This is a non-controlled version.
**Note: controlled versions of this document are subject to change without notice.*

Nimbus-assisted 96-well PCR-enriched Library Construction for Illumina Sequencing v2

I. Purpose

To provide specific guidelines for 96-well PCR-enriched library construction for Illumina Paired-End Sequencing.

II. Scope

All procedures are applicable to the BCGSC Library Core and Library TechD 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 Quality Systems associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Group Leader 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
Sample Preparation for Paired-End Sample Prep Kit from Illumina	Version 1.1 (from Prep Kit)

VI. Related Documents

Document Title	Document Number
Automated DNA Quantification using the dsDNA Quant-iT High Sensitivity Assay Kit and VICTOR3V/VICTOR X3	LIBPR.0108
Operation of the Covaris LE220	LIBPR.0097
Operation and Maintenance of the Agilent 2100 Bioanalyzer for DNA samples	LIBPR.0017
Operation and Maintenance of the Caliper Labchip GX for DNA samples using the High Sensitivity Assay	LIBPR.0051

Document Title	Document Number
Quantifying DNA samples using the Qubit 4 Fluorometer	LIBPR.0153
JANUS G3 Normalization and Pooling of DNA Samples	LIBPR.0146
Normalization of Nucleic Acid Concentration using the JANUS Automated Workstation	LIBPR.0113

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 safety data sheet (SDS) for additional information.

VIII. Materials and Equipment

Name	Supplier	Number: #	Model or Catalogue #	
NEB Paired-End Sample Prep Premix Kit – End Repair	NEB	E6875B-GSC		✓
NEB Paired-End Sample Prep Premix Kit – A Tail	NEB	E6876B-GSC		✓
NEB Paired-End Sample Prep Premix Kit – Ligation	NEB	E6877B-GSC		✓
NEBNext Ultra II Q5 Master Mix	NEB	M0544L		✓
Fisherbrand Textured Nitrile gloves – various sizes	Fisher	270-058-53		✓
Ice bucket	Fisher	11-676-36		✓
Wet ice	In house	N/A	N/A	N/A
Covaris E220e	Covaris	E220e	✓	
Covaris LE220 with WCS and Chiller	Covaris	LE220	✓	
DNA *AWAY*	Molecular BioProducts	21-236-28		✓
AB1000 Plates	Thermo Scientific	SP-5201/150		✓
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 DFL10	Mandel Scientific	GF -F171203		✓
Diamond Filter tips DF30	Mandel Scientific	GF-F171303		✓
Diamond Filter tips DF200	Mandel Scientific	GF-F171503		✓
Diamond Filter tips DF1000	Mandel Scientific	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		✓
Aluminum Foils seals	VWR	60941-126		✓
Aluminum foil tape, 3"x 60 yds	Scotch/3M	34000740		✓
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		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	CommercialAlcohols	00023878		✓
IKA Works Vortexer	Agilent	MS2S9-5065-4428	✓	
22R Microfuge Centrifuge	Beckman	22R Centrifuge	✓	

Name	Supplier	Number: #	Model or Catalogue #	
PCRMax Alpha Cycler 4	Froggabio/Cole Parmer	AC496	✓	
Microlab NIMBUS	NIMBUS	Hamilton	✓	
Eppendorf Benchtop Centrifuge	Eppendorf	5810 R	✓	
70% Ethanol	In house	N/A	N/A	N/A
Qiagen Buffer EB – 250 mL	Qiagen	19086		✓
UltraPure Distilled Water	Invitrogen	10977-023		✓
TruSeqV3 (Short)	IDT		Custom, see sequence below	N/A
TruSeqV3 (Short) with UMIs	IDT	N/A	xGen Duplex Seq Adapter—Tech Access	N/A
IDT Dual Index Plate (primers)	IDT	N/A	N/A	N/A
AmpErase Uracil N-Glycosylase	Applied Biosystems	N8080096		✓
96 Low Profile Reservoir, Pyramid bottom	Thomas Scientific	1149J14		✓
Ampure XP Beads, 450 mL	Agencourt	A63882		✓
PCR Clean DX (ALINE beads)	ALINE Biosciences	C-1003-450		✓
USER Enzyme	NEB	M5505L		✓
MagMax express 96 Deep Well plates	Applied Biosystems	4388476		✓
ABgene Storage Plate 96-well, 1.2 mL square well, U-bottomed	Thermo Scientific	AB1127		✓
Adhesive foil -96 ONE TAB NS CS100).	VWR	60941-126		✓
ALPS 50V Microplate Heat Sealer	Thermo Scientific	AB-1443	✓	
EZPierce 20 um Thermal foil	ThermoFisher	AB1720		✓

TruSeqV3 (Short) /5Phos/GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
*** is a phosphorothioate bond**

TruSeqV3 (Short) with UMIs are pre-made adapters by adding 3-4bp UMIs at both ligation ends to the TruSeqV3 (Short) —Tech Access (no cat#).

IX. Introduction and Guidelines

1. General Guidelines

1.1. Ensure proper personal protective equipment is used when handling sample plates, reagents and equipment. Treat everything with clean PCR techniques.

1.2. Wipe down the assigned workstation, pipettes, tip boxes, and small equipment with DNA *AWAY*. Ensure you have a clean working surface before you start.

1.3. Pre-PCR and Post-PCR work should be performed on the 5th Floor and 6th floor respectively.

1.4. Acronyms: NA stands for Not Applicable. Pre-LC refers to Pre-Library Construction. Post-LC refers to Post-Library Construction. BC refers to Bead Clean.

1.5. Colour code: red fonts designate exceptions or protocol-specific steps.

1.6. Discuss with the APC/PC/designated trainer the results of every QC step. Report and record equipment failures and/or malfunctions and variations in reaction well volumes.

2. General Plate Guidelines

2.1. To avoid cross-well contamination, reaction plates should never be vortexed and plate seals should never be re-used. Use Nimbus for mixing.

2.2. Use only VWR foil seals for both short term storage and tetrad incubations, Adhesive foil EZPierce 20µM Thermal Foil (Cat. No. AB1720) for UNG digestion/PCR, and 3M aluminum foil seal for long term storage.

