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QIAGEN GeneRead® Fast Sequencing Q Kit Handbook

2 x 100

For preparation of DNA sequencing for next-generation sequencing (NGS) applications using the QIAGEN GeneReader™ instrument

For research use only

Not for use in diagnostic procedures

REF 185221

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Sample to Insight

QIAGEN

Contents

Intended Use

The QIAGEN GeneRead Fast Sequencing Q Kit is intended for research use only. 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 to adhere to the National Institutes of Health (NIH) guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Introduction

Next-generation sequencing (NGS) is a driving force for numerous applications, including cancer research, stem cell research, metagenomics, population genetic and medical research. The GeneRead Fast Sequencing Q Kit consists of easy-to-use buffers and reagents that only need to be mixed together before being loaded onto the GeneReader instrument, saving time and preventing handling errors. Optimized enzyme and buffer compositions ensure high-quality sequencing performance, perfect for research applications that require precise data insight. Streamlined GeneRead sequencing protocols enable straightforward automation of sequencing-by-synthesis technology on the GeneReader.

Principles of the Procedure

The GeneReader sequencing workflow includes the following 5 processes:

- "Protocol: Sequencing Primer hybridization"* on page 11
- "Protocol: Flow cell preparation" on page 14
- "Protocol: Reagent preparation" on page 17
- "Protocol: Loading and running the GeneReader" on page 19
- "Protocol: Unloading reagents and flow cells" on page 27

All protocols are essential for starting a run on the GeneReader and should be followed as indicated in this handbook. Refer to the *QIAGEN GeneReader User Manual for Advanced Process Flow (APF) Instrument Configuration* for additional instrument information. The principle behind the GeneReader sequencing chemistry is shown below in Figure 1.

* The "Protocol: Sequencing Primer hybridization" contains an additional 3-step "Optional procedure: Processing samples stored for longer than 48 hours" which is used only for treating beads that have been stored longer than 48 hours in Sequencing Primer.

The GeneReader sequencing-by-synthesis technology consists of the incorporation of unique, reversible terminated and fluorescent-labeled dNTPs ("labeled nucleotides") first, followed by unlabeled reversible terminated dNTPs ("dark nucleotides"). The GeneReader sequencing chemistry uses four dye colors for labeling with each color indicating a different base (A, C, G or T) that is incorporated onto the DNA fragment. Furthermore, the reversible terminators facilitate the addition of only one engineered nucleotide at a time to the growing strand of all DNA templates.

Upon signal detection from each bead the fluorescent labeling as well as the terminators are removed allowing for a new cycle of incorporation, ensuring highly accurate and cost-effective next-generation sequencing.

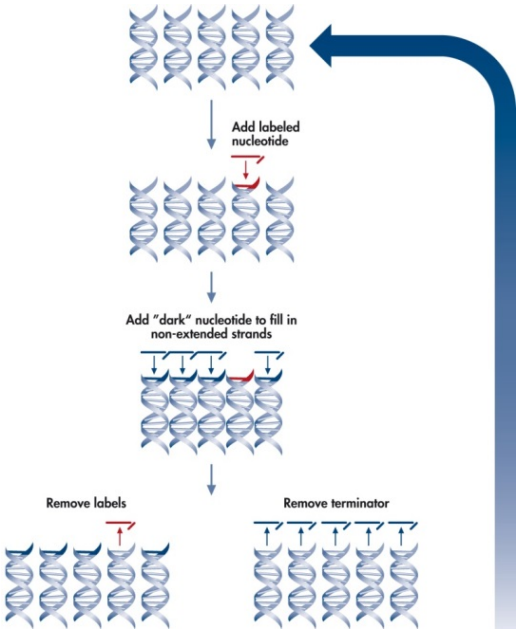


Figure 1. Principle behind the sequencing-by-synthesis technology on the GeneReader.

DNA libraries are clonally amplified on beads using the GeneRead QIAcube® to become a sequencing template. After hybridization of a sequencing primer the primer-template carrying beads are immobilized via direct bead-glass interaction to produce a high-density array on a GeneReader flow cell. To read out the content of templates on each bead, the array of fragments is first subjected to reagents containing uniquely engineered dNTPs, as described above These bases are incorporated by a modified DNA polymerase to the end of the growing strand of DNA in accordance with the base on the complementary strand (as shown in Figure 1). The array is subsequently scanned by a high-resolution digital camera and the fluorescent output of each of the four dye colors at each array position is measured and recorded. Finally, the array is exposed to cleavage chemistry to break off the fluorescent dyes and reversible terminators that will then allow additional bases to be added. This cycle is then repeated on the GeneReader.

Materials Provided

The GeneRead Fast Sequencing Q Kit (cat. no. 185221) contains reagents, two disposable flow cells and wash buffers. Reagent volumes are sufficient to perform two single flow cell runs with 100 cycles of sequencing on each flow cell.

Components of the GeneRead Fast Sequencing Q Kit are delivered in four boxes (see Figure 2).

Kit contents

Box 1

GeneRead Fast Sequencing Q Buffers		
Catalog number		**1104795***
Color	**Identity**	**Volume**
Pink lid label	Extend Premix A1	2 x 13.9 ml
Blue lid label	Extend Premix B1	2 x 13.9 ml
Green lid label	Cleave Premix	2 x 14.2 ml
White lid label	Buffer P	2 x 2 ml
White lid label	Buffer T	2 x 2 ml

* Not for individual sale; to order reagents, see cat. no. 185221.

Box 2

GeneRead Fast Sequencing Q Add-Ons		
Catalog number		**1104805†**
Color	**Identity**	**Volume**
Red cap	Extend A2	2 x 142 µl
Blue cap	Extend B2	2 x 142 µl
Clear cap	Pol Extend	2 x 354 µl
Gray lid label	Image Premix	2 x 14.2 ml
Blue cap	Sequencing Primer	1 x 20 µl

† Not for individual sale; to order reagents, see cat. no. 185221.

Box 3

GeneRead Fast Sequencing Q Flow Cell		
Catalog number		**1104788***
Color	**Identity**	**Number**
N/A	Flow cell	2
N/A	Sealing tapes	2
N/A	Cleave Additive	2 x 0.149 g

* Not for individual sale; to order reagents, see cat. no. 185221.
N/A: Not applicable.

