Watchmaker DNA Library Prep Kit

for Fragmented Double-Stranded DNA

Product Description

Watchmaker DNA Library Prep Kits support robust and flexible library preparation from fragmented DNA templates for a wide variety of sequencing applications. DNA fragments generated by biologically occurring DNA fragmentation such as cell-free DNA (cfDNA), mechanical (e.g., Covaris®) or enzymatic fragmentation methods, as well as PCR products, are suitable as input into the workflow. The kit generates A-tailed, 5' phosphorylated DNA fragments during the End Repair and A-tailing step, which are then ligated to T-tailed adapters in a rapid single-tube workflow. The kit is compatible with starting DNA quantities from 0.5 ng to 1000 ng.

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. 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	96 rxn
Watchmaker DNA Library Prep Kit (PCR-free)	**7K0101-024** (24 rxns) **7K0101-096** (96 rxns)	ER/AT Buffer	185 µL	840 µL
		ER/AT Enzyme Mix	80 µL	360 µL
		Ligation Buffer	660 µL	3.0 mL
		Ligation Enzyme	135 µL	600 µL
Watchmaker DNA Library Prep Kit (w/o primers)	**7K0103-024** (24 rxns) **7K0103-096** (96 rxns)	Includes all components listed for **7K0101**, and: Equinox Amplification Master Mix (2X)	690 µL	3.0 mL
Watchmaker DNA Library Prep Kit	**7K0102-024** (24 rxn) **7K0102-096** (96 rxn)	Includes all components listed for **7K0103**, and: P5/P7 Primer Mix (10X)	144 µL	600 µL

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

Product Applications

This workflow was developed to address the performance needs in next-generation sequencing (NGS) applications such as liquid biopsy, ultra-low variant detection, bisulfite sequencing, 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:

- Circulating tumor DNA (ctDNA) and cell-free DNA (cfDNA) analysis
- Methyl-seq (in combination with the Equinox Uracil Tolerant Library Amplification Kit)
- Whole-genome sequencing (including PCR-free workflows)
- Targeted sequencing protocols employing hybridization capture
- Amplicon sequencing (for post-amplification adapter ligation)
- High- and low-complexity genomes, and genomes with extreme (15 – 85%) GC content
- Preparation of libraries from FFPE tissue
- Mechanically or enzymatically sheared double-stranded DNA, plasmid DNA, and long PCR products
- Workflows that employ unique molecular identifiers (UMIs) for improved sensitivity

Storage and Handling

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

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

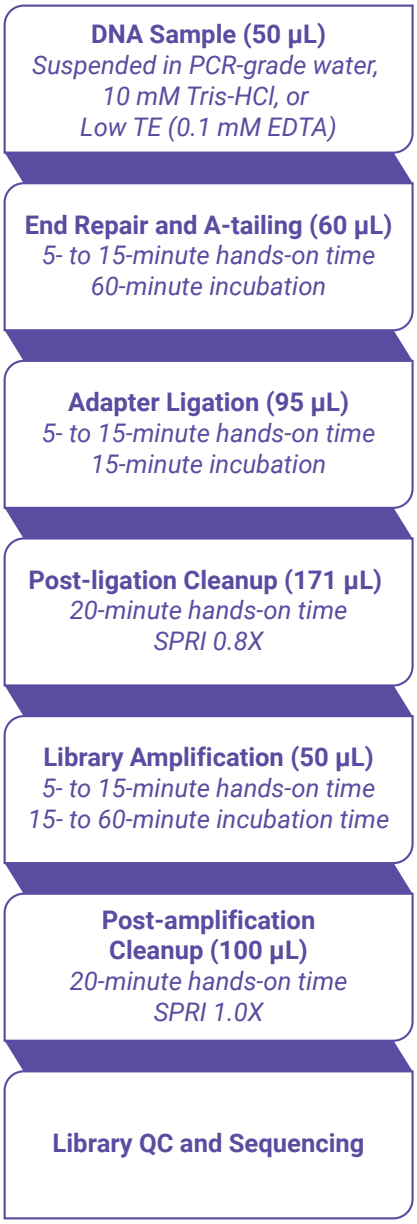
When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

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)
- 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)
- 10 mM Tris-HCl, pH 8.0
- Magnetic rack compatible with 0.2 mL PCR tubes and/or 96-well plate
- 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.

