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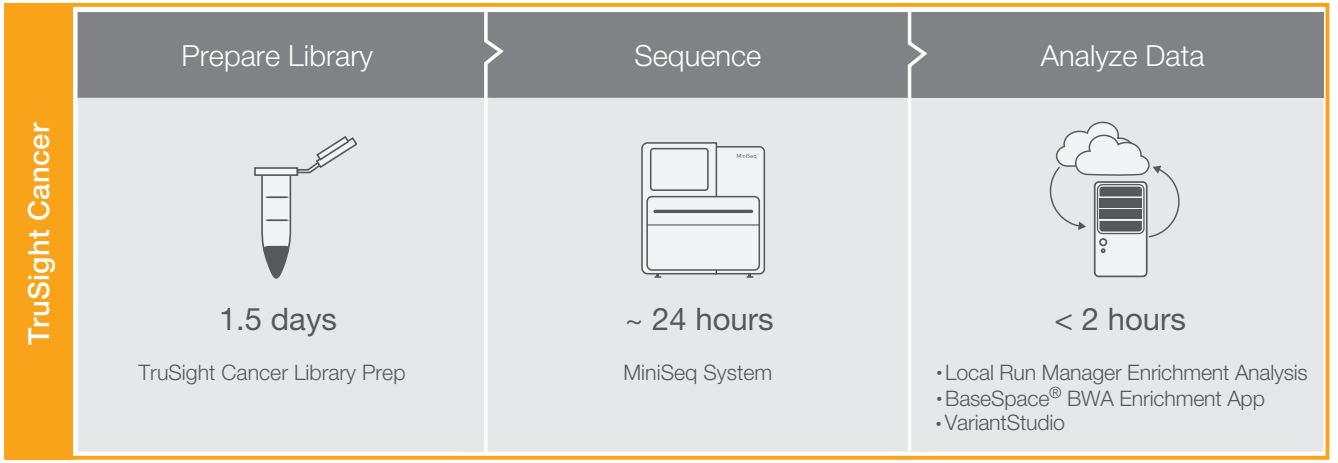
TruSight® Cancer Workflow on the MiniSeq™ System



This checklist supports the following workflow choices.

Sequencing Instrument:	MiniSeq System
Setup Option	Local Run Manager
Library Preparation Kit:	TruSight Rapid Capture
Indexing:	Dual Indexing
Reagent Kits:	MiniSeq Kit
Analysis Workflow:	DNA Enrichment
Analysis Software:	Local Run Manager

MiniSeq sequencing using TruSight Rapid Capture libraries_

Set Run Parameters

- ☐1 Log in to Local Run Manager.
- ☐2 Click **Create Run**, and select **DNA Enrichment**.
- ☐3 Enter a run name that identifies the run.
- ☐4 [Optional] Enter a run description.
- ☐5 From the Library Kit drop-down list, select TruSight Enrichment Panels.
- ☐6 Specify the number of index reads.
- ☐7 Specify a read type: **Single Read** or **Paired End**.
- ☐8 Enter the number of cycles for the run.
- ☐9 Select an alignment method.
- ☐10 Select a variant calling method.
- ☐11 Specify the **Manifest Padding** threshold.
- ☐12 Enable or disable the Flag PCR Duplicates and Indel Realignment settings.
- ☐13 Click **Show advanced module settings** and enable or disable Picard HS metrics.
- ☐14 Click **Import Manifests**.
- ☐15 Navigate to the manifest file.
- ☐16 Enter a unique sample ID.
- ☐17 [Optional] Enter a sample description.
- ☐18 Select an Index 1 adapter.
- ☐19 Select an Index 2 adapter.
- ☐20 Select a manifest file.
- ☐21 Click **Save Run**.

Tagment Genomic DNA

- ☐1 Quantify gDNA using a fluorometric method.
- ☐2 Dilute gDNA in Tris-HCl 10 mM, pH 8.5 to a final volume of 10 µl at 5 ng/µl.
- ☐3 Add the following to a new plate.
  - Normalized gDNA (10 µl)
  - TD (25 µl)
  - TDE1 (15 µl)
- ☐4 Shake at 1800 rpm for 1 minute.
- ☐5 Centrifuge at 280 × g for 1 minute.
- ☐6 Place on the 58°C microheating system with the lid closed for 10 minutes.
- ☐7 Add 15 µl ST.
- ☐8 Shake at 1800 rpm for 1 minute.
- ☐9 Centrifuge at 280 × g for 1 minute.
- ☐10 Incubate at room temperature for 4 minutes.

