

SureSelect Max PCR-Free Whole Genome Library Preparation with Enzymatic Fragmentation

For Illumina Platform NGS

Protocol

Version A1 April 2026

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In this Guide...

This guide provides an optimized protocol for PCR-free whole genome DNA library preparation, using the SureSelect Max enzymatic DNA fragmentation system and full-length SureSelect Max UDI adaptors. Prepared libraries are ready for multiplexed whole-genome sequencing (WGS) on the Illumina platform.

1 Before You Begin

This section contains information that you should read and understand before you start an experiment.

2 PCR-Free DNA Library Preparation with Enzymatic Fragmentation Protocol

This section describes the steps to prepare gDNA sequencing libraries using enzymatic fragmentation and PCR-free library construction with full-length SureSelect Max UDI Adaptors for WGS. The prepared libraries are quantified by qPCR and qualified by automated electrophoresis prior to pooling for multiplex whole genome NGS.

3 Appendix: NGS Guidelines

This section provides guidelines for downstream NGS on the Illumina platform.

4 Reference

This section contains reference information, including reagent kit contents, adaptor UDI sequences, and troubleshooting information.

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Before You Begin

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Make sure you read and understand the information in this section and have the necessary equipment and reagents before you start an experiment.

Overview of the Workflow

The SureSelect Max system offers flexible workflow options for NGS library preparation in a modular format. This publication provides optimized protocols for PCR-free whole genome DNA library preparation, using enzymatic DNA fragmentation. The workflow is summarized in [Figure 1](#). The SureSelect Max enzymatic fragmentation kit uses a streamlined protocol with DNA fragmentation, end-repair and dA-tailing in a single reaction. Libraries are then constructed by ligation of full-length adaptors that include unique dual indexes (UDIs). The libraries are then purified, qualified and quantified to pool for multiplex NGS on an Illumina platform. For detailed protocols see [“PCR-Free DNA Library Preparation with Enzymatic Fragmentation Protocol”](#) on page 10.

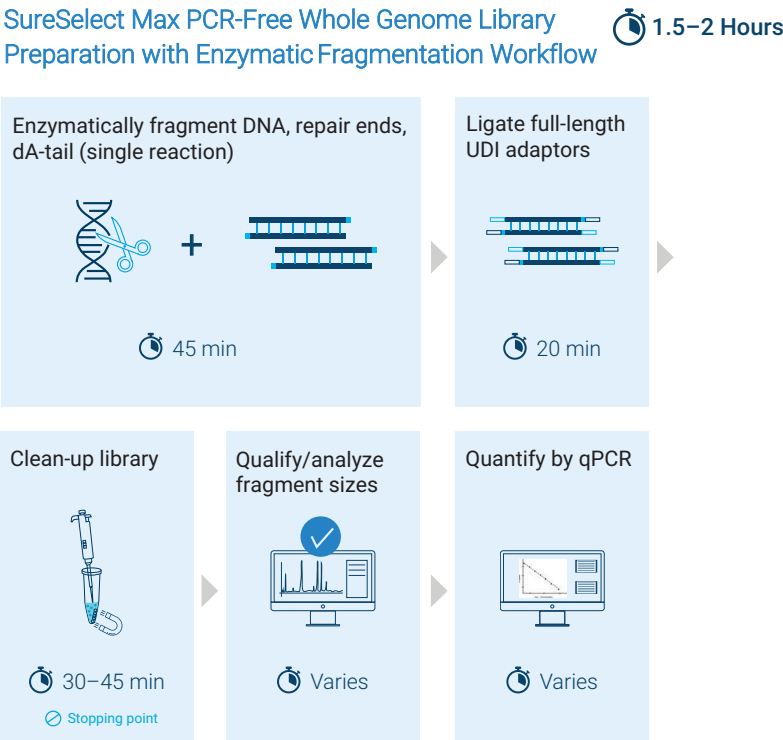


Figure 1 Summary of the SureSelect Max PCR-free whole genome DNA library preparation with enzymatic fragmentation workflow. The estimated time requirements and optional stopping points are provided in this diagram for reference. Estimates are guidelines for 16 reaction runs using high-quality input DNA. Timing for runs using different parameters may vary.

SureSelect Max Modules Used in the Workflow

This publication provides optimized protocols for PCR-free whole genome DNA library preparation, using enzymatic DNA fragmentation. Agilent's SureSelect Max reagents required to complete the protocols are summarized in [Table 1](#).

Table 1 Reagent Kits for SureSelect Max PCR-free WGS Library Prep with Enzymatic Fragmentation

Module Description	16 Reaction Kits [*]	96 Reaction Kits [†]
SureSelect Max Enzymatic Fragmentation Library Prep Kit, PCR-free	G9619A	G9619B
SureSelect Max UDI Adaptors for WGS, PCR-free (Select One): with ILM UDI Adaptors 1-16 with ILM UDI Adaptors 1-96	G9620A	G9620B
SureSelect Max Purification Beads	G9962A (5 mL)	G9962B (30 mL)

* 16-reaction kits contain enough reagents for 2 runs containing 8 samples per run.

† 96-reaction kits contain enough reagents for 4 runs containing 24 samples per run.

NOTE

AMPure XP Beads can replace SureSelect Max Purification Beads in the protocols provided in this user guide (see [Table 3](#) for ordering information).

Both types of magnetic purification beads have similar overall performance. The bead types are not, however, identical in composition and the average fragment length and yield results may differ slightly. Use a single bead source in validated protocols.

Additional Materials Used in the Workflow

The protocol requires 100–500 ng DNA input. See [Table 2](#) for recommended systems for the preparation and qualification of DNA samples based on sample type. See [Table 3](#) for additional reagents and equipment used in other workflow segments.

Table 2 Recommended DNA Sample Isolation and Qualification Systems

Description	Vendor and Part Number	Usage Notes
For preparation of DNA samples from fresh or fresh-frozen sources		
QIAamp DNA Mini Kit	Qiagen	Recommended reagents for high-quality DNA sample preparation prior to library preparation.
50 Samples	p/n 51304	
250 Samples	p/n 51306	
For preparation of FFPE DNA samples		
QIAamp DNA FFPE Tissue Kit, 50 Samples	Qiagen p/n 56404	Recommended reagents for FFPE gDNA sample preparation prior to library preparation.
Deparaffinization Solution	Qiagen p/n 19093	
FFPE DNA integrity assessment system:		Recommended systems for FFPE gDNA qualification prior to library preparation. See Table 3 for Agilent TapeStation instrument and accessory ordering information.
Agilent NGS FFPE QC Kit	Agilent	
16 reactions	p/n G9700A	
96 reactions	p/n G9700B	
OR		
TapeStation Genomic DNA Analysis Consumables:	Agilent	
Genomic DNA ScreenTape	p/n 5067-5365	
Genomic DNA Reagents	p/n 5067-5366	

Table 3 Ordering Information for Additional Reagents and Equipment

Description	Vendor and Part Number	Usage Notes
1X Low TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	Thermo Fisher Scientific p/n 12090-015, or equivalent	Recommended solvent for gDNA sample preparation and dilution. Do not use standard TE buffer containing >0.1 mM EDTA; higher EDTA concentrations inhibit the fragmentation reaction.
Nuclease-free Water	Thermo Fisher Scientific p/n AM9930	Water should not be DEPC-treated.
Qubit BR dsDNA Assay Kit, 100 assays	Thermo Fisher Scientific p/n Q32850	Use with Thermo Fisher Scientific's Qubit Fluorometer/Assay Tubes (p/n Q33238/Q32856)
Optional: AMPure XP Kit (5 mL)	Beckman Coulter Genomics p/n A63880	Optional alternative to SureSelect Max Purification Beads (See Table 1)
100% Ethanol (Ethyl Alcohol, 200 proof)	Millipore p/n EX0276	—
Thermal Cycler with 96-well, 0.2 mL block	Various suppliers	—
Plasticware compatible with the selected thermal cycler: 96-well plates or 8-well strip tubes Tube cap strips	Consult the thermal cycler manufacturer's recommendations	—
Magnetic separator	Thermo Fisher Scientific p/n 12331D or equivalent	Select a magnetic separator configured to collect magnetic particles on one side of each well. Do not use a magnetic separator configured to collect the particles in ring formation.
DNA LoBind Tubes, 1.5-mL PCR clean, 250 pieces	Eppendorf p/n 022431021 or equivalent	—
Microcentrifuge	Eppendorf microcentrifuge, model 5417C or equivalent	—
Plate or strip tube centrifuge	Labnet International MPS1000 Mini Plate Spinner, p/n C1000, or equivalent	Requires adapter, p/n C1000-ADAPT, for use with strip tubes
Multichannel and single channel pipettes	Rainin Pipet-Lite Multi Pipette or equivalent	—
Sterile, nuclease-free aerosol barrier pipette tips, vortex mixer, ice bucket, and powder-free gloves	General laboratory supplier	—
Agilent TapeStation system 4200/4150 TapeStation Instrument Consumables: 96-well sample plates 96-well plate foil seals 8-well tube strips 8-well tube strip caps D5000 ScreenTape D5000 Reagents	Agilent p/n G2991AA/G2992AA p/n 5042-8502 p/n 5067-5154 p/n 401428 p/n 401425 p/n 5067-5588 p/n 5067-5589	Automated electrophoresis system for library fragment sizing QC prior to downstream NGS.
NGS Library Quantification Kit (qPCR-based)	Various suppliers	Required for quantification of PCR-free whole genome libraries prior to NGS. Select a kit designed for Illumina library quantification.