Box 4

GeneRead Sequencing Q Wash Buffers		
Catalog number		**1106081†**
Color	**Identity**	**Volume**
N/A	Wash Buffer 9	1 x 267.9 ml
N/A	Wash Buffer 11 Premix	2 x 249.6 ml
N/A	Wash Buffer 11 Add-On	2 x 27.7 ml
N/A	Maintenance Buffer	1 x 170 ml

† Not for individual sale; to order reagents, see cat. no. 185221.
N/A: Not applicable.

Figure 2. Components of the GeneRead Fast Sequencing Q Kit are delivered in four boxes. Box 1 contains reagents in 50 ml tubes; Box 2 contains reagents in 50 ml tubes and 2 ml tubes; Box 3 contains flow cells in aluminum foil packages, adhesive tape and reagent in 2 ml tubes; Box 4 contains wash buffers in 1 liter bottles.

Materials Required but Not Provided

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), available from the product supplier.

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

Starting material

- Enriched bead samples

  For further information, see GeneRead Clonal Amp Q Kit (cat. no. 185001) at **www.qiagen.com**.

General laboratory equipment

- Dedicated pipets (adjustable) (1–10 µl; 10–100 µl; 100–1000 µl)
- Nuclease-free, aerosol-resistant, sterile PCR pipet tips with hydrophobic filters
- 2 ml capped LoBind tubes (Eppendorf® cat. no. 022431048)
- Two instruments capable of heating and mixing (Eppendorf ThermoMixer®, or equivalent)
- Vortex mixer
- Microcentrifuge
- Optional: Spectrophotometer capable of reading the optical density (OD) at 550 nm or 600 nm and corresponding labware (plates or cuvettes) for spectrophotometer

  Note: This equipment is not necessary if bead yield is determined using the color chart supplied in Box 3 of the GeneRead Clonal Amp Q Kit (cat. no. 185001).
- Magnetic rack (equivalent to Life Technologies® DynaMag™-2 rack; cat. no. 12321D)
- Low retention filter tips for depositing beads into flow cell (USA Scientific 200 µl TipOne® RPT; cat. no. 1180-8810, or equivalent)
- Alcohol wipes or lint-free wipes
- 0.2 M NaOH solution
- Laboratory timer
- 50 ml conical tubes
- Deionized water

Additional equipment

- GeneReader instrument (cat. no. 9002312)

Warnings and Precautions

Warnings

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please 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.

WARNING **Risk of personal injury**

Do not add bleach or acidic solutions to the sample preparation waste.

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 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.

Reagent Storage and Handling

The GeneRead Fast Sequencing Q Kit is delivered in 4 boxes (see Figure 2).

- GeneRead Fast Sequencing Q Buffers (Box 1) are shipped and stored at room temperature (15°C to 25°C).
- GeneRead Fast Sequencing Q Add-Ons (Box 2) are shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer (not frost-free).
- GeneRead Fast Sequencing Q flow cells (Box 3) are shipped on cool packs and should be stored immediately upon receipt at 2°C to 8°C.
- GeneRead Sequencing Q Wash Buffers (Box 4) are shipped on cool packs and should be stored immediately upon receipt at 2°C to 8°C.

Kit components should be protected from light exposure. We recommend storing all reagents in the dark. If stored under these conditions, the GeneRead Fast Sequencing Q Kit is stable until the date indicated on the QC label inside the kit lid.

Procedure

Protocol: Sequencing Primer hybridization

Important points before starting

- Pipets should be used to measure correct volumes.
- All volumes are for 1 sample.
- The acceptable range of beads required for Sequencing Primer hybridization is 140–300 x 10^6 beads
- For samples stored in Sequencing Primer longer than 48 hours, see "Optional procedure: Processing samples stored for longer than 48 hours", page 12, before proceeding to step "Wash beads with Buffer T", page 13.
- Additional Buffer T is provided in the GeneRead Clonal Amp Q Kit (cat. no. 185001).

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 *QIAGEN GeneRead Clonal Amp Q Handbook*, or by using the Color Chart provided in Box 3 of the GeneRead Clonal Amp Q Kit.
- Power ON GeneReader by pressing the power switch on 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 of 10 µM Sequencing Primer which must be prepared fresh from the stock.

Add Sequencing Primer

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

   Note: Dilute 1 mM Sequencing Primer stock to 10 µM in Buffer T.
4. Pulse-vortex and then pulse-centrifuge the tube.
5. Incubate for 5 minutes at 95°C on the thermomixer at 950 rpm.

6. Incubate for 10 minutes at 25°C on the thermomixer at 950 rpm.
7. Vortex for 5 seconds and then pulse-centrifuge the tube.

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

IMPORTANT: For samples stored less than 48 hours, proceed to step "Wash beads with Buffer T" on page 13.

IMPORTANT: For samples stored longer than 48 hours, proceed to step "Optional procedure: Processing samples stored for longer than 48 hours" before proceeding to step "Wash beads with Buffer P", page 14.

Optional procedure: Processing samples stored for longer than 48 hours

Note: This protocol is for samples stored longer than 48 hours.

Denaturation with NaOH

1. Place the tube on the magnetic rack, and wait at least 30 seconds for a pellet to form.
2. Remove and discard the supernatant.
3. Add 500 µl 0.2 M NaOH solution.
4. Vortex for 5 seconds, and then pulse-centrifuge the tube.
5. Incubate for 5 minutes at room temperature (15°C to 25°C) off the magnet.
6. Place the tube on the magnetic rack, and wait at least 30 seconds for a pellet to form.
7. Remove and discard the supernatant.
8. Add 500 µl Buffer T.
9. Vortex for 5 seconds, and then pulse-centrifuge the tube.
10. Place the tube on the magnetic rack, and wait at least 30 seconds for a pellet to form.
11. Remove and discard the supernatant.
12. Add 500 µl Buffer T.
13. Vortex for 5 seconds, and then pulse-centrifuge the tube.
14. Place the tube on the magnetic rack, and wait at least 30 seconds for a pellet to form.
15. Remove and discard the supernatant.

Primer re-hybridization

Note: ONLY for samples stored longer than 48 hours.

16. Add 100 µl 10 µM Sequencing Primer.

    Note: Dilute 1 mM Sequencing Primer stock to 10 µM with Buffer T.

17. Pulse-vortex and then pulse-centrifuge the tube.
18. Incubate for 5 minutes at 95°C on a thermomixer at 950 rpm.
19. Incubate for 10 minutes at 25°C on a thermomixer at 950 rpm.
20. Vortex for 5 seconds, and then pulse-centrifuge the tube.

