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

Histone Modification Primer QC

I. Purpose

The purpose of this work instruction is to outline the method for QC'ing a new batch of Histone Modification Primers.

II. References

Reference Title	Reference Number
N/A	

III. Related Documents

Document Title	Document Number
Chromatin Immunoprecipitation (ChIP)	LIBPR.0015
Native ChIP Using 100,000 Cells	LIBPR.0138
qPCR of ChIP Samples	LIBPR.0075
Resuspension of PE 1.0, PE 2.0 and Index Primers for Library Construction	LIBPR.0084
Operation of the Invitrogen E-Gel iBase Power System	LIBPR_WorkInst.0012

IV. Procedure

Note: The following instructions provide details of validating primers of human origin. If primers of mouse origin are to be validated, prepare mouse (eg.Hepa) gDNA as a template.

1. Preparing HL60 gDNA template for PCR (can be done in ChIP room)

1.1. Retrieve a '1µg' aliquot of HL60 gDNA + Spike-In from rac166064 located in equ2007 (F4-40 4°C fridge).

1.2. Dilute HL60 gDNA to 1ng/µl with EB.

1.3. Place on ice for transport to 5th floor.

OFFICIAL

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

NB The following steps are to take place in the 5th Floor PCR clean room

2. Reconstitution of Stock and Preparation of Dilutions of Primers

2.1. Follow LIBPR.0084 Step 2 to resuspend the lyphilized primer to a 100µM stock concentration. See Table 1 for a list of the histone modification primers and their sequences.

2.2. Dilute the 100µM stock to a working concentration of 10µM (see Step 2.3.). In this case, a 10µM mix of forward and reverse primers in the same tube. Store the remainder of the 100µM stock in the -80°C freezer for up to 18 months.

2.3. Make 200µL of working stock by adding 20µl of 100µM forward primer and 20µL of 100µM reverse primer to 160µL of EB Buffer in a 1.5mL microfuge tube. Label the tube with primer name, species specificity, date and that it is a forward and reverse primer mix. Vortex the tube to mix and quick spin in a mini centrifuge.

2.4. Make 50µL aliquots of the above working stock and store in the -20°C for up to 6 months.

Table 1. Histone Modification Primer Sequences and Target Species

Primer Name	Sequence	Species
GAPDH_genic_H3K36me3_F	5'-AGGCAACTAGGATGGTGTGG-3'	human
GAPDH_genic_H3K36me3_R	5'-TTGATTTTGGAGGGATCTCG-3'	human
Six1_promoter_H3K27me3_F	5'-CATGCAGCCTCTGGAAGAGT-3'	human
Six1_promoter_H3K27me3_R	5'-GGGGGAGGACAGTGGTAGTT-3'	human
ZNF333_genic_H3K9me3_F	5'-AGCCTTCAATCAGCCATCATCCCT-3'	human
ZNF333_genic_H3K9me3_R	5'-TCTGGTATGGGTTCGCAGATGTGT-3'	human
H3K27me3_mu_hu_F	5'-ACTGAAGTAATGAAGGCAGTGTCGT-3'	human/mouse
H3K27me3_mu_hu_R	5'-GCAGCAYCAGAACTGGTCGGTG-3'	human/mouse
H3K9me3_mu_F	5'-GTGGAAAGAACAGACGGGATT-3'	mouse
H3K9me3_mu_R	5'-GTGGTCGAGAGCAAGGGTT-3'	mouse
GAPDH2_F (mouse)	5'-CTG GGT CCG GCT TGC ACA CTT CG-3'	mouse
GAPDH2_R (mouse)	5'-ATC GCG GAG TGG GCC GCA GGA G-3'	mouse
GAPDH_F (human)	5'-TACTAGCGGTTTTACGGGCG-3'	human
GAPDH_R (human)	5'-TCGAACAGGAGGAGCAGAGAGCGA-3'	human

OFFICIAL

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

3. Set up PCR Reaction:

3.1. Set up reactions and scale up accordingly using Table 2 as a guide.

Table 2. PCR Reaction

Reagent	Volume (µl)/reaction
H_2O	12.4
5x Phusion HF Buffer	4
10mM dNTPs	0.4
Phusion DNA Polymerase	0.2
Total Volume	**17**

