Element Biosciences

# Cloudbreak Freestyle Dx™ Sequencing

## User Guide

**FOR USE WITH**

AVITI Dx™ System
AVITI Dx Operating Software v4.0
Cloudbreak Freestyle Dx™ Sequencing Kits

**ELEMENT BIOSCIENCES**

**The AVITI Dx System product family delivers precise, high-quality raw DNA sequencing data from a variety of biological samples. The product is intended solely as a data generation tool and does not provide diagnostic interpretations or direct clinical recommendations.**

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# Overview

The Cloudbreak Freestyle Dx sequencing workflow uses a Cloudbreak Freestyle Dx kit to sequence libraries on an AVITI Dx System.

- **Cloudbreak Freestyle Dx**—Provides multiple read lengths and output options to meet a diversity of applications. Cloudbreak Freestyle Dx kits enable direct loading of linear libraries without library conversion, including third-party libraries.

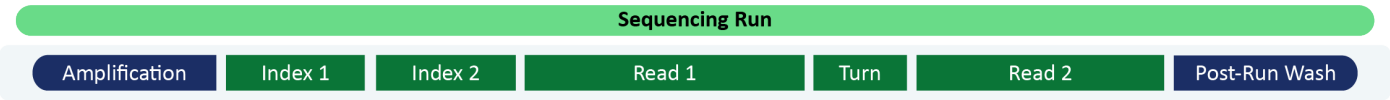
All Cloudbreak Freestyle Dx kits are designed for minimal waste and easy disposal. Reagent overage supports the extra cycles that index sequences and unique molecular identifiers (UMIs) need to identify samples with high confidence. For a list of kit configurations and catalog numbers, see *Cloudbreak Freestyle Dx Sequencing Kits* on page 22.

Cloudbreak Freestyle Dx kits are compatible with a variety of library preparation workflows. For more information on compatibility, see the *Product Compatibility* page on the Element website. Cloudbreak Freestyle Dx kits can only be used when the AVITI Dx System is operating in IVD mode. Before initiating a sequencing run, verify that the instrument is in the correct operating mode. For instructions on switching between RUO and IVD modes, refer to the *AVITI Dx System User Guide (MA-00088)*.

## Sequencing Run Stages

AVITI Dx Operating Software (AVITI Dx OS) generates a recipe based on the run parameters entered during run setup. The recipe governs each stage of a run. A run is complete when the recipe is executed and primary analysis is finished. The following stages comprise a sequencing run:

- **Amplification**—Hybridizes the library to the flow cell and performs amplification to form polonies, each containing multiple copies of the same sequence from the library.
- **Sequencing**—Performs each read in the run, including imaging and primary analysis.
- **Post-run wash**—Automatically flushes buffer from the sequencing cartridge through the fluidic system to remove salts and residual library.




## Reads in a Sequencing Run

Up to four reads comprise a sequencing run: Index 1, Index 2, Read 1, and Read 2.

- **Index reads**—A run can include one, two, or no index reads.
  - » **Index 1** sequences the Index 1 sequence.
  - » **Index 2** sequences the Index 2 sequence.
  - » A dual-index run sequences Index 1 and Index 2.
- **Read 1 and Read 2**—All runs must have a Read 1.
  - » **Read 1** sequences the forward strand of the DNA insert.
  - » **Read 2** sequences the reverse strand.
  - » A paired-end run sequences Read 1 and Read 2, including a paired-end turn before Read 2 to generate the complementary strand.

# Number of Cycles

Read length is the total number of cycles performed in a run. For bioinformatics purposes, adding one extra cycle to each read is recommended. The additional cycle improves the accuracy of the Q score for the 150th cycle. The software and chemistry used for the run prescribe a minimum number of cycles. Read 1 requires at least five cycles and at least 25 cycles to generate all run metrics. The maximum number of cycles depends on the kit:

- A 2 x 75 kit performs up to 184 cycles, supporting one 2 x 76 run with indexing and unique molecular identifiers (UMIs).
- A 2 x 150 kit performs up to 334 cycles, supporting one 2 x 151 run with indexing and UMIs.

When operating the instrument in RUO mode, refer to the Cloudbreak Sequencing User Guide for supported read lengths and expected run times.

# Run Times

| Kit Output Configuration | Read Length | Run Time[1] |
|---|---|---|
| High output | 2 x 75 | ≥25 hours |
| | 2 x 150 | ≥40 hours |
| Low output | 2 x 150 | ≥28 hours |

[1] Individually addressable lanes and custom recipes can extend run times.

# Read Counts and Output on AVITI Dx System

Read counts and output on the AVITI Dx System vary depending on sequencing kit configuration and run setup.

| Kit Configuration | Kit Size | Target Read Counts | Output (Gb) |
|---|---|---|---|
| Cloudbreak Freestyle Dx High Output | 2 x 75 | ≥1.2 billion | 180 |
| | 2 x 150 | ≥1.2 billion | 360 |
| Cloudbreak Freestyle Dx Low Output | 2 x 150 | ≥300 million | 90 |

# Library Considerations

Some libraries have special considerations for sequencing. Make sure to follow the applicable requirements for your library.

## Low-Diversity Amplicon Libraries

For low-diversity, high-multiplex libraries, such as a 16S amplicon library, Element recommends that you enable the Low-Diversity High-Multiplex setting during run setup. This setting requires a library pool that meets the following requirements:

- Third-party libraries
- High plexity of ≥ 64 unique dual indexed (UDI) libraries
- A 1–5% spike-in of PhiX Control Library

**CAUTION**
Exceeding a 5% spike-in can reduce the index diversity of the pool, leading to a reduction in quality.

## Bead-Based Normalization

PCR is required when sequencing a library pool that has undergone the bead-based normalization protocol. Before diluting to the target loading concentration, use amplification and Qubit kits to amplify and quantify the library pool.

