

Reducing Run-to-Run Carryover on the MiSeq Using Dilute Sodium Hypochlorite Solution

Run-to-Run Carryover

The MiSeq uses a fixed template loading fluidic pathway to load sequencing template onto the flow cell before a run. It is possible that low numbers of template molecules remain in this instrument fluidic pathway after the flow cell is loaded. These remaining molecules can be washed onto the flow cell in a subsequent run and can appear as clusters. This run-to-run sample carryover (often referred to as "carryover") can cause some reads to be incorrectly assigned in a consecutive run. Other possible causes of misassignment are sample cross-contamination during library preparation or bioinformatics issues during the demultiplexing process.

Measurement of Sample Carryover

Sample carryover is measured as the ratio of reads from the previous run to the total reads in a current run. To measure sample carryover, the two runs must use different sample types to allow reads to be assigned to a sample. A simple example of this is the case where PhiX is sequenced on the first run and a human sample is sequenced on the subsequent run. The rate of sample carryover would be the number of reads aligning to PhiX in the second run divided by the total number of reads in the second run.

Implications of Sample Carryover for Highly Sensitive Applications

MiSeq instruments that are maintained according to Illumina recommendations typically have sample carryover rates below 0.1% (one read in a thousand). For most applications, these rare reads are discarded and do not pose a problem for analysis. However, for a few highly sensitive applications (such as somatic variant calling), a 0.1% carryover rate could potentially cause false variant calling, particularly in runs with highly divergent call rates and which are run sequentially (for example, an all-tumor sequencing run followed by a non-tumor run where rare variants are the focus of the assay).

Benefits of a Post-Run Wash That Incorporates Sodium Hypochlorite

It has been demonstrated that a post-run wash incorporating freshly diluted sodium hypochlorite (bleach) can reduce run-to-run carryover levels to as low as 0.001% (one read per 100,000). This run-to-run carryover level is 100-fold lower than levels achieved using standard water washes. The bleach wash achieves these levels by chemically modifying any template DNA remaining in the instrument lines so that it can no longer be amplified or form clusters. This document describes how to perform this wash on a MiSeq. This wash is optional and is only recommended for customers running applications in which the typical low levels of sample carryover can affect analysis.

Steps to Perform a Carryover Decontamination Post-Run Wash

Use the following steps to perform a carryover decontamination post-run wash on the MiSeq.

Load the Carryover Decontamination PostRunWash.xml Recipe Fragment into the Recipe

The carryover decontamination wash can be performed within any recipe. All that is required is replacing the current PostRunWash.xml recipe fragment file inside the "Wash" folder with the PostRunWash.xml provided for carryover decontamination.

1. Open the desired recipe folder.
2. Open the "Wash" folder with the recipe folder.

3. Rename the current PostRunWash.xml to OriginalPostRunWash.xml.
4. Copy the provided carryover decontamination PostRunWash-carryover.xml into the wash folder, and remove the "-carryover" extension from the name.

For more information on the recipe steps the MiSeq performs, see Table 1 on page 3.

Make the Carryover Wash the Default Post-Run Wash (Optional)

If you want the instrument default post -run wash to be the carryover decontamination wash (i.e., running a post-run wash directly from the home screen, or running a wash after opening MCS and before starting a run) you need to repeat steps 1-4 above for the c:\Illumina\MiSeqControlSoftware\Recipe\V1\Default\Wash folder and the c:\Illumina\MiSeq Control Software\Recipe\V2\Default\Wash. The software automatically defaults to the washes in these recipes unless the post-run wash is executed after the run is complete and before turning off or restarting MCS.

Prepare the Dilution of Sodium Hypochlorite

1. Prepare a fresh 1 ml solution of 0.01% Sodium Hypochlorite. *Make this solution fresh each day.*
For standard Clorox bleach (6% sodium hypochlorite), add 30 µl of bleach to 870 µl of PW1 or reagent grade water (1:30 dilution). **DO NOT** use tap water.
2. Dilute again by adding 50 µl of 1:30 dilution to 950 µl of PW1 or reagent grade water in a small MiSeq tube (Illumina PN: 15021932). Mix well by vortexing carefully (avoid splashing) or pipetting up and down with a P1000 set to 900 µl.

