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QIAGEN GeneRead[®] UMI Advanced Sequencing Q Kit Handbook

For preparation of DNA sequencing for NGS applications
using the QIAGEN GeneReader[®] instrument

For research use only. Not for use in diagnostic procedures.



185251



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Contents

Kit Contents	3
Storage	6
Intended Use	7
Safety Information	7
Quality Control	8
Equipment and Reagents to be Supplied by User	9
Introduction	11
Principles and procedures	11
Protocol: Sequencing Primer Hybridization for Bead Samples Stored Less than 72 Hours ..	13
Protocol: Sequencing Primer Rehybridization for Hybridized Bead Samples Stored Less than 48 Hours	16
Things to do before starting	17
Protocol: Flow Cell Preparation	20
Protocol: Reagent Preparation	27
Protocol: Loading and Running the GeneReader	34
Protocol: Unloading Reagents and Flow Cells	44
Troubleshooting Guide	47
Appendix: Performing Routine Maintenance after a Run	49
Routine maintenance (DI water wash)	49
Weekly maintenance	52
Ordering Information	63
Document Revision History	64

Kit Contents

The GeneRead® Advanced Sequencing Q Kit contains reagents, 3 disposable flow cells, and wash buffers. Sufficient reagents are provided to sequence 3 flow cells with up to 150 (150+7) cycles of sequencing on each flow cell.

Components of the GeneRead UMI Advanced Sequencing Q Kit are delivered in 4 boxes:

- GeneRead UMI Advanced Sequencing Q Buffers — reagents in 50 ml and 2 ml tubes
- GeneRead UMI Advanced Sequencing Q Add-Ons — reagents in 50 ml and 2 ml tubes
- GeneRead UMI Advanced Sequencing Q flow cell — flow cells in aluminum foil packages, adhesive tape, and reagent in 2 ml tubes
- GeneRead UMI Advanced Sequencing Q Accessories (3) — reagents in 3 ml and 2 ml tubes
- GeneRead UMI Advanced Sequencing Q Wash Buffers (9) — wash buffers in 1 liter bottles, **ordered separately**; this kit contains sufficient reagents to sequence 9 flow cells in total

GeneRead UMI Advanced Sequencing Q Buffers	(3)
Catalog no.	1108487*
Component (lid label color)	Volume
Cleave Premix (green)	3 x 16.3 ml
FPF Buffer (purple)	6 x 16.3 ml
Extend Additive P (white)	1 x 140 µl
Buffer P (white)	3 x 2 ml
Buffer T (white)	3 x 2 ml

* Not for individual sale; to order reagents, use cat. no. 185251.

GeneRead UMI Advanced Sequencing Q Add-Ons	(3)
Catalog no.	1108488*
Component (color)	Volume
Extend Premix A1 UMI (pink lid label)	3 x 15.97 ml
Extend A2 (red cap)	3 x 162 µl
Extend Premix B1 UMI (light blue lid label)	3 x 15.97 ml
Extend B2 (blue cap)	3 x 162 µl
Pol Extend (clear cap)	3 x 354 µl
Image Premix (yellow lid label)	3 x 16.3 ml
Sequencing Primer (blue cap)	1 x 20 µl

* Not for individual sale; to order reagents, use cat. no. 185251.

GeneRead UMI Advanced Sequencing Q Flow Cell	(3)
Catalog no.	1108489*
Component	Quantity
Flow Cell	3
Sealing Tape	3
Cleave Additive M	3

* Not for individual sale; to order reagents, use cat. no. 185251.

GeneRead UMI Advanced Sequencing Q Accessories	(3)
Catalog no.	1115135*
Component	Volume
EDC	3 x 3 ml
NHS	3 x 3 ml
Buffer P	3 x 1.6 ml

* Not for individual sale; to order reagents, use cat. no. 185251.

GeneRead UMI Advanced Sequencing Q Wash Buffers	(9)
Catalog no.	185905*
Component	Volume
Wash Buffer 9	1 x 650 ml
Wash Buffer 11	1 x 1000 ml
Maintenance Wash Buffer	1 x 900 ml

* Must be ordered separately.

Storage

The GeneRead UMI Advanced Sequencing Q Kit is delivered in 4 boxes.

The buffers in the GeneRead UMI Advanced Sequencing Q Buffers box are stored at room temperature (15–25°C).

The reagents in the GeneRead UMI Advanced Sequencing Q Add-Ons box are shipped on dry ice and should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer (not frost free).

The flow cells and Cleave Additive M in the GeneRead UMI Advanced Sequencing Q Flow Cell box are shipped on cool packs and should be stored immediately upon receipt at 2–8°C.

The reagents in the GeneRead UMI Advanced Sequencing Q Accessories box are shipped on dry ice. EDC and NHS should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer (not frost free). Buffer P is stored at room temperature.

The wash buffers ordered separately in the GeneRead UMI Advanced Sequencing Q Wash Buffers box are stored at room temperature.

Kit components should be protected from light exposure. We recommend storing all reagents in the dark. When stored correctly, the GeneRead UMI Advanced Sequencing Q Kit is good until the expiration date printed on the QC label inside the kit lid.

Intended Use

The QIAGEN GeneRead UMI Advanced Sequencing Q Kit (cat. no. 185251) is intended for research use only and not intended for use in diagnostic procedures.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products adhere to the NIH guidelines that have been developed for recombinant DNA experiments or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs). These are available online in PDF format at www.qiagen.com/safety where you can find, view and print the SDS for each QIAGEN kit and kit component.

Make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.

<p>WARNING</p> 	<p>Risk of personal injury</p> <p>Do not add bleach or acidic solutions to the sample preparation waste.</p>
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All buffers and reagents should be handled using suitable personal protective equipment including disposable gloves, a lab coat, and eye protection. Disposal of wastes must be in accordance with all national, state, and local health and safety regulations.

Cleave Additive M is a strong reducing agent and should be handled with care. If liquid containing this agent is spilt, clean with suitable laboratory detergent and water.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the GeneRead UMI Advanced Sequencing Q Kit is tested against predetermined specifications to ensure consistent product quality.

Equipment and Reagents to be Supplied by User

The following materials are required but are not included in this kit.

- Bead samples

For preparing enriched beads, use the GeneRead Clonal Amp Q Kit (cat. no. 185001) and follow the instructions in the *GeneRead Clonal Amp Q Handbook*, www.qiagen.com/HB-2027.

Specific laboratory equipment

- GeneReader® instrument (cat. no. 9002312)
- **Optional:** Spectrophotometer capable of reading the optical density (OD) at 550 nm or 600 nm and corresponding labware (plates or cuvettes)

Note: This equipment is not necessary if bead yield is determined using the color chart supplied in the GeneRead Clonal Amp Q Kit.

General laboratory equipment and materials

- GeneRead QIAcube® for automated emulsion making
- Dedicated pipettes: 1–10 µl, 10–100 µl, and 100–1000 µl
- Nuclease-free, sterile PCR pipette tips with hydrophobic filters
- 2 ml capped LoBind® tubes (Eppendorf® cat. no. 022431048)
- Two thermomixers capable of heating and mixing
- Vortex mixer
- Microcentrifuge
- Magnetic rack (equivalent to Invitrogen® DynaMag™-2 rack, cat. no. 12321D)

-
- Low-retention filter tips for depositing beads into flow cells (USA Scientific 20 μ l TipOne® RPT, cat. no. 1180-1810; Sarstedt® Biosphere™, cat. no. 70.760.213; or equivalent)
 - Alcohol wipes or lint-free wipes
 - 0.2 M NaOH solution
 - Laboratory timer
 - Reagent tubes, 50 ml
 - Deionized water

Introduction

Next-generation sequencing (NGS) is a driving force for numerous applications, including cancer research, stem cell research, metagenomics, population genetics, and medical research. The GeneRead UMI Advanced Sequencing Q Kit consists of easy-to-use buffers and reagents that need to be mixed before loading onto the GeneReader instrument. This format saves time and reduces handling errors. Optimized enzyme and buffer compositions ensure high-quality sequencing performance, suited for research applications that required precise data insight. Streamlined GeneRead sequencing protocols enable straightforward automation of sequencing-by-synthesis (SBS) technology on the GeneReader.

Principles and procedures

The QIAGEN GeneReader Platform uses an SBS approach for deciphering the DNA sequence of specific gene targets. Each cycle of SBS involves 3 major steps: DNA extension, signal detection, and cleavage.

During the extension step, the DNA polymerase incorporates reversible, terminated fluorescent-labeled deoxyribonucleotide triphosphates (dNTPs) to the DNA template. The reversible terminators enable the addition of only one fluorescence-labeled nucleotide at a time to the growing strand of the DNA template. This step is followed by a signal detection step carried out by the GeneReader scanner and imaging system, where signal from each bead is recorded. The cycle is completed with a cleavage step, in which the fluorescence dyes and the terminators are cleaved from the incorporated nucleotides, allowing the next cycle of incorporation. This technique ensures highly accurate and cost-effective NGS.

The GeneReader sequencing workflow includes the following processes:

1. "Protocol: Sequencing Primer Hybridization", pages 13 and 16.
2. "Protocol: Flow Cell Preparation", page 20.
3. "Protocol: Reagent Preparation", page 27.
4. "Protocol: Loading and Running the GeneReader", page 34.
5. "Protocol: Unloading Reagents and Flow Cells", page 44.

All protocols are essential for completing a run on the GeneReader and should be followed as indicated in this handbook. Refer to the *GeneReader APF/HP Configuration User Manual*, www.qiagen.com/HB-2503, for additional instrument information.

Protocol: Sequencing Primer Hybridization for Bead Samples Stored Less than 72 Hours

Important: This protocol is only for use with bead samples that have not been hybridized to the sequencing primer and have been stored at 4°C for up to 72 h.

Once the enriched beads are recovered from the GeneRead QIAcube after clonal amplification (see the *GeneRead Clonal Amp Q Handbook*), they can be stored at 4°C for up to 72 h.

Once the sequencing primer has been hybridized to the beads, they can only be stored at 4°C for up to 48 h. In this case, it is necessary to remove and rehybridize the sequencing primer immediately prior to sequencing.

If the samples have already been hybridized to the sequencing primer and then stored at 4°C for up to 48 h, see “Protocol: Sequencing Primer Rehybridization for Hybridized Bead Samples Stored Less than 48 Hours”, page 16, for sequencing primer removal and rehybridization.

Note: Do not use beads that have been stored longer than 72 h.

Important points before starting

- Pipettes should be used to measure volumes accurately.
- All volumes listed are for 1 sample.
- The acceptable range of beads required for sequencing primer hybridization is 140 million to 300 million beads.
- This protocol is for unhybridized beads samples that are stored in Buffer T for less than 72 h at 4°C.