- For Cloudbreak Freestyle Dx chemistry with third-party libraries, use the KAPA HiFi HotStart Library Amplification Kit with Primer Mix. Follow manufacturer instructions.

## Short Insert or Long Insert Libraries

Sequencing short insert or long insert libraries require that you specify the preloaded custom recipe during run setup.

| | |
|---|---|
| **Short insert libraries** | • If you are using Cloudbreak Freestyle Dx chemistry and libraries with < 100 bp inserts, the short insert recipe is required.<br>• For libraries with 100–300 bp inserts, the short insert recipe is recommended.<br>• The short insert recipe is compatible with 2 x 75 and 2 x 150 kits. |
| **Long insert libraries** | • If you are using Cloudbreak Freestyle Dx chemistry and libraries with > 750 bp inserts, the long insert recipe is required.<br>• The long insert recipe is compatible with 2 x 75 and 2 x 150 kits. |

# Input Recommendations

The recommended input for sequencing is ≥ 1 nM library. The input library is normalized to 1 nM, denatured into single strands, and diluted to the target loading concentration. When starting with a 0.2–1 nM library, the library is denatured and diluted but not normalized. Library pools that start at < 0.2 nM are not supported.

## PhiX Control Library Spike-In

For most applications, Element recommends a spike-in of PhiX Control Library. The following recommendations for spike-in percentages optimize the benefits of PhiX Control Library for specific experiments.

| Experiment | Spike-In (%) |
|---|---|
| QC and error rate reporting | > 2 |
| Low-complexity indexing (≤ 2-plex) | > 2 |
| Libraries that use Low-Diversity High-Multiplex setting | 1–5 |
| Other low-diversity libraries | ≥ 5 |

## Loading Concentration

Use the following recommendations as a starting point to determine your optimal loading concentration. Loading concentration recommendations depend on your library prep workflow, the kit chemistry, output level, and kit size.

- Polony counts increase as the loading concentration increases. Lower polony counts promote higher data quality but lower the amount of data output. Some libraries require a higher or lower concentration than the indicated ranges.
- Average library size is the full length of the library, including DNA insert and adapters. Pooled libraries must contain similar size distributions.

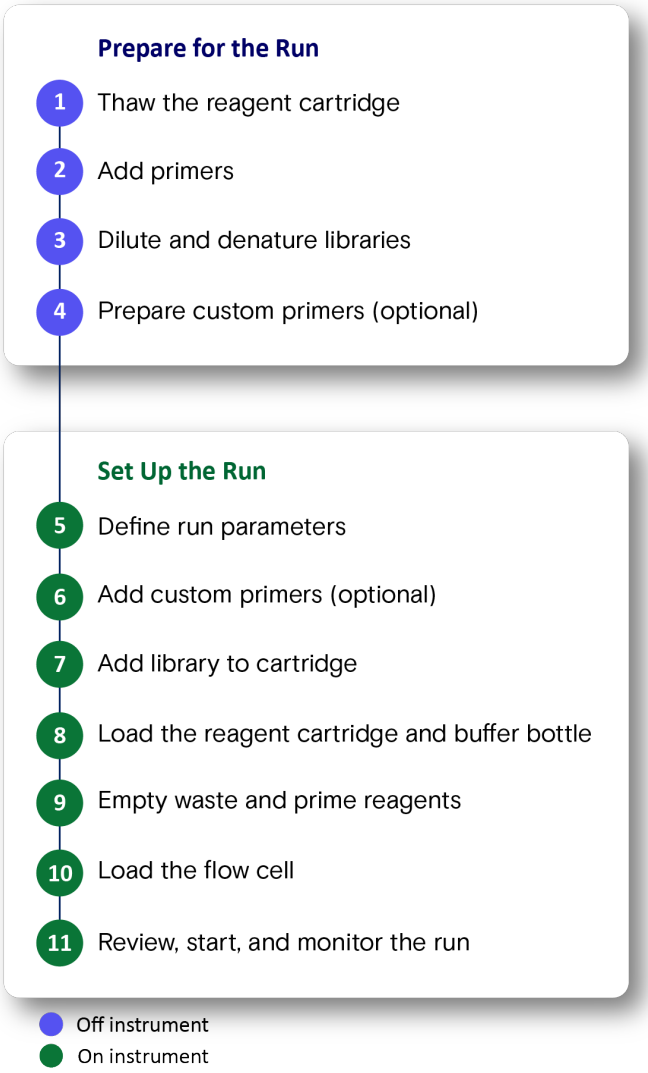
### AVITI Dx with AVITI Dx OS v4.0

**Cloudbreak Freestyle Dx Chemistry, 2 x 75 and 2 x 150 Kits**

| Average Library Size | Third Party PCR-Free | Third Party PCR-Plus |
|---|---|---|
| Small (250–450 bp) | 7.5–11.25 pM | 8.75–12.5 pM |
| Medium (450–700 bp) | 8.75–12.5 pM | 11.25–15 pM |
| Large (≥ 700 bp) | 8.75–12.5 pM | 11.25–15 pM |

# Cloudbreak Freestyle Dx Workflow Summary

Performing a Cloudbreak Freestyle Dx sequencing run includes steps to prepare reagents and dilute the library to the appropriate volume and concentration for sequencing. For more information, see *Loading Concentration* on page 7.



