



SureSelect Automated Library Prep and Capture System

**SureSelect^{XT} Automated
Target Enrichment for Illumina
Paired-End Multiplexed
Sequencing**

Protocol

Version E.2, September 2012

**SureSelect platform manufactured with Agilent
SurePrint Technology**

**Research Use Only. Not for use in Diagnostic
Procedures.**



Agilent Technologies

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Acknowledgement

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

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In this Guide...

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

This protocol is specifically developed and optimized to capture the genomic regions of interest using Agilent's SureSelect system to enrich targeted regions of the genome from repetitive sequences and sequences unrelated to the research focus prior to sample sequencing. Sample processing steps are automated using the SureSelect Automated Library Prep and Capture System.

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 Using the Agilent NGS Workstation for SureSelect Target Enrichment

This chapter contains an orientation to the Agilent NGS Workstation, an overview of the SureSelect target enrichment protocol, and considerations for designing SureSelect experiments for automated processing using the Agilent NGS Workstation.

3 Sample Preparation

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

4 Hybridization

This chapter describes the steps to hybridize and capture samples.

5 Indexing

This chapter describes the steps to amplify, purify, and assess quality of the sample libraries. Samples are pooled by mass prior to sequencing.

6 Appendix: Optional DNA Quantitation Protocol

This chapter describes how to use the optional automated DNA quantitation protocols provided in VWorks software.

6 Reference

This chapter contains reference information.

What's New in Version E.2

- Target enrichment protocol updated to include on-bead post-capture PCR. Related updates include revised automation protocols for DNA Capture (SureSelectCapture&Wash_v1.5.rst, starting on [page 90](#)) and Post-Capture Indexing (Post-CaptureIndexing_XT_Illumina_v1.5.pro, starting on [page 100](#)).
- Target enrichment protocol updated for use of 750 ng prepared DNA library in the hybridization step
- Updates to hybridization automation protocol for improved temperature control of master mix source wells
- Support for NGS Workstation error messages that may be encountered during run setup ([page 25](#))
- Support for use of Agilent's SureCycler 8800 thermal cycler in the workflow (see [page 10](#) and [page 15](#))

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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.

CAUTION

This Protocol supports the SureSelect Target Enrichment workflow with on-bead post-capture PCR, using version 1.5 (**v1.5**) VWorks SureSelect automation protocols. If your VWorks SureSelect setup form displays earlier versions of the automation protocols, including SureSelect Capture&Wash_v1.1.rst and Post-CaptureIndexing_XT_Illumina_v1.0.pro, which support in-solution post-capture PCR, please contact service.automation@agilent.com for assistance.

NOTE

This protocol describes automated sample processing using the Agilent NGS Workstation. For non-automated sample processing procedures for Agilent's SureSelect^{XT} Target Enrichment Kit for Illumina Multiplex Sequencing, see publication G7530-90000.

NOTE

This protocol differs from other SureSelect protocols at several steps. Pay close attention to the primers used for each amplification step and the blocking agents used during hybridization.



Procedural Notes

- Certain protocol steps require the rapid transfer of sample plates between the Bravo deck and a thermal cycler. Locate your thermal cycler in close proximity to the Agilent NGS Workstation to allow rapid and efficient plate transfer.
- Prepare and load the Agilent NGS Workstation as detailed in each of the protocol steps before initiating each automated protocol run. When loading plates in the workstation's Labware MiniHub, always place plates in the orientation shown in [Figure 3](#) on page 38.
- Use of Agilent's SureCycler 8800 thermal cycler for incubation of samples in the MicroAmp 96-well half-skirted plates used in the automation protocols requires the use of two compression mats to ensure that wells are tightly sealed during incubation. After transferring the MicroAmp plate to the SureCycler thermal block, place a MicroAmp Optical Film Compression Pad over the MicroAmp plate. In addition, place a SureCycler 8800 Compression Mat over the MicroAmp compression pad before closing the thermal cycler lid and starting the incubation step. See the footnote to [Table 4](#) on page 14 for compression device ordering information.
- To prevent contamination of reagents by nucleases, always wear powder-free laboratory gloves and use dedicated solutions and pipettors with nuclease-free aerosol-resistant tips.
- Maintain a clean work area.
- Do not mix stock solutions and reactions containing gDNA on a vortex mixer. Instead, gently tap the tube with your finger to mix the sample.
- Avoid repeated freeze-thaw cycles of stock and diluted gDNA solutions. 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 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

Description	Vendor and part number
Agilent SureSelect ^{XT} Capture Library *	Select one library from Table 2 or Table 3
Agilent SureSelect ^{XT} Automation Reagent Kit*†	
HiSeq platform (HSQ), 96 reactions	Agilent p/n G9641B
HiSeq platform (HSQ), 480 reactions	Agilent p/n G9641C
MiSeq platform (MSQ), 96 reactions	Agilent p/n G9642B
MiSeq platform (MSQ), 480 reactions	Agilent p/n G9642C
Herculase II Fusion DNA Polymerase, 400 reactions (includes dNTP mix and 5x Buffer)	Agilent p/n 600679
Agilent QPCR NGS Library Quantification Kit (Illumina GA)	Agilent p/n G4880A
Nuclease-free Water (not DEPC-treated)	Ambion Cat #AM9930
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
60 mL	p/n A63881
450 mL	p/n A63882
Quant-iT dsDNA BR Assay Kit, for use with the Qubit fluorometer	Life Technologies
100 assays, 2-1000 ng	Cat #Q32850
500 assays, 2-1000 ng	Cat #Q32853
Dynabeads MyOne Streptavidin T1	Life Technologies
2 mL	Cat #65601
10 mL	Cat #65602
100 mL	Cat #65603
100% Ethanol, molecular biology grade	Sigma-Aldrich p/n E7023

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

† Each 96-reaction kit contains sufficient reagents for 96 reactions used in runs that include at least 3 columns of samples per run.

Table 2 Agilent SureSelect^{XT} Automation Custom Capture Libraries

Custom Capture Library Size	96 Reactions	96 Reactions for Reorder	480 Reactions	480 Reactions for Reorder
1 kb up to 499 kb	5190-4808	5190-4813	5190-4810	5190-4815
0.5 Mb up to 2.9 Mb	5190-4818	5190-4823	5190-4820	5190-4825
3 Mb up to 5.9 Mb	5190-4828	5190-4833	5190-4830	5190-4835
6 Mb up to 11.9 Mb	5190-4838	5190-4843	5190-4840	5190-4845
12 Mb up to 24 Mb	5190-4898	5190-4903	5190-4900	5190-4905

Table 3 Agilent SureSelect^{XT} Automation Catalog Capture Libraries

Catalog Capture Library	96 Reactions	480 Reactions
Human All Exon v4	5190-4633	5190-4635
Human All Exon v4 + UTRs	5190-4638	5190-4640
Human All Exon 50 Mb	5190-4628	5190-4630
Human DNA Kinome	5190-4648	5190-4650
Mouse All Exon	5190-4643	5190-4645

Required Equipment

Table 4 Required Equipment

Description	Vendor and part number
Agilent NGS Workstation for the Agilent SureSelect Automated Library Prep and Capture System, with VWorks software version 11.0.1.1032 or later.	Contact Agilent Automation Solutions for ordering information: Customerservice.automation@agilent.com
Robotic Pipetting Tips (Sterile, Filtered, 250 µL)	Agilent p/n 19477-022
Eppendorf twin.tec full-skirted 96-well PCR plates	Eppendorf p/n 951020401 or 951020619
Thermo Scientific Reservoirs	Thermo Scientific p/n 1064156
Nunc DeepWell Plates, sterile, 1.3-mL well volume	Thermo Scientific p/n 260251
Axygen 96 Deep Well Plate, 2.2 mL, Square Well (waste reservoirs)	Axygen p/n P-2ML-SQ-C E & K Scientific p/n EK-2440
MicroAmp Optical 96-well plates, half-skirted	Life Technologies p/n N8010560
MicroAmp 96-well base (black adapter for MicroAmp plates)	Life Technologies p/n N8010531
DNA Analysis Platform and Consumables	
Agilent 2100 Bioanalyzer Laptop Bundle	Agilent p/n G2943CA
Agilent 2100 Bioanalyzer Electrophoresis Set	Agilent p/n G2947CA
Agilent DNA 1000 Kit	Agilent p/n 5067-1504
Agilent High Sensitivity DNA Kit	Agilent p/n 5067-4626
OR	
Agilent 2200 TapeStation	Agilent p/n G2964AA or G2965AA
Agilent D1K ScreenTape	Agilent p/n 5067-5361
Agilent D1K Reagents	Agilent p/n 5067-5362
Agilent High Sensitivity D1K ScreenTape	Agilent p/n 5067-5363
Agilent D1K Reagents	Agilent p/n 5067-5364
Qubit Fluorometer	Life Technologies p/n Q32857
Qubit assay tubes	Life Technologies p/n Q32856
Covaris Sample Preparation System, S-series or E-series model	Covaris

Table 4 Required Equipment (continued)

Description	Vendor and part number
Covaris sample holders	
96 microTUBE plate (E-series only)	Covaris p/n 520078
microTUBE for individual sample processing	Covaris p/n 520045
Thermal cycler	Life Technologies Veriti Thermal Cycler *
DNA LoBind Tubes, 1.5-mL PCR clean, 250 pieces	Eppendorf p/n 022431021 or equivalent
Centrifuge	Eppendorf Centrifuge model 5804 or equivalent
P10, P20, P200 and P1000 pipettes	Pipetman P10, P20, P200, P1000 or equivalent
Vacuum concentrator	Savant SpeedVac, model DNA120, with 96-well plate rotor, model RD2MP, or equivalent
Magnetic separator	DynaMag-50 magnet, Life Technologies p/n 123-02D or equivalent
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
NucleoClean Decontamination Wipes	Millipore p/n 3097
Ice bucket	
Powder-free gloves	
Sterile, nuclease-free aerosol barrier pipette tips	
Vortex mixer	
Timer	

* Protocols are also compatible with Agilent's SureCycler 8800 thermal cycler, when used with a SureCycler 8800 Compression Mat (Agilent p/n 410187) AND a MicroAmp Optical Film Compression Pad (Life Technologies p/n 4312639) for incubation of samples in MicroAmp 96-well half-skirted plates. See [page 10](#) for additional information.

1 Before You Begin

Required Equipment



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Using the Agilent NGS Workstation for SureSelect Target Enrichment

About the Agilent NGS Workstation 18

Overview of the SureSelect Target Enrichment Procedure 28

Experimental Setup Considerations for Automated Runs 31

This chapter contains an orientation to the Agilent NGS Workstation, an overview of the SureSelect^{XT} target enrichment protocol, and considerations for designing SureSelect experiments for automated processing using the Agilent NGS Workstation.



About the Agilent NGS Workstation

About the Bravo Platform

The Bravo platform is a versatile liquid handler with a nine plate-location platform deck, suitable for handling 96-well, 384-well, and 1536-well plates. The Bravo platform is controlled by the VWorks Automation Control software. Fitted with a choice of seven interchangeable fixed-tip or disposable-tip pipette heads, it accurately dispenses fluids from 0.1 μL to 250 μL .

CAUTION

Before you begin, make sure that you have read and understand operating, maintenance and safety instructions for using your Bravo platform. Refer to the *Bravo Platform User Guide* (G5409-90004) and the *VWorks Software User Guide* (G5415-90002).

Bravo Platform Deck

The protocols in the following sections include instructions for placing plates and reagent reservoirs on specific Bravo deck locations. Use [Figure 1](#) to familiarize yourself with the location numbering convention on the Bravo platform deck.

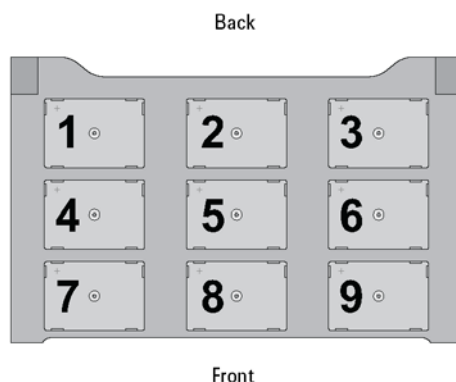


Figure 1 Bravo platform deck

Setting the Temperature of Bravo Deck Heat Blocks

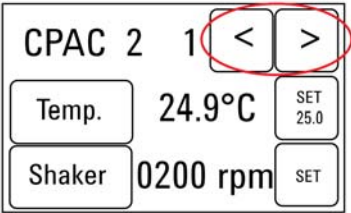
Bravo deck positions 4 and 6 are equipped with Inheco heat blocks, used to incubate sample plates at defined temperatures during the run. Runs that include high- (85°C) or low- (4°C) temperature incubation steps may be expedited by pre-setting the temperature of the affected block before starting the run.

Bravo deck heat block temperatures may be changed using the Inheco Multi TEC Control device touchscreen as described in the steps below. See [Table 5](#) for designations of the heat block-containing Bravo deck positions on the Multi TEC control device.

Table 5 Inheco Multi TEC Control touchscreen designations

Bravo Deck Position	Designation on Inheco Multi TEC Control Screen
4	CPAC 2 1
6	CPAC 2 2

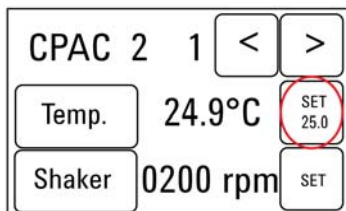
- 1 Using the arrow buttons, select the appropriate block (CPAC 2 block 1 or CPAC 2 block 2).



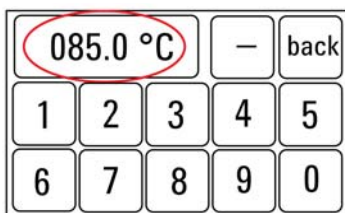
2 Using the Agilent NGS Workstation for SureSelect Target Enrichment

About the Bravo Platform

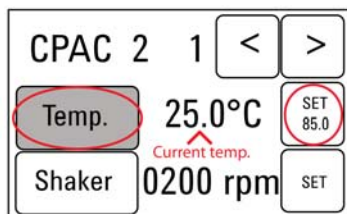
- 2 To set the temperature of the selected block, press the SET button.



- 3 Using the numeral pad, enter the desired temperature. The entered temperature appears in the top, left rectangle. Once the correct temperature is displayed, press the rectangle to enter the temperature.



- 4 Press the Temp button until the new temperature is displayed on the SET button and until the Temp button is darkened, indicating that the selected heat block is heating or cooling to the new temperature setting. The current temperature of the block is indicated in the center of the display.



Setting the Temperature of Bravo Deck Position 9 Using the ThermoCube Device

Bravo deck position 9 is equipped with a ThermoCube thermoelectric temperature control system, used to incubate components at a defined temperature during the run. During protocols that require temperature control at position 9, you will be instructed to start and set the temperature of the ThermoCube device before starting the run.

ThermoCube temperature settings are modified using the control panel (LCD display screen and four input buttons) on the front panel of the device using the following steps.

- 1** Turn on the ThermoCube and wait for the LCD screen to display **TEMP.**
- 2** Press the **UP** or **DOWN** button to change **SET TEMP 1** to the required set point.
- 3** Press the **START** button.

The ThermoCube will then initiate temperature control of Bravo deck position 9 at the displayed set point.

VWorks Automation Control Software

VWorks software, included with your Agilent NGS Workstation, allows you to control the robot and integrated devices using a PC. The Agilent NGS Workstation is preloaded with VWorks software containing all of the necessary SureSelect system liquid handling protocols. General instructions for starting up the VWorks software and the included protocols is provided below. Each time a specific VWorks protocol is used in the SureSelect procedure, any settings required for that protocol are included in the relevant section of this manual.

NOTE

The instructions in this manual are compatible with VWorks software version 11.0.1.1032 or later, including SureSelect^{XT} automation protocols version 1.5.

If you have questions about VWorks version compatibility, please contact service.automation@agilent.com.

Logging in to the VWorks software


- 1 Double-click the VWorks icon or the SureSelect_XT_Illumina.VWForm shortcut on the Windows desktop to start the VWorks software.
- 2 If User Authentication dialog is not visible, click **Log in** on the VWorks window toolbar.
- 3 In the User Authentication dialog, type your VWorks user name and password, and click **OK**. (If no user account is set up, contact the administrator.)

VWorks protocol and runset files

VWorks software uses two file types for automation runs, .pro (protocol) files and .rst (runset) files. Runset files are used for automated procedures in which the workstation uses more than one automation protocol during the run.

Using the SureSelect_XT_Illumina_v1.5.VWForm to setup and start a run

Use the VWorks form SureSelect_XT_Illumina_v1.5.VWForm, shown below, to set up and start each SureSelect automation protocol or runset.



SureSelect^{XT}
with on-bead PCR
for Illumina sequencers

Parameters

1) Select Protocol to Run

2) Select additional Parameters
 AMPureXP_XT_Illumina_v1.5.pro Only

a. Select DNA Product to Cleanup

b. Select Plate type containing DNA for cleanup

3) Select Number of Columns of Samples*
 Columns selected
*needs to be 1, 2, 3, 4, 6, or 12 columns

4) Click button below to Display Initial Workstation Setup

Controls

Once you have loaded labware according to Workstation Setup on right, Click "Run Selected Protocol" to start run.

Elapsed Time: 00:00:00

Advanced Settings

☐ TESTING ONLY: Reduces all incubation times and/or mix cycles

Workstation Setup

MiniHub	MiniHub Cassette 1	MiniHub Cassette 2	MiniHub Cassette 3	MiniHub Cassette 4
Shelf 5				
Shelf 4				
Shelf 3				
Shelf 2				
Shelf 1				

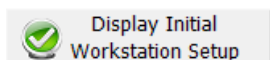
Bravo Deck

<Position 1>	<Position 2>	<Position 3>
<Pos 4: Peltier>	<Pos 5: Shaker>	<Pos 6: Peltier>
<Pos 7: Magnetic>	<Position 8>	<Pos 9: Chilled>

BenchCel


BenchCel Stacker 1	BenchCel Stacker 2	BenchCel Stacker 3	BenchCel Stacker 4

- 1 Open the form using the SureSelect_XT_Illumina_v1.5.VWForm shortcut on your desktop.
- 2 Use the drop-down menus on the form to select the appropriate SureSelect workflow step and number of columns of samples for the run.
- 3 Once all run parameters have been specified on the form, click **Display Initial Workstation Setup**.



2 Using the Agilent NGS Workstation for SureSelect Target Enrichment VWorks Automation Control Software

- 4 The Workstation Setup region of the form will then display the required placement of reaction components and labware in the NGS Workstation for the specified run parameters.



SureSelect^{XT}
with on-bead PCR
for Illumina sequencers

Parameters

1) Select Protocol to Run

2) Select additional Parameters
 AMPureXP_XT_Illumina_v1.5.pro Only
 a. Select DNA Product to Cleanup

 b. Select Plate type containing DNA for cleanup

3) Select Number of Columns of Samples*
 1 Columns selected
*needs to be 1, 2, 3, 4, 6, or 12 columns

4) Click button below to Display Initial Workstation Setup

Controls

Once you have loaded labware according to Workstation Setup on right, Click "Run Selected Protocol" to start run.

