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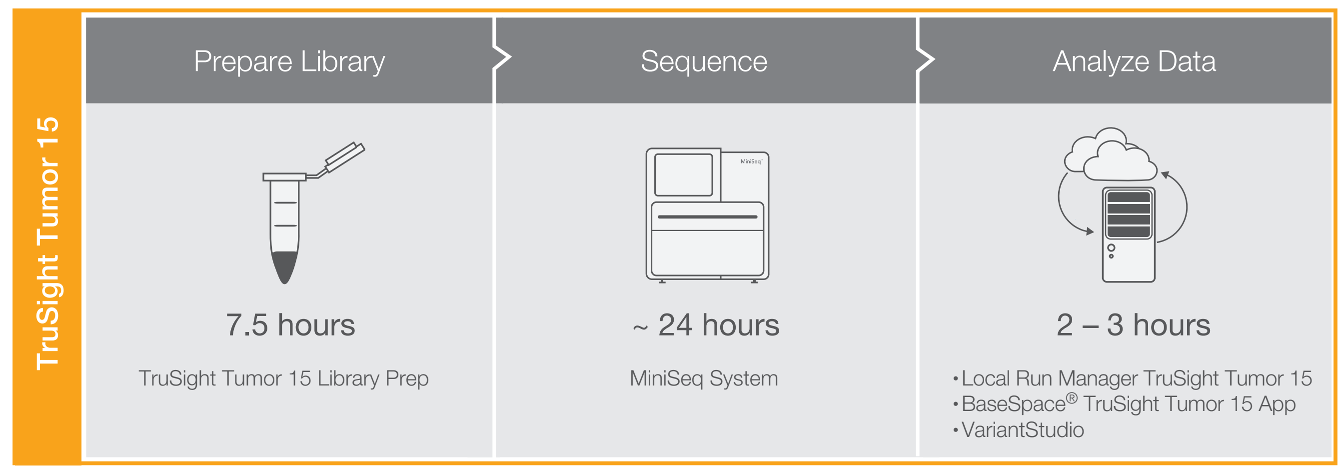
# TruSight® Tumor 15 Workflow on the MiniSeq™ System



This checklist supports the following workflow choices.

| | |
|---|---|
| **Sequencing Instrument:** | MiniSeq System |
| **Setup Option** | Local Run Manager |
| **Library Preparation Kit:** | TruSight Tumor 15 |
| **Indexing:** | Dual Indexing |
| **Reagent Kits:** | MiniSeq Kit |
| **Analysis Workflow:** | TruSight Tumor 15 |
| **Analysis Software:** | Local Run Manager |

MiniSeq sequencing using TruSight Tumor 15 libraries.

## Set Run Parameters

- ☐1 Log in to Local Run Manager.
- ☐2 Click **Create Run**, and select **TruSight Tumor 15**.
- ☐3 Enter a run name that identifies the run.
- ☐4 [Optional] Enter a run description.
- ☐5 Enter a unique sample ID.
- ☐6 [Optional] Enter a sample description.
- ☐7 Enter index adapters for Mix A.
- ☐8 Enter index adapters for Mix B.
- ☐9 Click **Save Run**.

## Amplify and Tag Targets

- ☐1 Quantify the sample DNA.
- ☐2 Dilute each sample DNA to 2 ng/µl in a final volume of 12.5 µl.
- ☐3 Combine the following reagents in separate microcentrifuge tubes to create PCR master mixes for TPA and TPB.

| PCR Component | Per Well | Per 24 Samples |
|---|---|---|
| TTM | 5.875 µl | 141 µl |
| TPA or TPB | 6.25 µl | 150 µl |
| TTE | 0.375 µl | 9 µl |

- ☐4 Pipette to mix.
- ☐5 Add 10 µl of each PCR master mix.
  - Master Mix A—Rows A and C
  - Master Mix B—Rows B and D
- ☐6 Add 5 µl of 2 ng/µl DNA.
  - Samples 1–12—Rows A and B
  - Samples 13–24—Rows C and D
- ☐7 Pipette to mix.
- ☐8 Centrifuge at 1000 × g for 1 minute.
- ☐9 Immediately place on a thermal cycler and run the TST15 PCR1 program.

**SAFE STOPPING POINT**

If you are stopping, seal the plate and store at 2°C to 8°C for up to 3 days. Alternatively, leave on the thermal cycler overnight.

## Index Targets

- ☐1 Arrange Index 1 (i7) adapters in the top row.
- ☐2 Arrange Index 2 (i5) adapters in rows A–B.
- ☐3 Place the plate on the TruSeq Index Plate Fixture.
- ☐4 For samples 1–12, add 4 µl of each Index 2 (i5) adapter across rows A and B.
- ☐5 For samples 1–12, add 4 µl of each Index 1 (i7) adapter (R701–R709, R711–R712, R749) to each column of rows A and B.
- ☐6 For samples 13–24, add 4 µl of each Index 2 (i5) adapter across rows C and D.
- ☐7 For samples 13–24, add 4 µl of each Index 1 (i7) adapter (R725–R736) to each column of rows C and D.
- ☐8 Add 27 µl TAM.
- ☐9 Pipette to mix.
- ☐10 Centrifuge at 1000 × g for 1 minute.
- ☐11 Immediately place on a thermal cycler and run the TST15 PCR2 program.