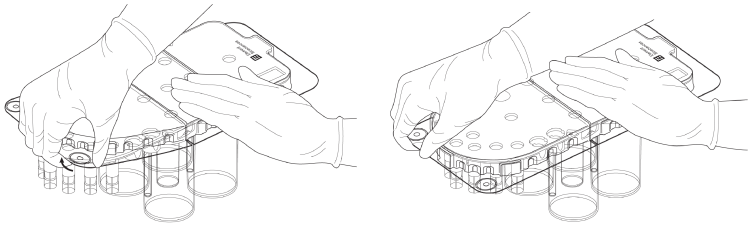
# Run Preparation

Run preparation includes thawing the sequencing cartridge and adding appropriate primers, if applicable. The subsequent library dilution procedure includes the option to store a normalized library. If you intend to store a library, do not prepare the cartridge until you are ready to sequence. Prepare the cartridge within a day of sequencing.

## Thaw Reagents

1. If the cartridge includes a shipping cover, remove the shipping cover:

   a. While supporting the cartridge, lift the removal tab at the left corner until it releases from the cartridge.



   b. Moving across the front edge of the shipping cover, repeatedly lift the edge until the cover is fully released.

   c. Pull to remove the remainder of the shipping cover from the cartridge.

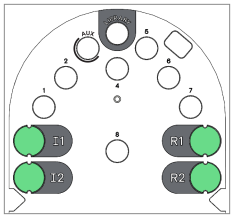
2. Thaw the sequencing cartridge. Protect the cartridge from light until loading onto the instrument.

| Cartridge | Room Temperature Water Bath | Refrigerator |
|---|---|---|
| 2 x 75 | 1.5 hours | 8 hours |
| 2 x 150 | 2.5 hours | 24 hours |

3. Make sure reagents are *fully* thawed. Inspect each well as reagents thaw at varying rates.
4. If any ice remains, continue thawing.
5. Set aside the thawed cartridge at room temperature and initiate the run within 2 hours. If you do not immediately initiate the run, place the thawed cartridge at 2°C to 8°C. You can store overnight at 2°C to 8°C for use the next day.

## Add Primer Tubes

1. If you are using a Cloudbreak Freestyle Dx sequencing kit without custom primers, skip the following steps and proceed to *Dilute Library and Custom Primers* on page 10.
2. Remove applicable primer set from -25°C to -15°C storage.
3. To replace the primer tubes in the cartridge with primer set, twist the primer tubes in wells labeled I1, I2, R1, and R2 toward the left to unlock.




4. Remove the primer tubes from the cartridge and discard per the SDS.

   If you have trouble removing the tubes, peel the labels off and twist the tubes as you push upwards.

5. Insert the tubes from the primer set into the vacated wells. Match the abbreviation on the tube label to the well label.
6. Twist each tube right until it locks into place.

# Dilute Library and Custom Primers

To prepare for sequencing, the input library is normalized to 1 nM, denatured with NaOH, neutralized, and further diluted. The dilution procedures prepare 1.4 ml diluted library at the target loading concentration with an optional spike-in. Custom primers are diluted as applicable.

If you are using the Individually Addressable Lanes add-on, follow the applicable procedures for both libraries. Both libraries use the same denature and dilution methods, resulting in a total volume of 1.4 ml for each library.

Gather the following consumables:

- 0.2 M Tris-HCl buffer, pH 7.0
- 1 N NaOH
- 2 ml DNA LoBind tubes (4–7)
- 10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA (low TE buffer)
- Nuclease-free water

## Prepare the Library

For bead-normalized libraries, perform amplification and quantification before proceeding. See *Bead-Based Normalization* on page 6.

1. Remove the following components from -25°C to -15°C storage and thaw on ice:
   - » Library Loading Buffer
   - » Experimental library
   - » [Optional] PhiX Control Library
2. Pulse vortex the thawed libraries and briefly centrifuge.
3. If the experimental library is ≥ 1 nM, normalize:
   a. In a new DNA LoBind tube, use low TE buffer to dilute the library to 1 nM.
   b. Proceed immediately or cap the tube, store the 1 nM library at -25°C to -15°C.

## Calculate Library Loading Concentration

Calculate the experimental library and PhiX Control Library loading concentrations and volumes for sequencing using a total loading volume of 1.4 ml. You will need the target loading concentration, relative amounts of each library, and spike-in percentage of PhiX Control Library to perform the calculations.

**NOTE**
The experimental and control library concentrations do not need to match.

1. Calculate the loading concentration of each library based on the target loading concentration as follows:

| Library | Calculation | Resulting Loading Concentration (pM) |
|---|---|---|
| Experimental | Target loading concentration in pM * (100% – spike-in %) | _____ |
| Control | Target loading concentration in pM * spike-in % | _____ |

2. Calculate the volume for each library based on the calculated loading concentration, starting library concentration, and a 1.4 ml loading volume as follows:

| Library | Calculation | Resulting Loading Volume (µl) |
|---|---|---|
| Experimental | (Library loading concentration in pM * 1400 µl) / library starting concentration in pM | _____ |
| Control | (Control library loading concentration in pM * 1400 µl) / control library starting concentration in pM | _____ |

**NOTE**
If the volume calculated for the control library is < 1 µl, dilute control library in low TE buffer to use a volume ≥ 1 µl for accurate pipetting.

