Element Biosciences

User Guide

Element Elevate™ Library Prep Workflow

Third-Party Protocols

FOR USE WITH

Elevate Long UDI Adapter Kit Set A, catalog # 830-00010
Elevate Index and Adapter Kit, catalog # 830-00005

ELEMENT BIOSCIENCES
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Introduction

The Element Elevate Library Prep Workflow prepares dual-index libraries for sequencing on the Element AVITI™ System. A third-party protocol supports the Elevate Long UDI Adapter Kit Set A and Elevate Index and Adapter Kit, which integrate with third-party library prep kits. Each Elevate kit supports up to 96 reactions.

Third-party protocols replace third-party indexes and adapters with Elevate indexes and adapters to generate a linear Elevate library that is compatible with onboard circularization. To prepare Elevate libraries using only Element kits, see the applicable guide:

- *Element Elevate Library Prep Workflow User Guide for the Mechanical Protocol (MA-00004)*
- *Element Elevate Library Prep Workflow User Guide for the Enzymatic Protocol (MA-00010)*

Elevate Library Structure

An Elevate library includes Read 1 and Read 2 sequencing primers and SP5 and SP27 outer primers, regardless of kit or protocol. The following figure depicts the library structure. The indexes add three random and diverse bases to the 9 bp Index 1 sequence. RC indicates a reverse complement.

Figure 1: Components of an Elevate library

Amplification Options

The Elevate kit and corresponding adapter type, long or stubby, determine compatibility with a third-party PCR-free or PCR-plus protocol. The recommended quantification method for the final library depends on whether you amplify.

Protocol	Kits	Adapter Type	Quantification
Third-party PCR-free	• Elevate Long UDI Adapter Kit Set A • Third-party library prep kit	Long	qPCR
Third-party PCR-plus	• Elevate Index and Adapter Kit • Third-party library prep kit	Stubby	Qubit

Low-Diversity Amplicon Library

When preparing 16S or other low-diversity amplicon libraries for sequencing with a 2 x 300 kit, meet the following requirements:

- An insert size of > 200 bp
- A 1–5% spike-in of PhiX Control Library

Sequencing Compatibility

Elevate libraries prepared with the third-party protocol are compatible with both Cloudbreak™ and Cloudbreak Freestyle™ sequencing kits on the Element AVITI System. For more compatibility information, see go.elembio.link/product-compatibility.

Safety Data Sheets

When using the Elevate Long UDI Adapter Kit Set A, Elevate Index and Adapter Kit, 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.

Kit Contents and Storage

Each kit is packaged in one box and shipped on dry ice. When you receive your kit, promptly store reagents at the proper temperature.

In addition to the kits, third-party protocols require user-supplied quantification materials. Any material that you have tested and demonstrates equivalent performance is acceptable. A PCR-free protocol also requires user-supplied surface primer at 10 µM per primer and 1 nM qPCR standards with specific sequences. For library prep materials, consult the third-party documentation.

Kit	Reagent	Quantity	Storage Temperature
Elevate Long UDI Adapter Kit Set A	Elevate Long UDI Adapter Plate, Set A 15 µM in 10 µl	1	-25°C to -15°C
Elevate Index and Adapter Kit	Element Adapter Mix	1	-25°C to -15°C
	Element Biosciences Index Pairs – Unique, Set A	1	-25°C to -15°C

User-Supplied Quantification Materials

Supplier	Material	Catalog #
qPCR Quantification for PCR-Free		
General lab supplier	96-well qPCR plates	Not applicable
Bio-Rad	CFX96 Touch Real-Time PCR Detection System	Catalog # 1845096
	Microseal 'C' Film, optical	Catalog # MSC1001
Roche	KAPA SYBR FAST qPCR Kit	Catalog # 07959397001
Teknova	Tris-HCl 10 mM with 0.05% Tween-20, pH 8.0	SKU # T1485
Qubit Quantification for PCR-Plus		
Thermo Fisher Scientific	Either kit: • Qubit dsDNA BR Assay Kit • Qubit dsDNA HS Assay Kit	The corresponding part #: • Catalog # Q32853 • Catalog # Q32854
	Either fluorometer: • Qubit 3 Fluorometer • Qubit 4 Fluorometer • Qubit Flex Fluorometer	The corresponding catalog #: • Q33216 • Q33238 • Q33327

