qPCR of Native ChIP Libraries

I.   Purpose

To provide instructions on how to perform qPCR quality control (QC) testing to determine the quality of Native ChIP libraries.

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 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 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

Document Title	Document Number
CFX96 Touch™, CFX96 Touch Deep Well™, CFX Connect™, and CFX384 Touch™ Real-Time PCR Detection Systems Instruction Manual	IM.0274

VI.   Related Documents

Document Title	Document Number
Native ChIP In 100,000 Cells	LIBPR.0138
Nimbus-assisted 96-well PCR –enriched Library Construction for Illumina Sequencing	LIBPR.0137

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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	Fisher Scientific	270-058-53		✓
Disposable Lab Coat				
Wet ice	In house	N/A	N/A	N/A
DNA Away	Molecular Bioproducts	21-236-28		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	People Soft ID: 23878		✓
Large Kimwipes	Fisher Scientific	06-666-117		✓
Black ink permanent marker pen	VWR	52877-310		✓
Small Autoclave waste bags 10"X15"	Fisher Scientific	01-826-4		✓
Gilson P2 pipetman	Mandel	GF-44801		
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P20 pipetman	Mandel	GF23600		✓
Gilson P200 pipetman	Mandel	GF-23601		✓
Diamond Filter tips DFL10	Mandel	GF-F171203		✓
Diamond Filter tips DF30	Mandel	GF-F171303		✓
Diamond Filter tips DF200	Mandel	GF-F171503		✓
1.5 ml Eppendorf tube	Ambion	12400		✓
Eppendorf BenchTop Refrigerated Centrifuge 5810R	Fisher Scientific	5810R	✓	
Galaxy mini-centrifuge	VWR	52877-310		
Vortex				
Tube Rack				
Ultrapure Water	Gibson	10977		✓
KAPA SYBR FAST qPCR Master Mix (2x) Universal qPCR Master Mix	Roche	07959389001		✓
Hard-Shell Thin-Wall 384-Well Skirted PCR Plates	Biorad	HSP3865		✓
Microseal 'B' Adhesive Seals	Biorad	MSB1001		✓
CFX384 TOUCH RT PCR SYSTEM	Biorad	C1000 Touch	✓	
Clear Tape Sealer	Qiagen	19570		✓
Aluminum foil tape 3"x 60 yards	Scotch/3M	34000740		✓

Janus	Perkin Elmer	AJM8M01	✓	
Perkin Elmer Robo Rack 25 µL	D-Mark Biosciences	6000689	✓	
Perkin Elmer Robo Rack 175 µL Filtered Tips	D-Mark Biosciences	6000685	✓	
Bench Coat (Bench Protection Paper)	Fisher	12-007-186		✓
JANUS G3	Perkin Elmer	G3	✓	

IX. Procedure

Note: Since the template is a constructed library that has been PCR amplified, it can only be aliquoted in the Post-PCR Amplified designated area of the 6th floor lab and dispersed using the JANUS G3. The brew is prepared in the Blood Room and aliquoted using the JANUS liquid handler, both located in the 5th floor lab. If the JANUS instrument is out of order or fails QC, the brew is to be dispensed manually on the fifth floor. If the JANUS G3 instrument is out of order or fails QC, the template is to be dispensed manually on the 6th floor in the Post-PCR Amplified area.

1. Worksheet Set-Up

1.1. Create a New folder in \Library Core\Epigenomics\qPCR. Name the folder with the date the qPCR reaction will be run, in the format YYMMDD.

1.2. Save a copy of the qPCR worksheet template in the folder created in Step 1.1 with the date of the run. The template can be found in \Library Core\ Epigenomics\qPCR\Templates\Native ChIP_qPCR_worksheet_template.xls. This template contains multiple tabs for setting up the brews, the templates, the CFX Manager software, the JANUS set-up, JANUS G3 and for calculating the average CTs.

1.3. A couple of options are available for the plate and run set up as noted in the table below. Proceed to sections as documented for specific type of set up selected by the user.

Table 1: qPCR setup options

	Brew	Template	Sections to Follow
A	Manual	Manual	2,5-7
B	JANUS	JANUS G3	3-7

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2. Manual Set Up (Brew and Template)

2.1. Under the qPCR Set Up tab in the qPCR worksheet, select 'Manual Set Up' from the drop down menu of the Brew Dispense Method highlighted in yellow. Fill out the name of the project and the date.

2.2. Enter the following information under the yellow highlighted headings, i.e. see Figure 1 and enter IP ID info where denoted (this can be copied and pasted from the plate layout info sheet):

      A. Well ID of template (Library plate)
      B. Sample name
      C. Antibody type

As the table is filled out, the correct primers are assigned to each sample. See Appendix A to ensure/double check that the correct positive and negative primer is associated with each antibody. Ensure each sample for each antibody has an associated Input as a control.

Figure 1: The qPCR Set Up tab in the qPCR worksheet

2.3. The brew calculator for each primer set self populates as the IP information is entered as well. See Figure 2 for an example.

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Figure 2: qPCR brew calculator

2.4. Save the file and print out a copy of the brew set up.

2.5. Click on the Plate Layout tab. The plate layout self populates as the sample IDs are entered. Save the file and print out a copy.

2.6. Click on the CFX384 Set-Up Table tab to set up the qPCR run on the instrument. The excel sheet auto populates as the library ID info was filled in. Copy the entire yellow highlighted table (all 384 rows). Open a new excel file and paste (paste special-values) the table. Save as a CSV (comma delimited) file. Name it with the date of the run into the same folder created in Step 1.1. Proceed to Step 5 and Appendix C to setting up CFX software.

2.7. Retrieve the 10nM normalized Native ChIP library constructed plate. This is the template source plate. Allow the template plate to thaw on ice in the post PCR area on the 6th floor while preparing and dispensing the brew into the qPCR plate.

2.8. Blood Room Prep (5th Floor):

   a. Retrieve a 384-well Biorad Hardshell PCR plate from the 6th floor (located by the CFX384 PCR Instrument). Handle it with gloves and seal it temporarily until ready to use. Transfer it to the 5th floor Blood Room.
   b. Put on a clean pair of gloves and a lab coat.
   c. Turn on the BSC and follow guidelines of the room.
   d. Wipe down the work bench, small equipment, with DNA Away and 70% ethanol.
   e. Leave hood on for 10 minutes minimum before using.
   f. Change gloves.

2.9. Preparing qPCR Brew Mixes:

a. Retrieve the 10µM Forward Primer and Reverse Primer mixes from the -20ºC freezer and place on a rack to thaw in the laminar flow hood in the 5th floor blood room. See Appendix B for instructions on how to prepare 10µM Primers mixes from 100µM stocks.

b. Retrieve the KAPA SYBR FAST qPCR Master Mix (2x) from the -20ºC freezer (located in the PCR Clean Room) and place in the hood. Please note that the Master Mix contains SYBr green, and hence it is light sensitive. It is advisable to turn off the light of the room but leave the BSC light on.

c. The brews can be set up at room temp. In a Diamed 1.5mL tube, prepare the qPCR brew according to the brew calculator prepared in Step 2.3 using ultrapure water.

d. Invert the tube several times to mix and pulse-spin.

e. Return the primer stocks and the SYBr Green Master Mix to their respective -20°C freezers.

2.10. For manual dispersal of brew, use a P10 to pipette 9µL of the brew into the wells of a 384-well plate according to the plate layout prepared in Step 2.5. Do not dispense brew past the first ejection of the pipette as this will introduce bubbles in the well which will impede with the qPCR run.

2.11. For manual addition of the template, add 1µL of template following the plate layout set up sheet. Pipette up and down twice. This can be done on the bench at room temperature in the post PCR area on the 6th floor.

2.12. Cover the plate with a temporary seal and spin down the plate at 1000rpm, at 4ºC, for 1 minute.

2.13. Proceed to Step 6 for loading and running the qPCR machine or to Step 5 if the CFX software has not yet been set up.

3. Automated Brew Addition Using the JANUS Instrument

3.1. Referring to the worksheet in Step 1.1. Under the qPCR Set Up Tab, select '1. JANUS setup' from the Brew Dispense Method drop down menu. Fill out the name of the project and the date as well.

3.2. Enter the column number of the waste plate in the red box of the qPCR Set Up Sheet. The waste plate is a 384-well plate where waste brew will be dispensed. This plate can be used

for multiple runs. Mark off the particular column number on the plate after each use to ensure that the same column will not be used again.

