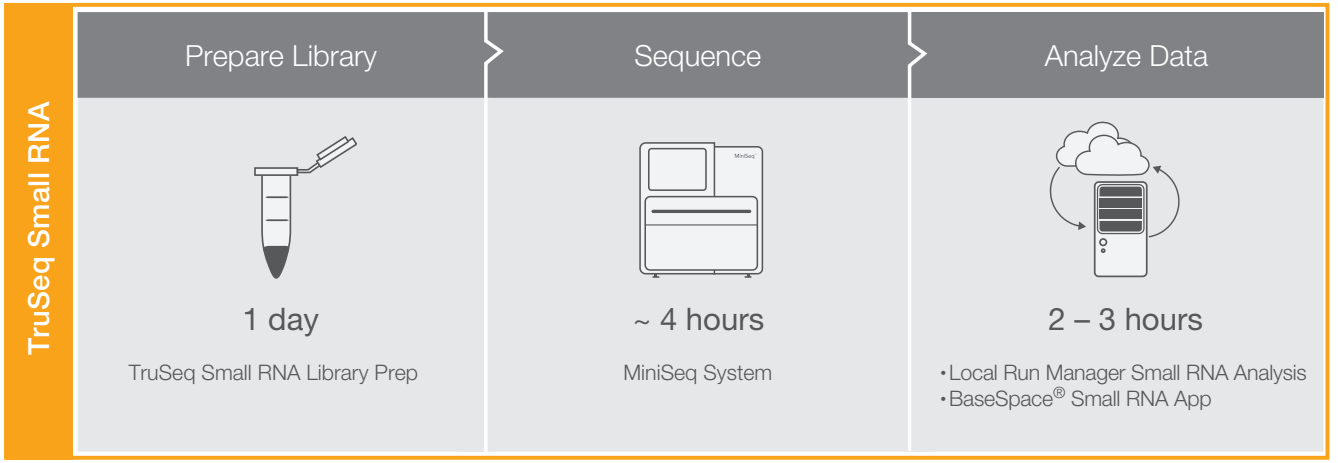
illumina®

TruSeq® Small RNA Workflow on the MiniSeq™ System



This checklist supports the following workflow choices.

Sequencing Instrument:	MiniSeq System
Setup Option	Local Run Manager
Library Preparation Kit:	TruSeq Small RNA Library Prep
Indexing:	Single Indexing
Reagent Kits:	MiniSeq Kit
Analysis Workflow:	Small RNA
Analysis Software:	Local Run Manager

MiniSeq sequencing using TruSeq Small RNA libraries

Set Run Parameters

- ☐1 Log in to Local Run Manager.
- ☐2 Click **Create Run**, and select **Small RNA**.
- ☐3 Enter a run name that identifies the run.
- ☐4 [Optional] Enter a run description.
- ☐5 Click **1** to specify a single-indexed run.
- ☐6 Enter the number of cycles for the run.
- ☐7 Select a reference genome from the Genome Folder drop-down list.
- ☐8 Enter a unique sample ID.
- ☐9 [Optional] Enter a sample description.
- ☐10 Select an Index 1 adapter.
- ☐11 Click **Save Run**.

