SureSelect Max for MGI DNA Library Preparation with Enzymatic Fragmentation

For MGI Platform NGS

Protocol

Version B0 May 2025

SureSelect platform manufactured with Agilent SurePrint technology.

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Manual Part Number

G9697-90000

Edition

Version B0 May 2025

Agilent Technologies, Inc.
5301 Stevens Creek Blvd
Santa Clara, CA 95051 USA

Technical Support

For US and Canada

Call (800) 227-9770 (option 3,4,4)

Or send an e-mail to:
ngs.support@agilent.com

For all other regions

Agilent's world-wide Sales and Support Center contact details for your location can be obtained at www.agilent.com/en/contact-us/page.

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In this Guide...

This guide provides an optimized protocol for preparation of MGI paired-end DNA sequencing libraries using the SureSelect Max Enzymatic Fragmentation Library Preparation Module. The SureSelect Max workflow segment supported by this guide includes enzymatic fragmentation of gDNA samples through library preparation using MGI adaptors and unique dual indexes (UDIs). Prepared libraries are ready for target enrichment as described in separate guides for later workflow segments.

1 Before You Begin

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

2 DNA Library Preparation with Enzymatic Fragmentation Protocol

This section describes the steps to prepare dual-indexed DNA sequencing libraries for the MGI platform using the SureSelect Max Enzymatic Fragmentation Library Preparation Module, featuring reagents for single-reaction DNA fragmentation, end-repair and dA-tailing. Libraries are prepared with molecular barcoded (MBC) MGI adaptors and using unique dual indexes (UDIs) designed for the MGI system.

3 Reference

This section contains reference information, including component kit contents, troubleshooting information and an abbreviated quick-reference protocol for experienced users.

Whats New in Version B0:

- UDI sequence availability advisory added to page 34 and page 35

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1
Before You Begin

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

NOTE

Agilent guarantees performance and provides technical support for the SureSelect Max reagents required for this workflow only when used as directed in this Protocol.

Overview of the Workflow

The SureSelect Max system offers flexible workflow options for preparation and target enrichment of DNA or RNA libraries for NGS, with kits for specific workflow segments and platforms in a modular format. This publication provides optimized protocols for the MGI DNA library preparation workflow segment, using enzymatic DNA fragmentation, summarized in Figure 1.

The SureSelect Max enzymatic fragmentation module uses a streamlined protocol with DNA fragmentation, end-repair and dA-tailing in a single reaction. The next step is ligation of MGI Adaptor Oligos containing duplex molecular barcodes (MBCs) used for deduplication. Samples are then amplified using 384 unique dual indexing (UDI) primers designed for the MGI system.

For detailed protocols see “DNA Library Preparation with Enzymatic Fragmentation Protocol” on page 11. The protocol for downstream target enrichment is provided in a separate publication.

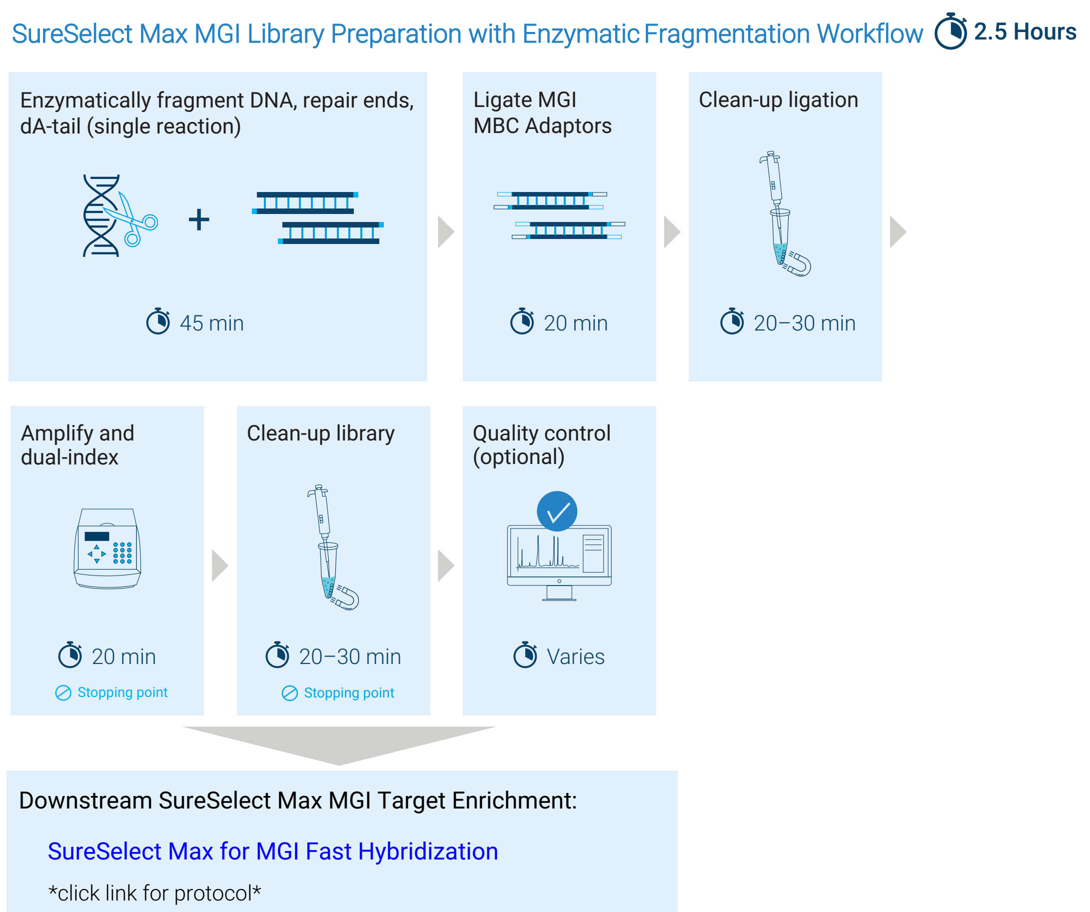

Figure 1 Summary of the SureSelect Max DNA library preparation using enzymatic fragmentation workflow for downstream target enrichment and MGI sequencing. The estimated time requirements and optional stopping points are provided in this diagram for reference. Estimates are guidelines for 16 reaction runs using 200 ng 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 the library preparation workflow segment, using enzymatic DNA fragmentation. Agilent's SureSelect reagents required to complete the protocols are summarized in Table 1.

SureSelect Max reagents are not interchangeable with SureSelect XT HS2 or other SureSelect system reagents.

Table 1 SureSelect Max Kits Used in the DNA Library Prep with Enzymatic Fragmentation Workflow

Module Description	16 Reaction Kits*	96 Reaction Kits†
SureSelect Max Enzymatic Fragmentation Library Prep Kit	G9660A	G9660B
SureSelect Max Adaptors and UDI Primers Kit for MGI (Select One):		
MBC Adaptors and UDI Primers 1-16	G9697A	
MBC Adaptors and UDI Primers 1-96		G9698A
MBC Adaptors and UDI Primers 97-192		G9698B
MBC Adaptors and UDI Primers 193-288		G9698C
MBC Adaptors and UDI Primers 289-384		G9698D
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.

‡ May be substituted with AMPure XP beads (see Table 2).

Additional Materials Used in the Workflow

See Table 2 through Table 4 for additional reagents and equipment used in the workflow.

