

revvity

LabChip User Guide

DNA NGS 3K Assay User Guide

For LabChip[®] GXII Touch

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CE



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Specifications

Assay Specifications

NOTE



All specifications pertaining to DNA fragments were determined using ladder as sample in TE buffer. All specifications pertaining to DNA smears were determined using Covaris sheared control genomic DNA (human male) in TE buffer.

Table 1. Assay Specifications

Sizing Range	50 - 3000 bp
Sizing Resolution ^a	± 10% from 200 - 1000 bp ± 15% from 50 - 200 bp, 1000 - 2000 bp ± 20% from 2000 - 3000 bp
Sizing Accuracy	± 10%
Sizing Precision	5% CV
Starting Sample Linear Concentration Range	<ul style="list-style-type: none"> • 50 - 5000 pg/uL for smears • 5 - 500 pg/uL per fragment from 50 to 2000 bp • 20 - 500 pg/uL per fragment from 2000 to 3000 bp
Linear Concentration Range (on plate after dilution with marker at 1:10 ratio)	<ul style="list-style-type: none"> • 5 - 500 pg/uL for smears • 0.5 - 50 pg/uL per fragment from 50 to 2000 bp • 2 - 50 pg/uL per fragment from 2000 to 3000 bp
Sensitivity	25 pg/μL for smears 2 pg/μL per fragment
Maximum Starting DNA Concentration	5000 pg/uL total , 500 pg/uL per fragment
Quantitation Accuracy	± 30% for smear samples and fragment peaks > 2 pg/uL (concentration on plate after 1:10 dilution)
Quantitation Precision	20% CV
Carry-Over	< 0.5%

Table 1. Assay Specifications (Continued)

Analysis Time	68 seconds per sample (~2.5 hours for 96 samples)
Samples per Chip Prep	Up to 192 samples per HT chip prep Up to 48 samples per LT chip prep
Chip Preps per Reagent Kit	10 HT chip preps or 20 LT chip preps

- a. Resolution is defined as half height or better separation of two peaks. Actual separation performance can depend on the sample and application. Peaks that are resolved less than half height can still be accurately identified by the system software. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity.

Sample Conditions

Table 2. Sample Conditions

Sample Buffer	Performance of the assay is dependent on sample buffer. DNA should be in low salt buffer (up to 10 mM Tris +/- up to 1 mM EDTA, or water). Higher salt concentrations, alternative buffers, and presence of detergents or other additives can result in decreased signal or assay failure. Non-aqueous solvents are not compatible with DNA LabChip protocols and may result in chip failure.
Particulates	Sample plates should be free from particles such as magnetic beads or precipitates. If samples have been purified on magnetic beads, they should be cleared by a magnet when taking an aliquot for analysis with the LabChip. Spin down all prepared sample plates prior to placing on the instrument for analysis.
Maximum Salt Concentration ^a	10 mM Tris, 1 mM EDTA

- a. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity.







DNA NGS 3K Reagent Kit Contents

The DNA NGS 3K Reagent Kit (P/N CLS960013) contains the reagents and consumables listed in the tables below.

Note: Use only consumables that are within their expiration date.

Storage: Store reagents at 2-8°C.

Table 3. Reagents

Reagent	Vial	Quantity
DNA NGS 3K Dye Concentrate	Blue 	1 vial, 0.09 mL
DNA Chip Storage Buffer	White 	9 vials, 1.8 mL each
DNA Hi Sens/NGS3K Gel Matrix	Red 	5 vials, 1.1 mL each
DNA NGS 3K Ladder	Yellow 	1 vial, 0.26 mL each
DNA NGS 3K Marker ^a	Orange 	1 centrifuge tube, 45 mL (packaged separately)
NGS 3K Marker Booster	Purple 	2 vials, 1.1 mL each

a. **Note:** DNA NGS 3K Marker can be ordered separately (P/N CLS920012).

Table 4. Consumable Items

Item	Supplier and Catalog Number	Quantity
Spin Filters	Costar [®] , Cat. # 8160	10
Detection Window Cleaning Cloth	VWR [®] , Cat. # 21912-046	1
Swab	ITW Texwipe [®] , Cat. # TX758B	3
Ladder Tubes, 0.2 mL	(Not sold separately)	20
Buffer Tubes, 0.75 mL	(Not sold separately)	20

Compatible LabChips

The DNA NGS 3K assay requires one of the chip types below. Note that HT chips are not compatible with GX Touch 24 or GXII Touch 24 instruments.

Storage: When not in use, store chips at 2-8°C. If using a prepared chip again within 24 hours, the chip can be stored at room temperature.

Table 5. DNA NGS 3K LabChips

Item	Part Number	Samples per Chip ^a
HT X-Mark DNA LabChip (GX Touch/GXII Touch HT)	CLS144006	1000
24 X-Mark LabChip (GX Touch/GXII Touch HT or 24)	CLS145331	500

a. Expected chip lifetime is based on use under normal laboratory conditions and adherence to Revvity chip preparation protocols, recommended sample composition, instrument maintenance procedures, and recommended chip and reagent storage. Individual results may vary.

Compatible Software

LabChip GX Touch software version 1.4 or higher, and LabChip GX Reviewer software version 5.2 or higher

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. All chemicals should be considered potentially hazardous. When handling chemical reagents, wear suitable protective clothing, such as laboratory overalls, safety glasses, and gloves. Avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

WARNING!



DNA NGS 3K Dye contains DMSO. Avoid contact with skin and eyes.

Usage

The DNA NGS 3K Assay is for use with LabChip GX Touch/GXII Touch instruments. LabChip GX Touch/GXII Touch instruments are for research use only and not for use in diagnostic procedures.

Preparation Procedures

Additional Items Required

- 18 megohm, 0.22- μ m filtered water (Milli-Q[®] or equivalent)
- 70% isopropanol solution in DI water
- Bio-Rad Hard-Shell[®] 384-well Skirted PCR Plates, Cat. # HSP-38XX (recommended)
- Bio-Rad Hard-Shell[®] 96-well Skirted PCR Plates, Cat. # HSP-96XX (recommended)

Preparing the Gel-Dye Solution

The volume of Gel-Dye solution prepared is the amount required for **2 High-Throughput** chips preps (for up to 192 samples each) or **4 Low-Throughput** chip preps (for up to 48 samples each).

Warning: *The dye and marker are light sensitive. Do not expose the Marker, Dye or Gel-Dye solution to light for any length of time. Keep the prepared Gel-Dye solution in the dark.*

- 1 Allow the chip and reagents to equilibrate to room temperature for at least 30 minutes before use. Protect the Dye and Marker from light while warming.

Note: *The DNA NGS 3K Dye contains DMSO and must be thawed completely and vortexed before use.*

- 2 Vortex the thawed **DNA NGS 3K Dye Concentrate** (blue cap ●) for 10-15 seconds and spin briefly to bring contents to the bottom of the tube.
- 3 Transfer **13 μ L** of **DNA NGS 3K Dye Concentrate** (blue cap ●) to **1 vial** of **DNA Hi Sens/NGS3K Gel Matrix** (red cap ●).
- 4 Vortex and invert the tube several times until the solution is well mixed and then spin down the mixture for a few seconds.
- 5 Transfer the Gel-Dye solution into **two spin filters** (approximately 550 μ L each).
- 6 Centrifuge the solutions in the spin filters at 9300 rcf for 7.5 minutes at room temperature.
- 7 Discard the filters.
- 8 Label and date the tubes.
- 9 Store in the dark at 2-8°C. Use within 3 weeks.

Preparing the Marker

A new optional NGS 3K Marker Booster is now included in the DNA NGS 3K Reagent Kit.

The DNA NGS 3K Assay is often used to measure DNA extract samples to assess size, purity and quantity for use in research applications because of the high sensitivity and throughput of the assay. Each extraction kit employs proprietary buffer chemistries optimized for each vendor. As such, each extraction kit yields samples that may vary slightly in final buffer formulation, ethanol contamination, salt carry-over, and protein contamination. In some cases, upper marker injection can be affected. This was commonly found for samples derived from extraction kits to isolate cell-free DNA. If the upper marker height is significantly low (lower than the lower marker height), then the **NGS 3K Marker Booster** can be added to the **DNA NGS 3K Marker** prior to sample preparation.

If not using the optional NGS 3K Marker Booster, the NGS 3K Marker is used undiluted as provided in the reagent kit.

Marker Preparation with NGS 3K Marker Booster (Optional)

It is recommended to mix the NGS 3K Marker Booster immediately prior to running the assay (with scaling to the required total volume of marker solution).

To prepare the DNA NGS 3K Marker with NGS 3K Marker Booster:

- 1 Add **1 mL** of **NGS 3K Marker Booster** (purple cap ●) to each **27 mL** of **DNA NGS 3K Marker** (orange cap ●) and mix well.
- 2 Vortex well and protect from light.
- 3 Continue with preparation as described in [Preparing the DNA Samples, Ladder, and Buffer Tube on page 10](#).

