q33238</li> </ul>
	SYBR Green PCR Master Mix	Catalog # 4364346 <sup>2</sup>

<sup>1</sup> Element has validated SPRIselect, 60 ml (Beckman Coulter, catalog # B23318).

<sup>2</sup> Consumables that you have tested and demonstrate equivalent performance are acceptable.

<sup>3</sup> Deepwell plates facilitate cleanup procedures. When cleaning up libraries, you can instead use 0.2 ml tubes or strip tubes.

## User-Supplied Equipment

Supplier	Equipment	Catalog # <sup>1</sup>
General lab supplier	Centrifuge, multipurpose	Not applicable
	Ice bucket	Not applicable
	Pipettes, single- or multi-channel	Not applicable
	Vortex mixer	Not applicable
	[Optional] Speed vac	Not applicable
Agilent	Either system: <ul style="list-style-type: none"> <li>• 4150 TapeStation System</li> <li>• 4200 TapeStation System</li> </ul>	The corresponding part #: <ul style="list-style-type: none"> <li>• G2992AA</li> <li>• G2991BA</li> </ul>
Bio-Rad	CFX96 Touch Real-Time PCR Detection System	Catalog # 1845096 <sup>1</sup>
	Either thermal cycler: <ul style="list-style-type: none"> <li>• C1000 Touch Thermal Cycler</li> <li>• T100 Thermal Cycler</li> </ul>	The corresponding catalog #: <ul style="list-style-type: none"> <li>• Catalog # 1851197</li> <li>• Catalog # 1861096</li> </ul>

Supplier	Equipment	Catalog # <sup>1</sup>
Thermo Fisher Scientific	The applicable magnet: <ul style="list-style-type: none"> <li>• DynaMag-96 Side Magnet for cleanup with tubes</li> <li>• Magnetic Stand-96 for cleanup with plates</li> </ul>	The corresponding catalog #: <ul style="list-style-type: none"> <li>• 12331D</li> <li>• AM10027</li> </ul>
	Either fluorometer: <ul style="list-style-type: none"> <li>• Qubit 3 Fluorometer</li> <li>• Qubit 4 Fluorometer</li> </ul>	The corresponding catalog #: <ul style="list-style-type: none"> <li>• Q33216</li> <li>• Q33238</li> </ul>
QInstruments	[Optional] BioShake XP	Order # 1808-0505

<sup>1</sup> Any equipment that you have tested and demonstrates equivalent performance is acceptable.

# Input Requirements

The Adept Workflow supports a double-stranded DNA (dsDNA) linear library prepared per third-party instructions. Accordingly, you must prepare a linear library and perform a quality control (QC) check **before** starting end polishing or any of the protocols.

Prepare the linear library from RNA, complementary DNA (cDNA), or genomic DNA (gDNA). For more information, see [Library Compatibility on page 5](#).

## Library Amount

The Element Adept Library Compatibility Kit v1.1 accepts input of 6.67–16.67 nM in 30  $\mu$ l low TE buffer or similar solution, which is equivalent to 0.2–0.5 pmol. The concentration of ethylenediaminetetraacetic acid (EDTA) in the library cannot exceed 1 mM.

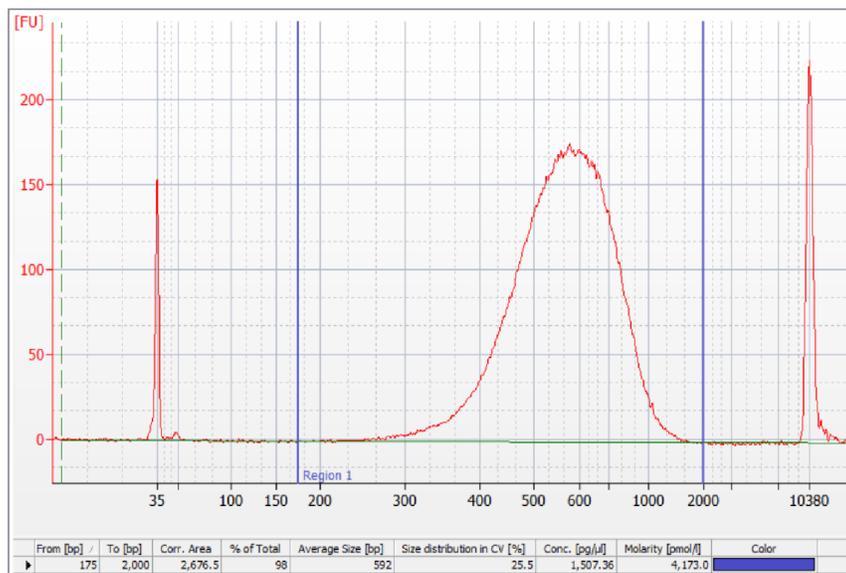
Determine the input library concentration with a Qubit fluorometer, qPCR, or equivalent, following supplier instructions. When reducing the input amount to < 0.5 pmol, accurate quantification is crucial.

