Watchmaker DNA Library Prep Kit

with Fragmentation

Product Description

The Watchmaker DNA Library Prep Kit with Fragmentation is designed for the highly efficient conversion of DNA, from both high- and low-quality samples, into Illumina®-compatible sequencing-ready libraries. The chemistry and streamlined, single-tube protocol have been optimized to produce libraries from 1 ng – 500 ng DNA while minimizing bias and artifacts. The integrated enzymatic fragmentation, end-repair and A-tailing module produces fragmented, A-tailed DNA in a single reaction with a high degree of consistency. The ligation module adds full-length or truncated Illumina adapters (not included) to DNA fragments with industry-leading efficiency and without the need for a prior cleanup step.

The kit supports PCR-free workflows for input DNA of sufficient mass and quality. For workflows where library amplification is desirable or required, the kit includes the Equinox Amplification Master Mix (2X). This ready-to-use mix contains an optimized PCR buffer and hot start enzyme formulation that enables library amplification with high fidelity and efficiency across a broad range of GC content and in the presence of a variety of paramagnetic beads. The hot start functionality inhibits both the polymerase and exonuclease activities of the enzyme, ensuring no loss of performance when amplification reactions are set up at room temperature.

Table of Contents

Kit Contents

Kit	Kit Code	Description	Component Volume	
			24 rxn kit	96 rxn kit
Watchmaker DNA Library Prep Kit with Fragmentation (PCR-free)[1]	**7K0013-024** (24 rxns) **7K0013-096** (96 rxns)	Frag/AT Buffer	110 µL	480 µL
		Frag/AT Enzyme Mix[2]	160 µL	720 µL
		Ligation Master Mix	530 µL	2.4 mL
Watchmaker DNA Library Prep Kit with Fragmentation (w/o primers)[3]	**7K0022-024** (24 rxns) **7K0022-096** (96 rxns)	Includes all components listed for **7K0013**, and:		
		Equinox Amplification Master Mix (2X)[4]	690 µL	3.0 mL
Watchmaker DNA Library Prep Kit with Fragmentation[5]	**7K0019-024** (24 rxns) **7K0019-096** (96 rxns)	Includes all components listed for **7K0022**, and:		
		P5/P7 Primer Mix (10X)	144 µL	600 µL

[1]Previously named **Library Preparation Kit – Enzymatic Fragmentation**
[2]Previously named **Frag/AT Enzymes**
[3]Previously named **Library Preparation and Amplification Kit – Enzymatic Fragmentation (w/o primers)**
[4]Previously named **Library Amplification Hot Start Master Mix (2X)**
[5]Previously named **Library Preparation and Amplification Kit – Enzymatic Fragmentation**

For custom formats, contact the **Sales Team** at sales@watchmakergenomics.com.

Product Applications

This workflow was developed to address unmet performance needs in next-generation sequencing (NGS) applications such as rare variant detection, single-cell analysis, and hybrid capture. These applications require high conversion efficiency, high library complexity, low bias, uniform coverage, and minimal experimental artifacts in order to support robust sensitivity and specificity.

This library preparation kit is ideally suited for:

- High-quality genomic DNA, DNA extracted from FFPE tissue, plasmid DNA and long PCR products
- A broad range of DNA inputs (1 ng – 500 ng)
- High- and low-complexity genomes, and genomes with extreme (15 – 85%) GC content
- Whole-genome sequencing (including PCR-free workflows). See **Appendix B** for specific recommendations.
- Targeted sequencing protocols employing hybridization capture
- Workflows that employ unique molecular identifiers (UMIs) for improved sensitivity
- Amplicon sequencing

