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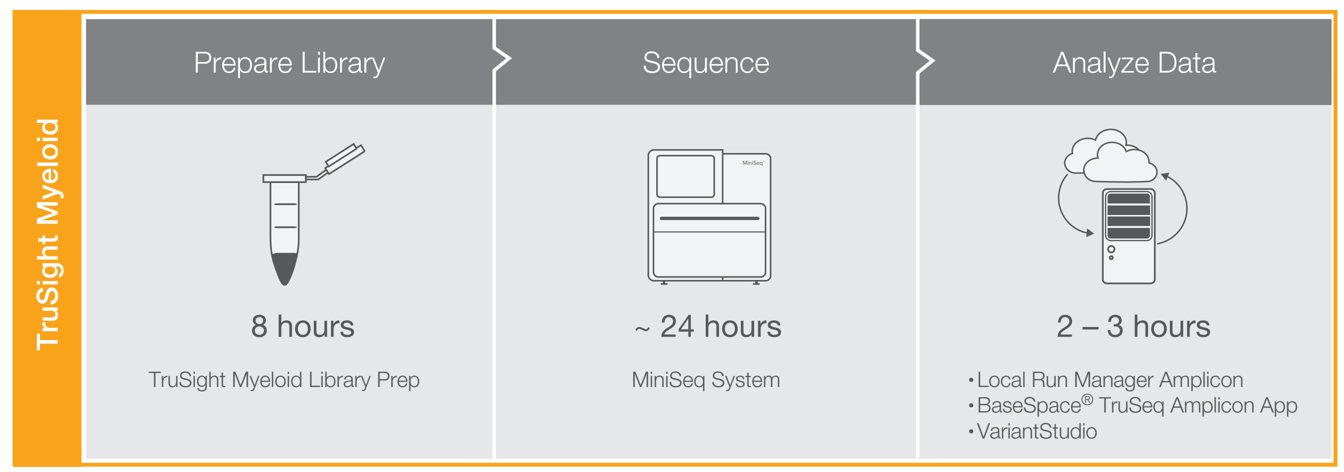
TruSight® Myeloid Workflow on the MiniSeq™ System



This checklist supports the following workflow choices.

Sequencing Instrument:	MiniSeq System
Setup Option	Local Run Manager
Library Preparation Kit:	TruSight Myeloid Sequencing Panel
Indexing:	Dual Indexing
Reagent Kits:	MiniSeq Kit
Analysis Workflow:	Amplicon
Analysis Software:	Local Run Manager

MiniSeq sequencing using TruSight Myeloid Sequencing Panel libraries

Set Run Parameters

- ☐1 Log in to Local Run Manager.
- ☐2 Click **Create Run**, and select **Amplicon**.
- ☐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 Amplicon Panels.
- ☐6 Specify the number of cycles for the run.
- ☐7 Select a variant calling method.
- ☐8 Click **Show advanced module settings** and specify the Read Stitching and Variant Quality Filter settings.
- ☐9 Click **Import Manifests**.
- ☐10 Navigate to the manifest file.
- ☐11 Enter a unique sample ID.
- ☐12 [Optional] Enter a sample description.
- ☐13 Select an Index 1 adapter.
- ☐14 Select an Index 2 adapter.
- ☐15 Select a manifest file.
- ☐16 Select a reference genome.
- ☐17 Click **Save Run**.

Hybridize Oligo Pool

- ☐1 Add 5 µl ACD1 and 5 µl TE or water to 1 well of the HYP plate.
- ☐2 Add 10 µl gDNA to each remaining well.
- ☐3 Add 5 µl TSO to each well containing gDNA.
- ☐4 Centrifuge at 1000 × g for 1 minute.
- ☐5 Add 35 µl OHS2. Pipette to mix.
- ☐6 Centrifuge at 1000 × g for 1 minute.
- ☐7 Place on the preheated heat block and incubate for 1 minute.
- ☐8 Reset the temperature to 40°C and incubate for 80 minutes.

Remove Unbound Oligos

- ☐1 Make sure that the heat block has cooled to 40°C.
- ☐2 Remove from the heat block.
- ☐3 Centrifuge at 1000 × g for 1 minute.
- ☐4 Transfer each sample to the FPU plate.
- ☐5 Cover and centrifuge at 2400 × g for 5 minutes.
- ☐6 Wash 2 times with 45 µl SW1.
- ☐7 Reassemble the FPU plate.
- ☐8 Add 45 µl UB1.
- ☐9 Cover and centrifuge at 2400 × g for 5 minutes.

Extend and Ligate Bound Oligos

- ☐1 Add 45 µl ELM4 to the FPU plate.
- ☐2 Incubate at 37°C for 45 minutes.

