LabChip User Guide

pDNA Assay
Assay User Guide

For LabChip® GXII Touch

 PN CLS160246 Rev A
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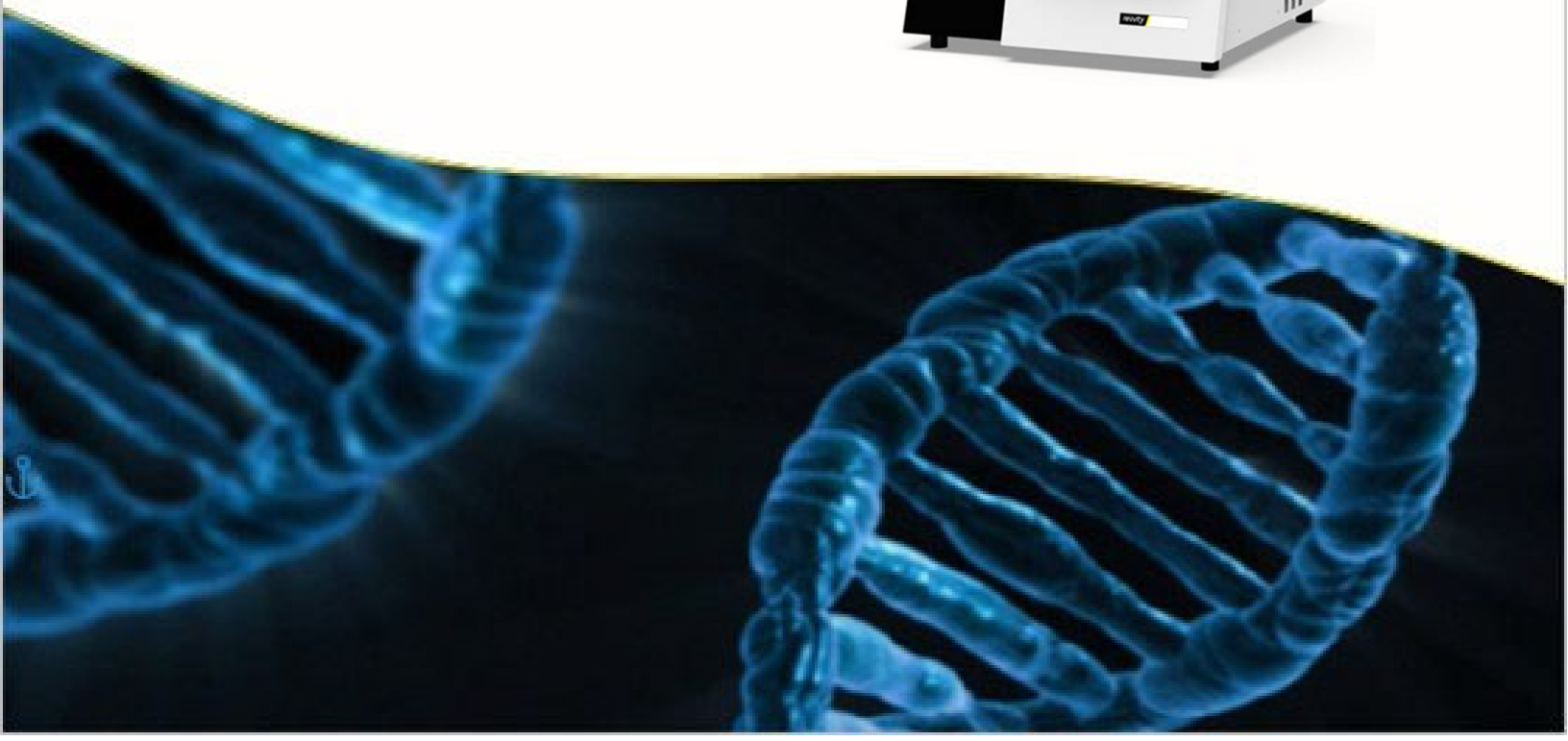

Contents

Specifications

Assay Specifications

Table 1. Assay Specifications

Minimum diluted sample volume on plate	20uL (384 well plate) 40uL (96 well plate)
Plasmid size range*	3 -13 kbp
Sizing Accuracy*	< 15% (3-13 kbp, SC) < 20% (3-4 kbp, Linear) < 15% (4-10 kbp, Linear) < 20% (10-13 kbp, Linear)
Input sample Concentration range	25 pg/µL – 500 pg/µL diluted in Revvity sample buffer
Maximum sample concentration	500 pg/µL
Linear concentration range	50 pg/µL – 500 pg/µL (Plasmid DNA)
Sensitivity (LOD)	25 pg/µL
Percent purity (CV)	< 10%
Carryover	< 0.5%
Time per sample	157 seconds
Samples per chip prep	24
Chip Lifetime	480 samples
Chip preps per reagent kit	20 Chip preps

*Note: 2-3 kbp plasmid isoforms can be analyzed for percent purity analysis

Sample Conditions

Table 2. Sample Conditions

Additives	Revvity recommends that BSA and detergents exceeding 0.05 mg/mL and 0.01% (v/v) respectively in concentration not be used. Higher concentrations can result in chip failure. In addition, non-aqueous solvents are not compatible with DNA LabChip protocols.
Particulates	All sample plates should be spun down prior to analysis. All buffers should be filtered with a 0.22 µm cellulose acetate filter.
Salt Concentration	Total salt concentration must not exceed 125 mM.

Kit Contents

Storage: When not in use, store chips and reagents refrigerated at 2 - 8°C until next use. **During first use of the pDNA ladder, aliquot the ladder using (12 µL) fractions and store at -20 °C.** If using the chip again within 24 hours, it may be left at room temperature. Allowing the chip wells to dry may lead to changes in chip performance.

pDNA Reagent Kit Part Number: CLS160450

Each kit contains enough reagents for 10 chip preparations.

Table 3. Reagent Kit Contents, PN CLS160450

Reagent	Vial	Quantity
DNA Dye Concentrate	Blue	4 vials, 0.09 mL
DNA Chip Storage Buffer	White	9 vials, 1.8 mL each
pDNA Gel Matrix	Red	2 vials, 1.1 mL each
pDNA Ladder, 10X	Yellow	1 vial, 0.26 mL
pDNA Marker	Green	1 vial, 1.5 mL
pDNA Gel Dilution Buffer	1 bottle	25 mL
pDNA Sample Buffer	1 bottle	30 mL

Table 4. Consumable Items

Item	Supplier and Catalog Number	Quantity
Spin Filters	Costar®, Cat. # 8160	20
Ladder Tubes	Genemate, Cat. #C-3258-1	40, 0.2 mL
Wash Buffer Tubes	E&K Scientific, Cat. #697075-NC	32, 0.75 mL
Detection Window Cleaning Cloth	VWR®, Cat. # 21912-046	2
Swab	ITW Texwipe®, Cat. # TX758B	6

pDNA LabChip

Table 5. pDNA LabChip

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Item	Catalog Number
pDNA Chip for use with GX and GXII Touch HT	P/N CLS160538

Safety and Usage

Safety Warnings and Precautions

CAUTION

We recommend that this product and components be handled only by those who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

WARNING!