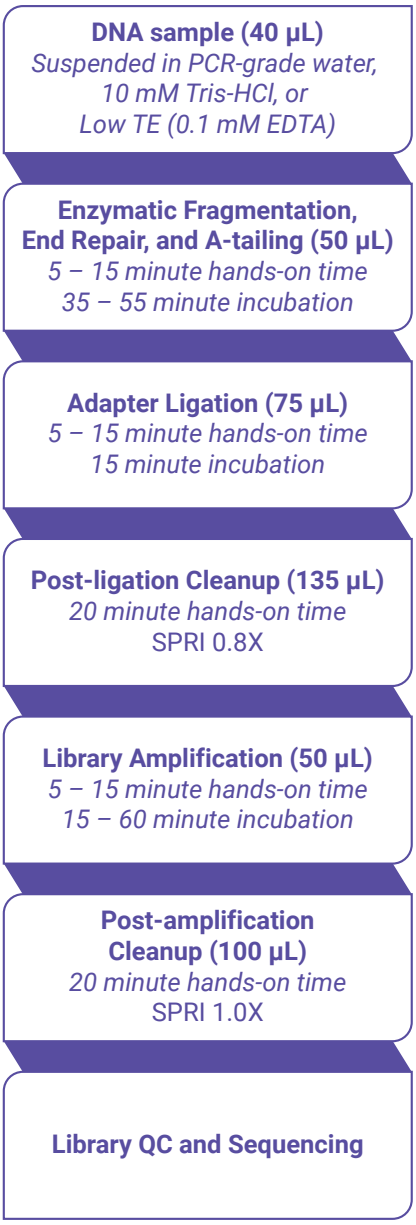
Storage and Handling

The Watchmaker DNA Library Prep Kit with Fragmentation is shipped on cold packs. Upon receipt, store all components at -20 ± 5°C.

Keep all components and reaction mixes on ice or a cooled reagent block during routine use. Many components are viscous; therefore, take care to homogenize solutions thoroughly before use and during reaction setup. Frag/AT Buffer and P5/P7 Primer Mix (10X) should be vortexed for 5 sec before use. The Frag/AT Enzyme Mix, Ligation Master Mix and Equinox Amplification Master Mix (2X) should be inverted ten times prior to reaction setup. The combined fragmentation/end-repair and A-tailing reaction must be set up on ice. The ligation and library amplification reactions may be set up at room temperature.

When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

Workflow Overview



Required Materials not Included

- Adapters (see **Prior to Starting** for more detail)
- Adapter diluent (e.g., 10 mM Tris-HCl, pH 8.0, 10 mM NaCl)
- Magnetic rack compatible with 0.2 mL PCR tubes and/ or 96-well plate
- Ampure® XP Beads (Beckman Coulter, Inc. #A63881) or equivalent (see **Prior to Starting** for more detail)
- 80% Ethanol
- 200 µL thin-wall PCR tubes compatible with thermocycler, or 96-well 0.2 mL PCR Plates and Plate-Seals
- 1 mL, 2 mL, 5 mL tubes (nuclease-free)
- PCR-grade water or Low TE (0.1 mM EDTA); optional
- 10 mM Tris-HCl, pH 8.0
- Thermocycler
- Vortex mixer
- Fragment Analyzer™ e.g., Bioanalyzer® or TapeStation (Agilent Technologies, Inc.), or similar instrument and consumables

These protocols are designed for use with the specified labware, consumables, and calibrated equipment.

Prior to Starting

Input DNA

This kit is compatible with a broad range of input amounts (1 ng – 500 ng), from both high- and low-quality DNA, including FFPE.

The quality of FFPE DNA can vary greatly depending on factors such as fixation, storage, and extraction method and this variation can have a significant impact on the fragmentation reaction. Due to the significant damage incurred by fixation and extraction, conversion efficiencies for FFPE DNA samples will most likely be lower and may be more variable sample to sample. We do still recommend performing fragmentation on DNA from FFPE samples as we have seen that very poor quality FFPE samples benefit from fragmenting for a short amount of time.

NOTE: Libraries have been successfully constructed using less than 1 ng of input DNA with the protocol outlined below.

PCR-grade water, 10 mM Tris-HCl pH 8.0, and Low TE (0.1 mM EDTA) are the recommended buffers for DNA suspension. Higher EDTA concentrations will significantly slow the enzymatic fragmentation reaction.

Fluorescence-based methods such as Qubit or PicoGreen are recommended for accurate input DNA quantification over spectrophotometric methods, such as NanoDrop.

Adapters

This kit is compatible with adapters that have a 3' overhanging T to facilitate adapter orientation during dsDNA ligation. Ensure that adapters are adequately duplexed and at the appropriate concentration prior to use. Adapter quality has an impact on the efficiency of the ligation reaction and library yield. High-quality adapters may be sourced from a variety of reliable vendors in convenient, ready-to-use formats.

