March 2017

QIAGEN GeneRead® Fast Sequencing Q Kit: Supplementary Protocol

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

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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

The *QIAGEN GeneRead Fast Sequencing Q Kit: Supplementary Protocol* is intended to be used in conjunction the *QIAGEN GeneRead Fast Sequencing Q Kit Handbook*.

The *QIAGEN GeneRead Fast Sequencing Q Kit Handbook* contains details of kit contents, materials and equipment needed, reagent storage and handling, safety information, protocols for Sequencing Primer hybridization, flow cell preparation, unloading reagents and flow cells, and the troubleshooting guide.

This Supplementary Protocol provides detailed instructions on reagent preparation and flow cell loading for a 2-flow cell run using software version 1.4.40. Running a 2-flow cell workflow with the GeneRead Fast Sequencing Q Kit is not the standard recommended workflow. The instructions for the standard workflow can be found in the *QIAGEN GeneRead Fast Sequencing Q Kit Handbook*.

Procedure

Protocol: Reagent preparation

Important points before starting

- "Protocol: Reagent preparation" (this protocol) is performed during step 8 of "Protocol: Flow cell preparation" in the *GeneRead Fast Sequencing Q Kit Handbook*.
- 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 2 tubes 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.
- Perform the steps in "Protocol: Sequencing Primer hybridization" in the *GeneRead Fast Sequencing Q Kit Handbook*.
- Perform steps 1–8 of "Protocol: Flow cell preparation" in the *GeneRead Fast Sequencing Q Kit Handbook*.

Reagent preparation for a 2-flow cell run (100 cycles)

The GeneRead Fast Sequencing Q Kit is delivered in 4 boxes.

- GeneRead Fast Sequencing Q Buffers (Box 1) are stored at room temperature (15°C to 25°C).
- GeneRead Fast Sequencing Q Add-Ons (Box 2) are stored at –30°C to –15°C in a constant-temperature freezer (not frost-free).
- GeneRead Fast Sequencing Q flow cells (Box 3) are stored at 2°C to 8°C.
- GeneRead Sequencing Q Wash Buffers (Box 4) are stored at 2°C to 8°C.

To perform a run with 2 flow cells, use 2 tubes of each component from the GeneRead Fast Sequencing Q Kit from Box 1 and Box 2, 2 flow cells and 2 tubes of Cleave Additive from Box 3 as well as 2 bottles/tubes of Wash 11 Buffer Premix and Add-On from Box 4.

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

Table 1. Reagent preparation for GeneRead Fast Sequencing Q Kit for running 2 flow cells

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

1. Add 142 µl Extend A2 and 142 µl Pol Extend to each Extend Premix A1. Mix the contents in the tube by inverting the tube at least 10 times. Pool the two tubes of Extend Premix A1 and invert at least 10 times to mix.

   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 each Extend Premix B1. Mix the contents in the tube by inverting the tube at least 10 times. Pool the two tubes of Extend Premix B1 and invert at least 10 times to mix.
3. Pour 0.149 g Cleave Additive into each of the tubes containing 14.2 ml Cleave Premix and mix by vortexing. To remove residual Cleave Additive from the 2 ml tube, pipet 500 µl Cleave Buffer into each of the 2ml Cleave Additive tubes, cap and vortex to mix. Transfer the 500 µl into the tubes containing Cleave Buffer. Pool the two tubes of Cleave Buffer and invert at least 10 times to mix.
4. Add 27.7 ml Wash Buffer 11 Add-On to each of the Wash Buffer 11 Premix bottles. Mix the contents in the bottles by inverting the bottle at least 10 times. Pool the two bottles of Wash Buffer 11 and invert at least 10 times to mix.
5. Proceed with step 9 of "Protocol: Flow cell preparation" in the *GeneRead Fast Sequencing Q Kit Handbook*.

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 available for your planned run (see Table 2, below)

Table 2. Minimum recommended volumes of Wash Buffer 9 for a flow cell run

	Target fill volume (ml)	
Buffer	**1 Flow cell**	**2 Flow cells**
Wash Buffer 9	100	200

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" in the *GeneRead Fast Sequencing Q Kit Handbook*.
- Perform the "Protocol: Reagent preparation", page 3.

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.

