SureSelect Max for MGI Target Enrichment using Fast Hybridization

For NGS using the MGI Platform

Protocol

Version B0 May 2025

SureSelect platform manufactured with Agilent SurePrint technology.

For Research Use Only. Not for use in diagnostic procedures.

Notices

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In this Guide...

This guide provides an optimized protocol for target enrichment of MGI paired-end DNA sequencing libraries using SureSelect Max Fast Hybridization Modules and a SureSelect probe. Libraries can be pooled for NGS using either pre-capture or post-capture pooling. The target-enriched library pools are ready for NGS using a suitable MGI instrument. Before starting this protocol, SureSelect Max libraries must be prepared as described in separate guides for earlier workflow segments.

1 Before You Begin

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

2 Target Enrichment with Fast Hybridization Protocol

This section describes the steps to hybridize and capture targeted fragments in a SureSelect Max DNA library using a SureSelect Probe, using fast (2-3 hour) hybridization conditions.

3 Appendix: NGS Guidelines

This section provides guidelines for downstream NGS sample preparation through analysis.

4 Reference

This section contains reference information, including component kit contents, troubleshooting information and a 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 libraries for NGS, with kits for specific workflow segments in a modular format. This publication provides optimized protocols for the target enrichment workflow segment, using fast hybridization conditions for target enrichment of prepared SureSelect Max DNA libraries for MGI sequencing using a compatible SureSelect XT HS probe, summarized in Figure 1. The fast hybridization protocol includes options for post-capture or pre-capture library pooling and a two-day workflow option using an optional overnight hold (up to 16 hours).

For detailed protocols see "Target Enrichment with Fast Hybridization Protocol" on page 10. Protocols for the upstream DNA library preparation module options are provided in separate publications with links provided below.

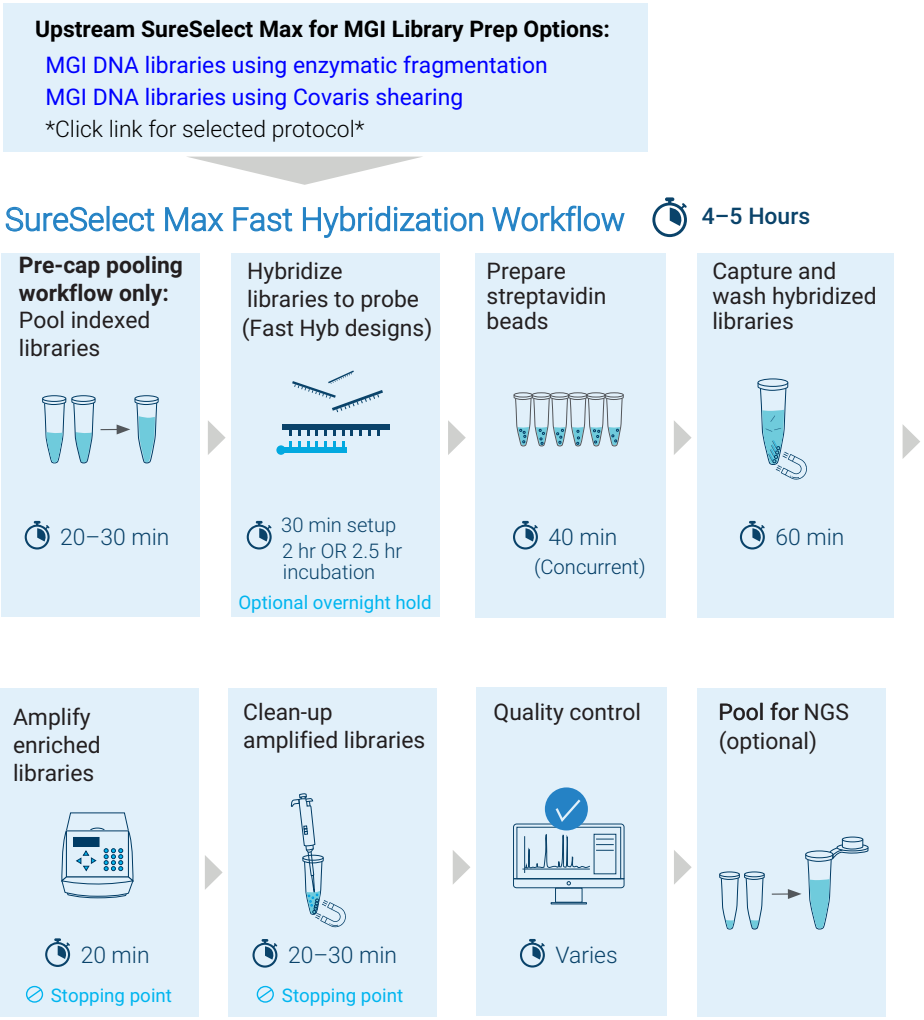

Figure 1 Summary of the SureSelect Max for MGI Fast Hyb target enrichment workflow segment, including optional stopping points and estimated time requirements. Estimates are guidelines for 16 reaction runs, with 2 hour or 2.5 hour hybridization time dependent on the specific probe design. Timing for runs using different protocol parameters may vary.

SureSelect Max Modules and Probes Used in the Workflow

This publication provides optimized protocols for the target enrichment workflow segment, using fast hybridization conditions for target enrichment of prepared SureSelect Max DNA libraries using a suitable SureSelect probe for downstream NGS on the MGI platform. Agilent's SureSelect reagents required to complete the protocols are summarized in Table 1 and Table 2.

Table 1 SureSelect Max kits used in the Target Enrichment with Fast Hybridization workflow

Kit Description	16 Hybridization Kits*	96 Hybridization Kits†
SureSelect Max Fast Hyb Kit‡	G9689A	G9689B
SureSelect Max Blockers and Primers Kit for MGI	G9696A	G9696B
SureSelect Max Purification Beads**	G9962A (5 mL)	G9962B (30 mL)
SureSelect XT HS Probe	See Table 2 for ordering information. Choose 90 min Hyb/HS design probes for use in the SureSelect Max Fast Hybridization workflow. Select the appropriate formulation and format for your indexed library pooling workflow choice (post-capture or pre-capture pooling).	

* 16 Hyb Kits contain enough reagents for 2 runs of 8 Hybs each, using either a post-capture pooling or a pre-capture pooling workflow.

† 96 Hyb Kits contain enough reagents for 4 runs of 24 Hybs each, using either a post-capture pooling or a pre-capture pooling workflow.

‡ Includes SureSelect Streptavidin Beads. Separate purchase not required.

**May be substituted with AMPure XP beads (see Table 3 on page 8).

Table 2 Probe ordering information

Probe Description	Design ID	Ordering Information
Recommended Pre-designed Probes		
SureSelect XT HS Human All Exon V8	S33266340	Please visit the Agilent.com probe webpages or the SureDesign website to obtain ordering information for pre-designed probes formulated for either post-capture pooling or pre-capture pooling. Please contact Sales or your local representative if you need assistance.
SureSelect XT HS Human All Exon V8+UTR	S33613271	
SureSelect XT HS Human All Exon V8+NCV	S33699751	
SSel XT HS and XT Low Input Human All Exon V7	S31285117	
SureSelect XT HS Clinical Research Exome V4	S34226467	
Custom Probes		
SureSelect Custom Tier1 1–499 kb		Please visit the SureDesign website to design Custom SureSelect probes and obtain ordering information for custom probes formulated for either post-capture pooling or pre-capture pooling. Contact the SureSelect support team (see page 2) or your local representative if you need assistance.
SureSelect Custom Tier2 0.5 –2.9 Mb		
SureSelect Custom Tier3 3 –5.9 Mb		
SureSelect Custom Tier4 6 –11.9 Mb		
SureSelect Custom Tier5 12–24 Mb		
Agilent Community Designs: Please visit the Community Designs (NGS) webpages at Agilent.com for information on custom panels developed in collaboration with experts in various fields.		Design details and ordering information are available at the SureDesign website on the *Published Designs* tab. Contact the SureSelect support team (see page 2) or your local representative if you need assistance.