- For primer-hybridized bead samples that are stored less than 48 h, see “Protocol: Sequencing Primer Rehybridization for Hybridized Bead Samples Stored Less than 48 Hours”, page 16.
- Additional Buffer T is provided in the GeneRead Clonal Amp Q Kit.

Things to do before starting

- After the clonal amplification workflow, evaluate bead concentration by following the procedure “Determination of Bead Concentration using OD” in the *GeneRead Clonal Amp Q Handbook* or by using the color chart provided in the GeneRead Clonal Amp Q Kit.
- Power on the GeneReader by pressing the power switch at the back of the instrument.
- Set one thermomixer to 95°C and a second thermomixer to 25°C.
- Thaw the sequencing primer (provided as 1 mM stock solution) and dilute with Buffer T to a final concentration of 10 µM (1:100 dilution). Each sample requires 100 µl 10 µM sequencing primer, which must be prepared fresh from the stock.

Sequencing primer hybridization

Important: This procedure is only for enriched beads samples that have not been hybridized and that have been stored at 4°C for less than 72 h.

1. Place the tube with the enriched bead sample on the magnetic rack and wait for at least 30 s for a pellet to form.
2. Remove and discard the supernatant.
3. Add 100 µl 10 µM sequencing primer.

Note: Dilute 1 mM sequencing primer stock to 10 µM in Buffer T before use.

4. Pulse-vortex and then pulse-centrifuge the tube.
5. Incubate for 5 min at 95°C on the thermomixer at 950 rpm.
6. Incubate for 10 min at 25°C on the thermomixer at 950 rpm.

7. Vortex for 5 s and then pulse-centrifuge the tube.

Note: After this step, samples can be stored at 4°C for up to 48 h.

Wash beads with Buffer T

8. Place the tube on the magnetic rack and wait for at least 30 s for a pellet to form.
9. Remove and discard the supernatant.
10. Add 500 µl Buffer T.

Wash beads with Buffer P

11. Place the tube on the magnetic rack and wait for at least 30 s for a pellet to form.
12. Remove and discard the supernatant.
13. Add 500 µl Buffer P.
14. Pulse-vortex and then pulse-centrifuge the tube.
15. Repeat steps 11–14 once.
16. Proceed with “Protocol: Flow Cell Preparation” on page 20.

Protocol: Sequencing Primer Rehybridization for Hybridized Bead Samples Stored Less than 48 Hours

If the sequencing primer has been hybridized to the beads, they can only be stored at 4°C for up to 48 h. In this case, it is necessary to remove and rehybridize the sequencing primer immediately prior to sequencing.

This protocol is for bead samples that have been hybridized with the sequencing primer after clonal amplification and then stored for up to 48 h. If the samples have not been hybridized with the sequencing primer and have been stored for less than 72 h, see “Protocol: Sequencing Primer Hybridization for Bead Samples Stored Less than 72 Hours”, page 13.

Note: Do not use hybridized beads that have been stored longer than 48 h.

Important points before starting

- Pipettes should be used to measure volumes accurately.
- All volumes listed are for 1 sample.
- The acceptable range of beads required for sequencing primer hybridization is 140 million to 300 million beads.
- This protocol is for samples that have been hybridized to the sequencing primer and stored for less than 48 h.
- Additional Buffer T is provided in the GeneRead Clonal Amp Q Kit.

Things to do before starting

- After the clonal amplification workflow, evaluate bead concentration by following the procedure “Determination of Bead Concentration using OD” in the *GeneRead Clonal Amp Q Handbook* or by using the color chart provided in the GeneRead Clonal Amp Q Kit.
- Power on the GeneReader by pressing the power switch at the back of the instrument.
- Set one thermomixer to 95°C and a second thermomixer to 25°C.
- Thaw sequencing primer (provided as 1 mM stock solution) and dilute with Buffer T to a final concentration of 10 µM (1:100 dilution). Each sample requires 100 µl 10 µM sequencing primer, which must be prepared fresh from the stock.

Denaturation with NaOH and primer rehybridization

1. Place the tube on the magnetic rack and wait for at least 30 s for a pellet to form.
2. Remove and discard the supernatant.
3. Add 500 µl 0.2 M NaOH solution.
4. Vortex for 5 s, and then pulse-centrifuge the tube.
5. Incubate for 5 min at room temperature off the magnet.
6. Place the tube on the magnetic rack and wait for at least 30 s for a pellet to form.
7. Remove and discard the supernatant.
8. Add 500 µl Buffer T.
9. Vortex for 5 s, and then pulse-centrifuge the tube.
10. Place the tube on the magnetic rack and wait for at least 30 s for a pellet to form.
11. Remove and discard the supernatant.
12. Add 500 µl Buffer T.
13. Vortex for 5 s, and then pulse-centrifuge the tube.
14. Place the tube with the enriched bead sample on the magnetic rack and wait for at least 30 s for a pellet to form.

15. Remove and discard the supernatant.

16. Add 100 μ l 10 μ M sequencing primer.

Note: Dilute 1 mM sequencing primer stock to 10 μ M in Buffer T before use.

17. Pulse-vortex and then pulse-centrifuge the tube.

18. Incubate for 5 min at 95°C on the thermomixer at 950 rpm.

19. Incubate for 10 min at 25°C on the thermomixer at 950 rpm.

20. Vortex for 5 s and then pulse-centrifuge the tube.

Note: After this step, samples can be stored at 4°C for up to 48 h.

Beads concentration measurement

Important: This procedure is only for hybridized samples stored less than 48 h.

21. Place the tube on the magnetic rack and wait for at least 30 s for a pellet to form.

22. Remove and discard the supernatant.

23. Add 500 μ l Buffer T.

24. Pulse-vortex the tube.

25. Either follow the protocol “Determination of Bead Concentration using OD” in the *GeneRead Clonal Amp Q Handbook* or refer to the Color Chart provided in the GeneRead Clonal Amp Q Kit.

Important: If the total amount of beads is in the range of 140 million to 300 million, proceed to step “Wash beads with Buffer P”. If the amount is outside the recommended range, QIAGEN is unable to guarantee optimal results.

Wash the beads with Buffer P

26. Place the tube on the magnetic rack and wait for at least 30 s for a pellet to form.
27. Remove and discard the supernatant.
28. Add 500 μ l Buffer P.
29. Pulse-vortex and then pulse-centrifuge the tube.
30. Repeat steps 26–29 once.
31. Proceed with “Protocol: Flow Cell Preparation”, page 20.

Protocol: Flow Cell Preparation

Important points before starting

- Pipettes should be used to measure volumes accurately.
- Following sequencing primer hybridization, samples must be transferred to Buffer P before proceeding with flow cell preparation. If samples are stored in Buffer T, binding will not occur.
- Reagent preparation (“Protocol: Reagent Preparation”, page 27) is performed during incubation step of this protocol.
- A USA Scientific 20 µl TipOne (cat. no. 1180-1810), Sarstedt Biosphere 2–20 µl (cat. no. 70.760.213), or equivalent pipette is recommended for depositing beads into the flow cell.

Things to do before starting

- Remove flow cells (cat. no. 1107269) from storage at 2–8°C.
- Samples must be hybridized with the sequencing primer and in Buffer P before depositing beads.

Flow cell setup

There are 3 options to generate a flow cell setup in the GeneReader NGS System:

- Option 1 : QIAGEN Clinical Insight (QCI®) Analyze
- Option 2: GeneRead Link
- Option 3: GeneReader software

Note: Options 1 and 2 are required in combination for the automatic flow cell setup procedure (“Automatically import flow cells”, page 38), whereas Option 3 is required for the manual flow cell setup (“Manually import flow cells”, page 39).

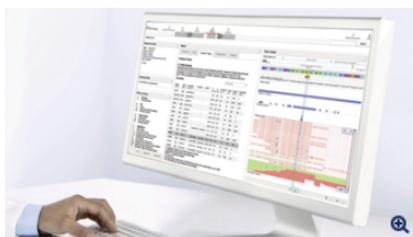
Option 1: QCI Analyze web interface

QCI Analyze is a locally installed software with a browser-based interface for analyzing NGS data. It comes fully integrated with the QIAGEN GeneReader and GeneRead Link; flow cell plans have been preincluded and can be set up using either the GeneRead Link or the QCI Analyze built-in GeneRead Planner. QCI Analyze offers automated data import and start of secondary analysis using preinstalled analysis workflows tailored for the QIAGEN GeneReader panels.

For information on how to set up a flow cell using QCI Analyze, please refer to the *QIAGEN Clinical Insight Analyze User Manual* found in www.qiagen.com/QCI-Analyze > **Product Resources** > **Software User Guides**:

QIAGEN Clinical Insight (QCI) Analyze

 Print



The bioinformatic complement to the GeneReader NGS System

- Optimized easy-to-use, pre-designed secondary data analysis pipelines
- Role-based permissions for running, editing, reporting and customizing workflows
- Fast and reliable globally-recognized algorithms
- Unique user capability to filter, confirm or reject variants within the review process
- Concise transparent reporting

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Option 2: GeneRead Link web interface

The GeneRead Link QIAGEN middleware software provides bidirectional connectivity with a laboratory information management system and specific QIAGEN instruments as well as additional software used within the GeneReader NGS System workflow.

For more information on how to generate a flow cell set up, please refer to the GeneRead Link User Manual found in www.qiagen.com/GeneRead-Link > **Product Resources** > **Software User Guides**:

GeneRead Link Software

 Print



For automated data transfer between the GeneReader NGS System and LIMS

- Automated transfer of data from LIMS to the GeneReader NGS System and of results back to LIMS
- Reduced time and effort for checking results
- Traceability of all sample-related data
- Proven performance with the GeneReader NGS System

GeneRead Link is a middleware solution that provides a complete chain of custody for your samples in the GeneReader NGS System workflow, from sample preparation to data analysis. It provides a searchable archive of all relevant data from the GeneReader NGS System including FASTQ files, variant calling files (VCF), instrument reports, result files and user notes. GeneRead Link automates data transfer between the GeneReader NGS System including the GeneRead QIAcube, GeneReader, QIAGEN Clinical Insight (QCI) bioinformatics platform and LIMS improving the efficiency and productivity of your laboratory workflow.

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Option 3: GeneReader software

The GeneReader software can also be used specifically for flow cell setup but only if the software is not connected to an external experiment planning system, see “Protocol: Loading and Running the GeneReader”, page 34. These steps guide users on how to add a flow cell manually via the GeneReader software user interface.

Important: Seamless data tracking and automated analysis of FASTQ files are not possible when the flow cells are manually imported. The user must enable analysis of FASTQ files by integrating data analysis with QCI Analyze via the GeneReader Link web interface.

Depositing beads on flow cells

1. Place the tube on the magnetic rack and wait for at least 30 s for a pellet to form.
2. Remove and discard the supernatant.
3. Add 13 μ l Buffer P, and gently pipet the entire volume up and down 30 times to mix.
4. Disinfect the benchtop with an alcohol wipe or lay down a layer of fresh lint-free wipe.
5. Open an aluminum foil package containing a flow cell (cat. no. 1107269), and place the flow cell on the disinfected bench top or lint-free wipe.