3.3. Check the brew layout on the JANUS under Source Rack Layout tab. This is auto-populated when the information is entered on the qPCR Set-Up sheet tab. Save the file and print a copy as a reference for the actual set up.

3.4. The JANUS instrument runs off of a CSV file. Create a CSV run file for JANUS.

3.5. Click on the 'JANUS CSV' tab of the qPCR worksheet. Refresh the page to allow the sheet to self populate.

3.6. The current file type is an "Excel Workbook". The file type needs to be changed to "CSV (comma delimited)" in order for the JANUS instrument to recognize it. Copy the yellow highlighted columns and paste them to a NEW excel file. It is important to 'Paste Special' with 'Values' selected.

3.7. Save the file as type "CSV (comma delimited)". Name the file with the date of the qPCR run with the user's initials and save the file in \LibraryCore\Epigenomics\qPCR\YYMMDD folder that is specific to that qPCR run, created in Step 1.1.

3.8. Prepare the qPCR Brew on the 5th floor PCR free room as described in Steps 2.7-2.9.

3.9. Transport the brew mixes to the JANUS area in a tube rack.

3.10. Log into the computer linked to the instrument. Password is 'barracuda'.

3.11. Click on the JANUS Application Assistant icon on the desktop.

3.12. Once the software is initialized, the type of protocol to be run is to be selected. Select "LibCore - ChIP qPCR Brew Set Up". See Figure 3.

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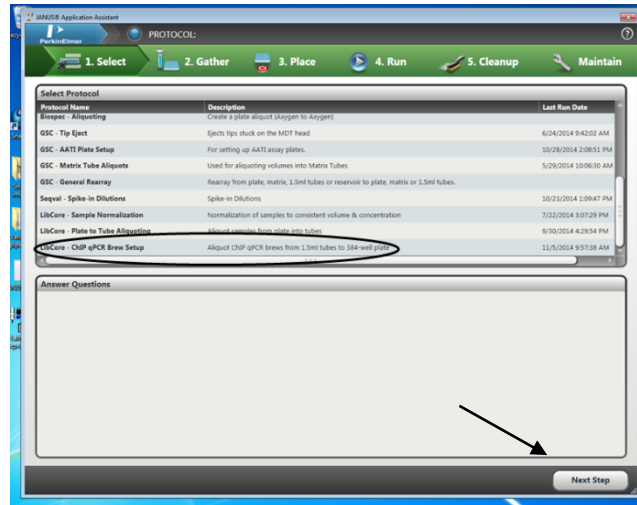

Figure 3: Protocol selection window

3.13. Click 'Next Step' on the lower right corner after the protocol has been selected.

3.14. "Step 2. Gather" prompts the user to assemble all accessories/parts and tips for the brew distribution (Figure 4). It is not necessary to gather all the parts at this time. Click 'Next Step'.
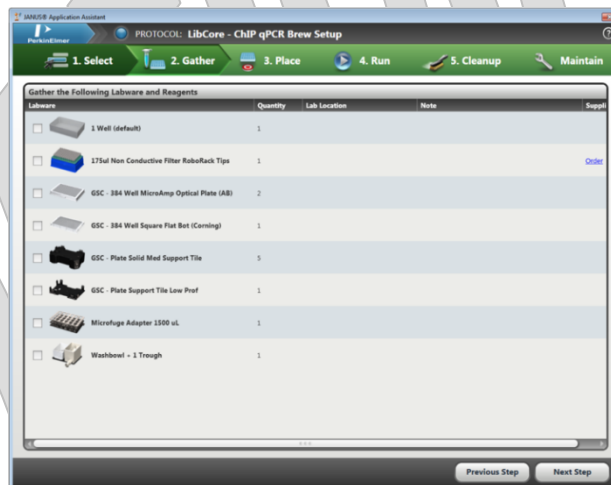

Figure 4: Accessory and part requirements for brew distribution

3.15. Empty the water waste container if it is full (Figure 5).

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Figure 5: Waste water container

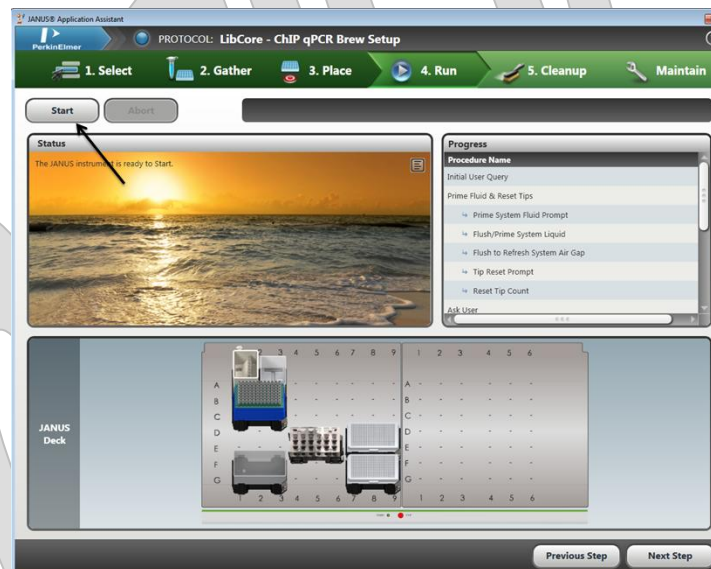3.16. Click 'Start' as indicated by the arrow in Figure 6.

Figure 6: qPCR brew setup program start

3.17. The next window will appear as illustrated in Figure 7. If the user is the first person to use the instrument in the day, it is necessary to flush the lines with water and also to ensure no bubbles are present in the capillary lines. Click the "Check if bubbles are visible in liquid lines. If so, enable this for a flush/prime of system fluid (a) and ensure the 'Prime System

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Fluid Prompt' is highlighted. Note the arrows in Figure 7 as indicators of the correct steps. Click 'Continue' icon once ready (b).
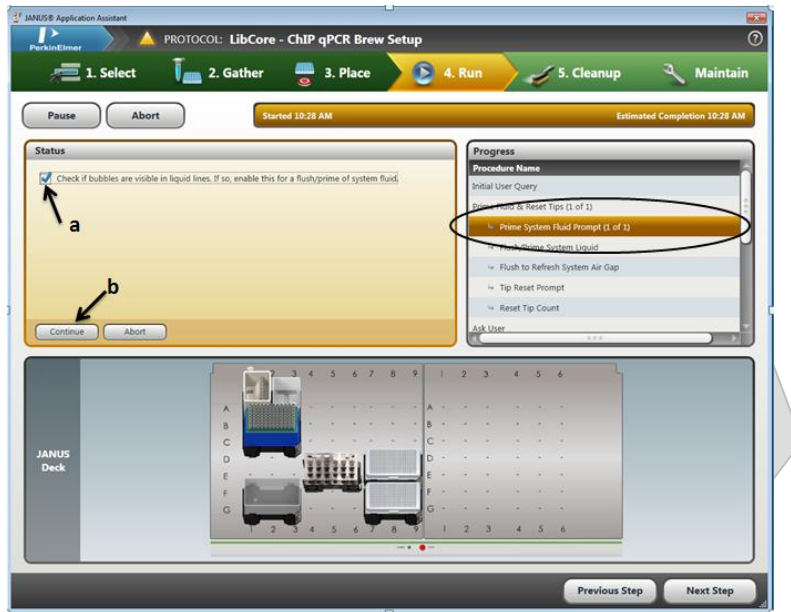

Figure 7: Capillary line bubble elimination step

3.18. Once the lines have been flushed, the user will be prompted to enter the column number containing a full column (8) of pipette tips in the green 175μL tip box. See Figure 8A for an example of the pipette tip box orientation on the deck. See Figure 8B for an image of the software screen.
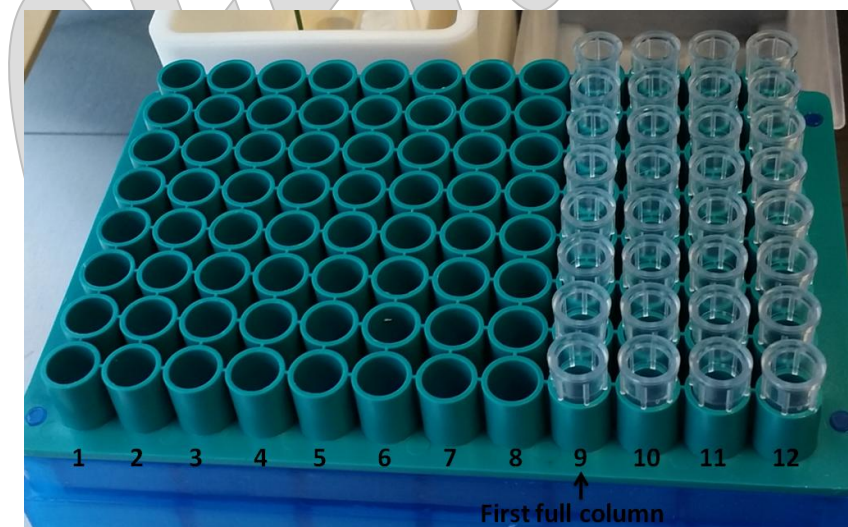

