MCCRD-SOP0023: KAPA Hyper Low Input Whole Exome Library Construction

Effective Date: 08/31/2018

Please check for revision status of the SOP at

https://pdmr.cancer.gov/sops/

PDMR NCI Patient-Derived Models Repository
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APPROVALS

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VERSION INFORMATION

1. Change History

Revision	Description
08/31/2018	Standardize SOP for posting to PDMR internal site for use by designated NCI intramural laboratories

2. Related SOPs and Reference Documentation

Document Number	Title
KR0961	KAPA Hyper Prep Kit (96 libraries) https://www.kapabiosystems.com/document/kapa-hyper-prep-kit-tds/?dl=1
G7530-90000	Agilent SureSelect XT Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library http://www.agilent.com/cs/library/usermanuals/public/G7530-90000.pdf
G2991-90030	4200 TapeStation System D1000 ScreenTape Assay Quick Guide http://www.agilent.com/cs/library/usermanuals/Public/4200-TapeStation_D1000_QG.pdf
G2991-90130	4200 TapeStation System High Sensitivity D1000 ScreenTape Assay Quick Guide http://www.agilent.com/cs/library/usermanuals/Public/4200-TapeStation_HS-D1000_QG.pdf
MCCRD-SOP0007	Illumina Sequencing Library Quantitation using Droplet Digital PCR (ddPCR) Protocol

1.0 PURPOSE/SCOPE

This Standard Operating Procedure (SOP) describes manual DNA Library Preparation with the KAPA Biosystems KAPA Hyper Prep Kit used/performed by the Molecular Characterization and Clinical Assay Development Laboratory (MoCha) at the Frederick National Laboratory for Cancer Research. **This SOP is for research purposes only and no clinical samples will be processed using this SOP.**

This procedure is to be used to prepare whole exome libraries from DNA samples for use on the Illumina HiSeq Sequencing platform. The resulting DNA libraries are suitable for paired-end, multiplexed sequencing applications.

2.0 SAFETY

2.1 Lab coats, safety glasses, and gloves must be worn at all times when handling hazardous or sensitive equipment, samples, reagents, and materials. These safety measures must also be followed when in close proximity to those who are working with these items.

3.0 REAGENTS & EQUIPMENT

3.1 Reagents

Description	Model#	Vendor
TapeStation D1000 ScreenTape	5067-5582	Agilent
TapeStation D1000 Reagents	5067-5583	Agilent
TapeStation High Sensitivity D1000 ScreenTape	5067-5584	Agilent
TapeStation High Sensitivity Reagents	5067-5585	Agilent
KAPA Hyper Prep Kit	KK8504	KAPA Biosystems
SureSelect XT Reagent Kit, HSQ, 96	G9611B	Agilent
SureSelect XT Human All Exon V5 baits, 96 rxns	5190-6209	Agilent
Herculase II Fusion DNA Polymerase	600679	Agilent
Nuclease-free Water	various	various
Agencourt AMPure XP Kit	A63881	Beckman Coulter
1X Low TE Buffer	12090-015	Life Technologies
Ethanol, 100% for molecular biology	E7023	Sigma-Aldrich
Elution Buffer (EB)	19086	Qiagen
Dynabeads MyOne Streptavidin T1	65602	Life Technologies
1.5-mL LoBind Tube	022431021	Eppendorf
Eppendorf™ 96-Well twin.tec™ PCR Plates	E951020346	Fisher Scientific

Description	Model#	Vendor
Hard-Shell 96 well PCR plate	HSP9631	Bio-Rad
200-1000 µL Aerosol Barrier Pipette Tips	30389213	Rainin
20-200 µL Aerosol Barrier Pipette Tips	30389240	Rainin
0.2-20 µL Aerosol Barrier Pipette Tips	30389226	Rainin
Microamp Adhesive plate seals	4306311	Applied Biosystems
96 microTUBE-50 AFA Fiber Plate	520168	Covaris
Optical Tube Strips	401428	Agilent Technologies

*Or comparable/equivalent (include unless must use listed items)

3.2 Equipment

Description	Model #	Vendor
Multi-Channel Pipettes (LTS 10, 20, 200,1000)	Variable	Rainin
Single-Channel Pipettes (LTS 2, 10, 20, 200, 1000)	Variable	Rainin
Vortex	58816-121	VWR
MiniFuge	93000-196	VWR
Plate Centrifuge	022628203	Eppendorf
C1000 Touch Thermal Cycler	1851196	BioRad
PCR Workstation	AC648LFUVC	AirClean Systems
4200 TapeStation System	G2991AA	Agilent
Vacuum concentrator	SpeedVac	Savant
96-well plate mixer	5382 000.015	Eppendorf
Magnetic Separator	12331D	ThermoFisher Scientific
Covaris Ultrasonicator	E220	Covaris

*Or comparable/equivalent (include unless must use listed items)

4.0 PROCEDURE

4.1 Sample Preparation

4.1.1 Prepare each DNA sample for shearing by diluting 50 ng with 1X Low TE Buffer to a final volume of 50 µL.

4.1.2 Vortex well to mix, then spin briefly to collect the liquid. Keep samples on ice.

4.2 Shear DNA using Covaris LE220 Ultrasonicator

Notes: The volume of sample (DNA+1X Low TE buffer) necessary for use with the Covaris microtube plate is 50 µL. If lower volumes are used, an air space may form in the fluid, partitioning the sample and resulting in a broad peak.

Fragment size generation is affected by treatment duration. A longer duration will produce smaller fragments

Bath water should be clean distilled, deionized water as foreign materials such as algae and particulates may scatter the high frequency focused acoustic beam, resulting in a shift to larger mean fragment size.

Insufficient degas levels within the bath may result in poor acoustic coupling and may shift the mean fragment size. System degas pumps should be run at least 60 minutes before running the instrument.

Warmer temperatures will cause a shift toward larger mean fragment size. Bath temperature should be closely monitored.

Ensure the water level needed for the program is consistent throughout the process.

4.2.1 Prepare the Covaris for shearing:

4.2.1.1 Open the Sonolab program on the computer connected to the LE220 Covaris.

4.2.1.2 Ensure the water temperature is 7°C.

4.2.1.3 Push the green door button and pull the handle to access the water chamber.

4.2.1.4 Fill the water tank to the -2 level mark on the measurement scale on the right hand side. (note: this level is marked with tape and label on front of tank, 50 uL)

4.2.1.5 Confirm that Degas On option is selected. Water should be degassed for a minimum of 60 minutes prior to start of sample processing. **Note: If Degas option is On, click the Degas button to Off, then back On. Always freshly degas the water before starting a run.**

4.2.1.6 Input settings below to shear to roughly 180 bp in a 50 µL total volume:

SHEARING CONDITIONS	
Peak Incident Power	450W
Duty Factor (%)	20
Cycles Per Burst	1000
Treatment Time (sec)	420
X and Y Dithering	0.5 mM each, X and Y @ 10mm/sec

4.2.1.7 Select Method Editor and click and drag the rows of samples to be sheared to the plate template.

4.2.2 Prepare Covaris plate for shearing

4.2.2.1 Label a new 96 microTUBE -50 AFA Fiber plate (PN 520168) (can also use a previous plate with unused wells).

4.2.2.2 Gently pierce the foil with the pipette tips and pipette each 50 µL sample prepared in step 7.1 into the corresponding well in the Covaris plate column-wise. Slowly dispense the samples into the plate to not introduce bubbles.