Ligate Adapters

- ☐1 Combine the following volumes in a 200 µl PCR tube on ice:
  - RA3 (1 µl)
  - 1 µg total RNA in nuclease-free water (5 µl)
- ☐2 Pipette to mix, and then centrifuge briefly.
- ☐3 Place on the thermal cycler.
- ☐4 Incubate at 70°C for 2 minutes.
- ☐5 Remove from the thermal cycler and place on ice.
- ☐6 Combine the following volumes in a new 200 µl PCR tube on ice. Multiply each volume by the number of samples. Make 10% extra reagent for multiple samples.
  - HML (2 µl)
  - RNase Inhibitor (1 µl)
  - T4 RNA Ligase 2, Deletion Mutant (1 µl)
- ☐7 Pipette to mix, and then centrifuge briefly.
- ☐8 Add 4 µl to the RA3/total RNA mixture.
- ☐9 Pipette to mix.
- ☐10 Place on the thermal cycler.
- ☐11 Incubate at 28°C for 1 hour.
- ☐12 Add 1 µl STP and pipette to mix.
- ☐13 Continue incubating at 28°C for 15 minutes.
- ☐14 Remove from the thermal cycler and place on ice.
- ☐15 Add 1.1 × N µl RA5 to a 200 µl PCR tube.
- ☐16 Place on the thermal cycler.
- ☐17 Incubate at 70°C for 2 minutes.
- ☐18 Remove from the thermal cycler and place on ice.
- ☐19 Add 1.1 × N µl 10mM ATP to the RA5.
- ☐20 Pipette to mix.
- ☐21 Add 1.1 × N µl T4 RNA Ligase to the RA5/ATP mixture.
- ☐22 Pipette to mix.
- ☐23 Add 3 µl to the RA3 mixture.
- ☐24 Pipette to mix.

☐25 Place on the preheated thermal cycler.
☐26 Incubate at 28°C for 1 hour.
☐27 Remove from the thermal cycler and place on ice.

Reverse Transcribe and Amplify Libraries

☐1 Combine the following volumes in the 12.5 mM dNTP Mix tube to dilute to 12.mM. Multiply each volume by the number of samples. Prepare 10% extra reagent for multiple libraries.
- 25 mM dNTP Mix (0.5 µl)
- Ultrapure water (0.5 µl)

☐2 Pipette to mix, and then centrifuge briefly.
☐3 Set aside on ice.
☐4 Add 6 µl each RNA library to a 200 µl PCR tube.
☐5 Add 1 µl RNA RT Primer to the RNA.
☐6 Pipette to mix, and then centrifuge briefly.
☐7 Place on the thermal cycler.
☐8 Incubate at 70°C for 2 minutes.
☐9 Remove from the thermal cycler and place on ice.
☐10 Combine the following volumes in a 200 µl PCR tube on ice. Multiply each volume by the number of libraries. Make 10% extra reagent for multiple libraries.
- 5X First Strand Buffer (2 µl)
- 12.5 mM dNTP Mix (0.5 µl)
- 100 mM DTT (1 µl)
- RNase Inhibitor (1 µl)
- SuperScript II Reverse Transcriptase (1 µl)

☐11 Pipette to mix, and then centrifuge briefly.
☐12 Add 5.5 µl to the RNA/primer mix.
☐13 Pipette to mix, and then centrifuge briefly.
☐14 Incubate at 50°C for 1 hour.
☐15 Remove from the thermal cycler and place on ice.
☐16 Combine the following reagents in a 200 µl PCR tube on ice. Multiply each volume by the number of libraries. Make 10% extra reagent for multiple libraries with the same index.
- Ultrapure water (8.5 µl)
- PML (25 µl)
- RP1 (2 µl)
- RPIX (2 µl)

☐17 Pipette to mix, and then centrifuge briefly.
☐18 Place on ice.
☐19 Add 37.5 µl PCR master mix to the adapter-ligated RNA mixture.
☐20 Pipette to mix, and then centrifuge briefly.
☐21 Place on ice.
☐22 Place on the thermal cycler.
☐23 Incubate using the following program on the thermal cycler:
- Choose the preheat lid option and set to 100°C.
- 98°C for 30 seconds
- 11 cycles of:
  - 98°C for 10 seconds
  - 60°C for 30 seconds
  - 72°C for 15 seconds
- 72°C for 10 minutes
- 4°C hold

☐24 Run each library on a High Sensitivity DNA chip.