For assistance with adapter compatibility, please contact the **Scientific Support Team** at support@watchmakergenomics.com.

Truncated ('stubby') adapters

The workflow is compatible with truncated or 'stubby' adapters, where sample indexes are added during subsequent library amplification, though purification bead ratios may need to be tuned to account for molecular length differences. When using 'stubby' adapters, user-supplied, uniquely indexed PCR primers are required for the amplification of each library to be sequenced on the same flow cell. 'Stubby' adapters may be added at higher

concentrations than full-length adapters to increase conversion efficiency of unique library molecules with minimal adapter dimer formation.

Full-length adapters

This workflow has been verified using full-length Illumina®-compatible adapters, where the P5 and P7 cluster generation sequences are added at the ligation step.

When using full-length adapters, a unique sample (sequencing) barcode is required for all samples to be sequenced on the same flow cell. Refer to the technical documentation provided by the adapter vendor for recommendations on optimal pooling.

Library Amplification Primers

The P5/P7 Primer Mix (10X) is supplied at a concentration of 20 µM each primer and is appropriate for the amplification of full-length adapter-ligated libraries. An annealing temperature of 60°C is recommended for standard Illumina® P5 and P7 primers.

- P5: AATGATACGGCGACCACCGA
- P7: CAAGCAGAAGACGGCATACGAGAT

User-supplied Amplification Primers

When using truncated, or 'stubby', adapters in multiplexed sequencing workflows, a uniquely barcoded primer mix will be required (and must be added individually) for each library to be sequenced on the same flow cell.

Primers should always use equimolar concentrations of the forward and reverse primers. A primer premix containing 20 µM of each primer (resulting in a final concentration of 2 µM each in the amplification reaction) is recommended.

Primers may also incorporate chemical modifications (e.g., one or more 3'-phosphorothioate bonds) to improve specificity.

Use a buffered solution, such as 10 mM Tris-HCl, pH 8.0, to store and dilute primers. Limit the number of freeze-thaw cycles.

For assistance with amplification primer compatibility, please contact the **Scientific Support Team** at support@watchmakergenomics.com.

Library Amplification Optimization

Annealing temperature

For the truncated adapter scheme detailed in Glenn et. al. 2019,[1] use an annealing temperature of 55°C. For other primers, an annealing temperature gradient (55°C to 70°C) may be performed to determine the optimal condition for amplification.

Extension Time

Longer extension times may be employed to ensure efficient amplification of longer-insert libraries. A 30-sec extension is sufficient for libraries with a mode fragment size up to 500 bp; a 45-sec extension time is recommended for libraries with mode fragment sizes >500 bp. The optimal condition for each application may have to be determined empirically.

Cycle Number

This protocol provides a starting point for PCR cycle number optimization based on DNA input into library preparation. FFPE and other degraded samples may require additional cycles. Adapter-ligated libraries may be quantified by qPCR or estimated by other means or methods to determine the optimal number of amplification cycles for the desired library yield.

SPRI Purification Beads

The protocol outlined below assumes the use of AMPure XP (Beckman Coulter) reagent for bead purification steps. Other SPRI bead brands may be used, assuming they are nuclease-free and deliver equivalent sizing performance at the given bead ratios (e.g., 0.8X). Otherwise, bead purification ratios will need to be optimized for the bead brand used.

Ensure beads are equilibrated to room temperature and thoroughly resuspended via vortexing prior to use.

[1]Glenn TC, Nilsen RA, Kieran TJ, et al. Adapterama I: universal stubs and primers for 384 unique dual-indexed or 147,456 combinatorially-indexed Illumina libraries (iTru & iNext). *PeerJ.* 2019;7:e7755. Published 2019 Oct 11. doi:10.7717/peerj.7755]

Library Construction Protocol

Recommendations

- Keep all sample tubes, buffers, and enzymes on ice unless stated otherwise.
- Ensure all the buffers are fully thawed on ice before use. Once thawed, invert the tubes several times, and vortex for at least 5 sec to ensure the reagent is fully mixed.
- Place enzymes and the Equinox Amplification Master Mix (2X) on ice before use. Invert the tubes 10 times to mix.
- Where possible, centrifuge briefly to remove any excess liquid from the tube and collect all liquid from the tube lids prior to opening a tube.
- We recommend making master mixes for each reaction step with a 10% excess to account for loss during pipetting.
- Ensure SPRI purification beads are fully equilibrated to room temperature and thoroughly resuspended by vortexing prior to use.

