



Caliper LifeSciences

Seeplex[®] LabChip[®] Kit LabChip Dx User Guide

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Specifications

Sizing Range	100 – 5000 bp
Sizing Accuracy	± 10%
Sizing Precision	5% CV
Carry-Over	< 0.5%
Number of Samples per Chip Prep	Up to 384 samples
Number of Samples per Chip Lifetime	Based on selection of Seeplex chip

Sample Conditions

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 125mM.

Kit Contents

Seeplex[®] Reagent Kit Part Number 760644

Item	Vial	Quantity
Dye Concentrate	Blue	1 vial
Ladder	Yellow	1 vial
Marker	Green	1 vial
Gel Matrix	Red	5 vials
Chip Storage Buffer	White	7 vials
Spin Filters	-	10
Ladder Tubes	-	20, 0.2 mL PCR tubes
Buffer Tubes	-	20, 0.75 mL tubes
Swab	-	3
Detection Window Cleaning Cloth	-	1

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Seeplex[®] 400 LabChip

Part Number 760585

(Chip Lifetime¹ = 400 samples)

Item	Quantity
Seeplex [®] 400 Chip	1

Seeplex[®] 800 LabChip

Part Number 760589

(Chip Lifetime¹ = 800 samples)

Item	Quantity
Seeplex [®] 800 Chip	1

Seeplex[®] 1600 LabChip

Part Number 760590

(Chip Lifetime¹ = 1600 samples)

Item	Quantity
Seeplex [®] 1600 Chip	1

¹ Expected chip lifetime is based on use under normal laboratory conditions and adherence to Caliper preparation protocols, sample guidelines and storage conditions. Individual results may vary.

Safety Warnings and Precautions

! WARNING ! For Research Use Only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

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.

Storage: When not in use, store chips and reagents refrigerated at 4 °C.
Do not leave chips and reagents unrefrigerated overnight.

Additional Items Required

Instruments

- Vortex mixer
- Microcentrifuge
- Plate Centrifuge
- Vacuum line
- Positive-displacement pipettes (recommended only)

Consumables

- Skirted 96-well or 384-well plates

Recommended Plates

Vendor	Well Number	Catalog Number	Description
Bio-Rad	96	MSP-9601	Microseal 96-well Skirted PCR Plates
Bio-Rad	96	HSP-9601	Hard-Shell Low-Profile 96-Well Skirted PCR Plates
Axygen Scientific	384	PCR-384-C	384 Well PCR Microplate

Note: All plate type definitions should be verified on the LabChip Dx before testing.

Deep well plates (such as the Applied Biosystems MicroAmp[®] 96-Well Plate) are not compatible with the LabChip Dx.

Reagents

- MilliQ water: Molecular biology grade or better, 0.22-micron filtered
- 70%-isopropanol solution in DI water

Preparation Procedures

Chip Preparation

Note: Allow the chip and all reagents to equilibrate to room temperature 20 minutes before use. The Dye Concentrate contains DMSO and **must be thawed** completely before use.

Preparing the Gel-Dye Solution

1. Gently vortex the thawed DNA Dye Concentrate (blue cap ●) before use and spin down. Avoid exposure of dye concentrate to light for long periods of time.
2. Transfer **13 μ L** of DNA Dye Concentrate (blue cap ●) to **1 vial** of DNA Gel Matrix (red cap ●). One DNA Gel Matrix vial is sufficient for 4 chip preps.
3. Vortex the solution at high speed for 15 seconds and then spin down for another 15 seconds.
4. Transfer the mixture to two spin filters (**525 μ L** each).
5. Centrifuge at **9400 rcf for 8 minutes at room temperature**. Discard filters, label and date the tubes. Use within 3 weeks. Store in the dark at 4 °C.

Loading the Chip

Note: Keep the chip in its container during preparation and when carrying from one location to another.

When pipetting reagents into the chip place pipette tip at the bottom and center of the well. Pipette slowly to prevent introduction of bubbles at bottom of the wells. For more details about this pipetting technique refer to the LabChip Kit Essential Practices section of this user guide.

1. Use a pipette tip attached to a vacuum line to aspirate all fluid from the chip wells. **DO NOT** run the tip over the central region of the detection window. See **Figure 1**. (For details on how to setup a vacuum line refer to the Chip Well Aspiration Using a Vacuum section of this user guide.)
2. Rinse and thoroughly aspirate each active chip well (1, 3, 4, 7, 8 and 10) once with molecular biology grade water. Do not allow active wells to remain dry for long periods of time.

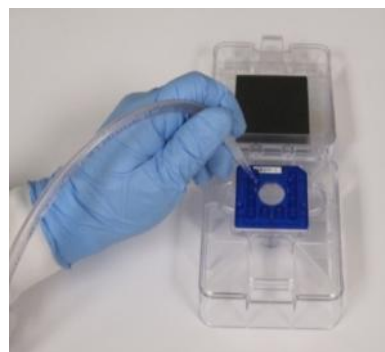


Figure 1. Chip aspiration and vacuum line.

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3. **If analyzing ≤ 96 samples**, pipette **50 μL** of Gel-Dye in wells 3, 7, 8 and 10. Then pipette **50 μL** of Marker solution (green cap ●) in well 4. See **Figure 2**.
4. **If analyzing > 96 samples**, pipette **50 μL** of Gel-Dye in wells 3, 7 and 8. Pipette **75 μL** of Gel-Dye in well 10. Then add **90 μL** of Marker solution (green cap ●) in well 4 when analyzing up to 192 samples and add **140 μL** when analyzing up to 384 samples. See **Figure 3**.
5. Ensure well 1 is empty before placing chip on LabChip Dx.



Figure 2. Chip preparation for ≤ 96 samples.

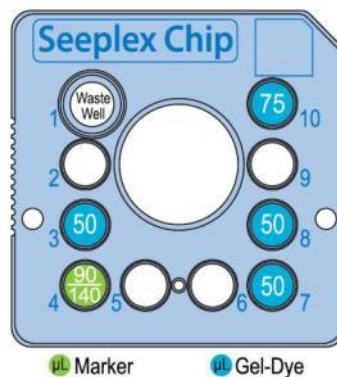



Figure 3. Chip preparation for **97 to 384 samples**. Pipette **90 μL** of Marker Solution if analyzing up to 192 samples and **140 μL** for up to 384 samples.

