SureSelect^{XT2} Target Enrichment System for Illumina Paired-End Multiplexed Sequencing

Featuring Pre-Capture Indexing Reagents and Protocols

Protocol

Version F0, December 2016

SureSelect platform manufactured with Agilent SurePrint Technology

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

Agilent Technologies

Notices

Manual Part Number

G9630-90000

Edition

Version F0, December 2016

Printed in USA

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Santa Clara, CA 95051 USA

Acknowledgement

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In this Guide...

This guide describes an optimized protocol for Illumina paired-end multiplexed library preparation using the SureSelect^{XT2} Library Prep and Capture System.

This protocol is specifically optimized to enrich targeted regions of the genome from repetitive sequences and sequences unrelated to the research focus prior to sample sequencing using the Illumina platform. The SureSelect^{XT2} Library Prep and Capture System features pre-capture indexing reagents and protocols.

1 Before You Begin

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

2 Sample Preparation (1 µg DNA Samples)

This chapter describes the steps to prepare index-tagged libraries for target enrichment from 1-µg gDNA samples.

3 Sample Preparation (100 ng DNA Samples)

This chapter describes the steps to prepare index-tagged libraries for target enrichment from 100-ng gDNA samples.

4 Hybridization

This chapter describes the steps to pool indexed libraries and then hybridize and capture the pooled DNA.

5 Post-Capture Sample Processing for Multiplexed Sequencing

This chapter describes the steps to amplify, purify, and assess quality and quantity of the captured libraries. Guidelines for post-capture pooling and sequencing setup are provided.

6 Reference

This chapter contains reference information, including component kit contents and index sequences.

What's New in Version F0

- Support for Clinical Research Exome V2 Capture Libraries (see Table 2 on page 13).
- Removal of reference information for obsolete kits containing indexed adaptors 1–16 or 1–96, provided in clear-capped tubes (typically received before February 2015). If you need assistance with kits containing these obsolete index components, please contact Technical Support.
- Removal of instructions and reference information for obsolete kits containing End Repair Master Mix (typically received before February 2015) or containing vials labeled as *SureSelect End Repair Oligo Mix*.
- Update to instructions for testing prolonged hybridization conditions to use water as test fluid (see Caution on page 49).
- Support for Agilent 4200 TapeStation (see Table 5 on page 16)
- Updates to Covaris instrument settings for DNA shearing including optional two-round shearing and simplified instructions that include only the current SonoLab software version (see page 19 and page 35).
- Updates to supplier name for materials purchased from Thermo Fisher Scientific (see Table 1 on page 12, Table 4 on page 14, and Table 5 on page 16)
- Kit use-by information removed. See product labels and the Certificate of Analysis for each component for expiration date information.

What's New in Version E2

- Updated product labeling statement.

Content

1 Before You Begin

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 or instruments to process samples for enrichment.

Overview of the Workflow

The SureSelect^{XT2} target enrichment workflow is summarized in Figure 1.

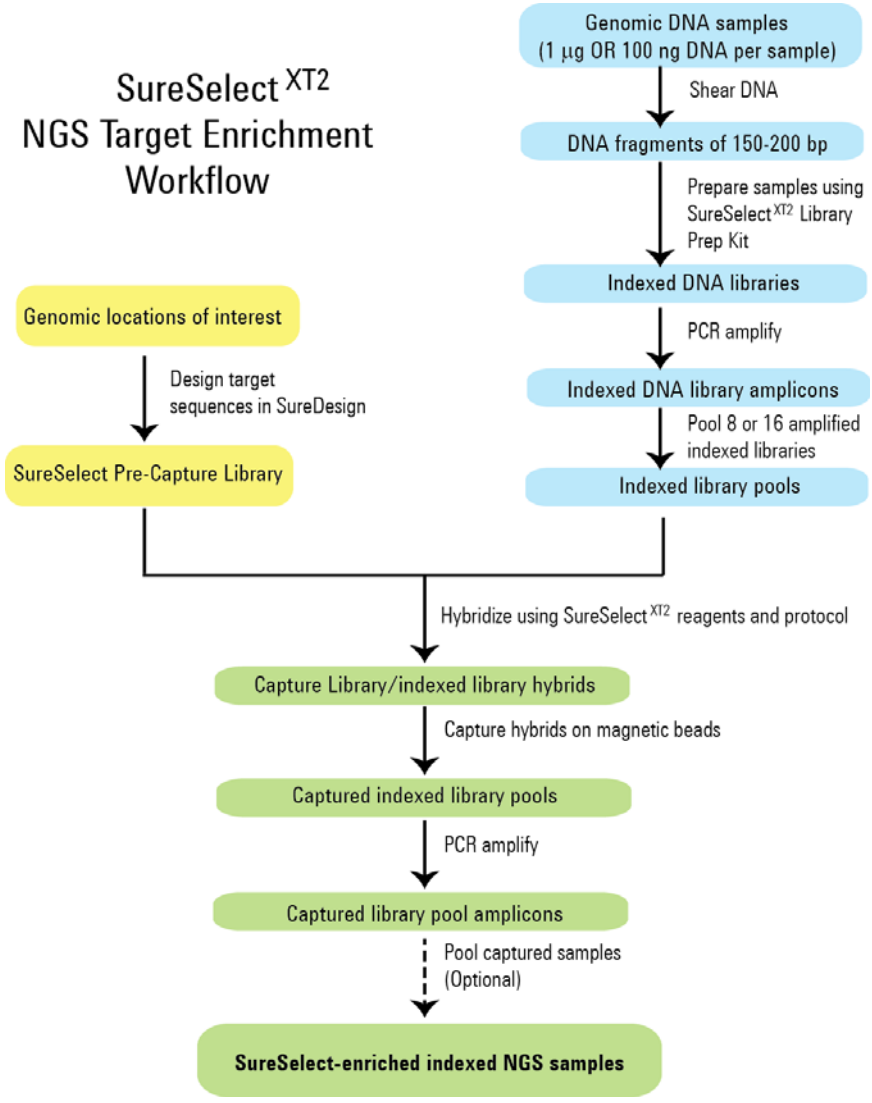

Figure 1 Overall target-enriched sequencing sample preparation workflow.

Procedural Notes

- This User Guide includes protocols for library preparation using either 1 µg DNA samples (see Chapter 2 on page 17) or 100 ng DNA samples (see Chapter 3 on page 33). Make sure that you are following the appropriate protocol for your DNA input amount. After the prepared libraries are amplified, both DNA input options use the same protocol for hybridization and post-capture processing.
- 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 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. Possible stopping points, where gDNA samples may be stored overnight at 4°C, are marked in the protocol. When storing samples for >24 hours, store the samples at –20°C, but do not subject the samples to multiple freeze/thaw cycles.
- When preparing 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 vials used during an experiment on ice or in a cold block.
  4. Library Preparation Master Mixes should not be frozen and thawed more than five times. If you plan to use the reagents in more than five experiments, aliquot to multiple vials to minimize freeze/thaw cycles for each vial.
- 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 SureSelect^{XT2} Target Enrichment

Description	Vendor and part number
SureSelect or ClearSeq Capture Library	Select one library from Table 2 or Table 3
SureSelect^{XT2} Reagent Kit*	Agilent
HiSeq platform (HSQ), 16 Samples	p/n G9621A
HiSeq platform (HSQ), 96 Samples	p/n G9621B
HiSeq platform (HSQ), 480 Samples	p/n G9621C
MiSeq platform (MSQ), 16 Samples	p/n G9622A
MiSeq platform (MSQ), 96 Samples	p/n G9622B
MiSeq platform (MSQ), 480 Samples	p/n G9622C
DNA 1000 Kit	Agilent p/n 5067-1504
High Sensitivity DNA Kit	Agilent p/n 5067-4626
Nuclease-free Water (not DEPC-treated)	Ambion Cat #AM9930
1X Low TE Buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA)	Life Technologies p/n 12090-015, or equivalent
Agencourt AMPure XP Kit	Beckman Coulter Genomics
5 ml	p/n A63880
60 ml	p/n A63881
450 ml	p/n A63882
Dynabeads MyOne Streptavidin T1	Thermo Fisher Scientific
2 ml	Cat #65601
10 ml	Cat #65602
Quant-iT dsDNA BR Assay Kit, for the Qubit fluorometer	Thermo Fisher Scientific
100 assays, 2-1000 ng	Cat #Q32850
500 assays, 2-1000 ng	Cat #Q32853
Qubit assay tubes	Thermo Fisher Scientific p/n Q32856
100% Ethanol, molecular biology grade	Sigma-Aldrich p/n E7023

* HiSeq and MiSeq Reagent Kits are also compatible with the NextSeq 500 platform.

