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

I. Purpose

To provide specific guidelines for operating and maintaining the Caliper LabChip GX. This is achieved using the reagents and instructions provided by Caliper to determine the quality and quantity of DNA samples in either 96 well plate format or 384 well plate format for up to 192 samples.

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 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 team to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
LabChip GX High Sensitivity DNA Assay Quick Guide	AM.0025
HT High Sensitivity DNA LabChip Kit, Version 2 LabChip GX/GXII User Guide	IM.0198

VI. Related Documents

Document Title	Document Number
N/A	N/A

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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 #	
Small Autoclave waste bags 10"X15"	Fisher Scientific	01-826-4		✓
Ice bucket – Blue	Fisher	11-676-36		✓
wet ice	In house	N/A		N/A
Gilson P2 pipetman	Mandel	GF-44801		✓
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P1000 pipetman	Mandel	GF-23602		✓
Galaxy mini-centrifuge	VWR	37000-700		✓
VX-100 Vortex Mixer	Labnet	S-0100		✓
Large Kimwipes	Fisher	06-666-117		✓
Black ink permanent marker pen	VWR	52877-310		✓
Eppendorf BenchTop Refrigerated Centrifuge 5424R	Eppendorf	5424 R		✓
UltraPure DNase/RNase Free water	Gibco	10977-015		✓
Vacuum pump	Millipore			✓
HT 1K/12K LabChip	Caliper	760517		✓
HT High Sensitivity DNA Reagent Kit		760568		✓
LabChip GX		GX1016N0210		✓
Thermo Scientific ABgene SuperPlate 96-Well PCR Plate	Abgene	AB2800		✓
Thermofast 96 skirted	Abgene	AB1000		✓
Tape Pads	Qiagen	19570		✓
Armadillo High Performance 384-Well PCR Plate	Thermo	AB2384		✓
70% Isopropanol in Nuclease-free water	In-house			
Biomek	Beckman		✓	
P165B tips for Biomek	Ultident	24-FXF-180-LRS		✓
P50 tips for Biomek	Beckman	A21586		✓
384 well shallow reservoirs	Ultident	24-RES-SW384-LP		✓

Diamond Filter tips DF1000 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171703		✓
Diamond Filter tips DFL10	Mandel Scientific	GF-F171203		✓
Diamond Filter tips DF200 (10 Tipacks of 96 racked filter tips)	Mandel Scientific	GF-F171503		✓
Medicom Safetouch Nitrile gloves- large	Ultident	1137-D		
Axygen 96 FSC PCR plates	Fisher Scientific	14222327		

IX. Procedure

1. Preparing the Gel-Dye Solution

1.1. Allow the HT 1K/12K labchip and HT High sensitivity DNA reagent kit to equilibrate to room temperature before use for approximately 30 minutes.

1.2. Gently vortex thawed dye concentrate for 10 seconds before use.

Note: Verify that the dye has completely thawed as it contains DMSO.

1.3. Transfer 6.25µL of HT DNA Dye concentrate (blue cap) to the centrifuge tubes provided with the reagent kit. Add 500µL of HT DNA Gel Matrix (red cap) using a **reverse Pipetting technique** (refer to Appendix A).

1.4. Vortex the solution until it is well mixed and spin in the microfuge for a few seconds.

1.5. Transfer the mixture to a spin filter. **Note**: Verify that the dye has completely thawed as it contains DMSO.

1.6. Centrifuge at 9200 rcf for 10 minutes at room temperature.

1.7. Discard filter and store filtered gel matrix in the dark at 4°C. Date the filtered gel matrix tube. Filtered gel matrix can be saved and used up to three weeks later.

2. Preparing the DNA samples and DNA ladder

2.1. In the provided 0.2mL Ladder tube, add 12µL of HT HS DNA ladder to 108µL of Qiagen EB Buffer (or the same buffer as your DNA samples). Mix thoroughly by pipetting the solution up and down several times.
Note: The Ladder tube can be re-used for a maximum of 5 times.

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2.2. Insert the ladder tube into the ladder slot on the LabChip GX instrument. This can be done by first pressing the EJECT button on the instrument front panel.

2.3. Prepare the appropriate dilution plate according to the appropriate pipeline to load on the chip and add Qiagen EB Buffer to a final volume of 30µL for 96 well plates or 15uL for 384 well plates.

2.4. Up to 2 full quadrants of a 384 well plate (192 samples) can be analyzed per Caliper run. The following biomek programs can be used to dilute samples to run on the Caliper:

P165B_from_384Axygen_to_AB1000_1Plate
P50B_LIBPR-Transfer to AB1000LIBPR-Caliper Setup in 384Well PCR (user can select 1 or 2 plates to transfer)

Please be aware that the minimum volume the biomek can accurately transfer is 2µL.