6. Transfer the entire volume of beads (15 μ l) into the port opposite the notch (shown in Figure 1), while holding the pipette vertically, as shown in Figure 2.

Note: It is recommended to use a USA Scientific 20 μ l TipOne RPT USA Scientific 20 μ l TipOne (cat. no. 1180-1810), Sarstedt Biosphere 2–20 μ l (cat. no. 70.760.213) or equivalent for depositing beads into the flow cell.



Figure 1. Location of the notch.



Figure 2. Correct dispensing technique for transferring beads to the flow cell port.

7. Seal both flow cell ports with the sealing tape as shown in Figure 3.

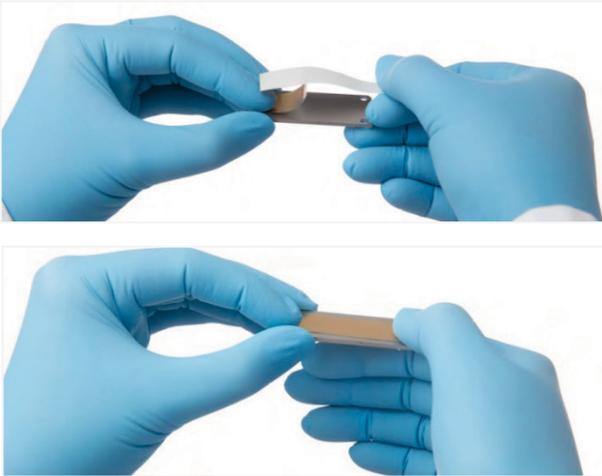


Figure 3. Sealing flow cell ports.

8. Incubate the flow cell at room temperature for 20 min. The flow cell should be lying flat on the disinfected benchtop or lint-free wipe, with the sealing tape facing up.

Important: During incubation, proceed with “Protocol: Reagent Preparation”, page 27. During the last 5 min of incubation, proceed to step 9, as described below.

Note: EDC/NHS will deactivate quickly following Buffer P addition. Please proceed with “Preparation of coupling master mix” only during the last 5 min of incubation.

Preparation of coupling master mix

9. Add 750 μ l Buffer P to EDC and briefly vortex.
10. Add 750 μ l of Buffer P to NHS and briefly vortex.
11. Combine EDC and NHS into one vial and vortex for 10 s.

Note: One coupling mixture will be sufficient for up to 6 flow cells.

12. Remove the seal tape and retain it for step 15 below.

-
13. Carefully pipet 200 μ l coupling mixture into the flow cell port opposite the notch for each flow cell (Figure 1).
 14. Gently absorb liquid from the other port using a lint-free wipe.
 15. Reseal the flow cell with the seal tape and incubate for 20 min.

Flow cell wash

16. Once incubation is complete, remove the seal tape.
17. Carefully pipet 200 μ l of Buffer P twice, for a total of 400 μ l, into a single flow cell port to remove any unbound beads.
Note: Pipet the Buffer P slowly.
18. Gently absorb liquid from the other port using a lint-free wipe.
19. Proceed with "Protocol: Loading and Running the GeneReader", page 34.

Protocol: Reagent Preparation

Important points before starting

- This protocol is performed during step 8 of “Depositing beads on flow cells”, page 23.
- Pipettes should be used to measure volumes accurately.
- Extend A2 should be shielded from light.
- Extend A2 and B2 should be thawed and stored at 4°C or on ice until use, shielded from light.
- Extend premixes should be stored at 4°C after thawing.
- Prepared Extend Mix should be stored at 4°C until use, shielded from light.

Things to do before starting

- Check wash buffers (Wash Buffer 9 and Wash Buffer 11) for precipitates before use, and, if necessary, redissolve by incubating at 37°C until dissolved.
- Perform “Protocol: Sequencing Primer Hybridization”, page 13.
- Perform “Protocol: Flow Cell Preparation”, page 19.

Sequencing reagents preparation

Follow instructions below according to the number of flow cells to be used in the run.

Run with 1 flow cell

1. Remove the components from the GeneRead UMI Advanced Sequencing Q Kit as listed in Table 1.

Table 1. Reagent preparation for GeneRead UMI Advanced Sequencing Q Kit for running 1 flow cell

Kit component	Number of tubes	Volume per tube	Component(s) to be added
Extend Premix A1 UMI	1	15.97 ml	162 µl Extend A2 162 µl Pol Extend 15 µl Extend Additive P*
Extend Premix B1 UMI	1	15.97 ml	162 µl Extend B2 162 µl Pol Extend 15 µl Extend Additive P*
Cleave Premix	1	16.3 ml	1 tube Cleave Additive M powder
FPF buffer	2	16.3 ml in each	No additive required
Image Premix	1	16.3 ml	No additive required

* Extend Additive P is shipped and stored at ambient temperature.

2. To the Extend Premix A1 UMI tube, add 162 µl Extend A2, 162 µl Pol Extend, and 15 µl Extend Additive P. Mix the contents by inverting the tube at least 10 times. Visually inspect the mix and make sure that all components are completely dissolved. If required, mix it again by inverting the tube.

Note: Store the remaining of the Extend Additive P at ambient temperature.

Note: If Extend Mix will not be used immediately after preparation, store at 4°C shielded from light. Use as soon as possible.

3. To the Extend Premix B1 UMI tube, add 162 µl Extend B2, 162 µl Pol Extend, and 15 µl Extend Additive P. Mix the contents in the tube by inverting the tube at least 10 times. Visually inspect the final mix and make sure that all components are completely dissolved. If required mix it again by inverting the tube.

Note: Store the remaining of the Extend Additive P at ambient temperature.

Note: If Extend Mix will not be used immediately after preparation, store at 4°C shielded from light. Use as soon as possible.

4. Pour the Cleave Additive M (powder) into the tube containing 16.3 ml Cleave Premix and mix by vortexing. To remove residual Cleave Additive, pipet 500 µl prepared Cleave Premix into the 2 ml Cleave Additive M tube, cap, and vortex to mix. Transfer the 500 µl into the tube containing prepared Cleave Premix.
5. The 2 tubes of FPF Buffer are ready for loading: no additive required (see Figure 6 for loading instruction).
6. The image premix is ready: no additive required (make sure that the image buffer is completely thawed).
7. Proceed to step 16 on page 26.

Run with 2 flow cells

1. Remove the components from the GeneRead UMI Advanced Sequencing Q Kit listed in Table 2.

Table 2. Reagent preparation for GeneRead UMI Advanced Sequencing Q Kit for running 2 flow cells

Kit component	Number of tubes	Volume per tube	Component(s) to be added/mixed
Extend Premix A1 UMI	2	15.97 ml/tube	2 x 162 µl Extend A2 2 x 162 µl Pol Extend 30 µl Extend Additive P*
Extend Premix B1 UMI	2	15.97 ml/tube	2 x 162 µl Extend B2 2 x 162 µl Pol Extend 30 µl Extend Additive P*
Cleave Premix	2	16.3 ml	2 tubes Cleave Additive M powder
FPF Buffer	4	16.3 ml	No additive required
Image Premix	2	16.3 ml	No additive required

* Provided in the kit as a single tube.

- To each of the 2 Extend Premix UMI A1 tubes, add 162 μ l Extend A2 and 162 μ l Pol Extend. Gently mix the contents by inverting the tubes 5 times. Combine the contents of the 2 tubes into one tube and add 30 μ l Extend Additive P (Figure 4). Mix by inverting the tube at least 10 times. Store the remaining of Extend Additive P at ambient temperature.

Note: If Extend Mix will not be used immediately after preparation, store at 4°C shielded from light. Use as soon as possible.

- To each of the 2 Extend B1 Premix UMI tubes, add 162 μ l Extend B2 and 162 μ l Pol Extend. Gently mix the contents by inverting the tubes 5 times. Combine the contents of the 2 tubes into 1 tube and add 30 μ l Extend Additive P (Figure 4). Mix by inverting the tube at least 10 times. Store the remaining Extend Additive P at ambient temperature.

Note: If Extend Mix will not be used immediately after preparation, store at 4°C shielded from light. Use as soon as possible.

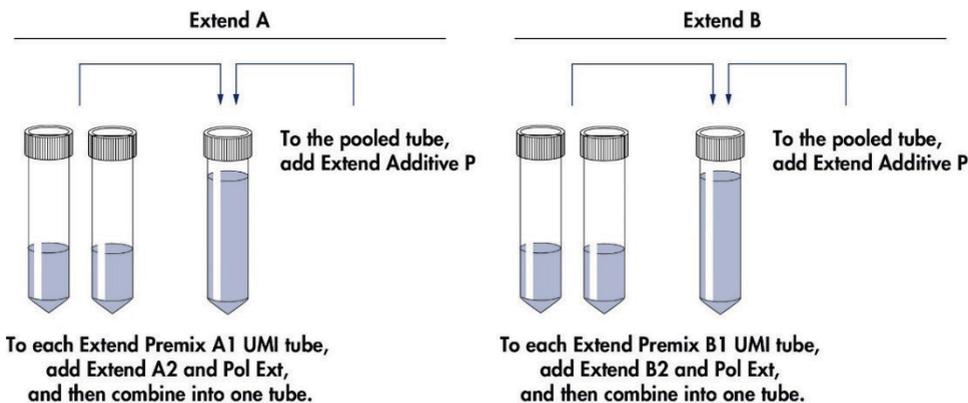


Figure 4. Extend buffer preparation for 2 flow cell run.

- To each 2 Cleave Premix tubes, add the Cleave Additive M (powder) and mix by vortexing for approximately 30 s.

5. To remove residual Cleave Additive, pipet 500 µl prepared Cleave Premix into each of the Cleave Additive M tubes, cap, and vortex for about 30 s. Transfer 500 µl from each tube to the tube containing prepared Cleave Premix.
6. Combine the contents of the 2 Cleave Premix tubes into a single tube and mix by vortexing for about 30 s.
7. Thaw 2 image buffer tubes at 25°C. When completely thawed, gently mix by inverting each tube 5 times. Combine the contents of the 2 tubes into one tube
8. Remove 4 FPF Buffer tubes from the kit, and then combine 2 FPF Buffer tubes into 1 tube each (total number of pooled tubes will be 2).

Run with 3 flow cells

1. Remove the components from the GeneRead UMI Advanced Sequencing Q Kit listed in Table 3.

Table 3. Reagent preparation for GeneRead UMI Advanced Sequencing Q Kit for running 3 flow cells

Kit component	Number of tubes	Volume per tube	Component(s) to be added/mixed
Extend Premix A1 UMI	3	15.97 ml/tube	3 x 162 µl Extend A2 3 x 162 µl Pol Extend 45 µl Extend Additive P*
Extend Premix B1 UMI	3	15.97 ml/tube	3 x 162 µl Extend B2 3 x 162 µl Pol Extend 45 µl Extend Additive P*
Cleave Premix	3	16.3 ml	3 x tube Cleave Additive M powder
FPF Buffer	6	16.3 ml	No additive required
Image Premix	3	16.3 ml	No additive required

* Provided in the kit as a single tube.