3. Record the total volume of diluted experimental and control library in µl.

**Example Calculation**

An example calculation using a target loading concentration of 9 pM with a 2% spike-in of PhiX Control Library and a starting experimental library concentration of 1 nM (1000 pM) is shown in the following table. The PhiX Control Library was diluted and used at 100 pM concentration to allow for ≥ 1 µl volume for accurate pipetting.

| Library | Loading Concentration (pM) | Loading Volume (µl) |
|---|---|---|
| Experimental | 9 * 98% = 8.82 pM | (8.82 * 1400)/1000 = 12.3 µl |
| Control | 9 * 2% = 0.18 pM | (0.18 * 1400)/100 = 2.5 µl |

## Denature the Library with NaOH

This procedure uses equal volumes of library, 0.2 N NaOH, and 0.2 M Tris-HCl buffer, pH 7.0. Therefore, the volume recorded during the calculations step is used in two subsequent steps

1. Combine the following reagents to prepare 0.2 N NaOH. Use 0.2 N NaOH within the day and discard.

| Reagent | Volume |
|---|---|
| 1 N NaOH | 20 µl |
| Nuclease-free water | 80 µl |
| **Total** | **100 µl** |

2. In a new DNA LoBind tube, combine the experimental and control library volumes calculated in step 3 of the calculations step.
3. Add an equal volume of freshly prepared 0.2 N NaOH.
4. Vortex the tube to mix and briefly centrifuge.
5. Incubate the tube at room temperature for 5 minutes to denature the library.
6. Vortex the tube to mix and briefly centrifuge.
7. Add 0.2 M Tris-HCl buffer, pH 7.0 at an equal volume of 0.2 N NaOH to neutralize the reaction.
8. Vortex the tube to mix and briefly centrifuge.
   —The library is denatured, neutralized, and at 1/3 the input concentration in 3x input volume.—
9. Add a sufficient volume of Library Loading Buffer to the combined library volume to reach a total volume of 1.4 ml:

   $$\textit{buffer volume in } \mu l = 1400 \text{ µl} - 3 * \textit{library volume in } \mu l$$

10. Vortex the tube to mix and briefly centrifuge.
11. Place the diluted sequencing library on ice. Use within the day.

## Prepare Custom Primers

1. If you are not using custom primers, skip the following steps and proceed to *Run Setup* on page 13.
2. In a new DNA LoBind tube, prepare each applicable custom primer using low TE buffer:

| Custom Primer | Volume | Concentration |
|---|---|---|
| Index 1 | 19 µl | 100 µM |
| Index 2 | 19 µl | 100 µM |
| Read 1 | 32.4 µl | 100 µM |
| Read 2 | 19 µl | 100 µM |

3. Set aside the 100 µM custom primers on ice. Use within the same day.

# Run Setup

Run setup for sequencing prompts you to define run parameters, load sequencing consumables, and empty the waste bottle. Before initiating a run, review the overview, software, troubleshooting, and safety information in the user guide for your instrument.

## Initiate a Sequencing Run

1. Gather the following materials:
   - » Buffer bottle
   - » Cartridge
   - » Cartridge basket
   - » Towel or wipe
   - » Used flow cell

   —A used flow cell might already be present on the instrument.—
2. If applicable, stage run manifests for import:
   - » If setting up the run manually, save the manifest on a USB and connect the USB drive to an instrument USB port.
   - » Alternatively, you can save the manifest to the specified SMB storage connection.
3. On the Home screen, select **New Run**.
4. If AVITI Dx OS prompts that the flow cell is missing, load a ***used*** flow cell:
   a. Select **Open Nest**.
   b. Place the used flow cell onto the nest and close the lid.
   c. Select **Close Nest**.
5. Select **Sequencing**.
6. Select the side for sequencing:
   - » **Side A**—Set up a run on side A.
   - » **Both**—Set up runs on sides A and B.
   - » **Side B**—Set up a run on side B.
7. Proceed to *Define Manual Run Parameters* on page 13.

### Define Manual Run Parameters

1. In the Run Name field, enter a unique name to identify the run.

   —The field accepts 1–64 alphanumeric characters, hyphens, and underscores.—
2. If applicable, select **Browse** and import the run manifest.
3. [Optional] In the Description field, enter a description that represents the run.

   —The field accepts ≤ 500 alphanumeric characters, hyphens, underscores, spaces, and periods.—
4. In the Storage drop-down menu, select a storage location:
   - » To output run data to the default storage location, leave the default selection.
   - » To override the default storage location for the current run, select a storage connection.
5. In the Sequencing Kit drop-down menu, select the kit you are using. For information on kit compatibility, see the *Product Compatibility* page on the Element website.

   —The kits listed depend on compatibility with the instrument type, and the selected library type and library structure.—
6. Select a Low-Diversity High-Multiplex option.

» **Yes**—Sequence low-diversity high-multiplex libraries. This option requires at least 4 cycles for Index 1.
» **No**—Sequence other libraries.

7. Select the number of library pools.
8. In the Cycles fields, enter the number of cycles to perform in each read.
   » Do not exceed the maximum number of cycles for the sequencing kit. See *Number of Cycles* on page 5.
   » Add one cycle to the desired number of Read 1 and Read 2 cycles. For example, enter **151** in the Read 1 field to perform 150 cycles in Read 1.
   » To skip a read, enter **0**.
   » See the following table for minimum and default cycle values. Aside from the minimum cycle limitations, AVITI Dx OS lets you distribute the available cycles among reads as necessary. However, performance specifications for each kit are based on sequencing Element control libraries using default read length settings specific to the kit.

| Library Type | Kit Size | Minimum Values | | | | Default Values | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Index 1 | Index 2 | Read 1 | Read 2 | Index 1 | Index 2 | Read 1 | Read 2 |
| Third Party | 2 x 75 | 0 | 0 | 5 | 0 | Blank | Blank | 76 | 76 |
| | 2 x 150 | 0 | 0 | 5 | 0 | Blank | Blank | 151 | 151 |

9. Select **Next** to proceed to the Run Side B or Prepare Reagents screen.
10. If applicable, repeat steps **1**–**9** to set up a dual start run.

# Inspect and Mix Reagents

1. Inspect each cartridge well to make sure reagents are fully thawed.
2. Make sure the cartridge contains the appropriate primers.
3. Make sure the tubes in the I1, I2, R1, and R2 wells are secure. If necessary, twist each tube to the right.
4. Gently invert the cartridge ***10 times*** to mix reagents.