Clean Up Tagmented DNA

- ☐1 Add 65 µl SPB.
- ☐2 Shake at 1800 rpm for 1 minute.
- ☐3 Incubate at room temperature for 8 minutes.
- ☐4 Centrifuge at 280 × g for 1 minute.
- ☐5 Place on a magnetic stand until liquid is clear.
- ☐6 Remove and discard all supernatant.
- ☐7 Wash 2 times with 200 µl 80% EtOH.
- ☐8 Use a 20 µl pipette to remove residual EtOH.
- ☐9 Air-dry on the magnetic stand for 10 minutes.
- ☐10 Remove from the magnetic stand.
- ☐11 Add 22.5 µl RSB.
- ☐12 Shake at 1800 rpm for 1 minute.
- ☐13 Incubate at room temperature for 2 minutes.
- ☐14 Centrifuge at 280 × g for 1 minute.
- ☐15 Place on a magnetic stand until liquid is clear.
- ☐16 Transfer 20 µl supernatant.

Amplify Tagmented DNA

- ☐1 Arrange Index 1 (i7) adapters in columns 1–12.
- ☐2 Arrange Index 2 (i5) adapters in rows A–H.
- ☐3 Place the plate on the TruSeq Index Plate Fixture.
- ☐4 Add 5 µl of each Index 1 adapter down each column.
- ☐5 Add 5 µl of each Index 2 adapter across each row.
- ☐6 Add 20 µl NLM.
- ☐7 Shake at 1200 rpm for 1 minute.
- ☐8 Centrifuge at 280 × g for 1 minute.
- ☐9 Place on the thermal cycler and run the NLM AMP program.

SAFE STOPPING POINT

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

Clean Up Amplified DNA

- ☐1 Centrifuge at 280 × g for 1 minute.
- ☐2 Transfer 50 µl supernatant.
- ☐3 Add 90 µl SPB.
- ☐4 Shake at 1800 rpm for 1 minute.
- ☐5 Incubate at room temperature for 10 minutes.
- ☐6 Centrifuge at 280 × g for 1 minute.
- ☐7 Place on a magnetic stand until liquid is clear.
- ☐8 Remove and discard all supernatant.
- ☐9 Wash 2 times with 200 µl 80% EtOH.
- ☐10 Use a 20 µl pipette to remove residual EtOH.
- ☐11 Air-dry on the magnetic stand for 10 minutes.
- ☐12 Add 27.5 µl RSB.
- ☐13 Shake at 1800 rpm for 1 minute.
- ☐14 Incubate at room temperature for 2 minutes.
- ☐15 Centrifuge at 280 × g for 1 minute.
- ☐16 Place on a magnetic stand until liquid is clear.
- ☐17 Transfer 25 µl supernatant.
- ☐18 Quantify the library using a fluorometric method.

SAFE STOPPING POINT

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

Hybridize Probes

- ☐1 Combine 500 ng of each DNA library. Make sure that each library has a unique index.
  - For total volume > 40 µl, concentrate the pooled sample to 40 µl.
  - For total volume < 40 µl, increase the volume to 40 µl with RSB.
- ☐2 Use 500 ng of each DNA library quantified by QuantiFluor.
- ☐3 Add the following to a new plate.
  - Library pool (40 µl)
  - EHB (50 µl)
  - CSO (10 µl)
- ☐4 Shake at 1200 rpm for 1 minute.
- ☐5 Centrifuge at 280 × g for 1 minute.
- ☐6 Place on the thermal cycler and run the NRC HYB program.
- ☐7 Keep at the 58°C holding temperature for at least 90 minutes and up to 24 hours.

Capture Hybridized Probes

- ☐1 Centrifuge at 280 × g for 1 minute.
- ☐2 Transfer all volumes.
- ☐3 Add 250 µl SMB.
- ☐4 Shake at 1200 rpm for 5 minutes.
- ☐5 Incubate at room temperature for 25 minutes.
- ☐6 Centrifuge at 280 × g for 1 minute.
- ☐7 Place on a magnetic stand until liquid is clear.
- ☐8 Remove and discard all supernatant.
- ☐9 Remove from the magnetic stand.
- ☐10 Wash 2 times with 200 µl EWS.
- ☐11 Mix 28.5 µl EE1 and 1.5 µl 2 N NaOH, and then vortex.
- ☐12 Add 23.5 µl elution premix.
- ☐13 Shake at 1800 rpm for 2 minutes.
- ☐14 Incubate at room temperature for 2 minutes.
- ☐15 Centrifuge at 280 × g for 1 minute.
- ☐16 Place on a magnetic stand until liquid is clear.
- ☐17 Transfer 21 µl supernatant.
- ☐18 Add 4 µl ET2.
- ☐19 Shake at 1200 rpm for 1 minute.
- ☐20 Centrifuge at 280 × 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 7 days.

