Watchmaker DNA Library Prep Kit with Fragmentation for library construction on the Singular G4™ Platform

Table of Contents

Introduction

Our Watchmaker DNA Library Prep Kit with Fragmentation, combined with the G4™ Universal Adapters, provides an efficient library preparation solution for sequencing on the Singular Genomics G4™ Platform. Watchmaker DNA Library Prep Kits with Fragmentation offer highly efficient conversion of DNA into sequencing-ready libraries. The chemistry and streamlined, single-tube, automation-friendly protocol have been optimized to produce libraries while minimizing bias and artifacts. When combined with the G4 Sequencing Platform, the accuracy, speed, flexibility and power enable multiple sequencing applications with high quality.

Workflow Details

The Watchmaker DNA Library Prep Kit with Fragmentation provides an integrated enzymatic fragmentation, end-repair, and A-tailing module which produces fragmented, A-tailed DNA in a single reaction with a high degree of consistency. The ligation module adds the Singular stem-loop adapters to the DNA fragments, which is the first step to introduce the nucleotide sequences necessary for cluster generation and sequencing. The Equinox Amplification Master Mix (2X) combined with the Singular primers, enables library amplification with high fidelity and efficiency.

The Singular adapters and primers used during library preparation with the Watchmaker DNA Library Prep Kit with Fragmentation add several functional nucleotide tags to each end of the inserts to be sequenced on the G4 instrument (Figure 1). At the 5' ends, Singular's proprietary platform sequences S1 and S2 are attached as anchors for the formation of clusters on the flow cell. The SP1 and SP2 tags, which are identical to the SP1 and SP2 sequencing primers used in many existing applications, are positioned directly adjacent to the insert. When constructing libraries for multiplexed reads, index 1 and index 2 sequences are placed in between S1 and SP1, and S2 and SP2, respectively.

Required Materials

The following consumables are required to perform library construction:

Description	Part Numbers	Consumable	Supplier
DNA Library Prep Kit with Enzymatic Fragmentation	7K0022-024 (24 rxns) 7K0022-096 (96 rxns)	Watchmaker DNA Library Prep Kit with Fragmentation (w/o primers)	Watchmaker Genomics
Adapters, Primers, and Cleave Reagents (Only one row is required)	700110	SG Universal Library Prep Adapter + UDI Primers Set (1 – 96)	Singular Genomics
	700111	SG Universal Library Prep Adapter + UDI Primers Set A (1 – 24)	Singular Genomics
	700112	SG Universal Library Prep Adapter + UDI Primers Set B (25 – 48)	Singular Genomics
	700118 and 700119	G4 Universal Library Prep Adapter (24 rxns) G4 Non-Indexed Library Prep Primers (24 rxns)	Singular Genomics
	700117 and 700119	G4 Universal Library Prep Adapter (96 rxns) G4 Non-Indexed Library Prep Primers (24 rxns)	Singular Genomics
SPRI Purification Beads	A63881	AMPure XP[1]	Beckman Coulter

[1]Other SPRI bead brands may be used, assuming they are nuclease-free and deliver equivalent sizing performance.

