



Trinity™ Sequencing

User Guide

FOR USE WITH

AVITI™ System, catalog # 880-00001

AVITI24™ System, catalog # 880-00004

AVITI Operating Software v3.3.0 or later

Trinity Sequencing Kits

ELEMENT BIOSCIENCES

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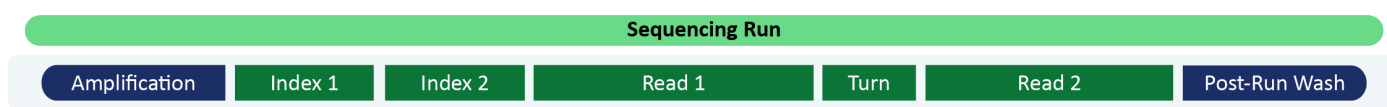
Overview

This guide provides instructions for performing a Trinity sequencing run using libraries prepared from a Trinity hybridization reaction.

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**—Hybridizes the library to the flow cell and performs amplification to form colonies, 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 or two 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 and how to distribute the total cycles depends on your experiment. For bioinformatics purposes, adding one extra cycle to each read is recommended. For example, a 2 x 150 cycle run ideally includes 2 x 151 cycles. 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.

Trinity Sequencing Kits

The Trinity workflow requires a Trinity sequencing kit to sequence the hybridized reaction on an AVITI™ or an AVITI24™ System. Each kit includes a flow cell, a buffer bottle, a reagent cartridge, the library loading buffer, and the Trinity sequencing reagent.

Trinity Sequencing User Guide

NOTE

The fast hybridization workflow requires the Trinity Fast Hyb Loading Buffer, catalog # 830-00030, in place of the library loading buffer.

Trinity kits are available in two sizes: a 2 x 75 kit and a 2 x 150 kit. See [Trinity Sequencing Kits on page 15](#).

Trinity PhiX Control

A Trinity PhiX Control, catalog # 830-00031, is available as an optional spike-in for the sequencing run. The addition of the PhiX control is a valuable tool for quality control in the sequencing process, as it provides an error rate estimation for the run. Without the PhiX control, no error rate data are available for the sequencing run. See [Add Sequencing Solution to the Cartridge on page 10](#).

Additional Documentation

For end-to-end workflow instructions including library preparation, see the following protocol guides:

- [xGen Exome Sequencing Kit Trinity for Element AVITI System \(MA-00056\)](#)
- [Twist for Element Exome 2.0 + Library Preparation and Standard Hybridization With Trinity Sequencing Workflow \(MA-00054\)](#)
- [Twist for Element Exome 2.0 + Library Preparation and Fast Hybridization With Trinity Sequencing Workflow \(MA-00055\)](#)

