

Quick Guide for the Adept™ Rapid PCR-Free Protocol

Element Adept Library Compatibility Workflow

Introduction

This quick guide provides concise instructions for the Adept PCR-Free Protocol, which uses the Element Adept Library Compatibility Kit v1.1. For comprehensive information and detailed instructions, see the *Element Adept Library Compatibility Workflow User Guide for the Rapid PCR-Free Protocol (MA-00033)*.

Anneal Splint Oligos

- Using 10 mM Tris buffer pH 7.5–8.5 or similar, prepare 24 µl 4.2–20.8 nM linear library.
- Add 24 µl library to a PCR plate.
- Add 3 µl 1 M NaOH.
- Incubate at room temperature for 5 minutes.
- Combine the following reagents, allowing 10–15% overage.

Reagent	Volume per Reaction (µl)
1 M Tris-HCl, pH 7.0	3
Adept Annealing Mix 2	13

- Add 16 µl master mix.
- Run the following program:

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

Circularize Library

- Combine the following reagents, allowing 10–15% overage.

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

- Add 7 µl master mix to each reaction and mix.
- Run the following program.

Step	Temperature	Time
Volume set to 50 µl		
Lid set to 45°C		
1	37°C	10 minutes
2	4°C	Hold

Digest Linear DNA

- Combine the following reagents, allowing 10–15% overage.

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

- Add 4 µl master mix.
- Run the following program:

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

- Add 2 µl 500 mM EDTA, pH 7.5.
- Transfer to a DNA LoBind tube.
- Proceed to [Quantify Library](#) and sequencing or store at -25°C to -15°C for ≤ 15 days.

Quantify Library

Prepare Dilutions

- Set aside ~20 µl dilution buffer (10 mM Tris-HCl, pH 8.0 with 0.05% Tween 20) as an NTC.
- Combine the following reagents and label **200 pM qPCR Standard 2**.

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

- From 200 pM qPCR Standard 2, make 1:10 serial 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

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

Prepare Master Mix and Assay Plate

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

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

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

Perform a qPCR Run

- Run the following program on the qPCR instrument.

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

- Analyze the results:

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