Procedural and Safety Notes

- To prevent contamination of reagents by nucleases, always wear powder-free laboratory gloves and use dedicated solutions and pipettors with nuclease-free aerosol-resistant tips.
- Use of the following best-practices is recommended to prevent PCR product and nuclease contamination of samples throughout the workflow:
 - 1 Assign an isolated work area, such as a laminar flow hood, and dedicated materials for PCR-free workflows when PCR amplification and product processing are performed in the same laboratory, to prevent contamination of samples or reagents with amplified DNA.
 - 2 Maintain clean work areas. Clean the surfaces that pose the highest risk of contamination daily using a 10% bleach solution, or equivalent.
 - 3 Use dedicated pipettors with nuclease-free aerosol-resistant tips.
 - 4 Wear powder-free gloves. Use good laboratory hygiene, including changing gloves after contact with any potentially-contaminated surfaces.
- Several reagent solutions used in the SureSelect Max protocols are highly viscous. Follow the mixing instructions provided in the protocols.
- Avoid introducing bubbles into reaction mixtures during mixing steps. Before adding sample vials to the thermal cycler for incubation or PCR steps, verify the absence of bubbles at the bottom of the sample wells. If present, spin samples briefly to release the bubbles.
- For each protocol step that requires removal of tube cap strips, reseal the tubes with a fresh strip of caps. Cap deformation may result from exposure of the cap strips to the heated lid of the thermal cycler and from other procedural steps. Reuse of strip caps can cause sample loss, sample contamination, or imprecision in sample temperatures during thermal cycler incubation steps.
- In general, follow Biosafety Level 1 (BSL1) safety rules.
- Possible stopping points, where samples may be stored at 4°C or –20°C, are marked in the protocol. Do not subject the samples to multiple freeze/thaw cycles.

CAUTION

Wear appropriate personal protective equipment (PPE) when working in the laboratory.

2 PCR-Free DNA Library Preparation with Enzymatic Fragmentation Protocol

- Step 1. Prepare and qualify genomic DNA samples [12](#)
- Step 2. Fragment, end-repair, and 3'-dA-tail the DNA (Frag/A-Tail) [14](#)
- Step 3. Ligate full-length UDI adaptors [15](#)
- Step 4. Purify libraries using magnetic purification beads [16](#)
- Step 5. QC and quantify the libraries [19](#)

The library preparation protocol is compatible with both high-quality gDNA prepared from fresh or fresh-frozen samples and lower-quality DNA prepared from FFPE samples. The protocol requires 100 to 500 ng of input DNA, with adjustments to the recommended range of input DNA based on sample quality and intended NGS read length (see [Table 4](#)). For optimal sequencing results, use the maximum amount of input DNA available within the recommended range.

Library preparation begins with single-reaction enzymatic fragmentation, end-repair and dA-tailing of the DNA fragments. Next, the dA-tailed fragments are ligated to full-length adaptors that include unique dual indexes (UDIs). The library fragments are then purified using procedures for optimal size selection tailored to specific library types. The final prepared DNA libraries are qualified and quantified using methods appropriate for PCR-free whole genome sequencing (WGS).

For each sample to be sequenced, an individual dual-indexed library is prepared. To process multiple samples together, the protocol includes steps for preparation of reagent mixtures for 8 or 24 samples with overage, followed by distribution to individual DNA samples.

Table 4 DNA input amount recommendations

Sample type	NGS read length	Amount input DNA
High-quality (non-FFPE) samples	2 × 150 reads	100–500 ng
	2 × 250 reads	200–500 ng
FFPE samples	2 × 150 reads*	100–500 ng (at least 200 ng for low integrity samples; see page 13)

* All FFPE sample libraries are prepared using 2 x 150 read-length NGS conditions.

This workflow segment uses the components listed in [Table 5](#). Remove the listed reagents from cold storage and prepare as directed just before use (see the *Where Used* column).

Before you begin, determine the appropriate SureSelect Max UDI Adaptor assignment for each sample. Use a different UDI number for each sample to be sequenced in the same lane. See [page 30](#) for sequences of the UDIs added to the library fragments in this step.

Table 5 Reagents prepared before use in protocol

Storage Location	Kit Component	Preparative Steps	Where Used
SureSelect Max Enzymatic Fragmentation Library Preparation Module, PCR-free, stored at -20°C	Frag/A-Tail Buffer (yellow cap)	Thaw on ice then keep on ice, vortex to mix.	page 14
	Frag/A-Tail Enzyme Mix (green cap)	Place on ice just before use. Mix thoroughly by inversion 10X.	page 14
	Ligation Master Mix (blue cap or bottle)	Thaw on ice then keep on ice. Mix thoroughly by inversion 10X.	page 15
SureSelect Max UDI Adaptors for WGS, PCR-free, ILM stored at -20°C	Adaptor with UDIs 1-8 in white 8-well strip OR Adaptor with UDIs 9-16 in black 8-well strip OR Adaptor with UDIs 1-96 in clear 96-well plate	Thaw the appropriate range of adaptors on ice then keep on ice, vortex to mix.	page 15
+4°C	SureSelect Max Purification Beads OR AMPure XP Beads	Equilibrate at room temperature (RT) for at least 30 minutes before use, vortex to mix.	page 15

Step 1. Prepare and qualify genomic DNA samples

Enzymatic DNA fragmentation conditions have been optimized for DNA samples prepared in either 1X Low TE Buffer (recommended) or nuclease-free water. DNA samples prepared in nuclease-free water may produce slightly shorter insert sizes compared to samples prepared in 1X Low TE Buffer.

Do not substitute the 1X Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) with standard TE buffer containing >0.1 mM EDTA; higher EDTA concentrations inhibit the fragmentation reaction.

Preparation of high-quality gDNA from fresh biological samples

- 1 Prepare high-quality gDNA using a suitable purification system, such as Qiagen's QIAamp DNA Mini Kit, following the manufacturer's protocol.

NOTE

Make sure genomic DNA samples are of high quality with an OD 260/280 ratio ranging from 1.8 to 2.0.

- 2 Use the Qubit BR dsDNA Assay Kit to determine the concentration of each gDNA sample. Follow the manufacturer's instructions for the instrument and assay kit.
- 3 In wells of a thermal cycler-compatible strip tube or PCR plate, dilute the appropriate amount of each gDNA sample in 1X Low TE Buffer or nuclease-free water to a final volume of 40 μ L.

The protocol requires 100–500 ng of input DNA for 2 x 150 read length NGS or 200–500 ng of input DNA for 2 x 250 read length NGS.

Keep the samples on ice.

Additional qualification of DNA samples is not required for DNA derived from fresh biological samples. Proceed to ["Step 2. Fragment, end-repair, and 3'-dA-tail the DNA \(Frag/A-Tail\)"](#) on page 14.

Preparation and qualification of gDNA from FFPE samples

All FFPE samples use 2 x 150 read-length library preparation conditions.

- 1 Prepare gDNA from FFPE tissue sections using a suitable purification system, such as Qiagen's QIAamp DNA FFPE Tissue Kit and Qiagen's Deparaffinization Solution, following the manufacturer's protocol. Elute the final gDNA samples from the MinElute column in two rounds, using 30 μ L Buffer ATE in each round, for a final elution volume of approximately 60 μ L.