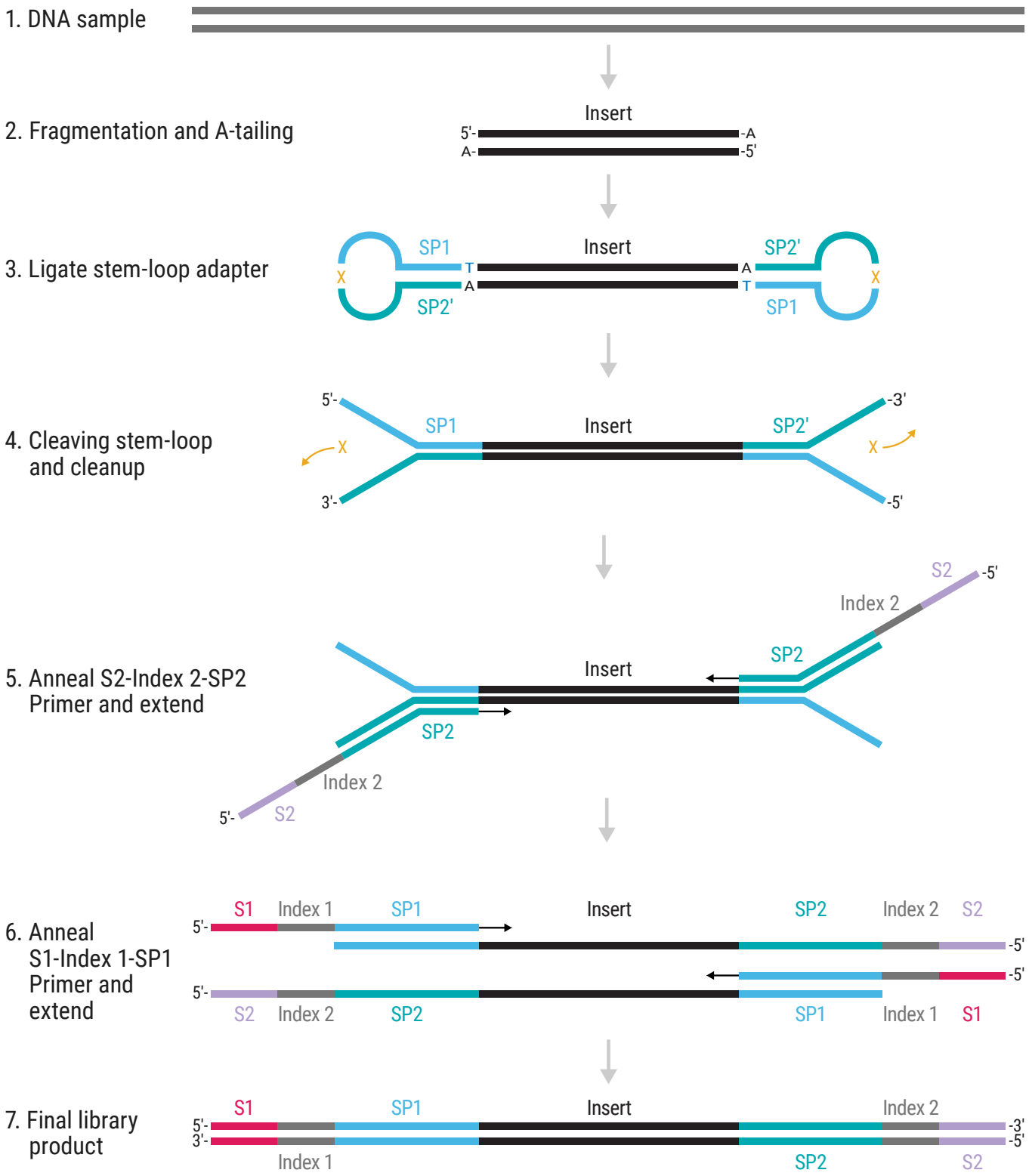

Figure 1: Watchmaker DNA Library Prep Kit with Fragmentation library construction outline for sequencing on the Singular G4 Sequencing Platform. The **(1)** input DNA sample is enzymatically fragmented **(2)**, followed by the addition of an A base to the 3' ends. The stem-loop adapter, containing the standard SP1 and SP2 sequencing primer binding sites, is then ligated to the fragment **(3)** and the stem-loop is cleaved using Cleave Enzyme **(4)**, followed by a cleanup step. Cleaving the backbone opens the loops and results in a linear fragment with the SP1 sequence at the 5' end, and the SP2 sequence at the 3' end. During library amplification, these sequences are then used to add the Singular S1 cluster sequence and index 1 at the SP1 end, and Singular S2 cluster sequence and index 2 at the SP2 end **(5 and 6)** to generate the final indexed library fragment **(7)**. The nucleotides tags are used to sequence the inserts and indices in the following order: Index 1 (S1), Insert Read 1 (SP1), Index 2 (S2), Insert Read 2 (SP2).

Protocol Adjustments

Refer to Watchmaker DNA Library Prep Kit with Fragmentation User Guide (v3.0 or later) for the library construction protocol, employing these specific modifications:

<table>
<tr><th>Parameter</th><th>Protocol Adjustment</th><th>Step</th></tr>
<tr><td>Input DNA</td><td>1 ng – 500 ng in a total volume of 40 µL
Inputs <0.1 ng are possible, but may require additional optimization.</td><td>N/A</td></tr>
<tr><td>Fragmentation, End-repair, and A-tailing</td><td>Program the thermocycler to:
37°C for 20 minutes when targeting 200 bp inserts
30°C for 4 minutes when targeting 500 bp inserts