   **CAUTION**
   Inadequately mixed reagents can cause run failure.

5. Tap the cartridge base on the benchtop to remove any large droplets from the tube tops.
6. Inspect the small tubes to make sure reagents are settled at the bottom.
7. Place the cartridge into a clean cartridge basket and lock the clips. Wipe any excess moisture.

# Add Custom Primers to the Cartridge

1. If you are not using custom primers, skip the following steps and proceed to *Add Library to the Cartridge*.
2. Using a new 1 ml pipette tip, pierce the center of the applicable I1, I2, R1, and R2 wells to create one hole. Push the foil to the edges.
3. Discard the pipette tip.
4. Add the applicable volume of 100 µM custom primer to each pierced well.

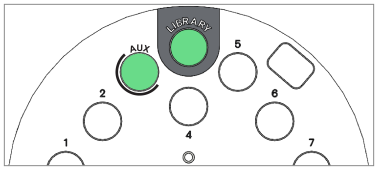
| Custom Primer | Volume | Well |
|---|---|---|
| Index 1 | 19 µl | I1 |
| Index 2 | 19 µl | I2 |
| Read 1 | 32.4 µl | R1 |
| Read 2 | 19 µl | R2 |

—The final concentration of each custom primer is 1 µM.—

5. Pipette the content of each tube 15 times to mix. Avoid losing existing primer volume.

## Add Library to the Cartridge

1. Using a new 1 ml pipette tip, pierce the center of the Library well to create one hole. Push the foil to the edges.




2. Discard the pipette tip.
3. Briefly centrifuge the diluted sequencing library to remove bubbles and foam from the tube lid.
4. Transfer the entire volume of diluted sequencing library to the Library well, dispensing along the well wall.
   - » Avoid aspirating any foam or dispensing air.
   - » Do not allow the library to contact the foil.
   - » Make sure the tube contains ≥ 1.3 ml diluted sequencing library.
5. If you are using the Individually Addressable Lanes add-on, repeat steps **1**–**4** with the AUX well and the second library.

   —The library for the AUX well contains the samples for Lane 2 in the run manifest.—

   **CAUTION**
   Transferring a library to the AUX well of an incompatible cartridge damages the library and the cartridge. For more information on Individually Addressable Lanes add-on compatibility, see the user guide for your instrument.
6. Inspect the Library well through the window at the front of the basket.
   - » Make sure the library is free of foam and that bubbles are minimal.
   - » If an air gap appears below the surface, use a new pipette tip to remove it.
7. If the cartridge include shipping locks, twist each shipping lock left to unlock and remove them from the cartridge lid.

## Confirm Reagent Preparation

1. Select the **Invert cartridge** checkbox to confirm that reagents are mixed.
2. Select the **Insert into basket** checkbox to confirm that the cartridge is in the cartridge basket.
3. Select any load library checkboxes to confirm that the cartridge contains diluted library.
4. Select **Next** to proceed to the Load Reagents screen.

## Load Reagents and Buffer

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Slide the basket containing the thawed cartridge into the reagent bay until it stops.
4. Support the buffer bottle with both hands and slide it into the reagent bay until it stops.
5. Close the reagent bay door, and then select **Next** to proceed.

## Empty Waste

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.

3. Remove the waste bottle from the waste bay and close the transport cap.

   **CAUTION**
   Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.

4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:

   a. Position the bottle over the funnel or waste receptacle.
      - If you inserted a funnel, align the handle to the inner edge of the funnel.
      - If you did not insert a funnel, center the handle over the waste receptacle.

   b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.

   c. If necessary, wipe liquid off the bottle.

7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.

# Prime Reagents

1. Bring a new Cloudbreak Freestyle Dx flow cell to room temperature:

   a. Remove a flow cell pouch from 2°C to 8°C storage. ***Do not open the pouch.***

   b. Set aside the pouch for at least 5 minutes.

   **NOTE**
   Before priming, if you need to discard the run setup, remove the cartridge, correct the run setup as needed, and reload the same cartridge. Proceed to the priming step.

2. Select **Next** to ***automatically*** start priming.

   Priming takes ~ 5 minutes or up to 8 minutes at high elevations.

3. When priming is complete, select **Next** to proceed to the Load Flow Cell screen.

   AVITI Dx OS moves the nest forward and opens the nest bay door. A brief delay is normal.

   **NOTE**
   After priming and prior to loading a new flow cell, if you need to discard the run setup, remove the cartridge, correct the run setup as needed, and reload the same cartridge. Proceed to loading the flow cell.

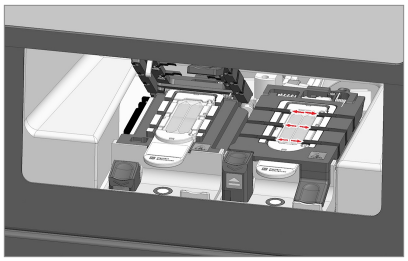
# Load the Flow Cell

1. Make sure the nest status light is blue.
2. To open the lid, fully press down on the button to the left of the nest until it reaches the bottom. Failure to fully press down on the button can cause errors when closing the lid or aligning the flow cell.
3. Remove the used flow cell from the nest. Discard or store at room temperature for use with priming or washes.
4. Unpackage the Cloudbreak Freestyle Dx flow cell before loading onto the nest. Ensure the flow cell is used within 2 hours.

   Handle the flow cell by the gripper only. If any debris contacts the flow cell surface during or after package opening, gently wipe the flow cell surface with an ethanol wipe and then a lens wipe.

