Element Biosciences

Cloudbreak™ Sequencing

User Guide

FOR USE WITH

AVITI™ System, catalog # 880-00001
AVITI™ System LT, catalog # 880-00003
AVITI24™ System, catalog # 880-00004
AVITI Operating Software v3.1.0 or later
Cloudbreak, Cloudbreak Freestyle™, and Cloudbreak UltraQ™ Sequencing Kits

ELEMENT BIOSCIENCES
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Overview

The Cloudbreak sequencing workflow uses a Cloudbreak Freestyle, Cloudbreak, or Cloudbreak UltraQ kit to sequence DNA libraries on an AVITI System or an AVITI24 System.

- **Cloudbreak Freestyle**—Provides multiple read lengths and output options to meet a diversity of applications. Cloudbreak Freestyle kits enable direct loading of linear libraries without library conversion, including third-party libraries.
- **Cloudbreak**—Provides the same read length and output options as Cloudbreak Freestyle with potential requirements for library circularization.
- **Cloudbreak UltraQ**—Provides high-quality Q40 and Q50 data for highly sensitive assays.

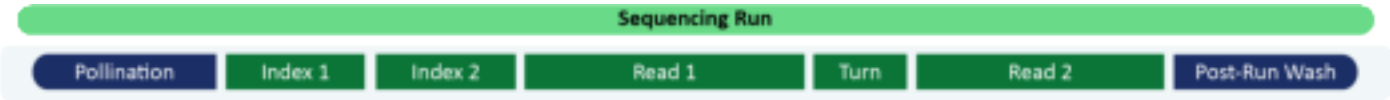
All Cloudbreak kits are designed for minimal waste and easy disposal. Reagent overage supports the extra cycles that index sequences and unique molecular identifiers (UMIs) need to identify samples with high confidence.

Cloudbreak kits are compatible with a variety of library preparation workflows. For more information on compatibility, see the *Product Compatibility* page on the Element website. For a list of kit configurations and catalog numbers, see *Cloudbreak Sequencing Kits* on page 24.

Sequencing Run Stages

AVITI Operating Software (AVITI 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 and pollination**—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. The optimal number of cycles to perform depends on your experiment and you can distribute the total cycles depending on experimental design.

Each kit specifies the number of cycles it performs. For bioinformatics purposes, it is recommended to add one extra cycle to each read. For instance, a 2×150 cycle run should ideally include 2×151 cycles. The additional cycle improves the accuracy of the Q score for the 150th cycle.

The software and chemistry 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 sequences up to 184 cycles, supporting one 2 x 76 run with indexing and unique molecular identifiers (UMIs).
- A 2 x 150 kit sequences up to 334 cycles, supporting one 2 x 151 run with indexing and UMIs.
- A 2 x 300 kit sequences up to 634 cycles, supporting one 2 x 301 run with indexing and UMIs.

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:

- Adept™ libraries or 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 chemistry with Adept libraries, use the Adept Rapid PCR-Plus Kit for amplification.
- For Cloudbreak Freestyle 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

Short or long insert libraries require that you specify the use of preloaded custom recipes during run setup.

- **Short insert libraries**—If you are using Cloudbreak Freestyle chemistry and libraries with ≤ 100 bp DNA inserts, such as miRNA libraries, use the preloaded custom recipe for short insert libraries. The recipe is only compatible with 2 x 75 and 2 x 150 sequencing kits.
- **Long insert libraries**—If you are using Cloudbreak or Cloudbreak Freestyle chemistry and libraries with > 1000 bp DNA inserts, use the preloaded custom recipe for long insert libraries. The recipe only applies to 2 x 75 and 2 x 150 sequencing kits. Using a 2 x 300 sequencing kit already accounts for long insert conditions.

For more information, see *Configure Advanced Run Settings* on page 15 and contact Element Technical Support.

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

* For Adept and third-party workflows, the first four cycles of Read 1 require high diversity. Index 1 includes high diversity for Elevate™ workflows.

Custom Primers

You can sequence any combination of I1, I2, R1, and R2 custom primers for third-party libraries with Cloudbreak Freestyle chemistry and Adept libraries with Cloudbreak chemistry. The custom primers must be HPLC-purified and prepared using the applicable method:

- **Spike-in**—Spike-in custom primers into the Cloudbreak Freestyle cartridge or the Adept Primer Set Cloudbreak tubes.
- **Replacement**—Replace the primers in the cartridge with buffer tubes from the Custom Primer Set Cloudbreak Freestyle or Adept Custom Primer Set Cloudbreak and add custom primers.

Sequencing Primer Compatibility

- For Cloudbreak Freestyle chemistry, Element oligonucleotides include sequencing primers that are compatible with standard Nextera, TruSeq, and small RNA libraries.
- For original Cloudbreak chemistry, sequencing primers are only compatible with standard Nextera and TruSeq libraries.
- Libraries with sequencing primer binding sites that do not meet these requirements *must* use custom primers.