Preparing the DNA Samples, Ladder, and Buffer Tube

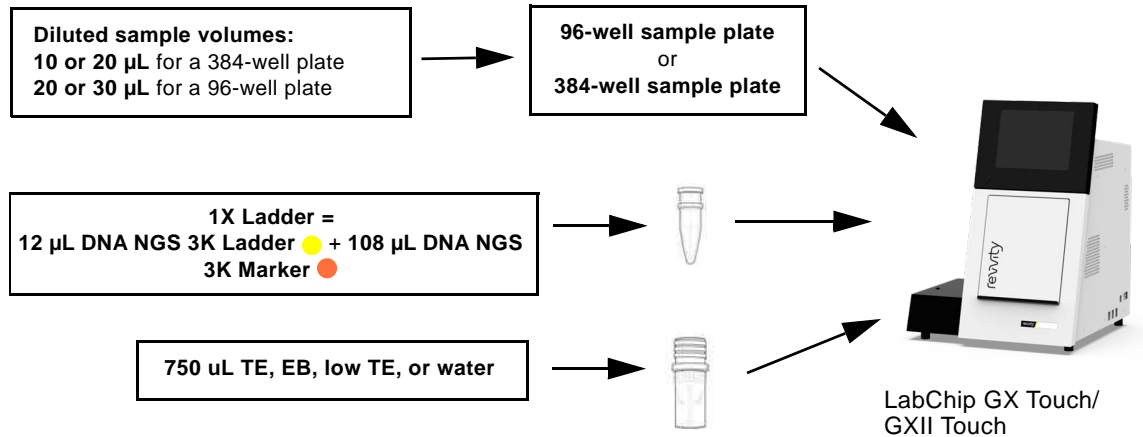


Figure 1. Sample Workflow

DNA Samples

NOTES



- **Samples must be diluted 1:10 with DNA NGS 3K Marker prior to analysis.** Use appropriate volumes of diluted samples based on the plate type and the total number of samples to be analyzed. Suggested volumes are shown in the table below. For longer runs (> 96 samples) or under low humidity conditions, use higher volumes of diluted sample as shown in [Table 6](#).
- **Ensure that the concentration of samples are within the linear concentration range.** Overloading the assay with samples containing pre-dilution concentrations > 5 ng/ μL will result in poor performance. If samples exceed 5 ng/ μL , either pre-dilute the samples in TE prior to adding them to the DNA NGS 3K Marker or increase the amount of DNA NGS 3K Marker used for dilution.

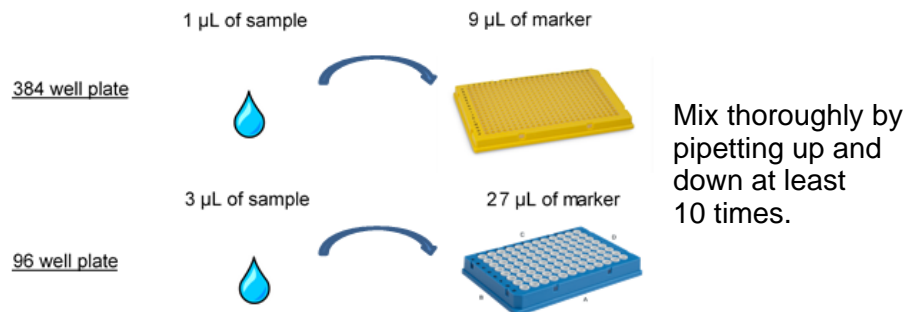


Figure 2. Typical Sample Preparation

To prepare samples:

- 1 Determine the volume of sample, volume of DNA NGS 3K Marker, and type of plate using the table below:

Table 6. Sample and Marker Volumes

Final Volume	Sample Volume	Marker Volume	Plate Type	Recommended Samples/Run
10 µL	1 µL	9 µL	384	≤ 96
20 µL	2 µL	18 µL	384	≤ 192
20 µL	2 µL	18 µL	96	≤ 48
30 µL	3 µL	27 µL	96	≤ 192 ^a

a. 192 = 96 samples x 2 plate cycles.

- 2 Pipet the appropriate volume of **DNA NGS 3K Marker** (orange cap ●) (or Marker mixed with Marker Booster) into each well.
- 3 Add the appropriate volume of **sample** to each well.
Note: If samples have been exposed to magnetic beads, place samples on magnet prior to removing the aliquot for analysis. Any carryover of magnetic beads may compromise assay performance.
- 4 Mix the sample and Marker thoroughly by pipetting up and down at least ten times.
- 5 Cover with PCR cap strips and spin down the plate at 3000 rpm for 5 minutes to remove air bubbles.
- 6 Remove the PCR cap strips and place the sample plate on the LabChip GX Touch/GXII Touch instrument.

DNA Ladder

- 1 Gently vortex the **DNA NGS 3K Ladder** (yellow cap ●) for 10 seconds. Briefly spin the ladder vial.
- 2 Transfer **12 µL** of **DNA NGS 3K Ladder** to the 0.2 mL Ladder Tube provided with the reagent kit.
- 3 Add **108 µL** of **DNA NGS 3K Marker** (orange cap ●) to the Ladder. Mix thoroughly by pipetting the solution up and down several times. Ensure there are no air bubbles in the Ladder Tube.
- 4 Insert the Ladder Tube into the ladder slot on the LabChip GX Touch/GXII Touch instrument (see [Figure 3](#)).

DNA Buffer Tube

- 1 Add **750 μL** of **DNA buffer** (TE, water, EB, or low TE) 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 (see [Figure 3](#)).

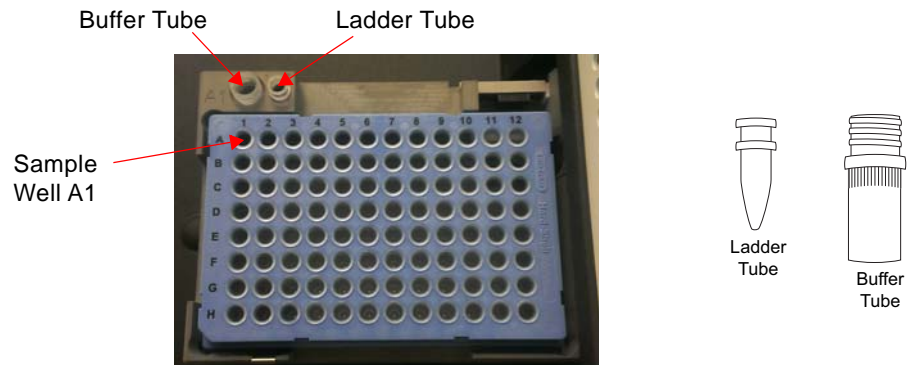


Figure 3. Buffer Tube and Ladder Tube in the GX Touch/GXII Touch

- 3 After loading the plate, buffer tube, and ladder tube, touch the **Load Plate** button on the Home window to retract the sample plate.

Preparing the Chip

NOTE



Unlike other DNA assays, chip well 4 is left empty during chip preparation. The DNA NGS 3K Marker is mixed with the sample directly on the sample plate. Remove any liquid from chip well 4 during chip preparation.

- 1 Allow the chip to equilibrate to room temperature for at least 30 minutes before use.
- 2 Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells (see [Figure 4](#)). For details on how to set up a vacuum line, see [page 42](#).

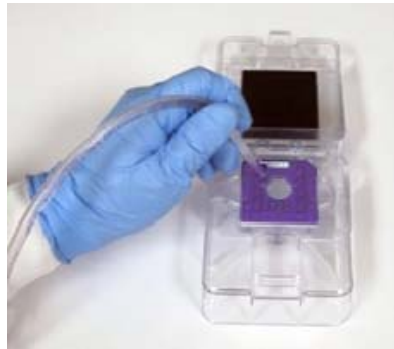


Figure 4. Using a vacuum to aspirate the chip wells is more effective than using a pipette

- 3 Rinse and completely aspirate each active chip well (1, 3, 7, 8, and 10) twice with water (Milli-Q[®] or equivalent). Do not allow active wells to remain dry.
- 4 If any water spills onto the top or bottom of the chip surfaces during rinsing, aspirate using the vacuum line. DO NOT run the tip over the detection window. Use the provided Detection Window Cleaning Cloth dampened with water (Milli-Q[®] or equivalent) to clean the chip detection window as needed.
- 5 Using a reverse pipetting technique, add **Gel-Dye solution** to chip wells **3, 7, 8, and 10** as shown in [Figure 5](#) (Low-throughput) or [Figure 6](#) (High-throughput).

