May 2021

# QIAprep&amp CRISPR and CRISPR-Q™ Custom Kits Handbook

For characterization of CRISPR editing events in cultured cells

Sample to Insight

QIAGEN

# Contents

# Kit Contents

| **QIAprep&amp CRISPR**<br>**Catalog no.**<br>**No. of reactions** | **(250)**<br>**232101**<br>**250*** | **(1000)**<br>**232102**<br>**1000*** |
|---|---|---|
| Cell Lysis Buffer | 12 ml | 4 x 12 ml |
| Proteinase K | 250 µl | 4 x 250 µl |
| AllTaq® Master Mix, 4x | 1.25 ml | 4 x 1.25 ml |
| Master Mix Tracer, 125x | 50 µl | 4 x 50 µl |
| Q-Solution®, 5x | 2 ml | 4 x 2 ml |
| CRISPR-Q Control PCR Assay | 100 µl | 4 x 100 µl |
| RNAse-Free Water | 2 x 1.9 ml | 8 x 1.9 ml |
| Quick-Start Protocol | 1 | 4 x 1 |

* The number of reactions is dependent on the experimental setup (e.g., culture plate format, cell type, and cell number). The number of PCR reactions is based on a reaction volume of 20 µl.

| **CRISPR-Q Custom PCR Assay**<br>**Catalog no.**<br>**No. of reactions** | **(20x)**<br>**232103**<br>**250** |
|---|---|
| CRISPR-Q Custom PCR Primer Mix, lyophilized | 1 vial |
| Product Sheet | 1 |

| **CRISPR-Q Sanger Primers**<br>**Catalog no.**<br>**No. of reactions** | **(10 µM)**<br>**232104**<br>**55*** |
|---|---|
| CRISPR-Q Sanger Primer, lyophilized | 1 vial |
| Product Sheet | 1 |

* The number of reactions is dependent on the Sanger sequencing method and the service provider. The number of reactions is calculated based on a primer input of 25 pmol per sequencing reaction.

# Shipping and Storage

The QIAprep&amp CRISPR Kit is shipped on dry ice. It should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. After the first thawing, the Cell Lysis Buffer should be stored at 2–8°C. Proteinase K is stored at 15–25°C or at 2–8°C to prolong shelf life.

The CRISPR-Q Custom PCR Assay and CRISPR-Q Sanger Primers are shipped lyophilized at room temperature (15–25°C). The primers can be stored at 4–20°C. After reconstitution, it is recommended to store the primers in aliquots at –20°C to avoid repeated freeze-thaw cycles. Under these conditions, the components are stable until the expiry date printed on their vials without showing any reduction in performance and quality, unless otherwise indicated on the label.

# Intended Use

The QIAprep&amp CRISPR Kit, the CRISPR-Q Custom PCR Assay Kit, and the CRISPR-Q Sanger Primers Kit are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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 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, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAprep&amp CRISPR Kit, CRISPR-Q Custom PCR Assay Kit, and CRISPR-Q Sanger Primers Kit is tested against predetermined specifications to ensure consistent product quality.

# Product Specifications

The QIAprep&amp CRISPR Kit contains the following:

**Description of the components used for liquid-based sample preparation:**

| Component | Description |
|---|---|
| Cell Lysis Buffer | Buffer allowing fast and efficient lysis of cultured cells |
| Proteinase K | Additive to the Cell Lysis Buffer to enhance performance |

**Description of the components used for amplification of a gene of interest:**

| Component | Description |
|---|---|
| AllTaq Master Mix, 4x | Contains AllTaq PCR Buffer and additives enabling fast cycling and direct loading of the reactions onto agarose gels or QIAxcel® Advanced. Also contains dNTP-Mix and AllTaq DNA Polymerase. The polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*. AllTaq DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. The enzyme is activated at 95°C. |
| Master Mix Tracer, 125x | Orange dye allowing tracking of master mix addition and monitoring of agarose gel loading and electrophoresis. The dye runs at approx. 50 bp on a 1% agarose gel. |
| Q-Solution | 5x concentrated |
| CRISPR-Q Control PCR Assay | 20x concentrated control primers enabling discrimination between raw cell lysate and assay issues. Control applicable to cultured cells derived from human, mouse, and rat. |
| RNase-Free Water | Ultrapure quality, PCR-grade |

The CRISPR-Q Custom PCR Assay Kit contains the following:

**Description of the assays used for the amplification of a gene of interest:**

| Component | Description |
|---|---|
| CRISPR-Q Custom PCR Assay | Two custom-designed primers specific for the target to be analyzed. The primers are flanking the CRISPR-edited site resulting in a PCR product of 400–600 bp. |

The CRISPR-Q Sanger Primers Kit contains the following:

**Description of the primers used for the sequencing of a gene of interest:**

| Component | Description |
|---|---|
| CRISPR-Q Sanger Primers | Custom designed primers specific for sequencing of CRISPR-edited gene sites. |

# Introduction

The QIAprep&amp CRISPR Kit is an innovative liquid-based method optimized for the characterization of CRISPR editing events. It combines fast sample processing and an amplification of CRISPR-edited targets without the need for laborious purification.

The kit is particularly suited for the liquid-based sample preparation from cultured cells derived from human, mouse, and rat.

The QIAprep&amp CRISPR Kit accelerates time to results, drastically reduces hands-on time compared to other methods, and streamlines the analysis of gene-editing events in cultured cells. By eliminating the need for DNA purification, the CRISPR kits allow PCR amplification directly from raw cell lysates. In combination with the CRISPR-Q Custom PCR Assay Kit, the CRISPR-Q Sanger Primers Kit, and the CRISPR-Q Sanger Sequencing Analysis tool, it covers the whole CRISPR editing detection workflow.

High specificity and sensitivity are achieved by the combination of the Cell Lysis Buffer and the AllTaq PCR chemistry. The CRISPR-Q Custom PCR Assays have been optimized for use with the QIAprep&amp CRISPR Kit and are not restricted to certain predefined targets. The generated PCR products are compatible with several downstream analyses such as Sanger sequencing or T7 endonuclease assays. The amplification of the edited region of interest can take place on any PCR cycler.