1. Enzymatic Fragmentation, End Repair and A-tailing (Frag/AT)

1.1 Program a thermocycler as indicated below, and initiate the run to cool the block:

NOTE: Insert sizes can be adjusted through modifying the Fragmentation, end repair and A-tailing incubation time and temperature. Optimal conditions vary depending on the DNA input quality. See Options 1 and 2 for more details.

Step	Temperature	Time
Lid temperature	105°C	N/A
Pre-cooling	4°C	HOLD
Fragmentation, end repair and A-tailing	See Options 1 and 2: either 37°C or 30°C may be used.	
	65°C	30 min
HOLD	4°C	HOLD

For high quality genomic DNA, PCR products, and plasmids, fragmentation may be performed at **either** 37°C (Option 1) **OR** at 30°C (Option 2) according to the tables below. Fragmentation temperature does not impact data quality.

One temperature may be preferred over the other based on application or for operational reasons.

For FFPE DNA, we recommend fragmentation at 30°C for 3 – 5 minutes.

If your workflow is intended for whole genome sequencing, refer to **Appendix B** for specific recommendations.

Option 1: 37°C Incubation*

Time (min)	5	10	20	30
Mode insert size (bp)	300	250	200	150

OR

Option 2: 30°C Incubation*

Time (min)	3	4	5	10	15
Mode insert size (bp)	575	525	425	300	250

*Recommended fragmentation times/sizes are a starting point and may require optimization for different sample types and applications. Fragmentation time is independent of DNA input amount when working within the recommended range of the kit.

1.2 On ice, prepare input DNA in a total volume of 40 µL. Dilute DNA in PCR-grade water, 10 mM Tris-HCl pH 8.0, or Low TE (0.1 mM EDTA). Higher concentrations of EDTA will significantly slow the enzymatic fragmentation reaction.

1.3 Prepare the 5X Frag/AT master mix as follows:

NOTE: The 5X Frag/AT master mix can be stored at 4°C for up to four hours.

Component	Volume (µL)[1]
Frag/AT Buffer	4
Frag/AT Enzyme Mix	6

[1]When preparing a small number of reactions, it is acceptable to add Frag/AT Buffer and Frag/AT Enzyme Mix directly to each reaction without preparing a master mix.

1.4 Mix on a touch vortexer (or 3,000 rpm) for 4 sec. Briefly centrifuge to collect all liquid in the bottom of the tube.

1.5 To each tube, add the Frag/AT master mix as specified below on ice:

Component	Volume (µL)
Input DNA	40
5X Frag/AT master mix	10

1.6 Vortex each reaction to homogenize the reaction. The consistency of this mixing step from tube to tube and experiment to experiment is important for consistent fragmentation results between samples. Briefly centrifuge to collect the sample at the bottom of the tube, then place back on ice.

NOTE: Complete mixing is critical to achieve desired fragment lengths. Mixing by pipetting is also acceptable. On ice, pipette 25 µL of the reaction up and down 10 times to mix completely.

1.7 Place the tubes into the chilled thermocycler (programmed in **Step 1.1**). Advance the thermocycler from the initial 4°C hold to start the 30°C or 37°C incubation.

1.8 Proceed immediately to **Adapter Ligation** after the program has finished and the samples have returned to 4°C.

2. Adapter Ligation

2.1 Program a thermocycler as indicated below:

Step	Temperature	Time
Lid temperature	OFF	N/A
Ligation	20°C	15 min[1]

[1]Ligation time may be extended to a maximum of 16 hours. Library quality decreases with overnight ligation.

2.2 Prepare an appropriate volume of each adapter at the concentration specified in **Table 1** using 10 mM Tris-HCl pH 8.0. 5 µL of adapter at the appropriate concentration is required per ligation reaction.

NOTE: Storing adapter solutions at concentrations <10 µM for extended periods of time is not recommended.

