Infinium HD
FFPE Restore Protocol

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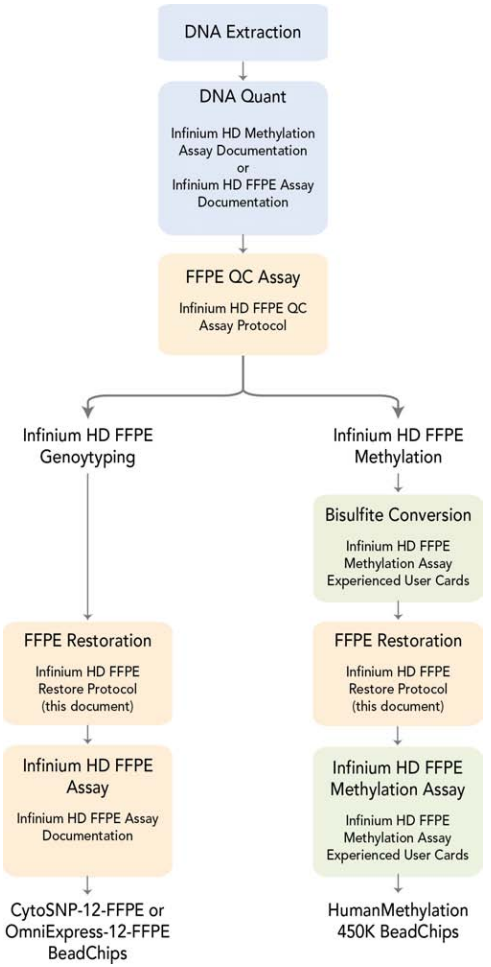
Infinium HD FFPE Workflow

The following figure illustrates the correct workflows for both Infinium HD FFPE assays (genotyping and methylation). This document describes the FFPE Restoration step of the workflow and assumes that all previous steps in the workflow have been completed.

Figure 1 FFPE Workflow, FFPE Restoration Step



Introduction

The Infinium HD FFPE Restore protocol restores degraded FFPE DNA to a state that is amplifiable by the Infinium HD FFPE genotyping or Infinium HD FFPE methylation whole genome amplification protocol.

DNA samples suitable for DNA Restoration are selected using the Illumina Infinium HD FFPE QC assay.

NOTE

Make sure your centrifuge equipment has buckets deep enough to hold the Zymo-Spin MIDI plate assembly. Some types of centrifuge equipment do not provide buckets deep enough or block access to two stacked plates.

Estimated Time

Hands-on time: ~2 hours

Incubation time: 2 hours

Consumables

Item	Quantity	Storage	Supplied By
Amp Mix Restore Reagent (AMR)	1 tube per 24 FFPE DNA samples	-15° to -25°C	Illumina
Convert Master Mix Reagent (CMM)	1 tube per 24 FFPE DNA samples	-15° to -25°C	Illumina
Elution Restore Buffer Reagent (ERB)	1 tube per 24 FFPE DNA samples	-15° to -25°C	Illumina
Primer Pre Restore Reagent (PPR)	1 tube per 24 FFPE DNA samples	-15° to -25°C	Illumina
Pre Restore (PRS) plate, 96-well 0.8 ml microtiter plate (MIDI)	1 plate	-15° to -25°C	User
Restore (RST) plate, 96-well 0.8 ml microtiter plate (MIDI)	1 plate	-15° to -25°C	User
Zymo™ Purification Kit (ZR-96 DNA Clean & Concentrator™-5)	1 plate per 48 FFPE DNA samples		User
MSA5 plate, 96-well 0.8 ml microtiter plate (MIDI)	1 plate		User
0.1N NaOH	4 µl per well containing sample	2° to 8°C	User
Cap mat	3		User
Troughs	Up to 7 (if using a multi-channel pipette)		User
Foil Adhesive Seal			User
DiH_2O	4 µl per well containing sample	-15° to -25°C	User
2.5 L Ice bucket	1		User

NOTE

Thaw all reagents completely at room temperature and allow to equilibrate. Once thawed, gently invert each tube several times to thoroughly mix the reagent. Pulse centrifuge each tube to 280 xg to eliminate bubbles and collect reagent at the bottom of the tube.

Preparation

- Prepare the wash buffer from the Zymo Purification Kit. Dispense 192 ml 100% ethanol to the 48 ml of DNA Wash Buffer concentrate as described in the Zymo instruction manual.
- Preheat one heat block to 37°C and one heat block to 95°C and allow the temperature to equilibrate.
- Apply a PRS barcode label to a new MIDI plate.
- Thaw the PPR and AMR reagent tubes to room temperature. Vortex to mix the contents. Use 1 tube each per 24 FFPE DNA samples.

Steps

1 Prepare the PRS plate by transferring 4 to 8 µl of sample genomic DNA to each well to reach a final DNA amount of at least 100 ng.

NOTE
If available, up to 250 ng may be used.

NOTE
If you are using bisulfate converted samples for the Infinium HD FFPE methylation assay, take the full elution volume (8 µl) from the bisulfate conversion purification step.

2 Prepare the 0.1N NaOH solution:
   a Dispense 4 µl of 0.1N NaOH to each well containing genomic DNA.
   b Incubate at room temperature for 10 minutes.

3 Dispense 34 µl of PPR to each sample well in the PRS plate.

4 Dispense 38 µl of AMR to each sample well in the PRS plate; seal the plate with a cap mat.

5 Invert the plate ten times and then centrifuge at 280 xg for 1 minute.

6 Place the sealed PRS plate on the preheated heat block and close the lid. Incubate the plate at 37°C for 1 hour.

7 At the end of the 1-hour incubation, remove and thaw the ERB and CMM reagent tubes to room temperature. Vortex to mix the contents. Use 1 tube each per 24 FFPE DNA samples.