Additional Materials Used in the Workflow

See Table 3 and Table 4 for additional reagents and equipment used in the workflow.

CAUTION Sample volumes exceed 0.2 mL in certain steps of this protocol. Make sure that the plasticware used with the selected thermal cycler holds ≥0.25 mL per well (see Table 3).

Table 3 Ordering Information for Additional Reagents and Equipment

Description	Vendor and Part Number	Usage Notes
Thermal Cyclers (2) with 96-well, 0.2 mL blocks	Various suppliers	Protocols may require two thermal cyclers to complete a reagent pre-warming step during certain sample incubation steps. See *Note* on page 12 for more information.
Plasticware compatible with the selected thermal cycler: 8-well strip tubes Tube cap strips (flat or domed)*	Consult the thermal cycler manufacturer's recommendations	Runs using 1–3 strip tubes per run are recommended to facilitate efficient sample handling and to minimize the number of required thermal cyclers. Runs may also be completed using 96 well plates.
Vacuum concentrator	Savant SpeedVac, model DNA120, or equivalent	Required only for pre-capture pooling workflows†
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 device 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	—
Nucleic acid analysis system	See Table 4 on page 9	—
1X Low TE Buffer	Thermo Fisher Scientific p/n 12090-015, or equivalent	10 mM Tris-HCl, pH 7.5-8.0, 0.1 mM EDTA
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: AMPure XP Kit (5 mL)	Beckman Coulter Genomics p/n A63880	Optional alternative to SureSelect Max Purification Beads (See Table 1 on page 7)

* Consult the thermal cycler manufacturer's recommendations for use of either flat or domed strip caps and for any accessories (e.g., compression mats) required for optimal performance with the selected caps. Ensure that the combination of selected thermal cycler and plasticware provides complete sealing of sample wells and optimal contact between the instrument heated lid and vial cap for heat transfer.

† Library pools may require dehydration prior to hybridization, where prepared library pools are added in volume of 12 µL. If a vacuum concentrator is not available in your laboratory, see *Troubleshooting* on page 40 for suggested protocol modifications.

Table 4 Recommended Nucleic Acid Analysis Systems

Analysis System	Vendor and Part Number Information	Usage Notes
Agilent 4200/4150 TapeStation Instrument Consumables: 8-well tube strips 8-well tube strip caps High Sensitivity D1000 ScreenTape High Sensitivity D1000 Reagents	Agilent p/n G2991AA/G2992AA p/n 401428 p/n 401425 p/n 5067-5584 p/n 5067-5585	Recommended system for final library qualification (see Agilent Fragment Analyzer system below for alternative).*
Agilent 5200/5300/5400 Fragment Analyzer Instrument Consumables: HS NGS Fragment Kit (1-6000 bp)	Agilent p/n M5310AA/M5311AA/M5312AA p/n DNF-474-0500	Recommended system for final library qualification (see Agilent TapeStation system above for alternative).*
Qubit dsDNA HS Assay (100 Assays)	Thermo Fisher Scientific p/n Q32851	Used for final library quantification prior to sequencing.

* Libraries may also be qualified using the Agilent 2100 Bioanalyzer instrument, p/n G2939BA, and the High Sensitivity DNA Kit (p/n 5067-4626).

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.
- 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.

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Target Enrichment with Fast Hybridization Protocol

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In this workflow segment, SureSelect Max DNA libraries are hybridized with a target-specific probe. After hybridization, the targeted molecules are captured on streptavidin beads and then PCR-amplified. The libraries can be pooled for multiplex NGS after hybridization (see page 11 for post-capture pooling workflow setup) or prior to hybridization (see page 12 for pre-capture pooling workflow setup).

The standard single-day SureSelect Max Fast Hyb workflow includes the fast hybridization step immediately followed by capture and amplification steps. If required, the hybridized samples may be held overnight with capture and amplification steps completed the following day by using the simple protocol modifications noted on page 13.

The hybridization and capture workflow segments use the components listed in Table 5. Prepare as directed before use (refer to the *Where Used* column).

Table 5 Reagents for Hybridization and Capture

Storage Location	Kit Component	Preparative Steps	Where Used
–80°C	SureSelect Probe	Thaw on ice before starting Hyb setup on page 12 and keep on ice; vortex to mix	page 14
SureSelect Max Blockers and Primers Module for MGI, stored at –20°C	Blocker Mix, MGI (blue cap)	Thaw and keep on ice, vortex to mix	page 13
SureSelect Max Target Enrichment Kit Fast Hyb Module Box 2, stored at –20°C	SureSelect RNase Block (purple cap)	Thaw and keep on ice, vortex to mix	page 14
	SureSelect Max Fast Hyb Buffer (bottle)	Thaw and keep at room temperature	page 14
+4°C	SureSelect Streptavidin Beads (clear cap)	Remove from 4°C just before use, vortex to mix	page 16
SureSelect Max Target Enrichment Kit Fast Hyb Module Box 1, stored at RT	SureSelect Binding Buffer (bottle)	Ready to use	page 16
	SureSelect Wash Buffer 1 (bottle)	Ready to use	page 17
	SureSelect Wash Buffer 2 (bottle)	Ready to use	page 16

Step 1. Place libraries or library pools in the hybridization wells

Option 1: Plate individual libraries (post-capture pooling workflow)

The hybridization reaction requires prepared library samples in 12 µL of nuclease-free water. See Table 6 for the recommended and required amounts of prepared library sample input for hybridization. To maximize complexity of the final enriched NGS library, add the full 12 µL of prepared library sample to the hybridization reaction.

Agilent recommends retaining the remaining prepared library solution volume for any later troubleshooting QC needs.

Table 6 Hybridization input guidelines

Recommended amount	Minimum amount*
12 µL of undiluted prepared gDNA library solution (pre-capture QC optional)	≥500 ng library DNA brought to 12 µL with nuclease-free water

* For workflows that include the optional pre-capture QC step, use at least the minimum amount of library shown here in the hybridization reactions. When library normalization in the run is not required, using the maximum amount of each library (12 µL of undiluted sample) is recommended to maximize complexity.

1 Prepare the hybridization strip(s) by placing the appropriate amount of each prepared library into a separate sample well.

2 Bring the volume of each library sample well to 12 µL with nuclease-free water, if required.

Keep the samples on ice until use in step 2 on page 13.

Option 2: Plate library pools (pre-capture pooling workflow)

1 Pool the indexed library samples as directed in Table 7 using equal amounts of 8 or 16 libraries, based on the probe design. For each pool, combine the appropriate volume of each purified library sample in one well of a strip tube.