Table 1. Adapter concentration by DNA input amount into library prep

DNA input (ng)	Adapter concentration (µM)
>10	15
1 – 10	3
<1	0.6

2.3 Remove the reaction tubes (from **Step 1.7**) from the thermocycler.

2.4 Add 5 µL of appropriately diluted adapter to each tube.

2.5 Add 20 µL of Ligation Master Mix to each reaction. The Ligation Master Mix is viscous. Carefully pipette a minimum of 40 µL up and down 10 times to ensure proper mixing.

NOTE: Ensure the reaction is homogeneous prior to proceeding.

2.6 Place the sample tubes in the thermocycler and initiate the Ligation incubation program (programmed in **Step 2.1**).

2.7 Once the program has completed, proceed immediately to **Post-ligation Cleanup**.

3. Post-Ligation Cleanup

3.1 Freshly prepare at least 0.4 mL of an 80% ethanol solution for each reaction.

3.2 Vortex **room temperature** SPRI beads to thoroughly mix. Add 60 µL (0.8X) of beads to each ligation reaction and mix thoroughly by pipetting.

NOTE: SPRI-to-sample bead ratios may be optimized for different applications or adapter configurations. A ratio of 0.8X is recommended as a starting point when using high-quality DNA and full-length adapters. If your workflow is intended for:

– FFPE samples, consider using a 0.65X or 0.5X SPRI ratio

– Whole genome sequencing, refer to **Appendix B**

Adjustments in SPRI ratios may require additional amplification cycles to account for reduced library yield.

3.3 Mix on a touch vortexer (or 3,000 rpm) for 4 sec. Briefly centrifuge to collect all liquid in the bottom of the tube.

3.4 Incubate the library-bead mixtures at room temperature for at least 5 min to maximize library recovery.

3.5 Place sample tubes on a magnet for at least 5 min, or until all beads have been collected on the tube wall and the solution is clear.

3.6 Carefully remove and discard the supernatant from each tube.

3.7 Add 200 µL of freshly prepared 80% ethanol to each tube, taking care to not disturb the bead pellet on the tube wall.

3.8 Incubate tubes at room temperature for at least 30 sec without removing the tube from the magnet or disturbing the bead pellet. Carefully remove and discard the ethanol.

3.9 Repeat **Steps 3.7 – 3.8** for a total of two washes.

OPTIONAL: Tubes can be briefly spun to pull down excess ethanol prior to removing with a p10 pipette.

3.10 Allow remaining ethanol to evaporate by allowing the pellets to air dry for 3 – 5 min.

NOTE: Beads have been sufficiently dried when cracks appear in the surface of the bead pellet and no liquid is visible in the tube. Residual trace ethanol in the library amplification reaction may decrease performance.

3.11 Remove sample tubes from the magnet and carefully resuspend each bead pellet in 22 µL of 10 mM Tris-HCl pH 8.0. Pipetting carefully will minimize bubbling and allow for greater library recovery.

3.12 Incubate tubes at room temperature for at least 2 min before placing back on the magnet.

3.13 Leave sample tubes on the magnet for at least 2 min, or until all beads have collected on the tube wall and the solution is clear.

3.14 Carefully transfer 20 µL of each library-containing supernatant to a new, labeled tube. If desired, library amplification may be carried out in the same tube in the presence of SPRI beads (See **Library Amplification** for reaction setup and **Appendix A** for details regarding PCR in the presence of beads).

3.15 Libraries not requiring amplification can proceed into QC and sequencing.

NOTE: We recommend using qPCR to quantify libraries and capillary electrophoresis to assess quality and sizing prior to preparing the libraries for sequencing. If using full-length adapters with a PCR-free workflow, libraries will not migrate true to size using capillary electrophoresis based platforms.

3.16 Libraries generated from low inputs and/or with truncated ('stubby') adapters will require amplification (**Step 4**) prior to qPCR-based library quantification and/or sequencing.

Safe stopping point. Samples can be stored at 4°C for up to 1 week and at -20°C for up to 1 month.

4. Library Amplification

4.1 Adapter-ligated libraries (recovered in **Step 3.14**) can be used as template DNA in the library amplification reaction described below.

NOTE: If your workflow requires library amplification in the presence of paramagnetic beads, refer to **Appendix A** on bead compatibility.