- To each of the three Extend Premix A1 UMI tube, add 162 μ l Extend A2 and 162 μ l Pol Extend (Figure 5). Gently mix the contents by inverting the tubes 5 times. Combine the contents of the 3 tubes into one tube. Add 45 μ l Extend Additive P, and mix by inverting the tube 10 times.

Note: If Extend Mix will not be used immediately after preparation, store at 4°C shielded from light. Use as soon as possible.

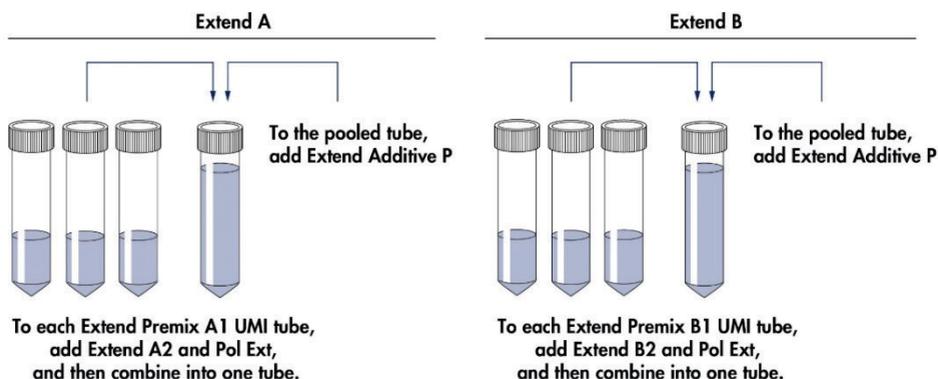


Figure 5. Extend buffer preparation for 3 flow cell run.

- To each of the three Extend Premix B1 UMI tubes, add 163 μ l Extend B2 and 162 μ l Pol Extend (Figure 5). Gently mix the contents by inverting the tubes 5 times. Combine the contents of the 3 tubes into one tube. Add 45 μ l Extend Additive P, and mix by inverting the tubes 10 times.

Note: If Extend Mix will not be used immediately after preparation, store at 4°C shielded from light. Use as soon as possible.

- To each of the 3 Cleave Premix tubes, add the Cleave Additive M (powder) and mix by vortexing for about 30 s.

-
5. To remove residual Cleave Additive, pipet 500 μ l prepared Cleave Premix into each of the Cleave Additive M tubes, cap, and vortex for about 30 s. Transfer the 500 μ l from each back into the tube containing prepared Cleave Premix.
 6. Pool the 3 Cleave Premix tubes into a single tube and mix by vortexing for about 30 s.
 7. Thaw 3 image buffer tubes at 25°C. When completely thawed, gently mix by inverting each tube 5 times. Pool the contents of both tubes into one tube.
 8. Remove 6 FPF Buffer tubes from the kit, and then combine 3 FPF Buffer tubes into 1 tube each (total number of pooled tubes will be 2).

Protocol: Loading and Running the GeneReader

Important points before starting

- All reagents should be prepared and ready before loading the GeneReader.
- Priming (see “Priming”, page 41) is performed for 20 min prior to loading the flow cells.
- After priming, the flow cell must be loaded within 1 h. Save the priming flow cell for later use.
- Save the cap from Wash Buffer 9 for later use.
- Check to see that you have sufficient Wash Buffer 9 available for your planned run.

Note: 1 bottle of 650 ml Wash Buffer 9 is provided in the kit. This amount is sufficient for 9 flow cells.

Note: If performing more than one run, remove the Wash 9 bottle from the Gene Reader at the end of the run, cap the bottle and store at ambient temperature for future use.

Things to do before starting

- If the GeneReader has been idle, make sure that all vessels in the GeneReader (1 liter bottles and 50 ml tubes) contain deionized water. If the GeneReader has not been idle, perform a routine maintenance wash (see “Appendix: Performing Routine Maintenance after a Run”, page 49).
- Make sure that the computer system has at least 1.5 TB of free space available on its D drive (i.e., **D:**).
- Perform “Protocol: Flow Cell Preparation”, page 20.
- Perform the “Protocol: Reagent Preparation”, page 27.

Starting the GeneReader software



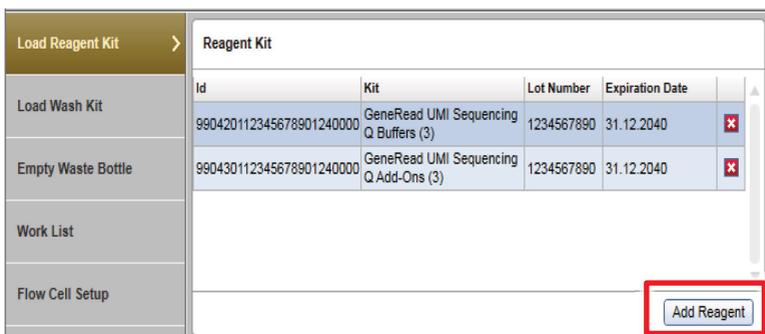
1. Start up the GeneReader software by clicking the GRS icon:

Note: The GeneReader application launches in approximately 45 s. During start-up, the software checks configuration files, performs a self-test, and searches for old flow cells.

2. Click the **Run Setup** icon.

Loading reagents

3. The hood opens automatically. Make sure to push the hood all the way up before opening the fluidic drawer.
4. Click **Add Reagent** twice.



5. Scan the GeneRead UMI Advanced Sequencing Q Buffers into the ID column in the reagent kit table.
6. Scan the GeneRead UMI Advanced Sequencing Q Add-Ons into the ID column in the reagent kit table.

7. Load the 50 ml tubes with prepared reagents (see “Protocol: Reagent Preparation”, page 27) into the cooling compartment of the GeneReader as shown in Figure 6, page 36.

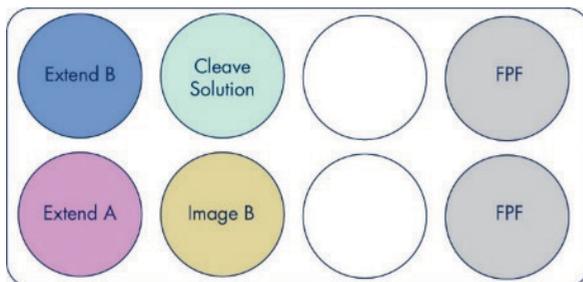


Figure 6. Reagent placement in the cooling compartment. **Extend A** (Extend Premix A1 UMI, Pol Extend, and Extend Additive P); **Extend B** (Extend B2, Pol Extend, and Extend Additive P); **Image B**: Image buffer (no additive required); **Cleave Solution**: Cleave Premix (Cleave Premix and Cleave Additive); **Empty**: no reagent in this location; **FPF**: FPF Buffer (no additive required). Scan the IDs of the GeneRead Sequencing Q wash buffers using the handheld scanner.

8. Load the two 1 liter wash bottles in the ambient deck area (Figure 7).

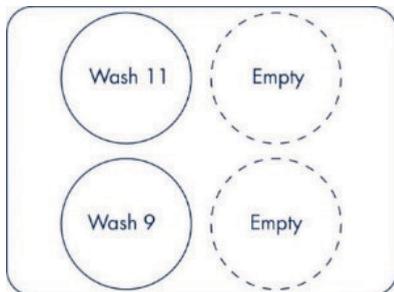


Figure 7. Wash buffer placement in the 1 liter compartment.

Note: The wash bottle 1 scan will direct positioning of Wash Buffer 9 into position 9, and the wash bottle 2 scan will direct positioning of Wash Buffer 11 into position 11.

Important: Save the caps of Wash Buffer 9 and Wash Buffer 11 for later use.

Load Reagent Kit	Wash Kit		
Load Wash Kit >	Wash Buffer 9	Lot Number	Expiration Date
	81700891234567890124000001	1234567890	31-Dez-2040
Empty Waste Bottle	Wash Buffer 11	Lot Number	Expiration Date
	81700901234567890124000003	1234567890	31-Dez-2040
Work List			
Flow Cell Setup			

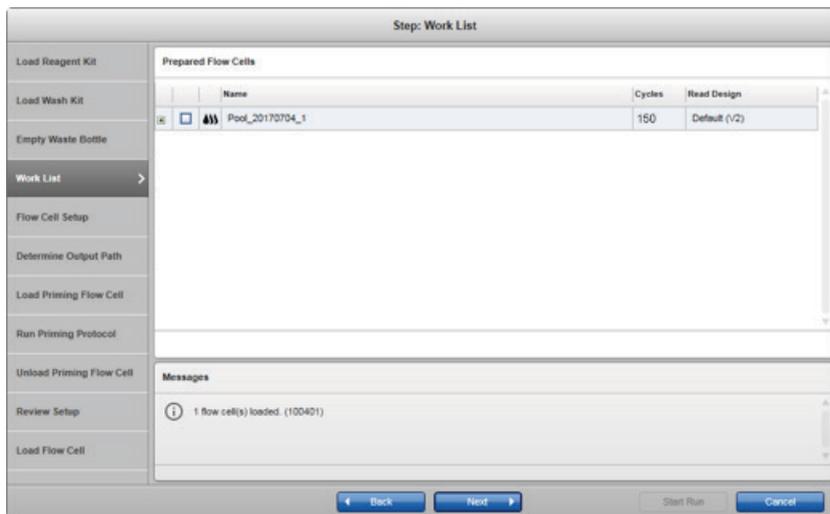
- Click **Done** and then **Next**.
- Check the liquid waste bottle. If it is full, empty the liquid waste bottle. (Disposal of wastes must be in accordance with all national, state, and local health and safety regulations.)
- Close the fluidic drawer by manually pushing it closed until it clicks.
Note: The fluidic drawer must remain locked during the entire run.
- Close the hood by manually pulling it down.
- Click **Next**.

Flow cell setup

- Start the flow cell setup process.
Important: The flow cell setup process can be performed either automatically or manually.

Automatically import flow cells

15. Select the flow cells to be imported in the **Work List** tab if the software is connected to QCI Analyze or GeneRead Link. Click **Next** and the selected flow cells are displayed as imported flow cells in the **Flow Cell Setup** tab. Proceed to “Determine output path”, page 41.



Note: The **Work List** tab is only visible if a connection is successfully established to QCI Analyze or GeneRead Link. Using this option, the data of a GeneReader sequencing run are automatically transferred to the external experiment planner system.