NOTE

If tissue lysis appears incomplete after one hour of digestion with Proteinase K, add an additional 10 μ L of Proteinase K and continue incubating at 56°C, with periodic mixing, for up to three hours.

Do not substitute Buffer ATE with any buffer containing >0.1 mM EDTA; higher EDTA concentrations inhibit the fragmentation reaction.

Store the gDNA samples on ice for same-day library preparation, or at –20°C for later processing.

- 2 Use the Qubit BR dsDNA Assay Kit to determine the concentration of each gDNA sample. Follow the manufacturer's instructions for the instrument and assay kit.
- 3 Assess the quality (DNA integrity) for each FFPE DNA sample using one of the methods below.

Option 1: Qualification using the Agilent Genomic DNA ScreenTape assay DIN score

The Agilent TapeStation Genomic DNA ScreenTape assay provides a quantitative electrophoretic assay for DNA sample integrity determination. This assay reports a DNA Integrity Number (DIN) score for each sample which is used to estimate the appropriate normalization of DNA input required for low-integrity DNA samples.

- a Analyze a 1- μ L aliquot of each FFPE gDNA sample using the Genomic DNA ScreenTape assay. Follow the instructions provided in the [assay Quick Guide](#).
- b See [Table 6](#) for DIN score-based input DNA input guidelines.

Table 6 DNA input guidelines based on DNA Integrity Number (DIN)

DIN Score	DNA Input Guidelines*
DIN ≥ 3	100 ng to 500 ng DNA, based on Qubit Assay quantification
DIN < 3	200 ng to 500 ng DNA, based on Qubit Assay quantification

* Use the DNA concentration determined by the Qubit Assay, instead of the concentration determined by ScreenTape assay, to calculate the input DNA volume.

Option 2: Qualification using the Agilent NGS FFPE QC Kit

The Agilent NGS FFPE QC Kit provides a qPCR-based assay for DNA sample integrity determination. Results include a $\Delta\Delta Cq$ DNA integrity score and the precise quantity of amplifiable DNA in the sample, allowing direct normalization of DNA input for each sample.

- a Analyze a 1- μ L aliquot of each FFPE gDNA sample using the Agilent NGS FFPE QC Kit. Follow the instructions provided in the [kit user manual](#).
- b Use the $\Delta\Delta Cq$ score-based guidelines below (summarized in [Table 7](#)) to determine the appropriate input DNA amount and quantification method for your sample:

For all samples with $\Delta\Delta Cq$ DNA integrity score ≤ 1 (more intact FFPE DNA samples), use the Qubit-based gDNA concentration to determine the volume of input DNA needed for the 100–500 ng DNA.

For all samples with $\Delta\Delta Cq$ DNA integrity score > 1 (less intact FFPE DNA samples), use the qPCR-based concentration of amplifiable gDNA, reported by the Agilent NGS FFPE QC Kit results, to determine the volume of input DNA needed for 100–500 ng DNA.

Table 7 DNA input guidelines based on $\Delta\Delta Cq$ DNA integrity score

$\Delta\Delta Cq$ Score	DNA Input Guidelines
$\Delta\Delta Cq \leq 1$	100 ng to 500 ng DNA, based on Qubit Assay quantification
$\Delta\Delta Cq > 1$	200 ng to 500 ng DNA, based on qPCR quantification

- 4 In wells of a thermal cycler-compatible strip tube or PCR plate, dilute 100 to 500 ng of each FFPE gDNA sample in 1X Low TE Buffer or nuclease-free water to a final volume of 40 μ L.

Keep the samples on ice.

Proceed to [“Step 2. Fragment, end-repair, and 3'-dA-tail the DNA \(Frag/A-Tail\)”](#) on page 14.

Step 2. Fragment, end-repair, and 3'-dA-tail the DNA (Frag/A-Tail)

- 1 Preprogram a thermal cycler as shown in [Table 8](#). Set the heated lid to 105°C. Hold at 4°C until samples are added in [step 4](#) on [page 15](#).

Table 8 Thermal cycler program for Frag/A-Tail reaction (50 µL vol)

Segment	Temperature	Time
1	4°C	Hold
2	30°C	Select fragmentation duration based on DNA quality and intended NGS read length (see Table 9)
3	65°C	30 minutes
4	4°C	Hold

Table 9 Fragmentation duration based on DNA quality and NGS read length

Input type	NGS read length requirement	Fragmentation duration (step 2 of PCR program)
Intact DNA	2 ×150 reads	5 minutes
	2 ×250 reads	2 minutes
FFPE DNA	2 ×150 reads	6 minutes

- 2 Prepare the appropriate volume of Frag/A-Tail master mix using the steps below:
 - a Vortex the thawed Frag/A-Tail Buffer at high speed for 5–10 seconds. Spin briefly and keep on ice.
 - b Invert the thawed Frag/A-Tail Enzyme Mix 10 times to homogenize. Spin briefly and keep on ice.
 - c Combine the volume of each reagent listed in [Table 10](#) in a 1.5-mL tube. Seal the tube and mix well by vortexing at high speed for 5 seconds. Spin briefly and keep on ice.

Table 10 Preparation of Frag/A-Tail master mix

Reagent	Volume for 1 reaction	Volume for 8 reactions (includes excess)*	Volume for 24 reactions (includes excess)†
Frag/A-Tail Buffer (yellow cap)	4 µL	36 µL	104 µL
Frag/A-Tail Enzyme Mix (green cap)	6 µL	54 µL	156 µL
Total	10 µL	90 µL	260 µL

* 16-reaction kits contain enough reagents for 2 runs of 8 samples each using the indicated excess volume. Reagents may be depleted after fewer than 16 reactions when using smaller run sizes.

† 96-reaction kits contain enough reagents for 4 runs of 24 samples each using the indicated excess volume. Reagents may be depleted after fewer than 96 reactions when using smaller run sizes.

- 3 Keep the DNA sample strip or plate on ice while adding 10 µL of the Frag/A-Tail master mix to each sample well containing 40 µL of DNA. Mix by pipetting up and down 15–20 times using a pipette set to 50 µL or cap the wells and vortex at high speed for 5–10 seconds.

- 4 Briefly spin the samples, then immediately place the plate or strip tube in the thermal cycler held at 4°C. Resume the thermal cycling program in [Table 8](#), advancing to the 30°C fragmentation step.

NOTE

Remove the magnetic purification beads from cold storage and equilibrate to room temperature (RT) for use on [page 16](#). Keep beads at RT for least 30 minutes before use.

Step 3. Ligate full-length UDI adaptors

CAUTION

The Ligation Master Mix used in this step is viscous and requires thorough mixing and careful pipetting. Make sure to follow the mixing instructions below.

Pipette the liquid slowly, ensuring that the full volume is aspirated and dispensed. After addition to the reaction mix, rinse the master mix tip with the sample solution.

- 1 Once the thermal cycling program in [Table 8](#) reaches the final 4°C Hold step, transfer the samples to ice. Preprogram the cycler as show in [Table 11](#) with heated lid off.

Table 11 Thermal cycler program for ligation (75 µL vol)

Segment	Temperature	Time
2	20°C	15 minutes
3	4°C	Hold

- 2 Keeping the samples on ice, add 5 µL of the assigned full-length SureSelect Max UDI Adaptor. Mix by pipetting up and down 15–20 times using a pipette set to 50 µL or cap the wells and vortex at high speed for 5–10 seconds.
- 3 Invert the thawed Ligation Master Mix (blue cap or bottle) 10 times to homogenize. Spin briefly. Add 20 µL of Ligation Master Mix to each sample well. Mix by pipetting up and down slowly at least 15–20 times using a pipette set to 50 µL.

NOTE

Make sure to add the Adaptor and the Ligation Master Mix to the samples in separate addition steps, mixing after each addition, as directed above. Adding these components together may decrease kit performance.

- 4 Briefly spin the samples, then immediately place the plate or strip tube in the thermal cycler and resume the program in [Table 11](#), advancing to the 20°C ligation step.

Step 4. Purify libraries using magnetic purification beads

NOTE

Before you begin, verify that the SureSelect Purification Beads or AMPure XP Beads were kept at RT for least 30 minutes before use in the protocol below.