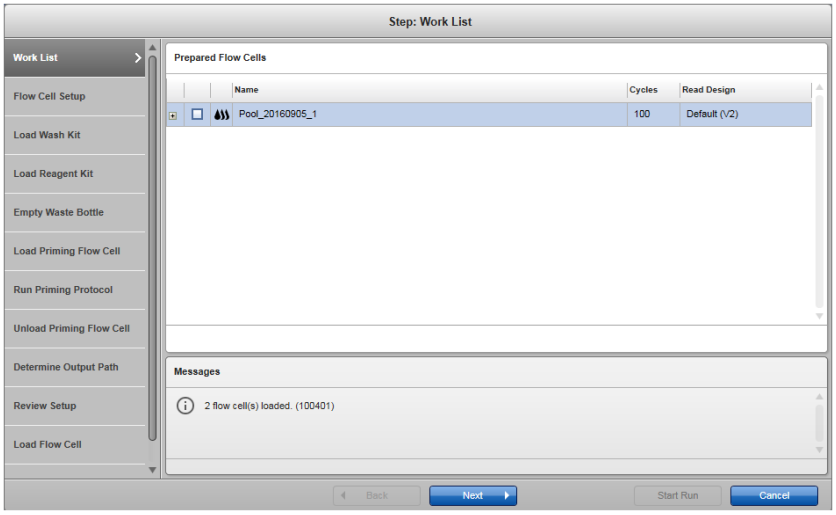
   The Flow Cell Setup Wizard will open.

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 8, "Loading reagents", page 9.



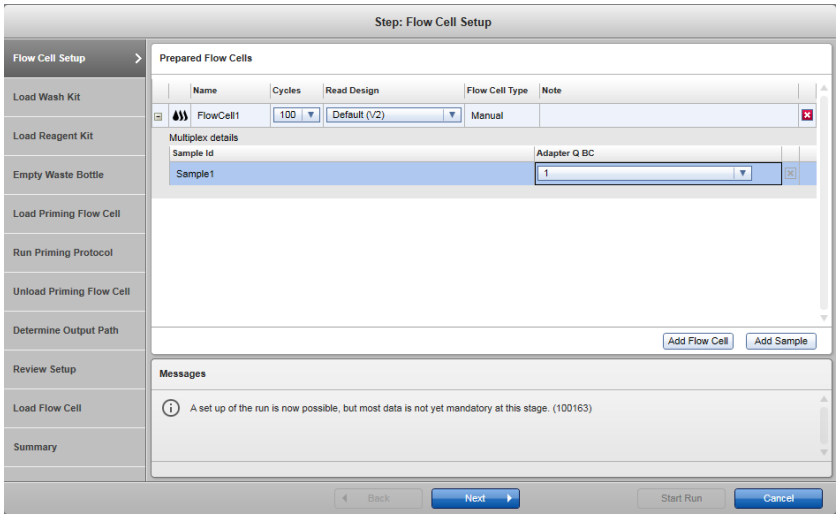
Note: The **Work List** tab is only visible if a connection is successfully established to QCI-A or GeneRead Link. Using this option, the data of a GeneReader sequencing run are automatically transferred to the external experiment planner system.

Option 2: Add flow cell manually

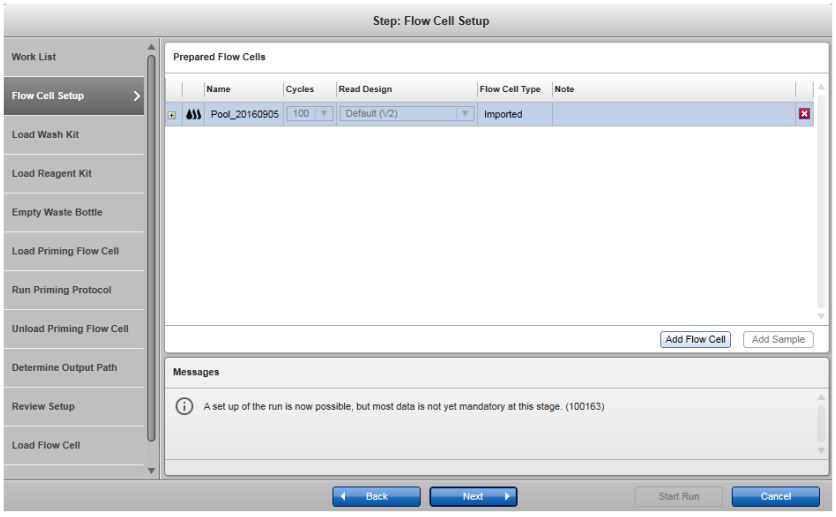
If the software is not connected to an external experiment planning system, click **Add Flow Cell** in the **Flow Cell Setup** tab and enter the flow cell parameters manually by following the Flow Cell Setup Wizard, as shown below and described in steps 4–6.

