SureSelect Target Enrichment System for Sequencing on Ion Proton

Protocol

Version B0, June 2015

SureSelect platform manufactured with Agilent SurePrint Technology

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

Before you begin, view hands-on videos of SureSelect procedures at http://www.agilent.com/genomics/protocolvideos.

Agilent Technologies

Notices

Manual Part Number

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For US and Canada, call (800) 227-9770 (option 3,4,4). For other countries, find your support center telephone numbers at www.agilent.com/chem/contactus.

Or send an e-mail to:
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Notice to Purchaser

SureSelect capture libraries and reagents must be used within one year of receipt.

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CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

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In this Guide...

This guide describes the recommended operational procedures to capture the genomic regions of interest using the Agilent SureSelect Target Enrichment system for sequencing on the Ion Proton System. This protocol is specifically developed and optimized to use biotinylated RNA oligomer libraries to enrich targeted regions of the genome from repetitive sequences and sequences unrelated to the research focus, specifically adjusted to provide high performance with the Ion Proton Sequencer.

This guide uses an optimized protocol for Ion Xpress Plus fragment library preparation.

1 Before You Begin

This chapter contains information (such as procedural notes, safety information, required reagents and equipment) that you should read and understand before you start an experiment.

2 Sample Preparation

This chapter describes the steps to prepare the DNA samples for target enrichment.

3 Hybridization

This chapter describes the steps to prepare and hybridize samples.

4 Post-Hybridization Amplification

This chapter describes the steps to amplify, purify, and assess quality of the sample libraries.

5 Reference

This chapter contains information on alternative equipment that can be used with this protocol.

What's New in Version B0

- Updated product labeling statement.

What's New in Version A5

- Support for SureSelect^{XT} Inherited Disease and SureSelect^{XT} Focused Exome capture libraries.

What's new in Version A4

- Support for SureSelect^{XT} Clinical Research Exome capture libraries.
- Added hybridization duration and temperature information.

What's new in Version A2/A3

- Updated Table 14, "Components for PCR mix," on page 27 to include PCR primer mix.

What's new in Version A1

- Updated part number for Target Enrichment kit modules.
- Updated names of SureSelect block and PCR primer mix.
- Updated Required Reagents list.

1 Before You Begin

Make sure you have the most current protocol. Go to genomics.agilent.com and search for G7530-90005.

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

NOTE This protocol differs from the Ion Xpress Plus Fragment Library Kit sequencing manual and other SureSelect protocols at several steps. Pay close attention to the primers used for each amplification step and the blocking agents used during hybridization.

NOTE Agilent cannot guarantee the use of the SureSelect Target Enrichment kits with non-Agilent protocols nor provide technical support for non-Agilent protocols to process samples for enrichment.

Procedural 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.
- Maintain a clean work area.
- Do not mix stock solutions and reactions containing gDNA on a vortex mixer. Instead, gently tap the tube with your finger to mix the sample.
- Avoid repeated freeze-thaw cycles of stock and diluted gDNA solutions.
- When preparing frozen reagent stock solutions for use:
  1. Thaw the aliquot as rapidly as possible without heating above room temperature.
  2. Mix briefly on a vortex mixer, then spin in a centrifuge for 5 to 10 seconds to drive the contents off of walls and lid.
  3. Store on ice or in a cold block until use.
- In general, follow Biosafety Level 1 (BL1) safety rules.

Safety Notes

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

Required Reagents

Table 1 Required Reagents for Library Prep and Post-Hybridization Amplification

Description	Vendor and part number
For use with 2100 Bioanalyzer:	
DNA 1000 Kit	Agilent p/n 5067-1504
High Sensitivity DNA Kit	Agilent p/n 5067-4626
Herculase II Fusion DNA Polymerase (includes dNTP mix and 5x Buffer)	Agilent
200 reactions	p/n 600677
400 reactions	p/n 600679
Nuclease-free Water (not DEPC-treated)	Ambion Cat #AM9930
Agencourt AMPure XP Kit	Beckman Coulter Genomics
5 mL	p/n A63880
60 mL	p/n A63881
450 mL	p/n A63882
1X Low TE Buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA)	Life Technologies p/n 12090-015 or equivalent
E-Gel SizeSelect 2% Agarose Gel	Life Technologies p/n G6610-02
Qubit dsDNA BR Assay Kit	
100 assays, 2-1000 ng	Life Technologies p/n Q32850
500 assays, 2-1000 ng	Life Technologies p/n Q32853
1000 assays, 2-1000 ng	Life Technologies p/n Q33130
Ion Xpress Plus Fragment Library Kit	Life Technologies p/n 4471269
Ion Xpress Barcode Adapters Kit	
1-16 kit	Life Technologies p/n 4471250
50-bp ladder	Life Technologies p/n 10416-014
Ethanol, 100% for molecular biology (for SPRI purifications)	Sigma-Aldrich p/n E7023

Table 2 Required Equipment and Reagents for Template Preparation and Sequencing

Description	Vendor and part number
Ion OneTouch 2 System	Life Technologies p/n 4474779
Ion PI Template OT2 200 Kit v2	Life Technologies p/n 4485146
Ion PI Sequencing 200 Kit v2	Life Technologies p/n 4485149

Table 3 SureSelect Reagent Kit*

Reagent Kits	16 Reactions	96 Reactions
SureSelect TE Reagent Kit, PTN	G9605A	G9605B

* SureSelect reagents must be used within one year of receipt.

Table 4 SureSelect Capture Library (select one)*

Capture Libraries	16 Reactions	96 Reactions	480 Reactions
SureSelect^{XT} Focused Exome	5190-7787	5190-7788	
SureSelect^{XT} Focused Exome Plus 1	5190-7790	5190-7791	
SureSelect^{XT} Clinical Research Exome	5190-7338	5190-7339	
SureSelect^{XT} Human All Exon V5	5190-6208	5190-6209	
SureSelect^{XT} Human All Exon V5+UTRs	5190-6213	5190-6214	
SureSelect^{XT} Human All Exon V5+lncRNA	5190-6446	5190-6447	
SureSelect^{XT} Human All Exon V5 Plus	5190-6211	5190-6212	
SureSelect^{XT} Human All Exon V4	5190-4631	5190-4632	5190-4634
SureSelect^{XT} Human All Exon V4+UTRs	5190-4636	5190-4637	5190-4639
SureSelect^{XT} Mouse All Exon	5190-4641	5190-4642	5190-4644
SureSelect^{XT} Inherited Disease	5190-7518	5190-7519	
SureSelect^{XT} Inherited Disease Plus	5190-7521	5190-7522	
SureSelect^{XT} Custom 1 kb up to 499 Kb (reorder)	5190-4806 5190-4811	5190-4807 5190-4812	5190-4809 5190-4814

Table 4 SureSelect Capture Library (select one)* (continued)

Capture Libraries	16 Reactions	96 Reactions	480 Reactions
SureSelect^{XT} Custom 0.5 Mb up to 2.9 Mb	5190-4816	5190-4817	5190-4819
(reorder)	5190-4821	5190-4822	5190-4824
SureSelect^{XT} Custom 3 Mb up to 5.9 Mb	5190-4826	5190-4827	5190-4829
(reorder)	5190-4831	5190-4832	5190-4834
SureSelect^{XT} Custom 6 Mb up to 11.9 Mb	5190-4836	5190-4837	5190-4839
(reorder)	5190-4841	5190-4842	5190-4844
SureSelect^{XT} Custom 12 Mb up to 24 Mb	5190-4896	5190-4897	5190-4899
(reorder)	5190-4901	5190-4902	5190-4904

* SureSelect capture libraries must be used within one year of receipt.

