Non-Controlled Version **Note: Controlled Versions of this document are subject to change without notice*

Multiplex Agilent Whole Exome or Target Capture

I. Purpose

To provide specific guidelines for Multiplex Whole Exome or Target Capture

II. Scope

All procedures are applicable to the BCGSC Library TechD and the Library Core groups.

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001). Do not copy or alter this document. To obtain a copy see a QS associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Library Core and Library Tech D Group Leaders to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Systems to audit this procedure for compliance and maintain control of this procedure.

V. References

Reference Title	Reference Number
SureSelect XT2 Target Enrichment for Illumina Multiplexed Sequencing. Featuring Pre-capture indexing reagents and protocols.	Version A, January 2012

VI. Related Documents

Document Title	Document Number
Operation and Maintenance of the Agilent 2100 Bioanalyzer for DNA samples	LIBPR.0017
Quantifying DNA Samples using the Qubit Fluorometer	LIBPR.0030
JANUS G3 Normalization and Pooling of DNA Samples	LIBPR.0146

VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the material safety data sheet (MSDS) for additional information.

VIII. Materials and Equipment

Name	Supplier	Number: #	Model or Catalogue #	
Fisherbrand Textured Nitrile gloves – various sizes	Fisher	270-058-53		✓
Ice bucket – Green	Fisher	11-676-36		✓
Wet ice	In house	N/A	N/A	N/A
AB1000 96-well 200µL PCR plate	Fisher	AB1000		✓
Gilson P2 pipetman	Mandel	GF-44801		✓
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P20 pipetman	Mandel	GF23600		✓
Gilson P200 pipetman	Mandel	GF-23601		✓
Gilson P1000 pipetman	Mandel	GF-23602		✓
Diamond Filter Tips 10uL	Mandel	GF-F171203		✓
Diamond Filter Tips 30uL	Mandel	GF-F171303		✓
Diamond Filter Tips 200uL	Mandel	GF-F171503		✓
Diamond Filter Tips 1000uL	Mandel	GF-F171703		✓
Galaxy mini-centrifuge	VWR	37000-700	✓	
VX-100 Vortex Mixer	Rose Scientific	S-0100	✓	
Black ink permanent marker pen	VWR	52877-310		✓
Clear Tape Sealer	Qiagen	19570		✓
Eppendorf BenchTop Refrigerated Centrifuge 5810R	Eppendorf	5810 R	✓	
Bench Coat (Bench Protection Paper)	Fisher	12-007-186		✓
Small Autoclave waste bags 10”X15”	Fisher	01-826-4		✓
DNA Away	VWR	53509-506		✓
Mussel Glycogen (20mg)	Roche Scientific	10 901 393 001		✓
3 M Sodium Acetate	Sigma	EC 211-162-9		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	People Soft ID: 23878		✓
Peltier Thermal Cycler	MJ Research	PTC-225	✓	
P2-20 Rainin Lite Manual 12-channel	Rainin	L12-20		✓
P20-200 Rainin Lite Manual 12-channel	Rainin	L12-200		✓
P200 Barrier Rainin tips	Rainin	RT-L200F		✓
P20 Barrier Rainin tips	Rainin	RT-L10F		✓
DynaI MyOne Streptavidin T1 Beads	Invitrogen	656-01 (2 mL)		✓