Elapsed Time: 00:00:01

Advanced Settings

☐ TESTING ONLY: Reduces all incubation times and/or mix cycles

Workstation Setup

MiniHub	MiniHub Cassette 1	MiniHub Cassette 2	MiniHub Cassette 3	MiniHub Cassette 4
Shelf 5	Empty Nunc DeepWell Plate			
Shelf 4				
Shelf 3		Empty Eppendorf Twin.tec Plate		
Shelf 2		Nuclease-free Water Reservoir	AmpureXP Beads in Nunc DeepWell	
Shelf 1		70% Ethanol Reservoir		

Bravo Deck

<Position 1> Waste Reservoir (Axygen 96DW)	<Position 2>	<Position 3>
<Pos 4: Peltier> 4°C DNA Plate for Cleanup (set in Parameter 2)	<Pos 5: Shaker>	<Pos 6: Peltier> RT
<Pos 7: Magnetic>	<Position 8> Empty Tip Box	<Pos 9: Chilled> RT

BenchCel

BenchCel Stacker 1	BenchCel Stacker 2	BenchCel Stacker 3	BenchCel Stacker 4
1 Tip Box	Empty	Empty	Empty

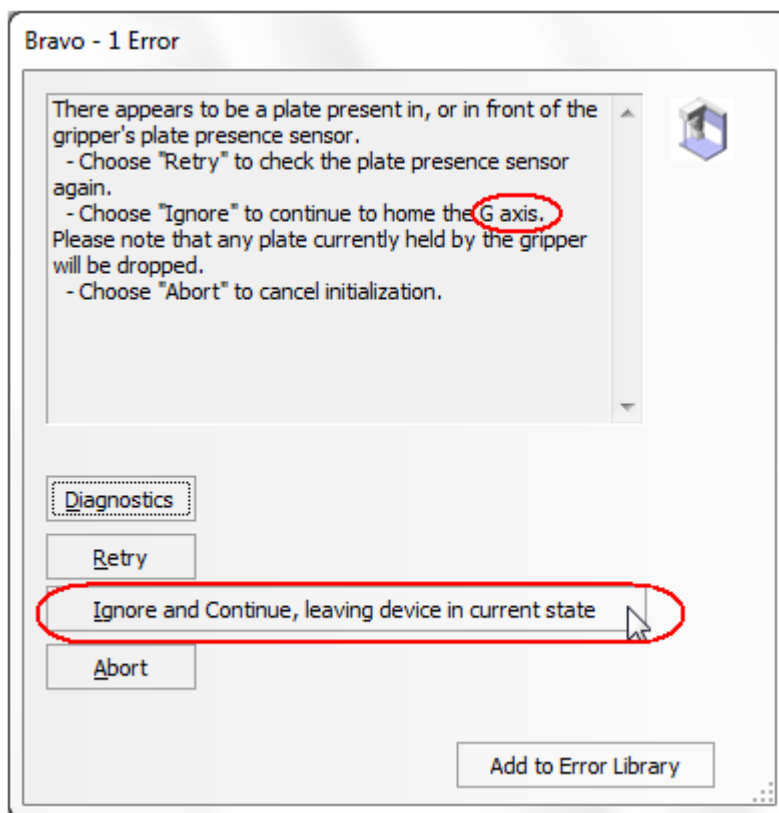
- 5 After verifying that the NGS Workstation has been set up correctly, click **Run Selected Protocol**.



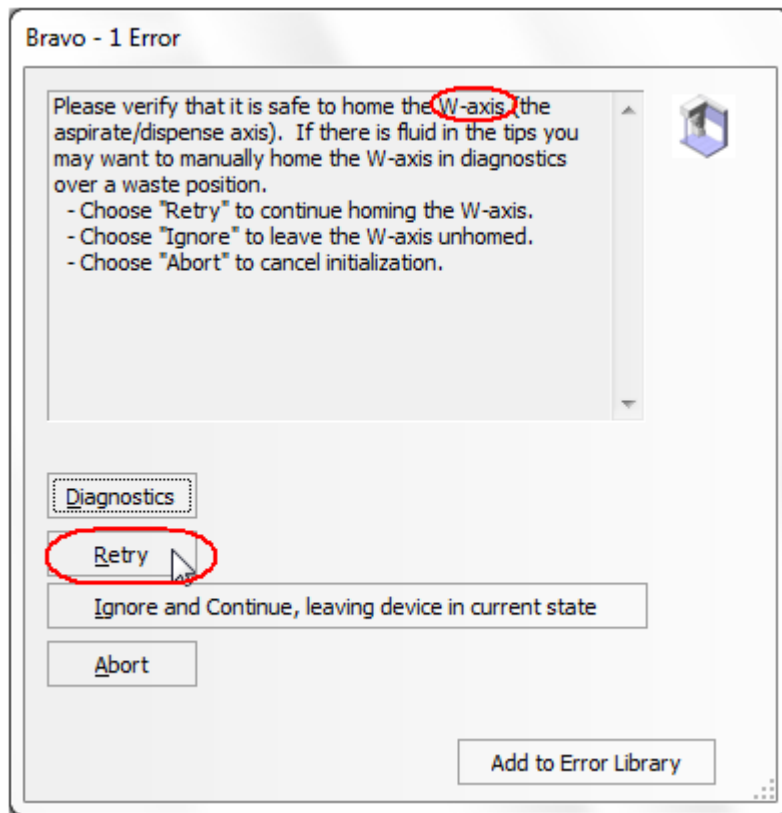
Error messages encountered at start of run

After starting the run, you may see the error messages displayed below. When encountered, make the indicated selections and proceed with the run. Encountering either or both of these error messages is not indicative of a problem with the NGS workstation or your run setup.

- 1 If you encounter the G-axis error message shown below, select **Ignore and Continue, leaving device in current state**.



- 2 If you encounter the W-axis error message shown below, select **Retry**.



Verifying the Simulation setting

VWorks software may be run in simulation mode, during which commands entered on screen are not completed by the NGS workstation. If workstation devices do not respond when you start a run, verify the simulation mode status in VWorks using the following steps.

- 1 Verify that **Simulation is off** is displayed on the status indicator (accessible by clicking **View > Control Toolbar**).



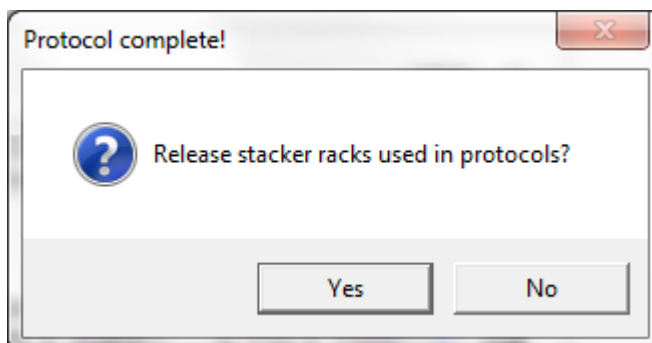
- 2 If the indicator displays **Simulation is on**, click the status indicator button to turn off the simulation mode.

NOTE

If you cannot see the toolbar above the SureSelect_XT_Illumina VWorks form, click the **Full Screen** button to exit full screen mode. If the toolbar is still not visible, right-click on the form and then select **Control Toolbar** from the menu.

Finishing a protocol or runset

The window below appears when each run is complete. Click **Yes** to release the BenchCel racks to allow removal of components used in the current run in preparation for the next .pro or .rst run.



Overview of the SureSelect Target Enrichment Procedure

[Figure 2](#) summarizes the SureSelect target enrichment workflow for samples to be sequenced using the Illumina paired-read sequencing platform. For each sample to be sequenced, individual library preparations, hybridizations, and captures are performed. The samples are then tagged by PCR with an index sequence. Depending on the target size of the SureSelect capture, up to 16 samples can be pooled and sequenced in a single lane using the Illumina-specified multiplex index tags that are provided with SureSelect Library Prep kits.

[Table 6](#) summarizes how the VWorks protocols are integrated into the SureSelect workflow. See [Sample Preparation](#), [Hybridization](#), and [Indexing](#) chapters for complete instructions for use of the VWorks protocols for sample processing.

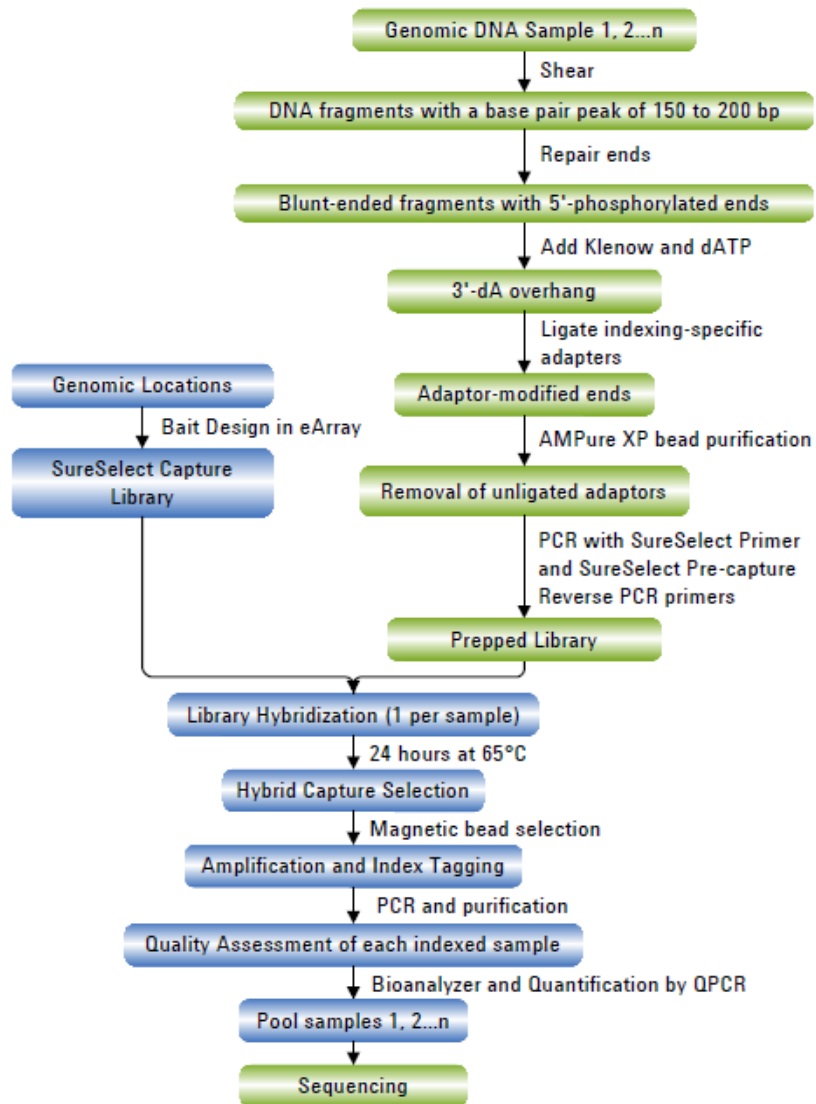


Figure 2 Overall sequencing sample preparation workflow.

2 Using the Agilent NGS Workstation for SureSelect Target Enrichment

Overview of the SureSelect Target Enrichment Procedure

Table 6 Overview of VWorks protocols and runsets used during the workflow

Workflow Step (Protocol Chapter)	Substep	VWorks Protocols Used for Agilent NGS Workstation automation
Sample Preparation	Purify DNA using AMPure XP beads	AMPureXP_XT_Illumina_v1.5.pro
	Prepare adaptor-ligated DNA	LibraryPrep_XT_Illumina_v1.5.rst
	Amplify adaptor-ligated DNA	Pre-CapturePCR_XT_Illumina_v1.5.pro
	Purify DNA using AMPure XP beads	AMPureXP_XT_Illumina_v1.5.pro
Hybridization	Aliquot 750-ng of prepped libraries for hybridization	Aliquot_Libraries_v1.5.pro
	Hybridize prepped DNA to Capture Library	Hybridization_v1.5.pro
	Capture and wash DNA hybrids	SureSelectCapture&Wash_v1.5.rst
Indexing	Add index tags by PCR	Post-CaptureIndexing_XT_Illumina_v1.5.pro
	Purify DNA using AMPure XP beads	AMPureXP_XT_Illumina_v1.5.pro

Experimental Setup Considerations for Automated Runs

Agilent SureSelect Automated Library Prep and Capture System runs may include 1, 2, 3, 4, 6, or 12 columns (equivalent to 8, 16, 24, 32, 48, or 96 wells) of gDNA samples to be enriched for sequencing on the Illumina platform. Plan your experiments using complete columns of samples.

Table 7 Columns to Samples Equivalency

Number of Columns Processed	Total Number of Samples Processed
1	8
2	16
3	24
4	32
6	48
12	96

The number of columns or samples that may be processed using the supplied reagents (see [Table 1](#)) will depend on the experimental design. For greatest efficiency of reagent use, plan experiments using at least 3 columns per run. Each 96-reaction kit contains sufficient reagents for 96 reactions configured as 4 runs of 3 columns of samples per run.

Considerations for Placement of gDNA Samples in 96-well Plates for Automated Processing

- The Agilent NGS Workstation processes samples column-wise beginning at column 1. gDNA samples should be loaded into 96-well plates column-wise, in well order A1 to H1, then A2 to H2, ending with A12 to H12. When processing partial runs with <12 sample columns, do not leave empty columns between sample columns; always load the plate using the left-most column that is available.
- At the hybridization step (see [Figure 2](#)), you can add a different SureSelect Capture Library to each row of the plate. Plan your experiment such that each prepared DNA library corresponds to the appropriate SureSelect Capture Library.
- For sample indexing after hybridization to the SureSelect library (see [Figure 2](#)), you will need to prepare a separate plate containing the indexing primers. Assign the wells to be indexed with their respective indexing primers during experimental design.
- For post-capture amplification (see [Figure 2](#)), different SureSelect Capture Libraries can require different amplification cycle numbers, based on sizes of the captured targets. It is most efficient to process similar-sized Capture Libraries on the same plate. See [Table 55](#) on page 110 to determine which Capture Libraries may be amplified on the same plate.

Considerations for Equipment Setup

- Some workflow steps require the rapid transfer of sample plates between the Bravo deck and a thermal cycler. Locate your thermal cycler in close proximity to the Agilent NGS Workstation to allow rapid and efficient plate transfer.
- Several workflow steps require that the sample plate be sealed using the PlateLoc thermal microplate sealer on the Agilent NGS Workstation, and then centrifuged to collect any dispersed liquid. To maximize efficiency, locate the centrifuge in close proximity to the Agilent NGS Workstation.



3 Sample Preparation

- Step 1. Shear DNA 34
- Step 2. Purify sheared DNA using AMPure XP beads 37
- Step 3. Assess sample quality and DNA fragment size 42
- Step 4. Modify DNA ends for target enrichment 44
- Step 5. Amplify adaptor-ligated libraries 53
- Step 6. Purify amplified DNA using AMPure XP beads 62
- Step 7. Assess Library DNA quantity and quality 66

This section contains instructions for gDNA library preparation specific to the Illumina paired-read sequencing platform and to automated processing using the Agilent NGS Workstation. For each sample to be sequenced, individual library preparations, hybridizations, and captures are performed in separate wells of a 96-well plate. The samples are then tagged by PCR with an index sequence. Depending on the target size of the SureSelect capture, up to 16 samples can be pooled and sequenced in a single lane using the Illumina-specified index tags that are provided with SureSelect^{XT} target enrichment kits.

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 Illumina's protocol *Preparing Samples for Paired-End Sequencing* (p/n 1005361), or the appropriate Illumina protocol for more information.



Step 1. Shear DNA

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. Make sure the gDNA is of high quality (non-degraded, A_{260}/A_{280} is 1.8 to 2.0).

Follow the instructions for the instrument.

- 2 Dilute 3 μg of high-quality gDNA with 1X Low TE Buffer in a 1.5-mL LoBind tube to a total volume of 130 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 at least 30 minutes, 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

When using a Covaris E-series instrument to prepare multiple gDNA samples in the same experiment, you can also use the 96 microTube plate (see [Table 4](#) on page 14) for the DNA shearing step.

- 5 Use a tapered pipette tip to slowly transfer the 130 μL DNA sample through the pre-split septa.

Be careful not to introduce a bubble into the bottom of the tube.
- 6 Secure the microTube in the tube holder and shear the DNA with the settings in [Table 8](#) or [Table 9](#), depending on the Covaris instrument SonoLab software version used.

The target peak size is 150 to 200 bp.

Table 8 Shear settings for Covaris instruments using SonoLab software version 7 or newer

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

Table 9 Shear settings for Covaris instruments using SonoLab software prior to version 7

Setting	Value
Duty Cycle	10%
Intensity	5
Cycles per Burst	200
Time	6 cycles of 60 seconds each
Set Mode	Frequency sweeping
Temperature	4° to 7° C

- 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.

3 Sample Preparation

Step 1. Shear DNA

- 9 Transfer the sheared DNA into the wells of a 96-well Eppendorf plate, column-wise for processing on the Agilent NGS Workstation, in well order A1 to H1, then A2 to H2, ending with A12 to H12.

NOTE

SureSelect Automated Library Prep and Capture System runs may include 1, 2, 3, 4, 6, or 12 columns of the plate. See [Using the Agilent NGS Workstation for SureSelect Target Enrichment](#) for additional sample placement considerations.

- 10 Seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 11 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to remove air bubbles.

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

Step 2. Purify sheared DNA using AMPure XP beads

In this step, the Agilent NGS Workstation transfers AMPure XP beads and gDNA samples to a Nunc DeepWell plate and then collects and washes the bead-bound DNA.

Prepare the workstation and reagents

- 1 Clear the Labware MiniHub and BenchCel of all plates and tip boxes.
- 2 Gently wipe down the Labware MiniHub, Bravo deck, and BenchCel with a NucleoClean decontamination wipe.
- 3 Let the AMPure XP beads come to room temperature for at least 30 minutes.
- 4 Mix the bead suspension well so that the reagent appears homogeneous and consistent in color. *Do not freeze.*
- 5 Prepare a Nunc DeepWell source plate for the beads by adding 240 μL of homogenous AMPure XP beads per well, for each well to be processed.
- 6 Prepare a Thermo Scientific reservoir containing 15 mL of nuclease-free water.
- 7 Prepare a separate Thermo Scientific reservoir containing 45 mL of freshly-prepared 70% ethanol.