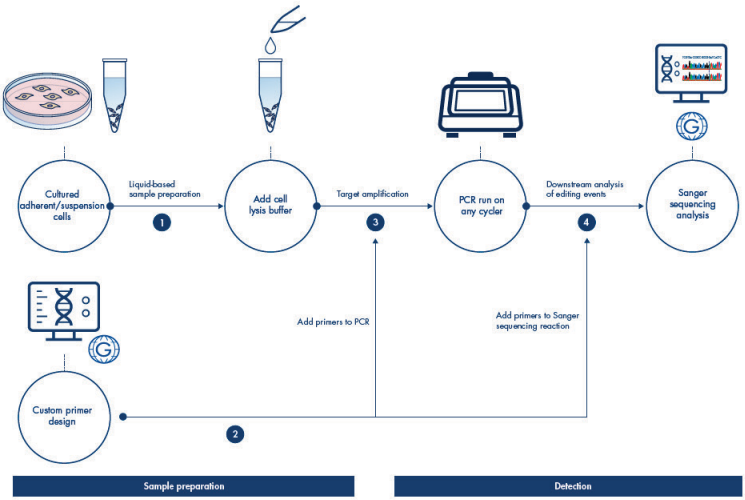



**Figure 1. Overview of the CRISPR gene-editing characterization workflow.** Cultured cells are processed and lysed (1) and can be directly used for the amplification of the region of interest (3). Target-specific assays for the PCR and Sanger sequencing primers can be designed on GeneGlobe® (2). For the quantification of editing efficiency, the PCR product obtained in step 3 can be further analyzed by Sanger sequencing and analyzed on GeneGlobe (4).

## Principle and procedure

The characterization of CRISPR editing events in cultured cells with the QIAprep&amp CRISPR Kit, CRISPR-Q Custom PCR Assay Kit, and CRISPR-Q Sanger Primers Kit comprises only 4 steps: liquid-based sample preparation, custom primer design, amplification of the region of interest, and quantification of editing efficiency.

### Cell processing and sample preparation

Cultured CRISPR-edited cells are briefly washed to remove cell culture medium, extracellular material released by living cells, and intracellular material released by any dead, lysed cells. Removal of such material is recommended because it can interfere with the downstream sample preparation and amplification processes.

For the CRISPR-edited genomic DNA preparation, the Cell Lysis Buffer supplemented with Proteinase K is directly added to the cells.

### Cell Lysis Buffer

Cell Lysis Buffer included in the QIAprep&amp CRISPR Kit is optimized for efficient cell lysis, increased lysate stability, and high compatibility with the AllTaq PCR chemistry. A wide range of cell numbers can be lysed. The lysis reaction takes place either in a tube or in the culture plate at room temperature and is stopped at 80°C.

Raw cell lysate can be directly used as DNA input into the PCR reaction without any intermediary purification step.

### Assays for the amplification of genomic regions of interest

The CRISPR-Q Custom PCR Assays can be designed using the free design tool available in GeneGlobe at **www.geneglobe.qiagen.com/customize/crispr/**. This tool generates several target-specific assays based on the genomic location and the sequence of the gRNA used for the CRISPR gene-editing events in cells.

### PCR amplification of genomic regions of interest

To determine whether CRISPR gene editing has been successful in the cells, the genomic region of interest can be amplified via PCR. The QIAprep&amp CRISPR Kit provides not only a solution for efficient cell lysis but also a highly compatible PCR chemistry. Target genes can be reliably amplified from raw cell lysates produced with the Cell Lysis Buffer using the AllTaq PCR chemistry and the CRISPR-Q Custom PCR Assays.

### AllTaq Master Mix

The AllTaq Master Mix provides a convenient format for highly sensitive and specific hot-start PCR using any DNA template. The master mix containing the AllTaq DNA Polymerase, AllTaq

PCR Buffer, and dNTPs is ready to use. The 4x concentrated master mix allows for a higher sample input.

### AllTaq DNA Polymerase, hot-start antibody mechanism

AllTaq DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *T. aquaticus*. At low temperatures, AllTaq DNA Polymerase is kept in an inactive state by an antibody and a novel guard additive, which stabilizes the complex. This improves the stringency of the hot-start and prevents any enzymatic activity at ambient temperature. The enzyme is fully activated by incubation at 95°C and starts amplifying with high specificity from the first cycle.

The hot-start procedure eliminates extension from nonspecifically annealed primers and primer-dimers in the first cycle, ensuring highly specific and reproducible PCR. The hot-start mechanism enables complete reaction setup at room temperature.

### AllTaq PCR Buffer

The innovative AllTaq PCR Buffer facilitates the amplification of specific PCR products. During the annealing step of every PCR cycle, the buffer allows a high ratio of specific-to-nonspecific primer binding. The verified buffer composition is adapted to ultra-fast cycling conditions and simultaneously provides stringent primer-annealing conditions over a wide range of annealing temperatures. It also ensures perfect duplex capabilities. Optimization of PCR by varying the $Mg^{2+}$ concentration is not required.

### Q-Solution

Q-Solution is an additive that facilitates amplification of difficult templates by modifying the melting behavior of nucleic acids. Q-Solution often enables or improves suboptimal PCR caused by DNA templates that have a high degree of secondary structure or that are GC-rich. For further information, please read Appendix D on page 35.

### Master Mix Tracer

The Master Mix Tracer is an orange dye that enables visual tracking during PCR setup and serves as a loading dye for agarose gels. The dye runs at approximately 50 bp on a 1% agarose gel. The 125x concentrate can be added either to the PCR reaction mix or directly to the master mix stock vial to obtain a 1x final concentration.

### CRISPR-Q Control PCR Assay

The CRISPR-Q Control PCR Assay is provided as a 20x concentrate and can be used as a positive control. It amplifies a conserved target region in human, mouse, and rat and timely reports insufficient DNA input or insufficient lysate quality. The size of the PCR product is 261 bp.

### CRISPR-Q Custom PCR Assay

The CRISPR-Q Custom PCR Primers can be designed using the free design tool available in GeneGlobe at **www.geneglobe.qiagen.com/customize/crispr/**. This tool generates several target specific primer pairs based on the genomic location and the sequence of the gRNA used for the introduction of CRISPR gene editing events in cells.

### Primers for Sanger sequencing of genomic regions of interest

The CRISPR-Q Sanger Primers can be designed using the free design tool available in GeneGlobe at **www.geneglobe.qiagen.com/customize/crispr/**. This tool generates several target specific primers based on the genomic location and the sequence of the gRNA used for the introduction of CRISPR gene editing events in cells.

## CRISPR-Q Sanger Sequencing Analysis tool

CRISPR editing efficiency can be determined by several analyses, one of which is Sanger sequencing of the region of interest. Obtained Sanger traces can be easily analyzed with QIAGEN's CRISPR-Q Sanger Sequencing Analysis tool offered on GeneGlobe at **www.geneglobe.qiagen.com/analyze**. Quantification is merely based on the Sanger traces and the sequence of the gRNA used for the CRISPR gene editing.

## Description of protocols

The following protocols for the characterization of CRISPR editing events in cultured cells are described in this handbook:

**Protocol: Liquid-based sample preparation from adherent cells**

Description of cell processing and raw cell lysate preparation. The protocol is optimized for cultured adherent cells.