Preparing the Chip (Continued)

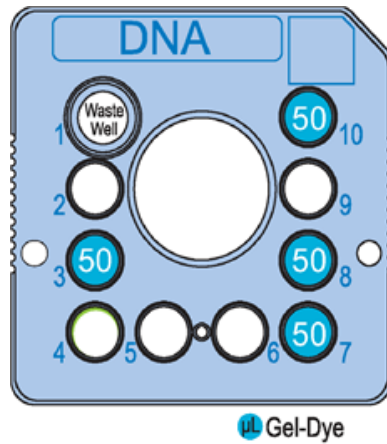


Figure 5. Reagent placement for Low-throughput (up to 48 samples)

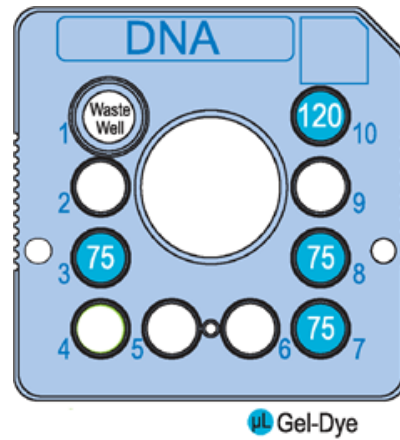


Figure 6. Reagent placement for High-throughput (up to 192 samples)

- 6 Make sure the rims of the chip wells are clean and dry.
- 7 **IMPORTANT:** Ensure chip **well 1** and **well 4** are empty before placing the chip into the instrument.

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

- 1 Before inserting the chip, close the instrument door and touch the **Purge Pressure Lines** button on the **Home** window to prevent any potential liquid or particles on the chip interface from getting into the chip during priming.

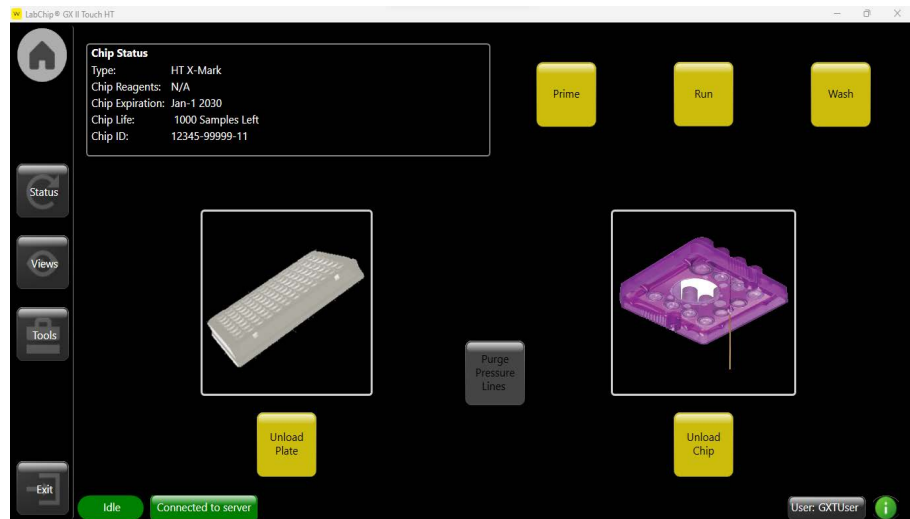


Figure 7. Home Window

- 2 Check that the sample plate, Buffer Tube, and Ladder Tube are placed on the instrument properly. **Ensure all of the samples, including the ladder, have been diluted in the DNA NGS 3K Marker.**
- 3 Touch the **Unload Chip** button on the **Home** window.
- 4 Clean the electrodes (shown in [Figure 8](#)) using the provided lint-free swabs dampened with DI water. Do not leave any visible droplets of water on the electrodes.

Inserting a Chip into the LabChip GX Touch/GXII Touch Instrument (Continued)

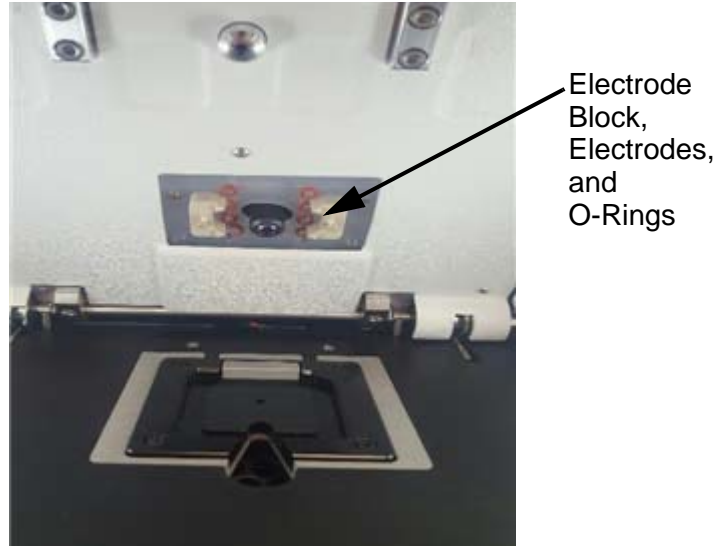


Figure 8. Chip Loading Area

- 5 Remove the chip from the chip storage container and inspect the detection window. Clean BOTH sides of the detection window with the supplied clean-room cloth dampened with a 70% isopropanol solution in DI water.
- 6 Place the chip on the chip heater block in the LabChip GX Touch/GXII Touch instrument ([Figure 9](#)) and close the chip door securely.

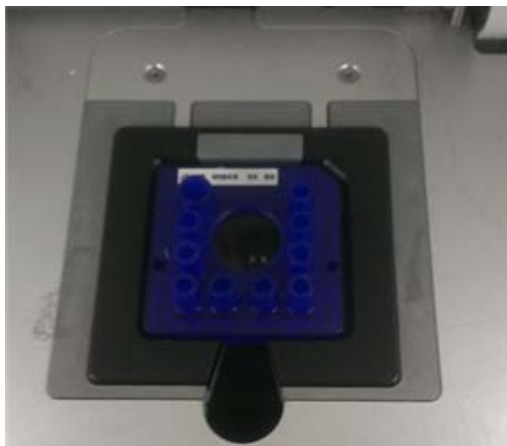


Figure 9. Chip in the LabChip GX Touch/GXII Touch Instrument

Note: Close the chip door promptly. Dye is sensitive to light and can be photobleached if the door is left open.

Priming the Chip

After a chip is placed in the instrument, the chip is automatically primed at the start of the first run.

Since priming is a lengthy process, the chip can be primed before starting the run. (To save time, the sample plate can be prepared while the chip is priming.)

To prime the chip without running an assay:

- 1 Insert the chip (see [page 15](#)).
- 2 Load a prepared Buffer Tube (see [page 12](#)). If running a test ladder, load a prepared Ladder Tube at the same time.
- 3 Touch the **Prime** button on the Home window. The Prime window opens (see [Figure 10](#)).

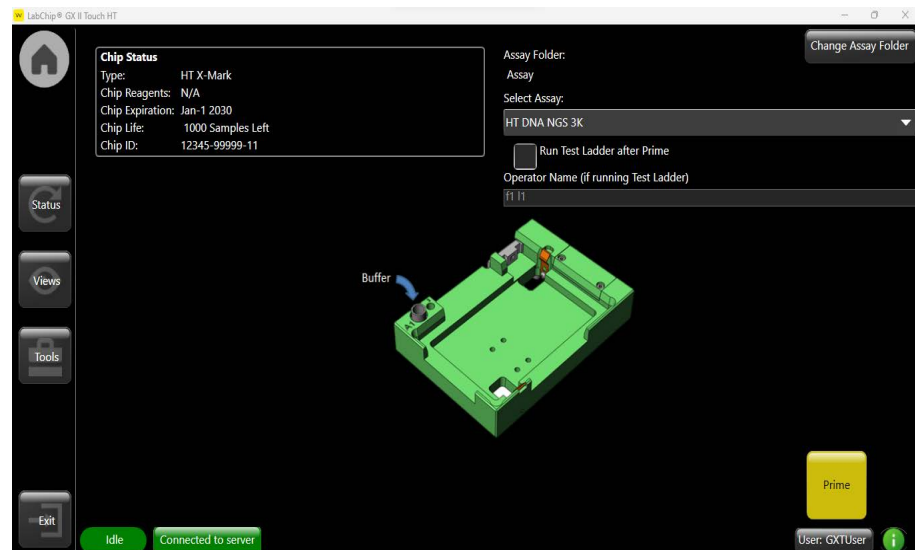


Figure 10. Prime Window

- 4 Select the assay for which to prime the chip in the **Assay** drop-down list.
- 5 If desired, select the **Run Test Ladder after Prime** check box to run one ladder after the prime is complete
- 6 Touch the **Prime** button to start the prime.

Operating Procedures

Running the Assay

To run an assay:

- 1 Touch the **Run** button on the **Home** window (see [Figure 7](#) on [page 15](#)). The **Select Wells** tab opens (see [Figure 11](#)).