Figure 8A: Pipette tip box orientation

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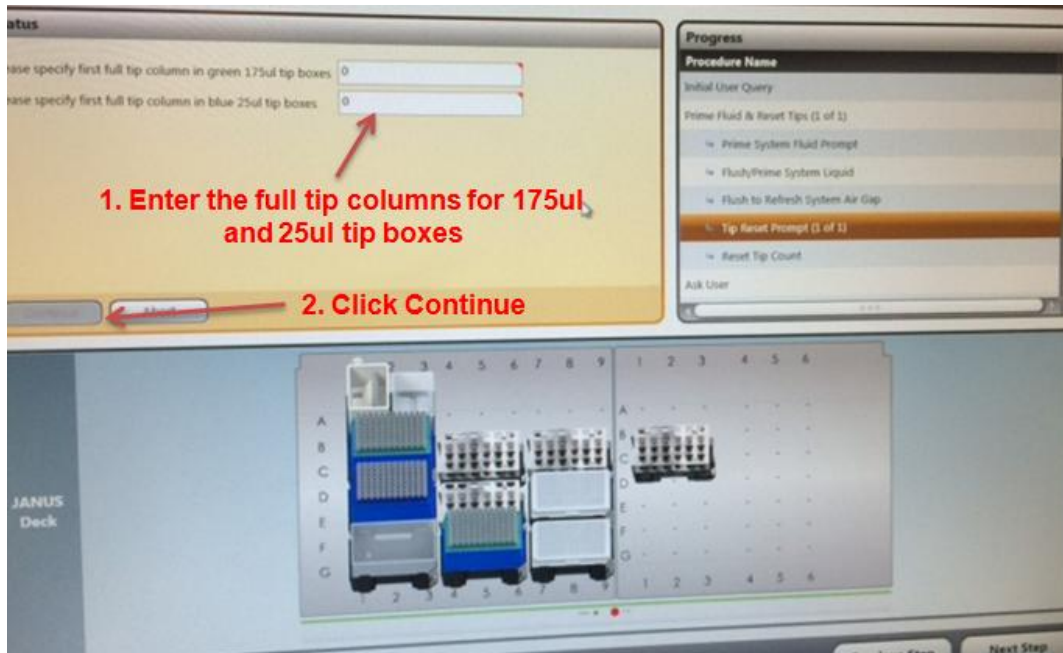

Figure 8B: Pipette tip box setup

3.19. The orientation of the tips within the tip box is important, more specifically the horizontal (column) orientation of the tips. If using a full tip box, enter 1 in Figure 8B. This indicates that the entire tip box is full of tips. If using a partial tip box, position the box so all empty columns are to the left. The tip box shown in Figure 8A has column 9 as the first full tip column. If there are a few tips mixing in sporadic spots, re-position them so no partial columns are present. **ALL COLUMNS must be complete with 8 tips as shown above, AS should all columns following the column number entered.**

3.20. Click 'Continue' once the first full tip column is entered for both 25µL and 175µL tip boxes.

3.21. Click "Browse…" and select the CSV file that was created in Step 3.8. Click 'Open' once the CSV file has been selected (Figure 9).

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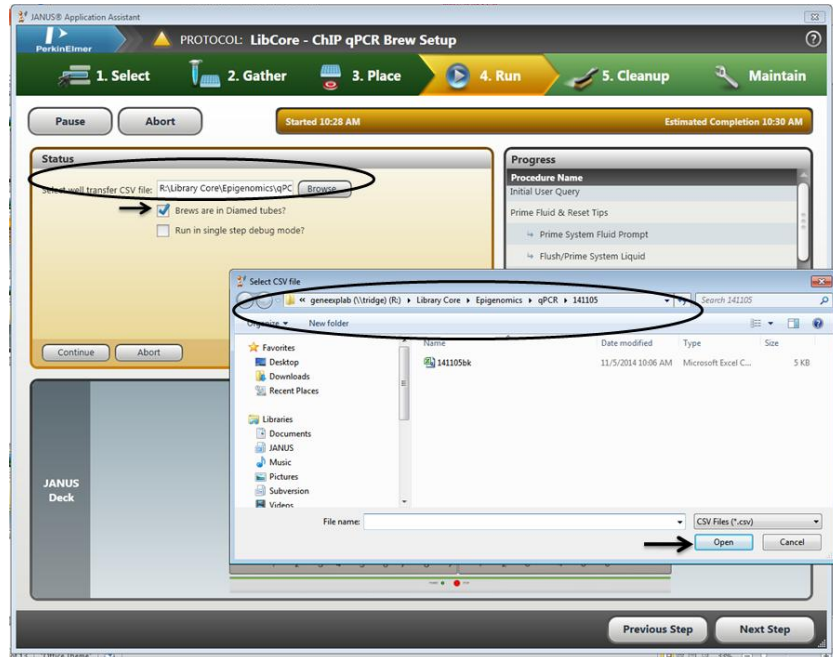

Figure 9: Selecting the CSV file

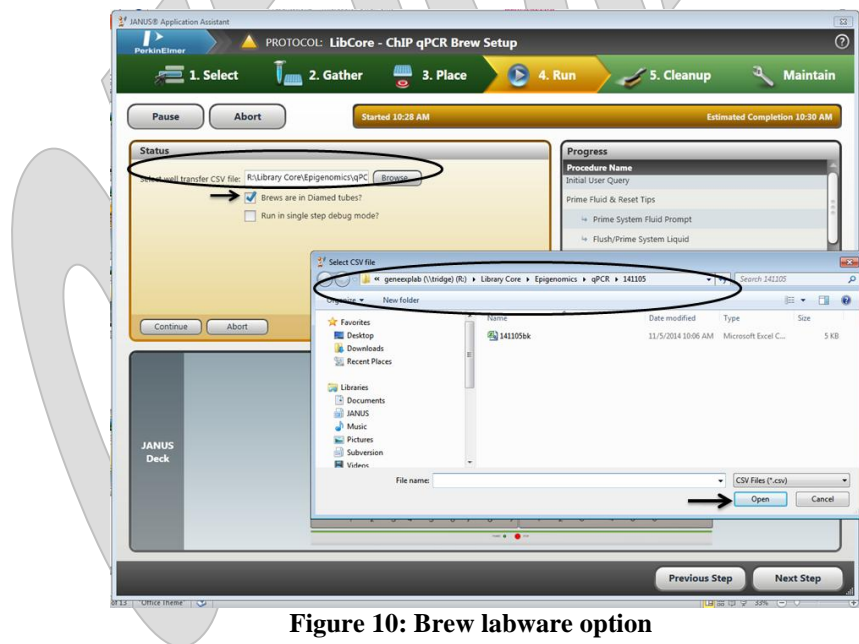3.22. Click on the checkbox, as indicated in Figure 10 for the brews are made in Diamed tubes.