3. Preparing the Buffer Tube

3.1. Add 750µL of Qiagen EB Buffer (or the same buffer as your DNA samples) to the 0.75mL Buffer Tube provided.
Note: The buffer tube can be re-used for a maximum of 5 times.

3.2. Insert the buffer tube into the buffer slot on the LabChip GX instrument. This can be done by first pressing the EJECT button on the instrument front panel.

4. Preparing the Chip

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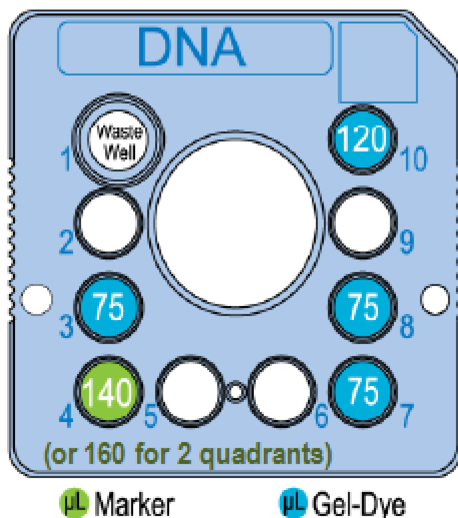

Figure 1; DNA Chip

4.1. Allow the Chip to come to room temperature and remove the parafilm cover from the chip wells.

4.2. Ensure that the top and bottom surfaces of the chip are dry using the vacuum.

4.3. Use a fresh non-filtered pipette tip attached to a vacuum line to thoroughly aspirate the buffer in each active well (1, 3, 4, 7, 8, and 10) while the chip is still in the container and the sipper is immersed in buffer. Do not run the pipette tip over the central region of the detection window.

4.4. Rinse and aspirate each active well twice with 100µL of Molecular biology grade water. **Do not allow the active wells to remain dry**.

4.5. Add 75µL of Gel-Dye solution to Wells 3, 7, and 8 using a **reverse pipetting technique** (refer to Appendix A). Add 120µL of Gel-Dye solution to well 10 using a reverse pipetting technique. **Ensure that there are no bubbles in these wells.**

4.6. Add HT DNA Marker (green cap) to chip well 4. **For up to 96 samples**, add 140uL of marker. **For 97 to 192 samples (384 well plate)**, add 160uL of marker.

Please note that the marker will need to be replenished if two or more full plates are run consecutively or if the chip is idle on the instrument for an extended period of time. If starting a second plate, top up well 4 with 50uL HT DNA Marker per 96 samples previously run, and empty waste well 1 by aspiration.

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4.7. Ensure that the rims are free of adhesive residue.

4.8. The Chip is now ready to be inserted into the LabChip GX.

5. Inserting a Chip into the LabChip GX Instrument

5.1. Check that the sample plate, Buffer tube and Ladder tube are placed in the correct positions on the instrument.

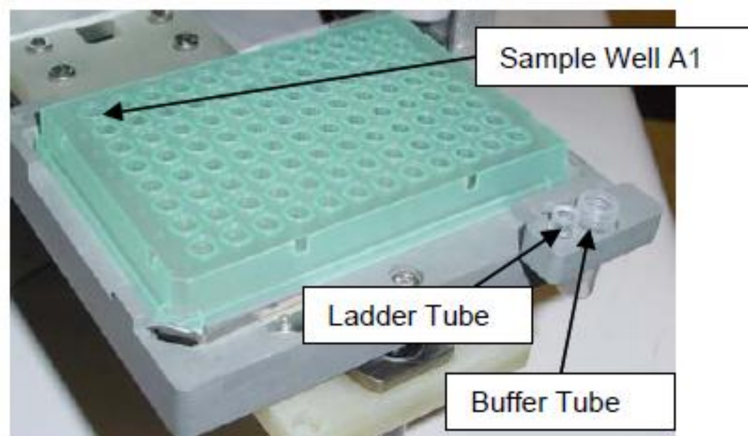

Figure 2: Sample Plate, Buffer Tube, and Ladder Tube Layout

5.2. Carefully remove the chip from the chip storage container and inspect the detection window on the chip by holding it up to the light. The detection window should be free of any dust particles or smudges. Clean both sides of the detection window with the Caliper supplied cleanroom cloth dampened with 70% Isopropanol.

5.3. Eject the chip cartridge by pressing the **CHIP** button on the instrument front panel.

5.4. Release the cartridge latch, insert the chip into the LabChip instrument, refasten the latch and push the cartridge into the instrument.

5.5. Press the **EJECT** button on the instrument front panel to retract the sample plate and send the sipper to the buffer tube.