Table 2 SureSelect^{XT2} Capture Libraries

Capture Library	16 Samples	96 Samples	480 Samples
SureSelect^{XT2} Clinical Research Exome V2*	5190-9500	5190-9501	—
SureSelect^{XT2} Clinical Research Exome V2 Plus 1*	5190-9503	5190-9504	—
SureSelect^{XT2} Clinical Research Exome V2 Plus 2*	5190-9506	5190-9507	—
SureSelect^{XT2} Clinical Research Exome*	5190-7345	5190-7346	—
SureSelect^{XT2} Focused Exome*	5190-7797	5190-7798	—
SureSelect^{XT2} Focused Exome Plus 1*	5190-7800	5190-7806	—
SureSelect^{XT2} Focused Exome Plus 2*	5190-7808	5190-7809	—
SureSelect^{XT2} Human All Exon v6*	5190-8872	5190-8873	—
SureSelect^{XT2} Human All Exon v6 + UTRs*	5190-8884	5190-8885	—
SureSelect^{XT2} Human All Exon v6 + COSMIC*	5190-9310	5190-9311	—
SureSelect^{XT2} Human All Exon v6 Plus 1*	5190-8875	5190-8876	—
SureSelect^{XT2} Human All Exon v6 Plus 2*	5190-8878	5190-8879	—
SureSelect^{XT2} Human All Exon v5*	5190-6216	5190-6217	—
SureSelect^{XT2} Human All Exon v5 + UTRs*	5190-6221	5190-6222	—
SureSelect^{XT2} Human All Exon v5 + lncRNA*	5190-6452	5190-6453	—
SureSelect^{XT2} Human All Exon v5 Plus*	5190-6219	5190-6220	—
SureSelect^{XT2} Human All Exon v4 *	5190-4666	5190-4667	5190-4669
SureSelect^{XT2} Human All Exon v4+ UTRs*	5190-4671	5190-4672	5190-4674
SureSelect^{XT2} Mouse All Exon*	5190-4681	5190-4682	5190-4684
SureSelect^{XT2} Custom 1 kb up to 499 kb† (reorder)	5190-4846 (5190-4851)	5190-4847 (5190-4852)	5190-4849 (5190-4854)
SureSelect^{XT2} Custom 0.5 Mb up to 2.9 Mb† (reorder)	5190-4856 (5190-4861)	5190-4857 (5190-4862)	5190-4859 (5190-4864)
SureSelect^{XT2} Custom 3 Mb up to 5.9 Mb† (reorder)	5190-4866 (5190-4871)	5190-4867 (5190-4872)	5190-4869 (5190-4874)
SureSelect^{XT2} Custom 6 Mb up to 11.9 Mb† (reorder)	5190-4876 (5190-4881)	5190-4877 (5190-4882)	5190-4879 (5190-4884)
SureSelect^{XT2} Custom 12 Mb up to 24 Mb† (reorder)	5190-4886 (5190-4891)	5190-4887 (5190-4892)	5190-4889 (5190-4894)

* Eight gDNA samples are enriched in one capture reaction after sample pooling. Capture Libraries are provided for the number of capture reactions needed to enrich the indicated number of samples.

† Sixteen gDNA samples are enriched in one capture reaction after sample pooling. Capture Libraries are provided for the number of capture reactions needed to enrich the indicated number of samples.

Table 3 Compatible ClearSeq Capture Libraries

Capture Library	16 Reactions	96 Reactions	480 Reactions
ClearSeq Comprehensive Cancer XT2*	5190-8017	5190-8018	5 × 5190-8018
ClearSeq Comprehensive Cancer Plus XT2*	5190-8020	5190-8021	5 × 5190-8021
ClearSeq Inherited Disease XT2†	5190-7524	5190-7525	5 × 5190-7525
ClearSeq Inherited Disease Plus XT2†	5190-7527	5190-7528	5 × 5190-7528
ClearSeq DNA Kinome XT2*	5190-4676	5190-4677	5190-4679

* Sixteen gDNA samples are enriched in one capture reaction after sample pooling. Capture Libraries are provided for the number of capture reactions needed to enrich the indicated number of samples.

† Eight gDNA samples are enriched in one capture reaction after sample pooling. Capture Libraries are provided for the number of capture reactions needed to enrich the indicated number of samples.

Required Equipment

Table 4 Required Equipment for SureSelect^{XT2} Target Enrichment

Description	Vendor and part number
SureCycler 8800 Thermal Cycler	Agilent p/n G8800A
96 well plate module for SureCycler 8800 Thermal Cycler	Agilent p/n G8810A
SureCycler 8800-compatible 96-well plates	Agilent p/n 410088
Optical strip caps	Agilent p/n 401425
Tube cap strips, domed	Agilent p/n 410096
Compression mats	Agilent p/n 410187
2100 Bioanalyzer Laptop Bundle	Agilent p/n G2943CA
2100 Bioanalyzer Electrophoresis Set	Agilent p/n G2947CA
Covaris Sample Preparation System, E-series or S-series	Covaris
Covaris sample holders	
96 microTUBE plate (E-series only)	Covaris p/n 520078
microTUBE for individual sample processing	Covaris p/n 520045

Table 4 Required Equipment for SureSelect^{XT2} Target Enrichment

Description	Vendor and part number
DNA LoBind Tubes, 1.5-ml PCR clean, 250 pieces	Eppendorf p/n 022431021 or equivalent
Centrifuge	Eppendorf Centrifuge model 5804 or equivalent
Qubit Fluorometer	Thermo Fisher Scientific p/n Q32857 or equivalent
Magnetic separator for 96-well plates	DynaMag-96 magnet, Thermo Fisher Scientific p/n 120-27 or equivalent
Nutator plate mixer	BD Diagnostics p/n 421105 or equivalent
Multichannel pipette	Pipetman or equivalent
P10, P20, P200 and P1000 pipettes	Pipetman P10, P20, P200, P1000 or equivalent
Vortex mixer	
Vacuum concentrator	Savant SpeedVac, model DNA120, with 96-well plate rotor, model RD2MP, or equivalent
Ice bucket	
Powder-free gloves	
Sterile, nuclease-free aerosol barrier pipette tips	
Timer	
Water bath or heat block suitable for incubation temperatures up to 65°C	

Optional Reagents and Equipment

Table 5 Optional Reagents and Equipment

Description	Vendor and part number
Agilent 4200 TapeStation*	Agilent p/n G2991AA
4200 TapeStation consumables	
D1000 ScreenTape	Agilent p/n 5067-5582
D1000 Reagents	Agilent p/n 5067-5583
High Sensitivity D1000 ScreenTape	Agilent p/n 5067-5584
High Sensitivity D1000 Reagents	Agilent p/n 5067-5585
Labnet MPS1000 Mini Plate Spinner	Labnet International p/n C1000
Magnetic separator for conical vials	DynaMag-50 magnet, Thermo Fisher Scientific p/n 123-02D or equivalent
QPCR NGS Library Quantification Kit (Illumina)	Agilent p/n G4880A
Mx3005P Real-Time PCR System	Agilent p/n 401449 or equivalent
Mx3000P/Mx3005P 96-well tube plates	Agilent p/n 410088 or equivalent
Mx3000P/Mx3005P optical strip caps	Agilent p/n 401425 or equivalent

* DNA samples may also be analyzed using the Agilent 2200 TapeStation, p/n G2964AA or G2965AA. ScreenTapes and associated reagents listed in this table are compatible with both platforms.

2 Sample Preparation (1 µg DNA Samples)

This section contains instructions for the preparation of indexed gDNA libraries from 1 µg DNA samples. For lower input (100 ng) DNA samples, see the library preparation protocol on page 33.

For each sample to be sequenced, an individual indexed library is prepared. For an overview of the SureSelect^{XT2} target enrichment workflow, see Figure 1 on page 10.

The sample preparation protocol is used to prepare DNA libraries for sequencing using the Illumina paired-read sequencing platform. The steps in this section differ from the Illumina protocol in the use of the Covaris system for gDNA shearing, smaller target shear size, elimination of size selection by gel purification, implementation of AMPure XP beads for all purification steps, and primers used for PCR. Refer to the Illumina protocol *Preparing Samples for Multiplexed Paired-End Sequencing* (p/n1005361) or the appropriate Illumina protocol for more information.

Step 1. Shear DNA

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

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

1. Use the Qubit dsDNA BR Assay to determine the concentration of your gDNA sample.

   Follow the instructions for the instrument.

2. Dilute 1 µg of high-quality gDNA with 1X Low TE Buffer in a 1.5-ml LoBind tube to a total volume of 50 µl.

3. Set up the Covaris E-series or S-series instrument.

   a. Check that the water in the Covaris tank is filled with fresh deionized water to the appropriate fill line level according to the manufacturer's recommendations for the specific instrument model and sample tube or plate in use.

   b. Check that the water covers the visible glass part of the tube.

   c. On the instrument control panel, push the Degas button. Degas the instrument for least 2 hours before use, or according to the manufacturer's recommendations.

   d. Set the chiller temperature to between 2°C to 5°C to ensure that the temperature reading in the water bath displays 5°C.

   e. *Optional.* Supplement the circulated water chiller with ethylene glycol to 20% volume to prevent freezing.

   Refer to the Covaris instrument user guide for more details.

4. Put a Covaris microTUBE into the loading and unloading station.

   Keep the cap on the tube.

NOTE You can use the 96 microTUBE plate (see Table 4 on page 14) for the DNA shearing step when preparing multiple gDNA samples in the same experiment.

5. Use a tapered pipette tip to slowly transfer the 50-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 6.

The target DNA fragment size is 150 to 200 bp.

Table 6 Shear settings for Covaris instruments (SonoLab software v7 or later)

Setting	Value
Duty Factor	10%
Peak Incident Power (PIP)	175
Cycles per Burst	200
Treatment Time	360 seconds*
Bath Temperature	4° to 8° C

* For more complete shearing, the 360-second treatment time may be completed in two rounds of 180 seconds each. After completing the first round of shearing for 180 seconds, spin the microTUBE briefly to collect the liquid, then shear the DNA for an additional 180 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 each 50-µl sheared DNA sample to a separate well of a SureCycler 8800 96-well plate.

CAUTION

Use SureCycler 8800-compatible 96-well plates (see Table 4 on page 14) for all SureSelect^{XT2} protocol steps done in 96-well plates.

Protocols were optimized using the specified plates; use of other plasticware may negatively impact your results and is not supported by Agilent.