4.2.2.3 Re-seal the wells with foil adhesive by cutting the seal to cover only the wells with sample in them.

4.2.3 Load Covaris plate and perform shearing

4.2.3.1 Push the green button and pull the handle to access the water chamber and loading arm.

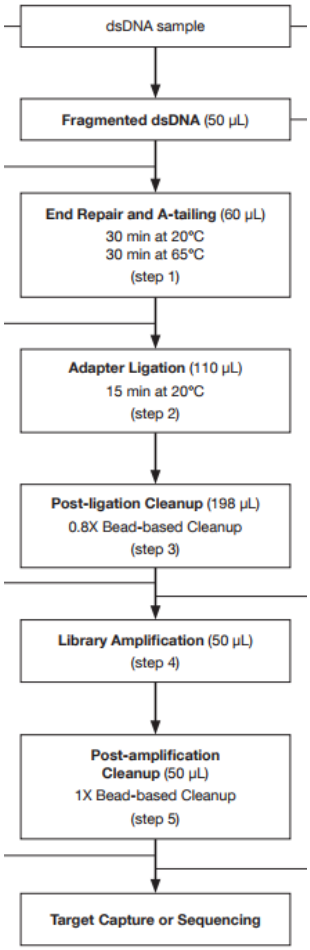
4.2.3.2 On the Laptop, select “Load Position”. Orient the plate so the notch is at the top right hand corner and A1 matches A1 on the loading arm.

4.2.3.3 Close the chamber door and ensure all shearing settings are correct. Select “Start Position” and after plate has moved to the starting position, verify that there are no bubbles underneath the plate against the ampules. If there are, lift closest edge of the plate out of the water and slowly lower it back in, repeating as necessary until all large bubbles are gone.

4.2.3.4 Press Run. When run is complete, press “Load Position” button to bring the arm back up to remove the plate.

4.2.3.5 Carefully transfer the sheared samples to a new PCR strip tube or plate. Continue to Repair procedure.

4.3 KAPA Hyper Prep Workflow



4.4 KAPA Hyper Prep – End Repair and A-tailing

4.4.1 Thaw the End Repair and A-Tailing reagents on ice.

4.4.2 Program the thermal cycler to the following program with heated lid at 85°C:

End Repair and A-Tailing	
Temperature	**Time**
20°C	HOLD
20°C	30 minutes
65°C	30 minutes
4°C or 20°C	HOLD

4.4.3 Ensure the AMPure XP beads have equilibrated to room temperature for at least 30 minutes.

4.4.4 Prepare the End Repair and A-Tailing Reaction MasterMix according to the number of reactions needed:

END REPAIR & A-TAILING MASTERMIX			
Reactions	**1**	**8**	**8**
Reagent	**Volume (uL)**	**Volume (uL)**	**Volume with 10% overage (uL)**
End Repair & A-Tailing Buffer	7	56	61.6
End Repair & A-Tailing Enzyme Mix	3	24	26.4
TOTAL	**10**	**80**	**88**

Note: The values above illustrate an example recipe for 1 or 8 samples and may not reflect those required for your specific run.

4.4.5 Add 10 µL of the End Repair & A-Tailing Reaction MasterMix to each 50 µL sheared DNA sample from the previous step. Gently mix by pipette, seal with an adhesive seal or strip caps, and spin down briefly.

4.4.6 Place the samples in the pre-heated thermal cycler. Press skip step and run the program in step 7.4.2.

4.4.7 During the incubation, thaw the Adapter Ligation reagents on ice in preparation for next step. There is no post end repair & A-tail cleanup.

4.5 KAPA Hyper Prep – Adapter Ligation

4.5.1 Program the thermal cycler to 20°C with the lid open.

4.5.2 Prepare the Adapter Ligation Reaction MasterMix according to the number of reactions needed:

ADAPTER LIGATION MASTERMIX			
Reactions	**1**	**8**	**8**
Reagent	**Volume (uL)**	**Volume (uL)**	**Volume with 10% overage (uL)**
SureSelect Adapter Oligo mix (undiluted)	5	40	44
Nuclease free water	5	40	44
Ligation Buffer	30	240	264
DNA Ligase	10	80	88
TOTAL	**50**	**400**	**440**

Note: The values above illustrate an example recipe for 1 or 8 samples and may not reflect those required for your specific run.

4.5.3 After the End Repair & A-Tailing incubation, add 50 µL of the Adapter Ligation Reaction MasterMix to each 60 µL sample from the previous step. Gently mix by pipette, seal with an adhesive seal or strip caps, and spin down briefly.

4.5.4 Place the samples in the pre-heated thermal cycler and incubate at 20°C for 15 minutes with the lid open.

4.5.5 During the incubation, thaw the Pre-Capture Library Amplification reagents on ice in preparation for next step.

4.5.6 Post Adapter Ligation cleanup with 0.8X AMPure XP beads:

4.5.6.1 Remove the PCR strip tube or plate from the thermal cycler.

4.5.6.2 Add 88 µL of AMPure XP beads to each 110 µL sample. Gently mix thoroughly by pipette at least 15-20 times.

4.5.6.3 Incubate the samples for 5 minutes at room temperature.

4.5.6.4 After incubation, place the PCR strip tube or plate on a magnet for 5 minutes or until solution is clear.

4.5.6.5 With the PCR strip tube or plate still on the magnet, carefully remove and discard the supernatant without disturbing the bead pellet.

4.5.6.6 With the PCR strip tube or plate still on the magnet, add 150 µL of fresh 80% ethanol to each sample. Incubate 30 seconds, then remove and discard the supernatant without disturbing the bead pellet.

4.5.6.7 Repeat the ethanol wash in the previous step for a total of two ethanol washes.

4.5.6.8 Carefully remove any residual ethanol with a P20 pipette.

4.5.6.9 With the PCR strip tube or plate still on the magnet, air dry the pellet by incubating the samples at room temperature for 5 minutes. *Note: Over drying beads can affect sample yield*

4.5.6.10 Remove the PCR strip tube or plate from the magnet and add 25 µL of elution buffer (EB) to each bead sample. Gently mix thoroughly to resuspend the beads.

4.5.6.11 Incubate the samples at room temperature for 2 minutes.

4.5.6.12 Place the PCR strip tube or plate back on the magnet for 3 minutes or until solution is clear.

4.5.6.13 Carefully transfer the supernatant (~20 µL) to a new, clean PCR strip tube or plate. Supernatant is eluted library.