Amplify Libraries

- ☐1 Arrange the Index 1 (i7) adapters in columns 1–12.
- ☐2 Arrange the Index 2 (i5) adapters in rows A–H.
- ☐3 Place the IAP plate on a TruSeq Index Plate Fixture.
- ☐4 Use a multichannel pipette to add 4 µl of each Index 1 (i7) adapter to each row.
- ☐5 Use a multichannel pipette to add 4 µl of each Index 2 (i5) adapter to each column.
- ☐6 Add 56 µl TDP1 to 2.8 ml PMM2.
- ☐7 Invert to mix.
- ☐8 When incubation is complete, remove the FPU plate from the incubator and remove the seal.
- ☐9 Cover and centrifuge at 2400 × g for 5 minutes.
- ☐10 Use a multichannel pipette to add 25 µl 50 mM NaOH.
- ☐11 Incubate at room temperature for 5 minutes.
- ☐12 Transfer 22 µl PMM2/TDP1 master mix to the IAP plate.
- ☐13 Transfer samples eluted from the FPU plate to the IAP plate.
- ☐14 Centrifuge at 1000 × g for 1 minute.
- ☐15 Transfer to the post-amplification area.
- ☐16 Determine the required number (X) of PCR cycles using the following table:

Number of Amplicons in TSO	Number of PCR Cycles (X)
< 96 amplicons	33
97–384 amplicons	28
385–768 amplicons	27
769–1536 amplicons	26

- ☐17 Perform PCR on a thermal cycler using the following program:

- 95°C for 3 minutes
- X cycles of:
  - 95°C for 30 seconds
  - 66°C for 30 seconds
  - 72°C for 60 seconds
- 72°C for 5 minutes
- Hold at 10°C

SAFE STOPPING POINT

If you are stopping, leave the plate on the thermal cycler at 2°C to 8°C overnight.

Clean Up Libraries

- ☐1 Centrifuge the IAP plate at 1000 × g for 1 minute.
- ☐2 Run an aliquot of the libraries on 4% agarose gel (5 µl) or Bioanalyzer (1 µl).
- ☐3 Add 45 µl AMPure XP beads to the CLP plate.
- ☐4 Transfer all the supernatant from the IAP plate to the CLP plate.
- ☐5 Shake at 1800 rpm for 2 minutes.
- ☐6 Incubate at room temperature for 10 minutes.
- ☐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 Remove from the magnetic stand and air-dry for 10 minutes.
- ☐12 Add 30 µl EBT.
- ☐13 Shake at 1800 rpm for 2 minutes.
- ☐14 Incubate at room temperature for 2 minutes.
- ☐15 Place on a magnetic stand until liquid is clear.
- ☐16 Transfer 20 µl supernatant from the CLP plate to the LNP plate.
- ☐17 Centrifuge at 1000 × g for 1 minute.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 3 days. Alternatively, store at -25°C to -15°C for up to 7 days.

Normalize Libraries

- ☐1 Add 4.4 µl LNA1 per library to a new 15 ml conical tube.
- ☐2 Use a P1000 pipette to resuspend LNB1.
- ☐3 Transfer 800 µl LNB1 to the tube of LNA1.
- ☐4 Add the LNA1/LNB1 mix to a trough.
- ☐5 Add 45 µl LNA1/LNB1 to the LNP plate.
- ☐6 Shake at 1800 rpm for 30 minutes.
- ☐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 45 µl LNW1.
- ☐11 Remove residual LNW1.
- ☐12 Remove from the magnetic stand.
- ☐13 Add 30 µl fresh 0.1 N NaOH.
- ☐14 Shake at 1800 rpm for 5 minutes.
- ☐15 Place the LNP plate on a magnetic stand until liquid is clear.
- ☐16 Add 30 µl LNS2 to the SGP plate.
- ☐17 Transfer 30 µl supernatant from the LNP plate to the SGP plate.
- ☐18 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 30 days.

Pool Libraries

- ☐1 Transfer 5 µl to an 8-tube strip.
- ☐2 Seal the plate and store at -25°C to -15°C.
- ☐3 Transfer the contents of the 8-tube strip to the PAL 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 Thaw the Hybridization Buffer at room temperature.
- ☐2 Vortex briefly before use.
- ☐3 Preheat the incubator to 98°C.
- ☐4 Combine the 5 µl pooled libraries and 995 µl prechilled Hybridization Buffer in a microcentrifuge tube.
- ☐5 Vortex briefly and then centrifuge at 280 × g for 1 minute.
- ☐6 Transfer 250 µl diluted library to a new microcentrifuge tube.
- ☐7 Add 250 µl prechilled Hybridization Buffer.
- ☐8 Vortex briefly and then centrifuge at 280 × g for 1 minute.
- ☐9 Place the tube on the preheated incubator for 2 minutes.
- ☐10 Immediately cool on ice.
- ☐11 Leave on ice for 5 minutes.
- ☐12 [Optional] Denature and dilute a PhiX control to 1.8 pM and a 1% spike-in to the final library.
- ☐13 Clean the foil seal covering reservoir #**16** using a low-lint tissue.
- ☐14 Pierce the seal with a clean 1 ml pipette tip.
- ☐15 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.