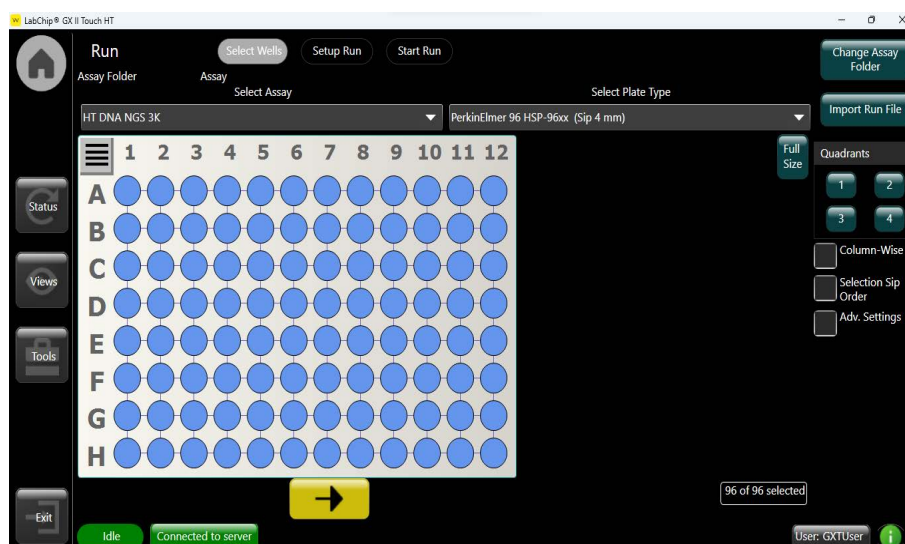


Figure 11. Select Wells Tab

- 2 Select the desired assay type, plate type, well pattern, and sip order (column or row). If desired, click **Adv. Settings** to specify the number of times each well is sampled.
- 3 Touch the **Green Arrow** button. The **Setup Run** tab ([Figure 12](#) on [page 19](#)) opens.

Running the Assay (Continued)

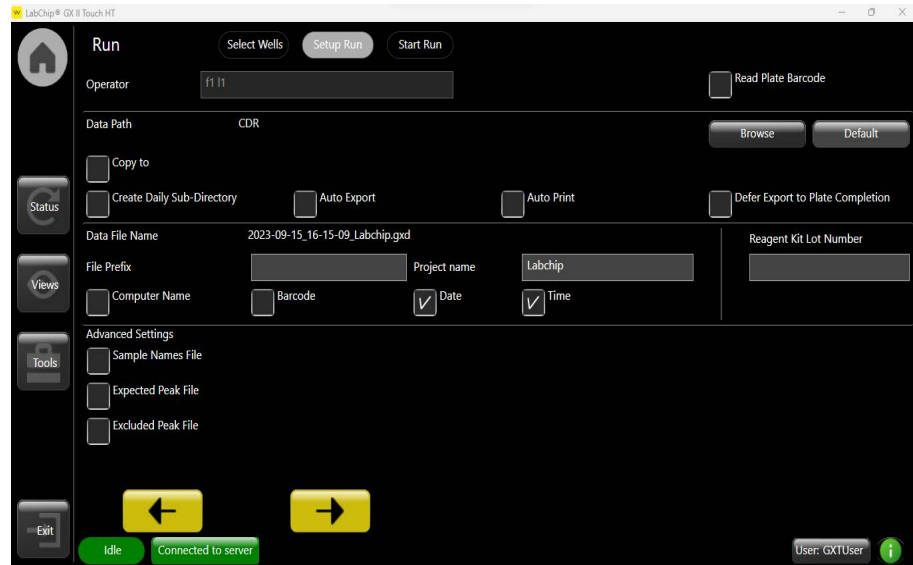


Figure 12. Setup Run Tab

- 4 Specify the operator name, the option to read the barcode, the destination of the file, the use of sample names, expected peaks, and excluded peaks files, the filename convention, and auto export option.
- 5 Touch the **Green Arrow** button. The Start Run tab ([Figure 13 on page 20](#)) opens.

Running the Assay (Continued)

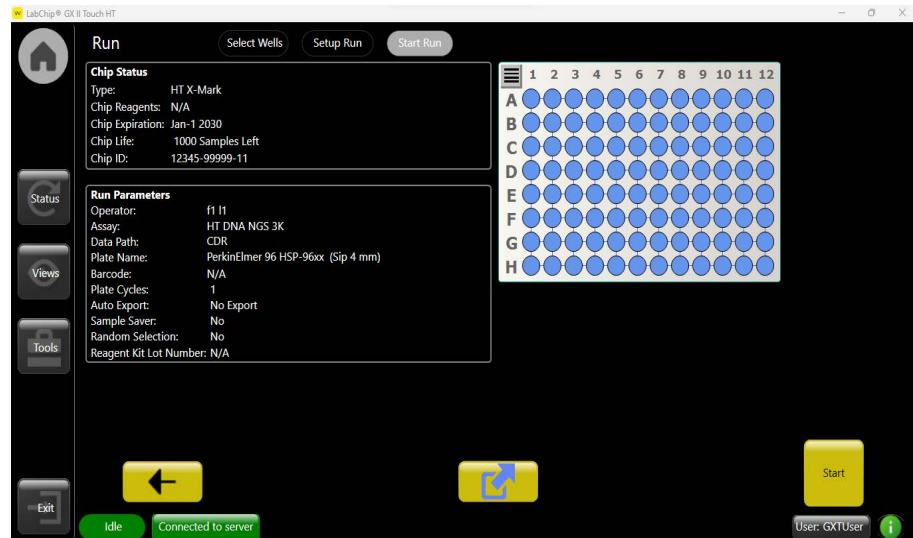


Figure 13. Start Run Tab

- 6 Touch **Start** to begin the run. If the chip has not been primed yet, the chip is primed automatically at the start of the first run.

Repriming Chips

If air bubbles or clogs in the chip channels are suspected, the chip can be reprimed to help remove air bubbles or clogs.

- 1 Touch the **Unload Chip** button on the **Home** window to open the instrument door. The software automatically resets to require priming prior to running the chip again.
- 2 Place the chip into the instrument.
- 3 Place a Buffer Tube with **750 μ L** of sample buffer or water (Milli-Q[®] or equivalent) in the buffer slot.
- 4 Close the chip door securely.
- 5 Touch the **Prime** button on the **Home** window. The Prime window opens.
- 6 Touch the **Prime** button on the Prime window to reprime the chip.

Washing and Repriming Chips

Washing the chip clears all reagents from the chip channels. The chip can be immediately reprimed to help remove air bubbles, clogs, particulates, or residue.

- 1 Touch the **Unload Chip** button on the **Home** window to open the instrument door.
- 2 Remove the chip from the instrument and place the chip into the chip storage container. Verify the sipper is submerged in the fluid reservoir.
- 3 Thoroughly aspirate all fluid from each chip well using vacuum.
- 4 Rinse and completely aspirate each active well (1, 3, 7, 8, and 10) twice with water (Milli-Q[®] or equivalent). Do not allow active wells to remain dry.
- 5 Add **100 µL** of **DNA Chip Storage Buffer** to each active well.
- 6 Place the chip in the LabChip GX Touch/GXII Touch instrument.
- 7 Place a Buffer Tube with **750 µL** of water (Milli-Q[®] or equivalent) in the buffer slot.
- 8 Close the chip door securely.
- 9 Touch the **Wash** button on the **Home** window. The Wash window opens.
- 10 Touch the **Wash** button on the Wash window to start the chip wash.
- 11 After the completion of the wash cycle, touch the **Unload Chip** button on the **Home** window to open the instrument door.
- 12 Return the chip to the chip storage container, ensuring the sipper is submerged in the fluid reservoir.
- 13 Thoroughly aspirate all fluid from the chip wells using vacuum.
- 14 Rinse and completely aspirate each active well (1, 3, 7, 8, and 10) twice with water (Milli-Q[®] or equivalent). Do not allow active wells to remain dry.
- 15 Prepare the chip as described on [page 13](#).
- 16 Place the chip into the LabChip GX Touch/GXII Touch instrument and close the door securely.
- 17 Touch the **Run** or **Prime** button on the **Home** window.

Cleaning and Storing the Chip

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

- 1 Place the chip into the chip storage container. Verify the sipper is submerged in the fluid reservoir.
- 2 Remove the reagents from each well of the chip using the vacuum.
- 3 Rinse and completely aspirate each active well (1, 3, 7, 8, and 10) twice with water (Milli-Q[®] or equivalent).
- 4 Add **100 µL** of **DNA Chip Storage Buffer** (white cap ○) to the active wells.
- 5 Place the chip in the LabChip GX Touch/GXII Touch.
- 6 Place a Buffer Tube with **750 µL** of water (Milli-Q[®] or equivalent) in the buffer slot.
- 7 Touch the **Wash** button on the Home window.
- 8 Touch the **Wash** button on the Wash window to start the chip wash.
- 9 When the chip wash is complete, remove the chip from the instrument and place the chip into the chip storage container.
- 10 Add an additional **50 µL** of **DNA Chip Storage Buffer** to well 1.
- 11 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, the chip can be stored at room temperature. Storing a chip with dry wells may clog the chip.