Step 2. Assess quality (optional)

NOTE You can use Agilent's 4200 TapeStation or 2200 TapeStation for rapid analysis of multiple samples at this step. For analysis of 1 µg sheared DNA samples, use the D1000 ScreenTape (p/n 5067-5582) and associated reagents. For more information, see the appropriate TapeStation User Manual at www.genomics.agilent.com.

This step is optional.

Use a Bioanalyzer DNA 1000 chip and reagent kit for analysis of the 1 µg sheared DNA samples using the 2100 Bioanalyzer. See the *DNA 1000 Kit Guide* at www.genomics.agilent.com for more information on doing this step.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
2. Open the 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, using 1 µl of each sample for the analysis.
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 DNA 1000 assay from the drop down list.
6. Start the run. Enter sample names and comments in the Data and Assay context.
7. Check that the electropherogram shows an average DNA fragment size of 150 to 200 bp. A sample electropherogram is shown in Figure 2.

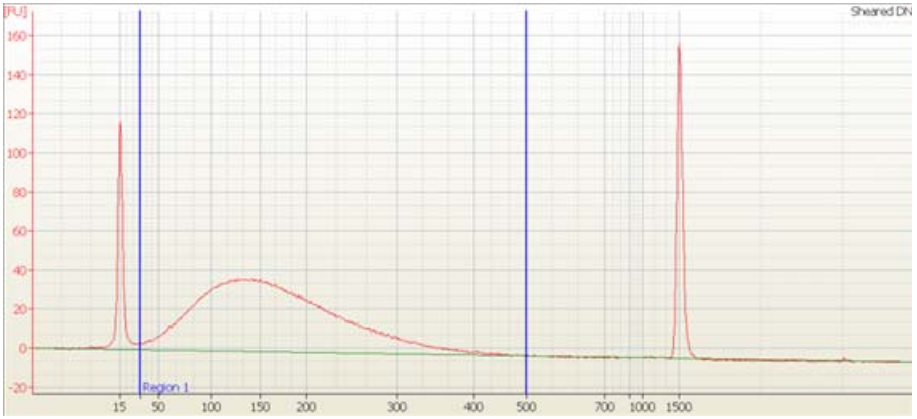

Figure 2 Analysis of sheared DNA using a DNA 1000 Bioanalyzer assay.

Step 3. Repair the ends

Use the SureSelect^{XT2} Library Prep Kit. Hold samples on ice while setting up the repair reaction.

1 Prepare the appropriate volume of End Repair Reaction Mix, as described in Table 7, on ice. Mix well on a vortex mixer.

Table 7 Preparation of SureSelect End Repair Reaction Mix

Reagent	Volume for 1 reaction	Volume for 16 reactions (includes excess)
SureSelect End Repair Enzyme Mix	40 µl	660 µl
SureSelect End Repair Nucleotide Mix	10 µl	165 µl
Total	**50 µl**	**825 µl**

2 To each 50-µl sheared DNA sample well, add 50 µl of the SureSelect End Repair Reaction Mix prepared in step 1.

3 Mix well by pipetting up and down or by gentle vortexing.

4 Incubate the plate in the SureCycler thermal cycler and run the program in Table 8. Do not use a heated lid.

Table 8 End-Repair Thermal Cycler Program

Step	Temperature	Time
Step 1	20°C	30 minutes
Step 2	4°C	Hold

Step 4. Purify the sample using AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes. *Do not freeze the beads at any time.*
2. Prepare 400 µl of 70% ethanol per sample, plus excess, for use in step 8.

NOTE The freshly-prepared 70% ethanol may be used for subsequent purification steps run on the same day. The complete Library Preparation protocol requires 1.2 ml of fresh 70% ethanol per sample.

3. Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.
4. Add 180 µl of homogeneous AMPure XP beads to each 100-µl end-repaired DNA library sample in the PCR plate. Pipette up and down 10 times to mix.
5. Incubate samples for 5 minutes at room temperature.
6. Put the plate into a magnetic separation device, such as the Dynal magnetic separator. Wait for the solution to clear (approximately 3 to 5 minutes).
7. Keep the plate in the magnetic stand. Carefully remove and discard the cleared solution from each well. Do not touch the beads while removing the solution.

NOTE If some magnetic beads remain suspended in solution after 5 minutes, carefully remove and discard 100 µl of cleared solution from near the bottom of the tube, and continue incubating the tube in the magnetic stand for an additional 3 minutes. After the remaining suspension has cleared, remove and discard the remaining cleared solution (approximately 180 µl) from the well.

8. Continue to keep the plate in the magnetic stand while you dispense 200 µl of 70% ethanol in each sample well.

   Use fresh 70% ethanol for optimal results.
9. Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.
10. Repeat step 8 to step 9 step once.

11 Seal the wells with strip caps, then briefly spin the plate to collect the residual ethanol. Return the plate to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.

12 Dry the samples on the SureCycler thermal cycler, set to hold samples at 37°C, for 5 to 10 minutes or until the residual ethanol completely evaporates.

13 Add 22 µl nuclease-free water to each sample well.

14 Seal the wells with strip caps, then mix well on a vortex mixer and briefly spin the plate in a centrifuge or mini-plate spinner to collect the liquid.

15 Incubate for 2 minutes at room temperature.

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

17 Remove the cleared supernatant to a fresh SureCycler 8800 PCR plate well. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, seal the plate and store at –20°C.

Step 5. Adenylate the 3' end of the DNA fragments

1 Add 20 µl of SureSelect dA-Tailing Master Mix to each end-repaired, purified DNA sample (approximately 20 µl).

2 Mix well by pipetting up and down or by gentle vortexing.

CAUTION SureSelect^{XT2} master mixes are viscous and thorough mixing is required to combine these mixtures with other solutions.

3 Incubate the plate in the SureCycler thermal cycler and run the program in Table 9. Do not use a heated lid.

Table 9 dA-Tailing Thermal Cycler Program

Step	Temperature	Time
Step 1	37°C	30 minutes
Step 2	4°C	Hold

Step 6. Ligate the pre-capture indexing adaptor

See the Reference section for sequences of the index portion of the indexing adaptors that are ligated to gDNA libraries in this section.

Be sure to keep the sample plate at 4°C or on ice while doing step 1 and step 2 sequentially as outlined below.

1. Add 5 µl of SureSelect Ligation Master Mix to each A-tailed DNA sample.
2. Add 5 µl of the appropriate SureSelect Pre-capture Indexed Adaptor solution to each sample.
3. Seal the wells then mix thoroughly by vortexing for 5 seconds. Briefly spin the plate, then keep the plate on ice until it is placed in the thermal cycler in step 4.

CAUTION SureSelect^{XT2} master mixes are viscous and thorough mixing is required to combine these mixtures with other solutions.

4. Incubate the plate in the SureCycler thermal cycler and run the program in Table 10. Do not use a heated lid.

Table 10 Ligation Thermal Cycler Program

Step	Temperature	Time
Step 1	20°C	15 minutes
Step 2	4°C	Hold

Step 7. Purify the indexed DNA using AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes. *Do not freeze the beads at any time.*
2. Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.
3. Add 60 µl of homogeneous AMPure XP beads to each 50-µl indexing adaptor-ligated DNA sample in the PCR plate. Pipette up and down to mix.
4. Incubate samples for 5 minutes at room temperature.
5. Put the plate into a magnetic separation device. Wait for the solution to clear (approximately 3 to 5 minutes).
6. Keep the plate in the magnetic stand. Carefully remove and discard the cleared solution from each well. Do not touch the beads while removing the solution.
7. Continue to keep the plate in the magnetic stand while you dispense 200 µl of freshly-prepared 70% ethanol in each sample well.
8. Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.
9. Repeat step 7 and step 8 step once.
10. Seal the wells with strip caps, then briefly spin the plate to collect the residual ethanol. Return the plate to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.
11. Dry the samples on the SureCycler thermal cycler, set to hold samples at 37°C, for 5 minutes or until the remaining ethanol completely evaporates.
12. Add 50 µl nuclease-free water to each sample well.
13. Seal the wells with strip caps, then mix well on a vortex mixer and briefly spin the plate in a centrifuge or mini-plate spinner to collect the liquid.
14. Incubate for 2 minutes at room temperature.
15. Put the plate in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
16. Remove the cleared supernatant (approximately 50 µl) to a fresh SureCycler 8800 PCR plate well. You can discard the beads at this time.

Step 8. Amplify the indexed library

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

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.

1 Prepare the appropriate volume of pre-capture PCR reaction mix, as described in Table 11, on ice. Mix well on a vortex mixer.

Table 11 Preparation of SureSelect Pre-Capture PCR Reaction Mix

Reagent	Volume for 1 reaction	Volume for 16 reactions (includes excess)
XT2 Primer Mix	1 µl	16.5 µl
Herculase II PCR Master Mix	25 µl	412.5 µl
Total	**26 µl**	**429 µl**

2 In separate wells of a SureCycler 8800 PCR plate, combine 26 µl of the amplification mixture prepared in Table 11 and 24 µl of each indexed gDNA library sample.

Mix by pipetting. Change pipette tips between samples.

3 Run the program in Table 12 in a SureCycler thermal cycler.

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Table 12 Pre-Capture PCR Thermal Cycler Program

Segment	Number of Cycles	Temperature	Time
1	1	98°C	2 minutes
2	5	98°C	30 seconds
		60°C	30 seconds
		72°C	1 minute
3	1	72°C	10 minutes
4	1	4°C	Hold

NOTE

Different library preparations can produce slightly different results, based on varying DNA quality. In most cases, five cycles will produce an adequate yield for subsequent capture without introducing bias or non-specific products. If yield is too low, or too high (where non-specific high molecular weight products are observed), adjust the number of cycles accordingly to amplify the remaining indexed library.

