Element Biosciences

Element Adept™ Library Compatibility

Workflow Guide

FOR USE WITH

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

ELEMENT BIOSCIENCES

For Research Use Only. Not for Use in diagnostic procedures.

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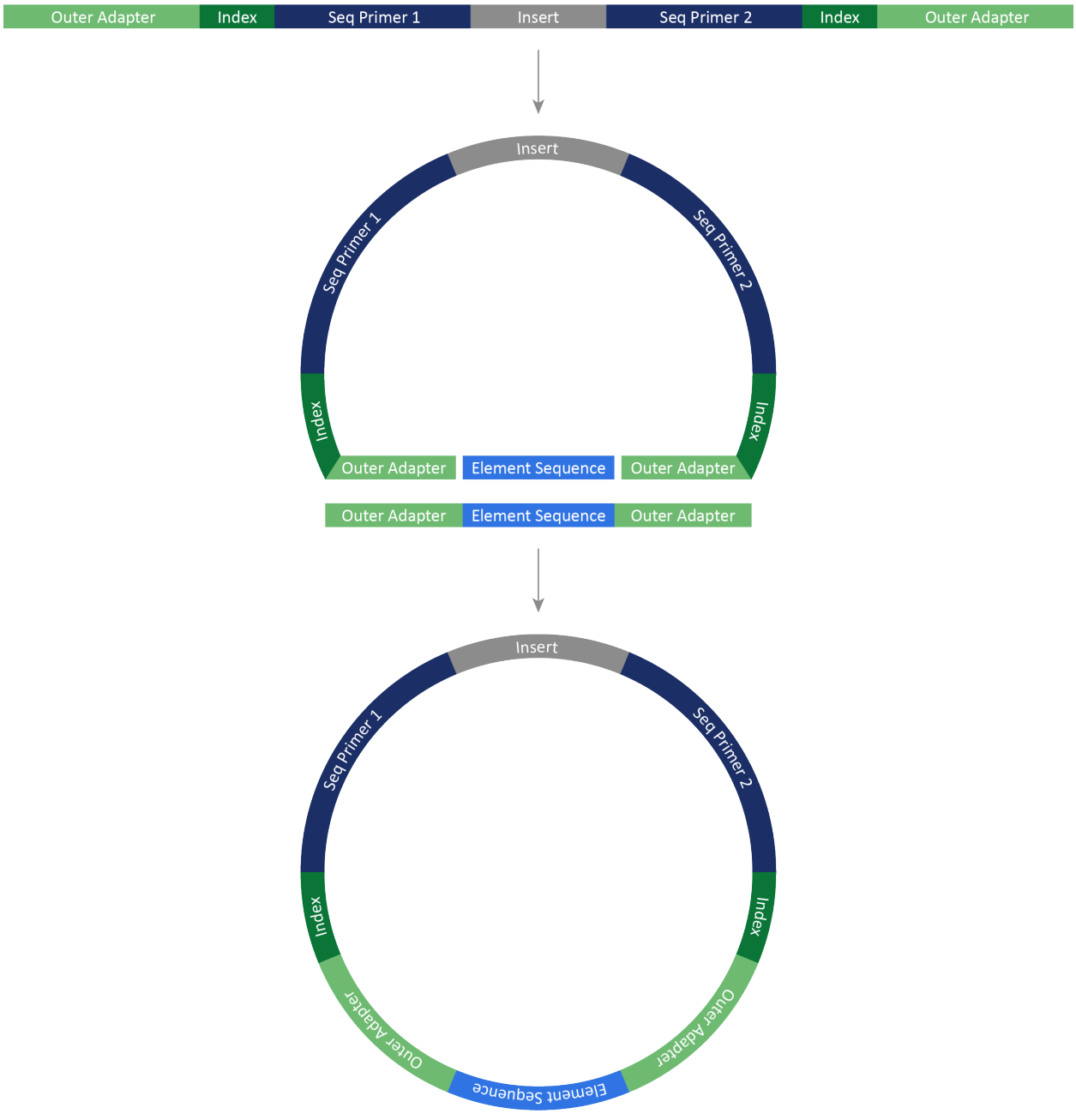
Introduction

The Element Adept Library Compatibility Workflow leverages the Element Adept Library Compatibility Kit v1.1 to adapt linear libraries from supported assays into a structure compatible with the Element AVITI™ System. Each kit supports up to 24 reactions and each reaction supports one linear library or a pool of indexed linear libraries as input.