Custom primers require special consideration and planning. To determine if your library requires custom primers and ensure a run with custom primers meets specifications, contact Element Technical Support early in experiment planning. For more information on Cloudbreak Freestyle custom primer recommendations, see *Cloudbreak Freestyle Compatibility with Third-Party Libraries*.

Loading Concentration

The following tables provide recommendations for a starting point to determine your optimal loading concentration. When reviewing the recommendations, note the following considerations:

- The recommendations are based on libraries prepared for Element and depend on your chemistry, kit size, library prep workflow, and other lab-specific factors. Some libraries require a higher or lower concentration than the indicated ranges.
- Library size refers to the full length of the library, including the DNA insert and adapters.
- If you are sequencing pooled libraries, the pool must contain libraries with similar size distributions.

Cloudbreak Chemistry, 2 x 75 and 2 x 150 Kits

Average Library Size (bp)	Adept v1.1 (pM)	Adept Rapid PCR-Plus (pM)	Elevate PCR-Free (pM)	Elevate PCR-Plus (pM)
Small (250–450)	4–6	10–14	6–10	8–11
Medium (450–700)	6–10	10–14	7–11	9–12
Large (≥ 700)	10–14	10–14	7–11	9–12

Cloudbreak Chemistry, 2 x 300 Kits

Average Library Size (bp)	Adept v1.1 (pM)	Adept Rapid PCR-Plus (pM)	Elevate PCR-Free (pM)	Elevate PCR-Plus (pM)
Medium (450–700)	4–6	5–8	3–5	4–6
Large (≥ 700)	6–8	5–8	3–5	4–6

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

Average Library Size (bp)	Elevate PCR-Free (pM)	Elevate PCR-Plus (pM)	Third Party PCR-Free (pM)	Third Party PCR-Plus (pM)
Small (250–450)	5–9	7–10	6–9	7–10
Medium (450–700)	6–10	8–11	7–10	9–12
Large (≥ 700)	6–10	8–11	7–10	9–12

Cloudbreak Freestyle Chemistry, 2 x 300 Kits

Average Library Size (bp)	Elevate PCR-Free (pM)	Elevate PCR-Plus (pM)	Third Party PCR-Plus (pM)	Third Party PCR-Free (pM)
Medium (450–700)	3–5	4–8	6–9	4–6
Large (≥ 700)	3–5	4–8	8–12	4–6

Cloudbreak UltraQ Chemistry, 2 x 150 Kit

Average Library Size (bp)	Elevate Libraries (pM)
450–550	5–6

Target Polony Counts

The target total polony counts associated with the loading concentration recommendations depend on your kit chemistry, output level, and size. The polony count increases as the loading concentration increases, which can compromise data quality. Low polony counts promote high data quality but lower the amount of data output.

Cloudbreak and Cloudbreak Freestyle Chemistry

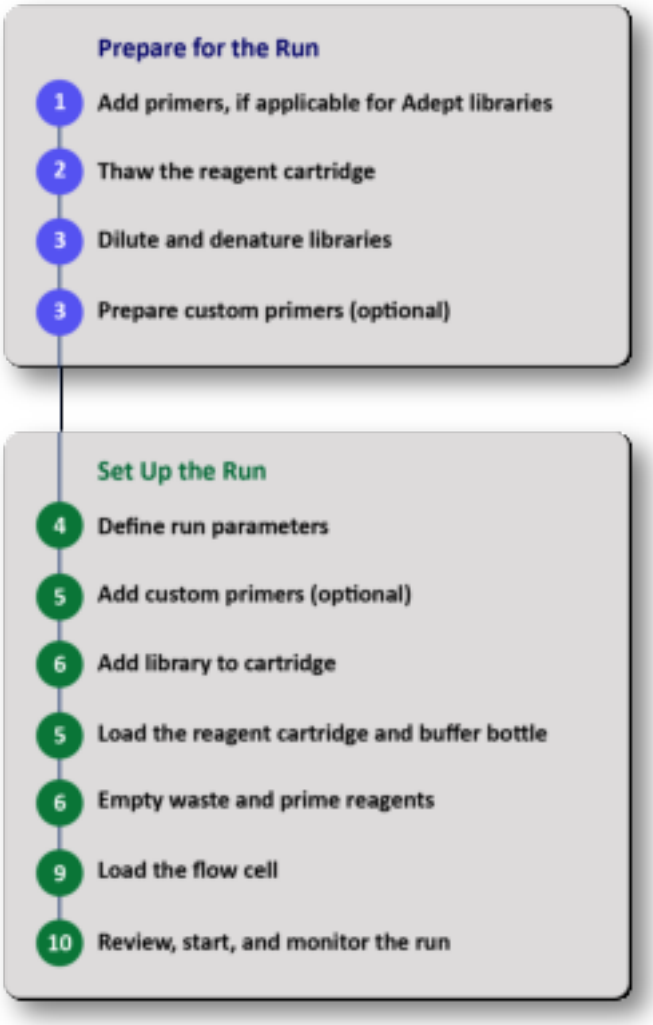
Output Level	Kit Size	Target Read Counts	Output (Gb)
High	2 x 75	1 billion	150
	2 x 150	1 billion	300
	2 x 300	300 million	180
Medium	2 x 75	500 million	75
	2 x 150	500 million	150
	2 x 300	100 million	60
Low	2 x 150	250 million	75