**Protocol: Liquid-based sample preparation from suspension cells**

Description of cell processing and raw cell lysate preparation. The protocol is optimized for cultured suspension cells.

**Protocol: PCR amplification of genomic regions of interest**

Description of target amplification procedure on unpurified DNA from raw cell lysates.

**Protocol: Quantification of editing efficiency**

Description of downstream CRISPR target analysis.

# Equipment and Reagents to Be Supplied by User

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.

- Heating block, PCR cycler, or water bath (capable of reaching 80°C)
- Microcentrifuge tubes or PCR plates or strips compatible with the abovementioned heating device
- PCR tubes, strips, or plates and appropriate sealing foil
- Pipettes and pipette tips
- Microcentrifuge
- Vortexer
- CRISPR-Q Custom PCR Assay (cat. no. 232103) or other primer assay for target region
- To assess PCR outcome: Equipment for agarose gel electrophoresis or a QIAxcel system with associated equipment and reagents (refer to Appendix E on page 36 for detailed information)
- In case of quantification of editing events by Sanger Sequencing, a PCR purification solution is required [e.g., QIAquick PCR Purification Kit (cat. no. 28104), MinElute® PCR Purification Kit (cat. no. 28004), or MinElute Gel Extraction Kit (cat. no. 28604)]

# Important Notes

- The QIAprep&amp CRISPR Kit is an innovative liquid-based method optimized for sample preparation and detection of CRISPR editing events in cultured cells.
- The QIAprep&amp CRISPR sample preparation and target amplification are very robust against inhibitors such as transfection reagents, coating agents, as well as secreted intracellular and extracellular material carried over into the lysis reaction by residual culture medium. Nevertheless, due to the inhibitory potential for sample preparation and target amplification, it is advisable to carefully remove any culture medium before cells are being processed. Refer to Appendix B and Appendix C for additional information.
- A wide range of cells can be processed using the QIAprep&amp CRISPR Kit. The applicable number of cells optimally ranges from 10 to 3000 cells/µl. The number of cells to be lysed is dependent on several factors such as cell type, cultivation conditions, and experimental setup. An increased number of cells per microliter Cell Lysis Buffer might negatively affect sample preparation. In case of the lysate being too viscous or containing aggregates, lysate should be further diluted by the addition of inactivated Cell Lysis Buffer. Refer to Appendix C for additional information.
- Depending on the experimental CRISPR editing procedure, only a few cells are available for processing and target amplification. Sensitivity of the AllTaq PCR chemistry provided with the QIAprep&amp CRISPR Kit allows target amplification from a single cell. Lysate volume added to the PCR reaction can make up to 70% of the PCR reaction volume. Please note that amplification from very few cells and considerably increased lysate volumes in the PCR reaction might need additional optimization depending on the CRISPR target of interest and the chosen editing method.
- Depending on the target region to be amplified, 100,000 lysed cells can be tolerated in the AllTaq PCR reaction without affecting PCR performance and specificity. However, to reduce potentially inhibitory effects, it is recommended to add lysate corresponding to a maximum of 40,000 cells to a 20 µl PCR reaction.

- Samples can be kept at room temperature during preparation steps and reaction setup. The assay setup can be done at room temperature and should be processed immediately after sample addition.
- The CRISPR-Q Control PCR Assay is intended to report whether the PCR was technically successful and the lysate contains sufficient intact DNA material.

# Protocol: Liquid-Based Sample Preparation from Adherent Cells

This protocol is optimized for sample preparation from adherent cells cultured in 96-well plates and derived from human, mouse, or rat.

## Important points before starting

- Culture plate format is dependent on the cell type as well as the CRISPR procedure used for introducing gene edits. When using another format as 96-well plates, follow this protocol and also refer to Appendix B (page 31) to find out about the appropriate number of cells to seed and the appropriate Cell Lysis Buffer volumes to use. Optimally, the lysate should contain no more than 3000 cells/µl. Exceeding the upper limit of cells can negatively affect lysate preparation and downstream PCR performance.
- If growing cells in suspension or processing trypsinized cells, follow "Protocol: Liquid-Based Sample Preparation from Suspension Cells" on page 20.
- If working on semi-adherent cells, follow this protocol for the attached cell fraction and "Protocol: Liquid-Based Sample Preparation from Suspension Cells" on page 20 for the floating cell fraction.
- It is recommended to carefully wash cells before lysis because the culture medium might negatively affect cell lysis and the downstream steps in the characterization workflow.

## Things to do before starting

- Thaw Cell Lysis Buffer at room temperature or at 2–8°C.
- Add 80 µl Proteinase K to 12 ml Cell Lysis Buffer, mix well, and store at 2–8°C. Tick the check box on the Cell Lysis Buffer bottle to indicate that Proteinase K has been added. Be sure to briefly shake the supplemented Cell Lysis Buffer before each use.

## Procedure

1. Preheat a thermo block, a thermal cycler, or a water bath to 80°C.
2. Aspirate the cell culture medium, wash the cells with 1 x PBS, and aspirate all liquids and discard.

   **Note**: No enzymatic treatment of cells (e.g., by trypsin) is required.
3. Lyse cells by adding 50 µl Cell Lysis Buffer to the wells of the 96-well plate.

   **Note**: Agitation of the plate is not required. Lysis status of the cells can be monitored under the microscope.
4. Incubate for 15 min at room temperature.
5. After incubation, pipet the cell lysate 10–15 times up and down.
6. Transfer the cell lysate into an appropriately sized reaction tube or PCR plate.
7. Incubate for 10 min at 80°C, and briefly spin down.
8. The cell lysate can be directly used for target amplification described in “Protocol: PCR Amplification of Genomic Regions of Interest” on page 22 or stored at –20°C.

   **Note**: When storing the lysate at –20°C, refer to Appendix C on page 32 for further information.

# Protocol: Liquid-Based Sample Preparation from Suspension Cells

This protocol is optimized for sample preparation from cultured suspended or trypsinized cells derived from human, mouse, and rat.

## Important points before starting

- The cell number to be processed is dependent on several factors such as cell type, culture conditions, and experimental CRISPR editing. The volume of Cell Lysis Buffer added to the cells is dependent on the cell number. Optimally, the lysate should contain no more than 3000 cells/µl buffer. Exceeding the upper limit of cells can negatively affect lysate preparation and downstream PCR performance. Refer to Appendix B and Appendix C (pages 31 and 32, respectively) to find out about appropriate Cell lysis Buffer volumes to use.
- If growing adherent cells, follow "Protocol: Liquid-Based Sample Preparation from Adherent Cells" on page 18.
- If working on semi-adherent cells, follow this protocol for cells being in suspension and "Protocol: Liquid-Based Sample Preparation from Adherent Cells" on page 18 for the cells sticking to the culture plate.
- It is recommended to carefully wash cells before lysis since the culture medium might negatively affect cell lysis and the downstream steps in the characterization workflow.

