

SEQQX® OnePot Pro DNA Library Prep Kit

Cat. no. LM801003

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SEQQX® OnePot Pro DNA Library Prep Kit

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Product Information

Cat. No.	Product name	Pack size
LM801003	SEQQX® OnePot Pro DNA Library Prep Kit	8 T
		24 T
		96 T
		96 T (automation-ready plate format)

Source item numbers: 12972ES08 / 12972ES24 / 12972ES96 / 12972ES97 (plate format). One test (T) corresponds to one library-preparation reaction. Please state the required pack size when ordering.

Kit Contents

Component	8 T	24 T	96 T	96 T (plate)
Smearase® Buffer 4.0	80 µL	240 µL	960 µL	8 × 150 µL
Smearase® Enzyme 4.0	80 µL	240 µL	960 µL	8 × 150 µL
Ligation Enhancer 4.0	240 µL	720 µL	3 × 960 µL	8 × 420 µL
Rapid DNA Ligase 4.0	80 µL	240 µL	2 × 480 µL	8 × 140 µL
2× Ultima HF Amplification Mix	200 µL	600 µL	3 × 800 µL	8 × 340 µL

If the recommended short adapters are used, no separate Primer Mix is required. If long adapters are used, a Primer Mix is required for amplification but is not included in this kit and must be ordered separately (Cat. no. 12190, DNA Library Prep Primer Mix for Illumina®; Cat. no. 12191, DNA Library Prep Primer Mix for MGI®).

Storage

All components of the kit can be stored for up to 12 months at –25 °C to –15 °C.

Introduction

The SEQQX® OnePot Pro DNA Library Prep Kit is a next-generation enzymatic-fragmentation library preparation kit for the Illumina® and MGI® high-throughput sequencing platforms. Compared with conventional library preparation methods, this kit uses a high-quality fragmentation enzyme that eliminates the laborious sonication step. DNA fragmentation and end-repair are combined into a single module, substantially reducing both library preparation time and cost. The kit is suitable for 1 ng–1 µg of standard animal, plant or microbial genomic DNA and other sample types, giving fairly uniform fragmentation across different species with minimal inter-species variability. DNA fragmentation, end repair and dA-tailing are completed in a single tube. The kit can be paired with Illumina® or MGI® adapters and primers for sequencing on the corresponding platform.

Features

1. Compatible with 1 ng–1 µg of genomic DNA, full-length cDNA and similar sample types.

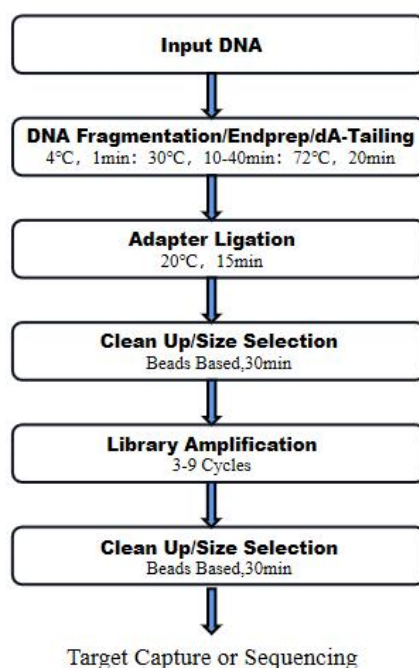
2. High-quality fragmentation enzyme randomly cleaves double-stranded DNA with low sequence-dependent bias.
3. Fragmentation, end repair and dA-tailing are completed in a single step.
4. High-fidelity amplification mix with strong amplification efficiency markedly improves library quality and yield.
5. Validated across a wide range of species and sample types.
6. Stringent batch-to-batch performance and stability quality control.

Notes Please be sure to read this precaution before using the kit.