COT human DNA 1 mg (1 mg/mL)	Roche	15279-101		✓
RNase Zap	Ambion	9780		✓
20x SSPE	Ambion	AM9767		✓
50x Denhardt's solution	Invitrogen	750018		✓
EDTA, 0.5 M	In house	n/a	n/a	n/a
SDS, 20%	Ambion	AM9820		✓
SUPERase-In, 20 U/uL	Ambion	AM2696		✓
Axygen PCR tubes, 200 uL, not autoclaved	Axygen	AM12225		✓
Applied Biosystems GeneAmp 9700	ABI	N8050200	✓	
Nutating Mixer	VWR	82007-202	✓	
NEBNext Ultra II Q5 Master Mix	NEB	M0544L		✓
5' IDX CAP (10 uM)	IDT	custom		✓
3' IDX CAP (10 uM)	IDT	custom		✓
Savant Speedvac Plus SC210A	Savant	SC210A	✓	
96-well Optical Reaction Plate	ABI	4306737		✓
MicroAmp Optical Adhesive Film	ABI	4311971		✓
Isotemp Heating Block	Fisher	11-715-1250	✓	
MinElute PCR Purification Kit	Qiagen	28004		✓
Aluminum Foil	VWR	60941-126		✓
SureSelectXT Custom 0.5-2.9Mb library	Agilent	5190-4817		✓
PCR 0.2mL strip tubes	VWR	80087-122		✓
PCR tube strip caps	VWR	80087-132		✓
SureSelect Human All Exon probes V6+UTR	Agilent	5190-8881		✓

IX. Procedure

1. Introduction and Upstream Set Up

Note: The Agilent Probes, (e.g. V6 + UTRs) are RNA-based therefore the appropriate precautions must be taken when handling the probe mix and other reagents that will be added to this probe mix. When setting up the following reactions, wipe down equipment with RNase Zap. Also note that the hybridization reaction has been qualified using the Applied Biosystems 9700 Thermocycler and **non autoclaved, nuclease free** Axygen 200uL PCR tubes.

The input materials for the multiplex capture are constructed libraries which carry full length Illumina primer sequences, so precaution must be taken to prevent cross-contamination!

Capture can be done on up to 12 pools in parallel.

1.1. Put on a clean pair of gloves and a lab coat. Wipe down the assigned specific workstation, pipetman, and small equipment with RNase zap. Wipe down all metal objects with 70% EtOH to prevent corrosion from RNase zap.

1.2. Place new bench coat down in your designated work area bench top.

Day 1: Library pooling and lyophilization, buffer brew preparation and hybridization.

2. Pool Genomic Libraries for Multiplex Capture

2.1. Ask your supervisor for pooling instructions. Currently, up to 24 libraries may be pooled per probe set. A minimum of 3 libraries are required for pooling. In cases where only 1 or 2 libraries are pooled for capture, supervisor will provide specific instructions.

Pool libraries using JANUS G3 according to the pooling map provided by your supervisor. Your supervisor will provide you with IX numbers for the hybrid mixture of the Multiplex Capture libraries. **A total of 1ug input library is required per capture.** Divide 1ug by the number of libraries that are being pooled for capture to determine how much library to use per library. In some cases, different total input library amount will be used, supervisor will advise in those cases.

LIBPR.0146 JANUS G3 Normalization and Pooling of DNA Samples

2.2. Mix well by vortexing, spin down.

2.3. The pool will be used for multiplex capture. Repeat the pooling process for the entire plate. Store the capture fractions at -20ºC until ready for use.

3. Reduce volume of Pooled Multiplex Libraries for Multiplex Capture

3.1. Pooled libraries (for example a pool of 24 libraries) may require reducing volume for Multiplex Capture. Samples prepared for capture are reduced to 17.5 uL by SpeedVac. Note that Agilent SureSelect XT2 manual supports the use of moderate heat < 47°C for this process. **Do not allow the sample to completely dry as this can reduce the capture efficiency of the library to the probe.**

3.2 Place tubes with lids open in the Savant SpeedVac Plus SC210A, making sure that the tubes are balanced in the machine. Close the lid. Turn the heat to medium and turn on the concentrator for 2 minutes to allow it to reach full speed. With the bleed valve open, turn on the vacuum pump and then close the bleed valve. Check that a vacuum has formed by attempting to raise the lid of the concentrator. Set a timer to count down for 15 minutes. After the 15 minute period, prepare to check the progress of the desiccation.

Open the bleed valve and turn off the pump (in that order) but keep the concentrator running until the pressure inside the concentrator has reached ambient air pressure. Test by lifting the lid slightly. When pressure has equilibrated it is safe to turn the concentrator off. For volumes above 100uL, a 30 minute time may be necessary.