Table 5 Required Reagents for Hybridization

Description	Vendor and part number
Dynabeads MyOne Streptavidin T1	Life Technologies
2 mL	Cat #65601
10 mL	Cat #65602
100 mL	Cat #65603
Nuclease-free Water (not DEPC-treated)	Ambion Cat #AM9930

Optional Reagents

Table 6 Optional Reagents

Description	Vendor and part number
Ethylene glycol	American Bioanalytical p/n AB00455
Tween 20	Sigma-Aldrich p/n P9416-50ML

Required Equipment

Table 7 Required Equipment for Library Prep and Post-Hybridization Amplification

Description	Vendor and part number
2100 Bioanalyzer	Agilent p/n G2938C
Thermal cycler	Agilent SureCycler, Life Technologies Veriti Thermal Cycler, BioRad (MJ Research) DNA Engine PTC-200, or equivalent
Microcentrifuge	Eppendorf Microcentrifuge Model 5417C or equivalent
1.5-mL LoBind Tube	Eppendorf p/n 022431021 or equivalent
Qubit Fluorometer	Life Technologies p/n Q32857
E-Gel iBase and E-Gel Safe Imager Combo Kit or Safe Imager Real-Time Transilluminator *and* E-Gel iBase Power System	Life Technologies p/n G6465 Life Technologies p/n G6500 Life Technologies p/n G6400
Dynal DynaMag-2 magnetic stand	Life Technologies p/n 123-21D or equivalent
P10, P20, P200 and P1000 pipettes	Pipetman P10, P20, P200, P1000 or equivalent
Vacuum concentrator	Savant SpeedVac or equivalent
Ice bucket	
Powder-free gloves	
PCR tubes, strips, or plates	
Sterile, nuclease-free aerosol barrier pipette tips	
Timer	
Vortex mixer	
Heat block at 37°C	

Table 8 Required Equipment for Hybridization

Description	Vendor and part number
MicroAmp Clear Adhesive Film	Life Technologies p/n 4306311
BD Clay Adams Nutator Mixer	BD Diagnostics p/n 421105 or equivalent
Dynal DynaMag-2 magnetic stand	Life Technologies p/n 123-21D or equivalent
P10, P20, P200 and P1000 pipettes	Pipetman P10, P20, P200, P1000 or equivalent
Pipet-Light Multichannel Pipette, 12 channels	Rainin p/n L12-20 or equivalent
PCR tubes, strips, or plates	
Sterile, nuclease-free aerosol barrier pipette tips	
Thermal cycler	Agilent SureCycler, Life Technologies Veriti Thermal Cycler, BioRad (MJ Research) DNA Engine PTC-200, or equivalent
Timer	
Vortex mixer	
Water bath or heat block set to 65°C	

Optional Equipment

Table 9 Optional Equipment for Hybridization

Description	Vendor and part number
Tube-strip capping tool	Agilent p/n 410099
PlateLoc Thermal Microplate Sealer *and* Peelable Aluminum Seal	Agilent p/n G5402A *and* Agilent p/n 24210-001

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2 Sample Preparation

This section contains instructions for prepped library production specific to the Ion Proton sequencing platform. For each sample to be sequenced, individual library preparations, hybridizations, and captures are performed.

The steps in this section differ from the Ion Xpress protocol in the use of the Hercules II enzyme for amplification.

Refer to *Ion Xpress Plus Fragment Library Kit* (p/n 4471269) for more information.

NOTE Make sure genomic DNA samples are of high quality with an OD 260/280 ratio ranging from 1.8 to 2.0. Use the Qubit system to quantify genomic DNA before library preparation.

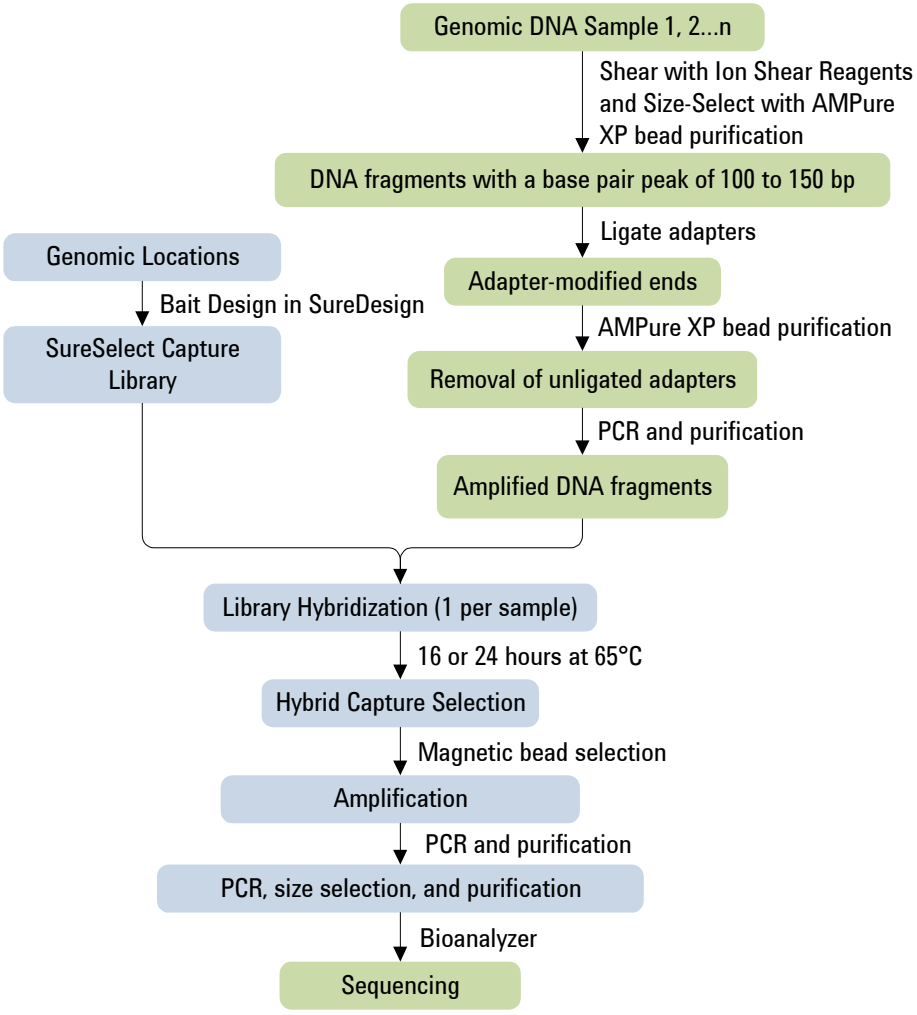

Figure 1 Overall sequencing sample preparation workflow.

Table 11 Overview and time requirements

Step	Time
Ion Xpress Plus Prepped library Production	1 day
Library Hybridization	17 to 25 hours
Bead preparation	30 minutes
Capture Selection and Washing	2 hours
Post-Hybridization Amplification	1 hour
PCR purification	30 minutes
E-gel size selection	30 minutes
Bioanalyzer QC	1 hour

Step 1. Fragment the DNA with Ion Shear Plus Reagents

For each DNA sample to be sequenced, prepare 1 library.

Use the reagents from the Ion Xpress Plus Fragment Library Kit (Life Technologies p/n 4471269).

1 Use the Qubit dsDNA BR Assay Kit to determine the concentration of your gDNA sample. Make sure the gDNA is of high quality (non-degraded, A_{260}/A_{280} is 1.8 to 2.0).

   Follow the instructions for the instrument.

2 Dilute gDNA in nuclease-free water to 100 ng/µL for 1 µg of gDNA.

CAUTION The final EDTA concentration must be < 0.1 mM in the gDNA dilution.

3 For each sample, combine the reagents in Table 12.

Table 12 Ion Shear reagents

Component	Volume
nuclease-free water	25 µL
diluted gDNA	10 µL
Ion Shear Plus 10x Reaction Buffer	5 µL
Total	**40 µL**

4 Add 10 µL of Ion Shear Plus Enzyme Mix to the reaction and mix well by pipetting.

CAUTION Do not create bubbles.

5 Incubate in a heat block or water bath at 37 °C for 50 minutes. Adjust incubation time between 50 to 60 minutes to optimize for your laboratory conditions for approximately 130 bp peaks.

6 Add 5 µL of Ion Shear Plus Stop Buffer immediately after incubation time, and mix thoroughly on a vortex mixer for 5 seconds.

