Standard Operating Procedure (SOP) for Normalization of Whole Genome Amplification Samples

I. SCOPE AND PURPOSE

Whole genome amplified (WGA) samples are shipped from Qiagen at varying concentrations. Because the samples contain the raw components of the amplification reaction, they cannot be accurately quantified via spectrophotometry and the normalization procedure must be modified accordingly.

Any deviation from this Standard Operating Procedure (SOP) will be noted on the WGA documentation sheet, normalization sheet, and the sample record in LabVantage. The number of the samples affected by the deviation will be noted as well.

This procedure is meant to establish a protocol to accurately and reproducibly normalize the whole genome amplification products received from Qiagen. There are two methods; an automated method and a manual method to normalize the samples. If the robot is down for repair the manual method may be used to normalize and dilute the samples.

II. PROCEDURE

A. Safety precautions

1. Use universal safety precautions, including the use personal protective equipment (PPE).

B. Required Equipment, Supplies and Reagents

1. "WGA plate" - WGA plate received from Qiagen
2. Water, RNase- and DNase-free (Sigma, #W4502)
3. Centrifuge capable of spinning 96 well plates.
4. Non-skirted 96-well plate (Fisher, AB-0600L)

For the Automated method:

5. Biomek FXp
6. Tip barrier Span8 P250 (VWR, #379503)
7. Tip barrier Span8 P20 (VWR, # 379506)
8. Greiner UV-Star® 96 well plates (no substitution, Fisher, #NC0532986)

For the Manual Method:

9. Pipettes
10. Pipette tips, assorted sizes

Notes:
It is possible to substitute disposable materials and certain equipment from other vendors, as long as they are equivalent to the items described above.

In the case that a reagent or disposable item either becomes contaminated or is suspected of being contaminated, it must be discarded.