Sample, Ladder and Buffer Preparation

Preparing the DNA Samples

1. Spin down sample plate containing at least 20 μ L of PCR product per well, at 1200 rcf for 1 minute.
2. Carefully remove the plate seal and place on the plate tray of the LabChip Dx instrument. See **Figure 4**.

Preparing the DNA Ladder

1. In the provided 0.2 mL Ladder Tube, add **12 μ L** of DNA Ladder (yellow cap ) to **108 μ L** of molecular biology grade water. Mix thoroughly by pipetting the solution up and down several times.
2. Insert the Ladder Tube into the ladder slot on the LabChip Dx instrument plate tray. See **Figure 4**.

Preparing the Buffer Tube

1. Add **750 μ L** of molecular biology grade water to the 0.75 mL Buffer Tube provided with the reagent kit.
2. Insert the Buffer Tube into the buffer slot on the LabChip Dx instrument plate tray. See **Figure 4**.

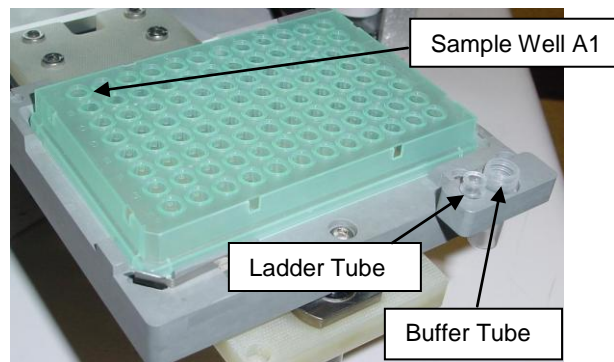


Figure 4. LabChip Dx plate tray.

Inserting a Chip into the LabChip Dx Instrument

Note: Be sure to inspect and clean the O-rings on the top plate of the chip interface on the LabChip Dx instrument, as described in the Chip Cartridge Cleaning section of this user guide.

1. Eject the chip cartridge by pressing the **CHIP** button on the instrument front panel.
2. Release the cartridge latch, remove chip from the storage container and insert the chip into the cartridge. Refasten the latch and push the cartridge into the instrument. See **Figure 5**.
3. Press the **EJECT** button on the instrument front panel to retract the sample plate and send the sipper to the Buffer Tube. (If not starting a run soon after inserting the chip, be sure to retract the plate tray so that the sipper is immersed in the Buffer Tube. Do not let the sipper dry out.)

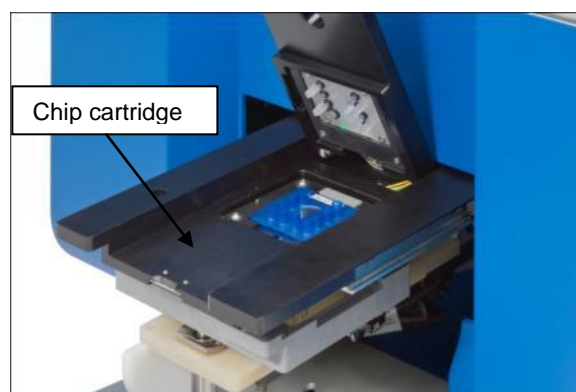


Figure 5. LabChip Dx chip cartridge.

Running the Seeplex Assay

1. Start the LabChip Dx software.
2. Ensure that the chip, sample plate, ladder tube and buffer tube have been loaded properly on the LabChip Dx instrument.
3. On the main screen, click the **Run** button in the upper left corner of the LabChip Dx software. See **Figure 6**.

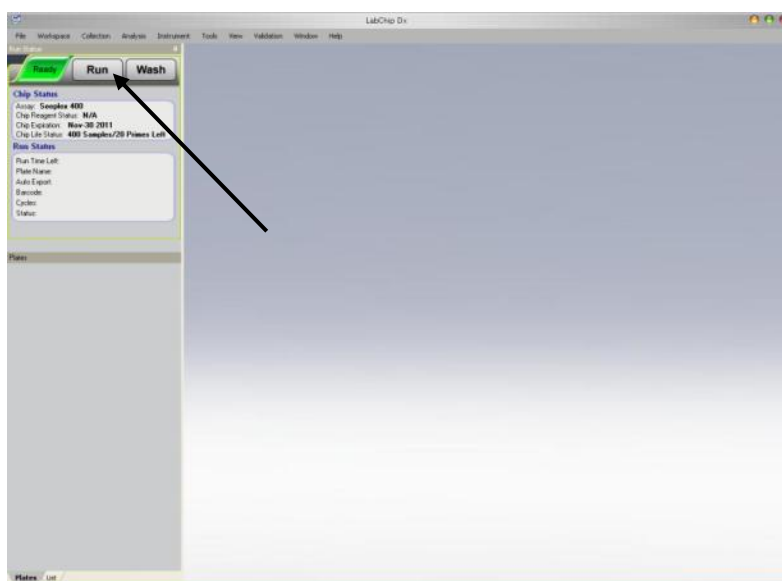


Figure 6. LabChip Dx GUI.

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4. The **Start Run** dialog box will pop up with tabs listed as **Run**, **Output** and **Advanced**. See **Figure 7**.
5. In the **Run** tab, select the appropriate **Assay Type**, **Operator Name**, **Plate Name**, **well pattern**, **barcode option** and **Sipping Order**. For selection of the appropriate **Assay Type (Seeplex 28S, Seeplex 40S or Seeplex 60S)** refer to **Table 1**.

