



SureSelect Target Enrichment System for SOLiD Fragment and Paired-End RNA-Seq Library Prep

SureSelect RNA Capture Kits

Protocol

Version 1.1, May 2011

**SureSelect platform manufactured with Agilent
SurePrint Technology**

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In this Guide...

This guide describes Agilent's recommended operational procedures to capture transcripts of interest using Agilent's SureSelect RNA Capture Kits, and sample preparation kits 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 transcriptome.

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, and quantify the sample library after target enrichment hybridization.

5 Reference

This chapter contains reference information.

What's New in Version 1.1

- Reagent cap colors are listed where available.
- More details given for the reagent kits to use for each step.
- More details given to fragment the RNA.

What's New in Version 1.0.1

- Primers for the PCR Mix were corrected in the cDNA amplification step.

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Before You Begin

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Make sure you read and understand the information in this chapter and have the necessary equipment and reagents 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 RNA 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 RNA 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 RNA 6000 Pico Kit	Agilent p/n 5067-1513
Agilent High Sensitivity DNA Kit	Agilent p/n 5067-4626
Nuclease-free Water (not DEPC-treated)	Ambion Cat #AM9930
SOLiD Total RNA-Seq Kit	Applied Biosystems p/n 4445374
1X Low TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	Applied Biosystems p/n 4389764
Agencourt AMPure XP Kit	Beckman Coulter Genomics
5 mL	p/n A63880
60 mL	p/n A63881
450 mL	p/n A63882
E-Gel SizeSelect 2% Agarose Gel	Invitrogen p/n G6610-02
50-bp ladder	Invitrogen p/n 10416-014
Platinum PCR SuperMix High Fidelity	Invitrogen p/n 12532-016
PureLink PCR Micro Kit	Invitrogen p/n K3100-50
Ribominus Concentration Module (6 preps)	Invitrogen p/n K1550-05
MinElute PCR Purification Kit (50)	Qiagen p/n 28004
100% Ethanol, molecular biology grade	Sigma-Aldrich p/n E7023

1 Before You Begin

Required Reagents

Table 2 Agilent SureSelect RNA Capture Enrichment System Kits (select one)

Reactions	Up to 0.2 Mb (Level MP1)	Up to 0.5 Mb (Level MP2)	Up to 1.5 Mb (Level MP3)	Up to 3.0 Mb (Level MP4)	Up to 6.0 Mb (Level MP0)	Human Kinome
5						G7580A*
10	G7581B*	G7582B*	G7583B*	G7584B*	G7585B*	G7580B*
25	G7581C*	G7582C*	G7583C*	G7584C*	G7585C*	G7580C*
50	G7581D*	G7582D*	G7583D*	G7584D*	G7585D*	G7580D*
100	G7581E*	G7582E*	G7583E*	G7584E*	G7585E*	G7580E*
250	G7581F*	G7582F*	G7583F*	G7584F*	G7585F*	G7580F*
500	G7581G*	G7582G*	G7583G*	G7584G*	G7585G*	G7580G*
1,000	G7581H*	G7582H*	G7583H*	G7584H*	G7585H*	G7580H*
2,000	G7581J*	G7582J*	G7583J*	G7584J*	G7585J*	G7580J*
5,000	G7581K*	G7582K*	G7583K*	G7584K*	G7585K*	G7580K*
10,000	G7581L*	G7582L*	G7583L*	G7584L*	G7585L*	G7580L*

* Order option 002.

Table 3 Required Reagents for Hybridization

Description	Vendor and part number
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

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	Applied Biosystems Veriti Thermal Cycler, BioRad (MJ Research) DNA Engine PTC-200, or equivalent
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
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 plates	
PCR strip caps or equivalent	
Sterile, nuclease-free aerosol barrier pipette tips	
Timer	
Vortex mixer	
Heat block at 37°C	

1 Before You Begin Optional Equipment

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
PCR plates	
PCR strip caps or equivalent	
Sterile, nuclease-free aerosol barrier pipette tips	
Thermal cycler	Applied Biosystems 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 6 Optional Equipment for Hybridization