Mode insert sizes of ~200 bp were found to be optimal, however insert lengths are tunable by adjusting fragmentation time and temperature.</td><td>Step 1.1</td></tr>
<tr><td>Adapter Ligation and Cleaving:
Thermocycling</td><td>Program a thermocycler as indicated below:
Incubate at 20°C for 15 min (ligation), followed immediately by 37°C for 15 min (cleaving).</td><td>Step 2.1</td></tr>
<tr><td>Adapter Ligation and Cleaving:
Adapter Concentration</td><td>Add 5 µL of appropriately diluted Singular Genomics G4 Universal Library Prep Adapter to each tube.
<table>
<tr><th>Input</th><th>Adapter Concentration</th></tr>
<tr><td>10 – 500 ng</td><td>15 µM</td></tr>
<tr><td>5 ng</td><td>3 µM</td></tr>
<tr><td>1 ng</td><td>1 µM</td></tr>
<tr><td><1 ng</td><td>0.1 – 0.5 µM</td></tr>
</table></td><td>Step 2.4</td></tr>
<tr><td>Adapter Ligation and Cleaving:
Cleave Enzyme</td><td>Add 3 µL Cleave Enzyme regardless of input amount, alongside the addition of the Ligation Master Mix for a total volume of 78 µL. No addition of Cleave Reaction Buffer is required.</td><td>Step 2.5</td></tr>
<tr><td>Post-ligation Cleanup</td><td>Follow the standard protocol for a 0.8X SPRI cleanup.</td><td>Step 3</td></tr>
<tr><td>Library Amplification:
Thermocycling and Amplification Cycle Number</td><td>Program a thermocycler as indicated below:
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr>
<tr><td>Initial Denaturation[1]</td><td>98°C</td><td>5 min</td><td>1</td></tr>
<tr><td>Denaturation</td><td>98°C</td><td>15 sec</td><td rowspan="4"><table>
<tr><th>Inputs</th><th>Cycles</th></tr>
<tr><td>10 – 500 ng</td><td>4</td></tr>
<tr><td>5 – 10 ng</td><td>8</td></tr>
<tr><td>1 ng</td><td>12</td></tr>
<tr><td><1 ng</td><td>16</td></tr>
</table></td></tr>
<tr><td>Annealing</td><td>60°C</td><td>30 sec</td></tr>
<tr><td>Extension</td><td>72°C</td><td>30 sec</td></tr>
<tr><td>Final Extension</td><td>72°C</td><td>60 sec</td><td>1</td></tr>
</table>
[1]Initial denaturation can be extended to 10 min to increase library yield, if required.</td><td>Step 4.3</td></tr>
<tr><td>Library Amplification:
Primer Concentration</td><td>Singular Primers:
Add 10 µL of the S1/SP1 + S2/SP2 PCR Primers (5 µM each) to the library amplification reaction, together with 25 µL Equinox Amplification Master Mix (2X).</td><td>Step 4.4</td></tr>
<tr><td>Post-amplification Cleanup</td><td>Follow the standard protocol for a 1X SPRI cleanup.

For inputs ≤10 ng, a 0.9X SPRI cleanup may be performed (instead of 1X) to remove excess adapter dimer while still retaining library yield.</td><td>Step 5.3</td></tr>
</table>

Library Quality Control

After post-amplification cleanup, assess the quality and concentration of the library using the Qubit 1X dsDNA HS Kit and by electrophoretic analysis (BioAnalyzer, TapeStation, or equivalent method) to visualize size distribution. There should be no peaks of higher or lower molecular weight than the expected library size as shown in Figure 2.

Note that the non-indexed primers are 62 bp long, while the indexed primers are 75 bp long. To calculate expected library fragment size, take the expected insert size and add 124 bp for non-indexed runs or 150 bp for indexed runs.

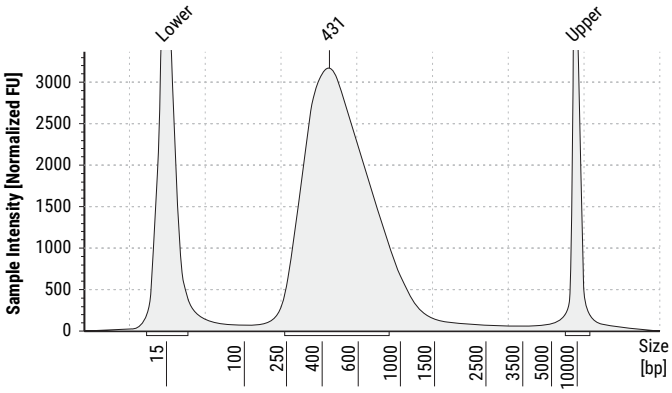

Figure 2: Example of NGS library on TapeStation instrument, optimized for sequencing on the Singular G4 platform.

Optional: Dilute each library in water to a standard concentration (in nM) for pooling and loading on the Singular G4 Sequencing Platform. We recommend storing libraries at -20°C for up to 4 weeks, at a concentration of ≥10 nM. Note that Concentration [in nM] = (Concentration [in ng/µL] * 1,000,000) / (660 * Size).

Demonstrated Compatibility

Watchmaker DNA Library Prep Kits with Fragmentation seamlessly integrate with the Singular Genomics G4 Sequencing Platform, providing accessible benchtop sequencing. This workflow delivers tunable insert sizes and excellent sequence accuracy across a range of applications, including whole genome, whole exome and targeted sequencing. In this poster, we demonstrate the utility of this workflow to generate high-quality libraries to perform metagenomic and human copy number variation (CNV) analysis.

Revision History

Version	Description	Date
1.0	• First release	09/2023

WATCHMAKER GENOMICS

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