Workflow Overview



Prior to Starting

Input DNA

This kit is compatible with a broad range of input amounts (0.5 ng – 1000 ng) from both high- and low-quality DNA, including FFPE. DNA fragments generated by biologically occurring DNA fragmentation such as cell-free DNA (cfDNA), mechanical (e.g., Covaris®) or enzymatic fragmentation methods, as well as PCR products, are suitable as input into the workflow. DNA input refers to the input into the End Repair and A-tailing reaction.

NOTE: Libraries have been successfully constructed using less than 0.5 ng of input DNA with the protocol outlined below.

If DNA was quantified prior to fragmentation, and/or DNA was further purified before end repair and A-tailing, the actual input into library preparation may be lower. This should be considered when evaluating library preparation efficiency and during library amplification cycle number optimization.

This kit is compatible with DNA extracted from formalin fixed paraffin-embedded tissues (FFPE). 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. The quality of FFPE DNA can vary greatly depending on factors such as fixation, storage, and extraction method. This variation can have a significant impact on the library preparation efficiency.

PCR-grade water, 10 mM Tris-HCl pH 8.0, and Low TE (0.1 mM EDTA) are the recommended buffers for input DNA suspension.

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

Adapters

Watchmaker DNA Library Prep Kits are 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.

Adapter concentrations may need to be optimized based on input mass, quality, fragment size, and adapter type. Adapter concentration recommendations are provided for full-length as well as truncated adapter types.

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

Truncated ('stubby') adapters

- When using 'stubby' adapters where sample indexes are added during subsequent library amplification, user-supplied, uniquely indexed PCR primers will be required for the amplification of each library to be sequenced on the same flow cell.
- 'Stubby' adapters provide improved library conversion efficiency due to the ability to include them at increased concentrations in the ligation reaction. We strongly recommend the use of 'stubby' adapters for maximum performance.
- For all dsDNA types—such as DNA sheared using Covaris instruments, as well as cell-free DNA—we recommend the same adapter concentration of 15 µM, irrespective of DNA input amount.

Full-length adapters

- This workflow is also compatible with full-length adapters where sample indexes are added during ligation. When using full-length adapters, a unique sample index 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.
- For full-length adapters, the recommended stock adapter concentration varies by sample type and input amount. For cfDNA, an adapter concentration of 15 µM is recommended. For sonicated DNA (such as DNA sheared using Covaris AFA instruments), or for samples with fragment sizes of approximately 150 to 500 base pairs, refer to **Table 1**.

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.

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.
- Vortex mixing is recommended for master mix generation and subsequent addition to sample. Pipette mixing is an acceptable alternative so long as care is taken to ensure a completely homogeneous reaction.
- 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 via vortexing prior to use.

1. End Repair and A-tailing

1.1 Program a thermocycler as indicated below:

Step	Temperature	Time
Lid temperature	85°C[1]	N/A
End repair	20°C	30 min
A-tailing	65°C	30 min
HOLD	4°C	HOLD

[1]A heated lid is required for this incubation. If possible, set the temperature of the lid at 85°C, instead of the usual ~105°C.

1.2 On ice, prepare input DNA in a total volume of 50 µL. Dilute DNA in PCR-grade water, 10 mM Tris-HCl pH 8.0, or Low TE (0.1 mM EDTA).

1.3 Prepare the End Repair and A-tailing (ER/AT) master mix as follows, on ice:

NOTE: The ER/AT master mix can be stored at 4°C for up to two hours.

Component	Volume (µL)
ER/AT Buffer	7
ER/AT Enzyme Mix	3

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 End repair and A-tailing master mix as specified below on ice:

Component	Volume (µL)
Input DNA	50
End repair and A-tailing master mix	10

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

1.7 Place the tubes in the thermocycler and initiate the end repair and A-tailing program (programmed in **Step 1.1**).

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

2. Adapter Ligation

NOTE: See **Prior to Starting** for considerations in adapter selection and design.

