# SureSelect Max Target Enrichment using Fast Hybridization

For NGS using the Illumina Platform

## Protocol

**Version B0 January 2026**

SureSelect platform manufactured with Agilent SurePrint technology.

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

# Notices



## Manual Part Number

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## Acknowledgment



## Technical Support

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# In this Guide...

This guide provides an optimized protocol for target enrichment of Illumina paired-end multiplexed DNA or RNA 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 Illumina instrument. Before starting this protocol, SureSelect Max libraries must be prepared as described in separate guides for earlier workflow segments.

## What's New in Version B0

- Added details to footnotes for Table 1 on page 9, Table 10 on page 17, and Table 11 on page 18 regarding the kit size and run configuration descriptions.
- Update to process for adding RNase Block to hybridization mix (see step 4 on page 17). Note that the amount of RNase Block in the final hybridization reaction is unchanged.
- Minor protocol updates including:
  - Revised preparative steps for the supplied Amplification Master Mix (Table 12 on page 21)
  - New *Note* on page 19 regarding pre-labeling the streptavidin bead strip(s)
  - Revised instructions for mixing the hybridization mixtures with the streptavidin capture beads (step 1 on page 19)
  - Updated list of probes that use the extended hyb program (see step 1 and Table 8 on page 16) to include SureSelect XT HS Human All Exon V7
- Addition of the SureSelect Cancer CGP Assay probes to list of recommended pre-designed probes in Table 2 on page 10.
- Update to footnote for Table 3 on page 11 regarding dehydration of library pools.
- Minor updates to instructions for obtaining NGS support for SureSelect Max for ILM library sequencing guidance (page 30).
- New entry in the "Troubleshooting Guide" with advice on shortening the capture of hybridized libraries (page 45).
- Fully updated "Analysis Pipeline Guidelines" on page 30.
- New *Note* below Table 1 on page 9 explaining use of AMPure XP Beads as a replacement for SureSelect Max Purification Beads.

# Content

# 1
# Before You Begin



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 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 or RNA libraries 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 13. Protocols for the upstream DNA or RNA library preparation modules are provided in separate publications with links provided below.

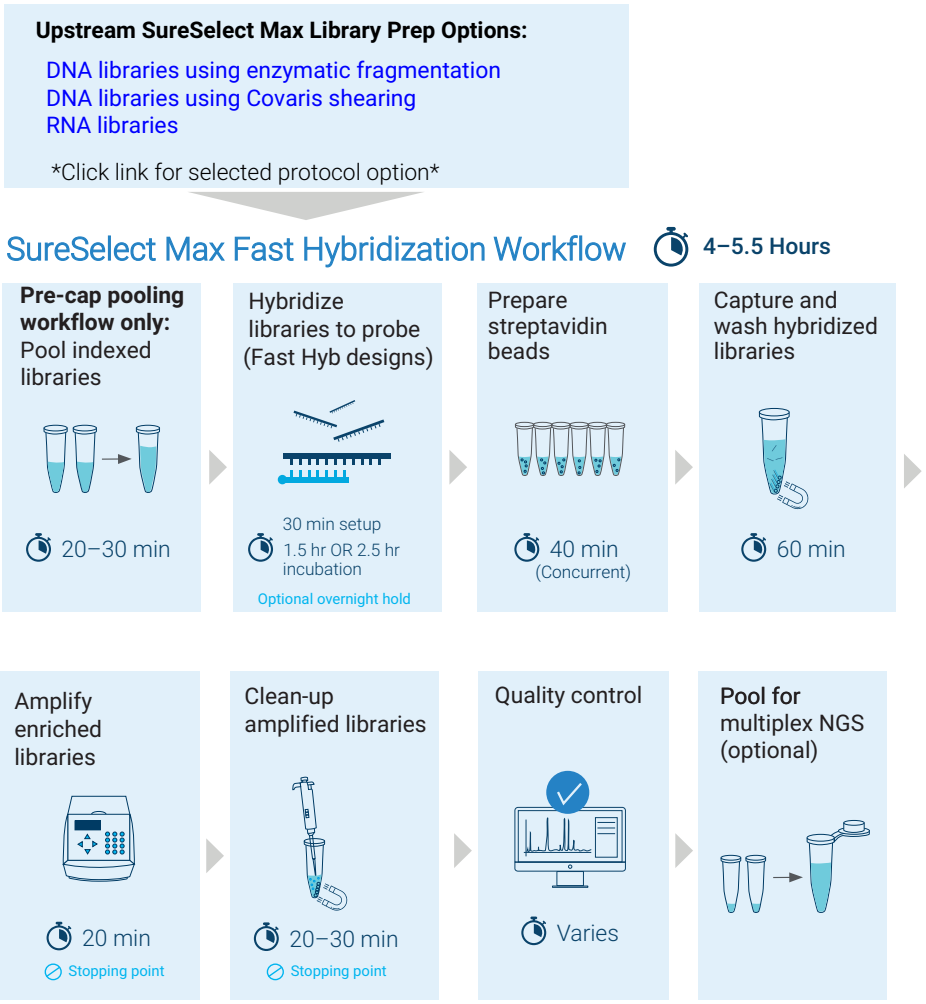


**Figure 1** Summary of the SureSelect Max Fast Hyb target enrichment workflow segment, including optional stopping points and estimated time requirements. Estimates are guidelines for 16 reaction runs, with 1.5 hour or 2.5 hour hybridization time dependent on the specific probe design. Timing for runs using different protocol parameters may vary.

The SureSelect Max system features several improvements over earlier SureSelect platforms:

- Streamlined enzymatic fragmentation library prep protocol with 1-step DNA fragmentation and dA-tailing, enhanced chemistry, and support for lower concentration samples
- Enhanced amplification chemistry and master mixed reagents
- Optional pre-capture QC, with support for capture of undiluted library samples
- Enhanced Fast Hyb chemistry and streamlined capture process
- Faster overall turnaround time with shorter, simplified protocol steps

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

# 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 or RNA libraries using a suitable SureSelect probe. 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 ILM | G9699A | G9699B |
| 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.

**NOTE**

AMPure XP Beads can replace SureSelect Max Purification Beads in the protocols provided in this user guide (see Table 3 on page 11 for ordering information).

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

**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 | |
| SureSelect Cancer CGP RNA Assay | A3370051 | |
| SureSelect Cancer CGP DNA Assay | A3416642 | |
| **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:<br>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 require two thermal cyclers to complete a reagent pre-warming step during certain sample incubation steps. See *Note* on page 16 for more information. |
| Plasticware compatible with the selected thermal cycler:<br>8-well strip tubes<br>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 12 | — |
| 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 9) |

* 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 44 for suggested protocol modifications.

**Table 4** Recommended Nucleic Acid Analysis Platforms*

| Analysis System | Vendor and Part Number Information |
|---|---|
| Agilent 4200/4150 TapeStation Instrument | Agilent p/n G2991AA/G2992AA |
| Consumables: | |
| 8-well tube strips | p/n 401428 |
| 8-well tube strip caps | p/n 401425 |
| High Sensitivity D1000 ScreenTape | p/n 5067-5584 |
| High Sensitivity D1000 Reagents | p/n 5067-5585 |
| Agilent 5200/5300/5400 Fragment Analyzer Instrument | Agilent p/n M5310AA/M5311AA/M5312AA |
| Consumables: | |
| HS NGS Fragment Kit (1-6000 bp) | p/n DNF-474-0500 |

* Libraries may also be analyzed 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.

# 2
# Target Enrichment with Fast Hybridization Protocol



In this workflow segment, SureSelect Max DNA or cDNA 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 14 for post-capture pooling workflow setup) or prior to hybridization (see page 15 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 17.

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 16 and keep on ice; vortex to mix | page 17 |
| SureSelect Max Blockers and Primers Module for ILM, stored at –20°C | Blocker Mix, ILM (blue cap) | Thaw and keep on ice, vortex to mix | page 17 |
| 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 17 |
| | SureSelect Max Fast Hyb Buffer (bottle) | Thaw and keep at room temperature | page 17 |
| +4°C | SureSelect Streptavidin Beads (clear cap) | Remove from 4°C just before use, vortex to mix | page 19 |
| SureSelect Max Target Enrichment Kit Fast Hyb Module Box 1, stored at RT | SureSelect Binding Buffer (bottle) | Ready to use | page 19 |
| | SureSelect Wash Buffer 1 (bottle) | Ready to use | page 20 |
| | SureSelect Wash Buffer 2 (bottle) | Ready to use | page 19 |

# 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

| Type of prepared library | Recommended amount | Minimum amount* |
|---|---|---|
| gDNA library (DNA input) | 12 µL of undiluted prepared gDNA library solution (pre-capture QC optional) | ≥500 ng library DNA brought to 12 µL with nuclease-free water |
| cDNA library (RNA input) | 12 µL of undiluted prepared cDNA library solution (pre-capture QC optional) | ≥200 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 17.

## 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 |
| | 8 | cDNA (RNA input) | 1.6 µg | 200 ng |
| SureSelect XT HS PreCap Custom Probes | 16 | DNA | 4 µg | 250 ng |
| | 16 | cDNA (RNA input) | 1.6 µg | 100 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 44.

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 17.

# Step 2. Hybridize libraries to the SureSelect probe

**NOTE**

Two thermal cyclers are required for the Hybridization and Capture protocols in the following sections, to complete reagent pre-warming steps during two sample incubation steps: 1) capture bead pre-warming in step 6 on page 19 during hybridization and 2) Wash Buffer 2 pre-warming during the capture incubation (see step 2 and step 3 on page 20). 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.