Chip Cartridge Cleaning

Daily

- 1 Inspect the inside of the chip cartridge and O-rings for particles or debris.
- 2 If a long run has been performed, or if the chip has been left on the instrument overnight, touch the **Purge Pressure Lines** button. The purge is complete when the Run Status displays “Purge successfully completed” and the instrument status returns to Idle.
- 3 Use the provided lint-free swab dampened with water (Milli-Q[®] or equivalent) to clean the electrodes and 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.

Monthly

- 1 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 Touch instrument. Soak the O-rings in water (Milli-Q[®] or equivalent) for a few minutes. Clean the O-ring faces by rubbing between two gloved fingers.
- 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 water (Milli-Q[®] or equivalent).
- 3 Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

Results

DNA NGS 3K Ladder Result

The electropherogram of a typical DNA NGS 3K ladder is shown in [Figure 14](#). Between the upper and lower markers, peaks in order of increasing migration time correspond to the ladder fragments of 50, 100, 150, 200, 300, 400, 500, 600, 700, 1000, 1500, 2000, and 3000 bp.

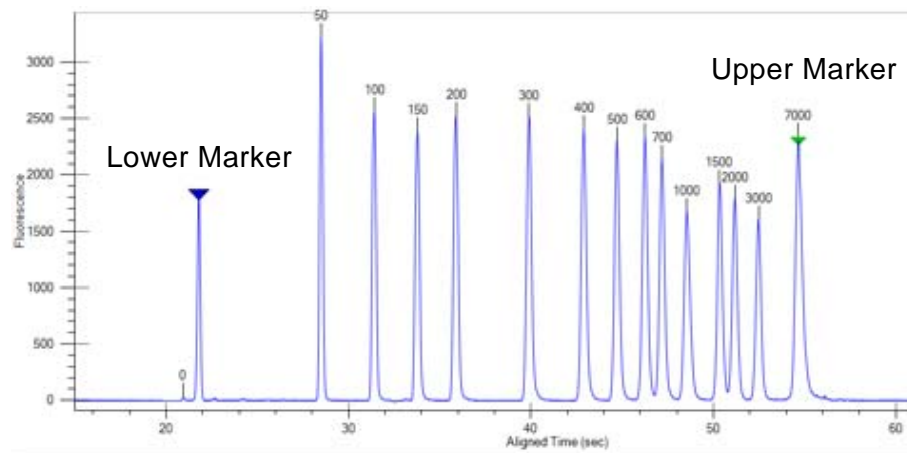


Figure 14. DNA NGS 3K Ladder Electropherogram

Concentration Accuracy

To obtain accurate concentration values from the NGS 3K assay, it is important to ensure proper dilution and mixing of samples. In addition, sample concentrations must be within the linear concentration range of the assay. The specifications shown on [page 3](#) serve as guidelines for expected performance. When analyzing smears or libraries, however, the specific distribution of fragments may affect the concentration range. Broadly distributed libraries may be analyzed at pre-marker dilution concentrations of up to 5 ng/ μ L, whereas more narrowly distributed libraries will have a lower linear range more similar to the range for individual fragments. Overloading samples will result in inaccurate concentration values.

Obtaining Accurate Concentration Values

- 1 Note that the assay returns values for both undiluted sample concentration and on-plate sample concentration. The value for undiluted sample concentration is calculated based on the “Dilution Correction” value specified in the **Analysis** tab of the **Analysis Settings** window. By default this value is set to 10, assuming a 1:10 dilution of sample with DNA NGS 3K Marker. If another dilution ratio is used, this value must be edited to obtain the correct undiluted sample concentration.
- 2 If the LabChip GX Touch/GXII Touch does not give concentration values as expected, the samples may not have been pipetted accurately or mixed adequately during dilution. Pipetting accuracy is crucial when diluting the samples in the DNA NGS 3K Marker, especially when working with small volumes, and it is important to ensure the samples are well mixed in the DNA NGS 3K Marker and the mixture is homogenous. If inadequate mixing is suspected, remove the sample plate and remix the samples by pipetting up and down. Spin down the plate again at 3000 rpm for 5 minutes. If this does not fix the problem, it may be necessary to use a larger volume of sample to ensure accurate dilution.

Obtaining Accurate Concentration Values (Continued)

- 3** Overloading the sample will also affect concentration accuracy, as dye depletion may occur within the separation channel. Dye depletion will show as a saddle shape in a broad smear or a sharp dip in a narrower smear (see [Figure 20](#) and [Figure 21](#) in the [Troubleshooting](#) section). Samples with narrow fragment distributions, such as those seen with some amplicon libraries or size selected libraries, will have a higher risk for dye depletion compared to samples with broad fragment distributions. Smear samples should not be run at on-plate concentrations exceeding 500 pg/μL (concentration after dilution with marker). For samples with narrow fragment distributions, the maximum load will be lower, approaching 50 pg/μL (on-plate concentration) as migration of the library begins to resemble migration of an individual fragment. Note that smears with narrow distributions will also show a lower limit of detection and can be analyzed down to concentrations approaching 0.5 pg/μL.
- 4** When mixing sample with DNA NGS 3K Marker, the recommended sample dilution is 1:10. However, to be in the linear concentration range, other dilutions (1:5, 1:20, 1:40 etc.) may be used. The sample can also be pre-diluted in TE to be in the linear concentration range once diluted 1:10 in DNA NGS 3K Marker.
- 5** If desired, a standard curve can be generated and used in place of the ladder to calculate sample concentrations. To apply a standard curve, several known concentrations of a standard sample should be included on a sample plate. During data analysis with Reviewer software, the Titer function of the Analysis Settings is used to define the standard curve and apply it to sample analysis. The standard curve can be generated from either a smear or an individual fragment.

Troubleshooting

This section contains:

- [Symptom: No Ladder, Sample or Marker peaks detected on page 28](#)
- [Symptom: Ladder detected but no sample peaks on page 29](#)
- [Symptom: No ladder peaks but sample peaks are present on page 30](#)
- [Symptom: Unexpected sharp peaks on page 30](#)
- [Symptom: Ladder and sample peaks/sample present, but no marker peaks on page 31](#)
- [Symptom: Dye Depletion: Smear sample shows an extra hump or sample peaks have a negative dip right after them on page 33](#)
- [Symptom: Delayed migration, slow migration of peaks on page 34](#)
- [Symptom: Smaller lower marker peak than expected on page 35](#)
- [Symptom: Ladder peaks appearing in sample wells on page 36](#)
- [Symptom: Lower Marker is present, but Upper Marker is missing on page 37](#)

Symptom: No Ladder, Sample or Marker peaks detected

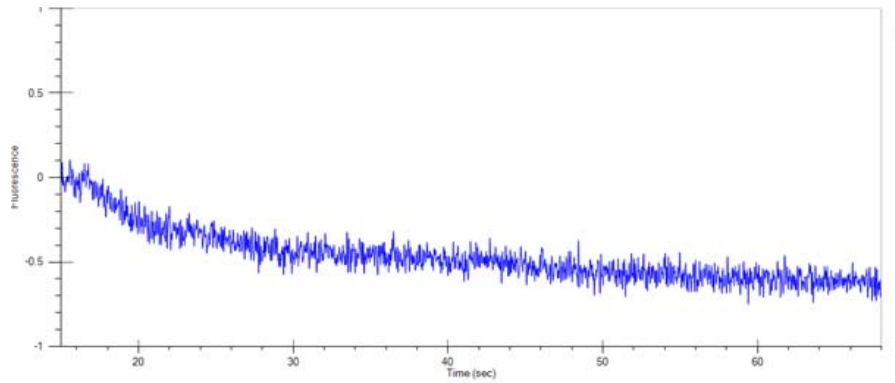


Figure 15. Profile of clogged sipper

Possible causes:

- 1 The sipper is blocked. An air bubble or particulates may have been introduced during priming or sampling.

What to do:

- 1 To remove an air bubble, reprime the chip (see [page 17](#)).
- 2 If the blockage remains after a reprime, follow the steps below to apply a vacuum to the sipper:
 - a Fill well 1 with **200 μ L of DNA Chip Storage Buffer**.
 - b Suction the sipper with a vacuum line, as shown in [Figure 16](#), 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 tip of the pipet to make a wider opening.

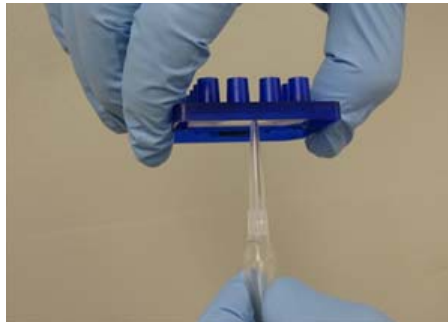


Figure 16. Removing an air bubble or clog by suctioning the sipper with a vacuum line

- 3 After clearing the sipper, empty well 1 and reprime the chip.

Symptom: Ladder detected but no sample peaks**Possible causes:**

- 1 Low volume in the sample plate due to evaporation or pipetting errors. If missing sample peaks are only found in a few wells, air bubbles may be present in those wells.