## Things to do before starting

- Thaw Cell Lysis Buffer at room temperature or at 4°C.
- Add 80 µl Proteinase K to 12 ml Cell Lysis Buffer, mix well, and store at 2–8°C. Tick the check box on the Cell Lysis Buffer bottle to indicate that Proteinase K has been added. Be sure to briefly shake the supplemented Cell Lysis Buffer before each use.

## Procedure

1. Preheat a thermo block, a thermal cycler, or a water bath to 80°C.
2. Harvest CRISPR-edited cells in an appropriate vessel at 500 x *g* for 5 min at 4°C.

   **Note**: Centrifugation speed and time might need to be adjusted depending on cell type and cell number.
3. Aspirate the cell culture medium, wash the cells with 1x PBS.
4. Determine the cell number and calculate the volume of Cell Lysis Buffer needed to obtain a lysate containing 10–3000 cells/µl.

   **Example**: A total of 100 µl Cell Lysis Buffer is added to 1 x $10^5$ cells to obtain a lysate with approximately 1000 cells/µl.
5. Pellet the cells once again and aspirate all liquids.
6. Resuspend pelleted cells in the appropriate amount of Cell Lysis Buffer.

   **Note**: Cell lysis can take place in any appropriately sized reaction tube or PCR plate.
7. Incubate for 15 min at room temperature.
8. After incubation, pipet the cell lysate 10–15 times up and down.

   **Optional**: If not yet done, transfer lysate into a tube or plate that can be placed in the corresponding heating device.
9. Incubate for 10 min at 80°C, and briefly centrifuge.
10. The cell lysate can be directly used for target amplification described in protocol "Protocol: PCR Amplification of Genomic Regions of Interest" on page 22 or stored at –20°C.

    **Note**: When storing the lysate at –20°C, refer to Appendix C on page 32 for further information.

# Protocol: PCR Amplification of Genomic Regions of Interest

This protocol has been optimized for amplification using the CRISPR-Q Custom PCR Assays.

## Important points before starting

- The protocol focuses on target amplification from unpurified raw cell lysate.
- AllTaq DNA Polymerase requires a heat activation step at 95°C.
- It is not necessary to keep PCR tubes on ice because nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of AllTaq DNA Polymerase.
- The QIAprep&amp CRISPR Kit is provided with Q-Solution, which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich. When using Q-Solution for the first time with a particular primer-template system, it is recommended to run parallel reactions with and without Q-Solution to find out whether Q-Solution is beneficial. Also refer to Appendix D on page 35 for additional information.
- A negative PCR control without lysate, a positive PCR control with lysate, and the provided CRISPR-Q Control PCR Assay should be included in every experiment. If the control PCR product is not detected, this may indicate issues with the lysate or the PCR per se.
- It is always recommended to start with the cycling conditions and primer concentrations specified in this protocol.
- PCR amplification can be performed on any PCR cycler.

## Things to do before starting

- If using CRISPR-Q Custom PCR Assays, resuspend the PCR assays: spin down the tube before opening it for the first time. Add 275 µl nuclease-free water or Buffer TE (not provided) to the tube to obtain a 20x stock and leave at room temperature for 20 min. Mix and briefly spin down.
- Optional: When planning to analyze the target PCR product on an agarose gel, add the 125x concentrated Master Mix Tracer to the AllTaq Master Mix stock vial to obtain a 1x final concentration.

## Procedure

1. Thaw all PCR components listed in Table 1 and mix thoroughly before use.
2. Prepare a reaction mix according to Table 1.

   **Note**: Prepare a volume of reaction mix greater than that required for the total number of reactions to be performed. It is not necessary to keep samples on ice during reaction setup or while programming the cycler.

**Table 1. PCR reaction setup**

| Component | Volume/reaction | Final concentration |
|---|---|---|
| AllTaq Master Mix, 4x | 5 µl | 1x |
| CRISPR-Q Custom PCR Assay, 20x | 1 µl* | 1x |
| Raw cell lysate (template) | 5 µl | 10–40,000 cells/reaction† |
| Optional: Master Mix Tracer, 125x | 0.16 µl | 1x |
| Optional: Q-Solution, 5x | 4 µl‡ | 1x |
| RNase-Free Water | Variable | – |
| **Total reaction volume** | **20 µl** | |

* When using CRISPR-Q Control PCR Assay (20x), please add 1 µl per reaction. When using other assays, please refer to the *AllTaq PCR Core and Master Mix Kits Handbook* for more information.

† Number of cells per reaction is dependent on lysis protocol, cell type, plate format, and target of choice. Cell number may need further optimization. See Appendix B for more information.

‡ When using Q-Solution for the first time with a particular primer-template system, always perform parallel reactions with and without Q-Solution.

3. Add the lysate to the reaction. Mix gently but thoroughly and dispense 20 µl into PCR tubes or wells of a PCR plate.

   **Important**: Make sure that the mixture is homogenous before aliquoting to PCR tubes or PCR plate.

4. Place the PCR tubes or plates in a PCR cycler and start the PCR program as outlined in Table 2.

   **Note**: After amplification, samples can be stored at –20°C for longer storage.

**Table 2. PCR cycling conditions***

| Step | Time | Temperature | Comment |
|---|---|---|---|
| Initial PCR activation | 3 min | 95°C | This heating step activates AllTaq DNA Polymerase |
| **3-step cycling** | | | |
| Denaturation | 30 s | 95°C | |
| Annealing | 30 s | 60°C | Temperature might differ dependent on the $T_m$ of primers |
| Extension | 45 s | 72°C | |
| Number of cycles | 40 | | |
| Final extension | 5 min | 72°C | |

* Cycling conditions are optimized for CRISPR-Q Custom PCR Assays and the CRISPR-Q Control PCR Assay. Depending on the PCR cycler used for amplification, initial PCR activation and denaturation step may need further adjustment. Refer to the Troubleshooting Guide section on page 27 for further information.

5. To assess PCR specificity and efficiency, PCR reactions can be analyzed either via agarose gel electrophoresis or via capillary electrophoresis with the QIAxcel Advanced System.

# Protocol: Quantification of Editing Efficiency

This protocol has been optimized for the assessment of editing efficiency using the CRISPR-Q Sanger Primers and QIAGEN's CRISPR-Q Sanger Sequencing Analysis tool.