The Whole Genome Library Preparation protocol uses different purification conditions optimized for specific library types as summarized in [Table 12](#). After reviewing the table, follow the detailed protocol steps provided below the table.

Table 12 Magnetic purification bead cleanup parameter summary

Step/Parameter	High-quality (fresh/fresh frozen) sample input DNA		FFPE sample input DNA
	2×150 read fragments	2×250 read fragments	2×150 read fragments
Dilute libraries to 100 µL prior to purification	Yes (add 25 µL nuclease-free water to each 75 µL sample)	Yes (add 25 µL nuclease-free water to each 75 µL sample)	No (retain 75 µL sample volume)
Rounds of purification required	Two (serial)	Two (serial)	One
Volume of fresh 70% ethanol to prepare (includes excess)	8-sample run: 7 mL 16-sample run: 14 mL	8-sample run: 7 mL 16-sample run: 14 mL	8-sample run: 3.5 mL 16-sample run: 7 mL
Round 1 bead volume	70 µL	60 µL	60 µL
Round 1 elution conditions	100 µL nuclease-free water	100 µL nuclease-free water	25 µL 1X Low TE Buffer
Round 2 bead volume	70 µL	60 µL	Not applicable
Round 2 elution conditions	25 µL 1X Low TE Buffer	25 µL 1X Low TE Buffer	
Final eluted purified library transferred to fresh well	Approximately 24 µL in 1X Low TE Buffer	Approximately 24 µL in 1X Low TE Buffer	Approximately 24 µL in 1X Low TE Buffer

- 1 Move all sample strips from the thermal cycler to room temperature. If required for your sample type, dilute samples prior to purification as described below:

For high-quality DNA libraries: Add 25 µL of nuclease-free water to the 75 µL library samples.

For FFPE DNA libraries: Do not dilute; proceed using 75 µL library samples.

- 2 Prepare fresh 70% ethanol for use in washes in [step 9](#) and [step 25](#) (where applicable). See [Table 12](#) for volume required for your sample type and run size.

- 3 Using SureSelect Max Purification Beads or AMPure XP Beads equilibrated to RT, mix the beads well until homogeneous and consistent in color.

- 4 Add the appropriate volume of bead suspension to each sample well:

For high-quality DNA libraries fragmented for 2 x 150 NGS: Add 70 µL of beads to the 100 µL diluted library samples

For high-quality DNA libraries fragmented for 2 x 250 NGS: Add 60 µL of beads to the 100 µL diluted library samples

For FFPE DNA libraries: Add 60 µL of beads to the 75 µL library samples

- 5 Mix by pipetting up and down 15–20 times or cap the wells and vortex at high speed for 5–10 seconds then spin briefly to collect the samples, being careful not to pellet the beads.
- 6 Incubate the bead suspensions for 10 minutes at room temperature.

- 7 Put the plate or strip tube into a magnetic separation device. Wait for the solution to clear (approximately 5 minutes).
- 8 Keep the plate or strip tube in the magnetic stand. Carefully remove and discard the cleared solution from each well. Do not touch the beads while removing the solution.
- 9 Continue to keep the plate or strip tube in the magnetic stand while you dispense 200 µL of fresh 70% ethanol in each sample well.
- 10 Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.
- 11 Repeat [step 9](#) and [step 10](#) once for a total of two washes.
- 12 Cap the wells, then briefly spin the samples to collect the residual ethanol. Return the samples to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.

NOTE

In the drying step below, do not dry the bead pellet to the point that the pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.

- 13 Dry the samples by keeping the unsealed plate or strip tube at room temperature for 2–5 minutes or until the residual ethanol has just evaporated.
- 14 Elute the library DNA by adding the appropriate solvent to each sample well:
 - For high-quality DNA libraries:** add 100 µL of nuclease-free water per well
 - For FFPE DNA libraries:** add 25 µL of 1X Low TE Buffer per well
- 15 Mix by pipetting up and down 10–15 times or cap the wells and vortex at high speed for 5 seconds. Verify that all beads have been resuspended, with no visible clumps in the suspension or bead pellets retained on the sides of the wells. If samples were vortexed, spin briefly to collect the liquid, being careful not to pellet the beads.
- 16 Incubate for 2–5 minutes at room temperature. Use of longer incubation times may improve recovery, especially for longer DNA fragments.
- 17 Put the plate or strip tube in the magnetic stand and leave until the solution is clear (up to 5 minutes).
- 18 Transfer each cleared supernatant to a fresh well and proceed as appropriate for your library type. You can discard the beads after collecting the supernatants.
 - For high-quality DNA libraries:** Transfer approximately 100 µL of each supernatant to a fresh strip well and proceed to [step 19](#) for the second round of purification.
 - For FFPE DNA libraries:** Transfer approximately 24 µL of each supernatant to a fresh strip well and keep the strip on ice. Proceed to [“Step 5. QC and quantify the libraries”](#) on page 19.

Round 2 purification for high-quality DNA input libraries only:

- 19 Mix the room-temperature purification beads well until homogeneous and consistent in color.
- 20 Add the appropriate volume of bead suspension to each sample well containing approximately 100 µL of library supernatant:
 - For high-quality DNA libraries fragmented for 2 x 150 NGS:** Add 70 µL of beads per well
 - For high-quality DNA libraries fragmented for 2 x 250 NGS:** Add 60 µL of beads per well
- 21 Mix by pipetting up and down 15–20 times or cap the wells and vortex at high speed for 5–10 seconds then spin briefly to collect the samples, being careful not to pellet the beads.
- 22 Incubate the bead suspensions for 10 minutes at room temperature.

- 23 Put the plate or strip tube into a magnetic separation device. Wait for the solution to clear (approximately 5 minutes).
- 24 Keep the plate or strip tube in the magnetic stand. Carefully remove and discard the cleared solution from each well. Do not touch the beads while removing the solution.
- 25 Continue to keep the plate or strip tube in the magnetic stand while you dispense 200 μ L of fresh 70% ethanol in each sample well.
- 26 Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.
- 27 Repeat [step 25](#) and [step 26](#) once for a total of two washes.
- 28 Cap the wells, then briefly spin the samples to collect the residual ethanol. Return the samples to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.

NOTE

In the drying step below, do not dry the bead pellet to the point that the pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.

- 29 Dry the samples by keeping the unsealed plate or strip tube at room temperature for 2–5 minutes or until the residual ethanol has just evaporated.
- 30 Elute the library DNA by adding 25 μ L of 1X Low TE Buffer to each sample well.
- 31 Mix by pipetting up and down 10–15 times or cap the wells and vortex at high speed for 5 seconds. Verify that all beads have been resuspended, with no visible clumps in the suspension or bead pellets retained on the sides of the wells. If samples were vortexed, spin briefly to collect the liquid, being careful not to pellet the beads.
- 32 Incubate for 2–5 minutes at room temperature. Use of longer incubation times may improve recovery, especially for longer DNA fragments.
- 33 Put the plate or strip tube in the magnetic stand and leave until the solution is clear (up to 5 minutes).
- 34 Transfer approximately 24 μ L of each supernatant to a fresh strip well and keep the strip on ice. You can discard the beads after collecting the supernatants. Proceed to [“Step 5. QC and quantify the libraries”](#) on page 19.

Stopping Point

If you do not continue to QC and library pooling on the same day, seal the wells and store at 4°C overnight or at –20°C for prolonged storage.

Step 5. QC and quantify the libraries

Library qualification with average fragment size determination

Analyze a sample of each library using the Agilent TapeStation system with the D5000 ScreenTape Assay (see the user guide link in [Table 13](#) for instructions).

Table 13 Library size qualification assay overview

Analysis platform	Assay used at this step	Amount of library sample to analyze
Agilent 4200/4150 TapeStation system	Agilent D5000 ScreenTape Assay	1 µL

Representative TapeStation electropherograms are provided in [Figure 2](#) and [Figure 3](#) to illustrate typical results.

Using the 100 bp to 3500 bp region of the electropherogram, determine the average fragment size. See [Table 14](#) for fragment size range guidelines.