4. Add a flow cell name, select **100** cycles and **Default (V2)** for the **Read Design** as stated in the panel kit handbook. Additionally, 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. To add another flow cell, click **Add Flow Cell**, and repeat steps 4–5 for each additional flow cell.
7. After adding all flow cells and their corresponding sample IDs, click **Next** and proceed to step "Loading reagents", below.



Loading reagents

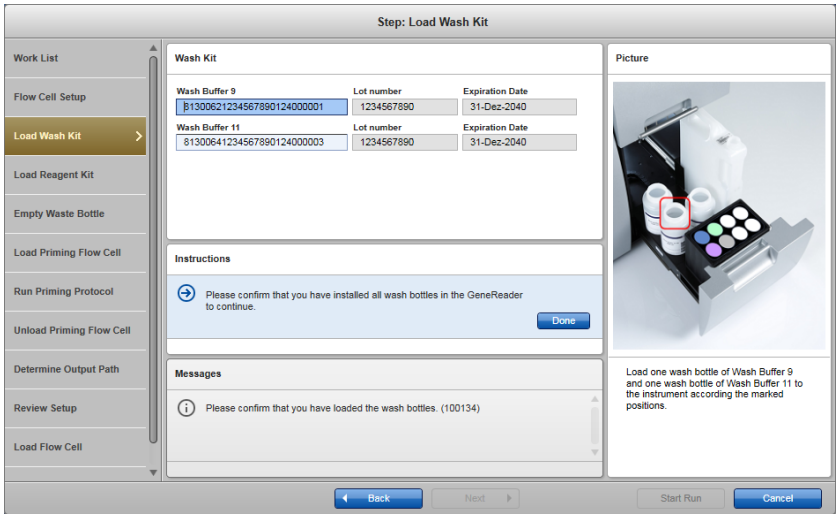
The hood opens automatically. Make sure to push the hood all the way up before opening the fluidic drawer.

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

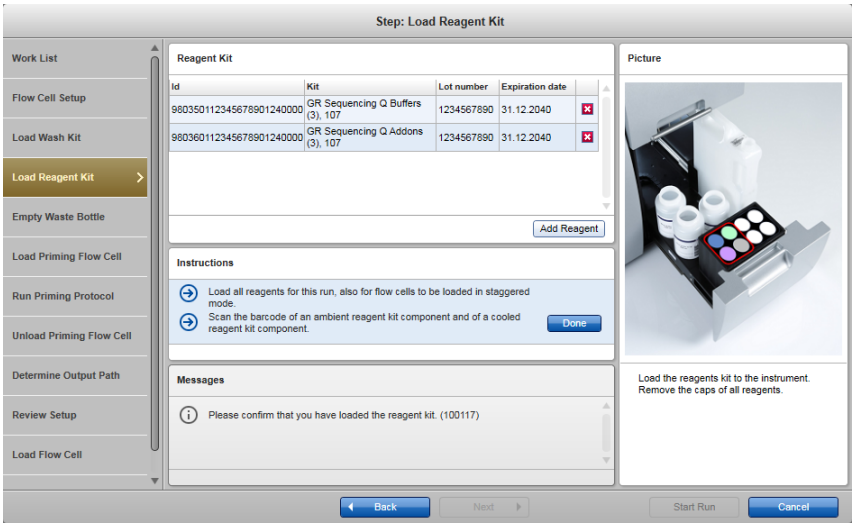
   Note: The wash bottle 1 scan will direct positioning of Wash Buffer 9 into position 9, and the wash bottle 2 scan will direct positioning of Wash Buffer 11 with Add-On into position 11.

   Note: The third wash bottle position is now redundant.

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



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



12. 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).
13. 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).
14. Load the 50 ml tubes with prepared reagents (see Table 1) into the cooling compartment of the GeneReader as shown in Figure 1.