1 Preprogram a thermal cycler with the program in Table 8 for the SureSelect XT HS Human All Exon Probes (V7/V8/V8+UTR/V8+NCV) and SureSelect XT HS Clinical Research Exome V4 Probe, or with the program in Table 9 for all other probes. Set the heated lid to 105°C. Prewarm the instrument before samples are loaded in step 3 on page 17.

**CAUTION**

During the hybridization program, an operator must be present and perform the actions described in the final column of Table 8 or Table 9 below.

For the single-day workflow, it is important to have washed and pre-warmed streptavidin beads ready for capture at the end of hybridization (Segment 5 in Table 8 or Segment 4 in Table 9).

**Table 8** Hyb program for **SureSelect XT HS Human All Exon V7/V8/V8+UTR/V8+NCV or Clinical Research Exome V4 Probes** (30 µL vol)

| Segment | Cycles | Temperature | Time | Operator Action Required during Segment(s) |
|---|---|---|---|---|
| 1 | 1 | 95°C | 5 minutes | Prepare Probe Hybridization Mix (see step 4 on page 17) |
| 2 | 1 | 65°C | 10 minutes | |
| 3 | 1 | 65°C | Hold | Add Probe Hybridization Mix to library + Blocker wells on cycler (see step 5 on page 18) |
| 4 | 60 | 68°C | 1 minute | Prepare reagents for hybrid capture (see page 19). Requires approximately 45 minutes. |
| | | 37°C | 3 seconds | |
| 5 | 1 | 68°C | 60 minutes | |
| 6 | 1 | 68°C | Hold **briefly** | Begin capture (see page 19) as quickly as possible after the hybridization in Segment 5 is complete. |

**Table 9** Hyb program for **all other SureSelect XT HS probes** (30 µL vol)

| Segment | Cycles | Temperature | Time | Operator Action Required during Segment(s) |
|---|---|---|---|---|
| 1 | 1 | 95°C | 5 minutes | Prepare Probe Hybridization Mix (see step 4 on page 17) |
| 2 | 1 | 65°C | 10 minutes | |
| 3 | 1 | 65°C | Hold | Add Probe Hybridization Mix to library + Blocker wells on cycler (see step 5 on page 18) |
| 4 | 60 | 68°C | 1 minute | Prepare reagents for hybrid capture (see page 19). Requires approximately 45 minutes. |
| | | 37°C | 3 seconds | |
| 5 | 1 | 68°C | Hold **briefly** | Begin capture (see page 19) as quickly as possible after the hybridization in Segment 4 is complete. |

**NOTE**

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

- In the final segment of the thermal cycler program (Table 8 or Table 9), replace the final brief 68°C Hold step with a 21°C Hold step. The hybridized samples may be held at 21°C for up to 16 hours.
- During Segments 4-5 in Table 8 or Segment 4 in Table 9, omit the hybrid capture reagent preparation steps described on page 19. Instead begin the capture preparation steps on day 2.

2 To each library sample well, add 5 µL of Blocker Mix, ILM (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 or Table 9, 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 as described in 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 10 for probes ≥3 Mb or Table 11 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 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 | 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 11** 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 library + 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 19 during the hybridization incubation 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 or Table 9 on page 16). Start the final capture bead pre-warming step (step 6 below) at 10 minutes before the end of the program.

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 70°C on page 20.

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 20.
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 5 in Table 8 or Segment 4 in Table 9), pre-warm the streptavidin capture beads in Binding Buffer by incubating the strip tube in a thermal cycler at 68°C for 10 minutes.

# Step 4. Capture the hybridized libraries

1. Immediately after the hybridization program is complete (Table 8 or Table 9 on page 16), 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 mix by pipetting up and down 8–10 times using the same tips; 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 19 (200 µL/well) to a thermal cycler held at 70°C. Pre-warm the buffer until used for washes in step 8.

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 70°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 70°C incubation step.

Do not use a tissue incubator, or other devices with significant temperature fluctuations, for the 70°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 70°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 or 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 70°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 on page 22.

**NOTE**

Captured DNA or cDNA 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 12. Prepare as directed before use (refer to the *Where Used* column).

**Table 12** 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 22 |
| SureSelect Max Blockers and Primers Module for ILM, stored at –20°C | SureSelect Post-Capture Primer Mix (clear cap) | Thaw and keep on ice, vortex to mix. | page 22 |
| +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 23 |

1 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 22.

**Table 13** Post-Capture PCR thermal cycler program (50 µL vol)

| Segment | Number of Cycles | Temperature | Time |
|---|---|---|---|
| 1 | 1 | 98°C | 45 seconds |
| 2 | 10–16 (see Table 14 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 14** Post-capture PCR cycle number recommendations

| Probe Design Size | Cycles | |
|---|---|---|
| | DNA input libraries | RNA input libraries |
| Probes <0.2 Mb | 16 cycles | 16 cycles |
| Probes 0.2–3 Mb | 12–16 cycles | 14 cycles |
| Probes 3–5 Mb | 11–12 cycles | 13 cycles |
| Probes >5 Mb (including Human All Exon and Exome probes) | 10–11 cycles | 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 15, on ice. Mix well on a vortex mixer.

**Table 15** 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 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 15 to each sample well containing 24 µL of bead-bound target-enriched DNA or cDNA.

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 13.

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 AmpPure XP Beads. Critical purification protocol parameters are summarized for experienced users in Table 16.

**Table 16** 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 or cDNA 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 26.

**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

Analyze a sample of each library using one of the platforms listed in Table 17. Follow the instructions in the linked user guide provided for each assay.

**Table 17** Post-capture 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 | High Sensitivity D1000 ScreenTape | Agilent High Sensitivity D1000 Assay Quick Guide | 2 µL |
| 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 |

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

Using the 150 bp to 1000 bp region of the electropherogram, determine the average fragment size and the library concentration. See Table 18 for fragment size distribution guidelines.

**Table 18** Post-capture expected library fragment size guidelines

| Hybridization input | Expected average fragment size (150–1000 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 | 380 to 480 bp |
| DNA library prepared from FFPE DNA fragmented for 2x100 or 2x150 NGS | 250 to 390 bp |
| cDNA library prepared from intact RNA | 380 to 480 bp |
| cDNA library prepared from FFPE RNA | 250 to 390 bp |

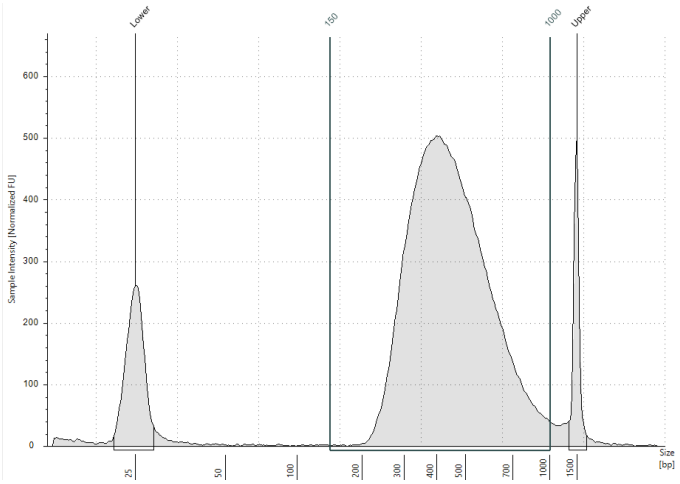


**Figure 2** Post-capture library prepared from a high-quality gDNA sample analyzed using a High Sensitivity D1000 ScreenTape assay.

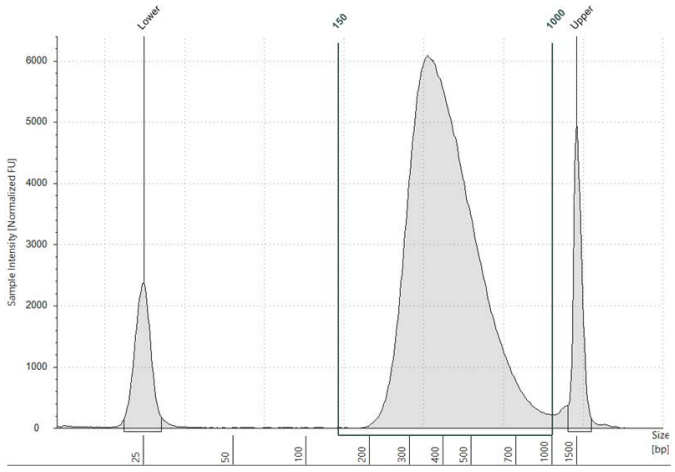


**Figure 3** Post-capture library prepared from an FFPE gDNA sample analyzed using a High Sensitivity D1000 ScreenTape assay.

**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 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 per sample for your research design. Calculate the number of indexes that can be combined per lane, according to the capacity of your platform and the amount of sequencing data required per sample.

**NOTE**

SureSelect Max UDI strip and plate layouts are designed to provide the proper color balance for Illumina's two-channel and four-channel systems. A minimum plexity of four is recommended to ensure that library pools are color balanced. Pools containing any four consecutive SureSelect Max UDIs meet Illumina's guidance for optimal color balance and sequencing performance. Consult Illumina's guidelines for additional color balance and pooling strategy information including two-plex or three-plex pooling considerations.

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

**Method 1:** Dilute each sample to be pooled to the same final concentration (typically 4–15 nM, or the concentration of the most dilute sample) then combine equal volumes of all samples to create the final NGS pool.

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

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

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

*C(f)* is the desired final concentration of all the DNA or cDNA in the pool (typically 4 nM–15 nM or the concentration of the most dilute sample)

*#* is the number of indexes, and

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

Table 19 shows an example of the amount of 4 index-tagged samples (of different concentrations) and Low TE needed for a final volume of 20 µL at 10 nM DNA or cDNA.