Table 14 Expected library fragment size guidelines

Input type	NGS read length used to select fragmentation duration	Expected reported average fragment size (100–3500 bp region)
Intact DNA	2 × 150 reads	800 to 1300 bp
	2 × 250 reads	1100 to 1600 bp
FFPE DNA	2 × 150 reads	700 to 1200 bp

Adjustments to reported average fragment sizes

The full-length adaptors used for PCR-free library prep contain single-stranded regions that can retard migration during TapeStation electrophoresis, resulting in a larger apparent size profile compared to the actual library fragment size distribution. The reported average fragment size values should be adjusted before use in qPCR-based library quantification using the conversion provided for your sample type in [Table 15](#).

Table 15 Size adjustment for enzymatically-fragmented DNA libraries with full-length adaptors

Sample Type	TapeStation-reported library size adjustment guidelines
High-quality DNA processed using enzymatic fragmentation	Adjusted average fragment size (bp) = $[0.82 \times \text{reported average size (bp)}] - 100$ bp
FFPE DNA processed using enzymatic fragmentation	Assume actual average library fragment size of ~400 bp for all FFPE libraries

Proceed to [page 21](#) for qPCR quantification guidelines.

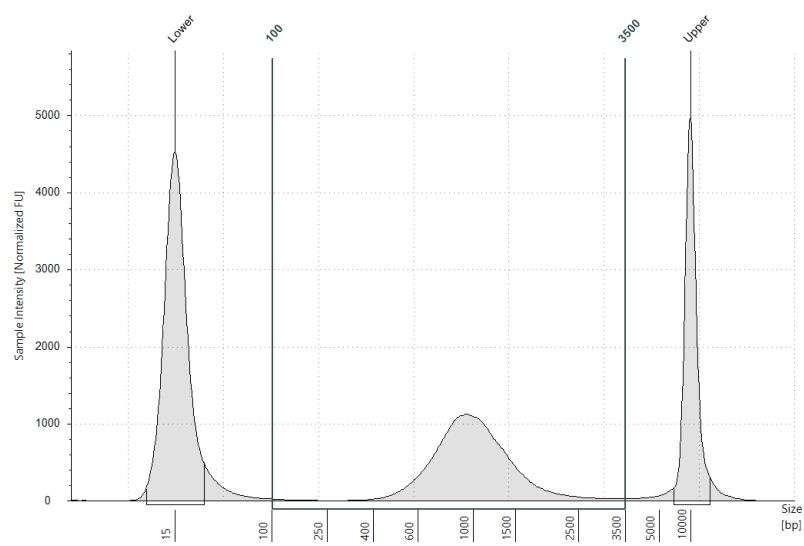


Figure 2 Library prepared from 100 ng of a high-quality gDNA sample, fragmented using 2x150 read length conditions.

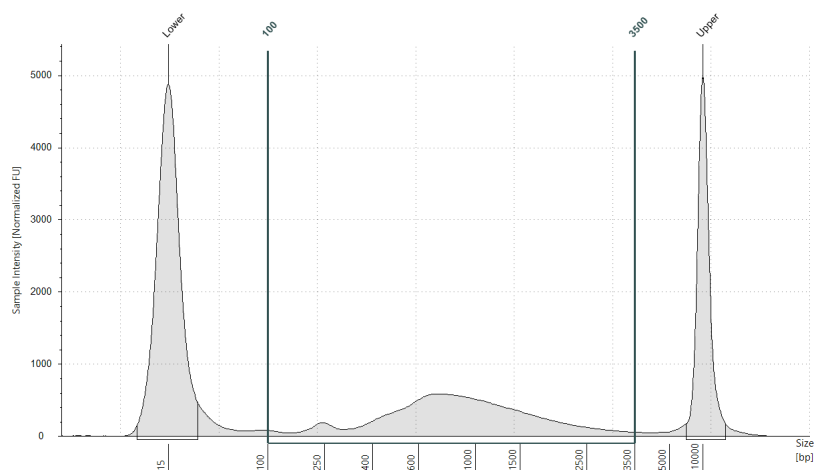


Figure 3 Library prepared from 100 ng of a medium-quality FFPE gDNA sample, fragmented using 2x150 read length conditions.

qPCR-based library quantification

Determine the concentration of each library using an appropriate qPCR-based NGS Library Quantification Kit designed to quantify fragments containing Illumina adaptor sequences.

Follow the manufacturer's protocols for standard curve generation and pM library concentration determination based on cycle threshold (Ct) values. The approximate library concentration from the TapeStation assay can be used to determine the appropriate library dilution scheme for qPCR quantification.

The qPCR quantification system should include instructions for correcting the pM library concentration value from the qPCR assay based on the relative lengths of the qPCR Standard and the library fragments. In these correction calculations, use the **adjusted** average fragment size (bp) determined for each library as described in [Table 15](#) on page 19.

If you have not already corrected the qPCR-determined library concentration values based on fragment size, use the formula below for this correction:

$$\text{Adjusted [Library]} = \text{Measured [Library]} \times (\text{qPCR Standard bp} / \text{Adjusted average library fragment size bp})$$

Stopping Point If you do not continue to library pooling on same day, seal the sample wells and store at 4°C overnight or at -20°C for prolonged storage.

3

Appendix: NGS Guidelines

Library Pooling Guidelines for Multiplex WGS [23](#)

Sequencing Setup and Run Guidelines [24](#)

Analysis Pipeline Guidelines [25](#)

This appendix provides guidelines for SureSelect Max PCR-free Whole Genome NGS using the Illumina platform.

Library Pooling Guidelines for Multiplex WGS

The SureSelect Max whole genome libraries are ready for pooling for multiplex WGS. Libraries multiplexed for sequencing in the same lane should be combined into a normalized pool using a plexity, concentration, and volume appropriate for your sequencing platform and research design.

Pool plexity: The number of indexed libraries that may be multiplexed is determined by the output specifications of the sequencer, together with the amount of sequencing data required per sample for your research design. Calculate the number of indexes that can be combined per lane, according to the capacity of your platform and the amount of sequencing data required per sample.

Normalized pool concentration: The required normalized pool concentration is determined by the optimal final loading concentration for PCR-free whole genome libraries for your selected sequencing platform and any pre-loading dilution steps in your NGS workflow.

For example, for NovaSeq 6000 Xp workflow runs, if the optimized final loading concentration is 100 pM, normalized library pools should be prepared at 0.5 nM to allow for pre-loading dilutions. Consult Illumina’s resources for more information for your NGS system.

NOTE

Accurate optimized loading concentrations can vary for libraries quantified with different qPCR quantification kits. The optimal final loading concentration should be titrated for the specific combination of sequencing and quantification systems used in your workflow.

An example of pooling conditions optimized for Agilent’s SureSelect Max ILM PCR-free WGS libraries for one combination of sequencing and quantification systems is provided in [Table 16](#). Optimal conditions for your quantification and NGS workflow may vary.

Table 16 Example pool concentration and volume determinations

qPCR Quantification Kit Manufacturer	Illumina Sequencing System	Desired Final Loading Concentration	Required Library Pool Concentration*
Takara	NovaSeq 6000 (Xp Workflow)	50–150 pM	0.25–0.75 nM

* For the NGS system in this example, libraries need to be pooled at a concentration 5× the intended final sequencer loading concentration (seeding concentration).

Pool volume: Consult Illumina’s support resources to determine the required pool volume for your sequencing platform and flow cell.

Normalized library pooling instructions

Combine the library samples such that each indexed library is present in equimolar amounts in the NGS pool using one of the following methods. Use the diluent specified by your sequencing provider, such as Low TE, for the dilution steps.

Method 1: Dilute each sample to be pooled to the same final concentration. Then combine equal volumes of all diluted samples to create the final NGS pool at the final volume required for your platform.

For example, if multiplexing 4 indexes with 0.5 nM pool concentration and 30 µL pool volume, dilute all libraries to 0.5 nM then combine 7.5 µL of each library.

Method 2: Starting with samples at different concentrations, add the appropriate volume of each sample to achieve equimolar concentration in the pool, then adjust the pool to the final volume required for your platform. The formula below is provided for determination of the amount of each indexed sample to add to the pool.

$$\text{Volume of Index} = \frac{V(f) \times C(f)}{\# \times C(i)}$$

where $V(f)$ is the final desired volume of the pool,

$C(f)$ is the desired final concentration of all DNA in the pool

$\#$ is the number of indexes, and

$C(i)$ is the initial concentration of each indexed sample

Table 17 shows an example of the amount of 4 indexed samples of different concentrations and amount of Low TE needed for a final volume of 30 μL at 0.5 nM DNA.