Table 7 Pre-capture pooling recommendations

Probe description	Number of indexes per pool	Library input type	Total amount libraries per pool	Amount of each library
SureSelect XT HS PreCap Human All Exon V7 or V8 (including V8+UTR/V8+NCV) or SureSelect XT HS PreCap Clinical Research Exome V4	8	DNA	4 µg	500 ng
SureSelect XT HS PreCap Custom Probes	16	DNA	4 µg	250 ng

2 Bring the volume of each library pool to 12 µL with nuclease-free water.

If the pool volume exceeds 12 µL, use a vacuum concentrator held at ≤ 45°C to dehydrate the prepped library pools to volume <12 µL without completely drying the samples. Measure the final volume in each well and then adjust each volume to 12 µL with nuclease-free water. If a vacuum concentrator is not available in your laboratory, see *Troubleshooting* on page 40.

Cap the wells, then vortex the sample strip vigorously for 5–10 seconds. Spin briefly to collect the liquid, then keep the samples on ice until use in step 2 on page 13.

Step 2. Hybridize libraries to the SureSelect probe

NOTE

Two thermal cyclers may be required in this section to complete the library hybridization/capture steps concurrently with certain reagent pre-warming steps.

For single strip tube runs, sample incubation and reagent pre-warming steps can be performed simultaneously in a single thermal cycler.

For larger runs, two thermal cyclers are required for the following concurrent steps: 1) capture bead pre-warming in step 6 on page 16 during hybridization and 2) Wash Buffer 2 pre-warming during the capture incubation (see step 2 and step 3 on page 17). If two thermal cyclers are not available in your laboratory, the reagent pre-warming steps may be completed using an alternative temperature control device. Reaction mixtures that include the library samples should be incubated using a thermal cycler where directed.

In this step, the prepared MGI sequencing libraries are hybridized to the SureSelect probe using a thermal cycler program that includes hybridization at 68°C for 90 or 120 minutes, depending on the probe design.

1 Preprogram a thermal cycler with the hybridization program in Table 8. Set the heated lid to 105°C. Prewarm the instrument before samples are loaded in step 3 on page 14.

CAUTION

During the hybridization program, an operator must be present and perform the actions described in the final column of Table 8 below.

For the single-day workflow, it is important to have washed and pre-warmed streptavidin beads ready for capture at the end of the hybridization incubation (Segment 4).

Table 8 MGI library hybridization program (30 µL vol)

Segment Number	Temperature	Duration		Operator Action Required during Segment(s)
		Time	Probe Design*	
1	95°C	5 minutes	All probes	Prepare Probe Hybridization Mix (see step 4 on page 14)
2	65°C	10 minutes	All probes	
3	65°C	Hold	All probes	Add Probe Hybridization Mix to DNA + Blocker wells on cycler (see step 5 on page 15)
4	68°C	120 minutes	SureSelect XT HS Human All Exon Probes (V7/V8/V8+UTR/V8+NCV)	Prepare reagents for hybrid capture (see page 16). Requires approximately 45 minutes.
		120 minutes	SureSelect XT HS Clinical Research Exome V4 Probe	
		90 minutes	All other probes	
5	68°C	Hold **briefly**	All probes	Begin capture (see page 17) as quickly as possible after the hybridization in Segment 4 is complete.

* The fast hybridization program provided in this table is optimal for SureSelect XT HS probe designs. The overnight hybridization protocol recommended for SureSelect XT probe designs is not available for the SureSelect Max for MGI system at the time of this publication. Contact the SureSelect Support team or your local representative for additional guidelines.

NOTE

The protocol may be modified for a 2-day workflow with an overnight pause by making the following changes:

- In segment 5 of the thermal cycler program (Table 8), replace the brief 68°C Hold with an elongated 21°C Hold step. The hybridized samples may be held at 21°C for up to 16 hours.
- During segment 4, omit the hybrid capture reagent preparation steps described on page 16. Instead begin the capture preparation steps on day 2.

2 To each library sample well, add 5 µL of Blocker Mix, MGI (blue cap). Seal the wells then vortex at high speed for 5 seconds. Spin briefly to collect the liquid and release any bubbles.

CAUTION

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

3 Transfer the sample strip to the thermal cycler and run the thermal cycler program in Table 8, allowing the cycler to finish Segments 1–2 and proceed into the Segment 3 Hold.

Important: Additional reagents are added to sample wells in the thermal cycler during the Segment 3 Hold step. During Segments 1 and 2, prepare the Probe Hybridization Mix (see step 4 below). If needed, you can finish this preparation step while the thermal cycler holds samples at 65°C in Segment 3.

NOTE

Prepare the mixture described in step 4 below at room temperature at the time of use. Do not keep solutions containing the probe at room temperature for extended periods.

For larger run sizes, hybridization reactions can be started using a multichannel pipette after dividing the master mix volume prepared in step 4 into wells of a strip tube before adding to wells in step 5. Using this protocol modification, which includes additional pipetting losses, may require preparation of a larger reagent volume mixture.

4 Prepare the Probe Hybridization Mix appropriate for your probe design size. Use Table 9 for probes ≥3 Mb or Table 10 for probes <3 Mb. For custom probes, see the probe tube label for design size range.

Combine the listed reagents at room temperature. Mix well by vortexing at high speed for 5 seconds then spin down briefly. Keep at room temperature until added to wells in step 5.

Table 9 Preparation of Probe Hybridization Mix for **probes ≥3 Mb**

Reagent	Volume for 1 Hyb	Volume for 8 Hybs (includes excess)*	Volume for 24 Hybs (includes excess)†
Nuclease-free water	1.5 µL	13.5 µL	37.5 µL
SureSelect RNase Block (purple cap)	0.5 µL	4.5 µL	12.5 µL
SureSelect Max Fast Hyb Buffer	6 µL	54 µL	150 µL
Probe (with design ≥3 Mb)	5 µL	45 µL	125 µL
Total	13 µL	117 µL	325 µL

* 16-Hyb Target Enrichment Kits contain enough reagents for 2 runs of 8 Hybs each using the indicated excess volumes. Reagents may be depleted after fewer than 16 reactions when using smaller run sizes.

† 96-Hyb Target Enrichment Kits contain enough reagents for 4 runs of 24 Hybs each using the indicated excess volumes. Reagents may be depleted after fewer than 96 reactions when using smaller run sizes.

Table 10 Preparation of Probe Hybridization Mix for **probes <3 Mb**

Reagent	Volume for 1 Hyb	Volume for 8 Hybs (includes excess)*	Volume for 24 Hybs (includes excess)†
Nuclease-free water	4.5 µL	40.5 µL	112.5 µL
SureSelect RNase Block (purple cap)	0.5 µL	4.5 µL	12.5 µL
SureSelect Max Fast Hyb Buffer	6 µL	54 µL	150 µL
Probe (with design <3 Mb)	2 µL	18 µL	50 µL
Total	13 µL	117 µL	325 µL

* 16-Hyb Target Enrichment Kits contain enough reagents for 2 runs of 8 Hybs each using the indicated excess volumes. Reagents may be depleted after fewer than 16 reactions when using smaller run sizes.

† 96-Hyb Target Enrichment Kits contain enough reagents for 4 runs of 24 Hybs each using the indicated excess volumes. Reagents may be depleted after fewer than 96 reactions when using smaller run sizes.

5 After the thermal cycler starts the Segment 3 Hold, while keeping the DNA + Blocker samples in the cycler, transfer 13 µL of the room-temperature Probe Hybridization Mix from step 4 to each sample well.

   Mix well by pipetting up and down slowly 8 to 10 times.