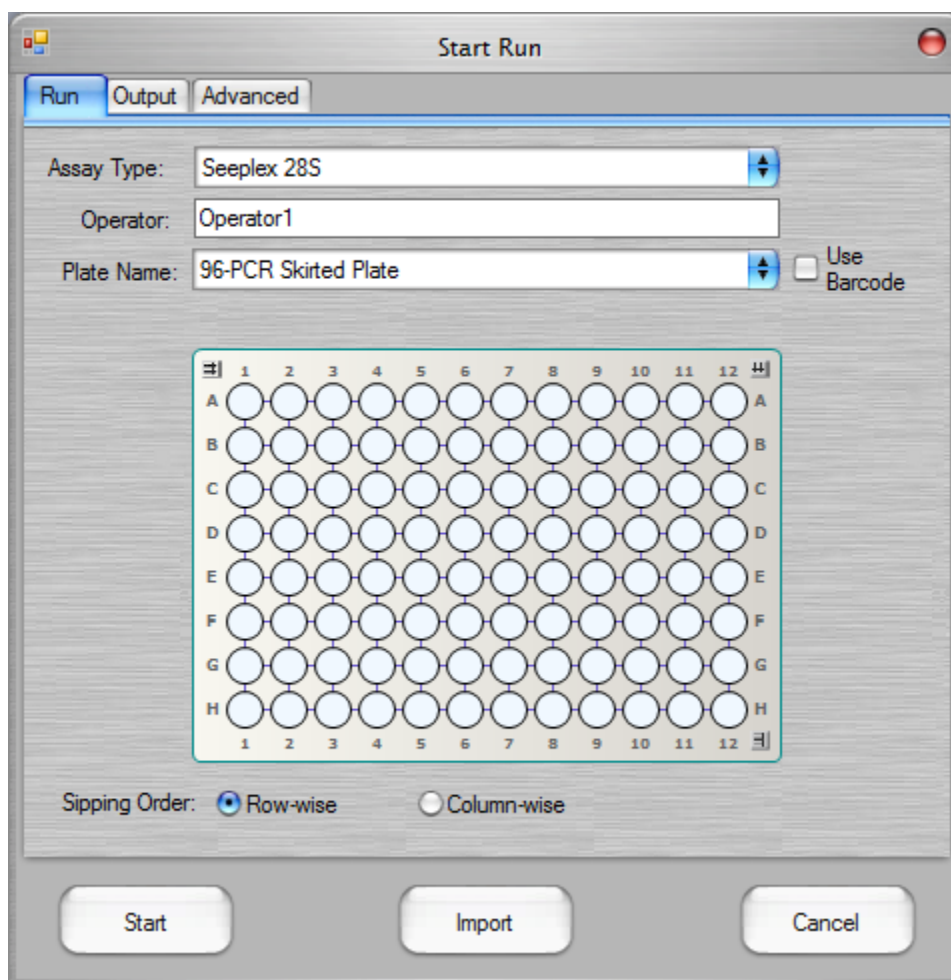


Figure 7. Run tab of Start Run window.

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Seeplex Kit	LabChip DX Assay Type
STD4D ACE STD6 ACE	Seeplex 28S
Diarrhea ACE Meningitis ACE HPV6 ACE RV7 RV5 ACE RV15 ACE RV15 OneStep ACE PneumoBacter ACE MTHFR MTB Nested ACE MTB/NTM ACE MTB ACE	Seeplex 40S
RV12 ACE	Seeplex 60S

Table 1. Seeplex kits and corresponding LabChip DX assay types.

6. In the **Output** tab select the destination of the file, the filename convention and any additional data to auto export. See **Figure 8**.

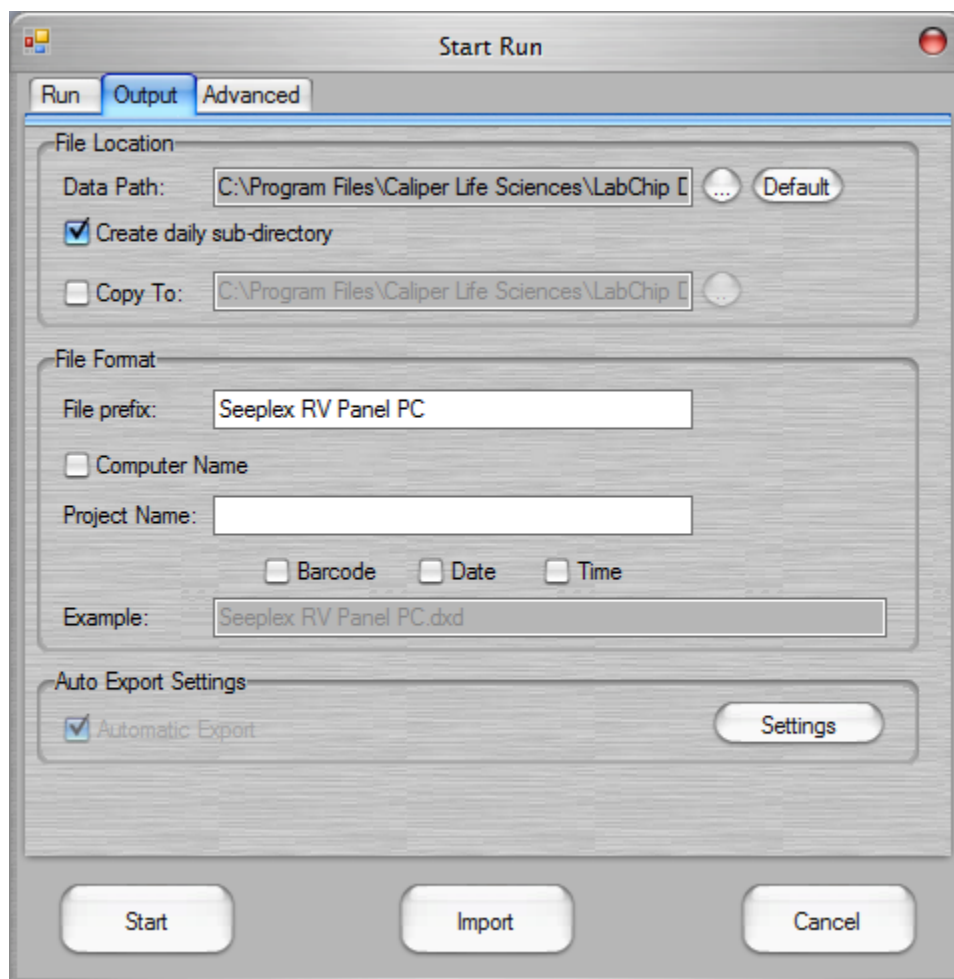


Figure 8. Output tab of Start Run window.