Table 17 Example pooling volume calculations

Component	V(f)	C(f)	# of indexes	C(i)	Volume to use (μL)
Sample 1	30 μL	0.5 nM	4	0.5 nM	7.5
Sample 2	30 μL	0.5 nM	4	0.75 nM	5.0
Sample 3	30 μL	0.5 nM	4	1.0 nM	3.75
Sample 4	30 μL	0.5 nM	4	1.5 nM	2.5
Low TE					11.25

Store the library pool under the conditions specified by your sequencing provider. Typically, libraries should be stored at -20°C for short-term storage.

Sequencing Setup and Run Guidelines

The pooled libraries are ready for sequencing using standard Illumina paired-end primers and chemistry. Each fragment in the prepared library contains one target insert surrounded by sequence motifs required for multiplexed sequencing (see [Figure 4](#)) including a unique pair of 8-bp P5 and P7 indexes suitable for Illumina sequencing platforms. See [“SureSelect Max Full-length Adaptor UDI Information”](#) on page 28 for additional index information.

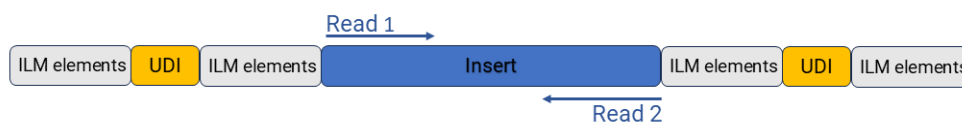


Figure 4 Content of SureSelect Max ILM PCR-free WGS libraries. Each fragment contains one target insert (blue), along with Illumina paired-end sequencing elements (gray) and unique dual indexes (gold).

Proceed to cluster amplification using the appropriate Illumina Paired-End Cluster Generation Kit. Final loading concentration (or seeding concentration) should be optimized for your selected sequencing platform and qPCR quantification kit (see [page 23](#)). Follow Illumina's recommendation for a PhiX low-concentration spike-in for improved sequencing quality control.

Set up the sequencing run to generate Read 1 and Read 2 FASTQ files for each sample using the instrument's software in standalone mode or using an Illumina run management tool such as Local Run Manager (LRM), Illumina Experiment Manager (IEM) or BaseSpace. Enter the appropriate **Cycles** or **Read Length** value for your library read length and using 8-bp dual index reads. See [Table 18](#) showing example settings for 2 x 150 bp sequencing.

Table 18 Run settings for 2 x 150 bp sequencing

Run Segment	Cycles/Read Length
Read 1	151*
Index 1 (i7)	8
Index 2 (i5)	8
Read 2	151*

* Follow Illumina's recommendation to add one (1) extra cycle to the desired read length.

Follow Illumina's instructions for each platform and setup software option, incorporating the additional setup guidelines below:

- Each of the sample-level indexes (i7 and i5) requires an 8-bp index read. For complete index sequence information, see [page 30](#).
- No custom primers are used for SureSelect Max library sequencing. Leave all *Custom Primers* options for *Read 1*, *Read 2*, *Index 1* and *Index 2* primers cleared/deselected during run setup.
- For runs set up using Illumina's LRM, IEM, or BaseSpace applications, refer to Illumina's instructions and support resources for setting up runs with custom library prep kits and index kits in the selected software. If you need additional SureSelect Max for ILM library sequencing guidance, contact the SureSelect support team (see [page 2](#)) or your local representative.

Analysis Pipeline Guidelines

Guidelines are provided below for typical NGS read processing and analysis pipeline steps for SureSelect Max PCR-free whole genome DNA libraries. Your NGS analysis pipeline may vary.

- Demultiplex using Illumina's bcl2fastq, BCL Convert or DRAGEN software to generate paired end reads based on the dual indexes and remove sequences with incorrectly paired P5 and P7 indexes.
- Sequencing adaptors can be trimmed using Illumina's demultiplexing software by specifying adaptor trimming in the sample sheet or using a suitable tool designed to trim the demultiplexed reads. Use the following sequences for adaptor trimming:
 - Read 1:** AGATCGGAAGAGCACACGTCTGAACTCCAGTCA
 - Read 2:** AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
- Align the trimmed reads using a suitable tool such as BWA-MEM.

The resulting BAM files are ready for downstream analysis including variant discovery.

4 Reference

Reagent Kit Contents [27](#)

SureSelect Max Full-length Adaptor UDI Information [28](#)

Troubleshooting Guide [32](#)

Quick Reference Protocol: PCR-free Whole Genome Library Prep [34](#)

This section contains reference information, including reagent kit contents, index sequences, troubleshooting information and quick-reference protocols for experienced users.

Reagent Kit Contents

SureSelect Max PCR-free whole genome DNA library preparation with enzymatic fragmentation uses the kits listed in [Table 19](#). Detailed contents of the multi-part component kits are shown in [Table 20](#) and [Table 21](#).

Table 19 Kits for SureSelect Max PCR-free WGS Library Preparation with Enzymatic Fragmentation

Reagent Kit Modules	Part Number		Storage Condition
	16 Reactions	96 Reactions	
SureSelect Max Enzymatic Fragmentation Library Preparation Module, PCR-free	5280-0148	5280-0149	–20°C
SureSelect Max UDI Adaptors for WGS, PCR-free, ILM	5280-0098 (Index 1-16)	5280-0099 (Index 1-96)	–20°C
SureSelect Max Purification Beads	5282-0225	5282-0226	+4°C

Component kit details

Table 20 SureSelect Max Enzymatic Fragmentation Library Preparation Module, PCR-free content

Kit Component	16 Reaction Kit	96 Reaction Kit
Frag/A-Tail Enzyme Mix	tube with green cap	tube with green cap
Frag/A-Tail Buffer	tube with yellow cap	tube with yellow cap
Ligation Master Mix	tube with blue cap	bottle

Table 21 SureSelect Max UDI Adaptors for WGS, PCR-free for ILM content

Kit Component	16 Reaction Kit Format	96 Reaction Kit Format
SureSelect Max UDI Adaptors for WGS, PCR-free, ILM*	White 8-well strip tube (index pairs 1-8) AND Black 8-well strip tube (index pairs 9-16)	Clear 96-well plate (index pairs 1–96)

* See [page 28](#) for index strip and plate position maps; see [page 30](#) and [page 31](#) for index pair sequence information.

CAUTION

The SureSelect Max UDI Adaptors are provided in single-use aliquots. To avoid cross-contamination of libraries, use each well in only one library preparation reaction. Do not retain and re-use any residual volume for subsequent experiments.

SureSelect Max Full-length Adaptor UDI Information

The SureSelect Max ILM UDI Adaptors for PCR-free WGS are provided in pre-combined index pairs. Each adaptor UDI well includes a unique 8-bp P5 index paired with a unique 8-bp P7 index, resulting in uniquely dual-indexed NGS libraries after adaptor ligation. Each well of the provided 8-well strip tubes contains a single-use aliquot of a specific index pair. Adaptor UDI strip and plate maps are provided below.

Nucleotide sequences of the indexes are provided in [Table 23](#) on page 30 and [Table 24](#) on page 31. Index sequences can also be obtained by downloading the [SureSelect Max UDI Adaptors for ILM WGS Index Sequence Resource](#) Excel spreadsheet from Agilent.com.

NOTE

Clicking the Excel spreadsheet link from Agilent.com automatically downloads the index spreadsheet to the default folder for downloaded files saved by your web browser. Locate the file in the folder and open it in Microsoft Excel or another compatible spreadsheet program. The first tab of the spreadsheet provides instructions for use of the spreadsheet contents.

In [Table 23](#) and [Table 24](#) and in the downloadable Excel spreadsheet, P7 indexes are shown in forward orientation, applicable to any of the supported Illumina platforms. P5 indexes are shown in both forward and reverse complement orientations. Refer to Illumina support documentation and resources to determine the correct P5 index orientation for your application.

NOTE

The indexes in the SureSelect Max ILM UDI Adaptors are shared with indexes used in other Agilent NGS library preparation systems. Different index numbers can have the same index sequence when comparing across these systems.

Before combining the SureSelect Max PCR-free WGS libraries with other types of SureSelect or Avida libraries in the same sequencing run, compare the **index sequences** to make sure that each index pair sequence is unique. Do not pool and run libraries together if they share index sequences.