Bead concentration measurement

Note: ONLY for samples stored longer than 48 hours.

21. Place the tube on the magnetic rack, and wait at least 30 seconds for a pellet to form.
22. Remove and discard the supernatant.
23. Add 500 µl Buffer T.
24. Pulse-vortex the tube.
25. Follow either the protocol "Determination of Bead Concentration using OD" in the *QIAGEN 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 140–300 x 10^6, proceed to step "Wash beads with Buffer P". If the amount is outside the recommended range, QIAGEN is unable to guarantee optimal results.

Wash beads with Buffer T

Note: ONLY for samples stored less than 48 hours.

1. Place the tube on the magnetic rack, and wait at least 30 s for pellet to form.
2. Remove and discard the supernatant.
3. Add 500 µl Buffer T.
4. Pulse-vortex and then pulse-centrifuge the tube.

Wash beads with Buffer P

Note: For ALL samples before flow cell preparation.

5. Place the tube on the magnetic rack, and wait at least 30 seconds for a pellet to form.
6. Remove and discard the supernatant.
7. Add 500 µl Buffer P.
8. Pulse-vortex and then pulse-centrifuge the tube.
9. Repeat steps 5–8 once.
10. Proceed with "Protocol: Flow cell preparation", page 14.

Protocol: Flow cell preparation

Important points before starting

- Pipets should be used to measure correct volumes.
- 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 (see "Protocol: Reagent preparation", page 17) should be started during incubation (step 8, below) of flow cell preparation.
- USA Scientific 200 µl TipOne RPT (cat. no. 1180-8810), or equivalent, is recommended for depositing beads into the flow cell.

Things to do before starting

- Remove flow cells (mat. no. 1077727) from storage at 2°C to 8°C.
- Perform the steps in "Protocol: Sequencing Primer hybridization", page 11.
- Samples must be in Buffer P before beginning to deposit beads.

Flow cell setup

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

- QCI™ Analyze (QCI-A)
- GeneRead Link
- GeneReader software

QCI Analyze web interface

QCI Analyze is a browser-based system for analyzing NGS data. Underneath QCI Analyze, a CLC Genomics Server is running the analyses, storing data, and handling various processes such as queuing. QCI Analyze can import NGS data, analyze it, display results for inspection and export result data in VCF format. In addition, it can connect directly to QCI Interpret for interpretation and reporting.

For more information on how to generate a flow cell setup, please refer to the *QIAGEN Clinical Insight Analyze 1.2 User Manual,* page 12.

GeneRead Link web interface

GeneRead Link is QIAGEN middleware software that is intended to provide bidirectional connectivity with a Laboratory Information Management System (LIMS) and specific QIAGEN instruments and software used within the GeneReader NGS System workflow.

For more information on how to generate a flow cell set up, please refer to section 7.3.1.4 in the *GeneRead Link v1.2 User Manual*, page 87.

GeneReader software

GeneReader software can be directly used for flow cell setup. See "Protocol: Loading and running the GeneReader" steps 4–6, below, for a step-by-step guide on how to add a flow cell manually using the GeneReader software user interface.

IMPORTANT: If GeneReader software is used for flow cell setup, there will be no automatic data analysis of FastQ files generated by the sequencer through QCI Analyze. In order to integrate data analysis in QCI Analyze, please use the QCI Analyze or the GeneRead Link web interfaces.

Depositing beads on flow cells

1. Place the tube on the magnetic rack, and wait at least 30 seconds for a pellet to form.
2. Remove and discard the supernatant.
3. Add 27 µl 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 (mat. no. 1077727), and place the flow cell on the disinfected bench top or lint-free wipe.
6. Gently transfer the entire volume of beads (27 µl) into either of the 2 ports of the flow cell (see Figure 3).

Note: It is recommended to use a USA Scientific 200 µl TipOne RPT (cat. no. 1180-8810), or equivalent, for depositing beads into the flow cell.

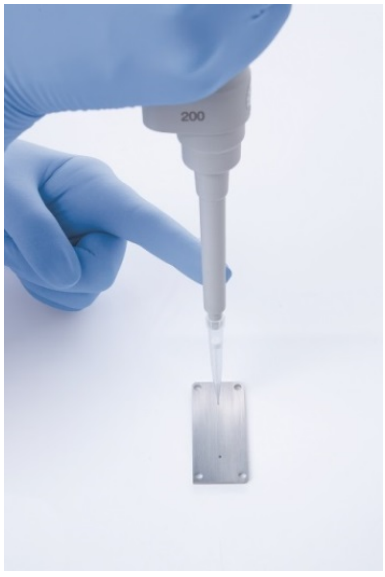


Figure 3. Correct dispensing technique for transferring beads to the flow cell port.

7. Seal both flow cell ports with the sealing tape (see Figure 4).

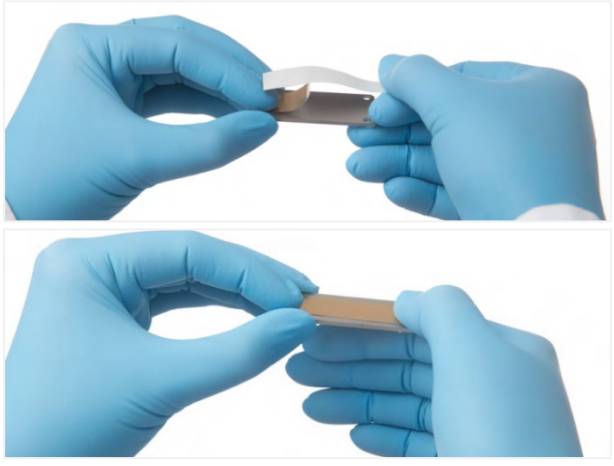


Figure 4. Sealing flow cell ports.

8. Incubate the flow cell at room temperature (15°C to 25°C) for 1 hour. 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 17.

9. Once incubation is complete, gently peel off the sealing tape.
10. Wipe the flow cell surface with a lint-free wipe.
11. Carefully pipet 2 x 200 µl (total of 400 µl) of Buffer P into a single flow cell port to remove any unbound beads.

    Note: Do not pipet all 400 µl at one time.
12. Gently absorb liquid from the other port using a lint-free wipe.
13. Proceed with "Protocol: Loading and running the GeneReader", page 19.

Protocol: Reagent preparation

Important points before starting

- "Protocol: Reagent preparation" (this protocol) is performed during step 8, above, of "Protocol: Flow cell preparation", page 14.
- Pipets should be used to measure correct volumes.
- Extend A2 should be shielded from light.