7 Store the reaction tube on ice.

Step 2. Purify and size-select the sample using Agencourt AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes.
2. Mix the reagent well so that the reagent appears homogeneous and consistent in color.
3. Add 60.5 µL of homogeneous AMPure XP beads to a 1.5-mL LoBind Tube, and add the sheared DNA library (~55 µL). Mix well on a vortex mixer and incubate for 5 minutes.
4. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
5. Keep the tube in the magnetic stand. Remove approximately 110 uL of the cleared solution to a fresh 1.5-mL LoBind Tube. You can discard the beads at this point.
6. Add 38.5 µL of homogeneous AMPure XP beads to the collected solution. Mix well on a vertex mixer and incubate for 5 minutes.
7. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
8. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
9. Continue to keep the tube in the magnetic stand while you dispense 200 µL of 70% Ethanol in each tube.

   Use fresh 70% Ethanol for optimal result.
10. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the Ethanol.
11. Repeat step 9 and step 10 once.
12. Spin briefly in a centrifuge. Return the tube to the magnetic stand for 30 seconds. Remove residual Ethanol with a P20 pipette.
13. Dry the samples on the 37°C heat block for 5 minutes or until the residual Ethanol completely evaporates.

    Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
14. Add 27 µL nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.

15 Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.

16 Remove approximately 27 µL of the supernatant to a fresh 1.5-mL LoBind Tube. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, store the samples at -20°C.

Step 3. Assess quality with the 2100 Bioanalyzer

Quality assessment can be done with the 2100 Bioanalyzer instrument.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
2. Open the Agilent 2100 Expert Software (version B.02.02 or higher), turn on the 2100 Bioanalyzer and check communication.
3. Prepare the chip and ladder as instructed in the reagent kit guide. For 1 µg of input DNA, load 1 µL of the sheared DNA.
4. Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
5. Within the instrument context, choose the appropriate assay from the drop down list.
6. Start the run. Enter sample names and comments in the Data and Assay context.
7. Verify the results.

   Check that the electropherogram shows a distribution between 50 to 250 nucleotides. If the concentration is more than 15 ng/µL, adjust the concentration to 15 ng/µL and use 25 µL of size selected library in "Step 4. Ligate the adaptor".

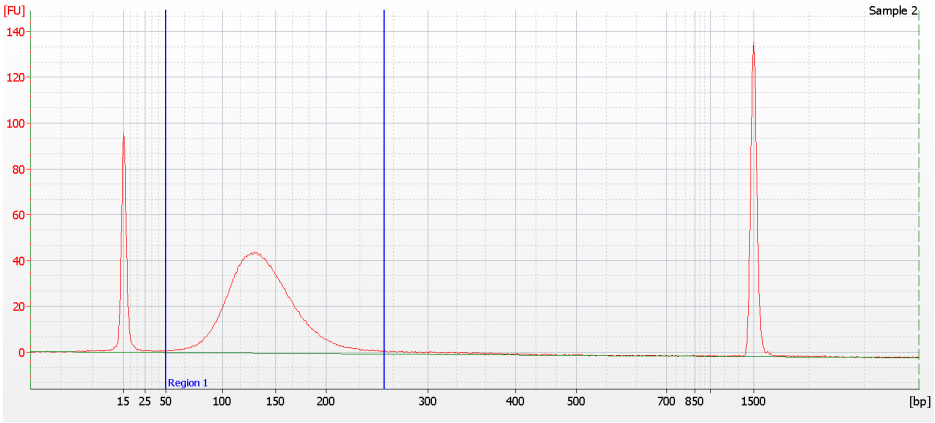

Figure 2 Analysis of sheared DNA using a DNA 1000 Bioanalyzer assay. The electropherogram shows a distribution between 50 to 250 nucleotides.

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

Step 4. Ligate the adaptor

Use the Ion Xpress Plus Fragment Library Kit (Life Technologies p/n 4471269) and the Ion Xpress Barcode Adapters Kit.

1 Prepare the reaction mix in Table 13 on ice.

2 Mix by pipetting. Change pipette tips between samples.

Table 13 Ligation master mix

Reagent	Volume for 1 reaction (µL)	Volume for 12 reactions (includes excess) (µL)
Nuclease-free Water	31	387.5
10x Ligase Buffer	10	125
Ion Xpress P1 Adapter*	10	125
Nick Repair Polymerase	8	100
DNA Ligase	4	50
dNTP Mix	2	25
Total Volume	**65**	**812.50**

* Included in the Ion Xpress Barcode Adapters Kit.

3 Divide the Ligation master mix into aliquots of 65 µL.

4 Add 10 µL of one of Ion Xpress Barcode 1 through 16 (from the Ion Xpress Barcode Adapters Kit) to each aliquot of Ligation master mix.

5 Add 25 µL of DNA sample to each mix.

6 Incubate in a thermal cycler. Do not use a heated lid.

- 25°C for 15 minutes
- 72°C for 5 minutes
- 4°C (hold)

If the heated lid of your thermal cycler cannot be turned off, incubate the sample with lid open.

7 Continue immediately to the next step.

Step 5. Purify the sample using Agencourt AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes.
2. Mix the reagent well so that the reagent appears homogeneous and consistent in color.
3. Add 180 µL of homogeneous AMPure XP beads to a 1.5-mL LoBind Tube, and add the ligated library (~100 µL). Mix well on a vortex mixer and incubate for 5 minutes.
4. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
5. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
6. Continue to keep the tube in the magnetic stand while you dispense 0.5 mL of 70% Ethanol in each tube.

   Use fresh 70% Ethanol for optimal result.
7. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the Ethanol.
8. Repeat step 6 and step 7 once.
9. Spin briefly in a centrifuge. Return the tube to the magnetic stand for 30 seconds. Remove residual Ethanol with a P20 pipette.
10. Dry the samples on the 37°C heat block for 5 minutes until the residual Ethanol is completely evaporated.

    Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
11. Add 50 µL of nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
12. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
13. Remove the supernatant (~50 µL) to a fresh 1.5-mL LoBind Tube. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, store the samples at -20°C.

Step 6. Amplify adaptor-ligated library

Use reagents from these kits:

- SureSelect Target Enrichment Kit PTN Hyb Module Box #2
- Herculase II Fusion DNA Polymerase (Agilent)

This protocol uses half of the adaptor-ligated fragments for amplification. The remainder can be saved at 20°C for future use, if needed.

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

1 Prepare reaction mix and DNA samples:

   a Prepare the reaction mix in Table 14, on ice. Mix well on a vortex mixer.

   b Add 25 µL of the reaction mix to each well or tube.

   c Add 25 µL of each DNA sample to each well or tube. Mix by pipetting. Change pipette tips between samples.

NOTE The optimal amount of adaptor-ligated library in the PCR is <200 ng.

Table 14 Components for PCR mix

Reagent	Volume for 1 reaction	Volume for 12 reactions (includes excess)
Nuclease-free water	11 µL	137.5 µL
5× Herculase II Rxn Buffer (clear cap)*	10 µL	125 µL
SureSelect PTN PCR primer mix (clear cap)†	2.5 µL	31.2 µL
Herculase II Fusion DNA Polymerase (red cap)*	1 µL	12.5 µL
100 mM dNTP Mix (green cap)*	0.5 µL	6.3 µL
Total	**25 µL**	**312.5 µL (25 µL/reaction)**

* Included in the Herculase II Fusion DNA Polymerase (Agilent) kit. *Do not use the buffer or dNTP mix from any other kit.*

† Included in the SureSelect Target Enrichment Kit PTN Hyb Module Box #2.

2 Run the program in Table 15 in a thermal cycler.

Table 15

Step	Temperature	Time
Step 1	98°C	2 minutes
Step 2	98°C	30 seconds
Step 3	60°C	10 seconds
Step 4	72°C	1 minute
Step 5		Repeat Step 2 through Step 4 for a total of 7 to 9 times.
Step 6	72°C	10 minutes
Step 7	4°C	Hold

NOTE

Different library preparations can produce slightly different results, based on varying DNA quality. In most cases, 8 cycles will produce an adequate yield for subsequent capture without introducing bias or non-specific products. If non-specific high molecular weight products are observed, consider dilution of the remaining extra library template or decrease the number of PCR cycles to prevent high molecular weight products. The small amount of high molecular weight PCR products around 400 bp does not have a significant impact on the capture performance.