Step 9. Purify the amplified library with AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes. *Do not freeze the beads at any time.*
2. Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.
3. Add 60 µl of homogeneous AMPure XP beads to each 50-µl amplified DNA sample in the PCR plate. Pipette up and down to mix.
4. Incubate samples for 5 minutes at room temperature.
5. Put the plate into a magnetic separation device. Wait for the solution to clear (approximately 3 to 5 minutes).
6. Keep the plate in the magnetic stand. Carefully remove and discard the cleared solution from each well. Do not touch the beads while removing the solution.
7. Continue to keep the plate in the magnetic stand while you dispense 200 µl of freshly-prepared 70% ethanol in each sample well.
8. Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.
9. Repeat step 7 and step 8 step once.
10. Seal the wells with strip caps, then briefly spin the plate to collect the residual ethanol. Return the plate to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.
11. Dry the samples on the SureCycler thermal cycler, set to hold samples at 37°C, for 5 minutes or until the residual ethanol completely evaporates.
12. Add 50 µl nuclease-free water to each sample well.
13. Seal the wells, then mix well on a vortex mixer and briefly spin the plate in a centrifuge or mini-plate spinner to collect the liquid.
14. Incubate for 2 minutes at room temperature.
15. Put the plate in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
16. Remove the cleared supernatant (approximately 50 µl) to a fresh SureCycler 8800 PCR plate well. You can discard the beads at this time.

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

Step 10. Assess quality with the 2100 Bioanalyzer DNA 1000 Assay

NOTE You can use Agilent's 4200 TapeStation or 2200 TapeStation for rapid analysis of multiple samples at this step. For analysis of indexed DNA amplicons prepared from 1-µg gDNA samples, use the D1000 ScreenTape (p/n 5067-5582) and associated reagents. For more information, see the appropriate TapeStation User Manual at www.genomics.agilent.com.

Use a Bioanalyzer DNA 1000 chip and reagent kit for analysis of indexed DNA amplicons prepared from 1-µg gDNA samples. See the *DNA 1000 Kit Guide* at www.genomics.agilent.com for more information on doing this step.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
2. Open the 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, using 1 µl of each sample for the analysis.
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 DNA 1000 assay from the drop down list.
6. Start the run. Enter sample names and comments in the Data and Assay context.
7. Verify that the electropherogram shows a distribution with an average DNA fragment size of approximately 250 to 275 bp. A sample electropherogram is shown in Figure 3.
8. Measure the concentration of the library by integrating under the peak. For accurate quantification, make sure that the concentration falls within the linear range of the assay.

After determining the DNA concentration for each sample, proceed to "Hybridization" on page 49.

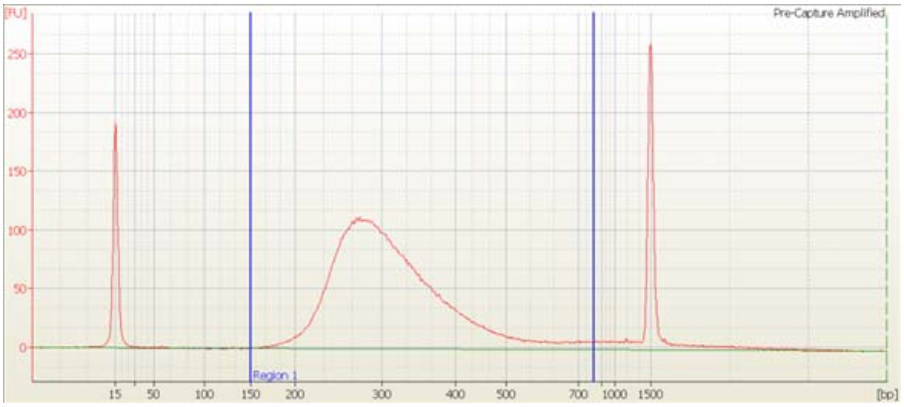

Figure 3 DNA 1000 Assay analysis of amplified library DNA prepared using the 1-µg DNA input Sample Preparation protocol.

3 Sample Preparation (100 ng DNA Samples)

This section contains instructions for the preparation of indexed gDNA libraries from 100 ng DNA samples. For higher input (1 µg) DNA samples, see the library preparation protocol on page 17.

For each sample to be sequenced, an individual indexed library is prepared. For an overview of the SureSelect^{XT2} target enrichment workflow, see Figure 1 on page 10.

The sample preparation protocol is used to prepare DNA libraries for sequencing using the Illumina paired-read sequencing platform. The steps in this section differ from the Illumina protocol in the use of the Covaris system for gDNA shearing, smaller target shear size, elimination of size selection by gel purification, implementation of AMPure XP beads for all purification steps, and primers used for PCR. Refer to the Illumina protocol *Preparing Samples for Multiplexed Paired-End Sequencing* (p/n1005361) or the appropriate Illumina protocol for more information.

Step 1. Shear DNA

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

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

1. Use the Qubit dsDNA BR Assay to determine the concentration of your gDNA sample.

   Follow the instructions for the instrument.

2. Dilute 100 ng of high-quality gDNA with 1X Low TE Buffer in a 1.5-ml LoBind tube to a total volume of 50 µl.

3. Set up the Covaris E-series or S-series instrument.

   a. Check that the water in the Covaris tank is filled with fresh deionized water to the appropriate fill line level according to the manufacturer's recommendations for the specific instrument model and sample tube or plate in use.

   b. Check that the water covers the visible glass part of the tube.

   c. On the instrument control panel, push the Degas button. Degas the instrument for least 2 hours before use, or according to the manufacturer's recommendations.

   d. Set the chiller temperature to between 2°C to 5°C to ensure that the temperature reading in the water bath displays 5°C.

   e. *Optional.* Supplement the circulated water chiller with ethylene glycol to 20% volume to prevent freezing.

   Refer to the Covaris instrument user guide for more details.

4. Put a Covaris microTUBE into the loading and unloading station.

   Keep the cap on the tube.

NOTE You can use the 96 microTUBE plate (see Table 4 on page 14) for the DNA shearing step when preparing multiple gDNA samples in the same experiment.

5. Use a tapered pipette tip to slowly transfer the 50-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 13.

The target DNA fragment size is 150 to 200 bp.

Table 13 Shear settings for Covaris instruments (SonoLab software v7 or later)

Setting	Value
Duty Factor	10%
Peak Incident Power (PIP)	175
Cycles per Burst	200
Treatment Time	360 seconds*
Bath Temperature	4° to 8° C

* For more complete shearing, the 360-second treatment time may be completed in two rounds of 180 seconds each. After completing the first round of shearing for 180 seconds, spin the microTUBE briefly to collect the liquid, then shear the DNA for an additional 180 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 each 50-µl sheared DNA sample to a separate well of a SureCycler 8800 96-well plate.

CAUTION

Use SureCycler 8800-compatible 96-well plates (see Table 4 on page 14) for all SureSelect^{XT2} protocol steps done in 96-well plates.

Protocols were optimized using the specified plates; use of other plasticware may negatively impact your results and is not supported by Agilent.

Step 2. Assess quality (optional)

NOTE You can use Agilent's 4200 TapeStation or 2200 TapeStation for rapid analysis of multiple samples at this step. For analysis of 100 ng sheared DNA samples, use the High-Sensitivity D1000 ScreenTape (p/n 5067-5584) and associated reagents. For more information, see the appropriate TapeStation User Manual at www.genomics.agilent.com.

This step is optional.

Use a High Sensitivity DNA Assay kit for analysis of the 100 ng sheared DNA samples using the 2100 Bioanalyzer. See the *High Sensitivity DNA Kit Guide* at www.genomics.agilent.com, for more information on doing this step.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
2. Open the 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, using 1 µl of each sheared DNA sample for the analysis.
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 High Sensitivity DNA assay from the drop down list.
6. Start the run. Enter sample names and comments in the Data and Assay context.
7. Check that the electropherogram shows an average DNA fragment size of 150 to 200 bp. A sample electropherogram is shown in Figure 4.

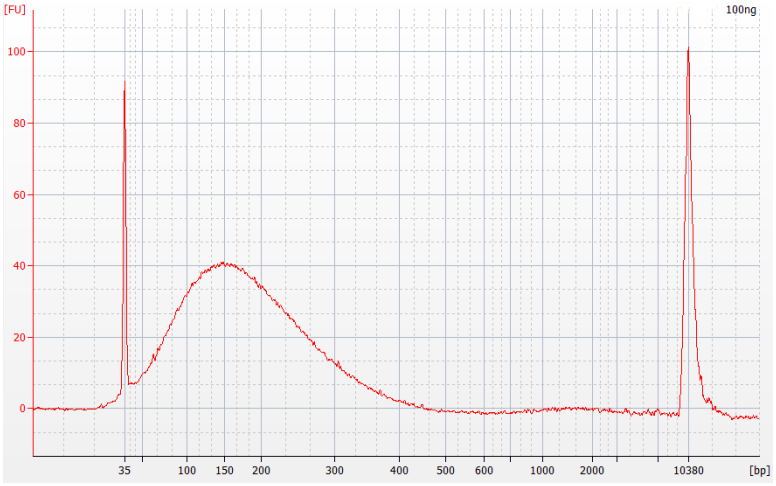

Figure 4 Analysis of 100 ng sheared DNA sample using a High-Sensitivity DNA Bioanalyzer assay.

Step 3. Repair the ends

Use the SureSelect^{XT2} Library Prep Kit. Hold samples on ice while setting up the repair reaction.

1 Prepare the appropriate volume of End Repair Reaction Mix, as described in Table 14, on ice. Mix well on a vortex mixer.