Things to do before starting

- Check wash buffers for precipitates before use, and re-dissolve at 37°C if necessary.
- Remove 1 tube each of Extend A2 (shield from light), Extend B2, Pol Extend and Image Premix from storage at –30°C to –15°C and place on ice.

  Note: 1 tube of each is required for the run.
- Perform the steps in "Protocol: Sequencing Primer hybridization", page 11.
- Perform steps 1–8 of "Protocol: Flow cell preparation", page 14.

Reagent preparation for a single flow cell run (100 cycles)

To perform a run with a single flow cell, use the following components from the GeneRead Fast Sequencing Q Kit.

- From Box 1: One tube Extend Premix A1, one tube Extend Premix B1 and one tube Cleave Premix (stored at room temperature 15°C to 25°C)
- From Box 2: One tube Pol Extend and one tube Image Premix (stored at –30°C to –15°C)
- From Box 3: One flow cell and one tube Cleave Additive (stored at 2°C to 8°C)
- From Box 4: One bottle Wash Buffer 11 Premix and one Wash Buffer 11 Add-On (stored at 2°C to 8°C)

See Table 1 (below) and the following steps, for correct reagent mixing instructions.

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

Kit component	Volume	Component(s) to be added/mixed
Extend Premix A1 (Box 1)	1 x 13.9 ml	1 x 142 µl Extend A2 (Box 2) 142 µl Pol Extend (Box 2)
Extend Premix B1 (Box 1)	1 x 13.9ml	1 x 142µl Extend B2 (Box 2) 142 µl Pol Extend (Box 2)
Cleave Premix (Box 1)	1 x 14.2 ml	1 x 0.149 g Cleave Additive (Box 3)
Image Premix (Box 2)	1 x 14.2 ml	No additive required
Wash Buffer 11 Premix (Box 4)	1 x 249.6 ml	1 x 27.7 ml of Wash Buffer 11 Add-On (Box 4)

1. Add 142 µl Extend A2 and 142 µl Pol Extend to Extend Premix A1. Mix the contents in the tube by inverting the tube at least 10 times.

   Note: If Extend Mix will not be used within 1 hour, store at 4°C shielded from light.
2. Add 142 µl extend B2 and 142 µl Pol Extend to Extend Premix B1. Mix the contents in the tube by inverting the tube at least 10 times.
3. Pour 0.149 g Cleave Additive into the tube containing 14.2 ml Cleave Premix and mix by vortexing. To remove residual Cleave Additive from 2 ml tube, pipet 500 µl prepared Cleave Buffer into the 2ml Cleave Additive tube, cap and vortex to mix. Transfer the 500 µl into the tube containing prepared Cleave Buffer.
4. Add 27.7 ml Wash Buffer 11 Add-On to the Wash Buffer 11 Premix bottle. Mix the contents by inverting the bottle at least 10 times.
5. Proceed with step 9, above, of “Protocol: Flow cell preparation”, page 14.

Protocol: Loading and running the GeneReader

Important points before starting

- All reagents should be prepared and ready for loading onto GeneReader.
- Priming is performed for 20 minutes prior to loading the flow cells.
- After priming, the flow cell must be loaded within 1 hour. 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 (100 ml) available for your planned run.

Things to do before starting

- If the GeneReader has been idle, make sure that sippers are sitting in deionized water. If the GeneReader has not been idle, perform a routine maintenance wash. See the instrument user manual for details.
- Make sure that the data drive has at least 1 TB of free space available.
- Perform the “Protocol: Flow cell preparation”, page 14.
- Perform the “Protocol: Reagent preparation”, page 17.

Starting the GeneReader and flow cell setup

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

   Note: The GeneReader application will launch in approximately 45 seconds. During startup, the software will check for configuration files, perform a self-test, and search for old flow cells.
2. Click the **Run Setup** icon.

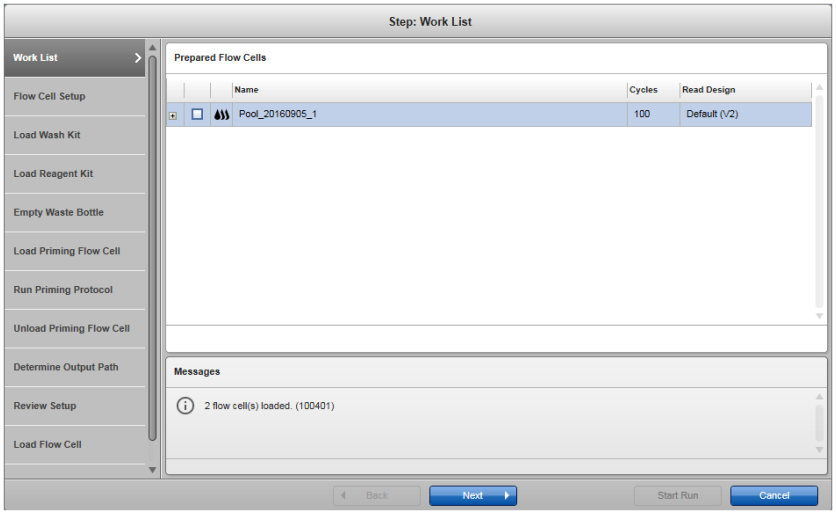
   

   The Flow Cell Setup Wizard will open.
3. Start the setup process.

IMPORTANT: There are 2 options for starting the setup process.

Option 1: Import flow cells

Select the flow cells to be imported in the **Work List** tab if the software is connected to QCI-A or GeneRead Link. Click **Next** and the selected flow cells are displayed as imported flow cells in the **Flow Cell Setup** tab. Proceed to step 7, "Loading reagents", page 22.