Table 2 Ordering Information for Additional Reagents and Equipment

Description	Vendor and Part Number	Usage Notes
gDNA isolation and qualification systems	Select from Table 3 on page 9	Select the preparation and qualification systems appropriate for your sample type.
Nucleic acid analysis system	Select from Table 4 on page 9	Prepared library QC is optional for workflows that exclude normalization in downstream target enrichment (see page 22). Systems may also be used for FFPE sample qualification.
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)
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	—
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	—
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.
Nuclease-free Water	Thermo Fisher Scientific p/n AM9930	Water should not be DEPC-treated.
100% Ethanol (Ethyl Alcohol, 200 proof)	Millipore p/n EX0276	—
Optional: 1X Low TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	Thermo Fisher Scientific p/n 12090-015, or equivalent	Optional 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.
Optional: AMPure XP Kit (5 mL)	Beckman Coulter Genomics p/n A63880	Optional alternative to SureSelect Max Purification Beads (see Table 1)

Table 3 Recommended DNA Sample Isolation and Qualification Systems

Description	Vendor and Part Number	Usage Notes
For preparation of high-quality DNA samples		
QIAamp DNA Mini Kit 50 Samples 250 Samples	Qiagen p/n 51304 p/n 51306	Recommended reagents for high-quality DNA sample preparation prior to library preparation.
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: Agilent NGS FFPE QC Kit 16 reactions 96 reactions OR TapeStation Genomic DNA Analysis Consumables: Genomic DNA ScreenTape Genomic DNA Reagents	 Agilent p/n G9700A p/n G9700B Agilent p/n 5067-5365 p/n 5067-5366	Recommended systems for FFPE gDNA qualification prior to library preparation. See Table 4 for Agilent TapeStation instrument and accessory ordering information.

Table 4 Recommended Nucleic Acid Analysis Systems

Analysis System	Vendor and Part Number Information	Usage Notes
Agilent 4200/4150 TapeStation Instrument Consumables: 96-well sample plates 96-well plate foil seals 8-well tube strips 8-well tube strip caps D1000 ScreenTape D1000 Reagents	Agilent p/n G2991AA/G2992AA p/n 5042-8502 p/n 5067-5154 p/n 401428 p/n 401425 p/n 5067-5582 p/n 5067-5583	Recommended systems for optional QC of libraries prior to Target Enrichment. (Libraries may also be analyzed using the Agilent 2100 Bioanalyzer instrument and DNA 1000 Kit, p/n 5067-1504.)
Agilent 5200/5300/5400 Fragment Analyzer Consumables: NGS Fragment Kit (1-6000 bp)	Agilent p/n M5310AA/M5311AA/M5312AA p/n DNF-473-0500	

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 separate pre-PCR and post-PCR work areas and use dedicated equipment, supplies, and reagents in each area. In particular, never use materials designated to post-PCR work areas for pre-PCR segments of the workflow.
  2. Maintain clean work areas. Clean the surfaces that pose the highest risk of contamination daily using a 10% bleach solution, or equivalent.
  3. Always use dedicated pre-PCR pipettors with nuclease-free aerosol-resistant tips to pipette dedicated pre-PCR solutions.
  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
DNA Library Preparation with Enzymatic Fragmentation Protocol

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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 10 ng to 200 ng of input DNA, with adjustments to DNA input amount or quantification method required for some FFPE samples. 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 MGI adaptors tagged with duplex molecular barcodes (MBCs). After purification, the library fragments are amplified using unique dual indexing (UDI) primer pairs. After a final purification step, the prepared DNA libraries are ready for downstream target enrichment and NGS workflow segments. Guidelines are provided at the end of this section for optional QC of the prepared libraries prior to target enrichment.

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.

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).

Table 5 Reagents thawed before use in protocol

Storage Location	Kit Component	Preparative Steps	Where Used
SureSelect Max Enzymatic Fragmentation Library Preparation Module, stored at –20°C	Frag/A-Tail Buffer (yellow cap)	Thaw on ice then keep on ice, vortex to mix.	page 16
	Frag/A-Tail Enzyme Mix (green cap)	Place on ice just before use. Mix thoroughly by inversion 10X.	page 16
	Ligation Master Mix (blue cap or bottle)	Thaw on ice then keep on ice. Mix thoroughly by inversion 10X.	page 17
	Amplification Master Mix (red cap or bottle)	Thaw on ice then keep on ice. Mix thoroughly by inversion 10X. Do not vortex.	page 19
SureSelect Max Adaptors and UDI Primers Kit for MGI, stored at –20°C	SureSelect Max MBC Adaptor Oligo Mix for MGI (brown cap)	Thaw on ice then keep on ice, vortex to mix.	page 17
	SureSelect Max UDI Primers for MGI (select the specific set of indexes to be used in the run): Index Pairs 1-8 (green strip) Index Pairs 9-16 (black strip) Index Pairs 1-96 (clear plate) Index Pairs 97-192 (green plate) Index Pairs 193-288 (red plate) Index Pairs 289-384 (blue plate)	Thaw on ice then keep on ice, vortex to mix.	page 20
+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. Beads may be retained at RT for both purification steps performed on same day.	page 17 and page 20

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 or nuclease-free water. DNA samples prepared in nuclease-free water may produce slightly smaller 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 Place 10–200 ng of each DNA sample in 40 µL of 1X Low TE Buffer or nuclease-free water into wells of a thermal cycler-compatible strip tube or PCR plate and hold 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 15.

Preparation and qualification of gDNA from FFPE samples

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 µL Buffer ATE in each round, for a final elution volume of approximately 60 µL.

NOTE If tissue lysis appears incomplete after one hour of digestion with Proteinase K, add an additional 10 µL 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-µL aliquot of each FFPE gDNA sample using the Genomic DNA ScreenTape assay. Follow the instructions provided in the assay Quick Guide.

b Consult Table 6 for DIN score-based input DNA input guidelines.

Table 6 DNA input modifications based on DNA Integrity Number (DIN) score

Protocol Parameter	non-FFPE Samples	FFPE Samples		
		DIN > 8*	DIN 3–8	DIN<3
DNA input for Library Preparation	10 ng to 200 ng DNA, quantified by Qubit Assay	10 ng to 200 ng DNA, quantified by Qubit Assay	Use at least 15 ng for more intact samples and at least 40 ng for less intact samples. Use the maximum amount of DNA available, up to 200 ng, for all samples. Quantify by Qubit Assay.	Use at least 50 ng for more intact samples and at least 100 ng for the least intact samples. Use the maximum amount of DNA available, up to 200 ng, for all samples. Quantify by Qubit Assay.

* FFPE samples with DIN>8 should be treated like non-FFPE samples for DNA input amount determinations.

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 ΔΔCq DNA integrity score and the precise quantity of amplifiable DNA in the sample, allowing direct normalization of DNA input for each sample.

c Analyze a 1-µL aliquot of each FFPE gDNA sample using the Agilent NGS FFPE QC Kit. Follow the instructions provided in the kit user manual to determine the concentration of amplifiable DNA.

d Use the ΔΔCq score-based guidelines below (summarized in Table 7) to determine the appropriate input DNA quantification method for your sample:

For all samples with ΔΔCq DNA integrity score ≤1 (more intact FFPE DNA samples), use the Qubit-based gDNA concentration to determine volume of input DNA needed for the protocol.

For all samples with ΔΔCq 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 amounts of input DNA for the protocol.

Table 7 DNA input guidelines based on ΔΔCq DNA integrity score

ΔΔCq Score	DNA Input Guidelines
ΔΔCq≤1*	10 ng to 200 ng DNA, based on Qubit Assay quantification
ΔΔCq>1	10 ng to 200 ng of amplifiable DNA, based on qPCR quantification

* FFPE samples with ΔΔCq scores ≤1 should be treated like non-FFPE samples for DNA input amount determinations. For samples of this type, make sure to use the DNA concentration determined by the Qubit Assay, instead of the concentration determined by qPCR, to calculate the volume required for 10–200 ng DNA.

