



Intermountain Forensics

SOP #

MNT-207

Revision #

01

Forensic DNA Technical Leader Approval

Issue Date

11/30/2020

MiSeq FGx Maintenance

1. Purpose

To describe the maintenance procedures for the MiSeq FGx instrument.

2. Summary

Regular maintenance is required post run, weekly, and on an as needed basis. Post run wash uses a bleach dilution and nuclease free water and is required before additional steps can be taken on the instrument. Weekly Maintenance washes and Standby washes require Tween 20 and nuclease free water.

3. Procedure

Maintenance Frequency

Activity	Daily	Weekly	As Needed
Post Run Wash	After Every Run		
Maintenance Wash		X	
Standby Wash			To Prepare for ≥ 7 days unused
Instrument Shutdown			X

Performing a Post-Run Wash

- Always perform an instrument wash after completing a sequencing run. Follow the software prompts to load the wash components and perform the wash. The post-run wash takes approximately 30 minutes.

NOTE: The post-run wash contains bleach. Do not use sodium hypochlorite for a maintenance wash or a standby wash.

- The software cannot proceed to the run setup steps for a subsequent run until a wash has been performed.

NOTE: Leave the used flow cell on the instrument. A flow cell must be loaded on the instrument to perform an instrument wash.

Procedure

- Prepare fresh 1:30 bleach wash solution with nuclease-free water by combining 30 μ l of 6% bleach with 870 μ l nuclease-free water.
- Add 50 μ l of the 1:30 bleach dilution to 950 μ l of nuclease-free water in a MiSeq wash tube or "Optional" tube removed from a reagent cartridge.

NOTE: The percentage of sodium hypochlorite is important. If the concentration is too high, it can make cluster generation fail in subsequent runs. If 6% sodium hypochlorite is not available, make a 1 ml solution of 0.01% sodium hypochlorite in nuclease-free water.

- Fill each reservoir of the wash tray with water except for position 17.
- Insert the tube containing the bleach wash solution into position 17 of the wash tray until the neck of the tube is flush with the tray.
- Add 350 ml water to the 500 ml wash bottle.



Intermountain Forensics

SOP #

MNT-207

Revision #

01

Forensic DNA Technical Leader Approval

Issue Date

11/30/2020

NOTE: Inserting the wash tube into any position other than position 17 can make cluster generation fail in a subsequent run and damage the MiSeq FGx fluidics system.

8. From the post-run wash screen, select **Start Wash**. The software automatically raises the sippers in the reagent chiller. Wait several seconds to make sure that the sippers are fully raised before continuing.
9. Open the reagent compartment door and reagent chiller door and slide the used reagent cartridge from the chiller.
10. Slide the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.
11. Raise the sipper handle in front of the SBS Solution (PR2) bottle and waste bottle until it locks into place.
12. Remove the SBS Solution (PR2) bottle and replace it with the wash bottle.

NOTE: Discard the SBS Solution (PR2) bottle after each run. Do not reuse any remaining solution.

13. Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
14. Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
15. Close the reagent compartment door.
16. Select **Next**. The post-run wash begins.
17. When the wash is complete, leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument. A small amount of wash solution remains in the wash tube in position 17.

NOTE: The sippers remain in the down position, which is normal. Leave the unused wash solution in the wash tray and wash bottle to prevent the sippers from drying out and air from entering the system.

Performing a Maintenance Wash

18. Perform a maintenance wash every 7 days to ensure optimal performance. The maintenance wash includes a series of three wash steps using a wash solution of water mixed with Tween 20. Allow approximately 90 minutes to complete the wash.

Procedure

19. Make sure that a used flow cell is loaded on the instrument.
20. From the Welcome screen, select **Perform Wash**.
21. From the Perform Wash screen, select **Maintenance Wash**. The software automatically raises the sippers in the reagent chiller.

Perform First Wash

22. Prepare fresh wash solution with Tween 20 and water as follows:
 - a. Add 5 ml 100% Tween 20 to 45 ml water. These volumes result in 10% Tween 20.
 - b. Add 25 ml 10% Tween 20 to 475 ml water. These volumes result in a 0.5% Tween 20 wash solution.
 - c. Invert several times to mix.
23. Fill each reservoir of the wash tray with wash solution.
24. Add remaining wash solution (at least 350 ml) to the 500 ml wash bottle.
25. Load the wash tray and wash bottle onto the instrument:
 - a. Open the reagent compartment door and reagent chiller door and slide the used reagent cartridge or wash tray from the chiller.
 - b. Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
 - c. Raise the sipper handle in front of the SBS Solution (PR2) bottle and waste bottle until it locks into place and replace the SBS Solution (PR2) bottle with the wash bottle.



Intermountain Forensics

SOP #

MNT-207

Revision #

01

Forensic DNA Technical Leader Approval

Issue Date

11/30/2020

NOTE: Discard the SBS Solution (PR2) bottle after each run. Do not reuse any remaining solution.

