

Quick Guide for the Enzymatic Protocol

Element Elevate™ Library Prep

Introduction

This quick guide provides concise instructions for the Elevate Enzymatic Library Prep Kit and Elevate Long UDI Adapter Kit Set A. For comprehensive information and detailed instructions, see the *Elevate Library Prep User Guide for the Enzymatic Protocol (MA-00010)*.

Fragment Sample and A-Tail

1. Start the following program. **Pause** at step 1.

Step	Temperature	Time
Volume set to 50 µl		
Lid set to 85°C		
1	4°C	Hold
2	See next table	See next table
3	65°C	30 minutes
4	4°C	Hold

Target Library Size	Temperature	Time
250–450 bp	37°C	20 minutes
450–700 bp	30°C	4 minutes
700–900 bp	30°C	2.5 minutes

2. Dilute gDNA in 40 µl nuclease-free water. Keep on ice.
 - » For the PCR-free workflow, dilute 200–500 ng.
 - » For the PCR-plus workflow, dilute 1–500 ng.
3. Combine the following reagents, allowing 10–15% overage.

Reagent	Volume per Sample
ELP EZ Frag/AT Buffer	4 µl
ELP EZ Frag/AT Enzyme Mix	6 µl

4. Place a PCR plate on ice.
5. Add 10 µl master mix.
6. Add 40 µl ice-cold sample.
7. Resume the paused program.

Ligate Adapters

1. For input 1–10 ng, use low TE buffer to dilute 15 µM adapters to 3 µM.
2. Add 5 µl adapter or diluted adapter.
3. Add 20 µl ELP EZ Ligation Master Mix to each reaction.
4. Run the following program.

Step	Temperature	Time
Volume set to 75 µl		
Heated lid turned off		
1	20°C	30 minutes
2	4°C	Hold

Clean Up Library

1. Transfer the entire volume to a deepwell plate or tubes.
2. Add 60 µl beads (0.8x) to each reaction.
3. Mix beads and library:
 - » For a plate, shake at 1800 rpm for 2 minutes.
 - » For tubes, pipette 10 times.
4. Incubate at room temperature for 5 minutes.
5. Place on the magnet and wait until supernatant clears.
6. Remove the entire volume of supernatant.
7. Wash the content of each well or tube:
 - a. Add 200 µl 80% ethanol and incubate 30 seconds.
 - b. Remove and discard ethanol.
 - c. Repeat steps **a–b** one time.
 - d. Remove residual ethanol.
8. Air-dry unsealed or uncapped 2–3 minutes.
9. Remove the plate or tubes from the magnet.
10. Add 53 µl low TE buffer.
11. Resuspend the beads:
 - » For plates, shake at 1800 rpm for 2 minutes.

- » For tubes, pipette ≥ 10 times.
12. Incubate at room temperature for a total of 4 minutes.
 13. Place on the magnet and wait until supernatant clears.
 14. Transfer 50 μl library to a deepwell plate or tubes.

Perform Size Selection

1. Add the applicable volume of beads.

Target Library Size	Bead Volume	Ratio
250–450 bp	30 μl	0.6x
450–700 bp	25 μl	0.5x
700–900 bp	23 μl	0.46x

2. Mix beads and library:
 - » For a plate, shake at 1800 rpm for 2 minutes.
 - » For tubes, pipette 10 times.
3. Incubate at room temperature for 5 minutes.
4. Place on the magnet and wait until supernatant clears.
5. Transfer supernatant to a deepwell plate or tubes.
6. Add the applicable volume of beads.

Target Library Size	Bead Volume	Supernatant Volume	Final Ratio
250–450 bp	10 μl	80 μl	0.8x
450–700 bp	10 μl	75 μl	0.7x
700–900 bp	8 μl	73 μl	0.62x

7. Mix beads and library:
 - » For a plate, shake at 1800 rpm for 2 minutes.
 - » For tubes, pipette 10 times.
8. Incubate at room temperature for 5 minutes.
9. Place on the magnet and wait until supernatant clears.
10. Remove and discard supernatant.
11. Wash the content of each well or tube:
 - a. Add 200 μl 80% ethanol and incubate 30 seconds.
 - b. Remove and discard ethanol.
 - c. Repeat steps **a–b** one time.
 - d. Remove residual ethanol.
12. Air-dry unsealed or uncapped 2–3 minutes.
13. Remove the plate or tubes from the magnet.
14. Add 23 μl low TE buffer.
15. Resuspend the beads:
 - » For a plate, shake at 1800 rpm for 2 minutes.
 - » For tubes, pipette ≥ 10 times.