4 After qualification using either method, place 10–200 ng of each FFPE DNA sample in 40 µL of 1X Low TE Buffer or nuclease-free water into wells of a thermal cycler-compatible strip tube or PCR plate and hold on ice.

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

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

CAUTION The SureSelect Max enzymatic fragmentation and library preparation protocols use different reagents and conditions compared to earlier SureSelect protocols. Adhere to the instructions detailed in this publication, including thermal cycler programs and reagent mixing instructions, which differ from those used with earlier SureSelect platforms.

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 16.

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

Segment	Temperature	Time
1	4°C	Hold
2	37°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 ×100 reads	20 minutes
	2 ×150 reads	10 minutes
FFPE DNA	2 ×100 OR 2 ×150 reads	15 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 40 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 37°C fragmentation step.

NOTE

Remove the magnetic purification beads from cold storage and equilibrate to room temperature (RT) for use on page 17. Keep beads at RT for least 30 minutes before use; beads can be kept at RT through the final pre-capture library purification step on page 20.

Step 3. Ligate the adaptor

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. Hold at 4°C until samples are added in step 4.

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

Segment	Temperature	Time
1	4°C	Hold
2	20°C	15 minutes
3	4°C	Hold

2 To each dA-tailed DNA sample (50 µL), add 5 µL of SureSelect Max MBC Adaptor Oligo Mix for MGI (brown cap).

Mix by pipetting up and down 10 times using a pipette set to 40 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 Oligo Mix and the Ligation Master Mix to the samples in separate addition steps with mixing after each addition, as directed above. Adding these components together may increase adaptor-dimer formation and 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

CAUTION

The bead volume used at this step differs in various SureSelect system protocols. Adhere to the instructions provided here; do not use protocols provided for other SureSelect kits.

Once the thermal cycler program in Table 11 reaches the final 4°C hold step, purify the libraries using room-temperature (RT) SureSelect Max Purification Beads or AMPure XP Beads. Critical purification protocol parameters are summarized for experienced users in Table 12.

Table 12 Magnetic purification bead cleanup parameters after adaptor ligation

Parameter	Value
Volume of RT purification bead suspension added to each sample well	60 µL
Final elution solvent and volume	21 µL nuclease-free water
Amount of eluted sample transferred to fresh well	Approximately 20 µL

1 Prepare 400 µL of 70% ethanol per sample, plus excess, for use in step 8.

NOTE The freshly-prepared 70% ethanol may be used for all purification steps run on the same day.

2 Mix the room-temperature purification beads well until homogeneous and consistent in color.

3 Transfer the DNA samples from the thermal cycler to room temperature, then add 60 µL of the bead suspension to each sample well.

4 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.

5 Incubate the bead suspensions for 5 minutes at room temperature.

6 Put the plate or strip tube into a magnetic separation device. Wait for the solution to clear (approximately 5 to 10 minutes).

7 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.

8 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.

9 Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.

10 Repeat step 8 and step 9 once for a total of two washes.

11 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.

12 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.

13 Elute the library DNA by adding 21 µL of nuclease-free water to each sample well.

14 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.

15 Incubate for 2–5 minutes at room temperature. Use of longer incubation times may improve recovery, especially for longer DNA fragments.

16 Put the plate or strip tube in the magnetic stand and leave until the solution is clear (up to 5 minutes).

17 Transfer the cleared supernatant (approximately 20 µL) to a fresh well. Keep on ice.

You can discard the beads at this time.

Step 5. Amplify and index the libraries

1 Determine the appropriate SureSelect Max UDI Primer assignment for each sample. See page 26 for information on the UDI for MGI primers used to amplify the libraries in this step.

Use a different UDI number for each sample to be sequenced in the same lane.

CAUTION The UDI primers are provided in single-use aliquots. To avoid cross-contamination of libraries, do not retain and re-use any residual volume for subsequent experiments.

2 Preprogram a thermal cycler as shown in Table 13. Set the heated lid to 105°C. Prewarm the instrument before samples are loaded in step 6 on page 20.

Table 13 Library indexing/amplification thermal cycler program (50 µL vol)

Segment	Number of Cycles	Temperature	Time
1	1	98°C	45 seconds
2	7 to 13 based on input DNA quality and quantity (see Table 14)	98°C	15 seconds
		60°C	30 seconds
		72°C	30 seconds
3	1	72°C	1 minute
4	1	4°C	Hold

Table 14 Cycle number recommendations

Quality of Input DNA	Quantity of Input DNA	Cycles
Intact DNA from fresh sample	200 ng	7 cycles
	100 ng	8 cycles
	50 ng	9 cycles
	10 ng	11 cycles
FFPE sample DNA	100 to 200 ng*	11 cycles
	50 ng*	12 cycles
	10 ng*	13 cycles

* For samples qualified by qPCR, use the qPCR-determined quantity of amplifiable DNA to choose cycle number. For samples qualified by DIN value, use the Qubit Assay-determined quantity. See page 13 to page 14 for FFPE sample DNA quantification and qualification details.

NOTE To avoid cross-contamination, it is recommended to set up PCR reactions (all components except the library DNA) in a dedicated clean area or PCR hood with UV sterilization and positive air flow.

3 Mix the thawed Amplification Master Mix (red cap or bottle) thoroughly by inversion 10X then spin briefly.

4 Add 25 µL of the Amplification Master Mix to each sample well containing purified DNA library fragments (20 µL).

5 Add 5 µL of the assigned SureSelect Max UDI Primers for MGI to each reaction.

Cap the wells then vortex at high speed for 5 seconds. Spin the plate or strip tube briefly to collect the liquid and release any bubbles.

6 Place the sample plate or strip tube in the thermal cycler and run the program in Table 13.

CAUTION The lid of the thermal cycler is hot and can cause burns. Use caution when working near the lid.

Stopping Point If you do not continue to the next step, seal the sample wells and store at 4°C overnight or at –20°C for prolonged storage.

Step 6. Purify amplified libraries using magnetic purification beads

Once the thermal cycler program in Table 13 reaches the 4°C hold step, purify the libraries using room-temperature SureSelect Max Purification Beads or AMPure XP Beads.

Critical purification protocol parameters are summarized for experienced users in Table 15.

Table 15 Magnetic purification bead cleanup parameters after amplification

Parameter	Value
Volume of RT purification bead suspension added to each sample well	50 µL
Final elution solvent and volume	15 µL nuclease-free water
Amount of eluted sample transferred to fresh well	Approximately 14 µL

1 Prepare 400 µL of 70% ethanol per sample, plus excess, for use in step 8.

2 Mix the room-temperature purification beads well until homogeneous and consistent in color.

3 Transfer the amplified library samples from the thermal cycler to room temperature, then add 50 µL of the bead suspension to each sample well.

4 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.

5 Incubate the bead suspensions for 5 minutes at room temperature.

6 Put the plate or strip tube into a magnetic separation device. Wait for the solution to clear (approximately 5 minutes).

7 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.

8 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.

9 Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.

10 Repeat step 8 and step 9 once for a total of two washes.

11 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.

12 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.

13 Elute the library DNA by adding 15 µL of nuclease-free water to each sample well.

14 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.

15 Incubate for 2–5 minutes at room temperature. Use of longer incubation times may improve recovery, especially for longer DNA fragments.

16 Put the plate or strip tube in the magnetic stand and leave until the solution is clear (up to 5 minutes).

17 Transfer the cleared supernatant (approximately 14 µL) to a fresh well. Keep on ice.

You can discard the beads at this time.

Stopping Point If you do not plan to continue to the hybridization workflow segment on same day, seal the wells and store at 4°C overnight or at –20°C for prolonged storage. Remove an aliquot for QC analysis before storage, if appropriate.

