Non Controlled Version

**Note: Controlled Versions of this document are subjected to change without notice*

Tethered Conformation Capture

I. Purpose

To provide details on the experimental outline of tethered conformation capture.

II. Scope

All procedures are applicable to the BCGSC Library Core group.

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 QA 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 Assurance Management to audit this procedure for compliance and maintain control of this procedure.

V. References

Reference Title	Reference Number
N/A	N/A

VI. Related Documents

Document Title	Document Number
SLX-PET Protocol for Illumina Sample Prep	LIBPR.0031
Quantifying DNA Samples Using the Qubit Fluorometer	LIBPR.0030
Operation and Maintenance of the Agilent 2100 Bioanalyzer for DNA Samples	LIBPR.0017
Operation of Covaris E-Series	LIBPR.0041

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 #	
Small size safetouch nitrile gloves	Ultident	296359683		✓
wet ice	In house	N/A	N/A	N/A
Ice bucket	Fisher	11-675-58		✓
1.5 ml Microtubes	Diamed	PRE150-B		✓
15ml Conical Tubes	BD Falcon	352097		✓
50ml Conical Tubes	BD Falcon	352070		
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 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171203		✓
Diamond Filter tips DFL30 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171303		✓
Diamond Filter tips DFL200 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171503		✓
Diamond Filter tips DFL1000 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171703		✓
Galaxy mini-centrifuge	VWR	37000-700		✓
Large Kimwipes (Kimberly Clark/Kimtech)	Fisher Scientific	06-666-1A		✓
Black ink permanent marker pen	VWR	52877-310		✓
Small Autoclave waste bags 10”X15”	Fisher Scientific	01-826-4		✓
10ml serological pipettes	Costar	CS004488		✓
Portable Pipet Aid, Multispeed XP, rechargeable	Fisher Scientific	13-681-15E		✓
Ultra Pure Water (Rnase/Dnase free)	Invitrogen	10977-023		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohols	People Soft ID: 23878		✓
DNA away	Molecular Bioproducts	7010		✓
T4 DNA Ligase	NEB	M020T		

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DNA Polymerase I, Large(Klenow) fragment	NEB	M0201S		✓
1M Tris Solution, pH 8.0	Ambion	AM9856		✓
Triton® X-100, laboratory grade	Sigma	X100-100ML		✓
20% SDS Solution	Ambion	AM9820		✓
Deoxynucleotide Solution Set	NEB	N0446S		✓
EDTA, Disodium salt,	Fisher Scientific	S311-3		✓
Exonuclease III (E.coli)	NEB	M0206S		✓
T4 DNA Ligase Reaction Buffer	NEB	B0202S		✓
Rnase-free Non-stick tubes, 1.5ml,	Ambion	AM12450		✓
Centrifuge, Eppendorf 5417R, refrigerated high-speed, 115V	Fisher Scientific	5417 R	✓	
NEBuffer 2	NEB	B7002S		✓
Bovine Serum Albumin (BSA), Buffers, 10mg/ml	NEB	B9001S		✓
Non-stick 2ml tubes	Ambion	AM12475		✓
Syringe, 1ml, Gauge: 26	Fisher Scientific	333-309625		✓
Syringe,3ml,Gauge:21	Fisher Scientific			
LabQuake Shaker/Rotator with Clips	Barnstead	415110	✓	
HindIII	NEB	R0104S		
Klenow Fragment (3' to 5' exo)	NEB	M0212L		
QIAquick PCR Purification Kit, 50rxn	Qiagen	28104		✓
Proteinase K (20µg/µl)	Invitrogen	2553049		✓
2ml phase lock gel heavy tubes	Inter Medico	5P2302830		✓
Phenol/Chloroform/Isoamyl Alcohol	Fisher Scientific	BP1752-400		✓
Sodium Acetate, 3M, pH 5.5	Ambion	AM9740		✓
Quick Ligation Kit	M2200S	NEB		✓
VX-100 Vortex Mixer	Rose Scientific	S-0100	✓	
Dynabeads® MyOne™ Streptavidin C1	Invitrogen	65002		✓
Rnase, Dnase-free, 500µg	Roche	11119915001		✓
Eppendorf Thermomixer 1.5 mL	Eppendorf	21516-166	✓	
Parafilm	Fisher Scientific	13-374-12		✓
Mussel Glycogen	Roche	10 901 393 001		✓
Water Bath	Fisher Scientific	Isotempn 220	✓	
5M NaCl	Ambion	AM9760G		✓
EZ-Link Iodoacetyl-PEG2-Biotin	Thermo Scientific	21334		✓
Biotin-14-dCTP	Invitrogen	19518-018		✓
2'-Deoxyguanosine-5'-O-(1-thiotriphosphate).Sodium Salt,Spisomer	Axxora	BLG-D31		✓
Dynabeads® MyOne™ Streptavidin T1	Invitrogen	65602		✓
MinElute PCR Purification Kit	Qiagen	28004		✓
beta-mercaptoethanol	Sigma	M7522-100ml		✓
1M DTT	Invitrogen	P2325		✓
NEB Paired End Library Construction Kit tube based	NEB	E6000B-YJ		✓

