MCCRD-SOP0006: RNA Access Automated Target Enrichment for Illumina Paired-End Sequencing Library

Effective Date: 9/14/2018

Please check for revision status of the SOP at

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PDMR NCI Patient-Derived Models Repository
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APPROVALS

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VERSION INFORMATION

1. Change History

Revision	Description
	Internal SOP used by PDMR In Vitro Laboratory
12/1/2014	Standardize SOP for posting to PDMR internal site for use by designated NCI intramural laboratories
9/14/2018	TapeStation assay added

2. Related SOPs and Reference Documentation

Document Number	Title
RS-301-9001DOC	TruSeq RNA Access Library Prep Guide http://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_truseq/truseqrnaaccess/truseq-rna-access-library-prep-guide-15049525-b.pdf
G2991-90030	Agilent D1000 ScreenTape Assay Quick Guide for 4200 TapeStation System www.agilent.com%2Fcs%2Flibrary%2Fusermanuals%2FPublic%2FG2964-90032_ScreenTape_D1000_QG.pdf&usg=AFQjCNHyLNSteB1M2kS0M7tVQpxWVTaW3A&sig2=fYxtuCt6wqKfwZu1HGPK6w&bvm=bv.150475504,d.eWE
G2964-90131 Rev. B	Agilent High Sensitivity D1000 ScreenTape System Quick Guide https://www.agilent.com/cs/library/usermanuals/Public/G2964-90131_ScreenTape_HSD1000_QG.pdf

Document Number	Title
G2964-90121 Rev. D	Agilent High Sensitivity RNA ScreenTape System Quick Guide http://www.agilent.com/cs/library/usermanuals/Public/ScreenTape_HSRNA_QG.pdf
4375799 Rev. E	Applied Biosystems Veriti Thermal Cycler User Guide https://tools.thermofisher.com/content/sfs/manuals/cms_042832.pdf
15042173	TruSeq Sample Preparation Pooling Guide
470-2014-001	Technical Note: Evaluating RNA Quality from FFPE Samples http://www.illumina.com/content/dam/illumina-marketing/documents/products/technotes/evaluating-rna-quality-from-ffpe-samples-technical-note-470-2014-001.pdf
N/A	PDXRNAAccesstemplate20171212

1.0 PURPOSE/SCOPE

This Standing Operating Procedure (SOP) describes Automated RNASeq Library Preparation using Illumina's RNA Access Library Prep kit performed by the Molecular Characterization Laboratory (MoCha) at the Frederick National Laboratory for Cancer Research. This SOP is intended for processing up to 94 PDX samples with two positive controls (Universal Human Reference RNA (UHR) and Human Brain Reference RNA (HBR)). **This SOP is for research purposes only and no clinical samples will be processed using this SOP.**

This procedure is to be used to process RNA samples for sequencing on the Illumina HiSeq Sequencing platform. The resulting 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.

2.2 First Strand Synthesis Act D mix contains Actinomycin D, a toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear appropriate personal protective equipment when handling.

3.0 DEFINITIONS

Abbreviation/Term	Definition
UHR	Universal Human Reference RNA
HBR	Human Brain Reference RNA
PCR	Polymerase Chain Reaction
RSB	Resuspension Buffer

4.0 REAGENTS & EQUIPMENT

4.1 Reagents

Description	Model#	Vendor
High Sensitivity RNA ScreenTape	5067-5579	Agilent
High Sensitivity RNA ScreenTape Sample Buffer	5067-5580	Agilent
High Sensitivity RNA ScreenTape Ladder	5067-5581	Agilent
High Sensitivity D1000 ScreenTape	5067-5584	Agilent
High Sensitivity D1000 Reagents	5067-5585	Agilent
High Sensitivity D1000 Ladder	5067-5587	Agilent
Microseal 'B' adhesive seal	MSB1001	Bio-Rad
D1000 ScreenTape	5067-5582	Agilent
D1000 Reagents	5067-5583	Agilent
D1000 Ladder	5067-5586	Agilent
Nuclease-free Water	various	various
Agencourt AMPure XP Kit	A63881	Beckman Coulter
Ethanol, 100% for molecular biology	E7023	Sigma-Aldrich
TruSeq RNA Access Library Prep Kit – Set A or B	RS-301-2001 or RS-301-2002	Illumina
1.5-mL LoBind Tube	022431021	Eppendorf
Fisherbrand 96 DeepWell 1 mL plate	12-566-120	Fisher Scientific
Eppendorf™ 96-Well twin.tec™ PCR Plates	E951020346	Fisher Scientific
0.2 mL PCR tube with lid	Various	various
Centrifuge tubes, 250 mL	430776	Corning
Disposable Standard Serological Pipets, 25 mL capacity	13-678-14B	Fisher Scientific
Disposable Standard Serological Pipets, 10 mL capacity	13-678-12E	Fisher Scientific
Centrifuge tubes, 50 mL	43082	Corning
DEPC-treated water	CG480	Hardy Diagnostics
Hard-Shell 96 well PCR plate	HSP9631	Bio-Rad

Description	Model#	Vendor
150 uL Barrier Sterile 96 Rack tips for Sciclone	111426	Perkin Elmer
NaCL PEG solution; 20% polyethylene glycol, 2.5M NaCL	P4136	Teknova
Seahorse single cavity polypropylene reservoir, 170ml, 12 column	200686-100	Agilent Technologies
MicroAmp® Optical 96-Well Reaction Plate	4316813	ThermoFisher Scientific
384 well U bottom plate	6008890	Perkin Elmer
Plate lids	600030	Perkin Elmer
96 well storage plates, 0.8 mL (MIDI)	AB-0859	Fisher Scientific
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

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

4.2 Equipment

Description	Model #	Vendor
Multi Channel Pipettes (LTS 20,200,1000)	Variable	Rainin
Vortex	58816-121	VWR
MiniFuge	93000-196	VWR
Plate Centrifuge	022628203	Eppendorf
Veriti 96 well thermal cycler	4375786	ABI
PCR Workstation	AC648LFUVC	AirClean Systems
Vacuum concentrator	SpeedVac	Savant
Argos Technologies Omega Single-Channel Pipet Controller	03-391-253	Fisher Scientific
Microplate shaker	VX2500	VWR
Sciclone G3 Automated Workstation	Sciclone G3	Perkin Elmer
Agilent 4200 TapeStation	G2991A	Agilent Technologies

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

5.0 PROCEDURE

5.1 Quantify and Assess Quality of RNA Samples using Agilent's High Sensitivity RNA ScreenTape Kit for the TapeStation.

5.1.1 Allow High Sensitivity RNA ScreenTapes, High Sensitivity RNA Sample buffer, and High Sensitivity RNA Ladder to equilibrate to room temperature for 30 minutes prior to use.