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



2 Sample Preparation

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



2 Sample Preparation

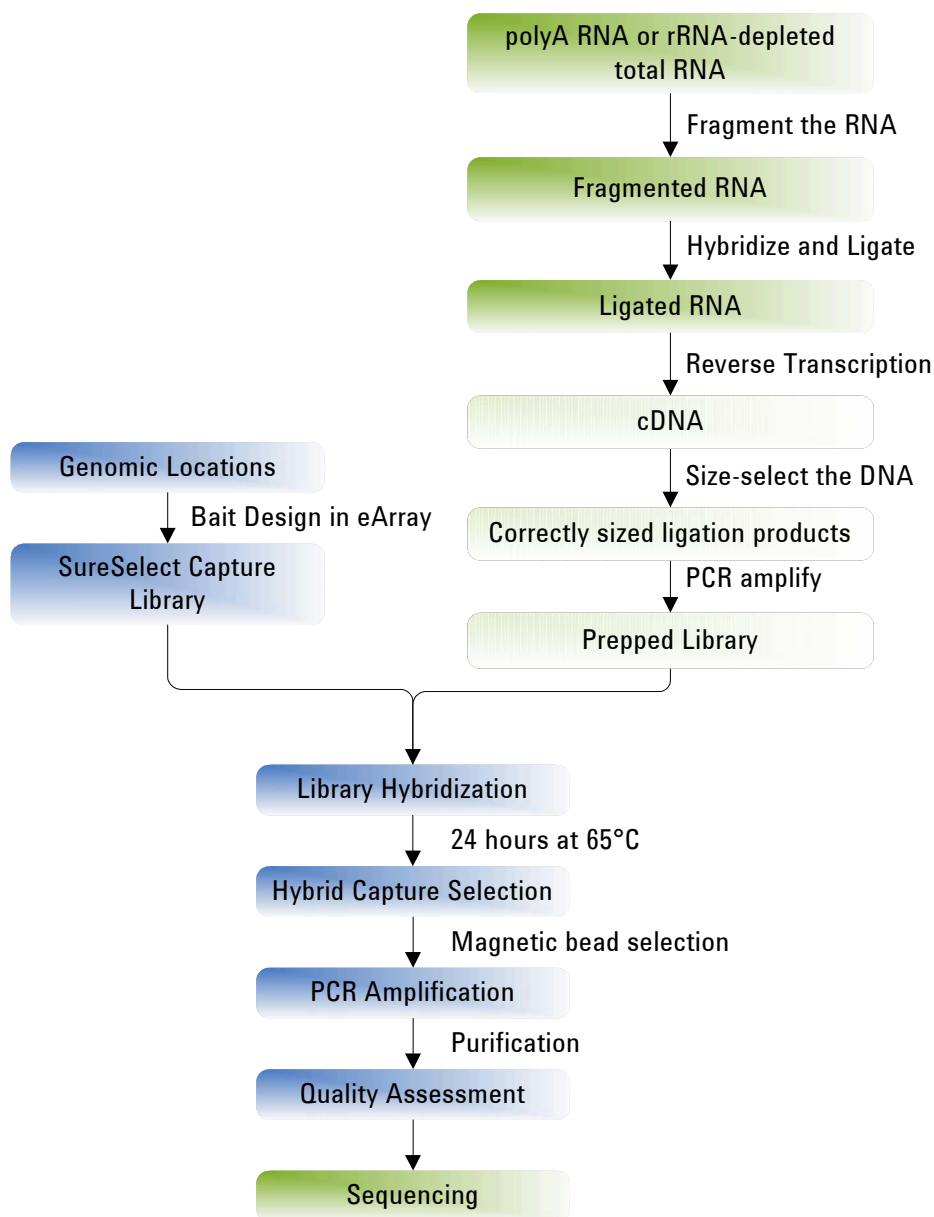


Figure 1 Overall sequencing sample preparation workflow.

Table 7 Overview and time requirements

Step	Time
AB SOLiD Fragment Library Production	2 days
Library Hybridization	25 hours (optional 72 hours)
Bead preparation	30 minutes
Capture Selection and Washing	2 hours
DNA Purification	30 minutes
Post-Hybridization Amplification	1 hour
PCR Purification	30 minutes
Bioanalyzer QC	1 hour

Step 1. Fragment the RNA

Use the [SOLiD Total RNA-Seq Kit](#) (Applied Biosystems p/n 4445374).

1 Prepare your RNA sample.

For poly(A) RNA:

- Do two rounds of oligo(dT) selection of the poly(A) RNA. For example, use the Applied Biosystems MicroPoly(A)Purist™ Kit.
- Confirm the absence of 18S and 28S rRNA. For example, check the profile of the poly(A) RNA on an Agilent 2100 Bioanalyzer.

For rRNA-depleted total RNA:

- Remove rRNA from total RNA for transcriptome analysis. Use the Invitrogen RiboMinus Eukaryote Kit for RNA-Seq or the Invitrogen RiboMinus Plant Kit for RNA-Seq.

Use only high-quality RNA as your starting material. FirstChoice Total RNA and Poly(A) RNA provide high-quality, intact RNA isolated from a variety of sources.

2 For each RNA reaction, mix the components on ice.

Table 8 Fragmentation Mix

Component	Volume
RNA Sample:	
Poly(A) RNA: 100 to 500 ng <i>or</i>	100 ng to 500 ng
rRNA-depleted total RNA	250 to 500 ng
WT Control RNA	0.5 µL
Nuclease-free water	Enough to complete 8 µL total with RNA sample and WT Control RNA
10X RNase III Buffer	1 µL
RNase III	1 µL
Total	10 µL

3 Gently tap the tube, or pipette the mix up and down to mix, then spin briefly.

- 4** Incubate the reaction in a preheated PCR thermal cycler at 37°C for 10 minutes.
- 5** Immediately add 90 µL of nuclease-free water, then put the fragmented RNA on ice.

Continue to the next step immediately, or leave the fragmented RNA on ice for one hour or less.

2 Sample Preparation

Step 2. Purify the RNA with the Ribominus Concentration Module

Step 2. Purify the RNA with the Ribominus Concentration Module

Use reagents from the [RiboMinus Concentration Module \(Invitrogen p/n K1550-05\)](#):

- [Wash Buffer \(W5\)](#)
- [Binding Buffer \(L3\)](#)
- [RNase-Free Water](#)

- 1 Mix the components in [Table 9](#) to prepare the Wash Buffer (W5), then store at room temperature.

Table 9 Wash Buffer Mix

Component	Volume
100% ethanol	6 mL
Wash Buffer (W5)	1.5 mL
Total	7.5 mL

- 2 Add the components in [Table 10](#) to the fragmented RNA and mix.

Table 10

Component	Volume
Binding Buffer (L3)	100 μ L
100% ethanol	250 μ L

- 3 Put the spin column in a clean 1.5-mL wash tube.
- 4 Put 450 μ L of the RNA sample that contains [Binding Buffer \(L3\)](#) and ethanol onto the spin column.
- 5 Spin the column in a centrifuge at $12,000 \times g$ for 1 minute.
- 6 Discard the flow-through.
- 7 Return the spin column to the same wash tube.

Step 2. Purify the RNA with the Ribominus Concentration Module

- 8** Add 500 μ L of **Wash Buffer (W5)** with ethanol to the spin column.
 - 9** Spin the column in a centrifuge at $12,000 \times g$ for 1 minute.
 - 10** Discard the flow-through.
 - 11** Return the spin column to the same wash tube.
 - 12** Spin the column in a centrifuge at $20,000 \times g$ or higher for 2 minutes.
 - 13** Put the spin column in a clean recovery tube.
 - 14** Add 12 μ L of the **RNase-Free Water** to the center of the spin column.
 - 15** Wait 1 minute, then spin the column at $20,000 \times g$ or higher for 1 minute.
- Typical recovery of fragmented RNA is approximately 10 μ L.

Step 3. Quantitate and assess size distribution

Use the Quant-iT RNA Assay Kit with the Qubit Fluorometer (Invitrogen) and the RNA 6000 Pico Chip Kit with the Agilent 2100 Bioanalyzer (Agilent).

- 1** Quantitate the yield of fragmented RNA using the Quant-it RNA Assay Kit with the Qubit Fluorometer or a Nanodrop Spectrophotometer
- 2** Dilute 1 μL of the sample 1:10 with nuclease-free water.
- 3** Open the Agilent 2100 Expert Software (version B.02.07 or higher), turn on the 2100 Bioanalyzer and check communication.
- 4** Prepare the chip, samples and ladder as instructed in the reagent kit guide.
- 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 around 100 to 200 nt.
- 9** If the amount of fragmented RNA in 3 μL is:
 - <50 ng poly (A) RNA
 - <250 ng rRNA-depleted total RNAthen:
 - a** Dry 50 to 250 ng of the RNA completely in a centrifugal vacuum concentrator at low or medium heat ($\leq 40^{\circ}\text{C}$). This process takes 10 to 20 minutes.
 - b** Resuspend in 3 μL nuclease-free water.