Figure 10: Brew labware option

3.23. Click "Continue" (Figure 10)

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3.24. Set up the deck as illustrated in Figure 11. The deck illustration informs the user of the type of base to be used as well as the location on the JANUS, it has been placed in. Ensure the correct type of base is being used. Move the pop up window out of the way if it is blocking the set up view. **Clicking OK will initiate the run.** Ensure the bases for the plates and brew tubes are in the correct orientation and position. This is easily determined as the deck of the instrument is a grid. The grid positions can be visualized through the base (Figure 12).
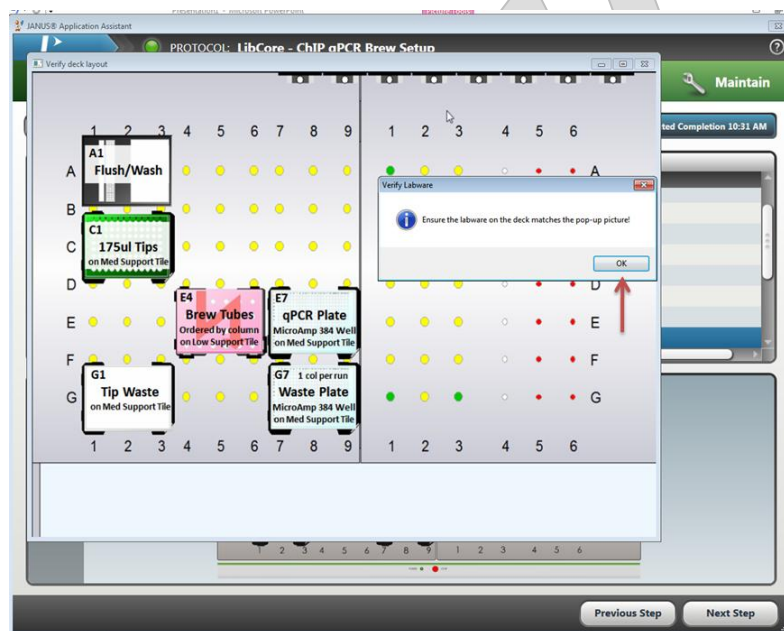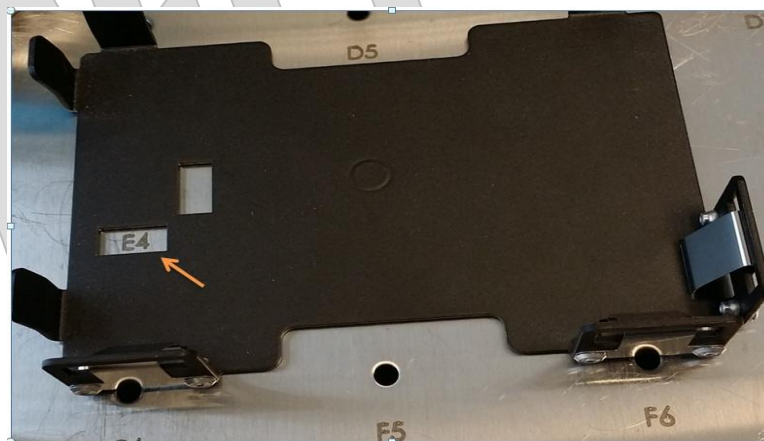

Figure 11: Deck layout

Figure 12: Correct grid position on the base

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3.25. Snap the 384 well plates, including the waste plate into the base with Well A1 in the top left corner. Be sure that the plate is snug but not buckling at the corners. Use a mini screwdriver to adjust the tension to make sure the plates are secure. See Figure 13 for where to adjust the tension, indicated by the red arrows.

Figure 13: Adjusting plate tension

3.26. Ensure the brew tubes are positioned firmly into place including the lids (Figure 14A). Note the tube orientation as well. Brew tubes are loaded as per the Source Rack Layout tab in the qPCR worksheet (Figure 14B).

Figure 14A: Brew tube position

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Brews		1	2	3
	A	GAPDH_hu		
	B	ZNF333_genic_H3K9me3_hu		
	C	H3K27me3_hu_mu		
	D	genic_H3K36me3_hu		

Figure 14B: The Source Rack Layout tab in the qPCR worksheet

3.27. Ensure the deck is set up correctly according to the deck layout illustrated in the JANUS Assistant software (Figure 11). Figure 15, is a snap shot of a correct layout.
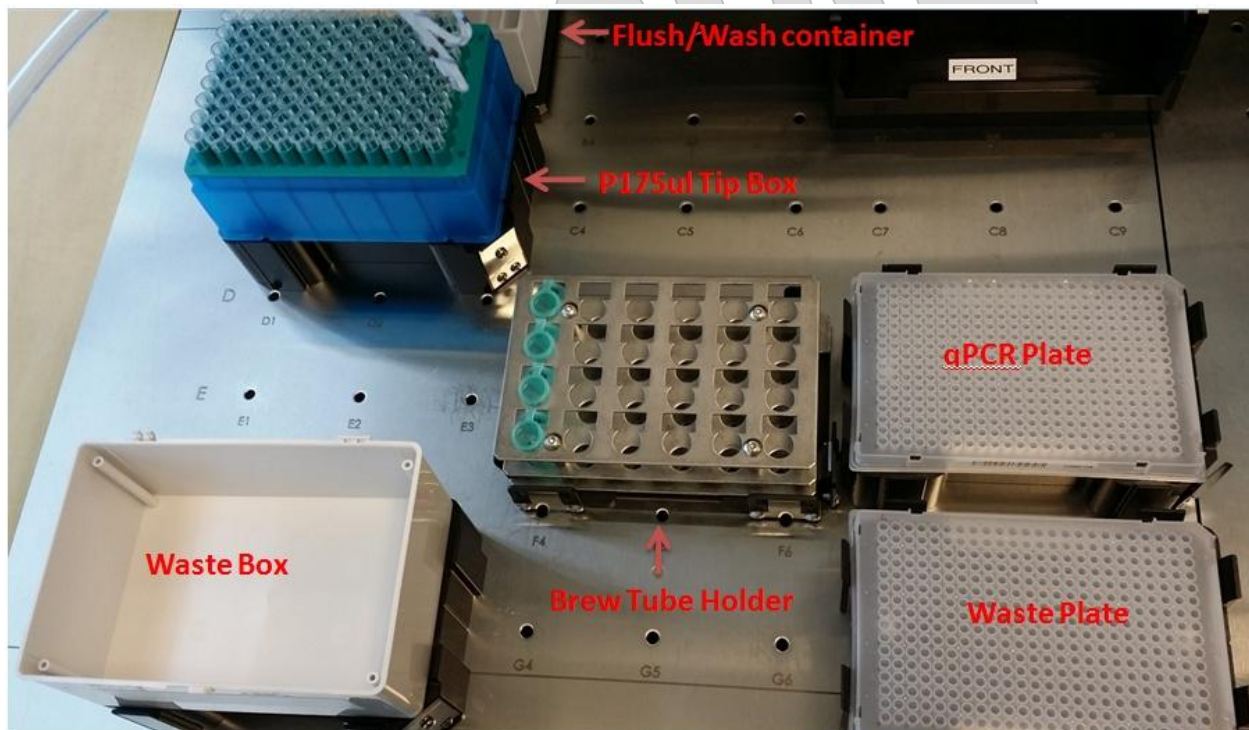

Figure 15: Snap shot of a correct setup on the deck

3.28. Once the deck is laid out, click the OK button on the pop up window to initialize the run.

3.29. Once the instrument has completed aliquoting the brews and templates, seal the plate with a tape seal.

3.30. Discard the brew tubes, and waste tips.

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3.31. Mark the column used for waste with an 'X' on the waste plate to ensure that is not reused again. Cover it with a tape seal for future use. Place it on a shelf next to the JANUS.

3.32. Close the JANUS assistant application software and log out of the computer.

3.33. Transport the plate to the 6th floor Post-PCR area.

3.34. Spin down the plate at 1500rpm, for 1min, at 4ºC.

3.35. Proceed to Step 4 for automated addition of template using JANUS G3.

4. Automated Addition of the Template using JANUS G3

4.1. The JANUS G3 instrument will be used to add 1μL the template to the brew plate from step 3.35.

4.2. To set up the software, refer to the qPCR worksheet created in Steps 1. Once the worksheet in the qPCR-Set Up tab is filled out, the information to load the JANUS G3 instrument will self-populate under the JANUS G3 CSV tab.

4.3. Click on the JANUS G3 CSV tab.

4.4. Copy the yellow highlighted columns and paste special 'values' into a new excel file. Save the file with the date of the run and user initials as a CSV file, in the same folder created in Step 1.1.

4.5. Spin down thawed 10nM template source plate from section 2.7, at 1500rpm, for 1 minute, at 4ºC.

4.6. Log into the desktop linked to JANUS G3 with the password 'janus'.

4.7. Start the JANUS G3 Software by double clicking the JANUS Application icon on the desktop.

4.8. Empty the waste bottle and top up the source water bottle, both located near/under the bench, with distilled water as, and if, needed. Never use tap water.

4.9. Once the software is initialized, select the protocol to be run (highlighted in black) i.e. "LibCore - G3 qPCR Sample Addition (see Figure 16).