2.3. After completion of every incubation step, quick spin the plate(s) at 4ºC for 1 minute at 2000g.

2.4. Sample plates can be stored at -20ºC overnight after every step except post "A" addition. **"A" addition and adapter ligation reactions must be performed on the same day.**

2.5. The reaction plates should be placed on ice throughout the day when not being worked on.

3. Positive and Negative Controls

3.1. The positive control template to be used for this protocol is HL60 genomic DNA or UHR cDNA. The yield of library products constructed from positive controls is expected to differ from those of collaborators' samples. However, the yield should not differ significantly from that of previously constructed positive controls.

3.2. The negative control template to be used for this protocol is Qiagen Elution Buffer. This control will ensure the absence of background products that result from the library construction process.

3.3. The PCR negative control to be used for this protocol is Ultrapure water.

4. General Brew Preparation Guidelines

4.1. Double check the QA release and expiry date of each reagent and enzyme.

4.2. Thaw required reagents and place them on ice. Enzymes should be left in the freezer until ready to use. Each premix tube can be freeze thawed three times.

4.3. Reagents and enzymes should be well mixed, the former by pulse-vortexing and the latter by gentle flicking. After mixing, quick spin down in a mini-centrifuge.

4.4. All premixed and prepared brews should be well mixed by gentle, repeated pulse-vortexing to ensure equal distribution of all components and thus uniformity of enzymatic reactions across a plate. The end-repair and ligation brews are particularly viscous.

5. Nimbus Handling Guidelines

5.1. The Nimbus adds DNA/cDNA to the brew plate and it is therefore crucial that the required brew volume is accurately pre-dispensed by the technician (there should not be any dead volume). However, a dead volume is required for the indexing primer plate (5µL/well).

5.2. The dead volume required by the Nimbus in the 96-well reservoir is 25mL.

5.3. Confirm that the plate and tip box locations on the Nimbus deck match the software deck layout on the computer screen.

5.4. Ensure that plate seals are removed before starting the Nimbus program.

6. General notes on Nimbus programs

Note: If you are unsure of which Nimbus protocol version to use, please consult your supervisor.

The following steps are generally followed:

A. Start Hamilton Run control

B. Open File/Production/LibraryConstruction/LibraryConstruction-Scheduler.wfl
*Note that file type must have the.wfl extension.

C. Select the correct starting material/library type: ss-cDNA, ChIP DNA etc

The Nimbus bead cleanup modules employed in this SOP are based on the following conditions:

Bead Binding Time (mins)	1st Magnet Clearing Time (mins)	2 X 70% EtOH Wash Vol (µL)	Ethanol Air-dry Time (mins)	Elution Volume (µL)	Elution time (mins)	2nd Magnet Clearing time (mins)
15	7	150	5	10-52	3	2

Notes: Bead to reaction ratio is 1.8:1 for pre-ligation purifications and 1:1 for post-ligation steps. Ethanol and beads must be warmed to room temperature for at least 30 minutes prior to use.

X. Procedure

Note: ALINE beads (PCR Clean DX) and Ampure XP beads can be used interchangeably in the magnetic bead clean up steps.

This SOP is applicable to the following pipelines:

Pipeline	Radio Button	Input amount (ng)
Small Gap Capture	Small Gap v2	500-1000
ChIP	ChIP v2	n/a
Strand Specific RNA Seq	ss-cDNA v2	n/a
Ribodepletion	ss-cDNA v2	n/a
Small Gap Genome Low Input	Low Input SG v2	1-100

1. Initial QC

1.1. For each gDNA 96-well stock plate, quantify according to the following SOP:

LIBPR.0108 Automated DNA Quantification using the dsDNA Quant-iT High Sensitivity Assay Kit and VICTOR3V/VICTOR X3

Note: this does not apply to ChIP DNA, ss-cDNA, or if the libraries have previously been quantified.

1.2. Normalize input as directed by your supervisor and according to the following SOP:

LIBPR.0113 Normalization of Nucleic Acid Concentration using the JANUS Automated Workstation

2. Shearing (applicable to all pipelines EXCEPT ChIP)

A. To transfer DNA/cDNA into the Covaris plate, log into the following Nimbus program:

Nimbus: File > Production> LibraryConstruction> Library Construction-Schedulerv.wfl >*Protocol X >**Shearing Setup**

*Select protocol type: e.g. ss-cDNA, Small Gap

B. Refer to the following SOP for shearing:

LIBPR.0097 Operation of the Covaris LE220

Make sure that you have performed the shearing twice with a spin in between according to the SOP above.

3. Agilent HS DNA QC after shearing – Spot Check

3.1. For each 96-well plate of sheared samples, use 1µL from 11 random samples (ensure that at least two of these samples are a positive and negative control) to spot check on a High Sensitivity DNA Agilent Assay. Refer to the SOP below. QC after shearing is not needed for ss-cDNA samples.

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

3.2. The average size from the sheared material for small gap is 250bp and for small gap low input is 300bp. Send the results to the APC for approval.

4. Transferring DNA/cDNA out of Covaris Plate

4.1. To transfer DNA/cDNA from Covaris plate to reaction plate (i.e. 96-well AB1000 plate), log into the following Nimbus program:

Nimbus: File > Production> LibraryConstruction > Library Construction-Scheduler.wfl > Protocol X > **Transfer out of Covaris**

4.2. Visually inspect the source and destination wells to ensure that all of the sheared material has been transferred. Repeat the transfer out of Covaris procedure if template is remaining in the Covaris tubes.

5. Post-shearing cleanup (applicable to Small Gap Capture pipeline only)

A. For ss-cDNA or ChIP DNA, there is no cleanup, therefore, proceed to End Repair.

B. For small gap, log into the following Nimbus program:

Nimbus: File > Production> LibraryConstruction > Library Construction-Schedulerv.wfl >Small Gap v2 >**Bead clean sheared DNA**

5.1. This is a safe stopping point. Samples can be stored at -20ºC until continuing library construction.

6. Post-shearing size selection (applicable to Low Input Small Gap pipeline only)

6.1. Libraries are size selected after shearing to enrich for 300bp targets and to normalize the fragment size across a plate of samples.

6.2. Large fragments are first excluded by a low ratio of beads to sample and then the supernatant containing the smaller fragments are transferred to a new plate. Additional beads are added to the supernatant and the fragments of interest are captured by the beads. The size selected fragments are eluted after two ethanol washes.