What to do:

- 1 Check the volume in the sample wells and, if necessary, add more sample and/or DNA NGS 3K Marker to the well. Be sure to check near the edge of the plate where evaporation is fastest.
- 2 If the missing sample peaks occurred only in a few wells of the plate, check those wells for air bubbles. When using 384-well plates, it is common to trap a bubble at the bottom of the well. If present, remove air bubbles by manually inserting a larger volume pipette tip (~100 μ L) into the sample well and dislodging the bubble. Spin the plate at 3000 rpm for 5 min, then retest these sample wells.
- 3 The incorrect plate definition may have been selected, such that the sip height is not deep enough in the well. For 384 well plates with 10 μ L sample, and for 96 well plates with 20 μ L sample, the sip height should be 2 mm.
- 4 Debris from the sample or sample preparation is clogging the sipper. If there may be debris, spin the sample plate down in a centrifuge. Unclog the sipper by repriming the chip or vacuuming the sipper as described in [Symptom: No Ladder, Sample or Marker peaks detected on page 28](#).
- 5 The instrument alignment may be incorrect, preventing the sipper from entering the well properly. If misalignment is suspected, contact technical support.

Symptom: No ladder peaks but sample peaks are present

Possible causes:

- 1 Low sample volume in the ladder tube due to evaporation or pipetting error.

What to do:

- 1 Add more ladder (diluted as described in [Preparing the DNA Samples, Ladder, and Buffer Tube on page 10](#)) to the Ladder Tube and restart the run. The recommended minimum ladder volume is 100 μ L. Preparing 120 μ L of ladder should allow for evaporation during the course of the day under normal laboratory conditions.
- 2 Ensure the provided tube has been used for the ladder preparation and that the ladder tube is inserted properly in the correct position on the plate holder.

Symptom: Unexpected sharp peaks

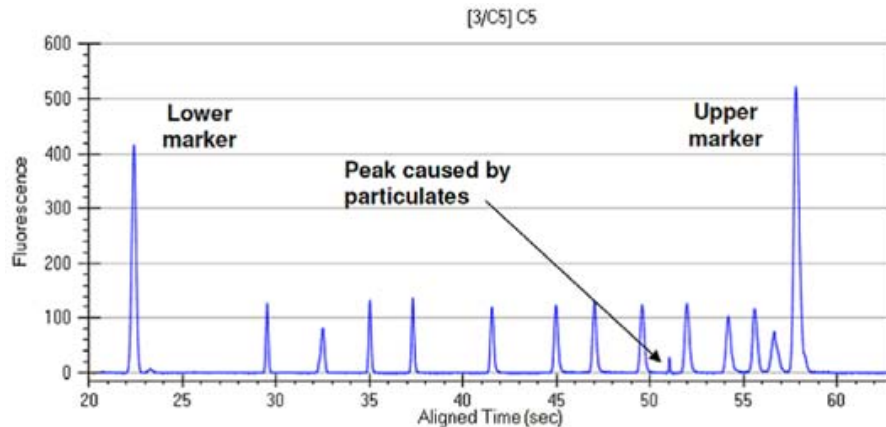


Figure 17. Electropherogram showing sharp random peaks

Possible causes:

- 1 Dust or particulates introduced to the chip from sample or reagents.

What to do:

- 1 Replace water (Milli-Q[®] or equivalent) used for chip preparation. Replace 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.

- 2 Ensure that the electrodes and O-rings on the instrument are clean and that users are following recommended daily maintenance procedures (see [Chip Cartridge Cleaning on page 23](#)).
- 3 In the GX Reviewer software, exclude extraneous peaks if they interfere with analysis by right clicking on the peak and clicking the Exclude Peak option.

Symptom: Ladder and sample peaks/sample present, but no marker peaks

Possible causes:

- 1 The samples or ladder have not been diluted with DNA NGS 3K Marker.

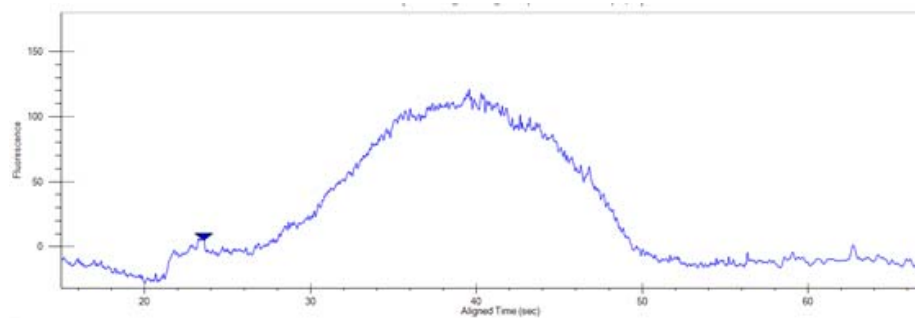


Figure 18. Smear sample diluted in TE but NO DNA NGS 3K Marker

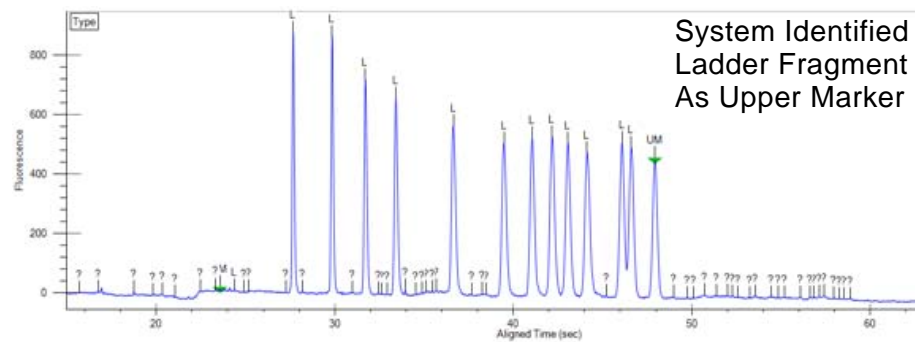


Figure 19. Ladder diluted in TE instead of DNA NGS 3K Marker

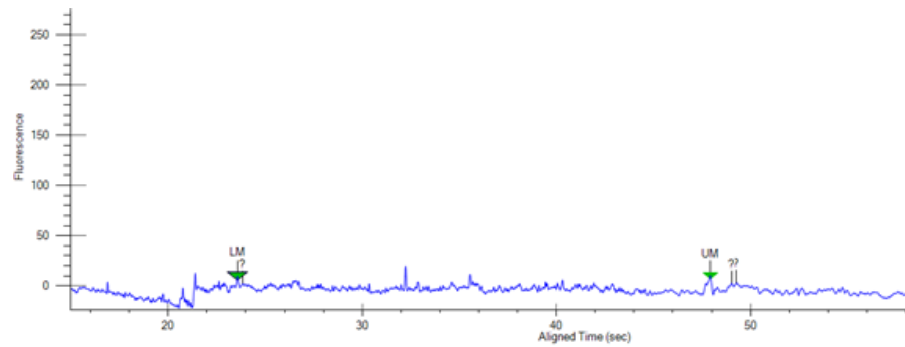


Figure 20. Blank Sample with no DNA NGS 3K Marker

What to do:

- 1 Lower marker and upper marker should be observed at 20~22 s and 50~55 s, respectively. Make sure all of the samples in the run and the ladder are diluted in the DNA NGS 3K Marker. A main difference between DNA NGS 3K assay and other LabChip DNA assays is that in the DNA NGS 3K assay, samples and ladder need to be further diluted with the provided DNA NGS 3K Marker, while in other DNA assays, samples and ladder are mixed with marker on the chip.

Symptom: Dye Depletion: Smear sample shows an extra hump or sample peaks have a negative dip right after them

Possible causes:

- 1 Sample concentration is too high creating inefficient staining of the library sample that causes a hump or dip in the signal. (see [Figure 21](#) and [Figure 22](#)).