Table 14 Preparation of SureSelect End Repair Reaction Mix

Reagent	Volume for 1 reaction	Volume for 16 reactions (includes excess)
SureSelect End Repair Enzyme Mix	40 µl	660 µl
SureSelect End Repair Nucleotide Mix	10 µl	165 µl
Total	**50 µl**	**825 µl**

2 To each 50-µl sheared DNA sample well, add 50 µl of the SureSelect End Repair Reaction Mix prepared in step 1.

3 Mix well by pipetting up and down or by gentle vortexing.

4 Incubate the plate in the SureCycler thermal cycler and run the program in Table 15. Do not use a heated lid.

Table 15 End-Repair Thermal Cycler Program

Step	Temperature	Time
Step 1	20°C	30 minutes
Step 2	4°C	Hold

Step 4. Purify the sample using AMPure XP beads

1 Let the AMPure XP beads come to room temperature for at least 30 minutes. *Do not freeze the beads at any time.*

2 Prepare 400 µl of 70% ethanol per sample, plus excess, for use in step 8.

NOTE The freshly-prepared 70% ethanol may be used for subsequent purification steps run on the same day. The complete Library Preparation protocol requires 1.2 ml of fresh 70% ethanol per sample.

3 Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.

4 Add 180 µl of homogeneous AMPure XP beads to each 100-µl end-repaired DNA library sample in the PCR plate. Pipette up and down 10 times to mix.

5 Incubate samples for 5 minutes at room temperature.

6 Put the plate into a magnetic separation device, such as the Dynal magnetic separator. Wait for the solution to clear (approximately 3 to 5 minutes).

7 Keep the plate in the magnetic stand. Carefully remove and discard the cleared solution from each well. Do not touch the beads while removing the solution.

NOTE If some magnetic beads remain suspended in solution after 5 minutes, carefully remove and discard 100 µl of cleared solution from near the bottom of the tube, and continue incubating the tube in the magnetic stand for an additional 3 minutes. After the remaining suspension has cleared, remove and discard the remaining cleared solution (approximately 180 µl) from the well.

8 Continue to keep the plate in the magnetic stand while you dispense 200 µl of 70% ethanol in each sample well.

Use fresh 70% ethanol for optimal results.

9 Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.

10 Repeat step 8 and step 9 step once.

11 Seal the wells with strip caps, then briefly spin the plate to collect the residual ethanol. Return the plate to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.

12 Dry the samples on the SureCycler thermal cycler, set to hold samples at 37°C, for 5 to 10 minutes or until the residual ethanol completely evaporates.

13 Add 22 µl nuclease-free water to each sample well.

14 Seal the wells with strip caps, then mix well on a vortex mixer and briefly spin the plate in a centrifuge or mini-plate spinner to collect the liquid.

15 Incubate for 2 minutes at room temperature.

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

17 Remove the cleared supernatant to a fresh SureCycler 8800 PCR plate well. You can discard the beads at this time.

Stopping Point If you do not continue to the next step, seal the plate and store at –20°C.

Step 5. Adenylate the 3' end of the DNA fragments

1 Add 20 µl of SureSelect dA-Tailing Master Mix to each end-repaired, purified DNA sample (approximately 20 µl).

2 Mix well by pipetting up and down or by gentle vortexing.

CAUTION SureSelect^{XT2} master mixes are viscous and thorough mixing is required to combine these mixtures with other solutions.

3 Incubate the plate in the SureCycler thermal cycler and run the program in Table 16. Do not use a heated lid.

Table 16 dA-Tailing Thermal Cycler Program

Step	Temperature	Time
Step 1	37°C	30 minutes
Step 2	60°C	10 minutes
Step 3	4°C	Hold

NOTE The thermal cycling program for dA-Tailing of 100 ng samples differs from the 1 µg-input dA-Tailing thermal cycling program. Be sure to include the 10-minute incubation at 60°C when preparing 100 ng DNA samples.

Step 6. Ligate the pre-capture indexing adaptor

See the Reference section for sequences of the index portion of the indexing adaptors that are ligated to gDNA libraries in this section.

Be sure to keep the sample plate at 4°C or on ice while doing step 1 through step 3 sequentially as outlined below.

1. For each of the SureSelect Pre-capture Indexed Adaptor solutions to be used in the run, prepare a 1:5 dilution in nuclease-free water.
2. Add 5 µl of SureSelect Ligation Master Mix to each A-tailed DNA sample.
3. Using the diluted indexing adaptor solutions prepared in step 1 above, add 5 µl of the appropriate indexed adaptor dilution to each sample.
4. Seal the wells then mix thoroughly by vortexing for 5 seconds. Briefly spin the plate, then keep the plate on ice until it is placed in the thermal cycler in step 5.

CAUTION SureSelect^{XT2} master mixes are viscous and thorough mixing is required to combine these mixtures with other solutions.

5. Incubate the plate in the SureCycler thermal cycler and run the program in Table 17. Do not use a heated lid.

Table 17 Ligation Thermal Cycler Program

Step	Temperature	Time
Step 1	20°C	15 minutes
Step 2	4°C	Hold

Step 7. Purify the indexed DNA using AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes. *Do not freeze the beads at any time.*
2. Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.
3. Add 50 µl of homogeneous AMPure XP beads to each 50-µl indexing adaptor-ligated DNA sample in the PCR plate. Pipette up and down to mix.
4. Incubate samples for 5 minutes at room temperature.
5. Put the plate into a magnetic separation device. Wait for the solution to clear (approximately 3 to 5 minutes).
6. Keep the plate in the magnetic stand. Carefully remove and discard the cleared solution from each well. Do not touch the beads while removing the solution.
7. Continue to keep the plate in the magnetic stand while you dispense 200 µl of freshly-prepared 70% ethanol in each sample well.
8. Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.
9. Repeat step 7 and step 8 step once.
10. Seal the wells with strip caps, then briefly spin the plate to collect the residual ethanol. Return the plate to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.
11. Dry the samples on the SureCycler thermal cycler, set to hold samples at 37°C, for 5 minutes or until the residual ethanol completely evaporates.
12. Add 25 µl nuclease-free water to each sample well.
13. Seal the wells with strip caps, then mix well on a vortex mixer and briefly spin the plate in a centrifuge or mini-plate spinner to collect the liquid.
14. Incubate for 2 minutes at room temperature.
15. Put the plate in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
16. Remove the cleared supernatant (approximately 25 µl) to a fresh SureCycler 8800 PCR plate well. You can discard the beads at this time.

Step 8. Amplify the indexed library

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.

1 Prepare the appropriate volume of pre-capture PCR reaction mix, as described in Table 18, on ice. Mix well on a vortex mixer.

Table 18 Preparation of SureSelect Pre-Capture PCR Reaction Mix

Reagent	Volume for 1 reaction	Volume for 16 reactions (includes excess)
XT2 Primer Mix	1 µl	16.5 µl
Herculase II PCR Master Mix	25 µl	412.5 µl
Total	**26 µl**	**429 µl**

2 In separate wells of a SureCycler 8800 PCR plate, combine 26 µl of the amplification mixture prepared in Table 18 and 24 µl of each indexed gDNA library sample. Mix by pipetting. Change pipette tips between samples.

3 Run the program in Table 19 in a SureCycler thermal cycler.

Table 19 Pre-Capture PCR Thermal Cycler Program

Segment	Number of Cycles	Temperature	Time
1	1	98°C	2 minutes
2	8	98°C	30 seconds
		60°C	30 seconds
		72°C	1 minute
3	1	72°C	10 minutes
4	1	4°C	Hold

Step 9. Purify the amplified library with AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes. *Do not freeze the beads at any time.*
2. Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.
3. Add 50 µl of homogeneous AMPure XP beads to each 50-µl amplified DNA sample in the PCR plate. Pipette up and down to mix.
4. Incubate samples for 5 minutes at room temperature.
5. Put the plate into a magnetic separation device. Wait for the solution to clear (approximately 3 to 5 minutes).
6. Keep the plate in the magnetic stand. Carefully remove and discard the cleared solution from each well. Do not touch the beads while removing the solution.
7. Continue to keep the plate in the magnetic stand while you dispense 200 µl of freshly-prepared 70% ethanol in each sample well.
8. Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.
9. Repeat step 7 and step 8 step once.
10. Seal the wells with strip caps, then briefly spin the plate to collect the residual ethanol. Return the plate to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.
11. Dry the samples on the SureCycler thermal cycler, set to hold samples at 37°C, for 5 minutes or until the residual ethanol completely evaporates.
12. Add 50 µl nuclease-free water to each sample well.
13. Seal the plate wells, then mix well on a vortex mixer and briefly spin the plate in a centrifuge or mini-plate spinner to collect the liquid.
14. Incubate for 2 minutes at room temperature.
15. Put the plate in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
16. Remove the cleared supernatant (approximately 50 µl) to a fresh SureCycler 8800 PCR plate well. You can discard the beads at this time.

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

Step 10. Assess quality with the 2100 Bioanalyzer DNA 1000 Assay

NOTE

You can use Agilent's 4200 TapeStation or 2200 TapeStation for rapid analysis of multiple samples at this step. For analysis of indexed DNA amplicons prepared from 100-ng gDNA samples, use the D1000 ScreenTape (p/n 5067-5582) and associated reagents. For more information, see the appropriate TapeStation User Manual at www.genomics.agilent.com.

Use a Bioanalyzer DNA 1000 chip and reagent kit for analysis of indexed DNA amplicons prepared from 100-ng gDNA samples. See the *DNA 1000 Kit Guide* at www.genomics.agilent.com for more information on doing this step.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
2. Open the 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, using 1 µl of each sample for the analysis.
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 DNA 1000 assay from the drop down list.
6. Start the run. Enter sample names and comments in the Data and Assay context.
7. Verify that the electropherogram shows a distribution with an average DNA fragment size of approximately 250 to 275 bp. A sample electropherogram is shown in Figure 5.
8. Measure the concentration of the library by integrating under the peak. For accurate quantification, make sure that the concentration falls within the linear range of the assay.

