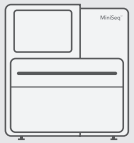
illumina®

TruSeq® Targeted RNA Workflow on the MiniSeq™ System

TruSeq Targeted RNA

Design Content	Prepare Library	Sequence	Analyze Data
			
< 0.5 days	1.5 days	~ 7 hours	~ 1 hour
For Custom Panels Only	TruSeq Targeted RNA Library Prep	MiniSeq System	•Local Run Manager Targeted RNA Analysis •BaseSpace® TruSeq Targeted RNA App

This checklist supports the following workflow choices.

Sequencing Instrument:	MiniSeq System
Setup Option	Local Run Manager
Library Preparation Kit:	TruSeq Targeted RNA Expression
Indexing:	Dual Indexing
Reagent Kits:	MiniSeq Kit
Analysis Workflow:	Targeted RNA
Analysis Software:	Local Run Manager

MiniSeq sequencing using TruSeq Targeted RNA Expression libraries

Set Run Parameters

- ☐1 Log in to Local Run Manager.
- ☐2 Click **Create Run**, and select **Targeted RNA**.
- ☐3 Enter a run name that identifies the run.
- ☐4 [Optional] Enter a run description.
- ☐5 Enter the number of cycles for the run.
- ☐6 Click **Import Manifests**.
- ☐7 Navigate to the manifest file.
- ☐8 Enter a unique sample ID.
- ☐9 Enter a sample name.
- ☐10 [Optional] Enter a sample description.
- ☐11 Select an Index 1 adapter.
- ☐12 Select an Index 2 adapter.
- ☐13 Select a manifest file.
- ☐14 Enter a list of gene names to be used for normalization. Separate each gene name with a semi-colon.
- ☐15 Click **Save Run**.

Synthesize cDNA

- ☐1 Vortex RCS1 for 5 seconds.
- ☐2 Centrifuge at 600 × g for 5 seconds.
- ☐3 Dilute according to your input RNA:
  - Dilute 50 ng intact total RNA with nuclease-free water to 5 µl.
  - Dilute ≥ 200 ng degraded RNA with nuclease-free water to 3 µl.
- ☐4 Add diluted RNA to a plate:
  - Add 5 µl diluted intact total RNA to the CDP1 plate.
  - Add 3 µl diluted degraded RNA to the CDP plate.
- ☐5 Combine the following volumes in a 1.7 ml microcentrifuge tube. Multiply each volume by the number of samples being prepared.

Reagent	Intact Total RNA Volume (µl)	Degraded RNA Volume (µl)
RCS1	4.4	4.4
ProtoScript II Reverse Transcriptase	1.1	2.2
10X DTT (0.1M)*	0	1.1
Total volume per pool	5.5	7.7

* Included with ProtoScript II Reverse Transcriptase reagent.

- ☐6 Invert to mix.
- ☐7 Centrifuge at 600 × g for 5 seconds.
- ☐8 Distribute evenly into an 8-tube strip.
- ☐9 Add the volume for your plate:
  - Add 5 µl to the CDP1 plate.
  - Add 7 µl to the CDP plate.
- ☐10 Shake at 1600 rpm for 20 seconds.
- ☐11 Centrifuge at 280 × g for 1 minute.

☐12 Place on the thermal cycler and run the CDNASYN1 or CDNASYN2 program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 7 days. Alternatively, leave on the thermal cycler overnight.

Hybridize Oligo Pool

☐1 Combine the following volumes in a 1.7 ml microcentrifuge tube. Multiply each volume by the number of reactions being prepared.
- Top (5.5 µl)
- Additional TOP or TE buffer (5.5 µl)

☐2 Vortex for 5 seconds.
☐3 Centrifuge at 600 × g for 5 seconds.
☐4 Distribute into an 8-tube strip.
☐5 Add 10 µl to the CDP or CDP1 plate.
☐6 Shake at 1600 rpm for 20 seconds.
☐7 Incubate at room temperature for 1 minute.
☐8 Vortex OB1 for 5 seconds.
☐9 Add 30 µl OB1 to the CDP or CDP1 plate.
☐10 Shake at 1600 rpm for 1 minute.
☐11 Place on the thermal cycler and run the ANNEAL program.
☐12 Centrifuge briefly.