5.1.2 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.

5.1.3 Flick the High Sensitivity RNA ScreenTapes to eliminate bubbles in the separation channel. Bubbles in the separation chambers may interfere with sample loading.

5.1.4 Load a High Sensitivity RNA ScreenTape in the processing bucket and provide tips. Load the required number of additional High Sensitivity RNA ScreenTapes in the holding rack. Select Eukaryotic RNA protocol.

5.1.5 Vortex and spin down High Sensitivity RNA ScreenTape reagents prior to use.

5.1.6 If necessary, thaw RNA samples on ice.

5.1.7 Prepare diluted Ladder solution by adding 10 µL RNase free water to the High Sensitivity RNA Ladder vial and mixing thoroughly.

5.1.8 If running 15 samples or fewer, prepare 3 µl of diluted ladder by mixing 2 µl of High Sensitivity RNA ladder with 1 µl High Sensitivity RNA Sample Buffer and place in a strip tube. If running more than 15 samples, combine 14 µl of High Sensitivity RNA ladder (yellow cap) with 7 µl of High Sensitivity RNA sample buffer (green cap) and place in a strip tube.

5.1.8.1 Cap the tube strip.

5.1.8.2 Vortex mix the tube strip on the IKA vortexer with adapter at 2000 rpm for 1 minute.

5.1.8.3 Spin the plate down.

5.1.9 Dilute 2 µl of each sample with 1 µl of HS RNA sample buffer and load on an Eppendorf skirted PCR plate.

5.1.9.1 Seal the sample plate with pierceable foil.

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

5.1.9.3 Spin the plate down.

5.1.10 Ladder/Sample denaturation

5.1.10.1 Heat ladder and samples at 72 °C (162 °F) for 3 min

5.1.10.2 Place ladder and samples on ice for 2 min

5.1.10.3 Spin down to position the sample at the bottom of the tube

5.1.11 Load sample plate onto the TapeStation instrument, lower the lid, and click *Start*.

5.1.12 Perform a region analysis to determine the percentage of RNA fragments that are greater than 200 nucleotides in length. This measurement yields the DV200 value that will be used to determine RNA input for library preparation.

5.2 Pre-program the Thermal Cyclers

5.2.1 Pre-program the thermal cycler with the following program and save as **Elution 2 -Frag – Prime**:

- Choose the pre-heat lid option and set to 100°C
- Pre-heat to 94°C & hold
- 94°C for 8 minutes
- 4°C hold

5.2.2 Pre-program the thermal cycler with the following program and save as **Synthesize 1st Strand**:

- Choose the pre-heat lid option and set to 100°C
- Pre-heat to 25°C & hold
- 25°C for 10 minutes
- 42°C for 15 minutes
- 70°C for 15 minutes
- 4°C hold

5.2.3 Pre-program the thermal cycler with the following program and save as **Synthesize 2nd Strand**:

- Choose the pre-heat lid option and set to 100°C
- Pre-heat to 16°C & hold
- 16°C for 60 minutes
- 16°C hold

5.2.4 Pre-program the thermal cycler with the following program and save as **First PCR Amplification**:

- Choose the pre-heat lid option and set to 100°C

- Hold at 98°C
- 98°C for 30 seconds
- 15 cycles of:
  - 98°C for 10 seconds
  - 60°C for 30 seconds
  - 72°C for 30 seconds
  - 72°C for 5 minutes
- Hold at 4°C

5.3 RNA Sample Preparation

5.3.1 Fill in the information for columns A through H of the Initial Quant Setup Data sheet within the PDXRNAAccesstemplateYYYYMMDD file.

5.3.2 RNA sample input amount is based on sample quality. Illumina recommends using the percentage of RNA fragments > 200nt, known as DV200, as a reliable determinant of RNA quality.

5.3.2.1 Use the table below with smear analysis from the TapeStation to determine the appropriate RNA input amount for each sample.

5.3.2.2 Pipette the required amount of RNA for each sample into wells on a 96 well plate.

RNA Quality	DV200	Input Requirement per Library Prep
High	>70	10 ng
Low	0-70%	100 ng

Note: Low quality samples with DV200 values less than or equal to 70% are prepped in quadruplicate using 40 ng per reaction.

5.3.3 Normalize the RNA samples to a final volume of 8.5 µL using DEPC-treated water in each well of the 96-well plate.

5.4 Automated cDNA Library Preparation on the SciClone

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

5.4.1 Allow the AMPure XP beads to come to room temperature for at least 30 minutes.

5.4.2 Remove the following reagents from -15°C--25°C storage:

- Elute, Prime, Fragment High Mix
- Resuspension Buffer (can be stored at 4°C after the initial thaw)

- First Strand Synthesis Act D Mix
- Second Strand Marking Master Mix
- A-Tailing Mix (place on ice after thawing)
- Stop Ligation Buffer
- RNA Adapter Indexes
- PCR Master Mix (place on ice after thawing)
- PCR Primer Cocktail (place on ice after thawing)

5.4.3 Remove the following reagents from -15°C--25°C storage and store on ice until use:

- SuperScript II Reverse Transcriptase
- Ligation Mix

5.4.4 Open Maestro software on the SciClone computer.

5.4.4.1 Open ***Illumina RNA Access Workbook*** worksheet in Excel (shortcut is available on the desktop).

5.4.4.2 Enter the number of columns containing samples to be processed in tabs ***1 cDNA Library Prep*** and ***2 Post PCR SPRI*** of the ***Illumina RNA Access Workbook***. Save the file.