Adaptor index pair strip tube and plate maps

SureSelect Max UDI Adaptors for WGS, PCR-free, ILM (UDI 1–16) are supplied in a set of two 8-well strip tubes as detailed below.

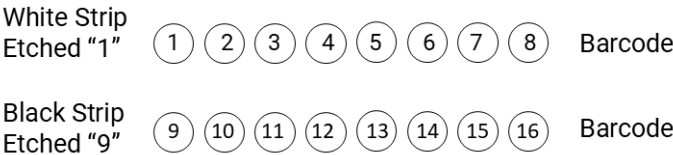


Figure 5 Map of the provided UDI Adaptor strips

The white strip contains adaptors with index pairs 1–8, with pair #1 supplied in the well proximal to the numeral **1** etched on the strip's plastic end tab.

The black strip contains adaptors with index pairs 9–16, with pair #9 supplied in the well proximal to the numeral **9** etched on the strip's plastic end tab.

Pierce the foil seal of the appropriate well with a pipette tip just before pipetting the solution.

Table 22 Plate map for SureSelect Max UDI Adaptors for WGS, PCR-free, ILM 1-96, provided in clear plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

SureSelect Max full-length adaptor index sequences

Index sequences can also be obtained by downloading the [SureSelect Max UDI Adaptors for ILM WGS Index Sequence Resource](#) Excel spreadsheet from Agilent.com.

Table 23 SureSelect Max full-length adaptor UDIs 1–48, provided in clear 96-well plate (1–16 also provided in strip tubes)

Primer Pair #	Well	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement	Primer Pair #	Well	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement
1	A01	CGTTCGTC	GCAGTTAG	CTAACTGC	25	A04	CGCGCAAT	CAGAGCAG	CTGCTCTG
2	B01	AATGCTAG	ATACTCGC	GCGAGTAT	26	B04	GCCTTCAT	AACGGTCA	TGACCGTT
3	C01	AAGTGGAC	TACGTAGC	GCTACGTA	27	C04	GATGCCAA	TTAGTTGC	GCAACTAA
4	D01	GCACACAT	AATACGCG	CGCGTATT	28	D04	CGTCTGTG	TTAACGTG	CACGTTAA
5	E01	AGAACCGT	TACGGTTG	CAACCGTA	29	E04	GTGCGCTT	CATCGACG	CGTCGATG
6	F01	TGCGCTCT	GTCGCCTA	TAGGCGAC	30	F04	GTACGGAC	GCACGATG	CATCGTGC
7	G01	CTGAGCTA	GCACAGTA	TACTGTGC	31	G04	AACGCATT	ATAGTGAC	GTCACTAT
8	H01	TAGGACAT	CATCGGAA	TTCCGATG	32	H04	ATATTCCG	GACAATTG	CAATTGTC
9	A02	TCAGCATC	AAGGAGCG	CGCTCCTT	33	A05	GATCGCCT	TGTATCAC	GTGATACA
10	B02	CTTGCGAT	CTGAGCTA	TAGCTCAG	34	B05	TCTGAGTC	ATCATGCG	CGCATGAT
11	C02	CGTAGTTA	GTAGACTG	CAGTCTAC	35	C05	TCTACCTC	ATATTCCG	CGGAATAT
12	D02	GTAGTCAT	AACTCTTG	CAAGAGTT	36	D05	CGCTTCCA	TCGAACTG	CAGTTCGA
13	E02	TCCGAACT	TCATACCG	CGGTATGA	37	E05	GAGTGCCT	TCGCGTCA	TGACGCCA
14	F02	CGTCAGAT	CGTAGTTA	TAACACG	38	F05	GAGGCTCT	CTAGTGTA	TACACTAG
15	G02	ATGGTAGC	GTCGATCG	CGATCGAC	39	G05	AACACCGA	CGATGTGC	CGACATCG
16	H02	TCGCCTGT	TCTGAGTC	GAATCAGA	40	H05	CGAATTCA	TTGGCATG	CATGCCAA
17	A03	AGCTACAT	GATCCATG	CATGGATC	41	A06	AGATACGA	CATACTTG	CAAGTATG
18	B03	GAAGGTAT	CGACTACG	CGTAGTCG	42	B06	TGTCGTCG	GTTATCGA	TCGATAAC
19	C03	AATGGAAC	GTTGCGGA	TCCGCAAC	43	C06	GCCAACAA	GTAATACG	CGTATTAC
20	D03	AGCAGTGA	CACAGGTG	CACCTGTG	44	D06	CATCAGTC	TTATCTCG	CGAGATAA
21	E03	TCAGCTCA	TATGCCGC	GCGGCATA	45	E06	CTAGTGTA	AACAGCCG	CGGCTGTT
22	F03	CAACCGTG	AAGTGGAC	GTCCACTT	46	F06	CAGTCATT	TCGAAGGA	TCCTTCGA
23	G03	CAGCAATA	ATTAGGAG	CTCCTAAT	47	G06	GAATTGAC	GCATCATA	TATGATGC
24	H03	TGGATTGA	TAAGAGTG	CACTCTTA	48	H06	GTCCACCT	AGTTGCCG	CGGCAACT

Table 24 SureSelect Max full-length adaptor UDIs 49–96, provided in clear 96-well plate

Primer Pair #	Well	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement	Primer Pair #	Well	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement
49	A07	TCTGTCAG	CGTAAGCA	TGCTTACG	73	A10	GCGAATTA	CTTGATCA	TGATCAAG
50	B07	CTTCACGT	GAATCGCA	TGCGATTG	74	B10	GTCGAAGT	CAGTGAGC	GCTCACTG
51	C07	CTTGATCA	CAATGCTG	CAGCATTG	75	C10	CATTGCAT	CGACGGTA	TACCGTCG
52	D07	TGCACATC	GAGTGTAG	CTACACTC	76	D10	TTCCAACA	GCTTAGCG	CGCTAAGC
53	E07	GCATGCCT	CGTAACTG	CAGTTACG	77	E10	AATACGCG	CAGGTCTG	CAGACCTG
54	F07	CACAACTT	AGGTGAGA	TCTCACCT	78	F10	TCTAGAGC	GATAGAGA	TCTCTATC
55	G07	CTGCCTTA	GCATGGAG	CTCCATGC	79	G10	TAAGAGTG	AGAAGACG	CGTCTTCT
56	H07	TCGATACA	TTAGTGCG	CGCACTAA	80	H10	AGTCTTCA	CAGTATCA	TGATACTG
57	A08	CAATAGAC	ATGACTCG	CGAGTCAT	81	A11	CAAGTTAC	TATTCCTG	CAGGAATA
58	B08	CGCCAATT	ATGGTAGC	GCTACCAT	82	B11	CGTAACTG	TGTCGTCTG	CGACGACA
59	C08	TGCCTCGA	CAGTTGCG	CGCAACTG	83	C11	GCTTAGCG	ATAAGCTG	CAGCTTAT
60	D08	GAGCACTG	GAATTGTG	CACAATTC	84	D11	TCAACTGC	GCTACGCA	TGCGTAGC
61	E08	GCCGATAT	AGACCTGA	TCAGGTCT	85	E11	AAGAGACA	TGCTTACG	CGTAAGCA
62	F08	CACAGACT	TCTGTCAG	CTGACAGA	86	F11	CGGTCTCT	GAGATCGC	GCGATCTC
63	G08	CTTAAGAC	GA CTGACG	CGTCAGTC	87	G11	TCGAGCAT	CGTTAACG	CGTTAACG
64	H08	AAGTACTC	GATAGGAC	GTCCTATC	88	H11	TCACAAGA	TTGTGTAC	GTACACAA
65	A09	TCGACAAC	CTCTCGTG	CACGAGAG	89	A12	TTGGATCT	TGCCGTTA	TAACGGCA
66	B09	GCCAGTAG	GTTGAGC	GCTCGAAC	90	B12	AGAGTAAG	GAGACACG	CGTGTCTC
67	C09	ATACCTAC	TCCTTGAG	CTCAAGGA	91	C12	AACCTAAC	CGTCTGTG	CACAGACG
68	D09	CATGAATG	TCTCCTTG	CAAGGAGA	92	D12	AGTACGTT	AGAGTAAG	CTTACTCT
69	E09	CACACATA	CTCGTACG	CGTACGAG	93	E12	GTTTCATT	GTCGGTAA	TTACCGAC
70	F09	TCCTGAGA	G TAGAGCA	TGCTCTAC	94	F12	AACTCTTG	AAGCCATC	GATGGCTT
71	G09	ATTGAGCT	TCACTGTG	CACAGTGA	95	G12	TCCAGACG	TGGCTTAG	CTAAGCCA
72	H09	TCTATGCG	GCGGTATG	CATACCGC	96	H12	CAATCCGA	CACATTCTG	CGAATGTG