Cloudbreak UltraQ Chemistry

Output Level	Kit Size	Target Read Counts	Output (Gb)
High	2 x 150	800 million	240

Cloudbreak Workflow Summary

Performing a Cloudbreak 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

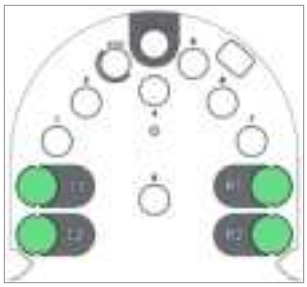
Run preparation includes adding appropriate primers, if applicable, and thawing the sequencing cartridge. The subsequent dilution procedure includes the option to store a normalized library. If you intend to store a library, do not prepare the cartridge until you are ready to sequence. Prepare the cartridge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

Add Primer Tubes

1. If one of the following conditions applies, skip the following steps and proceed to *Thaw Reagents* on page 10.
   - » You are using a Cloudbreak Freestyle sequencing kit without custom primers.
   - » You are sequencing Elevate libraries.
2. Remove a cartridge and applicable primer set from -25°C to -15°C storage.

Chemistry and Library	Primer Strategy	Primer Set
Cloudbreak, Adept	No custom primers	Adept Primer Set Cloudbreak
	Custom primers (spike-in method)	Adept Primer Set Cloudbreak
	Custom primers (replacement method)	Adept Custom Primer Set Cloudbreak
Cloudbreak Freestyle, Third Party	Custom primers	Custom Primer Set Cloudbreak Freestyle

   —Subsequent procedures prepare custom primers and load them into the cartridge.—
3. Twist the primer tubes in wells labeled I1, I2, R1, and R2 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.

   —For example, insert Adept Custom Index 1 (**I1**) Buffer Cloudbreak into the **I1** well.—
6. Twist each tube right until it locks into place.

Thaw Reagents

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

Cartridge	Room Temperature Water Bath	Refrigerator
2 x 75	90 minutes	8 hours
2 x 150, 2 x 300	2.5 hours	24 hours

2. Make sure reagents are *fully* thawed. Inspect each cartridge well as reagents thaw at varying rates.

3. If any ice remains, continue thawing.
4. Set aside the thawed cartridge at room temperature. If not immediately initiating the run, place the thawed cartridge at 2°C to 8°C. Do not exceed 3 hours.
5. Proceed to *Run Setup* on page 13.

Dilute Library and Custom Primers

The library 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.

Prepare the Library

For bead-normalized libraries, perform amplification and quantification before proceeding. See *Bead-Based Normalization* on page 5.

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

Reagent	Volume (µl)
1 N NaOH	20
Nuclease-free water	80
Total	100

3. Remove the following components from -25°C to -15°C storage and thaw on ice:
   - » Library Loading Buffer
   - » Experimental library
   - » [Optional] PhiX Control Library
4. Pulse vortex the thawed libraries and briefly centrifuge.
5. 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°, and sequence within the allotted time.

Denature the Library with NaOH

1. Calculate the loading concentration of each library, experimental and control, based on the target loading concentration and relative amount of each library:

   $$\textit{loading concentration in pM} = \textit{target loading concentration in pM} * \textit{library amount in \%}$$

   —For example, if the target loading concentration is 9 pM with a 2% spike-in: the experimental library concentration is 8.82 pM (9 pM * 98%) and the control library concentration is 0.18 pM (9 pM * 2%).—

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

2. Calculate the experimental library volume based on the calculated loading concentration and a 1.4 ml loading volume:

   $$\textit{library volume in } \mu l = (\textit{library loading concentration in pM} * 1400\ \mu l)/\textit{library starting concentration in pM}$$

   —Continuing the preceding example and assuming a 1 nM starting concentration, the library volume is 12.3 µl: (8.82 pM * 1400 µl)/1000 pM.—
3. If you are adding a spike-in, calculate the control library volume based on the loading concentration and a 1.4 ml loading volume:

   $$\textit{control library volume in } \mu l = (\textit{control library loading concentration in pM} * 1400\ \mu l)/\textit{control library concentration in pM}$$

   —Continuing the preceding example and assuming a 1 nM PhiX Control Library, the control library volume is 0.25 µl: (0.18 pM * 1400 µl)/1000 pM.—
4. If step 3 calculated a volume < 1 µl, dilute PhiX Control Library in low TE buffer to use a volume ≥ 1 µl for accurate pipetting.
5. Record the total volume of diluted sequencing library (experimental and control) in µl.