3.3 Measure the volume of each sample and if extra time is required, estimate the length of additional time needed and do an additional concentration step. Some samples may have less than 17.5uL. In that case, bring the volume up to 17.5uL with nuclease free water. Re-suspend by vortexing sample for 30 seconds, give a quick spin in a microfuge and then store on ice. A white precipitate may appear due to salt in the samples. If precipitate is detected, leave the 17.5uL solution at room temperature for 20 minutes to allow precipitate to go into solution.

4. Prepare Blocking Brew and Add to Pooled Library Mixture.

4.1. Generate the Blocking Brew Mix calculator using LIMS:

LIMS: Mix Standard Solution > MXC_Blocking_Brew > follow the prompts > Save Standard Solution

Retrieve both the brew barcode and reagent check list label. Place both in your lab notebook.

4.2. On the 6th floor PPGP bench, prepare enough blocking buffer for the number of pooled reactions performed.

Table 1: Multiplex Capture Blocking Brew

Reagent	Per capture
Human Cot-1 DNA, 20 ug/uL	2.5
PE1/PE2 primer, 20 uM each	5
Total volume	**7.5**

4.3. Mix well by pipetting and store on ice.

4.4. Label non-autoclaved, nuclease-free 0.2mL PCR tubes with the IX number corresponding to your pooled libraries.

4.5. Transfer 7.5uL of Blocking Brew to the labeled 0.2mL PCR tubes; then add 17.5uL of the pooled library to the Blocking Brew (total volume should be 25ul).

4.6. Mix blocking brew and pooled library by vortexing, quick spin and then store on ice.

5. Prepare 2X Hybridization Buffer

5.1. Generate the 2X Hyb Brew Mix calculator using LIMS:

LIMS: Mix Standard Solution > MXC_2X_Hyb_Brew > follow the prompts > Save Standard Solution

Retrieve both the brew barcode and reagent check list label. Place both in your lab notebook.

5.2. On the 6th floor PPGP bench at room temperature, prepare hybridization buffer for the number of pooled reactions required (see Table 2 below). Retrieve 50x Denhardt's Solution from -20°C freezer and thaw on ice before use. Vortex each reagent and spin down before taking an aliquot. Store buffer at room temperature until use. You may need to heat buffer mixture to 65ºC for 5 minutes if you notice a precipitate forming. After heating, vortex, quick-spin and then continue to store at room temperature until needed.

Table 2: Multiplex Capture 2X Hybridization Buffer

Reagent	**Per Capture**	**Final [ ]**
20x SSPE	17.5	10x
50x Denhardt's Solution	7	10x
50 mM EDTA	7	10mM
2% SDS	3.5	0.20%
Total	**35**	

6. Prepare RNA Oligonucleotide Probe

6.1. Prepare aliquots in the 6th floor Biological Safety Cabinet after it has been sprayed down with RNase Zap. Label tubes clearly with the probe name, *eg.* Agilent V6 + UTRs, the date and your initials, and solution number. Store unused aliquots in a box labeled with the correct barcode in -80°C freezer.

6.2. Take appropriate number of aliquots out of the -80ºC freezer and immediately store it on ice. While the probe melts **on ice**, prepare Superase.In (RNase Block) dilution. Store the dilution on ice.

Table 3: Superase.In dilution

Reagent	**Per Rxn**
Superase.In, 20 U/uL	1
Nuclease free water	1
Total	**2**

6.3. After diluting Superase.In, aliquot appropriate volume (refer to table 4 below: 2uL or 5uL) into labeled, non-autoclaved, nuclease-free 0.2mL PCR tubes. Add appropriate volume of probe (refer to the note and table 4 below: 5uL or 2uL) to the 0.2mL PCR tubes containing the diluted Superase.In. Mix well by pipetting, quick spin, then **store on ice**.

