MiSeq v3 Upgrade
Frequently Asked Questions

1. What do I need to upgrade my MiSeq?

   The new MiSeq upgrade requires a new version of MiSeq Control Software (MCS) and new MiSeq v3 reagent kits.

2. Is a hardware upgrade required?

   No new hardware is required.

Reagents and Flow Cells

1. Are flow cells provided in MiSeq v3 kits different from flow cells provided in v2 kits?

   No. The flow cells are identical. However, the upgrade to MCS v2.3 enables imaging of the flow cell in 19 tiles per surface.

2. Are there changes to reagents in MiSeq v3 kits?

   Yes. There are two new reagents in MiSeq v3 kits:

   - New incorporation mix (IMT)
   - New scan mix (USM)

   The PR2 volume is increased to 500 ml to support longer runs. In the near future, all MiSeq PR2 bottles will have a 500 ml fill to avoid confusion when starting a run. Look for announcements on the Illumina Tech Support Bulletin Board regarding this change.

Software

1. Where can I download the software required for the MiSeq v3 kits?

   You can download the software free of charge through BaseSpace or on the MiSeq support page on the Illumina website.

2. I am currently finishing a project using MiSeq v2 reagents. Can I upgrade my software to MCS v2.3 and still finish my project with v2 reagents?

   Yes, the new MiSeq software package is backward compatible with v2 and v1 reagents. Using the RFID feature, the MiSeq automatically recognizes which kit

version is loaded for the run and chooses the appropriate Q-table. There are no changes to v1 or v2 workflows.

Ordering

1. I have an existing order for MiSeq v2 kits. Can I convert my order to MiSeq v3 kits?

   Yes, you can convert your v2 orders to v3 orders on a dollar for dollar basis. For more information, contact Customer Service at: customerservice@illumina.com.

2. What are the MiSeq v3 kit catalog numbers?

Kit Name	Catalog #
MiSeq Reagent Kit v3, 600 Cycles	MS-102-3003
MiSeq Reagent Kit v3, 150 Cycles	MS-102-3001

3. When will MiSeq v3 kits begin shipping?

   Shipping of MiSeq v3 kits begins August 14, 2013.

Compatibility

1. Are MiSeq v3 kits compatible with all Illumina sample prep kits?

   Yes. Just like v2 kits, MiSeq v3 kits are compatible with all Illumina sample prep kits. Some sample prep guides require slight changes to the protocol to reach optimum clusters. Check the Illumina Tech Support Bulletin Board for the latest information on sample prep.

2. I have a MiSeqDx. Can I use the MiSeq v3 kits?

   No, the MiSeqDx software is not compatible with MiSeq v3 kits.

Workflow

1. How many cycles will MiSeq v3 kits support?

   MiSeq v3 kits are kitted for 150-cycle and 600-cycle runs.

2. How long does it take to complete a 600-cycle run on the MiSeq?

   A 600-cycle run takes approximately 65 hours.

3. How long does it take to complete a 150-cycle run on the MiSeq?

   A 150-cycle run takes approximately 24 hours.

4. Are there changes to run times with MCS v2.3 and MiSeq v3 reagents?

   Run times with are as follows:

   ~2 minutes for chemistry
   ~4 minutes for imaging
   ~6 minutes total per cycle
   ~70 minutes for cluster generation
   ~50 minutes for paired-end turnaround

5. Are there changes to the number of cycles required for template generation?

   Yes. The number of cycles required for template generation is increased to 7 cycles. Density metrics are not reported until ~cycle 20.

6. What is the size of the output data folder?

   You can expect the output data folder size to be 25–35 GB.

7. Are there changes to analysis?

   Software supports overlapping reads using read stitching for FASTQ generation and the TruSeq Amplicon workflow. For more information, see the *MiSeq Sample Sheet Quick Reference Guide (part # 15028392)*. This setting will be an available option in IEM.

8. What are the Illumina performance specifications for MiSeq v3 kits?

Run Length	Feature	Specification
2 x 300	Run Length	< 72 hours
	Yield (Gb)	Up to 15 Gb
	Reads PF (M)	22–25 M
	Total % ≥ Q30	> 70%
2 x 75	Run Length	< 24 hours
	Yield (Gb)	Up to 3.75 Gb
	Reads PF (M)	22–25 M
	Total % ≥ Q30	> 85%

9. Are MiSeq v3 data sets available?

   Data sets will be available in BaseSpace. Check the MiSeq support page for more information.

10. Can I expect changes when using Sequence Analysis Viewer (SAV)?

    Intensity (Data by cycle) plots appear different due to non-linear exposure ramping. Non-linear ramping prevents exposure damage early in the read, which provides a boost later in the read when it is more necessary.