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Operation and Maintenance of the LabChipGX for RNA samples using the HT RNA Assay

I. Purpose

To provide guidelines for operating and maintaining the Caliper LabChip GX and for running the HT RNA assay. Note RNA Std Sens js for detection of RNA in 25ng/µl to 250ng/µl range; HiSens is for detection of RNA in 5-50ng/µL. This is achieved using the reagents and instructions provided by Caliper to determine the quality and quantity of RNA samples in 96 well plate format.

II. Scope

All procedures are applicable to the BCGSC Library Construction Core group and Library Technology Development 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

Document Title	Document Number
HT RNA LabChip Kit, Version 2 LabChip GX/GXII User Guide LabChip GX RNA Assay Quick Guide	IM.0193

VI. Related Documents

Document Title	Document Number

Document Title	Document Number
N/A	N/A

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	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		✓
Diamond Filter Tips 10ul	Mandel	GF-F171203		✓
Diamond Filter Tips 1000ul	Mandel	GF-F171703		✓
Galaxy mini-centrifuge	VWR	37000-700		✓
VX-100 Vortex Mixer	Rose Scientific	S-0100		✓
Large Kimwipes	Fisher	06-666-117		✓
Black ink permanent marker pen	VWR	52877-310		✓
Eppendorf BenchTop Refrigerated Centrifuge 5810R	Eppendorf	5810 R		✓
Nuclease free water 0.2uM filtered	Ambion	AM9938/AM9937		✓
Vacuum pump	Millipore			✓
Centrifuge	Eppendorf	5810 R		✓
Pipet-Lite Multichannel 1-10uL	Rainin	L-12-10		✓
Pipet-Lite 20-200ul	Rainin	L-12-200		✓
20ul Rainin Tips	Rainin	RT-L10F		
200ul Rainin Tips	Rainin	RT-L200F		✓
HT RNA LabChip Kit, version 2	Caliper	760435		✓
HT RNA Reagent Kit LabChip GX	Caliper	760410		✓
Computer	Caliper	GX1016N0210		
Thermofast 96 skirted	Abgene	AB1000		

Thermo Scientific ABgene SuperPlate 96-Well PCR PlateN	Abgene	AB2800		✓
Disposable troughs	VWR	21007-972		✓
200µl Tips (non-barrier)	VWR	30128-378		✓
Heat Block ISOTEMP 125D	Fisher Scientific			✓
Aluminum Foil Seal	VWR	60941-126		✓
Tape Pads	Qiagen	19570		✓
70% Isopropanol in Nuclease-free water	In-house			
Sorvall Legend RT Centrifuge	Mandel		✓	
MJR-96 DNA Engine Thermal Cycler	MJ Research		✓	
NIMBUS 50 uL CORE Sterile Filter tips	Hamilton	235979		
NIMBUS 300 uL CORE Sterile Filter tips	Hamilton	235938		
Hamilton Microlab NIMBUS96	Hamilton			

IX. Procedure

1. Preparing the Gel-Dye Solution (6th floor location)

1.1. Allow the chip and all reagents 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 75µL of HT RNA Dye concentrate (blue cap) to the 2ml centrifuge tubes provided with the reagent kit. Add 425µL of HT RNA Gel Matrix (red cap) using **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. Label and date the tube.

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

1.7. Discard filter. Date the tube and store filtered gel matrix in the dark. Filtered gel matrix can be saved in the dark at 4°C. Use within 5 days.

2. Preparing the RNA samples (5th floor RNA area and 6th floor locations)

2.1. Manual Preparation

2.1.1. Take RNA Sample Buffer Concentrate (purple cap) to 5th floor RNA Area. Prepare sample buffer by adding 620µl RNA Sample Buffer Concentrate to 5580µl DEPC treated water. Save approximately 1ml of the prepared RNA Sample Buffer for step 4.1.

Note: This volume is enough for one full 96- well plate run (includes the samples, ladder, and buffer). You may adjust accordingly for partial plates or prepare a larger batch for later use. The ratio of RNA Sample Buffer Concentrate to DEPC treated water is 1:9.