Note: for Exome Capture, use 5uL of probe per capture

Capture Size	**Volume of SureSelect Probe**	**RNase Block Dilution (Parts RNase block: Parts water)**	**Volume of RNase Block Dilution to Add**
< 3.0Mb	2uL	1:9 (10%)	5uL
≥ 3.0Mb	5uL	1:3 (25%)	2uL

Table 4: Probe volume for different target sizes

7. Heat Target Libraries

7.1. Retrieve the pooled libraries/blocking brew samples from step 4.6. Run the program "Target Capture" on the 9700 Thermal cycler.

Note: The lid will heat up to 103°C before the program starts, which will take ~10mins. Allow the Thermal cycler to ramp up to 93°C, then pause, place the pooled libraries/blocking brew samples in the Thermal cycler, close the lid and press Resume.

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

Table 5: Thermocycler program - Target Capture

7.2. Set the hybridization volume to 50 uL (maximum setting for machine). The final volume will be 64 uL + 25 uL mineral oil (total volume should be 89uL).

7.3. Once the thermal cycler reaches 65°C, set a timer to count up to ensure target libraries are held at 65°C for at least 2 minutes or longer. **Make sure that target libraries are held at 65°C for at least 2 minutes - longer is okay**. This will ensure that the temperature has equilibrated to 65°C.

8. Add Hybridization Buffer to RNA Probe

8.1. Transfer 32 uL of 2X hybridization buffer held at room temperature to the 7uL SureSelect probe/Superase.In mixture from step 6.3 (total volume should be 39uL).

8.2. Mix well by pipetting. Quick spin and store at ROOM TEMPERATURE until it is used in step 9.1.

9. Hybridize Libraries to RNA Probe

9.1. Add all of the capture probe/hybridization mixture (39 uL) from step 8 to each library. Mix well by pipetting slowly 8-10 X. Note: use multichannel pipettor if needed.

9.2. Overlay with 25 uL of mineral oil to prevent evaporation. Seal the lid. Repeat for all of the pools of libraries. Double check that all lids are shut securely to prevent evaporation. **Extensive evaporation will reduce hybridization efficiency.**

9.3. Incubate multiplexed libraries for 24 hours at 65°C. Place a tape label on top of the thermal cycler to indicate that you are performing a 24-hour incubation and start your timer.

Day 2: Hybrid Capture and Capture Library Bead Clean Up

Notes: Wash Buffer #2 from Agilent kit must be pre-warmed to 65°C to ensure capture specificity. Do not use this buffer at room temperature.

The DNA is eluted from the biotinylated RNA probe by NaOH digestion of the RNA probe. NaOH is hygroscopic and readily absorbs CO_2 from the air. **The NaOH Elution Buffer should not be exposed to air** otherwise the pH may be altered and elution efficiency may decrease. Take an appropriate aliquot (650 uL for 12 pools of libraries) and immediately recap the aliquot and stock tube to avoid exposure to air. Store the aliquot at room temperature.

10. Prepare Streptavidin Beads for Exome Capture

In a heating block set at 65°C, pre-warm Wash Buffer #2. Each capture reaction requires 1.2mL of pre-warmed Wash Buffer #2. Pre-heat one aliquot of Wash Buffer # 2 for each capture reaction processed.

10.1 On the 6th floor, vigorously resuspend DynaI MyOne Streptavidin T1 magnetic beads by extensive vortexing. Once homogenous, make a 50 uL aliquot of beads for each capture reaction into a **non-stick, nuclease-free, 1.5 mL tube**. Alternatively, batch-wash as described below in Step 10.7. Close tubes and transfer aliquots to the post-PCR lab area on the 6th floor.

10.2. In the 6th floor PPGP area, wipe down pipettors, microfuge, nutator, and magnetic stand with RNase Zap and then place a fresh bench coat down.

10.3. Wash the beads by adding **200 uL of binding buffer** to the beads. Mix individual tubes by vortexing for 5 seconds. Spin down and place tubes into a magnetic separator.