3 Sample Preparation

Step 2. Purify sheared DNA using AMPure XP beads

8 Load the Labware MiniHub according to [Table 10](#), using the plate orientations shown in [Figure 3](#).

Table 10 Initial MiniHub configuration for AMPureXP_XT_Illumina_v1.5.pro

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty Nunc DeepWell plate	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty Eppendorf Plate	Empty	Empty
Shelf 2	Empty	Nuclease-free water reservoir from step 6	AMPure XP beads in Nunc DeepWell plate from step 5	Empty
Shelf 1 (Bottom)	Empty	70% ethanol reservoir from step 7	Empty	Empty

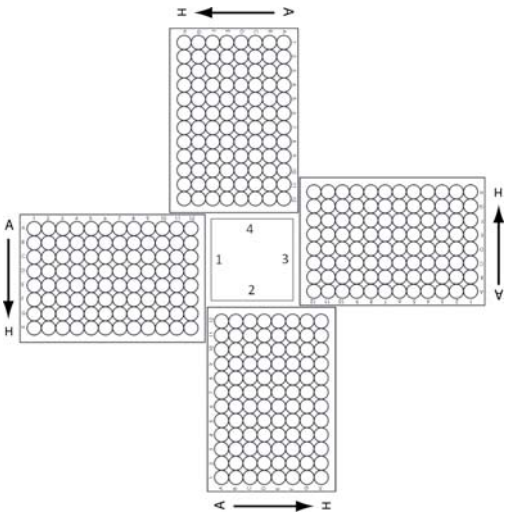


Figure 3 Agilent Labware MiniHub plate orientation. For Thermo Scientific reservoirs, place the notched corner facing the center of the hub.

- 9 Load the BenchCel Microplate Handling Workstation according to [Table 11](#).

Table 11 Initial BenchCel configuration for AMPureXP_XT_Illumina_v1.5.pro

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	2 Tip boxes	Empty	Empty	Empty
4	2 Tip boxes	Empty	Empty	Empty
6	3 Tip boxes	Empty	Empty	Empty
12	6 Tip boxes	Empty	Empty	Empty

- 10 Load the Bravo deck according to [Table 12](#).

Table 12 Initial Bravo deck configuration for AMPureXP_XT_Illumina_v1.5.pro

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
4	Eppendorf plate containing sheared gDNA samples, oriented with well A1 in the upper-left
8	Empty tip box

Run VWorks protocol AMPureXP_XT_Illumina_v1.5.pro

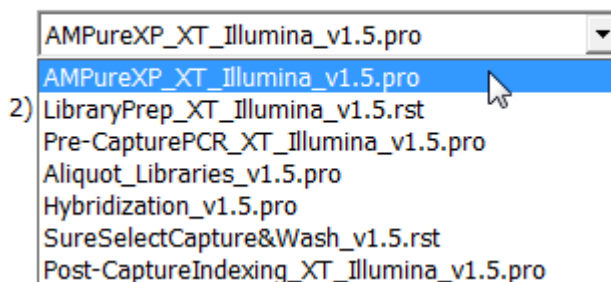
- 11 Open the SureSelect setup form using the SureSelect_XT_Illumina.VWForm shortcut on your desktop.
- 12 Log in to the VWorks software.

3 Sample Preparation

Step 2. Purify sheared DNA using AMPure XP beads

13 On the setup form, under **Select Protocol to Run**, select **AMPureXP_XT_Illumina_v1.5.pro**.

1) Select Protocol to Run



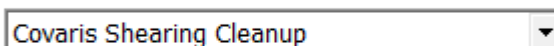
AMPureXP_XT_Illumina_v1.5.pro
AMPureXP_XT_Illumina_v1.5.pro
LibraryPrep_XT_Illumina_v1.5.rst
Pre-CapturePCR_XT_Illumina_v1.5.pro
Aliquot_Libraries_v1.5.pro
Hybridization_v1.5.pro
SureSelectCapture&Wash_v1.5.rst
Post-CaptureIndexing_XT_Illumina_v1.5.pro

14 Under **Select additional Parameters**, select the step **Covaris Shearing Cleanup** and the plate type **96 Eppendorf Twin.tec PCR**.

2) Select additional Parameters

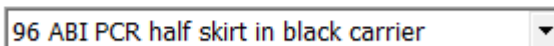
AMPureXP_XT_Illumina_v1.5.pro Only

a. Select DNA Product to Cleanup



Covaris Shearing Cleanup

b. Select Plate type containing DNA for cleanup



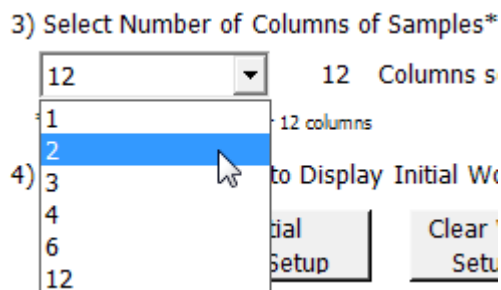
96 ABI PCR half skirt in black carrier

3) Select 96 Eppendorf Twin.tec PCR

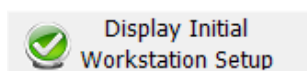
NOTE

The AMPureXP_XT_Illumina_v1.5.pro protocol is used during multiple steps of the SureSelect automation workflow. Be sure to select the correct workflow step when initiating the automation protocol.

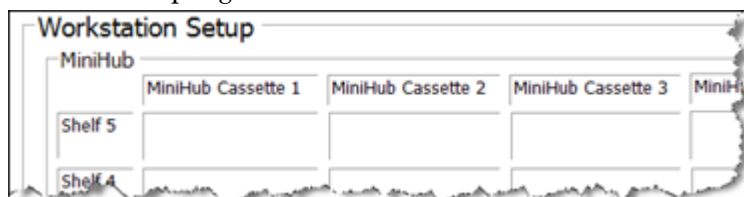
- 15** Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.



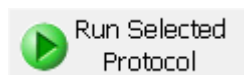
- 16** Click **Display Initial Workstation Setup**.



- 17** Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



- 18** When verification is complete, click **Run Selected Protocol**.



NOTE

If workstation devices do not respond when you start the run, but activity is recorded in the Log, verify that VWorks is not running in Simulation mode. See [page 27](#) for more information.

Running the AMPureXP_XT_Illumina_v1.5.pro protocol takes approximately 45 minutes. Once complete, the purified DNA samples are located in the Eppendorf plate at position 7 of the Bravo deck.

Step 3. Assess sample quality and DNA fragment size

Option 1: Analysis using the Agilent 2100 Bioanalyzer and DNA 1000 Assay

Use a Bioanalyzer DNA 1000 chip and reagent kit. For more information to do this step, see the *Agilent DNA 1000 Kit Guide*.

- 1 Set up the 2100 Bioanalyzer as instructed in the reagent kit guide.
- 2 Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 3 Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
- 4 Prepare the chip, samples and ladder as instructed in the reagent kit guide, using 1 μ L of each sample for the analysis.
- 5 Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
- 6 Verify that the electropherogram shows an average DNA fragment size of 150 to 200 bp. A sample electropherogram is shown in [Figure 4](#).

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.

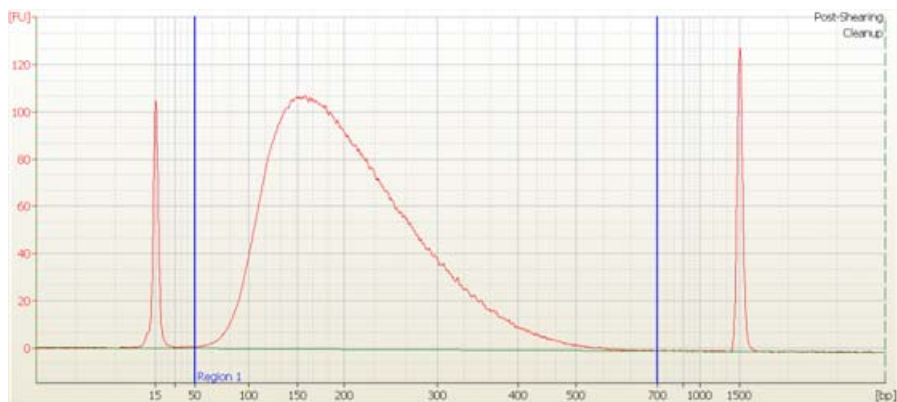


Figure 4 Analysis of sheared DNA using a DNA 1000 Bioanalyzer assay. The electropherogram shows an average peak size between 150 to 200 bp.

Option 2: Analysis using the Agilent 2200 TapeStation and D1K ScreenTape

You can use Agilent's 2200 TapeStation for rapid analysis of multiple samples. Use a D1K ScreenTape (p/n 5067-5361) and associated reagent kit (p/n 5067-5362) to analyze the sheared DNA. For more information to do this step, see the *Agilent 2200 TapeStation User Manual*.

- 1 Seal the sheared DNA sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 2 Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
- 3 Prepare the TapeStation samples as instructed in the *Agilent 2200 TapeStation User Manual*. Use 1 µL of each sheared DNA sample diluted with 3 µL of D1K sample buffer for the analysis.
- 4 Load the sample plate or tube strips from [step 3](#), the D1K ScreenTape, and loading tips into the 2200 TapeStation as instructed in the *Agilent 2200 TapeStation User Manual*. Start the run.
- 5 Verify that the electropherogram shows an average DNA fragment size of 150 to 200 bp. A sample electropherogram is shown in [Figure 5](#).

Stopping Point

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

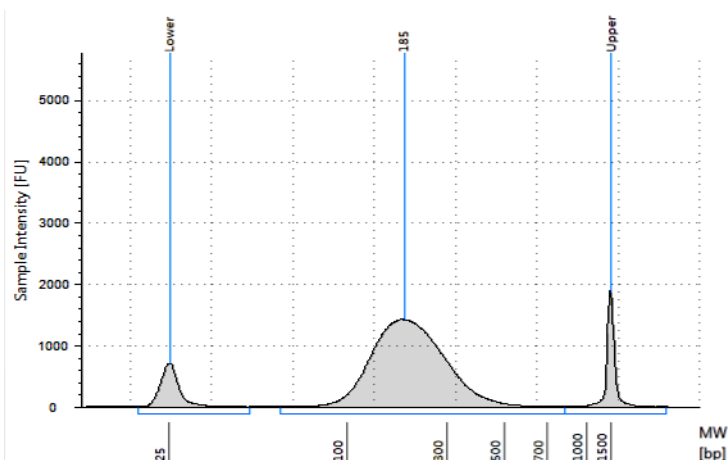


Figure 5 Analysis of sheared DNA using the 2200 TapeStation with a D1K ScreenTape. The electropherogram shows an average DNA fragment size between 150 to 200 bp.

Step 4. Modify DNA ends for target enrichment

In this step, the Agilent NGS Workstation completes the DNA end modification steps required for SureSelect target enrichment, including GA end-repair, A-tailing, and adaptor ligation. After each modification step, the Agilent NGS Workstation purifies the prepared DNA using AMPure XP beads.

Before starting the run, you need to prepare master mixes (with overage) for each step, without the DNA sample. Master mixes for runs that include 1, 2, 3, 4, 6, and 12 columns (including overage) are shown in each table.

Prepare each master mix on ice.

Prepare the workstation

- 1** Turn on the ThermoCube, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.
- 2** Clear the Labware MiniHub and BenchCel of all plates and tip boxes.

Prepare the SureSelect DNA end-repair master mix

- 3** Prepare the appropriate volume of end-repair master mix, according to [Table 13](#). Mix well using a vortex mixer and keep on ice.

Table 13 Preparation of End-Repair Master Mix

SureSelect^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	35.2 µL	448.8 µL	748.0 µL	1047.2 µL	1346.4 µL	1944.8 µL	3889.6 µL
10X End-Repair Buffer	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105.0 µL
dNTP mix	1.6 µL	20.4 µL	34.0 µL	47.6 µL	61.2 µL	88.4 µL	176.8 µL
T4 DNA polymerase	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
Klenow DNA polymerase	2.0 µL	25.5 µL	42.5 µL	59.5 µL	76.5 µL	110.5 µL	221.0 µL
T4 Polynucleotide Kinase	2.2 µL	28.1 µL	46.8 µL	65.5 µL	84.2 µL	121.6 µL	243.1 µL
Total Volume	52 µL	663 µL	1105 µL	1547 µL	1989 µL	2873 µL	5746 µL

3 Sample Preparation

Step 4. Modify DNA ends for target enrichment

Prepare the A-tailing master mix

- 4 Prepare the appropriate volume of A-tailing master mix, according to [Table 14](#). Mix well using a vortex mixer and keep on ice.

Table 14 Preparation of A-Tailing Master Mix

SureSelect ^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	11.0 µL	187.0 µL	280.5 µL	374.0 µL	467.5 µL	654.5 µL	1262.3 µL
10x Klenow DNA Polymerase Buffer	5.0 µL	85.0 µL	127.5 µL	170.0 µL	212.5 µL	297.5 µL	573.8 µL
dATP	1.0 µL	17.0 µL	25.5 µL	34.0 µL	42.5 µL	59.5 µL	114.8 µL
Exo (-) Klenow DNA Polymerase	3.0 µL	51.0 µL	76.5 µL	102.0 µL	127.5 µL	178.5 µL	344.3 µL
Total Volume	20 µL	340 µL	510 µL	680 µL	850 µL	1190 µL	2295 µL

Prepare the adaptor ligation master mix

- 5 Prepare the appropriate volume of adaptor ligation master mix, according to [Table 15](#). Mix well using a vortex mixer and keep on ice.

Table 15 Preparation of Adaptor Ligation Master Mix

SureSelect ^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	15.5 µL	197.6 µL	329.4 µL	461.1 µL	592.9 µL	856.4 µL	1712.8 µL
5X T4 DNA Ligase Buffer	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105.0 µL
SureSelect Adaptor Oligo Mix*	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105.0 µL
T4 DNA Ligase	1.5 µL	19.1 µL	31.9 µL	44.6 µL	57.4 µL	82.9 µL	165.8 µL
Total Volume	37.0 µL	471.8 µL	786.3 µL	1100.8 µL	1415.3 µL	2044.3 µL	4088.5 µL

* Previously labeled as InPE Adaptor Oligo Mix.

Prepare the master mix source plate

- 6 In a Nunc DeepWell plate, prepare the master mix source plate containing the master mixes prepared in steps 3 to 5. Add the volumes indicated in [Table 16](#) of each master mix to all wells of the indicated column of the Nunc DeepWell plate. Keep the master mixes on ice during the aliquoting steps. The final configuration of the master mix source plate is shown in [Figure 6](#).

Table 16 Preparation of the Master Mix Source Plate for LibraryPrep_XT_Illumina_v1.5.rst

Master Mix Solution	Position on Source Plate	Volume of Master Mix added per Well of Nunc Deep Well Source Plate					
		1-Column Runs	2-Column Runs	3-Column Runs	4-Column Runs	6-Column Runs	12-Column Runs
End Repair Master Mix	Column 1 (A1-H1)	76.4 µL	131.6 µL	186.9 µL	242.1 µL	352.6 µL	711.8 µL
A-Tailing Master Mix	Column 2 (A2-H2)	40.0 µL	61.3 µL	82.5 µL	103.8 µL	146.3µL	284.4 µL
Adaptor Ligation Master Mix	Column 3 (A3-H3)	54.3 µL	93.7 µL	133.0 µL	172.3 µL	250.9 µL	506.4 µL

3 Sample Preparation

Step 4. Modify DNA ends for target enrichment

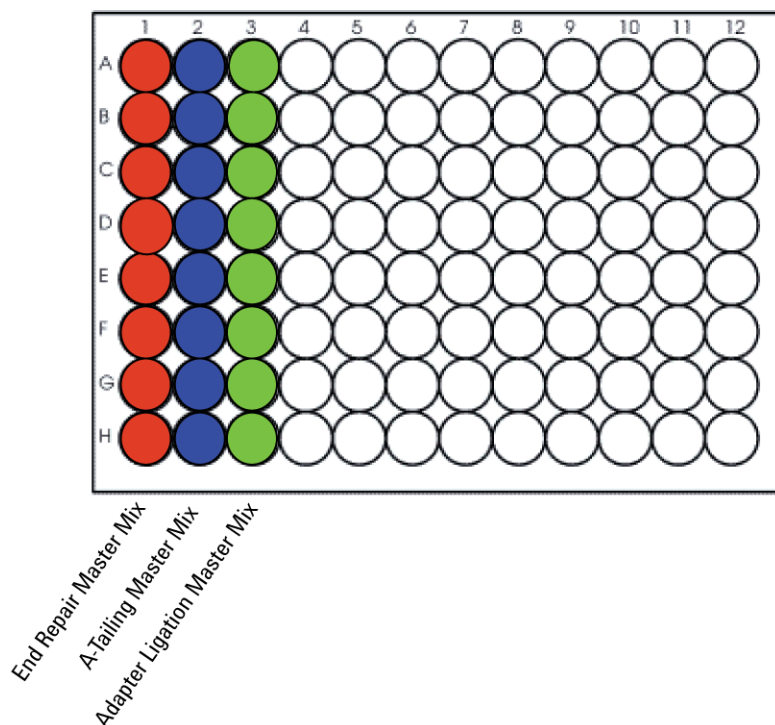


Figure 6 Configuration of the master mix source plate for LibraryPrep_XT_Illumina_v1.5.rst

- 7** Seal the master mix source plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 8** Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate any bubbles. Keep the master mix source plate on ice.

NOTE

The presence of bubbles in source plate solutions may cause inaccurate volume transfer by the Bravo liquid handling platform. Ensure that the source plate is sealed and centrifuged prior to use in a run.

Prepare the purification reagents

- 9 Verify that the AMPure XP bead suspension is at room temperature.
- 10 Mix the bead suspension well so that the reagent appears homogeneous and consistent in color. *Do not freeze.*
- 11 Prepare a separate Nunc DeepWell source plate for the beads by adding 370 μ L of homogenous AMPure XP beads per well, for each well to be processed.
- 12 Prepare a Thermo Scientific reservoir containing 20 mL of nuclease-free water.
- 13 Prepare a separate Thermo Scientific reservoir containing 150 mL of freshly-prepared 70% ethanol.

Load the Agilent NGS Workstation

- 14 Load the Labware MiniHub according to [Table 17](#), using the plate orientations shown in [Figure 3](#).