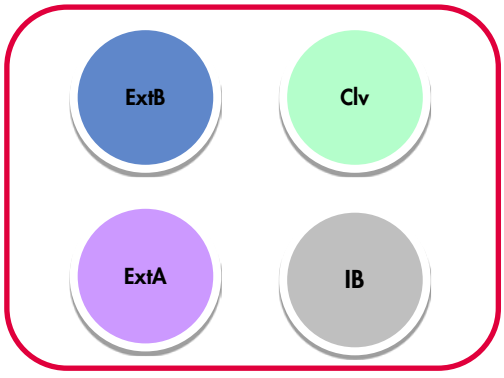


Figure 1. Reagent placement in the cooling compartment. Extend A (Ext A): Extend Premix A1 + Extend A2 + Pol Extend; Extend B (Ext B): Extend Premix B1 + Extend B2 + Pol Extend; Cleave Buffer (Clv): Cleave Premix + Cleave Additive; Image Buffer (IB): Image Premix (no additive required).

15. Click **Done** and then **Next**.
16. 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.)

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

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

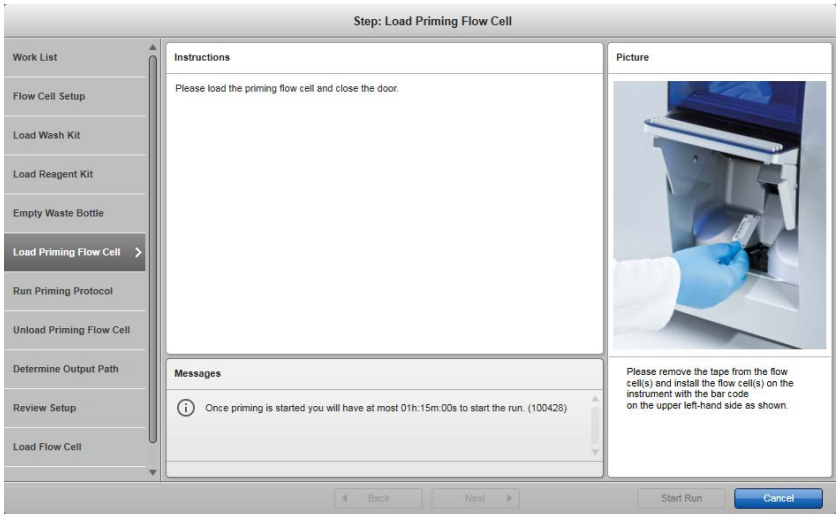
18. Close the hood by manually pulling it down.

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

Priming

20. 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.



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

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

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

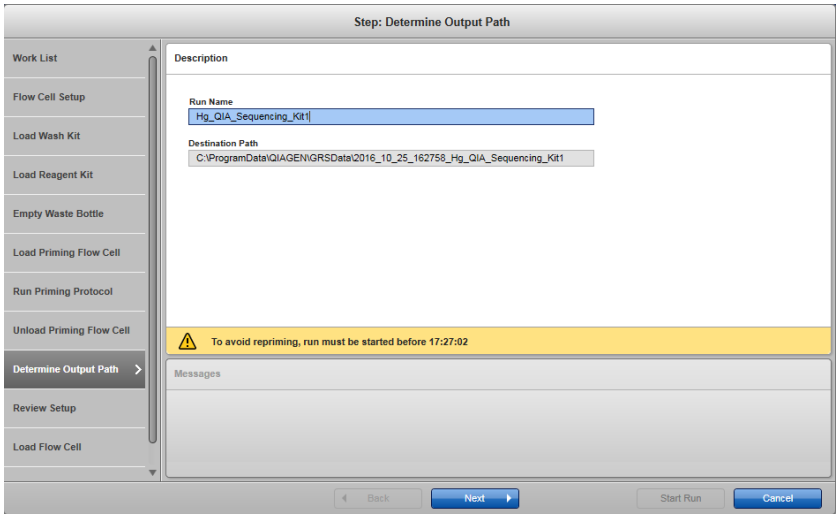
23. Unload the priming flow cell when priming is complete, and then close the flow cell door as guided by the wizard.

    IMPORTANT: Start run within 1 h after priming.