7. In the **Advanced** tab, select the number of times each well is sampled, the sample names and any expected peaks. See **Figure 9**.

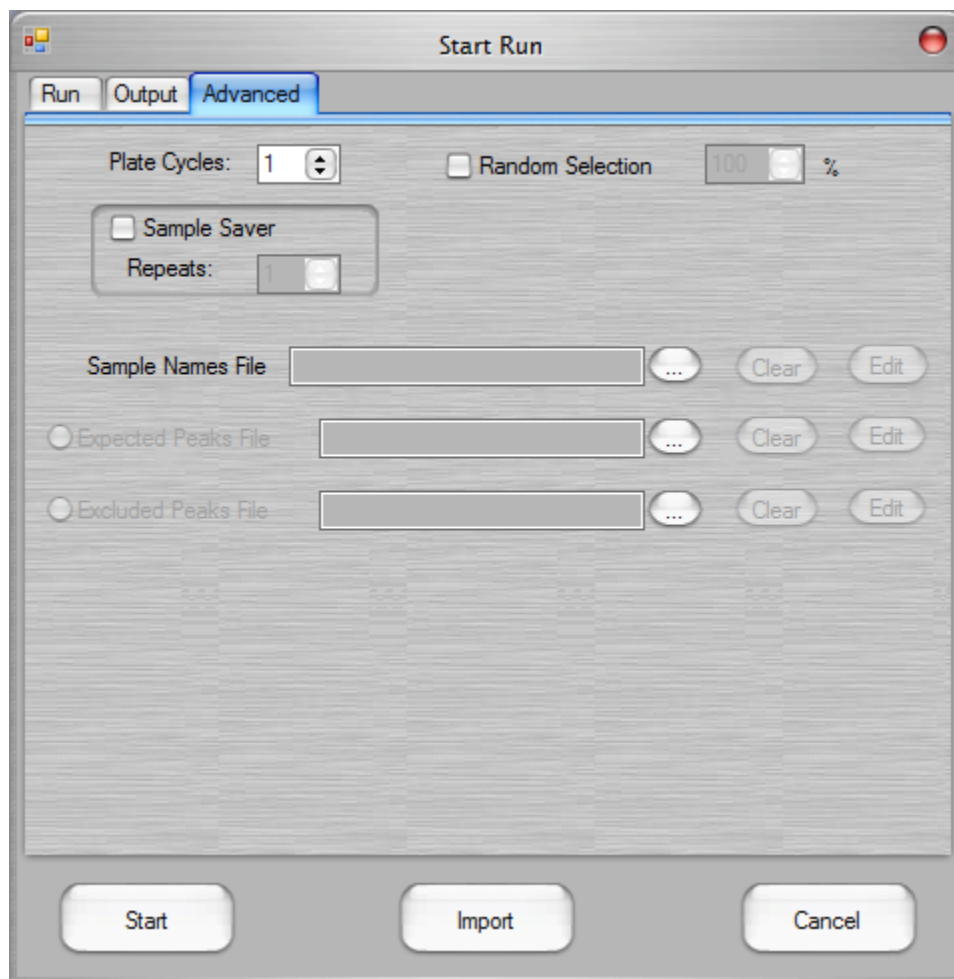



Figure 9. Advanced tab of Start Run window.

8. Click **Start** to begin the run.

Storing the DNA Chip

After use, the chip must be cleaned and stored in the chip container. The procedure below can be conducted the following day when running overnight.

1. Remove the reagents from each well of the chip, using a vacuum.
2. Each active well (1, 3, 4, 7, 8, and 10) should be rinsed and aspirated once, using molecular biology grade water.
3. Add **75 µL** of Chip Storage Buffer (white cap ) to the active wells.
4. Place the chip in the LabChip Dx instrument and push the cartridge into the instrument.
5. Insert a Buffer Tube with **750 µL** of molecular grade biology water into the buffer slot of the LabChip Dx instrument.
6. Click the **Wash** button in the left corner of the LabChip Dx software.
7. After wash is completed, remove the chip from the instrument and place it in the plastic storage container with the matching chip serial number. Add an additional **50 µL** of storage buffer to fill wells 1 and 4. Cover the wells with 2 layers of Parafilm and store at 4 °C.

Note: For the 2 layers of Parafilm leave the wax-backing on. For the first layer face the Parafilm-side down to cover the wells and prevent buffer evaporation. Storage of a chip with dry wells may cause it to become clogged. For the second layer face the wax-side down on top of the first layer of Parafilm to prevent the chip from sticking to the lid when opening the chip case.

Chip Cartridge Cleaning

Weekly

1. Inspect the inside of the chip cartridge and O-rings for debris.
2. If debris is observed, use the provided lint free swab dampened with DI water or 70%-isopropanol solution in DI water 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.

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 Dx instrument. Soak O-rings in DI water for a few minutes. Clean the O-ring faces by rubbing between two 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 DI water.
3. Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

Results

The electropherogram in **Figure 10** is of a typical DNA Ladder using the **Seplex 28S assay script**. Between the upper and lower markers, peaks in order of increasing migration time correspond to ladder fragments of 100, 300, 500, 700, 1100, 1900, 2900, and 4900 bp.