## Important points before starting

- This protocol describes the determination of CRISPR editing efficiency by Sanger sequencing.
- Sanger sequencing can be performed at any sequencing provider offering this service.
- It is recommended to sequence both strands of each PCR product particularly in the case of highly repetitive regions.
- PCR products need to be purified before sequencing. Recommended kits include the QIAquick® PCR Purification, the QIAquick Gel Extraction, and the MinElute PCR Purification Kits.
- Always include sequencing of a PCR product amplified from a nonedited lysate (e.g., wild type cells). This control sequence trace is required for the calculation of editing efficiency.

## Things to do before starting

- If using CRISPR-Q Sanger Primers, reconstitute the primers: spin down the tube before opening it for the first time. Add 137.5 µl nuclease-free water to the tube to obtain a 10 µM stock and leave at room temperature for 20 min. Mix and briefly spin down.

  **Important**: Do not reconstitute the primers with Buffer TE. The buffer contains EDTA which interferes with the sequencing reaction. Buffer EB (not provided) can be alternatively used to nuclease-free water. Stock concentration might need to be adjusted depending on the Sanger sequencing provider. Increase or decrease volume of nuclease-free water added to the stock accordingly.

## Procedure

1. Purify target PCR product using a PCR purification or gel extraction kit by following the respective protocol.

   **Note**: It is recommended to check PCR performance and specificity via agarose gel or capillary electrophoresis before starting with the purification. See Appendix E on page 36 for further information.

2. Prepare sequencing reaction according to the protocol indicated by the respective sequencing service provider.
3. Analyze the sequencing traces and quantify editing efficiency using the CRISPR-Q Sanger Sequencing Analysis tool on GeneGlobe at **www.geneglobe.qiagen.com/analyze**.

# 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 in 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 (for contact information, visit **support.qiagen.com**).

| | | Comments and suggestions |
|---|---|---|
| **Little or no PCR product detected** | | |
| a) | Lysate is too dilute | Increase the amount of lysate in the PCR reaction stepwise. Up to 14 µl lysate can be added to the PCR reaction. Alternatively, reduce cell lysis buffer volume or increase cell number to be lysed in the sample preparation step. |
| b) | Lysate is too concentrated | An increased number of cells in the PCR can negatively affect amplification performance. Reduce volume of lysate in the PCR or dilute lysate (e.g., 1:2, 1:5, and 1:10, and repeat the PCR reaction). |
| c) | Lysate quality is not sufficient | Ensure proper preparation and storage of lysate until use in PCR. Preferably use lysate timely after preparation. |
| d) | GC-rich region of interest | Add Q-Solution to the PCR reaction. It can also be beneficial to use 98°C instead of 95°C in initial activation step and in denaturation step during cycling. |
| e) | Target region with strong secondary structures | Use 98°C instead of 95°C in initial activation and denaturation step during PCR cycling. |
| f) | High concentration of PCR inhibitors | Ensure that cell culture medium is removed and cells are washed before lysis. Dilute lysate potentially containing PCR inhibitors and repeat experiment. |
| g) | Incomplete cell lysis | Follow all steps in the sample preparation protocols closely and make sure to stick to incubation times. |
| h) | Pipetting errors or missing reagent | Check the concentrations and storage conditions of the reagents, including primers and lysate samples. Repeat the PCR. |
| i) | Incorrect cycling conditions | Always start with the optimized cycling conditions specified in the protocol. Ensure that the PCR cycling conditions include the initial step for activation of AllTaq DNA Polymerase (95°C for 3 min) and the specified times and temperatures for denaturation and annealing/extension. |

| | | Comments and suggestions |
|---|---|---|
| j) | Primer concentration not optimal | Using 1 µl of reconstituted CRISPR-Q Custom or Control PCR Assay per reaction is strongly recommended. When using other primers please refer to concentrations stated in the *AllTaq PCR Core and Master Mix Kits Handbook*. |
| k) | Assay design not optimal | When using other assays than CRISPR-Q Custom PCR Assays please refer to *AllTaq PCR Core and Master Mix Kits Handbook* for primer design information and ensure to use according annealing temperature during cycling. |
| l) | Insufficient number of cycles | Increase the number of cycles. We recommend 40 cycles. |
| **Nonspecific PCR product(s)** | | |
| a) | Target region dependency | Addition of Q-Solution might be beneficial for the PCR performance. Performance can also be enhanced by increasing the temperature of the initial activation step and the denaturation step during cycling from 95°C to 98°C. |
| b) | Primer design not optimal | It is recommended to test up to 3 different assays per target region. |
| c) | Too much template | Dilute lysate, e.g., 1:10 and repeat PCR reaction. |
| d) | Insufficient lysate quality | Ensure proper preparation and storage of lysate until use in PCR. Preferably use lysate timely after preparation. |
| **CRISPR-Q Control PCR and target-specific PCR assay give no product** | | |
| a) | Lysate quality insufficient | Repeat liquid-based sample preparation |
| b) | Lysate input too high | Reduce template input into PCR by reducing lysate volume or by diluting lysate before addition to the PCR reaction. |
| c) | Insufficient template | Stepwise increase the amount of lysate in the PCR reaction. Alternatively, reduce cell lysis buffer volume or increase cell number to be lysed in the sample preparation step. |
| **CRISPR-Q Control PCR Assay is positive but assay for region of interest gives no product** | | |
| a) | Amount of template not optimal | Increase or reduce amount of lysate in the PCR reaction or dilute lysate if too concentrated. |
| b) | Secondary structures in template, GC-rich region of interest | Use 98°C instead of 95°C in initial activation and denaturation step during PCR cycling. |
| c) | Assay design not optimal | Try alternative assay design. We recommend trying 3 different assay designs. |
| **PCR product in negative PCR control** | | |
| a) | Contamination during reaction setup | Take appropriate precautions during reaction setup such as using aerosol-barrier pipette tips. |
| b) | Contamination of reagents | Discard all components of the assay (e.g., master mix, primers, RNase-Free Water) and repeat the PCR reaction with new components. |

# Appendix A: CRISPR Edit Types Covered by the QIAprep&amp CRISPR Kit and the CRISPR-Q Custom PCR Assay Kit

Genes can be disrupted in many different ways using the CRISPR technology. DNA insertions, large or small, can also be stably integrated into the genome. The characterization of the resulting knock-out or knock-in cells requires different approaches depending on the editing strategy. The QIAprep&amp CRISPR Kit covers the most commonly used approaches for the generation of those cell lines, whereas the CRISPR-Q Custom PCR Assay Kits are optimized for the characterization of edits introduced, for example, with 1 gRNA.