See [Figure 2](#) for typical results of fragmentation of whole transcriptome RNA.

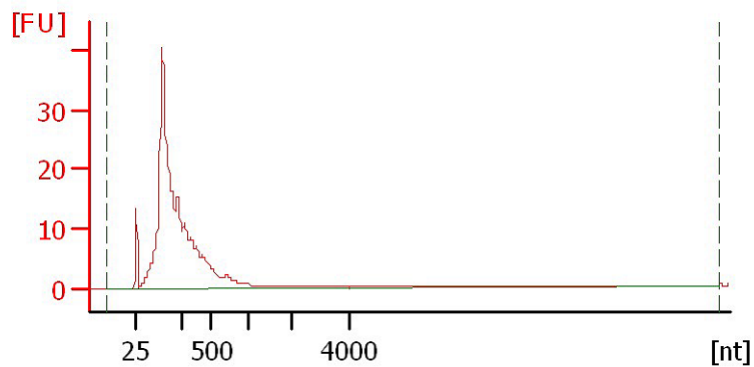


Figure 2 Size distribution of fragmented rRNA-depleted total RNA

Step 4. Hybridize and ligate the RNA

Use the SOLiD Total RNA-Seq Kit (Applied Biosystems p/n 4445374).

- SOLiD Adaptor Mix
- Hybridization Solution
- Nuclease-free Water
- 2X Ligation Buffer
- Ligation Enzyme Mix

1 Prepare the Hybridization Master Mix in [Table 11](#) on ice.

Table 11 Hybridization Master Mix

Component	Volume per reaction*
SOLiD Adaptor Mix	2 μ L
Hybridization Solution	3 μ L
Total volume per reaction	5 μL

* To prepare for multiple samples, include 5% to 10% excess volume.

2 Transfer 5 μ L hybridization master mix to 3 μ L fragmented RNA sample:

- Fragmented poly(A) RNA: 50 ng
- Fragmented rRNA-depleted total RNA: up to 250 ng
- Fragmented WT Control RNA: 100 ng

3 Slowly pipet up and down a few times to mix well, then spin briefly.

4 Run the hybridization reaction in a thermal cycler:

Table 12 PCR program

Temperature	Time
65°C	10 minutes
16°C	5 minutes

- 5 Add the RNA ligation reagents to each 8- μ L hybridization reaction in the order listed in Table 13:

Table 13 RNA ligation reagents

Component	Volume
2X Ligation Buffer	10 μ L
Ligation Enzyme Mix	2 μ L

NOTE

If you see white precipitate in the 2X Ligation Buffer, warm the tube at 37°C for 2 to 5 minutes or until the precipitate is dissolved. 2X Ligation Buffer is very viscous. Pipette slowly to dispense it accurately.

- 6 Flick the tube, or slowly pipet up and down a few times, to mix well, then spin briefly.
- 7 Incubate the 20- μ L ligation reaction in a thermal cycler at 16 °C for 16 hours.

If possible, set the temperature of the thermal cycler lid to match the block temperature. Otherwise, incubate the reaction with the heated lid turned off, or do not cover the reaction tubes with the heated lid.

Step 5. Reverse transcription

Use the SOLiD Total RNA-Seq Kit (Applied Biosystems p/n 4445374).

- Nuclease-free Water
- 10X RT Buffer
- dNTP Mix
- SOLiD RT Primer
- ArrayScript Reverse Transcriptase

- 1 Prepare RT Master Mix in Table 14. Do not add the ArrayScript Reverse Transcriptase at this time.

Table 14 RT Master Mix

Component	Volume per reaction*
Nuclease-free Water	11 µL
10X RT Buffer	4 µL
dNTP Mix	2 µL
SOLiD RT Primer	2 µL
Total volume per reaction	19 µL

* To prepare for multiple samples, include 5% to 10% excess volume.

- 2 Incubate the RT master mix with the ligated RNA sample:
 - a Add 19 µL of RT master mix to each 20-µL ligation reaction.
 - b Pipette up and down a few times to mix, then spin briefly.
 - c Incubate in a thermal cycler with a heated lid at 70 °C for 5 minutes, then snap-cool on ice.

- 3** Do the reverse transcription reaction:
- a** Add 1 μ L **ArrayScript Reverse Transcriptase** to each ligated RNA sample.
 - b** Mix gently but thoroughly on a vortex mixer, then spin briefly.
 - c** Incubate in a thermal cycler with a heated lid at 42 °C for 30 minutes.

If not used immediately, store the cDNA at -20 °C for up to a few weeks, or -80°C long-term.

Step 6. Purify the cDNA

Use the [MinElute PCR Purification Kit](#) (Qiagen p/n 28004 or 28006):

- [Buffer PBI](#)
- [MinElute Spin Column](#)
- [Buffer PE](#)
- [Buffer EB](#)

1 Add nuclease-free water and [Buffer PBI](#) to the cDNA:

- a** Transfer all of the cDNA (40 μ L) to a clean 1.5-mL microcentrifuge tube.
- b** Add 60 μ L of nuclease-free water.
- c** Add 500 μ L of [Buffer PBI](#), then mix well.

You do not need to add pH Indicator to [Buffer PBI](#) before use.

2 Load the cDNA onto the [MinElute Spin Column](#):

- a** Load 600 μ L of the sample containing [Buffer PBI](#) onto the MinElute column.
- b** Spin the column at $13,000 \times g$ for 1 minute.
- c** Discard the supernatant.

3 Wash the cDNA:

- a** Return the [MinElute Spin Column](#) to the microcentrifuge tube.
- b** Add 750 μ L of [Buffer PE](#) to the [MinElute Spin Column](#).
- c** Spin the column at $13,000 \times g$ for 1 minute.
- d** Discard the supernatant.
- e** Return the [MinElute Spin Column](#) to the microcentrifuge tube.
- f** Spin the column at $13,000 \times g$ for 1 minute.

4 Elute the cDNA in a clean microcentrifuge tube:

- a** Place the [MinElute Spin Column](#) in a clean microcentrifuge tube.
- b** Add 20 μ L of [Buffer EB](#) to the center of the [MinElute Spin Column](#).
- c** Wait 1 minute, then spin the column at $13,000 \times g$ for 1 minute.