5.4.4.3 Entering the number of columns will update the volumes for the reagent recipes.

5.4.4.4 Open the ***1 RNA Access cDNA Library Preparation*** method in the Illumina RNA Access folder.

5.4.4.5 Prepare indexing setup

5.4.4.5.1 Determine pooling strategy. Refer to the TruSeq Library Preparation Pooling Guide and the table below for details.

Note: RNAseq samples will be pooled 4 samples per pool. Assign low quality replicate samples different indices. Low quality replicate libraries constructed from the same original DNA should be pooled in the same pool.

Table 17 Single-Indexed Pooling Strategies for 2–4 Samples with RNA and ChIP Kits

Plexity	Option	Set A Only	Set B Only
2	1	AR006 and AR012	Not recommended
	2	AR005 and AR019	
3	1	AR002 and AR007 and AR019	AR001 and AR010 and AR020
	2	AR005 and AR006 and AR015	AR003 and AR009 and AR025
	3	2-plex options with any other adapter	AR008 and AR011 and AR022
4	1	AR005 and AR006 and AR012 and AR019	AR001 and AR008 and AR010 and AR011
	2	AR002 and AR004 and AR007 and AR016	AR003 and AR009 and AR022 and AR027
	3	3-plex options with any other adapter	3-plex options with any other adapter

5.4.4.5.2 Click on the Indexing sheet within the Illumina RNA Access Workbook

5.4.4.5.3 In the Index well column (column B) located to the right of the Sample well column, enter in the well location of the desired index for the sample using the index well corresponding to the index names located in columns H and I.

5.4.4.5.4 The workbook will automatically calculate the necessary volumes of indexes needed and autofill these values in the 1 cDNA library prep sheet.

5.4.4.5.5 Record which index was assigned to each sample as well as each sample's projected pool on the PDXRNAAccesstemplate20171212 file.

5.4.4.6 Prepare 1st Strand, 2nd Strand, A-Tailing, and Ligation reaction mixes as well as the index aliquots on ice according to the Illumina RNA Access Workbook. Use the tables found under the 1 cDNA Library Prep tab.

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.

5.4.4.6.1 Mix well using a P1000 pipette.

1st Strand Master Mix	per well	total
1st Strand Master Mix	9	87.1 uL
SuperScriptII	1	9.7 uL
Total	10	96.8 uL

2nd Strand Master Mix	per well	total
2nd Strand Marker Mix	20	190.1 uL
Resuspension Buffer (RSB)	5	47.5 uL
Total	25	237.6 uL

A-Tailing Mix	Per Reaction	Total
A-Tailing Mix (ATL)	12.5	132.0 uL
Resuspension Buffer (RSB)	2.5	26.4 uL
Total	15	158.4 uL

Ligation Mix	Per Reaction	Total
Ligase Mix	2.5	57.2 uL
Resuspension Buffer	2.5	57.2 uL
Total	5	114.4 uL

PCR MIX	Per Reaction	Total
PCR Master Mix (PMM)	25	238.0 uL
PCR Primer Mix (PPC)	5	47.6 uL
Total	30	285.6 uL

5.4.4.6.2 Place unused cDNA preparation reagents in -20°C storage. Maintain prepared reagent mixes on ice until prompted.

Note: Combine the PCR mix reagents during the ligation step and add to plate when prompted.

5.4.4.7 Prepare approximately 8 mL of 80% ethanol per column of library prep samples using nuclease-free water (plus a dead volume of 2 mL)

5.4.4.8 Prepare reagent plates with aliquots of the mixes in the plate type specified in the top left of the workbook table.

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

Master Mixes in PCR Plate (Bio-Rad HSP-96 PCR Plate)
Sciclone Deck Location: A4

	1st Strand Master Mix (w/SSII added)	2nd Strand Master Mix	2nd Strand Master Mix	EPH
	1	2	3	4
A	62	152	27	66
B	62	152	27	66
C	62	152	27	66
D	62	152	27	66
E	62	152	27	66
F	62	152	27	66
G	62	152	27	66
H	62	152	27	66

Reagent Plate (Bio-Rad HSP-96 PCR Plate)
Sciclone Deck Location: D2

	A-Tailing Mix	Ligation Mix (Ligase + RSE)	Stop Ligase Buffer	ID001-008	ID009-016	ID017-024	ID025-032				PCR Master Mix	PCR Master Mix
	1	2	3	4	5	6	7				11	12
A	108	61	52	26ul ID001	26ul ID009		0				185	35
B	108	61	52	26ul ID002	26ul ID010	0					185	35
C	108	61	52	26ul ID003	26ul ID011	26ul ID019	26ul ID027				185	35
D	108	61	52	26ul ID004	26ul ID012	0					185	35
E	108	61	52	26ul ID005	0	0					185	35
F	108	61	52	26ul ID006	0	26ul ID022					185	35
G	108	61	52	26ul ID007	0	0					185	35
H	108	61	52	26ul ID008	26ul ID016						185	35

5.4.4.9 Broadcast reagents to match the number of sample columns for AMPure XP beads, PEG buffer, and Resuspension Buffer according to the workbook table.

5.4.4.10 Begin the Elution 2 -Frag – Prime program on the thermal cycler.

5.4.5 Prepare the SciClone automation deck with boxes of tips and the reagent plates previously prepared.

5.4.5.1 Press the Play button and follow prompts for placement of consumables and reagent plates within the Maestro software.

5.4.5.2 Once completed, place RNA samples on the SciClone deck.

5.4.5.3 Click Finish to complete preparation and begin automation.

Note: Only perform fragmentation of samples with DV200 values greater than 70. Remove lower quality samples from the reaction plate following the addition of EPH and store on ice during the EPF incubation. Return samples to the library preparation plate after the EPF step.

5.5 Elute, Prime, and Fragment RNA

5.5.1 The SciClone will broadcast 8.5 µL of EPH to a 384 well plate and then to each column of sample.

5.5.2 Once prompted, seal the plate with a Microseal 'B' adhesive seal.

5.5.3 Shake the plate on a microplate shaker at 1600 rpm for 20 seconds.

5.5.4 Place the sealed plate on the pre-programmed thermal cycler. Close the lid and select 'skip step' to continue the Elution 2 - Frag - Prime program and fragment the RNA.