After determining the DNA concentration for each sample, proceed to "Hybridization" on page 49.

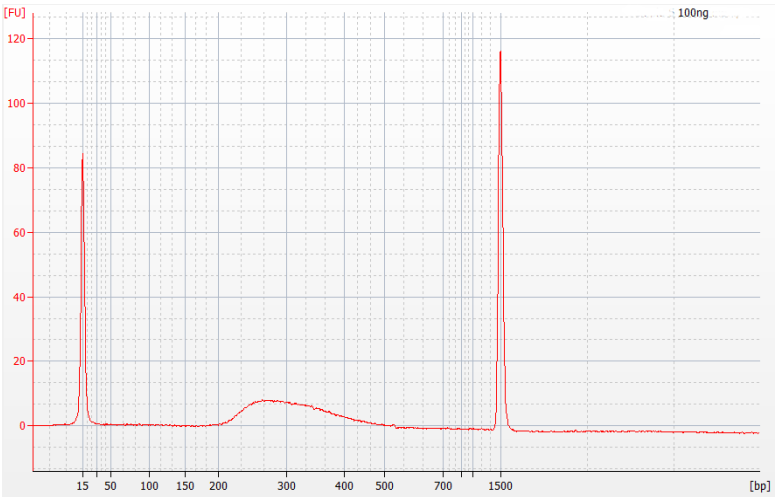

Figure 5 DNA 1000 Assay analysis of amplified library DNA prepared using the 100-ng DNA input Sample Preparation protocol.

4 Hybridization

This chapter describes the steps to pool indexed gDNA libraries and then hybridize the pooled gDNA libraries with a SureSelect or ClearSeq Capture Library. Pools of 8 or 16 indexed samples are hybridized to the appropriate Capture Library and the targeted molecules are captured for sequencing.

The size of your Capture Library determines the number of indexes that may be combined for hybridization. See Table 20 for the recommended number of indexes for each Capture Library.

CAUTION The ratio of Capture Library to indexed gDNA library is critical for successful capture.

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 duration of hybridization >24 hours, first test the conditions. Incubate 60 µl of water 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 8 µl.

Step 1. Pool indexed DNA samples for hybridization

In this step, you pool the indexed gDNA samples, before hybridization to the SureSelect or ClearSeq Capture Library.

Each hybridization reaction requires a total of 1500 ng indexed gDNA, made up of equal amounts of 8 or 16 individual libraries. See Table 20 for recommended gDNA library pool composition, based on the specific Capture Library used for hybridization.

Table 20

Capture Library	Number of indexed gDNA libraries per pool	Amount of each indexed gDNA library in pool
SureSelect Custom Capture Libraries	16	93.75 ng
ClearSeq Comprehensive Cancer	16	93.75 ng
ClearSeq DNA Kinome	16	93.75 ng
SureSelect Human or Mouse All-Exon	8	187.5 ng
SureSelect Clinical Research Exome	8	187.5 ng
SureSelect Focused Exome	8	187.5 ng
ClearSeq Inherited Disease	8	187.5 ng

1. For each capture reaction pool, combine the appropriate volume of each indexed gDNA library sample in one well of a SureCycler 8800 PCR plate.

   Each final capture reaction pool should contain 1500 ng indexed gDNA.

2. Use a vacuum concentrator, held at ≤ 45°C, to reduce the volume in each well to <7 µl.

   Avoid completely drying the sample. Over-drying the indexed library pool negatively impacts target enrichment.

3. Add sufficient nuclease-free water to each concentrated gDNA pool to bring the final well volume to 7 µl.

4. Cap the wells, then vortex the plate vigorously for 30 seconds. Spin in a centrifuge or mini-plate spinner to collect the liquid at the bottom of the wells.

Step 2. Hybridize gDNA library pools to the Capture Library

1 To each 7-µl indexed gDNA pool, add 9 µl of SureSelect XT2 Blocking Mix. Pipette up and down to mix.

2 Cap the wells, then transfer the sealed plate to the thermal cycler and run the program shown in Table 21.

Use a heated lid, set at 105°C, to hold the temperature at 65°C.

Make sure that the plate is held at 65°C for at least 5 minutes before the gDNA library/Block mixtures are used in step 7 below.

Table 21 Thermal cycler program used for sample denaturation prior to hybridization

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

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

3 Prepare the appropriate dilution of SureSelect RNase Block, based on the size of your Capture Library, according to Table 22. Prepare the amount required for the number of hybridization reactions in the run, plus excess.

Table 22 Preparation of RNase Block dilution

Capture Library Size	RNase Block dilution (parts RNase Block:parts water)	Volume of dilute RNase Block Required per hybridization reaction
<3.0 Mb	10% (1:9)	5 µl
>3.0 Mb	25% (1:3)	2 µl

4 In a PCR plate (kept on ice), for each hybridization reaction well, combine the indicated volumes of SureSelect or ClearSeq Capture Library and dilute RNase Block, according to Table 23. Mix well by pipetting.

Table 23 Preparation of Capture Library/RNase Block mixture

Capture Library Size	Volume of Capture Library per hybridization reaction	Volume of dilute RNase Block per hybridization reaction
<3.0 Mb	2 µl	5 µl of 10% solution
>3.0 Mb	5 µl	2 µl of 25% solution

5 To each well containing 7 µl of Capture Library/RNase Block mix, add 37 µl of SureSelect XT2 Hybridization Buffer. Mix well by pipetting.

NOTE

If precipitate is present in the Hybridization Buffer, warm the solution to 65°C for 5 minutes before use.

6 Cap the wells, then briefly spin the plate in a centrifuge or mini-plate spinner. Keep the plate at room temperature until it is used in step 7.

7 Maintain the gDNA pool plate at 65°C while you use a multi-channel pipette to transfer the entire 44-µl of Capture Library mixture from step 5 to each sample well of the gDNA pool plate. Mix well by slowly pipetting up and down 8 to 10 times.

The hybridization reaction wells now contain approximately 60 µl.

8 Seal the wells with domed strip caps. Make sure that all wells are completely sealed. Place a compression mat over the PCR plate in the thermal cycler.

CAUTION

Wells must be adequately sealed to minimize evaporation, or your results can be negatively impacted.

Before you do the first experiment, make sure the plate and capping method are appropriate for the thermal cycler. Check that no more than 8 µl is lost to evaporation under the conditions used for hybridization.

9 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 you must verify that the extended hybridization does not cause extensive evaporation in the sample wells.

Step 3. Prepare streptavidin-coated magnetic beads

1. Prewarm SureSelect XT2 Wash 2 at 65°C in a water bath or heat block for use in "Step 4. Capture the hybridized DNA using streptavidin beads".
2. Vigorously resuspend the Dynabeads MyOne Streptavidin T1 magnetic beads on a vortex mixer. The magnetic beads settle during storage.
3. For each hybridization sample, add 50 µl of the resuspended beads to wells of a SureCycler 8800 PCR plate.
4. Wash the beads:
   a. Add 200 µl of SureSelect XT2 Binding Buffer.
   b. Mix by pipetting up and down until the beads are fully resuspended.
   c. Put the plate into a magnetic separator device.
   d. Wait for the solution to clear, then 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 XT2 Binding Buffer.

NOTE

For runs that include multiple sample capture wells, the streptavidin beads may be batch-washed in an Eppendorf tube or conical vial. Start the batch-washing procedure using excess bead solution. After resuspending the washed beads in the appropriate volume of SureSelect Binding Buffer, aliquot 200 µl of the washed beads to each well to be used for hybridization capture.

Step 4. Capture the hybridized DNA using streptavidin beads

1 Estimate and record the volume of hybridization solution that remains after the 24 hour incubation.

CAUTION Excessive evaporation, such as when less than 52 µl remains after hybridization, can indicate suboptimal capture performance.

2 Maintain the hybridization plate at 65°C while you use a multichannel pipette to transfer the entire volume (approximately 60 µl) of each hybridization mixture to the plate wells containing 200 µl of washed streptavidin beads.

Mix well by slowly pipetting up and down 3 to 5 times.

3 Cap the wells, then incubate the capture plate on a Nutator mixer or equivalent for 30 minutes at room temperature.

Make sure the samples are properly mixing in the wells.

4 Briefly spin the plate in a centrifuge or mini-plate spinner.

5 Put the plate in a magnetic separator to collect the beads from the suspension. Remove and discard the supernatant.

6 Resuspend the beads in 200 µl of SureSelect XT2 Wash 1. Mix by pipetting up and down until the beads are fully resuspended.

7 Briefly spin in a centrifuge or mini-plate spinner.

8 Put the plate in the magnetic separator.

9 Wait for the solution to clear, then remove and discard the supernatant.

CAUTION It is important to maintain bead suspensions at 65°C during the washing procedure below to ensure specificity of capture.

Make sure that the SureSelect XT2 Wash 2 is pre-warmed to 65°C before use.

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

10 Wash the beads with SureSelect XT2 Wash 2:

- **a** Resuspend the beads in 200 µl of 65°C prewarmed SureSelect XT2 Wash 2. Pipette up and down until the beads are fully resuspended.
- **b** Incubate the sample plate for 5 minutes at 65°C on the SureCycler thermal cycler.
- **c** Briefly spin the plate in a centrifuge or mini-plate spinner.
- **d** Put the plate in the magnetic separator.
- **e** Wait for the solution to clear, then remove and discard the supernatant.
- **f** Repeat step a through step e for a total of 6 washes.