The CRISPR-Q Custom PCR Assays can be designed using the free design tool available in GeneGlobe at **www.geneglobe.qiagen.com/customize/crispr/**. This tool generates three target-specific assays based on the genomic location and the sequence of the gRNA used for the CRISPR gene-editing events in cells. Assay designs are available for all genomic locations in human, mouse, or rat species and are not restricted to pre-designed targets. Because assay design is based on the genomic location of where the gene edit is predicted, the CRISPR-Q design algorithm allows primer generation for a variety of Cas9 and Cas12/Cpf1 enzymes.

The following list is highlighting CRISPR editing strategies compatible with the QIAprep&amp CRISPR Kit and CRISPR-Q Custom PCR Assay Kits. Please note that the listed examples represent the most common editing approaches and are not exhaustive.

- **Generation of knock-out cells using one gRNA (NHEJ)**: Cells that were edited with one gRNA and the DNA break repaired by nonhomologous end joining (NHEJ) can be genetically characterized using QIAprep&amp CRISPR Kit as well as the CRISPR-Q PCR assays and Sanger primers.

- **Generation of knock-out cells using two gRNAs (NHEJ)**: Cells that were edited with two gRNAs for the same gene can be partly characterized using QIAprep&amp CRISPR Kit as well as the CRISPR-Q PCR assays and Sanger primers. If the gRNAs are positioned in a distance of maximally 100 bp to each other, the CRISPR-Q primer design tool can be used by indicating just one of the genomic locations and the gRNA sequences in GeneGlobe. In case of the gRNAs being more than 100 bp apart, only the QIAprep&amp CRISPR Kit is applicable but not the CRISPR-Q products.

  When editing multiple genes at once, each gene is treated separately and the above-mentioned applies.
- **Generation of knock-out cells using one gRNA (HDR)**: Cells that were edited with one gRNA and the DNA break repaired by homology-directed repair (HDR) can be genetically characterized using QIAprep&amp CRISPR Kit as well as the CRISPR-Q PCR assays and Sanger primers in case of small insertions (approx. 10 bp). When generating knock-out cells by inserting larger fragments, please refer to "Generation of knock-in cells using one gRNA (HDR)".
- **Generation of knock-in cells using one gRNA (HDR)**: Cells that were edited with one gRNA and the DNA break repaired by HDR can be genetically characterized using QIAprep&amp CRISPR kit as well as the CRISPR-Q PCR assays and Sanger primers in case of insertions of a maximum size of 400 bp. When inserting larger fragments only the QIAprep&amp CRISPR Kit is applicable.
- **Introduction of point mutations using base editing techniques**: Cells that were edited using one gRNA can be genetically characterized with QIAprep&amp CRISPR kit as well as the CRISPR-Q PCR assays and Sanger primers. The same holds true when using two gRNAs with a greater distance than 500 bp to each other. In that case each editing event can be treated separately.

# Appendix B: Seeding and Processing Cells for Different Plate Formats

The protocol on page 18 is for use with 96-well plates with 5000 cells per well. If using another type of plate, refer to Table 3 for the number of cells to seed per well and the volumes of Cell Lysis Buffer to add per well. Cells are grown according to the corresponding procedure or until confluent.

**Table 3. Cell number and Cell Lysis Buffer volumes for different plate formats**

| Plate format | Number of cells per well* | Volume of Cell Lysis Buffer per well (µl)† |
|---|---|---|
| 96-well plate | $1 \times 10^4$ | 40–50 |
| 24-well plate | $5 \times 10^4$ | 150–200 |
| 6-well plate | $3 \times 10^5$ | 200–500 |

* The values given are only suggestions. The number of cells per well depends on factors such as cell type, culture conditions, and CRISPR method used to introduce gene edits.

† Cell Lysis Buffer is supplemented with Proteinase K as described in "Things to do before starting". Depending on the cell number in each well, the buffer volume may need to be adjusted.

# Appendix C: Preparation of Cell Lysates for Target Amplification and Storage

Important factors for successful characterization include proper cell processing and sample preparation and the use of appropriate concentrated raw cell lysate for target amplification.

## Preparation of raw cell lysates

A wide range of cell numbers can be lysed using the QIAprep&amp CRISPR Cell Lysis Buffer. The lysate should optimally contain 10–3000 cells/µl. Increased cell numbers might lead to incomplete cell lysis and very viscous lysates containing aggregates hampering transfer to the PCR reaction. When observing aggregates in the lysate, addition of inactivated lysis buffer might help reducing aggregation and viscosity. To do so, Cell Lysis Buffer supplemented with Proteinase K is incubated for 10 min at 80°C before being used as diluent.

## Preparation of raw cell lysates from suspended cells

Cells in suspension need to be harvested before lysis. Optimally, the cell number resulting in a visible cell pellet is processed. The volume of Cell Lysis Buffer added to the cell pellet is calculated accordingly to obtain a lysate containing 10–3000 cells/µl. The centrifugation settings are dependent on the cell type. Recommended centrifugation speed from the respective cell line provider should be followed.

## Potential factors that may affect sample preparation and target amplification

Culture medium that might contain, depending on the experimental CRISPR setup, transfection/transduction reagents, coating agents, as well as secreted intracellular and extracellular material can influence the lysis reaction and subsequent PCR reactions. The effects

of several substances listed in Table 4 have been tested in HeLa, HEK 293T, and Jurkat cells. The tolerated quantity is displayed in Table 4 as well. Note that, depending on the cell line and the CRISPR target to be analyzed, these values may not be applicable.

**Table 4. Substances tested to be tolerated by the QIAprep&amp CRISPR workflow**

| Tested Substance | Tolerated Concentration |
|---|---|
| DMEM complete medium | Up to 25% of lysate volume |
| Polybrene (Hexadimethrine Bromide) | Up to 1 µg/ml* |
| Lipofectamine CRISPRMAX™ | Concentration recommended by the manufacturer† |
| Lipofectamine 2000® | Concentration recommended by the manufacturer† |
| DharmaFECT® Duo | Concentration recommended by the manufacturer† |
| Effectene® | Concentration recommended by the manufacturer† |
| Corning® Cell-Tak | 3,5 µg/cm² in culture vessel‡ |
| Poly-L-Lysine | 100 µg/ml‡ |
| Fibronectin | 10 µg/ml‡ |
| Gelatin | 0.1% (w/v)‡ |
| Gelatin from cold water fish skin | 2% (w/v)‡ |

* Final concentration in target amplification reaction that did not influence PCR outcome.

† Concentration that was used during treatment of cells and that did not negatively influence cell processing and PCR amplification of targets.

‡ Concentration used for coating of cell culture vessels prior cultivation of cells that did not affect cell lysate preparation and PCR amplification of targets.

