Element Biosciences

Quick Guide

Element AVITI™ Sequencing

Introduction

This quick guide provides concise sequencing instructions for an Element AVITI instrument and is intended for experienced operators who have completed several runs. Before sequencing, review the user guide for your instrument for safety information and comprehensive instructions.

Add Primer Tubes

1. For the following libraries, proceed to *Thaw the Cartridge*.
   - » Elevate™ libraries
   - » Third-party libraries with a Cloudbreak Freestyle™ (CB Freestyle) kit and no custom primers
2. Remove the primer tubes from the cartridge.
3. Insert primer tubes from a set. Twist each tube right.

Thaw the Cartridge

1. Thaw the cartridge using either of the following methods. Protect from light.

Method	Cartridge	Approximate Thaw Time
Water bath	2 x 75	90 minutes
	2 x 150 or 2 x 300	2.5 hours
Refrigerator	2 x 75	8 hours
	2 x 150 or 2 x 300	24 hours

2. Make sure reagents are fully thawed.
   - » If ice remains after a water bath, return to the bath until fully thawed.
   - » If ice remains after a refrigerator thaw, thaw in a room-temperature water bath:
     - For a 2 x 75 cartridge, thaw for ≤ 1 hour.
     - For a 2 x 150 or 2 x 300 cartridge, thaw for ≤ 20 minutes.
3. Set aside at room temperature.

Denature the Library

1. Calculate the loading concentration of each library:

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

2. Calculate the experimental library volume:

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

3. If adding a spike-in, calculate the control library volume:

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

4. Combine the volumes calculated in steps 2 and 3 in a DNA LoBind tube.
5. Add an equal volume of 0.2 N NaOH.
6. Incubate at room temperature for 5 minutes.
7. Add 0.2 M Tris-HCl buffer, pH 7.0. Use the same volume as 0.2 N NaOH in step 5.
8. Add Library Loading Buffer to reach 1.4 ml:

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

9. Place on ice.

Prepare Custom Primers

1. Prepare custom primers 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

Initiate a Sequencing Run

1. On the Home screen, select **New Run**.
2. Select the side to sequence on, and then select **Sequence**.
3. Select the type of run, and then select **Next**.
   - » For a **Planned Run**, select the run and storage. Select **Next**, and then proceed to *Inspect and Mix Reagents*.
   - » For a **Manual Run**, proceed to *Define Run Parameters*.

Define Run Parameters

1. In the Run Name field, enter a unique name.
2. If applicable, select **Browse** and import the run manifest.
3. Complete Description and Storage as applicable.
4. Select a Library Type, Library Structure, and Sequencing Kit.
5. If applicable, select options for low-diversity high-multiplex libraries and library pools.
6. Enter the number of cycles, and then select **Next**.

Inspect and Mix Reagents

1. Make sure primer tubes are correct and secure.
2. Gently invert the cartridge *10 times*.
3. Tap the base on the benchtop.
4. Place into a sequencing basket and lock the clips.

Add Custom Primers

1. With a 1 ml pipette tip, pierce the applicable I1, I2, R1, and R2 wells.
2. Add 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

3. Pipette 15 times.

Add the Library

1. Using a 1 ml pipette tip, pierce the Library well.
2. Briefly centrifuge the library.
3. Transfer the entire volume to the Library well.
4. For the Individually Addressable Lanes add-on, repeat 1–3 with the AUX well and second library.
5. Remove both shipping locks from the cartridge lid.

Confirm Reagent Preparation

1. For Adept™, select the **Swap primer tubes** checkbox.
2. Select the **Invert cartridge** checkbox.
3. Select any load library checkboxes.
4. Select the **Insert into basket** checkbox, and then select **Next**.

Load Reagents and Buffer

1. Open the reagent bay door and remove any materials.
2. Slide the basket into the reagent bay.
3. Slide the buffer bottle into the reagent bay until it stops.
4. Close the reagent bay door, and then select **Next**.

Empty Waste and Prime Reagents

1. Open the waste bay door.
2. Remove the waste bottle and close the transport cap.
3. Open the transport and vent caps and empty the waste.
4. Close the vent cap and reload the waste bottle.
5. Select **Next** to start priming.
6. During priming, bring a flow cell to room temperature. Keep packaged.

Load the Flow Cell

1. Remove the used flow cell from the nest.
2. Unpackage the new flow cell and load it onto the nest.
3. Select **Close Nest**, and then select **Next**.

Review and Start the Run

1. Review the run, and then select **Run**.
2. Monitor run metrics as they appear onscreen.

Dispose of Reagents

1. Remove the cartridge lid.
2. Remove the wells marked hazardous.
3. Enlarge the hole in each foil seal to form a triangle.
4. Empty each well into hazardous waste or other appropriate container.
5. Remove the cartridge from the basket.
6. Remove the remaining wells and form triangular holes.
7. Empty each well into the appropriate container.
8. Discard the cartridge and buffer bottle.
9. Rinse the basket in nuclease-free water. Dry upside down.