10.4. Let the plate/tubes stand for 1minute (or until clear) and then remove and discard the supernatant. Remove from the magnetic separator.

10.5. Repeat steps 10.3-10.4 for **a total of 3 washes**.

10.6. Resuspend the beads in 200 uL of binding buffer. **Label tubes with the designated IX number, the date and your initials.** Let tubes stand at room temperature until use at step 11.2.

10.7. For batch processing, Streptavidin beads can be washed with the same ratio of bead:buffer. For 12 samples, 3 batches of 4 reactions can be prepared (205 uL of MyOne beads: 820 uL of binding buffer). After the three washes, resuspend in 800 uL of binding buffer and aliquot 200 uL into individual tubes. Label tubes with IX number corresponding to the pooled libraries used in the capture. Note that you may perform the capture in 0.3 mL plates with corresponding plate magnet.

11. Capture Hybridized DNA Libraries with Streptavidin Beads

11.1 Estimate volume loss due to evaporation. Total volume entering hybridization is 64uL. A loss of 10% is acceptable. If more than 20% is lost report this information in your lab notebook and notify your supervisor.

11.2. Maintain the hybridization reaction at 65ºC until you transfer the entire volume (approximately 89uL) including the mineral oil to **pre-labeled** tubes containing 200 uL

of pre-washed Streptavidin beads. Quick spin the PCR tube and transfer any remaining library to the bead mix.

11.3. Mix by pipetting slowly 3-5 times.

11.4. Cap tubes and incubate reaction at room temperature on a nutator for 30 minutes. Invert tubes 3 times at 10 minute intervals to ensure contact between the beads and the supernatant (beads may settle).

11.5. Quick spin tubes and transfer to a magnetic separator. Let stand at least 2 minutes (until the supernatant appears clear). Remove supernatant. Remove tubes from the magnet.

11.6. Resuspend beads in **200 uL of Wash Buffer #1**. Mix by vortexing then quick spin.

11.7. Return tubes to a magnetic separator. Let stand at least 1 minute or until supernatant appears clear. Remove supernatant and then remove from magnetic stand.

11.8. Resuspend beads in **200 uL of 65°C pre-warmed Wash Buffer # 2**. Cap tubes, vortex for 5 seconds and immediately incubate at 65°C for 5 minutes. **It is important to maintain 65ºC to ensure specificity of capture. WASH BUFFER #2 MUST BE PRE-WARMED.**

11.9. Return tubes to magnetic separator, let stand until supernatant clears (approximately 30 seconds) and then remove supernatant.

11.10. Repeat steps 11.8-11.9 for **a total of 6 washes**.

11.11. Remove all residual Wash Buffer #2 from the beads.

11.12. Add 50 uL of Elution Buffer. Resuspend by capping tubes and vortexing 5 seconds. Incubate at room temperature for 10 minutes. Briefly vortex after 5 minutes to assist in the elution. **DO NOT EXCEED 10 MINUTES INCUBATION IN ELUTION BUFFER.**

11.13. During the incubation, label new tubes with the designated IX number, 'Post-capture', the date and your initials. Add 50uL of neutralization buffer.

11.14. After the 10 minute incubation, quick spin the tubes and then return the tubes to the magnetic separator. Let tubes stand at 1-2 minutes (until supernatant is clear). **Transfer the supernatant to the appropriate pre-labeled tubes containing the neutralization buffer.**

11.15. Cap tubes and mix by vortexing briefly. Quick spin and proceed to Minelute column clean up, step 12.

12. MinElute Column Cleanup of Post Capture Material (PPGP area)

12.1. When the neutralization is complete, purify and concentrate using the MinElute Kit components but following the instructions below.

12.2. Pre-heat elution buffer to 65°C by placing the Qiagen elution buffer aliquot in the 65°C heat block. For each post capture PCR product, label a fresh 1.5mL tube with library name, date, your initials and "post capture". Set tubes aside until later (step 12.6).