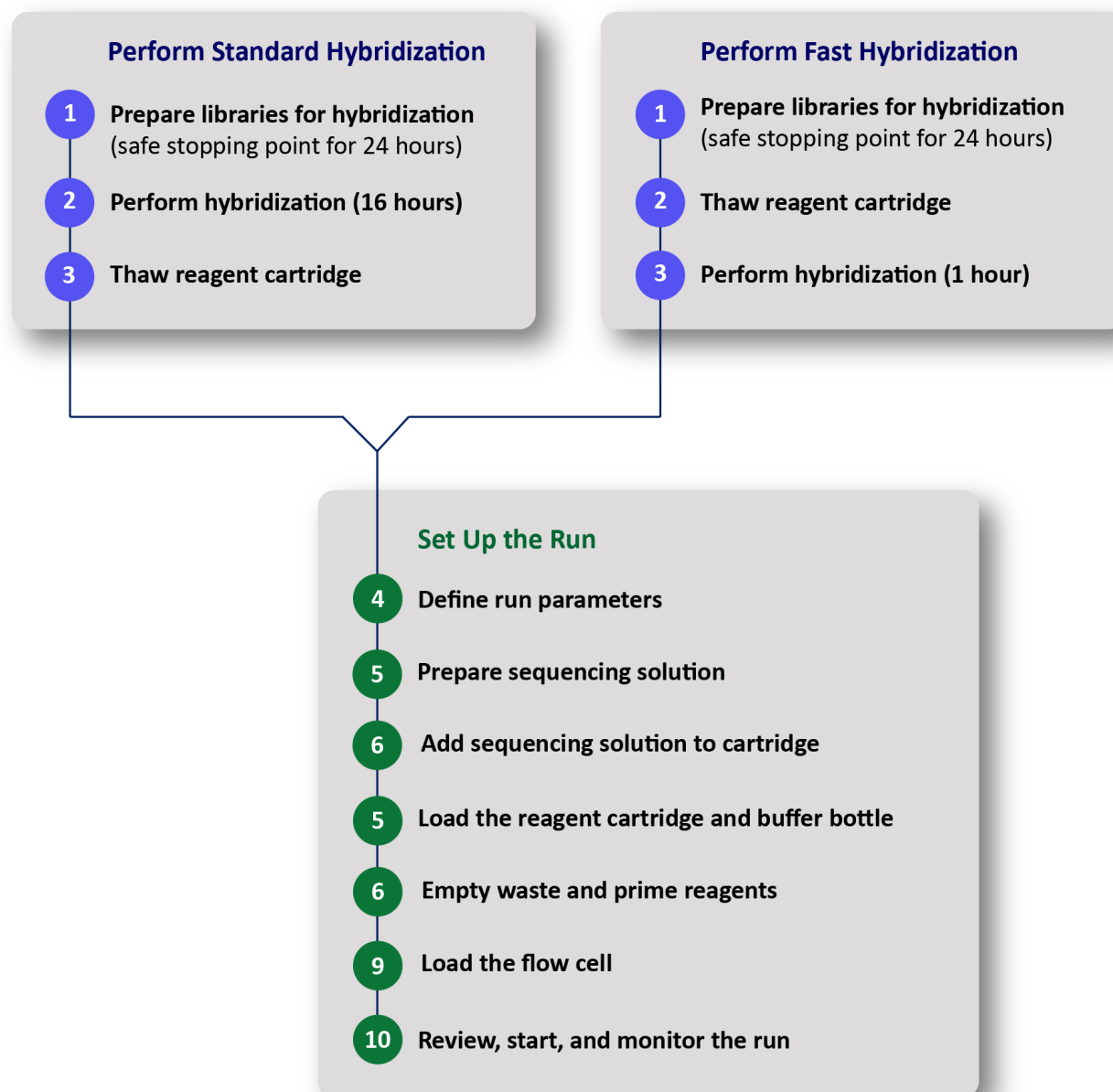
For experienced user documentation for the hybridization and run setup steps, see the following quick guides:

- [xGen Exome Hybridization & Trinity Run Setup Quick Guide \(MA-00061\)](#)
- [Twist Exome Hybridization & Trinity Run Setup Quick Guide \(MA-00064\)](#)
- [Twist Exome Fast Hybridization & Trinity Run Setup Quick Guide \(MA-00065\)](#)

Trinity Workflow Summary

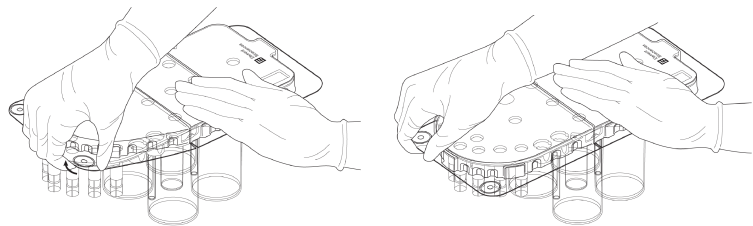
Preparing for a Trinity sequencing run includes steps to prepare a standard hybridization reaction or fast hybridization reaction. For hybridization protocols, see [Additional Documentation on page 5](#).

Following the hybridization step, run setup includes steps to prepare the sequencing solution and add it to the reagent cartridge before loading consumables onto the instrument.