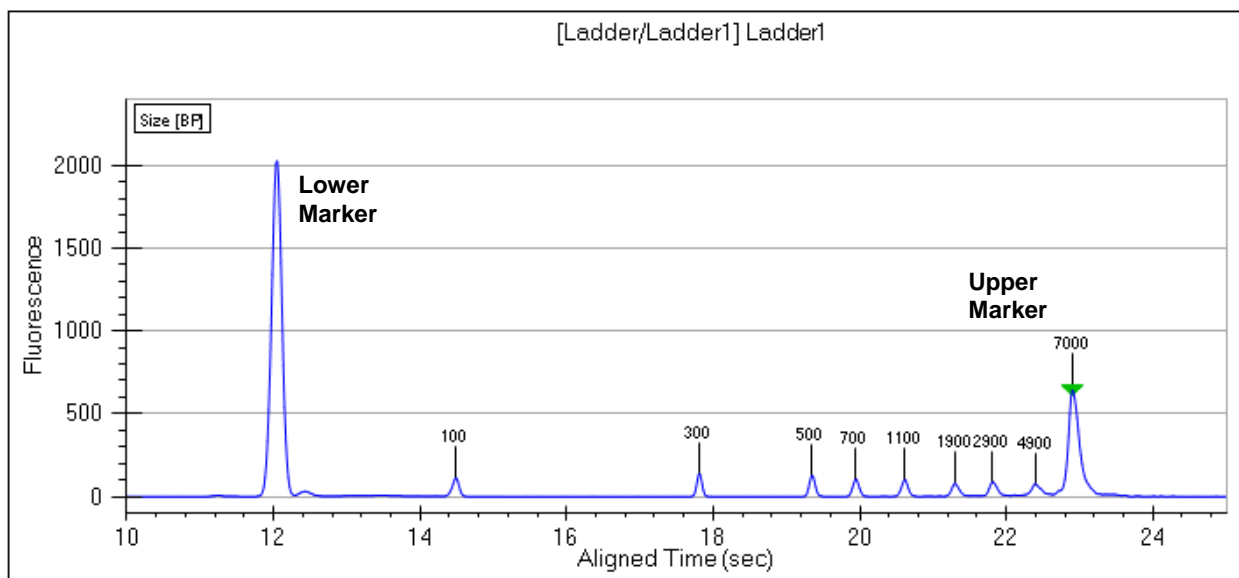


Figure 10. Electropherogram of Ladder using the Seplex 28S assay script.

The electropherogram in **Figure 11** is of a typical DNA Ladder using the **Seeplex 40S assay script**.

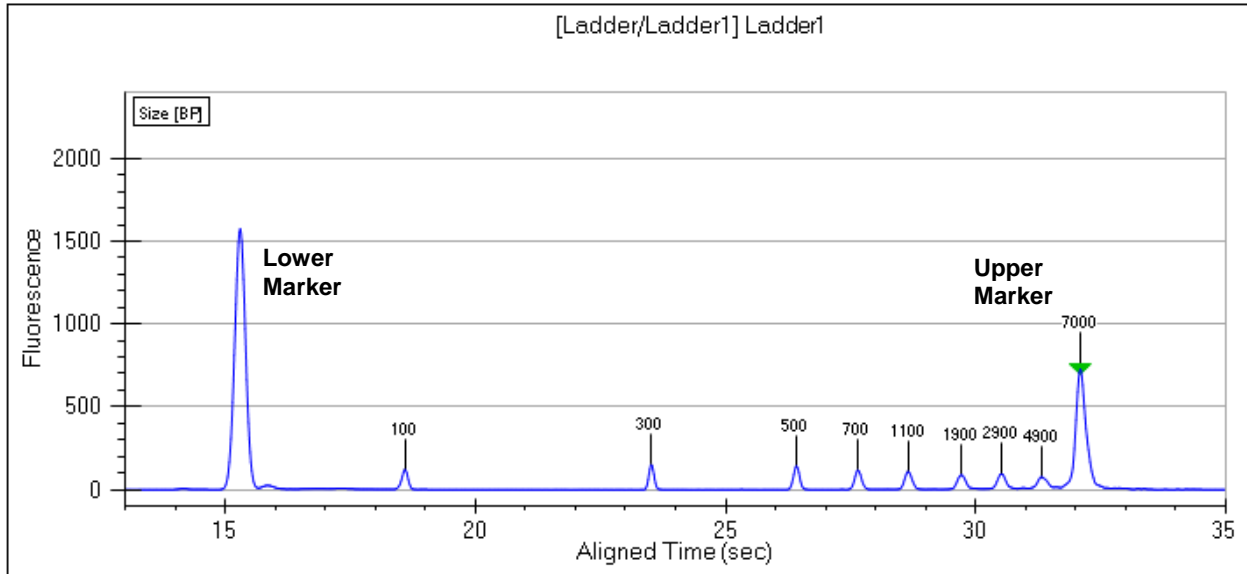


Figure 11. Electropherogram of Ladder using the Seeplex 40S assay script.

The electropherogram in **Figure 12** is of a typical DNA Ladder using the **Seeplex 60S assay script**.

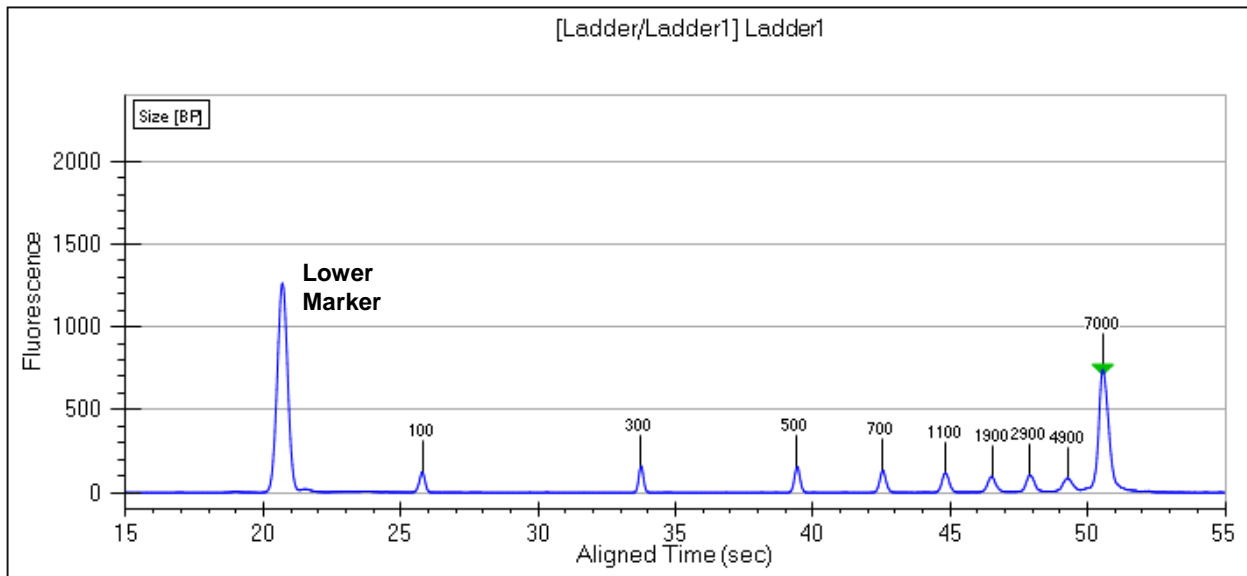


Figure 12. Electropherogram of Ladder using the Seeplex 60S assay script.