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 the Ligation master mix as follows:

NOTE: The Ligation master mix can be stored at 4°C for up to two hours.

Component	Volume (µL)
Ligation Buffer	25
Ligation Enzyme	5

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

2.4 Using an appropriate diluent (e.g., 10 mM Tris-HCl, pH 8.0, 10 mM NaCl), prepare the required volume of each adapter (stubby OR full-length) at the concentration specified below. 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.

Stubby adapters:
For DNA of any source (including FFPE and cfDNA), an adapter concentration of 15 µM is recommended, irrespective of DNA input amount.

NOTE: This is the minimum recommended adapter concentration. However, concentrations up to 50 µM have been tested and may result in improved performance.

Full-length adapters:
For cfDNA, an adapter concentration of 15 µM is recommended.

For non-cfDNA (including FFPE), the following adapter concentrations are recommended (**Table 1**):

Table 1. Recommended full-length adapter concentrations into library prep for non-cfDNA[1]

DNA Input (ng)	Adapter Concentration (µM)
>50	15.0
25 – 50	7.5
10 – 24	3.0
2 – 9	1.5
<2	0.3

[1]Adapter concentrations may need to be optimized based on input mass, quality (FFPE), fragment size, and adapter source. Use the maximum concentration of adapter while minimizing adapter dimer product.

2.5 Remove the reaction tubes (from **Step 1.8**) from the thermocycler.

2.6 To each tube, add the diluted adapter and Ligation master mix as specified below on ice:

Component	Volume (µL)
End repair and A-tailed DNA	60
Adapter	5
Ligation master mix	30

2.7 The Ligation master mix is viscous. Mix the ligation reaction on a touch vortexer (or 3,000 rpm) for 4 sec or carefully pipette a minimum of 50 µL up and down ≥10 times to ensure proper mixing. Briefly centrifuge if needed to collect all liquid in the bottom of the tube.

NOTE: Ensure the reaction is homogeneous prior to proceeding.

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

2.9 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 76 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. When 'stubby' adapters are used, the SPRI bead ratio may be increased to 1X (95 µL) to improve performance.

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.

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[1]	Temperature	Time	Cycles
Lid temperature	105°C	N/A	N/A
Initial denaturation	98°C	45 sec	1
Denaturation	98°C	15 sec	See **Table 2**
Annealing	P5/P7 primers: 60°C[2] Indexed primers: 55°C[3]	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.

[2]Appropriate temperature for P5/P7 Primer Mix (10X).

[3]For indexes used with the truncated adapter scheme detailed in Glenn, et. al. 2019, use 55°C. For the IDT xGen™ Stubby Adapter-UDI Primers, an annealing temperature of 64°C is recommended. For other adapter/primer configurations optimization may be required (see **Prior to Starting**).

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 – 40 nM Library	1 µg Library
1000	0	1 – 2
500	0	2 – 4
100	2 – 3	6 – 7
50	3 – 5	7 – 8
10	6 – 8	10 – 12
5	7 – 9	11 – 13
1[3]	9 – 11	13 – 15
0.5[3]	10 – 12	14 – 16

[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 until all beads have collected on the tube wall and 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-HCl, pH 8.0 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, the 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 A details the various paramagnetic bead types evaluated.

Table A. Paramagnetic purification bead types

Bead Type	Vendor	Catalog Number	Compatibility/amount tested[1]
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[2]	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

[1]Volume of slurry or mass of beads per 50 µL amplification reaction. [2]Used in xGen Hybridization and Wash Kit (Integrated DNA Technologies).

Revision History

Version	Description	Date
1.0	• First protocol release	03/2023
1.1	• Stubby adapter concentration recommendations consolidated for all DNA types in Step 2.4 • Full-length adapter concentration recommendations added for cfDNA in Step 2.4 • Full-length adapter concentration recommendations for other DNA types included in Step 2.4, Table 1	06/2023
2.0	• Step 2.5: Mixing instructions rewritten for clarification purposes • Step 4.3: Primer annealing temperature recommendations modified and lid temperature in thermocycler post-ligation amplification program added • Trademark updated	10/2024

WATCHMAKER GENOMICS

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