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 that cleans up the circular library.

Figure 1: Library circularization



Protocol Options

The Adept Workflow supports rapid and standard circularization protocols. Both protocols result in a circular library and have the same guidelines for quantification and loading concentration. The difference is the method of creating the library:

- The rapid protocol uses sodium hydroxide (NaOH) to denature the library and the standard protocol uses heat.
- The rapid protocol uses ethylenediamine tetraacetic acid (EDTA) to stop enzyme activity.
- The standard protocol includes a bead cleanup procedure.
- The ligation and digestion procedures in the standard protocol include high-temperature steps.

Optional Amplification

An optional amplification procedure makes an unsupported linear library compatible with the Adept Workflow. The procedure occurs before circularization and is compatible with both protocols.

To determine whether amplification is appropriate for your library, see *Input Requirements* on page 10.

Quantification Methods

Accurate quantification of the final circular library ensures the appropriate input for sequencing. Element recommends quantitative PCR (qPCR) because it detects only circular library, ensuring consistent and accurate quantification.

Qubit is an alternative quantification method that requires testing and modifications to effectively quantify circular libraries. For more information, see the *Accurate Quantification of Circular Libraries for Sequencing on the Element AVITI System Technical Note (LT-00009)*. The technical note compares quantification methods and provides Qubit guidance.

Custom Primers

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

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 go.elembio.link/sds.

Workflow Summary

The following figures summarize the rapid and standard circularization protocols, including the approximate duration of each procedure and reagents.

- The rapid protocol takes ~40 minutes, including ~10 minutes of hands-on time.
- The standard protocol takes ~75 minutes, including ~25 minutes of hands-on time.

Rapid Circularization

	Procedure	Duration	Kit Reagents	User-Supplied Reagents
1	**Anneal splint oligos**	10 minutes	Adept Annealing Mix 2, Elution Buffer	1 M NaOH, 1 M Tris-HCl, pH7.0
2	**Circularize library**	15 minutes	Ligation Buffer, Ligation Enzyme 1, Ligation Enzyme 2	
3	**Digest linear DNA**	15 minutes	Digestion Enzyme 1, Digestion Enzyme 2	500 mM EDTA, pH 7.5
4	**Quantify library**		qPCR Primer Mix 2, qPCR Standard 2	SYBR Green PCR Master Mix; Tris-HCl 10 mM with 0.05% Tween-20, pH 8.0

Standard Circularization

	Procedure	Duration	Kit Reagents	User-Supplied Reagents
1	**Anneal splint oligos**	15 minutes	Adept Annealing Mix 2, Elution Buffer	
2	**Circularize library**	25 minutes	Ligation Buffer, Ligation Enzyme 1, Ligation Enzyme 2	
3	**Digest linear DNA**	17 minutes	Digestion Enzyme 1, Digestion Enzyme 2	
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

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 Element-supplied reagents in the kit, circularizing libraries requires the third-party materials listed in the following sections. Although the protocols specify processing libraries in a plate, 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 #
Circularization and qPCR Consumables		
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[1,2]
Agilent	Agilent High Sensitivity DNA Kit	Part # 5067-4626[2]

Supplier	Consumable	Catalog #
Bio-Rad	Microseal 'B' Film, adhesive	Catalog # MSB1001[2]
	Microseal 'C' Film, optical	Catalog # MSC1001[2]
Eppendorf	DNA LoBind Tubes, 1.5 ml	Catalog # 022431021
Teknova	10 mM Tris-HCl with 0.05% Tween-20, pH 8.0	SKU # T1485[2]
Thermo Fisher Scientific	96-well 0.8 ml deepwell storage plates	Catalog # AB0765[2,3]
	SYBR Green PCR Master Mix	Catalog # 4364346
Additional Consumables Required for the Rapid Protocol		
Teknova	1 M Tris-HCl, pH 7.0	SKU # T1070[2]
	1 N Sodium Hydroxide (NaOH)	SKU # H0224[2]
	500 mM EDTA, pH 7.5	SKU # E0375[2]
As-Needed Consumable for the Amplification Procedure		
Roche	Either PCR kit: • KAPA Library Amplification Kit with primer mix (50 reactions) • KAPA Library Amplification Kit with primer mix (250 reactions)	The corresponding catalog #: • KK2620 • KK2621

[1] Element has validated SPRIselect, 60 ml (Beckman Coulter, catalog # B23318).

[2] Consumables that you have tested and demonstrate equivalent performance are acceptable.