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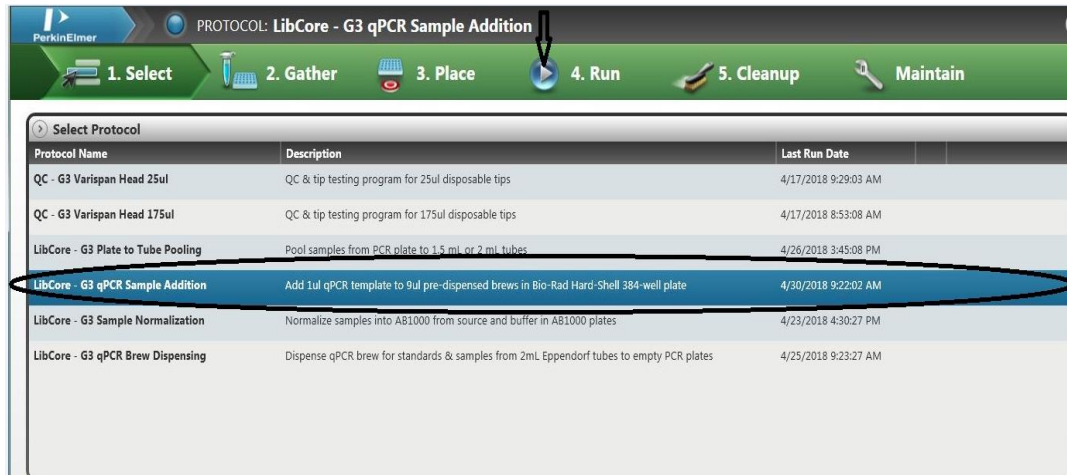

Figure 16: Selecting the protocol for qPCR sample addition

4.10. Click on the run button. Another window appears on the screen. Click on the start icon to begin the protocol as shown in Figure 17.
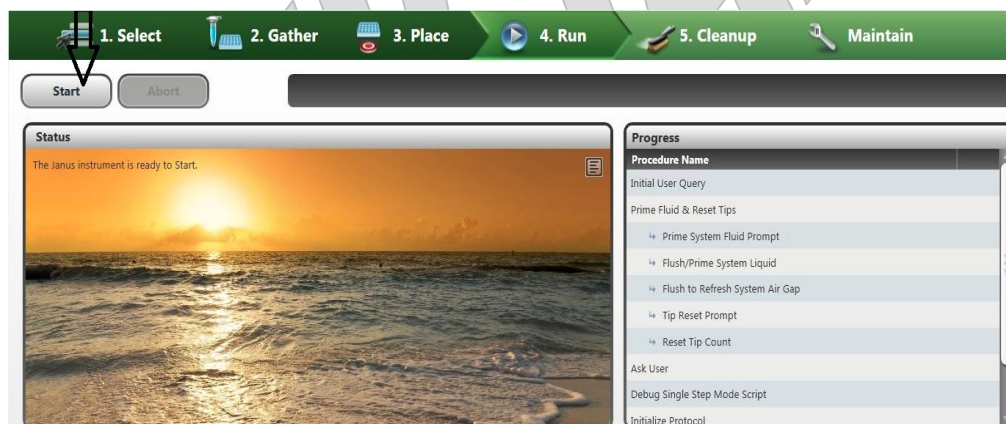

Figure 17: The protocol initiation

4.11. The next window will appear as illustrated in Figure 18. If the user is the first person to use the instrument in the day, it is necessary to flush the lines with water and also to ensure that there are no bubbles present in the capillary lines. Click the "Check if bubbles are visible in liquid lines. If so, enable this for a flush/prime of system fluid (a) and ensure the 'Prime System Fluid Prompt' is highlighted. Note the arrows in Figure 18 as indicators of the correct steps to follow. Click 'Continue' icon once ready (b) to proceed to the next step.

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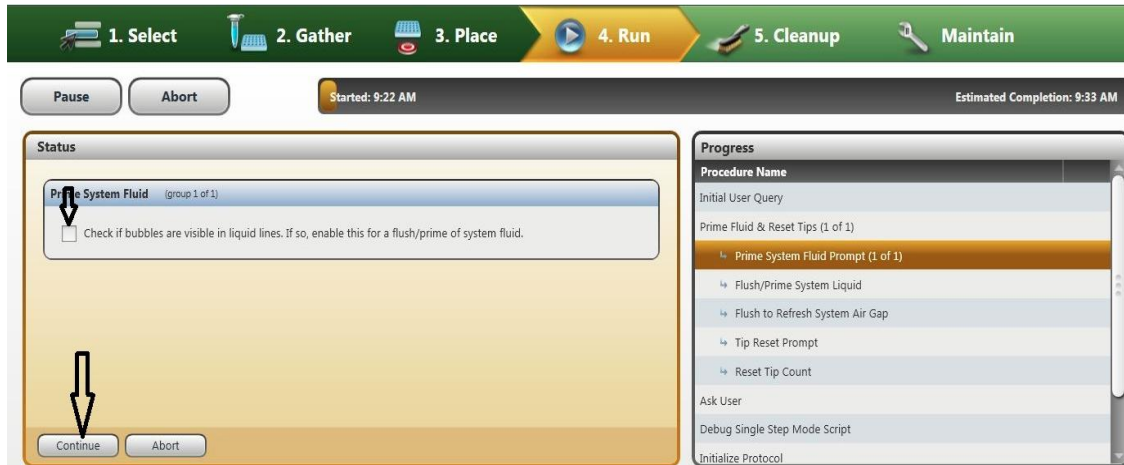

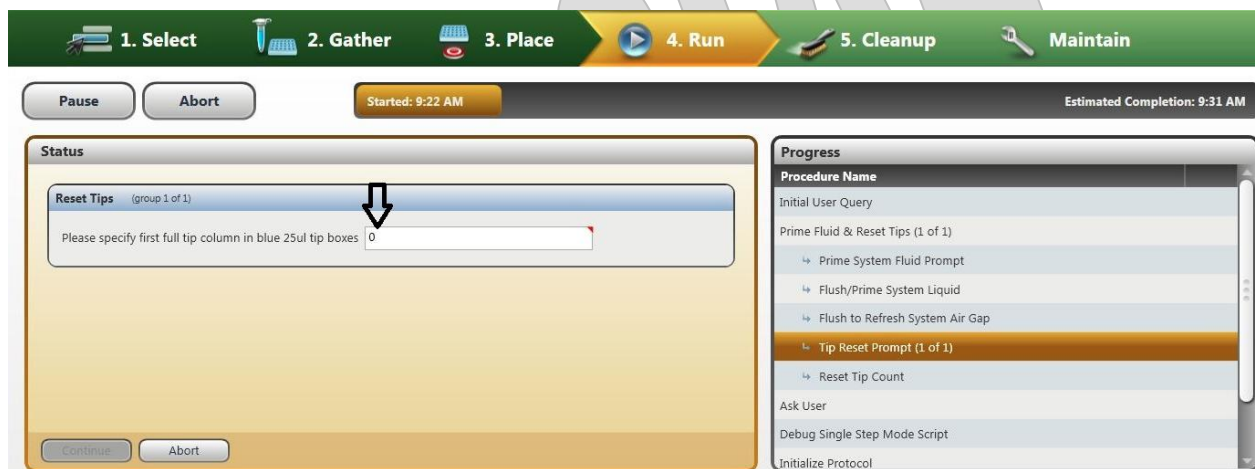**Figure 18: Capillary line bubble elimination step**

Figure 19: Defining the first full column of tips

4.12. Enter the first full column of tips in the next popup window, as shown in Figure 19. If you indicate 1 this means that the entire tip box is full (see Figure 20).
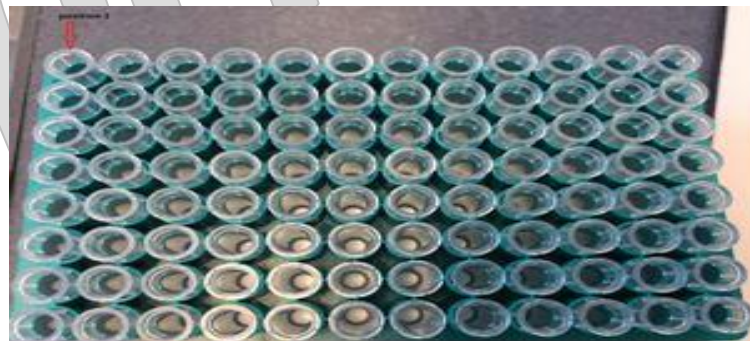