2.1.2. Keep the RNA samples on the plate chiller to keep it cold while preparing the caliper plate. Your supervisor will let you know what volume to use for QC and any dilutions needed. Pipette sample into individual wells on AB1000 96 well microtiter plate. Note: If using 1µl sample then pre-dispense 1µl DEPC water before adding 1µl sample into well. Add 1µl DEPC water using multichannel Rainin P10. This is 2X fold dilution on caliper machine, and will need to be calculated into final concentration reading. **It is important that all wells to be analyzed on the LabChip GX contain sample. Failure to ensure this will cause the sipper to draw air into the channels, which will damage the chip. Add DEPC water to wells not being used as sample needs to be present in each well.**

2.1.3. Seal sample plate with Aluminum Foil seal and quick spin**.**

2.1.4. Heat on tetrad for 2 minutes at 70°C.

2.1.5. Snap cool the plate by placing plate on ice for 5minutes.

2.1.6. Spin plate for 1 minute to bring down any condensation.

2.1.7. Use multichannel pipette to transfer 46µl of prepared sample buffer to samples. Mix by pipetting up and down 10 times. Change tips between rows. Proceed to step 2.3.

2.2. Preparation on the Nimbus

Note: For Caliper QC on Standard Assay, the Nimbus will use 2 µL of sample and 46 µL of buffer. For Caliper QC on HiSens Assay, the Nimbus will use 2 µL of sample diluted with 4 µL DEPC water (6 µL total) and 19 µL buffer. The quantitative range for the Standard Assay is 25-250ng/µL and for the HiSens Assay it is 5-50ng/µL.

2.2.1. Take RNA Sample Buffer Concentrate (purple cap) to 5th floor RNA Area. Prepare sample buffer by adding 2.5 mL RNA Sample Buffer Concentrate to 22.5 mL DEPC treated water. Save approximately 1ml of the prepared RNA Sample Buffer for step 4.1. Do not discard partially used tubes of RNA Sample Buffer Concentrate; keep for future use.

2.2.2. Log on to Nimbus and start the Hamilton Run Control software. Open:

> *Open File*: **Production** *toggle to workflow* > **mRNA Isolation v2.2** > **mRNA Isolation v2.2 – Scheduler.wfl** > **Caliper Transfer** >*select either* **Standard** *or* **High Sensitivity**

2.2.3. When prompted by the Nimbus program "Heat denature, chill and spin down" proceed with the following:

2.2.4. Seal sample plate with Aluminum Foil seal and quick spin**.**

2.2.5. Heat on tetrad for 2 minutes at 70°C.

2.2.6. Snap cool the plate by placing plate on ice for 5minutes.

2.2.7. Spin plate for 1 minute to bring down any condensation.

2.2.8. Return plate to Nimbus deck for the addition of sample buffer. When the Nimbus program is complete, proceed with Step 2.3.

2.3. Cover plate with tape seal and bring plate upstairs to location of caliper machine. Also bring remaining diluted RNA Sample Buffer to the 6th floor to prepare RNA ladder and Buffer tube.

2.4. Spin down plate at 1250g for 1 minute at room temperature to remove air bubbles.

2.5. Note: Can spin at 3000g for 5 minutes if particulate debris in sample preparation is a concern.

2.6. Carefully remove tape seal.

2.7. Insert sample plate into LabChip GX Instrument when ready to run assay. This can be done by first pressing the EJECT button on the instrument front panel.

3. Preparing RNA ladder (on 6th floor)

3.1. Take a 4µl aliquot HT RNA Ladder aliquot out of -80 C freezer and allow to heat on thermoblock for 2 minutes at 70°C. RNA ladder should be snap chilled on ice for 5 minutes after 70°C/2min denaturation.

Note: When opening a new kit, allow the HT RNA Ladder (yellow cap) to thaw on ice and prepare five 4µl aliquots for individual use. Keep one for present use. Store the remaining aliquots (label as "RNAcaliper") in -80°C freezer.