4.6 KAPA Hyper Prep – Pre-Capture Library Amplification

4.6.1 Program the thermal cycler to the following program:

PRE-CAPTURE PCR PROTOCOL		
Temperature	**Time**	**Cycles**
98°C	HOLD	HOLD
98°C	45 sec	1
98°C	15 sec	10-14
65°C	30 sec	
72°C	30 sec	
72°C	5 min	1
4°C	HOLD	HOLD

4.6.2 Choose the cycling conditions based on the type of DNA sample and input DNA amount into library preparation:

PREHYB PCR CYCLING CONDITIONS	
Quantity of Input DNA	**Cycles**
Blood, Fresh Frozen, Cell Culture DNA	
50 ng	12
25 ng	12
10 ng	14
FFPE DNA	
50 ng	12
25 ng	13
10 ng	14

Note: If plate is mixed with different DNA input amounts and DNA types, multiple thermal cyclers must be used. Once PCR mastermix is added to each sample, split the plate into new, clean plates per cycling condition. Then combine samples back to one plate after PCR for the PCR purification step.

4.6.3 Prepare the Pre-Capture Library Amplification Reaction MasterMix according to the number of reactions needed:

PRE-CAPTURE PCR MASTERMIX			
Reactions	**1**	**8**	**8**
Reagent	**Volume (uL)**	**Volume (uL)**	**Volume with 10% overage (uL)**
2X KAPA HiFi HotStart ReadyMix	25	200	220
SureSelect Primer (brown cap)	4	32	35.2
SureSelect ILM Indexing Pre Capture PCR Reverse Primer (clear cap)	4	32	35.2
TOTAL	**33**	**240**	**290.4**

Note: The values above illustrate an example recipe for 1 or 8 samples and may not reflect those required for your specific run.

4.6.4 Add 33 µL of the Pre-Capture Library Amplification Reaction MasterMix to each 20 µL adapter ligated sample from the previous step. Gently mix thoroughly by pipette, seal with an adhesive seal or strip caps, and spin down briefly.

4.6.5 Place the samples in the pre-heated thermal cycler, press skip step and run the PCR program in step 7.6.1.

4.6.6 Pre-Capture Library Amplification cleanup with 1X AMPure XP beads:

4.6.6.1 Remove the PCR strip tube or plate from the thermal cycler.

4.6.6.2 Add 53 µL of AMPure XP beads to each sample. Gently mix thoroughly by pipette at least 15-20 times.

4.6.6.3 Incubate the samples for 5 minutes at room temperature.

4.6.6.4 After incubation, place the PCR strip tube or plate on a magnet for 5 minutes or until solution is clear.

4.6.6.5 With the PCR strip tube or plate still on the magnet, carefully remove and discard the supernatant without disturbing the bead pellet.

4.6.6.6 With the PCR strip tube or plate still on the magnet, add 150 µL of fresh 80% ethanol to each sample. Incubate 30 seconds, then remove and discard the supernatant without disturbing the bead pellet.

4.6.6.7 Repeat the ethanol wash in the previous step for a total of two ethanol washes.

4.6.6.8 Carefully remove any residual ethanol with a P20 pipette.

4.6.6.9 With the PCR strip tube or plate still on the magnet, air dry the pellet by incubating the samples at room temperature for 5 minutes.

Note: Over drying beads can affect sample yield

4.6.6.10 Remove the PCR strip tube or plate from the magnet and add 30 µL of nuclease-free water to each bead sample. Gently mix thoroughly to resuspend the beads.

4.6.6.11 Incubate the samples at room temperature for 2 minutes.

4.6.6.12 Place the PCR strip tube or plate back on the magnet for 3 minutes or until solution is clear.

4.6.6.13 Carefully transfer the supernatant (~30 µL) to a new, clean PCR strip tube or plate. Supernatant is eluted amplified library.

Stopping Point – can store libraries at 4°C overnight or -20°C long term

4.7 Quality assessment of pre-hybridization amplified libraries using the 4200 TapeStation D1000 ScreenTape

4.7.1 Prepare the 4200 TapeStation:

4.7.1.1 Ensure D1000 ScreenTape and D1000 reagents have equilibrated to room temperature for at least 30 minutes.

4.7.1.2 Launch the Agilent 4200 TapeStation Controller software on the laptop or computer connected to the TapeStation.

4.7.1.3 Highlight the number and location of samples to be assessed on the plate diagram. *Note: The TapeStation reads samples in columns.*

4.7.1.4 Before loading the D1000 ScreenTape, gently flick the ScreenTape and load it into the TapeStation instrument. The instrument will scan the ScreenTape and show on the screen the amount of reagents and supplies needed for the run.

4.7.1.5 The software will show on the screen if additional ScreenTapes are needed. If additional ScreenTapes are needed, flick and load them in the rack in the back left hand corner of the instrument.

4.7.1.6 The software will show on the screen how many loading tips are needed for the run. Ensure enough loading tips have been placed in the instrument.

4.7.1.7 The software will show on the screen how much D1000 ladder with Sample buffer to prepare. Proceed to next steps to prepare reagents and samples.

4.7.2 Prepare D1000 ladder:

4.7.2.1 In a strip tube, in position A1, dilute the D1000 ladder with D1000 Sample Buffer according to the TapeStation software in the previous step.

4.7.2.2 Cover the strip tube with caps and vortex on high speed for 5 seconds. Spin down briefly to collect liquid to the bottom of the tube and ensure there are no bubbles.

4.7.2.3 Open the TapeStation and place the strip tube in the strip tube holder oriented with the ladder at the back. Remove and discard the strip caps.