Dye Concentrate contains DMSO. S24/25: Avoid contact with skin and eyes.

Usage

The pDNA assay is for use with LabChip™ GX Touch/GXII Touch instruments. The LabChip™ GX Touch/GXII Touch instruments are for research use only and not for use in diagnostic procedures.

Preparation Procedures

Additional Items Required

- 1N NaOH solution (Sigma Aldrich, 55881-1Kg) filtered using 0.22 µm filter (Millipore, SLGPR33RS)
- 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- 70% isopropanol solution in DI water.
- Revvity Hard-Shell thin-wall 96-well skirted PCR plate (blue), Cat. # 6008870 (recommended)
- Bio-Rad Hard-Shell® 384-well Skirted PCR Plates, Cat. #6008910 (recommended)
- Centrifuge tubes (2.0 mL), Eppendorf Cat. #022431048

Note: *Allow the chip and reagents to equilibrate to room temperature for at least 30 minutes before use.*

Preparing the Gel-Dye Solution

Notes:

- *The Dye Concentrate contains DMSO and must be thawed completely before use.*
- *Gel-Dye should be prepared fresh on the day of the experiment.*
- *The pDNA chip should be base washed on same day prior to a run.*

1. Prepare the **Working Gel** by diluting 133 µL Plasmid DNA Gel Concentrate 🔴 (reverse pipetting) with 1867 µL of Gel Dilution Buffer (Falcon Tube) for a final 2 mL volume. Amounts of DNA gel concentrate, and dilution buffer can be modified proportionally to desired final volume.

   Note: **The Working Gel** (without dye concentrate) can be stored for up to 3 weeks at 2-8 °C.

2. Vortex the thawed **DNA Dye Concentrate** (blue cap 🔵) for 10-15 seconds before use.

3. Transfer 500 uL of the Working Plasmid DNA Gel (step 1) to a spin filter. Add 15 uL of DNA dye concentrate 🔵 into the solution. Vortex and mix well.

4. Centrifuge at 9300 rcf for 9 minutes at room temperature.

5. Ensure all the gel/dye mixture passes through the filter and then discard the filter.

Preparing the Plasmid DNA Samples, Ladder and the Buffer Tube

Notes:

- *Aliquot the ladder into 12 µL fractions using 0.2 mL PCR tubes, store aliquots at -20 ºC.*

- *When handling the pDNA ladder and/or supercoiled plasmid samples, proceed by pipetting up and down. Do not vortex.*

Standard Sample Workflow

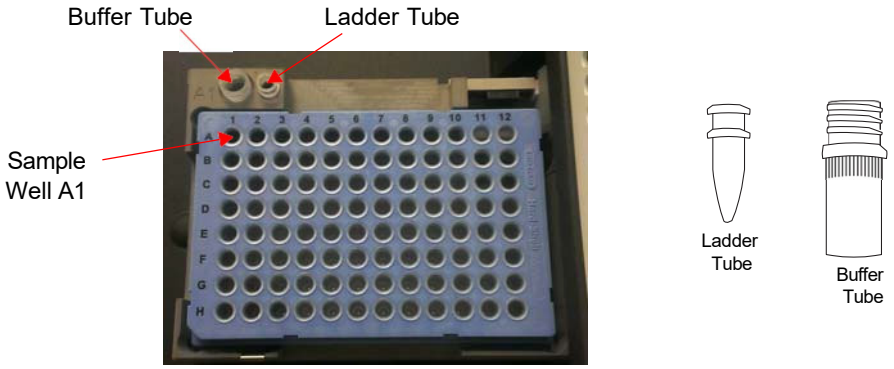

Figure 1. Locations of the Buffer Tube and Ladder Tube in the GX Touch/GXII Touch instrument.

Preparing the Plasmid DNA Samples

1 Dilute the plasmid DNA samples to ~500 pg/µL using the pDNA Sample Buffer. Pipette the samples onto 96-well (40 µL) or 384-well (20 µL) plates for analysis.

2 Spin down the plate at 9300 rcf for 5 minutes at room temperature.

Preparing the Ladder Tube

3 In the provided 0.2 mL Ladder Tube, add 12 µL of pDNA Ladder to 108 µL of pDNA sample buffer solution. Mix thoroughly by gently pipetting the solution up and down several times. Ensure there are no air bubbles in the Ladder Tube. **Do not vortex**.

4 Insert the Ladder Tube into the ladder slot on the LabChip GX Touch/GXII Touch instrument.

Preparing the Buffer Tube

1 Add 750 µL of pDNA Sample Buffer solution to the 0.75 mL Buffer Tube provided with the reagent kit. Ensure there are no air bubbles in the Buffer Tube.

2 Insert the Buffer Tube into the buffer slot on the LabChip GX Touch/GXII Touch instrument.

Cleaning the pDNA Lab Chip before a run

Important: Prior to each use, pDNA LabChips only should be cleaned following the instructions below.

1 Rinse and completely aspirate each well (1,2,3,4,7,8,9 and 10) with water (Milli-Q® or equivalent)

2 Repeat the water rinse for wells (1,2,3,4,7,8,9 and 10).

3 Add 100 µL of 1N NaOH solution to the active well (1,3,4,7,8 and 10)

4 Place the chip into the LabChip GX/GXII Touch

5 Place a Buffer Tube with 750 µL of water (Milli-Q® or equivalent) in the buffer slot.

6 Touch the wash button on the home screen.

7 When the wash is complete, remove the chip from the instrument and place the chip into the chip storage container.

8 Rinse and completely aspirate each active well (1,3,4,7,8 and 10) with water (Milli-Q® or equivalent)

9 Repeat the water rinse for wells (1,3,4,7,8 and 10).

10 Add 120 µL of water (Milli-Q® or equivalent) to each active well, place the chip back into the LabChip GX/GXII Touch and press wash.

11 When the wash is complete, press wash again to repeat water wash. Once second wash completed, remove the chip from the instrument and proceed to chip preparation steps (Loading the Gel-Dye Section, page 10).

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Loading the Gel-dye into the Chip

1 Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells (see Figure 2). For more details on how to set up a vacuum line see page 35.

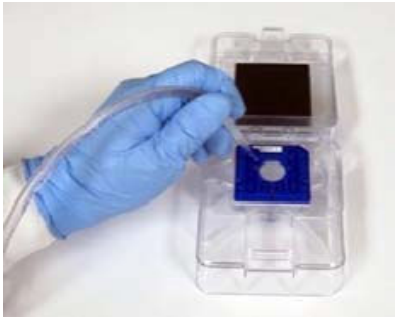

Figure 2. Using a vacuum to aspirate the chip wells is more effective than using a pipette.