Table 17 Initial MiniHub configuration for LibraryPrep_XT_Illumina_v1.5.rst

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty Nunc DeepWell plate	Empty Nunc DeepWell plate	Empty Nunc DeepWell plate	Empty
Shelf 4	Empty	Empty Eppendorf plate	Empty Eppendorf plate	Empty
Shelf 3	Empty	Empty	Empty	Empty Eppendorf plate
Shelf 2	Empty tip box	Nuclease-free water reservoir from step 12	AMPure XP beads in Nunc DeepWell plate from step 11	Empty
Shelf 1 (Bottom)	New tip box	70% ethanol reservoir from step 13	Empty	Empty tip box

3 Sample Preparation

Step 4. Modify DNA ends for target enrichment

15 Load the BenchCel Microplate Handling Workstation according to [Table 18](#).

Table 18 Initial BenchCel configuration for LibraryPrep_XT_Illumina_v1.5.rst

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	2 Tip boxes	Empty	Empty	Empty
2	4 Tip boxes	Empty	Empty	Empty
3	5 Tip boxes	Empty	Empty	Empty
4	7 Tip boxes	Empty	Empty	Empty
6	10 Tip boxes	Empty	Empty	Empty
12	11 Tip boxes	8 Tip boxes	Empty	Empty

16 Load the Bravo deck according to [Table 19](#).

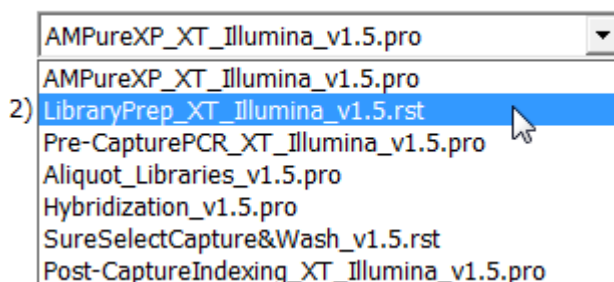
Table 19 Initial Bravo deck configuration for LibraryPrep_XT_Illumina_v1.5.rst

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
6	Empty Eppendorf plate, oriented with well A1 in the upper-left
7	Eppendorf plate containing purified gDNA samples, oriented with well A1 in the upper-left
9	DNA End Modification Master Mix Source Plate

Run VWorks runset LibraryPrep_XT_Illumina_v1.5.rst

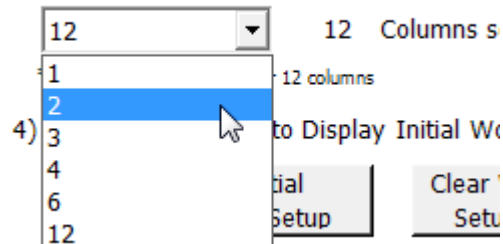
17 On the SureSelect setup form, under **Select Protocol to Run**, select **LibraryPrep_XT_Illumina_v1.5.rst**.

1) Select Protocol to Run

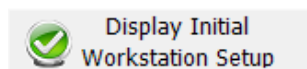


18 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

3) Select Number of Columns of Samples*



19 Click **Display Initial Workstation Setup**.



3 Sample Preparation

Step 4. Modify DNA ends for target enrichment

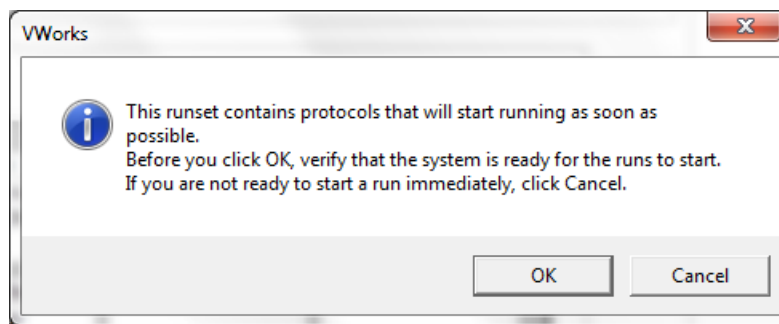
- 20 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



- 21 When verification is complete, click **Run Selected Protocol**.



- 22 When ready to begin the run, click **OK** in the following window.



Running the LibraryPrep_XT_Illumina_v1.5.rst runset takes approximately 4 hours. Once complete, the purified, adaptor-ligated DNA samples are located in the Eppendorf plate at position 7 of the Bravo deck.

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 5. Amplify adaptor-ligated libraries

In this step, the Agilent NGS Workstation completes the liquid handling steps for amplification of the adaptor-ligated DNA samples. Afterward, you transfer the PCR plate to a thermal cycler for amplification.

In this protocol, one half of the adaptor-ligated DNA sample is removed from the Eppendorf sample plate for amplification. The remainder can be saved at 4°C for future use or amplification troubleshooting, if needed. Store the samples at –20°C for long-term storage.

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 the workstation

- 1 Turn on the ThermoCube, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.
- 2 Leave tip boxes on shelves 1 and 2 in cassette 1 of the Labware MiniHub from the previous LibraryPrep_XT_Illumina_v1.5.rst run. Otherwise, clear the remaining positions of the MiniHub and BenchCel of plates and tip boxes.
- 3 Pre-set the temperature of Bravo deck position 6 to 4°C using the Inheco Multi TEC control touchscreen, as described in [Setting the Temperature of Bravo Deck Heat Blocks](#). Bravo deck position 6 corresponds to CPAC 2, position 2 on the Multi TEC control touchscreen.

3 Sample Preparation

Step 5. Amplify adaptor-ligated libraries

Prepare the pre-capture PCR master mix and master mix source plate

- 4 Prepare the appropriate volume of pre-capture PCR Master Mix, according to [Table 20](#). Mix well using a vortex mixer and keep on ice.

Table 20 Preparation of Pre-Capture PCR Master Mix

SureSelect ^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	21.0 µL	267.8 µL	446.3 µL	624.8 µL	803.3 µL	1160.3 µL	2320.5 µL
Herculase II 5X [*] Reaction Buffer	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105 µL
dNTP mix [*]	0.5 µL	6.4 µL	10.6 µL	14.9 µL	19.1 µL	27.6 µL	55.3 µL
SureSelect Primer [†] (Forward)	1.25 µL	15.9 µL	26.6 µL	37.2 µL	47.8 µL	69.1 µL	138.1 µL
SureSelect Indexing Pre-Capture PCR (Reverse) Primer [‡]	1.25 µL	15.9 µL	26.6 µL	37.2 µL	47.8 µL	69.1 µL	138.1 µL
Herculase II Polymerase	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
Total Volume	35 µL	446.3 µL	743.8 µL	1041.3 µL	1338.8 µL	1933.8 µL	3867.5 µL

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

[†] Previously labeled as InPE Primer 1.0. Included in SureSelect^{XT} Library Prep Kit-ILM.

[‡] Included in SureSelect^{XT} Automated Hybridization Kit Box #2. Ensure that the correct primer is selected from Box #2 at this step (do not use the SureSelect Indexing Post-Capture PCR Forward Primer).

- 5 Using the same Nunc DeepWell master mix source plate that was used for the LibraryPrep_XT_Illumina_v1.5.rst run, add the volume of PCR Master Mix indicated in Table 21 to all wells of column 4 of the master mix source plate. The final configuration of the master mix source plate is shown in Figure 7.

Table 21 Preparation of the Master Mix Source Plate for Pre-CapturePCR_XT_Illumina_v1.5.pro

Master Mix Solution	Position on Source Plate	Volume of Master Mix added per Well of Nunc Deep Well Source Plate					
		1-Column Runs	2-Column Runs	3-Column Runs	4-Column Runs	6-Column Runs	12-Column Runs
Pre-Capture PCR Master Mix	Column 4 (A4-H4)	51.4 µL	88.6 µL	125.8 µL	163.0 µL	237.3 µL	479.1 µL

NOTE

If you are using a new DeepWell plate for the pre-capture PCR source plate (for example, when amplifying the second half of the adaptor-ligated DNA sample), leave columns 1 to 3 empty and add the PCR Master Mix to column 4 of the new plate.

3 Sample Preparation

Step 5. Amplify adaptor-ligated libraries

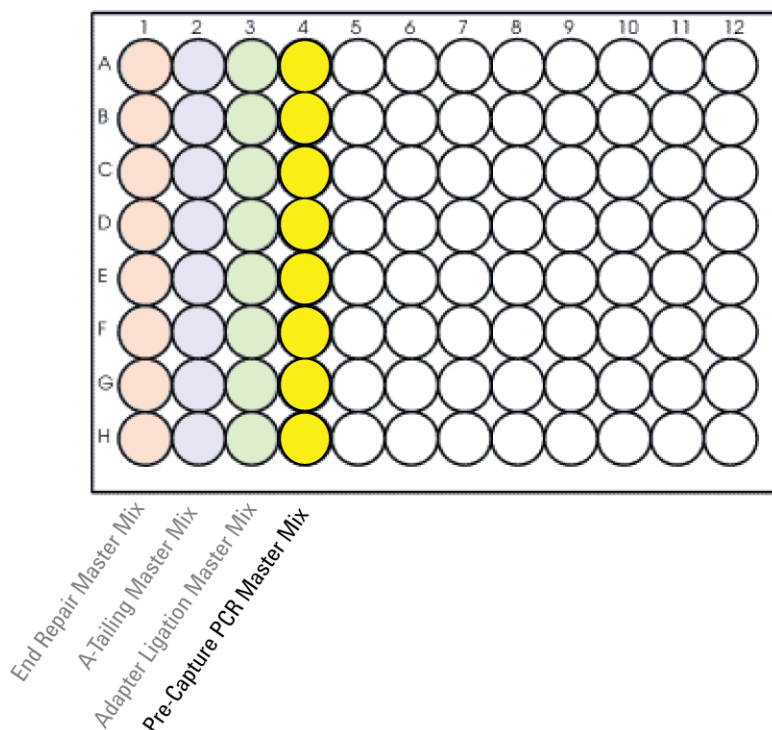


Figure 7 Configuration of the master mix source plate for Pre-CapturePCR_XT_Illumina_v1.5.pro. Columns 1-3 were used to dispense master mixes during the previous protocol.

- 6** Seal the master mix source plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 7** Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate any bubbles.

NOTE

The presence of bubbles in source plate solutions may cause inaccurate volume transfer by the Bravo liquid handling platform. Ensure that the source plate is sealed and centrifuged prior to use in a run.

Load the Agilent NGS Workstation

- 8 Load the Labware MiniHub according to [Table 22](#), using the plate orientations shown in [Figure 3](#).

Table 22 Initial MiniHub configuration for Pre-CapturePCR_XT_Illumina_v1.5.pro

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty	Empty	Empty	Empty ABI MicroAmp plate seated in black adapter
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty	Empty	Empty
Shelf 2	Waste tip box*	Empty	Empty	Empty
Shelf 1 (Bottom)	Clean tip box*	Empty	Empty	Empty tip box

* The waste tip box (Cassette 1, Shelf 2) and clean tip box (Cassette 1, Shelf 1) are retained from the LibraryPrep_XT_Illumina_v1.5.rst run and reused here.

NOTE

If you are using a new box of tips on shelf 1 of cassette 1 (for example, when amplifying the second half of the adaptor-ligated DNA sample), first remove the tips from columns 1 to 3 of the tip box. Any tips present in columns 1 to 3 of the tip box may be inappropriately loaded onto the Bravo platform pipette heads and may interfere with automated processing steps.

3 Sample Preparation

Step 5. Amplify adaptor-ligated libraries

- 9 Load the BenchCel Microplate Handling Workstation according to [Table 23](#).

Table 23 Initial BenchCel configuration for Pre-CapturePCR_XT_Illumina_v1.5.pro

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	1 Tip box	Empty	Empty	Empty
4	1 Tip box	Empty	Empty	Empty
6	1 Tip box	Empty	Empty	Empty
12	1 Tip box	Empty	Empty	Empty

- 10 Load the Bravo deck according to [Table 24](#).

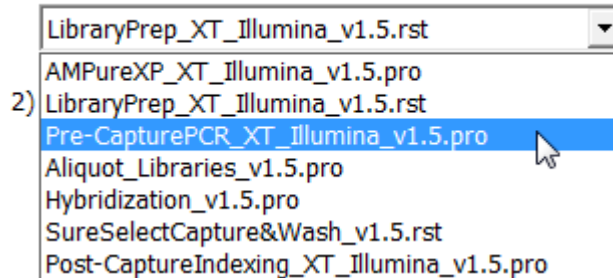
Table 24 Initial Bravo deck configuration for Pre-CapturePCR_XT_Illumina_v1.5.pro

Location	Content
7	Eppendorf plate containing adaptor-ligated DNA samples, oriented with well A1 in the upper-left
9	Master mix plate containing PCR Master Mix in Column 4

Run VWorks protocol Pre-CapturePCR_XT_Illumina_v1.5.pro

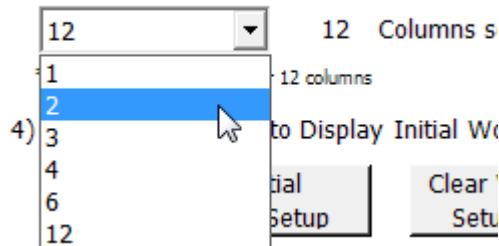
11 On the SureSelect setup form, under **Select Protocol to Run**, select **Pre-CapturePCR_XT_Illumina_v1.5.pro**.

1) Select Protocol to Run

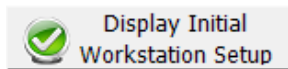


12 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

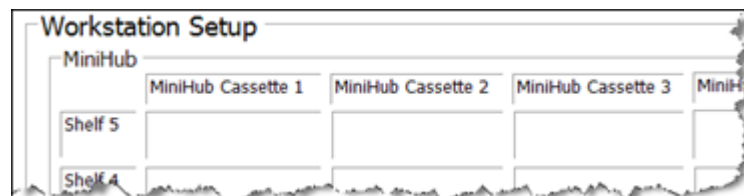
3) Select Number of Columns of Samples*



13 Click **Display Initial Workstation Setup**.



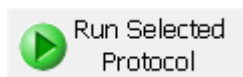
14 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



3 Sample Preparation

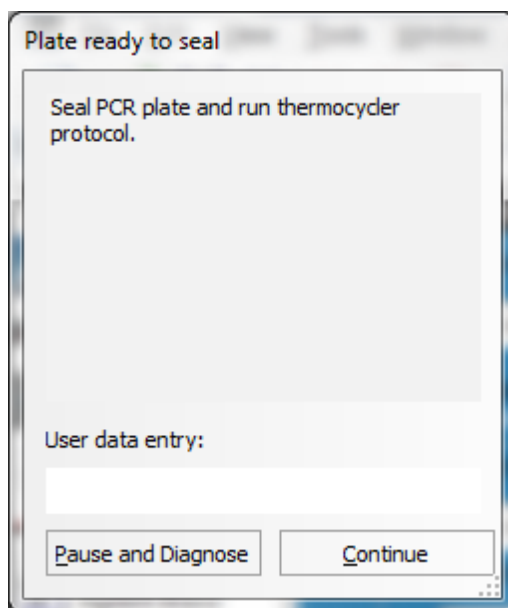
Step 5. Amplify adaptor-ligated libraries

15 When verification is complete, click **Run Selected Protocol**.



Running the Pre-CapturePCR_XT_Illumina_v1.5.pro protocol takes approximately 15 minutes. Once complete, the PCR-ready samples, containing prepped DNA and PCR master mix, are located in the ABI MicroAmp plate at position 5 of the Bravo deck. The Eppendorf plate containing the remaining prepped DNA samples, which may be stored for future use at 4°C overnight, or at -20°C for long-term storage, is located at position 7 of the Bravo deck.

16 When you see the following prompt, remove the PCR plate from position 5 of the Bravo deck and seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 3.0 seconds.



17 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate air bubbles.

18 Transfer the PCR plate to a thermal cycler and run the PCR amplification program shown in [Table 25](#).

Table 25 Pre-Capture PCR cycling program

Segment	Number of Cycles	Temperature	Time
1	1	98°C	2 minutes
2	4 to 6	98°C	30 seconds
		65°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, 5 cycles will produce an adequate yield for subsequent capture without introducing bias or non-specific products. If yield is too low or non-specific high molecular weight products are observed, adjust the number of cycles accordingly with the remaining library template.

Step 6. Purify amplified DNA using AMPure XP beads

In this step, the Agilent NGS Workstation transfers AMPure XP beads and amplified adaptor-ligated DNA to a Nunc DeepWell plate and then collects and washes the bead-bound DNA.

Prepare the workstation and reagents

- 1 Clear the Labware MiniHub and BenchCel of all plates and tip boxes.
- 2 Verify that the AMPure XP bead suspension is at room temperature. (If necessary, allow the bead solution to come to room temperature for at least 30 minutes.)
- 3 Mix the bead suspension well so that the reagent appears homogeneous and consistent in color. *Do not freeze.*
- 4 Prepare a Nunc DeepWell source plate for the beads by adding 92 µL of homogenous AMPure XP beads per well, for each well to be processed.
- 5 Prepare a Thermo Scientific reservoir containing 15 mL of nuclease-free water.
- 6 Prepare a separate Thermo Scientific reservoir containing 45 mL of freshly-prepared 70% ethanol.
- 7 Load the Labware MiniHub according to [Table 26](#), using the plate orientations shown in [Figure 3](#).

Table 26 Initial MiniHub configuration for AMPureXP_XT_Illumina_v1.5.pro

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty Nunc DeepWell plate	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty Eppendorf Plate	Empty	Empty
Shelf 2	Empty	Nuclease-free water reservoir from step 5	AMPure XP beads in Nunc DeepWell plate from step 4	Empty
Shelf 1 (Bottom)	Empty	70% ethanol reservoir from step 6	Empty	Empty

Step 6. Purify amplified DNA using AMPure XP beads

- 8** Load the BenchCel Microplate Handling Workstation according to [Table 27](#).

Table 27 Initial BenchCel configuration for AMPureXP_XT_Illumina_v1.5.pro

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	2 Tip boxes	Empty	Empty	Empty
4	2 Tip boxes	Empty	Empty	Empty
6	3 Tip boxes	Empty	Empty	Empty
12	6 Tip boxes	Empty	Empty	Empty

- 9** Load the Bravo deck according to [Table 28](#).

Table 28 Initial Bravo deck configuration for AMPureXP_XT_Illumina_v1.5.pro

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
4	Amplified DNA libraries in ABI MicroAmp plate seated in black adapter
8	Empty tip box

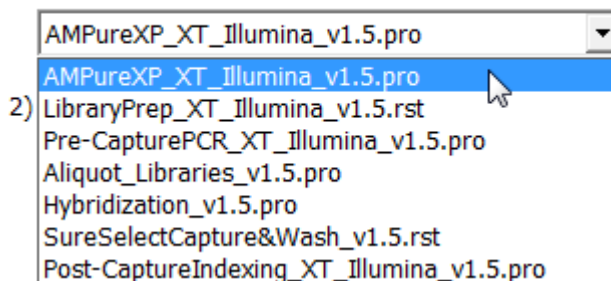
3 Sample Preparation

Step 6. Purify amplified DNA using AMPure XP beads

Run VWorks protocol AMPureXP_XT_Illumina_v1.5.pro

10 On the SureSelect setup form, under **Select Protocol to Run**, select **AMPureXP_XT_Illumina_v1.5.pro**.

1) Select Protocol to Run



AMPureXP_XT_Illumina_v1.5.pro

AMPureXP_XT_Illumina_v1.5.pro

LibraryPrep_XT_Illumina_v1.5.rst

Pre-CapturePCR_XT_Illumina_v1.5.pro

Aliquot_Libraries_v1.5.pro

Hybridization_v1.5.pro

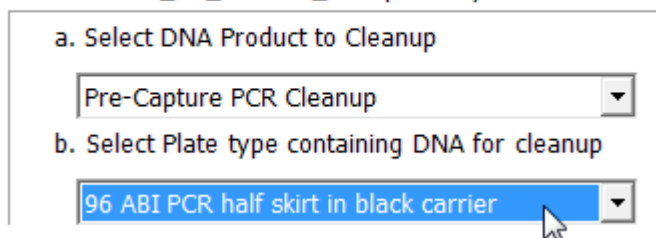
SureSelectCapture&Wash_v1.5.rst

Post-CaptureIndexing_XT_Illumina_v1.5.pro

11 Under **Select additional Parameters**, select the step **Pre-Capture PCR Cleanup** and the plate type **96 ABI PCR half skirt in black carrier**.