## Fragment Size

Use a 4150 TapeStation System, 4200 TapeStation System, or equivalent instrument to qualify the input library and identify short byproducts in electropherograms. Set the region to > 175 bp and determine the average fragment size. Library portions that contain > 1000 bp sequences might impact Q30 scores and require adjustment for density.

Avoid significant amounts of adapter dimer or other short byproducts (< 175 bp). If the input library contains short byproducts, Element recommends additional cleanup using sample purification beads. Reassess the purified library with the TapeStation System to confirm byproduct removal, and then requantify.

**Figure 3:** Example Bioanalyzer trace showing an average fragment size of 592 bp



## Pooling Guidelines

An Adept reaction processes one linear library or a pool of indexed linear libraries. When pooling, uniquely index each library in the pool and apply the following criteria to pool libraries with similar characteristics:

- Pool libraries that require the same run parameters.
- Do not pool Adept libraries with Elevate™ libraries.
- Balance the concentrations of libraries in a pool based on the throughput requirements for each sample. To maintain balance after library prep, make sure the libraries have similar size distributions.
- Review the *Element AVITI System User Guide (MA-00008)* for guidance on using the PhiX Control Library, which can improve color and nucleotide balancing and library complexity. Certain experiments require a spike-in.

# Adept Standard Protocol

Follow the protocol steps in the order listed using specified volumes and durations. Proceed immediately from one step to the next.

To avoid cross-contamination, use filtered pipette tips throughout the protocol. When adding or transferring reagents and libraries, change pipette tips between each reagent and each library.

## Prepare Reagents

Reagent preparation is a preliminary procedure. Start 20–30 minutes before proceeding with the protocol.

1. Make sure that you have all Element- and user-supplied consumables. For lists, see [Kit Contents and Storage on page 7](#).
2. Remove the Element Adept Library Compatibility Kit v1.1 reagents from -25°C to -15°C storage.
  - » Avoid unnecessary freeze-thaw cycles of Ligation Buffer:
    - If you are preparing ≤ 12 reactions, remove one tube.
    - If you are preparing 13–24 reactions, remove two tubes.
  - » If you are quantifying libraries immediately after circularization, remove qPCR Standard 2 and qPCR Primer Mix 2.
3. If necessary, remove the library from -25°C to -15°C storage and thaw on ice.
4. Fully thaw the reagents on ice. Keep on ice.
5. Combine the following volumes to prepare fresh 80% ethanol for each reaction. Discard leftover 80% ethanol after 1 day.

Reagent	Volume per Reaction (μl)
Absolute ethanol	400
Nuclease-free water	100
Total	500

## Anneal Splint Oligos

The anneal splint oligos procedure heat-denatures the input linear library and anneals splint oligos.

1. Gather the following consumables:
  - » 96-well PCR plate
  - » Microseal 'B'
  - » Adept Annealing Mix 2 (green cap)
  - » Elution Buffer
  - » Linear library
2. Make sure a dsDNA linear library is prepared per supplier instructions. Prepare 0.2–0.5 pmol linear library or indexed linear library pool in 30 μl.
  - » If the volume is < 30 μl, add Elution Buffer to reach 30 μl.
  - » If the volume is > 30 μl, use a speed vac or validated bead-based method to concentrate the library to 30 μl.  
—For example, start with 25 μl 20 nM library and add 5 μl Elution Buffer to reach 16.67 nM in 30 μl.—
3. Make sure that Adept Annealing Mix 2 is fully thawed.