2 Rinse and completely aspirate each active chip well (1, 3, 4, 7, 8 and 10) with water. Do not allow active wells to remain dry.

3 Repeat the water rinse for wells (1, 3, 4, 7, 8 and 10).

4 If any water spills onto the top and bottom chip surfaces during rinsing, aspirate using the vacuum line. DO NOT run the tip over the central region of the detection window. Use the provided Detection Window Cleaning Cloth dampened in water (Milli-Q® or equivalent) or alcohol to clean chip detection window as needed.

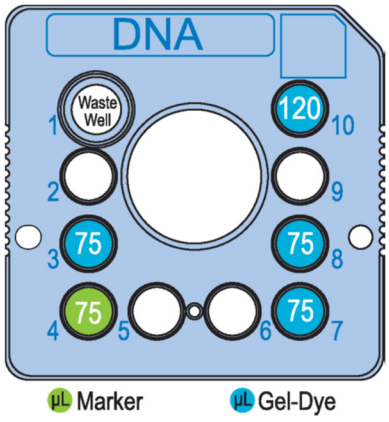

Figure 3. Reagent placement for pDNA Chip.

5 Using a reverse pipetting technique, add Gel-Dye solution to chip wells 3, 7, 8 and 10 as shown in Figure 3.

6 Add pDNA Marker (green cap ) to chip well 4 as shown in Figure 3.

7 **IMPORTANT:** Ensure chip well 1 (waste well) is empty before placing the chip into the instrument.

Inserting a Chip into the LabChip GX Touch/GXII Touch Instrument

1. Check that the sample plate, Buffer Tube, and Ladder Tube are properly placed on the instrument.
2. Remove the chip from the chip storage container and inspect the chip window. Clean BOTH sides of the chip window with the Revvity-supplied clean-room cloth dampened with a 70% isopropanol solution in DI water.
3. Touch the *Unload Chip* button on the *Home* screen.

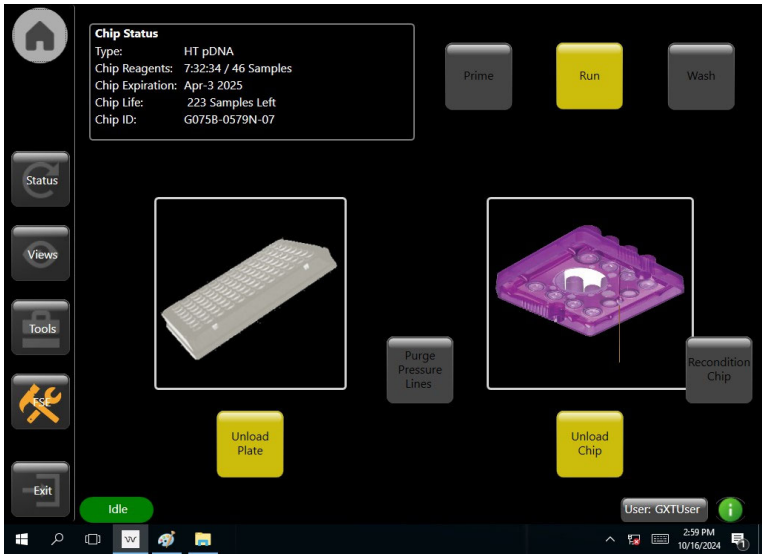

Figure 4. *Home* screen.

4. Insert the chip into the LabChip GX Touch/GXII Touch instrument (Figure 5) and close the chip door securely.
5. Touch the *Load Plate* button on the *Home* screen (Figure 4) to retract the sample plate and send the sipper to the Buffer Tube.

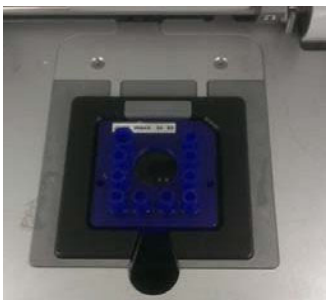

Figure 5. Chip in the LabChip GX Touch/GXII Touch instrument

Note: *Do not keep the chip door open for any length of time. Dye is sensitive to light and can be photobleached.*

Notes: *If performing multiple runs in a day, in between chip preparations the chip should be washed using 1N Sodium Hydroxide, as described in* Cleaning and Storing the Chip Section (page 17).

Be sure to periodically clean the O-rings on the top plate of the chip interface on the LabChip GX Touch/GXII Touch. Use the provided lint-free swab dampened with water (Milli-Q® or equivalent) to clean.
the O-rings using a circular motion. Allow the O-rings to dry before inserting a chip.

Running the Assay

Note**: Chips can be primed independently from running assays. Touch the Prime button on the Home screen. **Make sure the Buffer Tube is placed on the instrument.

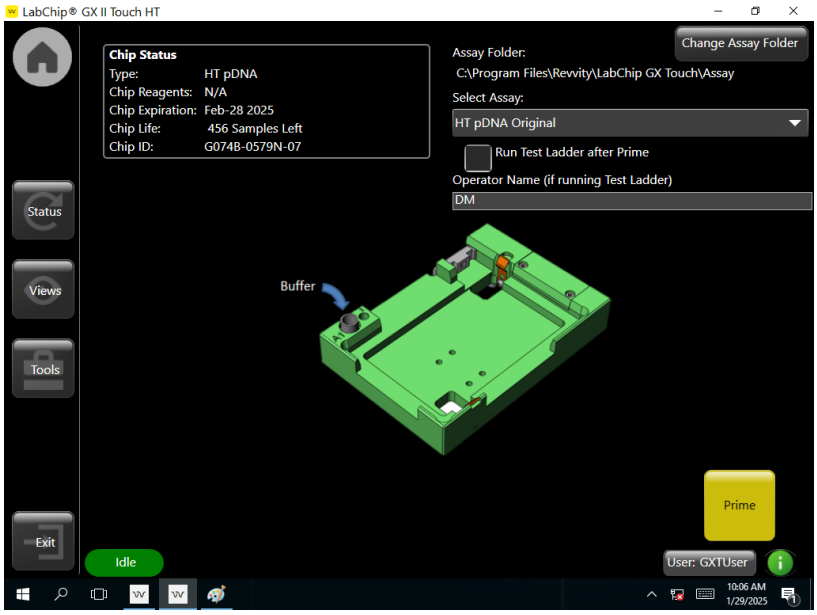

Figure 7. Chip priming screen.

1 Touch the *Run* button (see Figure 7).

2 Select the appropriate assay type (see Figure 7), plate name, well pattern, and whether to read wells in columns or rows. Select number of times each well is sampled under *Adv. Settings* (Figure 8). Touch the *yellow arrow* button.