4.7.3 Prepare samples to be assessed:

4.7.3.1 For each sample, pipette 3 µL D1000 Sample Buffer and 1 µL of sample into an Eppendorf skirted PCR plate.

4.7.3.2 Apply foil seal to the plate and vortex the plate using the IKA vortex at 2000 rpm for 1 minute.

4.7.3.3 Briefly spin down the plate to collect liquid to the bottom of the wells and ensure there are no bubbles.

4.7.3.4 Load the plate into the TapeStation with the foil seal still on.

4.7.4 Sample Analysis

4.7.4.1 Confirm all reagents and supplies for the TapeStation run are in the instrument.

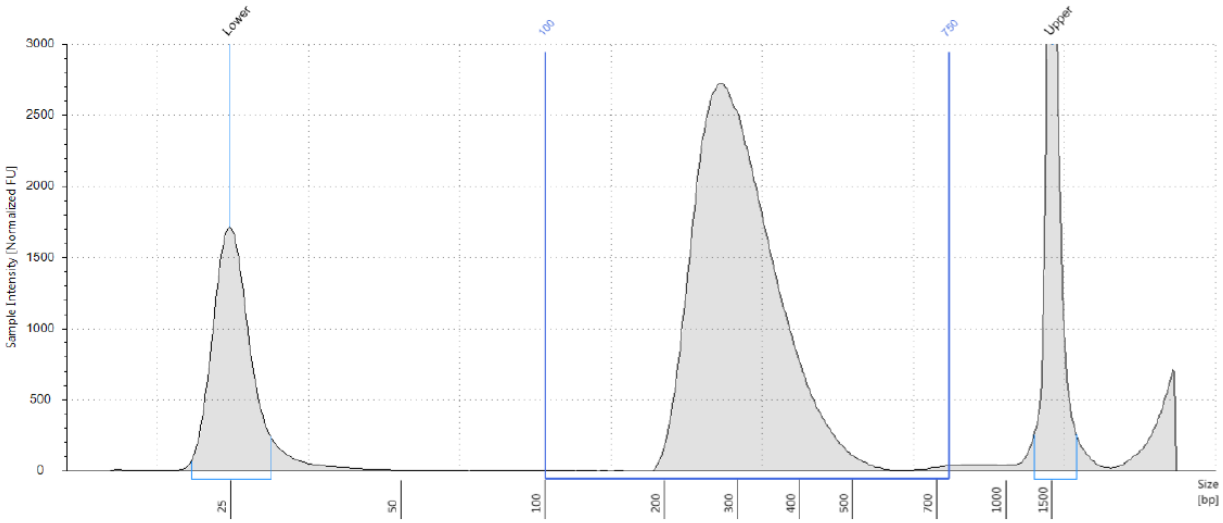
4.7.4.2 Ensure that the ScreenTape is not expired and the strip caps were taken off of the strip tube containing the ladder.

4.7.4.3 Click Start to start the run. Confirm the run continues after initialization.

4.7.4.4 After the run has completed, discard all used reagents. Partially used ScreenTapes can be saved and stored at +4°C.

4.7.4.5 Perform a region analysis of 100 bp to 700 bp.

4.7.4.6 Pre-hybridization libraries should have a target peak size of approximately **250 bp to 350 bp**, and **greater than 750 ng yield** to continue. A sample electropherogram is shown below:



<u>*Stopping Point – can store libraries at 4°C overnight or -20°C long term*</u>

4.8 Normalization of Samples for Hybridization

4.8.1 Open the ***Normalization_for_Hyb_Setup*** excel spreadsheet on the SciClone computer. Enter quantitation values from the TapeStation smear analysis.

4.8.1.1 Save the spreadsheet and close the file.

4.8.2 Open the SSXT Normalization for PreCapture Same Day Hyb method in the Maestro control software.

4.8.3 Begin the procedure by clicking the Start button.

4.8.4 The Maestro software will prompt user to enter number of columns of samples to be processed. Enter this number and click OK to continue.

4.8.5 The software will prompt the user to select a quantitation file.

4.8.5.1 Select the ***Normalization_for_Hyb_Setup*** file from the ***Workbooks*** Folder on the Desktop.

4.8.5.2 Click *OK* to continue.

4.8.6 Place a deep well reservoir containing nuclease-free water onto the SciClone deck according to the deck layout.

4.8.7 Place the sample plate (containing amplified, ligated, SPRI-purified samples from the ***SSXT Library Prep*** procedure) onto the SciClone deck according to the deck layout.

4.8.8 Place all additional labware onto SciClone deck according to the deck layout.

4.8.9 Press *Start* to begin procedure.

4.8.10 Automation will proceed with the following:

4.8.10.1 The predetermined amount of library and water will be added to wells in the destination plate individually; depending on the amount of samples normalized, can take upwards of 45 minutes.

4.8.11 When the automation steps are complete, the software will prompt the user to remove the normalized library plate from the SciClone.

4.8.11.1 Place a lid on the normalized plate or heat seal with a peelable seal.

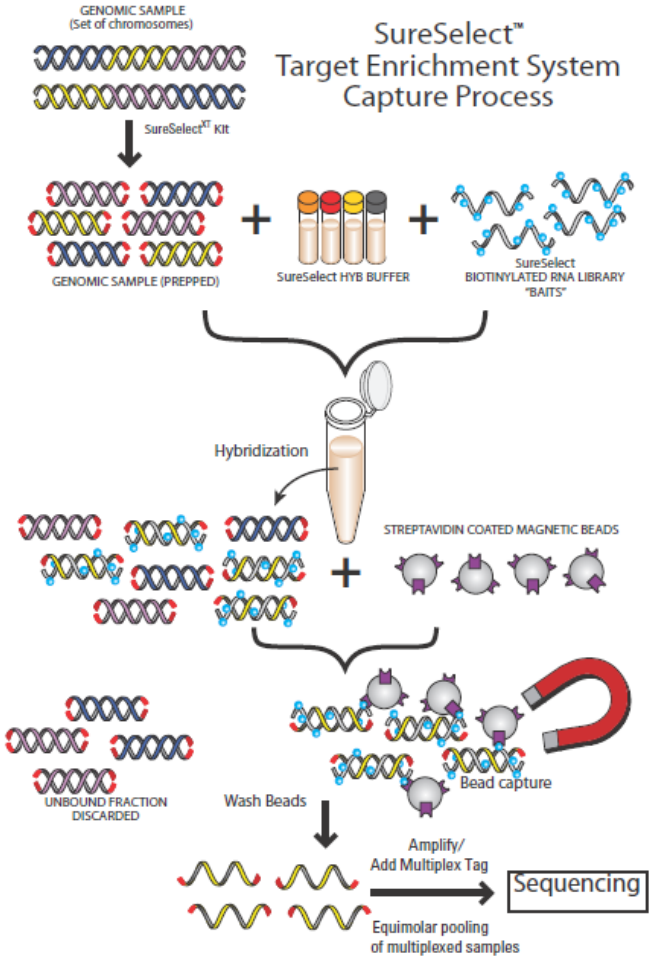
4.8.11.2 Place the plate on ice while preparing for the hybridization procedure.

4.8.12 When the automation steps are complete, the software will also prompt the user to remove the sample plate containing the plate of remaining ligated, amplified material.

4.8.12.1 Heat seal the plate with a peelable seal.

4.8.12.2 Store the plate at -20°C.

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



SureSelect Target Enrichment System Capture Process

4.9 Hybridization

4.9.1 Remove the following reagents from -20°C storage and thaw on ice:

4.9.1.1 SureSelect Indexing Block 1

4.9.1.2 SureSelect Block 2

4.9.1.3 SureSelect ILM Indexing Block 3

4.9.1.4 SureSelect RNase Block

4.9.2 Remove the SureSelect Hyb 3 from -20°C storage and warm to room temperature.

4.9.3 Remove the SureSelect Human All Exon V5 or V6+cosmic capture library probes from -80°C storage and thaw on ice.

4.9.4 On the Sciclone to be used, open the ***SureSelect XT Workbook*** excel file, and enter the number of columns of samples to be processed under the ***SS Hyb Setup tab***. Save the file.

4.9.5 Open the ***SSXT Hyb Setup*** method in the ***Maestro*** control software.

4.9.5.1 Press ***Start*** to begin the procedure.

Note: There will be a 30 minute wait time to allow the heat blocks to come to the correct temperature. Use this time to prepare hybridization reagent mixes.