SAFE STOPPING POINT

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

Purify cDNA Construct

- ☐1 Combine the following volumes in the 0.1X Pellet Paint tube. Multiply each volume by the number of libraries. Make 10% extra reagent for multiple libraries.
  - 1X Pellet Paint NF Co-Precipitant (0.2 µl)
  - Ultrapure water (1.8 µl)
- ☐2 Pipette to mix, and then centrifuge briefly.
- ☐3 Combine 2 µl CRL and 2 µl DNA loading dye in a 1.5 ml microcentrifuge tube.
- ☐4 Pipette to mix.
- ☐5 Combine 1 µl HRL and 1 µl DNA loading dye in a 1.5 ml microcentrifuge tube.
- ☐6 Pipette to mix.
- ☐7 Combine all amplified cDNA construct and 10 µl DNA Loading Dye in a 1.5 ml microcentrifuge tube.
- ☐8 Pipette to mix.
- ☐9 Load 2 gel lanes with 2 µl CRL/loading dye mixture.
- ☐10 Load 1 gel lane with 2 µl HRL/loading dye mixture.
- ☐11 Load 2 gel lanes with 25 µl each of amplified cDNA construct/loading dye mixture.
- ☐12 Run the gel for 60 minutes at 145 V or until the blue front dye leaves the gel.
- ☐13 Remove the gel from the unit.
- ☐14 Open the cassette and stain the gel with ethidium bromide for 2–3 minutes.
- ☐15 Place the gel breaker tube into a 2 ml microcentrifuge tube.
- ☐16 View the gel on a Dark Reader transilluminator or a UV transilluminator.
- ☐17 Using a razor blade, cut out the bands from the 2 lanes that correspond to the adapter-ligated constructs derived from the 22 nt and 30 nt small RNA fragments.
- ☐18 Place the band into the 0.5 ml gel breaker tube.
- ☐19 Centrifuge the nested tubes at 20,000 × g for 2 minutes.
- ☐20 If you are concentrating the final library, skip the next 4 steps and proceed to adding 300 µl Ultrapure Water to gel debris.
- ☐21 Add 200 µl ultrapure water to the gel debris.
- ☐22 Rotate for at least 2 hours.
- ☐23 Transfer the eluate and gel debris to the top of a 5 µm filter.
- ☐24 Centrifuge at 10 seconds at 600 × g.
- ☐25 Add 300 µl ultrapure water to the gel debris.
- ☐26 Rotate for at least 2 hours.
- ☐27 Transfer the eluate and gel debris to the top of a 5 µm filter.
- ☐28 Centrifuge at 600 × g for 10 seconds, and then discard the filter.
- ☐29 Add the following volumes to the eluate:
  - Glycogen (2 µl)
  - 3M NaOAc (30 µl)
  - [Optional] 0.1X Pellet Paint (2 µl)
  - 100% ethanol (2 µl)
- ☐30 Centrifuge at 20,000 × g at 20 minutes at 4°C.
- ☐31 Remove and discard the supernatant. Leave the pellet intact.
- ☐32 If the pellet becomes loose, centrifuge at 20,000 × g for 2 minutes.
- ☐33 Wash the pellet with 500 µl 70% ethanol.
- ☐34 Centrifuge at 20,000 × g for 2 minutes.
- ☐35 Remove and discard the supernatant. Leave the pellet intact.
- ☐36 With the lid open, place the tube in a 37°C heat block until the pellet is dry.
- ☐37 Resuspend the pellet in 10 µl 10 mM Tris-HC1, pH 8.5.

Check Libraries

- ☐1 Load 1 µl resuspended construct on an Agilent Technologies 2100 Bioanalyzer using a DNA-specific chip.
- ☐2 Check the size, purity, and concentration of the library.

Normalize Libraries

☐1 Normalize library concentration to 2 nM using Tris-HCl 10 mM, pH 8.5.
☐2 For storage, add Tween 20 for a final concentration of 0.1% Tween 20.

SAFE STOPPING POINT

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

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 50 µl of the 2 nM library pool to a new microcentrifuge tube.
☐7 Add 50 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.