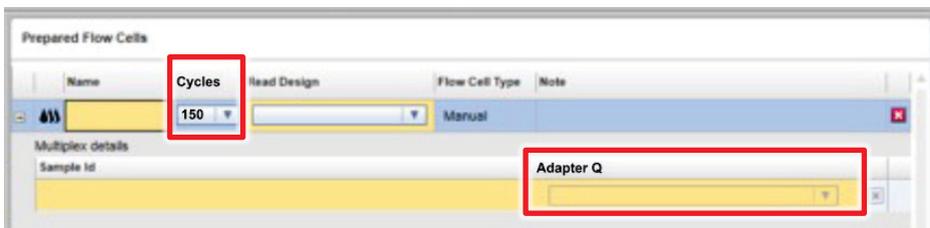
Manually import flow cells

16. If the software is not connected to an external experiment planning system, click **Add Flow Cell** in the **Flow Cell Setup** tab and enter the flow cell parameters manually by following the flow cell Setup Wizard, as shown below and described in the following steps.

Important: Seamless data tracking and automated analysis of FASTQ files are not possible when the flow cells are manually imported. The user must enable analysis of FASTQ files by integrating data analysis with QCI Analyze via the GeneReader Link web interface.

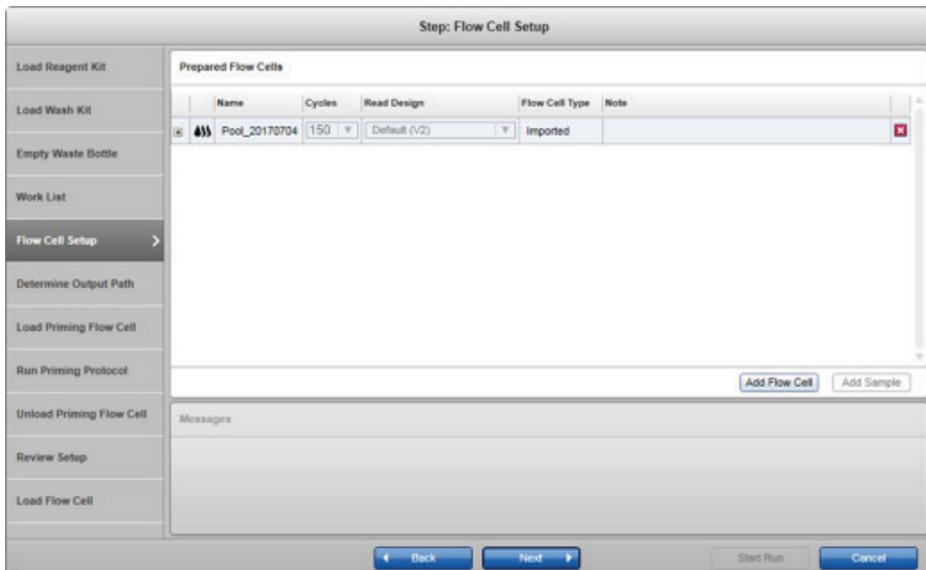
17. Add a flow cell name, and for Cycles, select **150**. Enter a sample ID and select **bar code** from the pull-down list under **Adapter Q**.

Important: The drop-down list also allows you to select 100 cycles run. For setting a 100-cycle run, make sure that 100 is selected for the number of cycles . All other settings are the same for runs of both 100 and 150 cycles. For bar coded samples, the numbers of bases in the Adapter Q will automatically be added to the run by the GeneReader software.



The screenshot shows a software interface titled "Prepared Flow Cells". It features a table with the following columns: Name, Cycles, Read Design, Flow Cell Type, and Note. The "Cycles" column has a dropdown menu currently set to "150". Below the table, there is a section for "Multiplex details" with a "Sample Id" field. To the right of the "Sample Id" field, there is a dropdown menu labeled "Adapter Q". Two red boxes highlight the "Cycles" dropdown and the "Adapter Q" dropdown.

- To add more samples, select **Add Sample** and enter the number of samples to be added. Enter a sample ID and add bar code(s) by selecting from the pull-down list under **Adapter Q**.
- After adding all flow cells and their corresponding sample IDs, click Next and proceed to step “Determine output path”, page 41.



Determine output path

20. Follow the wizard to enter a run-specific name.

Load Reagent Kit	Description
Load Wash Kit	Run Name Hg_QIA_Sequencing_Kit1
Empty Waste Bottle	Destination Path E:\QIAGENGRSData\2017_07_31_165651_Hg_QIA_Sequencing_Kit1
Work List	
Flow Cell Setup	
Determine Output Path >	

21. Click **Next** and the flow cell door will automatically open.

Priming

22. Open an aluminum foil package containing the priming flow cell (from the starter kit), and load the priming flow cell with the bar code toward the left side of the GeneReader.

Note: The flow cell door cannot be opened manually.



23. After loading the priming flow cell, manually close the flow cell door.

24. Click **Next** to start the priming protocol.

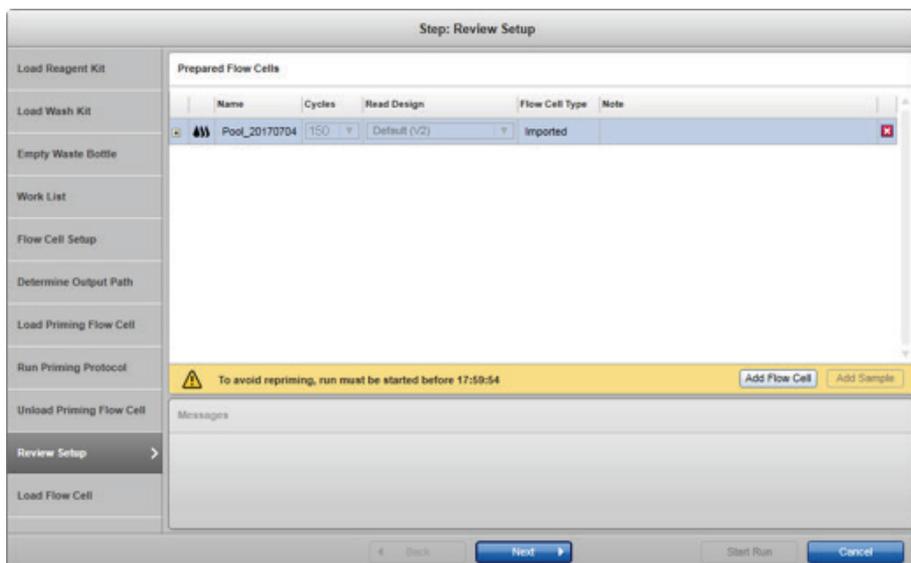
Note: Upon completion, the flow cell door will open automatically.

25. Unload the priming flow cell when priming is complete, and then close the flow cell door as guided by the wizard.

Important: Start run within 1 h after priming.

Important: Save the priming flow cell for later use.

26. Click **Next**. The **Review Setup** step is shown.

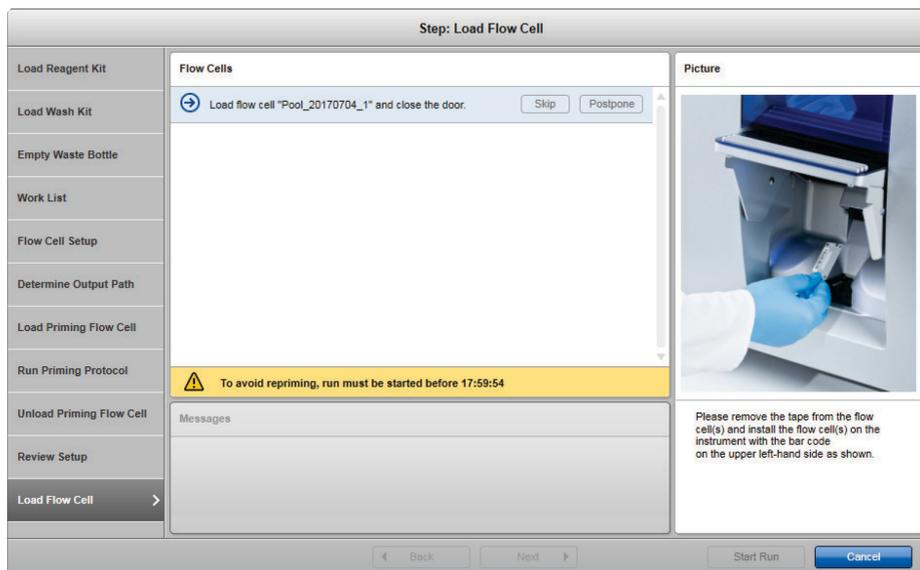


27. Click **Next** to begin loading flow cells.

Loading the flow cells

28. Before loading a flow cell, wipe the metal and glass surfaces with an alcohol wipe or lint-free wipe.
29. Load the specified flow cell into the GeneReader through the flow cell door, with the flow cell bar code toward the left side of the GeneReader.

Note: The flow cell door cannot be opened manually.



30. After loading the flow cell, close the flow cell door manually until the fastener snaps into place.

Important: The GeneReader will not function if the door is not completely closed.

31. Repeat steps 28–30 until all configured flow cells are loaded.
32. Click **Start Run**.
33. When the sequencing run is complete, proceed to "Protocol: Unloading Reagents and Flow Cells", page 44.

Protocol: Unloading Reagents and Flow Cells

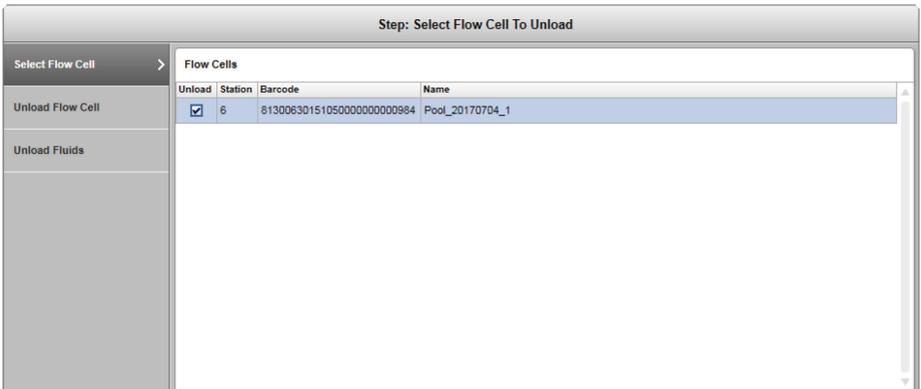
Important point before starting

- Make sure the sequencing run has completed before unloading reagents and flow cells.