4. Vortex Adept Annealing Mix 2 to mix and briefly centrifuge.
5. In each well of a new PCR plate, combine the following components.

Component	Volume per Library (μl)
Linear library	30
Adept Annealing Mix 2	13
Total	43

6. Set a pipette to 30 μl and pipette each reaction 10 times to mix.
7. Seal the plate and briefly centrifuge.
8. Place the plate in the thermal cycler.
9. Run the following ~10-minute program.

Step	Temperature	Time
Volume set to 43 μl		
Lid set to 105°C		
1	95°C	5 minutes
2	37°C	5 minutes
3	37°C	Hold

10. Remove the plate from the thermal cycler.
11. Briefly centrifuge the plate and immediately proceed.

## Circularize Library

The circularize library procedure phosphorylates the 5' end of the linear library and uses a ligation reaction to produce a circular library.

1. Gather the following consumables:
  - » 1.5 ml DNA LoBind tube
  - » Microseal 'B'
  - » Ligation Buffer
  - » Ligation Enzyme 1
  - » Ligation Enzyme 2
2. Make sure that all reagents are fully thawed.
3. Gently flick Ligation Enzyme 1 and Ligation Enzyme 2 to mix and briefly centrifuge. Place on ice.
4. Vortex Ligation Buffer to mix and briefly centrifuge.
5. Combine the following reagents to prepare fresh master mix, allowing 10–15% overage. Set a pipette to 70% of the master mix volume and pipette 10 times on ice to mix.

Reagent	Volume per Reaction (μl)
Ligation Buffer	5
Ligation Enzyme 1	1
Ligation Enzyme 2	1
Total	7

6. Add 7 μl master mix to each reaction.
7. Set a pipette to 38 μl and pipette each reaction 10 times to mix.
8. Seal the plate and briefly centrifuge.
9. Place the plate in the thermal cycler.
10. Run the following ~20-minute program.

Step	Temperature	Time
Volume set to 50 μl		
Lid set to 75°C		
1	37°C	10 minutes
2	65°C	10 minutes
3	4°C	Hold

11. Remove the plate from the thermal cycler.
12. Briefly centrifuge the plate and immediately proceed.

## Digest Linear DNA

The digestion procedure removes carryover linear DNA.

1. Gather the following consumables:
  - » 1.5 ml DNA LoBind tube
  - » Microseal 'B'
  - » Digestion Enzyme 1
  - » Digestion Enzyme 2
2. Gently flick Digestion Enzyme 1 and Digestion Enzyme 2 and briefly centrifuge. Place on ice.
3. Combine the following reagents to prepare fresh master mix, allowing 10–15% overage. Set a pipette to 70% of the master mix volume and pipette 10 times on ice to mix.

Reagent	Volume per Reaction (μl)
Digestion Enzyme 1	2
Digestion Enzyme 2	2
Total	4

4. Add 4  $\mu$ l master mix to each reaction.
5. Set a pipette to 41  $\mu$ l and pipette each reaction 10 times to mix.
6. Seal the plate and briefly centrifuge.
7. Place the plate in the thermal cycler.
8. Run the following ~12-minute program.

Step	Temperature	Time
Volume set to 54 $\mu$ l		
Lid set to 105°C		
1	37°C	10 minutes
2	80°C	2 minutes
3	4°C	Hold

9. Remove the plate from the thermal cycler.
10. Briefly centrifuge the plate and immediately proceed.

## Clean Up Circular Library

The cleanup circular library procedure removes small fragments, enzymes, and salts to purify the final library.