Sequences for PCR-Free Protocols

Component	Sequence
SP5	SP5 5′ Phosphate-CATGTAATGCACGTACTTTCAGGGT
SP27	SP27 5′ GATCAGGTGAGGCTGCGACGACT
qPCR standard	5’CGGAAGAGCACACGTCTGAACTCCAGTCACGTCGGCCGTTATCTCGTATGCCGTCTTCT GCTTGTCTCGCATGTAATGCACGTACTTTCAGGGTTAATTGATGAGCCACGTTAATGATAC GGCGACCACCGAGATCTACACCCACGTATTGACACTCTTTCCCTACACGACGCTCTTCCGA TCTATGTCGGAAGGTGTGCAGGCTACCGCTTGTCAACTGGCCTTAATCGAGCTGCGTCTCC ATCTCGAGACCAGTCATACGATACCATGTTGACTCTGTTCTATTGGCGTGCTGGATTGGCT CACCAGACACCTTCCGACATCTGTCTCTTATACACATCTCCGAGCCCACGAGACTCGCCTTA ATGTGCTAACGCGACTTCTCGTTGACTGGTCTCTATTCCGCCTCAAAACGAGCATCCGTCG GGCAGCTCAGAAATCAAGTCGTCGCAGCCTCACCTGATCTTAGATCGCTCGTCGGCAGCGT CAGATGTGTA-3’

Library Prep Guidelines

Follow third-party library prep guidelines for input, plexity, and yield. When preparing the libraries, use the volumes and other parameters indicated in the third-party instructions.

Supplement or replace the third-party materials with the kits and materials listed in *Kit Contents and Storage* on page 6.

PCR-Free Protocol

When performing a third-party PCR-free protocol, replace the third-party adapters and indexes with the long adapters in the Elevate Long UDI Adapter Kit Set A. Calculate the final concentration of each adapter in the ligation.

Element Reagent	Starting Concentration
Long adapter	15 µM

PCR-Plus Protocol

When performing a third-party PCR-plus protocol, replace the third-party PCR primers and adapters with the corresponding Element reagents from the Elevate Index and Adapter Kit. Calculate the final concentration of each primer in the amplification.

Third-Party Reagent	Element Reagent	Element Reagent Starting Concentration
PCR primers	Element Index Pair – Unique	10 µM per primer
Adapter	Element Adapter Mix	15 µM

Plate Preparation

When preparing an Elevate plate, apply the following best practices to avoid cross-contamination and otherwise ensure proper usage. For more information, see *Plate Layout* on page 9.

- Check the plate for defects. Do not use a plate with a loose seal, cracks, or chips.
- Fully thaw the plate on ice.
- Before use, confirm full thawing, centrifuge at 1500 rpm for 30 seconds, and clean the seal with an alcohol wipe.
- Pierce the seal covering the desired wells only. Each well is single-use.
  - » Avoid splashing the liquid in the wells.
  - » Change tips between each well piercing.
- After adding adapters to a library, pipette 10 times to mix.
  - » Change tips between each well.
  - » Pipette carefully to avoid well-to-well adapter transfer.

Pooling Libraries

Pooling combines libraries into one pool for multiplex sequencing. After sequencing, index sequences identify each library for demultiplexing and analysis.

When pooling, uniquely index each library and combine in a new 1.5 ml LoBind tube. Pool libraries with similar characteristics:

- Pool libraries that require the same run parameters.
- 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.
- See the *Run Manifest Documentation* for index sequences.