Step 7. QC and quantify the libraries (optional)

QC of the prepared libraries is optional, but quantification is required for hybridization workflows using library normalization, including pre-capture pooling. When normalization is not required, the SureSelect Max Fast Hyb for MGI Target Enrichment post-capture pooling workflow supports the use of up to 12 µL of unquantified library samples in hybridization.

For workflows that include pre-capture QC, analyze a sample of each library using one of the platforms in Table 16. Follow the instructions in the linked user guide for each assay.

Table 16 Library analysis options

Analysis platform	Assay used at this step	Link to assay instructions	Amount of library sample to analyze
Agilent 4200/4150 TapeStation system	D1000 ScreenTape	Agilent D1000 Assay Quick Guide	1 µL of five-fold dilution
Agilent 5200/5300/5400 Fragment Analyzer system	NGS Fragment Kit (1-6000 bp)	Agilent NGS Fragment Kit (1-6000 bp) Kit Guide	2 µL of five-fold dilution

Each analysis method provides an electropherogram showing the size distribution of fragments in the sample and tools for determining the concentration of DNA in the sample. See Table 17 for fragment size distribution guidelines. Representative electropherograms generated using the TapeStation system are provided in Figure 2 and Figure 3 to illustrate typical results.

Using the 180 bp to 1000 bp region of the electropherogram, determine the average fragment size and the library DNA concentration.

Table 17 Expected library fragment size guidelines

Input type	NGS read length used to select fragmentation duration	Expected average fragment size (180–1000 bp region)
Intact DNA	2 ×100 reads	250 to 450 bp
	2 ×150 reads	320 to 470 bp
FFPE DNA	2 ×100 OR 2 ×150 reads	250 to 350 bp

Observation of a low molecular weight peak, in addition to the expected library fragment peak, indicates the presence of adaptor-dimers in the library. It is acceptable to proceed to target enrichment with library samples for which adaptor-dimers are observed in the electropherogram at low abundance, similar to the examples shown on page 23. See *Troubleshooting* on page 39 for additional considerations.

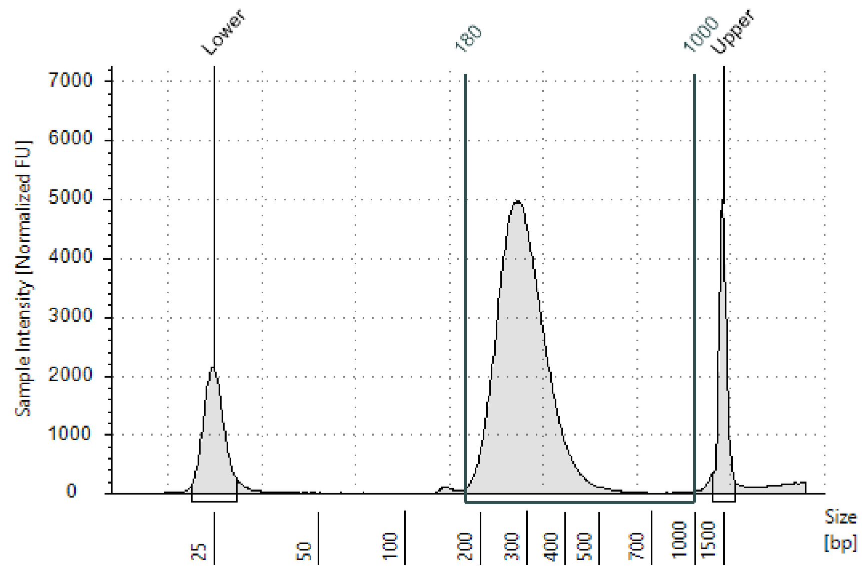

Figure 2 Library prepared from an FFPE gDNA sample analyzed using a D1000 ScreenTape assay.

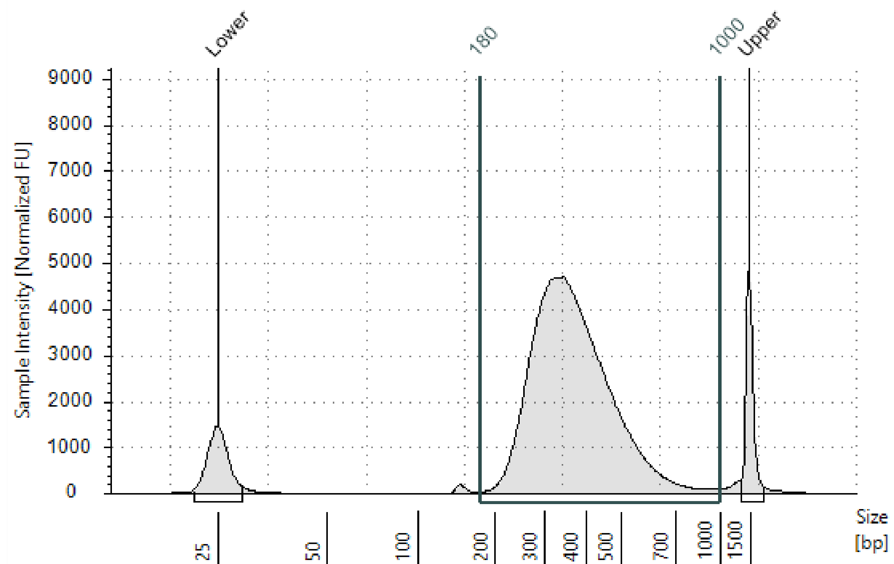

Figure 3 Library prepared from a high-quality gDNA sample analyzed using a D1000 ScreenTape assay.

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

The prepared DNA library fragments are ready for target enrichment. Proceed to the SureSelect Max for MGI Fast Hybridization user guide.

3
Reference

Reagent Kit Contents 25
SureSelect Max Library Composition 26
SureSelect Max UDI Primers Information 26
Troubleshooting Guide 38
Quick Reference Protocol: MGI Library Prep with Enzymatic Fragmentation 40

This section contains reference information, including Reagent Kit contents, index sequences, troubleshooting information and a quick-reference protocol for experienced users.

Reagent Kit Contents

SureSelect Max DNA Library Preparation with Enzymatic Fragmentation uses the kits listed in Table 18. Detailed contents of the multi-part component kits are shown in Table 19 through Table 21.

Table 18 Kits for SureSelect Max DNA Library Preparation with Enzymatic Fragmentation

Purchased Kit	Included Component Kits	Component Kit Part Number		Storage Condition
		16 Reactions	96 Reactions	
SureSelect Max Enzymatic Fragmentation Library Preparation Kit	SureSelect Max Enzymatic Fragmentation Library Preparation Module	5280-0063	5280-0064	−20°C
SureSelect Max Adaptors and UDI Primers Kit for MGI	SureSelect Max MBC Adaptor Oligo Mix for MGI	5282-0155	5282-0156	−20°C
	SureSelect Max UDI Primers for MGI	5280-0014 (Index 1-16)	5280-0015 (Index 1-96) 5280-0016 (Index 97-192) 5280-0017 (Index 193-288) 5280-0018 (Index 289-384)	−20°C
SureSelect Max Purification Beads		5282-0225	5282-0226	+4°C

Component Kit Details

Table 19 SureSelect Max Library Preparation Module content

Kit Component	16 Reaction Kit (p/n 5280-0063)	96 Reaction Kit (p/n 5280-0064)
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
Amplification Master Mix	tube with red cap	bottle

Table 20 SureSelect Max Adaptor Oligo Mix for MGI content

Kit Component	16 Reaction Kits	96 Reaction Kits
SureSelect Max MBC Adaptor Oligo Mix for MGI	tube with brown cap	tube with brown cap

Table 21 SureSelect Max UDI Primers for MGI options

Kit Component	16 Reaction Kit Format	96 Reaction Kit Format
SureSelect Max UDI Primers for MGI*	Green 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), OR Green 96-well plate (index pairs 97–192), OR Red 96-well plate (index pairs 193–288), OR Blue 96-well plate (index pairs 289–384)

* See page 27 through page 29 for index strip and plate position maps; see page 30 through page 37 for index pair sequence information.