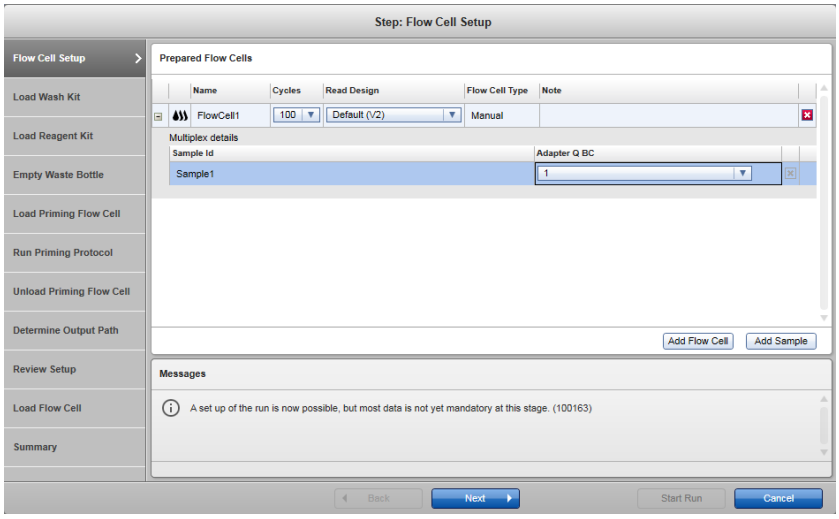
Note: The **Work List** tab is only visible if a connection is successfully established to QCI-A or GeneRead Link. Using this option, the data of a GeneReader sequencing run are automatically transferred to the external experiment planner system.

Option 2: Add flow cell manually

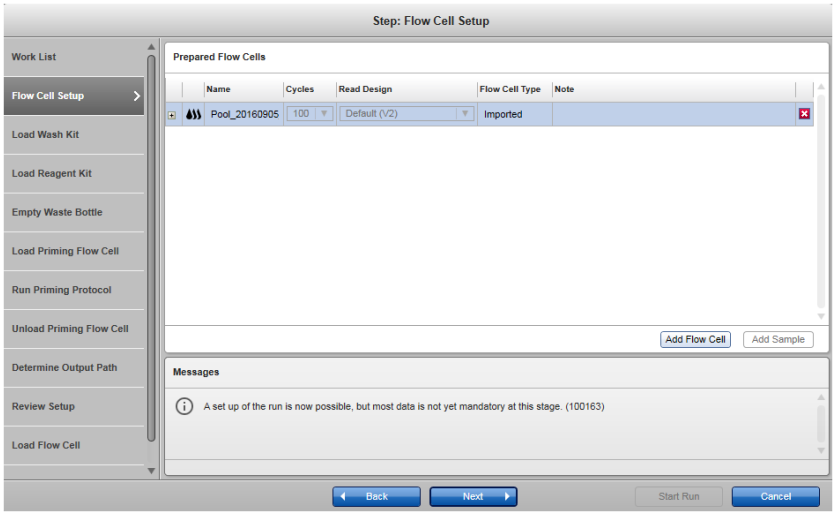
If the software is not connected to an external experiment planning system, click **Add Flow Cell** in the **Flow Cell Setup** tab and enter the flow cell parameters manually by following the Flow Cell Setup Wizard, as shown below and described in steps 4–6.

4. Add a flow cell name, select **100** from the **Cycles** drop-down list and **Default (V2)** for the **Read Design** as stated in the panel kit handbook. Enter a **Sample Id** and select bar code from the pull-down list under **Adapter Q BC**.

   IMPORTANT: The drop-down list allows you to select 100 cycles. As this procedure requires 100 cycles, make sure that 100 is selected for the number of cycles. For bar-coded samples, 7 cycles will automatically be added onto the run by the GeneReader software.



5. 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 BC**. It is possible to add up to 10 different bar-coded samples per flow cell.
6. After adding all flow cells and their corresponding sample IDs, click **Next** and proceed to step "Loading reagents", below.



Loading reagents

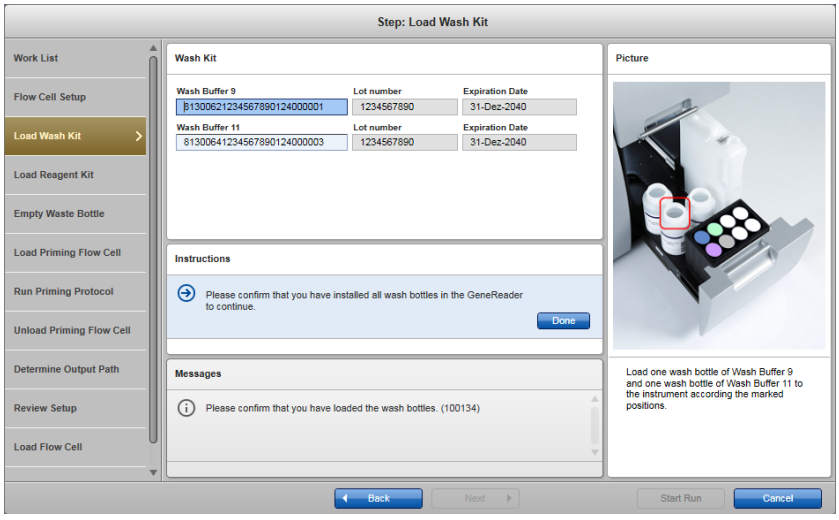
The hood opens automatically. Make sure to push the hood all the way up before opening the fluidic drawer.

7. Scan the IDs of the GeneRead Sequencing Q Wash Buffers (in Box 4) using the handheld scanner.
8. Load the two 1 liter wash bottles in the ambient deck area as shown by the electronic red square indicator.

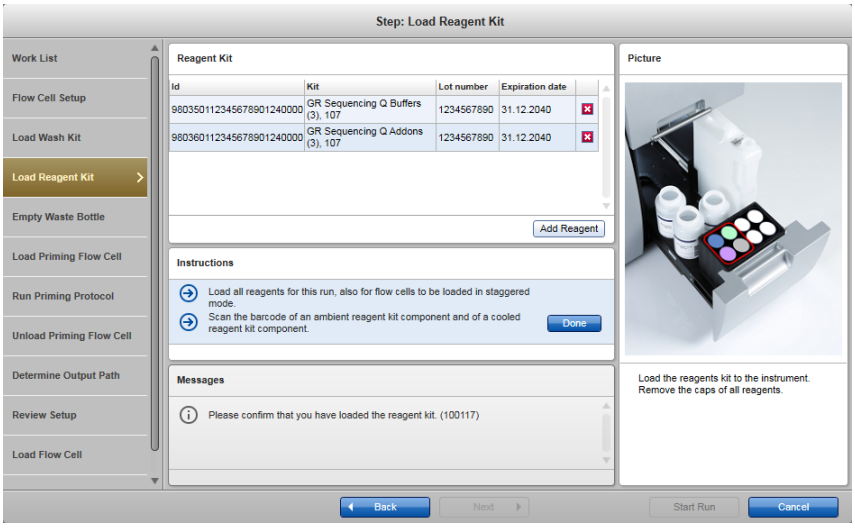
   Note: The wash bottle 1 scan will direct positioning of Wash Buffer 9 into position 9, and the wash bottle 2 scan will direct positioning of Wash Buffer 11 with Add-On into position 11.