16. Incubate at room temperature for 5 minutes.
17. Place on the magnet and wait until supernatant clears.
18. Transfer 20 μl library to a PCR plate for PCR-plus or a DNA LoBind tube for PCR-free.



SAFE STOPPING POINT

Store at 2°C to 8°C for ≤ 4 days.

Amplify Library

1. For PCR-free, skip amplification and go to [Prepare Dilutions for qPCR on page 3](#).
2. Combine the following reagents, allowing 10–15% overage.

Reagent	Volume per Library
ELP 2X PCR Master Mix	25 μl
ELP PCR Primer Mix	5 μl

3. Add 30 μl master mix.
4. Run the following program.

Temperature	Time	Number of Cycles
Volume set to 50 μl		
Lid set to 105°C		
98°C	45 seconds	1
98°C	15 seconds	See next table
60°C	30 seconds	
72°C	30 seconds*	
72°C	60 seconds	1
12°C	Hold	Not applicable


* For inserts ≥ 500 bp, increase to 45 seconds.

gDNA Input	Number of Cycles
500 ng	3–4
100 ng	5–7
50 ng	7–8
10 ng	9–11
5 ng	11–12
1 ng	13–14

Clean Up Amplified Library

1. Transfer the entire library to a deepwell plate or tubes.
2. Add 40 μl beads (0.8x).
3. Mix beads and library:
 - » For a plate, shake at 1800 rpm for 2 minutes.
 - » For tubes, pipette 10 times.

4. Incubate at room temperature for a total of 5 minutes.
5. Place on the magnet and wait until supernatant clears.
6. Remove the entire volume of supernatant.
7. Wash the content of each well:
 - a. Add 200 µl 80% ethanol and incubate 30 seconds.
 - b. Remove and discard ethanol.
 - c. Repeat steps **a–b** one time.
 - d. Remove residual ethanol.
8. Air-dry unsealed 2–3 minutes.
9. Remove the plate or tubes from the magnet.
10. Add 22 µl low TE buffer.
11. Resuspend the beads:
 - » For plates, shake at 1800 rpm for 2 minutes.
 - » For tubes, pipette ≥ 10 times.
12. Incubate at room temperature for a total of 4 minutes.
13. Place on the magnet and wait until supernatant clears.
14. Transfer 20 µl library to a PCR plate.
15. Proceed to [Perform Qubit Quantification](#).

 **SAFE STOPPING POINT**
Store at 2°C to 8°C for ≤ 4 days or -25°C to -15°C for ≤ 3 weeks.

Prepare Dilutions for qPCR

1. Set aside ~20 µl dilution buffer (10 mM Tris-HCl, pH 8.0 with 0.05% Tween 20) as an NTC.
2. Combine the following reagents and label **100 pM qPCR Standard 2**.

Reagent	Volume per Reaction
Dilution buffer	18 µl
1 nM qPCR Standard 2	2 µl

3. From 100 pM qPCR Standard 2, make 1:10 serial dilutions.

Standard	Concentration (pM)
Std 1	10
Std 2	1
Std 3	0.1
Std 4	0.01
Std 5	0.001
Std 6	0.0001

4. Using two 1:100 dilutions, dilute 2 µl library 1:10,000 in dilution buffer.

Prepare Master Mix and Assay Plate

1. Combine the following reagents, allowing for triplicates and 10–15% overage.

Reagent	Starting Concentration	Volume per Reaction
KAPA SYBR FAST qPCR Master Mix Universal	2x	5 µl
ELP PCR Primer Mix	20x	0.5 µl
Nuclease-free water	Not applicable	0.5 µl

2. Add 6 µl master mix to an assay plate.
3. Add 4 µl NTC, standard dilutions, or library dilutions to the master mix.
4. Repeat steps **2–3** to prepare triplicates.

Perform a qPCR Run

1. Run the following program on the qPCR instrument.

Step	Setting
	Volume set to 10 µl
	Lid set to 105°C
Activation	5 minutes at 95°C
PCR 35 cycles	30 seconds at 95°C
	45 seconds at 60°C*
	Plate read
Melt curve	65°C to 95°C with increments of 1°C every 5 seconds
	Plate read after each temperature step

* For libraries > 700 bp, increase to 90 seconds.

2. Analyze the results:

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

3. [Optional] Size 1 µl library using TapeStation.
4. Adjust the concentration:

$$\text{adjusted concentration} = (396 / \text{library size}) * \text{input library concentration in nM}$$

Perform Qubit Quantification

1. Quantify 2 µl library using Qubit.
2. Size 1 µl library using TapeStation.
3. Calculate the molar concentration:

$$\text{nM} = (\text{ng}/\mu\text{l}) / (660 * \text{average library size}) * 1,000,000$$