5.5.5 Remove the sealed plate from the thermal cycler when it reaches 4°C and centrifuge briefly.

5.5.6 Carefully unseal the plate and place it on the Sciclone deck as prompted.

5.6 First Strand Synthesis

5.6.1 Begin the ***Synthesize 1st Strand*** program on the thermal cycler.

5.6.2 Automation will add 8 µL of First Strand Synthesis and SuperScript II combination to a 384 well plate and then to each column of sample mixing thoroughly.

5.6.2.1 When prompted, seal the plate with a Microseal 'B' adhesive seal.

5.6.3 Shake the plate on a microplate shaker at 1600 rpm for 20 seconds.

5.6.4 Place the sealed plate on the pre-programmed thermal cycler. Close the lid and select *skip step* to continue the Synthesize 1st Strand program.

5.6.4.1 When the thermal cycler reaches 4°C, remove the sealed plate from the thermal cycler and centrifuge briefly.

5.6.5 Carefully unseal the plate and place it on the SciClone deck as prompted and proceed immediately with second strand synthesis. Discard 384 well plate on deck and replace with a new 384 well plate.

5.7 Second Strand Synthesis

5.7.1 Begin the ***Synthesize 2nd Strand*** program on the thermal cycler.

5.7.2 Automation will add 25 µL of Second Strand Synthesis and mix.

5.7.3 Incubate for 1 hour at 16°C.

5.8 SPRI Clean Up

5.8.1 Automation will proceed with the following:

5.8.1.1 Add 90 µL of AMPure XP beads to each sample.

5.8.1.2 Shake and incubate at room temperature for 10 minutes.

5.8.1.3 Place on magnet and incubate for 5 minutes.

5.8.1.4 Remove 135 µL of supernatant.

5.8.1.5 Add 150 µL of 80% EtOH to each well.

5.8.1.6 Incubate for 1 minute.

5.8.1.7 Remove ethanol.

5.8.1.8 Repeat steps 5 through 7.

5.8.1.9 Let the plate stand at 37°C for 3 minutes.

5.8.1.10 Add 15 µL of Resuspension Buffer.

5.8.1.11 Shake and incubate at room temperature for 3 minutes.

Note: This double stranded cDNA will remain in bead solution through library preparation.

5.9 A-Tailing

5.9.1 Automation will proceed with the following:

5.9.2 Add 15 µL of the A-Tailing and Resuspension Buffer mixture to a 384 well plate and then to each column of sample.

5.9.3 Incubate the sample plate at 37°C for 30 minutes, then 70°C for 5 minutes.

5.10 Ligation

5.10.1 Automation will proceed with the following:

5.10.1.1 Broadcast indices from pre-mix plate to the 384 well plate on deck.

5.10.1.2 Broadcast ligation mixture to the 384 well plate on deck.

5.10.1.3 Add 5 µL of the ligation mixture and 2.5 µL of the pre-determined index to each well. Pipette to mix.

5.10.1.4 Incubate the sample plate at 30°C for 10 minutes.

5.10.1.5 Add 5 µL of Stop Ligation buffer to each well. Pipette to mix.

5.10.1.6 Shake and incubate the plate at room temperature for 2 minutes.

5.11 SPRI Clean-Up

5.11.1 Automation will proceed with the following:

5.11.1.1 Add 25 µL of PEG Buffer.

5.11.1.2 Shake the plate at room temperature for 1 minute. Pipette to mix.

5.11.1.3 Incubate at room temperature for 10 minutes.

5.11.1.4 Place the plate on the magnet and incubate for 10 minutes.

5.11.1.5 Remove 79.5 µL of supernatant.

5.11.1.6 Add 150 µL of 80% ethanol to each well.

5.11.1.7 Incubate for 30 seconds.

5.11.1.8 Remove ethanol.

5.11.1.9 Repeat steps 5 through 7.

5.11.1.10 Incubate the plate at 37°C for 5 minutes.

5.11.1.11 Add 22.5 µL of Resuspension Buffer.

5.11.1.12 Shake and incubate at room temperature for 3 minutes.

5.11.1.13 Place the plate on magnet and incubate for 5 minutes.

5.12 First PCR Amplification

5.12.1 Begin the ***First PCR Amplification*** program on the thermal cycler to allow it to come to temperature.

5.12.2 Automation will proceed with the following:

5.12.2.1 Broadcast the PCR mixture.

5.12.2.2 Transfer 20 µL of purified sample from the plate on the magnet to the PCR plate and mix.

5.12.3 Seal the plate and centrifuge for 1 minute at 280 x g.

5.12.4 Place the plate in the thermal cycler and press *Skip step* to continue the ***First PCR Amplification*** program.

5.12.5 During cycling, prepare the SciClone deck by closing the ***1 RNA Access cDNA Library Preparation method*** and opening the ***2 RNA Access Post Library Amp Clean method***. Remove any partially used tip boxes and the used reagent plates.

5.13 Purify the Sample Using Agencourt AMPure XP beads on the SciClone

5.13.1 Bring AMPure XP beads and RSB to room temperature.

5.13.2 Enter the number of columns of samples to be processed in the ***2 PostPCRSPRI*** tab of the ***Illumina RNA Access Workbook***. Save the file.

5.13.3 Mix the AMPure XP beads well so that the reagent appears homogeneous and consistent in color. Do not freeze.

5.13.4 Add 55 µL of AMPure XP beads per well to be processed on a 96 well BioRad hard shell plate. Place the plate onto the SciClone deck according to the SciClone software's deck layout.

5.13.5 Add 80% ethanol to 12 well column plate as directed by the SciClone software and place on the SciClone deck according to the SciClone software's deck layout.

5.13.6 Add RSB to the appropriate labware and place on the SciClone deck according to the SciClone software's deck layout.