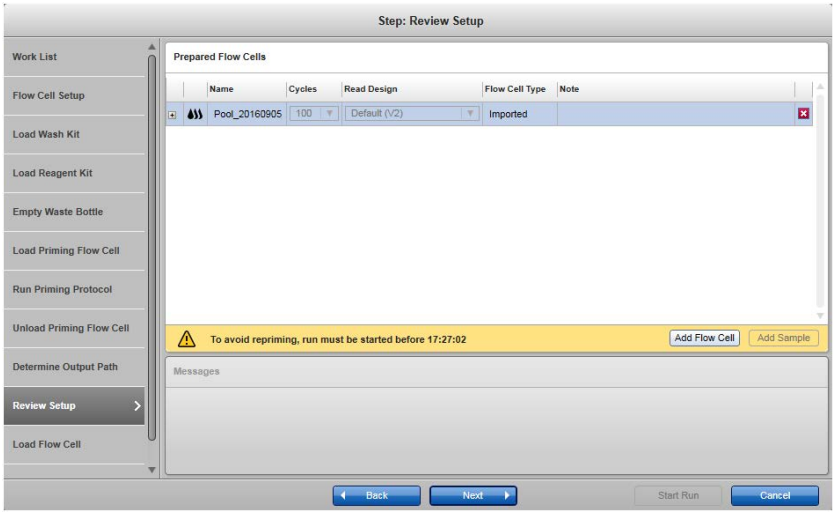
    IMPORTANT: Save the priming flow cell for later use.

24. Click **Next**.

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



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

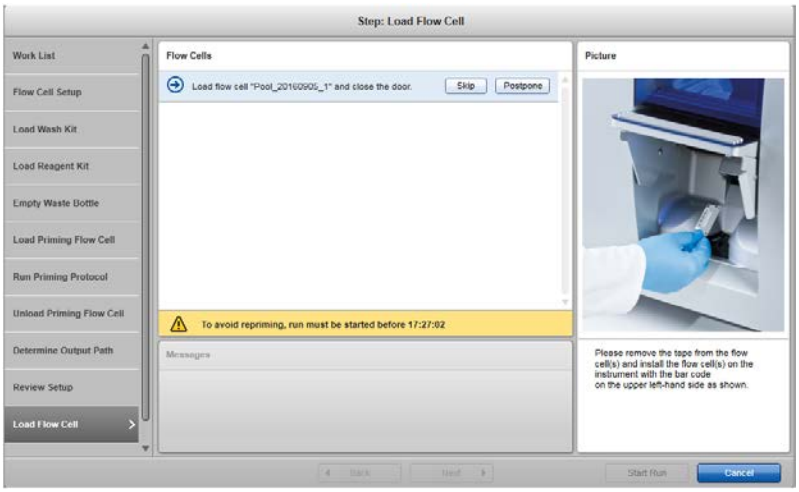


27. Click **Next** to begin loading flow cells.

Loading the flow cell

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

29. 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.

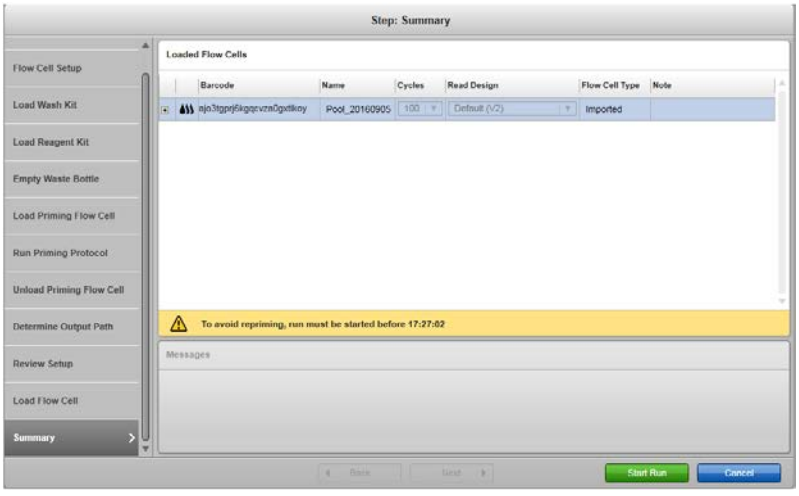
30. After loading the flow cell, close the flow cell door manually until the fastener snaps into place.

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

Note: If you set up more than one flow cell, the door will open again for each flow cell. Follow the on-screen instructions.

31. Click **Next**.

32. Click **Start Run**.



33. When the sequencing run is complete, proceed to "Protocol: Unloading reagents and flow cells" in the *GeneRead Fast Sequencing Q Kit Handbook*.

Symbols

The symbols in the following table include symbols used in this supplememtary protocol.

 <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		
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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