   —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 at this step is used in two subsequent steps.—
6. Combine the library volumes calculated in steps 2 and 3 in a new DNA LoBind tube.
7. Add an equal volume of freshly prepared 0.2 N NaOH.
8. Vortex the tube to mix and briefly centrifuge.
9. Incubate the tube at room temperature for 5 minutes to denature the library.
10. Vortex the tube to mix and briefly centrifuge.
11. Add 0.2 M Tris-HCl buffer, pH 7.0 at an equal volume of 0.2 N NaOH to neutralize the reaction.
12. Vortex the tube to mix and briefly centrifuge.

    —The library is denatured, neutralized, and at 1/3 the input concentration in 3x input volume.—
13. Add a sufficient volume of Library Loading Buffer to reach a total volume of 1.4 ml:

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

14. Vortex the tube to mix and briefly centrifuge.
15. 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 (µl)	Concentration (µM)
Index 1	19	100
Index 2	19	100
Read 1	32.4	100
Read 2	19	100

3. Set aside the 100 µM custom primers on ice. Use within the 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.
   - » If you planned the run in ElemBio Cloud, upload the manifest to the planned run.
3. On the Home screen, select **New Run**.
4. If AVITI 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. For chemistry type, select **Cloudbreak**, and then select **Next**.
8. Proceed as follows:
   - » For a planned run created in ElemBio Cloud, proceed to *Select a Planned Run*.
   - » For a manual run, proceed to *Define Manual Run Parameters* on page 14.

Select a Planned Run

1. Select **Planned Run**.

   AVITI OS displays a list of compatible planned runs for the instrument and run type. For more information on planned run compatibility, see *Run Planning* in the *Online Help*.
2. Select the run you want to use from the list of planned runs.
3. Review the run parameter fields to make sure they are correct.

   If you need to edit a planned run, modify it in ElemBio Cloud. For more information, see *Run Planning* in the *Online Help*.
4. In the Storage drop-down menu, select the storage location for the run.

5. Select **Next** to proceed to the Prepare Reagents or the Run Side B screen.
   - » After you proceed, the selected planned run becomes unavailable for other connected instruments.
   - » If you exit run setup before priming, the run returns to the list of available planned runs.
6. If applicable, repeat steps 2–5 to set up a dual start run with a second planned run.
7. Proceed to *Inspect and Mix Reagents* on page 15.

Define Manual Run Parameters

1. Make sure **Manual Run** is selected for the type of run.
2. In the Run Name field, enter a unique name to identify the run.

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

   —The field accepts ≤ 500 alphanumeric characters, hyphens, underscores, spaces, and periods (.).—
5. 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.
6. Select a Library Type:
   - » **Elevate**—Sequence libraries prepared with Elevate indexes and adapters.
   - » **Adept**—Sequence libraries prepared with the Adept Workflow. Only compatible with Cloudbreak sequencing kits.
   - » **Third Party**—Sequence libraries prepared with a third-party workflow. Only compatible with Cloudbreak Freestyle sequencing kits.
7. If applicable, select a Library Structure:
   - » **Circular**—Sequence libraries that complete circularization before loading.
   - » **Linear**—Sequence libraries prepared for on-instrument circularization.
8. 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.—
9. If you are using the Adept or Third Party library type, 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.
10. If you are using the Individually Addressable Lanes add-on and a compatible sequencing kit, select the number of library pools.
11. 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 OS lets you distribute the available cycles among reads as necessary.

Library Type	Kit Size	Minimum Values				Default Values			
		Index 1	Index 2	Read 1	Read 2	Index 1	Index 2	Read 1	Read 2
Adept or Third Party	2 x 75	0	0	5	0	Blank	Blank	76	76
	2 x 150	0	0	5	0	Blank	Blank	151	151
	2 x 300	0	0	5	0	Blank	Blank	301	301
Elevate	2 x 75	4	0	5	0	12	9	76	76
	2 x 150	4	0	5	0	12	9	151	151
	2 x 300	4	0	5	0	12	9	301	301

12. If you are using the Advanced Run Settings, select **Advanced Settings** and proceed to *Configure Advanced Run Settings*.
13. Select **Next** to proceed to the Run Side B or Prepare Reagents screen.
14. If applicable, repeat steps 2–13 to set up a dual start run.

Configure Advanced Run Settings

Use Advanced Run Settings to modify primary analysis and recipe configurations for a run. Available settings depend on kit compatibility. Some settings require the activation of an add-on. For more information, see the Advanced Run Settings and Add-On information in the user guide for your instrument.