   The hybridization reaction wells now contain approximately 30 µL.

6 Seal the wells with fresh strip caps. Make sure that all wells are completely sealed. Vortex briefly, then spin the strip briefly to remove any bubbles. Immediately return the hybridization strip to the thermal cycler.

7 Advance the thermal cycling program to Segment 4 to allow hybridization of the library to the probe.

   Complete the streptavidin bead and buffer preparation steps on page 16 during the hybridization incubation (Segment 4 in Table 8) unless you are using the 2-day workflow.

Step 3. Prepare streptavidin beads and buffers for capture

NOTE

If performing same-day hybridization and capture, begin capture reagent preparation at least 45 minutes before the end of the hybridization program (Table 8 on page 13). Start the final capture bead pre-warming step (step 6 below) at 10 minutes before the end of the hybridization incubation (Segment 4 in Table 8).

If performing next-day capture after an overnight hold at 21°C, begin the steps below on day 2.

Set the thermal cycler heated lid to 105°C for all thermal cycler incubation steps used for bead preparation and library capture, including the 6X capture wash incubations at 68°C on page 18.

1. Prepare 200 µL aliquots of Wash Buffer 2 for later use in capture wash steps. Using fresh strip tubes, aliquot 6 wells of Wash Buffer 2 for each sample in the run. Keep the aliquots at room temperature until directed to pre-warm for washes on page 17.
2. Vigorously resuspend the vial of SureSelect Streptavidin Beads on a vortex mixer. The magnetic beads settle during storage.
3. For each hybridization sample, add 50 µL of the resuspended beads to wells of a fresh PCR strip tube.

NOTE

Pre-label the streptavidin bead strip(s) with sample IDs. The prepared bead strips are pre-warmed then kept in a thermal cycler during sample addition, preventing later labeling.

4. Equilibrate the beads in SureSelect Binding Buffer using 3× washes at room temperature:
   a. Add 200 µL of SureSelect Binding Buffer.
   b. Mix by pipetting up and down 20 times or cap the wells and vortex at high speed for 5–10 seconds then spin down briefly.
   c. Put the strip tube into a magnetic separator device.
   d. Wait 5 minutes or until the solution is clear, then remove and discard the supernatant.
   e. Repeat step a through step d two more times for a total of 3 washes.
5. Resuspend the washed streptavidin beads in 200 µL of SureSelect Binding Buffer.
6. At 10 minutes before the end of hybridization (Segment 4 in Table 8), pre-warm the streptavidin capture beads in Binding Buffer by incubating the strip tube in a thermal cycler at 68°C for 10 minutes.

NOTE

For small run sizes using strip tubes, streptavidin bead pre-warming may be performed in empty columns of the hybridization thermal cycler block.

Step 4. Capture the hybridized libraries

1 Immediately after the hybridization program is complete (Table 8 on page 13), add the hybridization mixture to the pre-warmed capture beads as described below.

   a Using a multichannel pipette, transfer the entire volume (approximately 30 µL) of the hybridization mixtures to wells of pre-warmed beads, while keeping the bead strip in the thermal cycler at 68°C.

   b Change the pipette setting to 150 µL, then use the same tips to mix by pipetting up and down 8–10 times; make sure the beads are fully resuspended. Seal the wells with fresh caps.

2 Incubate the capture reaction strip in the thermal cycler at 68°C for 10 minutes.

3 Transfer the SureSelect Wash Buffer 2 aliquots prepared on page 16 (200 µL/well) to a thermal cycler held at 68°C. Pre-warm the buffer until used for washes in step 8.

NOTE For single-strip runs, wash buffer pre-warming may be performed in empty columns of the thermal cycler block used for capture.

4 When the 10 minute capture incubation (step 2 above) is complete, spin the samples briefly to collect the liquid.

5 Put the capture reaction strip in a magnetic separator to collect the beads. Wait until the solution is clear (approximately 1 to 2 minutes), then remove and discard the supernatant.

6 Resuspend the beads in 200 µL of SureSelect Wash Buffer 1 at room temperature. Mix by pipetting up and down 15–20 times, until beads are fully resuspended.

7 Return the sample strip to the magnetic separator. Wait for the solution to clear (approximately 1 minute), then remove and discard the supernatant.

   Spin the samples briefly to collect any residual Wash Buffer 1 and remove with a P20 pipette.

CAUTION To ensure specificity of capture, make sure that the SureSelect Wash Buffer 2 is pre-warmed to 68°C before use in the wash steps below.

During the washes in step 8, keep the sample strip at room temperature during the pipetting and vortexing steps prior to each 68°C incubation step.

Do not use a tissue incubator, or other devices with significant temperature fluctuations, for the 68°C incubation steps.

8 Wash the beads 6X with Wash Buffer 2, using the steps below. **Place the capture sample strip in a rack at room temperature before you begin.**

   a Resuspend the beads in 200 µL of 68°C pre-warmed Wash Buffer 2. **Make sure the beads are completely in suspension before proceeding.** To ensure thorough resuspension, Agilent recommends the following two-part mixing routine. Keep the capture reactions at room temperature while mixing.

      i) Using a P200 pipette set to 150 µL, pipette up and down 15–20 times, until beads are fully resuspended. Pipette slowly and gently to reduce bubble formation.

      ii) Seal the wells with fresh caps and vortex at high speed for 8 seconds. Spin the strip tube briefly to collect the liquid and release any bubbles without pelleting the beads.

b Incubate the samples for 5 minutes at 68°C in a thermal cycler.

c Put the sample strip in the magnetic separator at room temperature.

d Wait 1 minute for the solution to clear, then remove and discard the supernatant. Return samples to the rack at room temperature.

e Repeat step a through step d five more times for a total of 6 washes.

When the final wash is complete, spin the samples briefly to collect any residual Wash Buffer 2 and remove with a P20 pipette.

9 Add 24 µL of nuclease-free water to each sample well. Pipette up and down 8 times to resuspend the beads.

10 Keep the samples on ice until they are used in the PCR reactions below.

NOTE Captured DNA is retained on the streptavidin beads during the post-capture amplification step.

Step 5. Amplify the captured libraries

The post-capture amplification workflow segment uses the components listed in Table 11. Prepare as directed before use (refer to the *Where Used* column).

Table 11 Reagents for post-capture amplification

Storage Location	Kit Component	Preparative Steps	Where Used
SureSelect Max Target Enrichment Kit Fast Hyb Module Box 2, stored at –20°C	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 Blockers and Primers Module for MGI, stored at –20°C	SureSelect MGI Post-Capture Primer Mix (clear cap)	Thaw and keep on ice, vortex to mix.	page 19
+4°C	SureSelect Max Purification Beads OR AMPure XP Beads	Equilibrate at room temperature for at least 30 minutes before use, vortex to mix.	page 20

1 Preprogram a thermal cycler as shown in Table 12. Set the heated lid to 105°C. Prewarm the instrument before samples are loaded in step 6 on page 19.

Table 12 Post-Capture PCR thermal cycler program (50 µL vol)

Segment	Number of Cycles	Temperature	Time
1	1	98°C	45 seconds
2	12–16 (see Table 13 for probe design size-based cycle number recommendation)	98°C	15 seconds
		60°C	30 seconds
		72°C	30 seconds
3	1	72°C	1 minute
4	1	4°C	Hold

Table 13 Post-capture PCR cycle number recommendations

Probe Design Size	Cycles
Probes <0.2 Mb	16 cycles
Probes 0.2–3 Mb	13–16 cycles
Probes 3–5 Mb	12–13 cycles
Probes >5 Mb (including Human All Exon and Exome probes)	12 cycles

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

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

3 Prepare the appropriate volume of post-capture PCR reaction mix as described in Table 14, on ice. Mix well on a vortex mixer.