Plate Layout

The Elevate Workflow supports an adapter plate and an index plate. Although the contents are different, both plates have the same 96-well layout with 001 in well A1 through 096 in well H2.

- Each well of the adapter plate contains a ligation-based long adapter and unique dual indexes.
- Each well of the index plate contains a pair of index primers to add indexes via PCR. The corresponding stubby adapters are packaged separately.

Figure 2: Layout for both plate types

Adapter Plate Contents

The long adapter in each adapter plate well consists of two oligos. The oligos contain the PCR primer and index sequences: one oligo includes a unique Index 1 sequence and the other includes a unique Index 2 sequence. Thus, each library is tagged with a distinct index pair for unique dual indexing.

The set A plate provides adapters ELP_UA_001 through ELP_UA_096. The name of each adapter depends on the well. Well A1 contains ELP_UA_001, well B1 contains ELP_UA_002, and so on through well H12, which contains ELP_UA_096.

Index Plate Contents

Each well in the index plate contains two primers for dual indexing: one Index 1 primer and one Index 2 primer.

Set A provides index pairs EIP_UA_001 through EIP_UA_096. Well A1 contains index pair EIP_UA_001, which consists of primers Index1_001 and Index2_001. Well B1 contains index pair EIP_UA_002, which consists of primers Index1_002 and Index2_002, and so on.

Index Color Balance

Unique dual indexes in the adapter plate ***do not*** require index color balance. When using the index plate, however, select index primer pairs with diverse sequences to optimize color balance in a run.

The arrangement of index plate wells facilitates index selection and targets ~25–75% color balance. The following sections recommend combinations of index primer pairs.

Four-Plex Pool

For a 4-plex pool, use index plate wells A1–D1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	001	009	017	025	033	041	049	057	065	073	081	089
B	002	010	018	026	034	042	050	058	066	074	082	090
C	003	011	019	027	035	043	051	059	067	075	083	091
D	004	012	020	028	036	044	052	060	068	076	084	092
E	005	013	021	029	037	045	053	061	069	077	085	093
F	006	014	022	030	038	046	054	062	070	078	086	094
G	007	015	023	031	039	047	055	063	071	079	087	095
H	008	016	024	032	040	048	056	064	072	080	088	096

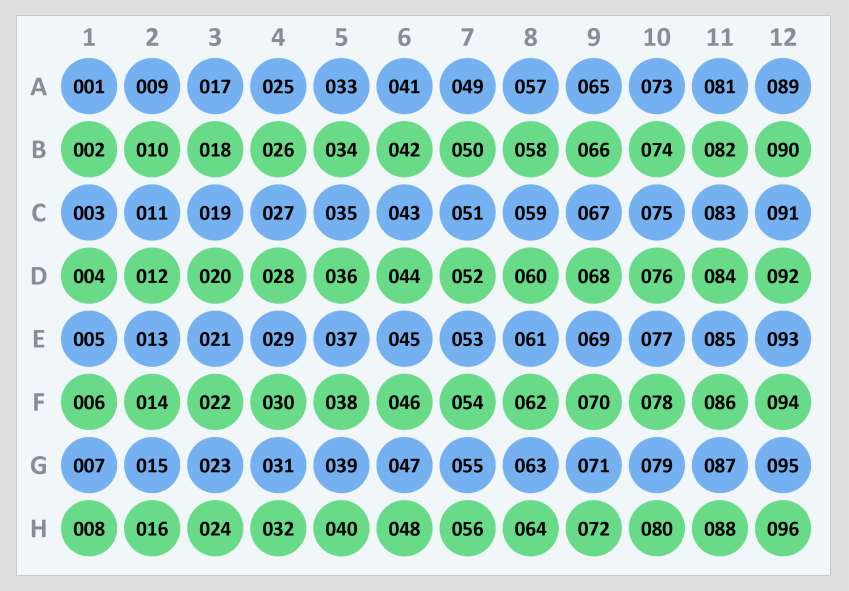
Eight-Plex Pool

For an 8-plex pool, use any index plate column except column 6. Column 6 is optimized for > 16-plex pools.