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III. PROCEDURE STEPWISE

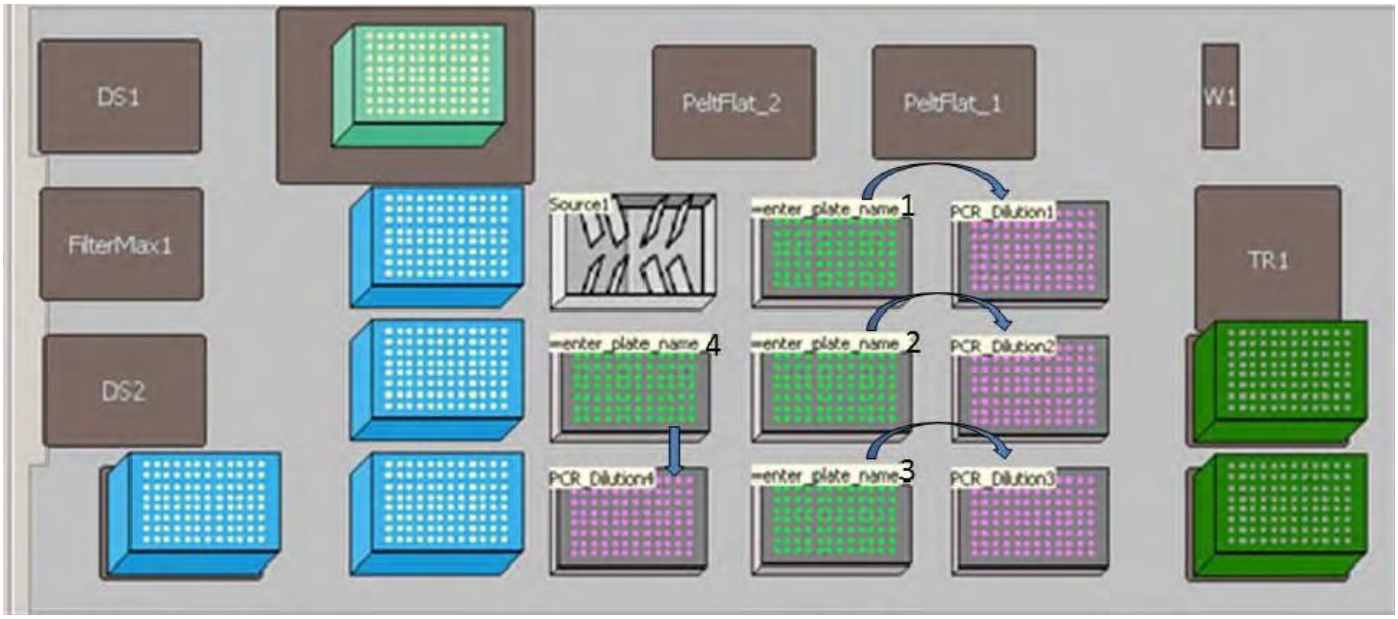
A. Calculations

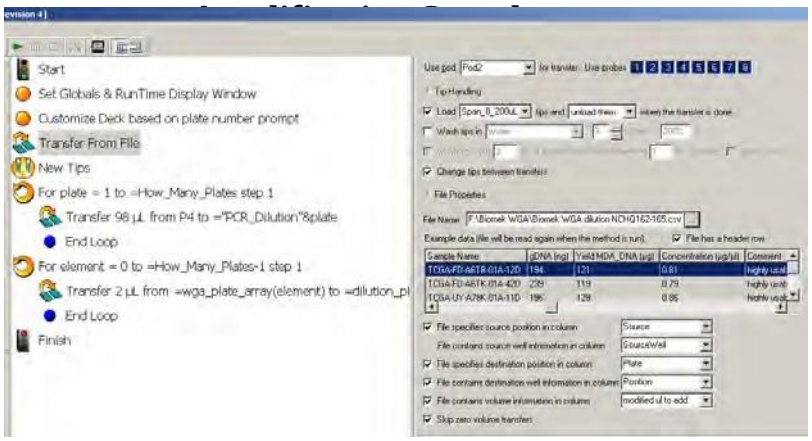
1. Insert the CD received from Qiagen. Click on "My Results" and open the Excel file. Print the "Report" tab on one or two pages (all columns for each sample should be included on a single page). Write the Plate ID, Batch number, and shipping location on the print-out (this print-out is the Repli_g_report).
2. Save the excel file as "Repli_g_report_#_#" in this folder: \\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Repli-G_Qiagen\Repli-g-reports.
3. Open the excel document and click on the Report_Table tab.
4. This information needs to be transferred to a dilution template file and saved on the Biomek. Open the Biomek WGA dilution template form on Sharepoint. (\\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Repli-G_Qiagen > Biomek WGA dilution template.xlsx)
5. Starting with cell A4 on the Repli_g_report, highlight up to four plates of data (columns A through G). If there are more than four plates on the Qiagen form, the additional plates will need to be saved to a separate file. The Biomek can only accommodate four plates at a time.
6. Paste this information "as values" into cell A2 of the Biomek WGA Dilution template.
7. An equation "F4/0.5*150-150" [(actual concentration/desired concentration)*sample volume-sample volume] has been added to column H named "µL to Add" for 0.5µg/µL. **The volumes calculated in row H cannot exceed 120µL**.
   (1) The plates can only accommodate 280µL of liquid, and the samples come back from Qiagen at 150µL each. To avoid any spillover and contamination, any sample that needs more than 120µL, will be adjusted to receive only 120µL maximum for normalization. A formula has been added to column K to change any volume greater than 120µL to 120µL maximum and any volume less than 0µL to 0µL (=IF((H2>120),120,IF(H2>0,H2,IF(H2<0,0))).
8. Save the Biomek WGA Dilution file in the folder on the SharePoint site as an Excel File and a CSV File under the name "Biomek WGA dilution NCHQXXX.xlsx" where the plate ID is inserted for the X's. (\\rex\BPC\TCGA-BCR\Shared Documents\Molecular Projects\Repli-G_Qiagen\Biomek WGA dilution data forms).
9. Print the Excel file with all columns on one page. This dilution document will be kept with the Excel report document printed in 1.
10. Open the CSV file created in Step 8. Filter by Sample (Column C) and filter the "QC", "0", negative values, or blank names. Delete all rows that have those sample names. Unfilter all columns. Click Save. Note the sample ID (Column C) and well position (Column B) of the first sample on the spreadsheet for comparison to the file that is in the program.

11. Copy this file onto a flashdrive and take to the Biomek computer. *Note: If performing the manual method, just print the file and continue to "Section C Manual Fluid Handling".*