As an alternative, you can prepare one PCR master mix as outlined in Table 14. Split the master mix into three small-scale 10 µL PCR reactions and run for 7, 8, or 9 cycles. Clean these PCR reactions using the AMPure XP protocol outlined in "Step 5. Purify the sample using Agencourt AMPure XP beads" with these modifications: Use 30 µL of AMPure XP beads and elute with 20 µL of nuclease-free water. Run these cleaned samples on the Bioanalyzer, as described in "Step 8. Assess quality and quantity with 2100 Bioanalyzer".

Use the optimal cycle number to repeat PCR at the 50 µL reaction scale.

Step 7. Purify the sample with Agencourt AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes.
2. Mix the reagent well so that the reagent appears homogeneous and consistent in color. *Do not freeze*.
3. Add 90 µL of homogeneous AMPure XP beads to a 1.5-mL LoBind Tube, and add the amplified library (~50 µL). Mix well on a vortex mixer and incubate for 5 minutes.
4. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
5. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
6. Continue to keep the tube in the magnetic stand while you dispense 500 µL of 70% Ethanol in each tube.

   Use fresh 70% Ethanol for optimal result.
7. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the Ethanol.
8. Repeat step 6 and step 7 once.
9. Spin briefly in a centrifuge. Return the tube to the magnetic stand for 30 seconds. Remove residual Ethanol with a P20 pipette.
10. Dry the samples on the 37°C heat block for 5 minutes or until the residual Ethanol completely evaporates.

    Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
11. Add 25 µL nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
12. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
13. Remove the supernatant (~25 µL) to a fresh 1.5-mL LoBind Tube. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, store the samples at -20°C.

Step 8. Assess quality and quantity with 2100 Bioanalyzer

Quality assessment can be done with the 2100 Bioanalyzer instrument.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
2. Open the Agilent 2100 Expert Software (version B.02.02 or higher), turn on the 2100 Bioanalyzer and check communication.
3. Prepare the chip, samples and ladder as instructed in the reagent kit guide.
4. Load the 1 µL of the amplified DNA.
5. Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
6. Within the instrument context, choose the appropriate assay from the drop down list.
7. Start the run. Enter sample names and comments in the Data and Assay context.
8. Verify the results. Check that the electropherogram shows a distribution with a peak size at approximately 220 bp. Measure the concentration of the library by integrating under the peak.

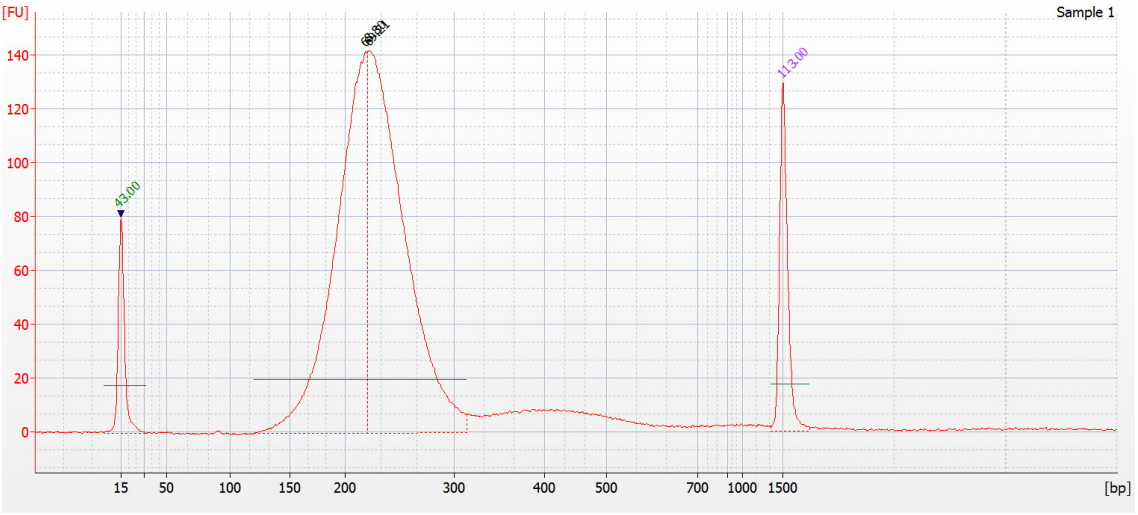

Figure 3 Analysis of amplified prepped library DNA using a DNA 1000 assay. The electropherogram shows a single peak around 220 bp. Note that high molecular weight PCR products around 400 bp do not affect the capture performance.

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

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3 Hybridization

This chapter describes the steps to combine the prepped library with the hybridization reagents, blocking agents and the SureSelect capture library. Each DNA library sample must be hybridized and captured individually.

CAUTION The ratio of SureSelect capture library to prepped library is critical for successful capture.

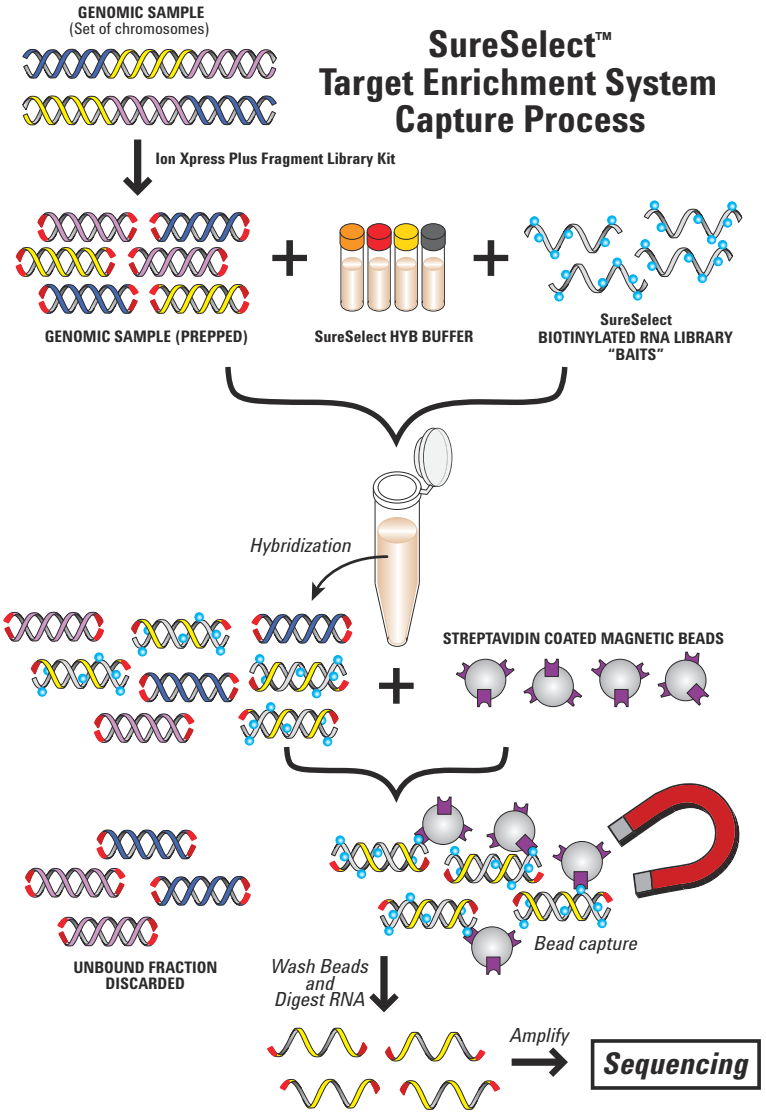

Figure 4 SureSelect Target Enrichment System Capture Process

Refer to "SureSelect Reagent Kit Content" on page 60 for a complete content listing of each SureSelect Target Enrichment kit.

CAUTION

You must avoid evaporation from the small volumes of the capture during the 16 or 24 hour incubation.

If you want to use a different combination of thermal cycler, lid temperature, plates or strips, and sealing method (strip caps or sealing tape), first test the conditions. Incubate 27 µL of SureSelect Hybridization Buffer (without DNA) at 65°C for 24 hours as a test. Include buffer in each well that you might use, including those in the center and those on the edges. Check that you do not get extensive evaporation. Evaporation should not exceed 3 to 4 µL.

For a partial list of tested options showing minimal evaporation, refer to "Alternative Capture Equipment Combinations" on page 63.

Step 1. Hybridize the library

For each sample library prepared, do one hybridization and capture.

The hybridization reaction requires 750 ng of DNA with a maximum volume of 3.4 µL.