SureSelect Max Library Composition

SureSelect Max library fragments prepared using Agilent's MGI-compatible kit modules are shown in Figure 4. Each fragment in the prepared library contains one target insert surrounded by sequence motifs used for MGI platform sequencing with standard MGI DNB chemistry.

Each library DNA fragment contains a pair of 10-bp sample indexes for multiplexed sequencing. See "SureSelect Max UDI Primers Information" below for additional information.

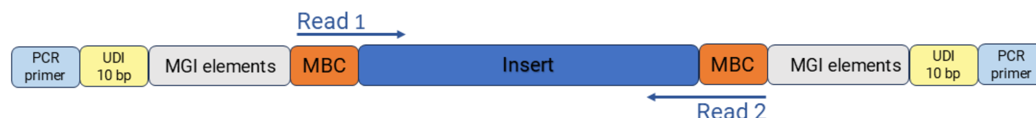

Figure 4 Content of SureSelect Max for MGI sequencing libraries. Each fragment contains one target insert (dark blue) surrounded by the following elements: MGI paired-end sequencing elements (gray), unique dual indexes (UDIs) for sample indexing (yellow), library PCR primers (light blue) and molecular barcode (MBC) sequences used as unique molecular identifiers for read deduplication (orange).

SureSelect Max UDI Primers Information

The SureSelect Max unique dual indexing (UDI) Primers are provided in pre-combined pairs of Index 1 and Index 2 primers, resulting in dual-indexed NGS libraries. One primer pair is provided per well of 8-well strip tubes in 16 reaction kits (see page 27 for map) or per well of 96-well plates in 96 reaction kits (see page 27 to page 29 for plate maps). Each well contains a single-use aliquot of a specific pair of forward plus reverse primers.

The nucleotide sequence of the 10-base index portion of each primer is provided in Table 26 on page 30 through Table 33 on page 37. Index sequences can also be obtained by downloading the SureSelect Max MGI 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 26 through Table 33 and in the downloadable Excel spreadsheet, index sequences are shown in the 5' to 3' orientation corresponding to the sequence of the 5'-phosphorylated strand used in MGI's DNB chemistry. Note that sample indexes may be referred to as *barcodes* in MGI publications and that MGI sample indexing *barcodes* are distinct from the SureSelect Max molecular barcodes (MBCs) added during adaptor ligation and used as unique molecular identifiers for read deduplication.

Index Primer Pair Strip Tube and Plate Maps

SureSelect Max UDI Primers 1-16 for MGI are supplied with 16 reaction kits in sets of two 8-well strip tubes as detailed below.

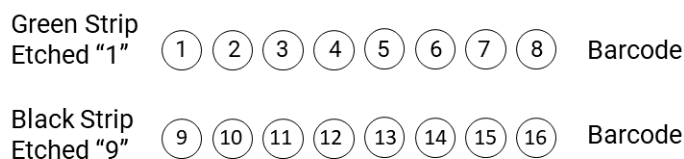

Figure 5 Map of the SureSelect Max UDI Primers for MGI strip tubes in 16 reaction kits

The green strip contains Index Primer 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 Index Primer Pairs 9-16, with pair #9 supplied in the well proximal to the numeral ***9*** etched on the strip's plastic end tab.

When using the strip tube-supplied index primer pairs in the library preparation protocol, pierce the foil seal of the appropriate well with a pipette tip just before pipetting the solution. If the foil seal for any unused wells is disrupted during use, the unused wells may be re-sealed using the provided fresh foil seal strips. The provided foil strips may also be used to re-seal used wells to prevent index pair cross-contamination during subsequent use.

Plate positions of the SureSelect Max UDI Primers for MGI provided with 96 reaction kits are shown in Table 22 through Table 25.

CAUTION The SureSelect Max UDI Primers 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.

Table 22 Plate map for SureSelect Max UDI Primers for MGI 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

Table 23 Plate map for SureSelect Max UDI Primers for MGI 97-192, provided in green plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	105	113	121	129	137	145	153	161	169	177	185
B	98	106	114	122	130	138	146	154	162	170	178	186
C	99	107	115	123	131	139	147	155	163	171	179	187
D	100	108	116	124	132	140	148	156	164	172	180	188
E	101	109	117	125	133	141	149	157	165	173	181	189
F	102	110	118	126	134	142	150	158	166	174	182	190
G	103	111	119	127	135	143	151	159	167	175	183	191
H	104	112	120	128	136	144	152	160	168	176	184	192

Table 24 Plate map for SureSelect Max UDI Primers for MGI 193-288, provided in red plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	193	201	209	217	225	233	241	249	257	265	273	281
B	194	202	210	218	226	234	242	250	258	266	274	282
C	195	203	211	219	227	235	243	251	259	267	275	283
D	196	204	212	220	228	236	244	252	260	268	276	284
E	197	205	213	221	229	237	245	253	261	269	277	285
F	198	206	214	222	230	238	246	254	262	270	278	286
G	199	207	215	223	231	239	247	255	263	271	279	287
H	200	208	216	224	232	240	248	256	264	272	280	288

Table 25 Plate map for SureSelect Max UDI Primers for MGI 289-384, provided in blue plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	289	297	305	313	321	329	337	345	353	361	369	377
B	290	298	306	314	322	330	338	346	354	362	370	378
C	291	299	307	315	323	331	339	347	355	363	371	379
D	292	300	308	316	324	332	340	348	356	364	372	380
E	293	301	309	317	325	333	341	349	357	365	373	381
F	294	302	310	318	326	334	342	350	358	366	374	382
G	295	303	311	319	327	335	343	351	359	367	375	383
H	296	304	312	320	328	336	344	352	360	368	376	384

SureSelect Max for MGI Index Sequences

Index sequences are shown in the 5' to 3' orientation corresponding to the sequence of the 5'-phosphorylated strand in MGI's DNB chemistry.

Table 26 SureSelect Max for MGI UDI 1–48, provided in clear 96-well plate or in strip tubes