5.13.7 Add tip boxes and necessary labware to the SciClone deck according to the SciClone software deck layout.

5.13.8 Press *Start* within the SciClone software to begin the automation procedure.

5.13.9 Automation will proceed with the following:

5.13.9.1 Add 55 ul of AMPure beads per well to be processed on a 96 well BioRad hard shell plate, pipette up and down to mix.

5.13.9.2 Shake for 10 minutes.

5.13.9.3 Move hard shell plate to magnet and incubate for 10 minutes.

5.13.9.4 Remove and discard supernatant.

5.13.9.5 Add 200 ul of 80% Ethanol to each well to be processed

5.13.9.6 After 30 second delay remove and discard ethanol

5.13.9.7 Repeat steps 7.13.9.5 – 7.13.9.6 for a total of 2 ethanol washes.

5.13.9.8 Incubate plate at 37°C for 5 minutes

5.13.9.9 Add 27.5 ul of RSB to each well to be processed and pipette up and down to mix.

5.13.9.10 Shaker for 3 minutes and then pipette up and down.

5.13.9.11 Incubate plate on magnet for 5 minutes.

5.13.9.12 Transfer 25 ul of supernatant to clean 96 well BioRad hard shell plate.

5.13.10 When the ***2 RNA Access Post Library Amp Cleanup*** method is complete, cover the sample plate with a lid and place the plate on ice while preparing up for library quality assessment.

Stopping Point - If you do not continue immediately to the next step, store the samples at -20°C

5.14 Assess Library Quality Using Agilent's D1000 ScreenTape Kit for the TapeStation

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

5.14.2 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.

5.14.3 Flick the D1000 Screen Tapes to eliminate bubbles in the separation channel. Bubbles in the separation chambers may interfere with sample loading.

5.14.3.1 Load the D1000 Screen Tape in the processing bucket and provide tips. Load the required number of additional D1000 ScreenTapes in the holding rack.

5.14.3.2 Vortex and spin down D1000 reagents prior to use.

5.14.4 If running 15 samples or fewer, prepare 4 µl of diluted ladder by mixing 3 µl D1000 Sample Buffer with 1 µl D1000 ladder and place in a strip tube. If running more than 15 samples, dilute 5 µl of D1000 ladder (yellow cap) with 15 µl of D1000 sample buffer (green cap) and place in a strip tube.

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

5.14.4.2 Spin the plate down.

5.14.5 Dilute 1 µl of each sample with 3 µl of D1000 sample buffer and load on an Eppendorf skirted PCR plate.

5.14.5.1 Seal the plate with pierceable foil.

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

5.14.5.3 Spin the plate down.

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

5.14.5.5 Record the concentration of each sample and calculate the total amount, in ng, of each sample in the ***PDXRNAAccesstemplate20171212*** file.

5.14.5.6 Assess library quality. Library peak sizes should be ~260 bp.

5.15 Pool and Normalize Library Samples

5.15.1 200 ng each of 4 samples with different indices will be pooled together with RSB.

Note: The volume of each pool should not exceed 45 µL.

If the total pooled volume is greater than 45 µL, pools must be concentrated using a vacuum concentrator with no heat at medium drying rate.

Note: Pool any low quality replicates in the same pool.

5.15.1.1 Enter pooling and normalization information into the ***3 Normalize and Pool Libraries*** tab on the ***Illumina RNA Access Workbook***. Save the file.

5.15.1.1.1 Copy and paste sample names into *Column B*.

5.15.1.1.2 Copy and paste concentration information as determined by TapeStation analysis into *Column F*.

5.15.1.1.3 Ensure that the desired samples will be pooled together. Consult the pooling guidelines publication for more information.

5.15.1.1.4 If the total pool volume listed in *Column N* is greater than 45 uL the pool must to be dried down using a SpeedVac.

5.15.1.2 Open the Maestro method RNA Access Normalize and Pool Library.

5.15.1.2.1 Select *OK* when the *Normalization with Pooling?* prompt appears.

5.15.1.2.2 The *Normalization Confirmation* screen will appear. Confirm that the pooling setup is correct.

5.15.1.2.3 Follow the prompts within the Maestro software to set up the SciClone deck with the appropriate reagents and consumables to complete the normalization.

5.15.1.3 Automation will proceed with the following:

5.15.1.3.1 Addition of appropriate amount of RSB to each well according to the workbook.

5.15.1.3.2 Addition of appropriate amount of library to appropriate pool according to the workbook.

Stopping Point - If you do not immediately continue to the next step, store the samples at -20°C

5.16 First Hybridization

Note: A 90 minute incubation is optimal for hybridization. Hybridizing longer than 2 hours results in a high degree of non-specific binding

5.16.1 Remove the following reagents from -25°C to -15°C storage and thaw at room temperature:

- Capture Target Buffer 3
- Coding Exome Oligos

5.16.2 If necessary, remove the plate of pooled samples from -25°C to -15°C storage and thaw on ice.

- Centrifuge the thawed plate at 280 × g for 1 minute.
- Remove the adhesive seal from the thawed plate.

5.16.3 Pre-program the thermal cycler with the following program and save as ***RNA HYB***:

- Choose the pre-heat lid option and set to 100°C
- 95°C for 10 minutes
- 18 cycles of 1 minute incubations, starting at 94°C, then decreasing 2°C per cycle
- 58°C for 90 minutes
- 58°C hold

5.16.4 Begin the ***RNA HYB*** program to pre-heat the thermal cycler.

5.16.5 Thoroughly vortex the Capture Target Buffer 3 tube until the solution is completely resuspended. Visually make sure that no crystal structures are present.

Note: If crystals and cloudiness are observed, vortex the Capture Target Buffer 3 tube until it is clear.

5.16.6 Open Maestro software on the SciClone.

5.16.7 Open the ***Illumina RNA Access Workbook*** worksheet in Excel (shortcut is available on the desktop).

5.16.8 Enter the number of columns of samples to be processed in tabs ***4 Hyb Setup***, ***5&6 Target Capture***, and ***7 Final Post-PCR SPRI*** of the ***Illumina RNA Access Workbook***. Save the file.