6.3. Dispense reagents according to the plate layout.

6.4. Log into the Nimbus and select the pipeline-specific Size Selection method:

NIMBUS: File> Production> LibraryConstruction > Library Construction-Scheduler.wfl> Low Input SG v2 > **Size Select Sheared DNA**

Upper Cut

Sonication DNA (µL)	Beads (µL)	80% Bead Mix (µL)	Supernatant (µL)
62.5	40	82	102.5

Lower Cut

Supernatant (µL)	Beads (µL)	80% Bead Mix Volume (µL)	Supernatant Volume (µL)	EB Elution Volume (µL)	Transfer Volume (µL)
102.5	20	98	122.5	37	35

6.5. This is a safe stopping point. Samples can be stored at -20ºC until continuing library construction.

7. End-Repair and Phosphorylation Reaction

7.1. The volume requirement for 1 reaction set up is as follows:

Solution	1 rxn (µL)
DNA	35
End Repair Premix	23.5
Reaction volume	**58.5**

7.2. Log into the Nimbus program as follows:

Nimbus: File > Production> LibraryConstruction > Library Construction-Schedulerv.wfl> Protocol X >**End Repair**

7.3. The brew plate is the "REACTION" and the DNA plate is the "DNA Sample." After Nimbus program completion, seal the plates and quick spin at 4ºC for 1 minute. Inspect the reaction plates for any variations in volume.

7.4. Incubate End-Repair reaction plate at 20ºC for 30 minutes. The total reaction volume is 58.5µL.

Tetrad Program: Run > LIBCOR > ER

Enter '58' for reaction volume.

8. Magnetic Bead Clean Up after End-Repair

8.1. Log into the following Nimbus program:

Nimbus: File > Production> LibraryConstruction > Library Construction-Schedulerv.wfl> Protocol X > **Bead Clean E.R.**

Note: Bead Clean E.R. ratio is 1.8:1 bead: sample ratio except for the low input small gap pipeline which uses a 1:1 bead: sample ratio.

End-repaired product can be stored at -20°C after the bead cleanup.

9. Addition of an 'A' Base (A-Tailing) Reaction

9.1. The volume requirement for 1 reaction set up is as follows:

Solution	1 rxn (µL)
End-Repair + BC DNA	15
Adenylation Brew	10
Reaction volume	**25**

9.2. Log into the following Nimbus program:

Nimbus: File > Production> LibraryConstruction > Library Construction-Schedulerv.wfl> Protocol X > **A-tailing**

9.3. The brew plate is the "REACTION" and the DNA plate is the "DNA Sample." After Nimbus program completion, seal the plates and quick spin at 4°C for 1 minute. Inspect the reaction plates for any variations in volume.

9.4. Incubate A-tailed reaction plate at 37°C for 30 minutes; 70°C for 5 minutes; 4°C for 5 minutes, hold at 4°C. Enter '25' for reaction volume.

Tetrad Program: Run > LIBCOR > ATAIL

9.5. After the incubation, store the template temporarily on ice. This is NOT a safe stopping point. Quick spin plate and store on ice while setting up the ligation reaction.

9.6. **Adenylated products are not bead cleaned prior to ligation.**

10. Illumina Adapter Ligation Reaction

10.1. Thaw the adapter stock aliquot in the laminar flow hood and immediately place on ice.

Note: For ChIP pipeline, use TSV3 (Short) UMI adapter; for all other pipelines, use TSV3 (Short) adapter.

10.2. For 5th floor set up: adapter ligation brew (minus the adapter) must be made in the PCR Clean Room laminar flow hood on the 5th floor (room 510). Addition of adapter to the brew must be done in the Blood Room laminar flow hood.

For 6th floor set up: adapter ligation brew (minus the adapter) must be made in the laminar flow hood. Addition of adapter to the brew must be done on the bench.

10.3. The volume requirement for 1 reaction set up is as follows:

Note: TSV3 (Short) or TSV3 (Short) UMI adapter (Y) and water (X) volumes vary depending on the pipeline.

Solution	**1 rxn (µL)**
Adenylated template	25
2X Ligation Premix	10.5
dH_2O	X
Adapter (10µM)	Y
Reaction volume	**37.5-39.5**

10.4. Ligation calculator for the pipelines:

Pipeline	**Ligation Calculator**
Small Gap Capture	0.5X_Ligation_Brew_40pmol
ChIP	0.5X_Ligation_Brew_4pmol
ss-cDNA	0.5X_Ligation_Brew_4pmol
Small Gap Genome Low Input	0.5X_Ligation_Brew_10pmol

10.5. Generate the Ligation-Brew Mix calculator using LIMS:

LIMS: Mix Standard Solutions > *X > *follow the prompts*> Save Standard Solution

***X**= 0.5X_Ligation_Brew_4pmol,
0.5X_Ligation_Brew_10pmol,
or 0.5X_Ligation_Brew_40pmol

To minimize adapter-adapter ligation, work quickly on ice and proceed as follows:

10.5.1. Prepare the ligation brew in an appropriate sized tube according to the chemistry calculator.

10.5.2. Add the adapter to the brew last, not more than 10 min before brew addition on Nimbus. Make sure the brew is on ice at all times.

10.5.3. Dispense the appropriate amount (12.5µL for ss-cDNA, small gap low input and ChIP; 14.5µL for small gap Capture) of brew into an AB1000 plate.

10.5.4. Cover the brew plate with plate seal and quick spin at 4ºC for 1 minute.

10.5.5. Keep plates on ice but *proceed quickly* to the next step.

10.5.6. Log into the following Nimbus program:

Nimbus: File > Production> LibraryConstruction > Library Construction-Schedulerv.wfl> Protocol X > **Adapter Ligation**

10.6. The brew plate is the "REACTION" and the DNA plate is the "DNA Sample." After Nimbus program completion, seal the plate and quick spin at 4ºC for 1 minute. Inspect the reaction plate for any variations in volume. Incubate the reaction plate at 20ºC for 15 minutes. Enter '38' or '40' for reaction volume. Set a timer for 15 minutes. As soon as the ligation reaction has completed, quick spin the plate and store on ice while preparing the Nimbus for post ligation bead clean up. **Bead clean up must occur immediately after ligation.**

Tetrad Program: LIBCOR> LIGATION

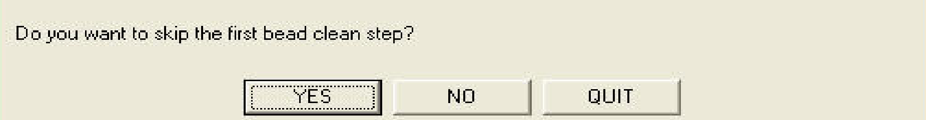
11. Magnetic Bead Clean Up after Adapter Ligation

11.1. The input volume for this step is 37.5-39.5µL per well.

11.2. Log into the following Nimbus program:

Nimbus: File > Production> LibraryConstruction > Library Construction-Schedulerv.wfl> Protocol X > **Bead clean Ligation (2x)**

11.3. Post-ligation bead cleanup is performed twice for all protocols and a safe stopping point is after the first bead clean. A prompt will appear asking "Do you want to skip the first bead clean? Yes, No or Quit". If you want to proceed to the first bead clean and pause, select "No". If you have already finished one round of bead clean and are continuing, select "Yes" (see Figure below).