## Lysate input into AllTaq PCR

Optimally, 10–40,000 cells are transferred to a 20 µl PCR reaction. An increased number of cells might have negative effects on the PCR. Depending on the target to be amplified, up to 100,000 cells per reaction can be tolerated. Due to the high sensitivity of the AllTaq PCR Master Mix, a single cell might be sufficient for amplification depending on the target. Input volume of lysates containing very few cells can be considerably increased. The lysate can take up a maximum of 70% of the PCR reaction volume. Input volume of lysates containing an

increased number of cells can be arbitrarily reduced. Note that, depending on the CRISPR target gene, this might not apply.

## Lysate stability

Raw cell lysates generated using the QIAprep&amp CRISPR Cell Lysis Buffer are particularly robust. Up to 15 freeze–thaw cycles have been tested to not affect lysate quality and downstream PCR performance. Note that stability might vary depending on the target of interest.

It is recommended to incubate the cells for 15 min with the Cell Lysis Buffer at room temperature. Extended lysis might affect PCR performance depending on the CRISPR target. Three-hour incubation at room temperature has been tested showing no significant effect on the downstream PCR. Prolonged lysate incubation at 80°C is not recommended.

## Lysate storage

Raw cell lysates can be long-term stored at –20°C in a constant-temperature freezer. To reduce repeated sample thawing, it is recommended to store the lysate in small aliquots. Storage in tubes is preferred over storage in plates. When storing in plates, proper plate sealing with an adhesive foil is crucial to avoid genetic material damages resulting in decreased lysate quality. When removing adhesive plate foil, make sure that the lysate is not spilling from one well to the other to avoid sample contamination.

# Appendix D: PCR using Q-Solution

Q-Solution changes the melting behavior of nucleic acids and can be used for PCR systems that do not work well under standard conditions. When using Q-Solution for the first time with a particular primer-template system, always perform parallel reaction with and without Q-Solution. PCR performance can differ in a target-dependent manner.

When using Q-Solution, the following effects may be observed depending on the individual PCR assay:

**Case A**: Q-Solution increases PCR specificity in certain primer-template systems. Q-Solution enables PCR that previously failed.

**Case B**: Q-Solution causes reduced efficiency or failure of a previously successful amplification reaction.

# Appendix E: Analysis of PCR Products Using the QIAxcel Advanced System

Following sample preparation and target amplification, it is recommended to assess PCR specificity and efficiency prior to subsequent steps in the CRISPR editing characterization workflow such as Sanger sequencing or T7 endonuclease I (T7EI) assay. Verification of obtained PCR product and monitoring of its quality is critical for obtaining accurate quantification of editing events via either Sanger sequencing or via T7EI assay. PCR products can be evaluated either by agarose gel electrophoresis or by capillary electrophoresis with the QIAxcel Advanced System. Moreover the QIAxcel system can be used to analyze T7EI cleavage reactions.

## Equipment and reagents

- 12-tube strips or 96-well plate containing samples to be analyzed
- Pipets and pipet tips
- QIAxcel DNA gel cartridge (we recommend the QIAxcel DNA High Resolution Kit, cat. no. 929002)
- QX Alignment Marker (for assessment of PCR products Alignment Marker 15 bp/5 kb, cat. no. 929524, and for analysis of T7EI cleavage reactions QX Alignment Marker 15 bp/1 kb, cat. no. 929521 can be used)
- QX DNA Size Marker (for assessment of PCR products QX DNA Size Marker 250 bp – 4 kb (50 µl) v2.0, cat. no. 929562, and for analysis of T7EI cleavage reactions QX DNA Size Marker 50–800 bp (50 µl) v2.0, cat. no. 929561 can be used)
- QIAxcel instrument and QIAxcel ScreenGel® Software, preferably version 1.6

## Important points before starting

- For verification of PCR products prior to clean up, we recommend using the sensitive QIAxcel 0L500 method to allow detection of even low-concentrated PCR products. To ensure accurate quantification of editing events, it is important to also detect additional PCR products that might have been unspecifically generated during target amplification. Hence, high sensitivity in detection is usually beneficial. However, in case of strong or saturated signal, methods 0M500 or 0H500 can be used. Alternatively, sample injection time can be accordingly adjusted. Please refer to the *QIAxcel DNA Handbook* for more detailed information on QIAxcel High Resolution methods.
- The choice of the QIAxcel size and alignment marker depends on the fragment size to be analyzed. For assessment of fragments directly after PCR or before Sanger sequencing QX Alignment Marker 15 bp/5 kb (cat. no. 929524) in combination with QX DNA Size Marker 250 bp – 4 kb (50 µl) v2.0 (cat. no. 929562) can be used. QX DNA Size Marker 250 bp – 4 kb v2.0 is used at a concentration of 10 ng/µl with a QIAcel DNA High Resolution Gel Cartridge using method 0L500. In case of determination of cleavage efficiency of mismatched heteroduplexes after, for example, T7EI cleavage, it is advisable to use a combination of markers covering smaller DNA fragments, such as QX Alignment Marker 15 bp/1 kb (1.5 ml) (cat. no. 929521) and QX DNA Size Marker 50–800 bp (50 µl) v2.0 (cat. no. 929561).
- For optimal performance, it is recommended to perform a long purge before each run as well as to frequently exchange the buffers in the QX Buffer Tray (refer to *QIAxcel Advanced User Manual* for additional information).
- The minimal sample volume required for analysis is 10 µl.

### Things to do before starting

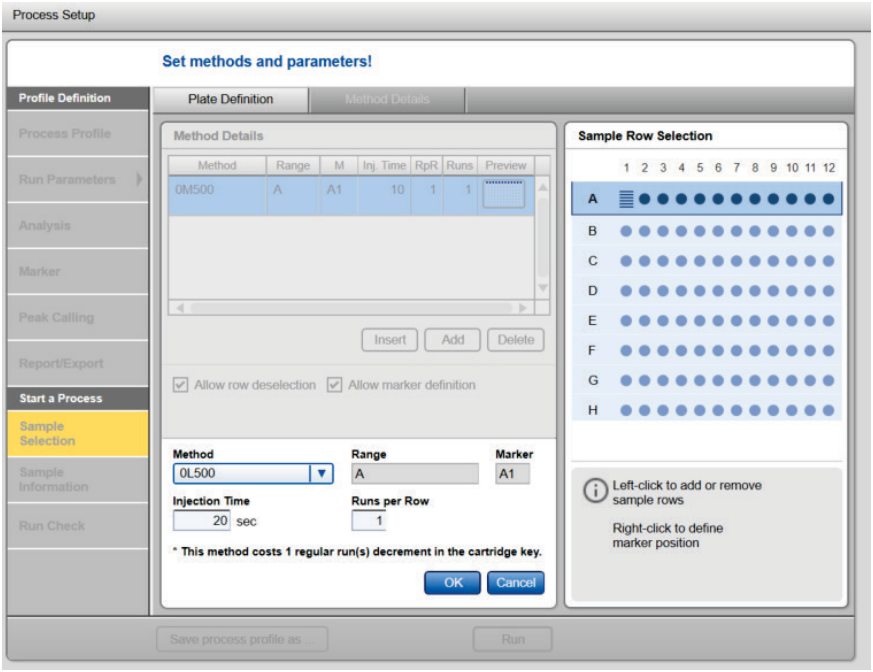
- Allow QIAxcel DNA High Resolution Gel Cartridge to equilibrate to room temperature for at least 20 minutes prior to use. If the QIAxcel DNA High Resolution Gel Cartridge is being used for the first time, intensity calibration needs to be performed (refer to the *QIAxcel DNA Handbook*).
- Prepare the buffer tray (refer to the *QIAxcel DNA Handbook*).
- Prepare QX Alignment Marker and QX DNA Size Markers as described in the *QIAxcel DNA Handbook*.