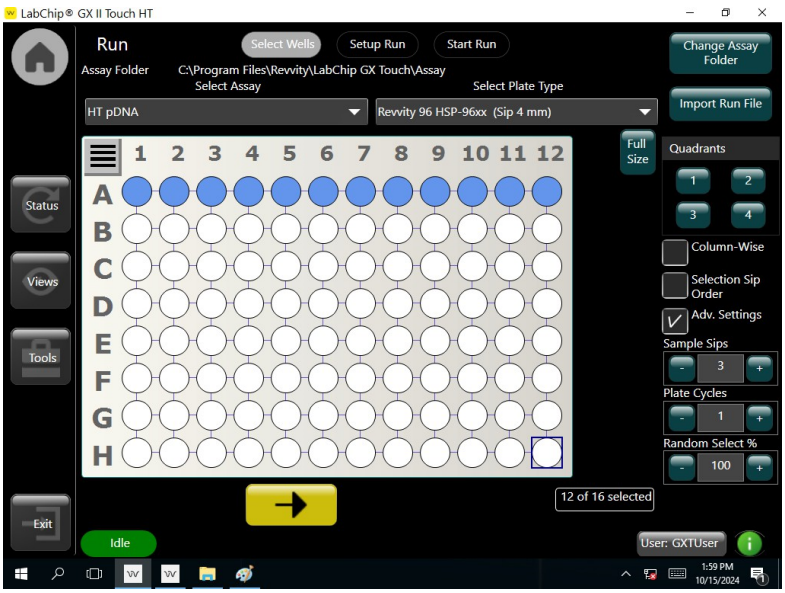

Figure 8. Selecting wells.

3 In the *Setup Run* tab, select the operator name, the option to read barcode, the destination of the file, the inclusion of sample names, expected peaks, and excluded peaks and the filename convention. Select *Auto Export* to export results tables automatically (Figure 9). Touch the *yellow arrow* button.

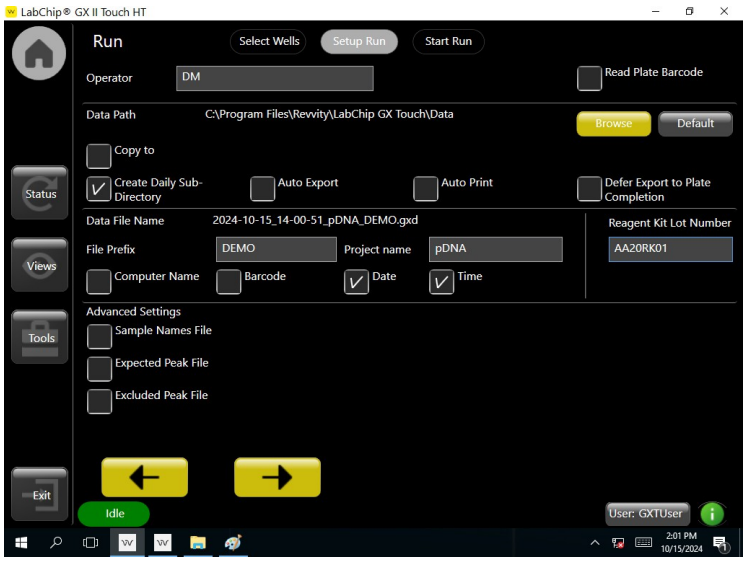

Figure 9. Run setup screen.

4 Touch *Start* to begin the run (Figure 10).

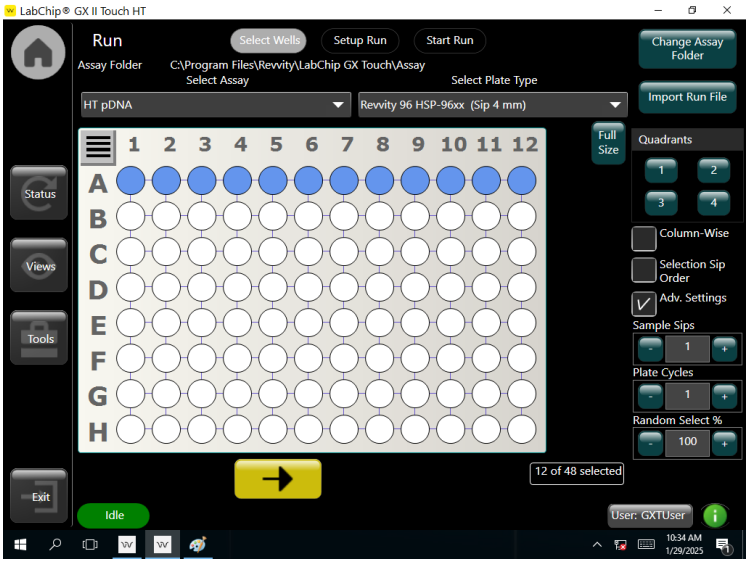

Figure 10. Starting a run.

Cleaning and Storing the Chip

Cleaning the chip after each run and preparing for storage

1. Place the chip into the chip storage container. Verify the sipper is submerged in the fluid reservoir. Remove the reagents from each chip using vacuum.
2. Rinse and completely aspirate each active well (1,3.4,7,8 and 10) with water (Milli-Q® or equivalent).
3. Repeat the water rinse of wells (1,3,4,7,8 and 10).
4. Add 120 µL of DNA Chip Storage Buffer (white cap ○) to each active well. Place the chip back into the LabChip™ GX/GXII Touch, and wash chip in the instrument.
5. When the washing on instrument is completed, press the wash button to run the washing step once more.
6. Remove chip from the Instrument, place the chip into the chip storage container. Verify the sipper is submerged in the fluid reservoir.
7. Cover the wells with Parafilm® to prevent evaporation and store at 2 - 8°C until next use. If using the chip again within 24 hours, it may be left at room temperature. Allowing the chip wells to dry may lead to changes in chip performance.

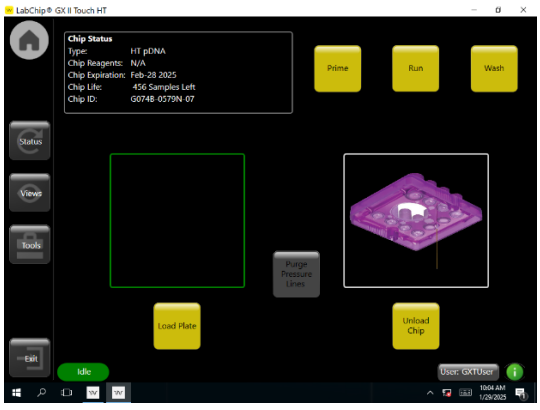

Figure 11. Wash screen

Chip Cartridge Cleaning

1 Daily

a Inspect the inside of the chip cartridge and O-rings for debris.

b Use the provided lint-free swab dampened with water (Milli-Q® or equivalent) to clean the O-rings using a circular motion. If the O-rings stick to the chip or a pressure leak is detected, perform the more extensive monthly cleaning procedure.