5.16.9 Open the ***4 RNA Access Hyb Setup*** method in the Illumina RNA Access folder.

5.16.10 Prepare the Enrichment Hyb Solution mix according to the ***Illumina RNA Access Workbook***.

5.16.10.1 Use the tables found under the ***4 Hyb Setup*** tab.

5.16.10.2 Mix the solution well using a P1000 pipette.

Note: Prepare the Enrichment Hyb Solution immediately before use.

5.16.11 Click *Play* in the ***RNA Access Hyb Setup*** method and prepare the Sciclone deck with consumables, reagents, and samples as directed.

5.16.12 Automation will then proceed with the following:

5.16.12.1 Addition of 55 uL of Enrichment Hyb Solution to 45 uL of sample.

5.16.12.2 Pipette to mix.

5.16.13 After the hybridization setup is complete, immediately seal the plate with two Microseal 'B' adhesive seals. Place in the pre-heated thermal cycler.

5.16.14 Press the *Skip step* button to continue the ***RNA HYB*** thermal cycler program.

5.17 First Capture

5.17.1 Remove the following reagents from -25°C to -15°C storage and thaw at room temperature:

- 2N NaOH
- Enrichment Elution Buffer 1
- Enrichment Wash Solution

5.17.2 Remove the following reagents from 2°C to 8°C storage and let equilibrate to room temperature:

- Elute Target Buffer 2
- Streptavidin Magnetic Beads

5.17.3 Open the Illumina RNA Access Workbook and select the 5&6 Target Capture sheet.

5.17.3.1 Update the number of columns of samples to be processed. Save the file.

5.17.3.2 Prepare the reagent mixes and reagent plates according to information in the tables.

5.17.4 Open the Maestro software on the SciClone.

5.17.5 Open the 5&6 Target Capture method in the Illumina RNA Access folder.

5.17.5.1 Select First Capture Only. Press *Play* and prepare the SciClone automation deck with consumables, plates, and samples as directed.

5.17.5.2 With approximately 5 minutes remaining before the 58°C hold step of the thermal cycler hybridization program, click *Finish* on the SciClone prompt to complete preparation and begin the automated washing of beads.

5.17.5.3 When prompted by the SciClone software, immediately transfer hybridization sample plate to the magnet on deck and carefully remove the seals.

5.17.6 Automation will proceed with the following:

5.17.6.1 Transfer the contents from each well of the RAH1 plate to the corresponding well of the 96-well MIDI plate labeled RAW1 containing 250 ul of Streptavidin Magnetic Beads. Pipette to mix.

5.17.6.2 Mix the beads and hybridized samples by pipetting up and down.

5.17.6.3 Let the RAW1 plate stand at room temperature for 25 minutes with intermittent mixing.

5.17.6.4 Place the RAW1 plate on the magnet for 2 minutes at room temperature.

5.17.6.5 Remove and discard all of the supernatant from each well of the RAW1 plate without disturbing the beads.

5.17.6.6 Remove the RAW1 plate from the magnet.

5.17.6.7 Add 150 ul of Enrichment Wash Solution to each well of the RAW1 plate.

5.17.6.8 Shake the RAW1 plate and pipette to mix.

5.17.6.9 Transfer the entire contents of the RAW1 plate to a new Hard Shell BioRad plate. Pipette to mix.

5.17.6.10 Place the BioRad plate on the magnetic stand for 2 minutes.

5.17.6.11 Remove and discard all of the supernatant from each well of the BioRad plate.

5.17.6.12 Aspirate 150 ul of Enrichment Wash Solution and dispense 30 ul of the solution to each well of the BioRad plate.

5.17.6.13 Move BioRad plate off the magnet to position A4. Dispense the remaining solution. Pipette to mix.

5.17.6.14 Incubate at 50°C for 20 minutes.

5.17.6.15 Place the plate on the magnet for 2 minutes.

5.17.6.16 Immediately remove and discard the supernatant from each well of the RAW1 plate.

5.17.6.17 Remove the RAW1 plate from the magnet.

5.17.6.18 Repeat steps 7.17.6.8 - 7.17.6.14 one time for a total of two Enrichment Wash Solution washes.

5.17.7 When prompted, place plate containing Elution Prep Mix, Elute Target Buffer 2, Enhanced PCR mix, and PCR Primer Cocktail on deck as instructed.

5.17.8 Automation will proceed with the following:

5.17.8.1 Add 23 µl of the Elution mix to each well of the BioRad plate.

5.17.8.2 Pipette to mix.

5.17.8.3 Let the plate stand at room temperature for 2 minutes.

5.17.8.4 Place the plate on the magnet for 2 minutes or until the liquid is clear.

5.17.8.5 Add 4 ul Elute Target Buffer 2 to a clean BioRad plate.

5.17.8.6 Transfer 24 µl of clear supernatant from each well of the plate to the corresponding well of the BioRad plate containing ETB2, pipette to mix.

5.17.8.7 Place on magnetic stand for 2 minutes.

5.17.8.8 Transfer 25 ul of supernatant to the new RAH2 plate.

Stopping Point - If you do not continue immediately to the next step, store the samples at -20°C

5.18 Second Hybridization

Note: A 90 minute incubation is optimal for hybridization. Hybridizing longer than 2 hours results in a high degree of non-specific binding

5.18.1 Remove the following reagents from -25°C to -15°C storage and thaw at room temperature:

- Capture Target Buffer 3
- Coding Exome Oligos

5.18.2 If necessary, remove the plate of samples (First Capture) from -25°C to -15°C storage and thaw on ice.

- Centrifuge the thawed plate at 280 × g for 1 minute.

- Remove the adhesive seal from the thawed plate.

5.18.3 Pre-program the thermal cycler with the following program and save as ***RNA HYB***:

- Choose the pre-heat lid option and set to 100°C
- 95°C for 10 minutes
- 18 cycles of 1 minute incubations, starting at 94°C, then decreasing 2°C per cycle
- 58°C for 90 minutes
- 58°C hold

5.18.4 Begin the ***RNA HYB*** program to pre-heat the thermal cycler.

5.18.5 Thoroughly vortex the Capture Target Buffer 3 tube until the solution is completely resuspended. Visually make sure that no crystal structures are present.

Note: If crystals and cloudiness are observed, vortex the Capture Target Buffer 3 tube until it is clear.