  Make sure all of the wash buffer has been removed during the final wash.

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

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

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

5 Post-Capture Sample Processing for Multiplexed Sequencing

This chapter describes the steps to amplify, purify, and assess quality and quantity of the captured libraries. Post-capture dilution and optional pooling instructions are provided to prepare the indexed samples for multiplexed sequencing.

Step 1. Amplify the captured libraries

In this step, the SureSelect-enriched indexed library DNA pools are PCR amplified. The protocol uses half of the bead-bound captured library pool for amplification. The remainder can be saved at –20°C for future use, if needed.

CAUTION To avoid cross-contaminating libraries, set up PCR master mixes in a dedicated clean area or PCR hood with UV sterilization and positive air flow.

Prepare 1 amplification reaction for each captured library pool.

1 Prepare the appropriate volume of PCR reaction mixture, according to Table 24. Mix well using a vortex mixer and keep on ice.

Table 24 Preparation of Post-Capture PCR Reaction Mix

SureSelect^{XT2} Reagent	Volume for 1 Amplification Reaction	Volume for 12 Amplification Reactions (includes excess)
Nuclease-free water	9 µl	112.5 µl
Herculase II Master Mix	25 µl	312.5 µl
XT2 Primer Mix	1 µl	12.5 µl
Total Volume	**35 µl**	**437.5 µl**

2 For each amplification reaction, place 35 µl of the PCR reaction mixture from step 1 in the wells of a SureCycler 8800 PCR plate.

3 Pipette each of the bead-bound captured library pool samples up and down to ensure that the bead suspension is homogeneous.

4 Add 15 µl of each captured library pool bead suspension to the appropriate PCR reaction mixture well. Mix thoroughly by pipetting until the bead suspension is homogeneous. Proceed immediately to thermal cycling in step 5.

5 Place the plate in a thermal cycler and run the PCR amplification program shown in Table 25 using the cycle number specified in Table 26.

Table 25 Post-Capture PCR cycling program

Segment	Number of Cycles	Temperature	Time
1	1	98°C	2 minutes
2	8-14 see Table 26	98°C	30 seconds
		60°C	30 seconds
		72°C	1 minute
3	1	72°C	10 minutes
4	1	4°C	Hold

Table 26 Recommended cycle number based on Capture Library size

Capture Library Size	Cycles
1 to 499 kb	12 to 14 cycles
0.5 to 1.49 Mb	9 to 11 cycles
> 1.5 Mb (including All Exon and Exome libraries)	8 to 10 cycles

NOTE Amplify the captured DNA using a minimal number of PCR cycles. If yield is too low, or too high (where non-specific high molecular weight products are observed), adjust the number of cycles accordingly with the remaining captured DNA template.

Step 2. Purify the amplified captured libraries using AMPure XP beads

1. Let the AMPure XP beads come to room temperature for at least 30 minutes. *Do not freeze the beads at any time.*
2. Prepare 400 µl of 70% ethanol per sample, plus excess, for use in step 9.
3. Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.
4. Add 90 µl of the homogeneous AMPure XP bead suspension to each sample well of the PCR plate, containing the 50-µl amplified captured library samples (also containing streptavidin beads used for capture).
5. Mix well on a vortex mixer. Briefly spin the plate in a centrifuge or mini-plate spinner to collect the liquid.
6. Incubate for 5 minutes at room temperature.
7. Put the plate into a magnetic separation device. Wait for the solution to clear (approximately 3 to 5 minutes).
8. Keep the plate in the magnetic stand. Carefully remove and discard the cleared solution from each well. Do not touch the beads while removing the solution.
9. Continue to keep the plate in the magnetic stand while you dispense 200 µl of 70% ethanol into each sample well.

   Use fresh 70% ethanol for optimal results.
10. Wait for 1 minute to allow any disturbed beads to settle, then remove the ethanol.
11. Repeat step 9 and step 10 step once.
12. Seal the wells with strip caps, then briefly spin the plate to collect the residual ethanol. Return the plate to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.
13. Dry the samples on the SureCycler thermal cycler, set to hold samples at 37°C, for 5 minutes or until the residual ethanol completely evaporates.
14. Add 30 µl nuclease-free water to each sample well then mix well on a vortex mixer. Briefly spin the plate in a centrifuge or mini-plate spinner to collect the liquid.
15. Incubate for 2 minutes at room temperature.

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

17 Remove the cleared supernatant (approximately 30 µl) to a fresh tube or plate well. You can discard the beads at this time.

18 Remove 1 µl of the purified captured library pool from the sample and combine with 9 µl of 1 X Low TE Buffer for Bioanalyzer analysis.

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

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

NOTE You can use Agilent's 4200 TapeStation or 2200 TapeStation for rapid analysis of multiple samples at this step. For analysis of captured DNA, use the High-Sensitivity D1000 ScreenTape (p/n 5067-5584) and associated reagents. For more information, see the appropriate TapeStation User Manual at www.genomics.agilent.com.

Use a High Sensitivity DNA Assay kit to assess sample quality and quantity using the 2100 Bioanalyzer. See the *High Sensitivity DNA Kit Guide* at www.genomics.agilent.com, for more information on doing this step.

NOTE Prior to Bioanalyzer analysis, dilute each amplified captured library sample ten- fold in TE buffer, as described in step 18 of the previous section.

1. Check that the 2100 Bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
2. Open the 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, using 1 µl of diluted captured library samples for the analysis.
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 High Sensitivity DNA 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 fragment size peak between approximately 275 to 300 bp. A sample electropherogram is shown in Figure 6.
8. Determine the concentration of each captured indexed library pool by integration under the peak in the electropherogram.

If the yield is too low or non-specific peaks are observed in the electropherogram, repeat the PCR with more or fewer cycles. The goal is to minimize cycles, while you produce enough library for application to the flow cell.

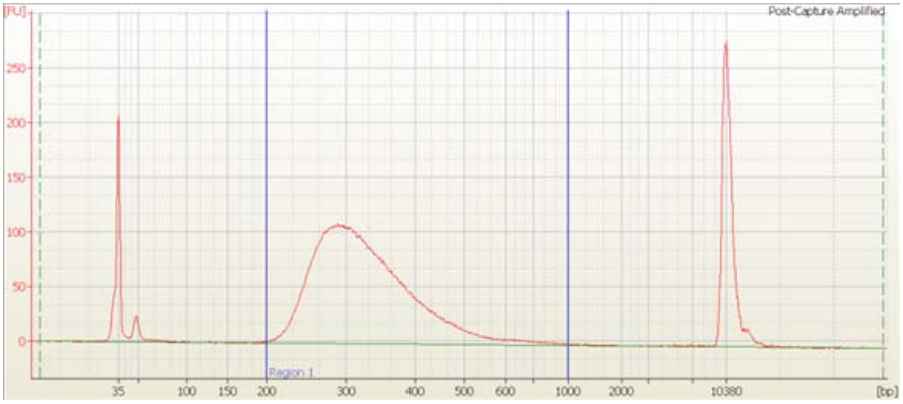

Figure 6 Analysis of amplified captured DNA using the High Sensitivity DNA Assay.

Step 4. Prepare samples for multiplexed sequencing

The final SureSelect^{XT2}-enriched samples contain pools of either 8 or 16 indexed libraries, based on the Capture Library used and resulting pre-capture pooling strategy. When appropriate for your sequencing platform, the 8-plex or 16-plex samples may be further multiplexed by post-capture pooling.

Determine whether to do post-capture pooling by calculating the number of indexes that can be combined per lane, according to the capacity of your platform, together with the amount of sequencing required to achieve the needed coverage for your specific Capture Library for each indexed sample.

If doing post-capture pooling, use the guidelines provided in "Step 6. Optional: Pool captured libraries for sequencing" on page 67. Prior to post-capture pooling, the DNA concentration of each sample may be accurately determined as described in "Step 5. Optional: Quantify captured library pools by QPCR" on page 66.

If samples will not be further combined in post-capture pools, proceed to cluster amplification using the Illumina Paired-End Cluster Generation Kit. Refer to the manufacturer's instructions for this step.

The optimal seeding concentration for SureSelect^{XT2} target-enriched libraries is 6 to 8 pM on HiSeq or MiSeq instruments and 1.2 to 1.3 pM on the NextSeq platform. Seeding concentration and cluster density may also need to be optimized based on the DNA fragment size range for the library and on the desired output and data quality. Follow Illumina's recommendation for a PhiX control in a low-concentration spike-in for improved sequencing quality control.

Sequencing run setup guidelines

Sequencing runs must be set up to perform an 8-bp index read. For complete 8-bp index sequence information, see the Reference chapter starting on page 69.

For the HiSeq 2500 and NextSeq 500 (v1) platforms, use the *Cycles* settings shown in Table 27. Cycle number settings can be specified on the *Run Configuration* screen of the instrument control software interface after choosing *Custom* from the index type selection buttons..

Table 27 Cycle Number settings for HiSeq and NextSeq platforms

Run Segment	Cycle Number
Read 1	100
Index 1 (i7)	9
Index 2 (i5)	0
Read 2	100

For the MiSeq platform, use the Illumina Experiment Manager (IEM) software to generate a Sample Sheet that includes the run parameters specified in Table 28.

Table 28 Run parameters for MiSeq platform Sample Sheet

Parameter	Entry
Workflow	GenerateFASTQ
Cycles for Read 1	100 for v2 chemistry 75 for v3 chemistry
Cycles for Read 2	100 for v2 chemistry 75 for v3 chemistry
Index 1 (i7) Sequence (enter in Data Section for each sample)	Type the 8-nt index sequence for each individual sample (see the index sequence tables in the Reference chapter starting on page 69).