2 Monthly

a To reduce pressure leaks at the chip interface, clean the O-rings frequently. Remove the O-rings from the top plate of the chip interface on the LabChip GX Touch/GXII instrument. Soak O-rings in water (Milli-Q® or equivalent) for a few minutes. Clean the O-ring faces by rubbing between two fingers. Wear gloves.

b 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 water (Milli-Q® or equivalent).

c Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

Results

pDNA Ladder and Isoform Resolution Result

The electropherogram of a typical pDNA ladder is shown in Figure 12. The ladder contains two markers and 6 ladder peaks. The first three peaks are supercoiled conformation with size 2686, 4361 and 6706 bp. The second three peaks have sizes 4180, 6500 and 13,300 bp respectively.

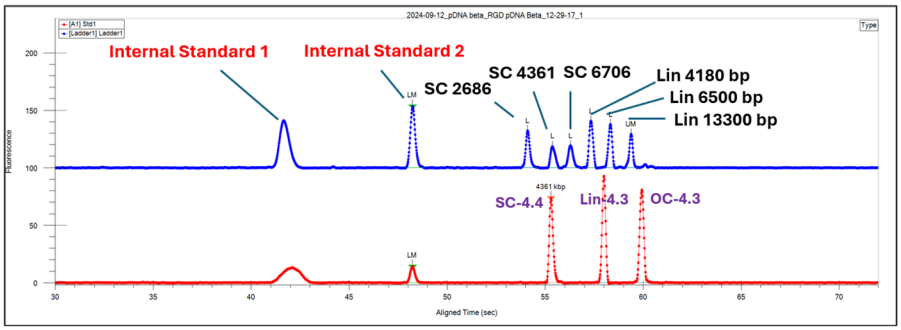

Figure 12. pDNA ladder (blue) and representative sample (red)

Frequently Asked Questions

1. How do I prepare the base wash solution used?

   Base wash solution is prepared by mixing sodium hydroxide with nuclease free water to a concentration of 1 N. Revvity uses sodium hydroxide (Sigma Aldrich, 55881-1kg) with purity > 98%. After dissolving, filter the solution through a 0.22 um filter (PES, polyethersulfone) syringe filter.

2. How do I use Reviewer Analysis settings to obtain and optimize sizing calculations for different pDNA isoforms?

   The Reviewer software contains in the **Analysis Settings → Analysis tab** options for the software to generate peak sizing values assuming either a supercoiled or linear conformation. When the SC button is selected, sample sizes are calculated based on calibration curve and fitting using the first three SC peaks of the ladder (peaks 1- 3). When the Linear button is selected, samples sizes are calculated using the linear ladder peaks (peaks 4-6). *Advanced users* may construct their own ladder and input the sizes into this table, however the first three peaks must be supercoiled conformation, and the second three peaks must be linear conformation. *Advanced users* may adjust fitting factors to perform sizing analysis outside of the range of supercoiled or linear ladder fragments. Calculation information for each sizing factor and is provided in the GX Reviewer Software User Manual. The Analysis tab is shown below with several fitting parameters. Please contact Revvity for assistance with assay development with custom ladders.

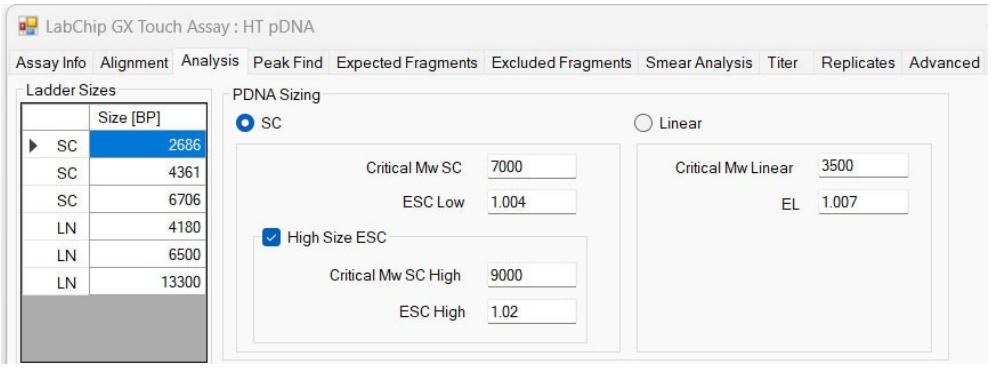

Figure 13. Analysis settings table.

Factors that may be adjusted by advanced users

Critical Mw SC –Increasing the Critical Mw SC can optimize fits for SC sizing for pDNA with size ~7000-9000.

ESC Low – a correction factor used for fitting between the Critical Mw SC and the critical Mw SC High value (7000-9000 bp above). May be adjusted in units of 0.001.

High Size ESC checkbox– when applied, is used for calculation of sizes above the Mw cut-off setting indicated (ex 9000 bp above).

Critical Mw SC High – molecular weight above which peaks can have a fitting factor ESC High applied.

ESC High – When High ESC is selected, a fitting factor used for calculation of SC sizes above the Critical Mw SC High. May be adjusted in units of ~0.005.

Critical Mw Linear – samples with molecular weight lower than this setting will have the EL fitting factor applied.

EL – a factor that is applied for the calculation of linear sizes below the critical MW Linear size entered (ex 4000 bp below). May be adjusted in units of 0.001.

3. **Can I perform pDNA analysis with higher concentration than 500 pg/µL concentration?**
   Yes, but the Assay Specifications of carry-over and run number may not reach the maximal limits. Samples up to 2000 pg/uL in Revvity buffer may be tested for sensitive impurity detection. Base chip washing recommended after 24 sips.

Troubleshooting

Note: *Some of the data examples shown in this section were generated with assays other than the assay described in this user guide.*

Symptom: The pDNA markers and pDNA sample peaks are delayed, and some expected peaks arrive at higher time and outside the assay time window.

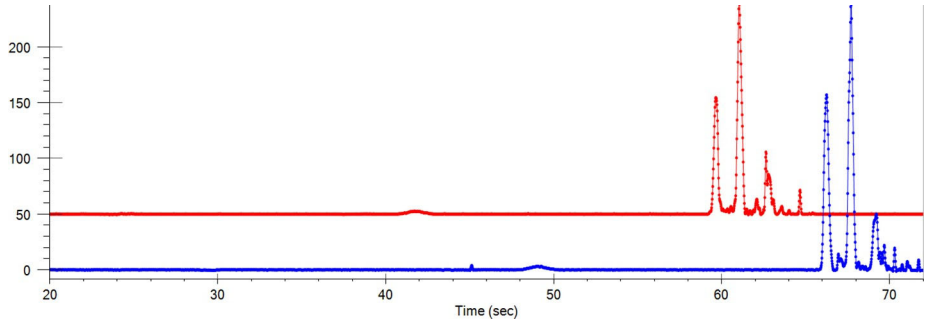

Figure 14 Electropherogram showing delayed migration (blue). The first marker peak is delayed ~8 s compared to the expected arrival time. The red electropherogram shows the result on same chip after performing the chip base wash procedure. The migration time is restored to the expected arrival time.