8 Remove the PRS plate from the heat block and centrifuge at 280 xg for 1 minute.

NOTE
If you are only using one heat block, set the temperature to 95°C.

9 Carefully remove the cap mat.

10 From the Zymo Purification Kit, take the Zymo-Spin™ I-96 Plate and place it on the Collection Plate.

11 Add 7 volumes (560 µl) of Zymo DNA Binding Buffer to each volume of DNA sample in the PRS plate.

NOTE
When using multi-channel pipettes, be careful to avoid cross-contamination between wells.

12 Pipette mix 5 times and then transfer sample mixtures to the wells of the Zymo-Spin I-96 Plate mounted on a Collection Plate.

13 Centrifuge the Zymo-Spin I-96 Plate at 2250 xg for 2 minutes. Discard the flow-through from the Collection Plate.

14 Add 600 µl of Zymo Wash Buffer (with ethanol added) to each well of the Zymo-Spin I-96 Plate mounted on a Collection Plate.

NOTE
The volume of Zymo Wash Buffer provided in this kit is limited. If you are using multi-channel pipettes and troughs keep all remaining Zymo Wash Buffer for use at step 33.

15 Centrifuge the Zymo-Spin I-96 Plate at 2250 xg for 2 minutes. Discard the flow-through from the Collection Plate.

16 Apply an RST barcode label to a new MIDI plate.

17 Transfer the Zymo-Spin I-96 Plate onto the RST plate and dispense 13 µl of ERB directly to the column matrix in each well.

18 Incubate the plate at room temperature for 5 minutes.

19 Centrifuge the Zymo-Spin I-96 RST plate assembly at 2250 xg for 1 minute to elute the DNA. There will be 10 µl of DNA solution generated.

NOTE
If you are processing less than 96 samples the unused wells can be used for following purification steps.

20 Seal the RST plate containing 10 µl of eluted DNA with a foil adhesive seal. Incubate for 2 minutes at 95°C in the heat block.

NOTE
Do not use a heat sealer to seal the RST plate. This may prevent the use of cap mats for plate sealing later in the protocol. We recommend using foil adhesive seal instead of a heat sealer.

21 During the 2-minute incubation, fill the 2.5 L ice bucket with ice.

22 Immediately after the 2-minute incubation, transfer the RST plate to the ice for 5 minutes. Press the RST plate into the ice to make sure the bottom of all wells contact the ice.

NOTE
If you are only using one heat block, set the temperature to 37°C. Make sure the temperature reaches 37°C before you begin the 1-hour incubation in step 26.

23 Leaving the RST plate on ice, remove the foil seal and add 10 µl of CMM to each well. Seal the plate with a new cap mat.

24 Vortex the RST plate for 1 minute at 1600 rpm.

25 Centrifuge the RST plate for 1 minute at 280 xg.

26 Incubate the RST plate at 37°C for 1 hour.

27 After the 1-hour incubation, centrifuge the RST plate for 1 minute at 280 xg.

28 Take a new Zymo-Spin I-96 Plate and place it on the Collection Plate.

NOTE
If you are processing 96 samples use a new plate from Zymo Purification Kit. If you're processing 48 samples or less use the unused wells from the previous purification step.

29 Remove the cap mat from the RST plate.

30 Add 7 volumes (140 µl) of Zymo DNA Binding Buffer to each volume of DNA sample in the RST plate.

NOTE
When using multi-channel pipettes, be careful to avoid cross-contamination between wells.

31 Pipette mix 5 times and then transfer sample mixtures to the wells of the Zymo-Spin I-96 Plate mounted on a Collection Plate.

32 Centrifuge the Zymo-Spin I-96 Plate at 2250 xg for 2 minutes. Discard the flow-through from the Collection Plate.

33 Add 600 µl of Zymo Wash Buffer (with ethanol added) to each well of the Zymo-Spin I-96 Plate mounted on a Collection Plate.

34 Centrifuge the Zymo-Spin I-96 Plate at 2250 xg for 2 minutes. Discard the flow-through from the Collection Plate.

35 Apply a MSA5 barcode label to a new MIDI plate.

36 Transfer the Zymo-Spin I-96 Plate onto the MSA5 plate and dispense 10 µl of DiH_2O directly to the column matrix in each well.

37 Incubate the plate at room temperature for 5 minutes.

38 Centrifuge the Zymo-Spin I-96 Plate at 2250 xg for 1 minute to elute the DNA. There will be 8 µl of purified DNA solution in the MSA5 plate.

NOTE
The MSA5 plate will be used in the Infinium HD FFPE genotyping or Infinium HD FFPE methylation assay as a DNA plate.

39 Do one of the following:

- Proceed to the Infinium HD FFPE genotyping or Infinium HD FFPE methylation assay documentation to begin processing your restored DNA.
- Seal the MSA5 plate with adhesive film and store at -20°C if you do not plan to proceed to the Infinium HD FFPE genotyping or Infinium HD FFPE methylation assay immediately.

Technical Assistance

For technical assistance, contact Illumina Customer Support.

Table 1 Illumina General Contact Information

Illumina Website	http://www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at http://www.illumina.com/msds.

Product Documentation

If you require additional product documentation, you can obtain PDFs from the Illumina website if PDFs are available. Go to http://www.illumina.com/support/documentation.ilmn. When you click on a link, you will be asked to log in to iCom. After you log in, you can view or save the PDF. To register for an iCom account, please visit https://icom.illumina.com/Account/Register.

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