   Note: The third wash bottle position is now redundant.

   IMPORTANT: Save the cap of Wash Buffer 9 for later use.



9. Click **Done** and then **Next**.
10. Click **Add Reagent** twice.



11. Position the cursor in the top box of the **Id** column in the **Reagent Kit** table and scan the GeneRead Fast Sequencing Q Buffers (Box 1; ambient temperature).
12. Position the cursor in the second box of the **Id** column in the **Reagent Kit** table and scan the GeneRead Sequencing Q Add-Ons (Box 2).
13. Load the 50 ml tubes with prepared reagents (see Table 1) into the cooling compartment of the GeneReader as shown in Figure 5.

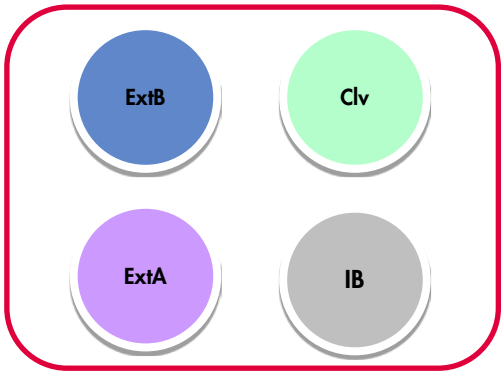


Figure 5. Reagent placement in the cooling compartment. Extend A (Ext A): Extend Premix A1 + Extend A2 + Pol Extend; Extend B (Ext B): Extend Premix B1 + Extend B2 + Pol Extend; Cleave Buffer (Clv): Cleave Premix + Cleave Additive; Image Buffer (IB): Image Premix (no additive required).

14. Click **Done** and then **Next**.
15. 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.)

16. Close the fluidic drawer by manually pushing it closed until it clicks.

    Note: The fluidic drawer must remain locked during the entire run.

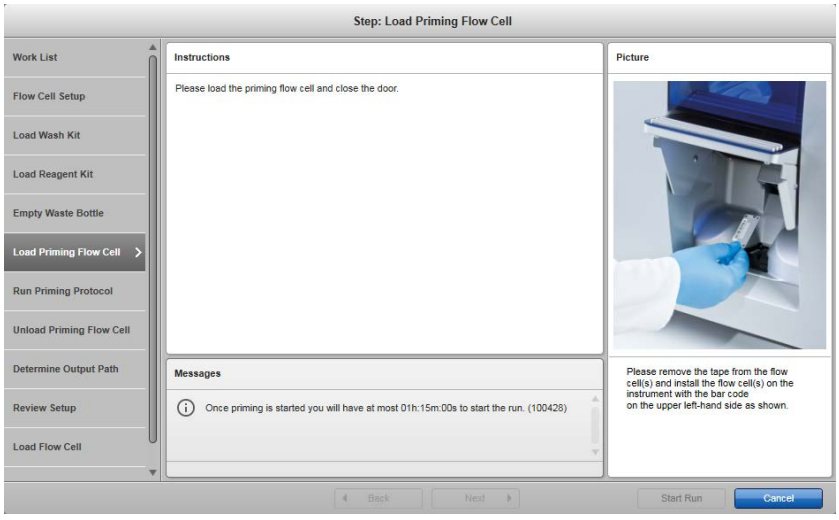
17. Close the hood by manually pulling it down.

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

Priming

19. Open an aluminum foil package containing the priming flow cell, and load the priming flow cell with the bar code towards the left side of the GeneReader.

    Note: The flow cell door cannot be opened manually.



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

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

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

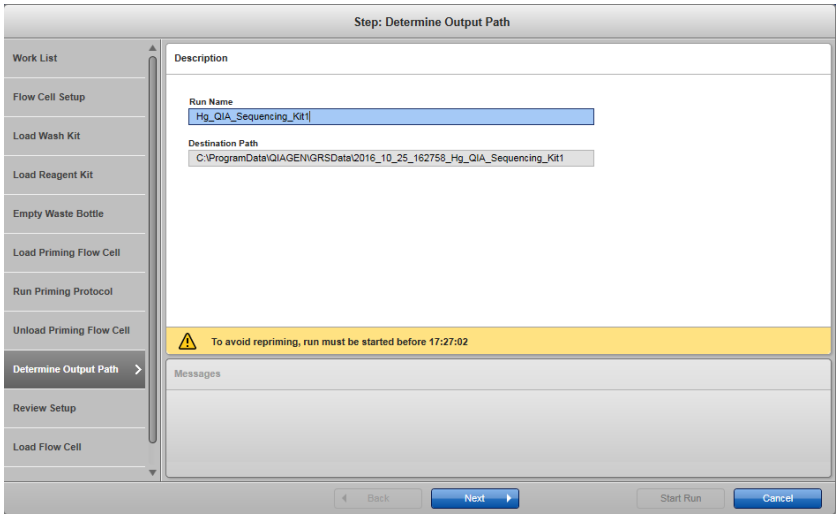
22. Unload the priming flow cell when priming is complete, and then close the flow cell door as guided by the wizard.

    IMPORTANT: Start run within 1 h after priming.

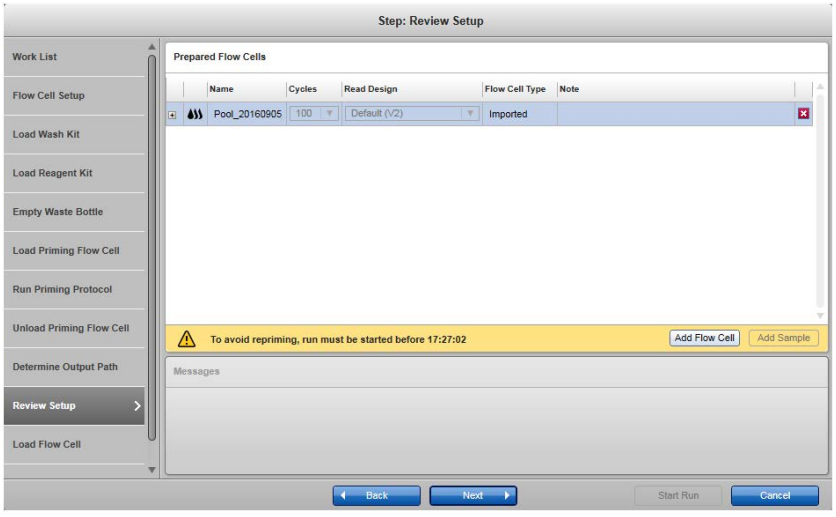
    IMPORTANT: Save the priming flow cell for later use.