Possible Causes:

1 pDNA is clogging the channel.

What to do:

2 Stop the run, open and close the chip door, and restart the run. This will re-prime the chip which may regain the expected arrival times.

3 If step 1 does not correct the delay migration, Perform the base wash procedure (page 9).

Symptom: Carry-over is observed in pDNA electropherogram.

Possible causes:

1 The sample is loaded at concentration greater than 500 pg/µL.

What to do:

1 Dilute the sample further using pDNA sample buffer. Typically, a 500 pg/µL sample will have ~100 rfu height.

2 Inserting a blank in between samples is one way to run samples at higher concentrations (up to 2000 pg/µL) while removing potential for carry-over.

Symptom: A poor ladder profile is obtained where only linear peaks are visible but no supercoiled peaks.

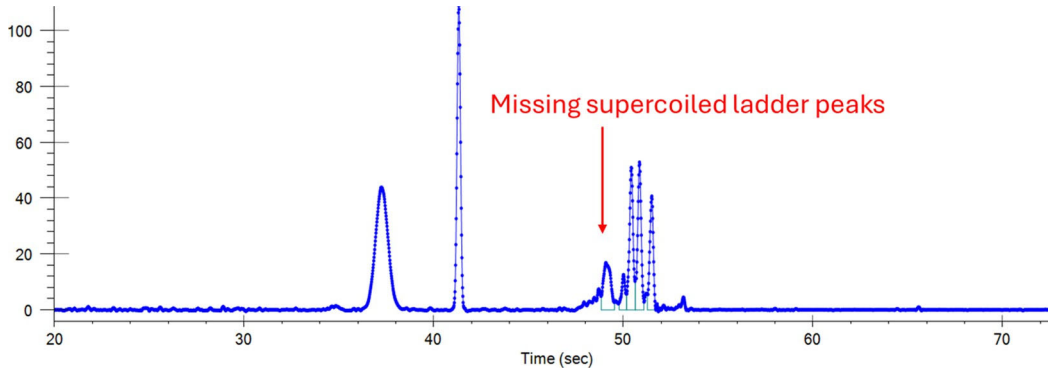

Figure 15. Electropherogram of pDNA ladder tested with low dye concentration. The first three supercoiled peaks are missing.

Possible causes:

1 During gel dye preparation, the final dye concentration is too low. Example: This may occur if one adds 15 µL of dye into 2 mL of diluted gel, instead of 500 µL diluted gel.

2 Partial chip clogging

What to do:

1 Prepare a new batch of gel dye by mixing 15 uL of dye into 500 uL of diluted gel. Run the assay again.

2 If new gel dye does not give expected ladder profile, a base wash on the chip is recommended.

Symptom: Supercoiled ladder peaks and sample peaks have low height or poor peak shape during run.

Possible cause:

1 The Gel-dye was prepared incorrectly or there is low dye concentration in the resulting solution.

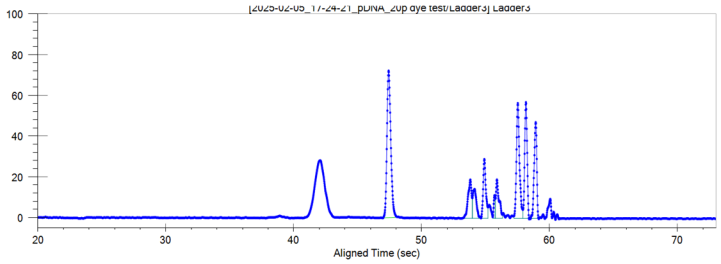

Figure 16. Ladder trace with splitting of supercoiled peaks.

What to do:

1 Prepare the gel dye solution again using 16.5 µL of dye added to 500 uL of working gel solution.

Symptom: Calculated sizes are shifted from the expected value and out of the assay's specification.

Possible cause:

1 A partial chip clog may change the elution spacing of the ladder peaks.

2 Procedures that remove digestion enzymes and salts could generate small conformational changes that affect mobility and sizing.

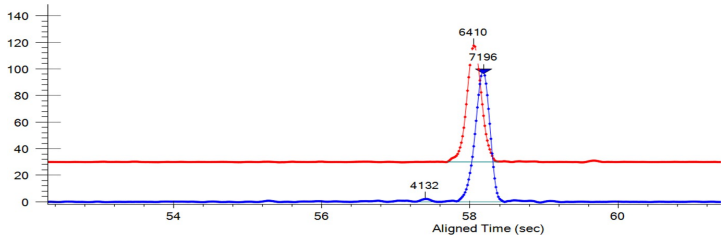

Figure 17. Red trace shows a 6700 bp pDNA that was linearized and run without cleaning. The blue trace shows the same sample run after QiaQuick pDNA clean-up.

What to do:

1 Compare the ladder demo data to the ladder obtained to determine if the peak spacing is similar. If noticeably different, it is recommended to base wash chip.

2 Adjust the apparent linear molecular weight ladder to correct for the sizing shift if consistent for a sample population. The apparent weights should be experimentally determined.

Symptom: Linear sizes are not shown in the Reviewer interface while a file is exported when the run is in progress.

Explanation:

1 This is due to the new software sizing capabilities. Once a run is completed and exported to Reviewer, both the SC and Linear sizing capabilities become active and correct sizing is visible.

Symptom: No pDNA ladder or sample peaks but marker peaks detected.

Note: *The lower marker peak height will most likely be greater than normal height.*

Possible causes:

1 Air bubble or other clog in sipper introduced during chip priming.

What to do:

1 Remove the chip from the instrument and manually Aspirate liquid from the sipper (as shown in Figure 19 and text). Note- for pDNA Assay, the aspiration may be done with gel still loaded in the caddies. Once liquid is seen to flow through the sipper, reinsert the chip onto the GX Touch and restart the run.

Symptom: Missing sample, ladder *and* marker peaks.

Possible causes:

1 Clog in sipper or marker channel of chip.

What to do:

1 Reprime the chip. See “LabChip Kit Essential Practices” on page 34 for instructions on how to reprime the chip.

Symptom: Ladder detected but no sample peaks.

Possible causes:

1 The sipper is not reaching the sample due to low sample volume in the well of the plate.

2 If the missing sample peaks occurred only in a few wells of the plate, check those wells for air bubbles.

3 The sipper is not reaching the sample due to an incorrect capillary height setting or incorrect plate definition.

4 If the plate has been uncovered for some time, sample evaporation might have occurred.

5 Debris from the sample or sample prep is clogging the sipper.

What to do:

1 Add more sample to the well.

2 Manually insert a larger volume pipette tip (~100 µL) into the sample well and dislodge the bubble. Rerun these sample wells.