2) Select additional Parameters

AMPureXP_XT_Illumina_v1.5.pro Only



a. Select DNA Product to Cleanup

Pre-Capture PCR Cleanup

b. Select Plate type containing DNA for cleanup

96 ABI PCR half skirt in black carrier

NOTE

The AMPureXP_XT_Illumina_v1.5.pro protocol is used during multiple steps of the SureSelect automation workflow. Be sure to select the correct workflow step when initiating the automation protocol.

Step 6. Purify amplified DNA using AMPure XP beads

- 12** Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

3) Select Number of Columns of Samples*

12 Columns s

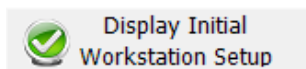
12 columns

4) to Display Initial W

Initial Setup

Clear Setup

- 13** Click **Display Initial Workstation Setup**.



- 14** Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.

Workstation Setup

MiniHub	MiniHub Cassette 1	MiniHub Cassette 2	MiniHub Cassette 3	MiniH
Shelf 5				
Shelf 4				

- 15** When verification is complete, click **Run Selected Protocol**.



The purification protocol takes approximately 45 minutes. When complete, the amplified DNA samples are in the Eppendorf plate located on Bravo deck position 7.

Step 7. Assess Library DNA quantity and quality

The hybridization protocol in the following section requires 750 ng of each amplified DNA library. Measure the concentration of each library using one of the methods detailed below. Once DNA concentration for each sample is determined, calculate the volume of the library to be used for hybridization using the following formula:

$$\text{Volume } (\mu\text{L}) = 750 \text{ ng/concentration (ng/}\mu\text{L)}$$

Option 1: Analysis using the Agilent 2100 Bioanalyzer and DNA 1000 Assay

Use a Bioanalyzer DNA 1000 chip and reagent kit. For more information to do this step, see the *Agilent DNA 1000 Kit Guide*.

- 1 Set up the 2100 Bioanalyzer as instructed in the reagent kit guide.
- 2 Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 3 Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
- 4 Prepare the chip, samples and ladder as instructed in the reagent kit guide, using 1 μL of each sample for the analysis.
- 5 Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
- 6 Verify that the electropherogram shows an average DNA fragment size of 250 to 275 bp. A sample electropherogram is shown in [Figure 8](#).
- 7 Determine the concentration of the library (ng/ μL) by integrating under the peak.

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.

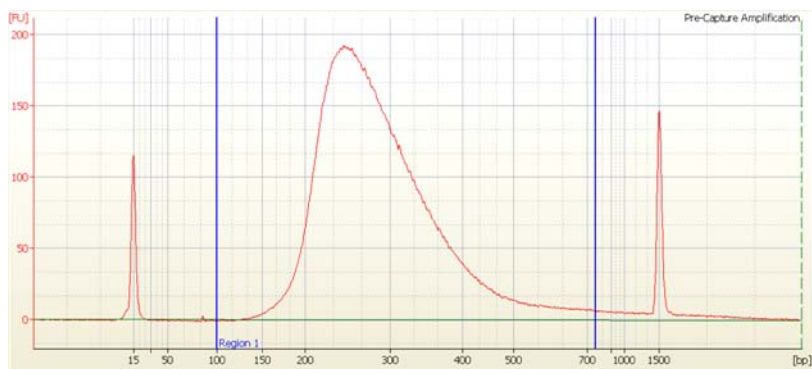


Figure 8 Analysis of amplified library DNA using a DNA 1000 assay. The electropherogram shows a single peak in the size range of 250 to 275 bp.

Option 2: Analysis using the Agilent 2200 TapeStation and D1K ScreenTape

Use a D1K ScreenTape (p/n 5067-5361) and associated reagent kit (p/n 5067-5362) to analyze the amplified libraries. For more information to do this step, see the *Agilent 2200 TapeStation User Manual*.

- 1 Seal the DNA sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 2 Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
- 3 Prepare the TapeStation samples as instructed in the *Agilent 2200 TapeStation User Manual*. Use 1 μ L of each amplified library DNA sample diluted with 3 μ L of D1K sample buffer for the analysis.
- 4 Load the sample plate or tube strips from [step 3](#), the D1K ScreenTape, and loading tips into the 2200 TapeStation as instructed in the *Agilent 2200 TapeStation User Manual*. Start the run.
- 5 Verify that the electropherogram shows an average DNA fragment size of 250 to 275 bp. A sample electropherogram is shown in [Figure 9](#).

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

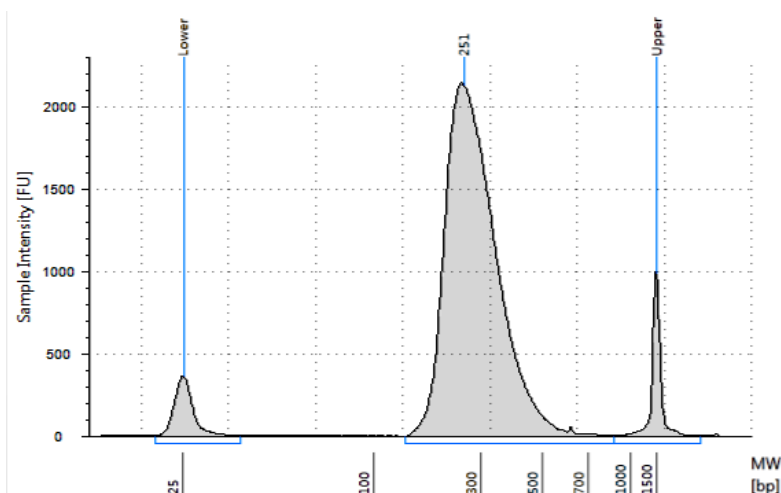


Figure 9 Analysis of amplified library DNA using the 2200 TapeStation with a D1K ScreenTape. The electropherogram shows an average DNA fragment size between 250 to 275 bp.



4 Hybridization

- Step 1. Aliquot prepped DNA samples for hybridization 70
- Step 2. Hybridize the gDNA library and SureSelect Capture Library 74
- Step 3. Capture the hybridized DNA 90

This chapter describes the steps to combine the prepped library with the blocking agents and the SureSelect capture library. Each DNA library sample must be hybridized and captured individually prior to addition of the indexing tag by PCR.

CAUTION

The ratio of SureSelect capture library to prepped 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 35 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.



Step 1. Aliquot prepped DNA samples for hybridization

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

Each hybridization reaction will contain 750 ng of the prepped gDNA sample. Before starting the hybridization step, you must create a table containing instructions for the Agilent NGS Workstation indicating the volume of each sample required for a 750-ng aliquot.

- 1 Create a .csv (comma separated value) file with the headers shown in [Figure 10](#). The header text must not contain spaces. The table may be created using a spreadsheet application, such as Microsoft Excel software, and then saved in .csv format. The file must include rows for all 96 wells of the plate.
- 2 Enter the information requested in the header for each DNA sample.
 - In the SourceBC field, enter the sample plate description or barcode. The SourceBC field contents must be identical for all rows.
 - In the SourceWell and DestinationWell fields, enter each well position for the plate. SourceWell and DestinationWell field contents must be identical for a given sample.
 - In the Volume field, enter the volume (in μL) equivalent to 750 ng DNA for each sample. These values are determined from the concentration values obtained from Bioanalyzer or TapeStation traces in the previous section. For all empty wells on the plate, enter the value 0, as shown in [Figure 10](#); **do not delete rows for empty wells**.

	A	B	C	D
1	SourceBC	SourceWell	DestinationWell	Volume
2	SamplePlateXYZ	A1	A1	5.35
3	SamplePlateXYZ	B1	B1	4.28
4	SamplePlateXYZ	C1	C1	4.76
5	SamplePlateXYZ	D1	D1	5.19
6	SamplePlateXYZ	E1	E1	5.49
7	SamplePlateXYZ	F1	F1	4.86
8	SamplePlateXYZ	G1	G1	5.05
9	SamplePlateXYZ	H1	H1	4.37
10	SamplePlateXYZ	A2	A2	0
11	SamplePlateXYZ	B2	B2	0
12	SamplePlateXYZ	C2	C2	0
13	SamplePlateXYZ	D2	D2	0

Figure 10 Sample spreadsheet for 750-ng sample aliquot for 1-column run.

NOTE

You can find a sample spreadsheet in the directory **C: > VWorks Workspace > NGS Option B > XT Illumina_1.5 > Aliquot Library Input Files > 750ng_transfer_full_plate_template.xlsx**.

The 750ng_transfer_full_plate_template.xlsx file may be copied and used as a template for creating the .csv files for each Aliquot_Libraries_v1.5.pro run. If you are using the sample file as a template for runs with fewer than 12 columns, be sure to retain rows for all 96 wells, and populate the Volume column with 0 for unused wells.

- 3 Load the .csv file onto the PC containing the VWorks software into a suitable folder, such as **C: > VWorks Workspace > NGS Option B > XT Illumina_1.5 > Aliquot Library Input Files**.
- 4 Turn on the chiller, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.
- 5 Load the Bravo deck according to [Table 29](#).

Table 29 Initial Bravo deck configuration for Aliquot_Libraries_v1.5.pro

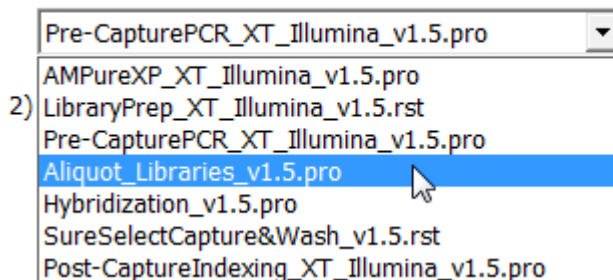
Location	Content
5	Empty ABI MicroAmp plate seated in black adapter (oriented with well A1 in the upper-left)
6	Empty tip box
8	New tip box
9	Prepped library DNA in Eppendorf plate (oriented with well A1 in the upper-left)

4 Hybridization

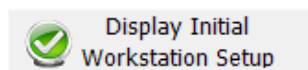
Step 1. Aliquot prepped DNA samples for hybridization

- 6 On the SureSelect setup form, under **Select Protocol to Run**, select **Aliquot_Libraries_v1.5.pro**.

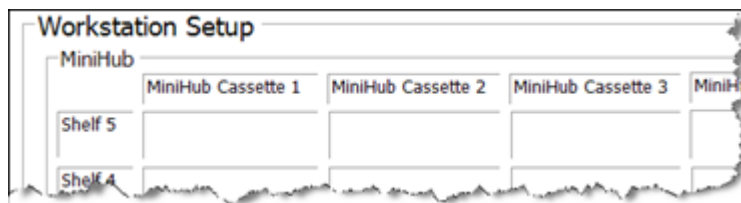
1) Select Protocol to Run



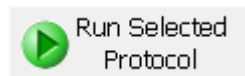
- 7 Click **Display Initial Workstation Setup**.



- 8 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.

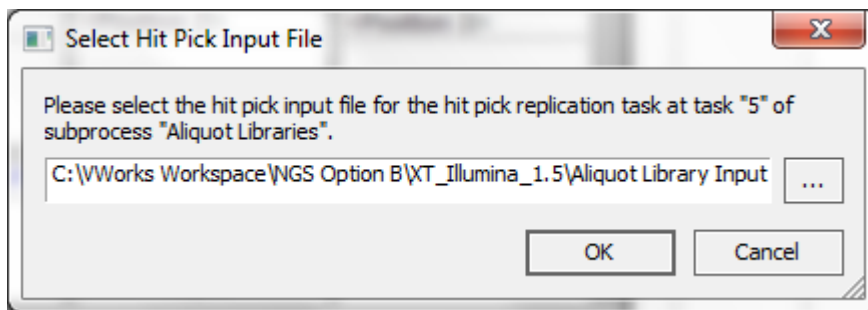


- 9 When verification is complete, click **Run Selected Protocol**.



Step 1. Aliquot prepped DNA samples for hybridization

- 10** When prompted by the dialog below, browse to the .csv file created for the source plate of the current run, and then click **OK** to start the run.



The library aliquoting protocol takes approximately 1 hour for 96 samples. When complete, the 750-ng samples are in the ABI MicroAmp plate located on Bravo deck position 5.

- 11** Remove the 750-ng sample plate from the Bravo deck and use a vacuum concentrator to dry the sample at $\leq 45^{\circ}\text{C}$.
- 12** Reconstitute each dried sample with 3.4 μL of nuclease-free water to bring the final concentration to 221 ng/ μL . Pipette up and down along the sides of each well for optimal recovery.
- 13** Seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 14** Vortex the plate for 30 seconds to ensure complete reconstitution, then centrifuge the plate for 1 minute to drive the well contents off the walls and plate seal.

Step 2. Hybridize the gDNA library and SureSelect Capture Library

In this step, the Agilent NGS Workstation completes the liquid handling steps in preparation for hybridization of the prepared DNA samples to one or more SureSelect capture libraries. Afterward, you transfer the sample plate to a thermal cycler, held at 65°C, to allow hybridization of the DNA sample to the SureSelect capture library.

Prepare the workstation

- 1 Clear the Labware MiniHub and BenchCel of all plates and tip boxes.
- 2 Gently wipe down the Labware MiniHub, Bravo decks, and BenchCel with a NucleoClean decontamination wipe.
- 3 Pre-set the temperature of Bravo deck position 4 to 85°C using the Inheco Multi TEC control touchscreen (see [Setting the Temperature of Bravo Deck Heat Blocks](#)). Bravo deck position 4 corresponds to CPAC 2, position 1 on the Multi TEC control touchscreen.
- 4 Turn on the ThermoCube and set to 25°C for position 9 of the Bravo deck.
- 5 Place the silver Nunc DeepWell plate insert on position 9 of the Bravo deck. This insert is required to facilitate heat transfer to DeepWell source plate wells during the Hybridization protocol.

Step 2. Hybridize the gDNA library and SureSelect Capture Library

Prepare the SureSelect Block master mix

- 6 Prepare the appropriate volume of SureSelect Block master mix, on ice, as indicated in [Table 30](#).

Table 30 Preparation of SureSelect Block Master Mix

SureSelect ^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	6.0 µL	76.5 µL	127.5 µL	178.5 µL	229.5 µL	331.5 µL	663.0 µL
SureSelect Indexing Block #1 (green cap)	2.5 µL	31.9 µL	53.1 µL	74.4 µL	95.6 µL	138.1 µL	276.3 µL
SureSelect Block #2 (blue cap)	2.5 µL	31.9 µL	53.1 µL	74.4 µL	95.6 µL	138.1 µL	276.3 µL
SureSelect Indexing Block #3 (brown cap)	0.6 µL	7.7 µL	12.8 µL	17.9 µL	23.0 µL	33.2 µL	66.3 µL
Total Volume	11.6 µL	147.9 µL	246.5 µL	345.1 µL	443.7 µL	640.9 µL	1281.9 µL

Prepare one or more SureSelect Capture Library master mixes

- 7 Prepare the appropriate volume of SureSelect capture library master mix for each of the capture libraries that will be used for hybridization as indicated in [Table 31](#) to [Table 34](#). Mix the components by pipetting. Keep the master mixes on ice during preparation and aliquoting.

NOTE

Each row of the prepped gDNA sample plate may be hybridized to a different SureSelect Capture Library. However, capture libraries of different sizes require different post-capture amplification cycles. Plan experiments such that similar-sized libraries are hybridized on the same plate.

For runs that use a single capture library for all rows of the plate, prepare the master mix as described in Step a ([Table 31](#) or [Table 32](#)) below.

For runs that use different capture libraries for individual rows, prepare each master mix as described in Step b ([Table 33](#) or [Table 34](#)) below.

4 Hybridization

Step 2. Hybridize the gDNA library and SureSelect Capture Library

- a For runs that use a single capture library for all rows**, prepare the SureSelect Capture Library Master Mix as listed in [Table 31](#) or [Table 32](#), based on the Mb target size of your design.

Table 31 Preparation of Capture Library Master Mix for target sizes <3.0 Mb, 8 rows of wells

Target size <3.0 Mb							
SureSelect ^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	4.5 µL	76.5 µL	114.8 µL	153.0 µL	191.3 µL	267.8 µL	516.4 µL
RNase Block (purple cap)	0.5 µL	8.5 µL	12.8 µL	17.0 µL	21.3 µL	29.8 µL	57.4 µL
SureSelect Capture Library	2.0 µL	34.0 µL	51.0 µL	68.0 µL	85.0 µL	119.0 µL	229.5 µL
Total Volume	7.0 µL	119.0 µL	178.6 µL	238.0 µL	297.6 µL	416.6 µL	803.3 µL

Table 32 Preparation of Capture Library Master Mix for target sizes >3.0 Mb, 8 rows of wells

Target size >3.0 Mb							
SureSelect ^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	1.5 µL	25.5 µL	38.3 µL	51.0 µL	63.8 µL	89.3 µL	172.1 µL
RNase Block (purple cap)	0.5 µL	8.5 µL	12.8 µL	17.0 µL	21.3 µL	29.8 µL	57.4 µL
SureSelect Capture Library	5.0 µL	85.0 µL	127.5 µL	170.0 µL	212.5 µL	297.5 µL	573.8 µL
Total Volume	7.0 µL	119.0 µL	178.6 µL	238.0 µL	297.6 µL	416.6 µL	803.3 µL

Step 2. Hybridize the gDNA library and SureSelect Capture Library

- b For runs that use different capture libraries in individual rows,** prepare a SureSelect Capture Library Master Mix for each capture library as listed in [Table 33](#) or [Table 34](#), based on the Mb target size of your design. The volumes listed in [Table 33](#) and [Table 34](#) are for a single row of sample wells. If a given capture library will be hybridized in multiple rows, multiply each of the values below by the number of rows assigned to that capture library.

Table 33 Preparation of Capture Library Master Mix for target sizes <3.0 Mb, single row of wells

Target size <3.0 Mb							
SureSelect ^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	4.5 µL	9.0 µL	13.8 µL	18.6 µL	23.3 µL	32.9 µL	64.0 µL
RNase Block (purple cap)	0.5 µL	1.0 µL	1.5 µL	2.1 µL	2.6 µL	3.7 µL	7.1 µL
SureSelect Capture Library	2.0 µL	4.0 µL	6.1 µL	8.3 µL	10.4 µL	14.6 µL	28.4 µL
Total Volume	7.0 µL	14.0 µL	21.4 µL	28.9 µL	36.3 µL	51.2 µL	99.5 µL

Table 34 Preparation of Capture Library Master Mix for target sizes >3.0 Mb, single row of wells

Target size >3.0 Mb							
SureSelect ^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	1.5 µL	3.0 µL	4.6 µL	6.2 µL	7.8 µL	11.0 µL	21.3 µL
RNase Block (purple cap)	0.5 µL	1.0 µL	1.5 µL	2.1 µL	2.6 µL	3.7 µL	7.1 µL
SureSelect Capture Library	5.0 µL	10.0 µL	15.3 µL	20.6 µL	25.9 µL	36.6 µL	71.1 µL
Total Volume	7.0 µL	14.0 µL	21.4 µL	28.9 µL	36.3 µL	51.2 µL	99.5 µL

4 Hybridization

Step 2. Hybridize the gDNA library and SureSelect Capture Library

Prepare the Hybridization Buffer master mix

- 8 Prepare the appropriate volume of Hybridization Buffer Master Mix, at room temperature, as indicated in [Table 35](#).