	1	2	3	4	5	6	7	8	9	10	11	12
A	001	009	017	025	033	041	049	057	065	073	081	089
B	002	010	018	026	034	042	050	058	066	074	082	090
C	003	011	019	027	035	043	051	059	067	075	083	091
D	004	012	020	028	036	044	052	060	068	076	084	092
E	005	013	021	029	037	045	053	061	069	077	085	093
F	006	014	022	030	038	046	054	062	070	078	086	094
G	007	015	023	031	039	047	055	063	071	079	087	095
H	008	016	024	032	040	048	056	064	072	080	088	096

Twelve-Plex Pool

For a 12-plex pool, use any row in the index plate.



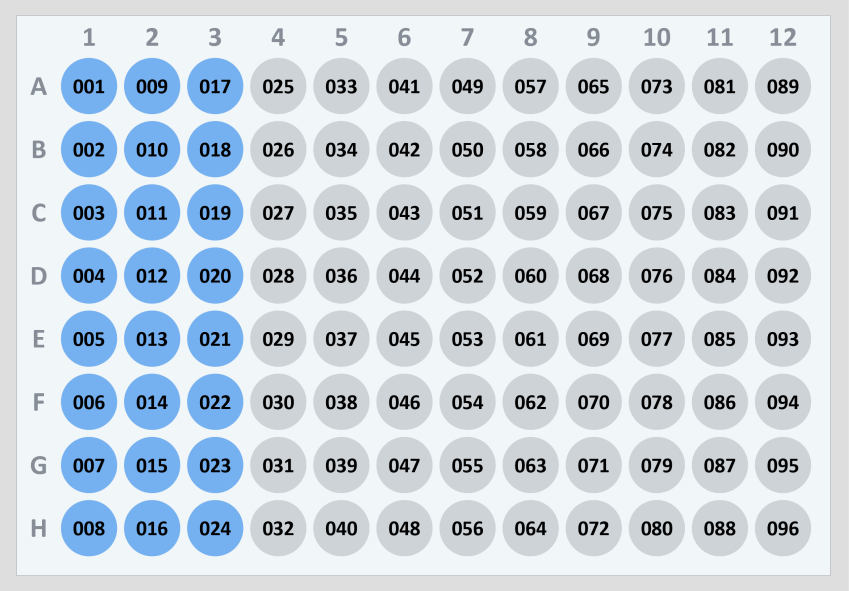
	1	2	3	4	5	6	7	8	9	10	11	12
A	001	009	017	025	033	041	049	057	065	073	081	089
B	002	010	018	026	034	042	050	058	066	074	082	090
C	003	011	019	027	035	043	051	059	067	075	083	091
D	004	012	020	028	036	044	052	060	068	076	084	092
E	005	013	021	029	037	045	053	061	069	077	085	093
F	006	014	022	030	038	046	054	062	070	078	086	094
G	007	015	023	031	039	047	055	063	071	079	087	095
H	008	016	024	032	040	048	056	064	072	080	088	096

Sixteen-Plex Pool

For a 16-plex pool, use index plate wells A1–H2.

Twenty-Four-Plex Pool

For a 24-plex pool, use index plate wells A1–H3.



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-00043 Rev. C	March 2024	• Added primer concentration and qPCR standard concentration. • Added requirements for low-diversity amplicon libraries. • Added information about sequencing kit compatibility.
Document # MA-00043 Rev. B	November 2023	• Added statement that the Elevate Workflow is not compatible with 2 x 300 sequencing of low-diversity amplicon libraries.
Document # MA-00043 Rev. A	September 2023	• Initial release

Element Biosciences
10055 Barnes Canyon Road, Suite 100
San Diego, CA 92121
elementbiosciences.com
Telephone: 619.353.0300
Email: info@elembio.com

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