Troubleshooting Guide

If yield of libraries is low

- ✓ Use only 1X Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) or nuclease-free water where specified in the protocol for sample preparation and dilution steps. Do not substitute with standard TE or other solvents containing >0.1 mM EDTA. Higher concentrations of EDTA inhibit the fragmentation reaction and reduce library yield.
- ✓ The library preparation protocol includes specific thawing, temperature control, pipetting, and mixing instructions which are required for optimal performance of the highly viscous buffer and enzyme solutions used in the protocol. Be sure to adhere to all instructions when setting up the reactions.
- ✓ DNA isolated from FFPE tissue samples may be over-fragmented or have modifications that adversely affect library preparation processes. Use the Agilent NGS FFPE QC Kit to determine the precise quantity of library-compatible DNA in the sample and allow direct normalization of input DNA amount.
- ✓ Yield from the magnetic bead purification step may be suboptimal. Consider the factors below:
 - Adhere to all of the bead and reagent handling steps in the protocol. In particular, make sure to equilibrate beads at room temperature for at least 30 minutes before use, and use freshly-prepared 70% ethanol (prepared on day of use) for each purification procedure.
 - Ensure that the magnetic beads are not over-dried just prior to sample elution (see [step 13 on page 17](#) and [step 29 on page 18](#)). Monitor the bead pellets during the drying incubation frequently and conclude the drying step immediately after the residual ethanol has evaporated.
 - Increasing the elution incubation time (up to 10 minutes) may improve recovery, especially for longer DNA fragments.

If library fragment size is different than expected or required in electropherograms

- ✓ If library fragments are longer than expected the gDNA samples may be under-fragmented. Use only 1X Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) or nuclease-free water where specified in the protocol for sample preparation and dilution steps. Do not substitute with standard TE or other solvents containing >0.1 mM EDTA. Higher concentrations of EDTA can inhibit DNA fragmentation.
- ✓ When preparing DNA samples for fragmentation, note that samples prepared in 1X Low TE Buffer typically produce slightly longer insert sizes compared to samples prepared in nuclease-free water.
- ✓ If library fragments are shorter than expected the gDNA samples may be over-fragmented. Make sure to keep DNA samples on ice while setting up the enzymatic fragmentation/end-repair/dA-tailing reactions in [step](#) on [page 14](#).
- ✓ DNA fragment size selection during magnetic bead purification depends upon using the correct ratio of sample to purification beads. Before removing an aliquot of beads for the purification step, mix the beads until the suspension appears homogeneous and consistent in color. Verify that you are using the recommended bead volume for your sample type.
- ✓ For libraries prepared from high-quality DNA (from fresh/fresh-frozen samples), the average library fragment size can be increased by adjusting the ratio of bead suspension to DNA sample volume in the library purification procedure on [page 16](#) as detailed below. **Note that this protocol modification will also decrease the overall library yield.**
 - For 2x150 NGS the standard protocol uses beads at 0.7X the library DNA sample volume. To increase the average fragment size in the library, decrease the bead volume ratio to 0.6X (with 60 µL of purification bead suspension added to each 100 µL sample). Use the adjusted ratio in both rounds of purification.
 - For 2x250 NGS the standard protocol uses beads at 0.6X the library DNA sample volume. To increase the average fragment size in the library, decrease the bead volume ratio to 0.5X (with 50 µL of purification bead suspension added to each 100 µL sample). Use the adjusted ratio in both rounds of purification.
- ✓ FFPE DNA libraries may have a smaller fragment size distribution due to the presence of DNA fragments in the sample input that are smaller than the targeted post-fragmentation size. Adhere to the DNA quality guidelines provided on [page 13](#).

If library concentration values reported by qPCR assay versus TapeStation assay are significantly different

- ✓ Accurate quantification of SureSelect Max PCR-free WGS library fragments prior to NGS requires use of a qPCR-based assay, as described on [page 21](#), in order to specifically quantify the NGS adaptor-containing DNA fragments. This concentration value may significantly differ from the value reported by automated electrophoresis, which includes all DNA fragments in the selected size range such as unmodified input DNA fragments. SureSelect workflows that include a PCR step can use automated electrophoresis for quantification prior to NGS due to the selective PCR amplification of the adaptor-containing fragments in library mixtures.

Quick Reference Protocol: PCR-free Whole Genome Library Prep

An abbreviated protocol summary is provided below for experienced users. Use the complete protocol on [page 11](#) to [page 21](#) until you are familiar with all of the protocol details.

Step	Summary of Conditions
Prepare and qualify DNA samples	Dilute 100–500 ng gDNA in 40 µL 1X Low TE Buffer (recommended) or nuclease-free H ₂ O> place in sample wells> keep on ice. See Table 4 on page 10 for DNA input amount requirements for specific workflows.
Prepare Frag/A-Tail master mix	Per 8 reactions: 36 µL Frag/A-Tail Buffer + 54 µL Frag/A-Tail Enzyme Mix Per 24 reactions: 104 µL Frag/A-Tail Buffer + 156 µL Frag/A-Tail Enzyme Mix Prepare on ice> vortex> spin> keep on ice.
Fragment, end-repair and dA-Tail the DNA samples	40 µL DNA sample + 10 µL Frag/A-Tail master mix Mix> spin> incubate in thermal cycler using program in Table 25 .
Ligate UDI adaptors	50 µL DNA fragments + 5 µL of assigned SureSelect Max UDI Adaptor> mix> spin. Add 20 µL Ligation Master Mix> pipette to mix> spin. Incubate in thermal cycler: Hold @ 4°C until samples loaded, 15 min @ 20°C, Hold @ 4°C.
High-quality DNA libraries only: dilute to 100 µL	For high-quality DNA input libraries only (non-FFPE), add 25 µL of nuclease-free H ₂ O per sample well for 100 µL final sample volume. Do not dilute FFPE input libraries.
Purify library DNA (Round 1) All libraries	For 2x150 high-quality libraries: 100 µL library DNA + 70 µL purification bead suspension For 2x250 high-quality libraries: 100 µL library DNA + 60 µL purification bead suspension For FFPE libraries: 75 µL library DNA + 60 µL purification bead suspension Mix> incubate 10 min> collect beads> discard supernatant. Wash beads 2X with 70% ethanol. Elute DNA in using appropriate solvent conditions for your library type: High-quality DNA libraries: 100 µL nuclease-free H ₂ O FFPE DNA libraries: 25 µL of 1X Low TE Buffer Add solvent> mix> incubate 2-5 minutes> collect beads> transfer library in supernatant to fresh well. For high quality DNA libraries proceed to round 2 purification steps below. FFPE library prep is complete; proceed to final QC steps.
Purify library DNA (Round 2) High-quality DNA libraries only	For 2x150 high-quality libraries: ~100 µL DNA supernatant + 70 µL purification bead suspension For 2x250 high-quality libraries: ~100 µL DNA supernatant + 60 µL purification bead suspension Mix> incubate 10 min> collect beads> discard supernatant. Wash beads 2X with 70% ethanol. Elute DNA in 25 µL of 1X Low TE Buffer > mix> incubate 2-5 minutes> collect beads> transfer library in supernatant to fresh well.
Quantify and qualify DNA	QC by TapeStation (see page 19 for size adjustment recommendations). Quantify library DNA for NGS using qPCR.

Table 25 Frag/A-Tail reaction thermal cycler program for PCR-free whole genome workflow (50 µL vol, heated lid at 105°C)

Segment	Temperature	Time
1	4°C	Hold--proceed to Segment 2 once samples added to block
2	30°C	Intact DNA for 2x150 NGS: 5 minutes Intact DNA for 2x250 NGS: 2 minutes FFPE DNA for 2x150 NGS: 6 minutes
3	65°C	30 minutes
4	4°C	Hold

In This Book

This guide provides instructions for SureSelect Max PCR-Free Whole Genome Library Preparation with Enzymatic Fragmentation for Illumina sequencing.