1. If you are using the Polony Density setting, select a Polony Density option.
   - » **Standard**—Uses the standard read output.
   - » **High Density**—Increases the read output.
2. If you are using the Filter Mask setting, enter a base mask to use for filtering.
   - » Use the base mask format. For more information, see *Base Masks* in the *Online Help*.
   - » If you do not use the Filter Mask setting, the default filter mask is `R1:Y15N*-R2:Y15N*`.
3. If you are using the Custom Recipes setting, import the custom recipe file from preloaded recipes or a USB drive:
   a. Select **Browse**.
   b. Select **Element Recipes** for preloaded recipes or **USB** to upload from a connected USB drive.
   c. Select the recipe file, and then select **Open**.
4. If you are using the PMG Shift setting, enter the number of cycles to skip.

   You cannot skip more than 20 cycles. The number of skipped cycles reduces the maximum number of cycles AVITI OS allows for the run.
5. Select **Next** to proceed.

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 (µl)	Well
Index 1	19	I1
Index 2	19	I2
Read 1	32.4	R1
Read 2	19	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 uses shipping locks, twist each shipping lock left to unlock and remove them from the cartridge lid.

8. [Optional] Set aside one shipping lock for use during reagent disposal.

Confirm Reagent Preparation

1. If you selected Adept, select the **Swap primer tubes** checkbox to confirm that the I1, I2, R1, and R2 wells contain Adept primers or custom primers.
2. Select the **Invert cartridge** checkbox to confirm that reagents are mixed.
3. Select the **Insert into basket** checkbox to confirm that the sequencing cartridge is in the cartridge basket.
4. Select any load library checkboxes to confirm that the sequencing cartridge contains diluted sequencing library.
5. 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 and Prime Reagents

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.

   NOTE
   Before priming, you can discard run setup and save the cartridge. Priming pierces reagent seals and prevents further use.
9. Select **Next** to ***automatically*** start priming.
10. During priming, which takes ~15 minutes, bring a new Cloudbreak 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 ≥ 5 minutes.
11. When priming is complete, select **Next** to proceed to the Load Flow Cell screen.

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

Load the Flow Cell

1. Make sure the nest status light is blue.
2. Press the button to the left of the nest to open the lid. Make sure to fully press down on the button.
   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.
4. Discard the used flow cell or store at room temperature to use for priming and washes.
5. Unpackage the new Cloudbreak flow cell. Handle the flow cell by the gripper only.
   CAUTION
   Touching the glass can introduce debris, smudges, and scratches, compromising data quality.
6. Face the label up and place the flow cell over the three registration pins on the nest.

7. Lower the tab on the right side of the lid until the lid snaps into place.
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. 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 data are output
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

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:
   - » If you removed a used cartridge and buffer bottle, follow the instructions in *Discard the Cartridge and Bottle* on page 21.
   - » If you removed a wash tray, follow the guidelines for wash tray maintenance 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 OS indicates the expected cycle during which metrics appear.
   —The expected cycles are approximate, and all metrics are estimates. Bases2Fastq generates the final metrics.—
3. Continue monitoring the run as AVITI 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 OS displays metrics for each library pool.
   - » AVITI OS refreshes the yield and reads metrics after cycle 15 of Read 2:
     - ◦ If Read 2 contains no cycles, the refresh occurs after cycle 15 of Read 1.
     - ◦ If Read 1 or Read 2 contain < 15 cycles, the refresh occurs when the last cycle of the read starts.
4. When the run is complete, leave all materials in the instrument.
   - » To return to the Details view, select **History**.
   - » To access run data, go to your storage location.

Initiate Flexible Start

Flexible start provides the option to initiate a run or recovery wash on one side of the instrument while a run is in progress on the other side. AVITI OS safely pauses the run in progress to enable set up of a run or recovery wash on the adjacent side. For more information on flexible start, see the user guide for your instrument.

1. On the Home screen, select **New Run**.
2. When prompted to request flexible start and pause the active run, select **New Run**. See the following table for estimated wait times to pause.

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 chemistry. For 2 x 300 runs, the wait time for a pause at the amplification step can exceed 2 hours.

3. Wait for the run to pause.
   - » To cancel flexible start while waiting, select **Cancel Request**.
   - » If the wait time to pause at the amplification step exceeds 5 hours, contact Element Technical Support.
   - » If the wait time to pause at other steps exceeds 1.5 hours, contact Element Technical Support.
4. When the run pauses, proceed through setting up and starting the second run or a recovery wash.

» For run setup instructions, proceed to *Initiate a Sequencing Run* on page 13.

» For recovery wash instructions, proceed to the instructions for a wash run in the user guide for your instrument.

5. To cancel setup of the second run or recovery wash:

   a. Select **Back** to return to the Home screen, and then select **Resume**.

