

SureSelect Target Enrichment System for the Applied Biosystems SOLiD System

**SureSelect Target Enrichment
for AB SOLiD Multiplexed
Sequencing**

Protocol

Version 1.1, July 2010

**SureSelect platform manufactured with Agilent
SurePrint Technology**

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In this Guide...

This guide describes Agilent's recommended operational procedures to capture genomic regions of interest using Agilent's SureSelect Target Enrichment System Kit for next-generation sequencing. This protocol is specifically developed and optimized to use Biotinylated RNA oligomer libraries, or Bait, to enrich targeted regions of the genome from repetitive sequences and sequences unrelated to the research focus.

This guide uses the Applied Biosystems SOLiD System for 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 contains instructions for prepped library production specific to the Applied Biosystems SOLiD System.

3 Hybridization

This chapter describes the steps to combine the prepped library with the blocking agents and the SureSelect capture library.

4 Post-Hybridization Amplification

This chapter describes the steps to amplify, purify, quantify, and pool the barcoded sample libraries after target enrichment hybridization.

5 Reference

This chapter contains reference information.

What's New in Version 1.1?

A QPCR quantification step, using the Agilent QPCR NGS Library Quantification Kit, was added to the post-hybridization amplification procedure.

Content

1	Before You Begin	7
	Procedural Notes	8
	Safety Notes	8
	Required Reagents	9
	Optional Reagents	11
	Required Equipment	12
	Optional Equipment	13
2	Sample Preparation	15
	Step 1. Quantify and shear DNA	18
	Step 2. Repair the ends	20
	Step 3. Purify the DNA with the SOLiD Library Column Purification Kit	21
	Step 4. Ligate the P1 and IA Adaptors to the DNA	22
	Step 5. Purify the DNA with the SOLiD Library Column Purification Kit	23
	Step 6. Size-select the DNA fragments with a SOLiD Library Size Selection gel	24
	Step 7. Purify the DNA with the SOLiD Library Column Purification Kit	26
	Step 8. Nick-translate and amplify the library	27
	Step 9. Purify the DNA with the SOLiD Library Column Purification Kit	28
	Step 10. Quantify the Library with the Agilent 2100 Bioanalyzer DNA 1000 assay	29
3	Hybridization	31
	Step 1. Hybridize the library	35
	Step 2. Prepare magnetic beads	41
	Step 3. Select hybrid capture with SureSelect	42
	Step 4. Purify the sample using the Agencourt AMPure XP beads	44
4	Addition of Barcode Tags by Post-Hybridization Amplification	45
	Step 1. Amplify the captured library to add barcode tags	46
	Step 2. Purify the sample using the Agencourt AMPure XP beads	49

Contents

Step 3. Assess quality with the Agilent 2100 Bioanalyzer High Sensitivity DNA assay 50

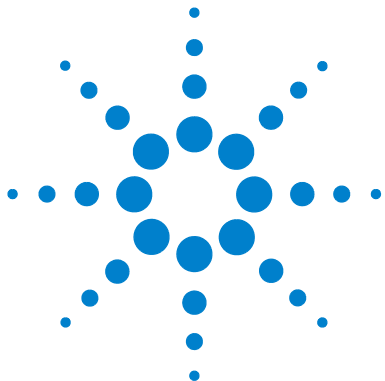
Step 4. Assess the quantity of each index-tagged library by QPCR 52

Step 5. Pool samples for Multiplexed Sequencing 53

Step 6. Do an emulsion PCR 55

5 Reference 57

Alternative Capture Equipment Combinations 58



1 Before You Begin

Procedural Notes	8
Safety Notes	8
Required Reagents	9
Required Equipment	12
Optional Equipment	13

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

Agilent cannot guarantee the SureSelect Target Enrichment kits and cannot provide technical support for the use of 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
Agilent DNA 1000 Kit	Agilent p/n 5067-1504
Agilent High Sensitivity DNA Kit	Agilent p/n 5067-4626
SureSelect AB Barcoding Library Kit	
10x16 reaction kit	Agilent p/n G3367A
25x16 reaction kit	Agilent p/n G3368A
50x16 reaction kit	Agilent p/n G3369A
Agilent QPCR NGS Library Quantification Kit (SOLiD)	Agilent p/n G4881A
AMPure Kit (SPRI XP beads)	Agencourt
5 mL	p/n A63880
60 mL	p/n A63881
450 mL	p/n A63882
Nuclease-free Water (not DEPC-treated)	Ambion Cat #AM9930
SOLiD Fragment Library Construction Kit with Size Selection Gels	Applied Biosystems p/n 4443471
1X Low TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	Applied Biosystems p/n 4389764
Quant-iT dsDNA BR Assay Kit, for use with the Qubit fluorometer	
100 assays, 2-1000 ng	Invitrogen p/n Q32850
500 assays, 2-1000 ng	Invitrogen p/n Q32853
Qubit assay tubes	Invitrogen p/n Q32856
100% Ethanol, molecular biology grade	Sigma-Aldrich p/n E7023
Isopropanol	Sigma-Aldrich p/n I9516
Herculase II Fusion DNA Polymerase (includes dNTP mix and 5x Buffer)	Stratagene
200 reactions	p/n 600677
400 reactions	p/n 600679
70% Ethanol (for SPRI clean-up)	

1 Before You Begin

Required Reagents

Table 2 Required Reagents for Hybridization

Description	Vendor and part number
SureSelect Target Enrichment System Kit	
10 reactions, <0.2 to 3.3 Mb (Level MP0 through MP4)	Agilent p/n G3360A*
25 reactions, 3 to 3.3 Mb (Level MP0)	Agilent p/n G3360B
50 reactions, 3 to 3.3 Mb (Level MP0)	Agilent p/n G3360C
100 reactions, 3 to 3.3 Mb (Level MP0)	Agilent p/n G3360D
250 reactions, 3 to 3.3 Mb (Level MP0)	Agilent p/n G3360E
500 reactions, 3 to 3.3 Mb (Level MP0)	Agilent p/n G3360F
1000 reactions, 3 to 3.3 Mb (Level MP0)	Agilent p/n G3360G
2000 reactions, 3 to 3.3 Mb (Level MP0)	Agilent p/n G3360H
5000 reactions, 3 to 3.3 Mb (Level MP0)	Agilent p/n G3360J
100 reactions, <0.2 Mb (Level MP1)	Agilent p/n G3360K
250 reactions, <0.2 Mb (Level MP1)	Agilent p/n G3360L
500 reactions, <0.2 Mb (Level MP1)	Agilent p/n G3360M
1000 reactions, <0.2 Mb (Level MP1)	Agilent p/n G3360N
100 reactions, 0.2 to 0.49 Mb (Level MP2)	Agilent p/n G3360O
250 reactions, 0.2 to 0.49 Mb (Level MP2)	Agilent p/n G3360P
500 reactions, 0.2 to 0.49 Mb (Level MP2)	Agilent p/n G3360Q
1000 reactions, 0.2 to 0.49 Mb (Level MP2)	Agilent p/n G3360R
100 reactions, 0.5 to 1.49 Mb (Level MP3)	Agilent p/n G3360S
250 reactions, 0.5 to 1.49 Mb (Level MP3)	Agilent p/n G3360T
500 reactions, 0.5 to 1.49 Mb (Level MP3)	Agilent p/n G3360U
1000 reactions, 0.5 to 1.49 Mb (Level MP3)	Agilent p/n G3360V
100 reactions, 1.5 to 2.99 Mb (Level MP4)	Agilent p/n G3360W
250 reactions, 1.5 to 2.99 Mb (Level MP4)	Agilent p/n G3360X
500 reactions, 1.5 to 2.99 Mb (Level MP4)	Agilent p/n G3360Y
1000 reactions, 1.5 to 2.99 Mb (Level MP4)	Agilent p/n G3360Z
Nuclease-free Water (not DEPC-treated)	Ambion Cat #AM9930
Dynabeads MyOne Streptavidin T1	Invitrogen
2 mL	Cat #656-01
10 mL	Cat #656-02
100 mL	Cat #656-03

* Use option 002 for AB SOLiD.