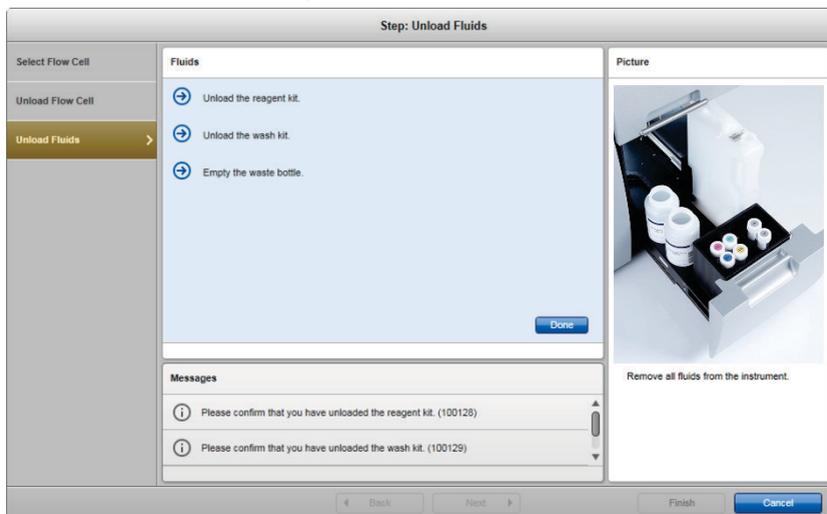
Procedure

1. Click **Unload** to unload flow cells.

Note: The **Run Finished** dialog also provides the option to directly transfer FASTQ and report files to another local or network directory by clicking **Transfer**.



2. Unload the flow cell and close the flow cell door. Repeat for all loaded flow cells.
3. After unloading the flow cell(s) and closing the flow cell door, click **Next**.
4. Click **Next** to unload the reagents.



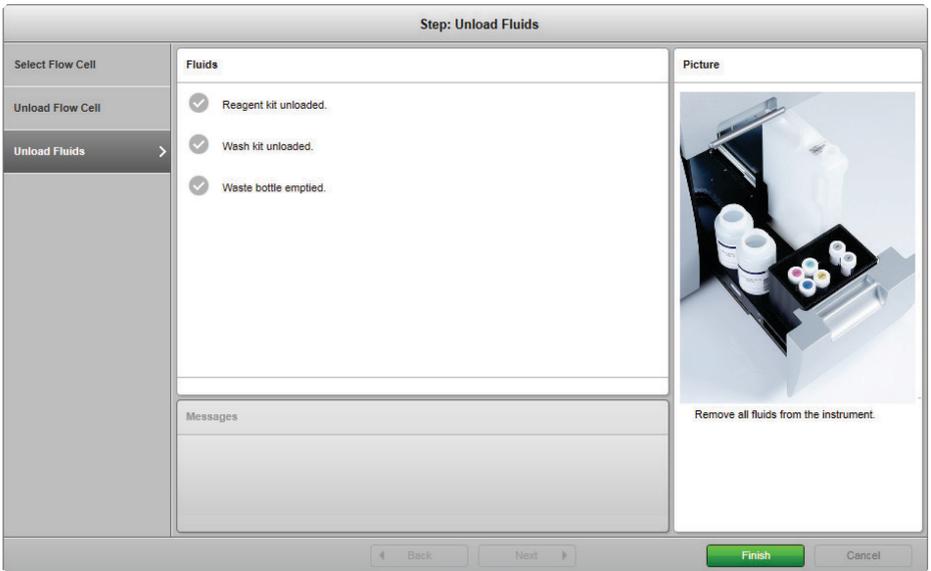
5. Remove and dispose of the 50 ml reagent tubes. Empty the liquid waste bottle following proper laboratory practice.

Note: Disposal of wastes must be in accordance with all national, state, and local health and safety regulations.

Important: If no other sequencing run is planned, remove Wash Buffer 9 and Wash Buffer 11, cap the bottles, and save for later use.

6. Perform a maintenance wash followed by deionized (DI) water wash after every 4 runs or once a week (whichever comes first). Follow the instructions in "Appendix: Performing Routine Maintenance after a Run", page 49.
7. If the instrument will be idle or no other sequencing run is planned, do not remove the flow cell, DI-containing bottles, and tubes from the GeneReader.
8. For deionized-water-wash instructions, see "Appendix: Performing Routine Maintenance after a Run", page 49.

9. Click **Done** to confirm, and then **Finish** to close the wizard.



Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit www.qiagen.com).

Comments and suggestions

No signal intensity or low signal intensity

- | | |
|--|--|
| a) No signal intensity on GeneReader software after 29 cycles | Check that Extend A2, Pol Extend, and Extend Additive P have been added to Extend Premix A1 UMI.
Check that Extend B2, Pol Extend, and Extend Additive P have been added to Extend Premix B1 UMI. |
| b) Low signal intensity on GeneReader software after 29 cycles | Check the color of image buffer. The color should be a light yellow-to-wheat color. If the buffer is in a brown color, use a new buffer or contact QIAGEN Technical Services. |
| c) Loss of beads on flow cell | Check that Cleave Additive M has been added to Cleave Premix for the Cleave Premix.
Check that the pH of the prepared Cleave Premix (with Cleave Additive M) is correct: the pH should be 9.5–9.6.
Check that a sufficient number of beads in Buffer P was deposited into the flow cell. The total amount of beads should be in the range of 140 million to 300 million beads. |

Comments and suggestions

GeneReader instrument failure or error

- | | |
|--|--|
| a) Instrument fails to start after loading the reagents | Remove all reagents from the instrument. Shut down the GeneReader software. Restart the GeneReader software, and start the run from the beginning. |
| b) Volume delivery failure | Reagent(s) may have been fully consumed during the run; check volumes. If any reagents are near full capacity, there is a volume delivery failure in that line. Contact QIAGEN Technical Services. |
| c) Error message indicating waste bottle is full | Empty the waste bottle. Disposal of waste must be in accordance with all national, state, and local health and safety regulations. |
| d) Error message indicating process finished with errors | Cancel the wizard; unload the flow cell. Contact QIAGEN Technical Services. |
| e) FASTQ files are not automatically analyzed | Ensure that the QCIA connections are maintained in the Connections tab. |

Appendix: Performing Routine Maintenance after a Run

This appendix describes 2 required maintenance procedures:

- **Routine maintenance (DI water wash)** must be performed when the GeneReader will be idle and/or when a run has completed and no additional runs are planned to follow immediately.
- **Weekly maintenance (using the Maintenance Wash Buffer)** must be performed every 4 runs or once a week (whichever comes first).

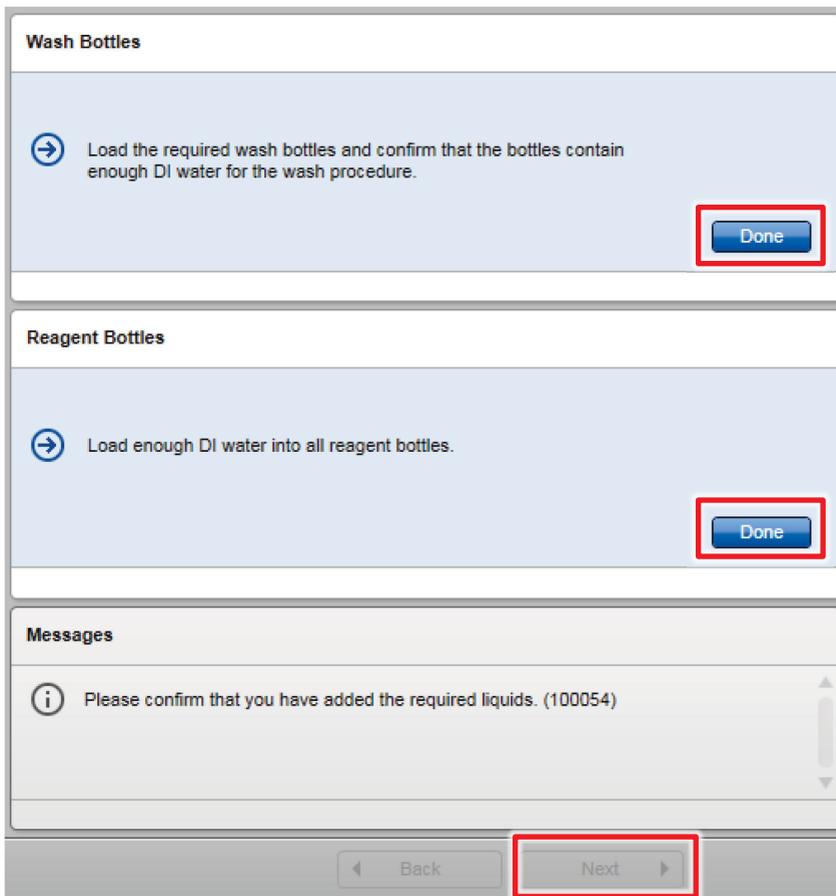
Performing these procedures ensures that the GeneReader is kept in good operating condition and helps prevent clogging of the fluidics system.

Routine maintenance (DI water wash)

After a sequencing run has finished and the GeneReader will be idle or no other sequencing run is planned, a DI water wash will prevent the formation of air pockets and the drying out of the fluidic lines inside the instrument.

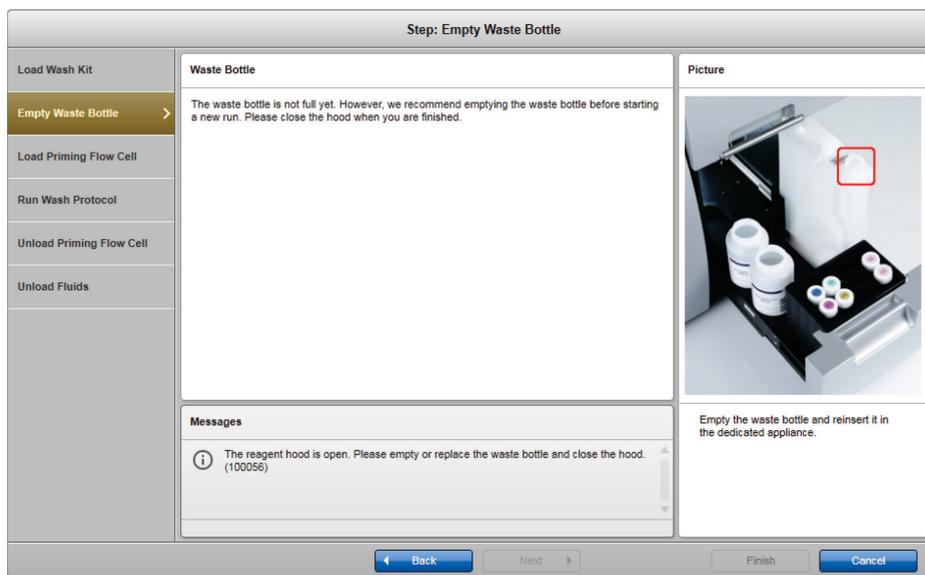
1. Open the GeneReader software. Click **Wash** in the navigation panel.
2. Select **DI Water Wash** and click **Next**.
3. The hood will open. Load 2 wash bottles (QIAGEN standard 1 liter bottle) containing 500 ml DI water into positions 9 and 11.