3.2. Retrieve reagents from the -20°C freezer located in the PCR Clean Room.

3.3. The brew mixes are to be set up in the PCR clean room.

3.4. Prepare the brew mix in a 1.5mL tube.

3.5. Aliquot 17µl of brew per 0.2mL PCR tube or PCR strip tubes. Label reaction tube with primer pair name.

3.6. Add 2µl of 10µM F/R primer mix of each primer set to the appropriate tube.

3.7. Place reaction tubes on ice and transport to Blood Room (on 5th floor).

3.8. Turn on template cabinet and wipe down with DNase Away.

3.9. Retrieve reactions tubes from ice and place in cabinet.

3.10. Add 1µl of 1ng/ul HL60 gDNA template to each reaction tube.

3.11. Gently finger flick reaction tubes or strip tubes and pulse spin.

3.12. Place reaction tubes on ice to transport to the 6th Floor.

NB The following steps are to take place on the 6th Floor

OFFICIAL

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

4. Load reaction tubes onto GE-GSC #2 Tetrad .Set up PCR using the following cycling conditions listed in Table 3: Program "CHIPPCRN".

Table 3. PCR Cycling Parameters

Stage	Temperature	Time	# of Cycles
1	98°C	30 sec	1
2	98°C	10 sec	30
2	59°C	30 sec	
2	72°C	30 sec	
3	72°C	5 min	1
4	4°C	Hold	1

4.1. Once PCR reaction is complete, run product on a gel to verify the size.

4.2. Run PCR products on an E-Gel (Invitrogen E-Gel EX 1% Agarose) on the 6th floor Post PCR designated area.

4.3. Refer to LIBPR_WorkInst.0012 for instructions on the use on E-Gels and the iBase Power System.

4.4. Prepare samples to run on an E-Gel as listed in Table 4.

Table 4. Sample Preparation for E-gel

Reagent	Volume(µl)
PCR product	5
EB	13
10x Loading Buffer	2
Total Volume	**20**

4.5. Dilute 100bp ladder (prepared with loading dye) 1:1 (volume:volume) with EB buffer. The final volume should be 20µl.

4.6. Load 20µl of sample and 20µl of ladder.

4.7. Load 20µl of EB in all EMPTY lanes.

OFFICIAL

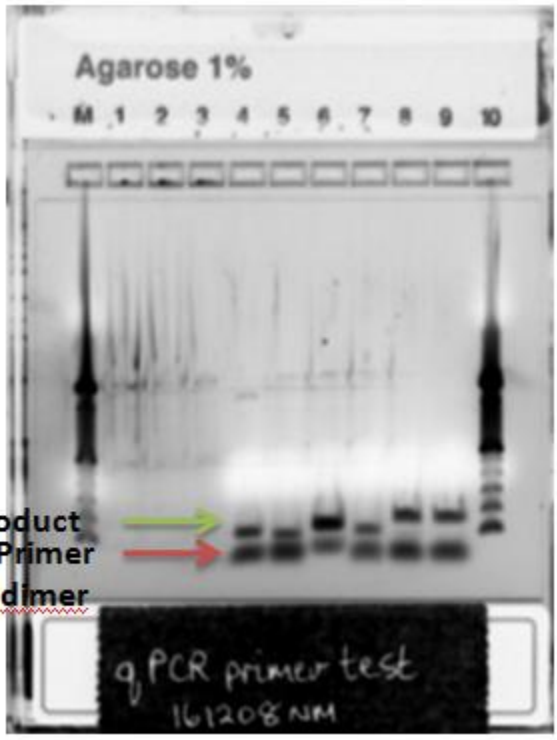
This is a non-controlled version.
****Note: controlled versions of this document are subject to change without notice.***

4.8. Select 'PROGRAM 7' for 10 min run.

4.9. Scan gel as per LIBPR.WorkInst.0012.

4.10. Capture the image and save it to an appropriate folder in R\\Library Core\Epigenomics.

An example of results may appear as seen in Figure 1:



Well	Sample
M	100bp Ladder
4	GAPDH_genic_H3K36me3
5	H3K27me3_hu_me
6	ZNF333_genic_H3K9me3
7	Six1_promoter_H3K27me3
8	GAPDH_hu
9	GAPDH_hu (current stock)
10	100bp Ladder

Figure 1 - An Example of Histone Modification Primer QC Gel Run

NB The top band shown in Figure 1 represents the PCR product (~150bp) and the lower bands represent dimerization of the primer pairs.

5. Discuss the results with APC/PC to ensure that the proper size band was amplified and that the primers have passed the QC.