4.9.6 Prepare SureSelect Block Mix, RNAse Block Mix, Hybridization Buffer, and SureSelect Capture Library probes mixes.

4.9.6.1 Prepare all mixes according to information in the ***SureSelect XT Workbook***, using tables found on the ***SSXT Hyb Setup*** sheet.

Note: The values below illustrate an example recipe for 1 column of samples (8 samples) and may not reflect those required for your specific run.

SureSelect Block Mix	
SureSelect Indexing Block #1 (green cap)	33.8
SureSelect Indexing Block #2 (blue cap)	33.8
SureSelect Indexing Block #3 (brown cap)	8.1
Total	75.7

Hybridization Buffer*	
SureSelect Hyb #1	61.0
SureSelect Hyb #2 (red cap)	2.4
SureSelect Hyb #3 (yellow cap)	24.4
SureSelect Hyb #4	31.7
Total	119.6

RNAse Block Mix	
Nuclease Free Water	13.8
RNAse Block (Purple Cap)	4.6
Total	18.4

SureSelect Capture Library	
SureSelect V5 Library	46
RNAse Block Mix (1:3)	18.4
Total	64.4

4.9.6.2 Prepare Capture Probes in Hyb Buffer according to the worksheet.

Capture Probes in Hyb Buffer	
Hybridization Buffer	119.6
SureSelect Capture Library	64.4
Total	184

4.9.7 Load the Block Mix and Hybridization Probe Plate with the SureSelect Block Mix and the Capture Probes in Hyb Buffer according to the worksheet.

4.9.7.1 Place the reagent plate onto the SciClone deck according to the deck layout.

4.9.8 Place the plate containing the Normalized DNA samples onto the SciClone deck according to the deck layout.

4.9.9 Click ***Start*** to begin procedure.

4.9.10 Automation will proceed with hybridization set-up

4.9.11 During the automated hybridization setup, begin a C1000 thermal cycler program set to 65 degrees, infinite hold with a lid temperature of 105 degrees.

4.9.12 When prompted by the SciClone software, heat seal the hybridization plate using a peelable seal.

4.9.13 Quickly transfer the hybridization plate onto the pre-programmed thermal cycler from step 7.9.11.

4.9.14 Return reagents to proper freezers. Be sure to store the Capture library probes at -80C.

4.9.15 Incubate the hybridization mixture for a minimum of 16 hours and a maximum of 24 hours at 65° with a heated lid at 105°C.

4.10 Post Hybridization Target Selection

4.10.1 Remove AMPure XP beads and Dynabeads MyOne Streptavidin T1 beads from 4°C storage and allow to equilibrate to room temperature for at least 30 minutes.

4.10.2 Load the following capture buffer reservoirs according to the ***SSXT Target Selection*** sheet of the ***SureSelect XT Workbook*** excel file:

4.10.2.1 Binding Buffer

4.10.2.2 Wash Buffer 1

4.10.2.3 Wash Buffer 2

4.10.2.4 Dynabead/10 mM Tris

4.10.3 Open the ***SSXT Target Selection*** method within the ***Maestro*** control software.

4.10.4 Load necessary reagent plates and consumables onto the SciClone deck according to the deck layout prompts.

4.10.5 Click ***Start*** to begin the procedure.

4.10.6 Automation will proceed with the target selection protocol.

4.10.7 In preparation for the PCR Enrichment, remove the following reagents from -15°C--25°C storage, thaw at room temperature, and then store on ice until use:

4.10.7.1 5x Herculase II Reaction Buffer

4.10.7.2 100mM dNTP Mix (25mM each dNTP)

4.10.7.3 SureSelect ILM Indexing Post-Capture Forward PCR Primer

4.10.7.4 SureSelect 8 bp Indexes (reverse primers) plate

4.10.8 In preparation for the PCR Enrichment, remove the Herculase II Fusion DNA Polymerase from -20°C storage and store on ice until use.

4.10.9 When the Dynabead washing process is complete, the software will prompt the user to remove the hybridization plate from the C1000 thermal cycler.

4.10.9.1 Remove the peel able seal from the hybridization plate.

4.10.9.2 Place the hybridization plate onto the SciClone deck according to the software prompt.

4.10.10 Automation will proceed with the target enrichment protocol.

4.10.11 During the final Wash 2 Buffer cleanup, prepare PCR Enrichment Master Mix according to the ***SSXT Target Selection*** sheet of the ***SureSelect XT Workbook*** excel file.

Note: The values below illustrate an example recipe for 1 sample and may not reflect those required for your specific run.

PCR Enrichment Mix	1 Sample	10% extra
SureSelect Indexing Post-Capture PCR (Forward)	1	1.1
Herculase II 5X Reaction Buffer	10	11
dNTP Mix	0.5	0.55
Herculase II Polymerase	1	1.1
Water	7.5	8.25
Total	20	22

4.10.12 When prompted by the SciClone software, place the PCR Master Mix plate, and the Index plate onto the SciClone deck according to the deck layout.

Note: Captured library is retained on the streptavidin beads during the post-capture amplification step. Half of the captured library will be used for the post capture PCR. The remaining half will be stored and used for amplification if the yield from the first amplification is insufficient.

4.10.13 Click ***OK*** to continue PCR setup.

4.10.14 During the automated PCR setup program a C1000 thermal cycler with the following program:

- Choose the pre-heat lid option and set to 100°C
- Hold at 98°C
- 98°C for 2 minutes
- 11 cycles of:
  - 98°C for 30 seconds
  - 57°C for 30 seconds
  - 72°C for 1 minute
- 72°C for 10 minutes
- Hold at 4°C

4.10.15 When automated washing is complete the Maestro software will prompt the user to heat seal the PCR plate with a peel able seal and transfer it to the pre-programed thermal cycler.

4.10.15.1 Click ***Skip step*** on the thermal cycler to start the amplification program.

4.10.16 Heat seal the plate containing the remaining library-bound beads with a peelable seal.

4.10.16.1 Store the sealed extra library plate in -20°C storage for future use, if needed.

4.10.17 When the PCR program has reached the 4°C hold step, remove the PCR plate from the thermal cycler.

4.10.17.1 Seal the plate with an adhesive seal or cover with a plate lid.

4.10.17.2 Place the plate on ice in preparation for final AMPure cleanup on SciClone.

4.11 Final Library AMPure Cleanup on the SciClone

4.11.1 Open the "***SSXT PostHyb PCR SPRI Cleanup***" workbook in Excel.

4.11.2 In cell D2, indicate how many columns of samples will be processed, then click save.

4.11.3 The workbook will automatically populate the values needed for the PCR cleanup.

4.11.4 Add the appropriate amount of AMPure XP beads to the Bead plate according to the ***"SSXT PostHyb PCR SPRI Cleanup"*** workbook. Ensure the AMPure beads are mixed well and have equilibrated to room temperature.

4.11.5 Add the appropriate amount of EB buffer to the elution buffer plate according to the ***"SSXT PostHyb PCR SPRI Cleanup"*** workbook.