Do you want to skip the first bead clean step?

11.4. Note that template will be eluted in 10µL of EB for subsequent full template iPCR.

12. Indexed PCR (iPCR) Amplification Reaction Or "USER Digestion with PCR" for ss-cDNA

12.1. Thaw the Indexing Primer Plate **(IDT Dual Index Primers)** in a working bench across from Nimbus on the 5th floor or in the 6th floor Library Construction Room, quick spin at 4ºC for 1 minute and immediately place on ice.

12.2. To keep track of freeze-thaw cycles, mark off the indexing primer plate each time the plate is thawed even if it is not used.

12.3. The maximum freeze-thaw cycles for the indexing primer plate are **5 times.**

12.4. Ensure there is enough volume including the Nimbus dead volume. Inspect the thawed index primer plate after spin down to ensure there are no cracked wells.

12.5. iPCR brew (minus the primers) must be made in the PCR Clean Room laminar flow hood on the 5th floor (room 510) or the hood in the Library Construction Room. Addition of the Indexing Primer is performed by the Nimbus.

12.6. The volume requirement for 1 reaction set up for ChIP, Small Gap and Low Input is as follows:

Solution	1 rxn (µL)
Adapter Ligated + BC DNA (Full Template)	10
2X Q5 Master Mix	12.5
IDT Dual Index Primers (20µM)	2.5
Reaction volume	**25**

12.7. The volume requirement for 1 reaction set up for ss-cDNA is as follows:

Solution	1 rxn (µL)
Adapter Ligated + BC DNA (Full Template)	10
2X Q5 Master Mix	12.5

USER	1.5
IDT Dual Index Primers (20 µM)	2.5
Reaction volume	**26.5**

PCR Brew + USER
(14µL)

12.8. Generate the PCR Brew Mix calculator using LIMS:

LIMS: Mix Standard Solutions > **0.5X_Q5_Indexing_PCR + USER Brew** > *follow the prompts* > Save Standard Solution

12.9. Obtain the 1D large Solution/Box/Kit Label and Chemistry Label. Prepare the brew in an appropriate sized tube according to the chemistry calculator. The indexing primers will be added to the DNA Plate using the Nimbus.

12.10. Dispense 12.5µL of 2X Q5 Master Mix (for ChIP and small gap) or 14µL PCR+USER brew (for ss-cDNA) into an AB1000 plate. Cover with plate seal and quick spin at 4°C for 1 minute.

12.11. Log into the following Nimbus program:

Nimbus: File > Production> LibraryConstruction > Library Construction-Schedulerv.wfl> Protocol X >**Index PCR**

12.12. Before starting the program, remove EB contents from the designated PCR Brew control well from the post BC ligation plate and replace it with 10µL of water.

12.13. The Nimbus program for iPCR setup for is as follows:

12.13.1. Addition of index primers to the DNA Source Plate (post BC ligation plate)

12.13.2. Transfer of DNA+ index primer to the brew plate.

12.14. After Nimbus program completion, seal the plate and quick spin at 4ºC for 1 minute. Inspect the reaction plate for any variations in volume.

*Heat seal the plate using Adhesive foil EZPierce 20µm Thermal foil (Cat. No. AB1720, Thermo Fisher). The equipment used for this is ALPS 50V Microplate Heat Sealer (Cat. No. AB-1443, Thermo Scientific). Please see Appendix B for instructions.

12.15. Run PCR program specified in the table below. Use a rubber pad on top of the reaction plate. For ss-cDNA libraries, you Supervisor will let you know the number of cycles to use.

PCR parameters for ss-cDNA:
(Your Supervisor will let you know the number of cycles to use)

- 37˚C 15 min
- 98˚C 1 min
- 98˚C 15 sec
- 65˚C 30 sec
- 72˚C 30 sec
- 72˚C 5min
- 4˚C ∞

*Total of 10, 11, 13 or 15 Cycles

PCR parameters for others:

- 98˚C 1 min
- 98˚C 15 sec
- 65˚C 30 sec
- 72˚C 30 sec
- 72˚C 5min
- 4˚C ∞

*# of cycles depends on pipeline

*The number of PCR cycles is dependent on each of the protocol:

Starting Material	**PCR cycles**	**Tetrad Program**
ss-cDNA (poly-A)	10	SSCDNA10
ss-cDNA (RBD; >50ng RNA input)	11 or 13	SSCDNA11 or SSCDNA13
ss-cDNA (RBD; ≤50ng RNA input)	15	SSCDNA15
Small gap Capture	6	LCPCR-6
100 ng Low Input Small gap	6	LCPCR-6
20 ng Low Input Small gap	8	LCPCR-8
5 ng Low Input Small gap	10	LCPCR-10
1 ng Low Input Small gap	12	LCPCR-12
ChIP (100k cells Native ChIP – except H3K27ac)	8	LCPCR-8
ChIP (100k cells Native ChIP – H3K27ac)	10	LCPCR-10
ChIP (Crosslinked ChIP)	13	LCPCR-13

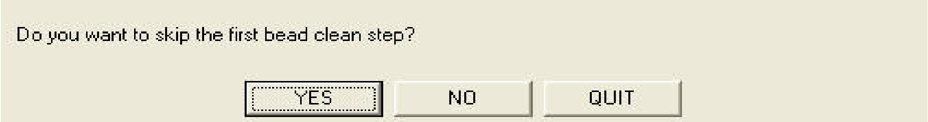
13. Post-LC Size Selection (2x 1:1)

13.1. The input volume for this step is 25µL per well.

13.2. Log into the following Nimbus program:

<ins>Nimbus</ins>: File > Production> LibraryConstruction > Library Construction-Schedulerv.wfl> Protocol X > **Bead clean iPCR (2x)**

13.3. Post-iPCR bead cleanup is performed twice for all protocols and a safe stopping point is after the first bead clean. A prompt will appear asking “Do you want to skip the first bead clean? Yes, No or Quit”. If you want to proceed to the first bead clean and pause, select “No”. If you have already finished one round of bead clean and are continuing, select “Yes” (see Figure below).