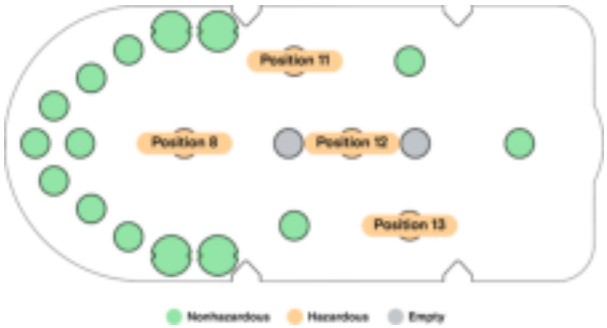
   b. When prompted to confirm that you want to resume the active run, select **Resume**.

—After you start or cancel the second run or recovery wash, AVITI OS resumes the active run.—

Discard the Cartridge and Bottle

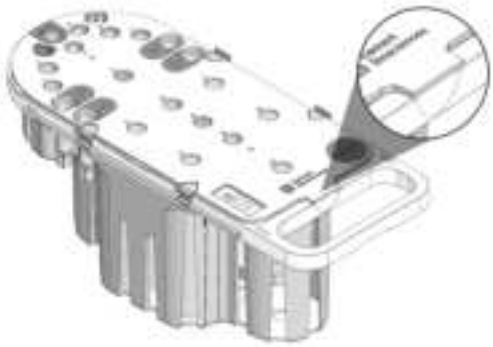
The cartridge and buffer bottle contain reagents with region-specific disposal requirements, which are described in the Safety Data Sheets (SDS) at elementbiosciences.com/resources. The amount of reagent remaining in each well after a run depends on how many cycles the run performed.

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. Grip the lid tab and ***quickly and forcefully*** pull off the lid. Expect resistance.



3. Remove the wells marked hazardous in from the cartridge.
   —The volume remaining in each well depends on the number of cycles performed.—
4. Using the nub on the shipping lock, 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.
8. Enlarge the hole in each foil seal as described in step **4** and 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, Cloudbreak Freestyle, or Cloudbreak UltraQ 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.
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 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.

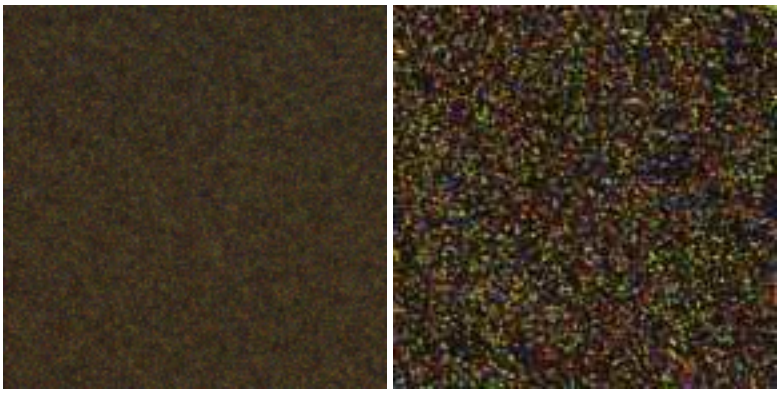
Sequencing Problems

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.	

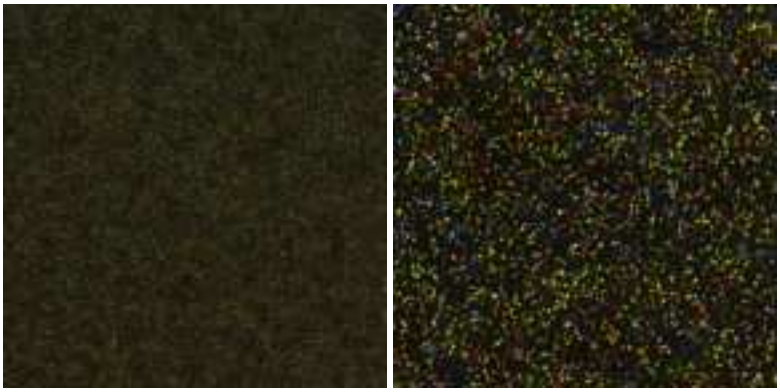
Thumbnail Image Troubleshooting

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.