5.18.6 Open Maestro software on the SciClone.

5.18.7 Open the ***Illumina RNA Access Workbook*** worksheet in Excel (shortcut is available on the desktop).

5.18.8 Enter the number of columns of samples to be processed in tabs ***4 Hyb Setup***, ***5&6 Target Capture***, and ***7 Final Post-PCR SPRI*** of the ***Illumina RNA Access Workbook***. Save the file.

5.18.9 Open the ***4 RNA Access Hyb Setup*** method in the Illumina RNA Access folder.

5.18.10 Prepare the Enrichment Hyb Solution mix according to the ***Illumina RNA Access Workbook***.

5.18.10.1 Use the tables found under the ***4 Hyb Setup*** tab.

5.18.10.2 Mix the solution well using a P1000 pipette.

Note: Prepare the Enrichment Hyb Solution immediately before use.

5.18.11 Click *Play* in the ***RNA Access Hyb Setup*** method and prepare the Sciclone deck with consumables, reagents, and samples as directed.

5.18.12 Automation will then proceed with the following:

5.18.12.1 Addition of 75 uL of Enrichment Hyb Solution to 25 uL of sample.

5.18.12.2 Pipette to mix.

5.18.13 After the hybridization setup is complete, immediately seal the plate with two Microseal ‘B’ adhesive seals. Place in the pre-heated thermal cycler.

5.18.14 Press the *Skip step* button to continue the ***RNA HYB*** thermal cycler program.

5.19 Second Capture and PCR Setup

5.19.1 Remove the following reagents from -25°C to -15°C storage and thaw on ice:

- Enhanced PCR Mix
- PCR Primer Cocktail

5.19.2 Remove the following reagents from -25°C to -15°C storage and thaw at room temperature:

- 2N NaOH
- Enrichment Elution Buffer 1
- Enrichment Wash Solution

5.19.3 Remove the following reagents from 2°C to 8°C storage and let equilibrate to room temperature:

- Elute Target Buffer 2
- Streptavidin Magnetic Beads

5.19.4 Open the Illumina RNA Access Workbook and select the 5&6 Target Capture sheet.

5.19.4.1 Update the number of columns of samples to be processed. Save the file.

5.19.4.2 Prepare the reagent mixes and reagent plates according to information in the tables.

5.19.5 Open the Maestro software on the SciClone.

5.19.6 Open the 5&6 Target Capture method in the Illumina RNA Access folder.

5.19.6.1 Select Second Capture. Press *Play* and prepare the SciClone automation deck with consumables, plates, and samples as directed.

5.19.6.2 With approximately 5 minutes remaining before the 58°C hold step of the thermal cycler hybridization program, click *Finish* on the SciClone prompt to complete preparation and begin the automated washing of beads.

5.19.6.3 When prompted by the SciClone software, immediately transfer hybridization sample plate to the magnet on deck and carefully remove the seals.

5.19.7 Automation will proceed with the following:

5.19.7.1 Transfer the contents from each well of the RAH1 plate to the corresponding well of the 96-well MIDI plate labeled RAW1 containing 250 ul of Streptavidin Magnetic Beads. Pipette to mix.

5.19.7.2 Let the RAW1 plate stand at room temperature for 25 minutes with intermittent mixing.

5.19.7.3 Place the RAW1 plate on the magnet for 2 minutes at room temperature.

5.19.7.4 Remove and discard all of the supernatant from each well of the RAW1 plate without disturbing the beads.

5.19.7.5 Remove the RAW1 plate from the magnet.

5.19.7.6 Add 150 ul of Enrichment Wash Solution to each well of the RAW1 plate.

5.19.7.7 Shake the RAW1 plate and pipette to mix.

5.19.7.8 Transfer the entire contents of the RAW1 plate to a new Hard Shell BioRad plate. Pipette to mix.

5.19.7.9 Place the BioRad plate on the magnetic stand for 2 minutes.

5.19.7.10 Remove and discard all of the supernatant from each well of the BioRad plate.

5.19.7.11 Aspirate 150 ul of Enrichment Wash Solution and dispense 30 ul of the solution to each well of the BioRad plate.

5.19.7.12 Move BioRad plate off the magnet to position A4. Dispense the remaining solution. Pipette to mix.

5.19.7.13 Incubate at 50°C for 20 minutes.

5.19.7.14 Place the plate on the magnet for 2 minutes.

5.19.7.15 Immediately remove and discard the supernatant from each well of the RAW1 plate.

5.19.7.16 Remove the plate from the magnet.

5.19.7.17 Repeat steps 7.17.6.8 through 7.17.6.14 one time for a total of two Enrichment Wash Solution washes.

5.19.8 When prompted, place plate containing Elution Prep Mix, Elute Target Buffer 2, Enhanced PCR mix, and PCR Primer Cocktail on deck as instructed.

5.19.9 Prepare the thermal cycler for the PCR with the following program and save as ***EPM AMP***.

- Choose the pre-heat lid option and set to 100°C
- Preheat to 98°C
- 98°C for 30 seconds
- 10 cycles of:
  - 98°C for 10 seconds
  - 60°C for 30 seconds
  - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 10°C

5.19.10 Automation will proceed with the following:

5.19.10.1 Add 23 µl of the elution mix to each well of the BioRad plate plate.

5.19.10.2 Pipette to mix.

5.19.10.3 Let the plate stand at room temperature for 2 minutes.

5.19.10.4 Place the plate on the magnet for 2 minutes.

5.19.10.5 Transfer 24 µl of clear supernatant from each well of the plate to the corresponding well of a new BioRad plate.

5.19.10.6 Add 4 µl Elute Target Buffer 2 to each well of the new BioRad plate containing samples to neutralize the elution.

5.19.10.7 Add 45 µl well-mixed AMPure XP beads to each well of the plate.

5.19.10.8 Pipette up and down to mix.

5.19.10.9 Incubate the plate at room temperature for 10 minutes.

5.19.10.10 Place the plate on the magnetic stand for 5 minutes.

5.19.10.11 Remove and discard the supernatant from each well of the plate.

5.19.10.12 While the plate is on the magnet, slowly add 150 µl freshly made 80% ethanol to each well without disturbing the beads.