Step 7. Size-select the DNA fragments with a E-Gel SizeSelect 2% Agarose gel

Step 7. Size-select the DNA fragments with a E-Gel SizeSelect 2% Agarose gel

Use the E-Gel SizeSelect 2% Agarose gel (Invitrogen p/n G6610-02).

- 1 Remove a E-Gel SizeSelect 2% Agarose 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 a well in the *top row*. Do not use the center well or outermost wells (to avoid edge effects). Do not load more than 1 μg of DNA.
If the sample volume < 20 μL , add nuclease-free water to the well for a total volume of 20 μL .
 - 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 3](#)).
- 6 Collect the solution from the sample well.
- 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](#) for a total of 50 μL .

See [Table 15](#) for expected lengths of the insert and PCR according to the excised cDNA length.

2 Sample Preparation

Step 7. Size-select the DNA fragments with a E-Gel SizeSelect 2% Agarose gel

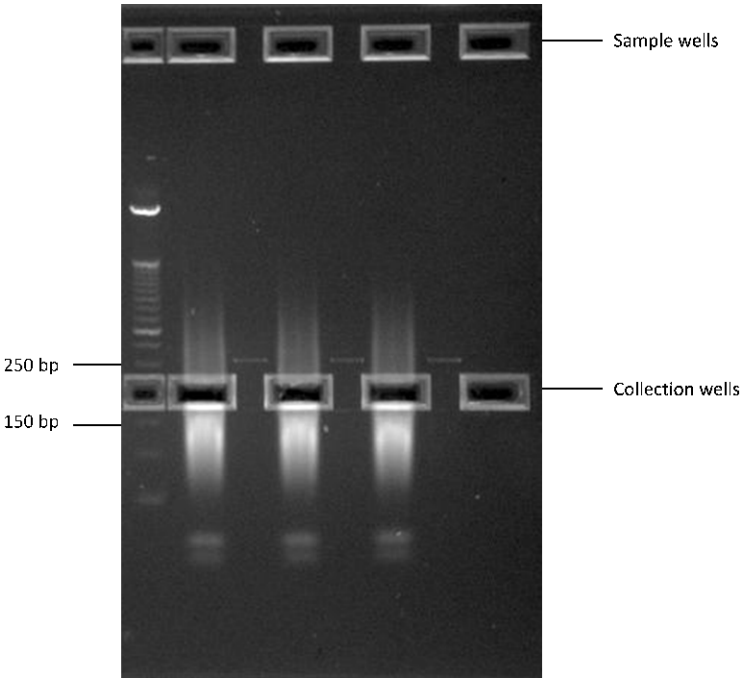


Figure 3 Elution of an approximately 200 bp region from SOLiD Library Size Selection gel. This image shows three samples on the same gel.

Table 15 Expected lengths of the insert and PCR according to the excised cDNA length

Excised cDNA length (nt)	Insert length (bp)	PCR product length (bp)
50	~0	~100
100	~50	~150
150	~100	~200
200	~150	~250
250	~200	~300

Step 8. Purify the size-selected cDNA

Use the [PureLink PCR Micro Kit \(Invitrogen p/n K3100-01\)](#):

- [PureLink PCR Spin Column](#)
- [PureLink PCR Collection Tube](#)
- [Binding Buffer \(B2\)](#)
- [Wash Buffer \(W1\)](#)
- [PureLink Elution Tube](#)
- [Elution Buffer; 10 mM Tris-HCl, pH 8.5 \(E1\)](#)

CAUTION

Do not use other PCR purification kits. Other purification kits are not as effective in the removal of unincorporated primers. Unincorporated primers can affect the final quantitation and emulsion PCR.

- 1 Prepare the sample:
 - a Add 50 μ L of nuclease-free water to the sample for a total of approximately 100 μ L.
 - b Add 400 μ L of [Binding Buffer \(B2\)](#) to the tube, then mix well.
- 2 Load the sample onto the [PureLink PCR Spin Column](#):
 - a Place the [PureLink PCR Spin Column](#) in a clean collection tube.
 - b Load 500 μ L of the sample containing [Binding Buffer \(B2\)](#) onto the column.
 - c Spin the column at $10,000 \times g$ for 1 minute.
 - d Discard the supernatant.
- 3 Wash the DNA:
 - a Return the column to the collection tube.
 - b Add 600 μ L of [Wash Buffer \(W1\)](#) to the column.
 - c Spin the column at $10,000 \times g$ for 1 minute.
 - d Discard the supernatant.
 - e Return the column to the collection tube.
 - f Spin the column at $14,000 \times g$ for 1 minute.

2 Sample Preparation

Step 8. Purify the size-selected cDNA

- 4 Elute the DNA in a clean **PureLink PCR Spin Column**:
 - a Place the column in a clean **PureLink PCR Spin Column**.
 - b Add 10 μ L of **Elution Buffer; 10 mM Tris-HCl, pH 8.5 (E1)** to the center of the membrane.
 - c Wait 1 minute, then spin the column at $14,000 \times g$ for 1 minute.

Step 9. Amplify the cDNA

Use the SOLiD Total RNA-Seq Kit (Applied Biosystems p/n 4445374).

- Nuclease-free Water
- 10X PCR Buffer
- dNTP Mix
- SOLiD 5' PCR Primer
- SOLiD 3' PCR Primer
- AmpliTaq DNA Polymerase

- 1 For each cDNA sample, prepare duplicate amplification reactions to generate sufficient cDNA for enrichment:

Table 16 PCR Mix

Component	Volume for one 100- μ L reaction	Volume for two 100- μ L reaction*
Nuclease-free Water	74.8 μ L	164.6 μ L
10X PCR Buffer	10.0 μ L	22.0 μ L
dNTP Mix	8.0 μ L	17.6 μ L
SOLiD 5' PCR Primer	2.0 μ L	4.4 μ L
SOLiD 3' PCR Primer	2.0 μ L	4.4 μ L
AmpliTaq DNA Polymerase	1.2 μ L	2.6 μ L
Total volume per reaction	98.0 μL	215.6 μL

* To prepare for multiple samples, include 5% to 10% excess volume.

- d Transfer 98 μ L PCR mix into each 0.2-mL PCR tube.
 - e Add 2 μ L of cDNA and mix thoroughly.
- 2 Run the PCR reactions in a thermal cycler as listed in Table 17.

