

Running the MiSeq® Enrichment Workflow



Requirements:

- Illumina Experiment Manager
- Nextera Enrichment Sample Prep Kit or TruSight Enrichment DNA Sample Prep Kit
- MiSeq Reagent Kit
- Enrichment manifest
- Reference Genome

Optional additions:

- PhiX v3 Control

1 Create Sample Sheet



- Create a sample sheet for the Enrichment workflow. ([Create Nextera or TruSight Sample Plates and Sample Sheets with IEM Quick Ref Card](#))
- Download an Enrichment manifest from the Illumina website.
- Copy the sample sheet to the sample sheet folder designated in MCS.
- Copy the manifest to the manifest folder designated in MCS.

2 Prepare Samples

Varies

Prepare enriched libraries using one of the following kits:

- Nextera Enrichment Sample Prep ([Nextera Enrichment Sample Prep Guide](#))
- TruSight Enrichment DNA Sample Prep ([TruSight Enrichment DNA Sample Prep Guide](#))

3 Prepare Cartridge

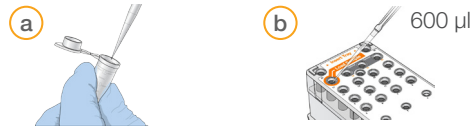
1 hour



- Thaw and prepare the reagent cartridge. ([MiSeq User Guide: Preparing Reagents](#))

3 Prepare Cartridge

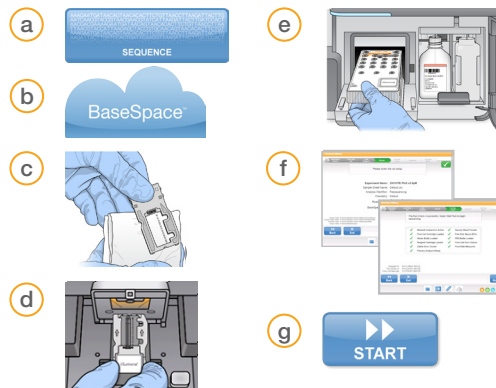
20 min.



- Denature and dilute libraries and optional PhiX control. ([Preparing Libraries for Sequencing on the MiSeq](#))
- Pierce foil at sample position and dispense libraries into sample reservoir.

5 Set Up Run

20 min.



- Select Sequence.
- (Optional) Log in to BaseSpace.
- Clean the flow cell and check gaskets at flow cell ports. ([MiSeq User Guide: Cleaning the Flow Cell](#))
- Load the flow cell. Confirm RFID read.
- Empty the waste bottle, and load the PR2 bottle and reagent cartridge. Confirm RFID reads.
- Confirm run settings and results of pre-run check.
- Start run.

6 Monitor Run

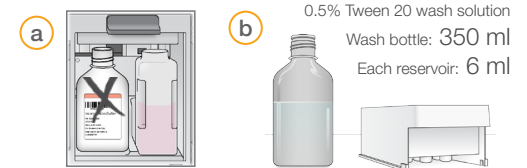
Up to 27 hours



- Check run metrics on MCS Sequencing screen:
 - After cycle 5, check cluster density and intensities.
 - After cycle 25, check estimated yield and Q-score distribution.
- (Optional) Use BaseSpace or SAV to monitor the run remotely. ([Sequencing Analysis Viewer User Guide](#))

7 Perform Post-Run Wash

20 min.



- Empty waste bottle in appropriate container and discard the used PR2 bottle. Leave the flow cell on the instrument.
- Perform a post-run wash. ([MiSeq User Guide: Performing a Post-Run Wash](#))

8 Secondary Analysis

~ 2 hours

- Allow MiSeq Reporter to complete analysis or requeue the job for analysis at a later time.
- Review reports in MiSeq Reporter. ([MiSeq Reporter User Guide: Enrichment Workflow](#))