Optional Reagents

Table 3 Optional Reagents

Description	Vendor and part number
SureSelect Target Enrichment, Demo	Agilent p/n G4459A
SOLiD Fragment Library Construction Kit	Applied Biosystems p/n 4443473
SOLiD Fragment Library Construction Reagents	Applied Biosystems p/n 4443713
E-Gel SizeSelect 2% Agarose	Invitrogen p/n G6610-02

Required Equipment

Table 4 Required Equipment for Library Prep and Post-Hybridization Amplification

Description	Vendor and part number
Agilent 2100 Bioanalyzer	Agilent p/n G2938C
Thermal cycler	BioRad (MJ Research) DNA Engine PTC-200, Applied Biosystems Veriti Thermal Cycler, or equivalent
Covaris S-series Single Tube Sample Preparation System, Model S2	Covaris
Covaris microTUBE with AFA fiber and snap cap	Covaris p/n 520045
Eppendorf Microcentrifuge Model 5417R	Eppendorf p/n 022621807 (120 V/60 Hz), Eppendorf p/n 022621840 (230 V/50 Hz) or equivalent
Eppendorf fixed-angle rotor with standard lid	Eppendorf p/n 022636006
DNA LoBind Tubes, 1.5-mL PCR clean, 250 pieces	Eppendorf p/n 022431021 or equivalent
E-Gel iBase and E-Gel Safe Imager Combo Kit	Invitrogen p/n G6465
Qubit Fluorometer	Invitrogen p/n Q32857
Dynal DynaMag-2 magnetic stand	Invitrogen 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	
Sterile, nuclease-free aerosol barrier pipette tips	
Timer	
Vortex mixer	
Heat block at 37°C	

Table 5 Required Equipment for Hybridization

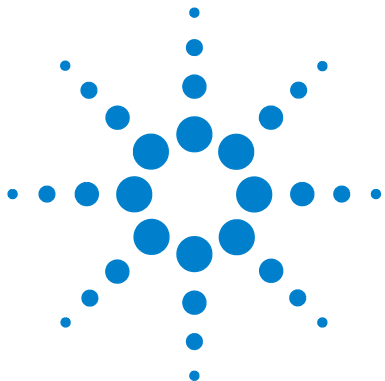
Description	Vendor and part number
Mx3000P/Mx3005P 96-well tube plates	Agilent p/n 410088 or equivalent
Mx3000P/Mx3005P optical strip caps	Agilent p/n 401425 or equivalent
MicroAmp Clear Adhesive Film	Applied Biosystems p/n 4306311 or equivalent
BD Clay Adams Nutator Mixer	BD Diagnostics p/n 421105 or equivalent
Dynal DynaMag-2 magnetic stand	Invitrogen 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
Sterile, nuclease-free aerosol barrier pipette tips	
Thermal cycler	BioRad (MJ Research) DNA Engine PTC-200, Applied Biosystems Veriti Thermal Cycler, or equivalent
Timer	
Vortex mixer	
Water bath or heat block set to 65°C	

Optional Equipment

Table 6 Optional Equipment for Hybridization

Description	Vendor and part number
Tube-strip capping tool	Agilent p/n 410099

1 Before You Begin
Optional Equipment



2 Sample Preparation

- Step 1. Quantify and shear DNA 18
- Step 2. Repair the ends 20
- Step 3. Purify the DNA with the SOLiD Library Column Purification Kit 21
- Step 4. Ligate the P1 and IA Adaptors to the DNA 22
- Step 5. Purify the DNA with the SOLiD Library Column Purification Kit 23
- Step 6. Size-select the DNA fragments with a SOLiD Library Size Selection gel 24
- Step 7. Purify the DNA with the SOLiD Library Column Purification Kit 26
- Step 8. Nick-translate and amplify the library 27
- Step 9. Purify the DNA with the SOLiD Library Column Purification Kit 28
- Step 10. Quantify the Library with the Agilent 2100 Bioanalyzer DNA 1000 assay 29

This chapter contains instructions for prepped library production specific to the Applied Biosystems SOLiD System.

NOTE

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



2 Sample Preparation

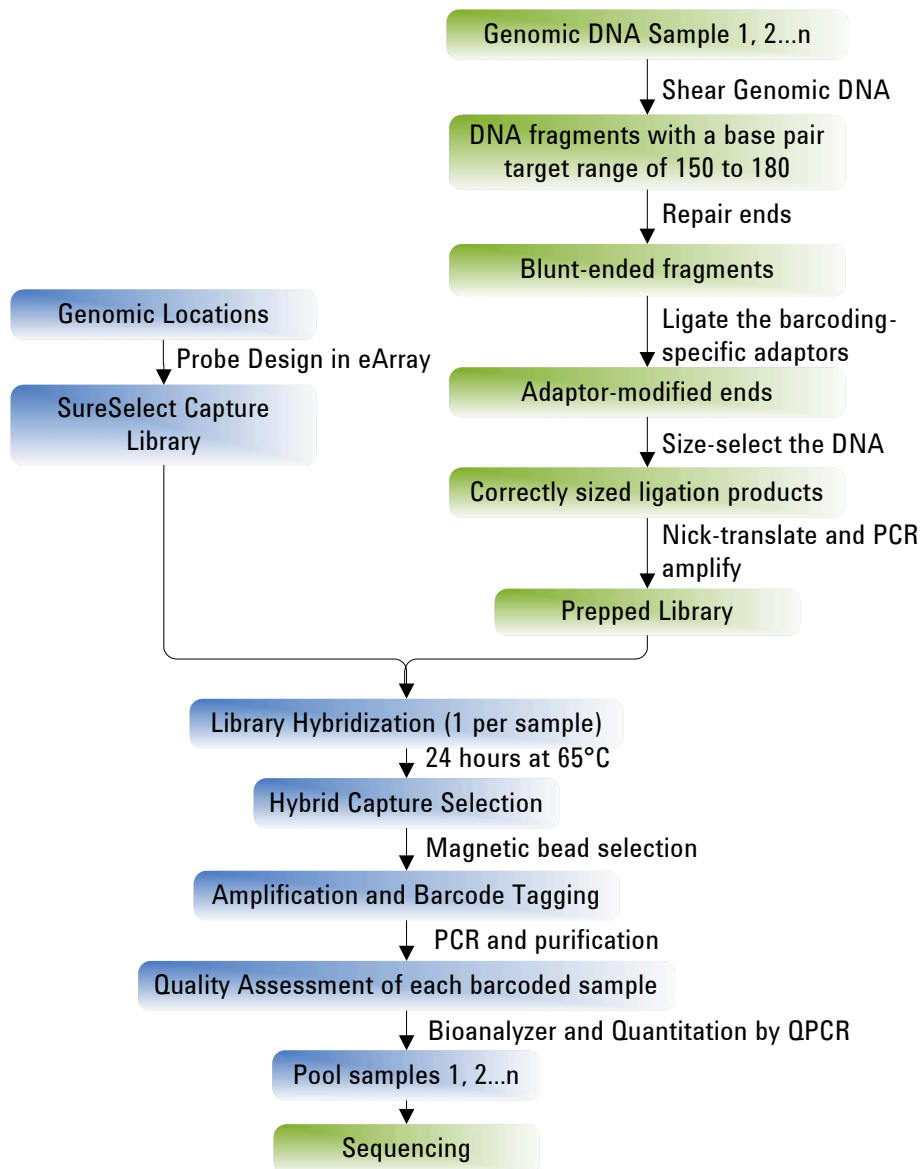


Figure 1 Overall sequencing sample preparation workflow.