5.19.10.13 Let the plate stand at room temperature for 1 minute.

5.19.10.14 Remove and discard the 80% ethanol from each well of the plate.

5.19.10.15 Repeat steps 7.18.5.12 through 7.18.5.14 one time for a total of two 80% ethanol washes.

5.19.10.16 Let the plate stand at room temperature for 7 minutes to dry.

5.19.10.17 Add 27.5 µl Resuspension Buffer to each well of the plate.

5.19.10.18 Pipette up and down to mix.

5.19.10.19 Incubate the plate at room temperature for 5 minutes.

5.19.10.20 Place the plate on the magnet for 5 minutes.

5.19.10.21 During the 5 minute incubation 20 ul of EPM and 5 ul of PPC will be broadcasted to a clean 96 well BioRad hard shell plate.

5.19.10.22 Transfer 25 µl of supernatant from each well of the plate to the corresponding well of the new PCR plate with 20 uL of EPM and 5 uL of PPC.

5.19.11 Seal the sample plate and place it in the pre-heated thermal cycler.

5.19.12 Press *Skip step* on the thermal cycler to continue with amplification.

5.20 Purify the Sample Using Agencourt AMPure XP beads on the SciClone

5.20.1 Allow AMPure XP beads and RSB to come to room temperature.

5.20.2 Mix the AMPure XP beads well so that the reagent appears homogeneous and consistent in color. Do not freeze.

5.20.3 In the ***Illumina RNA Access Workbook*** enter the number of columns of samples to be processed in the ***7 Final Post-PCR SPRI tab***. Save the file.

5.20.4 In the Maestro Software open the ***7 Final Post-PCR SPRI*** method.

5.20.4.1 Press *Start* to begin procedure.

5.20.4.2 For the wells being processed, add 90 µL of homogeneous AMPure XP beads to each well of a 96 well BioRad hard shell plate.

5.20.4.3 Place the plate onto the SciClone deck according to the deck layout.

5.20.4.4 Add the necessary volume of 80% ethanol to labware and place on the SciClone deck according to the deck layout.

5.20.4.5 Add the necessary volume of RSB to labware and place on the SciClone deck according to the deck layout.

5.20.4.6 Add tip boxes and additional labware to the SciClone deck according to the deck layout.

5.20.5 Automation will proceed with the following:

5.20.5.1 Add 90 ul AMPure beads to each well to be processed of the 96 well BioRad hard shell plate, pipette up and down to mix.

5.20.5.2 Shaker for 10 minutes.

5.20.5.3 Move hard shell plate to magnet at B4.

5.20.5.4 Incubate on the magnet for 10 minutes.

5.20.5.5 Remove and discard the supernatant.

5.20.5.6 While the plate is on the magnet, slowly add 150 µl freshly made 80% ethanol to each well without disturbing the beads.

5.20.5.7 After a 30 second delay remove and discard the ethanol.

5.20.5.8 Repeat steps 7.20.5.6 – 7.20.5.7 for a total of 2 ethanol washes.

5.20.5.9 Incubate the plate at 37°C for 5 minutes to dry the beads.

5.20.5.10 Add 32 ul RSB to each well to be processed, pipette up and down to mix.

5.20.5.11 Shaker for 3 minutes.

5.20.5.12 Move hard shell plate to the magnet at B4.

5.20.5.13 Incubate on the magnet for 5 minutes.

5.20.5.14 Transfer 30 ul of the supernatant to a clean 96 well BioRad hard shell plate.

5.20.6 When *7* ***Final Post-PCR SPRI program*** is complete, cover sample plate with lid. Place plate on ice while setting up for quality assessment.

Stopping Point - If you do not continue to the next step, store the samples at -20°C

5.21 Assess Quality of Final Library Pools Using Agilent's High Sensitivity D1000 ScreenTape Kit for the TapeStation

5.21.1 Allow High Sensitivity D1000 ScreenTapes, High Sensitivity D1000 sample buffer, and High Sensitivity D1000 ladder to equilibrate to room temperature for 30 minutes prior to use.

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

5.21.3 Flick the required number of D1000 ScreenTapes to eliminate bubbles in the separation channel. Bubbles in the separation chambers can interfere with sample loading.

5.21.4 Load a High Sensitivity D1000 ScreenTape in the processing bucket and provide tips. Load the required number of additional High Sensitivity D1000 ScreenTapes in the holding rack.

5.21.5 Vortex and spin down the ScreenTape reagents prior to use.

5.21.6 If running 15 samples or fewer, prepare 4 µl of ladder by mixing 2 µl D1000 Sample Buffer with 2 µl 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.

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

5.21.6.2 Spin the plate down.

5.21.7 Prepare 1:5 dilutions of final library pools by diluting 4 µl of each library pool with 16 µl buffer EB.

5.21.7.1 This 1:5 dilution will be used for TapeStation quality assessment as well as normalizations prior to ddPCR quantitation.

5.21.8 Dilute 2 µl of each final library pool sample with 2 µl of D1000 sample buffer and load on an Eppendorf skirted PCR plate.

5.21.8.1 Seal the plate with pierceable foil.

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

5.21.8.2.1 Spin the plate down.

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

5.21.10 Perform a region analysis for a 175 to 1000 bp region. Record the concentration and peak size of each sample in the ***PDXRNAAccesstemplate20171212*** file.

5.21.10.1 Assess library quality. Library size range should be ~200 to 1000 bp with a peak size of ~300-320 bp.

5.22 Quantify Final Library Pools Using ddPCR

5.22.1 Refer to SOP the titled PDX ddPCR Library Quantification Protocol, SOP document number MCCRD-SOP0007.

5.23 Dilute, Denature, and Cluster Final Library Pools for Sequencing

5.23.1 Refer to the SOP titled PDX Dilute Denature and Cluster Protocol, SOP document number MCCRD-SOP0008.

5.24 Sequence Final Library Pools

5.24.1 Refer to SOP titled Sequencing PDX Library Pools, SOP document number MCCRD-SOP0008 or MCCRD-SOP0010.