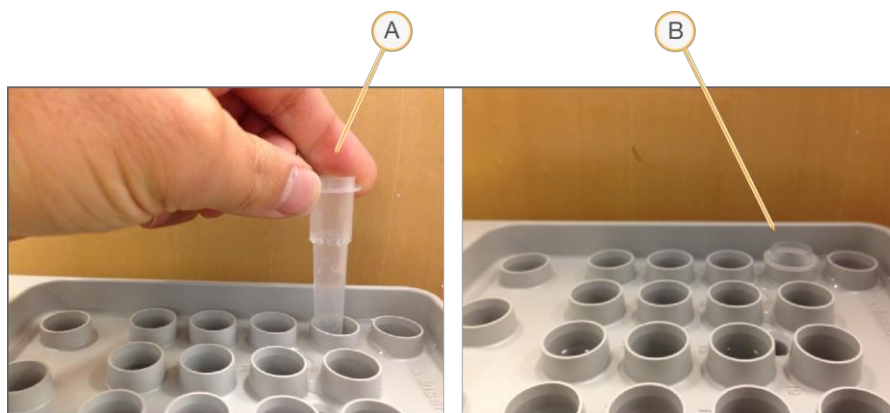
If using other stock concentrations of sodium hypochlorite, perform appropriate dilutions to achieve 1 ml of 0.01% sodium hypochlorite final.

Perform the Post-Run Wash

1. Fill a MiSeq wash tray with water as normally would be done for a post-run wash.
2. Insert the 0.01% sodium hypochlorite MiSeq tube into position 17 of the MiSeq wash tray. The tube displaces the water from position 17. Push the MiSeq tube down until the neck is flush with the tray in position 17.
3. Load the wash tray on to the instrument and perform the post-run wash.

After the wash is complete, a small amount of water will remain in the MiSeq tube in position 17. Total wash time takes approximately 30 minutes. Wash time includes the carryover wash as well as the default MiSeq post-run water wash.

Figure 1. Inserting the 0.01% Sodium Hypochlorite MiSeq Tube



- A. Insert the tube into position 17 of the MiSeq wash tray.
- B. Make sure the neck of the MiSeq tube is flush with the wash tray.

Recipe Details for PostRunWash-carryover.xml

The information in Table 1 describes the wash step sequence the MiSeq performs when it follows the PostRunWash-carryover.xml recipe. This series of recipe steps is intended to pump the decontamination solution through the system, and then uses multiple steps to deliver water to dilute the sodium hypochlorite solution in position 17 to minimal levels (as well as washing the common line). Following the decontamination steps, the recipe includes a standard MiSeq default post-run wash with optimized dispense speeds that takes approximately 10 minutes. The total time for both steps is ~30 minutes.

Table 1. Series of Recipe Steps the MiSeq Performs for a PostRunWash-carryover.xml

Step	Activity	Approx. NaOCl Dilution/ Approx. Total NaOCl Dilution
1	Prime system by pumping 1mL water from position 3	
2	Aspirate 1mL of carryover wash solution + 250uL air from position 17. 60uL of dead volume left behind	
3	Aspirate 1mL water from position 3 to push air and decontamination solution completely through the system to waste	
4	Dispense to flow cell (pump "backwards") 2.0mL of water from position 3 to position 17 in 250uL increments	30X / 30X
5	Aspirate 2mL of water from position 17 (and air) leaving 60uL dead volume behind, prime 1mL water from position 3 to push volume through system to waste	
6	Dispense to flow cell (pump "backwards") 2.0mL from position 3 to position 17 in 250uL increments	30X / 900X
7	Aspirate 2mL of water from position 17 (and air) leaving 60uL dead volume behind, prime 1mL water from position 3 to push volume through system to waste	
8	Dispense to flow cell (pump "backwards") 2.0mL from position 3 to position 17 in 250uL increments	30X / 27,000X
9	Continue with standard wash	