4.11.6 Prepare 50mL-100mL of 80% EtOH to be used in the cleanup.

4.11.7 Open the "***SSXT PostHyb PCR SPRI Cleanup***" application in Maestro.

4.11.8 Press start to initialize the instrument and start the deck layout setup.

4.11.9 Continue through the prompts to set up the deck with appropriate labware and reagents.

4.11.10 Ensure the deck layout is set up according to the software prompts and the ***SSXT PostHyb PCR SPRI Cleanup*** workbook.

4.11.11 Ensure all plates with reagent have no bubbles at the bottom of the wells. Spin down plates if there are bubbles present.

4.11.12 When the PCR is complete, briefly spin down the plate, remove the seal and place the plate on the SciClone deck according to the prompt.

4.11.13 Click ***Finish*** on the setup prompt to start the ***SSXT PostHyb PCR SPRI Cleanup*** application.

4.11.14 When the cleanup process is completed the software will prompt the user to remove the plate containing the final libraries.

4.11.15 Proceed to Final Library Quality Assessment.

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

4.12 Final Library Quality Assessment Using the Agilent TapeStation

4.12.1 Allow the HS D1000 ScreenTapes, HS D1000 Sample Buffer, and HS D1000 ladder to equilibrate to room temperature for 30 minutes prior to use.

4.12.2 Launch the TapeStation software. Select the plate well positions to be run on the virtual plate set-up. This will calculate the necessary number of tapes and amount of ladder necessary.

4.12.3 Flick the HS D1000 ScreenTapes to eliminate bubbles in the separation channel. Bubbles in the separation chambers may interfere with sample loading.

4.12.3.1 Load the HS D1000 ScreenTape in the processing bucket and provide tips. Load the required number of additional HS D1000 ScreenTapes in the holding rack.

4.12.3.2 Vortex and spin down the HS D1000 reagents prior to use.

4.12.4 If running 15 samples or fewer, prepare 4 µl of diluted ladder by mixing 2 µl HS D1000 Sample Buffer with 2 µl HS D1000 ladder and place in a strip tube. If running more than 15 samples, dilute 15 µl of D1000 ladder (yellow cap) with 15 µl of D1000 sample buffer (green cap) and place in a strip tube.

4.12.4.1 Cap the strip and vortex mix using the IKA vortexer and adapter at 2000 rpm for 1 minute.

4.12.4.2 Spin the plate down.

4.12.5 Mix 2 µl of each final library sample with 2 µl of HS D1000 sample buffer and load on an Eppendorf skirted PCR plate.

4.12.5.1 Seal the plate with pierceable foil.

4.12.5.2 Mix sample plate on the IKA vortexer with adapter at 2000 rpm for 1 minute.

4.12.5.3 Spin the plate down.

4.12.5.4 Load the sample plate into the TapeStation, close the lid, and click *Start*.

4.12.6 Using TapeStation Analysis software, perform a smear analysis to quantify the amount of amplified library.

4.12.6.1 Select TapeStation run

4.12.6.2 Click Region

4.12.6.3 Click Region Settings

4.12.6.4 Select a range starting at 100 bp and ending at 1000 bp. Apply to the whole file.

4.12.6.5 Export results to a csv file.

4.12.6.6 Click File

4.12.6.7 Click Export Data

4.12.6.8 Check the box to include the smear/region analysis.

4.12.6.9 Click Export

4.12.6.10 Open the exported smear analysis csv file. The column containing the molar concentration information will be used to dilute libraries prior to digital PCR quantitation.

4.12.7 Assess library quality. Library peak sizes should be between 250 and 350 bp.

4.12.8 Using EB, create at least 20 µl of each library diluted to a range of 5-10 nM based on TapeStation data.

4.13 Quantify the individual Final Libraries using ddPCR

4.13.1 Refer to the SOP titled ***ddPCR Library Quantification***, SOP document number MCCRD_SOP0007_PDX ddPCR Library Quantification.

4.13.1.1 Use the diluted final libraries from step 7.12.8 to perform quantitation.

4.14 Pool Final Libraries

4.14.1 Once all libraries have been quantified using digital PCR, pool the diluted library samples in equimolar amounts to a final concentration of 3nM.

4.14.1.1 Samples should be pooled such that pools contain libraries with similar starting concentrations and unique indexes

4.15 Quantify the pooled libraries using ddPCR

4.15.1 Refer to the SOP titled ***ddPCR Library Quantification***, SOP document number MCCRD_SOP0007_PDX ddPCR Library Quantification.

4.16 Dilute final pools

4.16.1 Each pool, prior to denaturing and clustering, should be diluted to a final concentration of 1.325 nM.

4.17 Dilute, Denature, and Cluster Final Pools for Sequencing

4.17.1 Refer to SOP titled PDX Dilute Denature and Cluster Protocol, MCCRD_SOP0008_PDX Sequencing library dilute_denature_cluster

4.17.2 Pools should be clustered at a concentration of 13.25 pM with a 1% phiX spike-in for each sample.

4.17.3 Samples are pooled together based on concentration and the type of enrichment baits used.

5.0 ATTACHMENTS

5.1 Appendix 1: Detailed SciClone Automation Steps.

5.1.1 "SureSelectXT Library Prep" Application.

5.1.1.1 Step 1 – Prepare End Repair Plate

5.1.1.2 Move lid from A3 to C2. Move lid from A4 to B2.

5.1.1.3 Load tips. Transfer 20 uL End Repair mix from col1 of A3 to Q1 of 384-well plate at A4. Eject tips.

5.1.1.4 Move lid from C2 to A3.

5.1.1.5 Load tips. Transfer 20 uL End Repair mix from Q1 of 384-well plate at A4 to sample plate at B4. Mix. Eject tips.

5.1.1.6 Step 2 – End Repair

5.1.1.7 Move End Repair plate B4 to shaker on D4. Move lid on D3 deepwell to plate on shaker at D4.

5.1.1.8 Incubate 30 minutes with shaking at 20C. During incubation:

5.1.1.9 Move lid at A3 to C2.

5.1.1.10 Load tips (single column). Transfer 20 uL of A-Tail mix from col 3/4 at A3 to Q2 of 384-well plate at A4. Eject tips.

5.1.1.11 Load tips (single column). Transfer 27 uL of ligation mix from col 5/6 at A3 to Q3 of 384-well plate at A4. Eject tips.