Perform Second Hybridization

- ☐1 Add the following.
  - RSB (15 µl)
  - EHB (50 µl)
  - CSO (10 µl)
- ☐2 Shake at 1200 rpm for 1 minute.
- ☐3 Centrifuge at 280 × g for 1 minute.
- ☐4 Place on the thermal cycler and run the NRC HYB program.
- ☐5 Keep at the 58°C holding temperature for at least 14.5 hours and up to 24 hours.

Perform Second Capture

- ☐1 Centrifuge at 280 × g for 1 minute.
- ☐2 Transfer supernatant.
- ☐3 Add 250 µl SMB.
- ☐4 Shake at 1200 rpm for 5 minutes.
- ☐5 Incubate at room temperature for 25 minutes.
- ☐6 Centrifuge at 280 × g for 1 minute.
- ☐7 Place on a magnetic stand until liquid is clear.
- ☐8 Remove and discard all supernatant.
- ☐9 Remove from the magnetic stand.
- ☐10 Wash 2 times with 200 µl EWS.
- ☐11 Mix 28.5 µl EE1 and 1.5 µl 2 N NaOH, and then vortex.
- ☐12 Add 23.5 µl elution premix.
- ☐13 Shake at 1800 rpm for 2 minutes.
- ☐14 Incubate at room temperature for 2 minutes.
- ☐15 Centrifuge at 280 × g for 1 minute.
- ☐16 Place on a magnetic stand until liquid is clear.
- ☐17 Transfer 21 µl supernatant.
- ☐18 Add 4 µl ET2.
- ☐19 Shake at 1800 rpm for 1 minute.
- ☐20 Centrifuge at 280 × g for 1 minute.

Clean Up Captured Library

- ☐1 Add 45 µl SPB.
- ☐2 Shake at 1800 rpm for 1 minute.
- ☐3 Incubate at room temperature for 10 minutes.
- ☐4 Centrifuge at 280 × g for 1 minute.
- ☐5 Place on a magnetic stand until liquid is clear.
- ☐6 Remove and discard all supernatant.
- ☐7 Wash 2 times with 200 µl 80% EtOH.
- ☐8 Use a 20 µl pipette to remove residual EtOH.
- ☐9 Air-dry for 10 minutes.
- ☐10 Add 27.5 µl RSB.
- ☐11 Shake at 1800 rpm for 1 minute.
- ☐12 Incubate at room temperature for 2 minutes.
- ☐13 Centrifuge at 280 × g for 1 minute.
- ☐14 Place on a magnetic stand until liquid is clear.
- ☐15 Transfer 25 µl supernatant.

SAFE STOPPING POINT

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

Amplify Enriched Library

- ☐1 Add 5 µl PPC.
- ☐2 Add 20 µl NEM.
- ☐3 Shake at 1200 rpm for 1 minute.
- ☐4 Centrifuge at 280 × g for 1 minute.
- ☐5 Place on the thermal cycler and run the NEM AMP12 program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days.

Clean Up Amplified Enriched Library

- ☐1 Centrifuge at 280 × g for 1 minute.
- ☐2 Transfer 50 µl.
- ☐3 Add 90 µl SPB.
- ☐4 Shake at 1800 rpm for 1 minute.
- ☐5 Incubate at room temperature for 10 minutes.
- ☐6 Centrifuge at 280 × g for 1 minute.
- ☐7 Place on a magnetic stand until liquid is clear.
- ☐8 Remove and discard all supernatant.
- ☐9 Wash 2 times with 200 µl 80% EtOH.
- ☐10 Use a 20 µl pipette to remove residual EtOH.
- ☐11 Air-dry on the magnetic stand for 10 minutes.
- ☐12 Add 32.5 µl RSB.
- ☐13 Shake at 1800 rpm for 1 minute.
- ☐14 Incubate at room temperature for 2 minutes.
- ☐15 Centrifuge at 280 × g for 1 minute.
- ☐16 Place on a magnetic stand until liquid is clear.
- ☐17 Transfer 30 µl supernatant.

SAFE STOPPING POINT

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

Check Enriched Libraries

- ☐1 Quantify using a fluorometric method.
- ☐2 If the concentration is higher than the quantitative range for the High Sensitivity DNA chip, dilute the library 1:10 with RSB.
- ☐3 Run 1 µl using a High Sensitivity DNA chip.

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.