B. Automated Fluid Handling Method

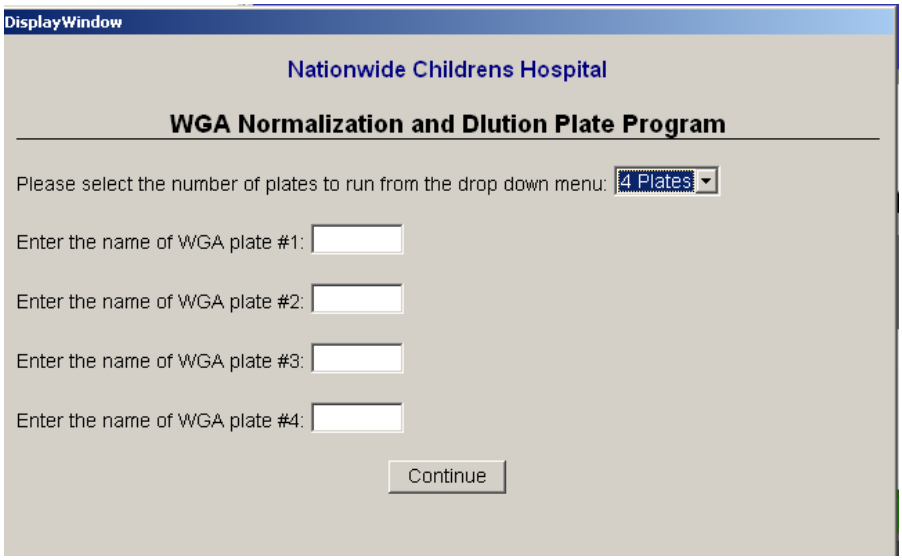
1. Remove the WGA Plate from the freezer and allow it to thaw at room temperature, checking every 20 minutes. Proceed with normalization as soon as samples are thawed.
2. Once thawed, spin the plate at 2000 x g for 1min.
3. Turn on the Biomek and Home All Axes.
4. Open the Biomek program "WGA Dilution HTML Prompt" and click on the Transfer File step. Open the correct WGA dilution plate file from the flashdrive.
   (1) The sampleID and well position noted in step 10 above should match the information shown in the Biomek software.
5. Make sure the correct drop-downs are selected by confirming with the picture below.
6. Very carefully, remove the heat sealed foil from each WGA plate without splashing and place in the appropriate position on the Biomek deck as prompted by the Biomek software. The picture below is an example of what the deck setup would look like if you had to dilute 4 WGA plates.
   a. The green and gray plates labeled "enter_plate_name_1 (through 4)" represent the WGA plates that need to be diluted.
   b. The pink and gray plates labeled "PCR_Dilution1 (through 4)" represent the SNP dilution plates being created for each WGA plate.
   c. Source 1 should be filled with H_2O up to the designated black mark on the reservoir.