5mL screw cap tubes	VWR (BD Falcon)	CA60819-295		✓
Chloroform	Fisher Scientific	C298-500		✓
Orbital Shaker	Barnstead/Lab line	2314	✓	
1M Hepes	Invitrogen	15630-080		✓
IGEPAL	Sigma	I8896-50mL		✓
Dounce Homogenizer	VWR	62400-595		✓
10% Tween-20	Bio-Rad	161-0781		✓
Slide-A-Lyzer Dialysis Cassette	Thermo Scientific	66003		✓
1M Magnesium Chloride (100mL)	Life Technologies	AM9530G		✓

IX. Procedure

1. Retrieval of reagents, sample(s) and equipment preparation (Day 1)

1.1. Put on a clean pair of gloves and lab coat.

1.2. Wipe down the work bench, small equipment, and ice bucket with DNA Away and 70% Ethanol.

1.3. Change gloves.

1.4. Retrieve fresh ice and all required reagents. Ensure all buffers are made and at the proper pH.

2. Cell Lysis and Chromatin Biotinylation

2.1. Thaw 25x10e6 (or approximately) cells on ice for 20min

2.2. Resuspend cells in 550uL of lysis buffer (Appendix A, recipe 1) and incubate on ice for 15min.

2.3. Transfer to sterile dounce homogenizer and apply 20 strokes with pestle.

2.4. Transfer lysate to 2mL tube.

2.5. Spin lysate down at 2500xg for 5min at RT. Discard supernatant.

2.6. Wash pellet 2x with cold wash buffer (Appendix A, recipe 2). Spin in between. Resuspend pellet in 250uL of same wash buffer.

2.7. Add 95uL of 2% SDS (Appendix A, Recipe 3) to pellet. Mix and incubate at 65C for 10min.

2.8. Cool suspension to room temperature (approximately 10 minutes).

2.9. Add 105uL of 25mM EZlink Iodoacetyl-PEG2-Biotin (IPB) (Appendix A, Recipe 4). Rock at room temperature for 1 houron the Orbital Shaker. Parafilm the tube to prevent leakage.

2.10. Add 1300uL of 1x NEB2 buffer. Incubate on ice for 5min.

2.11. Add 225uL of 10% TritonX-100 (Appendix A, Recipe 5). Incubate on ice for 10min, then at 37C for 10min on the thermomixer.

3. Digestion and Dialysis

3.1. Set up the HindIII digestion rxn in a 5mL screw cap tube :

Entire Sample from Part A, Step 12 above	xxxµl
10X NEB2 Buffer	100µl
1M DTT	5µl
200U/uL HindIII	10µl
Water	440µl

3.2. Parafilm tube and incubate O/N at 37°C in an incubator

END OF DAY 1

3.3. Aliquot 2µl to run on a DNA 7500 Agilent chip (LIBPR.0017) to ensure digestion is complete. Confirm with supervisor.

3.4. Dialyze digestion mixture by placing it on a 20kD cutoff Slide-A-Lyzer Dialysis Cassette. (SEE APPENDIX B FOR INSTRUCTIONS ON HOW TO SET UP THE CASSETTE)

a. Dialyze for 4 hour at RT against 1L of Dialysis Buffer (Appendix A, Recipe 6)
b. Renew the dialysis buffer after 3 hours.
c. the final volume of the dialyzed sample should be ~2500uL once removed from the cassette.
d. discard the cassette and dialysis buffer.