1. If the prepped library concentration is below 221 ng/µL, use a vacuum concentrator to concentrate the sample at ≤ 45°C.
   a. Add the entire 25 µL of prepped library to an Eppendorf tube. Poke one or more holes in the lid with a narrow gauge needle.

      You can also break off the cap, cover with parafilm, and poke a hole in the parafilm.

   b. Completely lyophilize. Use a vacuum concentrator on low heat (less than 45°C) to dehydrate.

   c. Reconstitute with nuclease-free water to bring the final concentration to 221 ng/µL (or greater if sample recovery is of concern). Pipette up and down along the sides of the tube for optimal recovery.

   d. Mix well on a vortex mixer and spin in a microfuge for 1 minute.
2. *Optional.* To test recovery after lyophilization, reconstitute the sample to greater than 221 ng/µL and check the concentration on a Bioanalyzer DNA 1000 chip. See "Step 8. Assess quality and quantity with 2100 Bioanalyzer" on page 30. After quantitation, adjust the sample to 221 ng/µL.

   Alternatively, concentrate a 750 ng aliquot at ≤ 45°C down to 3.4 µL. If the sample dries up completely, resuspend in 3.4 µL of water and mix on a vortex mixer. If processing multiple samples, adjust to equivalent volumes before concentrating.
3. Mix the components in Table 16 at room temperature to prepare the hybridization buffer.

Table 16 Hybridization Buffer

Reagent	Volume for 1 capture (µL), includes excess	Volume for 6 captures (µL), includes excess	Volume for 12 captures (µL), includes excess
SureSelect Hyb #1 (orange cap, or bottle)	25	125	250
SureSelect Hyb #2 (red cap)	1	5	10
SureSelect Hyb #3 (yellow cap)	10	50	100
SureSelect Hyb #4 (black cap, or bottle)	13	65	130
Total	**49 (40 µL needed)**	**245 (40 µL/sample)**	**490 (40 µL/sample)**

4 If precipitate forms, warm the hybridization buffer at 65°C for 5 minutes.

5 In a PCR plate, prepare the SureSelect capture library mix for target enrichment:

- **a** Keep capture library mix tubes on ice during preparation and until they are used in step 10.
- **b** For each sample, add the amount of SureSelect capture library as listed in Table 17, based on the Mb target size of your design.
- **c** Use nuclease-free water to prepare a dilution of the SureSelect RNase Block (purple cap) as listed in Table 17.

  Prepare enough RNase Block dilution for all samples, plus excess.
- **d** Add the amount of diluted SureSelect RNase Block listed in Table 17 to each capture library, and mix by pipetting.

Table 17 SureSelect Capture Library.

Capture Size	Volume of SureSelect Library	RNase Block Dilution (Parts RNase block: Parts water)	Volume of RNase Block Dilution to Add
< 3.0 Mb	2 µL	1:9 (10%)	5 µL
≥ 3.0 Mb	5 µL	1:3 (25%)	2 µL

6 Mix the contents in Table 18 to make the correct amount of SureSelect Block mix for the number of samples used.

Table 18 SureSelect Block Mix

Reagent	Volume for 1 reaction	Volume for 12 reactions (includes excess)
SureSelect PTN Block #1 (green cap)	2.5 µL	31.25 µL
SureSelect Block #2 (blue cap)	2.5 µL	31.25 µL
SureSelect PTN Block #3 (brown cap)	0.6 µL	7.5 µL
Total	**5.6 µL**	**70 µL**

7 In a separate PCR plate, prepare the prepped library for target enrichment.

- **a** Add 3.4 µL of 221 ng/µL prepped library to the "B" row in the PCR plate. Put each sample into a separate well.
- **b** Add 5.6 µL of the SureSelect Block Mix to each well in row B.
- **c** Mix by pipetting up and down.
- **d** Seal the wells of row "B" with caps and put the PCR plate in the thermal cycler. Do not heat the Hybridization Buffer or capture library yet, only the prepped library with blockers.
- **e** Start the thermal cycler program in Table 19.

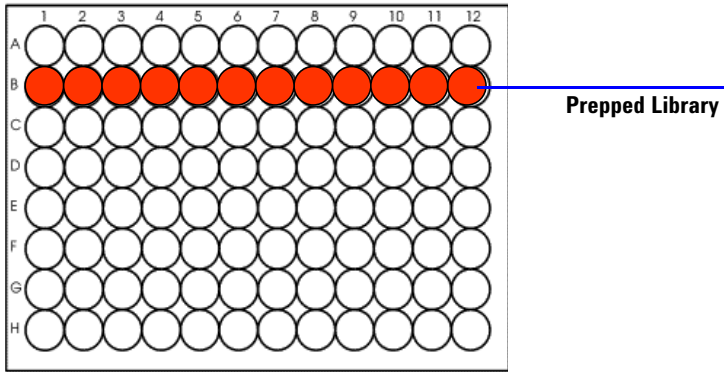

Figure 5 Prepped library shown in red

Table 19 PCR program

Step	Temperature	Time
Step 1	95°C	5 minutes
Step 2	65°C	Hold

8 Use a heated lid on the thermal cycler at 105°C to hold the temperature of the plate on the thermal cycler at 65°C.

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

9 Add the hybridization buffer to the PCR plate:

a Maintain the plate at 65°C while you load 40 µL of hybridization buffer per well into the "A" row of the PCR plate. The number of wells filled in Row A is the number of libraries prepared.

The example in Figure 6 is for 12 captures.

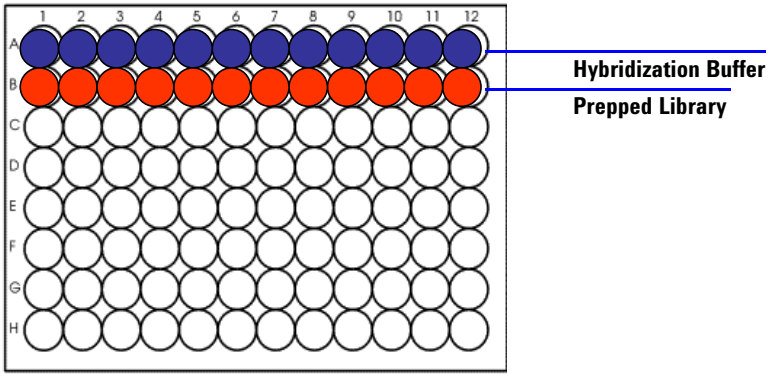

Figure 6 Hybridization buffer shown in blue

b Seal the wells with strip caps. Use a capping tool to make sure the fit is tight.

c Keep the plate at 65°C for a minimum of 5 minutes before you go to step 10.

10 Add the capture library mix from step 5 to the PCR plate:

a Add the capture library mix (7 µL) to the "C" row in the PCR plate.

For multiple samples, use a multi-channel pipette to load the capture library mix into the "C" row in the PCR plate.

Keep the plate at 65°C during this time.

b Seal the wells with strip caps. Use a capping tool to make sure the fit is tight.

c Incubate the samples at 65°C for 2 minutes.

11 Maintain the plate at 65°C while you use a multi-channel pipette to take 13 µL of Hybridization Buffer from the "A" row and add it to the SureSelect capture library mix contained in row "C" of the PCR plate for each sample. (See Figure 7.)

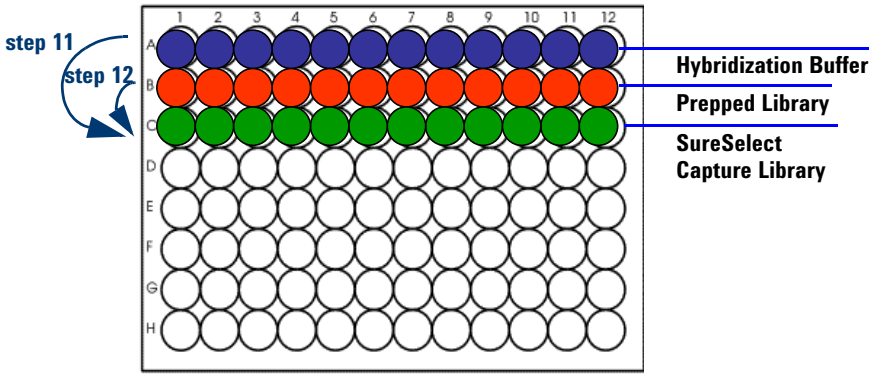

Figure 7 SureSelect Capture Library, or "Baits", shown in Green

12 Maintain the plate at 65°C while you use a multi-channel pipette to transfer the entire contents of each prepped library mix in row "B" to the hybridization solution in row "C". (See Figure 7.) Mix well by slowly pipetting up and down 8 to 10 times.