Table 14 Preparation of post-capture PCR reaction mix

Reagent	Volume for 1 reaction	Volume for 8 reactions (includes excess)	Volume for 24 reactions (includes excess)
Amplification Master Mix (red cap or bottle)	25 µL	225 µL	650 µL
SureSelect MGI Post-Capture Primer Mix (clear cap)	1 µL	9 µL	26 µL
Total	26 µL	234 µL	676 µL

4 Add 26 µL of the PCR reaction mix prepared in Table 14 to each sample well containing 24 µL of bead-bound target-enriched DNA.

5 Mix the PCR reactions well by pipetting up and down until the bead suspension is homogeneous. Avoid splashing samples onto well walls; do not spin the samples at this step.

6 Place the sample strip in the thermal cycler and run the program in Table 12.

NOTE The SureSelect Max Purification Beads or AMPure XP Beads used in the next section must be equilibrated to room temperature for at least 30 minutes before use.

7 When the PCR amplification program is complete, spin the amplified library strip briefly. Proceed to purification.

NOTE In the following purification protocol, the hybrid capture streptavidin beads remain mixed with the magnetic purification beads throughout the procedure.

If preferred, you can remove the streptavidin beads prior to purification by adding the following steps: 1) Place the amplified library strip tube on the magnetic stand at room temperature 2) Wait 2 minutes for the solution to clear 3) Transfer each supernatant (approximately 50 µL) to fresh strip tube wells and proceed with the purification procedure. The streptavidin beads can be discarded after collecting the library solution.

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 the final libraries using magnetic purification beads

Purify the amplified 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 post-capture PCR

Parameter	Value
Volume of RT purification bead suspension added to each sample well	50 µL
Final elution solvent and volume	25 µL Low TE Buffer
Amount of eluted sample transferred to fresh well	Approximately 24 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. Add 50 µL of the purification bead suspension to each amplified library sample well, containing approximately 50 µL of streptavidin bead suspension.
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 strip tube into a magnetic separation device. Wait for the solution to clear (approximately 2 to 5 minutes).
7. Keep the 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 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 strip tube at room temperature for 2–5 minutes or until the residual ethanol has just evaporated.
13. Elute the library by adding 25 µL of Low TE buffer 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 strip tube in the magnetic stand and leave until the solution is clear (up to 5 minutes).

17 Transfer the cleared supernatant (approximately 24 µL) to a fresh well. Keep on ice.

You can discard the beads at this time.

NOTE

If magnetic beads are carried over during the final elution step, samples can be placed on the magnetic stand while removing an aliquot for QC (below) and while removing an aliquot for pooling on page 24.

Stopping Point If you do not plan to continue through the library pooling for NGS step on same day, seal the wells and store at 4°C overnight or at –20°C for prolonged storage (remove aliquot for QC analysis before storage, if appropriate).

Step 7. QC and quantify final libraries

This workflow segment includes library qualification with fragment size analysis using an Agilent automated electrophoresis system and library DNA quantification using the Qubit dsDNA HS Assay.

Library Fragment Size Qualification

Analyze a sample of each library using one of the platforms listed in Table 16. Follow the instructions in the linked user guide provided for each assay. Combine 1 µL of sample with 4 µL of Low TE Buffer before running the assays.

Table 16 Post-capture library qualification options

Analysis platform	Assay used at this step	Link to assay instructions	Amount of library sample to analyze
Agilent 4200/4150 TapeStation system	High Sensitivity D1000 ScreenTape	Agilent High Sensitivity D1000 Assay Quick Guide	2 µL of 5-fold dilution
Agilent 5200/5300/5400 Fragment Analyzer system	HS NGS Fragment Kit (1-6000 bp)	Agilent HS NGS Fragment Kit (1-6000 bp) Kit Guide	2 µL of 5-fold dilution

Each analysis method provides an electropherogram showing the size distribution of fragments in the sample. See Table 17 for expected 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, record the average fragment size (bp) reported for each sample for use in library pooling calculations.

Table 17 Post-capture expected library fragment size guidelines

Hybridization input	Expected average fragment size (180–1000 bp region)
DNA library prepared from intact DNA fragmented for 2x100 NGS	350 to 450 bp
DNA library prepared from intact DNA fragmented for 2x150 NGS	400 to 500 bp
DNA library prepared from FFPE DNA fragmented for 2x100 or 2x150 NGS	250 to 390 bp

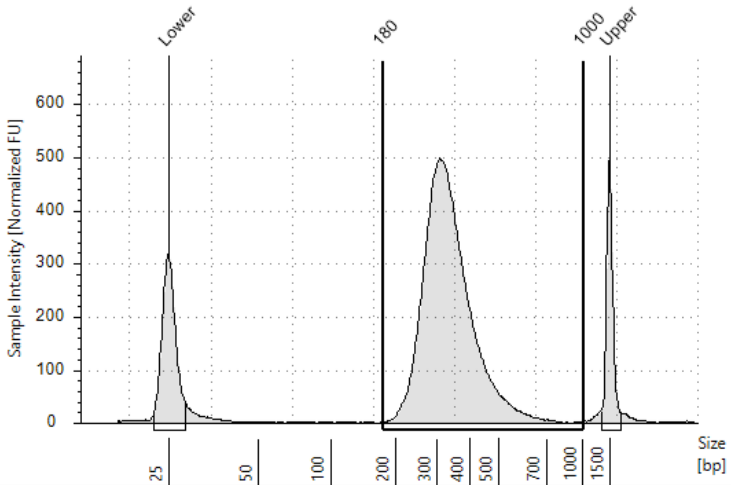

Figure 2 Post-capture library prepared from an FFPE-derived gDNA sample analyzed using a High Sensitivity D1000 ScreenTape assay.

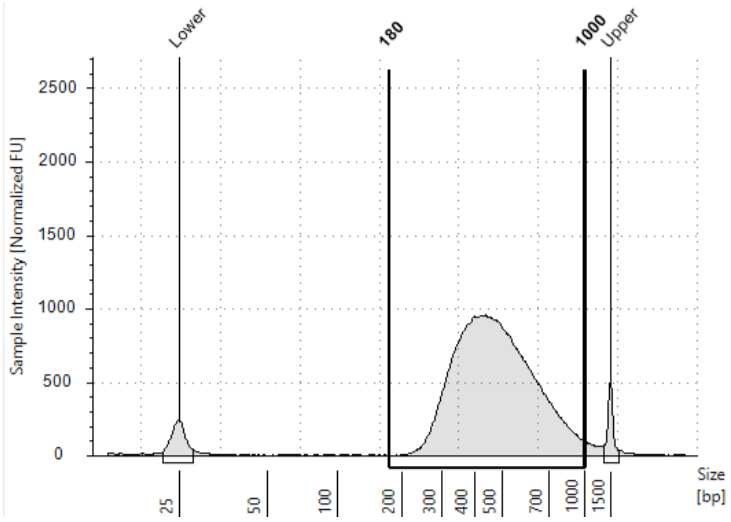

Figure 3 Post-capture library prepared from a high-quality gDNA sample analyzed using a High Sensitivity D1000 ScreenTape assay.