2 Sample Preparation
Step 9. Amplify the cDNA

Table 17 PCR Program

Step	Temp	Time
Hold	95 °C	5 minutes
Cycle (15 cycles)	95 °C	30 seconds
	54 °C	30 seconds
	72 °C	30 seconds
Hold	72 °C	7 minutes

Run 15 cycles if you start with 50 to 100 ng of fragmented RNA. If needed, adjust the number of cycles according to the amount of input fragmented RNA, but for optimal results run between 14 and 18 cycles. Too many cycles results in overamplification and changes the expression profile.

Step 10. Purify the amplified DNA

Use the [PureLink PCR Micro Kit](#) (Invitrogen p/n K3100-50):

- [Binding Buffer \(B2\)](#)
- [Wash Buffer \(W1\)](#)
- [Elution Buffer \(E5\)](#) (10 mM Tris-HCl, pH 8.5)
- [PureLink Micro Kit Column](#)
- [PureLink Elution Tube](#)
- [Collection Tube](#)

CAUTION

Do not use other PCR purification kits. Other purification kits are not as effective in the removal of unincorporated primers. Unincorporated primers can affect the final quantitation and emulsion PCR.

- 1 Prepare the sample:
 - a Combine the two 100- μ L PCR reactions in a new 1.5-mL tube.
 - b Add 800 μ L of [Binding Buffer \(B2\)](#) to the tube, then mix well.
- 2 Load the sample onto the [PureLink Micro Kit Column](#):
 - a Place the [PureLink Micro Kit Column](#) in a clean collection tube.
 - b Load 500 μ L of the sample containing [Binding Buffer \(B2\)](#) onto the column.
 - c Spin the column at $10,000 \times g$ for 1 minute.
 - d Discard the supernatant.
 - e Load the remaining 500 μ L of the sample containing [Binding Buffer \(B2\)](#) onto the column.
 - f Spin the column at $10,000 \times g$ for 1 minute.
 - g Discard the supernatant.

2 Sample Preparation

Step 10. Purify the amplified DNA

- 3 Wash the DNA:
 - a Return the column to the collection tube.
 - b Add 600 μ L of **Wash Buffer (W1)** to the column.
 - c Spin the column at $10,000 \times g$ for 1 minute.
 - d Discard the supernatant.
 - e Return the column to the collection tube.
 - f Spin the column at $14,000 \times g$ for 1 minute.
- 4 Elute the DNA in a clean **PureLink Elution Tube**:
 - a Place the column in a clean **PureLink Elution Tube**.
 - b Add 10 μ L of **Elution Buffer (E5)** (10 mM Tris-HCl, pH 8.5) to the center of the membrane.
 - c Wait 1 minute, then spin the column at $14,000 \times g$ for 1 minute.

Step 11. Quantify the Library with the Agilent 2100 Bioanalyzer DNA 1000 assay

- 1 Measure the concentration of the purified DNA with a NanoDrop spectrophotometer. If needed, dilute the DNA to <50 ng/ μ L for accurate quantitation with the DNA 1000 Kit.
- 2 Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
- 3 Open the Agilent 2100 Expert Software (version B.02.07 or higher), turn on the 2100 Bioanalyzer and check communication.
- 4 Prepare the chip, samples and ladder as instructed in the reagent kit guide. Use 1 μ L of sample.
- 5 Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
- 6 Start the run. Enter sample names and comments in the Data and Assay context.
- 7 Within the instrument context, choose the smear analysis to quantify the percentage of DNA that is 25 to 200 bp.
If the percentage of DNA is less than 20%, refer to the *SOLiD Total RNA-Seq Kit Protocol* for troubleshooting information.
- 8 Determine the median peak size (bp) and molar concentration (nM) of the cDNA library.

The mass concentration of the cDNA must be <50 ng/ μ L for accurate quantitation with the DNA 1000 Kit.

2 Sample Preparation

Step 11. Quantify the Library with the Agilent 2100 Bioanalyzer DNA 1000 assay

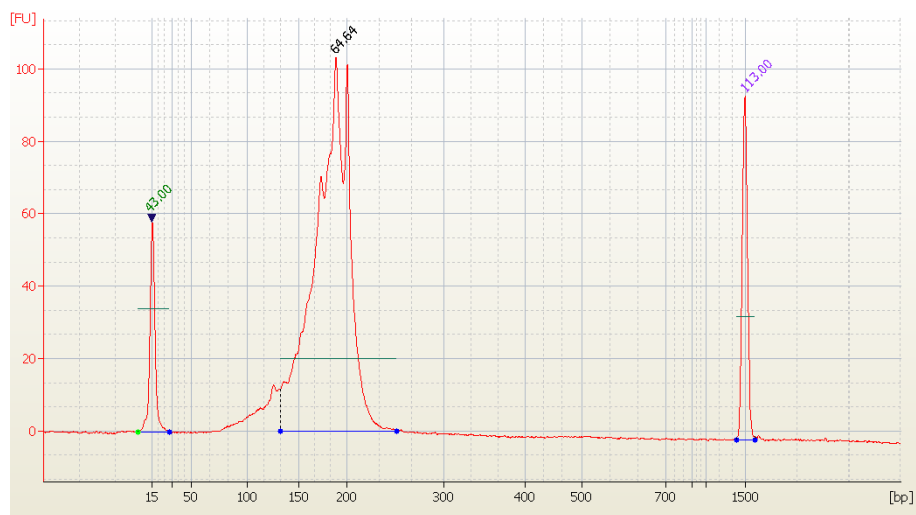


Figure 4 Analysis of amplified prepped library DNA using a DNA 1000 assay. The electropherogram shows a single peak in the size range of approximately 250bp \pm 10%.