1. Gather the following consumables:
  - » Deepwell plate, 0.2 ml PCR tubes, or strip tubes
  - » Microseal 'B'
  - » DNA LoBind tubes
  - » Elution Buffer
  - » Freshly prepared 80% ethanol
  - » Sample purification beads
2. Transfer each reaction (54  $\mu$ l) to a new deepwell plate or tubes.
3. Thoroughly vortex sample purification beads to resuspend. Make sure beads are not aggregated at the bottom of the bottle.
4. Add 108  $\mu$ l sample purification beads (2x) to each reaction.
  - » Aspirate and dispense beads slowly.
  - » Fully dispense beads from the pipette tip.
5. Mix beads and library using the applicable method:
  - » For a plate, seal and shake at 1500–1800 rpm for 2 minutes.
  - » For tubes, set a pipette to 113  $\mu$ l, pipette content 10 times, and cap.
6. Incubate beads and library at room temperature for a total of 5 minutes.
7. Place the plate or tubes on the magnet and wait until the beads settle and the supernatant clears (~3–5 minutes). **Keep on the magnet.**
8. Unseal the plate or uncap the tubes.

9. Remove and discard the entire volume of supernatant (~160  $\mu$ l).
  - » Do not disturb the bead pellets.
  - » Pipette carefully to avoid aspirating beads.
10. Wash the content of each well or tube:
  - a. Without resuspending the beads, add 200  $\mu$ l 80% ethanol to each reaction and incubate for 30–60 seconds.
  - b. Remove and discard ethanol.
  - c. Without resuspending the beads, add another 200  $\mu$ l 80% ethanol to each reaction and incubate for 30–60 seconds.
  - d. Remove and discard ethanol.
  - e. Using a 10  $\mu$ l or 20  $\mu$ l pipette, remove residual ethanol.
11. Air-dry the beads and library unsealed or uncapped for 2–3 minutes until the pellet loses shine. Do not overdry.  
 **CAUTION** Overdrying can reduce yield and compromise sequencing performance.
12. Remove the plate or tubes from the magnet.
13. Add 32  $\mu$ l Elution Buffer to each reaction.
14. Resuspend the beads in Elution Buffer using the applicable method:
  - » For a plate, seal the plate and shake at 1500–1800 rpm for 2 minutes.
  - » For tubes, set a pipette to 22  $\mu$ l, pipette content  $\geq$  10 times until fully resuspended, and cap.
15. Incubate the reactions at room temperature for 2 minutes.
16. Place the plate or tubes on the magnet and wait until the beads settle and the supernatant clears (~2 minutes).
17. Unseal the plate or uncap the tubes.
18. Transfer 30  $\mu$ l **supernatant** to a new DNA LoBind tube.  
—The tubes contain the final circular libraries.—
19. If you are not immediately quantifying or sequencing, cap the tubes and store at -25°C to -15°C for  $\leq$  15 days.  
—The *Element AVITI System User Guide (MA-00008)* contains sequencing instructions, including diluting the library to the loading concentration.—

## Quantify Library

The quantify library procedure uses qPCR to generate PCR amplicons over the ligated junctions and quantify a portion of the library in preparation for sequencing. The procedure requires standard and library dilutions run in triplicate qPCR reactions.

Each qPCR reaction is 10  $\mu$ l and includes the following components:

- 1  $\mu$ l 10x qPCR Primer Mix 2
- 4  $\mu$ l standard, library, or any positive or negative control diluted to assay-appropriate levels
- 5  $\mu$ l 2x SYBR Green PCR Master Mix

## Prepare Dilutions

1. Gather the following consumables:
  - » 1.5 ml DNA LoBind tube
  - » 96-well qPCR-compatible plate (assay plate)

- » Microseal 'C'
  - » 10 mM Tris-HCl with 0.05% Tween-20, pH 8.0 (dilution buffer)
  - » Circular library
  - » qPCR Primer Mix 2
  - » qPCR Standard 2
  - » SYBR Green PCR Master Mix
2. Prepare the library, qPCR Primer Mix 2, and qPCR Standard 2:
    - a. Thaw the library and reagents on ice.
    - b. Make sure the library and reagents are fully thawed.
    - c. Pulse vortex the library and reagents and briefly centrifuge.
  3. Set aside ~20  $\mu$ l dilution buffer as a no-template control (NTC).
  4. In a 1.5 ml DNA LoBind tube, combine the following reagents to prepare 200 pM qPCR Standard 2.