4.2 Thaw and equilibrate the Equinox Amplification Master Mix (2X) on ice. Once thawed, invert several times or swirl vigorously to mix (**DO NOT VORTEX**).

4.3 Program a thermocycler as indicated below:

Step	Temperature	Time	Cycles
Initial denaturation	98°C	45 sec	1
Denaturation	98°C	15 sec	See **Table 2**
Annealing	60°C[1]	30 sec	
Extension	72°C	30 – 45 sec[1]	
Final extension	72°C	60 sec	1
–	12°C	Hold	–

[1]See **Prior to Starting** for more information on library amplification optimization.

Table 2: Recommended PCR cycle numbers based on DNA input into library prep

DNA input into Library Preparation (ng)	PCR cycles to generate[1,2]	
	10 nM library	1 µg library
500	0	3 – 4
100	1 – 2	5 – 7
50	2 – 3	7 – 8
10	5 – 6	9 – 11
5	6 – 8	11 – 12
1	8 – 10	13 – 14
<1[3]	>10	>14

[1]Estimated final yields are based on a mode library insert size of ~300 bp (mode library fragment size of ~450 bp).

[2]See **Prior to Starting** for more information on library amplification optimization.

[3]Depending on the sample type and application, it is possible to reliably produce high-quality sequencing libraries from input amounts <1 ng. However, the quality and precise quantification of low-input samples greatly influences cycling parameters, and as a result may need to be optimized for specific applications.

4.4 Assemble each amplification reaction in the order specified below:

Component	Volume (µL)
Adapter-ligated library	20
P5/P7 primer mix (10X) or user-supplied primers[1]	5
Equinox Amplification Master Mix (2X)	25

[1]See **Prior to Starting** for more information on user-supplied amplification primers.

4.5 Mix on a touch vortexer (or 3,000 rpm) for 4 sec. Briefly centrifuge to collect all liquid in the bottom of the tube.

4.6 Place tubes in the thermocycler (programmed in **Step 4.3**) and initiate the PCR program.

4.7 Once the program has completed, proceed immediately to **Post-amplification Cleanup**.

5. Post-amplification Cleanup

5.1 Freshly prepare at least 0.4 mL of an 80% ethanol solution for each reaction.

5.2 Vortex **room temperature** SPRI beads to thoroughly mix.

5.3 Add 50 µL (1X) of beads to each amplification reaction.

5.4 Mix on a touch vortexer (or 3,000 rpm) for 4 sec. Briefly centrifuge to collect all liquid in the bottom of the tube.

5.5 Incubate the library-bead mixtures at room temperature for at least 5 min to maximize library recovery.

5.6 Place sample tubes on a magnet for at least 5 min, or all beads have collected on the tube wall and until the solution is clear.

5.7 Carefully remove and discard the supernatant from each tube.

5.8 Add 200 µL of freshly prepared 80% ethanol to each tube, taking care to not disturb the bead pellet on the tube wall.

5.9 Incubate tubes at room temperature for at least 30 sec without removing the tube from the magnet or disturbing the bead pellet. Carefully remove and discard the ethanol.

5.10 Repeat **Steps 5.8 – 5.9** for a total of two washes.

OPTIONAL: Tubes can be briefly spun to pull down excess ethanol prior to removing with a p10 pipette.

5.11 Allow remaining ethanol to evaporate by allowing the pellets to air dry for 3 – 5 min.

NOTE: Beads have been sufficiently dried when cracks appear in the surface of the bead pellet and no liquid is visible in the tube.

5.12 Remove sample tubes from the magnet and carefully resuspend each bead pellet in 22 µL of 10 mM Tris pH 8.0.

5.13 Incubate tubes at room temperature for at least 2 min before placing back on the magnet.

5.14 Leave sample tubes on the magnet for at least 2 min, or until all beads have collected on the tube wall and the solution is clear.

5.15 Carefully transfer 20 µL of each library-containing supernatant to a new tube.

5.16 At this point, libraries are ready for quantification, normalization, pooling, hybrid capture, and/or sequencing.

NOTE: We recommend quantifying libraries using qPCR and analyzing quality and sizing using capillary electrophoresis prior to preparing the libraries for sequencing.