**Table 19** Example of volume calculation for total volume of 20 µL at 10 nM concentration

| Component | V(f) | C(i) | C(f) | # | Volume to use (µL) |
|---|---|---|---|---|---|
| Sample 1 | 20 µL | 20 nM | 10 nM | 4 | 2.5 |
| Sample 2 | 20 µL | 10 nM | 10 nM | 4 | 5 |
| Sample 3 | 20 µL | 17 nM | 10 nM | 4 | 2.9 |
| Sample 4 | 20 µL | 25 nM | 10 nM | 4 | 2 |
| Low TE | | | | | 7.6 |

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

# 3
# Appendix: NGS and Analysis Guidelines



This appendix provides guidelines for completing NGS using the Illumina platform and for SureSelect Max library read processing steps.

# SureSelect Max Library Composition

The final SureSelect Max library fragments prepared using Agilent's Illumina-compatible kit modules are shown in Figure 4. Each fragment in the prepared library contains one target insert surrounded by sequence motifs required for multiplexed sequencing using Illumina sequencers. The libraries are ready for sequencing using standard Illumina paired-end primers and chemistry.

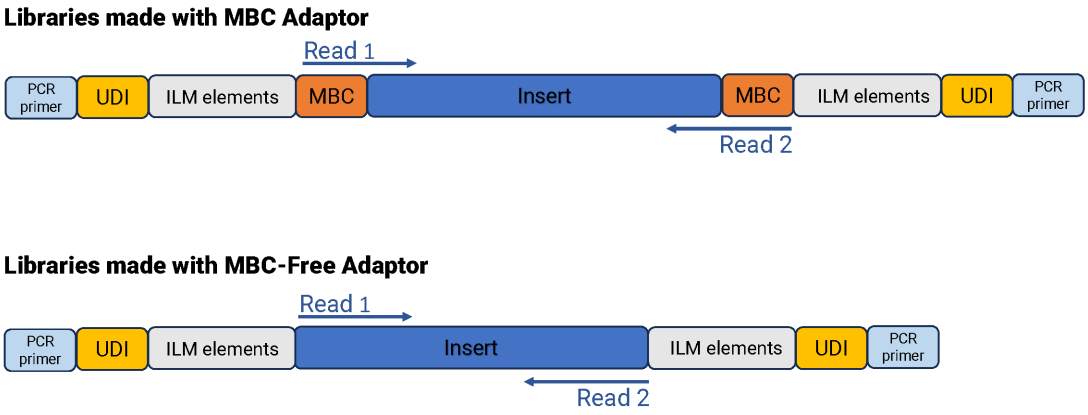


**Figure 4** Content of SureSelect Max for ILM sequencing libraries. Each fragment contains one target insert (dark blue) surrounded by the following elements: Illumina paired-end sequencing elements (gray), unique dual indexes (gold), library PCR primers (light blue) and optional molecular barcode (MBC) sequences. MBCs include a 3-bp degenerate barcode plus dark bases.

# Sequencing Setup and Run Guidelines

Proceed to cluster amplification using the appropriate Illumina Paired-End Cluster Generation Kit. Table 20 provides guidelines for use of several Illumina instrument and chemistry combinations suitable for processing the SureSelect Max target-enriched NGS libraries. For other Illumina NGS platforms, consult Illumina's documentation for kit configuration and seeding concentration guidelines.

**Table 20** Illumina Kit Configuration Selection Guidelines

| Instrument | Run Type | Read Length | SBS Kit Configuration | Chemistry | Seeding Concentration |
|---|---|---|---|---|---|
| MiSeq | All Runs | 2 × 100 bp or 2 × 150 bp | 300 Cycle Kit | v2 | 9–10 pM |
| | | | 600 Cycle Kit | v3 | 12–16 pM |
| iSeq 100 | All Runs | 2 × 100 bp or 2 × 150 bp | 300 Cycle Kit | v2 | 50–150 pM |
| NextSeq 500/550 | All Runs | 2 × 100 bp or 2 × 150 bp | 300 Cycle Kit | v2.5 | 1.2–1.5 pM |
| NextSeq 1000/2000 | All Runs | 2 × 100 bp or 2 × 150 bp | 200 or 300 Cycle Kit | Standard SBS | 650–1000 pM |
| | | | | XLEAP-SBS | 650–1000 pM |
| NovaSeq 6000 | Standard Workflow Runs | 2 × 100 bp or 2 × 150 bp | 200 or 300 Cycle Kit | v1.5 | 300–600 pM |
| NovaSeq 6000 | Xp Workflow Runs | 2 × 100 bp or 2 × 150 bp | 200 or 300 Cycle Kit | v1.5 | 200–400 pM |
| NovaSeq X | All runs | 2 × 100 bp or 2 × 150 bp | 200 or 300 Cycle Kit | v1 | 90–180 pM |

Seeding concentration and cluster density may also need to be optimized based on the fragment size range for the library and on the desired output and data quality. Begin optimization using a seeding concentration in the middle of the range listed in Table 20 or provided by Illumina. Follow Illumina's recommendation for a PhiX control in a low-concentration spike-in for improved sequencing quality control.

Set up the sequencing run to generate Read 1 and Read 2 FASTQ files for each sample using the instrument's software in standalone mode or using an Illumina run management tool such as Local Run Manager (LRM), Illumina Experiment Manager (IEM) or BaseSpace. Enter the appropriate **Cycles** or **Read Length** value for your library read length and using 8-bp dual index reads. See Table 21 showing example settings for 2x150 bp sequencing.

**Table 21** Run settings for 2x150 bp sequencing

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

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

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

- Each of the sample-level indexes (i7 and i5) requires an 8-bp index read. For complete index sequence information, see page 34.
- No custom primers are used for SureSelect Max library sequencing. Leave all *Custom Primers* options for *Read 1*, *Read 2*, *Index 1* and *Index 2* primers cleared/deselected during run setup.
- For MBC-tagged libraries, turn off any adaptor trimming tools included in Illumina's run setup and read processing software applications. Adaptors are trimmed in later processing steps to ensure proper processing of the degenerate MBC sequences in the adaptors.
- For runs set up using Illumina's LRM, IEM, or BaseSpace applications, refer to Illumina's instructions and support resources for setting up runs with custom library prep kits and index kits in the selected software. For use in these applications, the SureSelect Max index sequences can be obtained by downloading the SureSelect Max Index Sequence Resource Excel spreadsheet from Agilent.com. The provided sequences should be converted to .tsv/.csv file format or copied to a Sample Sheet according to Illumina's specifications for each application. If you need additional SureSelect Max for ILM library sequencing guidance, contact the SureSelect support team (see page 2) or your local representative.

# Analysis Pipeline Guidelines

Guidelines are provided below for typical NGS read processing and analysis pipeline steps appropriate for SureSelect Max DNA and RNA libraries prepared with either MBC-free or MBC-tagged adaptors. Your NGS pipeline may vary.

## MBC-free libraries

1. **Demultiplexing**: Generate demultiplexed FASTQ files for each sample using Illumina's bcl2fastq, BCL Convert, or a similar software application. This process generates paired-end reads based on the dual indexes and removes sequences with incorrectly paired P5 and P7 indexes.
2. **Adaptor trimming**: For MBC-free libraries, turn on the adaptor trimming tools in the selected Illumina demultiplexing software to complete adaptor trimming at this step.
3. **Alignment**: The trimmed reads should be aligned using a suitable tool such as BWA-MEM for DNA libraries or STAR for RNA libraries.

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

## MBC-tagged libraries

1 **Demultiplexing**: Generate demultiplexed FASTQ files for each sample using Illumina's bcl2fastq, BCL Convert, or a similar software application. This process generates paired-end reads based on the dual indexes and removes sequences with incorrectly paired P5 and P7 indexes.

   Turn off the MBC/UMI trimming options in Illumina's demultiplexing software to allow proper adaptor processing and use of the MBCs by downstream tools.

NOTE

If your libraries are MBC-tagged but your sequence analysis pipeline excludes MBCs, you can remove the first 5 bases from Read 1 and Read 2 by masking or trimming before proceeding to downstream analysis.

If demultiplexing using bcl2fastq, MBCs may be masked by including the base mask **N5Y*,I8,I8,N5Y*** (where * is replaced with the remaining read length after subtracting the 5 masked bases, e.g., use **N5Y146,I8,I8,N5Y146** for 2x150 NGS set up as shown in Table 21 on page 29). The sum of the values following N and Y must match the read length value in the RunInfo.xml file.

If demultiplexing using BCL Convert, MBCs may be trimmed by including the following string in the sample sheet header: **OverrideCycles,N5Y*;I8;I8;N5Y*** (where * is replaced with read length after trimming, e.g., use **N5Y146;I8;I8;N5Y146** for 2x150 NGS set up as shown in Table 21 on page 29). The sum of the values following N and Y must match the read length value in the RunInfo.xml file.

Alternatively, the first 5 bases may be trimmed from the demultiplexed FASTQ files using a suitable processing tool (e.g., fgbio).

2 **MBC-adaptor processing**: Use a suitable processing tool of your choice to trim and collect inline MBCs from each sequencing read. For example, MBC processing could be conducted with the fgbio best practice consensus pipeline, as described in: https://github.com/fulcrumgenomics/fgbio/blob/main/docs/best-practice-consensus-pipeline.md. The output includes trimmed, deduplicated reads in unaligned BAM format with single-stranded MBC consensus reads. For generation of double-stranded MBC consensus reads, refer to https://fulcrumgenomics.github.io/fgbio/tools/latest/CallDuplexConsensusReads.html.