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6. Generating Run Definition Files (384 well plate run only)

6.1. Open the "**Caliper 384 Well Assay Setup**" Excel form, located in the "R:\Library Core\QC\Caliper\384-well" folder. This form defines run parameters including well selection, plus sample names indicating the source plate and well (ex. "Tra12345_B6").

6.2. To enable macros, click **Options…** on the Security Warning notification bar at the top of the Excel sheet. Select **Enable this content**, then click **OK**. The form will load.

6.3. Select the appropriate quadrants and wells for the 96 well sample plates. Enter the Tra number of each 96 well sample plate as quadrant names.

6.4. Enter the run number (ex. DNA#345), and choose "AB-2384 Armadillo 384 2mm_production" as the plate type. Assay and operator can be specified either here or later in LabChip GX.

6.5. Click **Export Data Files** and save the two exported setup files in "R:\Library Core\QC\Caliper\384-well\Run setups". The run parameters will be saved as an XML file and the sample names will be saved as a CSV file.

7. Running the HT DNA Assay for 96 Well or 384 Well Plates

7.1. Start the LabChip GX software.

7.2. On the main screen, click the **RUN** button in the upper left corner of the LabChip GX software.

7.3. The *Start Run* dialogue box will pop up with tabs listed as *Output, Run and Advanced*.

7.4. In the *Run* tab:

7.4.1. **For 96 well plate runs**, select the well pattern and plate type. For AB1000 or AB2800 plates, choose "AB-1000 2.5mm_production" as plate type The Caliper machine has been calibrated to use these plate types; 2.5mm denotes sipper distance from well bottom.

7.4.2. **For 384 well plate runs**, the well pattern and plate type is imported from the generated run parameters XML file. Click **Import** and select the appropriate file to load these parameters. The sample names file will be applied automatically.

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7.4.3. **Ensure that only wells that contain sample are selected when editing the well pattern. Failure to do so will cause the sipper to draw air into the channels, which will damage the chip.**

7.4.4. Select the "HT DNA High Sens v16_production" assay type, and enter the operator name and assay plate name**.**

7.5. In the *Output* Tab, change the destination of the file to \Library Core\QC\Caliper\PCR Product. The file name should be in the format "DNA#X_Collaborator'sName_LibraryX_PCRcycles_date+initial". Select any additional data to autoexport.

Note: The *Advanced* Tab allows the user to select the number of times each is sampled, the inclusion of any sample names and any expected peaks. The user can use any of these options if necessary.

7.6. Click **START** to begin the run.

8. Clearing Sample Names (384 well plate run only)

8.1. Clear the sample name list once the run is complete so that incorrect sample names are not applied to the next run. This can be done by exiting the LabChip GX software or by clicking **Tools->Sample Name Editor** from the menu bar and selecting **Blank 96 Wells** or **Blank 384 Wells**.

9. Storing the DNA Chip

9.1. Eject the chip cartridge by pressing the **CHIP** button on the instrument front panel.

9.2. Release the cartridge latch and remove the chip from the LabChip instrument. Place the chip in the storage container.

9.3. Remove reagents from each well of the chip using the vacuum.

9.4. Each active well (1, 3,4,7,8 and 10) should be rinsed and aspirated twice using 100uL molecular biology grade water.

9.5. Add 100µL HT storage buffer (white cap) to the active wells.

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9.6. Hit the **EJECT** button to eject the stage. Place the chip in the LabChip GX instrument, refasten the latch and push the cartridge into the instrument. Click the **WASH** button on the left corner of the LabChip GX software.

9.7. Once the instrument has completed washing the chip, remove the chip from the instrument and place it in the plastic storage container. Add an additional amount of storage buffer to well 1(~30µL). Cover the wells with parafilm to prevent buffer evaporation and store at 4°C.

Note: Storage of the chip with dry wells may cause it to become clogged.

10. Cleaning and Storing the Ladder and Buffer tubes

10.1. Eject the chip cartridge by pressing the **CHIP** button on the instrument front panel.

10.2. Remove the solutions from each tube using the vacuum if saving the tubes. They can be discarded if the tubes are in excess.

10.3. Rinse/Aspirate the Ladder and Buffer tubes 2X using 150µL and 1mL of nuclease-free water respectively if saving the tubes. The tubes can be disposed of if they are in excess.

10.4. Mark the side of each tube with a permanent marker to indicate the number of plates the tube has been used for. The maximum number of plates a tube can be re-used for is 5 plates.

10.5. Place the Ladder and Buffer tubes in the storage tube rack.

11. Chip Cartridge cleaning

11.1. Every time after running a chip:

11.1.1. Use the provided lint free swab dampened with DI water or 70% Isopropanol to clean the O-rings using a circular motion.
11.1.2. If the O-rings stick to the chip or a pressure leak is detected, perform the more extensive monthly cleaning procedure.