- 9 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 (100 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 40
- Step 2. Prepare magnetic beads 46
- Step 3. Select hybrid capture with SureSelect 47
- Step 4. Purify the sample using Agencourt AMPure XP beads 49

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

CAUTION

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



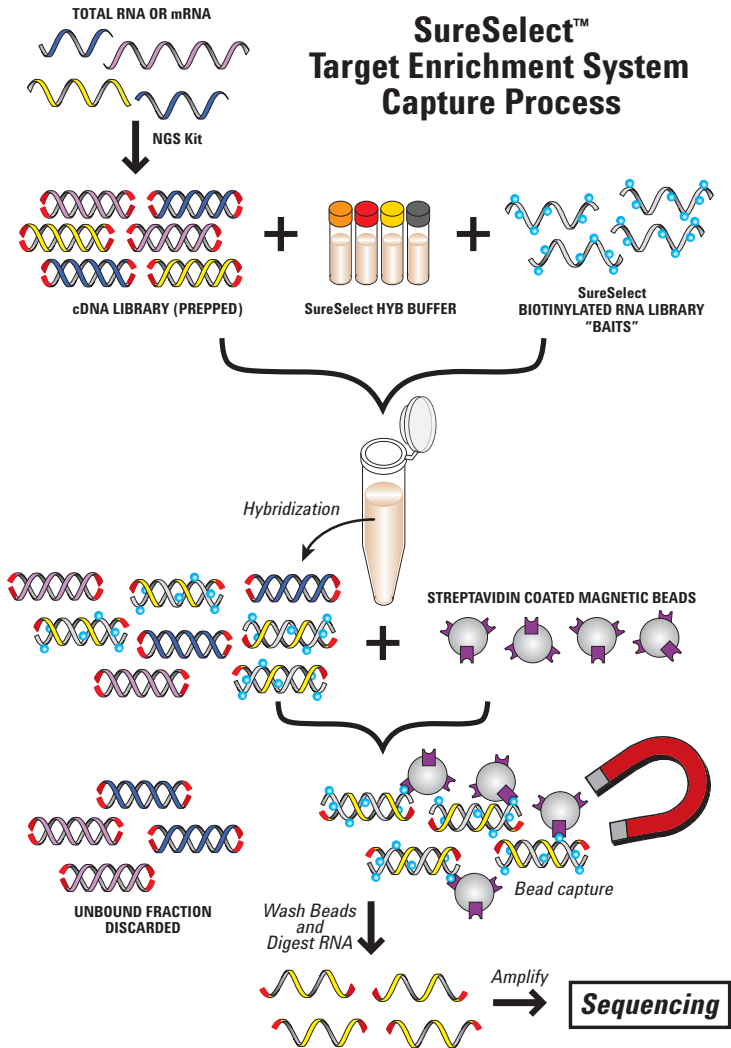


Figure 5 SureSelect RNA Capture Process

Refer to “[SureSelect RNA Capture Kit Content](#)” on page 58 for a complete content listing of each SureSelect RNA Capture kit.

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 63.

Step 1. Hybridize the library

The hybridization reaction requires 100 ng of cDNA with a maximum volume of 3.4 μ L.

- 1** If the prepped library concentration is below 30 ng/ μ L, use a vacuum concentrator to concentrate the sample at $\leq 45^{\circ}\text{C}$.
 - a** Add the entire volume 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 30 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 30 ng/ μ L and check the concentration on an Agilent Bioanalyzer DNA 1000 chip. See [“Step 11. Quantify the Library with the Agilent 2100 Bioanalyzer DNA 1000 assay”](#) on page 35. After quantitation, adjust the sample to 30 ng/ μ L.

Alternatively, concentrate a 100 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 18](#) at room temperature to prepare the hybridization buffer.

Table 18 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 needed)	490 (40 μL/sample)

- 4 If precipitate forms, warm the hybridization buffer at 65°C for 5 minutes.
- 5 In a PCR plate, strip tubes, or tubes, prepare the SureSelect capture library mix for target enrichment:
 - a Keep tubes on ice until [step 10](#).
 - b For each sample, add 5 μL of SureSelect capture library.
 - c For 1 library, combine 1 μL [SureSelect RNase Block \(purple cap\)](#) with 2 μL nuclease-free water. For multiple libraries, use 1 part [SureSelect RNase Block \(purple cap\)](#) to 2 parts nuclease-free water to make enough mix for 2 μL per capture library, plus excess.
 - d Add 2 μL of diluted [SureSelect RNase Block \(purple cap\)](#) to each capture library, and mix by pipetting.
- 6 Mix the contents in [Table 19](#) to make the correct amount of SureSelect Block mix for the number of samples used.

Table 19 SureSelect Block Mix

Reagent	Volume for 1 reaction	Volume for 12 reactions (includes excess)
SureSelect Block #1 (green cap)	2.5 µL	31.25 µL
SureSelect Block #2 (blue cap)	2.5 µL	31.25 µL
SureSelect Block #3-AB (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 30 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 Run the following thermal cycler program in [Table 20](#).

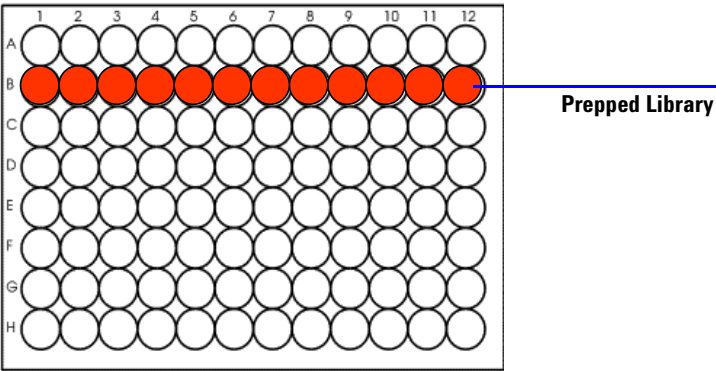


Figure 6 Prepped library shown in red

Table 20 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 7](#) is for 12 captures.

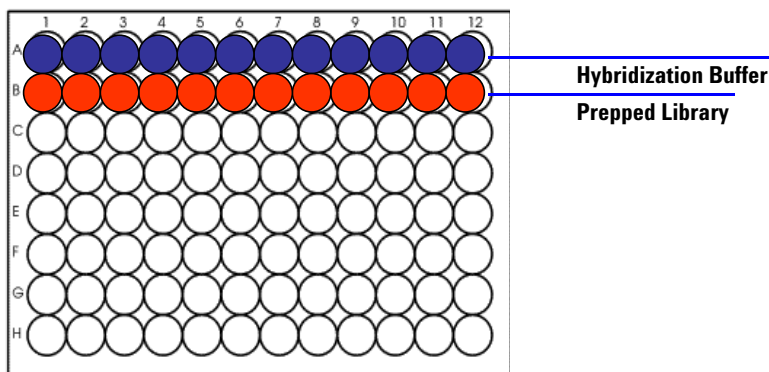


Figure 7 Hybridization buffer shown in blue

Make sure that the plate is at 65°C for a minimum of 5 minutes before you go to [step 10](#).