3.5. Transfer the sample to a 15mL conical tube.

4. Immobilization At Low Surface Coverage (Tethering)

4.1. Aliquot 400µl of MyOne Streptavidin T1 beads into 5 non-stick 2mL tubes each.

4.2. Wash the beads 3x with 400µl PBST (Appendix A, Recipe 7), capturing the beads on a magnetic rack for 1min in between washes.
4.3. Resuspend the beads in 400uL of PBST.

4.4. Add 500µl of sample (from Part B Step4/Digestion and Dialysis) to each tube of the beads. Parafilm the tubes.

4.5. Rock the bead:sample suspension for 30min at RT on an orbital shaker.

4.6. During incubation prepare the following:

Neutralize 150uL of IPB (Appendix A, Recipe 4) (EZ-Link Iodoacetyl-PEG2-Biotin) with **equimolar amount** of beta-mercaptoethanol. In a 1.5mL tube add 150µl of IPB and 262µl of beta-mercaptoethanol. Mix and keep at RT till needed.

4.7. Add 5uL of neutralized IPB to the bead:sample mix. Rock at RT for 15min on the orbital shaker. (NOTE: the sample is still divided amongst 5 tubes).

5. Filling DNA Ends and Blunt-end Ligation on Beads

5.1. Wash each bead:sample tube with 600uL of TPBS. (use a magnetic stand to collect beads, pull off S/N and resuspend in next buffer).

5.2. Wash each bead:sample tube with 600uL of TPBS. (use a magnetic stand to collect beads, pull off S/N and resuspend in next buffer).

5.3. Wash 1x with 600uL of Wash Buffer (Appendix A, recipe 8).

5.4. Resuspend in 100uL of same wash buffer.

5.5. To each of the bead: sample mix tubes add the following to set up the blunting reaction:

Water	63uL
1M $MgCl_2$	1uL
10X NEB 2 buffer	10uL
10mM ATP	0.7uL
10mM dTTP	0.7uL
10mM dGTP<S	0.7uL
0.4mM Biotin-14-dCTP	15uL
10% TritonX-100 (Appendix A, Recipe 5)	4uL
5U/uL Klenow	5uL

5.6. Rock the reaction for 40min at RT on the orbital shaker.

5.7. Stop the blunting reaction by adding 5uL of 0.5M EDTA to the suspension.

5.8. Wash the suspension 2x with 600uL with a buffer (Recipe 9) 50mM Tris HCl, pH7.4, 0.4% TritonX-100,0.1M EDTA).

5.9. Resuspend in 500uL of same buffer (recipe 9) and tranfer the bead/sample mix to a 15mL conical tube. There will be 5 15mL conical tubes.

5.10. Set up the ligation reaction as follows: (add directly to each bead/sample suspension in 15mL conical tube):

Water	4mL
10x NEB Ligase Buffer	250uL
20% TritonX-100 (Appendix A,Recipe 11)	90uL
1M Tris-HCl,pH7.4	100uL
100X BSA (NEB)	50uL
2000U/uL T4 DNA Ligase (NEB)	2uL

5.11. Parafilm the tubes and rock the reaction for at 16C overnight. Use a tube rotator to rock the reaction. Set up the reaction in the media prep room with the door shut. Make a note on the door and notify LabOps. Take an initial temperature reading using a thermometer from the Engineering group. Also take a reading at the end of the ligation as well.

END OF DAY 2

5.12. Stop the reaction by adding 200uL of 0.5M EDTA

6. DNA Extraction (Reverse Crosslinking and DNA Purification)

6.1. Quick spin the conical tube at 2000rpm for 2 min to pull the beads down. Aspirate the supernatant off and discard.

6.2. Add 400uL of extraction buffer (recipe 11) to each tube of the bead/sample mix.

6.3. Transfer the reaction to a non-stick microfuge tube.

6.4. Add 20uL of 20mg/ml Proteinase K (ProK).

6.5. Incubate O/N at 65°C on the thermomixer. Set up interval shaking at 1000rpm for 1min off/30sec off.

END OF DAY 3

6.6. Add 5uL of ProK to each tube and incubate for 2hrs at 65°C.

6.7. Place suspension on magnetic rack, aspirate the supernatant carefully.

6.8. TRANSFER THE SUPERNATANTS TO A NEW 1.5mL microfuge tube (There will be 5 tubes).

6.9. 2x equal volume PCI extraction using phase-lock tubes.
a. spin down phase-lock tube at 14,000 rpm for 1 min.
b. Add sample and equal volume PCI
c. Invert 5-6x to mix thoroughly
d. Spin at 14,000rpm for 5 minutes.
e. Transfer aqueous to a fresh 1.5mL tube.
f. Repeat 1x

6.10. 1x equal volume chloroform extraction.
a. same steps as 9a-9d, except use equal volume chloroform.
b. Transfer aqueous phase into a new 1.5mL microfuge tube.