### Procedure

1. Switch on QIAxcel instrument and computer and launch QIAxcel ScreenGel Software.
1. Install the QIAxcel DNA High Resolution Gel Cartridge and smart key (refer to the *QIAxcel User Manual*).
2. Load the buffer tray and the QX Alignment Marker into the buffer tray holder. Refer to Section 5.2.2 of the *QIAxcel User Manual* or the *QIAxcel Advanced User Manual* for more details.
3. Load the sample strips or a 96-well plate containing samples onto the sample tray holder.

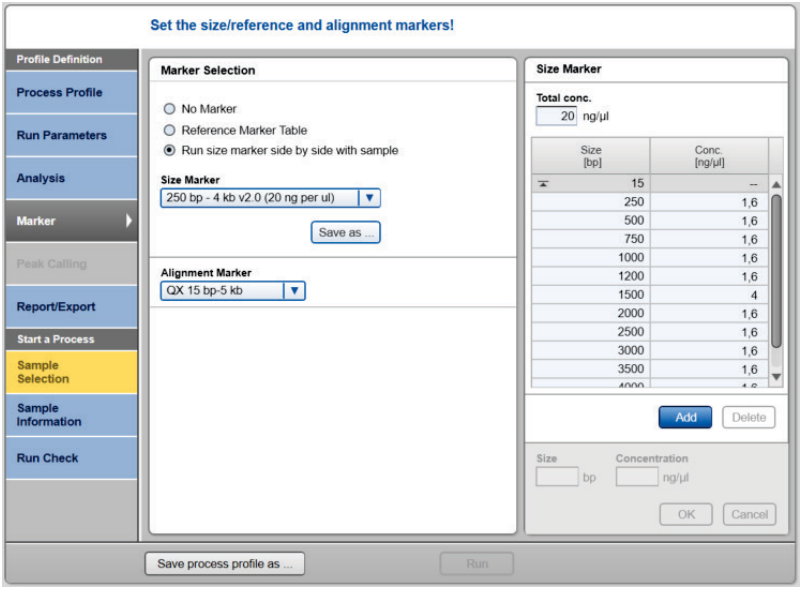
   **Note**: The cartridge door and sample door of the QIAxcel instrument must remain closed during operation of the instrument. Opening the cartridge door or sample door during operation will cause the system to immediately stop.
4. Select the following process profile from the drop-down list: **Default High Res**.

5. Go to **Run Parameters**, click on the plate **Preview** and change the method from **0M500** to **0L500** in the method drop-down list.




6. In the **Sample Row Selection** on the right (see above) activate the rows to be analyzed in the run by left-clicking and select marker position by right-clicking. Multiple rows can be activated by dragging over the corresponding rows. Click **OK** to save method and rows to be analyzed.

7. Go to **Marker** tab and chose the corresponding Size Marker and Alignment Marker in the drop-down menus. Adjust the total concentration of the Size Marker on the right side if necessary.




8. In the **Sample Information** tab, additional information on samples can be entered or uploaded.
9. Open the **Run Check** tab to confirm that the samples as well as both of the markers have been loaded correctly.
10. Click **Run** to start the analysis.

    **Note**: A report is automatically generated according to the settings in the selected process profile.
    Changes to the analysis settings can be performed by an advanced user.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QIAprep&amp CRISPR Kit (250) | 1 x 12 ml Cell Lysis Buffer, 1 x 1.25 ml AllTaq Master Mix (4x), 1 x 50 µl Master Mix Tracer (125x), 1 x 250 µl CRISPR-Q Control PCR Assay, and 2 x 1.9 ml RNase-Free Water. | 232101 |
| QIAprep&amp CRISPR Kit (1000) | 4 x 12 ml Cell Lysis Buffer, 4 x 1.25 ml AllTaq Master Mix (4x), 4 x 50 µl Master Mix Tracer (125x), 4 x 250 µl CRISPR-Q Control PCR Assay, and 8 x 1.9 ml RNase-Free Water. | 232102 |
| CRISPR-Q Custom PCR Assay | 2 premixed primers, dried down | 232103 |
| CRISPR-Q Sanger Primers | 2 custom designed primers, dried down | 232104 |
| **Related products** | | |
| QIAquick Gel Extraction Kit (50) | For gel extraction or cleanup of 50 reactions: 50 QIAquick spin columns, buffers, and collection tubes (2 ml) | 28704 |
| QIAquick 96 PCR Purification Kit (4) | For purification of 4 x 96 PCR reactions: 4 QIAquick 96 plates, buffers, collection microtubes (1.2 ml), and caps. | 28181 |
| MinElute PCR Purification Kit (50) | For purification of PCR products in low elution volumes: 50 MinElute Spin Columns, buffers, and collection tubes (2 ml) | 28004 |

| Product | Contents | Cat. no. |
|---|---|---|
| QIAxcel DNA High Resolution Kit (1200) | QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips | 929002 |
| QX Alignment Marker 15 bp/5 kb (1.5 ml) | Alignment marker with 15 bp and 5 kb fragments | 929524 |
| QX Alignment Marker 15 bp/1 kb (1.5 ml) | Alignment marker with 15 bp and 1 kb fragments | 929521 |
| QX DNA Size Marker 250 bp-4 kb (50 µl) v2.0 | DNA size marker with fragments of 250, 500, 750, 1000, 1200, 1500, 2000, 2500, 3000, 3500, and 4000 bp; concentration 100 ng/µl | 929562 |
| QX DNA Size Marker 50 bp-800 bp (50 µl) v2.0 | DNA size marker with fragments of 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, and 800 bp; concentration 100 ng/µl | 929561 |

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# Document Revision History

| Date | Changes |
|---|---|
| 05/2021 | Initial revision |

## Notes

## Notes

## Notes

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HB-2863-001 05/2021