3 Check the plate definitions.

4 Check the sample wells, especially around the edge of the plate where evaporation is fastest, and make a fresh plate if volumes are low.

5 If you suspect there may be debris in your samples, spin the sample plate down in a centrifuge (e.g. 3000 rcf for 5 minutes). Unclog the sipper by repriming the chip. See “LabChip Kit on page 34 for instructions on how to reprime the chip.

Symptom: No ladder peaks but sample peaks and marker peaks are present.

Possible causes:

1 Low or no ladder volume in the Ladder Tube.

What to do:

1 Add more ladder to the Ladder Tube and restart the run. Recommended standard ladder volume is 120 µL (minimum volume is 100 µL).

Symptom: No marker peaks but sample peaks are present.

Possible causes:

1 No marker added to chip well 4.

2 If there is marker solution in chip well 4, the problem may be due to a marker channel clog.

What to do:

1 This may be due to not filling marker well or chip remaining idle on instrument for extended period. Add or replenish the marker solution in the chip using the following procedure:

- Touch the *Unload Chip* button on the *Home* screen to open the chip door.
- Return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from chip well 4 using a vacuum line.
- Ensure that chip well 4 is rinsed and completely aspirated with water (Milli-Q® or equivalent).
- Repeat chip well rinsing with water.
- Add Marker Solution (green cap) to chip well 4.

- Reinsert the chip back into the instrument.
- Restart the run.

2 Perform a marker channel unclogging procedure by repriming the chip. See “LabChip Kit Essential Practices” on page 33 for instructions on how to reprime the chip.

Symptom: Ladder traces show up in the lanes following the ladders (delayed sip).

Possible causes:

1 Separation channel overloaded with sample.

2 Partial clog in the separation channel.

What to do:

1 Lower the starting sample concentration.

2 Reprime the chip. See “LabChip Kit Essential Practices” on page 34 for instructions on how to reprime the chip.

Symptom: Unexpected sharp peaks.

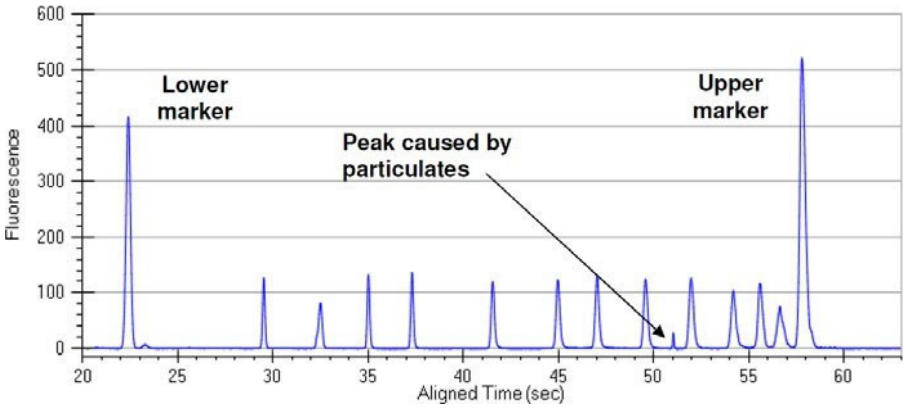

Figure 18. Unexpected sharp peak. Note-a DNA ladder from DNA HiSense Assay is shown here for illustrative purpose.

Possible causes:

- Dust or other particulates introduced through sample or reagents.

What to do:

1 Do one or all of the following:

- Replace the 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent) water used for chip preparation.
- Replace the buffer used for sample and reagent preparation.
- Use a 0.22-micron filter for all water and buffers used for chip, sample, and reagent preparation.
- Spin down sample plate to pellet any particulates.

Symptom: Peaks migrating much faster or slower than expected.

Note: *Some migration time variances between chips or within a plate is considered normal chip performance. All chips are QC tested at Revvity prior to shipment.*

Normal migration time windows for the markers are:

- *pDNA assay Lower Marker (45 - 51 seconds)*
- *pDNA assay Upper Marker (56 - 64 seconds)*

Possible causes:

1 Incorrect Gel-Dye ratio. Migration time is sensitive to dye concentration and peaks will migrate too fast or too slow if the dye concentration in the gel is too low or too high, respectively.

Note: *Excess dye within the separation channel will slow down migration, and less dye in the separation channel will make peaks migrate faster.*

2 Particulates from the samples may be clogging the separation channel (this will slow down migration).

3 Gel-Dye was not primed properly into the chip.

What to do:

1 Prepare a fresh Gel-Dye solution. Wash and reprime the chip with the new Gel-Dye mixture. See “LabChip Kit Essential on page 32 for instructions on how to wash and reprime the chip.

2 If fast or slow migration is observed repeatedly on a new chip, contact technical support to arrange return of the chip to Revvity. Please send a data file showing the failure along with the return request.

3 Minimize the loading of particulates in the sample by performing a centrifuge spin of the sample plate (e.g. 9300 rcf for 5 minutes) before starting a new run. The debris may be flushed out of the chip by washing and re-priming the chip. See page 34 for instructions on how to wash and reprime the chip.

4 Check the O-rings on the top surface of the chip interface and clean if necessary.

LabChip Kit Essential Practices

To ensure proper assay performance, please follow the important handling practices described below. Failure to observe these guidelines may void the LabChip Kit product warranty.[1]

Note: *It is important to keep particulates out of the chip wells, channels and capillary. Many of the following guidelines are designed to keep the chips particulate-free.*

For assay and instrument troubleshooting, refer to the LabChip GX Touch software Help file or call Revvity Technical Support at 1-800-762-4000.

General

- Allow the chip, sample plate, and all reagents to equilibrate to room temperature for at least 30 minutes before use.
- Clean the O-rings in the chip interface weekly and the electrodes daily. Refer to the Instrument Users Guide Maintenance and Service section for procedures.
- Avoid use of powdered gloves. Use only non-powdered gloves when handling chips, reagents, sample plates, and when cleaning the instrument electrodes and electrode block.
- Calibrate laboratory pipettes regularly to ensure proper reagent dispensing.
- Only the Revvity-supplied clean room cloth can be used on the chip to clean the detection window.
- Water used for chip preparation procedures must be 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- Using the “Reverse Pipetting Technique” (described next) will help avoid introducing bubbles into the chip when pipetting the gel.