[3] Deepwell plates facilitate cleanup procedures. When cleaning up circular libraries, you can instead use 0.2 ml tubes or strip tubes.

User-Supplied Equipment

Supplier	Equipment	Catalog #
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

Supplier	Equipment	Catalog #
Agilent	2100 Bioanalyzer Instrument	Catalog # G2939BA[1]
Bio-Rad	CFX96 Touch Real-Time PCR Detection System	Catalog # 1845096[1]
	Either thermal cycler: • C1000 Touch Thermal Cycler • T100 Thermal Cycler	The corresponding catalog #: • Catalog # 1851197[1] • Catalog # 1861096[1]
Thermo Fisher Scientific	DynaMag-96 Side Magnet	Catalog # 12331D[1,2]
	Magnetic Stand-96	Catalog # AM10027[1,3]
QInstruments	BioShake XP	Order # 1808-0505[1,3]

[1] Equipment that you have tested and demonstrates equivalent performance is acceptable.

[2] Required for cleanup with tubes.

[3] Required for cleanup with deepwell plates.

Input Requirements

The Element Adept Library Compatibility Kit v1.1 supports a double-stranded DNA (dsDNA) linear library prepared per third-party supplier instructions. Accordingly, you must prepare a linear library and perform a quality control (QC) check ***before*** starting circularization. Prepare the linear library from RNA, complementary DNA (cDNA), or genomic DNA (gDNA).

The Element website lists supported library prep and index kits at go.elembio.link/compatible. If your kit is unsupported due to the following factors, see *Amplify Linear Library* on page 21 to amplify the library before circularization:

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

Library Amount

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

The concentration of EDTA in the library cannot exceed 1 mM. Otherwise, the input amount depends on the circularization protocol.

Table 1: Input linear library

Protocol	Concentration	Volume in 10 mM Tris Buffer pH 7.5–8.5*	Amount
Rapid circularization	4.2–20.8 nM	24 µl	~0.1–0.5 pmol
Standard circularization	6.67–16.67 nM	30 µl	~0.2–0.5 pmol

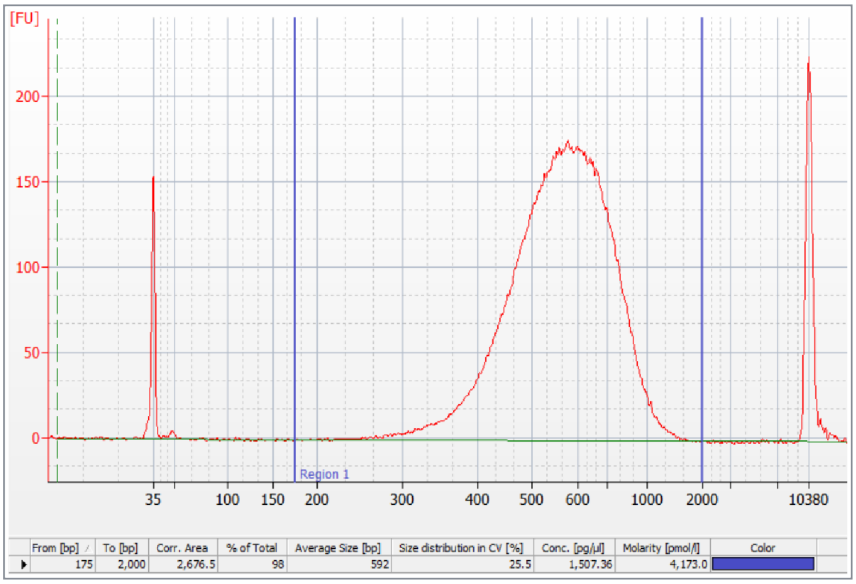
* Or similar solution

Fragment Size

Use a 2100 Bioanalyzer Instrument or equivalent 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 Bioanalyzer to confirm byproduct removal, and then requantify.

Figure 2: Example Bioanalyzer trace showing an average fragment size of 592 bp



Pooling Recommendations

An Adept reaction processes one linear library or a pool of ≤ 384 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 Workflow Guide (MA-00008)* for guidance on PhiX Control Library, which can improve library complexity and color and nucleotide balancing. Certain experiments require a spike-in.

Rapid Circularization Protocol

Follow the rapid circularization protocol steps in the order indicated using the 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. If your library is incompatible with the Adept Workflow, perform the amplification procedure. For instructions, see *Amplify Linear Library* on page 21.
3. 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.
4. If necessary, remove the library from -25°C to -15°C storage and thaw on ice.
5. Fully thaw the reagents on ice. Keep on ice until use.