6.11. Add 10% v/v 3M NaOAc.

6.12. Add 3 µl of glycogen.

6.13. Add 900uL of 100% EtOH. Incubate O/N at -20C or for 1hr at -80°C.

6.14. Pellet the DNA by centrifuging for 20min at 20000xg at 4C

6.15. Wash pellest 2x with 500uL of ice-cold 80% EtOH and decant.

6.16. Do not let the pellet dry completely and add 20uL of 10mM Tris-HCL, pH 8.0 (Appendix A, Recipe 12).

6.17. Pool all 5 aliquots to a final volume of 100uL.

6.18. RNase treat the DNA by adding 20µl of 0.5mg/ml RNAse A.

6.19. Incubate at 37C for 30min on the thermomixer (no shaking required).

6.20. Purify the DNA by using the QIAquick PCR purification kit.

6.21. Elute the DNA in 50uL of EB buffer.

6.22. Quantify the DNA on a HS Qubit Assay (LIBPR.0030) and run on an DNA 1000 Agilent Assay (LIBPR.0017). It is now safe to store the DNA at -20C indefinitely.

END OF DAY 4

7. Removal of Biotin from non-ligated DNA Ends and Shearing

7.1. Aliquot 5ug of purified DNA into a new 1.5mL non-stick microfuge tube.

7.2. Add 300units (3µl) of ExoIII enzyme (NEB) and 10X NEB1 buffer to a final volume of 90µl.

7.3. Incubate for 1hr at 37°C on the thermomixer. .

7.4. Stop reaction by adding 2uL of 0.5M EDTA and 2uL of 5M NaCl.

7.5. Inactivate enzyme by incubation at 70°C for 20min on the thermomixer.

7.6. Adjust the reaction volume to a final of 100uL by adding ultrapure water.

7.7. Shear to 100-500bp-covaris using Covaris E210, duty cycle 5%, intensity 5, cycles/burst at 200, 180sec (LIBPR.0041).

7.8. Take out 1uL and run on DNA 1000 Agilent assay (LIBPR.0017) to confirm size. Consult with supervisor.

7.9. Purify using QIAquick PCR purification kit and elute in 50µl of EB buffer.

7.10. Size selection depends on the agilent profile.

CAN END DAY 5 OR CAN PROCEED TO LIBRARY CONSTRUCTION

8. Library Construction On Beads: End-repair & Adding the A-Over hangs

Note: A-tailing (Step 8.4) and Ligation (Step 9) need to be done on the same day.

8.1. To the 50uL of sheared DNA add the following to repair the ends of the DNA fragments:

Klenow (NEB) 5 units	1µl
T4 DNA ligase (NEB) 15 units	5µL
T4 DNA kinase (NEB) 100units	10µL
10 mM dNTPs (0.4mM)	4µL
10x phosphorylation (end repair) buffer	10µl
Ultrapure Water	20µl
Total Volume	**100µl**

8.2. Incubate for 30min at 20°C on the thermomixer.

8.3. Purify the DNA using QIAquick PCR purification kit and elute in 40uL of EB buffer.

8.4. To the 40uL of purified DNA add the following to set up the addition of As:

Klenow-exo (15 units)	3µl
10x NEB2 buffer	50uL
dATP(1mM) 0.2mM final	20µl
Ultrapure Water	27µl

Total Volume	**100µl**

8.5. Incubate for 30min at 37°C on the thermomixer.

8.6. Stop reaction by adding 1uL of 0.5M EDTA.

8.7. Put the reaction on ice and proceed to next step.

9. Pull-down of Biotinylated DNA and ligation of Adapters

9.1. Aliquot 10uL of MyOne Streptavidin C1 beads into a 1.5ml non-stick tube.

9.2. Wash the beads 2x with 500uL of 1xBind/Wash Buffer (B&W) (dilute recipe 13 (Appendix A), 1x) Use the magnetic rack to collect the beads, pull off supernatant and add next buffer.