Wash, Extend, and Ligate Bound Oligos

☐1 Transfer all supernatant to the HYP plate.
☐2 Place on a magnetic stand until liquid is clear.
☐3 Remove and discard all of the supernatant.
☐4 Move from the magnetic stand to a bench.
☐5 Add 100 µl AM1 to each well.
☐6 Shake at 1800 rpm for 2 minutes.
☐7 Centrifuge at 280 × g for 5 seconds.
☐8 Unseal and place on a magnetic stand until liquid is clear.
☐9 Remove and discard all supernatant.
☐10 Move from the magnetic stand to a bench.
☐11 Add 175 µl UB1.
☐12 Shake at 1800 rpm for 2 minutes.
☐13 Centrifuge at 280 × g for 5 seconds.
☐14 Unseal and place on a magnetic stand until liquid is clear.
☐15 Invert ELM4.
☐16 Remove and discard all supernatant.
☐17 Move from the magnetic stand to a bench.
☐18 Add 40 of ELM4.
☐19 Shake at 1800 rpm for 2 minutes.
☐20 Centrifuge at 280 × g for 5 seconds.
☐21 Place on the 37°C preheated microheating system and incubate for 45 minutes.
☐22 Remove the adhesive seal from the plate.
☐23 Unseal and place on a magnetic stand until liquid is clear.
☐24 Remove and discard all supernatant.
☐25 Add 50 µl of UB1.

Amplify Libraries

- ☐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 4 µl of each Index 1 adapter down each column.
- ☐5 Add 4 µl of each Index 2 adapter across each row.
- ☐6 Remove and discard all supernatant from the HYP plate.
- ☐7 Remove from the magnetic stand.
- ☐8 Add 22.5 µl diluted HP3.
- ☐9 Shake at 1800 rpm for 30 seconds.
- ☐10 Incubate at room temperature for at least 5 minutes.
- ☐11 Create the amplification mix:
  - **96 libraries**—Add 56 µl TDP1 to 2.8 ml of PMM2.
  - **48 libraries**—Combine 28 µl TDP1 and 1.4 ml PMM2 in a 1.7 ml microcentrifuge tube.
  - **16 libraries**—Combine 9.2 µl TDP1 and 460 µl PMM2 in a 1.7 ml microcentrifuge tube.
- ☐12 Invert to mix.
- ☐13 Add 22 µl to the IAP plate.
- ☐14 Unseal the HYP plate.
- ☐15 Place on a magnetic stand until liquid is clear.
- ☐16 Transfer 20 µl supernatant from the HYP plate to the IAP plate.
- ☐17 Shake at 1600 rpm for 30 seconds.
- ☐18 Centrifuge at 280 × g for 1 minute.
- ☐19 Place on the thermal cycler and run the 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 Libraries

- ☐1 Add 85 µl AMPure XP Beads to the CLP plate.
- ☐2 Centrifuge the IAP plate at 280 × g for 1 minute.
- ☐3 Unseal the IAP plate.
- ☐4 Transfer all supernatant to the CLP plate.
- ☐5 Shake at 1800 rpm for 2 minutes.
- ☐6 Centrifuge the plate at 280 × g for 5 seconds.
- ☐7 Incubate room temperature for 15 minutes
- ☐8 Unseal and place on a magnetic stand until liquid is clear.
- ☐9 Remove and discard 135 µl supernatant.
- ☐10 Wash 2 times with 200 µl 80% EtOH.
- ☐11 Air dry on the magnetic stand for 15 minutes.
- ☐12 Remove from the magnetic stand.
- ☐13 Add 15 µl RSB.
- ☐14 Shake at 1800 rpm for 2 minutes.
- ☐15 Centrifuge at 280 × g for 5 seconds.
- ☐16 Return RSB to 2°C to 8°C storage.
- ☐17 Incubate the plate at room temperature for 2 minutes.
- ☐18 Unseal and place on a magnetic stand until liquid is clear.
- ☐19 Transfer 12.5 µl supernatant to the LNP plate.

SAFE STOPPING POINT

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

Pool and Quantify Libraries

- ☐1 Transfer 5 µl from the LNP plate to a 2 ml microcentrifuge tube.
- ☐2 Vortex for 5 seconds.
- ☐3 Centrifuge at 600 × g for 5 seconds.
- ☐4 Load 1 µl pooled library onto the Standard Sensitivity NGS Fragment Analysis Kit or DNA 1000 Kit.
- ☐5 Determine the concentration of the pooled library.
- ☐6 Select the **Region Analysis** tab.
- ☐7 Drag the blue region lines to capture the 100–300 bp region.
- ☐8 Dilute each pooled library to 4 nM using RSB.

SAFE STOPPING POINT

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

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.