   - Inline MBCs are added to both ends of the DNA inserts in the assay. To collect the MBCs and process them for analysis, one approach is to trim off 5 bases at the beginning of each read, take the first 3 of the 5 bases as MBC, and discard the remaining 2.

3 **Alignment**:

   - DNA library alignment can be completed as part of the fgbio best practice consensus pipeline described at the link above or aligned using another suitable alignment tool of your choice.
   - RNA library alignment should be performed after read trimming and deduplication using a suitable tool such as STAR.

The resulting aligned BAM files are ready for downstream analysis including variant and gene expression discovery.

## RNA strandedness guidelines

The SureSelect Max RNA sequencing library preparation method preserves RNA strandedness using dUTP second-strand marking. The sequence of read 1, which starts at the P5 end, matches the reverse complement of the poly-A RNA transcript strand. Read 2, which starts at the P7 end, matches the poly-A RNA transcript strand. When running analysis of this data to determine strandedness, it is important to include this information. For example, when using the Picard tools (https://broadinstitute.github.io/picard) to calculate RNA sequencing metrics, it is important to include the parameter *STRAND_SPECIFICITY= SECOND_READ_TRANSCRIPTION_STRAND* to correctly calculate the strand specificity metrics.

# SureSelect Max UDI Information

The SureSelect Max unique dual indexes (UDIs) are added to the library fragments during the library preparation workflow segment. Each fragment contains a unique 8-bp P5 and P7 index (see Figure 4 on page 28) suitable for Illumina sequencing platforms.

**NOTE** Agilent's SureSelect 8-bp dual indexes use a uniform numbering system across kit formats and between SureSelect Max for ILM and SureSelect XT HS2 platforms. Do not combine samples indexed with the same-numbered dual index pair from different kit formats for multiplex sequencing.

Index sequences are provided on page 34 through page 41. Index sequences can also be obtained by downloading the SureSelect Max Index Sequence Resource Excel spreadsheet from Agilent.com.

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

In Table 23 on page 34 through Table 30 on page 41 and in the downloadable Excel spreadsheet, P7 indexes are shown in forward orientation, applicable to any of the supported Illumina platforms. P5 indexes are shown in two orientations (forward and reverse complement) for use with different platforms and sequencing run setup and management tools, e.g., Local Run Manager and Instrument Run Setup. A selection of Illumina sequencing platforms and their P5 sequencing orientations are shown in Table 22. Correct representation of the P5 index orientation

in sample sheets or during sequencing run setup is crucial to successful demultiplexing. Refer to Illumina support documentation and resources to determine the correct P5 index orientation for your application.

**Table 22** P5 index sequencing orientation by Illumina platform

| P5 Index Orientation | Platform |
|---|---|
| Forward | MiSeq |
| Reverse Complement* | NovaSeq 6000 with v1.5 chemistry<br>NextSeq 500/550/1000/2000<br>iSeq 100<br>MiniSeq |

* Some run setup and management tools used with these platforms automatically create the reverse complement sequence for the P5 index sequence entered for the run. Be sure to consult Illumina's support documentation for the combination of platform and tools used in your pipeline to determine the correct index orientation to enter during run setup.

## SureSelect Max index sequences

Index sequences can also be obtained by downloading the SureSelect Max Index Sequence Resource Excel spreadsheet from Agilent.com.

**Table 23** SureSelect Max Index Primer Pairs 1–48, provided in orange 96-well plate or in strip tubes

| Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement | Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement |
|---|---|---|---|---|---|---|---|---|---|
| 1 | A01 | CAAGGTGA | ATGGTTAG | CTAACCAT | 25 | A04 | AGATGGAT | TGGCACCA | TGGTGCCA |
| 2 | B01 | TAGACCAA | CAAGGTGA | TCACCTTG | 26 | B04 | GAATTGTG | AGATGGAT | ATCCATCT |
| 3 | C01 | AGTCGCGA | TAGACCAA | TTGGTCTA | 27 | C04 | GAGCACTG | GAATTGTG | CACAATTC |
| 4 | D01 | CGGTAGAG | AGTCGCGA | TCGCGACT | 28 | D04 | GTTGCGGA | GAGCACTG | CAGTGCTC |
| 5 | E01 | TCAGCATC | AAGGAGCG | CGCTCCTT | 29 | E04 | AATGGAAC | GTTGCGGA | TCCGCAAC |
| 6 | F01 | AGAAGCAA | TCAGCATC | GATGCTGA | 30 | F04 | TCAGAGGT | AATGGAAC | GTTCCATT |
| 7 | G01 | GCAGGTTC | AGAAGCAA | TTGCTTCT | 31 | G04 | GCAACAAT | TCAGAGGT | ACCTCTGA |
| 8 | H01 | AAGTGTCT | GCAGGTTC | GAACCTGC | 32 | H04 | GTCGATCG | GCAACAAT | ATTGTTGC |
| 9 | A02 | CTACCGAA | AAGTGTCT | AGACACTT | 33 | A05 | ATGGTAGC | GTCGATCG | CGATCGAC |
| 10 | B02 | TAGAGCTC | CTACCGAA | TTCGGTAG | 34 | B05 | CGCCAATT | ATGGTAGC | GCTACCAT |
| 11 | C02 | ATGTCAAG | TAGAGCTC | GAGCTCTA | 35 | C05 | GACAATTG | CGCCAATT | AATTGGCG |
| 12 | D02 | GCATCATA | ATGTCAAG | CTTGACAT | 36 | D05 | ATATTCCG | GACAATTG | CAATTGTC |
| 13 | E02 | GACTTGAC | GCATCATA | TATGATGC | 37 | E05 | TCTACCTC | ATATTCCG | CGGAATAT |
| 14 | F02 | CTACAATG | GACTTGAC | GTCAAGTC | 38 | F05 | TCGTCGTG | TCTACCTC | GAGGTAGA |
| 15 | G02 | TCTCAGCA | CTACAATG | CATTGTAG | 39 | G05 | ATGAGAAC | TCGTCGTG | CACGACGA |
| 16 | H02 | AGACACAC | TCTCAGCA | TGCTGAGA | 40 | H05 | GTCCTATA | ATGAGAAC | GTTCTCAT |
| 17 | A03 | CAGGTCTG | AGACACAC | GTGTGTCT | 41 | A06 | AATGACCA | GTCCTATA | TATAGGAC |
| 18 | B03 | AATACGCG | CAGGTCTG | CAGACCTG | 42 | B06 | CAGACGCT | AATGACCA | TGGTCATT |
| 19 | C03 | GCACACAT | AATACGCG | CGCGTATT | 43 | C06 | TCGAACTG | CAGACGCT | AGCGTCTG |
| 20 | D03 | CTTGCATA | GCACACAT | ATGTGTGC | 44 | D06 | CGCTTCCA | TCGAACTG | CAGTTCGA |
| 21 | E03 | ATCCTCTT | CTTGCATA | TATGCAAG | 45 | E06 | TATTCCTG | CGCTTCCA | TGGAAGCG |
| 22 | F03 | GCACCTAA | ATCCTCTT | AAGAGGAT | 46 | F06 | CAAGTTAC | TATTCCTG | CAGGAATA |
| 23 | G03 | TGCTGCTC | GCACCTAA | TTAGGTGC | 47 | G06 | CAGAGCAG | CAAGTTAC | GTAACTTG |
| 24 | H03 | TGGCACCA | TGCTGCTC | GAGCAGCA | 48 | H06 | CGCGCAAT | CAGAGCAG | CTGCTCTG |