Library Quantification

Analyze a sample of each library using the Qubit dsDNA HS Assay. Follow the instructions in the manufacturer's user guide using 1 µL of each library sample combined with 199 µL of Qubit dsDNA HS Working Solution for each assay.

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.

Post-capture pooling guidelines

The downstream sequencing steps are performed using MGI's systems and protocols or your sequencing provider's services. Guidelines are provided in this section for library pooling in preparation for sequencing. Before designing your pooling strategy, verify the requirements for library DNA amounts and sample volumes using the specifications provided by MGI or by your sequencing provider.

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

NOTE

Make sure that all libraries pooled for multiplex sequencing are indexed with a different SureSelect Max UDI number.

Agilent recommends pooling at 8-plex or greater if compatible with the instrument lane capacity and sequencing depth requirements.

The initial MGI sequencing step of single-strand circularization requires at least 1 picomole (pmol) of post-capture library or library pool dsDNA fragment molecules. For optimal sequencing results, Agilent recommends pooling 1.2 to 1.5 pmol (total) library fragments where possible.

Combine the library samples into pools using equimolar amounts of each indexed library. An example method for equimolar pooling calculations is provided below.

1. For each sample, calculate the amount (ng) of library DNA corresponding to 1 pmol library fragments using the average fragment size (bp) values from the TapeStation or Fragment Analyzer assay (page 21), based on the following conversion:

   Mass (ng) of library DNA per 1 pmol = DNA fragment size (bp) x (660 ng/1000 bp)

2. Divide the mass (ng) values from step 1 by the number of indexed samples to be pooled.
3. Calculate the volume (µL) of each sample to include in the pool using the Qubit dsDNA HS assay concentration values (ng/µL) determined on page 23.

Table 18 shows an example of calculations for the amounts of indexed library samples needed for a 8-plex pool containing 1 pmol or 1.5 pmol library fragments.

Table 18 Volume calculations for 8-plex sequencing pool with 1 pmol or 1.5 pmol total fragments

Component	Average size (bp)	ng per 1 pmol	ng for 8-plex pool	Qubit Concentration (ng/µL)	Volume (µL) for 1 pmol pool	Volume (µL) for 1.5 pmol pool
Library 1	463	305.6	38.2	7.97	4.79	7.19
Library 2	445	293.7	36.7	7.11	5.16	7.75
Library 3	435	287.1	35.9	6.70	5.36	8.03
Library 4	432	285.1	35.6	8.26	4.31	6.47
Library 5	440	290.4	36.3	7.75	4.68	7.03
Library 6	438	289.1	36.1	7.97	4.53	6.80
Library 7	423	279.2	34.9	8.24	4.24	6.35
Library 8	438	289.1	36.1	7.70	4.69	7.04

4 Pool by combining the calculated amount of each library.

Adjust the pool to the volume required for the MGI single-strand circularization protocol (15–48 µL) or the volume required by your sequencing service provider.

Dilute using a provider-specified diluent such as Low TE. If the pool volume exceeds the allowed maximum, reduce the volume using a vacuum concentrator held at ≤ 45°C.

Store at –20°C or the conditions specified by your sequencing provider.

3
Appendix: NGS Guidelines

SureSelect Max Library Composition 27
Sequencing and Read Processing Guidelines 27
SureSelect Max for MGI UDI Information 29

This appendix provides guidelines for completing NGS using the MGI platform and for analysis of the SureSelect Max libraries.

SureSelect Max Library Composition

Components of the 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 paired-end sequencing with standard MGI DNB chemistry.

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

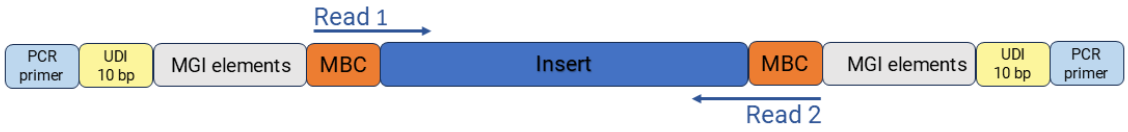

Figure 4 Content of SureSelect Max for MGI sequencing libraries. Each fragment contains one target insert (dark blue) surrounded by the following elements: MGI paired-end sequencing elements (gray), unique dual indexes (UDIs) for sample indexing (yellow), library PCR primers (light blue) and molecular barcode (MBC) sequences used as unique molecular identifiers for read deduplication (orange). MBCs include a 3-bp degenerate barcode plus linker bases.

Sequencing and Read Processing Guidelines

Guidelines are provided below for typical sequencing and read processing pipeline steps appropriate for SureSelect Max DNA libraries for MGI. Your NGS pipeline may vary. If you need additional guidance on downstream SureSelect Max MGI library sequencing, contact the SureSelect support team (see page 2) or your local representative.

- The dsDNA libraries need to be single-strand circularized and processed using MGI's standard DNBSEQ chemistry. Use MGI's systems and protocols or your sequencing provider's services to complete these steps. Follow the supplier's guidelines for library sample concentration and volume requirements and sequencing run parameters.
- Use MGI guidelines for run setup to generate Read 1 and Read 2 FASTQ files for each sample. Use the appropriate number of cycles for your library insert length.
- Each sample-level index (Index 1 and Index 2) requires an 10-bp index read. SureSelect Max index sequences can be obtained by downloading the SureSelect Max MGI Index Sequence Resource Excel spreadsheet from Agilent.com. For complete index sequence information, see page 29.
- The following library structure details may be requested by some sequencing providers: Read 1 and Read 2 begin with 5 bp of non-insert unique molecular identifier (UMI) sequence, composed of the 3-bp MBC, with random sequence at all three positions, followed by 2 bp of partially-randomized sequences. Dark cycling at base positions 4 and 5 is **not** required in the sequencing run setup.
- Include a 1% in-lane control spike-in DNA for improved sequencing quality control and color balance.
- Demultiplexing is typically performed by MGI's base-calling software demultiplexing tools.

- Decline any adaptor trimming tools or services available prior to obtaining the demultiplexed FASTQ files from the instrument or sequencing service. Adaptors are trimmed from demultiplexed FASTQ files in later processing steps to ensure proper processing of the adaptors, including the degenerate molecular barcodes (MBCs) adjacent to opposite-strand adaptor sequences.
- The demultiplexed FASTQ reads need to be pre-processed to extract MBC sequences for de-duplication while trimming the adaptors. Agilent's AGeNT toolkit, described below, can be used for these pre-processing steps.

NOTE Read pre-processing steps can also be completed using suitable commercial or open-source software tools after performance verification. Performance of non-Agilent tools should be verified for appropriate adaptor and MBC sequence processing on both strands. Some non-Agilent adaptor trimmers may fail to remove the MBC sequences accurately from the opposite adaptor, which may affect alignment quality.

- If your sequence analysis pipeline excludes MBCs, you can remove MBCs by trimming or masking the first five bases from each read.
- If your pipeline requires adaptor trimming outside of Agilent's AGeNT software, remove or filter the sequences below using a suitable tool.
  Read 1: AAGTCGGAGGCCAAGCGGTCTTAGGAAGACAA
  Read 2: AAGTCGGATCGTAGCCATGTCGTTCTGTGAGCCAAGGAGTTG

Using Agilent's AGeNT software for SureSelect Max read preprocessing

Agilent's AGeNT is a Java-based toolkit for library read processing steps, designed for users with bioinformatics expertise to enable building internal analysis pipelines. To download this toolkit, visit the AGeNT page at www.agilent.com. Use of the AGeNT read processing tools is outlined briefly below. See the AGeNT Best Practices and the AGeNT 3.1 FAQ documents for more information.