The hybridization mixture is now 27 to 29 µL, depending on degree of evaporation during the preincubations.

13 Seal the wells with strip caps or double adhesive film. Make sure all wells are completely sealed.

Use new adhesive seals or strip caps. The structural integrity of the seals and caps can be compromised during the previous incubation steps.

CAUTION Wells must be adequately sealed to minimize evaporation, or your results can be negatively impacted. You can use the PlateLoc Thermal Microplate Sealer to minimize evaporation.

If you use strip tubes, test for evaporation before you do the first experiment to make sure the tube/capping method is appropriate for the thermal cycler. Check that no more than 3 to 4 µL is lost to evaporation.

14 Incubate the hybridization mixture for 16 or 24 hours at 65°C with a heated lid at 105°C.

Step 2. Prepare magnetic beads

Use these reagents from the SureSelect Target Enrichment Kit PTN Hyb Module Box #1:

- SureSelect Binding Buffer
- SureSelect Wash 2

1. Prewarm SureSelect Wash 2 at 65°C in a circulating water bath or heat block for use in "Step 3. Select hybrid capture with SureSelect".
2. Vigorously resuspend the Dynabeads MyOne Streptavidin T1 on a vortex mixer. Magnetic beads settle during storage.
3. For each hybridization, add 50 µL of Dynabeads MyOne Streptavidin T1 to a 1.5-mL LoBind Tube.
4. Wash the beads:
   a. Add 200 µL of SureSelect Binding Buffer.
   b. Mix the beads on a vortex mixer for 5 seconds.
   c. Put the tubes into a magnetic device, such as Dynal magnetic separator (Life Technologies).
   d. Remove and discard the supernatant.
   e. Repeat step a through step d for a total of 3 washes.
5. Resuspend the beads in 200 µL of SureSelect Binding Buffer.

Step 3. Select hybrid capture with SureSelect

Use these reagents from the SureSelect Target Enrichment Kit PTN Hyb Module Box #1:

- SureSelect Wash 1
- SureSelect Wash 2

1. Estimate and record the volume of hybridization that remained after 16 or 24 hour incubation.
2. Keep the PCR plate or tubes at 65°C in the PCR machine while you add the hybridization mixture directly from the thermal cycler to the bead solution. Invert the tube to mix 3 to 5 times.

   Excessive evaporation, such as when less than 20 µL remains after hybridization, can indicate suboptimal capture performance. See Table 30 on page 63 for tips to minimize evaporation.
3. Incubate the hybrid-capture/bead solution on a Nutator or equivalent for 30 minutes at room temperature.

   Make sure the sample is properly mixing in the tube.
4. Briefly spin in a centrifuge.
5. Separate the beads and buffer on a magnetic separator and remove the supernatant.
6. Resuspend the beads in 500 µL of SureSelect Wash 1 by mixing on a vortex mixer for 5 seconds.
7. Incubate the samples for 15 minutes at room temperature. Occasionally mix on a vortex mixer.
8. Briefly spin in a centrifuge.
9. Separate the beads and buffer on a magnetic separator and remove the supernatant.
10. Wash the beads:
    a. Resuspend the beads in 500 µL of 65°C prewarmed SureSelect Wash 2 and mix on a vortex mixer for 5 seconds to resuspend the beads.
    b. Incubate the samples for 10 minutes at 65°C in a recirculating water bath, heat block or equivalent. Occasionally mix on a vortex mixer.

Do not use a tissue incubator. It cannot properly maintain temperature.

c Invert the tube to mix. The beads may have settled.

d Briefly spin in a centrifuge.

e Separate the beads and buffer on a magnetic separator and remove the supernatant.

f Repeat step a through step e for a total of 3 washes.

Make sure all of the wash buffer has been removed.

11 Mix the beads in 30 µL of nuclease-free water on a vortex mixer for 5 seconds to resuspend the beads.

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

4 Post-Hybridization Amplification

This chapter describes the steps to amplify, purify, assess quality of the library, and dilute the sample appropriately for cluster amplification.

Step 1. Amplify the captured library

Use reagents from:

- Herculase II Fusion DNA Polymerase (Agilent)
- SureSelect Target Enrichment Kit PTN Hyb Module Box #2

CAUTION Do not use amplification enzymes other than Herculase II Fusion DNA Polymerase. Other enzymes have not been validated.

CAUTION To avoid cross-contaminating libraries, set up PCR reactions (all components except the library DNA) in a dedicated clean area or PCR hood with UV sterilization and positive air flow.

Prepare 1 amplification reaction for each hybrid capture. Include a negative no-template control.

1 Prepare reaction mix and DNA samples:
   a Prepare the reaction mix in Table 20, on ice. Mix well on a vortex mixer.
   b Add 36 µL of the reaction mix to each well or tube.
   c Pipette each DNA sample up and down to make sure that the bead solution is homogeneous.
   d Use a pipette to add 14 µL of each DNA sample to each well or tube. Mix by pipetting. Change pipette tips between samples to avoid cross-contamination.

Table 20 Herculase II Master Mix

Reagent	Volume for 1 reaction	Volume for 12 reactions (includes excess)
Nuclease-free water	22.5 µL	281.25 µL
5× Herculase II Rxn Buffer (clear cap) *	10 µL	125 µL
SureSelect PTN PCR primer mix (clear cap)†	2 µL	25 µL
Herculase II Fusion DNA Polymerase (red cap)*	1 µL	12.5 µL
100 mM dNTP Mix (green cap)*	0.5 µL	6.25 µL
Total	**36 µL**	**450 µL (36 µL/reaction)**

* Included in the Herculase II Fusion DNA Polymerase (Agilent). *Do not use the buffer or dNTP mix from any other kit.*

† Included in the SureSelect Target Enrichment Kit PTN Hyb Module Box #2.

2 Put the tubes in a thermal cycler and run the program in Table 21.

Store the unused bead-bound captured DNA samples at 4°C overnight, or at -20°C long-term.

Table 21 PCR program

Step	Temperature	Time
Step 1	98°C	2 minutes
Step 2	98°C	30 seconds
Step 3	60°C	10 seconds
Step 4	72°C	1 minute
Step 5		• Repeat Step 2 through Step 4 for a total of 8 to 11 times.
Step 6	72°C	10 minutes
Step 7	4°C	Hold

As with the pre-capture PCR amplification, minimize the number of PCR cycles used to enrich the captured DNA. The use of only half of the captured DNA for amplification lets you adjust the number of cycles by repeating the PCR if needed.

Table 22 Number of cycles

Capture Size	Cycles
1 Mb up to 3.2 Mb	11 cycles
> 3.2 Mb	10 cycles
All Exon	9 cycles

As an alternative, you can prepare one PCR master mix as outlined in Table 20. Split this master mix into three small-scale 10-µL PCR reactions and cycle for 9, 10, 11, or 12 cycles. Clean these PCR reactions using the AMPure XP protocol outlined in "Step 2. Purify and size-select the sample using Agencourt AMPure XP beads" on page 20 with these modifications: use 30 µL of AMPure XP beads and elute with 20 µL of nuclease-free water. Run these cleaned samples on a High Sensitivity chip on the Bioanalyzer, as described in "Step 3. Assess quality with the 2100 Bioanalyzer" on page 22.

Use the optimal cycle number to repeat PCR at the 50-µL reaction scale. See Table 22 for approximate number of cycles for a given library size. Results may vary based on library content.

Step 2. Purify the sample using Agencourt AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes.
2. Mix the reagent well so that the reagent appears homogeneous and consistent in color. *Do not freeze*.
3. Add 90 µL of homogeneous AMPure beads to a 1.5-mL LoBind Tube, and add amplified library (~50 µL). Mix well on a vortex mixer and incubate for 5 minutes.
4. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
5. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
6. Continue to keep the tube in the magnetic stand while you dispense 500 µL of 70% Ethanol in each tube.