Figure 20: Full tip box

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4.13. If using a partial tip box, position the box such that all empty columns are to the left. If there are a few tips mixing in sporadic positions, re-position them so there are no partial columns present. **ALL COLUMNS must be full with 8 tips AND so should all columns following the column number entered.**

4.14. Click 'Continue' once the first full tip column number is entered.
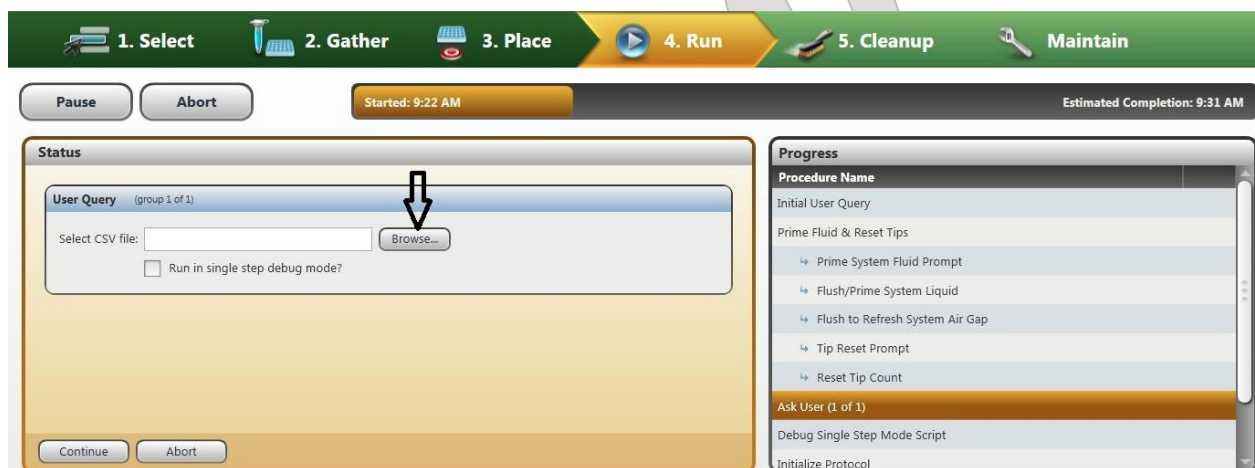
4.15. Another popup window appears as shown in Figure 21.

Figure 21: Selecting the CSV file

4.16. Click "Browse…" and select the CSV file that was created in Step 4.4. Click 'Open' once the CSV file has been selected.

4.17. Set up the deck as illustrated in Figure 22. Move the pop up window out of the way if blocking the set up view. **Clicking OK will initiate the run.**

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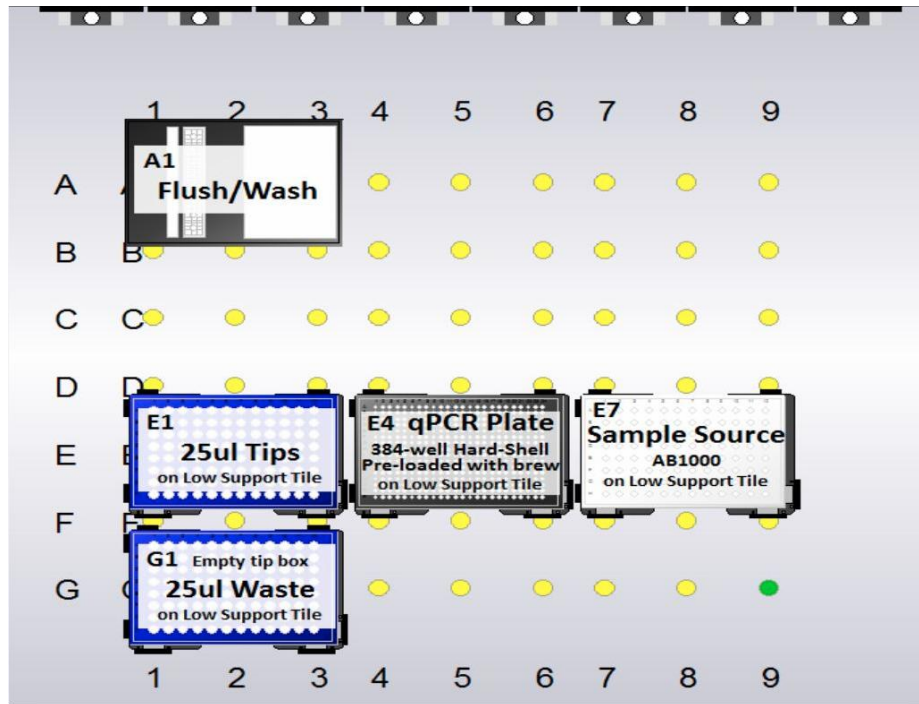

Figure 22: Deck layout

4.18. Seal the template and qPCR plates with a tape seal. Quickly spin down both plates. Foil seal the template plate and place back in the -20ºC freezer.

4.19. Proceed to Step 6 to load the plate on the qPCR machine or to Step 5 if the CFX software has not yet been set up.

5. Setting up the CFX Software

5.1. Double click on the CFX Manager Software icon on the desktop, or select it from the programs list.

5.2. Select the correct instrument type (CFX 384) and the run type as 'User-defined as shown in the Figure 23 below.
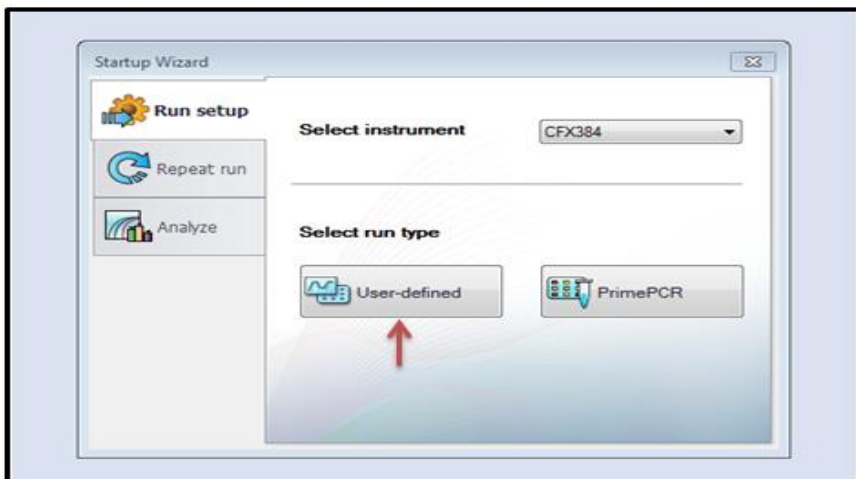
Figure 23: Selecting run type

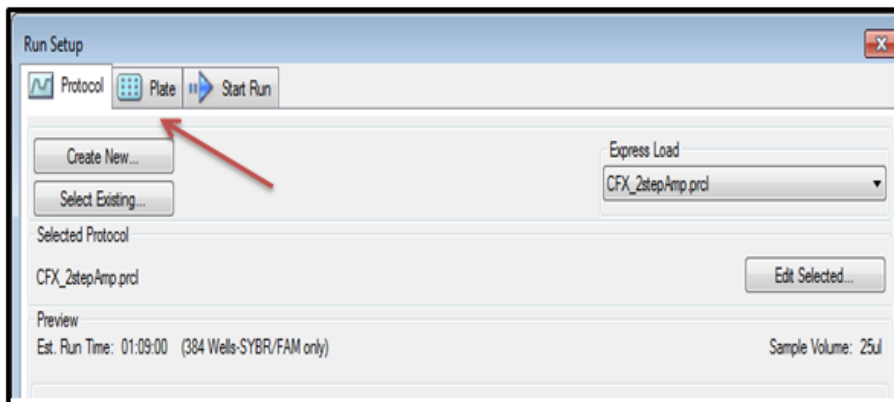5.3. Select the Plate tab, as shown in Figure 24, to set up the plate layout.

Figure 24: Setting up the plate layout

5.4. Ensure the type of plate is 'Quick Plate_384 wells_SYBR Only.pltd' under the Express Load tab. See Figure 25. Click on Edit Selected to upload the plate layout.