   **CAUTION**
   Touching the glass can introduce debris, smudges, and scratches, compromising data quality.

5. Face the label up and place the flow cell over the three registration pins on the nest.



6. Lower the tab on the right side of the lid until the lid snaps into place.
7. Make sure the six prongs are flush with the flow cell.

   —The nest status light turns green when the lid closes fully.—

   **NOTE**
   A red status light indicates the flow cell is improperly loaded — the lid is open or the nest is empty. Reload the flow cell to correct this. If the red status light persists after the flow cell is properly loaded, contact Element Technical Support.
8. Select **Close Nest** to close the nest bay door and retract the stage.
9. Select **Next** to proceed to the Run Summary screen.

# Review and Start the Run

1. On the Details page, review the run parameters:

| Parameter | Description |
|---|---|
| Library | The workflow that prepared the libraries and the library type |
| Sequencing Kit | The size and version of the sequencing kit |
| Storage | The location where sequencing output is stored |
| Manifest | The file name of the uploaded run manifest, if applicable |
| Cycles | The number of cycles in each read |
| Description | An optional description of the run |
| Advanced | If applicable, the advanced run settings for the run |

   **NOTE**
   If you notice any errors in run set up, refer to the guidance in Knowledge Base to discard the run, skip the priming step, and restart the run setup process.
2. Review the flow cell, cartridge, and buffer bottle information:

| Field | Description |
|---|---|
| Lot Number | The number assigned to the batch the consumable was manufactured with |
| Expires on | The year, month, and date that the consumable expires |
| Serial Number | The unique identifier or all zeros indicating an unscanned barcode |
| Part Number | The Element-assigned identifier for the consumable |

3. Select **Run** to start sequencing.
4. [Optional] If you imported run manifests from a USB drive, disconnect the USB drive:

   a. In the taskbar, select **USB Drive**, and then select **Eject**.
   b. Detach the USB drive from the instrument.
5. Process the materials removed from the reagent bay:

   » For a used cartridge and buffer bottle, follow the instructions in *Discard the Cartridge and Bottle* on page 19.

» For a wash tray, follow the guidelines in the user guide for your instrument. Residual liquid in the wash tray is normal.

## Monitor Run Metrics

1. If necessary, select **Details** to open run details.
2. Monitor run metrics as they appear onscreen. AVITI Dx OS indicates the expected cycle that metrics appear.

   —The expected cycles are approximate, and all metrics are estimates. Bases2FastqDx generates the final metrics.—
3. Continue monitoring the run as AVITI Dx OS refreshes the metrics.
   - » Each cycle refreshes the Q scores, error rates, base compositions, and index metrics.
   - » If you are using the Individually Addressable Lanes add-on, AVITI Dx OS displays metrics for each library pool.
   - » AVITI Dx OS refreshes the yield and reads metrics after cycle 15 of Read 2:
     - ◦ If Read 2 contains no cycles, metrics refresh after cycle 15 of Read 1.
     - ◦ If Read 1 or Read 2 contain fewer than 15 cycles, metrics refresh when the last cycle begins.
4. When the run is complete, leave all materials on the instrument.
   - » To return to the Details view, select **Overview**.
   - » To access run data, go to your storage location.

## Initiate Flexible Start

Flexible start provides the option to start a run or recovery wash while another run is in progress. AVITI Dx OS safely pauses the run on the adjacent side.

1. On the Home screen, select **New Run**.
2. When prompted to request flexible start and pause the active run, select **New Run**.

| Step | Estimated Wait Time to Pause* |
|---|---|
| Amplification | 2 hours |
| Index 1, Index 2, Read 1, or Read 2 | A few minutes |
| Turn | 30 minutes |
| Wash | 1 hour |

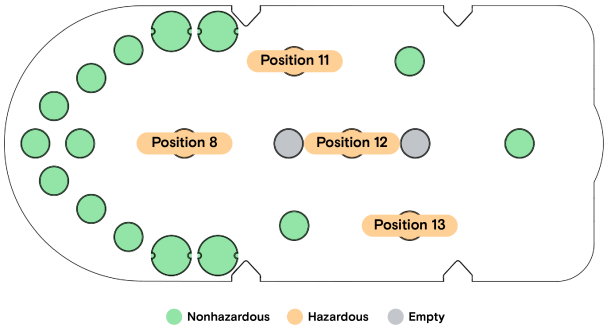
* Estimates are for 2 x 75 and 2 x 150 runs with Cloudbreak Freestyle Dx chemistry.

3. Wait for the run to pause.
   - » To cancel flexible start while waiting, select **Cancel Request**.
   - » Contact Element Technical Support if the wait time exceeds 5 hours at the amplification step or 1.5 hours at any other step.
4. When the run pauses, proceed through run setup and start the second run or recovery wash.
   - » For run setup instructions, proceed to *Initiate a Sequencing Run* on page 13.
   - » For recovery wash instructions, see the user guide for your instrument.
5. To cancel setup of the second run or recovery wash, select **Back** to return to the Home screen, and then select **Resume**.

# Discard the Cartridge and Bottle

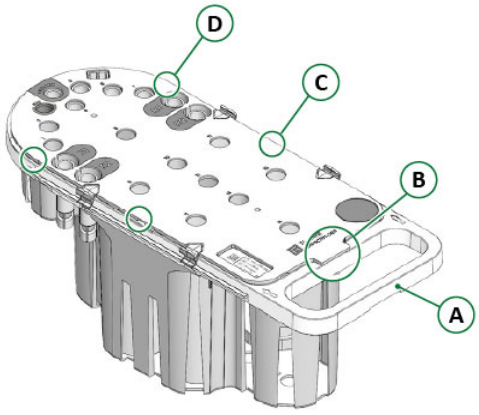
The cartridge and buffer bottle contain reagents with region-specific disposal requirements, which are described in the Safety Data Sheets (SDS) at elementbiosciences.com/resources. The amount of reagent remaining in each well after a run depends on how many cycles the run performed.