   Use fresh 70% Ethanol for optimal results.
7. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the Ethanol.
8. Repeat step 6 and step 7 once.
9. Spin briefly in a centrifuge. Return the tube to the magnetic stand for 30 seconds. Remove residual Ethanol with a P20 pipette.
10. Dry the samples on the 37°C heat block for 5 minutes or until the residual Ethanol is completely evaporated.

    Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
11. Add 20 µL of Nuclease-free Water or 1X Low TE Buffer, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
12. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
13. Remove the supernatant (~20 µL) to a fresh 1.5-mL LoBind Tube. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, store the samples at 4°C for up to a week, or at -20°C for longer periods.

Step 3. Assess quality

Use a Bioanalyzer High Sensitivity DNA Assay to assess the quality and size range.

You may need to dilute your sample accordingly. Refer to the *Agilent High Sensitivity DNA Kit Guide* at http://www.chem.agilent.com/en-US/Search/Library/_layouts/Agilent/PublicationSummary.aspx?whid=59504.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
2. Open the Agilent 2100 Expert Software (version B.02.07 or higher required to run the High Sensitivity Kit), turn on the 2100 Bioanalyzer and check communication.
3. Prepare the chip, samples and ladder as instructed in the reagent kit guide.
4. Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
5. Within the instrument context, choose the appropriate assay from the drop down list.
6. Start the run. Enter sample names and comments in the Data and Assay context.
7. Verify the results.

   Determine the concentration of the sample by integration under the peak.
8. If you see different peak sizes across multiple samples, do "Step 4. Pool samples for Multiplexed Sequencing" only after you do "Step 5. Size-select the DNA fragments" through "Step 7. Assess quality".

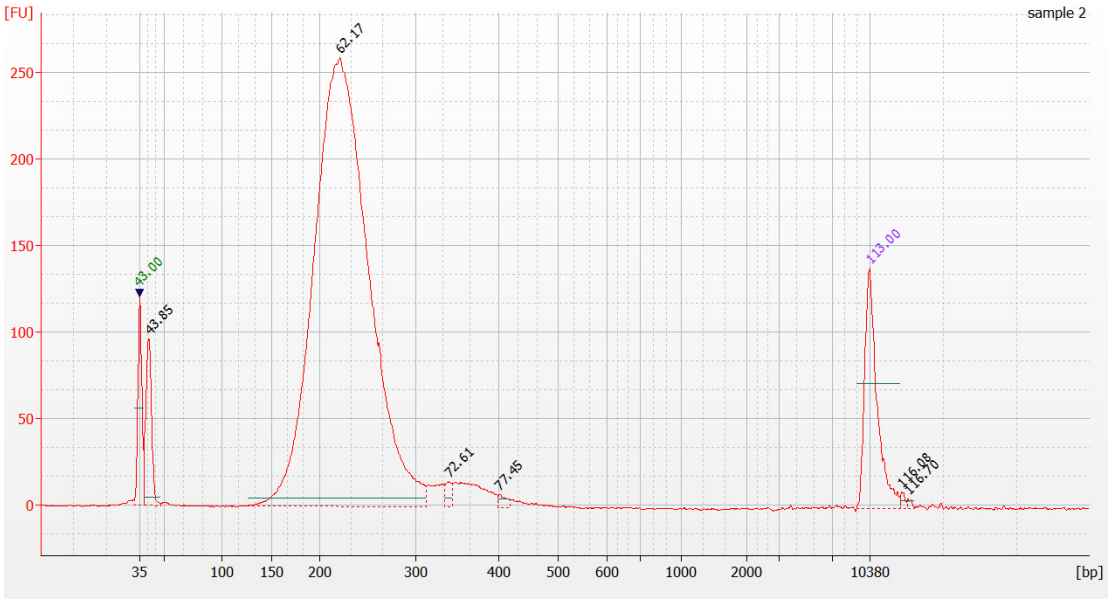

Figure 8 Analysis of Amplified Capture DNA using the High Sensitivity DNA Kit. The expected peak size is approximately 220 bp. Primer-dimer peaks can be expected around 40 bp and are removed during size-selection. See Figure 9 on page 57.

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

Step 4. Pool samples for Multiplexed Sequencing

1 Combine the libraries such that each barcode-tagged sample is present in equimolar amounts in the pool. For each library, use the formula below to determine the amount of barcoded sample to use.

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

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

C(f) is the desired final concentration of all the DNA in the pool, for example, 10 nM for the standard Ion Proton protocol. For enough visibility in E-gel, make sure that the final concentration is more than 10 nM.

is the number of samples to be combined, and

C(i) is the initial concentration of each barcoded sample.

Table 23 shows an example of the amount of 2 barcoded samples and 1X Low TE Buffer needed for a final volume of 20 µL at 10 nM.

Table 23 Example of barcode volume calculation for a total volume of 20 µL

Component	V(f)	C(i)	C(f)	#	Volume to use (µL)
Sample 1	20 µL	10 nM	10 nM	2	10
Sample 2	20 µL	12.5 nM	10 nM	2	8
1X Low TE Buffer					2

2 Adjust the final volume of the pooled library to the desired final concentration.

- If the final volume of the combined barcode-tagged samples is less than the desired final volume, V(f), add 1X Low TE Buffer to bring the volume to the desired level.
- If the final volume of the combined barcode-tagged samples is greater than the final desired volume, V(f), lyophilize and reconstitute to the desired volume.

3 If you store the library before sequencing, add Tween 20 to 0.1% v/v and store at -20°C short term.

Step 5. Size-select the DNA fragments

This topic describes the basic protocol to size-select by agarose gel electrophoresis with the use of the integrated E-Gel system.

1. Set up the integrated E-Gel system:
   a. Put the E-Gel iBase Power System on top of the Safe Imager Real-Time Transilluminator.
   b. Connect the short electrical cord of the from the Safe Imager Real-Time Transilluminator into the power inlet of the E-Gel iBase Power System.
   c. Plug the power cord from the Safe Imager Real-Time Transilluminator into an electrical outlet. Both units will turn on.
2. Use the arrow buttons on the E-Gel iBase Power System to select the **SizeSelect™ 2%** program. If the program is not available, upgrade the firmware. Refer to "Downloading Firmware Upgrades" in the *E-Gel User Manual*.
3. Remove an E-Gel SizeSelect 2% Agarose Gel from its package and gently remove both of the combs from the cassette.
4. Slide the cassette into the two electrode connections on the E-Gel iBase Power System so that the two electrodes on the right side of the gel cassette are in contact with the two connections on the E-Gel iBase Power System. Press down on the left side of the cassette to seat it.

   The LED illuminates with a steady red light when the cassette is properly inserted. Do not pre-run the gel.
5. Load 20 µL of each sample into one well. Do not use the wells on the edge of the gel as they tend to run slower and will affect the size selection. Do not use the wells next to the ladder.
6. Dilute the 1 µg/µL 50-bp ladder in 1X Low TE Buffer to 25ng/µL (1:40). Load 10 µL (250 ng) of the diluted DNA ladder into the smaller middle lane (Lane M).
7. Add 25 µL of nuclease-free water to all unused wells in the top row.
8. Add 25 µL of nuclease-free water to all of the large wells in the bottom row, and 10 µL to the smaller center well (Lane M).
9. Place the Amber filter unit on top of the E-Gel iBase Power System.
10. Press **Mode** to set the run time to 11 to 13 minutes. If you are not experienced with this system, select a shorter time.

11 Press **Go** on the E-Gel iBase Power System to start electrophoresis. The red light turns green.

12 Run the samples until the PCR product band reaches the reference line on the gel.

13 If the time runs out before the PCR product band reaches the reference line, run the gel for a few additional minutes by repeating step 10 through step 12 with run times of 2 to 3 minutes.

14 Press **Go** on the E-Gel iBase Power System to stop electrophoresis

15 Change the run time to 1 to 2 minutes and press **Go** to resume electrophoresis. Carefully monitor the migration of the PCR product and 200 bp band of the ladder. The target selection size is approximately 220 bp, which is similar to the expected PCR product size.