Do you want to skip the first bead clean step?

13.4. The final elution volume is 25µL.

14. Preparation of Diluted Library QC Plate

14.1. Prepare a 10x dilution QC plate using the following Nimbus program:

<ins>Nimbus</ins>: File > Production> LibraryConstruction > Library Construction-Schedulerv.wfl> Protocol X > **Dilute for QC**

The Nimbus will transfer 18µL of Qiagen EB to a new plate and then transfer 2µL of final library product to the EB plate. This 10x dilution will be used first for Quant-iT (2 µL) and the remaining 18µL will subsequently be used for Caliper.

15. Quant-iT/Qubit QC

15.1. Refer to the following SOPs for setting up the QC plate prior to normalization/pooling:

LIBPR.0108 Automated DNA Quantification using the dsDNA Quant-iT High Sensitivity Assay Kit and VICTOR3V/VICTOR X3
or
LIBPR.0153 Quantifying DNA Samples using the Qubit 4 Fluorometer

15.2. For Quant-iT, use the 10x dilution plate or undiluted library as source plates for the QC. Log into the following Nimbus program:

Nimbus: File > Production> LibraryConstruction > Library Construction-Schedulerv.wfl> Protocol X> **Quant-It**

15.3. For Qubit, use the undiluted DNA from post-library construction size selection.

16. Final HS Caliper QC or DNA1000 Agilent QC

For Caliper QC, run the 10x dilution QC plate on the Caliper GX according to the following SOP:

LIBPR.0051 Operation and maintenance of the Caliper LabChip GX for DNA Samples using the High Sensitivity Assay

16.1. For Agilent DNA1000 QC, run the undiluted DNA from post-library construction size selection according to the following SOP:

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

Calculate the nM quants using the average bp size from Caliper or Agilent and the concentration from Qubit or Quant-iT. Send the results to the APC for approval.

17. Normalization on JANUS G3 (if necessary)

17.1. Refer to the following SOP for normalization on JANUS G3:

LIBPR.0146 JANUS G3 Normalization and Pooling of DNA Samples

18. Pooling Samples into 1.5mL Tubes on JANUS G3 (if needed) or Rearray Unpooled Samples into 1.5mL Tubes

18.1. Refer to the following SOP for pooling on JANUS G3:

LIBPR.0146 JANUS G3 Normalization and Pooling of DNA Samples

19. Qubit QC on Pooled Samples Samples for submission

19.1. Refer to the following SOP:

LIBPR.0153 Quantifying DNA Samples using the Qubit 4 Fluorometer

20. Sequencing Submission

20.1. For each library or pooled libraries, determine the corrected final molar concentration for submission to sequencing. Use the average base pair size previously obtained from the Caliper HS DNA or Agilent DNA1000 profile and the results from the Qubit to obtain the final size-corrected nM quant. Minimum and maximum concentrations and volumes will vary by library type. The APC will confirm whether acceptable range for submission.

Appendix A: LIMS Protocol

1. Start of Plate Library Construction (Skip if doing ss-cDNA)

2. Bioanalyzer Run or Caliper Run – QC Category: Sonication QC (Skip if doing ChIP or ss-cDNA)

3. A-Library Construction – IDX pipeline

4. Plate_Indexed_PCR- IDX pipeline

5. Plate_PPBC_SizeSelection – IDX pipeline

6. Bioanalyzer Run or Caliper Run – QC Category: Post library construction size selection

   Note: For libraries going into multiplex capture, please select "Post-PCR QC" as the QC category instead. No need to enter attributes and please skip remaining steps.

   Enter the following attributes:

   - Library_size_distribution_bp (From Agilent or Caliper)
   - Avg_DNA_bp_size (From Agilent or Caliper)
   - DNA_concentration_ng_µL (From Quant-iT or Qubit)

7. If Pooling or normalizing: Action: Aliquot pooling volume into a new TRA

8. If Pooling: Pooling and/or Manual Rearray into tubes – IPE pipeline

9. Final_Submission – DITP pipeline (pooled)

Appendix B: ALPS 50V Microplate Heat Sealer

NOTE: The seals should be stored in the foil seal packaging to maintain proper orientation. Failure to orient the foil seal with the adhesive side down in the plate sealer will result in the seal adhering to the instrument rather than the plate.

1. Turn on the ALPS 50V heat sealer and allow the instrument to warm up. The Heat on/off LED will flash during this time and stay on once the desired temperature is reached. The sealer should be pre-set for 165°C, 3 second seal time.

2. Place the foil seal on top of the input plate (shiny side up).

3. Place the plate on the plate carrier so that well A1 is in the back left corner. Avoid touching the heating surface while loading the sample plate to prevent injury.

4. Grasp the handle and lower to thermally compress the foil seal onto the input plate. Do NOT apply more pressure to the handle than necessary. When the correct pressure is achieved, an audible tone will sound and the timer will count down to zero.

5. Once the timer reaches zero, another audible tone will sound. Raise the handle to release the heater plate.

6. Rotate the plate so that well A1 is in the front right corner (H12 will be in the back left corner) and repeat the sealing steps 4 and 5.