4. Load reagent tubes (QIAGEN standard 50 ml conical tubes) containing 30–35 ml DI water into positions 1, 2, 4, 5, 6, and 8 of the cooling compartment. Click **Done** twice and then click **Next**.



5. Empty the waste bottle and dispose of the contents according to local safety regulations. Reload the empty waste bottle into the instrument. Close the hood and click **Next**.

Important: Make sure to reload the waste bottle into the instrument in the correct orientation.



6. The flow cell door will open. Load an empty or used flow cell (priming flow cell) to allow liquid flow. Close the flow cell door and click **Next**.
7. Instrument will now prime with DI water. The routine maintenance wash will take about 15 min. Once complete, click **Next**.
8. The flow cell door will open. Unload the priming flow cell. Close the flow cell door and click **Next**.

9. The reagent hood will open. Unload the reagent and wash kit. Empty the waste bottle. Close the hood and click **Done**. Click **Finish** to close the wizard.

Note: If the GeneReader is not used immediately for another run, leave the reagent and wash the bottles containing DI water in the system. This is important to ensure that the fluidic lines of the system do not dry out.

Note: If no run is planned for **longer than 1 month**, perform the long-term storage procedure to prepare the GeneReader instrument for dry storage conditions (see the *GeneReader User Manual for use with Advanced Process Flow (APF/HP)* instrument configuration and GeneReader SW version 1.6).

Weekly maintenance

To make sure reagent lines remain clear and to prevent buildup of material that could potentially clog the lines, a maintenance wash must be performed every 4 runs or once per week (whichever occurs first). The maintenance wash uses the Maintenance Wash Buffer provided in the GeneRead UMI Sequencing Q Wash Buffer Kit (cat. no. 185905). Maintenance buffer preparation and the maintenance wash procedure are described below.

Preparation of Maintenance Wash Buffer (sufficient volume for 9 maintenance washes)

1. Take out the Maintenance Wash Buffer bottle from the GeneRead UMI Sequencing Q Wash Buffer Kit.
2. Add 100 ml DI water to the Maintenance Wash Buffer for a final volume of 1000 ml.
3. Label a second empty 1000 ml bottle (QIAGEN GeneReader 1 liter bottle) with a Maintenance Wash Buffer sticker.
4. Transfer 500 ml Maintenance Wash Buffer to the second bottle, so that each bottle contains 500 ml Maintenance Wash Buffer.
5. Label 6 reagent tubes (QIAGEN standard 50 ml conical tubes) with Maintenance Wash Buffer stickers.

6. Aliquot approximately 30–35 ml Maintenance Wash Buffer from one bottle to each of the 6 reagent tubes. This initial volume is sufficient for several maintenance wash runs.

Important: A refill of the maintenance wash reagent tubes will be required when the liquid level in the maintenance wash reagent tubes falls below the conical mark. Refer to the *GeneRead User Manual* for refill instructions.

7. Load the Maintenance Wash Buffer bottle that was used for aliquoting in the previous step (containing approximately 300 ml remaining volume) onto position 11 in the GeneReader. Load the bottle containing 500 ml Maintenance Wash Buffer onto position 9 (Figure 8).

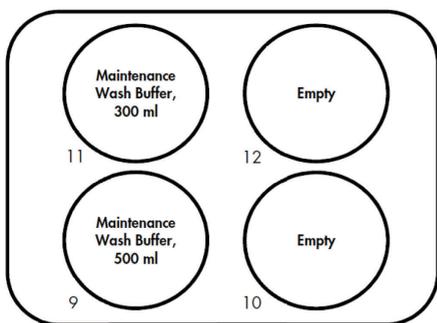


Figure 8. Loading 1 liter bottles containing Maintenance Wash Buffer.

8. Load the 6 reagent tubes containing the aliquoted Maintenance Wash Buffer (approximately 30–35 ml fill volume each) into the reagent cooler according to Figure 9 (positions 1, 2, 4, 5, 6 and 8), leaving positions 3 and 7 empty.

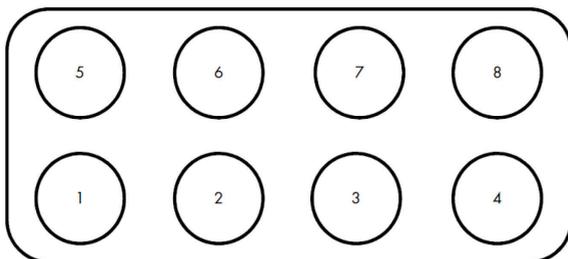


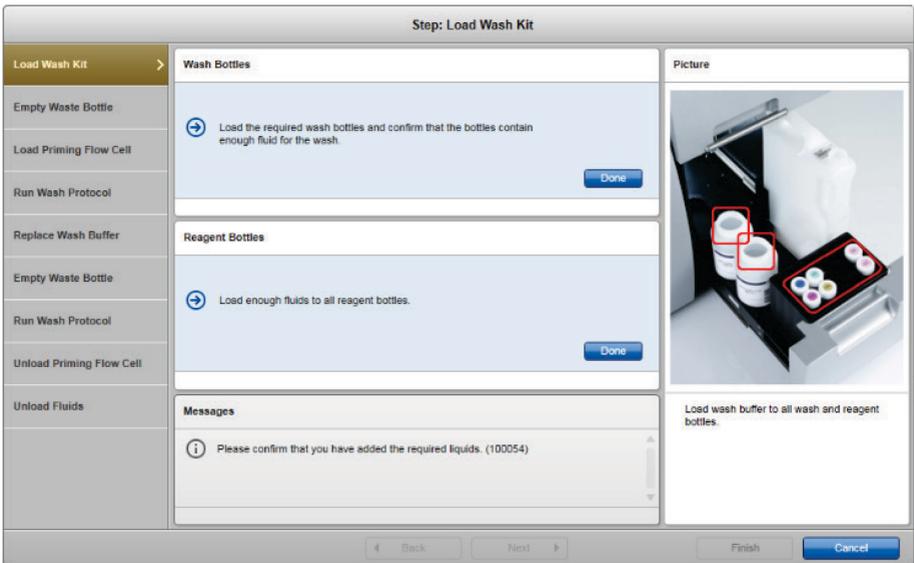
Figure 9. Loading 50 ml tubes containing Maintenance Wash Buffer.

Weekly maintenance procedure

1. If the sequencing run has just completed, make sure that all flow cells are removed, all reagents have been removed from the cooling compartment and discarded, and all wash buffers are removed and stored at room temperature.
2. If GeneReader software is not running, double-click the GeneReader icon to start the software. The GeneReader instrument will begin to scan for flow cells.
If prompted by the GeneReader, remove any flow cells, and close the flow cell door after removal (depending on the previous run, up to 3 flow cells may prompt for their removal from the GeneReader instrument).
3. Click **Wash** in the navigation panel.
4. Select **Maintenance Wash** and click **Next**. The reagent hood will open.
5. Load a 1 liter bottle (QIAGEN GeneReader 1 liter bottle) containing approximately 300 ml Maintenance Wash Buffer onto position 11 in the fluidic drawer.
6. Load a 1 liter bottle (QIAGEN GeneReader 1 liter bottle) containing 500 ml Maintenance Wash Buffer onto position 9 in the fluidic drawer.

7. Load 50 ml conical tubes (QIAGEN standard 50 ml conical tubes) containing approximately 30–35 ml Maintenance Wash Buffer onto positions 1, 2, 4, 5, 6, and 8 of the reagent cooler, leaving positions 3 and 7 empty, according to Figure 9.

Important: A refill of the maintenance wash reagent tubes will be required when the liquid level in the maintenance wash reagent tubes falls below the conical mark. Use Maintenance Wash Buffer bottle on position 11 for this refill.



8. Click **Done** twice when finished.
9. Click **Next**.

10. Empty the waste bottle and dispose the contents according to local safety regulations. Reload the empty waste bottle into the instrument. Close the reagent hood and click **Next**.

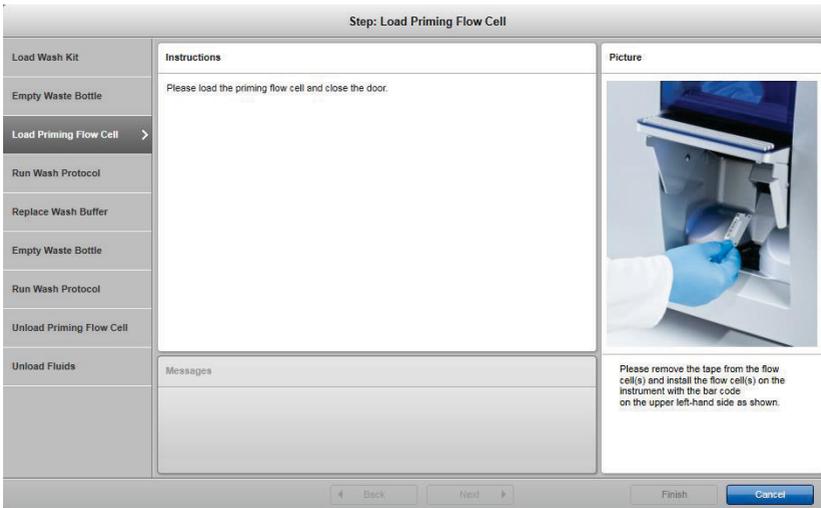
Important: Make sure to reload the waste bottle into the instrument in the correct orientation. An incorrect orientation may damage the instrument. Do not use force to close the hood. If blocked, check the waste bottle orientation and correct if necessary before retrying to close the reagent hood.

Step: Empty Waste Bottle

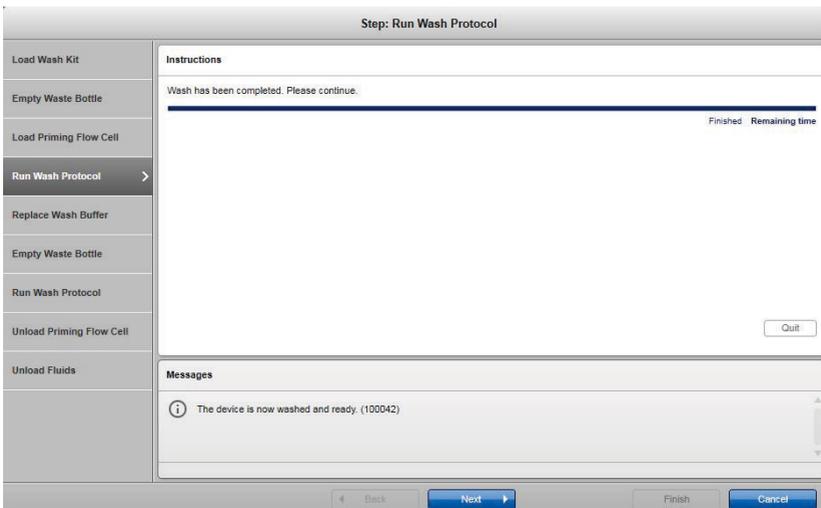
Load Wash Kit	Waste Bottle The waste bottle is not full yet. However, we recommend emptying the waste bottle before starting a new run. Please close the hood when you are finished.	Picture 
Empty Waste Bottle >		
Load Priming Flow Cell		
Run Wash Protocol		
Replace Wash Buffer		
Empty Waste Bottle		
Run Wash Protocol		
Unload Priming Flow Cell		
Unload Fluids	Messages <p> The reagent hood is open. Please empty or replace the waste bottle and close the hood. (100056)</p>	Empty the waste bottle and reinsert it in the dedicated appliance.

