Element Biosciences | TWIST BIOSCIENCE

Quick Guide for the Trinity Workflow

Twist Standard Hybridization & Trinity Run Setup

Introduction

Intended for the experienced user, this quick guide describes the Twist Exome standardhybridization protocol and Trinity sequencing run setup on an AVITI™ System.

This protocol supports up to a 24-plex hybridization reaction for one Trinity sequencing run.

Prepare Libraries for Hybridization

Prerequisite: Completion of the Twist for Element Library Preparation.

1. Use the concentration of each amplified, indexed library to calculate the volume (µl) of each library.
2. Divide the amount of each indexed library pool by the concentrations measured in ng/µl. Input per hybridization reaction is 4 µg.

Samples per Pool	Library per Pool
24	166.67 ng

3. Transfer the calculated volumes to a 0.2 ml PCR tube strip or 96-well PCR plate.
4. Pulse centrifuge the indexed library pool.
5. Dry the indexed pool using a SpeedVac at low or no heat (less than 40°C).

SAFE STOPPING POINT
If not proceeding to hybridization, store the dried indexed library pool at -25°C to -15°C for up to 24 hours.

Perform Hybridization

1. Gather the following components for hybridization:
   - » Indexed library pool
   - » Twist Exome 2.0 + Comp Spike, Trinity Compatible
   - » Hybridization Mix
   - » Hybridization Enhancer
   - » Blocker Solution
   - » Trinity Binding Reagent
2. Thaw reagents on ice, pulse vortex for 2 seconds, and then pulse centrifuge.
3. Prepare the probe solution in a 0.2 ml PCR tube strip or 96-well PCR plate. Flick the tubes to mix.

Component	Volume
Hybridization Mix	20 µl
Twist Exome 2.0 + Comp Spike, Trinity Compatible	2 µl
Water	6 µl
Total	**28 µl**

4. Resuspend the dried indexed library. Flick the tubes to mix.

Component	Volume
Dried indexed library	—
Blocker Solution	5 µl
Trinity Binding Reagent	5 µl
Water	2 µl
Total	**12 µl**

5. Heat the probe solution tube to 95°C for 2 minutes with a lid temperature of 105°C.
6. *Immediately* cool the probe solution on ice for 5 minutes.
7. Heat the library/binding reagent solution at 95°C with a lid temperature of 105°C.
8. Equilibrate the probe solution and library/binding solution to room temperature for 5 minutes.
9. Vortex and briefly centrifuge the probe solution.
10. Transfer the entire volume to the library/binding solution.
11. Vortex and briefly centrifuge the combined solution.
12. Add 30 µl Hybridization Enhancer to the top of the reaction and pulse centrifuge the tube.

13. Run the following thermal cycler program to incubate.

Temperature	Time
Lid set to 85°C	
72°C	16 hours
72°C	Hold

Thaw Reagents

1. Thaw the Trinity sequencing cartridge. Protect from light.

Cartridge	Water Bath	Refrigerator
2 x 75	90 minutes	8 hours
2 x 150	2.5 hours	24 hours

2. Make sure reagents are *fully* thawed.
3. Set aside at room temperature or keep at 2°C to 8°C.

Initiate a Sequencing Run

1. On the Home screen, select **New Run**.
2. Select **Sequencing**.
3. Select the side for sequencing:**Side A**, **Both**, or **Side B**.
4. For chemistry type, select **Trinity**, and then select **Next**.
5. For a **Manual Run**, proceed to *Define Run Parameters*.
   For a **Planned Run**, select the run and storage connection, and then select **Next**. Proceed to *Inspect and Mix Reagents*.

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 the Description and Storage fields as applicable.
4. Select a Trinity Sequencing Kit.
5. Select the panel **Twist for Element, Trinity Exome Workflow**.
6. Enter the number of cycles, and then select **Next**.

Inspect and Mix Reagents

1. Gently invert the cartridge ***10 times***.
2. Tap the base on the benchtop.
3. Place into a cartridge basket and lock the clips.

Prepare Sequencing Solution

1. Gather the following components:
   - » Trinity Sequencing Reagent
   - » Library Loading Buffer
2. Remove the hybridization reaction from the thermal cycler and briefly centrifuge.
3. *Immediately* add 130 µl Library Loading Buffer to dilute the 70 µl hybridization reaction. Pipette gently to mix.
4. Prepare the sequencing solution in a 5 ml tube. Pipette gently to mix.

Component	Volume
Library Loading Buffer	2053 µl
Trinity Sequencing Reagent	72 µl
Diluted hybridization reaction	75 µl
Total	**2200 µl**

Add Sequencing Solution to Cartridge

1. Using a 1 ml pipette tip, pierce the Library well.
2. Transfer 2200 µl sequencing solution to the Library well.

Confirm Reagent Preparation

1. Select the **Invert cartridge** checkbox.
2. Select the **Insert into basket** checkbox.
3. Select the **Load hybed reaction** checkbox. 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, remove the waste bottle, and close the transport cap.
2. Open the transport and vent caps and empty the waste.
3. Close the vent cap and reload the waste bottle.
4. Select **Next** to ***automatically*** start priming.
5. Bring a new Trinity flow cell to room temperature in the package.
6. When priming is complete, select **Next**.

Load the Flow Cell

1. Remove the used flow cell from the nest.
2. Unpackage the Trinity 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.