- Prior to variant discovery, demultiplexed SureSelect Max library FASTQ data are pre-processed to remove MGI sequencing adaptors and extract the MBC sequences using the AGeNT Trimmer module. An example invocation (on Linux/Mac) for SureSelect Max MGI reads is below:

```
agent.sh trim –adaptor MGI –mbc xths2 -fq1
    /path/to/fastq_input_dir/sample_R1.fastq.gz -fq2
    /path/to/fastq_input_dir/sample_R2.fastq.gz -out
    myOutputDirPath/myOutputFilePrefix
```

NOTE If reads are provided in legacy format using **syntax "/1" and "/2" in read names**, Trimmer runs may fail to complete. To resolve, turn on the "-IDEE_FIXE" option. See AGeNT 3.1 FAQ for details.

- The trimmed reads should be aligned and MBC tags added to the aligned BAM file using a suitable tool such as BWA-MEM.
- The AGeNT CReaK (Consensus Read Kit) tool can be used to generate consensus reads and mark or remove duplicates for the aligned reads based on the read MBCs. CReaK allows consensus generation in duplex, hybrid, or single-strand mode.
- If your pipeline requires positional deduplication, you can utilize a suitable tool such as Picard MarkDuplicates on aligned BAM files for read deduplication.

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

SureSelect Max for MGI UDI Information

The SureSelect Max unique dual indexes (UDIs) are added to the library DNA fragments during the library preparation workflow segment. Each fragment contains a unique pair of 10-bp Index 1 and Index 2 sequences suitable for MGI's DNB chemistry and demultiplexing tools (see Figure 4 on page 27).

Note that such sample indexes may be referred to as *barcodes* in MGI publications. MGI barcodes used for sample indexing are distinct from the SureSelect Max molecular barcodes (MBCs) added during adaptor ligation and used for read deduplication.

SureSelect Max index sequences are provided in Table 19 on page 30 through Table 26 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 19 through Table 26 and in the downloadable Excel spreadsheet, Index 1 and Index 2 sequences are shown in the 5' to 3' orientation corresponding to the sequence of the 5'-phosphorylated strand in MGI's DNB chemistry. Index 1 is located farthest from and Index 2 is located closest to the 5'-phosphorylated end of this strand. The provided orientation corresponds to the orientation to be included in the *barcode.csv* file used by the MGI sequencer base calling software for on-instrument demultiplexing. Index sequencing reads generated by MGI sequencers are the reverse complement of the Index 1 and Index 2 sequences provided by Agilent for run setup tasks.

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 19 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 20 **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 21 **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 22 **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 23 **SureSelect Max for MGI UDI 193–240, provided in red 96-well plate**

UDI #	Well Position	Index 1	Index 2	UDI #	Well Position	Index 1	Index 2
199							
200							

Sequences to be provided at future date

Table 24 **SureSelect Max for MGI UDI 241–288, provided in red 96-well plate**

UDI #	Well Position	Index 1	Index 2	UDI #	Well Position	Index 1	Index 2
247							
248							

Sequences to be provided at future date

Table 25 **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 26 **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

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Reference

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

Reagent Kit Contents

SureSelect Max Target Enrichment with Fast Hybridization workflow uses the kits listed in Table 27. Detailed contents of each of the multi-part component kits are shown in Table 28 through Table 30.

Table 27 Kits for SureSelect Max Target Enrichment with Fast Hybridization

SureSelect Max Kits and Included Component Kits	Component Kit Part Numbers		Storage Condition
	16 Hybridizations	96 Hybridizations	
SureSelect Max Fast Hyb Kit (G9689A/G9689B)			
SureSelect Max Target Enrichment Kit Fast Hyb Module, Box 1	5282-0128	5282-0130	Room Temperature
SureSelect Max Target Enrichment Kit Fast Hyb Module, Box 2	5282-0129	5282-0131	–20°C
SureSelect Streptavidin Beads	5191-5741	5191-5742	+4°C
SureSelect Max Blockers and Primers Module for MGI (G9696A/G9696B)	5282-0157	5282-0158	–20°C
SureSelect Max Purification Beads (G9962A/G9962B)	5282-0225	5282-0226	+4°C

Component Kit Details

Table 28 SureSelect Max Target Enrichment Kit Fast Hyb Module, Box 1 content

Kit Component	16 Hyb Kit (p/n 5282-0128)	96 Hyb Kit (p/n 5282-0130)
SureSelect Binding Buffer	bottle	bottle
SureSelect Wash Buffer 1	bottle	bottle
SureSelect Wash Buffer 2	bottle	bottle

Table 29 SureSelect Max Target Enrichment Kit Fast Hyb Module, Box 2 content

Kit Component	16 Hyb Kit (p/n 5282-0129)	96 Hyb Kit (p/n 5282-0131)
SureSelect Max Fast Hyb Buffer	bottle	bottle
SureSelect RNase Block	tube with purple cap	tube with purple cap
Amplification Master Mix	tube with red cap	bottle

Table 30 SureSelect Max Blockers and Primers Module for MGI content

Kit Component	16 Hyb Kit (p/n 5282-0157)	96 Hyb Kit (p/n 5282-0158)
Blocker Mix, MGI	tube with blue cap	tube with blue cap
SureSelect MGI Post-Capture Primer Mix	tube with clear cap	tube with clear cap

Troubleshooting Guide

If vacuum concentrator is not available for pre-capture pooling library volume reduction prior to hybridization

✓The standard pre-capture pooling hybridization protocol requires 12 µL samples containing 4 µg of pooled library DNA, using a vacuum concentrator to reduce the volume of the library pools where needed. If a vacuum concentrator is not available in your laboratory, use the protocol modifications below to generate concentrated library pools for hybridization. This modification may result in some loss of library complexity. Follow the steps below for pool concentration prior to hybridization:

- To minimize losses in library complexity, pool indexed libraries using 2.5X the amount of each library DNA given in Table 7 on page 12. For example, for gDNA libraries enriched with the SureSelect XT HS PreCap Human All Exon V8 probe, pool 1250 ng of each of 8 indexed DNA libraries for total pool of 10 µg library DNA.
- Concentrate each pool using an additional round of magnetic bead purification. Follow the purification instructions on page 20 to page 21, modifying step 3 to use a bead volume of 1.8X the total pool volume.
- Elute in 24 µL of nuclease-free water in step 13. Place 12 µL of each concentrated pool in a separate well for the hybridization reaction. The remaining concentrated pool volume is not used in the hybridization reaction.

If yield of post-capture libraries is low

✓The protocol includes specific thawing, temperature control, pipetting, and mixing instructions which are required for optimal performance. Be sure to adhere to all instructions when setting up the reactions.

✓PCR cycle number may require optimization. Repeat target enrichment for the sample, increasing the post-capture PCR cycle number by 1 to 2 cycles.

✓The probe used for hybridization may have been compromised. Verify the expiration date on the probe vial or Certificate of Analysis. Adhere to the recommended storage and handling conditions. Ensure that the probe hybridization mix is prepared at the time of use as directed on page 14, and that solutions containing the probe are not held at room temperature for extended periods.