← Back Next → Finish Cancel

11. Load an empty or used flow cell (priming flow cell) to allow liquid flow, close the flow cell door, and click **Next**.



12. The instrument will now start the wash. The maintenance wash will take about 40 min. The software will notify when the maintenance wash is complete; click **Next**.



-
13. The reagent hood will open.
 14. Remove the reagent tubes containing the Maintenance Wash Buffer, recap the reagent tubes, and store them at room temperature for subsequent maintenance wash procedure uses.

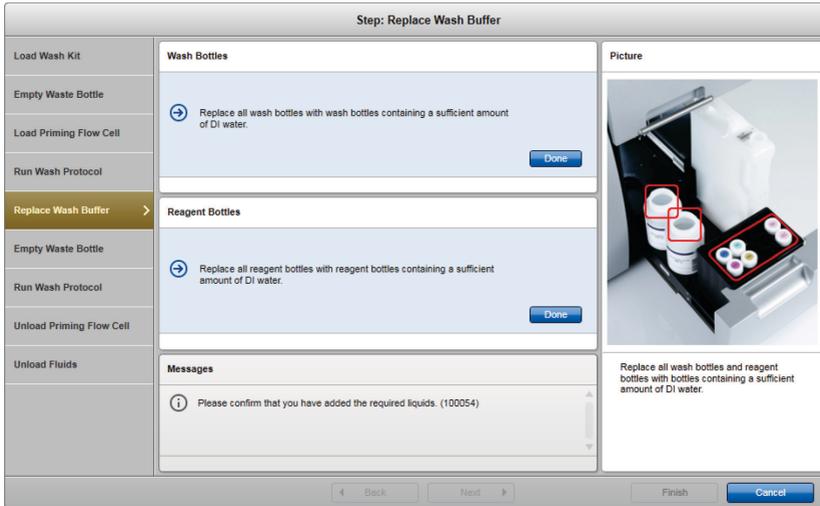
Important: The initial volume of 30–35 ml will be sufficient for 6–7 maintenance washes, and a refill will be required when the liquid level in the maintenance wash reagent tubes falls below the conical mark.

Use Maintenance Wash Buffer bottle on position 11 for this refill: prepare new when the liquid level in the maintenance wash reagent tubes falls below the conical mark.

15. Remove both 1 liter Maintenance Wash Buffer bottles, recap the bottles, and store them at room temperature for subsequent maintenance wash procedure uses.

Important: The initial volumes of 300 ml (wash buffer position 11) and 500 ml (wash buffer position 9), respectively, will be sufficient for 9 maintenance washes: prepare new when the Maintenance Wash Buffer bottle volume is less than 100 ml.

16. Load 50 ml conical tubes containing 30–35 ml DI water onto positions 1, 2, 4, 5, 6, and 8 of the cooling compartment.



17. Click **Done** twice when finished.
18. Click **Next**.

19. Empty the waste bottle and dispose the contents according to local safety regulations. Reload the empty waste bottle into the instrument. Close the reagent hood and click **Next**.

Important: Make sure to reload the waste bottle into the instrument in the correct orientation. An incorrect orientation may damage the instrument. Do not use force to close the reagent hood. If blocked, check the waste bottle orientation and correct if necessary before trying to close the hood again.

Step: Empty Waste Bottle

Load Wash Kit	Waste Bottle The waste bottle is not full yet. However, we recommend emptying the waste bottle before starting a new run. Please close the hood when you are finished.	Picture 
Empty Waste Bottle		
Load Priming Flow Cell		
Run Wash Protocol		
Replace Wash Buffer		
Empty Waste Bottle >		
Run Wash Protocol		
Unload Priming Flow Cell		
Unload Fluids		

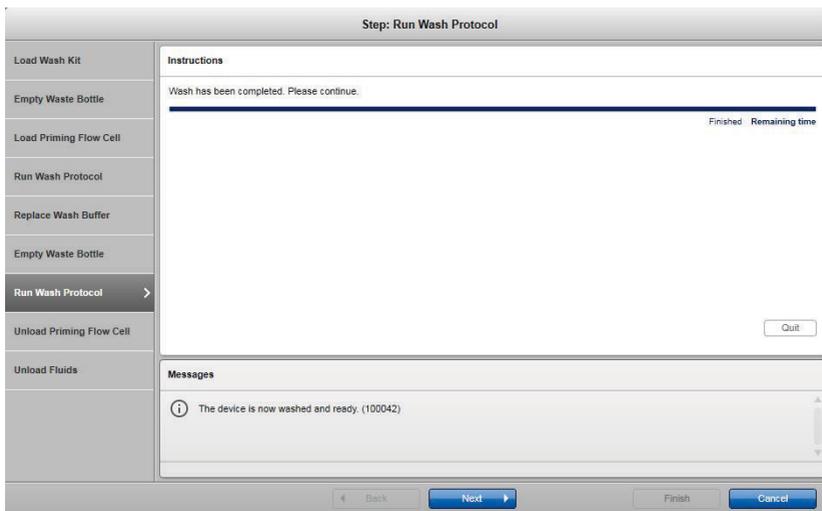
Messages

 The reagent hood is open. Please empty or replace the waste bottle and close the hood. (100056)

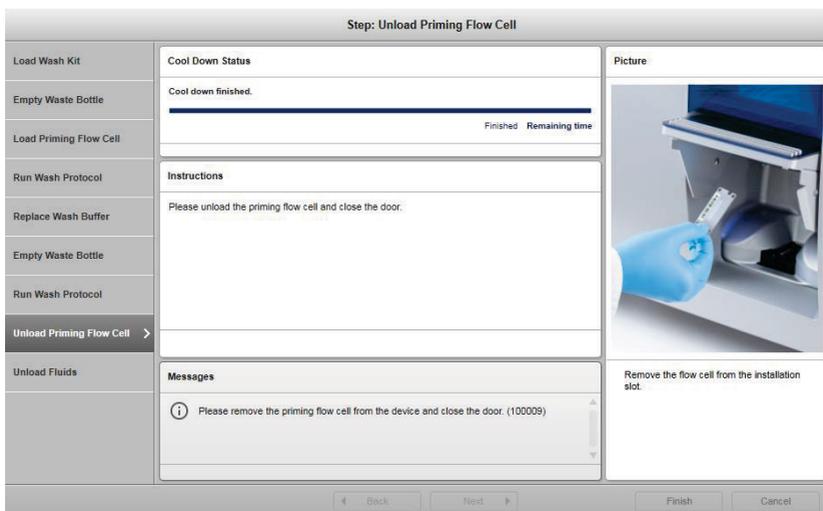
Empty the waste bottle and reinsert it in the dedicated appliance.

← Back Next → Finish Cancel

20. The instrument will now start the water wash. The DI water wash will take about 40 min. After the final wash is complete, click **Next**.



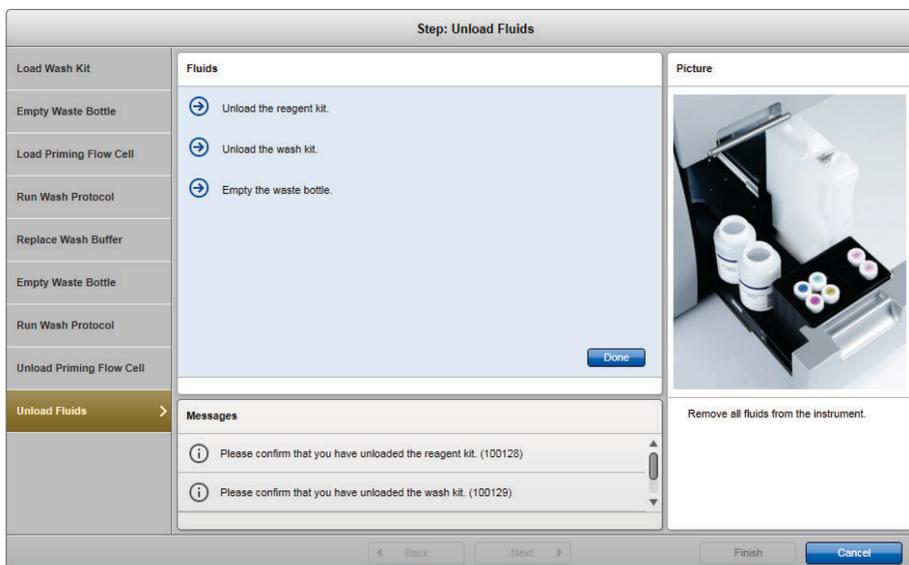
21. The flow cell door will open. Unload the flow cell, close the flow cell door, and click **Next**.



22. The reagent hood will open. Unload the reagent and wash kit. Empty the waste bottle. Close the reagent hood and click **Done**.

Note: If the GeneReader is not used immediately for another run, leave the reagent and wash bottles containing DI water in the system. This is important to ensure that the fluidic lines of the system do not dry out.

Note: If no run is planned for longer than 1 month, perform the long-term storage procedure to prepare the GeneReader instrument for dry storage conditions (see the *GeneReader APF/HP Configuration User Manual*/instrument configuration and GeneReader SW version 1.6).



23. Click **Finish** to close the wizard.

Ordering Information

Product	Contents	Cat. no.
QIAGEN GeneRead UMI Advanced Sequencing Q Kit (3)	Includes reagents and buffers, add-ons, accessories, and 3 flow cells supplied for up to 3 single flow cell runs on the GeneReader	185251
Related products		
GeneRead Clonal Amp Kit (4)	Clonal amplification of 4 library pools; reagents and buffers for library concentration normalization and pooling, emulsion making, breaking and pooling, and library enrichment	185001
GeneReader Platform	Next-generation sequencing instrument; includes installation and training, 1 year warranty on parts and labor	9002312

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Document Revision History

Date	Changes
01/2019	Updated kit contents. Added steps for preparation of coupling master mix and subsequent wash
09/2019	Updated instructions for storing Extend premixes. Updated Wash Buffer 9 consumption on page 34, "Important points before starting", bullet 5. Updated "Safety Information".
12/2020	Reduced the fill volume of Buffer P in the accessory kit.

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