**SAFE STOPPING POINT**

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

## Clean Up Libraries

- ☐1 Centrifuge at 1000 × g for 1 minute.
- ☐2 Add 40 µl SPB of a new midi plate.
- ☐3 Transfer 45 µl supernatant from the PCR plate to the midi plate.
- ☐4 Shake at 1800 rpm for 5 minutes.
- ☐5 Incubate at room temperature for 5 minutes.
- ☐6 Place on a magnetic stand until beads bind to the magnet.
- ☐7 Remove and discard all supernatant.
- ☐8 Wash 2 times with 200 µl 80% EtOH.
- ☐9 Using a 20 µl pipette, remove residual 80% EtOH.
- ☐10 Air-dry on the magnetic stand for 5 minutes.
- ☐11 Add 32 µl RSB.
- ☐12 Shake at 1800 rpm for 2 minutes.
- ☐13 Incubate at room temperature for 2 minutes.
- ☐14 Place on a magnetic stand until liquid is clear.
- ☐15 Transfer 30 µl supernatant to the PLP plate.
- ☐16 Centrifuge at 1000 × g for 1 minute.

**SAFE STOPPING POINT**

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

## Check Libraries

- ☐1 Quantify the library.
- ☐2 Calculate the volume of RSB required to adjust the library concentration to 5 ng/µl.
- ☐3 Add the required volume of RSB to the NLP plate.
- ☐4 Transfer 8 µl of each library to the NLP plate.
- ☐5 Run an aliquot of each normalized library on either of the following methods:
  - 15 µl on a 2% agarose gel
  - 1 µl on a Bioanalyzer using a DNA 1000 chip

**SAFE STOPPING POINT**

If you are stopping, seal the plate and store at -25°C to -15°C for up to 14 days.

## Pool Libraries

- ☐1 Centrifuge the NLP plate at 1000 × g for 1 minute.
- ☐2 Transfer 4 µl of each library to the PNL tube.
- ☐3 Vortex to mix, and then centrifuge briefly.
- ☐4 Add 41 µl RSB to the DNL tube.
- ☐5 Transfer 9 µl from the PNL tube to the DNL tube.

## Prepare Consumables

- ☐1 Remove the reagent cartridge from -25°C to -15°C storage.
- ☐2 Thaw reagents in a room temperature water bath for 90 minutes.
- ☐3 Invert the cartridge 5 times to mix reagents.
- ☐4 Gently tap on the bench to reduce air bubbles.
- ☐5 Remove a new flow cell package from 2°C to 8°C storage.
- ☐6 Set the unopened flow cell package aside at room temperature for 30 minutes.
- ☐7 Remove the flow cell from the foil package and flow cell container.
- ☐8 Clean the glass surface of the flow cell with a lint-free alcohol wipe.
- ☐9 Dry with a lint-free lens cleaning tissue.

## Denature, Dilute, and Load Libraries

- ☐1 Dilute 100 µl 1 N NaOH to 1 ml 0.1 N NaOH.
- ☐2 Invert the tube several times to mix.
- ☐3 Thaw the Hybridization Buffer at room temperature.
- ☐4 Vortex briefly before use.
- ☐5 Thaw the RSB at room temperature.
- ☐6 Transfer 25 µl of the 4 nM library pool to a new microcentrifuge tube.
- ☐7 Add 75 µl RSB to dilute to 1 nM.
- ☐8 Vortex briefly and then centrifuge at 280 × g for 1 minute.
- ☐9 Combine 5 µl library with 5 µl 0.1 N NaOH.
- ☐10 Vortex briefly and then centrifuge at 280 × g for 1 minute.
- ☐11 Incubate at room temperature for 5 minutes.
- ☐12 Add 5 µl 200 mM Tris-HCl, pH 7.0.
- ☐13 Vortex briefly and then centrifuge at 280 × g for 1 minute.
- ☐14 Add 985 µl of prechilled Hybridization Buffer.
- ☐15 Vortex briefly and then centrifuge at 280 × g for 1 minute.
- ☐16 Transfer 180 µl library to a new microcentrifuge tube.
- ☐17 Add 320 µl prechilled Hybridization Buffer.
- ☐18 Vortex briefly and then centrifuge at 280 × g for 1 minute.
- ☐19 [Optional] Denature and dilute a PhiX control to 1.8 pM and a 1% spike-in to the final library.
- ☐20 Clean the foil seal covering reservoir #**16** using a low-lint tissue.
- ☐21 Pierce the seal with a clean 1 ml pipette tip.
- ☐22 Add 500 µl prepared libraries into reservoir #**16**.

## Perform a Sequencing Run

- ☐1 From the Home screen, select **Sequence**.
- ☐2 Enter your user name and password.
- ☐3 Select **Next**.
- ☐4 Select a run name from the list of available runs.
- ☐5 Select **Next**.
- ☐6 Open the flow cell compartment door.
- ☐7 Press the release button to the right of the flow cell latch.
- ☐8 Place the flow cell on the flow cell stage over the alignment pins.
- ☐9 Close the flow cell latch to secure the flow cell.
- ☐10 Close the flow cell compartment door.
- ☐11 Open the reagent compartment door.
- ☐12 Slide the reagent cartridge into the reagent compartment until the cartridge stops.
- ☐13 Remove the spent reagents bottle from the compartment.
- ☐14 Discard the contents and slide the empty spent reagents bottle into the compartment.
- ☐15 Close the compartment door and select **Next**.
- ☐16 Confirm run parameters.
- ☐17 Select **Next**.
- ☐18 When the automated check is complete, select **Start**.
- ☐19 Monitor run progress, intensities, and quality scores as metrics appear on the screen.

## View Analysis Results

- ☐1 From the Local Run Manager dashboard, click the run name.
- ☐2 From the Run Overview tab, review the sequencing run metrics.
- ☐3 [Optional] Click the **Copy to Clipboard** icon for access to the output run folder.
- ☐4 Click the Sequencing Information tab to review run parameters and consumables information.
- ☐5 Click the Samples and Results tab to view the analysis report.
- ☐6 [Optional] Click the **Copy to Clipboard** icon for access to the Analysis folder.