1. This product is for research use only. Not for use in diagnostic procedures.
2. For your safety and health, wear a laboratory coat and disposable gloves when handling this product.
3. Thaw all kit components at room temperature before use, mix thoroughly by inverting several times, then briefly centrifuge and keep on ice. For the 96 T plate format (12972ES97), vortex to mix and centrifuge using a horizontal/swing-bucket centrifuge (Cat. no. 80162ES recommended) before use.
4. Mix reaction solutions by gentle pipetting or gentle vortexing; vigorous vortexing may reduce library yield.
5. To avoid cross-contamination, use filter tips and change tips between different samples.
6. Carry out all reaction steps in a thermal cycler with a heated lid, pre-heated to the reaction temperature before use.
7. PCR products are highly prone to aerosol contamination if handled improperly, which can compromise result accuracy. Physically separate the reaction set-up area from the post-PCR/clean-up area, use dedicated pipettes, and regularly clean all work areas (e.g. with 0.5% sodium hypochlorite or 10% bleach) to maintain a clean working environment.

Protocol

A. Workflow Overview



B. DNA Fragmentation / End Repair / dA-Tailing

This step fragments genomic DNA while simultaneously performing end repair and dA-tailing.

Component	Volume (μL)
Input DNA	x
Smearase® Buffer 4.0	10
Smearase® Enzyme 4.0	10
ddH ₂ O	up to 60

Lid / Temperature	Time
Heated lid 105 °C	On
4 °C	1 min*
30 °C	10–40 min**
72 °C	20 min
4 °C	Hold

* To ensure reproducible fragmentation and avoid over-digestion, the programme may be pre-set to hold at 4 °C; load the PCR tubes once the block has reached 4 °C.

** For intact, high-quality genomic DNA, use the fragmentation-time reference table below. These are recommended starting points; fine-tune empirically for your own sample type and desired insert size.

Fragmentation temperature	10 min	15 min	20 min	25 min	30 min	40 min
30 °C	700 bp	300–500 bp	200–400 bp	150–300 bp	150–250 bp	150–200 bp
35 °C	500–600 bp	200–300 bp	150–250 bp	150–200 bp	150 bp	130 bp

Approximate insert-size distributions obtained at each fragmentation time/temperature combination. See Application Cases (Figures 2 and 3) for the corresponding Bioanalyzer traces.

Protocol (continued)

C. Adapter Ligation

This step ligates Illumina® or MGI® adapters to the ends of the product from step B. Dilute the adapter to the appropriate concentration according to the recommended tables below, based on the Input DNA amount.

Component	Volume (μL)
dA-tailed DNA (product of step B)	60
Ligation Enhancer 4.0	30*
Rapid DNA Ligase 4.0	10
PE Adapter**	5
ddH ₂ O	up to 110

* Ligation Enhancer is viscous; invert and vortex thoroughly, then briefly centrifuge before use.

** Standard adapter concentrations are 15 μM for the Illumina® platform and 10 μM for the MGI® platform; dilute according to the Input DNA amount so that the adapter addition volume remains fixed at 5 μL (see tables below). "PE Adapter" denotes the universal short adapter without an index sequence; if long adapters are used, substitute the corresponding indexed long adapter.

Lid / Temperature	Time
Heated lid	Off
20 °C	15 min
4 °C	Hold

If Input DNA amount is low and ligation efficiency appears suboptimal, the ligation time may be doubled.

Recommended adapter dilution - Illumina® platform

Input DNA	Standard adapter dilution	Adapter concentration
1 ng	30×	0.5 μM
10 ng	7.5×	2 μM
100 ng	3×	5 μM
1000 ng	1.5×	10 μM

Recommended adapter dilution - MGI® platform

Input DNA	Standard adapter dilution	Adapter concentration
1 ng	20×	0.5 μM
10 ng	5×	2 μM
100 ng	2×	5 μM
1000 ng	Undiluted	10 μM

D. Post-Ligation Clean-Up / Size Selection

Size selection can be performed either after adapter ligation or after library amplification. If the Input DNA amount is ≥ 50 ng, selection after ligation is an option. Because Ligation Enhancer contains a high concentration of PEG that interferes with double-sided size selection, a clean-up step must precede any double-sided selection performed at this stage; if size selection is instead performed after library amplification, double-sided bead selection may be carried out directly.