3 Hybridization

Step 1. Hybridize the library

- 10** Add the capture library mix from [step 5](#) to the PCR plate:
- a** Add the capture library mix (7 μ L) 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 μ L 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 8](#).)

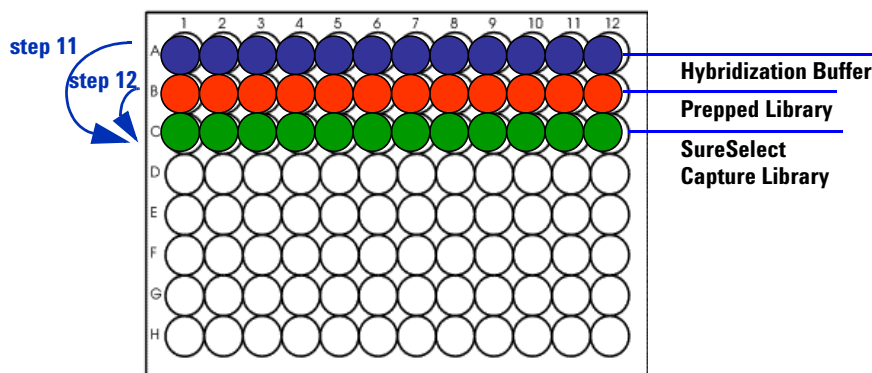


Figure 8 SureSelect Capture Library, or “Baits”, shown in Green

- 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 8](#).) Mix well by slowly pipetting up and down 8 to 10 times.
The hybridization mixture is now 27 to 29 μ L, 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.

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, do a test to make sure that evaporation is not extensive.

Step 2. Prepare magnetic beads

Use these reagents from the [SureSelect Target Enrichment Kit Box #1](#):

- [SureSelect Binding Buffer](#)
- [SureSelect Wash Buffer #2](#)

- 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

Use these reagents from the [SureSelect Target Enrichment Kit Box #1](#):

- [SureSelect Wash Buffer #1](#)
- [SureSelect Wash Buffer #2](#)
- [SureSelect Elution Buffer](#)
- [SureSelect Neutralization Buffer](#)

- 1 Estimate and record the volume of hybridization that remained after 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 33](#) 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 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 Separate the beads and buffer on a Dynal magnetic separator and remove the supernatant.

3 Hybridization

Step 3. Select hybrid capture with SureSelect

9 Wash the beads:

a Resuspend the beads in 500 μ L of 65°C 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 in a recirculating water bath, heat block or equivalent.

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

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

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.

10 Mix the beads in 50 μ L of **SureSelect Elution Buffer** on a vortex mixer for 5 seconds to resuspend the beads.

11 Incubate the samples for 10 minutes at room temperature.

12 Separate the beads and buffer on a Dynal magnetic separator.

13 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.

14 Add 50 μ L of **SureSelect Neutralization Buffer** to the captured DNA.

Step 4. 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 180 μ L of homogenous AMPure XP beads to a 1.5-mL LoBind tube, and add the sample library (\sim 100 μ 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 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](#) step once.
- 9 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.
- 10 Add 17 μ L 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 15 μ 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.

3 Hybridization

Step 4. Purify the sample using Agencourt AMPure XP beads



4 Post-Hybridization Amplification

- Step 1. Amplify the sample [52](#)
- Step 2. Purify the sample using Agencourt AMPure XP beads [53](#)
- Step 3. Quantify the library with the Agilent 2100 Bioanalyzer DNA 1000 assay [54](#)
- Step 4. Do an emulsion PCR [56](#)

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



4 Post-Hybridization Amplification

Step 1. Amplify the sample

Step 1. Amplify the sample

1 Prepare the PCR reaction mix (see [Table 21](#)).

Table 21 PCR reaction mix

Component	Volume (μL)
Platinum PCR SuperMix High Fidelity	100
SureSelect- Post Capture PCR Primers (clear cap) *	5
Eluted DNA	15
Total	120

* Included in the [SureSelect Target Enrichment Kit AB Box #2](#)

2 Run the PCR program in [Table 22](#).

Table 22 PCR Conditions

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

CAUTION

Minimize the amount of cycling to avoid overamplification and production of redundant molecules.

Make sure you add to the Wash Buffer the correct amount of ethanol, which is listed on the Wash Buffer bottle.

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 180 μL of homogenous AMPure beads to a 1.5-mL LoBind tube, and add the sample 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.

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.
- 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 purified DNA in Elution Buffer (E1) at 4°C.

Step 3. Quantify the library with the Agilent 2100 Bioanalyzer DNA 1000 assay

- 1 Run a Bioanalyzer DNA 1000 assay to confirm and quantitate DNA amplification. See “Step 10. Quantify the Library with the Agilent 2100 Bioanalyzer DNA 1000 assay” on page 31.
- 2 If amplification products are visible, continue at [step 5](#).
- 3 If amplification products are not visible, run a Bioanalyzer High Sensitivity DNA assay to confirm and quantitate DNA amplification.

Typically, amplification products are visible when you use the Bioanalyzer DNA 1000 assay.

You can also use the Bioanalyzer High Sensitivity DNA assay in conjunction with a lower number of post-hybridization amplification cycles.
- 4 If amplification products are still not visible:
 - a Add the components in [Table 21](#) on page 52 with the entire amount of eluate from “Step 2. Purify the sample using Agencourt AMPure XP beads” on page 53.
 - b Run the PCR cycling program in [Table 23](#).
 - c Repeat “Step 2. Purify the sample using Agencourt AMPure XP beads” on page 53.
 - d Run another Bioanalyzer High Sensitivity DNA assay to confirm and quantitate DNA amplification.
 - e Repeat [step a](#) through [step d](#) if needed, until amplification is observed.

Do not overamplify the sample. When you see even trace amplification, continue at “Step 4. Do an emulsion PCR” on page 56.

Table 23 PCR conditions

Stage	Step	Temp	Time
Cycling (2 cycles)	Denature	95 °C	15 sec
	Anneal	54 °C	15 sec
	Extend	70 °C	1 min
Holding	—	4 °C	∞

Step 3. Quantify the library with the Agilent 2100 Bioanalyzer DNA 1000 assay

5 *Optional.* Quantify the library by quantitative PCR.

For a TaqMan or SYBR qPCR protocol, see Appendix B of the *Applied Biosystems SOLiD 3 Plus System Library Preparation Guide* (p/n 4442697).