Table 7 Overview and time requirements

Step	Time
AB SOLiD Fragment Library Production	8 hours
Bioanalyzer QC	1 hour
Library Preparation and Hybridization	24.5 hours (optional 72 hours)
Bead Preparation	10 minutes
Capture Selection and Washing	2.5 hours
DNA Purification	30 minutes
Post-Hybridization Amplification	1 hour
PCR Purification	30 minutes
Bioanalyzer QC	1 hour

Step 1. Quantify and shear DNA

- 1 Use the Qubit dsDNA BR Assay 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 each 3 μg of high-quality gDNA with 1X Low TE Buffer (or EB buffer) in a 1.5-mL LoBind tube to a total volume of 120 μL .
- 3 Set up the Covaris instrument.
 - a Check that the water in the Covaris tank is filled with fresh deionized water to fill line level 12 on the graduated fill line label.
 - b Check that the water covers the visible glass part of the tube.
 - c Set the chiller temperature to between 2°C to 5°C to ensure that the temperature reading in the water bath displays 5°C.
 - d *Optional.* Supplement the circulated water chiller with ethylene glycol to 20% volume to prevent freezing.
 - e On the instrument control panel, push the Degas button. Degas the instrument for least 30 minutes before use.

Refer to the Covaris instrument user guide.

- 4 Put a Covaris microTube into the loading and unloading station.

Keep the cap on the tube.
- 5 Use a tapered pipette tip to slowly transfer the 120 μL DNA sample through the pre-split septa.

Be careful not to introduce a bubble into the bottom of the tube.
- 6 Secure the microTube in the tube holder and shear the DNA with the settings in [Table 8](#). The target peak for base pair size is 150 to 180 bp.

Table 8 Covaris shear settings

Setting	Value
Number of Cycles	6
Bath Temperature	5°C
Bath Temperature Limit	8°C
Mode	Frequency sweeping
Water Quality Testing Function	Off
Duty Cycle	10%
Intensity	5
Cycles per Burst	100
Time	60 seconds

- 7** Put the Covaris microTube back into the loading and unloading station.
- 8** While keeping the snap-cap on, insert a pipette tip through the pre-split septa, then slowly remove the sheared DNA.
- 9** Transfer the sheared DNA into a new 1.5- mL LoBind tube.

Step 2. Repair the ends

- 1 For 1 library:
 - In a PCR tube, strip tube, or plate, prepare the reaction mix in [Table 9](#), on ice. Mix well by gently pipetting up and down.
- 2 For multiple libraries:
 - a Prepare the reaction mix in [Table 9](#), on ice. Mix well on a vortex mixer.
 - b Add 80 μL of the reaction mix to each well or tube.
 - c Use a pipette to 120 μL of each DNA sample to each well or tube. Mix by pipetting. Change pipette tips between samples.

Table 9 End Repair

Reagent	Volume for 1 Library (μL)	Volume for 10 Libraries (μL), includes excess
Sheared DNA	120	
5X End-Polishing Buffer	40	420
dNTP mix*	8	84
End Polishing Enzyme 1, 10 U/ μL	4	42
End Polishing Enzyme 2, 5 U/ μL	16	168
Nuclease-free water	12	126
Total Volume	200	840

* Included in the SOLiD Fragment Library Construction Kit

- 3 Incubate the mixture at room temperature for 30 minutes.

Step 3. Purify the DNA with the SOLiD Library Column Purification Kit

- 1** Add 800 μL of Binding Buffer (B2-S) with 55% isopropanol to the end-repaired DNA.
Make sure you use the correct Binding Buffer, with the correct isopropanol concentration. Check the Binding Buffer bottles for the amount of isopropanol to add.
- 2** Apply about 700 μL of the end-repaired DNA in Binding Buffer (B2-S) to the column.
- 3** Let the column stand for 2 minutes at room temperature.
- 4** Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 1 minute and discard the flow-through.
- 5** Repeat [step 2](#) through [step 4](#) until the entire sample has been loaded onto the column. Put the SOLiD Library Column back into the same collection tube.
- 6** Add 650 μL of Wash Buffer (W1) to wash the column.
Make sure you add to the Wash Buffer the correct amount of ethanol, which is listed on the Wash Buffer bottle.
- 7** Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 2 minutes, then discard the flow-through. Repeat to remove residual wash buffer.
- 8** Air-dry the column for 2 minutes to completely evaporate residual ethanol. All traces of ethanol must be removed.
- 9** Transfer the column to a clean 1.5-mL LoBind tube.
- 10** Add 50 μL of Elution Buffer (E1) to the column to elute the DNA, then let the column stand for 2 minutes.
- 11** Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 1 minute.
- 12** Add the eluate from [step 11](#) back to the column, then let the column stand for 2 minutes. Repeat [step 11](#).

2 Sample Preparation

Step 4. Ligate the P1 and IA Adaptors to the DNA

Step 4. Ligate the P1 and IA Adaptors to the DNA

- 1 For 1 library:
 - In a PCR tube, strip tube, or plate, prepare the reaction mix in [Table 10](#), on ice. Mix well by gently pipetting up and down.
- 2 For multiple libraries:
 - a Prepare the reaction mix in [Table 10](#), on ice. Mix well on a vortex mixer.
 - b Add 152 μL of the reaction mix to each well or tube.
 - c Use a pipette to add 48 μL of each DNA sample to each well or tube. Mix by pipetting. Change pipette tips between samples.

Table 10 Ligation mix

Component	Volume (μL)	Volume for 10 Libraries (μL) includes excess
End-repaired DNA	48	
SureSelect P1-AB, 50 μM *	26	273
SureSelect IA-AB, 50 μM *	26	273
5X T4 Ligase Buffer	40	420
T4 Ligase, 5 U/ μL	10	105
Nuclease-free water	50	525
Total	200	1596

* Provided in the Agilent SureSelect AB Barcoding Library Kit. Note that adaptors provided in the Agilent SureSelect AB Barcoding Library Kit are truncated compared to the adaptors provided in the SOLiD Fragment Library Barcoding Kit Modules and are not interchangeable.

- 3 Incubate at room temperature for 15 minutes.

Step 5. Purify the DNA with the SOLiD Library Column Purification Kit

- 1 Add 800 μL of Binding Buffer (B2-L) with 40% isopropanol to the sample.
Make sure you use the correct Binding Buffer, with the correct isopropanol concentration. Check the Binding Buffer bottles for the amount of isopropanol to add.
- 2 Apply about 700 μL of the ligated DNA in Binding Buffer (B2-L) to the column.
- 3 Let the column stand for 2 minutes at room temperature.
- 4 Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 1 minute, then discard the flow-through.
- 5 Repeat [step 2](#) through [step 4](#) until the entire sample has been loaded onto the column. Put the SOLiD Library Column back into the same collection tube.
- 6 Add 650 μL of Wash Buffer (W1) to wash the column.
Make sure you add to the Wash Buffer the correct amount of ethanol, which is listed on the Wash Buffer bottle.
- 7 Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 2 minutes, then discard the flow-through. Repeat to remove residual wash buffer.
- 8 Air-dry the column for 2 minutes to completely evaporate residual ethanol. All traces of ethanol must be removed.
- 9 Transfer the column to a clean 1.5-mL LoBind tube.
- 10 Add 60 μL of Elution Buffer (E1) to the column to elute the DNA, then let the column stand for 2 minutes.
- 11 Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 1 minute.
- 12 Add the eluate from [step 11](#) back to the column, then let the column stand for 2 minutes. Repeat [step 11](#).

Stopping Point If you do not continue to the next step, store the purified DNA in Elution Buffer (E1) at 4°C.