23. Click **Next**.

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



25. Click **Next**. The **Review Setup** window opens.

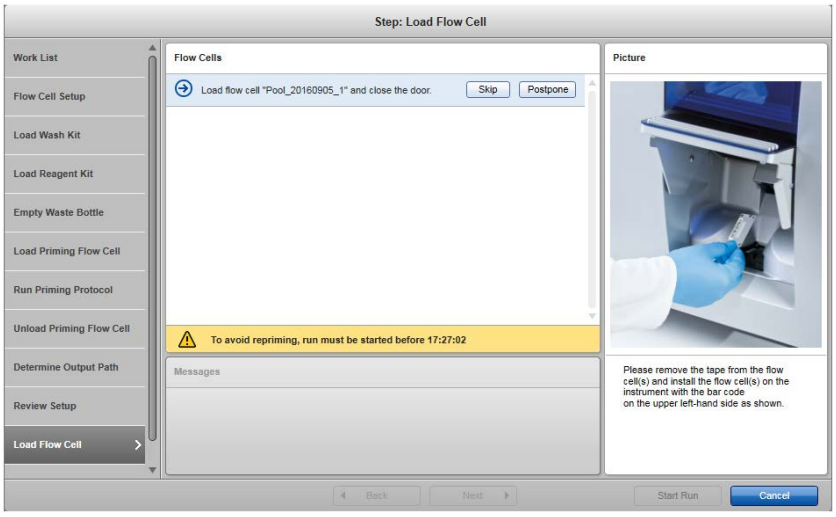


26. Click **Next** to begin loading flow cells.

Loading the flow cell

27. Before loading the flow cell, wipe the metal and glass surfaces with an alcohol or lint-free wipe.

28. Load the flow cell into the GeneReader through the flow cell door with the flow cell bar code towards the left side of the GeneReader.



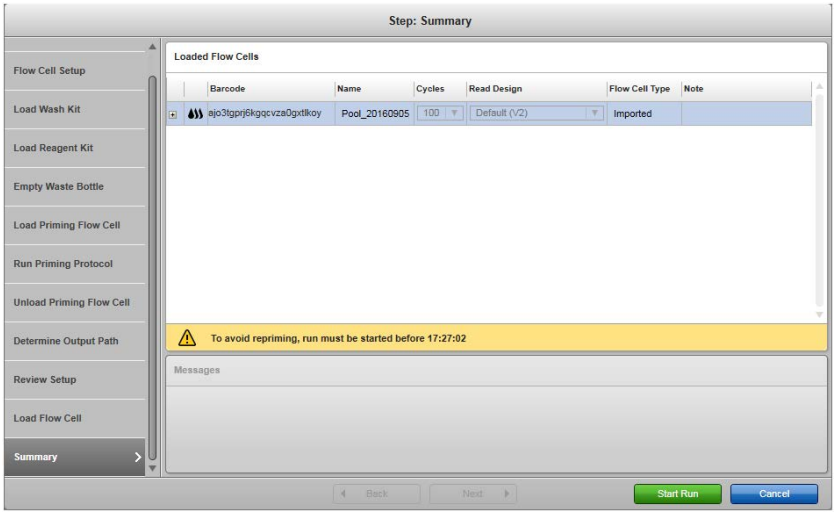
Note: The flow cell door cannot be opened manually.

29. 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.

30. Click **Next**.

31. Click **Start Run**.



32. When the sequencing run is complete, proceed to "Protocol: Unloading reagents and flow cells", page 27.

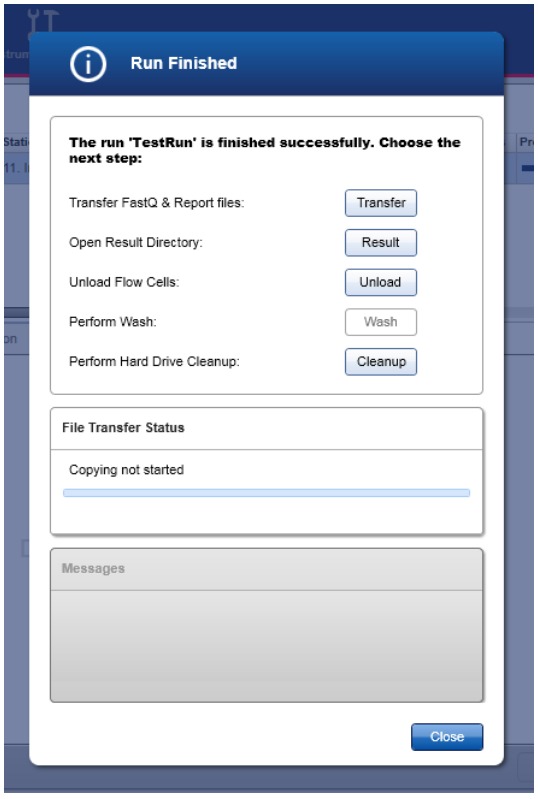
Protocol: Unloading reagents and flow cells

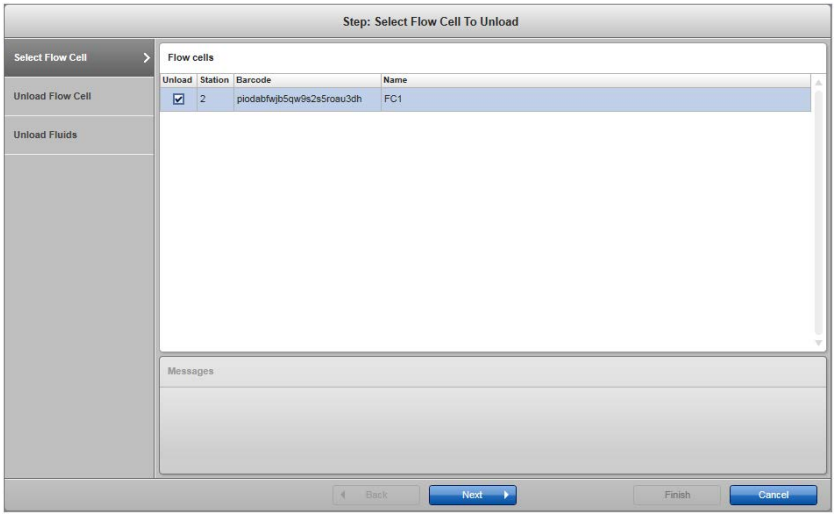
Things to do before starting

- Sequencing run must be completed.