UDI #	Well Position	Index 1	Index 2	UDI #	Well Position	Index 1	Index 2
1	A01	TAGAGGCGGT	TAGGTAACAG	25	A04	GAAGGTAGCG	ATCAGGATTC
2	B01	GCTTAACGTA	TAGAGGCGGT	26	B04	GTCCGAGATG	GAATATGGCT
3	C01	CTCACCGTAG	GCTTAACGTA	27	C04	AATTCCTCAG	GTCCGAGATG
4	D01	TGCTAGAAGA	CTAGCGTGTA	28	D04	CTATACCTGA	AATTCCTCAG
5	E01	CGTATAGACG	AAGCCTTCCG	29	E04	GCAATTCTGT	CTATACCTGA
6	F01	GCGTCACAGA	CGTATAGACG	30	F04	CATACTACTC	GCAATTCTGT
7	G01	CTAGCGTGTA	TGCTAGAAGA	31	G04	CTTCATTGCT	CATACTACTC
8	H01	TCTTCGGCCT	ATCGACAGAC	32	H04	CGCTCCATGT	CTTCATTGCT
9	A02	TTGAGACAGC	TCTTCGGCCT	33	A05	TTGAGTTCCA	CGCTCCATGT
10	B02	CAATAGACCG	TTGAGACAGC	34	B05	GCGGAATAGC	TTGAGTTCCA
11	C02	TACGCGTGTC	CAATAGACCG	35	C05	GCATCGCAAG	GCGGAATAGC
12	D02	AGGTCAACGT	TACGCGTGTC	36	D05	CTGCCGGTTA	GCATCGCAAG
13	E02	AACAGAACCT	AGGTCAACGT	37	E05	CAGTGCGATA	CTGCCGGTTA
14	F02	AACAGCAGAT	AACAGAACCT	38	F05	TTCTTGGTGC	CAGTGCGATA
15	G02	TCGTAGTGGC	AACAGCAGAT	39	G05	AAGCACCAAT	TTCTTGGTGC
16	H02	AGCTGTTCAG	TCGTAGTGGC	40	H05	ATTGCAGTTC	AAGCACCAAT
17	A03	ATAACGACCG	AGCTGTTCAG	41	A06	CTCGTAATTC	ATTGCAGTTC
18	B03	GAACGCACAC	ATAACGACCG	42	B06	TTACGTCCTT	CTCGTAATTC
19	C03	CTTCAACGGT	GAACGCACAC	43	C06	TCAGCGTTCG	TTACGTCCTT
20	D03	TGCTCGGAAC	CTTCAACGGT	44	D06	AGGCCATGTT	TCAGCGTTCG
21	E03	AGACTAGATG	TGCTCGGAAC	45	E06	TGTCAGTCTA	AGGCCATGTT
22	F03	GCATACCATA	AGACTAGATG	46	F06	TCGTTGCTGT	TGTCAGTCTA
23	G03	ATGTATCGGA	CAATACACTC	47	G06	TCATCACCAC	TCGTTGCTGT
24	H03	ATCAGGATTC	ATGTATCGGA	48	H06	CGATCATTAG	TCATCACCAC

Table 27 **SureSelect Max for MGI UDI 49–96, provided in clear 96-well plate**

UDI #	Well Position	Index 1	Index 2	UDI #	Well Position	Index 1	Index 2
49	A07	ATCACGGTAC	CGATCATTAG	73	A10	GACTAGCTAT	AGACTGCACA
50	B07	CATGTTACGC	ATCACGGTAC	74	B10	GAGACAGCGA	GACTAGCTAT
51	C07	GACGGAAGGT	CATGTTACGC	75	C10	TCGCCTAGAA	GAGACAGCGA
52	D07	TACTGCGCCT	GACGGAAGGT	76	D10	ATCATGTCTC	TCGCCTAGAA
53	E07	CTAACCTACT	TACTGCGCCT	77	E10	AAGGTCTCGT	ATCATGTCTC
54	F07	TCGTGTGTGC	CTAACCTACT	78	F10	TCGCGTTCGT	AAGGTCTCGT
55	G07	GCGCTTACCT	GCGGATTGGA	79	G10	GCGCCAAGAT	TCGCGTTCGT
56	H07	TCTTAACGGC	TAGCGCACGT	80	H10	CGCTTCGACA	GCGCCAAGAT
57	A08	CGCATGCTAC	TCTTAACGGC	81	A11	AGACGTTGTC	CGCTTCGACA
58	B08	CAGCAGTTCT	CGCATGCTAC	82	B11	TTAGTCCAGA	AGACGTTGTC
59	C08	GTGATCTAAG	CAGCAGTTCT	83	C11	GCGACTTGGT	TTAGTCCAGA
60	D08	AGTGACTGGT	GTGATCTAAG	84	D11	GCCTATTCCG	GCGACTTGGT
61	E08	CGCACAACTA	AGTGACTGGT	85	E11	CGCACTTCGA	GCCTATTCCG
62	F08	TCAAGGATGT	CGCACAACTA	86	F11	TTCAGCCGAG	CGCACTTCGA
63	G08	AAGATCTCAG	GAAGGTAGCG	87	G11	ATTCGGTTGA	TTCAGCCGAG
64	H08	GTTGCACGTG	TGCCGTTCCT	88	H11	TCATCCACGT	ATTCGGTTGA
65	A09	AACGCTCTTC	GTTGCACGTG	89	A12	GCATATAGTG	TCATCCACGT
66	B09	GCGGTCATGA	AACGCTCTTC	90	B12	AAGATTCGCA	GCATATAGTG
67	C09	AACACAGACG	GCGGTCATGA	91	C12	GTTACAGCAA	AAGATTCGCA
68	D09	GTTATCAGCT	AACACAGACG	92	D12	TGGAGCCTGA	GTTACAGCAA
69	E09	TCCGGTATTC	GTTATCAGCT	93	E12	GTCGGTTGCT	TGGAGCCTGA
70	F09	TAGCACAAGA	TCCGGTATTC	94	F12	CAGAGAGAGC	GTCGGTTGCT
71	G09	CATGAGGACT	TAGCACAAGA	95	G12	GTAACGGATA	CAGGCTCCAT
72	H09	AGACTGCACA	CATGAGGACT	96	H12	GAGAACCATC	TACCAGTGTA

Table 28 **SureSelect Max for MGI UDI 97–144, provided in green 96-well plate**

UDI #	Well Position	Index 1	Index 2	UDI #	Well Position	Index 1	Index 2
97	A01	GTGTGAGTCG	GAGAACCATC	121	A04	CGGTTGCTTA	TCCGTCCATT
98	B01	CGACTCCTTA	GTTGATGGCT	122	B04	GACTCCTACG	CGGTTGCTTA
99	C01	AACAAGGAAC	AGTCTCGACA	123	C04	CTTCCATGTA	GACTCCTACG
100	D01	AGTCTCATTC	AACAAGGAAC	124	D04	AGGCCTACAC	CTTCCATGTA
101	E01	TACTGTTGCG	AGTCTCATTC	125	E04	CAAGGCCGTT	AGGCCTACAC
102	F01	CAACCTCTCT	TACTGTTGCG	126	F04	TGCCTCACGT	CAAGGCCGTT
103	G01	TCAAGTGATG	CAACCTCTCT	127	G04	GTTGGATAGT	TGCCTCACGT
104	H01	TGGCGCTGTA	TCAAGTGATG	128	H04	ATACCTAGAC	GTTGGATAGT
105	A02	GTTGTGATAC	TGGCGCTGTA	129	A05	TAAGGCGGTG	ATACCTAGAC
106	B02	CGACAAGTAA	GTTGTGATAC	130	B05	CTAACGACTA	TAAGGCGGTG
107	C02	TCAACGTAAG	CGACAAGTAA	131	C05	ATTCTCGCAT	CTAACGACTA
108	D02	TTGACTGTGA	TCAACGTAAG	132	D05	AATAACGGCG	ATTCTCGCAT
109	E02	GCCTTACTAG	TTGACTGTGA	133	E05	TCGTATTCAG	AATAACGGCG
110	F02	CATCCAATGT	GCCTTACTAG	134	F05	GACTCTCGTA	TCGTATTCAG
111	G02	CGCAACGAGT	CATCCAATGT	135	G05	ATGATCTCCT	CGACTCCTTA
112	H02	AACGGTTAAC	CGCAACGAGT	136	H05	CTCTATGCGC	AGGAGAAGAC
113	A03	GTTGACTTGC	AACGGTTAAC	137	A06	GACCATCCAG	CTCTATGCGC
114	B03	AACCAGATCT	GTTGACTTGC	138	B06	CACACTTGTC	GACCATCCAG
115	C03	TAAGTTACCG	AACCAGATCT	139	C06	GAGCGCATAG	CACACTTGTC
116	D03	TAATCAGTGC	TAAGTTACCG	140	D06	AGCGGCTTAG	GAGCGCATAG
117	E03	CGGTGTGTCT	TAATCAGTGC	141	E06	GTCCTACACG	AGCGGCTTAG
118	F03	AGGCATTCCT	AGTGATCTTG	142	F06	CATACGAGGA	GTCCTACACG
119	G03	AGACATCCTC	GAGATCGACG	143	G06	CAGGAACAGA	CATACGAGGA
120	H03	TCCGTCCATT	AGACATCCTC	144	H06	CTATCCGGTG	CAGGAACAGA