2 Sample Preparation

Step 6. Size-select the DNA fragments with a SOLiD Library Size Selection gel

Step 6. Size-select the DNA fragments with a SOLiD Library Size Selection gel

- 1 Remove a SOLiD Library Size Selection gel from its package. Remove the combs from the *top* sample-loading wells and the *middle* collection wells. Set the E-Gel on the E-Gel iBase linked with the E-Gel Safe Imager.
- 2 Load the E-Gel as follows:
 - a Load 20 μL of the ligated, purified DNA into each of 3 wells on the *top row* of wells. If the sample volume < 20 μL , add nuclease-free water to the well for a total volume of 20 μL . Skip the center well (smaller well in the top center of the gel for the ladder). Skip the two outermost wells (to avoid edge effects). Do not load more than 1 μg of DNA per lane.
 - b Load 2 μL 50-bp ladder at 0.1 $\mu\text{g}/\mu\text{L}$ to the center top well. Add 15 μL of water to fill the well.
 - c Fill empty wells in the top row with 20 μL of nuclease-free water.
 - d Fill each of the collection wells in the *middle* of the gel with 25 μL of nuclease-free water. Add 20 μL of nuclease-free water to the *middle* center well.
- 3 Run the gel:
 - iBase program: **Run E-Gel DC**
 - Approximate run time: **13:45** (13 minutes and 45 seconds)Monitor the E-Gel in real-time with the E-Gel[®] Safe Imager.
- 4 If needed during the run, fill the middle collection wells with nuclease-free water.
- 5 When the 200-bp band from the marker lane is in the center of the collection well, stop the run if the run has not already stopped (see [Figure 2](#)).
- 6 Collect the solution from the wells and pool according to samples.
- 7 Wash each collection well with 25 μL of nuclease-free water, pipette up and down, then retrieve the wash solution and combine with the respective sample solution collected in [step 6](#).

Step 6. Size-select the DNA fragments with a SOLiD Library Size Selection gel

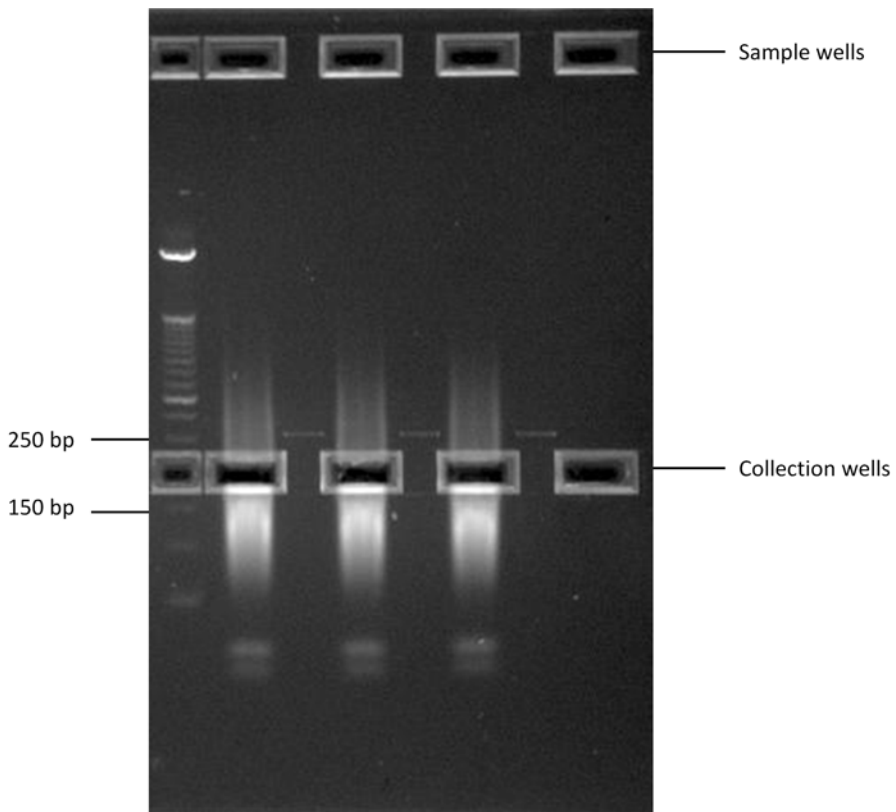


Figure 2 Elution of an approximately 200 bp region from SOLiD Library Size Selection gel.

2 Sample Preparation

Step 7. Purify the DNA with the SOLiD Library Column Purification Kit

Step 7. Purify the DNA with the SOLiD Library Column Purification Kit

- 1 Add 400 μL of Binding Buffer (B2-L) with 40% isopropanol to the size selected sample.

Make sure you use the correct Binding Buffer, with the correct isopropanol concentration. Check the Binding Buffer bottles for the amount of isopropanol to add.

- 2 Apply 500 μL of the size selected DNA in Binding Buffer (B2-L) to the column.
- 3 Let the column stand for 2 minutes at room temperature
- 4 Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 1 minute, then discard the flow-through.
- 5 Put the SOLiD Library Column back into the same collection tube.
- 6 Add 650 μL of Wash Buffer (W1) to wash the column.
Make sure you add to the Wash Buffer the correct amount of ethanol, which is listed on the Wash Buffer bottle.
- 7 Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 2 minutes, then discard the flow-through. Repeat to remove residual wash buffer.
- 8 Air-dry the column for 2 minutes to completely evaporate residual ethanol. All traces of ethanol must be removed.
- 9 Transfer the column to a clean 1.5-mL LoBind tube.
- 10 Add 50 μL of Elution Buffer (E1) to the column to elute the DNA, then let the column stand for 2 minutes.
- 11 Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 1 minute.
- 12 Add the eluate from [step 11](#) back to the column, then let the column stand for 2 minutes. Repeat [step 11](#).

Step 8. Nick-translate and amplify the library

- 1 Prepare a master mix for the number of reactions needed, plus one additional reaction for the negative control, in [Table 11](#).

Table 11 Amplify the gel eluate

Component	Volume for 1 Library	Volume for 10 Libraries (includes excess)
Platinum PCR Amplification Mix	380 μ L	3990 μ L
SureSelect Pre-Capture Primers, 50 μ M*	20 μ L	210 μ L
Total	400 μL	4200 μL

* Provided in the Agilent SureSelect AB Barcoding Library Kit

- 2 Add 400 μ L of master mix to 50 μ L of the purified gel eluate, then distribute into 4 PCR reaction tubes (approximately 112.5 μ L per tube).
- 3 Run the PCR program in [Table 12](#).

Table 12 PCR conditions to nick-translate and amplify the library

Stage	Step	Temp	Time
Holding	Nick translation	72°C	20 min
Holding	Denature	95°C	5 min
Cycling (12 cycles)	Denature	95°C	15 sec
	Anneal	54°C	45 sec
	Extend	70°C	1 min
Holding	Extend	70°C	5 min
Holding	—	4°C	∞

- 4 Pool respective PCR samples into a new 1.5-mL LoBind tube.

2 Sample Preparation

Step 9. Purify the DNA with the SOLiD Library Column Purification Kit

Step 9. Purify the DNA with the SOLiD Library Column Purification Kit

- 1 Add 1800 μL of Binding Buffer (B2-L) with 40% isopropanol to the sample. Use a 15-mL conical vial.

Make sure you use the correct Binding Buffer, with the correct isopropanol concentration. Check the Binding Buffer bottles for the amount of isopropanol to add.