- d. Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
- e. Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
- f. Close the reagent compartment door.

26. Select **Next** to begin the first wash.

Perform Second Wash

NOTE: Always use fresh wash solution for each wash step. Reusing wash solution from the previous wash can return waste to the fluidics lines.

27. Discard the wash solution remaining in the wash tray and repeat the first wash steps with wash solution from the wash bottle.

Perform Final Wash

28. When the second wash is complete, remove the wash tray and wash bottle, and discard the remaining wash solution.
29. Refill the wash components with water.
 - a. Add 6 ml to each reservoir of the wash tray.
 - b. Add 350 ml to the 500 ml wash bottle.
30. Load the wash tray and wash bottle, as follows:
 - a. Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
 - b. Load the wash bottle and slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
 - c. Close the reagent compartment door.

After the Wash

31. When the wash is complete, leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument.

NOTE: The sippers remain in the down position, which is normal. Leave the unused wash solution in the wash tray and wash bottle to prevent the sippers from drying out and air from entering the system.

Performing a Standby Wash

32. If there are no plans to use the instrument within the next seven days, prepare the instrument to sit idle by performing a standby wash. A standby wash performs two consecutive washes that flush each position of any remaining reagents or salt accumulation. Each wash takes approximately 60 minutes. Allow approximately 2 hours to complete the standby wash.
33. When the standby wash is complete, the instrument is in standby mode and a message appears on the Welcome screen stating the status of the instrument. When the instrument is in standby mode, a maintenance wash must be performed before a sequencing run can be initiated.

NOTE: Verogen recommends repeating the standby wash every 30 days that the instrument remains idle.

Procedure

34. Make sure that a used flow cell is loaded on the instrument.
35. From the Welcome screen, select **Perform Wash**.
36. From the Wash Options screen, select **Standby Wash**. The software automatically raises the sippers in the reagent chiller.

Perform First Wash

37. Prepare fresh wash solution with Tween 20 and nuclease-free water as follows:
 - a. Add 5 ml 100% Tween 20 to 45 ml nuclease-free water. These volumes result in 10% Tween 20.
 - b. Add 25 ml 10% Tween 20 to 475 ml nuclease-free water. These volumes result in a 0.5% Tween 20 wash solution.
 - c. Invert several times to mix.



Intermountain Forensics

SOP #

MNT-207

Revision #

01

Forensic DNA Technical Leader Approval

Issue Date

11/30/2020

38. Prepare the wash components with fresh 0.5% Tween 20 wash solution, as follows:
 - a. Add 6 ml wash solution to each reservoir of the wash tray.
 - b. Add 350 ml wash solution to the 500 ml wash bottle.
39. Load the wash tray and wash bottle onto the instrument:
 - a. Open the reagent compartment door and reagent chiller door and slide the used reagent cartridge or wash tray from the chiller.
 - b. Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
 - c. Raise the sipper handle in front of the SBS Solution (PR2) bottle and waste bottle until it locks into place and replace the SBS Solution (PR2) bottle with the wash bottle.

NOTE: Discard the SBS Solution (PR2) bottle after each run. Do not reuse any remaining solution.

- d. Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
 - e. Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
 - f. Close the reagent compartment door.
40. Select **Next**. The first wash begins.

Perform Second Wash

NOTE: Always use fresh wash solution for each wash step. Reusing wash solution from the previous wash can return waste to the fluidics lines.

41. When the first wash is complete, remove the wash tray and wash bottle, and discard the remaining wash solution.
42. Refill the wash components with water:
 - a. Add 6 ml to each reservoir of the wash tray.
 - b. Add 350 ml to the 500 ml wash bottle.
43. Load the wash tray and wash bottle, as follows:
 - a. Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
 - b. Load the wash bottle and slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
 - c. Close the reagent compartment door.
44. Select **Next**. The second wash begins.

After the Wash

45. When the wash is complete, leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument.

NOTE: The sippers remain in the down position, which is normal. Leave the unused wash solution in the wash tray and wash bottle to prevent the sippers from drying out and air from entering the system.

Shutting Down the Instrument

NOTE: It is best to leave the instrument on at all times. However, if the instrument must be turned off, use the following procedure to shut down Windows and prepare the fluidics lines.

46. Perform a maintenance wash.
47. Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
48. Close the reagent compartment door.
49. From the Manage Instrument screen, select **Shut Down**. This command shuts down the software.
50. Toggle the power switch to the OFF position.



Intermountain Forensics

SOP #

MNT-207

Revision #

01

Forensic DNA Technical Leader Approval

Issue Date

A handwritten signature in black ink, appearing to read "Dawn [unclear]".

11/30/2020

NOTE: Any time the instrument is turned off, wait a minimum of 61 seconds before turning the power switch back to the ON position.

4. *References*

ForenSeq™ DNA Signature Prep Reference Guide

5. *Definitions*

Clarify any terms used within the document