Table 35 Preparation of Hybridization Buffer Master Mix

SureSelect ^{XT} Reagent	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
SureSelect Hyb # 1	212.5 µL	212.5 µL	212.5 µL	425.0 µL	425.0 µL	850.0 µL
SureSelect Hyb # 2 (red cap)	8.5 µL	8.5 µL	8.5 µL	17.0 µL	17.0 µL	34.0 µL
SureSelect Hyb # 3 (yellow cap)	85.0 µL	85.0 µL	85.0 µL	170.0 µL	170.0 µL	340.0 µL
SureSelect Hyb # 4	110.5 µL	110.5 µL	110.5 µL	221.0 µL	221.0 µL	442.0 µL
Total Volume	416.5 µL	416.5 µL	416.5 µL	833.0 µL	833.0 µL	1666 µL

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

Prepare the master mix source plate

10 In a Nunc DeepWell plate, prepare the master mix source plate containing the master mixes prepared in [step 6](#) to [step 8](#) at room temperature. Add the volumes indicated in [Table 36](#) of each master mix to each well of the indicated column of the Nunc DeepWell plate. When using multiple capture libraries in a run, add each Capture Library Master Mix to the appropriate row(s) of the Nunc DeepWell plate. The final configuration of the master mix source plate is shown in [Figure 11](#).

Table 36 Preparation of the Master Mix Source Plate for Hybridization_v1.5.pro

Master Mix Solution	Position on Source Plate	Volume of Master Mix added per Well of Nunc Deep Well Source Plate					
		1-Column Runs	2-Column Runs	3-Column Runs	4-Column Runs	6-Column Runs	12-Column Runs
Block Master Mix	Column 1 (A1-H1)	17.0 µL	29.4 µL	41.7 µL	54.0 µL	78.7 µL	158.8 µL
Capture Library Master Mix	Column 2 (A2-H2)	14.0 µL	21.4 µL	28.9 µL	36.3 µL	51.2 µL	99.5 µL
Hybridization Buffer Master Mix	Column 3 (A3-H3)	50 µL	50 µL	50 µL	100 µL	100 µL	200 µL

4 Hybridization

Step 2. Hybridize the gDNA library and SureSelect Capture Library

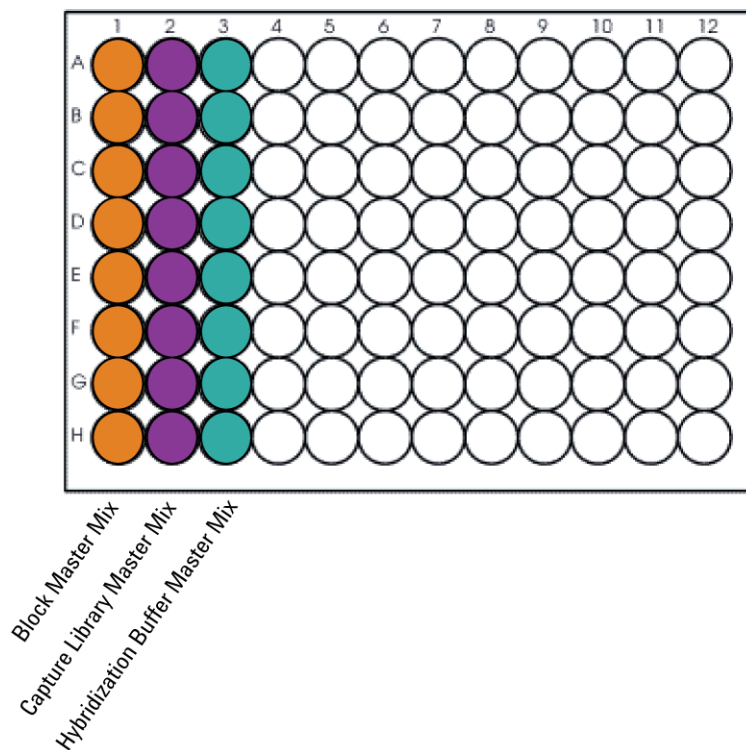


Figure 11 Configuration of the master mix source plate for Hybridization_v1.5.pro.

- 11** Seal the master mix source plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 12** Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate any bubbles. Keep the master mix plate at room temperature.

Step 2. Hybridize the gDNA library and SureSelect Capture Library

Load the Agilent NGS Workstation

13 Load the Labware MiniHub according to [Table 37](#), using the plate orientations shown in [Figure 3](#).

Table 37 Initial MiniHub configuration for Hybridization_v1.5.pro

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty	Empty	Empty
Shelf 2	Empty tip box	Empty	Empty	Empty
Shelf 1 (Bottom)	Empty	Empty	Empty	Empty

14 Load the BenchCel Microplate Handling Workstation according to [Table 38](#).

Table 38 Initial BenchCel configuration for Hybridization_v1.5.pro

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	2 Tip boxes	Empty	Empty	Empty
2	2 Tip boxes	Empty	Empty	Empty
3	2 Tip boxes	Empty	Empty	Empty
4	3 Tip boxes	Empty	Empty	Empty
6	3 Tip boxes	Empty	Empty	Empty
12	5 Tip boxes	Empty	Empty	Empty

4 Hybridization

Step 2. Hybridize the gDNA library and SureSelect Capture Library

15 Load the Bravo deck according to [Table 39](#).

Table 39 Initial Bravo deck configuration for Hybridization_v1.5.pro

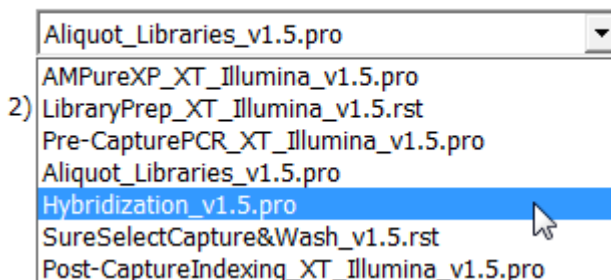
Location	Content
5	750-ng aliquots of prepped gDNA (reconstituted at 221 ng/μL), in ABI MicroAmp plate seated in black adapter
6	Empty Eppendorf plate
8	Empty tip box
9	Hybridization Master Mix source plate seated on silver insert

Step 2. Hybridize the gDNA library and SureSelect Capture Library

Run VWorks protocol Hybridization_v1.5.pro

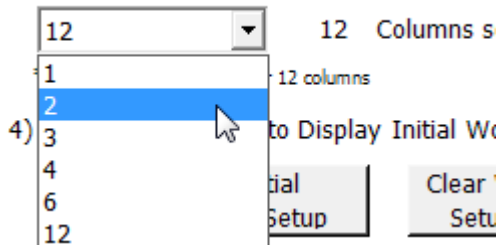
16 On the SureSelect setup form, under **Select Protocol to Run**, select **Hybridization_v1.5.pro**.

1) Select Protocol to Run

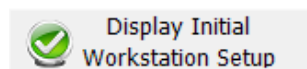


17 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

3) Select Number of Columns of Samples*



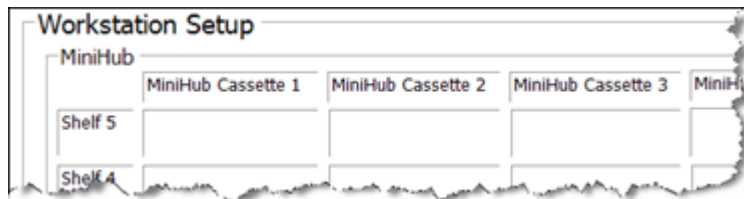
18 Click **Display Initial Workstation Setup**.



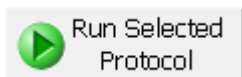
4 Hybridization

Step 2. Hybridize the gDNA library and SureSelect Capture Library

- 19 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



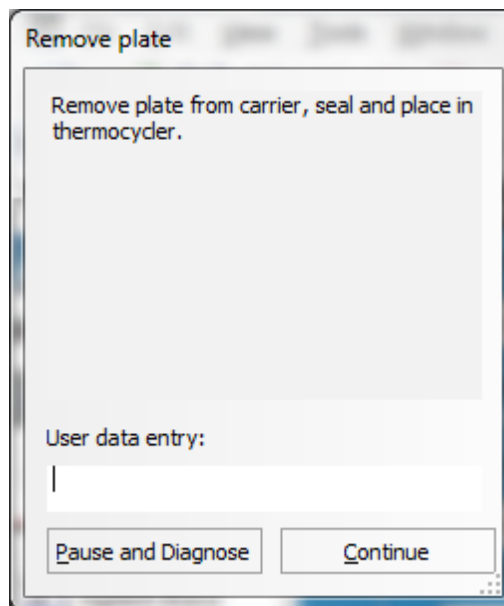
- 20 When verification is complete, click **Run Selected Protocol**.



The Agilent NGS Workstation transfers SureSelect Block Master Mix to the prepped gDNA-containing wells of the ABI MicroAmp plate. When this process is complete, you will be prompted to transfer the plate to the thermal cycler for sample denaturation prior to hybridization.

Step 2. Hybridize the gDNA library and SureSelect Capture Library

- 21** When prompted by VWorks as shown below, remove the ABI MicroAmp plate from position 5 of the Bravo deck, leaving the black adapter in place.



- 22** Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 3.0 sec.
- 23** Transfer the sealed plate to a thermal cycler and run the following program shown in [Table 40](#). After transferring the plate, click **Continue** on the VWorks screen.

Table 40 Thermal cycler program used for sample denaturation prior to hybridization

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

While the sample plate incubates on the thermal cycler, the Agilent NGS Workstation combines aliquots of the SureSelect Capture Library master mix and Hybridization Buffer master mix.

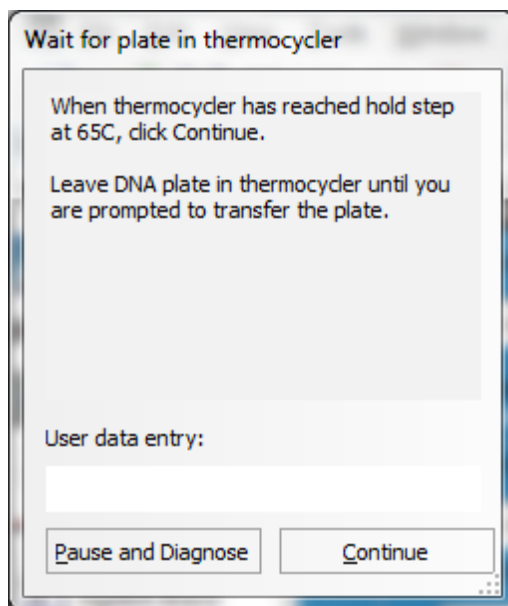
4 Hybridization

Step 2. Hybridize the gDNA library and SureSelect Capture Library

CAUTION

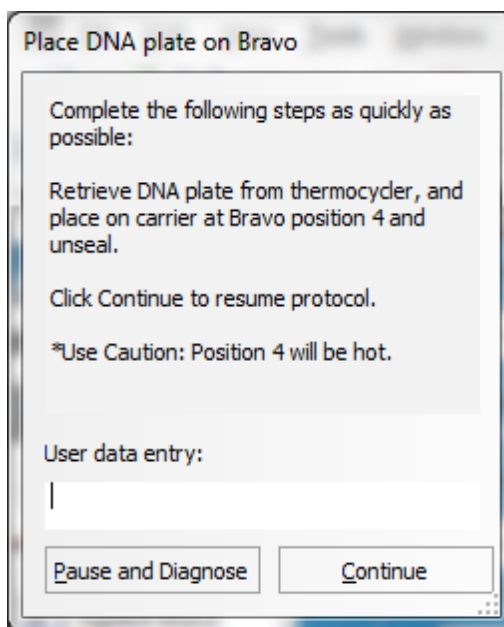
You must complete [step 24](#) to [step 28](#) quickly, and immediately after being prompted by the VWorks software. It is important that sample temperature remains approximately 65°C during transfers between the Agilent NGS Workstation and thermal cycler.

- 24** When the workstation has finished aliquoting the Capture Library and Hybridization Buffer master mixes, you will be prompted by VWorks as shown below. When the thermal cycler reaches the 65°C hold step, click **Continue**. Leave the sample plate in the thermal cycler until you are notified to move it.



Step 2. Hybridize the gDNA library and SureSelect Capture Library

- 25 When prompted by VWorks as shown below, quickly remove the sample plate from the thermal cycler, unseal the plate carefully to avoid splashing, and transfer the plate to position 4 of the Bravo deck, seated in the black ABI adapter. Click **Continue**.

**WARNING**

Bravo deck position 4 will be hot.

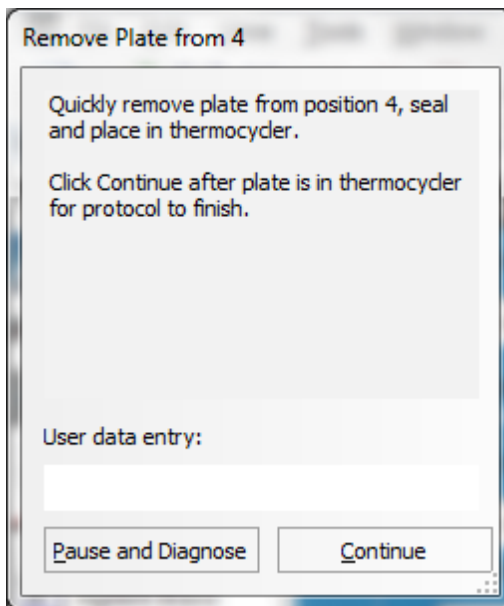
Use caution when handling components that contact heated deck positions.

The Agilent NGS Workstation transfers the capture library-hybridization buffer mixture to the wells of the ABI MicroAmp plate, containing the mixture of prepped gDNA samples and blocking agents.

4 Hybridization

Step 2. Hybridize the gDNA library and SureSelect Capture Library

- 26** When prompted by VWorks as shown below, quickly remove the ABI MicroAmp sample plate from Bravo deck position 4, leaving the black ABI adapter in place.



- 27** Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 3.0 sec.
- 28** Quickly transfer the plate back to the thermal cycler, held at 65°C. After transferring the plate, click **Continue** on the VWorks screen.
- 29** To finish the VWorks protocol, click **Continue** in the **Unused Tips** and **Empty Tip box** dialogs, and click **Yes** in the **Protocol Complete** dialog.

Step 2. Hybridize the gDNA library and SureSelect Capture Library

CAUTION

The temperature of the plate in the thermal cycler should be held at 65°C using a heated lid at 105°C. The lid of the thermal cycler is hot and can cause burns. Use caution when working near the lid.

30 Incubate the hybridization mixture in the thermal cycler 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.

NOTE

If you are using the SureCycler 8800 thermal cycler for this step, be sure to set up the incubation using the appropriate compression pads, as detailed in [“Procedural Notes”](#) on page 10.

Step 3. Capture the hybridized DNA

CAUTION

Instructions in this section are for automation of SureSelect capture using VWorks protocol SureSelect Capture&Wash_v1.5.rst, in preparation for on-bead PCR.

If the SureSelect setup form displays only the SureSelect Capture&Wash_v1.1.rst capture protocol, please contact service.automation@agilent.com for assistance.

In this step, the gDNA-capture library hybrids are captured using streptavidin-coated magnetic beads. This step is run immediately after the 24-hour hybridization period.

This step is automated by the NGS workstation using the SureSelectCapture&Wash_v1.5.rst runset, with a total duration of approximately 3 hours. A workstation operator must be present to complete two actions during the runset, at the time points in the table below. The times provided are approximate; each action is completed in response to a VWorks prompt at the appropriate time in the runset.

Table 41

Operator action	Approximate time after run start
Transfer hybridization reactions from thermal cycler to NGS workstation	<5 minutes
Replace PCR plate with red aluminum insert	5-10 minutes

Prepare the workstation

- 1 Clear the Labware MiniHub and BenchCel of all plates and tip boxes.
- 2 Gently wipe down the Labware MiniHub, Bravo decks, and BenchCel with a NucleoClean decontamination wipe.
- 3 Pre-set the temperature of Bravo deck position 4 to 85°C using the Inheco Multi TEC control touchscreen, as described in [Setting the Temperature of Bravo Deck Heat Blocks](#). Bravo deck position 4 corresponds to CPAC 2, position 1 on the Multi TEC control touchscreen.

Prepare the Dynabeads streptavidin beads

- 4 Vigorously resuspend the Dynabeads MyOne Streptavidin T1 magnetic beads on a vortex mixer. The beads settle during storage.
- 5 Wash the magnetic beads.
 - a In a conical vial, combine the components listed in [Table 42](#). The volumes below include the required overage.

Table 42 Components required for magnetic bead washing procedure

Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Dynabeads MyOne Streptavidin T1 bead suspension	50 µL	425 µL	825 µL	1225 µL	1.65 mL	2.5 mL	5.0 mL
SureSelect Binding Buffer	0.2 mL	1.7 mL	3.3 mL	4.9 mL	6.6 mL	10 mL	20 mL
Total Volume	0.25 mL	2.125 mL	4.125 mL	6.125 mL	8.25 mL	12.5 mL	25 mL

- b Mix the beads on a vortex mixer for 5 seconds.
- c Put the vial into a magnetic device, such as the Dynal magnetic separator.
- d Remove and discard the supernatant.
- e Repeat [step a](#) through [step d](#) for a total of 3 washes. (Retain the beads after each wash and combine with a fresh aliquot of the indicated volume of SureSelect Binding Buffer.)

4 Hybridization

Step 3. Capture the hybridized DNA

- 6 Resuspend the beads in SureSelect Binding buffer, according to [Table 43](#) below.

Table 43 Preparation of magnetic beads for SureSelect Capture&Wash_v1.5.rst

Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
SureSelect Binding Buffer	0.2 mL	1.7 mL	3.3 mL	4.9 mL	6.6 mL	10 mL	20 mL

- 7 Prepare a Nunc DeepWell source plate for the washed streptavidin bead suspension. For each well to be processed, add 200 μ L of the homogenous bead suspension to the Nunc DeepWell plate.
- 8 Place the streptavidin bead source plate at position 5 of the Bravo deck.