11.2. Monthly (or periodically if usage is infrequent)

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11.2.1. To reduce pressure leaks at the interface. Remove the O-rings from the top plate of the chip interface on the LabChip GX instrument. Soak the O-rings in DI water for a few minutes. Clean the O-rings by rubbing between fingers.
11.2.2. To reduce the occurrence of current leaks, clean the chip interface frequently. Clean the top plate of the chip interface using the provided lint free swab dampened with DI water.
11.2.3. Allow the O-rings and chip interface to air dry. Re-insert the O-rings into the chip cartridge.
11.2.4. Document that monthly cleaning of O rings has been completed in the Caliper logbook.

Note: The Caliper software keeps track of how many samples have been run on a chip. Each chip can run ~2000 samples. Once the number of samples has been reached, the chip will be flagged as “expired” by the software and a new chip will need to be used.

12. 384-Well Data Splitting

12.1. Open the “**Split Caliper well table by quadrant**” Excel spreadsheet stored in “R:\Library Core\QC\Caliper\384-well”

Do not insert or delete rows, columns or cells anywhere in this spreadsheet.

12.2. In the *Paste 384-well Caliper data* tab, paste the Caliper LabChip GX well table data including headings into cell B4 (highlighted yellow).

Note: this data can be copied directly from the LabChip GX *Well Table* tab by clicking the grey region to the left of the “Plate Name” heading to select the entire table, then pressing Ctrl-C to copy. Alternatively, click **File** -> **Export…**, select **Well Table** and click **Ok**, then open the resulting CSV file in Excel and copy the data from there.

12.3. In the *Quadrant data* tab, choose a quadrant (ie. 96-well sample plate), and choose **Rows** or **Columns** as the sorting order. Data from this tab can be copied directly into LIMS or another spreadsheet.

12.4. Check that the plate ID for the quadrant is correctly displayed in cell D2. If this is blank or incorrect, the plate ID can be manually entered into cell E2 and text in cell D2 can be hidden by setting the font colour to white.

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12.5. Save a copy of the spreadsheet in the same folder as the Caliper data file. Give the file the same name as the Caliper data file followed by "_WellTable_split". For example: **DNA#466_Hela_SMART_cDNA_130613bk_CALIPER-N3_2013-06-13_09-15-04_WellTable_split**

13. Creating a Caliper Run

13.1. All Caliper runs are now tracked in lims. To do this please export your caliper run images to \\isaac\labinstruments\Caliper_Run\Exports as .bmp files. Also you will need to export the well table to the current library core folder where the Caliper run was saved (eg \geneexplab\Library Core\QC\Caliper).

13.2. In LIMS, scan in your tray # and the equ# of the caliper. A run for your plate will be created. You will then need to enter the just chip ID (eg. DNA# 1020) into the field provided along with the dilution factor (if needed) and the QC category.

13.3. After the run is created, check the box for your run and click the "Refresh" button. This will link the well table csv file to your runs and copy over the relevant information from this file to your samples (eg Avg_bp).

13.4. Click on the actual run ID. This will give you a map of the wells on your plate. Select the wells to approve the runs (this is to approve that the chip ran well, not to approve the sample quality).

13.5. For some runs attributes will be linked to your samples. Confirm that the information is correct. You can edit the information if not correct. However once you approved the second time, you cannot go back and edit the attributes from this page. You will need to edit the attributes by scanning in the tray.

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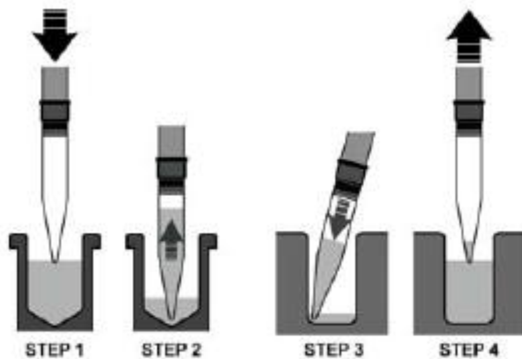
Appendix A

Reverse pipetting Technique:



1. **Depress the pipette plunger to the second stop.**
2. **Aspirate the selected volume plus an excess amount from the tube.**
3. **Dispense the selected volume into the corner of the well by depressing plunger to the first stop.**
4. **Withdraw the pipette from the well.**

Chip Considerations:

- **Handle chip with due care to prevent sipper damage.**
- **The sipper must be kept immersed in fluid at all times. And not be exposed to open environment for long periods of time.**
- **Entire chip surface must be dry before use.**
- **Chips can be prepared and left idle on instrument for up to 8 hours.**
- **If using the chip again within 24 hours it may be left at room temperature otherwise should store at 4C in the appropriate manner.**

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