Table 29 **SureSelect Max for MGI UDI 145–192, provided in green 96-well plate**

UDI #	Well Position	Index 1	Index 2	UDI #	Well Position	Index 1	Index 2
145	A07	TCCTGATGAC	CTATCCGGTG	169	A10	CTGAGATGAG	TAATCGTAGC
146	B07	TATCCGTAAC	TCCTGATGAC	170	B10	TAATCTCGCA	AAGATCGTCT
147	C07	CTGTCACGTC	TATCCGTAAC	171	C10	TGGAATACTC	CTCCAAGCCA
148	D07	TGCGAGTAAG	TACGACCAAT	172	D10	AATGGAAGTG	TGGAATACTC
149	E07	TACGGACTAT	AGATCGTAGT	173	E10	TACTCGGTAA	AATGGAAGTG
150	F07	GAGATACAAC	TACGGACTAT	174	F10	AGAGCCAATC	TACTCGGTAA
151	G07	AACGGTGCTG	GAGATACAAC	175	G10	CAACGGCCTA	AGAGCCAATC
152	H07	TTAGCGCACG	AACGGTGCTG	176	H10	TTGGACGCGT	CAACGGCCTA
153	A08	TCCTACCGCA	TTAGCGCACG	177	A11	AAGCACAGAC	TTGGACGCGT
154	B08	GTGAAGCTCA	TCCTACCGCA	178	B11	TCTACGACGA	AAGCACAGAC
155	C08	CTCCGTGTTG	TGTAAGAGTC	179	C11	TGCCGAAGCA	TCTACGACGA
156	D08	GCATACAACT	AGACGTGATT	180	D11	TGGTGTGATG	TGCCGAAGCA
157	E08	GTATACTACG	GCATACAACT	181	E11	CAGTCGGCTA	TGGTGTGATG
158	F08	GATAAGCGTA	GTATACTACG	182	F11	ATGAGTGAGC	CAGTCGGCTA
159	G08	ATTCGATGAG	GATAAGCGTA	183	G11	TTAGACATCG	TGTAGGACCA
160	H08	GAGTTGTCGA	ATTCGATGAG	184	H11	TCGCCATTAT	ATATCCATCG
161	A09	AGCTCCTACA	GAGTTGTCGA	185	A12	AATAGACCTG	TCGCCATTAT
162	B09	AACGCCAATA	AGCTCCTACA	186	B12	CAACTGCTGC	AATAGACCTG
163	C09	CTACCAGTCG	AACGCCAATA	187	C12	ATGTCCAGAT	AACTAGCGGA
164	D09	GTTCGTGAGC	CTACCAGTCG	188	D12	TCACGGTAGC	TGAGACCGAG
165	E09	GAGGAACTAT	TAGTGGTAGA	189	E12	GAGACGCACA	TCACGGTAGC
166	F09	ATGTGACTCA	AACTTGAGAG	190	F12	CGGTCTCATG	GAGACGCACA
167	G09	CTCAACACCG	ATGTGACTCA	191	G12	GTAACCGTTG	CGGTCTCATG
168	H09	TAATCGTAGC	CTCAACACCG	192	H12	GTGCTGGAAT	GTAACCGTTG

Table 30 **SureSelect Max for MGI UDI 193–240, provided in red 96-well plate**

Sequences to be provided at future date

Table 31 SureSelect Max for MGI UDI 241–288, provided in red 96-well plate

Sequences to be provided at future date

Table 32 **SureSelect Max for MGI UDI 289–336, provided in blue 96-well plate**

UDI #	Well Position	Index 1	Index 2	UDI #	Well Position	Index 1	Index 2
289	A01	CTCGAAGTAG	TGCCGTTATA	313	A04	GTCCATACCT	AGAACTCTAC
290	B01	GAGGTTCGGT	CTCGAAGTAG	314	B04	GATCCTGGAT	GTCCATACCT
291	C01	CTAACGCTCA	GAGGTTCGGT	315	C04	AGTGGCACGA	GATCCTGGAT
292	D01	CAAGCGCATA	CTAACGCTCA	316	D04	AGACGCGTAT	AGTGGCACGA
293	E01	TCGAACTCTC	CAAGCGCATA	317	E04	GATCGGATAT	AGACGCGTAT
294	F01	TCATGCTCAG	TCGAACTCTC	318	F04	CACTATCGAA	GATCGGATAT
295	G01	CGTATACTAG	TCATGCTCAG	319	G04	CTTCACAATG	GTAGCTCGTT
296	H01	GACATGCTCT	CGTATACTAG	320	H04	TGGACAGAAG	AAGCAGGCGA
297	A02	CGAGCCTCTA	CAGCGTACTA	321	A05	GACATGAAGA	TGGACAGAAG
298	B02	TGCCATCGTA	TCCTCAGCGA	322	B05	GCTTCGATGT	GACATGAAGA
299	C02	CAGACGAACG	TGCCATCGTA	323	C05	CGCAATACAC	GCTTCGATGT
300	D02	GCAAGTCGCT	CAGACGAACG	324	D05	TTCGCATCTG	CGCAATACAC
301	E02	AGATGCGTCG	GCAAGTCGCT	325	E05	TATAGCGCTG	TTCGCATCTG
302	F02	TCGAACGTGA	AGATGCGTCG	326	F05	AACTGCATCT	TATAGCGCTG
303	G02	AACGCTACAT	TCGAACGTGA	327	G05	GAGAAGTCCA	AACTGCATCT
304	H02	TAGCTTGATG	AACGCTACAT	328	H05	TGTTCAACCA	GAGAAGTCCA
305	A03	ATTAGCGCAA	TAGCTTGATG	329	A06	ATGAAGCCGA	TGTTCAACCA
306	B03	CGACCATTGA	ATTAGCGCAA	330	B06	CGCCTTAGAT	ATGAAGCCGA
307	C03	ATCTACCTCG	CGACCATTGA	331	C06	CATCAGTTGC	CGCCTTAGAT
308	D03	GCTACTCGTG	ATCTACCTCG	332	D06	TACTGCTGGA	CATCAGTTGC
309	E03	AGCCGCTCTA	GCTACTCGTG	333	E06	AATGACGCGC	TACTGCTGGA
310	F03	GCCAGAGAAG	AGCCGCTCTA	334	F06	AGCAAGCCAG	AATGACGCGC
311	G03	AAGTTCGCGA	GCCAGAGAAG	335	G06	CGCCTTGTTC	AGCAAGCCAG
312	H03	AGAACTCTAC	AAGTTCGCGA	336	H06	GAGGTGCTTC	CGCCTTGTTC