Prepare capture and wash solution source plates

- 9 Prepare a Thermo Scientific reservoir containing 15 mL of nuclease-free water.
- 10 Prepare an Eppendorf source plate labeled *Wash #1*. For each well to be processed, add 160 μ L of SureSelect Wash Buffer #1.
- 11 Prepare a Nunc DeepWell source plate labeled *Wash #2*. For each well to be processed, add 1150 μ L of SureSelect Wash Buffer #2.
- 12 Place the silver Nunc DeepWell plate insert on position 6 of the Bravo deck. This insert is required to facilitate heat transfer to DeepWell source plate wells during the Capture&Wash runset.
- 13 Place the *Wash #2* source plate on the silver insert at position 6 of the Bravo deck. Make sure the plate is seated properly on the silver DeepWell insert.

Load the Agilent NGS Workstation

14 Load the Labware MiniHub according to [Table 44](#), using the plate orientations shown in [Figure 3](#).

Table 44 Initial MiniHub configuration for SureSelect Capture&Wash_v1.5.rst

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty Eppendorf plate	Empty	Wash #1 Eppendorf source plate	Empty
Shelf 2	Empty	Nuclease-free water reservoir	Empty	Empty
Shelf 1 (Bottom)	Empty	Empty	Empty	Empty tip box

15 Load the BenchCel Microplate Handling Workstation according to [Table 45](#).

Table 45 Initial BenchCel configuration for SureSelectCapture&Wash_v1.5.rst

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	2 Tip boxes	Empty	Empty	Empty
2	3 Tip boxes	Empty	Empty	Empty
3	4 Tip boxes	Empty	Empty	Empty
4	5 Tip boxes	Empty	Empty	Empty
6	7 Tip boxes	Empty	Empty	Empty
12	10 Tip boxes	3 Tip boxes	Empty	Empty

4 Hybridization

Step 3. Capture the hybridized DNA

16 Load the Bravo deck according to [Table 46](#) (positions 5 and 6 should already be loaded).

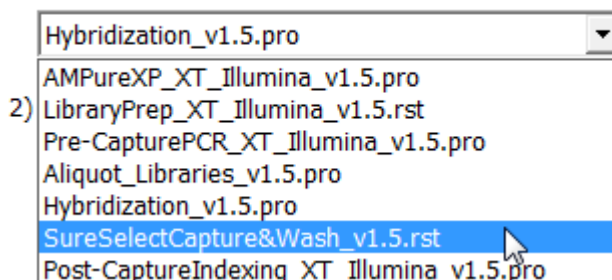
Table 46 Initial Bravo deck configuration for SureSelectCapture&Wash_v1.5.rst

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
4	Empty black ABI adapter
5	Dynabeads streptavidin bead DeepWell source plate
6	<i>Wash #2</i> DeepWell source plate seated on silver insert

Run VWorks runset SureSelectCapture&Wash_v1.5.rst

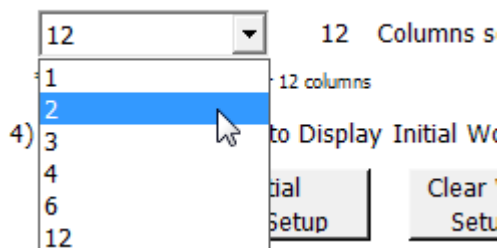
17 On the SureSelect setup form, under **Select Protocol to Run**, select **SureSelectCapture&Wash_v1.5.rst**.

1) Select Protocol to Run

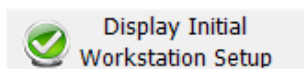


18 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

3) Select Number of Columns of Samples*



19 Click **Display Initial Workstation Setup**.



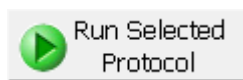
20 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



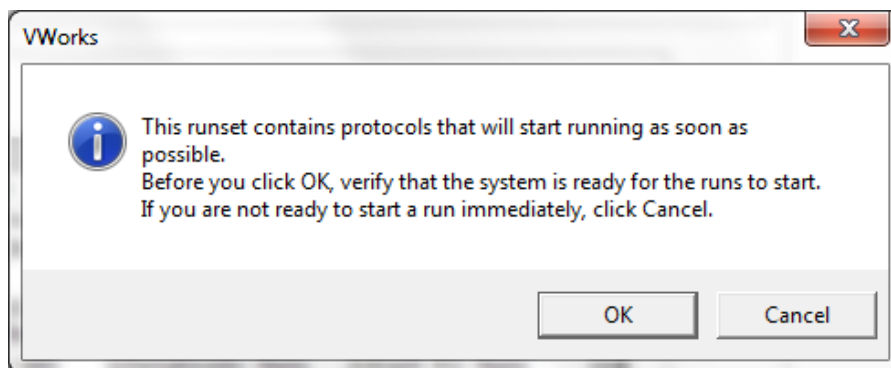
4 Hybridization

Step 3. Capture the hybridized DNA

21 When verification is complete, click **Run Selected Protocol**.



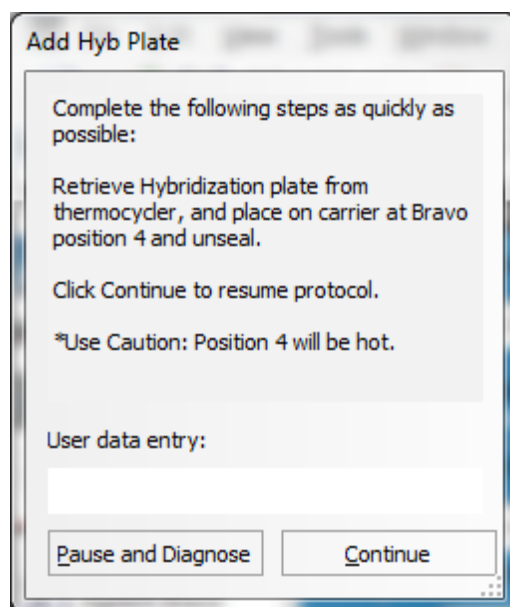
22 When ready to begin the run, click **OK** in the following window. If the temperature of Bravo deck position 4 was not pre-set to 85°C, the runset will pause while position 4 reaches temperature.



CAUTION

It is important to complete [step 23](#) quickly and carefully. Transfer the sample plate to the Bravo platform quickly to retain the 65°C sample temperature. Unseal the plate without tilting or jerking the plate to avoid sample splashing. Make sure that the Agilent NGS Workstation is completely prepared, with deck platforms at temperature and all components in place, before you transfer the sample plate to the Bravo deck.

23 When prompted by VWorks as shown below, quickly remove the ABI MicroAmp plate, containing the hybridization reactions held at 65°C, from the thermal cycler. Unseal the plate carefully to avoid splashing, and quickly transfer the plate to position 4 of the Bravo deck, seated in the black ABI adapter. Click **Continue** to resume the runset.

**WARNING**

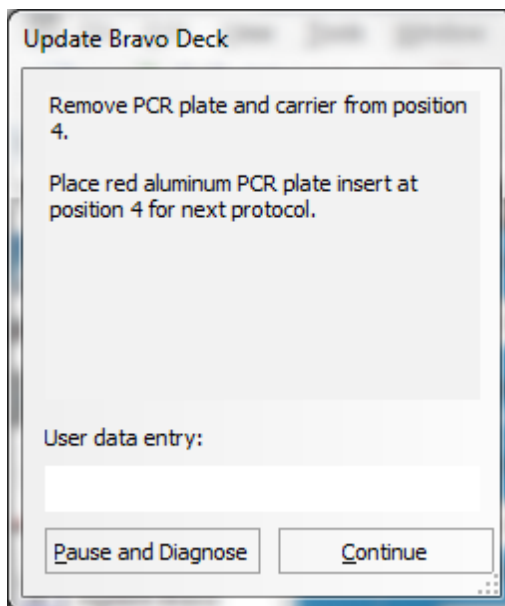
Bravo deck position 4 will be hot.

Use caution when handling components that contact heated deck positions.

4 Hybridization

Step 3. Capture the hybridized DNA

- 24 When prompted by VWorks as shown below, install the red aluminum insert at position 4. When finished, click **Continue** to resume the runset.



The remainder of the SureSelectCapture&Wash_v1.5.rst runset takes approximately 3 hours. Once the runset is complete, the captured, bead-bound DNA samples are located in the Eppendorf plate at position 9 of the Bravo deck

When the runset is complete, seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec and store the plate on ice while setting up the next automation protocol.

NOTE

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



5 Indexing

- Step 1. Amplify the captured libraries to add index tags [100](#)
- Step 2. Purify the amplified indexed libraries using Agencourt AMPure XP beads [111](#)
- Step 3. Assess indexed DNA quality [116](#)
- Step 4. Quantify each index-tagged library by QPCR [119](#)
- Step 5. Pool samples for Multiplexed Sequencing [120](#)

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



Step 1. Amplify the captured libraries to add index tags

CAUTION

Instructions in this section are for automation of post-capture indexing by on-bead PCR using VWorks protocol Post-CaptureIndexing_XT_Illumina_v1.5.pro.

If the setup form displays only the Post-CaptureIndexing_XT_Illumina_v1.0.pro post-capture PCR protocol, please contact service.automation@agilent.com for assistance.

In this step, the Agilent NGS Workstation completes the liquid handling steps for PCR-based addition of indexing tags to the SureSelect-enriched DNA samples. After the PCR plate is prepared by the Agilent NGS Workstation, you transfer the plate to a thermal cycler for amplification.

The size of your SureSelect Capture Library determines the amplification cycle number used for indexing. Plan your experiments for amplification of samples prepared using SureSelect Capture Libraries of similar sizes on the same plate. See [Table 55](#) for cycle number recommendations.

Assign indexes to DNA samples

Select the appropriate indexing primer for each sample. Nucleotide sequences of the 16 indexes provided with the SureSelect^{XT} system are provided in [Table 64](#) on page 127.

Use a different index primer for each sample to be sequenced in the same lane. The number of samples that may be combined per lane depends on the sequencing platform performance and the Capture Library size. See [Table 47](#) for sequence data requirement guidelines. Calculate the number of indexes that can be combined per lane based on these guidelines.

Table 47 Sequencing data requirement guidelines

Capture Library Size	Recommended Amount of Sequencing Data per Sample
1 kb up to 0.5 Mb	0.1 to 50 Mb*
0.5 Mb up to 2.9 Mb	50 to 290 Mb*
3 Mb up to 5.9 Mb	300 to 590 Mb*
6 Mb up to 11.9 Mb	600 to 1190 Mb*
12 Mb up to 24 Mb	1.2 to 2.4 Gb*
Human All Exon v4	4 Gb
Human All Exon v4 + UTRs	6 Gb
Human All Exon 50 Mb	5 Gb
Human DNA Kinome	320 Mb
Mouse All Exon	5 Gb

* For custom libraries, Agilent recommends analyzing 100X amount of sequencing data compared to the Capture Library size for each sample. Pool samples according to your expected sequencing output.

5 Indexing

Step 1. Amplify the captured libraries to add index tags

Prepare the workstation

- 1 Turn on the ThermoCube, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.
- 2 Clear the Labware MiniHub and BenchCel of plates and tip boxes.
- 3 Gently wipe down the Labware MiniHub, Bravo decks, and BenchCel with a Nucleoclean decontamination wipe.

Prepare indexing primers and PCR master mix

CAUTION

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

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.

- 4 Dilute each indexing primer to be used in the run according to [Table 48](#). The volumes below include the required excess.

Table 48 Preparation of indexing primer dilutions

SureSelect ^{XT} Reagent	Volume to Index 1 Sample	Volume to Index 12 Samples
Nuclease-free water	8.0 µL	100 µL
Index PCR primer (reverse)	1.0 µL	12.5 µL
Total Volume	9.0 µL	112.5 µL

- 5 In an ABI MicroAmp plate, aliquot 9 µL of the appropriate indexing primer dilution to the intended sample indexing well position. Keep the plate on ice.

Step 1. Amplify the captured libraries to add index tags

- 6 Prepare the appropriate volume of PCR master mix, according to Table 49. Mix well using a vortex mixer and keep on ice.

Table 49 Preparation of PCR Master Mix for Post-CaptureIndexing_XT_Illumina_v1.5.pro

SureSelect ^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	14.5 µL	184.9 µL	308.1 µL	431.4 µL	554.6 µL	801.1 µL	1602.3 µL
Herculase II 5X Reaction Buffer*	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105.0 µL
SureSelect Indexing Post-Capture PCR (Forward) Primer†	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
dNTP mix*	0.5 µL	6.4 µL	10.6 µL	14.9 µL	19.1 µL	27.6 µL	55.3 µL
Herculase II polymerase	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
Total Volume	27.0 µL	344.3 µL	573.8 µL	803.3 µL	1032.8 µL	1491.8 µL	2983.5 µL

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

† Included in SureSelect^{XT} Automated Hybridization Kit Box #2.

5 Indexing

Step 1. Amplify the captured libraries to add index tags

- 7 Using the same Nunc DeepWell master mix source plate that was used for the Hybridization_v1.5.pro protocol, add the volume of PCR master mix indicated in Table 50 to all wells of column 4 of the master mix source plate. The final configuration of the master mix source plate is shown in Figure 12.

Table 50 Preparation of the Master Mix Source Plate for Post-CaptureIndexing_XT_Illumina_v1.5.pro

Master Mix Solution	Position on Source Plate	Volume of Master Mix added per Well of Nunc Deep Well Source Plate					
		1-Column Runs	2-Column Runs	3-Column Runs	4-Column Runs	6-Column Runs	12-Column Runs
PCR Master Mix	Column 4 (A4-H4)	39.7 µL	68.3 µL	97.0 µL	125.7 µL	183.1 µL	369.6 µL

NOTE

If you are using a new DeepWell plate for the post-capture PCR source plate (for example, when amplifying the second half of the captured DNA sample), leave columns 1 to 3 empty and add the PCR Master Mix to column 4 of the new plate.

Step 1. Amplify the captured libraries to add index tags

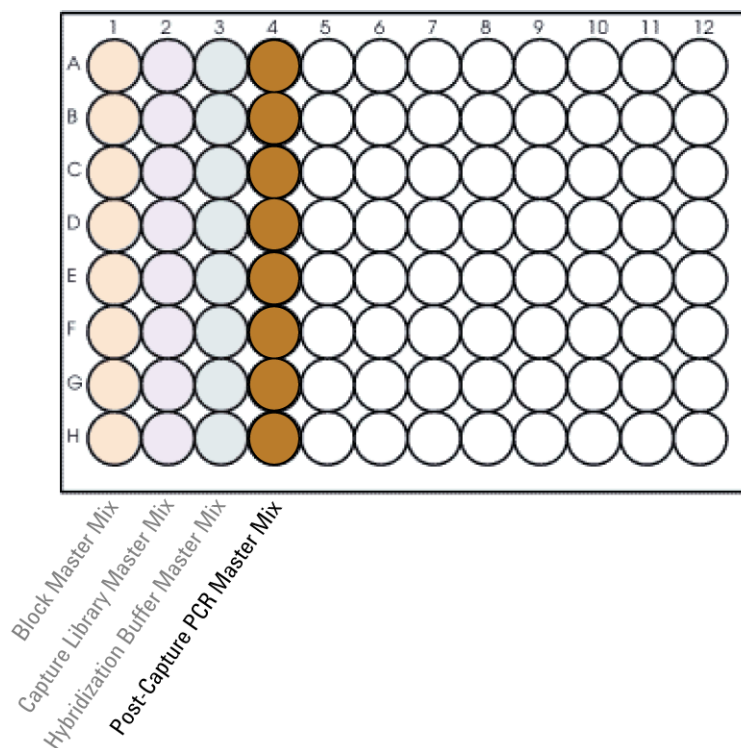


Figure 12 Configuration of the master mix source plate for Post-CaptureIndexing_XT_Illumina_v1.5.pro. Columns 1-3 were used to dispense master mixes for the Hybridization_v1.5.pro protocol.

- 8** Seal the master mix source plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 9** Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate any bubbles.

5 Indexing

Step 1. Amplify the captured libraries to add index tags

Load the Agilent NGS Workstation

10 Load the Labware MiniHub according to [Table 51](#), using the plate orientations shown in [Figure 3](#).

Table 51 Initial MiniHub configuration for Post-CaptureIndexing_XT_Illumina_v1.5.pro

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty	Empty	Empty	Indexing primers in ABI MicroAmp plate seated in black adapter
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty	Empty	Empty
Shelf 2	Empty tip box	Empty	Empty	Empty
Shelf 1 (Bottom)	New tip box	Empty	Empty	Empty tip box

11 Load the BenchCel Microplate Handling Workstation according to [Table 52](#).

Table 52 Initial BenchCel configuration for Post-CaptureIndexing_XT_Illumina_v1.5.pro

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	1 Tip box	Empty	Empty	Empty
4	1 Tip box	Empty	Empty	Empty
6	1 Tip box	Empty	Empty	Empty
12	1 Tip box	Empty	Empty	Empty

Step 1. Amplify the captured libraries to add index tags

12 Load the Bravo deck according to [Table 53](#).

Table 53 Initial Bravo deck configuration for Post-CaptureIndexing_XT_Illumina_v1.5.pro

Location	Content
4	Captured DNA bead suspensions in Eppendorf twin.tec plate
9	Master mix plate containing PCR Master Mix in Column 4 (Nunc DeepWell plate)

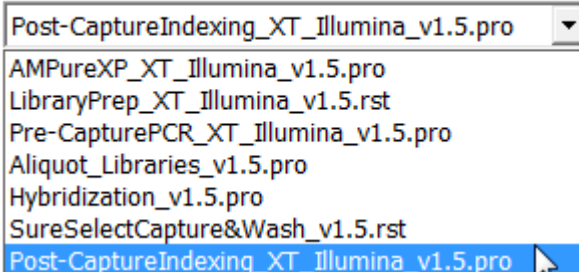
5 Indexing

Step 1. Amplify the captured libraries to add index tags

Run VWorks protocol Post-CaptureIndexing_XT_Illumina_v1.5.pro

13 On the SureSelect setup form, under **Select Protocol to Run**, select **Post-CaptureIndexing_XT_Illumina_v1.5.pro**.

1) Select Protocol to Run



Post-CaptureIndexing_XT_Illumina_v1.5.pro

AMPureXP_XT_Illumina_v1.5.pro

LibraryPrep_XT_Illumina_v1.5.rst

Pre-CapturePCR_XT_Illumina_v1.5.pro

Aliquot_Libraries_v1.5.pro

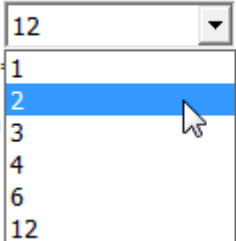
Hybridization_v1.5.pro

SureSelectCapture&Wash_v1.5.rst

Post-CaptureIndexing_XT_Illumina_v1.5.pro

14 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

3) Select Number of Columns of Samples*



12 Columns s

1 12 columns

2

3 to Display Initial W

4

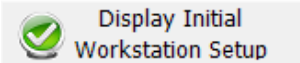
6

12

Display Initial Workstation Setup

Clear Setup

15 Click **Display Initial Workstation Setup**.



16 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



MiniHub	MiniHub Cassette 1	MiniHub Cassette 2	MiniHub Cassette 3	MiniHub Cassette 4
Shelf 5				
Shelf 4				

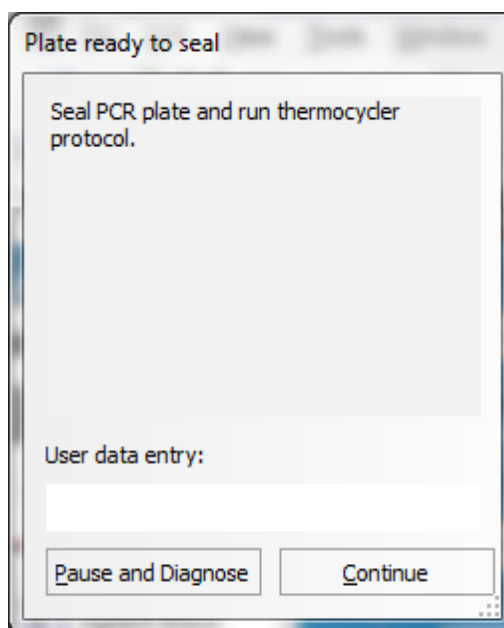
Step 1. Amplify the captured libraries to add index tags

- 17** When verification is complete, click **Run Selected Protocol**.