Thaw Reagents

- 1. 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 Trinity 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	2.5 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. If you do not immediately initiate the run, place the thawed cartridge at 2°C to 8°C. You can store overnight for use the next day.
- 6. Proceed to [Run Setup on page 8](#).

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 **Trinity**, 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 9](#).

Select a Planned Run

1. Select **Planned Run**.

AVITI OS displays a list of compatible planned runs for the instrument and run type. For 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. See [Run Planning](#) in the [Online Help](#).
4. In the Storage drop-down menu, select the storage connection 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 10](#).

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. In the Sequencing Kit drop-down menu, select the Trinity sequencing kit you are using.
 7. In the Panel drop-down menu, select one of the following panels:
 - » **Twist for Element, Trinity Exome Workflow**—For use with either the standard or fast hybridization protocols.
 - » **xGen Exome Kit for Trinity**—For use with the xGen Exome hybridization protocol.
 - » **Other**—For use with other panels not listed.
- NOTE**
When you select a panel, the standard bed file is copied to the run output folder for downstream analysis.
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 4](#).
 - » Add one cycle to the number of Read 1 and Read 2 cycles. For example, enter **151** in the Read 1 field to perform 150 cycles.

Kit Size	Index 1	Index 2	Read 1	Read 2
2 x 75	12	9	76	76
2 x 150	12	9	151	151

9. If you are using the Advanced Run Settings add-on, select **Advanced Settings** and proceed to [Configure Advanced Run Settings](#).
10. Select **Next** to proceed to Run Side B and repeat steps 2–10 or proceed to the Prepare Reagents screen.

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. 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.

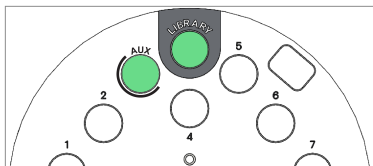
CAUTION

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 Sequencing Solution 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. [Optional] Add 8 µl Trinity PhiX Control to the sequencing solution. Pipette gently to mix. Estimate a 0.5–5% representation in the Trinity sequencing run.
4. Transfer 2200 µl sequencing solution to the Library well, dispensing along the well wall.
 - » Avoid aspirating any foam or dispensing air.
 - » Do not allow the sequencing solution to contact the foil.
5. Inspect the Library well through the window at the front of the basket.
 - » Make sure the sequencing solution 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.

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 the **Load hybrid reaction** checkbox to confirm that the cartridge contains the hybridized reaction and sequencing solution.
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 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.
9. Bring a new Trinity 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, you can discard run setup and save the cartridge. Priming pierces reagent seals and prevents further use.

10. Select **Next** to **automatically** start priming.
Priming takes ~ 5 minutes or up to 8 minutes at high elevations.
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

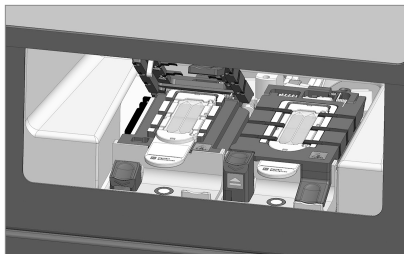
1. Make sure the nest status light is blue.

2. Press the button to the left of the nest to open the lid. 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 new Trinity flow cell. Handle the flow cell by the gripper only.

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. Select **Close Nest** to close the nest bay door and retract the stage.
8. 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
Application	The type of sequencing application for the run
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
Panel	The panel for a Trinity workflow
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:
 - » For a used cartridge and buffer bottle, follow the instructions in [Discard the Cartridge and Bottle on page 13](#).
 - » 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 OS indicates the expected cycle that 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.
 - » AVITI 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

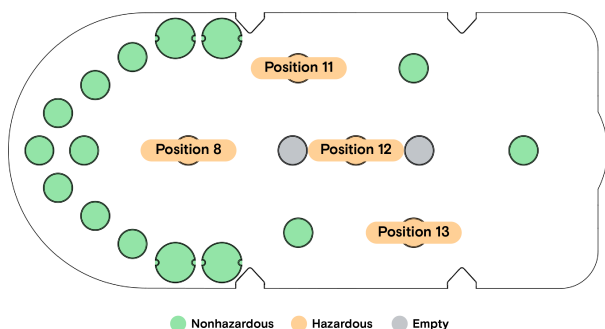
Flexible start provides the option to start a run or recovery wash while another run is in progress. AVITI OS safely pauses the run on the adjacent side.