**Table 24** SureSelect Max Index Primer Pairs 49–96, provided in orange 96-well plate

| Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement | Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement |
|---|---|---|---|---|---|---|---|---|---|
| 49 | A07 | TGAGGAGT | CGCGCAAT | ATTGCGCG | 73 | A10 | AACGCATT | ATAGTGAC | GTCACTAT |
| 50 | B07 | ATGACGAA | TGAGGAGT | ACTCCTCA | 74 | B10 | CAGTTGCG | AACGCATT | AATGCGTT |
| 51 | C07 | TACGGCGA | ATGACGAA | TTCGTCAT | 75 | C10 | TGCCTCGA | CAGTTGCG | CGCAACTG |
| 52 | D07 | AGCGAGTT | TACGGCGA | TCGCCGTA | 76 | D10 | AAGGCTTA | TGCCTCGA | TCGAGGCA |
| 53 | E07 | TGTATCAC | AGCGAGTT | AACTCGCT | 77 | E10 | GCAATGAA | AAGGCTTA | TAAGCCTT |
| 54 | F07 | GATCGCCT | TGTATCAC | GTGATACA | 78 | F10 | AAGAACCT | GCAATGAA | TTCATTGC |
| 55 | G07 | GACTCAAT | GATCGCCT | AGGCGATC | 79 | G10 | CTGTGCCT | AAGAACCT | AGGTTCTT |
| 56 | H07 | CAGCTTGC | GACTCAAT | ATTGAGTC | 80 | H10 | TACGTAGC | CTGTGCCT | AGGCACAG |
| 57 | A08 | AGCTGAAG | CAGCTTGC | GCAAGCTG | 81 | A11 | AAGTGGAC | TACGTAGC | GCTACGTA |
| 58 | B08 | ATTCCGTG | AGCTGAAG | CTTCAGCT | 82 | B11 | CAACCGTG | AAGTGGAC | GTCCACTT |
| 59 | C08 | TATGCCGC | ATTCCGTG | CACGGAAT | 83 | C11 | CTGTTGTT | CAACCGTG | CACGGTTG |
| 60 | D08 | TCAGCTCA | TATGCCGC | GCGGCATA | 84 | D11 | GCACGATG | CTGTTGTT | AACAACAG |
| 61 | E08 | AACTGCAA | TCAGCTCA | TGAGCTGA | 85 | E11 | GTACGGAC | GCACGATG | CATCGTGC |
| 62 | F08 | ATTAGGAG | AACTGCAA | TTGCAGTT | 86 | F11 | CTCCAAGC | GTACGGAC | GTCCGTAC |
| 63 | G08 | CAGCAATA | ATTAGGAG | CTCCTAAT | 87 | G11 | TAGTCTGA | CTCCAAGC | GCTTGGAG |
| 64 | H08 | GCCAAGCT | CAGCAATA | TATTGCTG | 88 | H11 | TTCGCCGT | TAGTCTGA | TCAGACTA |
| 65 | A09 | TCCGTTAA | GCCAAGCT | AGCTTGGC | 89 | A12 | GAACTAAG | ATACGAAG | CTTCGTAT |
| 66 | B09 | GTGCAACG | TCCGTTAA | TTAACGGA | 90 | B12 | AAGCCATC | GAGATTCA | TGAATCTC |
| 67 | C09 | AGTAACGC | GTGCAACG | CGTTGCAC | 91 | C12 | AACTCTTG | AAGCCATC | GATGGCTT |
| 68 | D09 | CATAGCCA | AGTAACGC | GCGTTACT | 92 | D12 | GTAGTCAT | AACTCTTG | CAAGAGTT |
| 69 | E09 | CACTAGTA | CATAGCCA | TGGCTATG | 93 | E12 | CTCGCTAG | GTAGTCAT | ATGACTAC |
| 70 | F09 | TTAGTGCG | CACTAGTA | TACTAGTG | 94 | F12 | AGTCTTCA | CAGTATCA | TGATACTG |
| 71 | G09 | TCGATACA | TTAGTGCG | CGCACTAA | 95 | G12 | TCAAGCTA | CTTCGTAC | GTACGAAG |
| 72 | H09 | ATAGTGAC | TCGATACA | TGTATCGA | 96 | H12 | CTTATCCT | TCAAGCTA | TAGCTTGA |

**Table 25** SureSelect Max Index Primer Pairs 97–144, provided in blue 96-well plate

| Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement | Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement |
|---|---|---|---|---|---|---|---|---|---|
| 97 | A01 | TCATCCTT | CTTATCCT | AGGATAAG | 121 | A04 | CAGGCAGA | AGACGCCT | AGGCGTCT |
| 98 | B01 | AACACTCT | TCATCCTT | AAGGATGA | 122 | B04 | TCCGCGAT | CAGGCAGA | TCTGCCTG |
| 99 | C01 | CACCTAGA | AACACTCT | AGAGTGTT | 123 | C04 | CTCGTACG | TCCGCGAT | ATCGCGGA |
| 100 | D01 | AGTTCATG | CACCTAGA | TCTAGGTG | 124 | D04 | CACACATA | CTCGTACG | CGTACGAG |
| 101 | E01 | GTTGGTGT | AGTTCATG | CATGAACT | 125 | E04 | CGTCAAGA | CACACATA | TATGTGTG |
| 102 | F01 | GCTACGCA | GTTGGTGT | ACACCAAC | 126 | F04 | TTCGCGCA | CGTCAAGA | TCTTGACG |
| 103 | G01 | TCAACTGC | GCTACGCA | TGCGTAGC | 127 | G04 | CGACTACG | TTCGCGCA | TGCGCGAA |
| 104 | H01 | AAGCGAAT | TCAACTGC | GCAGTTGA | 128 | H04 | GAAGGTAT | CGACTACG | CGTAGTCG |
| 105 | A02 | GTGTTACA | AAGCGAAT | ATTCGCTT | 129 | A05 | TTGGCATG | GAAGGTAT | ATACCTTC |
| 106 | B02 | CAAGCCAT | GTGTTACA | TGTAACAC | 130 | B05 | CGAATTCA | TTGGCATG | CATGCCAA |
| 107 | C02 | CTCTCGTG | CAAGCCAT | ATGGCTTG | 131 | C05 | TTAGTTGC | CGAATTCA | TGAATTCG |
| 108 | D02 | TCGACAAC | CTCTCGTG | CACGAGAG | 132 | D05 | GATGCCAA | TTAGTTGC | GCAACTAA |
| 109 | E02 | TCGATGTT | TCGACAAC | GTTGTCGA | 133 | E05 | AGTTGCCG | GATGCCAA | TTGGCATC |
| 110 | F02 | CAAGGAAG | TCGATGTT | AACATCGA | 134 | F05 | GTCCACCT | AGTTGCCG | CGGCAACT |
| 111 | G02 | ATTGATGC | AGAGAATC | GATTCTCT | 135 | G05 | ATCAAGGT | GTCCACCT | AGGTGGAC |
| 112 | H02 | TCGCAGAT | TTGATGGC | GCCATCAA | 136 | H05 | GAACCAGA | ATCAAGGT | ACCTTGAT |
| 113 | A03 | GCAGAGAC | TCGCAGAT | ATCTGCGA | 137 | A06 | CATGTTCT | GAACCAGA | TCTGGTTC |
| 114 | B03 | CTGCGAGA | GCAGAGAC | GTCTCTGC | 138 | B06 | TCACTGTG | CATGTTCT | AGAACATG |
| 115 | C03 | CAACCAAC | CTGCGAGA | TCTCGCAG | 139 | C06 | ATTGAGCT | TCACTGTG | CACAGTGA |
| 116 | D03 | ATCATGCG | CAACCAAC | GTTGGTTG | 140 | D06 | GATAGAGA | ATTGAGCT | AGCTCAAT |
| 117 | E03 | TCTGAGTC | ATCATGCG | CGCATGAT | 141 | E06 | TCTAGAGC | GATAGAGA | TCTCTATC |
| 118 | F03 | TCGCCTGT | TCTGAGTC | GACTCAGA | 142 | F06 | GAATCGCA | TCTAGAGC | GCTCTAGA |
| 119 | G03 | GCGCAATT | TCGCCTGT | ACAGGCGA | 143 | G06 | CTTCACGT | GAATCGCA | TGCGATTC |
| 120 | H03 | AGACGCCT | GCGCAATT | AATTGCGC | 144 | H06 | CTCCGGTT | CTTCACGT | ACGTGAAG |

**Table 26** SureSelect Max Index Primer Pairs 145–192, provided in blue 96-well plate

| Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement | Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement |
|---|---|---|---|---|---|---|---|---|---|
| 145 | A07 | TGTGACTA | CTCCGGTT | AACCGGAG | 169 | A10 | CGCTCAGA | CTAACAAG | CTTGTTAG |
| 146 | B07 | GCTTCCAG | TGTGACTA | TAGTCACA | 170 | B10 | TAACGACA | CGCTCAGA | TCTGAGCG |
| 147 | C07 | CATCCTGT | GCTTCCAG | CTGGAAGC | 171 | C10 | CATACTTG | TAACGACA | TGTCGTTA |
| 148 | D07 | GTAATACG | CATCCTGT | ACAGGATG | 172 | D10 | AGATACGA | CATACTTG | CAAGTATG |
| 149 | E07 | GCCAACAA | GTAATACG | CGTATTAC | 173 | E10 | AATCCGAC | AGATACGA | TCGTATCT |
| 150 | F07 | CATGACAC | GCCAACAA | TTGTTGGC | 174 | F10 | TGAAGTAC | AATCCGAC | GTCGGATT |
| 151 | G07 | TGCAATGC | CATGACAC | GTGTCATG | 175 | G10 | CGAATCAT | TGAAGTAC | GTACTTCA |
| 152 | H07 | CACATTCG | TGCAATGC | GCATTGCA | 176 | H10 | TGATTGGC | CGAATCAT | ATGATTCG |
| 153 | A08 | CAATCCGA | CACATTCG | CGAATGTG | 177 | A11 | TCGAAGGA | TGATTGGC | GCCAATCA |
| 154 | B08 | CATCGACG | CAATCCGA | TCGGATTG | 178 | B11 | CAGTCATT | TCGAAGGA | TCCTTCGA |
| 155 | C08 | GTGCGCTT | CATCGACG | CGTCGATG | 179 | C11 | CGCGAACA | CAGTCATT | AATGACTG |
| 156 | D08 | ATAGCGTT | GTGCGCTT | AAGCGCAC | 180 | D11 | TACGGTTG | CGCGAACA | TGTTCGCG |
| 157 | E08 | GAGTAAGA | ATAGCGTT | AACGCTAT | 181 | E11 | AGAACCGT | TACGGTTG | CAACCGTA |
| 158 | F08 | CTGACACA | GAGTAAGA | TCTTACTC | 182 | F11 | AGGTGCTT | AGAACCGT | ACGGTTCT |
| 159 | G08 | ATACGTGT | CTGACACA | TGTGTCAG | 183 | G11 | ATCGCAAC | AGGTGCTT | AAGCACCT |
| 160 | H08 | GACCGAGT | ATACGTGT | ACACGTAT | 184 | H11 | GCCTCTCA | ATCGCAAC | GTTGCGAT |
| 161 | A09 | GCAGTTAG | GACCGAGT | ACTCGGTC | 185 | A12 | TCGCGTCA | GCCTCTCA | TGAGAGGC |
| 162 | B09 | CGTTCGTC | GCAGTTAG | CTAACTGC | 186 | B12 | GAGTGCGT | TCGCGTCA | TGACGCGA |
| 163 | C09 | CGTTAACG | CGTTCGTC | GACGAACG | 187 | C12 | CGAACACT | GCATAAGT | ACTTATGC |
| 164 | D09 | TCGAGCAT | CGTTAACG | CGTTAACG | 188 | D12 | TAAGAGTG | AGAAGACG | CGTCTTCT |
| 165 | E09 | GCCGTAAC | TCGAGCAT | ATGCTCGA | 189 | E12 | TGGATTGA | TAAGAGTG | CACTCTTA |
| 166 | F09 | GAGCTGTA | GCCGTAAC | GTTACGGC | 190 | F12 | AGGACATA | TGGATTGA | TCAATCCA |
| 167 | G09 | AGGAAGAT | GAGCTGTA | TACAGCTC | 191 | G12 | GACATCCT | AGGACATA | TATGTCCT |
| 168 | H09 | CTAACAAG | AGGAAGAT | ATCTTCCT | 192 | H12 | GAAGCCTC | GACATCCT | AGGATGTC |