Appendix A: Amplification with Paramagnetic Purification beads

For some library preparation workflows, it may be preferred or advantageous to perform PCR in the presence of paramagnetic purification beads. For example, in hybrid capture workflows, post-capture amplification may be performed by adding PCR mix directly to the capture beads with bound library, eliminating an elution step. Alternatively, libraries may be eluted directly from SPRI beads in the PCR reaction mixture.

We have found paramagnetic purification beads to fall into three categories of compatibility with PCR and sequencing based on their surface chemistry: Group I—fully compatible, Group II—potentially inhibitory, and Group III—incompatible. While Group II beads are inhibitory to non-optimized PCR systems, Equinox Amplification Master Mix (2X) has been optimized to allow amplification in the presence of both Group I and Group II beads, with no observable loss in performance (e.g., efficiency, uniformity, or fidelity). Equinox Amplification Master Mix (2X) and other library amplification systems are not compatible with the extreme inhibition characterized by Group III beads. Table 3 details the various paramagnetic bead types evaluated.

Table 3. Paramagnetic purification bead types

Bead type	Vendor	Catalog number	Compatibility/ amount tested*
Group I (Tosyl-activated beads)			
Dynabeads™ M280 Streptavidin	Thermo Fisher	11205D	500 µg
Dynabeads™ MyOne™ Streptavidin T1	Thermo Fisher	65601	500 µg
Group II (Carboxylic acid-activated beads)			
Dynabeads™ M270 Streptavidin**	Thermo Fisher	65305	500 µg
SPRI	Various, incl. Beckman Coulter	A63882	100 µL
Dynabeads™ MyOne™ Streptavidin C1	Thermo Fisher	65001	500 µg
Group III (Not compatible with PCR)			
Dynabeads™ M270 Carboxylic Acid	Thermo Fisher	14305D	500 µg

*Volume of slurry or mass of beads per 50 µL amplification reaction.
**Used in xGen Hybridization and Wash Kit (Integrated DNA Technologies).

Appendix B: Recommendations for Whole Genome Sequencing

While there are different strategies for preparing DNA libraries, longer insert sizes are often preferred for whole genome sequencing (WGS) for several reasons including improved genome mapping for reference-based analysis, better sequencing economy, better detection of structural variants, and more accurate representation of complex regions of the genome.

Here we describe optimized conditions for generation of longer insert sizes (mode insert sizes: 400 – 450 bp), specifically for WGS applications. We recommend following the **Library Construction Protocol** (pg. 5), with the following modifications:

Step 1.1: Refer to Table 4 below for optimized Fragmentation, end repair, and A-tailing Cycling Conditions.

Step 3.2: Adjust post-ligation cleanup SPRI ratio to 0.5X (37.5 µL). We have shown that a lower SPRI bead ratio enriches for longer insert size fragments. This is sufficient for PCR-free sequencing when starting with a minimum of 75 ng of high-quality gDNA. For inputs less than 75 ng, PCR amplification is recommended (**Step 4.3**) with a post-amplification cleanup using a 1X SPRI bead ratio (**Step 5.3**).

Table 4: Optimized Fragmentation, end repair, and A-tailing Cycling Conditions

Step	Temp	Time
Lid temperature	105°C	N/A
Pre-cooling	4°C	HOLD
Fragmentation, end repair, and A-tailing	30°C	3 min
	65°C	30 min
HOLD	4°C	HOLD

Revision History

Version	Description	Date
1.1	• First protocol release	10/2021
2.0	• Protocol text was edited	10/2022
	• Enzymatic fragmentation recommendations (**Step 1.1**) were adjusted	
	• "Table 2: Recommended PCR cycle numbers based on DNA input into library prep" was adjusted	
3.0	• Appendix B: Recommendations for Whole Genome Sequencing was added	08/2023
	• FFPE-specific fragmentation conditions were included in **Step 1.1**	
	• Fragmentation conditions for high-quality gDNA, PCR products, and plasmids were specified in **Step 1.1**	
	• Recommendations for FFPE and WGS SPRI bead ratios were included in **Step 3.2**	

WATCHMAKER GENOMICS

For Technical Support, please contact
support@watchmakergenomics.com.

5744 Central Avenue, Suite 100
Boulder, CO 80301
www.watchmakergenomics.com

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