Table 33 **SureSelect Max for MGI UDI 337–384, provided in blue 96-well plate**

UDI #	Well Position	Index 1	Index 2	UDI #	Well Position	Index 1	Index 2
337	A07	AGGCCAATCA	GAGGTGCTTC	361	A10	CGGATGCACT	GCTCAATTGT
338	B07	CTCGTACCTG	AGGCCAATCA	362	B10	ATTCCGTACA	CGGATGCACT
339	C07	ATGCCTTAGC	CTCGTACCTG	363	C10	TAATGTCTGC	ATTCCGTACA
340	D07	GAAGGCTCGA	ATGCCTTAGC	364	D10	TACGGCAGCA	TAATGTCTGC
341	E07	TGTATCTGCG	GAAGGCTCGA	365	E10	AACTCTTCCT	TACGGCAGCA
342	F07	CTCCTAATAG	TGTATCTGCG	366	F10	GTTCATGCAG	AACTCTTCCT
343	G07	CATGGAATCA	GAGCCTTGAG	367	G10	TGGACATTGC	GTTCATGCAG
344	H07	GACTCGATTG	AGCAAGGCGA	368	H10	TCTCGTCTAT	TGGACATTGC
345	A08	CTGCATTCCA	GACTCGATTG	369	A11	AGATGTCCGA	TCTCGTCTAT
346	B08	AGATGAGTGA	CTGCATTCCA	370	B11	AATAGCTCTC	AGATGTCCGA
347	C08	AACAGATGAG	AGATGAGTGA	371	C11	CAATAGCAAG	AATAGCTCTC
348	D08	GATGTACTTG	AACAGATGAG	372	D11	TATGTGCTAG	CAATAGCAAG
349	E08	TCCGATAATG	GATGTACTTG	373	E11	ATACAGCCAG	TATGTGCTAG
350	F08	GTCCTGGTTA	TCCGATAATG	374	F11	CAACCATCTA	ATACAGCCAG
351	G08	CACGTTAGCT	GTCCTGGTTA	375	G11	CAGGTGCTGT	CAACCATCTA
352	H08	ATTCTCACCA	CACGTTAGCT	376	H11	TGCATCGTAT	CAGGTGCTGT
353	A09	GCTCAGCTTC	ATTCTCACCA	377	A12	CTATACACCA	TGCATCGTAT
354	B09	CACCAGTGAT	GCTCAGCTTC	378	B12	ATGAGCTAAC	CTATACACCA
355	C09	TTGGTCTTCT	CACCAGTGAT	379	C12	TCACCTTCTA	ATGAGCTAAC
356	D09	ATCGTTGATG	TTGGTCTTCT	380	D12	TGCGATGGAG	TCACCTTCTA
357	E09	CTGTCCATCA	CTTCACAATG	381	E12	GTCCTGCAGA	TGCGATGGAG
358	F09	GAACATGCTA	GCTCAACGAC	382	F12	ATGTGCACAA	GTCCTGCAGA
359	G09	CTATTGTAGC	GAACATGCTA	383	G12	CAACGAAGTT	ATGTGCACAA
360	H09	GCTCAATTGT	CTATTGTAGC	384	H12	GCTCCGTCAT	CAACGAAGTT

Troubleshooting Guide

If recovery of gDNA from samples is low

- ✓Using excess tissue for gDNA isolation can reduce yield. Use only the amount of each specific tissue type recommended by the gDNA isolation protocol.
- ✓Tissue sample lysis may not have been optimal during gDNA isolation. Monitor the extent of sample lysis during the Proteinase K digestion at 56°C by gently pipetting the digestion reaction every 20–30 minutes, visually inspecting the solution for the presence of tissue clumps. If clumps are still present after the 1-hour incubation at 56°C, add another 10 µL of Proteinase K and continue incubating at 56°C, with periodic mixing and visual inspections, for up to two additional hours. When the sample no longer contains clumps of tissue, move the sample to room temperature until lysis is complete for the remaining samples. Do not over-digest. Individual samples may be kept at room temperature for up to 2 hours before resuming the protocol. Do not exceed 3 hours incubation at 56°C for any sample.

If yield of libraries is low

- ✓Use only nuclease-free water or 1X Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) 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.
- ✓PCR cycle number may require optimization. Repeat library preparation for the sample, increasing the PCR cycle number by 1 to 2 cycles. If a high molecular weight peak (>500 bp) is observed in the electropherogram for a sample with low yield, the DNA may be over-amplified. Repeat library preparation for the sample, decreasing the PCR cycle number by 1 to 3 cycles.
- ✓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 amplifiable 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 12 on page 18 and page 21). Monitor the bead pellets frequently while drying 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.
- ✓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 3 on page 16.
- ✓When preparing DNA samples for fragmentation, note that samples prepared in nuclease-free water can produce slightly smaller insert sizes compared to samples prepared in 1X Low TE Buffer.
- ✓FFPE DNA pre-capture 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 14.

- ✓DNA fragment size selection during purification depends upon using the correct ratio of sample to magnetic purification bead solution. 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 at each stage of the protocol.

If low molecular weight adaptor-dimer peak is present in pre-capture library electropherograms

- ✓The presence of a low molecular weight peak, in addition to the expected peak, indicates the presence of adaptor-dimers in the library. It is acceptable to proceed to target enrichment with library samples for which adaptor-dimers are observed in the electropherogram at low abundance, similar to the samples analyzed on page 23. The presence of excessive adaptor-dimers in the samples may be associated with reduced yield of pre-capture libraries. If excessive adaptor-dimers are observed, verify that the adaptor ligation protocol is being performed as directed on page 17. In particular, ensure that the Adaptor Oligo Mix is mixed with the sample prior to adding the Ligation Master Mix to the mixture. Do not add the Ligation Master Mix and the Adaptor Oligo Mix to the sample in a single step.

Quick Reference Protocol: MGI Library Prep with Enzymatic Fragmentation

An abbreviated protocol summary is provided below for experienced users. Use the complete protocol on page 12 to page 23 until you are familiar with all of the protocol details.

Step	Summary of Conditions
Prepare and qualify DNA samples	Prepare 10–200 ng gDNA in 40 µL 1X Low TE Buffer or nuclease-free H_2O> place in sample wells> keep on ice. For FFPE DNA, qualify integrity and adjust input amount as directed on page 14.
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 34.
Ligate adaptor	50 µL DNA fragments + 5 µL SureSelect Max MBC Adaptor Oligo Mix for MGI> 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.
Purify DNA	75 µL DNA sample + 60 µL purification bead suspension> mix> incubate 5 min> collect beads> discard supernatant. Wash beads 2X with 70% ethanol. Elute DNA in 21 µL nuclease-free H_2O> mix> incubate 2-5 minutes> collect beads> transfer 20 µL supernatant to fresh well.
Index and amplify library	20 µL DNA sample + 25 µL Amplification Master Mix + 5 µL SureSelect Max UDI Primers for MGI Vortex> spin> amplify in thermal cycler using program in Table 35.
Purify amplified library DNA	50 µL amplified DNA + 50 µL purification bead suspension> mix> incubate 5 min> collect beads> discard supernatant. Wash beads 2X with 70% ethanol. Elute DNA in 15 µL nuclease-free H_2O> mix> incubate 2-5 minutes> collect beads> transfer 14 µL supernatant to fresh well.
Quantify and qualify DNA	Optional: Analyze quantity and quality using TapeStation or Fragment Analyzer System

Table 34 Frag/A-Tail reaction thermal cycler program (50 µL vol, heated lid at 105°C)

Segment	Temperature	Time
1	4°C	Hold--proceed to Step 2 once samples added to block
2	37°C	Intact DNA for 2 ×100 NGS: 20 minutes Intact DNA for 2 ×150 NGS: 10 minutes FFPE DNA for any read length NGS: 15 minutes
3	65°C	30 minutes
4	4°C	Hold

Table 35 Library indexing/amplification thermal cycler program (50 µL vol; heated lid at 105°C)

Segment	Number of Cycles			Temperature	Time
1	1			98°C	45 seconds
2	Intact DNA input 200 ng: 7 cycles 100 ng: 8 cycles 50 ng: 9 cycles 10 ng: 11 cycles	**OR**	FFPE DNA input 100–200 ng: 11 cycles 50 ng: 12 cycles 10 ng: 13 cycles	98°C 60°C 72°C	15 seconds 30 seconds 30 seconds
3	1			72°C	1 minute
4	1			4°C	Hold

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In This Book

This guide provides instructions for SureSelect Max DNA Library Preparation with Enzymatic Fragmentation for MGI sequencing.

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Version B0 May 2025

G9697-90000

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