The following wells contain hazardous reagents. The position numbers in the figure align with the position numbers in the SDS.




## Dispose of Reagents

1. Keep the cartridge in the basket with the clips locked.
2. Hold the basket handle with one hand and lift the cartridge lid tab with the other. Expect resistance at three points.




- **A** Basket handle
- **B** Cartridge lid tab and first point of resistance
- **C** Second point of resistance on both sides of the cartridge
- **D** Final point of resistance on both sides of the cartridge

3. Remove the hazardous wells from the cartridge.
   —The volume remaining in each well depends on the number of cycles performed.—
4. Using a pipette tip or a similar tool, enlarge the hole in each foil seal to form a triangle.



5. Empty each well into hazardous waste or other appropriate container per the SDS.
6. Unlock the clips and remove the cartridge from the basket.
7. Remove the remaining wells from the cartridge and enlarge the hole in each foil seal.
8. Empty each well into the appropriate container per the SDS.
9. Discard the cartridge and buffer bottle per the SDS.
10. Rinse the basket with nuclease-free water and dry upside down.

# Troubleshooting

The following troubleshooting information addresses problems that can occur during run setup and sequencing with a Cloudbreak Freestyle Dx kit. If a problem persists, contact Element Technical Support. For more information on troubleshooting, see the user guide for your instrument.

## Run Setup Problems

| Problem | Resolution |
|---|---|
| The flow cell is cracked, scratched, or otherwise damaged. | Contact Element Technical Support. |
| Small particulates are visible in the flow cell lane. | See *Cloudbreak Freestyle Dx Flow Cell Variations* on page 24. |
| The lid does not engage when a flow cell is on the nest. | Remove the flow cell and wipe the nest. Inspect the flow cell for large debris and wipe with an alcohol pad if necessary. Reload the flow cell. |
| AVITI Dx OS cannot detect a loaded cartridge or waste bottle. | Follow the onscreen prompt to reload the cartridge or waste bottle. Make sure the applicable bay, reagent or waste, is unobstructed, and that the cartridge is contained within a cartridge basket. |
| The system cannot scan or detect a barcode on the cartridge, buffer bottle, or flow cell. | Follow the onscreen prompt to reload the consumable or continue by manually entering consumable information. |
| The flow cell version is incompatible with the cartridge. | Load a flow cell that is the same version as the cartridge. |

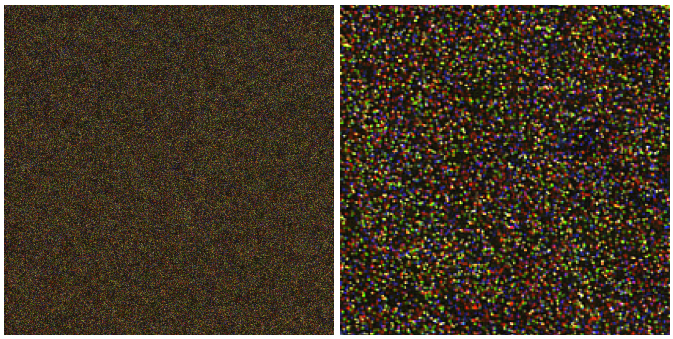
## Sequencing Problems

| Problem | Resolution |
|---|---|
| Polony density is lower or higher than expected. | Contact Element Technical Support or stop the run. For instructions on stopping a run, see the troubleshooting section of the user guide for your instrument. |
| The assigned or perfect match metrics are lower than expected. | Make sure that the index sequences recorded in the run manifest are correct. |
| The samples with low representation metric is higher than expected. | Select **Sample Details** to view the samples with low representation. Make sure that the index sequences recorded in the run manifest and the pooling concentration are correct. |
| The Q30 percentage is lower than expected. | Contact Element Technical Support. |
| The PhiX error rate is higher than expected. | |
| The flow cell contains very few polonies or no polonies. | |
| The user interface is frozen. | |

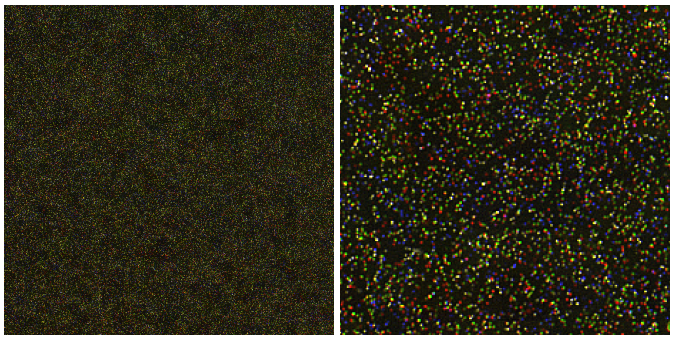
# Thumbnail Image Troubleshooting

The following figures show example thumbnail images for a standard flow cell, an underloaded flow cell, and an overloaded flow cell. If the thumbnail image for a run indicates an underloaded flow cell, increase the loading concentration. For an overloaded flow cell, reduce the loading concentration. If problems persist, contact Element Technical Support.