12.3. Add 500uLof PBI to each PCR reaction and mix thoroughly.

12.4. Apply the sample to the Qiagen MinElute spin column and centrifuge at room temperature for 1 minute at 13000rpm. Discard flow-through. Place the Qiagen MinElute spin column back into the same tube.

12.5. Add 0.75mL of Buffer PE to the column and centrifuge at room temperature for 1 minute at 13000rpm. Discard flow-through and place the column back into the same tube.

12.6. Perform a second spin at room temperature for 2 minutes at 13000rpm. Use a p10 pipette to aspirate any additional ethanol trapped on the inner rim in the MinElute column. Transfer column to a clean tube (from step 12.2).

12.7. Allow the column air dry for 1 minute before adding Elution Buffer.

12.8. Add 15µL of pre-warmed EB (Qiagen) to the centre of the column and let sit for 1 minute prior to centrifugation. Centrifuge at room temperature for 1 minute at 13,000rpm to elute DNA. Check volume of flow-through before discarding column. The yield should be approximately 14µL. Ensure the microfuge tube is accurately labeled before discarding the column.

13. Post-Capture Enrichment PCR Using Flanking Universal Primers

Note: You must use CAP primers which flank the index sequences introduced prior to capture. Failure to do so will lead to the loss of index tags.

13.1. Prepare the following reaction mixture in the 5th floor Biological Safety Cabinet.

Note: Prepare 2 reactions for each sample and 1 for no template control.

Generate the Post Capture PCR Brew Mix calculator using LIMS:

LIMS: Mix Standard Solution > Agilent_Q5_PostCap_PCR_Brew > follow the prompts > Save Standard Solution

Retrieve both the brew barcode and reagent check list label. Place both in your lab notebook.

Table 5: Multiplex Capture Post Cap PCR Brew

Reagent	Per reaction (uL)	# of reactions	Volume (uL)
2X Q5 Master Mix	12.5	13	162.5
5' IDX CAP (10uM)	1	13	13
3' IDX CAP (10uM)	1	13	13
Nuclease free water	7	13	91

DNA template: 3.5uL

13.2. Retrieve and thaw at room temperature Q5 Master Mix and 5' and 3' IX Cap primers. Once thawed, place all reagents on ice.

13.3. Prepare PCR brew in a 1.5mL tube according to the printed calculator label. Mix each reagent before addition to the brew. Mix the brew very well by gentle repeated pulse-vortexing.

13.4. In the 5th floor BSC aliquot 21.5 uL brew into labeled 0.2ml PCR tubes or 0.2mL PCR strip tubes. Include a "no template" control with 3.5 uL of nuclease free water. Place tubes on ice and transport them to the 6th floor.

13.5. Add 3.5 uL of enriched post-capture template from step 12.8 to corresponding tubes in the 6th floor BSC. Quick spin the tubes. Store the remaining 7 uL of post capture template in the appropriate post PCR template box at -20°C.

13.6. Run the TSPET10 program described below on the MJR Tetrad. If using a plate use a rubber pad on top of the plate. Note: Sometimes different number of PCR cycles will be needed, supervisor will advise in those cases.

TSPET10 PCR Parameters:

1. 98˚C 1 min
2. 98˚C 15 sec
3. 65˚C 30 sec
4. 72˚C 30 sec
   Go to step 2, 9 more times.
5. 72˚C 5min
6. 4˚C ∞

13.7. When the protocol is complete, you may proceed to Step 14 MinElute column cleanup or store the PCR product at 4°C over night. A post-PCR gel purification step is not required.

14. MinElute Column Cleanup of Post PCR Material (PPGP area)

14.1. When the amplification is complete, purify and concentrate using the MinElute Kit components by following the instructions below.

14.2. Pre-heat elution buffer to 65°C by placing the Qiagen elution buffer aliquot in the 65°C heat block. For each post capture PCR product, label a fresh 1.5mL tube with library name, date, your initials and the # of cycles. Set tubes aside until later (step 14.6).