For interpretation of sample results please refer to the Seegene Viewer software and guide.

Troubleshooting

1. **Symptom: No ladder fragments and/or sample peaks detected. See Figure 13 for an example.**

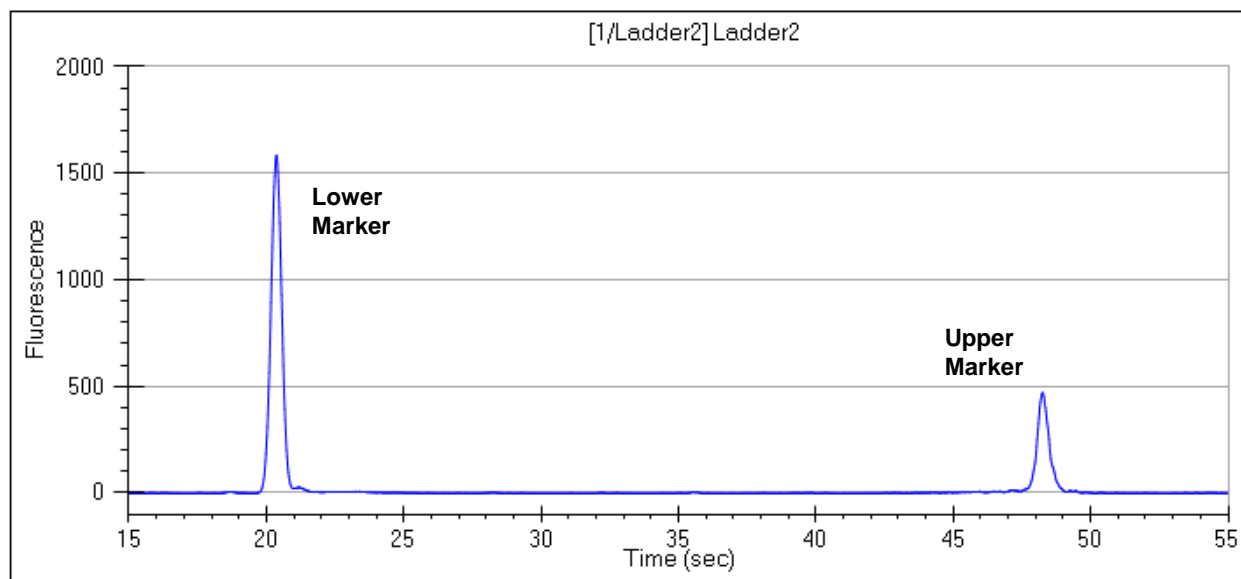


Figure 13. Example electropherogram of Ladder with marker peaks but no ladder peaks.

Possible causes:

- A) Low or no ladder volume in Ladder Tube.
- B) The sipper is not reaching the sample due to low sample volume in the well plate.
- C) Air bubble at the bottom of sample well and/or Ladder Tube is clogging the sipper.
- D) Air bubble or clog in sipper introduced during chip priming.

What to do:

- A) Ensure there is **120 μ L** of ladder in the Ladder Tube and restart the run. Long idle time on the instrument in dry climate can lead to evaporation.
- B) Ensure that there is at least 20 μ L of sample in each sample well. If the plate has been uncovered for some time, sample evaporation may have occurred.
- C) Inspect the bottom of sample wells (see **Figure 14**) and Ladder Tube for air bubbles. Spin the sample plate down at 1200 rcf for 1 minute. See **Figure 15** for an example of what samples should look like after spinning. Remove any air bubbles at the bottom of the Ladder Tube. Retest sample plate.

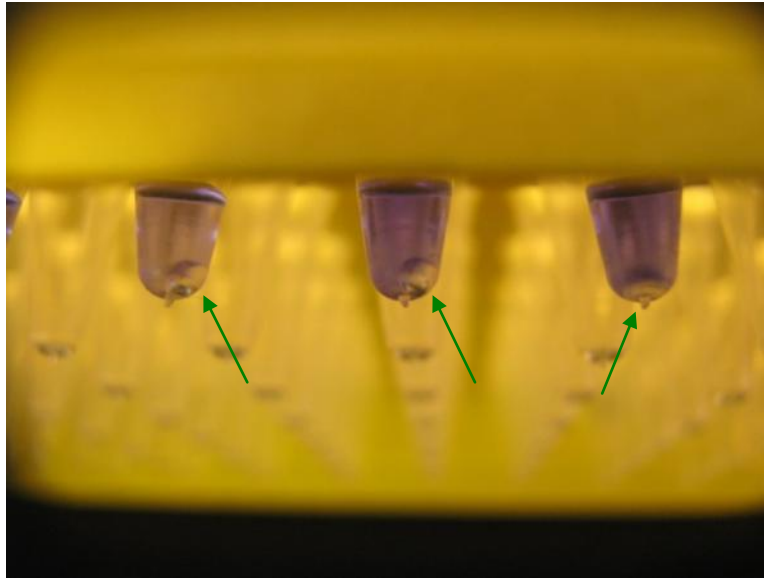


Figure 14. Example of air bubbles at the bottom of sample wells. Before testing these samples should be spun down at 1200 rcf for 1 minute.



Figure 15. Example of sample wells in **Figure 14** after spinning.

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- D) Remove the chip from the LabChip Dx. Ensure that the waste well contains at least ~100 μ L of fluid. If not, bring up the volume by adding molecular biology grade water to the waste well. To remove the air bubble or clog suction the sipper with a vacuum line, as shown in **Figure 16**, until a few droplets have flowed out the sipper. When suctioning the sipper be careful not to bend or break the sipper. To facilitate this, cut the tip of the vacuum line to widen the mouth. Afterward aspirate the fluid in well 1, and if needed, replenish the marker solution. Then rerun the chip.