5.1.1.12 Move lid from C2 to A3. Move lid from B2 to A4.

5.1.1.13 Move lid from D4 to D3. Move bead plate from C2 to D2.

5.1.1.14 Load tips. Mix beads at D2.

5.1.1.15 Transfer 90 uL beads from D2 to End Repair reaction on shaker at D4.

5.1.1.16 Move bead plate from D2 to C2.

5.1.1.17 Mix beads and sample at D4. Shake for 5 minutes.

5.1.1.18 Move sample/bead plate from D4 to the magnet at B4. Incubate 5 minutes.

5.1.1.19 Remove SN from beads at B4 to waste at A5. Eject tips.

5.1.1.20 Move beads from B4 to magnet with spacer at C4. Move lid B5 to C5.

5.1.1.21 Load tips. Wash beads with 150 uL of 80% EtOH. Eject tips. Repeat.

5.1.1.22 Move lid from C5 to B5. Move plate from C4 to D2 CPAC at 37C. Incubate 5 minutes to dry EtOH.

5.1.1.23 Move plate from D2 to D4. Move lid from D3 deepwell to C2.

5.1.1.24 Load tips. Transfer 30 uL elution buffer from D3 deepwell to beads at D4.

5.1.1.25 Move lid from C2 to D3.

5.1.1.26 Mix at D4 to resuspend beads. Shake for 3 minutes. Eject tips.

5.1.1.27 Wait for temp at D2 (20C). Move plate from D4 to D2

5.1.1.28 Step 3 – A-Tailing

5.1.1.29 Move lid from A4 to C2.

5.1.1.30 Load tips. Transfer 20 uL A-Tail mix from Q2 384 at A4 to samples on D2. Mix. Eject tips

5.1.1.31 Move samples from D2 to D4 at 37C for A-Tailing incubation. Move lid from D3 to D4.

5.1.1.32 Move lid from A2 to D3. Move Adapter Oligo plate from A2 to D2.

5.1.1.33 Incubate A-Tailing reaction for 30 minutes at 37C with shaking. During incubation:

5.1.1.34 Load tips (single column). Broadcast 10 uL of adapter oligos from plate at D2 to Q4 of 384-well plate at A4. Eject tips.

5.1.1.35 Dispose of plate at D2. Move lid from C2 to A4.

5.1.1.36 Move lid from D3 to A2. Move lid from D4 to D3.

5.1.1.37 Move lid from B2 to C2. Move PEG plate from B2 to D2.

5.1.1.38 Load tips. Transfer 90 uL PEG from D2 to D4. Move peg plate from D2 to B2.

5.1.1.39 Move lid from C2 to B2.

5.1.1.40 Mix samples/PEG at D4. Shake for 5 minutes.

5.1.1.41 Move samples/beads from D4 to magnet without spacer at B4, incubate 5 min.

5.1.1.42 Remove SN from B4 to waste at B5, eject tips.

5.1.1.43 Move beads B4 to magnet with spacer C4. Move lid from B5 to C5.

5.1.1.44 Load tips. Wash beads with 150 uL of 80% EtOH. Eject tips. Repeat.

5.1.1.45 Move lid from C5 to B5. Move plate from C4 to D2 at 37C. Incubate for 5 minutes.

5.1.1.46 Move plate from D2 to D4. Move lid from D3 deepwell to C2.

5.1.1.47 Load tips. Transfer 15 uL elution buffer from D3 deepwell to beads at D4.

5.1.1.48 Move lid from C2 to D3.

5.1.1.49 Mix at D4 to resuspend beads. Shake for 3 minutes. Eject tips.

5.1.1.50 Step 4 – Adapter Ligation

5.1.1.51 Dispose of lid from 384 at A4.

5.1.1.52 Load tips. Transfer 27 uL of ligation mix from Q3 and 10 uL adapter Q4 from A4 to D4. Mix. Eject tips.

5.1.1.53 Move lid from D3 to D4. Incubate for 15 minutes at 20C. During incubation:

5.1.1.54 Dispose of 384 plate from A4

5.1.1.55 Move lid from B2 to C2. Move PEG plate from B2 to D2. Move lid from D4 to D3.

5.1.1.56 Load tips. Transfer 90 uL PEG from D2 to D4.

5.1.1.57 Move plate from D2 to B2. Move lid from C2 to B2.

5.1.1.58 Mix at D4, shake for 5 minutes

5.1.1.59 Transfer to magnet without spacer at B4. Incubate for 5 minutes.

5.1.1.60 Remove SN from B4 to waste at B5, eject tips.

5.1.1.61 Move beads B4 to magnet with spacer C4. Move lid from B5 to C5.

5.1.1.62 Load tips. Wash beads with 150 uL of 80% EtOH. Eject tips. Repeat.

5.1.1.63 Move lid from C5 to B5. Move plate from C4 to D2 at 37C. Incubate for 5 minutes.

5.1.1.64 Move plate from D2 to D4. Move lid from D3 deepwell to C2.

5.1.1.65 Load tips. Transfer 30 uL elution buffer from D3 deepwell to beads at D4.

5.1.1.66 Move lid from C2 to D3.

5.1.1.67 Mix at D4 to resuspend beads. Shake for 3 minutes. Eject tips.

5.1.1.68 Step 5 – PCR Setup

5.1.1.69 Move plate from D4 to magnet without spacer at B4. Incubate for 5 min.

5.1.1.70 Prompt user to add PCR MM to col 7/8 of PCR plate on A3, re-lid, place again at A3.

5.1.1.71 Prompt user to place empty PCR plate on D2 CPAC at 4C.

5.1.1.72 Dispose of lid from A3.

5.1.1.73 Load tips (single column). Transfer 35 uL PCR mix from col 7/8 of A3 to clean plate on D2, eject tips.

5.1.1.74 Move bead plate from C2 to waste. Move clean plate from C2 to D4.

5.1.1.75 Load tips. Aspirate 30 uL sample from B4. Dispense 15 uL to clean plate at D4, 15 uL to PCR plate at D2. Mix at D2. Eject tips.

5.1.1.76 Dispose of plate from B4.

5.1.1.77 Prompt user to seal PCR plate, spin down, and run enrichment protocol on thermocycler.

5.1.1.78 Prompt user to remove plate from D4, seal, spin down, and store at -20C for future use.

5.1.1.79 Step 6 – Post PCR

5.1.1.80 Prompt user to replace 10 mM Tris Elution buffer in deepwell A3 with water in deepwell at A3. Ensure clean, lidded plate on A2.

5.1.1.81 Prompt user to place PCR/library plate at B4 and plate with beads at D4.

5.1.1.82 Dispose of lid at B2. Move plate from B2 to C2.

5.1.1.83 Transfer 50 uL PCR reactions from B4 to beads at D4.

5.1.1.84 Mix sample and beads at D4. Incubate 5 minutes.

5.1.1.85 Transfer to samples to magnet without spacer at B4. Incubate for 5 minutes.

5.1.1.86 Remove SN from B4 to waste at B5, eject tips.

5.1.1.87 Move beads B4 to magnet with spacer C4. Move lid from B5 to C5.

5.1.1.88 Load tips. Wash beads with 150 uL of 80% EtOH. Eject tips. Repeat.

5.1.1.89 Move lid from C5 to B5. Move plate from C4 to D2 at 37C. Incubate for 5 minutes.

5.1.1.90 Move plate from D2 to D4. Move lid from D3 deepwell to C2.

5.1.1.91 Load tips. Transfer 30 uL water from D3 deepwell to beads at D4.

5.1.1.92 Move lid from C2 to D3.

5.1.1.93 Mix at D4 to resuspend beads. Shake for 3 minutes. Eject tips.

5.1.1.94 Dispose of plate from B4. Move lid from C2 to D4.

5.1.1.95 Prompt user to remove final library plate from D4.

5.1.1.96 Application complete.

5.1.2 "SureSelectXT Normalization" Application

5.1.2.1 Load tips (single column). Eject tips to empty tip box at C5.

5.1.2.2 Load single tip from C5.

5.1.2.3 Aspirate water (sample-specific volume) from A2 deepwell. Aspirate sample (sample specific volume) from A3 source plate.