Step 5. Optional: Quantify captured library pools by QPCR

For accurate determination of the DNA concentration in each captured library pool, use the QPCR NGS Library Quantification Kit (for Illumina).

Refer to the protocol that is included with the QPCR NGS Library Quantification Kit (p/n G4880A) for more details to do this step.

1. Prepare a standard curve using the quantification standard included in the kit, according to the instructions provided in the user guide.
2. Dilute each captured library pool 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.
3. Prepare the QPCR master mix with Illumina adaptor-specific PCR primers according to instructions provided in the kit.
4. Add an aliquot of the master mix to PCR tubes and add template.
5. 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.
6. Use the standard curve to determine the concentration of each unknown captured library pool, in nM.

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

NOTE

In most cases, the cycle numbers in Table 25 will produce an adequate yield for sequencing without introducing bias or non-specific products. If yield is too low or non-specific products are observed, adjust the number of cycles accordingly with the remaining captured DNA template.

Step 6. Optional: Pool captured libraries for sequencing

See Table 29 on page 68 for post-capture pooling guidelines, based on your SureSelect or ClearSeq Capture Library size and sequencing design. Pooling instructions are provided below.

1 Combine the capture pools such that each index-tagged sample is present in equimolar amounts in the final sequencing sample pool. For each final pool, use the formula below to determine the amount of each capture pool to use.

$$\text{Volume of capture pool} = \frac{V(f) \times C(f)}{\# \times C(i)} \quad \text{where}$$

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

C(f) is the desired final concentration of all the DNA in the pool

is the number of capture pool samples to be combined, and

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

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

- If the final volume of the combined index-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 index-tagged samples is greater than the final desired volume, V(f), lyophilize and reconstitute to the desired volume.

Table 29 shows an example of the amount of 2 capture pool samples (of different concentrations) and Low TE needed for a final volume of 20 µl at 10 nM final DNA concentration.

Table 29 Example of capture pool volume calculations for a 20-µl final sequencing sample pool containing 10 nM DNA

Component	V(f)	C(i)	C(f)	#	Volume to use (µl)
Sample 1	20 µl	20 nM	10 nM	6	5.0
Sample 2	20 µl	15 nM	10 nM	6	6.7
Low TE					8.3

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

4 Proceed to template denaturation and flow cell preparation. Refer to the appropriate Illumina protocol.

Proceed to cluster amplification using the Illumina Paired-End Cluster Generation Kit; refer to the manufacturer's instructions for this step. The optimal seeding concentration for cluster amplification from SureSelect^{XT2} DNA libraries is approximately 6 to 8 pM.

NOTE The optimal seeding concentration may vary, depending on the method used for library quantification and fragment size distribution.

See page 64 for sequencing run setup guidelines for SureSelect^{XT2} libraries.

6 Reference

This chapter contains reference information, including component kit contents and index sequences.

Kit Contents

SureSelect^{XT2} Reagent Kits contain the following component kits:

Table 30 SureSelect^{XT2} Reagent Kit Content

Component Kits*	Storage Condition	16 Samples†	96 Samples‡	480 Samples**
SureSelect XT2 Library Prep Kit, ILM	–20°C	5500-0130	5500-0131	5 x 5500-0131
SureSelect XT2 Pre-Capture Box 1	Room Temperature	5190-4076	5190-4076	5190-4077
SureSelect XT2 Pre-Capture ILM Module Box 2	–20°C	5190-4461	5190-3940	5190-3942

* See Table 31 through Table 33 for a list of reagents included in each component kit.

† Kits contain reagents to prepare indexed libraries from 16 gDNA samples and to enrich the samples in 1 or 2 hybridization and capture reactions (as appropriate for the specific Capture Library size and sample pooling format).

‡ Kits contain reagents to prepare indexed libraries from 96 gDNA samples and to enrich the samples in 6 or 12 hybridization and capture reactions (as appropriate for the specific Capture Library size and sample pooling format).

** Kits contain reagents to prepare indexed libraries from 480 gDNA samples and to enrich the samples in 30 or 60 hybridization and capture reactions (as appropriate for the specific Capture Library size and sample pooling format).

The contents of each of the component kits listed in Table 30 are described in the tables below.

Table 31 SureSelect XT2 Library Prep Kit, ILM Content

Kit Component	16 Sample Kit	96 or 480 Sample Kit
SureSelect End Repair Enzyme Mix	tube with orange cap	bottle
SureSelect End Repair Nucleotide Mix	tube with green cap	tube with green cap
SureSelect dA-Tailing Master Mix	tube with yellow cap	bottle
SureSelect Ligation Master Mix	tube with purple cap	tube with purple cap
SureSelect Herculase II Master Mix	tube with red cap	bottle
XT2 Primer Mix	tube with clear cap	tube with clear cap
SureSelect Pre-Capture Indexed Adaptors*	Indexes A01 through H02, provided in16 tubes with white caps	Indexes A01 through H12, provided in blue 96-well plate†

* See Table 35 on page 73 for index sequences.

† See Table 34 on page 72 for a plate map.

Table 32 SureSelect XT2 Pre-Capture Box 1 Content

Kit Component	16 Sample Kit	96 Sample Kit	480 Sample Kit
SureSelect XT2 Binding Buffer	bottle	bottle	bottle
SureSelect XT2 Wash 1	bottle	bottle	bottle
SureSelect XT2 Wash 2	bottle	bottle	bottle

Table 33 SureSelect XT2 Pre-Capture ILM Module Box 2 Content

Kit Component	16 Sample Kit	96 Sample Kit	480 Sample Kit
SureSelect XT2 Blocking Mix	tube with blue cap	tube with blue cap	tube with blue cap
SureSelect XT2 Hybridization Buffer	tube with yellow cap	tube with yellow cap	bottle
SureSelect RNase Block	tube with purple cap	tube with purple cap	tube with purple cap

Table 34 Plate map for SureSelect Pre-Capture Indexed Adaptors A01 through H12 provided in blue plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	A12
B	B01	B02	B03	B04	B05	B06	B07	B08	B09	B10	B11	B12
C	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12
D	D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12
E	E01	E02	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12
F	F01	F02	F03	F04	F05	F06	F07	F08	F09	F10	F11	F12
G	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12
H	H01	H02	H03	H04	H05	H06	H07	H08	H09	H10	H11	H12

Nucleotide Sequences of SureSelect^{XT2} Indexes A01 to H12

Each index is 8 nt in length. Sequencing protocols must be run using the third-read protocol that is also used for 9-bp indexes. See page 64 for additional sequencing run setup information

Table 35 SureSelect^{XT2} Indexes, for indexing primers provided in blue 96-well plate

Index	Sequence	Index	Sequence	Index	Sequence	Index	Sequence
A01	ATGCCTAA	A04	AACTCACC	A07	ACGTATCA	A10	AATGTTGC
B01	GAATCTGA	B04	GCTAACGA	B07	GTCTGTCA	B10	TGAAGAGA
C01	AACGTGAT	C04	CAGATCTG	C07	CTAAGGTC	C10	AGATCGCA
D01	CACTTCGA	D04	ATCCTGTA	D07	CGACACAC	D10	AAGAGATC
E01	GCCAAGAC	E04	CTGTAGCC	E07	CCGTGAGA	E10	CAACCACA
F01	GACTAGTA	F04	GCTCGGTA	F07	GTGTTCTA	F10	TGGAACAA
G01	ATTGGCTC	G04	ACACGACC	G07	CAATGGAA	G10	CCTCTATC
H01	GATGAATC	H04	AGTCACTA	H07	AGCACCTC	H10	ACAGATTC
A02	AGCAGGAA	A05	AACGCTTA	A08	CAGCGTTA	A11	CCAGTTCA
B02	GAGCTGAA	B05	GGAGAACA	B08	TAGGATGA	B11	TGGCTTCA
C02	AAACATCG	C05	CATCAAGT	C08	AGTGGTCA	C11	CGACTGGA
D02	GAGTTAGC	D05	AAGGTACA	D08	ACAGCAGA	D11	CAAGACTA
E02	CGAACTTA	E05	CGCTGATC	E08	CATACCAA	E11	CCTCCTGA
F02	GATAGACA	F05	GGTGCGAA	F08	TATCAGCA	F11	TGGTGGTA
G02	AAGGACAC	G05	CCTAATCC	G08	ATAGCGAC	G11	AACAACCA
H02	GACAGTGC	H05	CTGAGCCA	H08	ACGCTCGA	H11	AATCCGTC
A03	ATCATTCC	A06	AGCCATGC	A09	CTCAATGA	A12	CAAGGAGC
B03	GCCACATA	B06	GTACGCAA	B09	TCCGTCTA	B12	TTCACGCA
C03	ACCACTGT	C06	AGTACAAG	C09	AGGCTAAC	C12	CACCTTAC
D03	CTGGCATA	D06	ACATTGGC	D09	CCATCCTC	D12	AAGACGGA
E03	ACCTCCAA	E06	ATTGAGGA	E09	AGATGTAC	E12	ACACAGAA
F03	GCGAGTAA	F06	GTCGTAGA	F09	TCTTCACA	F12	GAACAGGC
G03	ACTATGCA	G06	AGAGTCAA	G09	CCGAAGTA	G12	AACCGAGA
H03	CGGATTGC	H06	CCGACAAC	H09	CGCATACA	H12	ACAAGCTA

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In This Book

This guide contains information to run the SureSelect^{XT2} Target Enrichment System for Illumina Paired-End Multiplexed Sequencing protocol featuring pre-capture indexing.

Version F0, December 2016

p/n G9630-90000

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