Running the Post-CaptureIndexing_XT_Illumina_v1.5.pro protocol takes approximately 15 minutes. Once complete, the PCR-ready samples, containing captured DNA and PCR master mix, are located in the ABI MicroAmp plate at position 5 of the Bravo deck. The Eppendorf plate containing the remaining bead-bound captured DNA samples, which may be stored for future use at 4°C overnight, or at -20°C for longer-term storage, is located at position 4 of the Bravo deck.

- 18** When you see the following prompt, remove the PCR plate from position 5 of the Bravo deck and seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 3.0 seconds.



- 19** Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate air bubbles.

5 Indexing

Step 1. Amplify the captured libraries to add index tags

20 Transfer the PCR plate to a thermal cycler and run the PCR amplification program shown in [Table 54](#) using the cycle number specified in [Table 55](#).

Table 54 Post-Capture PCR cycling program

Segment	Number of Cycles	Temperature	Time
1	1	98°C	2 minutes
2	10-16 see Table 55	98°C	30 seconds
		57°C	30 seconds
		72°C	1 minute
3	1	72°C	10 minutes
4	1	4°C	Hold

Table 55 Recommended cycle number based on SureSelect Capture Library size

Size of SureSelect Capture Library	Cycles
<0.5 Mb	16 cycles
0.5 to 1.49 Mb	14 cycles
> 1.5 Mb	12 cycles
All Exon	10 to 12 cycles

NOTE

Amplify the captured DNA using a minimal number of PCR cycles. If yield is too low or 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 indexed libraries using Agencourt AMPure XP beads

In this step, the Agilent NGS Workstation transfers AMPure XP beads to the indexed DNA sample plate and then collects and washes the bead-bound DNA.

Prepare the workstation and reagents

- 1 Clear the Labware MiniHub and BenchCel of all plates and tip boxes.
- 2 Gently wipe down the Labware MiniHub, Bravo decks, and BenchCel with a Nucleoclean decontamination wipe.
- 3 Let the AMPure XP beads come to room temperature for at least 30 minutes.
- 4 Mix the bead suspension well so that the reagent appears homogeneous and consistent in color. *Do not freeze.*
- 5 Prepare a Nunc DeepWell source plate containing AMPure XP beads. For each well to be processed, add 92 μ L of homogenous AMPure XP beads per well to the Nunc DeepWell plate.
- 6 Prepare a Thermo Scientific reservoir containing 15 mL of nuclease-free water.
- 7 Prepare a separate Thermo Scientific reservoir containing 45 mL of freshly-prepared 70% ethanol.

5 Indexing

Step 2. Purify the amplified indexed libraries using Agencourt AMPure XP beads

- 8 Load the Labware MiniHub according to [Table 56](#), using the plate orientations shown in [Figure 3](#).

Table 56 Initial MiniHub configuration for AMPureXP_XT_Illumina_v1.5.pro

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty Nunc DeepWell plate	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty Eppendorf Plate	Empty	Empty
Shelf 2	Empty	Nuclease-free water reservoir from step 6	AMPure XP beads in Nunc DeepWell plate from step 5	Empty
Shelf 1 (Bottom)	Empty	70% ethanol reservoir from step 7	Empty	Empty

- 9 Load the BenchCel Microplate Handling Workstation according to [Table 57](#).

Table 57 Initial BenchCel configuration for AMPureXP_XT_Illumina_v1.5.pro

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	2 Tip boxes	Empty	Empty	Empty
4	2 Tip boxes	Empty	Empty	Empty
6	3 Tip boxes	Empty	Empty	Empty
12	6 Tip boxes	Empty	Empty	Empty

Step 2. Purify the amplified indexed libraries using Agencourt AMPure XP beads

10 Load the Bravo deck according to [Table 58](#).

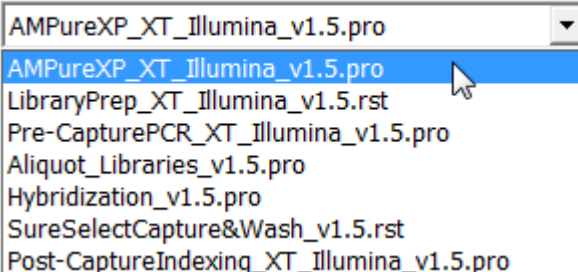
Table 58 Initial Bravo deck configuration for AMPureXP_XT_Illumina_v1.5.pro

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
4	Indexed library samples in ABI MicroAmp plate seated in black adapter
8	Empty tip box

Run VWorks protocol AMPureXP_XT_Illumina_v1.5.pro

11 On the SureSelect setup form, under **Select Protocol to Run**, select **AMPureXP_XT_Illumina_v1.5.pro**.

1) Select Protocol to Run



AMPureXP_XT_Illumina_v1.5.pro

AMPureXP_XT_Illumina_v1.5.pro

2) LibraryPrep_XT_Illumina_v1.5.rst

Pre-CapturePCR_XT_Illumina_v1.5.pro

Aliquot_Libraries_v1.5.pro

Hybridization_v1.5.pro

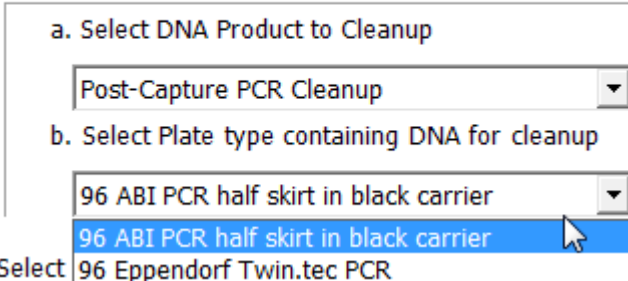
SureSelectCapture&Wash_v1.5.rst

Post-CaptureIndexing_XT_Illumina_v1.5.pro

12 Under **Select additional Parameters**, select the step **Post-Capture PCR Cleanup** and the plate type **96 ABI PCR half skirt in black carrier**.

2) Select additional Parameters

AMPureXP_XT_Illumina_v1.5.pro Only



a. Select DNA Product to Cleanup

Post-Capture PCR Cleanup

b. Select Plate type containing DNA for cleanup

96 ABI PCR half skirt in black carrier

96 ABI PCR half skirt in black carrier

3) Select 96 Eppendorf Twin.tec PCR

NOTE

The AMPureXP_XT_Illumina_v1.5.pro protocol is used during multiple steps of the SureSelect automation workflow. Be sure to select the correct workflow step when initiating the automation protocol.

Step 2. Purify the amplified indexed libraries using Agencourt AMPure XP beads

- 13** Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

3) Select Number of Columns of Samples*

12 Columns s

12 columns

4) 1 to Display Initial W

2

3

4

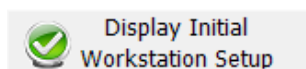
6

12

Initial Setup

Clear Setup

- 14** Click **Display Initial Workstation Setup**.



- 15** Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.

 A screenshot of the "Workstation Setup" form. It has a title bar "Workstation Setup". Below it, there's a "MiniHub" section with four columns: "MiniHub Cassette 1", "MiniHub Cassette 2", "MiniHub Cassette 3", and "MiniHub Cassette 4". Under each cassette column, there are two rows labeled "Shelf 5" and "Shelf 4". Each of these four cells contains a small white box with a green checkmark.

- 16** When verification is complete, click **Run Selected Protocol**.



The purification protocol takes approximately 45 minutes. When complete, the amplified DNA samples are in the Eppendorf plate located on Bravo deck position 7.

Step 3. Assess indexed DNA quality

Option 1: Analysis using the Agilent 2100 Bioanalyzer and High Sensitivity DNA Assay

- 1 Set up the 2100 Bioanalyzer as instructed in the High Sensitivity DNA Assay kit guide.

NOTE

Version B.02.07 or higher of the Agilent 2100 Expert Software is required for High Sensitivity DNA Assay Kit runs.

- 2 Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 3 Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
- 4 Prepare the chip, samples and ladder as instructed in the reagent kit guide, using 1 µL of each sample for the analysis.

NOTE

For some samples, Bioanalyzer results are improved by diluting 1 µL of the sample in 9 µL of 10 mM Tris, 1 mM EDTA prior to analysis. Be sure to mix well by vortexing at 2000 rpm on the IKA vortex supplied with the Bioanalyzer before analyzing the diluted samples.

- 5 Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
- 6 Verify that the electropherogram shows an average DNA fragment size of approximately 300 to 400 bp. A sample electropherogram is shown in [Figure 13](#).

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.

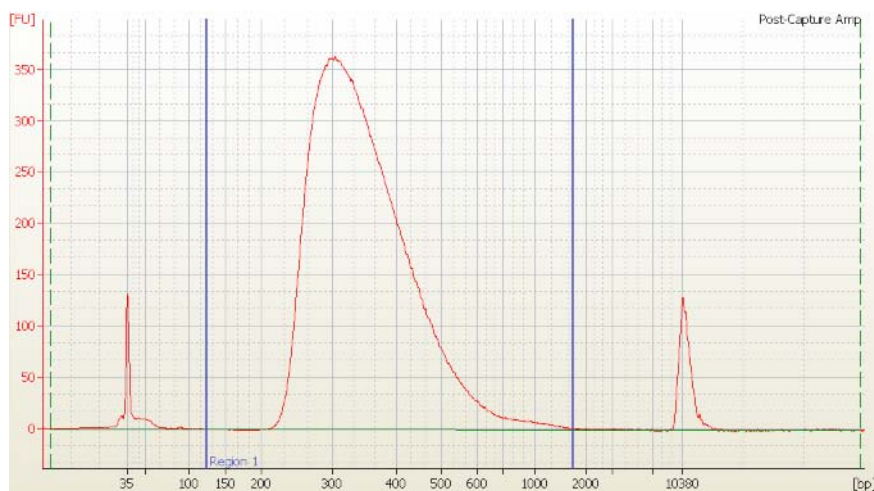


Figure 13 Analysis of indexed DNA using the High Sensitivity DNA Assay. The electropherogram shows a peak in the size range of approximately 300 to 400 bp.

Option 2: Analysis using the Agilent 2200 TapeStation and High Sensitivity D1K ScreenTape

Use a High Sensitivity D1K ScreenTape (p/n 5067-5363) and reagent kit (p/n 5067-5364) to analyze the indexed DNA. For more information to do this step, see the *Agilent 2200 TapeStation User Manual*.

- 1 Seal the DNA sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
- 2 Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
- 3 Prepare the TapeStation samples as instructed in the *Agilent 2200 TapeStation User Manual*. Use 2 µL of each indexed DNA sample diluted with 2 µL of D1K sample buffer for the analysis.
- 4 Load the sample plate or tube strips from [step 3](#), the High Sensitivity D1K ScreenTape, and loading tips into the 2200 TapeStation as instructed in the *Agilent 2200 TapeStation User Manual*. Start the run.
- 5 Verify that the electropherogram shows an average DNA fragment size of 300 to 400 bp. A sample electropherogram is shown in [Figure 14](#).

Stopping Point

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

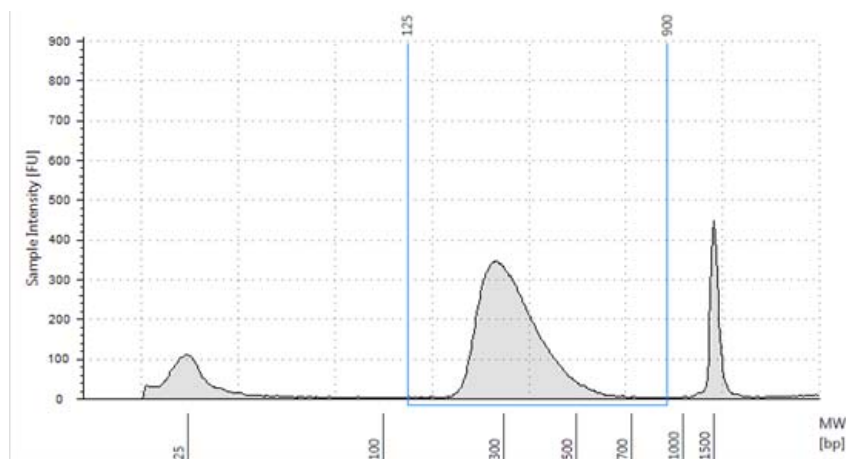


Figure 14 Analysis of indexed DNA using the 2200 TapeStation with a High Sensitivity D1K ScreenTape. The electropherogram shows an average DNA fragment size between 300 to 400 bp.

Step 4. Quantify each index-tagged library by QPCR

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

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

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

- 4 Prepare the QPCR master mix with Illumina adaptor-specific PCR primers according to instructions provided in the kit.
- 5 Add an aliquot of the master mix to PCR tubes and add template.
- 6 On a QPCR system, such as the Mx3005p, run the thermal profile outlined in the QPCR NGS Library Quantification kit user guide. Use the SYBR Green instrument setting.
- 7 Use the standard curve to determine the concentration of each unknown index-tagged library, in nM.

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

NOTE

In most cases, the cycle numbers in [Table 55](#) 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 5. Pool samples for Multiplexed Sequencing

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

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

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

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

$\#$ is the number of indexes, and

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

Table 59 shows an example of the amount of 4 index-tagged samples (of different concentrations) and Low TE needed for a final volume of 20 μL at 10 nM.

Table 59 Example of index volume calculation for a total volume of 20 μL

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

- 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.
- 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.

Exact library pool dilution and processing can vary based on the flow cell capacity and analysis pipeline versions being used. Refer to the appropriate Illumina user guide for instructions. This protocol has been validated with 36-base paired-end reads. However, read length can be adjusted to achieve the desired research goals.

Proceed to cluster amplification using the Illumina Paired-End Cluster Generation Kit.

NOTE

For Human All Exon captures, where average exonic length is 150 bp, use 2x76 base pair sequencing to get optimal performance and specificity. However 2x100 base pair sequencing can also be used with limited effects on data quality.

5 Indexing

Step 5. Pool samples for Multiplexed Sequencing



6 Reference

Kit Contents [124](#)

Nucleotide Sequences of SureSelect Indexes [127](#)

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



Kit Contents

NOTE

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

Each SureSelect^{XT} Automation Reagent Kit contains the following component kits:

Table 60 SureSelect^{XT} Automation Reagent Kit Contents

Product	Storage Condition	96 Reactions	480 Reactions
SureSelect ^{XT} Library Prep Kit - ILM	-20°C	5500-0075	5 x 5500-0075
SureSelect Target Enrichment-Box #1	Room Temperature	5190-4394	5190-4395
SureSelect ^{XT} Automated Hybridization Kit Box #2	-20°C	5190-3730	5190-3732

The contents of each of the component kits listed in [Table 60](#) are described in the tables below.

Table 61 SureSelect^{XT} Library Prep Kit-ILM Content

Kit Component	96 Reactions	480 Reactions
10X End Repair Buffer	tube with clear cap	bottle
10X Klenow Polymerase Buffer	tube with blue cap	bottle
5X T4 DNA Ligase Buffer	tube with green cap	bottle
T4 DNA Ligase	tube with red cap	tube with red cap
Exo(-) Klenow	tube with red cap	bottle
T4 DNA Polymerase	tube with purple cap	tube with purple cap
Klenow DNA Polymerase	tube with yellow cap	tube with yellow cap
T4 Polynucleotide Kinase	tube with orange cap	tube with orange cap
dATP	tube with green cap	tube with green cap
dNTP mix	tube with green cap	tube with green cap
SureSelect Adaptor Oligo Mix [*]	tube with brown cap	bottle
SureSelect Primer [†] (forward primer)	tube with brown cap	tube with brown cap
PCR Primer Index 1 through Index 16 (reverse primers)	tube with clear cap	tube with clear cap

* Previously labeled as InPE Adaptor Oligo Mix.

† Previously labeled as InPE Primer 1.0.

Table 62 SureSelect Target Enrichment-Box #1 Content

Kit Component	96 Reactions	480 Reactions
SureSelect Hyb # 1	tube with orange cap	bottle
SureSelect Hyb # 2	tube with red cap	tube with red cap
SureSelect Hyb # 4	tube with black cap	bottle
SureSelect Binding Buffer	bottle	bottle
SureSelect Wash Buffer 1	bottle	bottle
SureSelect Wash Buffer 2	bottle	bottle
SureSelect Elution Buffer*	bottle	bottle
SureSelect Neutralization Buffer*	bottle	bottle

* The provided SureSelect Elution Buffer and Neutralization Buffer are not used in the workflow described in this manual. These reagents are used in earlier versions of the Target Enrichment workflow, and are provided to support use of the earlier protocol versions for a limited time.

Table 63 SureSelect^{XT} Automated Hybridization Kit Box #2 Content

Kit Component	96 Reactions	480 Reactions
SureSelect Hyb # 3	tube with yellow cap	bottle
SureSelect Indexing Block #1	tube with green cap	tube with green cap
SureSelect Block #2	tube with blue cap	tube with blue cap
SureSelect Indexing Block #3	tube with brown cap	tube with brown cap
SureSelect RNase Block	tube with purple cap	tube with purple cap
SureSelect Indexing Pre-Capture PCR Reverse Primer	tube with clear cap	tube with clear cap
SureSelect Indexing Post-Capture PCR Forward Primer	tube with orange cap	tube with orange cap

Nucleotide Sequences of SureSelect Indexes

The nucleotide sequence of each SureSelect^{XT} Index is provided in the table below.

Table 64 SureSelect^{XT} Indexes 1-16

Index Number	Sequence
1	ATCACG
2	CGATGT
3	TTAGGC
4	TGACCA
5	ACAGTG
6	GCCAAT
7	CAGATC
8	ACTTGA
9	GATCAG
10	TAGCTT
11	GGCTAC
12	CTTGTA
13	AAACAT
14	CAAAAG
15	GAAACC
16	AAAGCA

In This Book

This guide contains information to run the SureSelect Automated Library Prep and Capture System protocol using a SureSelect^{XT} Automated Reagent Kit (HSQ or MSQ) and automation protocols provided with the Agilent NGS Workstation.

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