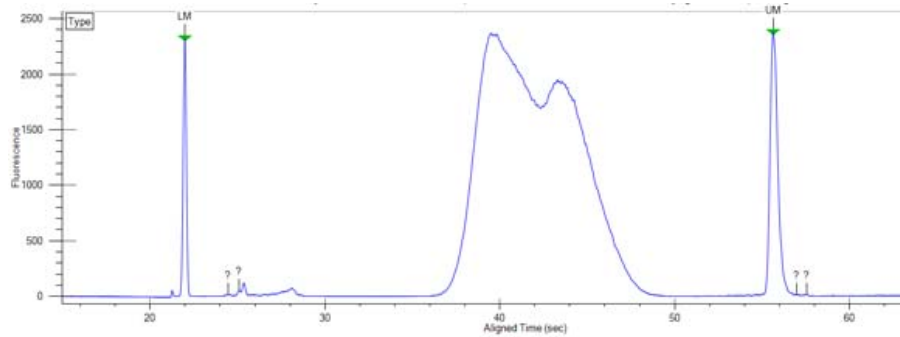


Figure 21. Overloaded Exome Library

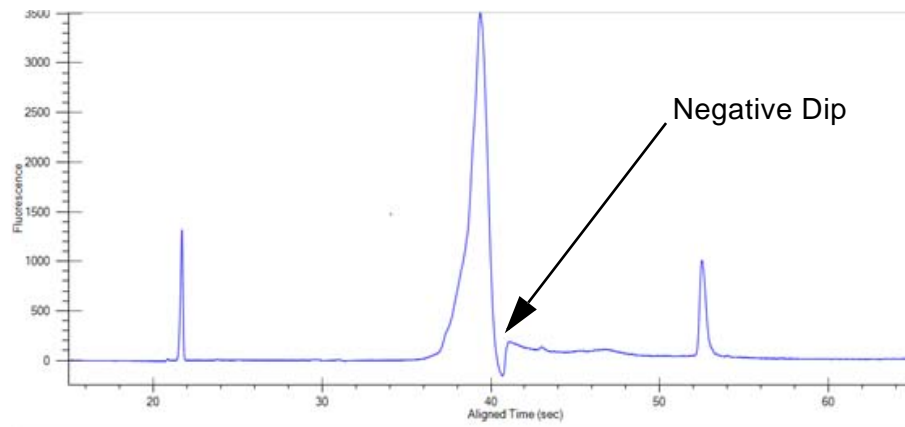


Figure 22. Overloaded Amplicon Library

- 2 A narrowly distributed smear, such as an amplicon library, will have a higher risk for dye depletion and a lower maximum concentration. See [Figure 22](#).

What to do:

- 1 Confirm samples are within the linear concentration range. Because maximum concentration for the DNA NGS 3K assay is 0.5 ng/μL on the plate, sample concentration before dilution in marker must be < 5 ng/μL.
- 2 For accurate concentration analysis, further dilute the samples to the middle of the linear concentration range. See [Concentration Accuracy on page 25](#) for more details.

Symptom: Delayed migration, slow migration of peaks

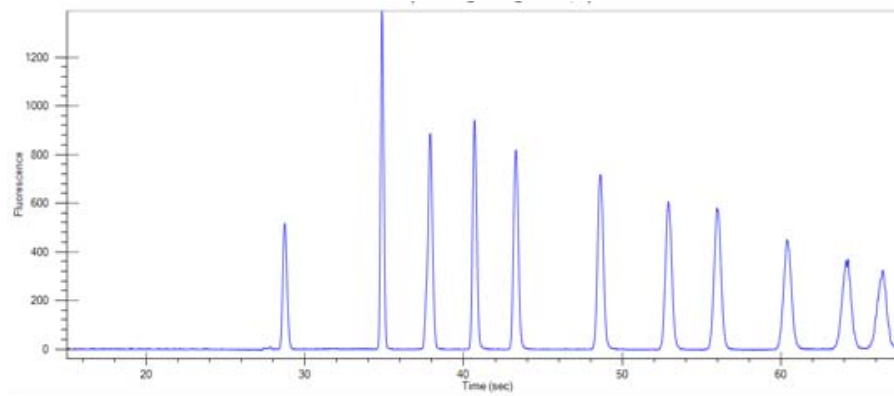


Figure 23. Delayed migration

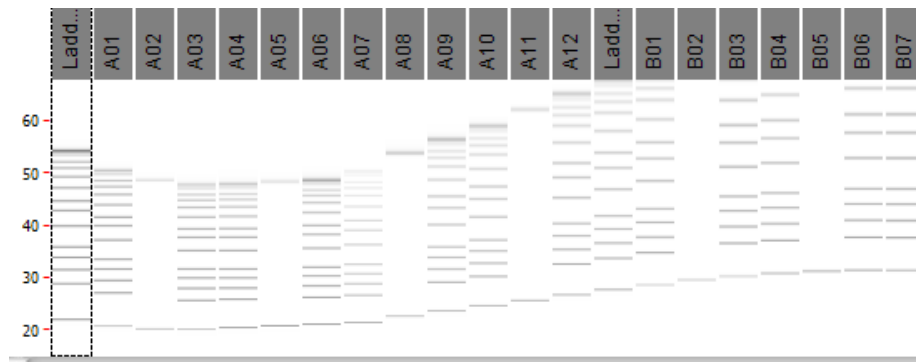


Figure 24. Gel View of run showing delayed migration. With *Analysis off*, a wave pattern can be observed as the upper marker migration time exceeds 60 sec. Normal upper marker migration time should be around 50-55 sec.

Possible causes:

- 1 Current leaks or particulates in the channels of the chip. To avoid this issue, it is VERY IMPORTANT to follow the instructions for proper instrument maintenance. Refer to [Chip Cartridge Cleaning on page 23](#).

What to do:

- 1 Remove the chip and clean the O-rings in the chip interface and the electrodes with water (Milli-Q® or equivalent) On the **Home** window, touch the **Purge Pressure Lines** button to remove any potential liquid or particles that may have been in the lines. Clean the electrodes with water (Milli-Q® or equivalent) again.
- 2 Run the instrument diagnostics to ensure that the instrument is functioning properly.

- 3 Ensure particulates are not introduced to the chip through the samples. Spin the plate at 3000 rpm for 5 min.
- 4 Wash the chip with the provided DNA Chip Storage Buffer and then re-prime and re-run the chip with fresh reagents. If the problem persists, try another chip on the instrument.
- 5 If migration delays are observed repeatedly on a new chip or observed on multiple chips, contact technical support to arrange for return of the chip to Revvity and troubleshooting assistance

Symptom: Smaller lower marker peak than expected

Figure 25 shows a broad range smear sample at 0.05 ng/μL diluted in DNA NGS 3K Marker stored under light-protected conditions (blue) overlaid with the same sample diluted in DNA NGS 3K Marker which been extensively exposed to light (red).

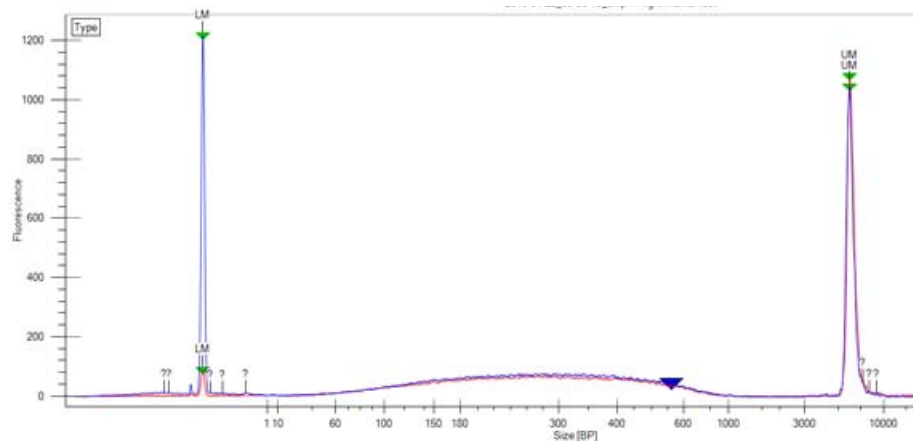


Figure 25. Lower Marker Photobleached

Possible causes:

- 1 The Lower Marker has photobleached due to extensive exposure of the DNA NGS 3K Marker to light.

What to do:

- 1 The DNA NGS 3K Marker can continue to be used provided the software is able to correctly identify the Lower Marker peak. Low signal from the Lower Marker will not have any effect on data analysis, as the peak is only used for alignment of migration times, not for quantitation of the sample.
- 2 If the software is unable to identify the Lower Marker, prepare the samples in fresh DNA NGS 3K Marker which has not been exposed to light.

Symptom: Ladder peaks appearing in sample wells

The Gel view in [Figure 26](#) shows sample data appearing during the ladder sip and ladder peaks overlapping with sample data in wells F2 and G2.

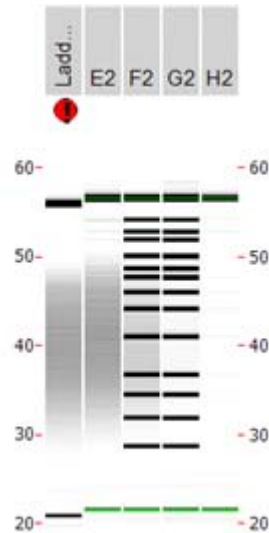


Figure 26. Ladder Peaks in Sample Wells

Possible causes:

- 1 Poor flow from the sipper to the waste well due to a partial blockage in the sipper or a bubble in the waste well channel.

What to do:

- 1 Ensure flow through the sipper by vacuuming the sipper as described in [Symptom: No Ladder, Sample or Marker peaks detected on page 28](#).
- 2 Reprime the chip.
- 3 Ensure that Well 1 is emptied immediately before placing the chip on the instrument. Do not leave Well 1 empty for extended periods of time.
- 4 If the problem persists, contact Technical Support ([page 43](#)) for assistance determining if there is an issue with the pressure line on the instrument.