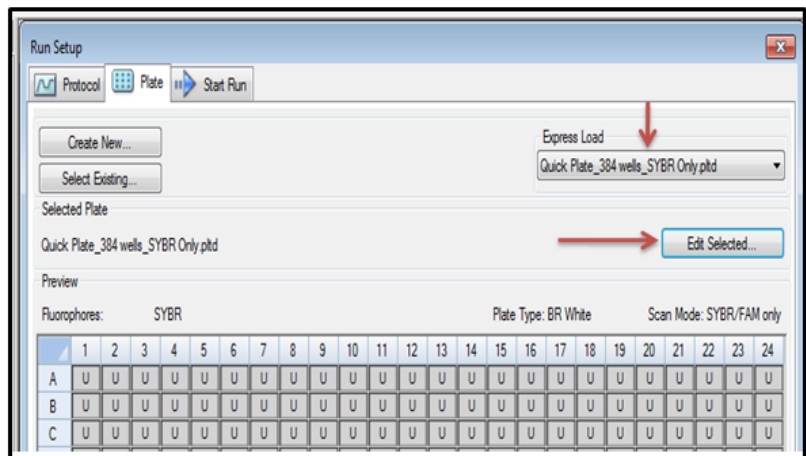*Non-Controlled Version* *Note: Controlled Versions of this document are subject to change without notice*

Figure 25: Selecting plate type

5.5. In the next window, click on 'Spreadsheet View/Importer' as shown in Figure 26.
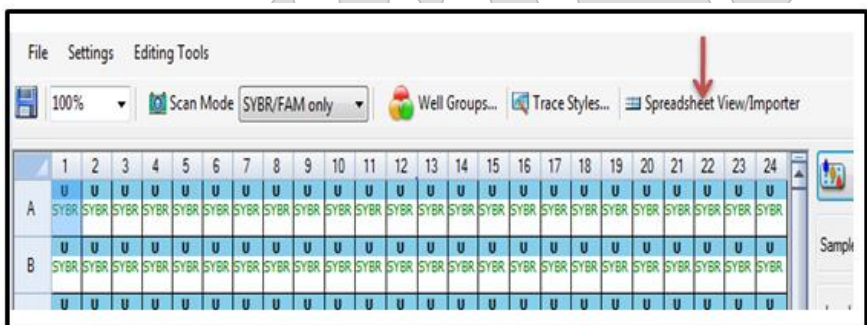

Figure 26: Spreadsheet View/Importer

5.6. In the next window click on 'Import' as shown in Figure 27.

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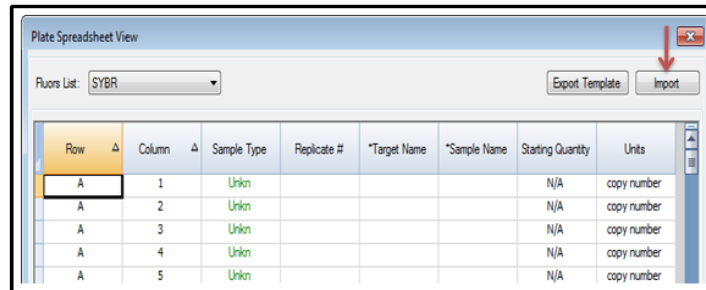

Figure 27: Importing the spreadsheet

5.7. Import the CSV file that was created in Step 2.6 as illustrated in Figure 28. Select the file and click OPEN at the bottom of the window.
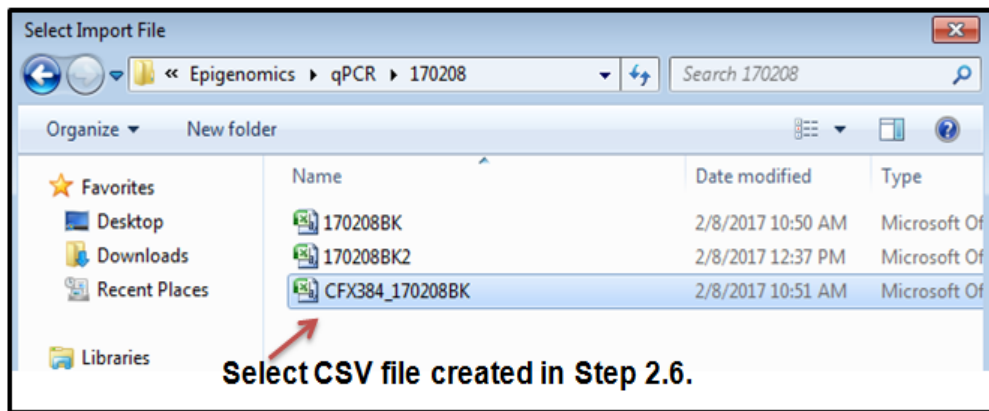

Figure 28: Selecting and importing the spreadsheet

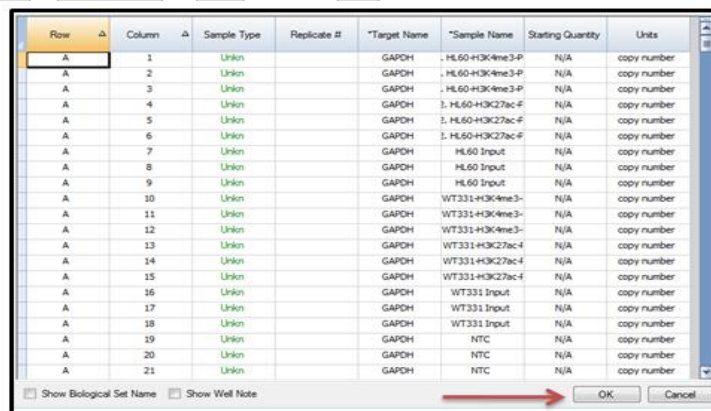5.8. The table will populate as shown in Figure 29. Click OK.

Figure 29: Imported plate layout

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5.9. It is important to clear all the wells that are not being used. Using CTRL+left click to select those wells that are empty. Click on 'Clear Wells' as shown in Figure 30 below.
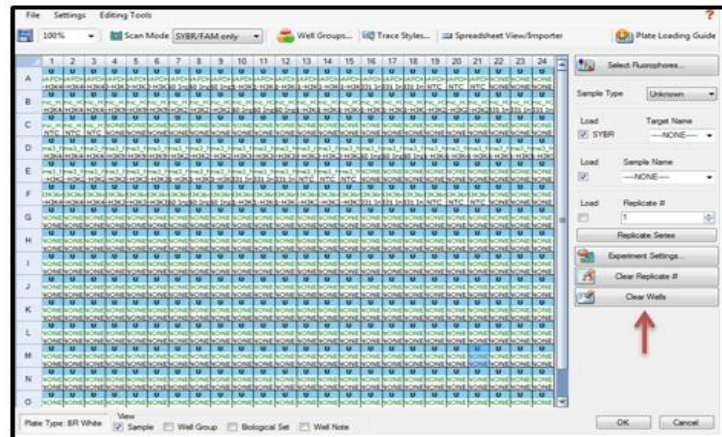

Figure 30: Clearing empty wells

5.10. Select the No Template Control (NTC) wells using the same method described in Step 4.9. Click on the Sample Type dropdown menu and select NTC as described in Figure 31.
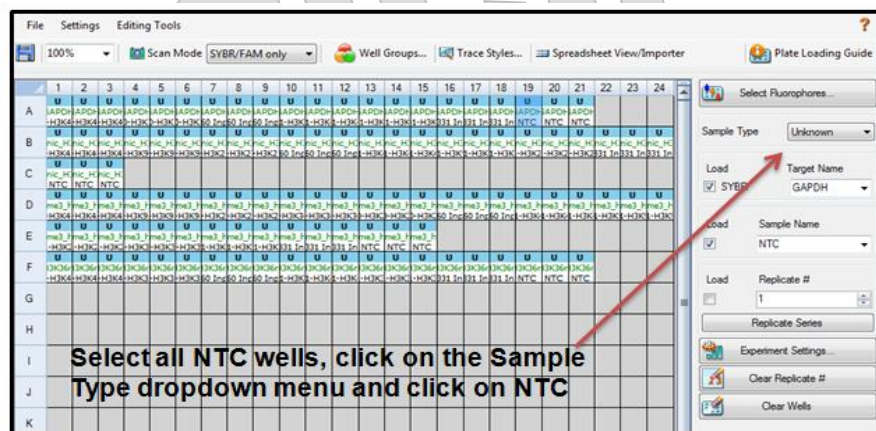

Figure 31: Defining no template control wells

5.11. The NTC wells will be highlighted in yellow and the Sample Type will change for 'U' to 'T' as shown in Figure 32. Click 'OK' once completed.