1. On the Home screen, select **New Run**.
2. When prompted to request flexible start and pause the active run, select **New Run**.
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 8](#).
 - » 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

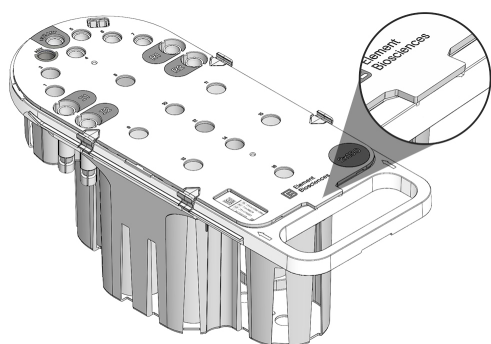
The cartridge and buffer bottle contain reagents with region-specific disposal requirements, which are described in the Safety Data Sheets (SDS) at elementbiosciences.com/resources. The amount of reagent remaining in each well after a run depends on how many cycles the run performed.

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



3. Remove the wells indicated as hazardous 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.

Trinity Sequencing Kits

The following tables list the kit contents and storage requirements for Trinity sequencing kits. Each kit is single-use and packaged in two boxes.

When you receive your kit, promptly store the components at the proper temperatures. For Safety Data Sheet (SDS) information, see elementbiosciences.com/resources.

Trinity 2x75 Sequencing Kit, # 860-00019

Part #	Component	Shipping	Storage
820-00030	Trinity 2x75 Cartridge	-25°C to -15°C	-25°C to -15°C
810-00015	Trinity Flow Cell	Room temperature	2°C to 8°C
830-00028	Trinity Sequencing Reagent	-25°C to -15°C	-25°C to -15°C
820-00002	AVITI Buffer Bottle (Universal Wash Buffer)	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

Trinity 2x150 Sequencing Kit, # 860-00020

Part #	Component	Shipping	Storage
820-00031	Trinity 2x150 Cartridge	-25°C to -15°C	-25°C to -15°C
810-00015	Trinity Flow Cell	Room temperature	2°C to 8°C
830-00028	Trinity Sequencing Reagent	-25°C to -15°C	-25°C to -15°C
820-00002	AVITI Buffer Bottle (Universal Wash Buffer)	Room temperature	Room temperature
820-00004	Library Loading Buffer	-25°C to -15°C	-25°C to -15°C

Trinity Binding Reagent, # 830-00029

Component	Storage
Trinity Binding Reagent	-25°C to -15°C

Trinity Fast Hyb Binding Reagent, # 830-00034

Component	Storage
Trinity Fast Hyb Binding Reagent	-25°C to -15°C

Trinity Fast Hyb Loading Buffer, # 830-00030

Component	Storage
Trinity Fast Hyb Loading Buffer	Room temperature

Trinity PhiX Control, # 830-00031

Component	Storage
Trinity PhiX Control	-25°C to -15°C

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Document History

Revision	Description of Change
April 2025 Document # MA-00059 Rev. C	• Updated expected priming time when using AVITI OS v3.3. • Updated storage time for thawed cartridges. • Added Universal Wash Buffer to AVITI Buffer Bottle in kit component list. • Removed the term pollination in the Run Stages description.
January 2025 Document # MA-00059 Rev. B	• Corrected step in Dispose of Reagents to use a pipette tip or similar tool to enlarge hole in foil seal. • Added a link to the hybridization protocols on the workflow summary page.
December 2024 Document # MA-00059 Rev. A	• Initial release.

Technical Support

Visit the [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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