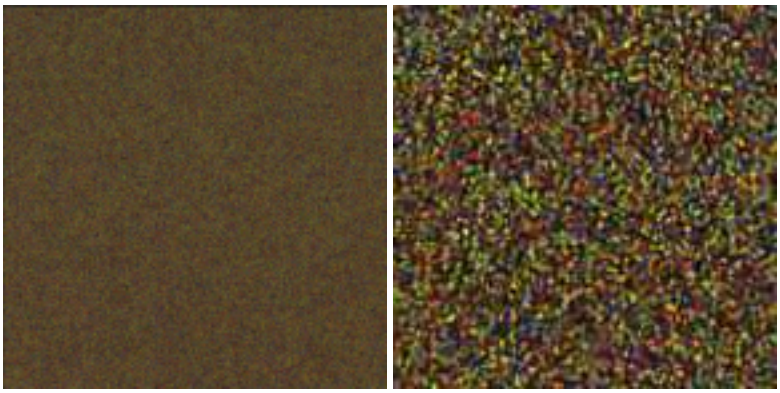
Example expected thumbnail image, full-size and zoomed



Example thumbnail image with underloading, full-size and zoomed



Example thumbnail image with overloading, full-size and zoomed



Cloudbreak Consumables

Cloudbreak 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 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 2x150 Sequencing Kit Cloudbreak UltraQ, # 860-00018

Part #	Component	Shipping	Storage
820-00026	AVITI 2x150 Cartridge Cloudbreak UltraQ	-25°C to -15°C	-25°C to -15°C
810-00008	AVITI Flow Cell Cloudbreak UltraQ	Room temperature	2°C to 8°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x75 Sequencing Kit Cloudbreak Freestyle High Output, # 860-00015

Part #	Component	Shipping	Storage
820-00022	AVITI 2x75 Cartridge Cloudbreak Freestyle High Output	-25°C to -15°C	-25°C to -15°C
810-00003	AVITI Flow Cell Cloudbreak Freestyle	Room temperature	2°C to 8°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x75 Sequencing Kit Cloudbreak Freestyle Medium Output, # 860-00014

Part #	Component	Shipping	Storage
820-00021	AVITI 2x75 Cartridge Cloudbreak Freestyle Medium Output	-25°C to -15°C	-25°C to -15°C
810-00003	AVITI Flow Cell Cloudbreak Freestyle	Room temperature	2°C to 8°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x75 Sequencing Kit Cloudbreak Freestyle Low Output, # 860-00034

Part #	Component	Shipping	Storage
820-00032	AVITI 2x75 Cartridge Cloudbreak Freestyle Low Output	-25°C to -15°C	-25°C to -15°C
810-00003	AVITI Flow Cell Cloudbreak Freestyle	Room temperature	2°C to 8°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x150 Sequencing Kit Cloudbreak Freestyle High Output, # 860-00013

Part #	Component	Shipping	Storage
820-00020	AVITI 2x150 Cartridge Cloudbreak Freestyle High Output	-25°C to -15°C	-25°C to -15°C
810-00003	AVITI Flow Cell Cloudbreak Freestyle	Room temperature	2°C to 8°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x150 Sequencing Kit Cloudbreak Freestyle Medium Output, # 860-00012

Part #	Component	Shipping	Storage
820-00019	AVITI 2x150 Cartridge Cloudbreak Freestyle Medium Output	-25°C to -15°C	-25°C to -15°C
810-00003	AVITI Flow Cell Cloudbreak Freestyle	Room temperature	2°C to 8°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x150 Sequencing Kit Cloudbreak Freestyle Low Output, # 860-00011

Part #	Component	Shipping	Storage
820-00018	AVITI 2x150 Cartridge Cloudbreak Freestyle Low Output	-25°C to -15°C	-25°C to -15°C
810-00003	AVITI Flow Cell Cloudbreak Freestyle	Room temperature	2°C to 8°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x300 Sequencing Kit Cloudbreak Freestyle High Output, # 860-00017

Part #	Component	Shipping	Storage
820-00024	AVITI 2x300 Cartridge Cloudbreak Freestyle High Output	-25°C to -15°C	-25°C to -15°C
810-00003	AVITI Flow Cell Cloudbreak Freestyle	Room temperature	2°C to 8°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x300 Sequencing Kit Cloudbreak Freestyle Medium Output, # 860-00016

Part #	Component	Shipping	Storage
820-00023	AVITI 2x300 Cartridge Cloudbreak Freestyle Medium Output	-25°C to -15°C	-25°C to -15°C
810-00003	AVITI Flow Cell Cloudbreak Freestyle	Room temperature	2°C to 8°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x75 Sequencing Kit Cloudbreak High Output, # 860-00004

Part #	Component	Shipping	Storage
820-00015	AVITI 2x75 Cartridge Cloudbreak High Output	-25°C to -15°C	-25°C to -15°C
810-00002	AVITI Flow Cell Cloudbreak	Room temperature	2°C to 8°C
820-00010	Adept Primer Set Cloudbreak	-25°C to -15°C	-25°C to -15°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x75 Sequencing Kit Cloudbreak Medium Output, # 860-00007

Part #	Component	Shipping	Storage
820-00014	AVITI 2x75 Cartridge Cloudbreak Medium Output	-25°C to -15°C	-25°C to -15°C
810-00002	AVITI Flow Cell Cloudbreak	Room temperature	2°C to 8°C
820-00010	Adept Primer Set Cloudbreak	-25°C to -15°C	-25°C to -15°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x150 Sequencing Kit Cloudbreak High Output, # 860-00003