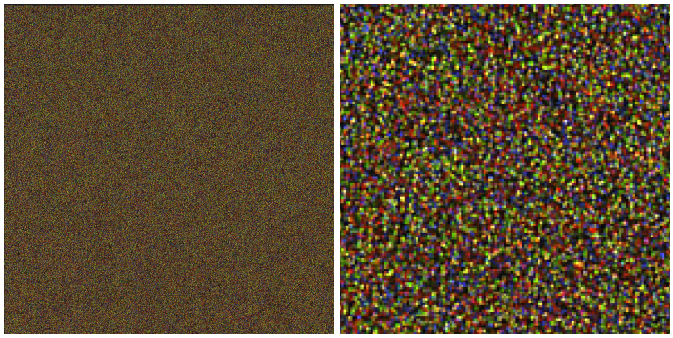
Example expected thumbnail image, full-size and zoomed



Example thumbnail image with underloading, full-size and zoomed



Example thumbnail image with overloading, full-size and zoomed



# Cloudbreak Freestyle Dx Consumables

Cloudbreak Freestyle Dx consumables include a sequencing kit and optional controls and custom primers. The workflow also requires user-supplied consumables. For a list of required equipment, see the site prep guide for your instrument.

## Cloudbreak Freestyle Dx Sequencing Kits

The following tables list the kit contents and storage requirements. Kits contain one of each part listed. The Library Loading Buffer pouch includes two tubes. For SDS information, see elementbiosciences.com/resources.

### AVITI Dx 2 x 150 Sequencing Kit CB Freestyle™ High Output (860-00052)

| Part # | Component | Shipping | Storage |
|---|---|---|---|
| 820-00043 | AVITI Dx 2 x 150 Cartridge CB Freestyle High Output | -25°C to -15°C | -25°C to -15°C |
| 810-00023 | AVITI Dx CB Freestyle Flow Cell | Room temperature | 2°C to 8°C |
| 820-00041 | AVITI Dx Buffer Bottle | Room temperature | Room temperature |
| 820-00042 | AVITI Dx Library Loading Buffer | -25°C to -15°C | -25°C to -15°C |

### AVITI Dx 2 x 150 Sequencing Kit CB Freestyle Low Output (860-00053)

| Part # | Component | Shipping | Storage |
|---|---|---|---|
| 820-00044 | AVITI Dx 2 x 150 Cartridge Cloudbreak Freestyle Low Output | -25°C to -15°C | -25°C to -15°C |
| 810-00023 | AVITI Dx CB Freestyle Flow Cell | Room temperature | 2°C to 8°C |
| 820-00041 | AVITI Dx Buffer Bottle | Room temperature | Room temperature |
| 820-00042 | AVITI Dx Library Loading Buffer | -25°C to -15°C | -25°C to -15°C |

### AVITI Dx 2 x 75 Sequencing Kit CB Freestyle High Output (860-00054)

| Part # | Component | Shipping | Storage |
|---|---|---|---|
| 820-00045 | AVITI Dx 2 x 75 Cartridge CB Freestyle High Output | -25°C to -15°C | -25°C to -15°C |
| 810-00023 | AVITI Dx CB Freestyle Flow Cell | Room temperature | 2°C to 8°C |
| 820-00041 | AVITI Dx Buffer Bottle | Room temperature | Room temperature |
| 820-00042 | AVITI Dx Library Loading Buffer | -25°C to -15°C | -25°C to -15°C |

## PhiX Control Library

PhiX Control Library is a color-balanced, ready-to-use library that adds diversity to low-complexity libraries. Each type of PhiX Control Library includes unique index sequences and has a concentration of 1 nM. For a list of sequences, see *Element Index Sequences*.

| Part # | Type | Format | Shipping and Storage |
|---|---|---|---|
| 830-00023 | Cloudbreak Freestyle PhiX Control, Third Party | Linear | -25°C to -15°C |

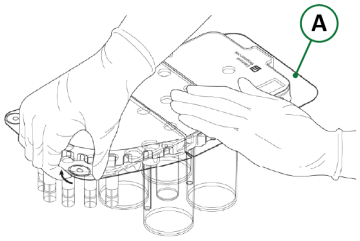
# User-Supplied Consumables and Equipment

| Consumables | Supplier |
|---|---|
| DNA LoBind Tubes, 2 ml | Eppendorf, # 022431021 |
| 0.2 M Tris-HCl, pH 7.0 | General lab supplier |
| 1 N NaOH | |
| 10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA (Low TE buffer) | |
| Filtered pipette tips | |
| Nuclease-free laboratory-grade water | |

# Cartridge Shipping Configuration

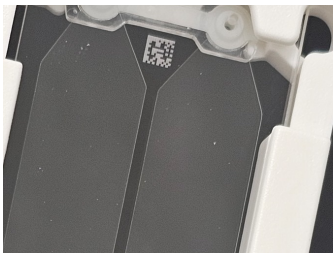
The Cloudbreak Freestyle Dx sequencing cartridge includes shipping protection in the form of shipping locks or a thermoform shipping cover.

- If your cartridge includes the thermoform shipping cover (**A**), remove the cover before thawing reagents.




# Cloudbreak Freestyle Dx Flow Cell Variations

Cloudbreak Freestyle Dx flow cells might have small particulate within the flow cell lane. These variations are normal and do not impact data quality.



# Document History

| Revision | Description of Change |
|---|---|
| July 2026<br>Document # MA-00087 Rev. A | • Initial release. |
| October 2026<br>Document # MA-00087 Rev. B | • Added red arrows to the flow cell loading image and a new step confirming the flow cell is properly seated in the Load the Flow Cell section; added note on red status light troubleshooting and when to contact Element Technical Support. |

# Technical Support

Visit the User Documentation page on the Element Biosciences website for additional guides and the most recent version of this guide. For technical assistance, contact Element Technical Support.

**Website:** www.elementbiosciences.com

**Email:**support@elembio.com

**Regional Phone Numbers:** www.elementbiosciences.com/contact




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**ELEMENT BIOSCIENCES**
**The AVITI Dx System product family delivers precise, high-quality raw DNA sequencing data from a variety of biological samples. The product is intended solely as a data generation tool and does not provide diagnostic interpretations or direct clinical recommendations.**