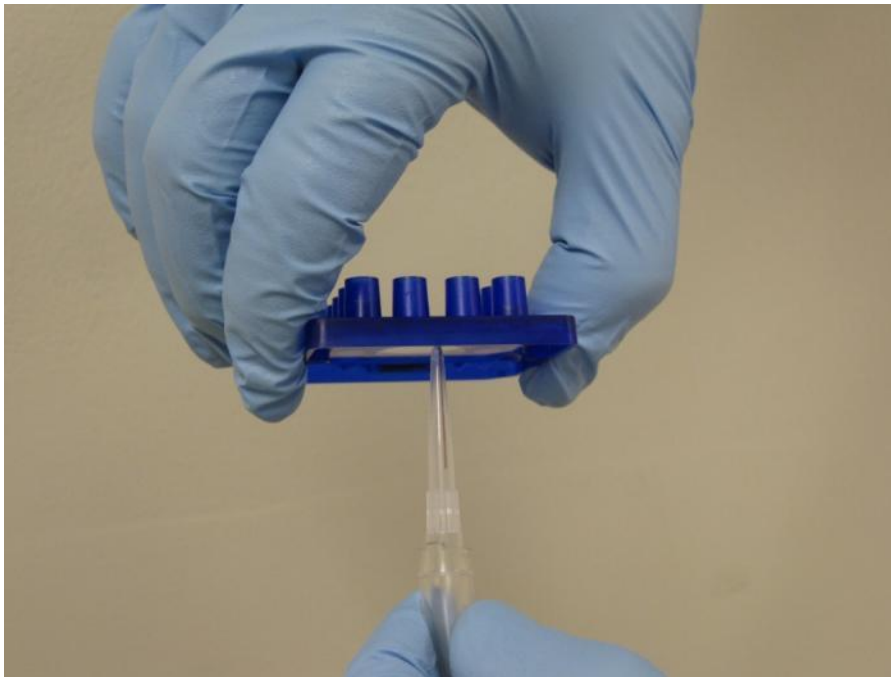


Figure 16. Removing an air bubble or clog by suctioning the sipper with a vacuum line.

2. **Symptom: No marker peaks but sample peaks are present. See Figure 17 for an example.**

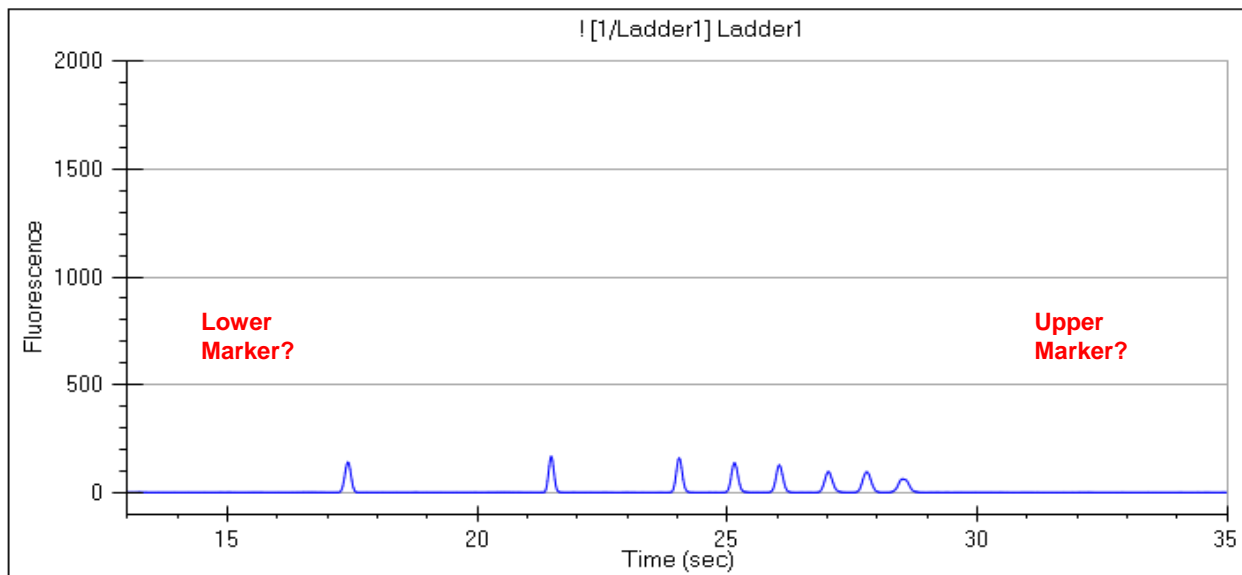



Figure 17. Example electropherogram of Ladder with ladder peaks but no marker peaks.

Possible causes:

- A) No or insufficient marker in chip well 4.
- B) If there is marker solution in well 4, the problem may be due to a marker channel clog.

What to do:

- A) This may be due to not filling marker well or chip remaining idle on instrument for extended period of time. Add or replenish the marker solution in the chip using the following procedure:
 - Remove the chip from the instrument.
 - Thoroughly aspirate all fluid from chip well 4 using a vacuum line.
 - Rinse and completely aspirate **well 4 only** once with molecular biology grade water.
 - Add Marker solution (green cap ) to well 4 as described in the Preparation Procedures section of this user guide.
 - Rerun the chip.
- B) Perform a marker channel unclogging procedure by suctioning the sipper as described in Troubleshooting case 1D in this section of the user guide.

3. Symptom: Run abort due to chip focusing failure. See Figure 18.

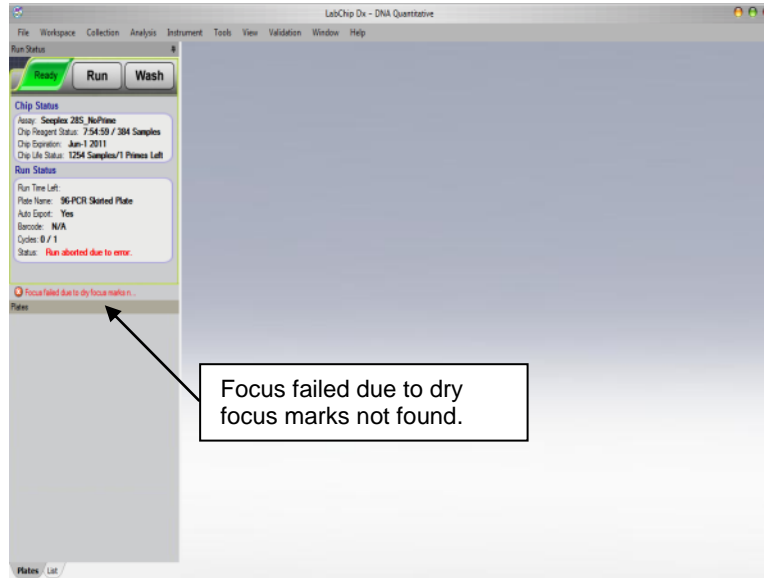


Figure 18. LabChip Dx GUI with run abort error message due to chip focusing failure.

