Element Biosciences

IDT INTEGRATED DNA TECHNOLOGIES

Quick Guide for the Trinity Workflow

xGen™ Exome Hybridization & Trinity Run Setup

Introduction

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

The protocol supports up to a 24-plex pooled hybridization reaction in one capture reaction.

Prepare Libraries for Hybridization

1. Gather the following components for hybridization:
   - » Elevate linear library, prepared using the xGen™ DNA Library Prep EZ UNI Kit
   - » xGen™ Hyb Capture Kit Trinity™ for Element
     - ◦ xGen™ Human Cot DNA
     - ◦ xGen™ 2X Hybridization Buffer
     - ◦ xGen™ Hybridization Buffer Enhancer
     - ◦ xGen™ Exome Hyb Panel
   - » Trinity Binding Reagent
2. Thaw reagents on ice and mix thoroughly.
3. Add the total library volume and the following reagents to a 1.5 ml LoBind tube or a 96-well plate. Vortex to mix and briefly centrifuge.

Component	Volume
Up to 24 indexed samples	5–6 µg total
xGen™ Human Cot DNA	5 µl
Trinity Binding Reagent	5 µl

4. Dry the indexed pool using a SpeedVac at low or no heat (less than 40°C).
5. If not proceeding to hybridization, store the dried-down library pool at -25°C to -15°C up to 1 week.

Perform Hybridization

1. Inspect the xGen™ 2X Hybridization Buffer for salt crystals. If present, heat the tube at 65°C and shake intermittently until the buffer is solubilized.
2. Prepare the hybridization master mix in a 1.5 ml tube. Multiply volumes by the number of samples and add 10% overage.

Component	Volume
xGen™ 2X Hybridization Buffer	8.5 µl
xGen™ Hybridization Buffer Enhancer	2.7 µl
xGen™ Exome Hyb Panel	4 µl
Water	1.8 µl
Total	**17 µl**

3. Vortex or pipette to mix the hybridization master mix solution and then briefly centrifuge.
4. Transfer 17 µl hybridization master mix to each tube or well containing dried libraries. Pipette to mix.
5. Incubate at room temperature for 5–10 minutes.
6. If using tubes up to this step, transfer the full volume to a 96-well PCR plate.
7. Cap the tube or seal the plate tightly to avoid evaporation. Vortex and then briefly centrifuge.
8. Run the following thermal cycler program to incubate.

Temperature	Time
Lid set to 100°C	
95°C	30 seconds
65°C	16 hours
65°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 **xGen Exome Kit for Trinity**.
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 183 µl Library Loading Buffer to dilute each xGen™ 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	2038 µl
Trinity Sequencing Reagent	72 µl
Diluted hybridization reaction	90 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.

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