✓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 20). 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 to up to 10 minutes may improve recovery, especially for longer library fragments (see step 15 on page 21).

If post-capture library fragment size is different than expected in electropherograms

✓Libraries prepared from FFPE DNA samples or ctDNA/cfDNA samples may have a smaller fragment size distribution due to the presence of fragments in the sample input that are smaller than the optimal fragment size for target enrichment. Adhere to the FFPE DNA quality guidelines provided in the SureSelect Max Library Preparation Module user guides.

✓Library fragment size selection during post-capture 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 and verify that you are using the bead volume recommended for post-capture purification on page 20.

If low percent on target is observed in library sequencing results

✓Stringency of post-hybridization washes may have been lower than required. Complete the wash steps as directed, paying special attention to the details of SureSelect Wash Buffer 2 washes listed below:

- Ensure that SureSelect Wash Buffer 2 is pre-warmed to 68°C before use (see page 17). Select a thermal cycler with a block configured for efficient heating of 0.2 mL liquid volumes; ensure that the plasticware containing the wash buffer is fully seated in the thermal cycler block wells, with minimal liquid volume visible above the block during the pre-warming step.
- Bead suspensions are mixed thoroughly during washes by pipetting up and down **and** vortexing (see page 17)

✓Minimize the amount of time that hybridization reactions are exposed to RT conditions during hybridization setup. Locate a vortex and plate spinner or centrifuge in close proximity to thermal cycler to retain the elevated sample temperature during mixing and transfer steps (step 6 on page 15).

Quick Reference Protocol: Max MGI Fast Hyb Target Enrichment

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
	Hybridization
Place libraries in hyb wells	Place prepared libraries or library pools (details below) into strip tube wells> keep on ice. **For post-cap pooling:** Use 12 µL of undiluted prepared library samples or use at least 500 ng library per well, brought to 12 µL with nuclease-free H_2O. **For pre-cap pooling:** Pool into wells according to Table 7 on page 12. Reduce pool volumes to <12 µL by vacuum concentration at ≤45°C. Bring well volumes to 12 µL with nuclease-free H_2O> vortex> spin.
Program thermal cycler	Input the appropriate hybridization program in Table 31 (next page). Pre-warm before loading samples.
Run pre-hybridization blocking protocol	Add 5 µL Blocker Mix, MGI to each sample well; mix> spin> place strip in cycler. Run thermal cycler program segments 1 through 3; prepare Probe/Hyb Mix while program runs.
Prepare Probe/Hyb Mix	Prepare the Probe/Hyb Mix for your probe design size--see Table 32 (next page). Prepare at RT> vortex> spin> keep briefly at RT.
Run the hybridization	With samples held in cycler (Segment 3 Hold), add 13 µL Probe/Hyb Mix to wells> mix by pipetting. Seal wells completely> vortex briefly> spin briefly> return strip to cycler. Resume cycler program, completing hybridization (Segment 4) and final 68°C Hold. Prep capture reagents during hybridization (unless using 2-day workflow with overnight 21°C Hold).
	Capture
Aliquot Wash Buffer 2	Aliquot 6 × 200 µL of SureSelect Wash Buffer 2 for each sample.
Prepare streptavidin beads in capture strip	For each sample, wash 50 µL SureSelect Streptavidin Beads 3× in 200 µL SureSelect Binding Buffer. After final wash, resuspend beads in 200 µL SureSelect Binding Buffer. Pre-warm beads/buffer in thermal cycler at 68°C for last 10 minutes of hybridization (Segment 4).
Capture hybridized libraries	Add hybridized samples (~30 µL) to 200 µL pre-warmed beads, keeping in cycler> mix well by pipetting. Incubate capture strip in thermal cycler at 68°C for 10 minutes. During incubation, pre-warm the 6 × 200 µL aliquots of SureSelect Wash Buffer 2 to 68°C.
Wash captured libraries	Spin capture strip briefly> collect beads using a magnetic stand> discard supernatant. Wash beads 1× with 200 µL SureSelect Wash Buffer 1 at RT. Wash beads 6× with 200 µL pre-warmed SureSelect Wash Buffer 2 (for each wash: add 200 µL 68°C Wash Buffer 2> mix well by pipetting and vortexing at RT> incubate 5 minutes at 68°C> collect beads at RT> discard supernatant). Resuspend washed beads in 24 µL nuclease-free H_2O> pipette to mix> keep on ice.
	Post-capture amplification
Program thermal cycler	Input the appropriate thermal cycler program in Table 33 (next page). Pre-warm before loading samples.
Prepare Post-capture PCR Mix	**Per 8 Hyb reactions:** 225 µL Amplification Master Mix + 9 µL SureSelect MGI Post-Capture Primer Mix Prepare on ice> mix> spin> keep on ice.
Amplify the bead-bound captured libraries	Add 26 µL Post-capture PCR Mix to each well containing 24 µL captured library bead suspension; pipette to mix (do not spin)> place strip in cycler> run thermal cycler program.
Purify amplified libraries	50 µL amplified library bead suspension + 50 µL purification bead suspension> mix> incubate 5 min> collect beads> discard supernatant. Wash beads 2X with 70% ethanol. Elute in 25 µL Low TE Buffer> mix> incubate 2-5 minutes> collect beads> retain supernatant.
Qualify and quantify libraries	Measure average fragment size using TapeStation or Fragment Analyzer System. Measure concentration using Qubit dsDNA HS Assay.

Table 31 Hybridization program (30 µL vol; heated lid at 105°C)

Segment	Temperature (Purpose)	Probe Design	Time	Two-day Workflow Adjustments
1	95°C (Pre-blocking)	All probes	5 minutes	—
2	65°C (Blocking)	All probes	10 minutes	—
3	65°C (Hyb reagent addition hold)	All probes	Hold	—
4	68°C (Hybridization)	SureSelect XT HS Human All Exon Probes (V7/V8/V8+UTR/V8+NCV) or Clinical Research Exome V4	120 minutes	Do not start streptavidin bead and capture reagent prep steps during Hyb. Begin at start of day 2.
		All other XT HS probes	90 minutes	
5	68°C (Hold for capture start)	All probes	Hold **briefly**	21°C Hold for up to 16 hours (overnight pause)

Table 32 Preparation of Probe/Hyb Mix

Reagent	Probe designs ≥3 Mb		Probe designs <3 Mb	
	Per Hyb Reaction	8 Hybs	Per Hyb Reaction	8 Hybs
Nuclease-free water	1.5 µL	13.5 µL	4.5 µL	40.5 µL
SureSelect RNase Block (purple cap)	0.5 µL	4.5 µL	0.5 µL	4.5 µL
SureSelect Max Fast Hyb Buffer	6 µL	54 µL	6 µL	54 µL
Probe	5 µL	45 µL	2 µL	18 µL
Total	13 µL	117 µL	13 µL	117 µL

Table 33 Post-Capture PCR thermal cycler program (50 µL vol; heated lid at 105°C)

Segment	Number of Cycles	Temperature	Time
1	1	98°C	45 seconds
2	Probes <0.2 Mb: 16 cycles Probes 0.2–3 Mb: 13–16 cycles Probes 3–5 Mb: 12–13 cycles Probes >5 Mb: 12 cycles	98°C	15 seconds
		60°C	30 seconds
		72°C	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 for MGI Target Enrichment using a Fast Hybridization workflow including either post-capture or pre-capture pooling of NGS libraries.

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