- 2 Apply about 700 μL of the nick-translated and amplified DNA in Binding Buffer (B2-L) to the column.
- 3 Let the column stand for 2 minutes at room temperature.
- 4 Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 1 minute, then discard the flow-through.
- 5 Repeat [step 2](#) through [step 4](#) until the entire sample has been loaded onto the column. Put the SOLiD Library Column back into the same collection tube.
- 6 Add 650 μL of Wash Buffer (W1) to wash the column.
Make sure you add to the Wash Buffer the correct amount of ethanol, which is listed on the Wash Buffer bottle.
- 7 Spin the column in a centrifuge at $\geq 13,000$ rpm ($17,900 \times g$) for 2 minutes, then discard the flow-through. Repeat to remove residual wash buffer.
- 8 Air-dry the column for 2 minutes to completely evaporate residual ethanol. All traces of ethanol must be removed.
- 9 Transfer the column to clean 1.5-mL LoBind tube.
- 10 Add 50 μL of Elution Buffer (E1) to the column to elute the DNA, then let the column stand for 2 minutes.
- 11 Centrifuge the column at $\geq 13,000$ rpm ($17,900 \times g$) for 1 minute.
- 12 Add the eluate from [step 11](#) back to the column, then let the column stand for 2 minutes. Repeat [step 11](#).

Stopping Point If you do not continue to the next step, store the purified DNA in Elution Buffer (E1) at 4°C.

Step 10. Quantify the Library with the Agilent 2100 Bioanalyzer DNA 1000 assay

- 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), 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. Check that the electropherogram shows a distribution with a peak size around 250 to 275 bp.

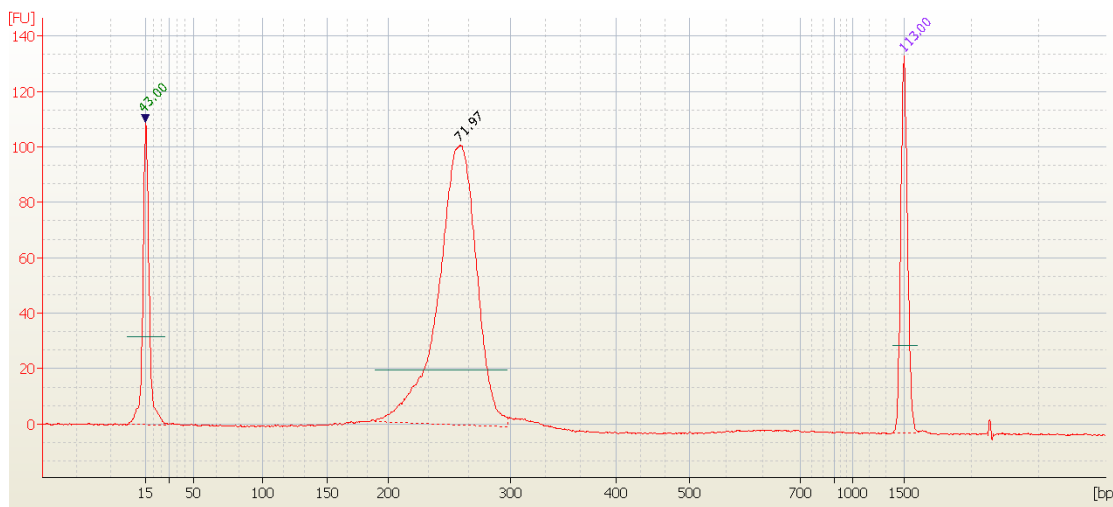


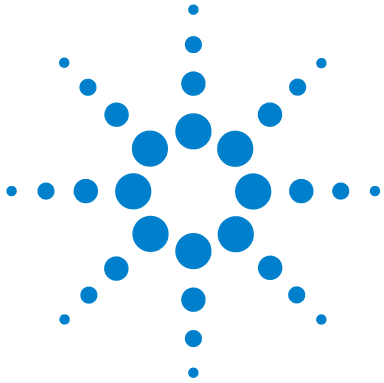
Figure 3 Analysis of amplified prepped library DNA using a DNA 1000 assay. The electropherogram shows a single peak in the size range of approximately 250bp \pm 10%.

2 Sample Preparation

Step 10. Quantify the Library with the Agilent 2100 Bioanalyzer DNA 1000 assay

- 8 If the concentration of your sample is greater than the high end of the dynamic range of the Agilent Bioanalyzer DNA 1000 assay ($> 50 \text{ ng}/\mu\text{L}$), use the Qubit Fluorometer to quantitate the library. Dilute your prepped library appropriately, and use the Agilent Bioanalyzer DNA 1000 assay to quantitate again. Use the concentration as determined by the Agilent Bioanalyzer DNA 1000 assay to calculate the volume of prepped library needed for hybridization (500 ng) in [Chapter 3](#).

Stopping Point If you do not continue at the next step, store the purified DNA in Elution Buffer (E1) at 4°C .



3 Hybridization

- Step 1. Hybridize the library [35](#)
- Step 2. Prepare magnetic beads [41](#)
- Step 3. Select hybrid capture with SureSelect [42](#)
- Step 4. Purify the sample using the Agencourt AMPure XP beads [44](#)

This chapter describes the steps to combine the prepped library with the blocking agents and the SureSelect capture library.

CAUTION

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



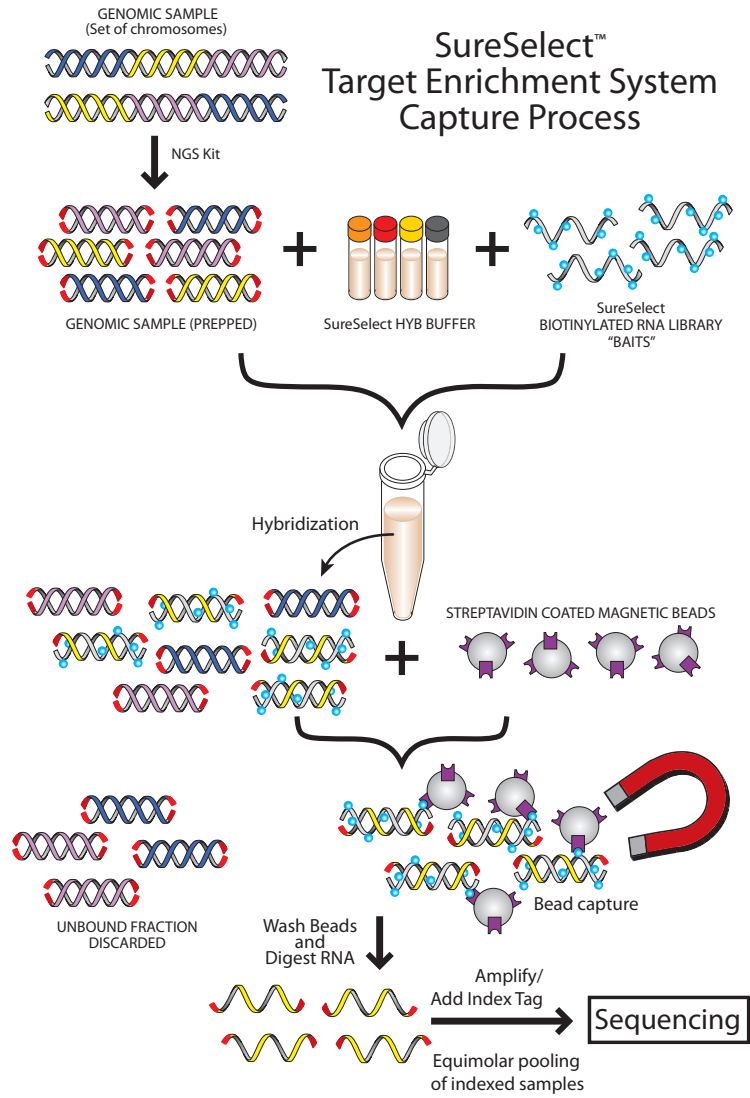


Figure 4 SureSelect Target Enrichment System Capture Process

Table 13 SureSelect Reagent Kit Components

Kit Component	250 RXN Kit	50 RXN Kit	Storage
SureSelect Hyb # 1	bottle	tube with orange cap	Room Temperature
SureSelect Hyb # 2	tube with red cap	tube with red cap	Room Temperature
SureSelect Hyb # 4	bottle	tube with black cap	Room Temperature
3M Sodium Acetate	tube with clear cap	tube with clear cap	Room Temperature
SureSelect Binding Buffer	bottle	bottle	Room Temperature
SureSelect Wash Buffer #1	bottle	bottle	Room Temperature
SureSelect Wash Buffer #2	bottle	bottle	Room Temperature
SureSelect Elution Buffer	bottle	bottle	Room Temperature
SureSelect Neutralization Buffer	bottle	bottle	Room Temperature
SureSelect Hyb # 3	tube with yellow cap	tube with yellow cap	-20°C
SureSelect Indexing Block #1	tube with green cap	tube with green cap	-20°C
SureSelect Block #2	tube with blue cap	tube with blue cap	-20°C
SureSelect Indexing Block #3 -AB	tube with brown cap	tube with brown cap	-20°C
SureSelect RNase Block	tube with purple cap	tube with purple cap	-20°C
SureSelect Oligo Capture Library	tube with red cap	tube with red cap	-80°C

The SureSelect reagents are packaged in separate kits. See [Table 14](#).