Symptom: Lower Marker is present, but Upper Marker is missing

The Sample electropherogram (with analysis off) in [Figure 27](#) shows normal migration of LM and smear sample, but no Upper Marker.

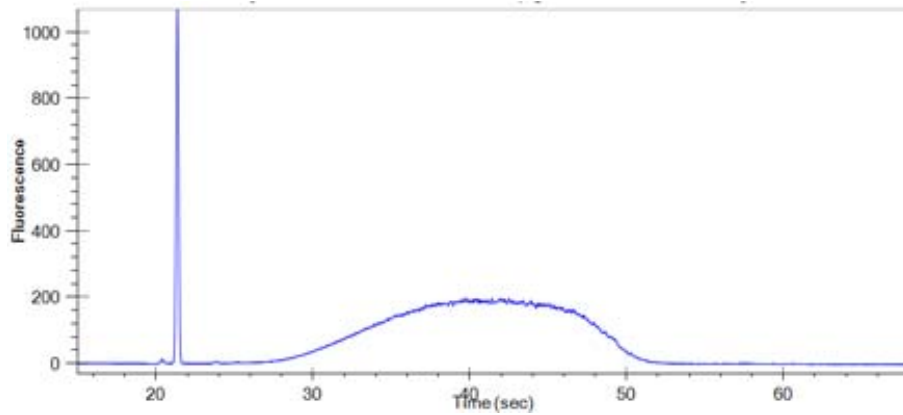


Figure 27. No Upper Marker

Possible causes:

- 1 The sample formulation has ionic strength and/or protein contamination that hinders loading of the Upper Marker into the chip separation channel.
- 2 The DNA NGS 3K Marker is contaminated and the Upper Marker is degraded. The Lower Marker is a dye molecule and is less sensitive to contamination. **Note:** It may be necessary to turn analysis off to obtain proper alignment of data. With analysis on, the problem may appear similar to delayed migration ([Figure 23](#)).

What to do:

- 1 Run the DNA NGS 3K Assay using NGS 3K Marker Booster mixed with DNA NGS 3K Marker (see [Preparing the Marker on page 9](#)).

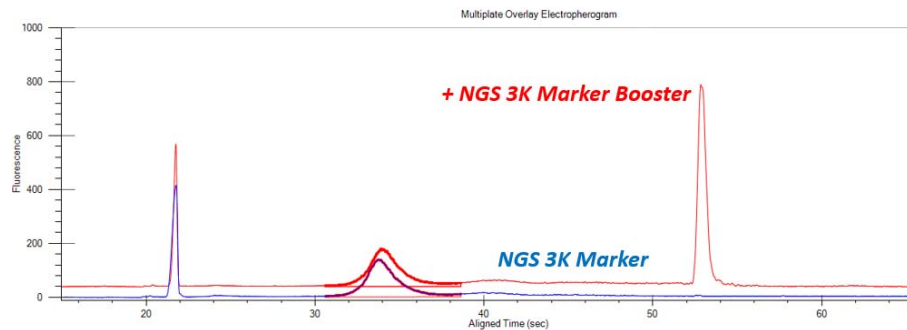


Figure 28. NGS 3K Marker only and NGS 3K Marker with Marker Booster

- 2** If the DNA NGS 3K Marker is contaminated:
 - If another tube of DNA NGS 3K Marker is available, prepare samples and ladder again with fresh DNA NGS 3K Marker.
 - Order new DNA NGS 3K Marker (P/N CLS920012).
 - Consider aliquoting the DNA NGS 3K Marker on first use into several smaller DNase free tubes.

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 contact Revvity Technical Support (see [page 43](#)).

General

- Allow the chip, sample plate, and all reagents to equilibrate to room temperature for at least 30 minutes before use.
- Use only consumables that are within their expiration date.
- Clean the O-rings in the chip interface weekly and the electrodes daily. Refer to [Chip Cartridge Cleaning on page 23](#) 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. Use of other, non-approved wipes may leave fluorescent debris, which can cause erratic focusing.
- Water used for chip preparation procedures must be 18 megohm, 0.22- μm filtered water (Milli-Q[®] or equivalent).
- Using the [Reverse Pipetting Technique on page 40](#) will help avoid introducing bubbles into the chip when pipetting the gel.

1. Revvity warrants that the LabChip Kit meets specification at the time of shipment, and is free from defects in material and workmanship. LabChip Kits are warranted for 90 days from the date of shipment. All claims under this warranty must be made within thirty days of the discovery of the defect.

Reverse Pipetting Technique

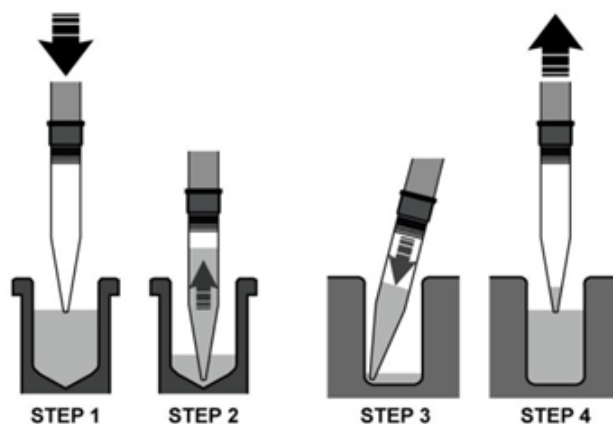


Figure 29. Reverse pipetting

- 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 the plunger to the first stop.
- 4 Withdraw the pipette from the well.

Reagents

- Store reagents at 2-8°C when not in use.
- All reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.
- The DNA NGS 3K Dye contains DMSO and must 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 DNA NGS 3K Marker, Dye, and Gel-Dye solution from light. Store in the dark at 2-8°C when not in use.

Chips

- Store chips at 2-8°C prior to first use.
- Cover the active chip wells with Parafilm® and store at 2-8°C until next use. If using the chip again within 24 hours, the chip can be stored at room temperature.
- 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 chip to dust by keeping the chip 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-prepare the chip as long as the maximum number of samples per chip prep is not exceeded.

Samples

- Prepared sample plates should be free of gas bubbles and particulate debris, both of which may inhibit sipper flow.
- Spin down sample plates containing gas bubbles and/or particulate debris at 3000 rpm for 5 minutes prior to analysis.

Chip Well Aspiration Using a 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 30A and B). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 31).

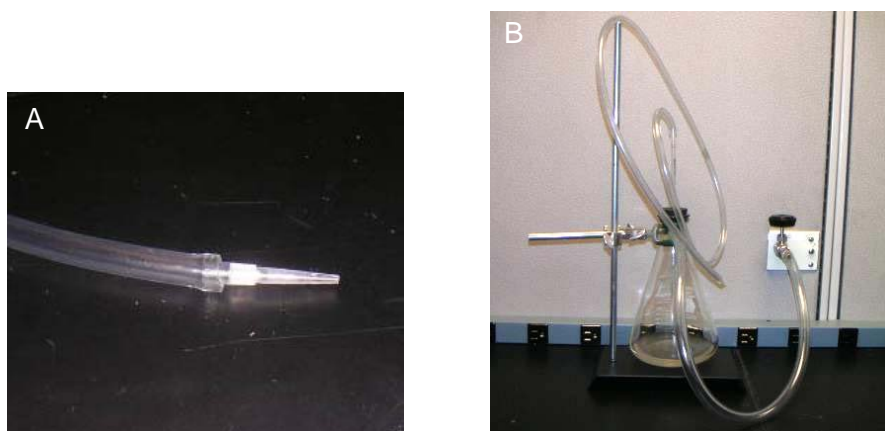


Figure 30. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap

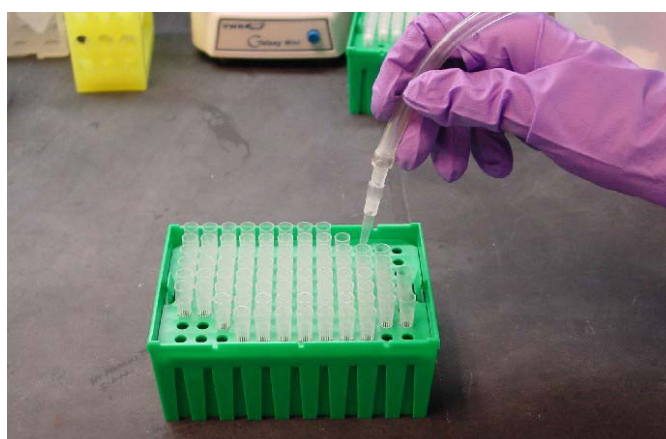


Figure 31. Replacing the disposable pipette tip

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Internet: www.Revvity.com

LabChip Chip QC test data portal:
<https://www.Revvity.com/tools/LabChipQCSearch>

LabChip Reagent CoA:
<https://www.Revvity.com/tools/COASearch>

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

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