Reagent	Volume per Reaction ( $\mu$ l)
Dilution buffer	18
2 nM qPCR Standard 2	2
Total	20

5. Vortex the tube to mix and briefly centrifuge.
6. Label the tube **200 pM qPCR Standard 2**.
7. From the 200 pM qPCR Standard 2, make 1:10 serial dilutions to prepare the following standard dilutions.

Standard	Concentration (pM)
Std 1	20
Std 2	2
Std 3	0.2
Std 4	0.02
Std 5	0.002
Std 6	0.0002

—Each standard requires 12  $\mu$ l for triplicate reactions.—

8. [Optional] Store unused 200 pM qPCR Standard 2 at -25°C to -15°C for  $\leq$  15 days. Avoid frequent freeze-thaw cycles.
9. Using two 1:100 dilutions, dilute 2  $\mu$ l library 1:10,000 in dilution buffer. If your expected yield is lower or higher than the typical yield, adjust the dilution.
 

—Libraries diluted to ~0.1–1 pM typically appear in the middle of the standard curve and provide the most accurate quantification. Proper dilution for an Adept library is 1:10,000.—
10. Return the remaining library to -25°C to -15°C storage.

## Prepare Master Mix and Assay Plate

1. Combine the following reagents to prepare fresh qPCR master mix with primers, allowing 10–15% overage.
  - » Set a pipette to 70% of the master mix volume and pipette the master mix 10 times on ice to mix.
  - » Prepare sufficient volume to run triplicate reactions of each NTC, standard dilution, and library dilution.

Reagent	Starting Concentration	Volume per Reaction (μl)
SYBR Green PCR Master Mix	2x	5
qPCR Primer Mix 2	10x	1
Total		6

2. Add 6 μl qPCR master mix with primers to the desired wells of a new assay plate.
3. Add 4 μl NTC, standard dilutions, or library dilutions to wells containing qPCR master mix with primers.  
—The assay volume is 10 μl per well. Mixing is not necessary.—
4. Repeat steps 2–3 to prepare triplicate reactions of each NTC, standard dilution, and library dilution.
5. Seal the plate and briefly centrifuge.

## Perform a qPCR Run

1. On the run setup page of the qPCR run software, edit the plate file:
  - a. Assign standard wells and set the corresponding concentrations.
  - b. Assign NTC wells.
  - c. Assign library wells and note the dilution factors.
  - d. Assign any reference libraries, positive controls, or negative controls to the appropriate wells.
2. Place the plate in the qPCR instrument.
3. Run the following > 1-hour program on the qPCR instrument. If you are not using the qPCR master mix and instrument specified in [Kit Contents and Storage on page 7](#), adjust the program settings.

Step	Setting
Volume set to 10 μl	
Lid set to 105°C	
Activation	10 minutes at 95°C
PCR 40 cycles	15 seconds at 95°C
	1 minute at 60°C
	Plate read
Melt curve	55°C to 95°C with increments of 1°C every 5 seconds
	Plate read after each temperature step

4. Follow vendor instructions to QC the run.

## Analyze Results

1. Analyze the results of the qPCR run:
  - » Exclude the data described in [Exclusion Criteria](#).
  - » Generate the standard curve as described in [Standard Curve Criteria](#).
2. Determine the library dilution concentrations in pM using either method:
  - » Use the starting quantity (SQ) mean values reported by the qPCR instrument software.
  - » Calculate mean values based on the standard curve.
3. Calculate the initial library concentration based on dilutions and measured concentrations:

$$\text{input library concentration in nM} = (\text{fold dilution} * \text{quantification mean in pM}) / 1000$$

—Size adjustment in quantification is not necessary.—

## Exclusion Criteria

- Outliers on the amplification and melt curves and failed wells per third-party qPCR instructions.
- Outliers with a difference > 0.5 Cq for standard dilution, library dilution, and control wells running replicate reactions.
- Standard dilutions that amplify < 3 Cq values ahead of the NTC. Any exclusion except Std 6 (0.0002 pM) requires a rerun.
- Any libraries that had all dilutions amplify outside the standard range require a rerun with, if necessary, an adjusted fold of dilution. The standard curve, which is generated from the standard dilutions that passed the first two exclusion criteria, determines the dynamic range.