**Table 27** SureSelect Max Index Primer Pairs 193–240, provided in green 96-well plate

| Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement | Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement |
|---|---|---|---|---|---|---|---|---|---|
| 193 | A01 | GTCTCTTC | GAAGCCTC | GAGGCTTC | 217 | A04 | GCGGTATG | CACGAGCT | AGCTCGTG |
| 194 | B01 | AGTCACTT | GTCTCTTC | GAAGAGAC | 218 | B04 | TCTATGCG | GCGGTATG | CATACCGC |
| 195 | C01 | AGCATACA | AGTCACTT | AAGTGACT | 219 | C04 | AGGTGAGA | TCTATGCG | CGCATAGA |
| 196 | D01 | TCAGACAA | AGCATACA | TGTATGCT | 220 | D04 | CACAACTT | AGGTGAGA | TCTCACCT |
| 197 | E01 | TTGGAGAA | TCAGACAA | TTGTCTGA | 221 | E04 | TTGTGTAC | CACAACTT | AAGTTGTG |
| 198 | F01 | TTAACGTG | TTGGAGAA | TTCTCCAA | 222 | F04 | TCACAAGA | TTGTGTAC | GTACACAA |
| 199 | G01 | CGTCTGTG | TTAACGTG | CACGTTAA | 223 | G04 | GAAGACCT | TCACAAGA | TCTTGTGA |
| 200 | H01 | AACCTAAC | CGTCTGTG | CACAGACG | 224 | H04 | AGTTCTGT | GAAGACCT | AGGTCTTC |
| 201 | A02 | AGAGTGCT | AACCTAAC | GTTAGGTT | 225 | A05 | GCAGTGTT | AGTTCTGT | ACAGAACT |
| 202 | B02 | TTATCTCG | AGAGTGCT | AGCACTCT | 226 | B05 | AGGCATGC | GCAGTGTT | AACACTGC |
| 203 | C02 | CATCAGTC | TTATCTCG | CGAGATAA | 227 | C05 | AAGGTACT | AGGCATGC | GCATGCCT |
| 204 | D02 | AAGCACAA | CATCAGTC | GACTGATG | 228 | D05 | CACTAAGT | AAGGTACT | AGTACCTT |
| 205 | E02 | CAGTGAGC | AAGCACAA | TTGTGCTT | 229 | E05 | GAGTCCTA | CACTAAGT | ACTTAGTG |
| 206 | F02 | GTCGAAGT | CAGTGAGC | GCTCACTG | 230 | F05 | AGTCCTTC | GAGTCCTA | TAGGACTC |
| 207 | G02 | TCTCATGC | GTCGAAGT | ACTTCGAC | 231 | G05 | TTAGGAAC | AGTCCTTC | GAAGGACT |
| 208 | H02 | CAGAAGAA | TCTCATGC | GCATGAGA | 232 | H05 | AAGTCCAT | TTAGGAAC | GTTCCTAA |
| 209 | A03 | CGGATAGT | CAGAAGAA | TTCTTCTG | 233 | A06 | GAATACGC | AAGTCCAT | ATGGACTT |
| 210 | B03 | CACGTGAG | CGGATAGT | ACTATCCG | 234 | B06 | TCCAATCA | GAATACGC | GCGTATTC |
| 211 | C03 | TACGATAC | CACGTGAG | CTCACGTG | 235 | C06 | CGACGGTA | TCCAATCA | TGATTGGA |
| 212 | D03 | CGCATGCT | TACGATAC | GTATCGTA | 236 | D06 | CATTGCAT | CGACGGTA | TACCGTCG |
| 213 | E03 | GCTTGCTA | CGCATGCT | AGCATGCG | 237 | E06 | ATCTGCGT | CATTGCAT | ATGCAATG |
| 214 | F03 | GAACGCAA | GCTTGCTA | TAGCAAGC | 238 | F06 | GTACCTTG | ATCTGCGT | ACGCAGAT |
| 215 | G03 | ATCTACCA | GAACGCAA | TTGCGTTC | 239 | G06 | GAGCATAC | GTACCTTG | CAAGGTAC |
| 216 | H03 | CACGAGCT | ATCTACCA | TGGTAGAT | 240 | H06 | TGCTTACG | GAGCATAC | GTATGCTC |

**Table 28** SureSelect Max Index Primer Pairs 241–288, provided in green 96-well plate

| Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement | Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement |
|---|---|---|---|---|---|---|---|---|---|
| 241 | A07 | AAGAGACA | TGCTTACG | CGTAAGCA | 265 | A10 | CAATGCTG | CATGAATG | CATTCATG |
| 242 | B07 | TAGCTATG | AAGAGACA | TGTCTCTT | 266 | B10 | CTTGATCA | CAATGCTG | CAGCATTG |
| 243 | C07 | TCTGCTAC | TAGCTATG | CATAGCTA | 267 | C10 | GCGAATTA | CTTGATCA | TGATCAAG |
| 244 | D07 | GTCACAGA | TCTGCTAC | GTAGCAGA | 268 | D10 | GTTCGAGC | GCGAATTA | TAATTCGC |
| 245 | E07 | CGATTGAA | GTCACAGA | TCTGTGAC | 269 | E10 | GCCAGTAG | GTTCGAGC | GCTCGAAC |
| 246 | F07 | GAGAGATT | CGATTGAA | TTCAATCG | 270 | F10 | AAGGTCGA | GCCAGTAG | CTACTGGC |
| 247 | G07 | TCATACCG | GAGAGATT | AATCTCTC | 271 | G10 | AGTGAAGT | CACTTATG | CATAAGTG |
| 248 | H07 | TCCGAACT | TCATACCG | CGGTATGA | 272 | H10 | GTTGCAAG | ATAACGGC | GCCGTTAT |
| 249 | A08 | AGAGAGAA | TCCGAACT | AGTTCGGA | 273 | A11 | AGCCGGAA | GTTGCAAG | CTTGCAAC |
| 250 | B08 | GATCGTTA | AGAGAGAA | TTCTCTCT | 274 | B11 | AACAGCCG | AGCCGGAA | TTCCGGCT |
| 251 | C08 | GCGCTAGA | GATCGTTA | TAACGATC | 275 | C11 | CTAGTGTA | AACAGCCG | CGGCTGTT |
| 252 | D08 | ATGACTCG | GCGCTAGA | TCTAGCGC | 276 | D11 | GAGGCTCT | CTAGTGTA | TACACTAG |
| 253 | E08 | CAATAGAC | ATGACTCG | CGAGTCAT | 277 | E11 | CTCCGCAA | GAGGCTCT | AGAGCCTC |
| 254 | F08 | CGATATGC | CAATAGAC | GTCTATTG | 278 | F11 | CGCTATTG | CTCCGCAA | TTGCGGAG |
| 255 | G08 | GTCAGAAT | CGATATGC | GCATATCG | 279 | G11 | GTGTTGAG | CGCTATTG | CAATAGCG |
| 256 | H08 | CATAAGGT | GCACTACT | AGTAGTGC | 280 | H11 | TCACCGAC | GTGTTGAG | CTCAACAC |
| 257 | A09 | TGTTGGTT | GATTCGGC | GCCGAATC | 281 | A12 | CGGTAATC | TCACCGAC | GTCGGTGA |
| 258 | B09 | ATACTCGC | TGTTGGTT | AACCAACA | 282 | B12 | GTGACTGC | CGGTAATC | GATTACCG |
| 259 | C09 | AATGCTAG | ATACTCGC | GCGAGTAT | 283 | C12 | CGACTTGT | GTGACTGC | GCAGTCAC |
| 260 | D09 | GCCTAGGA | AATGCTAG | CTAGCATT | 284 | D12 | GATAGGAC | CGACTTGT | ACAAGTCG |
| 261 | E09 | GCAACCGA | GCCTAGGA | TCCTAGGC | 285 | E12 | AAGTACTC | GATAGGAC | GTCCTATC |
| 262 | F09 | ATACTGCA | GCAACCGA | TCGGTTGC | 286 | F12 | GCTCTCTC | AAGTACTC | GAGTACTT |
| 263 | G09 | TCTCCTTG | ATACTGCA | TGCAGTAT | 287 | G12 | CTACCAGT | GCTCTCTC | GAGAGAGC |
| 264 | H09 | CATGAATG | TCTCCTTG | CAAGGAGA | 288 | H12 | GATGAGAT | CTACCAGT | ACTGGTAG |