Standard clean-up: add 88 μL (0.8×, Beads:DNA = 0.8:1) Hieff NGS®-equivalent DNA Selection Beads directly to the ligation product, incubate, separate on a magnetic rack, wash twice with freshly prepared 80% ethanol, air-dry briefly and elute in ddH₂O.

Recommended bead ratios for double-sided size selection

Library insert size	150–250 bp	200–300 bp	300–400 bp	400–500 bp	500–600 bp
Library fragment size	250–350 bp	350–450 bp	450–500 bp	500–550 bp	550–650 bp
Round 1 volume ratio (Beads:DNA)	0.80×	0.70×	0.60×	0.55×	0.50×
Round 2 volume ratio (Beads:DNA)	0.20×	0.20×	0.20×	0.15×	0.15×

Ratios are relative to the ligation-product volume carried into size selection (typically 100 μL). Example: for a 250 bp insert, round 1 uses $0.70 \times 100 \mu\text{L} = 70 \mu\text{L}$ of beads, and round 2 uses $0.20 \times 100 \mu\text{L} = 20 \mu\text{L}$.

Protocol (continued)

E. Library Amplification

This step amplifies the purified or size-selected, adapter-ligated product by PCR. The number of cycles must be controlled carefully: too few cycles give low library yield, while too many increase library bias, duplication rate, chimera formation and amplification-induced errors.

Component	Volume (μL)
Adapter-ligated DNA (product of step D)	20
2× Ultima HF Amplification Mix	25
Primer Mix*	5

* Use the Primer Mix matching the sequencing platform and adapter type in use. With complete Illumina® long adapters, use DNA Library Prep Primer Mix for Illumina® (Cat. no. 12190); with MGI® single-end long adapters, use DNA Library Prep Primer Mix for MGI® (Cat. no. 12191). If incomplete (short) adapters were used on either platform, amplify with the Index Primer supplied in the corresponding adapter kit.

Temperature	Time	Cycles
98 °C	45 sec	1
98 °C	15 sec	see recommended cycle table below
60 °C / 72 °C	30 sec / 30 sec	
72 °C	1 min	
4 °C	Hold	–

Input DNA	Recommended PCR cycles for 1 μg library yield
1000 ng	2–4
500 ng	2–4
250 ng	4–6
100 ng	5–7
50 ng	7–9
1 ng	12–14

If incomplete adapters were used, add 1–3 extra cycles to complete the adapter sequence. If size selection was performed during library construction, use the higher end of the recommended cycle range.

F. Post-Amplification Clean-Up / Size Selection

Purify the amplified library using the standard clean-up procedure described in step D, with a 1.0× bead ratio (Beads:DNA = 1:1). If size selection is required, follow the double-sided selection procedure described in step D.

G. Library Quality Analysis

Finished libraries are typically evaluated for concentration and size distribution. For concentration, use a double-stranded-DNA fluorescent dye-based method (e.g. Qubit®, PicoGreen®) or qPCR-based absolute quantification; spectrophotometric methods (e.g. NanoDrop®) are not recommended, as they cannot distinguish fully adapter-ligated, sequenceable libraries from singly-ligated or unligated background. qPCR-based absolute quantification is preferred, as it specifically quantifies library molecules with adapters on both ends. For size distribution, use a capillary electrophoresis or microfluidics-based instrument such as the Agilent Bioanalyzer 2100.

Application Cases

1. Insert-Size Distribution at Different Fragmentation Times

Using 250 ng of standard genomic DNA as template, fragmentation was performed at 30 °C or 35 °C for 10, 15, 20, 25, 30 or 40 min. Fragmented products were purified with 2.0× beads, eluted in 15 μL ddH₂O, quantified by Qubit®, and the recovered insert-size distribution was analysed by Bioanalyzer.

