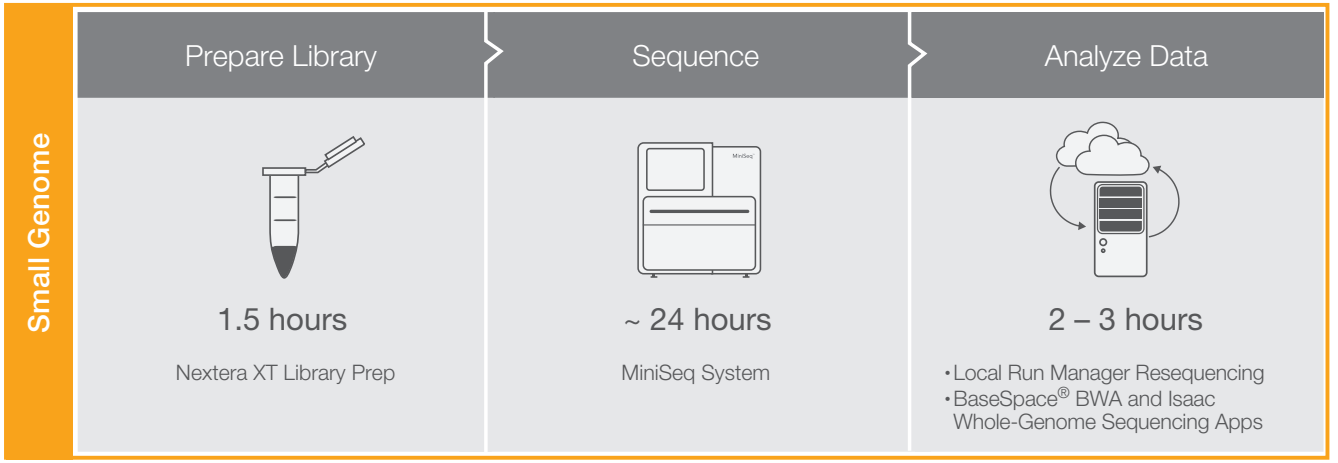
illumina®

Small Genome Workflow on the MiniSeq™ System



This checklist supports the following workflow choices.

Sequencing Instrument:	MiniSeq System
Setup Option	Local Run Manager
Library Preparation Kit:	Nextera XT DNA Sample Prep
Indexing:	Dual Indexing
Reagent Kits:	MiniSeq Kit
Analysis Workflow:	Resequencing
Analysis Software:	Local Run Manager

Small Genome Checklist

Set Run Parameters

- ☐1 Log in to Local Run Manager.
- ☐2 Click **Create Run**, and select **Resequencing**.
- ☐3 Enter a run name that identifies the run.
- ☐4 [Optional] Enter a run description.
- ☐5 From the Library Kit drop-down list, select Nextera XT or Nextera XT V2.
- ☐6 Click **2** to specify a dual-indexed run.
- ☐7 Specify a read type: **Single Read** or **Paired End**.
- ☐8 Enter the number of cycles for the run.
- ☐9 Select an alignment method.
  - **BWA-MEM**—(Default) Optimized for Illumina sequencing data and reads ≥ 70 bp.
  - **BWA-Backtrack Legacy**—Use with legacy data or reads < 70 bp.
- ☐10 Select a variant calling method.
  - **Starling**—(Default) Calls SNPs and small indels, and summarizes depth and probabilities for every site in the genome.
  - **GATK**—Calls raw variants for each sample, analyzes variants against known variants, and then calculates a false discovery rate for each variant.
- ☐11 Enable or disable the Export gVCF, Flag PCR Duplicates, and Indel Realignment settings.
- ☐12 Enter a unique sample ID.
- ☐13 [Optional] Enter a sample description.
- ☐14 Select an Index 1 adapter.
- ☐15 Select an Index 2 adapter.
- ☐16 Select a reference genome.
- ☐17 Click **Save Run**.