7. Use a roller seal to ensure that all wells are properly sealed.

8. Put a thermal pad on top of the output plate, then close and tighten the lid.

Appendix C: Manual PCR-enriched Library Construction

1. Shearing & QC (not suitable for ChIP)

1.1. Transfer all ss-cDNA or gDNA to Covaris LE220 vessels

1.2. Covaris LE220, LIBPR.0097

1.3. QC: Agilent HS DNA Assay (not needed for ss-cDNA)

2. Bead Clean Sheared gDNA (Small Gap only)

2.1. Ethanol and Magnetic beads must be incubated at room temperature for at least 30 minutes before use.

DNA volume (µL)	Bead Volume (µL)	Mixing Volume (µL)	Bead Binding Time (mins)	Magnet Clearing Time (mins)	Supernatant Volume (µL)	2x 70% EtOH Wash Vol (µL)	Ethanol Air Dry Time (mins)	EB Elution Volume (µL)	Elution Time (mins)	Magnet Elution Time (mins)	Transfer Volume (µL)
60	108	135	15	7	168	150	5	37	3	2	35

3. Size Select Sheared gDNA (Low Input Small Gap only)

Upper Cut

Sonication DNA (µL)	Beads (µL)	80% Bead Mix (µL)	Supernatant (µL)
62.5	40	82	102.5

Lower Cut

Supernatant (µL)	Beads (µL)	80% Bead Mix Volume (µL)	Supernatant Volume (µL)	EB Elution Volume (µL)	Transfer Volume (µL)
102.5	20	98	122.5	37	35

4. End Repair & Phosphorylation

Solution	1 rxn (µL)
DNA	35
NEB End Repair Premix	23.5

Solution	1 rxn (µL)
Reaction volume	**58.5**

4.1. Transfer 23.5µL of NEB End Repair Premix into wells of a destination plate.

4.2. Transfer 35µL of ss-cDNA, ChIP DNA or gDNA to End Repair Premix, mix using 80% volume, 10X.

4.3. Tetrad Program: LIBCOR>ER; 20°C for 30 minutes; hold 4°C.

4.4. Safe stopping point if stored at -20°C.

5. Bead Clean End Repaired & Phosphorylated Template

Ethanol and Magnetic beads must be incubated at room temperature for at least 30 minutes before use.

For all samples EXCEPT low input small gap

DNA volume (µL)	Bead Volume (µL)	Mixing Volume (µL)	Bead Binding Time (mins)	Magnet Clearing Time (mins)	Supernatant Volume (µL)	2x 70% EtOH Wash Vol (µL)	Ethanol Air Dry Time (mins)	EB Elution Volume (µL)	Elution Time (mins)	Magnet Elution Time (mins)	Transfer Volume (µL)
58.5	105	131	15	7	163.5	150	5	16	3	2	15

For low input small gap

DNA volume (µL)	Bead Volume (µL)	Mixing Volume (µL)	Bead Binding Time (mins)	Magnet Clearing Time (mins)	Supernatant Volume (µL)	2x 70% EtOH Wash Vol (µL)	Ethanol Air Dry Time (mins)	EB Elution Volume (µL)	Elution Time (mins)	Magnet Elution Time (mins)	Transfer Volume (µL)
58.5	58.5	94	15	7	117	150	5	16	3	2	15

5.1. Note: This is a safe stopping point. Do not proceed to adenylation unless you have adequate time to perform ligation reaction as well.

A-Tailing

Solution	1 rxn (µL)
End-Repair + BC DNA	15
NEB Adenylation Premix	10
Reaction volume	**25**

5.2. Transfer 10µL of NEB Adenylation Premix to 15µL of size selected and repaired/phosphorylated DNA.

5.3. Tetrad Program: LIBCOR>ATAIL

5.4. Proceed directly to in-tandem ligation (**do not bead clean after Adenylation**). Store on ice while preparing Ligation premix and adapters.

6. Adapter Ligation

Solution	**1 rxn (µL)**
Adenylated template	25
2X Ligation Premix	10.5
dH$_2$O	X
PE Adapter (10 µM)	Y
Reaction volume	**37.5-39.5**

Ligation calculator for pipelines:

Pipeline	**Ligation Calculator**
Small Gap Capture	0.5x_Ligation_Brew_40pmol
ChIP	0.5x_Ligation_Brew_4pmol
ss-cDNA	0.5x_Ligation_Brew_4pmol
Small Gap Genome Low Input	0.5x_Ligation_Brew_10pmol

6.1. Transfer 12.5µL (ChIP, Low Input Small Gap and ss-cDNA) or 14.5µL (small gap capture) of ligation brew to 25µL of adenylated template.

6.2. Reset pipette to 80% total volume, mix 10X.

6.3. Select tetrad program: LIBCOR>LIGATION

6.4. Set a timer for 15 minutes. Quick spin plate and store on ice immediately after the 15 minute ligation.

7. Double Bead Clean post Ligation

Bead clean #1

DNA volume (µL)	**Bead Volume (µL)**	**Mixing Volume (µL)**	**Bead Binding Time (mins)**	**Magnet Clearing Time (mins)**	**Supernatant Volume (µL)**	**2x 70% EtOH Wash Vol (µL)**	**Ethanol Air Dry Time (mins)**	**EB Elution Volume (µL)**	**Elution Time (mins)**	**Magnet Elution Time (mins)**	**Transfer Volume (µL)**
37.5-39.5	37.5	60	15	7	75-77	150	5	52	3	2	50

Bead clean #2

DNA volume (µL)	**Bead Volume (µL)**	**Mixing Volume (µL)**	**Bead Binding Time (mins)**	**Magnet Clearing Time (mins)**	**Supernatant Volume (µL)**	**2x 70% EtOH Wash Vol (µL)**	**Ethanol Air Dry Time (mins)**	**EB Elution Volume (µL)**	**Elution Time (mins)**	**Magnet Elution Time (mins)**	**Transfer Volume (µL)**
50	50	80	15	7	100	150	5	10	3	2	10

7.1. The ligated template can be stored at -20°C after the first or second bead clean up step.

8. PCR enrich adapter-ligated template

8.1. The volume requirement for 1 reaction set up for Small Gap, ChIP and Low Input is as follows:

Solution	**1 rxn (µL)**
Adapter Ligated + BC DNA (Full Template)	10
2X Q5 Master Mix	12.5
Indexed PCR primers (20µM)	2.5
Reaction volume	**25**

8.2. The volume requirement for 1 reaction set up for ss-cDNA is as follows:

Solution	**1 rxn (µL)**
Adapter Ligated + BC DNA (Full Template)	10
2X Q5 Master Mix	12.5
USER	1.5
Indexed PCR primers (20µM)	2.5
Reaction volume	**26.5**

} PCR Brew + USER (14µL)