9.3. Resuspend the beads in 50uL of 2x B&W Buffer (Appendix A, recipe 12). Add the A-tailed sample from Step 8.7.

9.4. Rock the sample at RT for 30min on the orbital shaker.

9.5. Wash the beads with 500uL of 1x B&W+0.1% TritonX-100 (Appendix A, recipe 14).

9.6. Wash the beads with 500uL of 10mM Tris-HCl,pH8 (Appendix A, Recipe 12). Steps 9.5. and 9.6. are to remove the non-biotinylated DNA fragments.

9.7. Resuspend the beads in 100uL of 1x Quick ligation buffer (dilute 2x in-house quick ligase buffer 1:1 with ultrapure water.

9.8. Add the following to set up the adapter ligation reaction:

Paired-end adapters (3uM)	3µl
2000U/µl T4 DNA Ligase	1.5µL

9.9. Incubate at RT for 20min with occasional shaking.

9.10. Stop the reaction by adding 6uL of 0.5M EDTA.

9.11. Wash the beads 2x with 500uL of 1X B&W buffer, using the magnetic stand to collect beads.

9.12. Wash the beads 2x with 500uL of TE (in-house stock), using the magnetic stand to collect beads.

9.13. Resuspend the beads in 50uL of water (template for PCR).

CAN END DAY 6 HERE OR PROCEED TO PCR

10. PCR Amplification

Use 10µl of template, PE1.0 and Indexed primers. Follow LIBPR.0031 SLX-PET Protocol for Illumina Sample Prep Steps

Consult with supervisor as to the number of PCR cycles to run.

10.1. Once the PCR is complete, put PCR tubes on mag stand and transfer the supernatant to a new 1.5mL tube. This contains the PCR product.
10.2. Add 20uL of water to the beads and KEEP. The biotinylated-adapter-ligated DNA template is still tethered to the beads and can be used as template again. Store at 4C.

10.2. Purify the PCR Product using the Qiagen Mini Elute Purification Kit and elute product in 13µl of EB.

10.3. Check quantity using HS Qubit Assay (LIBPR.0030) and the quality by running an aliquot on a DNA 1000 Agilent Assay (LIBPR.0017).

10.4. Consult with supervisor to determine whether more PCR should be done and potential pooling strategy.

10.5. Follow LIBPR.0031 for submission. Complete LIMS according to Appendix C.

APPENDIX A: RECIPES OF BUFFERS AND REAGENTS

TCC Recipe Buffers

Recipe 1: Lysis Buffer (Final volume 50mL)
10mM Hepes, pH 8
10mM NaCl
0.2% IGEPAL

IGEPAL: C_i=100% C_f=0.2%

$$V_1 = \frac{50(0.2mL)}{100} = 0.1mL$$

NaCl: C_i = 5M C_f = 10mM (0.01M)

$$V_i = \frac{0.01(50)}{5M} = 0.1mL$$

HEPES: C_i = 1M C_f = 10mM

$$V_i = \frac{10(50mL)}{1000mM} = 0.5mL$$

Upto 50mL with ultrapure water

Recipe 2: Wash Buffer (Final volume 100mL)
50mM Tris-HCl, pH8
50mM NaCl
1mM EDTA

Tris-HCl: C_i = 1M C_f = 50mM

$$V_i = \frac{50mM(100mL)}{1000mM} = 5mL$$

NaCl: C_i = 5M C_f = 50mM

$$V_i = \frac{50(100mL)}{5000mM} = 1mL$$

EDTA: C_i=0.5M C_f=1mM

$$V_i = \frac{1mM(100mL)}{500mM} = 0.2mL$$

Upto 100mL with ultrapure water

Recipe 3: 2% SDS (Final volume 50mL)
C_i = 20% C_f = 2%

$$V_i = \frac{2(50mL)}{20} = 5mL + 45mL\ H_2O$$

Recipe 4: EZlink Iodoacetyl-PEG2-Biotin
C_f = 25mM MM=542.43g/mol V_f = 1mL

mol=mol/L(L) = 0.025mol/L (0.001L) = 0.000025mol
mass = 542.43g/mol x 0.000025mol = 0.0135g (into 1mL of ultrapure water)

(use analytical balance to weigh out EZlink)

Recipe 5: 10% triton-100 (Final volume 50mL)