Anneal Splint Oligos

The anneal splint oligo procedure denatures the input linear library with NaOH and anneals splint oligos.

1. Gather the following consumables:
    - » 1.5 ml DNA LoBind tube
    - » 96-well PCR plate
    - » Microseal ‘B’
    - » 1 M NaOH
    - » 1 M Tris-HCl, pH 7.0
    - » Adept Annealing Mix 2 (green cap)
    - » Elution Buffer
    - » Linear library
2. Make sure a dsDNA linear library is prepared per supplier instructions. Prepare 24 µl 4.2–20.8 nM linear library or indexed linear library pool so the total input amount is ~0.1–0.5 pmol.
    - » If the volume is < 24 µl, add Elution Buffer to reach 24 µl.
    - » If the volume is > 24 µl, use a speed vac or validated bead-based method to concentrate the library to 24 µl.
3. Make sure that Adept Annealing Mix 2 is fully thawed.
4. Vortex Adept Annealing Mix 2 to mix and briefly centrifuge.
5. Add 24 µl library to each well of a new PCR plate.

6. Add 3 µl 1 M NaOH to each well.
7. Mix using either method:
   - » Seal the plate and vortex.
   - » Set a pipette to 19 µl, pipette each reaction 10 times to mix, and seal the plate.
8. Incubate at room temperature for 5 minutes.
9. During incubation, 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)
1 M Tris-HCl, pH 7.0	3
Adept Annealing Mix 2	13
Total	16

10. After incubation, add 16 µl master mix to each well.
11. Mix using either method:
    - » Seal the plate and vortex.
    - » Set a pipette to 30 µl, pipette each reaction 10 times to mix, and seal the plate.
12. Briefly centrifuge the plate.
13. Place the plate in the thermal cycler.
14. Run the following ~5-minute program:

Step	Temperature	Time
Volume set to 43 µl		
Lid set to 45°C		
1	37°C	5 minutes
2	37°C	Hold

15. Remove the plate from the thermal cycler.
16. 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 ~10-minute program.

Step	Temperature	Time
Volume set to 50 µl		
Lid set to 45°C		
1	37°C	10 minutes
2	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:
   - » DNA LoBind tubes
   - » Microseal ‘B’
   - » 500 mM EDTA, pH 7.5 (EDTA)
   - » 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.

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

4. Add 4 µl master mix to each reaction.
5. Set a pipette to 38 µl 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 ~10-minute program:

Step	Temperature	Time
Volume set to 54 µl		
Lid set to 45°C		
1	37°C	10 minutes
2	4°C	Hold

9. Remove the plate from the thermal cycler.
10. Vortex EDTA to mix and briefly centrifuge.
11. Add 2 µl EDTA to each reaction to neutralize.
12. Set a pipette to 39 µl and pipette each reaction 10 times to mix.
13. Seal the plate and briefly centrifuge.
14. Transfer each reaction (56 µl) to a new DNA LoBind tube.

    —The tubes contain the final circular libraries.—
15. If you are not immediately quantifying or sequencing, cap the tubes and store at -25°C to -15°C for ≤ 15 days.
    - » For quantification instructions, see *Quantify Circular Library* on page 21.
    - » For sequencing instructions, including diluting the library to the loading concentration, see the *Element AVITI System Workflow Guide (MA-00008)*.

Standard Circularization Protocol

Follow the standard circularization protocol steps in the order indicated using the 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. If your library is incompatible with the Adept Workflow, perform the amplification procedure. For instructions, see *Amplify Linear Library* on page 21.
3. 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.
4. If necessary, remove the library from -25°C to -15°C storage and thaw on ice.
5. Fully thaw the reagents on ice. Keep on ice until use.
6. 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 µl master mix to each reaction.
5. Set a pipette to 41 µl 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 µl		
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 µl) 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 µl 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 µl, 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 µl).
   - » 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 µl 80% ethanol to each reaction and incubate for 30–60 seconds.
    b. Remove and discard ethanol.
    c. Without resuspending the beads, add another 200 µl 80% ethanol to each reaction and incubate for 30–60 seconds.
    d. Remove and discard ethanol.
    e. Using a 10 µl or 20 µl 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 µl Elution Buffer to each reaction.
14. Resuspend the beads in Elution Buffer using the applicable method:
    - » For plates, seal the plate and shake at 1500–1800 rpm for 2 minutes.
    - » For tubes, set a pipette to 22 µl, pipette content ≥ 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 µl ***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 ≤ 15 days.
    - » For quantification instructions, see *Quantify Circular Library* on page 21.
    - » For sequencing instructions, including diluting the library to the loading concentration, see the *Element AVITI System Workflow Guide (MA-00008)*.