**Table 29** SureSelect Max Index Primer Pairs 289–336, provided in red 96-well plate

| Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement | Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement |
|---|---|---|---|---|---|---|---|---|---|
| 289 | A01 | AGATAGTG | GATGAGAT | ATCTCATC | 313 | A04 | AGCTACAT | GATCCATG | CATGGATC |
| 290 | B01 | AGAGGTTA | AGATAGTG | CACTATCT | 314 | B04 | CGCTGTAA | AGCTACAT | ATGTAGCT |
| 291 | C01 | CTGACCGT | AGAGGTTA | TAACCTCT | 315 | C04 | CACTACCG | CGCTGTAA | TTACAGCG |
| 292 | D01 | GCATGGAG | CTGACCGT | ACGGTCAG | 316 | D04 | GCTCACGA | CACTACCG | CGGTAGTG |
| 293 | E01 | CTGCCTTA | GCATGGAG | CTCCATGC | 317 | E04 | TGGCTTAG | GCTCACGA | TCGTGAGC |
| 294 | F01 | GCGTCACT | CTGCCTTA | TAAGGCAG | 318 | F04 | TCCAGACG | TGGCTTAG | CTAAGCCA |
| 295 | G01 | GCGATTAC | GCGTCACT | AGTGACGC | 319 | G04 | AGTGGCAT | TCCAGACG | CGTCTGGA |
| 296 | H01 | TCACCACG | GCGATTAC | GTAATCGC | 320 | H04 | TGTACCGA | AGTGGCAT | ATGCCACT |
| 297 | A02 | AGACCTGA | TCACCACG | CGTGGTGA | 321 | A05 | AAGACTAC | TGTACCGA | TCGGTACA |
| 298 | B02 | GCCGATAT | AGACCTGA | TCAGGTCT | 322 | B05 | TGCCGTTA | AAGACTAC | GTAGTCTT |
| 299 | C02 | CTTATTGC | GCCGATAT | ATATCGGC | 323 | C05 | TTGGATCT | TGCCGTTA | TAACGGCA |
| 300 | D02 | CGATACCT | CTTATTGC | GCAATAAG | 324 | D05 | TCCTCCAA | TTGGATCT | AGATCCAA |
| 301 | E02 | CTCGACAT | CGATACCT | AGGTATCG | 325 | E05 | CGAGTCGA | TCCTCCAA | TTGGAGGA |
| 302 | F02 | GAGATCGC | CTCGACAT | ATGTCGAG | 326 | F05 | AGGCTCAT | CGAGTCGA | TCGACTCG |
| 303 | G02 | CGGTCTCT | GAGATCGC | GCGATCTC | 327 | G05 | GACGTGCA | AGGCTCAT | ATGAGCCT |
| 304 | H02 | TAACTCAC | CGGTCTCT | AGAGACCG | 328 | H05 | GAACATGT | GACGTGCA | TGCACGTC |
| 305 | A03 | CACAATGA | TAACTCAC | GTGAGTTA | 329 | A06 | AATTGGCA | GAACATGT | ACATGTTC |
| 306 | B03 | GACTGACG | CACAATGA | TCATTGTG | 330 | B06 | TGGAGACT | AATTGGCA | TGCCAATT |
| 307 | C03 | CTTAAGAC | GACTGACG | CGTCAGTC | 331 | C06 | AACTCACA | TGGAGACT | AGTCTCCA |
| 308 | D03 | GAGTGTAG | CTTAAGAC | GTCTTAAG | 332 | D06 | GTAGACTG | AACTCACA | TGTGAGTT |
| 309 | E03 | TGCACATC | GAGTGTAG | CTACACTC | 333 | E06 | CGTAGTTA | GTAGACTG | CAGTCTAC |
| 310 | F03 | CGATGTCG | TGCACATC | GATGTGCA | 334 | F06 | CGTCAGAT | CGTAGTTA | TAACTACG |
| 311 | G03 | AACACCGA | CGATGTCG | CGACATCG | 335 | G06 | AACGGTCA | CGTCAGAT | ATCTGACG |
| 312 | H03 | GATCCATG | AACACCGA | TCGGTGTT | 336 | H06 | GCCTTCAT | AACGGTCA | TGACCGTT |

**Table 30** SureSelect Max Index Primer Pairs 337–384, provided in red 96-well plate

| Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement | Primer Pair # | Well | P7 Index Forward | P5 Index Forward | P5 Index Reverse Complement |
|---|---|---|---|---|---|---|---|---|---|
| 337 | A07 | TGAGACGC | GCCTTCAT | ATGAAGGC | 361 | A10 | CTGAGCTA | GCACAGTA | TACTGTGC |
| 338 | B07 | CATCGGAA | TGAGACGC | GCGTCTCA | 362 | B10 | CTTGCGAT | CTGAGCTA | TAGCTCAG |
| 339 | C07 | TAGGACAT | CATCGGAA | TTCCGATG | 363 | C10 | GAAGTAGT | CTTGCGAT | ATCGCAAG |
| 340 | D07 | AACACAAG | TAGGACAT | ATGTCCTA | 364 | D10 | GTTATCGA | GAAGTAGT | ACTACTTC |
| 341 | E07 | TTCGACTC | AACACAAG | CTTGTGTT | 365 | E10 | TGTCGTCG | GTTATCGA | TCGATAAC |
| 342 | F07 | GTCGGTAA | TTCGACTC | GAGTCGAA | 366 | F10 | CGTAACTG | TGTCGTCG | CGACGACA |
| 343 | G07 | GTTCATTC | GTCGGTAA | TTACCGAC | 367 | G10 | GCATGCCT | CGTAACTG | CAGTTACG |
| 344 | H07 | AAGCAGTT | GTTCATTC | GAATGAAC | 368 | H10 | TCGTACAC | GCATGCCT | AGGCATGC |
| 345 | A08 | ATAAGCTG | AAGCAGTT | AACTGCTT | 369 | A11 | CACAGGTG | TCGTACAC | GTGTACGA |
| 346 | B08 | GCTTAGCG | ATAAGCTG | CAGCTTAT | 370 | B11 | AGCAGTGA | CACAGGTG | CACCTGTG |
| 347 | C08 | TTCCAACA | GCTTAGCG | CGCTAAGC | 371 | C11 | ATTCCAGA | AGCAGTGA | TCACTGCT |
| 348 | D08 | TACCGCAT | TTCCAACA | TGTTGGAA | 372 | D11 | TCCTTGAG | ATTCCAGA | TCTGGAAT |
| 349 | E08 | AGGCAATG | TACCGCAT | ATGCGGTA | 373 | E11 | ATACCTAC | TCCTTGAG | CTCAAGGA |
| 350 | F08 | GCCTCGTT | AGGCAATG | CATTGCCT | 374 | F11 | AGACCATT | ATACCTAC | GTAGGTAT |
| 351 | G08 | CACGGATC | GCCTCGTT | AACGAGGC | 375 | G11 | CGTAAGCA | AGACCATT | AATGGTCT |
| 352 | H08 | GAGACACG | CACGGATC | GATCCGTG | 376 | H11 | TCTGTCAG | CGTAAGCA | TGCTTACG |
| 353 | A09 | AGAGTAAG | GAGACACG | CGTGTCTC | 377 | A12 | CACAGACT | TCTGTCAG | CTGACAGA |
| 354 | B09 | AGTACGTT | AGAGTAAG | CTTACTCT | 378 | B12 | GTCGCCTA | CACAGACT | AGTCTGTG |
| 355 | C09 | AACGCTGC | AGTACGTT | AACGTACT | 379 | C12 | TGCGCTCT | GTCGCCTA | TAGGCGAC |
| 356 | D09 | GTAGAGCA | AACGCTGC | GCAGCGTT | 380 | D12 | GCTATAAG | TGCGCTCT | AGAGCGCA |
| 357 | E09 | TCCTGAGA | GTAGAGCA | TGCTCTAC | 381 | E12 | CAACAACT | GCTATAAG | CTTATAGC |
| 358 | F09 | CTGAATAG | TCCTGAGA | TCTCAGGA | 382 | F12 | AGAGAATC | CTCTCACT | AGTGAGAG |
| 359 | G09 | CAAGACTA | CTGAATAG | CTATTCAG | 383 | G12 | TAATGGTC | AGACGAGC | GCTCGTCT |
| 360 | H09 | GCACAGTA | CAAGACTA | TAGTCTTG | 384 | H12 | GTTGTATC | TAATGGTC | GACCATTA |

# 4
# Reference



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 31. Detailed contents of each of the multi-part component kits are shown in Table 32 through Table 34.

**Table 31** 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 ILM (G9699A/G9699B)** | 5282-0136 | 5282-0137 | –20°C |
| **SureSelect Max Purification Beads (G9962A/G9962B)** | 5282-0225 | 5282-0226 | +4°C |

## Component Kit Details

**Table 32** 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 33** 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 34** SureSelect Max Blockers and Primers Module for ILM content

| Kit Component | 16 Hyb Kit (p/n 5282-0136) | 96 Hyb Kit (p/n 5282-0137) |
|---|---|---|
| Blocker Mix, ILM | tube with blue cap | tube with blue cap |
| SureSelect 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 or 1.6 µg of pooled library cDNA, 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 or cDNA given in Table 7 on page 15. 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 23 to page 24, 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 17, 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 23). 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 See page 23.).

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

✓Libraries prepared from FFPE DNA or RNA 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 or RNA 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 23.

### 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 70°C before use (see page 20). 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 20)

✓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 to step 7 on page 18).

### If low uniformity of coverage with high AT-dropout is observed in library sequencing results

✓High AT-dropout may indicate that hybridization conditions are too stringent to obtain the desired level of coverage for AT-rich targets.