16 When the PCR product band migrates in the collection well, press **Go** to stop electrophoresis.

17 Take care not to pierce the bottom of the wells as you use a pipette to collect approximately 10 µL of the solution from the collection well.

18 Adjust the total volume to 20 µL.

Step 6. Purify the sample using Agencourt AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes.
2. Mix the reagent well so that the reagent appears homogeneous and consistent in color. *Do not freeze*.
3. Add 1.8 × the volume of the sample (36 µL) of homogeneous AMPure XP beads to a 1.5-mL LoBind Tube, and add the pooled and size-selected library (20 µL). Mix well on a vortex mixer and incubate for 5 minutes.
4. Put the tube in the magnetic stand. Wait for the solution to clear (approximately 3 to 5 minutes).
5. Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared solution from the tubes.
6. Continue to keep the tube in the magnetic stand while you dispense 0.2 mL of 70% Ethanol in each tube.

   Use fresh 70% Ethanol for optimal result.
7. Let the tube sit for 1 minute to allow any disturbed beads to settle, and remove the Ethanol.
8. Repeat step 6 and step 7 once.
9. Spin briefly in a centrifuge. Return the tube to the magnetic stand for 30 seconds. Remove residual Ethanol with a P20 pipette.
10. Dry the samples on the 37°C heat block for 5 minutes until the residual Ethanol is completely evaporated.

    Do not dry the bead pellet to the point that the bead pellet appears cracked. Elution efficiency is significantly decreased when the bead pellet is excessively dried.
11. Add 25 µL of nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
12. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
13. Remove the supernatant (~25 µL) to a fresh 1.5-mL LoBind Tube. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, store the samples at -20°C.

Step 7. Assess quality

Use a Bioanalyzer High Sensitivity DNA Assay to assess the quality and size range.

You may need to dilute your sample accordingly. Refer to the *Agilent High Sensitivity DNA Kit Guide* at http://www.chem.agilent.com/en-US/Search/Library/_layouts/Agilent/PublicationSummary.aspx?whid=59504.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
2. Open the Agilent 2100 Expert Software (version B.02.07 or higher required to run the High Sensitivity Kit), turn on the 2100 Bioanalyzer and check communication.
3. Prepare the chip, samples and ladder as instructed in the reagent kit guide.
4. Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
5. Within the instrument context, choose the appropriate assay from the drop down list.
6. Start the run. Enter sample names and comments in the Data and Assay context.
7. Verify the results.

   Determine the concentration of the sample by integration under the peak. Check that the samples show similar peak sizes of approximate 220 bp.

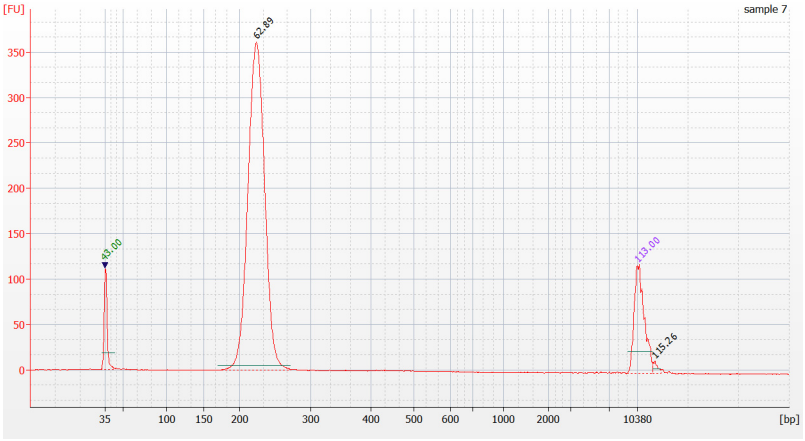

Figure 9 Analysis of Amplified Capture DNA using the High Sensitivity DNA Kit. The electropherogram shows a peak of approximately 220 bp.

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

Step 8. Prepare templates for sequencing

- Use the Ion OneTouch 2 System with the Ion PI Template OT2 200 Kit v2 and Ion PI Sequencing 200 Kit v2 to prepare the templates for the sequencer.

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5 Reference

This chapter contains reference information.

SureSelect Reagent Kit Content

NOTE SureSelect capture libraries and reagents must be used within one year of receipt.

Each SureSelect Reagent Kit contains one or more of each of these kits:

Table 24 SureSelect Reagent Kit Contents

Product	Storage Condition	16 Reactions	96 Reactions
SureSelect Target Enrichment Kit PTN Hyb Module Box #1	Room Temperature	5190-6542	5190-6543
SureSelect Target Enrichment Kit PTN Hyb Module Box #2	-20°C	5190-6514	5190-6515

The content of each of these kits are described in the next tables.

Table 25 SureSelect Target Enrichment Kit PTN Hyb Module Box #1

Kit Component
SureSelect Hyb #1 (orange cap, or bottle)
SureSelect Hyb #2 (red cap)
SureSelect Hyb #4 (black cap, or bottle)
SureSelect Binding Buffer
SureSelect Wash 1
SureSelect Wash 2

Table 26 SureSelect Target Enrichment Kit PTN Hyb Module Box #2

Kit Component
SureSelect Hyb #3 (yellow cap)
SureSelect PTN Block #1 (green cap)
SureSelect Block #2 (blue cap)
SureSelect PTN Block #3 (brown cap)
SureSelect RNase Block (purple cap)
SureSelect PTN PCR primer mix (clear cap)

Other Reagent Kits Content

These reagents are from kits other than the SureSelect Reagent kit. Make sure you use only the reagents listed here.

Table 27 Herculase II Fusion DNA Polymerase (Agilent)

Component
DMSO (green cap)
5× Herculase II Rxn Buffer (clear cap)
100 mM dNTP Mix (green cap)
Herculase II Fusion DNA Polymerase (red cap)

Table 28 Ion Xpress Plus Fragment Library Kit (Life Technologies p/n 4471269)

Components
Ion Shear Plus Enzyme Mix
Ion Shear Plus 10x Reaction Buffer
Ion Shear Plus Stop Buffer
10x Ligase Buffer
DNA Ligase
Adapters
Nick Repair Polymerase
dNTP Mix

Table 29 Ion Xpress Barcode Adapters Kit

Component
Ion Xpress P1 Adapter
Ion Xpress Barcode 1 through 16

Alternative Capture Equipment Combinations

Table 30 lists combinations of thermal cycler, lid temperature, plates or strips, and sealing method (strip caps or sealing tape) other than those used in this protocol that have shown minimal evaporation.

Refer to this list for additional of equipment combination options for hybridization. Note that minimal evaporation is needed to ensure good capture results.

Table 30 Tested options that show minimal evaporation

PCR Machine	Plate/Strips	Cover	Comments
Agilent Mx3005P QPCR	Mx3000P Strip Tubes (401428)	MX3000P Optical Strip Caps (401425)	Heated Lid
Agilent Mx3005P QPCR	MicroAmp Optical 96-well reaction plate (N801-0560)	MicroAmp Clear Adhesive Film (4306311)	Heated Lid; ABI compression pad (4312639) Use two layers of film.
ABI GeneAmp 9700	MicroAmp Optical 96-well Reaction Plate (N801-0560)	MicroAmp Caps (8caps/strip) (N801-0535)	Heated Lid
ABI Veriti (4375786)	MicroAmp Optical 96-well Reaction Plate (N801-0560)	MicroAmp Clear Adhesive Film (4306311)	Heated Lid; ABI compression pad (4312639) Use two layers of film.
Eppendorf Mastercycler	Eppendorf 8-Tube PCR Tubes	Attached lids	Lid heating set to 75°C
BioRad (MJ Research) PTC-200	Agilent strip tubes 410022 (Mx4000)	Agilent Optical cap 410024 (Mx4000)	Heated Lid
BioRad (MJ Research) PTC-200	Agilent strip tubes 410022 (Mx4000)	Agilent Optical cap 401425 (Mx3000/3005)	Heated Lid
BioRad (MJ Research) PTC-200	Agilent 96-well Plate 410088 (Mx3000/3005)	Agilent Optical cap 401425 (Mx3000/3005)	Heated Lid

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In This Book

This guide contains information to run the SureSelect Target Enrichment System for Sequencing on Ion Proton protocol.

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