Supplementary Procedures

The following procedures supplement both the rapid and standard circularization protocols as needed to amplify and quantify circular libraries.

Amplify Linear Library

The amplification procedure generates library ends that are compatible with the Adept Workflow.

1. Gather the following consumables:
   - » Microseal 'B'
   - » PCR plate
   - » KAPA Library Amplification Kit with primer mix
   - » Linear library
   - » Nuclease-free water
2. Remove the library from -25°C to -15°C storage and thaw on ice.
3. Download the *KAPA HiFi HotStart Library Amplification Kit Technical Data Sheet (KR0408)* from rochesequencingstore.com/wp-content/uploads/2017/10/KAPA-HiFI-Hotstart-Library-Amplification-Kit.pdf.
4. In each well of a new PCR plate, prepare 0.05 pmol linear library in 20 µl nuclease-free water.

   —The prepared library is equivalent to 2.5 nM.—
5. Follow the protocol described in the technical data sheet to amplify and clean up the library.
   - » For the denaturation, annealing, and extension steps, use 5 cycles.
   - » When cleaning up the library, elute in 30 µl elution buffer.
6. Immediately proceed to step 7 or seal the plate and store the amplified library at -25°C to -15°C for < 15 days.

   —These storage parameters override any KAPA storage parameters.—
7. Perform a QC check of 2 µl linear library:
   - » Determine the concentration with a Qubit fluorometer, qPCR, or equivalent, following vendor instructions.
   - » Determine the average fragment size and identify short byproducts in electropherograms with a 2100 Bioanalyzer Instrument or equivalent. Set the region to > 175 bp.

   —An average fragment size > 1000 bp might compromise sequencing efficiency.—
8. Proceed to *Rapid Circularization Protocol* on page 12 or *Standard Circularization Protocol* on page 16.

Quantify Circular 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 µl and includes the following components:

- 1 µl 10x qPCR Primer Mix 2
- 4 µl standard, library, or any positive or negative control diluted to assay-appropriate levels
- 5 µl 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'
   - » Tris-HCl 10 mM 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 µl 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 (µl)
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 µl for triplicate reactions.—

8. [Optional] Store unused 200 pM qPCR Standard 2 at -25°C to -15°C for ≤ 15 days. Avoid frequent freeze-thaw cycles.

9. Using two 1:100 dilutions, dilute 2 µl 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

Step	Setting
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:

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

   —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 ≥ 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 ≥ 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

Email: support@elembio.com

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

Document History

Document #	Date	Description of Change
Document # MA-00001 Rev. D	June 2023	• Added a rapid circularization protocol with consumables. • Added rapid input requirements and adjusted the standard input volume. • Added user-supplied reagents and the quantification procedure to the workflow diagram. • Added a section on quantification methods. • Updated bead drying time and cautioned against overdrying. • Updated pipette settings to 70% of a total volume. • Updated the link for accessing user guides. • Moved the amplification and qPCR procedures. • Removed the limitation of ≤ 384 libraries per pool.
Document # MA-00001 Rev. C	November 2022	• Updated fragment size requirements for input libraries.
Document # MA-00001 Rev. B	October 2022	• Replaced the Element Adept Library Compatibility Kit (catalog # 830-00003) with the Element Adept Library Compatibility Kit v1.1 (catalog # 830-00007). • Replaced Adept Annealing Mix with Adept Annealing Mix 2. • Replaced qPCR Standard with qPCR Standard 2. • Replaced qPCR Mix N and qPCR Mix T with qPCR Primer Mix 2, which supports quantification of all Adept libraries. • Removed the expected concentration from the library quantification procedure. • Increased the range of supported input amounts. • Added an amplification procedure and custom primer information. • Added user-supplied consumables and equipment for QC and amplification. • Updated the link to the compatible libraries web page. • Updated the pooling recommendations.
Document # MA-00001 Rev. A	June 2022	• Initial release

Element Biosciences
10055 Barnes Canyon Road
San Diego, CA 92121
elementbiosciences.com
Telephone: 619.353.0300
Email: info@elembio.com