3.2. Add 96µl of prepared sample buffer to the HT RNA ladder.

3.3. Mix well by pipetting up and down.

3.4. Transfer entire 100µl RNA-buffer solution to the provided 0.2ml ladder tube for caliper machine.

3.5. 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.

4. Preparing the Buffer Tube

4.1. Add 750µL of the Prepared RNA Sample Buffer from step 2.1 to the 0.75ml Buffer Tubeprovided.

4.2. 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.

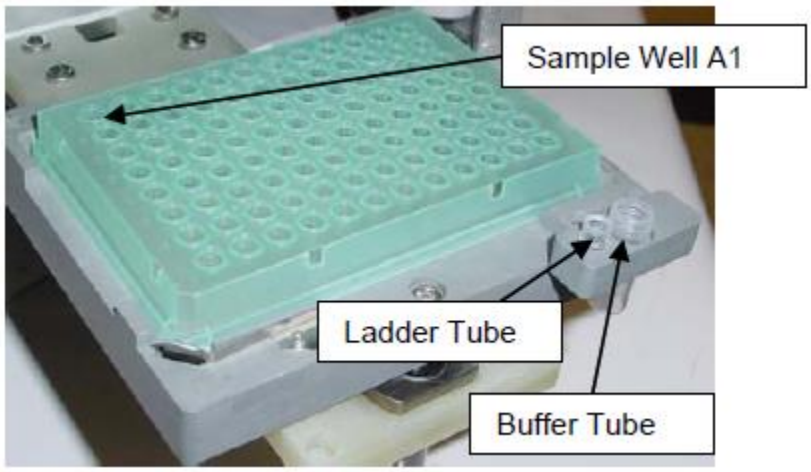


Figure 1. Plate, Ladder Tube and Buffer Tube

5. Preparing the Chip

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

5.2. Ensure that the top and bottom surfaces of the chip are dry using the vacuum pump next to the Caliper computer.

5.3. Use a fresh non-filtered p200 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 over the central region of the detection window.

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

5.5. Add 75µL of Gel-Dye solution to Wells 3, 7, 8, and add 120µl of Gel-Dye to chip well 10 using **a reverse pipetting** technique (refer to Appendix A). **Ensure that there are no bubbles in these wells.** (Please note that the gel-dye solution in well 10 may need to be replenished after 100 samples or if the chip had been left idle for more than 3 hours on the instrument.)

5.6. Add 120µL HT RNA marker (green cap) to chip well 4.

5.7. Ensure that the rims of the chip wells are free of adhesive residue.

5.8. The Chip is now ready to be inserted into the LabChip GX. The prepped chip can be used to run two 96 well plates.

Note: Recommend topping up topping up the Marker in well 4 (can be done by pipette without removing chip from instrument) after 100 samples or if Marker was placed in the chip more than 3 hours ago.

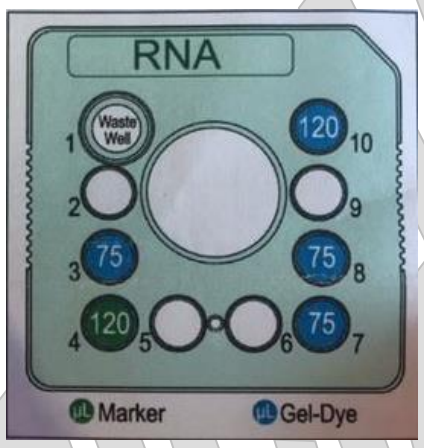


Figure 2. RNA chip

6. Inserting a Chip into the LabChip GX Instrument

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

6.2. Carefully remove the chip from the chip storage container (without bending the sipper) 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.