Table 14 Part number for reagent kits

Reagent Kit	50 reactions	250 reactions
Room temperature reagents	5190-1953	5190-1959
Cold reagents, -20°C	5190-2343	5190-2344

CAUTION

You must avoid evaporation from the small volumes of the capture during the 24 hour or greater 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 μ L of SureSelect Hybridization Buffer (without DNA) at 65°C for 24 hours (or longer, if applicable) 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 μ L.

For a partial list of tested options showing minimal evaporation, refer to [“Alternative Capture Equipment Combinations”](#) on page 58.

Step 1. Hybridize the library

For each sample library prepared, do one hybridization and capture. Do not pool samples at this stage.

- 1** If the prepped library concentration is below 147 ng/ μ L, use a vacuum concentrator to concentrate the sample at $\leq 45^{\circ}\text{C}$.
 - a** Add the entire 50 μ L 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 147 ng/ μ L (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 147 ng/ μ L and check the concentration on an Agilent Bioanalyzer DNA 1000 chip. See “[Step 10. Quantify the Library with the Agilent 2100 Bioanalyzer DNA 1000 assay](#)” on page 29. After quantitation, adjust the sample to 147 ng/ μ L.

Alternatively, concentrate a 500 ng aliquot at $\leq 45^{\circ}\text{C}$ down to 3.4 μ L. If the sample dries up completely, resuspend in 3.4 μ L of water and mix on a vortex mixer. If processing multiple samples, adjust to equivalent volumes before concentrating.
- 3** Mix the components in [Table 15](#) at room temperature to prepare the hybridization buffer.

3 Hybridization

Step 1. Hybridize the library

Table 15 Hybridization Buffer

Reagent	Volume for 1 capture (μL), includes excess	Volume for 12 captures (μL), includes excess
SureSelect Hyb # 1	25	250
SureSelect Hyb # 2 (red cap)	1	10
SureSelect Hyb # 3 (yellow cap)	10	100
SureSelect Hyb # 4	13	130
Total	49	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 tubes on ice until [step 10](#).
 - b For each sample, add the amount of SureSelect capture library as listed in [Table 16](#), based on the Mb target size of your design.
 - c Use nuclease-free water to prepare a dilution of the RNase Block (purple cap) as listed in [Table 16](#).
Prepare enough RNase Block dilution for all samples, plus excess.
 - d Add the amount of diluted RNase Block listed in [Table 16](#) to each capture library, and mix by pipetting.

Table 16 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	1:6	5 μL
\geq 3.0 Mb	5 μL	1:2	2 μL

- 6 Mix the contents in [Table 17](#) to make the correct amount of SureSelect Block mix for the number of samples used.

Table 17 SureSelect Block Mix

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

- 7** In a separate PCR plate, prepare the prepped library for target enrichment.
- a** Add 3.4 μ L of 147 ng/ μ L prepped library to the “B” row in the PCR plate. Put each sample into a separate well.
 - b** Add 5.6 μ L 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** Run the following thermal cycler program in [Table 18](#).

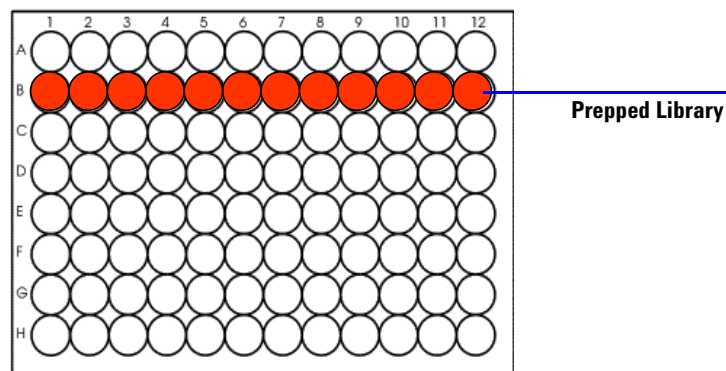


Figure 5 Prepped library shown in red

3 Hybridization

Step 1. Hybridize the library

Table 18 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** 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.

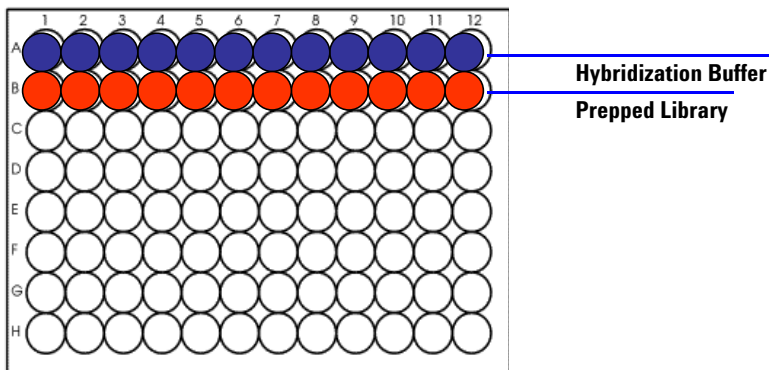


Figure 6 Hybridization buffer shown in blue

Make sure that the plate is 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:
- 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.
 - Seal the wells with strip caps. Use a capping tool to make sure the fit is tight.
 - 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](#).)

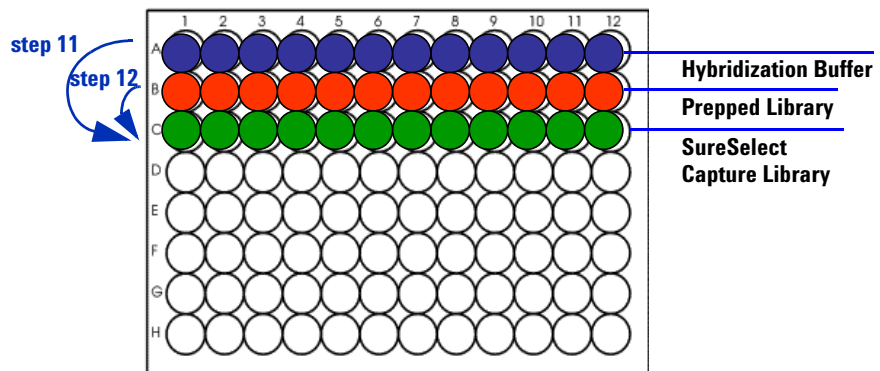


Figure 7 SureSelect Capture Library, or “Baits”, shown in Green

- 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.

3 Hybridization

Step 1. Hybridize the library

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 24 hours at 65°C with a heated lid at 105°C.

Samples may be hybridized for up to 72 hours, but when you hybridize at longer periods, check that there is no extensive evaporation.