8.3. Select Pipeline-specific tetrad program:

Starting Material	**PCR cycles**	**Tetrad Program**
ss-cDNA (poly-A)	10	SSCDNA10

ss-cDNA (RBD; >50ng RNA input)	11 or 13	SSCDNA11 or SSCDNA13
ss-cDNA (RBD; ≤50ng RNA input)	15	SSCDNA15
Small gap Capture	6	LCPCR-6
100 ng Low Input Small gap	6	LCPCR-6
20 ng Low Input Small gap	8	LCPCR-8
5 ng Low Input Small gap	10	LCPCR-10
1 ng Low Input Small gap	12	LCPCR-12
ChIP (100k cells Native ChIP – except H3K27ac)	8	LCPCR-8
ChIP (100k cells Native ChIP – H3K27ac)	10	LCPCR-10
ChIP (Crosslinked ChIP)	13	LCPCR-13

*Heat seal the plate using Adhesive foil EZPierce 20µm Thermal foil (Cat. No. AB1720, Thermo Fisher). The equipment used for this is ALPS 50 V Microplate Heat Sealer (Cat. No. AB-1443, Thermo Scientific). Please see Appendix B for instructions. PCR-enriched template can be stored at -20ºC or proceed immediately to bead clean PCR enriched template.

9. Double Bead Clean post iPCR

Bead clean #1

DNA volume (µL)	Bead Volume (µL)	Mixing Volume (µL)	Bead Binding Time (mins)	Magnet Clearing Time (mins)	Supernatant Volume (µL)	2x 70% EtOH Wash Vol (µL)	Ethanol Air Dry Time (mins)	EB Elution Volume (µL)	Elution Time (mins)	Magnet Elution Time (mins)	Transfer Volume (µL)
25	25	40	15	7	50	150	5	52	3	2	50

Bead clean #2

DNA volume (µL)	Bead Volume (µL)	Mixing Volume (µL)	Bead Binding Time (mins)	Magnet Clearing Time (mins)	Supernatant Volume (µL)	2x 70% EtOH Wash Vol (µL)	Ethanol Air Dry Time (mins)	EB Elution Volume (µL)	Elution Time (mins)	Magnet Elution Time (mins)	Transfer Volume (µL)
50	50	80	15	7	100	150	5	25	3	2	23

9.1. Template can be stored at -20ºC after the first or second bead clean up post PCR.

10. QC Final Library Products

10.1. Run 1µL of each final library product on Agilent DNA 1000 chip assay or dilute libraries 1 in 10 and perform a HS Caliper QC.

10.2. Quantify each final library product by Qubit HS DNA assay or Quant-iT.

10.3. If required, normalize and pool samples using JANUS G3 or manually and quantify pool by Qubit HS DNA assay.

Appendix D: Expert SOP: 96-well ss-cDNA library construction

Step	SOP; program name	Nimbus protocol: LibraryConstruction	LIMS protocols
1) **Transfer DNA to Covaris plate**		ss-cDNA v2> **Shearing Setup**	Aliquot to new TRA to set to IDX pipeline
2) **Shear DNA to 200-250 bp** ss-cDNA (LE220)	**Plate_130sec_cDNA.e1proc** (in 40 µL vol) LIBPR.0097		
4) **Transfer out of covaris plate**		ss-cDNA v2> **Transfer out of Covaris**	
5) **End Repair**	ER (tetrad)	ss-cDNA v2> **End Repair**	A-Library Construction – IDX pipeline
6) **Clean up End Repair**		ss-cDNA v2> **Bead Clean E. R.**	
7) **Adenylation**	ATAIL (tetrad)	ss-cDNA v2> **A-Tailing**	
8) **Ligation**	LIGATION (tetrad)	ss-cDNA v2> **Adapter Ligation**	*Ligation_Brew_4pmol*
9) **Adapter Clean up**		ss-cDNA v2> **Bead Clean Ligation(2X)**	
10) **Indexing PCR**	SSCDNA10 (PolyA); SSCDNA11 or SSCDNA13 (>50 ng RBD RNA input); SSCDNA15 (≤50 ng RBD RNA input)	**ss-cDNA v2> Index PCR**	Plate_Indexed_PCR – IDX pipeline
11) **Post PCR size selection** 2X, 1:1 bead:sample clean up		ss-cDNA v2> **Bead Clean iPCR(2X)**	Plate_PPBC_SizeSelection – IDX pipeline
12) **Quantify final libraries** Quant-iT HSDNA Assay or Qubit	LIBPR.0108 LIBPR.0153	ss-cDNA v2> **Quant-iT** (10x dilution) ss-cDNA v2> **Qubit** (undiluted)	
13) **QC Average size** Caliper HSDNA assay	LIBPR.0051	ss-cDNA v2> **Dilute for QC** (10X dil.)	Bioanalyser Run / Caliper Run- Post library construction size selection QC
14) **Option: JANUS G3 equal molar pooling**	LIBPR.0146	JANUS G3> LibCore – G3 Plate to Tube Pooling	Action: -Aliquot to create pooling TRA – rearray function to track IX pool
15) **Option: Quantify pooled**	LIBPR.0153		

libraries Qubit HS DNA assay			
16) **Submit libraries**			Final_Submission: DITP pipeline

Solutions: ***0.5X_****Ligation_Brew_4pmol*, ***0.5X_****Q5_Indexing_PCR + USER Brew*