Possible causes:

- A) Chip detection window is dirty. See **Figure 19** for detection window.

What to do:

- A) Clean BOTH sides of the chip window with the Caliper-supplied cleanroom cloth dampened with a 70%-isopropanol solution in DI water. Then restart the run.

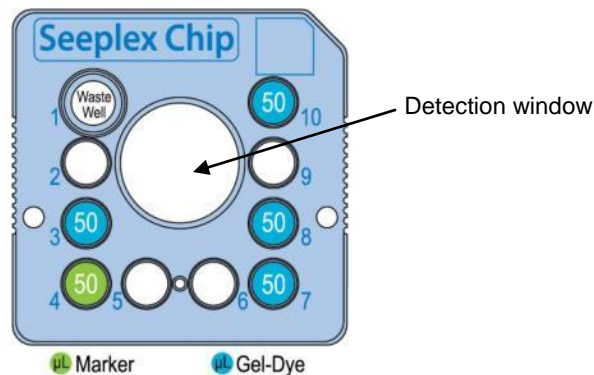


Figure 19. Chip detection window.

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.²

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 Dx Software Help file or call Caliper Technical Support at 1-877-LABCHIP.

General

- 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 Caliper-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 0.22-micron filtered deionized, molecular biology grade.

Reagents

- Store all reagents at 4 °C when not in use.
- Gently vortex all kit reagents before use.
- Positive-displacement pipettes are recommended for dispensing reagents into the chip to help prevent introducing bubbles into the chip wells. If using standard pipettes, dispense reagents into the chip as shown in **Figure 20**.

- Step 1. Insert the pipette tip at the bottom and center of the chip well.
- Step 2. Dispense the volume without introducing air bubbles by depressing the plunger slowly to the first stop only. Afterward there will be a small amount of reagent left in the tip. Remove the tip from the well and **do not dispense the remaining reagent**.

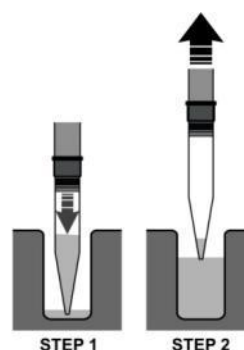


Figure 20. Pipetting technique for chip reagents.

² Caliper Life Sciences 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 60 days from the date of shipment. All claims under this warranty must be made within thirty days of the discovery of the defect.

Chips

- DNA chips should be stored refrigerated prior to first use.
- Cover the active wells on the chip with 2 layers of Parafilm and store at 4 °C. If using the chip again within 24 hours it may be left 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 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-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.
- Sample plates containing gas bubbles and/or particulate debris should be spun down (for example, **1200 rcf for 1 minute at room temperature**) prior to analysis.

Chip Well Aspiration Using a Vacuum

Aspirating with a pipette can leave used reagents in the chip wells. For this reason, Caliper recommends vacuuming the wells instead. This can be accomplished by attaching a permanent pipette tip to a house vacuum line with trap (**Figure 21** and **Figure 22**). To avoid contamination, use a new pipette tip over the permanent tip for each chip aspirated (**Figure 23**).

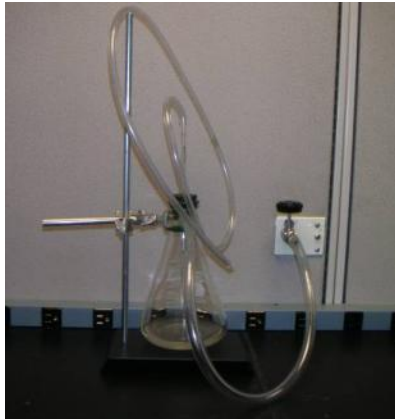


Figure 21



Figure 22

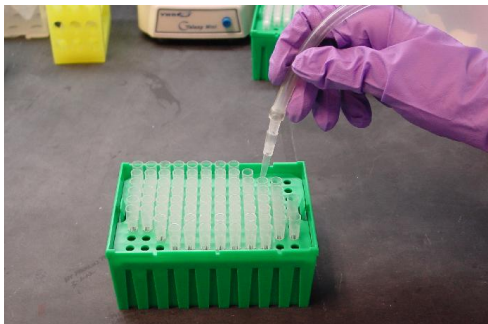


Figure 23

Reordering Information

<u>Product</u>	<u>Part Number</u>
Seeplex [®] 400 LabChip	760585
Seeplex [®] 800 LabChip	760589
Seeplex [®] 1600 LabChip	760590
Seeplex [®] Reagent Kit	760644
Buffer Tube	E&K Scientific 697075-NC
Ladder Tube	Genemate C-3258-1

Customer Technical Support

Caliper Life Sciences
 68 Elm Street
 Hopkinton, MA 01748-1668
 Phone: 1-877-LABCHIP (522-2447)
 Fax: 1-508-435-3439

For additional assay and instrument troubleshooting, refer to the LabChip Dx Software Help file. Call Caliper Technical Support at 1-877-LABCHIP.

The chip and reagents supplied with this kit are sold with limited rights of use. The chip may only be used with the specific quantity of reagents supplied with this kit. The purchaser has no right or license to refurbish, reuse, remanufacture, or otherwise use the chip with any other reagents than those specifically supplied in this kit. For more information on the terms and conditions of use of these chips and reagents, please read your LabChip Dx User Guide. Caliper, the Caliper logo, LabChip, and the LabChip logo are registered trademarks of Caliper Life Sciences.

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