- For libraries target-enriched using SureSelect XT HS Human All Exon V8-based probes or SureSelect XT HS Clinical Research Exome V4 using the hybridization program in Table 8 on page 16 (including segment with one-hour incubation at 68°C), repeat target enrichment without the one-hour incubation at 68°C segment.
- For other probes, repeat target enrichment at lower stringency using a modified thermal cycler program for hybridization, reducing the hybridization temperature in segments 4 and 5 from 68°C to 65°C or 60°C (see Table 9 on page 16).

✓Make sure custom probes used in the Fast Hyb protocol are designed in SureDesign for HS/90-min Hyb workflows and not for XT/Overnight Hyb workflows. Before use in the Fast Hyb protocol, custom Overnight Hyb designs should be redesigned using SureDesign's XT HS/90-min Hyb parameters.

### If your libraries are MBC-tagged but your sequence analysis pipeline excludes MBCs

✓You can remove the first 5 bases from Read 1 and Read 2 by masking or trimming before proceeding to downstream analysis using one of the options below:

- If demultiplexing using bcl2fastq, MBCs may be masked by including the base mask **N5Y*,I8,I8,N5Y*.** If demultiplexing using BCL Convert, MBCs may be trimmed by including the following string in the sample sheet header: **OverrideCycles,N5Y*;I8;I8;N5Y*.** For both methods, * is replaced with value equal to the remaining read length after masking or trimming. For example, use **N5Y146,I8,I8,N5Y146** for 2x150 NGS with 151 cycles (as shown in Table 21 on page 29). The sum of the values following N and Y must match the read length value in the RunInfo.xml file.
- The first 5 bases may be trimmed from the demultiplexed FASTQ files using the AGeNT Trimmer module while trimming adaptor sequences or using a suitable processing tool of your choice, such as seqtk. Non-Agilent adaptor trimmer performance should be verified for removal of the MBC sequences from the opposite adaptor (refer to Figure 4 on page 28); failure to remove MBC sequences from both strands may affect alignment quality.

### If the procedure for capturing the hybridized libraries must be shortened

✓The recommended process for capturing hybridized libraries (described in "Step 4. Capture the hybridized libraries" on page 19) calls for 6 washes with SureSelect Wash Buffer 2. You may get satisfactory capture using the alternative quick wash capture described below. Validation and optimization of the shortened procedure may be required for your specific Capture Probe Library.

### Modifications for quick wash capture of hybridized libraries

You can execute a shortened procedure for capture of the hybridized libraries by making the protocol modifications described in Table 35.

**Table 35** Modifications needed for simplified capture of hybridized libraries

| Step to be modified | Original procedure | Modified procedure |
|---|---|---|
| step 1 on page 19 | Prepare 6 wells of Wash Buffer 2 for each sample (200 µL/well). | Prepare 3 wells of Wash Buffer 2 for each sample (200 µL/well) AND 1 well of Wash Buffer 1 for each sample (200 µL/well). Make sure to clearly label the tubes of Wash Buffer 1 and Wash Buffer 2. |
| step 3 on page 20 | Pre-heat the aliquots of Wash Buffer 2 in a 70°C thermal cycler. | Pre-warm the aliquots of Wash Buffer 2 AND the aliquots of Wash Buffer 1 in a 70°C thermal cycler. |
| step 6 on page 20 | Resuspend the beads in 200 µL of room temperature Wash Buffer 1.<br>Close the strip tubes using the previously used tube cap strip.<br>After mixing, return the sample strip to the magnetic separator as described in step 7 on page 20. | Resuspend the beads in 200 µL of Wash Buffer 1 that was pre-warmed to 70°C.<br>Close the strip tubes using the previously used tube cap strip.<br>After mixing, vortex for 8 seconds, spin down briefly, then incubate in the 70°C thermal cycler for 5 minutes. After the 5-minute incubation, return the sample strip to the magnetic separator as described in step 7 on page 20. |
| step 8 on page 20 | Repeat the wash with Wash Buffer 2 for a total of 6 washes. | Repeat the wash with Wash Buffer 2 for a total of 3 washes.<br>The wash steps described in step a through step d do not require modification, only the number of washes is changed (from 6 to 3). |

# Quick Reference Protocol: Max Fast Hyb Target Enrichment

An abbreviated protocol summary is provided below for experienced users. Use the complete protocol on page 13 to page 24 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 in 12 µL of nuclease-free $H_2O$ into strip tube wells> keep on ice.<br>**For post-cap pooling:** Use 12 µL of undiluted prepared library samples or use at least 500 ng gDNA library or 200 ng cDNA library per well.<br>**For pre-cap pooling:** Pool into wells according to Table 7 on page 15. 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 36 (next page). Pre-warm before loading samples. |
| Run pre-hybridization blocking protocol | Add 5 µL Blocker Mix ILM to each sample well; mix> spin> place strip in cycler.<br>Run paused 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 37 (next page).<br>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.<br>Seal wells completely> vortex briefly> spin briefly> return strip to cycler. Resume cycler program, completing the remaining hybridization segment(s) and final 68°C Hold.<br>Prep capture reagents during hybridization (unless using two-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 | For each sample, wash 50 µL SureSelect Streptavidin beads 3× in 200 µL SureSelect Binding Buffer.<br>After final wash, resuspend beads in 200 µL SureSelect Binding Buffer.<br>Pre-warm beads/buffer in thermal cycler at 68°C for last 10 minutes of hybridization. |
| Capture hybridized libraries | Add hybridized samples (~30 µL) to pre-warmed beads (200 µL) held in cycler> mix well by pipetting.<br>Incubate capture strip in thermal cycler at 68°C for 10 minutes.<br>During incubation, pre-warm the 6 × 200 µL aliquots of SureSelect Wash Buffer 2 to 70°C. |
| Wash captured libraries | Spin capture strip briefly> collect beads using a magnetic stand> discard supernatant.<br>Wash beads 1× with 200 µL SureSelect Wash Buffer 1 at RT.<br>Wash beads 6× with 200 µL pre-warmed SureSelect Wash Buffer 2 (for each wash: add 200 µL 70°C Wash Buffer 2> mix well by pipetting and vortexing at RT> incubate 5 minutes at 70°C> collect beads at RT> discard supernatant).<br>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 38 (next page). Pre-warm before loading samples. |
| Prepare Post-capture PCR Mix | **Per 8 Hyb reactions:** 225 µL Amplification Master Mix + 9 µL SureSelect Post-Capture Primer Mix<br>**Per 24 reactions:** 650 µL Amplification Master Mix + 26 µL SureSelect Post-Capture Primer Mix<br>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. |

| Step | Summary of Conditions |
|---|---|
| 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.<br>Elute in 25 µL Low TE Buffer> mix> incubate 2-5 minutes> collect beads> retain supernatant. |
| Quantify and qualify libraries | Analyze quantity and quality using TapeStation or Fragment Analyzer System. |

**Table 36** Hybridization program (30 µL vol; heated lid at 105°C)

| Segment | Probe | Cycles | Temperature (Purpose) | Time | Two-day Workflow Adjustments |
|---|---|---|---|---|---|
| 1 | All probes | 1 | 95°C (Pre-blocking) | 5 minutes | — |
| 2 | All probes | 1 | 65°C (Blocking) | 10 minutes | |
| 3 | All probes | 1 | 65°C (Hyb reagent addition hold) | Hold | — |
| 4 | All probes | 60 | 68°C (Hyb cycles) | 1 minute | Do not start streptavidin bead and capture reagent prep steps during Hyb. Begin at start of day 2. |
| | | | 37°C (Hyb cycles) | 3 seconds | |
| 5 | SureSelect XT HS Human All Exon V7/V8/V8+UTR/V8+NCV or Clinical Research Exome V4 | 1 | 68°C (Extended hyb incubation- include for listed probes only) | 60 minutes | — |
| | | | 68°C (Hold for capture start) | Hold **briefly** | 21°C Hold for up to 16 hours (overnight pause) |
| | All other XT HS probes | 1 | 68°C (Hold for capture start) | Hold **briefly** | 21°C Hold for up to 16 hours (overnight pause) |

**Table 37** Preparation of Probe/Hyb Mix

| | Probe designs ≥3 Mb | | | Probe designs<3 Mb | | |
|---|---|---|---|---|---|---|
| **Reagent** | **Per Hyb Reaction** | **8 Hybs** | **24 Hybs** | **Per Hyb Reaction** | **8 Hybs** | **24 Hybs** |
| Nuclease-free water | 1.5 µL | 13.5 µL | 37.5 µL | 4.5 µL | 40.5 µL | 112.5 µL |
| SureSelect RNase Block (purple cap) | 0.5 µL | 4.5 µL | 12.5 µL | 0.5 µL | 4.5 µL | 12.5 µL |
| SureSelect Max Fast Hyb Buffer | 6 µL | 54 µL | 150 µL | 6 µL | 54 µL | 150 µL |
| Probe | 5 µL | 45 µL | 125 µL | 2 µL | 18 µL | 50 µL |
| Total | 13 µL | 117 µL | 325 µL | 13 µL | 117 µL | 325 µL |

**Table 38** 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 | gDNA libraries (DNA input):<br>Probes <0.2 Mb: 16 cycles<br>Probes 0.2–3 Mb: 12–16 cycles<br>Probes 3–5 Mb: 11–12 cycles<br>Probes >5 Mb: 10–11 cycles | **OR** | cDNA libraries (RNA input):<br>Probes <0.2 Mb: 16 cycles<br>Probes 0.2–3 Mb: 14 cycles<br>Probes 3–5 Mb: 13 cycles<br>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 Target Enrichment using a Fast Hybridization workflow including either post-capture or pre-capture pooling of NGS libraries.



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