5.1.2.4 Dispense water and sample to destination plate at A4. Eject tip.

5.1.2.5 Repeat steps 2-4 for remaining samples in column.

5.1.2.6 Repeat steps 1-5 for remaining columns of samples.

5.1.2.7 Prompt user to remove starting sample plate from A3 and seal for long-term storage.

5.1.2.8 Prompt user to remove the normalized library plate from A4 and seal for short-term storage in preparation for hybridization setup.

5.1.2.9 Prompt user to remove and discard the reservoir containing water from A2.

5.1.2.10 Application complete.

5.1.3 "SureSelectXT Hyb Setup" Application

5.1.3.1 Set temperatures for INHECO blocks and equilibrate for 30 minutes.

5.1.3.2 Load tips (single column). Transfer 5.6 uL of block mix from C4 column 1 to library plate at B4. Mix. Eject tips. Repeat for additional columns.

5.1.3.3 Move library plate from B4 to CPAC on A3 (95C). Incubate for 5 minutes without lid. During incubation:

5.1.3.4 Load tips (single column). Transfer 20 uL Capture Library in Hyb Buffer from C4 column 2/3 to plate on D4 (65C).

5.1.3.5 Repeat for number of columns processed. Eject tips.

5.1.3.6 Move denatured library plate from A3 to B4.

5.1.3.7 Load tips. Transfer remaining volume denatured library to plate at D4. Mix. Eject tips.

5.1.3.8 Prompt user to double seal the hybridization plate while on D4, then quickly transfer to pre-warmed thermocycler set to 65C for hybridization.

5.1.3.9 Application complete.

5.1.4 "SureSelectXT Target Selection" Application

5.1.4.1 Step 1 – Prepare Magnetic Beads

5.1.4.2 Move lid from C3 to B4 (This is to position lid correctly on B4).

5.1.4.3 Move empty bead washing plate from A2 to D4. Move lid from A4 to A2.

5.1.4.4 Load tips (single column). Transfer 50 uL of Streptavidin beads from A4 column 1 to plate at D4. Repeat for number of columns processed. Eject tips.

5.1.4.5 Move lid from A2 to A4. Move lid B5 to C5.

5.1.4.6 Load tips. Transfer 100 uL binding buffer from B5 reservoir to beads at D4. Mix. Shake for 2 minutes.

5.1.4.7 Move beads from D4 to magnet with spacer at C2. Incubate for 3 minutes.

5.1.4.8 Remove SN from beads on magnet at C2 to waste deep-well at A5.

5.1.4.9 Move bead plate from C2 to D4. Eject tips.

5.1.4.10 Load tips. Transfer 150 uL of Binding Buffer from B5 to the bead plate at D4. Mix. Shake for 2 min.

5.1.4.11 Move beads from D4 to the magnet at C2. Incubate for 3 minutes.

5.1.4.12 Remove SN from beads on magnet at C2 to waste deep-well at A5.

5.1.4.13 Move beads from C2 to D4. Eject tips.

5.1.4.14 Repeat steps 9-12 two more times.

5.1.4.15 Load tips. Transfer 125 uL of Binding Buffer to bead plate at D4. Mix. Eject tips. Shake for 2 minutes.

5.1.4.16 Move lid from C5 to B5.

5.1.4.17 Move head out of way.

5.1.4.18 Step 2 – Capture DNA

5.1.4.19 Prompt user to place hybridized samples plate on magnet at C4.

5.1.4.20 Load tips. Transfer samples from C4 to bead plate at D4. Mix. Shake for 10 minutes at 22C. Repeat mix and shake.

5.1.4.21 Dispose of sample plate from C4.

5.1.4.22 Move sample/bead plate from D4 to magnet C2. Incubate for 2 minutes.

5.1.4.23 During incubation on magnet, move lid B4 to C3. Move Wash Buffer II deepwell plate B4 to A3 to warm at 65C. Move lid C3 to A3.

5.1.4.24 Remove SN from beads at C2 to waste deep-well at A5. Eject tips.

5.1.4.25 Step 3 – Wash Buffer 1

5.1.4.26 Move lid from D3 to B2.

5.1.4.27 Load tips. Transfer 150 uL of Wash Buffer 1 from D3 to samples at D4. Mix. Shake at 22C for 10 min.

5.1.4.28 Move sample plate from D4 to magnet at C2. Incubate 1 min.

5.1.4.29 Remove SN from beads at C2 to waste deep-well at A5. Move plate from C2 to D4. Eject tips.

5.1.4.30 Repeat wash (steps 2-4).

5.1.4.31 Move lid from B2 to Wash Buffer 1 at D3.

5.1.4.32 Move samples from D4 to A2.

5.1.4.33 Step 4 – Wash Buffer 2

5.1.4.34 Load tips. Pick lid from A3. Aspirate 150 uL of Wash Buffer 2 from A3 deep-well. Place lid at A3.

5.1.4.35 Dispense 150 uL Wash Buffer 2 to sample plate at A2. Mix at A2.

5.1.4.36 Move sample plate from A2 to D2 CPAC at 65C. Mix.

5.1.4.37 Move lid from D3 to D2. Incubate for 5 minutes.

5.1.4.38 Move lid from D2 to D3.

5.1.4.39 Move plate to magnet at C2. Incubate for 1 minute.

5.1.4.40 Remove SN from beads at C2 to waste at A5

5.1.4.41 Move sample plate from C2 to A2. Eject tips.

5.1.4.42 Repeat wash 5 times (steps 1-8)

5.1.4.43 Move sample plate from A2 to D4.

5.1.4.44 Step 5 – PCR Set-up

5.1.4.45 Load tips (single column). Transfer 50 uL of TE Buffer from col 6 of deep-well A4 to sample plate at D4. Eject tips. Repeat for all sample columns.

5.1.4.46 Wait for temp 4C for Inheco at D2.

5.1.4.47 Prompt user to place PCR Master Mix plate at D2 and Pre-Arrayed Indexing Primers at C4.

5.1.4.48 Load tips (single column). Broadcast 20 uL PCR MM across plate at D2. Eject tips.

5.1.4.49 Load tips. Transfer indexed primers from C4 to D2 (total volume, 5 uL). Mix. Eject tips.

5.1.4.50 Dispose of primer plate from C4.

5.1.4.51 Load tips. Mix samples/beads at D4. Shake for 2 minutes. Mix again.

5.1.4.52 Transfer 25 uL of sample/beads from D4 to PCR MM plate at D2. Mix. Eject tips.

5.1.4.53 Prompt user to seal plate at D2 and place on thermocycler for Post-Capture PCR.

5.1.4.54 Prompt user to store the remaining sample in plate at D4 for future use.