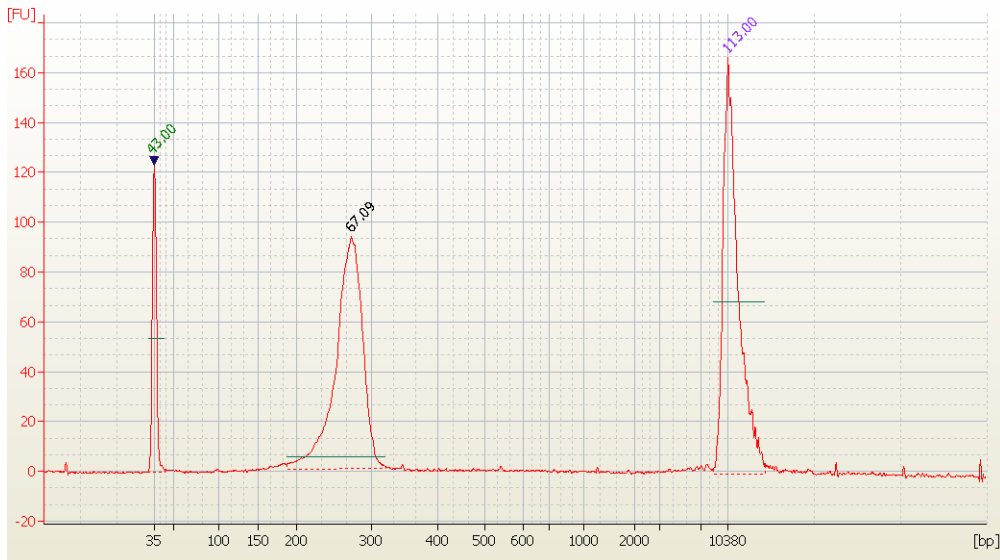


Figure 9 Analysis of amplified post-hybridized library DNA using a DNA 1000 assay. The electropherogram shows a single peak in the size range of approximately 275bp \pm 20%.

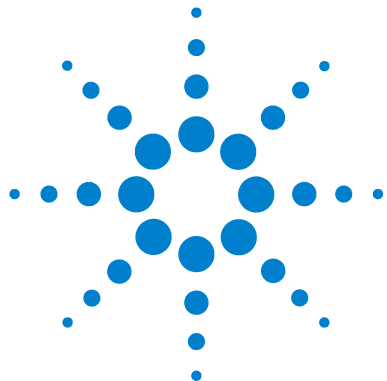
4 Post-Hybridization Amplification

Step 4. Do an emulsion PCR

Step 4. 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.



5 Reference

SureSelect RNA Capture Kit Content	58
Other Reagent Kits Content	60
Alternative Capture Equipment Combinations	63

This chapter contains reference information.



SureSelect RNA Capture Kit Content

Each SureSelect RNA Capture Kit contains one or more of each of these individual kits:

Table 24 SureSelect RNA Capture Kit Contents

Product5	Storage Condition	50 Reactions	250 Reactions
SureSelect Target Enrichment Kit Box #1	Room Temperature	5190-1953	5190-1959
SureSelect Target Enrichment Kit AB Box #2	-20°C	5190-1932	5190-1933
Capture Library	-80°C		

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

Table 25 SureSelect Target Enrichment Kit Box #1

Kit Component
SureSelect Hyb # 1 (orange cap, or bottle)
SureSelect Hyb # 2 (red cap)
SureSelect Hyb # 4 (black cap, or bottle)
3M Sodium Acetate (clear cap)
SureSelect Binding Buffer
SureSelect Wash Buffer #1
SureSelect Wash Buffer #2
SureSelect Elution Buffer
SureSelect Neutralization Buffer

Table 26 SureSelect Target Enrichment Kit AB Box #2

Kit Component
SureSelect Hyb #3 (yellow cap)
SureSelect Block #1 (green cap)
SureSelect Block #2 (blue cap)
SureSelect Block #3-AB (brown cap)
SureSelect RNase Block (purple cap)
SureSelect- Post Capture PCR Primers (clear cap)

Other Reagent Kits Content

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

Table 27 SOLiD Total RNA-Seq Kit (Applied Biosystems p/n 4445374)

Component
Nuclease-free Water
10X RNase III Buffer
RNase III
SOLiD Adaptor Mix
Hybridization Solution
2X Ligation Buffer
Ligation Enzyme Mix
10X RT Buffer
dNTP Mix
SOLiD RT Primer
ArrayScript Reverse Transcriptase
10X PCR Buffer
AmpliTaq DNA Polymerase
SOLiD 5' PCR Primer
SOLiD 3' PCR Primer
WT Control RNA

Table 28 SOLiD Total RNA-Seq Kit (Applied Biosystems p/n 4445374)

Component
Nuclease-free Water
10X RNase III Buffer
RNase III
SOLiD Adaptor Mix
Hybridization Solution
2X Ligation Buffer
Ligation Enzyme Mix
10X RT Buffer
dNTP Mix
SOLiD RT Primer
ArrayScript Reverse Transcriptase
10X PCR Buffer
AmpliTaq DNA Polymerase
SOLiD 5' PCR Primer
SOLiD 3' PCR Primer
WT Control RNA

Table 29 PureLink PCR Micro Kit (Invitrogen p/n K3100-50)

Component
Binding Buffer (B2)
Wash Buffer (W1)
Elution Buffer (E5) (10 mM Tris-HCl, pH 8.5)
PureLink Micro Kit Column
PureLink Elution Tube
Collection Tube

Table 30 RiboMinus Concentration Module (Invitrogen p/n K1550-05)

Binding Buffer (L3)
Wash Buffer (W5)
RNase-Free Water

Table 31 MinElute PCR Purification Kit (Qiagen p/n 28004 or 28006)

MinElute Spin Column
Buffer PBI
Buffer PE
Buffer EB

Table 32 PureLink PCR Micro Kit (Invitrogen p/n K3100-01)

Binding Buffer (B2)
Wash Buffer (W1)
PureLink PCR Spin Column
PureLink PCR Collection Tube
Elution Buffer; 10 mM Tris-HCl, pH 8.5 (E1)
PureLink Elution Tube

Alternative Capture Equipment Combinations

Table 33 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 33 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
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

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In This Book

This guide contains information to run the SureSelect Target Enrichment System for SOLiD Fragment and Paired-End RNA-Seq Library Prep protocol with the SureSelect RNA Capture Kits.

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