## Standard Curve Criteria

Generate the standard curve from standard dilutions that passed the first two exclusion criteria and plot Cq values against the log concentration. When assessing the standard curve, apply the following passing criteria: standard dilution amplification is 90–110%, which is equivalent to a slope of -3.6 to -3.1, and  $R^2 > 0.99$ .

- If the amplification efficiency and  $R^2$  value are out of range, reassess data points in the standard curve and exclude outliers. The remaining standard dilutions must have  $\geq 3$  dilution points. A dilution point is a set of duplicates or triplicates in one of the six standard dilutions.
- If the remaining standard dilutions do not have  $\geq 3$  dilution points, troubleshoot and repeat the qPCR run with freshly prepared dilutions and reagents. The resulting standard curve must meet all passing criteria.

# Technical Support

Visit the [User Documentation page](#) on the Element Biosciences website for additional guides and the most recent version of this guide. For technical assistance, contact Element Technical Support.

**Website:** [www.elementbiosciences.com](http://www.elementbiosciences.com)

**Email:** [support@elembio.com](mailto:support@elembio.com)

**Telephone:** +1 866.ELEMBIO (+1 866.353.6246)

# Document History

Document #	Date	Description of Change
Document # MA-00001 Rev. G	March 2024	<ul style="list-style-type: none"><li>• Added information about sequencing kit compatibility.</li><li>• Added requirements for low-diversity amplicon libraries.</li></ul>
Document # MA-00001 Rev. F	January 2024	<ul style="list-style-type: none"><li>• Corrected footer.</li></ul>
Document # MA-00001 Rev. E	November 2023	<ul style="list-style-type: none"><li>• Renamed the rapid protocol Adept Rapid PCR-Free and moved it to the <i>Element Adept Library Compatibility User Guide for the Adept PCR-Free Rapid Protocol (MA-00033)</i>.</li><li>• Replaced the 2100 Bioanalyzer Instrument with a 4150 or 4200 TapeStation System.</li><li>• Added requirements for long-read sequencing of low-diversity amplicon libraries.</li><li>• Updated pooling guidelines for linear and circular libraries.</li><li>• Updated the SDS link.</li></ul>
Document # MA-00001 Rev. D	June 2023	<ul style="list-style-type: none"><li>• Added a rapid circularization protocol with consumables.</li><li>• Added rapid input requirements and adjusted the standard input volume.</li><li>• Added user-supplied reagents and the quantification procedure to the workflow diagram.</li><li>• Added a section on quantification methods.</li><li>• Updated bead drying time and cautioned against overdrying.</li><li>• Updated pipette settings to 70% of a total volume.</li><li>• Updated the link for accessing user guides.</li><li>• Moved the amplification and qPCR procedures.</li><li>• Removed the limitation of <math>\leq 384</math> libraries per pool.</li></ul>
Document # MA-00001 Rev. C	November 2022	<ul style="list-style-type: none"><li>• Updated fragment size requirements for input libraries.</li></ul>
Document # MA-00001 Rev. B	October 2022	<ul style="list-style-type: none"><li>• Replaced the Element Adept Library Compatibility Kit (catalog # 830-00003) with the Element Adept Library Compatibility Kit v1.1 (catalog # 830-00007).</li><li>• Replaced Adept Annealing Mix with Adept Annealing Mix 2.</li><li>• Replaced qPCR Standard with qPCR Standard 2.</li><li>• Replaced qPCR Mix N and qPCR Mix T with qPCR Primer Mix 2, which supports quantification of all Adept libraries.</li><li>• Removed the expected concentration from the library quantification procedure.</li><li>• Increased the range of supported input amounts.</li><li>• Added an amplification procedure and custom primer information.</li><li>• Added user-supplied consumables and equipment for QC and amplification.</li><li>• Updated the link to the compatible libraries web page.</li><li>• Updated the pooling recommendations.</li></ul>
Document # MA-00001 Rev. A	June 2022	Initial release



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