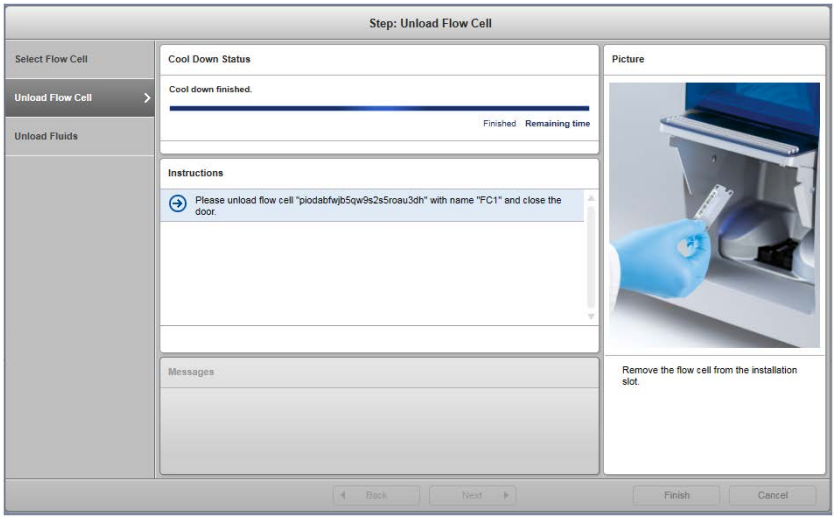
1. Click **Unload** to unload flow cells.

   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**.

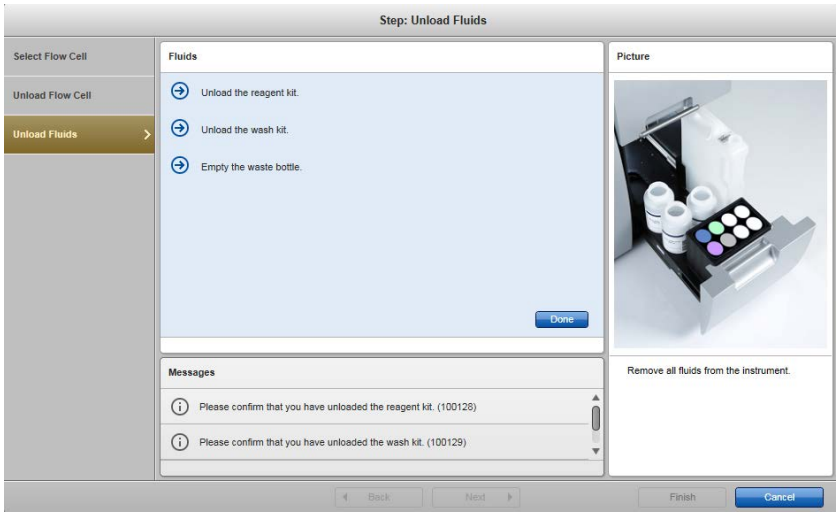




2. Unload the flow cell, and close the flow cell door. Repeat for all loaded flow cells.



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.

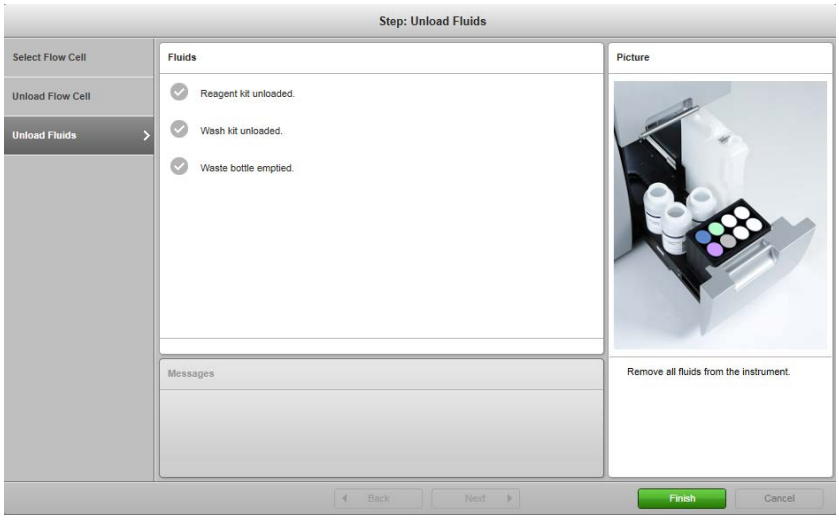
   Note: Disposal of wastes must be in accordance with all national, state and local health and safety regulations.

   IMPORTANT: If no other sequencing run is planned, remove Wash Buffer 9, cap the bottle, and save for later use. Remove and discard Wash Buffer 11.

   If the instrument will be idle, or no other sequencing run is planned, place sippers in 50 ml conical tubes with deionized water.

   For deionized water wash instructions, see section 6.2.1 "Maintenance wash: Routine maintenance" in the *QIAGEN GeneReader User Manual for Advanced Process Flow (APF) Instrument Configuration*.

6. 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 or protocols in this handbook, or sample and assay technologies. For contact information, see back cover or 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 and Pol Extend have been added to Extend Premix A1. Check that Extend B2 and Pol Extend have been added to Extend Premix B1.
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 a brown color, use a new buffer, or contact QIAGEN Technical Services.
c)	Loss of beads on flow cell	Check that Cleave Additive has been added to Cleave Premix for the Cleave Buffer. The pH of the prepared Cleave Buffer is incorrect; the pH should be 9.6–9.8. There was an insufficient number of beads in Buffer P deposited into the flow cell. The total amount of beads should be in the range of 140–300 x 10^6 beads (see page 13).
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.

		Comments and suggestions
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.

Quality Control

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

Symbols

The symbols in the following table include symbols used in this handbook.

 <N>	Contains reagents sufficient for <N> reactions
	Catalog number
	Material number (i.e., component labeling)
	Manufacturer

Ordering Information

Product	Contents	Cat. no.
GeneRead Fast Sequencing Q Kit (2)	Includes Reagents and Buffers, Add-Ons, and 2 flow cells supplied for up to 2 single flow cell runs on the GeneReader	185221
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

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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