C_i = 100% C_f = 10%

$V_i = \frac{10(50mL)}{100} = 5mL + 45mL\ H_2O$

Recipe 6: Dialysis Buffer (final volume 2L)
10mM Tris-HCl, pH8
1mM EDTA

10mM Tris-HCl: C_i = 1M, C_f = 10mM

$V_i = \frac{1\ (2L)}{1000mM} = 20mL$

1mM EDTA: C_i = 0.5M, C_f = 1mM

$V_i = \frac{1mM\ (2000L)}{500mM} = 4mL$

Top up to 2L with H_2O

Recipe 7: PBST = PBS + 0.01% Tween 20
PBS: Made 50mL using ChIP Stock
+0.1% Tween 20

C_i = 100% C_f = 0.1%

$V_i = \frac{0.1\ (50)}{100} = 50uL$

Recipe 8: Wash Buffer (removal of non biotinylated chromatin)
10mM Tris HCl, pH8
50mM NaCl
0.4% TritonX-100

V_f = 50mL

10mM Tris HCl, pH8 : C_i = 1M

$$V_i = \frac{10mM(50)}{1000mM} = 500ul$$

50mM NaCl: C_i = 5M

$$V_i = \frac{50(50)}{5000} = 500uL$$

0.4% TritonX-100: C_i = 100%

$$V_i = \frac{0.4(50)}{100} = 200uL$$

Top up to 50uL with H_2O
Make 50mL stock using 1M stock, pH8.0
pH to 7.4 (use pH meter)

Recipe 9:
50mM Tris-HCl, pH7.4
0.4% TritonX-100
0.1mM EDTA

50mM Tris-HCl, pH7.4: C_i = 1M

$$V_i = \frac{50(50mL)}{1000} = 2.5mL$$

0.4% TritonX-100: C_i = 100%

$$V_i = \frac{0.4\%\ (50mL)}{100} = 200uL$$

0.1mM EDTA: C_i = 0.5M EDTA

$$V_i = \frac{0.1M\ (50mL)}{500} = 10uL$$

20% TritonX-100 **V_f = 50mL**

C_i = 100%

$V_i = \frac{20(50mL)}{100}$ = 10mL + 40mL H_2O

Recipe 10: DNA Extraction Buffer V_f = 50mL

50mM Tris-HCl, pH8: C_i = 1M

$$V_i = \frac{50(50mL)}{1000} = 2.5mL$$

0.2% SDS: C_i = 20%

$$V_i = \frac{0.2(50mL)}{20} = 500uL$$

1mM EDTA: C_i = 0.5M

$$V_i = \frac{1mM(50mL)}{500mM} = 100uL$$

100mM NaCl: C_i = 1M

$$V_i = \frac{100(50mL)}{1000} = 5mL$$

Top up to 50mL with ultrapure water

Recipe 11: 10mM Tris-HCl, ph8 (Vf=10ml)

Ci=1M

$Vi=\frac{10mM(10mL)}{1M}$ =100uL + 9.9mL water

Recipe 12: 2x Bind/Wash Buffer (Vf=50mL)

(10mM Tris-HCl,pH7.5,1mM EDTA, 2M NaCl)

10mM Tris-HCl,pH 7.5
Ci=1M Vi=$\frac{0.01M(50mL)}{1M}$ =0.5mL

1mM EDTA
Ci=0.5M Vi=$\frac{1mM(50mL)}{500mM}$ =0.1mL (100uL)

2M NaCl
Ci=5M Vi=$\frac{2M(50mL)}{5M}$ =20mL

Upto 50mL with ultrapure water

Recipe 13: 1x B+W Buffer + 0.1% TritonX-100 (V=20mL)

20mL 1x B+W Buffer

10% Triton diluted to 0.1%: (0.1%*20mL)/10%=200uL of stock

APPENDIX B: INSTRUCTIONS ON HOW TO LOAD DIALYSIS CASSETTE

Hydrate Membrane

Perform the following steps for cassettes requiring hydration and for all cassettes used with low sample volumes:

1. Remove Slide-A-Lyzer Cassette from its pouch and slip into the groove of an appropriate size buoy.
2. Immerse cassette in dialysis buffer (Figure 1). Hydrate the 3.5-20K cassettes for 1-2 minutes and the 2K cassettes for at least 2 minutes.
3. Remove cassette from buffer and remove excess liquid by tapping the edge of the cassette gently on paper towels. DO NOT BLOT THE MEMBRANE.