Step 2. Prepare magnetic beads

- 1 Prewarm SureSelect Wash Buffer #2 at 65°C in a circulating water bath for use in “Step 3. Select hybrid capture with SureSelect”.
- 2 Vigorously resuspend the Dynal MyOne Streptavidin T1 (Invitrogen) magnetic beads on a vortex mixer. Dynal beads settle during storage.
- 3 For each hybridization, add 50 µL Dynal magnetic beads 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 the Dynal magnetic separator (Invitrogen).
 - 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

- 1** Estimate and record the volume of hybridization that remained after 24 hour incubation.
- 2** Add the hybridization mixture directly from the thermal cycler to the bead solution, and 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 24](#) on page 59 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 Dynal magnetic separator and remove the supernatant.
- 6** Resuspend the beads in 500 μL of SureSelect Wash Buffer #1 by mixing on a vortex mixer for 5 seconds.
- 7** Incubate the samples for 15 minutes at room temperature.
- 8** Briefly spin in a centrifuge.
- 9** Separate the beads and buffer on a Dynal magnetic separator and remove the supernatant.
- 10** Wash the beads:
 - a** Resuspend the beads in 500 μL of prewarmed SureSelect Wash Buffer #2 and mix on a vortex mixer for 5 seconds to resuspend the beads.
 - b** Incubate the samples for 10 minutes at 65°C.
 - c** Briefly spin in a centrifuge.
 - d** Separate the beads and buffer on a Dynal magnetic separator and remove the supernatant.
 - e** Repeat [step a](#) through [step d](#) for a total of 3 washes.
Make sure all of the wash buffer has been removed.
- 11** Mix the beads in 50 μL of SureSelect Elution Buffer on a vortex mixer for 5 seconds to resuspend the beads.
- 12** Incubate the samples for 10 minutes at room temperature.
- 13** Briefly spin in a centrifuge.

Step 3. Select hybrid capture with SureSelect

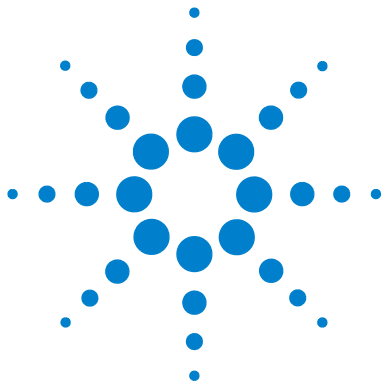
- 14** Separate the beads and buffer on a Dynal magnetic separator.
- 15** Use a pipette to transfer the supernatant to a new 1.5-mL LoBind tube.
The supernatant contains the captured DNA. The beads can now be discarded.
- 16** Add 50 μL of SureSelect Neutralization Buffer to the captured DNA.
- 17** Briefly mix on a vortex mixer.

3 Hybridization

Step 4. Purify the sample using the Agencourt AMPure XP beads

Step 4. Purify the sample using the Agencourt AMPure XP beads

- 1 Let the SPRI 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 180 μL of homogenous SPRI XP beads to a 1.5-mL LoBind tube, and add 100 μL of captured DNA library. 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.
- 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](#) step once.
- 9 Dry the samples on the 37°C heat block for 5 minutes or until the residual ethanol completely evaporates.
- 10 Add 50 μL nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
- 11 Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
- 12 Remove the supernatant ($\sim 50 \mu\text{L}$) to a fresh 1.5-mL LoBind tube. You can discard the beads at this time.



4 Addition of Barcode Tags by Post-Hybridization Amplification

- Step 1. Amplify the captured library to add barcode tags 46
- Step 2. Purify the sample using the Agencourt AMPure XP beads 49
- Step 3. Assess quality with the Agilent 2100 Bioanalyzer High Sensitivity DNA assay 50
- Step 4. Assess the quantity of each index-tagged library by QPCR 52
- Step 5. Pool samples for Multiplexed Sequencing 53
- Step 6. Do an emulsion PCR 55

This chapter describes the steps to add barcode tags by amplification, purify, assess quality and quantity of the libraries, and pool barcoded samples for multiplexed sequencing.



4 Addition of Barcode Tags by Post-Hybridization Amplification

Step 1. Amplify the captured library to add barcode tags

Step 1. Amplify the captured library to add barcode tags

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 For 1 library:

- In a PCR tube, strip tube, or plate, prepare the reaction mix in [Table 19](#), on ice. Mix well by gently pipetting up and down.

2 For multiple libraries:

- a** Prepare the reaction mix in [Table 19](#), on ice. Mix well on a vortex mixer.
- b** Add 46 μL of the reaction mix to each well or tube.
- c** Add 4 μL of the appropriate SureSelect Barcoding Primer from the Agilent SureSelect AB Barcoding Library Kit to each well and mix by pipetting.

Use a different barcode primer for each sample to be sequenced in the same quad. See [Table 20](#) for the optimal number of barcodes per sequencing quad.

- d** Use a pipette to add 50 μL of each DNA sample to each well or tube. Mix by pipetting. Change pipette tips between samples to avoid cross-contamination.

Table 19 Herculase II Master Mix

Reagent	Volume for 1 reaction	Volume for 12 reactions (includes excess)
Captured DNA	50 μ L	
Nuclease-free water	23 μ L	287.5 μ L
5X Herculase II Reaction Buffer *	20 μ L	250 μ L
dNTP mix (25 mM each) *	1 μ L	12.5 μ L
Herculase II Fusion DNA Polymerase	2 μ L	25 μ L
SureSelect Barcoding Primer (BC1 - BC16) †	4	
Total	100 μL	575 μL

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

† Use one of the 16 primers included in the Agilent SureSelect AB Barcoding Library Kit.

4 Addition of Barcode Tags by Post-Hybridization Amplification

Step 1. Amplify the captured library to add barcode tags

Table 20 Optimal number of barcodes per sequencing quad

Capture Size	Optimal number of barcodes per quad*
<0.2 Mb	16
0.2 to 0.49 Mb	12
0.5 to 1.49 Mb	10
1.5 to 2.99 Mb	8
3.0 to 3.3 Mb	6

* Values are approximate and depend on sequencing efficiency and flow cell capacity. These values refer to sequencing on 1 quad of the SOLiD 3 plus system and approximately 25 Mb of high quality uniquely mapped reads or approximately 1 Gb of mapped bases. More samples can be sequenced per quad with higher sequencing capacities.

3 Put the tubes in a thermal cycler and run the program in [Table 21](#).

Table 21 PCR program

Step	Temperature	Time
Step 1	95°C	5 minutes
Step 2	95°C	15 seconds
Step 3	54°C	45 seconds
Step 4	70°C	1 minute
Step 5		Repeat Step 2 through Step 4 , depending on the size of the capture: <ul style="list-style-type: none">• 0.2 Mb to 0.49 Mb: 12 cycles total• 0.5 to 1.49 Mb: 10 cycles total• 1.5 to 2.99 Mb: 9 cycles total• 3 to 3.3 Mb: 8 cycles total
Step 6	70°C	5 minutes
Step 7	4°C	Hold

Step 2. Purify the sample using the Agencourt AMPure XP beads

- 1 Let the SPRI 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 180 μL of homogenous SPRI beads to a 1.5-mL LoBind tube, and add amplified library ($\sim 100 \mu\text{L}$). 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.
- 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 Dry the samples on the 37°C heat block for 5 minutes or until the residual ethanol is completely evaporated.
- 10 Add 50 μL nuclease-free water, mix well on a vortex mixer, and incubate for 2 minutes at room temperature.
- 11 Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
- 12 Remove the supernatant ($\sim 50 \mu\text{L}$) 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 Agilent 2100 Bioanalyzer High Sensitivity DNA assay

Use a Bioanalyzer High Sensitivity DNA Assay to assess the quality and size range. You may need to dilute your sample accordingly.

- 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 primer-dimers are observed in the 50-75bp size range, repeat “[Step 2. Purify the sample using the Agencourt AMPure XP beads](#)” on page 49 with 90 μ L of homogenous SPRI XP Beads, then repeat “[Step 3. Assess quality with the Agilent 2100 Bioanalyzer High Sensitivity DNA assay](#)” on page 50.

