Element Biosciences | TWIST BIOSCIENCE

Quick Guide for the Trinity Workflow

Twist Fast Hybridization & Trinity Run Setup

Introduction

Intended for the experienced user, this quick guide describes the Twist Exome fast hybridization 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 Fast Hybridization

Prerequisite: Completion of the Twist for Element Library Preparation.

1. Gather the following components for fast hybridization:
   - » Amplified, indexed linear library
   - » Twist Exome 2.0 + Comp Spike, Trinity Compatible
   - » Twist Fast Hybridization reagents
   - » Blocker Solution
   - » Trinity Fast Hyb Binding Reagent
2. Thaw the following reagents on ice, pulse vortex for 2 seconds, and then pulse centrifuge.
   - » Blocker Solution
   - » Trinity Fast Hyb Binding Reagent
   - » Twist Exome 2.0 + Comp Spike, Trinity Compatible
3. Use the concentration of each amplified, indexed library to calculate the volume (µl) of each library.
4. 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

5. Transfer the calculated volumes to a 0.2 ml PCR tube strip or 96-well PCR plate.
6. Add the following reagents to each indexed library pool. Flick the tubes to mix.

Component	Volume
Blocker Solution	5 µl
Trinity Fast Hyb Binding Reagent	5 µl
Twist Exome 2.0 + Comp Spike, Trinity Compatible	2 µl
Total	**12 µl**

7. Pulse centrifuge the indexed library pool.
8. 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.

Thaw Reagents

1. Collect the following components:
   - » Trinity Sequencing Cartridge
   - » Fast Hybridization Mix
   - » Hybridization Enhancer
   - » Trinity Sequencing Reagent
   - » Trinity Fast Hyb Loading Buffer (catalog # 830-00030)
2. 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

3. Make sure reagents are *fully* thawed.
4. Set aside at room temperature or keep at 2°C to 8°C.
5. If preparing to immediately initiate the run, set aside the thawed cartridge at room temperature.
6. Thaw the following Twist reagents on ice:
   - » Fast Hybridization Mix
   - » Hybridization Enhancer
7. About 5–10 minutes before completion of the 1-hour hybridization, thaw the Trinity Sequencing Reagent on ice.

Perform Fast Hybridization

1. Program a thermal cycler with the following settings.

Step	Temperature	Time
	Lid set to 85°C	
1	95°C	Hold
2	95°C	5 minutes
3	71°C	1 hour
4	71°C	Hold

2. Heat the Fast Hybridization Mix to 65°C for 10 minutes or until all precipitate is dissolved.
3. Vortex to mix and proceed immediately.
4. Resuspend the dried indexed library pool and pre-hybridization solution in 20 µl Fast Hybridization Mix.
5. If transferring to another tube or plate for hybridization, do the following:
   a. Vortex for 5 seconds, briefly centrifuge, and incubate at 65°C for 5 minutes.
   b. Pulse centrifuge, and then transfer the solution to a PCR tube or 96-well plate.
6. Add 30 µl Hybridization Enhancer to the top of the reaction.
7. Cap the tube or seal the plate and pulse centrifuge to ensure all solution is at the bottom of the tube or wells.
8. Place the tube or plate in the programmed thermal cycler starting with step 2 of the program.
9. About 5–10 minutes before completion of the 1-hour hybridization, prepare the sequencing solution. See *Prepare Sequencing Solution*.

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. In the Library Type field, select **Elevate**.
5. Select a Trinity Sequencing Kit.
6. Select the panel **Twist for Element, Trinity Exome Workflow**.
7. 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
   - Trinity Fast Hyb Loading Buffer (catalog # 830-00030)
2. Remove the hybridization reaction from the thermal cycler and briefly centrifuge.
3. *Immediately* add 150 µl Trinity Fast Hyb Loading Buffer to dilute the hybridization reaction. Pipette gently to mix.

   Note: Store any unused diluted hybridization reaction at -25°C to -15°C for up to one week.
4. Prepare the sequencing solution on ice in a 5 ml tube. Pipette gently to mix.

Component	Volume
Trinity Fast Hyb Loading Buffer	2073 µl
Trinity Sequencing Reagent	72 µl
Diluted hybridization reaction	55 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. Bring a new Trinity flow cell to room temperature in the pouch.
5. Select **Next** to ***automatically*** start priming.
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.

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