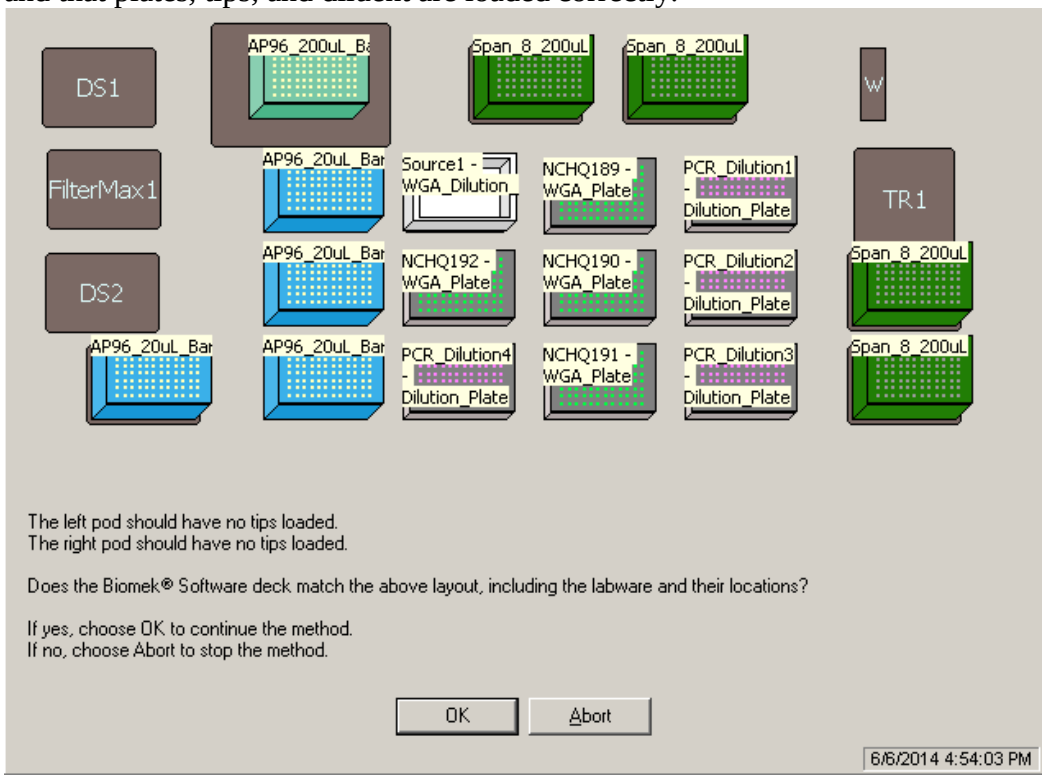


d. The dilution plate for each specific WGA stock plate must be positioned adjacent to the stock plate as shown by arrows in figure D above. This dilution plate will be a 96-well non-skirted plate labeled with the WGA plate ID, date (of dilution), and dilution value (1:50). Using the Repli_g_report, outline the plate to correspond with the wells containing samples on the WGA plate. The 96-well plate should be nestled and taped into a UV-Star 96-well plate.
e. The blue boxes represent the P250 barrier tips.
f. The green boxes represent the P20 barrier tips.

7. After the deck has been set up and all plates and tips are in the proper location, start the program by hitting the green "Play" button.
8. A pop-up window will prompt the tech to name each plate that will be normalized. On the drop down, select the number of plates to run. Enter each plate name to match the plate name on the WGA plate. For example "nchq131".



9. Click continue.
10. The user is now prompted to review and confirm the deck configuration. The plate names are now visible on the deck. Confirm that all positions were accurately named, and that plates, tips, and diluent are loaded correctly.



11. Click OK to confirm and start the dilution run: the program will execute two basic steps. First, it will normalize the concentration of the stock WGA plates by adding the volume of water specified in WGA Dilution Template column K. Then it will create a 10 ng/µL dilution plate for SNP genotype testing.
12. Once the program is complete, seal all plates with adhesive foil and store in a -80^{o}C freezer. Normalized stock WGA and 10 ng/ µL dilution plates are currently stored in the -80 Freezer BONO. Dilution plates can be prepared for shipping using SOP M018 "Shipping Analytes to Downstream Centers."
13. The 10 ng/µL dilution plates will undergo Tissue Matching by SNP Analysis (SOP M010). Passing samples are eligible to be called up to shipping (SOP M018 Shipping Analytes to Downstream Centers) if needed for the project.
14. File the Excel documents printed into the appropriate binders.

C. Manual Fluid Handling Method

1. Remove the WGA Plate from the freezer and allow it to thaw at room temperature, checking every 20 minutes. Proceed with normalization as soon as samples are thawed.
2. Once thawed, spin the plate at 2000 x g for 1min.

3. Using the "modified µL to Add" column K of the Excel spreadsheet as a reference, transfer the correct volume of RNase- and DNase-free water to the appropriate wells of the WGA Plate by piercing the film cover of the plate with the pipet tip.
4. Seal the plate with a second, new adhesive foil and mix by gentle inversion. Spin the plate at 2000 x g for 1 min.
5. Take an empty 96-well non-skirted plate and label it with the WGA plate ID, date, and dilution value (1:50). Using the Repli_g_report, outline the plate to correspond with the wells containing samples on the WGA plate.
6. Add 99 µL of RNase- and DNase-free water to each well in the dilution plate.
7. Add 1 µL of DNA from the WGA plate to the dilution plate.
8. Seal all plates with adhesive foil and store in a -80^{o}C freezer. Normalized stock WGA and 10 ng/µL dilution plates are currently stored in the -80 Freezer BONO. Dilution plates can be prepared for shipping using SOP M018 Shipping Analytes to Downstream Centers.
9. The 10 ng/µL dilution plates will undergo Tissue Matching by SNP Analysis (SOP M010). Passing samples are eligible to be called up to shipping (SOP M018 Shipping Analytes to Downstream Centers) if needed for the project.
10. File the Excel documents printed into the appropriate binders.

II. REFERENCES

A. BCR SOP M018 "Shipping Analytes to Downstream Centers"
B. BCR SOP M010 "Tissue Matching by SNP Analysis"

III. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date **8/16/2016**
1. Made title not all capitalized
2. Updated two instances where "non-skirted" PCR plate needed to be specified.

B. Changes made in Version 2, Effective Date 12/31/2014
1. Updated the location of where the Qiagen file is saved.
2. Updated the dilution template to have a fixed equation in place to eliminate typing in the equation each time it is used.
3. Updated formatting.
4. Added section "Automated Fluid Handling Method"
5. Added equipment, supplies, and reagents
6. Updated catalog numbers

C. Version 1, Effective Date 9/11/2012 - New

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Signatures

Approved by:	**Signature on file**	**Date on file**
	Julie M. Gastier-Foster **Principal Investigator**	**Date**