Tagment Genomic DNA

- ☐1 Add the following to a new PCR plate. Pipette to mix.

Item	Volume (µl)
TD	10
Normalized gDNA	5

- ☐2 Add 5 µl ATM. Pipette to mix.
- ☐3 Centrifuge at 280 × g at 20°C for 1 minute.
- ☐4 Place on the thermal cycler and run the tagmentation program.
- ☐5 Add 5 µl NT. Pipette to mix.
- ☐6 Centrifuge at 280 × g at 20°C for 1 minute.
- ☐7 Incubate at room temperature for 5 minutes.
- ☐8 [Optional] Run 1 µl sample on a High Sensitivity DNA chip.

Amplify Libraries

- ☐1 [24 libraries] Arrange the index primers as follows.
  - Arrange Index 1 (i7) adapters in columns 1–6.
  - Arrange Index 2 (i5) adapter in rows A–D.
- ☐2 [96 libraries] Arrange the index primers as follows.
  - Arrange Index 1 (i7) adapters in columns 1–12.
  - Arrange Index 2 (i5) adapter in rows A–H.
- ☐3 Add 5 µl of each Index 1 (i7) adapter down each column. Replace the cap on each i7 adapter tube with a new orange cap.
- ☐4 Add 5 µl of each Index 2 (i5) adapter across each row. Replace the cap on each i5 adapter tube with a new white cap.
- ☐5 Add 15 µl NPM. Pipette to mix.
- ☐6 Centrifuge at 280 × g at 20°C for 1 minute.
- ☐7 Place on the thermal cycler and run the PCR 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 Centrifuge at 280 × g at 20°C for 1 minute.
- ☐2 Transfer 50 µl PCR product.
- ☐3 Add 30 µl AMPure XP beads.
- ☐4 Shake at 1800 rpm for 2 minutes.
- ☐5 Incubate at room temperature for 5 minutes.
- ☐6 Place on a magnetic stand until liquid is clear.
- ☐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 15 minutes.
- ☐11 Remove from the magnetic stand.
- ☐12 Add 52.5 µl RSB.
- ☐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 50 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 Libraries

- ☐1 [Optional] Run 1 µl of undiluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip.

Normalize Libraries

- ☐1 Transfer 20 µl supernatant.
- ☐2 [96 samples] Add 4.4 ml LNA1 to a new 15 ml conical tube.
- ☐3 Thoroughly resuspend LNB1. Pipette to mix.
- ☐4 Transfer 800 µl LNB1 to the tube. Invert to mix.
- ☐5 Add 45 µl combined LNA1/LNB1.
- ☐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 Wash 2 times with 45 µl LNW1.
- ☐10 Add 30 µl 0.1 N NaOH.
- ☐11 Shake at 1800 rpm for 5 minutes.
- ☐12 During the 5 minute elution, label a new 96-well PCR plate SGP.
- ☐13 Add 30 µl LNS1 to the SGP plate. Set aside.
- ☐14 After the 5 minute elution, make sure that all samples are resuspended. Pipette to mix.
- ☐15 Shake at 1800 rpm for 5 minutes.
- ☐16 Place on a magnetic stand until liquid is clear.
- ☐17 Transfer the supernatant from the midi 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 7 days.

Pool Libraries

- ☐1 Centrifuge at 1000 × g at 20°C for 1 minute.
- ☐2 Transfer 5 µl from the SGP plate to a new PCR 8-tube strip.
- ☐3 Label a new Eppendorf tube PAL.
- ☐4 Transfer the contents of the PCR 8-tube strip to the PAL tube. Invert to mix.
- ☐5 Dilute pooled libraries to the loading concentration for the sequencing instrument you are using. See the denature and dilute libraries guide for your instrument.
- ☐6 Store unused pooled libraries in the PAL tube and SGP plate 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 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 1.8 pM 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.