Step 3. Assess quality with the Agilent 2100 Bioanalyzer High Sensitivity DNA assay

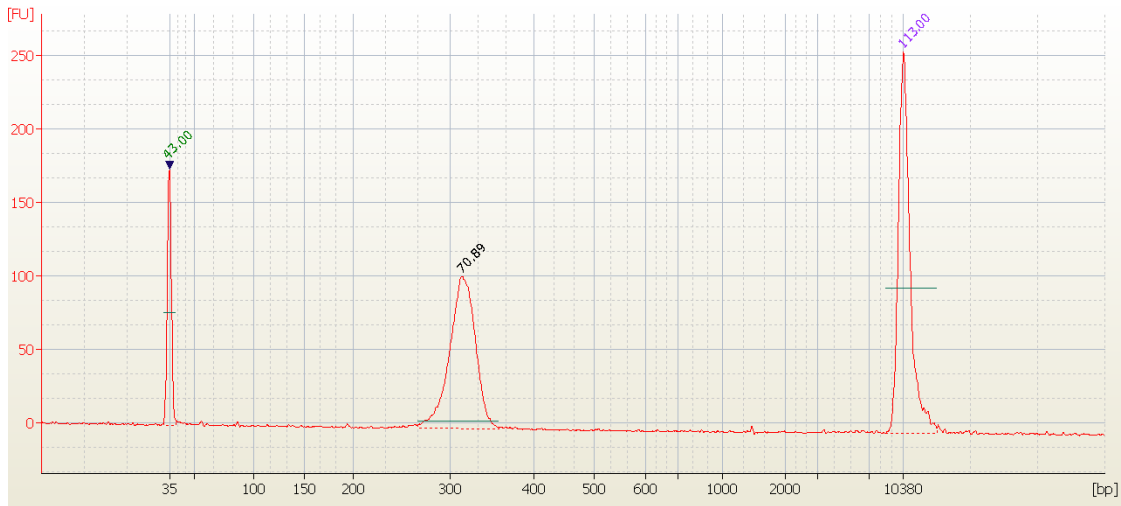


Figure 8 Analysis of Amplified Capture DNA using the High Sensitivity DNA Kit. The electropherogram shows a peak in the size range of approximately 270 to 350 nucleotides.

4 Addition of Barcode Tags by Post-Hybridization Amplification

Step 4. Assess the quantity of each index-tagged library by QPCR

Step 4. Assess the quantity of each index-tagged library by QPCR

Refer to the protocol that is included with the Agilent QPCR NGS Library Quantification Kit (p/n G4881A) for more details to do this step.

- 1 Use the Agilent QPCR NGS Library Quantification Kit (for SOLiD) to determine the concentration of each index-tagged captured library.
- 2 Prepare a standard curve using the quantification standard included in the kit, according to the instructions provided in the user guide.
- 3 Dilute each index-tagged captured library such that it falls within the range of the standard curve.

Typically this corresponds to approximately a 1:1000 to 1:10,000 dilution of the captured DNA.

- 4 Prepare the QPCR master mix with SOLiD adapter-specific PCR primers according to instructions provided in the kit.
- 5 Add an aliquot of the master mix to PCR tubes and add template.
- 6 On a QPCR system, such as the MX3005p, run the thermal profile outlined in the QPCR NGS Library Quantification kit user guide. Use the SYBR Green instrument setting.
- 7 Use the standard curve to determine the concentration of each unknown index-tagged library, in nM.

The concentration will be used to accurately pool samples for multiplexed sequencing.

Step 5. 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.

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

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, 500 pM for the standard SOLiD protocol

$\#$ is the number of barcode tags, and

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

See [Table 22](#) for the approximate volume of sample to use.

Table 22 Approximate volume of sample to use

SOLiD Sequencing Capacity	Approximate Sample Volume Needed	Final Concentration Needed
Octet	20 µL	500 pM
Quad	20 µL	500 pM
Full Slide	50 to 100 µL	500 pM

[Table 23](#) shows an example of the amount of 4 barcoded samples (of different concentrations) and Low TE needed for a final volume of 100 µL at 500 pM.

4 Addition of Barcode Tags by Post-Hybridization Amplification

Step 5. Pool samples for Multiplexed Sequencing

Table 23 Example of barcode volume calculation for a total volume of 100 μ L

Component	V(f)	C(i)	C(f)	#	Volume to use (μ L)
Sample 1	100 μ L	921 pM	500 pM	4	13.6
Sample 2	100 μ L	1050 pM	500 pM	4	11.9
Sample 3	100 μ L	1352 pM	500 pM	4	9.2
Sample 4	100 μ L	684 pM	500 pM	4	18.3
Low TE					47

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 Low TE 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 to 0.1% v/v and store at -20°C short term.

4 Proceed to emulsion PCR. Refer to the appropriate SOLiD protocol.

To get > 90% of targeted bases with a read depth of > 20 times, barcode tags can be blended together and sequenced in one quad (on the SOLiD 3 Plus sequencer or newer). Do not use more than 16 barcode tags per quad for 0.2 Mb libraries and 6 barcode tags for 3 Mb libraries.

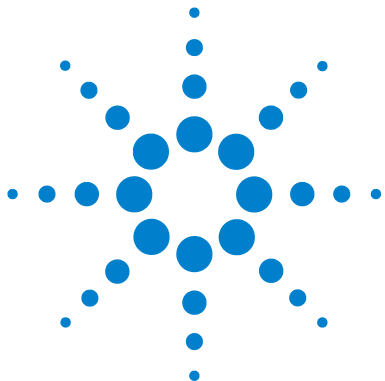
Step 6. Do an emulsion PCR

- Do an emulsion PCR as described in the *Applied Biosystems SOLiD 3 Plus System Templated Bead Preparation Guide* (p/n 4442695).

Stopping Point If you do not do an emulsion PCR at this time, store the DNA in Elution Buffer (E1) at 4°C.

4 Addition of Barcode Tags by Post-Hybridization Amplification

Step 6. Do an emulsion PCR



5 Reference

Alternative Capture Equipment Combinations 58

This chapter contains reference information.



Alternative Capture Equipment Combinations

Table 24 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 24 Tested options that show minimal evaporation

PCR Machine	Plate/Strips	Cover	Comments
Stratagene Mx3005P QPCR	Mx3000P Strip Tubes (401428)	MX3000P Optical Strip Caps (401425)	Heated Lid
Stratagene 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
ABI Veriti (4375786)	Stratagene strip tubes 410022 (Mx4000)	Stratagene Strip cap domed 410096 (Robocycler)	Heated Lid
ABI Veriti (4375786)	Stratagene strip tubes 410022 (Mx4000)	Stratagene Optical cap 401425 (Mx3000/3005)	Heated Lid
BioRad (MJ Research) PTC-200	Stratagene strip tubes 410022 (Mx4000)	Stratagene Optical cap 410024 (Mx4000)	Heated Lid
BioRad (MJ Research) PTC-200	Stratagene strip tubes 410022 (Mx4000)	Stratagene Optical cap 401425 (Mx3000/3005)	Heated Lid
BioRad (MJ Research) PTC-200	Stratagene 96-well Plate 410088 (Mx3000/3005)	Stratagene Optical cap 401425 (Mx3000/3005)	Heated Lid
BioRad (MJ Research) PTC-200	Stratagene 96-well Plate 410088 (Mx3000/3005)	Stratagene Plate sealers 400774-15	Heated Lid 2 layers of plate sealer

5 Reference
Alternative Capture Equipment Combinations

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In This Book

This guide describes Agilent's recommended operational procedures to capture genomic regions of interest using the Agilent [SureSelect Target Enrichment for AB SOLiD Multiplexed Sequencing](#) and sample preparation kits for next-generation sequencing.

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