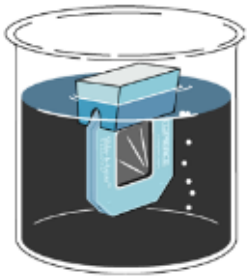

Figure 1. Membrane hydration

Add Sample

Note: Do not allow the needle to contact the membrane.

1. Fill the syringe with the sample, leaving a small amount of air in the syringe.
2. With the bevel sideways, insert the tip of the needle through one of the syringe ports located at a top corner of the cassette.
3. Inject sample slowly. Withdraw air by pulling up on the syringe piston (Figure 2).
4. Remove the syringe needle from the cassette while retaining air in the syringe.

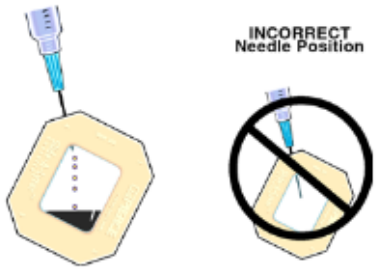

Figure 2. Sample addition

Remove Sample

Note: Use caution to avoid contacting the needle to the membrane.

1. Fill the syringe with a volume of air equal to the sample size. For low-volume samples, fill the syringe with a volume of air approximately equal to two times the sample volume.
2. With the bevel sideways, insert the tip of the needle through another syringe port located at a corner of the cassette. Inject air slowly into the cassette to separate the membranes.
3. Turn the unit so that needle is on the bottom and allow the sample to collect near the port. Withdraw the sample into the syringe (Figure 3).

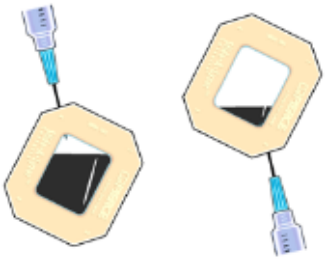

Figure 3. Sample removal

Appendix C: LIMS

1. LIMS: Start of Library Construction

    1.1. Under the Lib_Construction tab, scan in the samples. Save the tube set.

    1.2. Select "Start of Library Construction" from the dropdown menu and select "Continue Prep". Change the pipeline to IDX:Illumina Index. Select "Complete Step". A new barcode will be produced. Paste it into your lab book.

    1.3. Skip the starting DNA/RNA amount step.

2. LIMS: Tethered Chromatin Capture

    2.1. Under the Lib_Construction tab, scan the DNA samples. Save the tube set.

    2.2. Select "Tethered Chromatin Capture" from the dropdown menu and select "Continue Prep".

    2.3. Transfer to 1.5ml tube. This will be the final PCR product barcode.

    2.4. Click on "Decant Out". The Decant step will set the PCR product volume to 0uL.

    2.5. Resuspend and Store PCR product. Scan in Buffer EB. Enter the volume of the PCR product minus the Agilent volume and the Qubit QC volume.

3. LIMS: PET PPGP Dilution

Note: only run this protocol when the PCR product concentration is >25nM, otherwise, skip to step 4 for sample submission.

    3.1. Under the Lib_Construction tab, scan the DNA samples. Save the tube set.

    3.2. Select "PET PPGP Dilution" from the dropdown menu and select "Continue Prep".

    3.3. Aliquot DNA to 1.5ml tube: enter the volume of the stock PPGP PCR product that was aliquoted out to make the diluted sample for submission.

    3.4. Click on "Completed Decant". The Decant step will set the volume of the sample for submission to 0uL.

3.5. Scan in Buffer EB to resuspend submission sample. Enter the final volume of the sample for submission.

4. LIMS: Illumina Concentration Checked

4.1. Under the Lib_Construction tab, scan the DNA samples. Save the tube set.

4.2. Select "Illumina Concentration Checked" from the dropdown menu and select "Continue Prep".

4.3. Enter the rac number. This is the rac number of the next sequencing run box.

4.4. Enter the Final Concentration (nM) of the samples

4.5. Enter the Qubit Run ID. This is the ID of the Qubit Spreadsheet (eg. Q090202AA)

4.6. Click "Completed Enter Concentration"

4.7. Under View\Lib_Construction Views , select "SLX – Libraries Ready for Sequencing". Check that the submitted library is on the view and that the library information is correct.

4.8. Put sample into the "next run box" in F20-56.