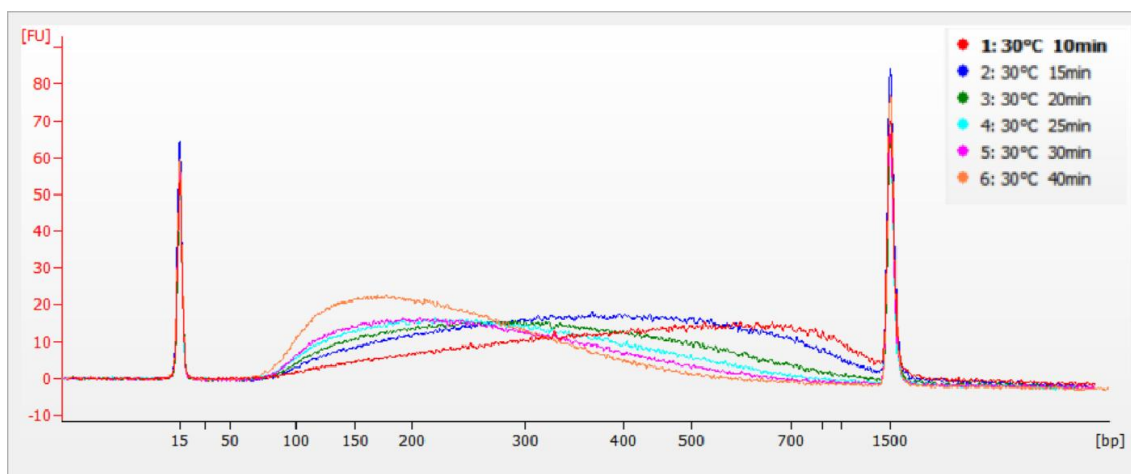


Figure 2. Bioanalyzer traces at 30 °C for different fragmentation times (10–40 min).

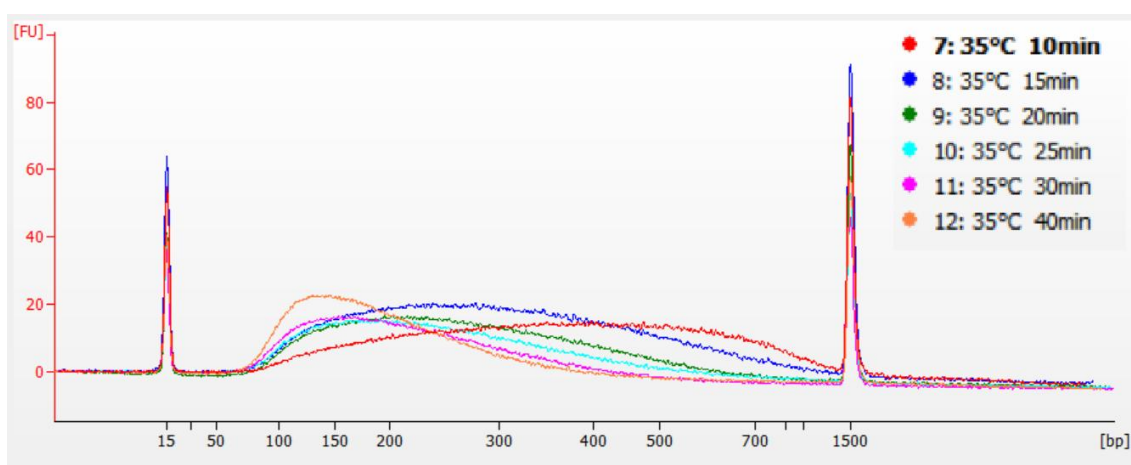


Figure 3. Bioanalyzer traces at 35 °C for different fragmentation times (10–40 min).

At 30 °C, longer fragmentation times progressively shift the insert-size distribution to smaller fragments, with a median insert size of roughly 700 bp at 10 min down to roughly 150–200 bp at 40 min. At 35 °C, the same trend is observed over a shorter time scale, reaching a median insert size of roughly 130 bp by 40 min. See the fragmentation-time reference table in Protocol section B for approximate target insert sizes.