Part #	Component	Shipping	Storage
820-00013	AVITI 2x150 Cartridge Cloudbreak High Output	-25°C to -15°C	-25°C to -15°C
810-00002	AVITI Flow Cell Cloudbreak	Room temperature	2°C to 8°C
820-00010	Adept Primer Set Cloudbreak	-25°C to -15°C	-25°C to -15°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x150 Sequencing Kit Cloudbreak Medium Output, # 860-00006

Part #	Component	Shipping	Storage
820-00012	AVITI 2x150 Cartridge Cloudbreak Medium Output	-25°C to -15°C	-25°C to -15°C
810-00002	AVITI Flow Cell Cloudbreak	Room temperature	2°C to 8°C
820-00010	Adept Primer Set Cloudbreak	-25°C to -15°C	-25°C to -15°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x150 Sequencing Kit Cloudbreak Low Output, # 860-00005

Part #	Component	Shipping	Storage
820-00011	AVITI 2x150 Cartridge Cloudbreak Low Output	-25°C to -15°C	-25°C to -15°C
810-00002	AVITI Flow Cell Cloudbreak	Room temperature	2°C to 8°C
820-00010	Adept Primer Set Cloudbreak	-25°C to -15°C	-25°C to -15°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x300 Sequencing Kit Cloudbreak High Output, # 860-00008

Part #	Component	Shipping	Storage
820-00016	AVITI 2x300 Cartridge Cloudbreak High Output	-25°C to -15°C	-25°C to -15°C
810-00002	AVITI Flow Cell Cloudbreak	Room temperature	2°C to 8°C
820-00010	Adept Primer Set Cloudbreak	-25°C to -15°C	-25°C to -15°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

AVITI 2x300 Sequencing Kit Cloudbreak Medium Output, # 860-00009

Part #	Component	Shipping	Storage
820-00017	AVITI 2x300 Cartridge Cloudbreak Medium Output	-25°C to -15°C	-25°C to -15°C
810-00002	AVITI Flow Cell Cloudbreak	Room temperature	2°C to 8°C
820-00010	Adept Primer Set Cloudbreak	-25°C to -15°C	-25°C to -15°C
820-00002	AVITI Buffer Bottle	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

Adept Primer Set Cloudbreak

Primers for I1, I2, R1, and R2 in Cloudbreak cartridges support Elevate libraries. Sequencing Adept libraries requires replacing the prepackaged primers with tubes from the Adept Primer Set Cloudbreak. The Adept Primer Set Cloudbreak, catalog # 820-00010, is not included or compatible with Cloudbreak Freestyle or Cloudbreak UltraQ kits.

Primer	Adept Primer Set Cloudbreak
Index 1	Adept Index 1 (I1) Primer Cloudbreak
Index 2	Adept Index 2 (I2) Primer Cloudbreak
Read 1	Adept Read 1 (R1) Primer Cloudbreak
Read 2	Adept Read 2 (R2) Primer Cloudbreak

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*.

Type	Format	Shipping and Storage
PhiX Control Library, Adept, # 830-00004	Circular	-25°C to -15°C
Cloudbreak PhiX Control Library, Elevate, # 830-00017	Linear	-25°C to -15°C
Cloudbreak Freestyle PhiX Control, Third Party, #830-00023	Linear	-25°C to -15°C

Custom Primer Sets

A custom primer set provides read-specific buffers for preparing custom primers for Adept libraries with Cloudbreak chemistry or third-party libraries with Cloudbreak Freestyle chemistry.

Custom Primer Set	Buffers	Shipping and Storage
Adept Custom Primer Set Cloudbreak, # 820-00009	Adept Custom Index 1 Buffer, Index First (I1)	-25°C to -15°C
	Adept Custom Index 2 Buffer, Index First (I2)	-25°C to -15°C
	Adept Custom Read 1 Buffer, Index First (R1)	-25°C to -15°C
	Adept Custom Read 2 Buffer, Index First (R2)	-25°C to -15°C
Custom Primer Set Cloudbreak Freestyle, # 820-00025	Custom Index 1 (I1) Buffer Cloudbreak Freestyle	-25°C to -15°C
	Custom Index 2 (I2) Buffer Cloudbreak Freestyle	-25°C to -15°C
	Custom Read 1 (R1) Buffer Cloudbreak Freestyle	-25°C to -15°C
	Custom Read 2 (R2) Buffer Cloudbreak Freestyle	-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	
Filtered pipette tips	
Low TE buffer	
Nuclease-free laboratory-grade water	

Document History

Revision	Description of Change
December 2024 Document # MA-00058 Rev. B	• Added 2x75 Cloudbreak Freestyle Low Output kit. • Updated name of sequencing basket to cartridge basket.
October 2024 Document # MA-00058 Rev. A	• Initial release of user guide.

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.

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