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Figure 32: Assigned no template control well

5.12. Name the file (pltd file type) with the date and user initials. Save the file in the same folder created in Step 1.1.

5.13. See Appendix C for setting up the cycling parameters under a Protocol file. The software runs off the protocol file and no data is collected on it. Therefore it is ok to create a protocol file template and load it for every run as the cycling parameters are constant.

6. Loading the Plate and Running qPCR

6.1. Ensure the plate has been spun down and it has been sealed with a Microseal 'B' adhesive seal. Avoid handling the top of the plate.

6.2. Drag plate (pltd) file created in Step 5 onto the desktop of the computer associated to the qPCR machine and open it.

6.3. Click on the Protocol tab and load Native ChIP Cycling Parameters file previously created as described in Appendix C.

6.4. Go the Start Run tab and select 'Open Lid' and place the 384-well optical plate inside the qPCR machine with well A1 at the top left.

6.5. Ensure that the lid is unobstructed, and select 'Close Lid'

6.6. Click on the Start Run button. You will be prompted for the run file name and destination. Enter 'raw data_DDMMYY' and save the file to the desktop.

6.7. Once the run is complete, drag the raw data file to the folder created in Step 1.1.

6.8. Remove the plate. Store at 4ºC until Supervisor has approved results. Shut down the qPCR machine.

7. Data Analysis

7.1. Open the raw data file and click on the Quantification Tab.

7.2. Set the threshold line of the Amplification Plot in the Log Scale. The threshold line should be set 3/4 the way up the linear portion of the curve before the run plateaus off. See Figure 33 for an example. Consult with supervisor if unsure.
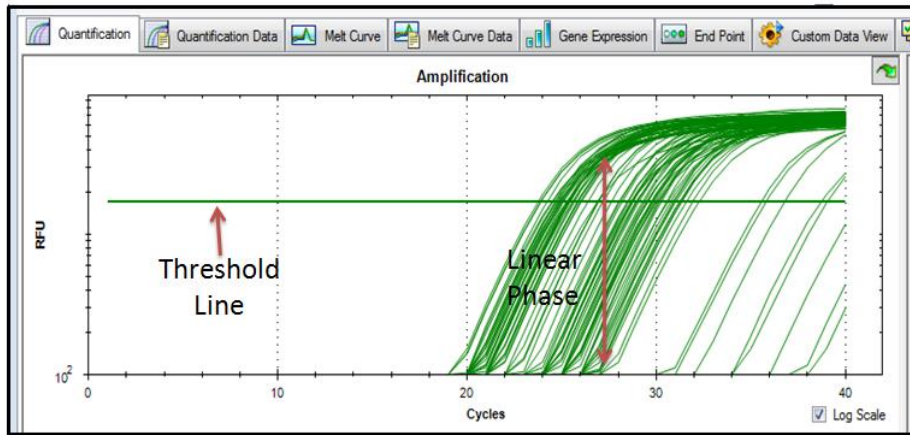

Figure 33: Threshold line example

7.3. Click on the Melt Curve Tab and scan the Melt Peak graph. A cluster of peaks at certain temperature represents amplification of a sample(s) at that particular target.

7.4. Click on the Quantification Data tab. Highlight and copy the table up to the Cq column. Paste special 'Values' into the Sheet 1 tab of the excel file created in Step 1.1.

7.5. Copy and paste the data by columns into the Average Cts Tab sheet. Ensure the correct information is associated to each well. The triplicate Ct values for each sample will auto average and standard deviation will be calculated. Print off the data.

7.6. Fold Enrichment represents the IP signal as the fold increase in signal relative to the background signal. Calculate fold enrichment vs. Input for both positive and negative targets for each sample as follows:

$$\text{Fold Enrichment} = 2^{\text{(Average Ct of Input at Mark-Average Ct of Sample at Mark)}}$$

7.7. Tabulate the results, for example as shown in Figure 34 below.

Library	Fold Enrichment vs Input at Target	
	Postive Target	Negative Target
HL60 H3K4me1	11.26	0.15
HL60 H3K4me3	67.49	11.18
HL60 H3K9me3	31.05	8.84
HL60 H3K27me3	11.90	0.41
HL60 H3K36me3	12.64	1.23
HL60 H3K27ac	189.58	0.01
WT331 H3K4me1	5.75	0.22
WT331H3K4me3	65.19	7.05
WT331 H3K9me3	36.84	2.57
WT331 H3K27me3	5.02	4.75
WT331 H3K36me3	2.21	1.49
WT331H3K27ac	48.28	0.59

Figure 34: Tabulated results

7.8. Email supervisors the results.

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Appendix A-Primer Targets and Sequences

Antibody	Positive Target-HUMAN	Negative Target-HUMAN	Positive Target-MOUSE	Negative Target-MOUSE
H3K4me1	genic_H3K36me3_hu	ZNF333_genic_H3K9me3_hu	GAPDH2	H3K9me3_mu
H3K4me3	GAPDH	H3K27me3_hu_mu	GAPDH2	H3K9me3_mu
H3K9me3	ZNF333_genic_H3K9me3_hu	H3K27me3_hu_mu	H3K9me3_mu	H3K27me3_hu_mu
H3K27me3	H3K27me3_hu_mu	ZNF333_genic_H3K9me3_hu	H3K27me3_hu_mu	H3K9me3_mu
H3K36me3	genic_H3K36me3_hu	H3K27me3_hu_mu	GAPDH2	H3K27me3_hu_mu
H3K27ac	GAPDH	H3K27me3_hu_mu	GAPDH2	H3K9me3_mu

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

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Appendix B - Diluting and Storing Primer Stocks

1. Follow LIBPR.0084 Step 2 to resuspend the lyphilized primer to a 100μM stock concentration.

2. Dilute the 100μM stock to a working concentration of 10μM. 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.

3. Make 200μL of working stock by adding 20μL of forward primer and 20μL of 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.

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

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Appendix C-Setting Up Cycling Parameters on CFX384 Software

1. Open the CFX Manager software.

2. Select the instrument as CFX384 and click on Run Type as User-defined.

3. Click on Create New.

4. Using the software tools in the next window, create the following cycling parameters:

Stage	Temperature (ºC)	Time	nº Cycles
1	95	10:00	1
2	95	0:10	40
2	59	0:30	40
2	72	0:30	40
3	72	5:00	1
4	95	0:15	1
4	59	0:15	1
4	95	0:15	1

Note: Stage 4 is the dissociation/melt curve.

5. Set the sample volume to 10 μL.

6. Below a snapshot of the cycling parameters:
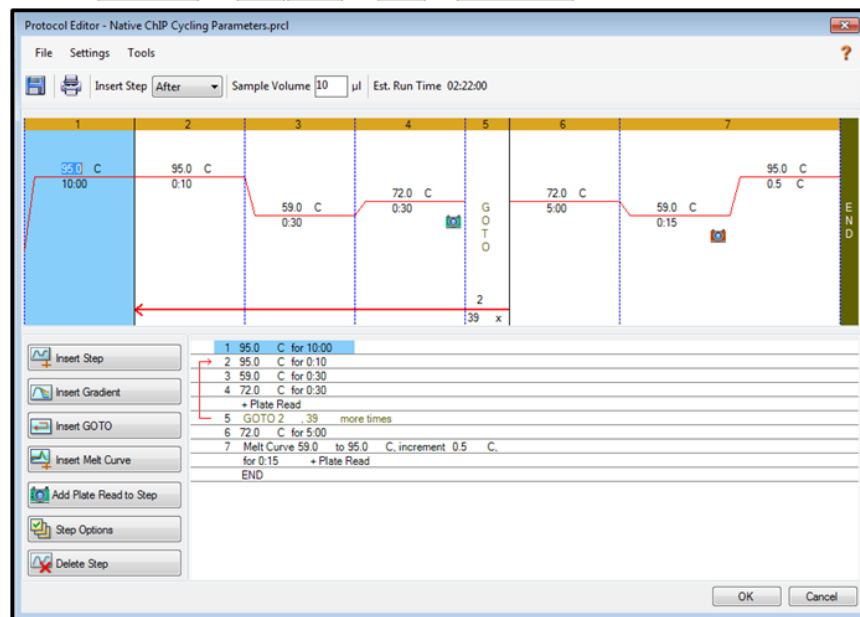
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7. Click OK once done.

8. A protocol template file called Native ChIP Cycling Parameters has been created and can be found in \Library Core\Epigenomics\qPCR\Templates.