Reverse Pipetting Technique

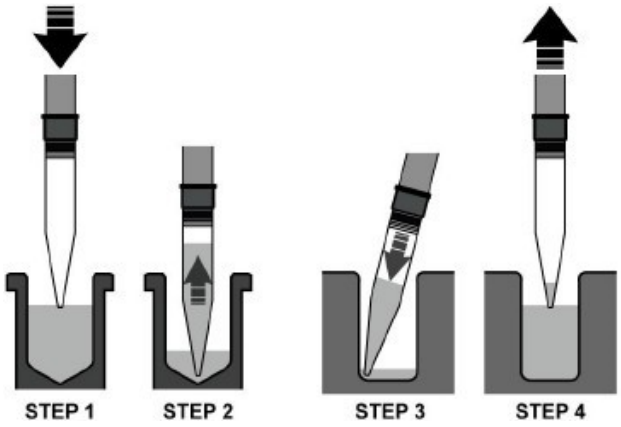

Figure 19. Reverse pipetting.

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.

Reagents

- Store reagents at 2 - 8°C when not in use.
- The LabChip dye contains DMSO and should be thawed completely before use. It is recommended that you prepare aliquots to reduce the time required for thawing.
- Gently vortex all kit reagents before use.
- Dispense reagents into chip wells slowly without introducing air bubbles. Insert the pipette tip vertically and to the bottom of the chip well.
- Protect the dye and Gel-Dye mixture from light. Store in the dark at 2 - 8°C when not in use.
- The Gel-Dye mixture should be prepared fresh on the day of use.

Chips

Removing Sipper Clogs

- If air bubbles are not dislodged after a reprime, apply a vacuum to the sipper. Perform this by filling all active wells with 100 µL of Chip Storage Buffer. Then suction the sipper with a vacuum line as shown in Figure 20 until droplets of fluid flow out from the sipper. When suctioning the sipper, be careful not to bend or break the sipper. To facilitate this, cut the end of the pipette tip attached to the vacuum line to widen the mouth.

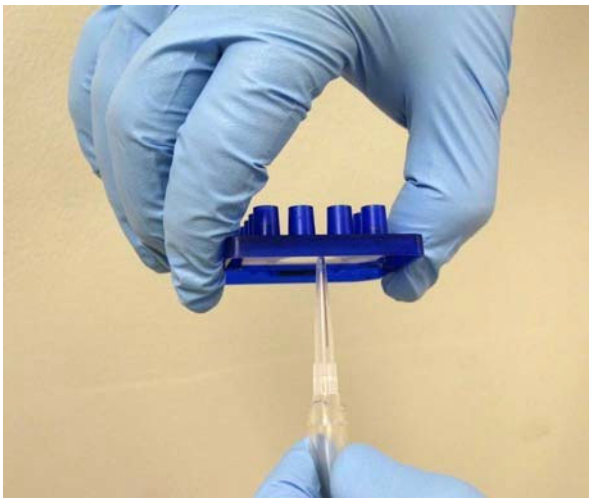

Figure 20. Removing an air bubble or clog by suctioning the sipper with a vacuum line.

Other Considerations:

- Chips should be stored at 2 - 8°C prior to first use.
- Cover the wells with Parafilm® to prevent evaporation and store at 2 - 8°C until next use. If using the chip again within 24 hours, it may be left at room temperature. Allowing the chip wells to dry may lead to changes in chip performance.
- Do not allow the liquid in the chip container to freeze, as this may lead to poor chip performance. Do not submerge the chip in any solution.
- The entire chip surface must be thoroughly dry before use.
- The sipper must be kept immersed in fluid at all times and should not be exposed to an open environment for long periods of time.
- Use care in chip handling to prevent sipper damage. Damage to the sipper can result in inconsistent sampling.
- Avoid exposing the chips to dust by keeping them in a closed environment such as in the chip container or in the instrument before and after chip preparation.

- Chips can be prepared and left idle on the instrument for up to 8 hours. This workflow allows analysis of samples as needed throughout the day without having to re-prep the chip as long as the maximum number of samples per chip prep is not exceeded.
- Revvity recommends the chip be re-prepared after it has been idle for 8 hours.Chip Well Aspiration Using a Vacuum
-

Chips Aspiration using Vacuum

- Aspirating with a pipette can leave used reagents in the chip wells. For this reason, Revvity recommends vacuuming the wells instead. This can be accomplished by attaching a permanent pipette tip to a house vacuum line with trap (Figure 21). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 21).

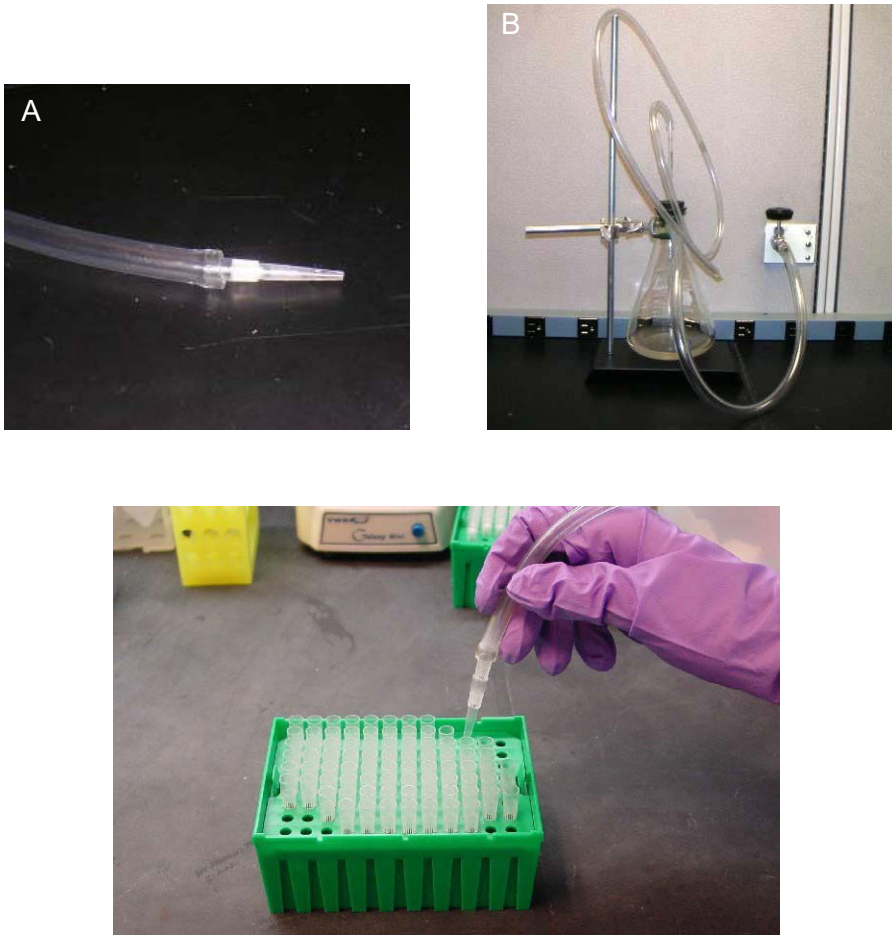

Figure 21. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap. C. Replacing the disposable pipette tip.

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For additional assay and instrument troubleshooting, refer to the LabChip GX Touch Software Help file.

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