14.3. Add 125µL of PBI to each PCR reaction and mix thoroughly.

14.4. Apply the sample to the Qiagen MinElute spin column and centrifuge at room temperature for 1 minute at 13,000rpm. Discard flow-through. Place the Qiagen MinElute spin column back into the same tube.

14.5. Add 0.75mL of Buffer PE to the column and centrifuge at room temperature for 1 minute at 13,000rpm. Discard flow-through and place the column back into the same tube.

14.6. Perform a second spin at room temperature for 2 minutes at 13,000rpm. Use a p10 pipette to aspirate any additional ethanol trapped on the inner rim in the MinElute column. Transfer column to a clean tube (from step 14.2).

14.7. Allow the column air dry for 1 minute before adding Elution Buffer.

14.8. Add 13µL of pre-warmed EB (Qiagen) to the centre of the column and let sit for 1 minute prior to centrifugation. Centrifuge at room temperature for 1 minute at 13,000rpm to elute DNA. Check volume of flow-through before discarding column.

The yield should be approximately 12µL. Ensure the microfuge tube is accurately labeled before discarding the column.

15. Agilent and Qubit QC

15.1. Run 1uL of the enriched, bead-cleaned libraries on an Agilent DNA High Sensitivity assay according to protocol:

LIBPR.0017 Operation and Maintenance of the Agilent 2100 Bioanalyzer for DNA Samples

Confirm that the amplified product is in the 220-850 bp range and that there are no primer or adapter peaks visible in the Agilent traces. Consult with your supervisor to determine if the profiles are acceptable for library submission. Refer to the figure below for expected results.

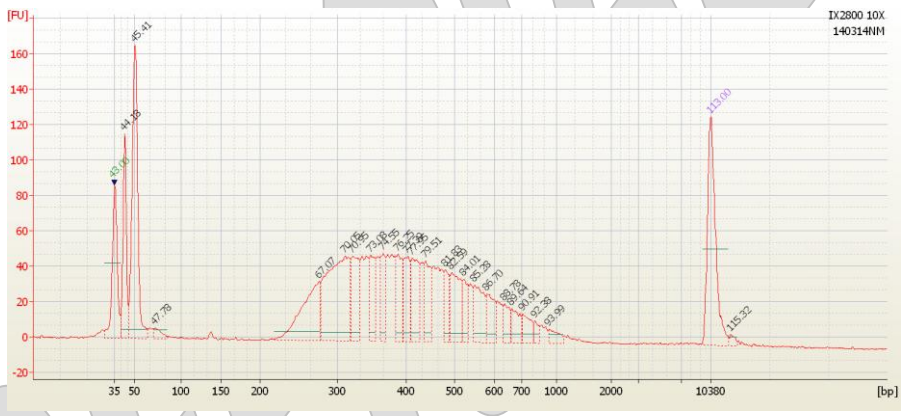

Figure 1: Post-capture PCR QC on Agilent

15.2. Quantify library DNA concentrations by Qubit Fluorometer according to protocol:

LIBPR.0030 Quantifying DNA Samples using the Qubit Fluorometer

Consult with your supervisor to determine if the library concentration is above the minimum submission concentration.

16. Dilution and Sample Submission

16.1. Use the stock values from step 15.2 to determine if dilution is required. The maximum submission concentration is 60nM. If dilution is required, re-quant the diluted sample by Qubit. Submit sample(s) for sequencing.

Appendix A- LIMS tracking

1. Samples rearray – IDX pipeline, 1.5mL tube

2. MXC

3. MXC_Post_Hyb_Clean_Up – IDX pipeline

4. MXC_Post_Cap_PCR – IDX pipeline

5. Bioanalyzer Run – Post Library Construction Size Selection QC
   Enter the following attributes:

   - DNA_concentration_ng_uL
   - Library_size_distribution_bp
   - Avg_DNA_bp_size

6. Final_Submission – IPE pipeline