Appendix E: Expert SOP: 96-well Small Gap library construction

Step	SOP; program name	Nimbus protocol: LibraryConstruction	LIMS protocols
1) **Transfer DNA to Covaris plate**		Small Gap v2> **Shearing Setup**	Start of plate library construction
2) **Shear DNA to 250 bp** Amplicon DNA (E220) gDNA (LE220)	LIBPR.0139 LIBPR.0097		
3) **QC sheared DNA:** QC all samples: Agilent HS DNA assay or Caliper HS DNA assay	LIBPR.0017 LIBPR.0051		Bioanalyser Run- Sonication QC Caliper Run – Sonication QC
4) **Transfer out of covaris plate**		Small Gap v2> **Transfer out of Covaris**	
5) **Bead clean sheared DNA**		Small Gap v2> **Bead clean sheared DNA**	
6) **End Repair**	ER (tetrad)	Small Gap v2> **End Repair**	A-Library Construction – IDX pipeline
7) **Clean up End Repair**		Small Gap v2> **Bead Clean E. R.**	
8) **Adenylation**	ATAIL (tetrad)	Small Gap v2> **A-Tailing**	
9) **Ligation**	LIGATION (tetrad)	Small Gap v2> **Adapter Ligation**	
10) **Adapter Clean up** 2X, 1:1 Ligation clean up		Small Gap v2> **Bead Clean Ligation**	
11) **Indexing PCR**	LCPCR-6 (tetrad)	Small Gap v2> **Index PCR**	Plate_Indexed_PCR – IDX pipeline
12) **Post PCR size selection** 2X, 1:1 bead:sample clean up		Small Gap v2> **Bead Clean iPCR (2X)**	Plate_PPBC_SizeSelection – IDX pipeline
13) **Quantify final libraries** Quant-iT HSDNA Assay Qubit HS DNA Assay	LIBPR.0108 LIBPR.0153	Small Gap v2> **Quant-iT (10x dil.)** Small Gap v2> **Qubit (undiluted)**	
14) **QC Average size** Caliper HSDNA assay Agilent DNA1000 assay	LIBPR.0051 LIBPR.0017	Small Gap v2> **Dilute for Caliper QC** (10X dil.) Small Gap v2> **undiluted for Agilent**	Bioanalyser Run / Caliper Run - Post library construction size selection QC
15) **Option: JANUS G3 equal molar pooling**	LIBPR.0146	JANUS G3> LibCore – G3 Plate to Tube Pooling	Action: Aliquot to create pooling TRA Rearray function to track IX pool

16) Option: Quantify pool Qubit HS DNA assay	LIBPR.0153		
17) Submit libraries			Final_Submission: DITP pipeline

Solutions: ***0.5X**_Ligation_Brew_40pmol*

Appendix F: Expert SOP: 96-well ChIP DNA library construction

Step	SOP; program name	Nimbus protocol: LibraryConstruction	LIMS protocols
1) **End Repair**	ER (tetrad)	ChIP DNA v2> **End Repair**	Start of Plate Library Construction
2) **Clean up End Repair**		ChIP DNA v2> **Bead Clean E. R.**	A-Library Construction - IDX pipeline
3) **Adenylation**	ATAIL (tetrad)	ChIP DNA v2> **A-Tailing**	
4) **Ligation**	LIGATION (tetrad)	ChIP DNA v2> **Adapter Ligation**	
5) **Adapter Clean up** 2X, 1:1 Ligation clean up		ChIP DNA v2> **Bead Clean Ligation(2X)**	
6) **Indexing PCR**	LCPCR-8,10 or 13 (tetrad)	ChIP DNA v2> **Index PCR**	Plate_Indexed_PCR - IDX pipeline
7) **Post PCR size selection** 2X, 1:1 bead:sample clean up		ChIP DNA v2> **Bead Clean iPCR(2X)**	Plate_PPBC_SizeSelection – IDX pipeline
8) **Quantify final libraries** Quant-iT HSDNA Assay Qubit HS DNA Assay	LIBPR.0108 LIBPR.0153	ChIP DNA v2> **Quant-iT (10x dilution)** ChIP DNA v2> **Qubit (undiluted)**	
9) **QC Average size** Caliper HSDNA assay Agilent DNA1000 assay	LIBPR.0051 LIBPR.0017	ChIP DNA v2> **Dilute for Caliper QC** ChIP DNA v2> **Undiluted for Agilent**	Bioanalyser Run / Caliper Run - Post library construction size selection QC
10) **Option: JANUS G3 equal molar pooling**	LIBPR.0146	JANUS G3> LibCore – G3 Plate to Tube Pooling	Action: Aliquot to create pooling TRA -Rearray function to track IX pool
11) **Option: Quantify pooled libraries** Qubit HS DNA assay	LIBPR.0153		
12) **Submit libraries**			Final_Submission – DITP pipeline

Solutions: *0.5X_Ligation_Brew_4pmol*

Appendix G: Expert SOP: 96-well Low Input Small Gap library construction

Step	SOP; program name	Nimbus protocol: LibraryConstruction	LIMS protocols
1) **Transfer DNA to Covaris plate**		Low Input SG v2> **Shearing Setup**	Start of plate library construction
2) **Shear DNA to 300 bp** gDNA (LE220)	LIBPR.0097		
3) **QC sheared DNA:** QC all samples: Agilent HS DNA assay or Caliper HS DNA assay	LIBPR.0017 LIBPR.0051		Bioanalyser Run- Sonication QC Caliper Run – Sonication QC
4) **Transfer out of covaris plate**		Low Input SG v2 > **Transfer out of Covaris**	
5) **Size Select sheared DNA**		Low Input SG v2 > **Size Select sheared DNA**	
6) **End Repair**	ER (tetrad)	Low Input SG v2 > **End Repair**	A-Library Construction – IDX pipeline
7) **Clean up End Repair**		Low Input SG v2> **Bead Clean E. R.**	
8) **Adenylation**	ATAIL (tetrad)	Low Input SG v2> **A-Tailing**	
9) **Ligation**	LIGATION (tetrad)	Low Input SG v2> **Adapter Ligation**	
10) **Adapter Clean up** 2X, 1:1 Ligation clean up		Low Input SG v2> **Bead Clean Ligation**	
11) **Indexing PCR**	PCR cycles depends on Input amount	Low Input SG v2> **Index PCR**	Plate_Indexed_PCR – IDX pipeline
12) **Post PCR size selection** 2X, 1:1 bead:sample clean up		Low Input SG v2> **Bead Clean iPCR (2X)**	Plate_PPBC_SizeSelection – IDX pipeline
13) **Quantify final libraries** Quant-iT HSDNA/Qubit HS DNA	LIBPR.0108 LIBPR.0153	Low Input SG v2> **Quant-iT (10x dil.)** Low Input SG v2> **Qubit (undiluted)**	
14) **QC Average size** Caliper HSDNA assay Agilent DNA1000 assay	LIBPR.0051 LIBPR.0017	Low Input SG v2> **Dilute for Caliper QC** (10X dil.) Low Input SG v2> **undiluted for Agilent**	Bioanalyser Run / Caliper Run - Post library construction size selection QC
15) **Option: JANUS G3 equal molar pooling**	LIBPR.0146	JANUS G3> LibCore – G3 Plate to Tube Pooling	Action: Aliquot to create pooling TRA Rearray function to track IX pool

16) Option: Quantify pool Qubit HS DNA assay	LIBPR.0153		
17) Submit libraries			Final_Submission: DITP pipeline

Solutions: *0.5x_Ligation_Brew_10pmol*