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

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

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

7. Running the HT RNA Assay

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, select

7.4.1. For standard Assay: **HT RNA Std Sens_production** assay type, operator name, plate name and well pattern. Choose AB1000_4mm_production* as plate type, this will work for AB2800 as well**.**

7.4.2. For Hisens Assay: **HT RNA HiSens** assay type, operator name, plate name and well pattern. Choose "AB1000_2.5mm_production" as the total volume is lower for the Caliper plate.

7.5. **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**. *Caliper machine has been calibrated to use ABgene AB1000 and AB2800 plate type; 4mm or 2.5mm denotes sipper distance from well bottom.

7.6. In the Output Tab select the destination of the file. Enter the file name in the format, RNA#xxx_Collaborator name or plate name_type of RNA_Date_initials.
Eg: RNA#138_CDouglas_CDR8_tot_RNA_130328SSC

7.7. In the *Advanced* Tab, can if wish, select the number of times each is sampled, the inclusion of any sample names and any expected peaks.

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

8. Cleaning and storing the RNA Chip

After use, the chip must be cleaned and stored in the chip container.

8.1. Transfer chip to chip container and remove the regents from each well of the chip using the vacuum.

8.2. Each active well (1, 3, 4, 7, 8, and 10) should be rinsed and aspirated twice using 100µL molecular biology nuclease free water.

8.3. Add 80µL HT RNA storage buffer (white cap) to the active wells.

8.4. Place the chip in the LabChip GX instrument and click the **WASH** button on the left corner of the LabChip GX software.

8.5. Once the instrument completes washing the chip, remove the chip from the instrument and place it in the plastic storage container. Add an additional 20µl of storage buffer to well 1. Cover (do not tightly seal) 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.

8.6. Discard buffer and ladder tubes.

8.7. Sample plate can be sealed and stored in -80°C freezer until data viewed and assessed. This is a precautionary measure in case run fails because of a chip issue or machine assay error. There is sufficient volume of sample in wells to re-load plate if required. The plate should be labeled and initialized.

9. Chip Cartridge cleaning

9.1. Daily - after use

9.1.1. Inspect the inside of the chip cartridge and O-rings for debris.
9.1.2. Cleaning of the O-rings is only done after use. Use the provided lint free swab dampened with DI water or 70% Isopropanol to clean the O-rings using a circular motion.
9.1.3. If the O-rings stick to the chip or a pressure leak is detected, perform the more extensive monthly cleaning procedure.

9.2. Monthly (or periodically if usage infrequent)

9.2.1. 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.

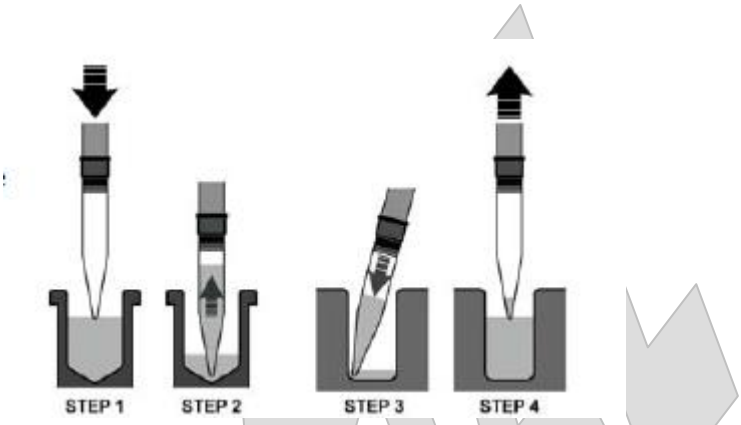
9.2.2. To reduce the occurrence of current leaks, clean the chip interface frequently. Clean the top plate of the chip interface using the provided lint free swab dampened with DI water.

9.2.3. Allow the O-rings and chip interface to air dry. Re-insert the O-rings into the chip cartridge.

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.

Appendix A

<u>Reverse pipetting Technique</u>:



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.**

<u>Chip Considerations</u>:

- **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.**
- **Can spin down plate at 3000g for 5 minutes if particulate debris in sample preparation is a concern before inserting sample plate into LabChIP GX instrument.**