Q Protocol

Guidelines for the QIAgility® forensic workflow using kits from suppliers other than QIAGEN

This document describes how to use the QIAgility to set up assays for quantitative real-time PCR, and STR analysis, and how to prepare daughter plates for subsequent sequence analysis by capillary electrophoresis. Three Q Protocols cover this complete forensic workflow, using the Quantifiler® Duo DNA Quantification Kit and the AmpFℓSTR® Identifiler® PCR Amplification Kit. The Q Protocols are intended to be run one after the other, enabling transfer of sample information along the workflow.

IMPORTANT: Before using the QIAgility, it is essential to read the QIAgility User Manual carefully and pay particular attention to the safety information.

Equipment and reagents

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.

Instruments

- QIAgility (cat no. 9001611)
- Applied Biosystems® 7500 Real-Time PCR System*
- GeneAmp® PCR System 9700 (Applied Biosystems)*
- ABI PRISM® 310, ABI PRISM 3100-*Avant*™/3100, Applied Biosystems 3130/3130*xl*, 3500™/3500xL Genetic Analyzers (Applied Biosystems)*

Kits and consumables

- Quantifiler Duo DNA Quantification Kit (cat. no. 4387746, Applied Biosystems)*
- AmpFℓSTR Identifiler PCR Amplification Kit (cat. no. 4322288, Applied Biosystems)*
- Hi-Di™ Formamide (cat. no. 4311320, Applied Biosystems)*
- GeneScan® 500 LIZ Size Standard (cat. no. 4322682, Applied Biosystems)*
- Allelic Ladder (cat. no. 4373672, Applied Biosystems)*
- 50 µl Conductive Filtered Tips (cat. no. 990512)
- 200 µl Conductive Filtered Tips (cat. no. 990522)

- 5 ml Tube; Graduated, Flat-Base (cat. no. 990552)
- 1.5 ml Flip Cap Tubes (generic)
- PCR Tubes, 200 µl (generic)
- MicroAmp® Optical 96-well Reaction Plate (cat. no. N801-0560, Applied Biosystems)*
- Thermo-Fast® 96 Non-skirted Plate (cat. no. AB-0600, Thermo-Fisher Scientific)†

* For more information, visit www.appliedbiosystems.com.

† For more information, visit www.thermoscientificbio.com.

Q protocols

- Quantifiler Duo Quantification Kit
- AmpFℓSTR Identifiler PCR Amplification Kit
- CE loading with the AmpFℓSTR Identifiler PCR Amplification Kit

Note: For detailed information about using and installing Q Protocols, see "Guidelines for using Q Protocols" and "Guidelines for installing Q Protocols".

Procedure

Set up assays using the Quantifiler Duo DNA Quantification Kit

1. **Manually dilute the Quantifiler Duo DNA Standard to a final concentration of 50 ng/µl.**
2. **Select the Quantifiler ABI DUO V1 Q Protocol in the QIAgility software.**
3. **Import sample names and IDs from a file (*.txt or *.csv format), or enter this information manually.**

   For more details about importing sample information, see the section "Importing and exporting reaction and sample data" in the *QIAgility User Manual*.
4. **Assign the samples to a predefined sample bank.**

   Note: The first 3 wells of the sample bank (i.e., wells A1, A2, and A3) must be empty to allow for controls and allelic ladder to be added later in the forensic workflow.
5. **Set up the QIAgility worktable and load the samples according to the instructions in the pre-run report.**
6. **Start the protocol.**
7. **When the protocol is finished, transfer the samples to the Applied Biosystems 7500 Real-Time PCR System.**
8. **Export the reaction data using the Applied Biosystems 7000/7500 SDS absolute quantification export mode.**

   For more details about exporting reaction data, see the section "Importing and exporting reaction and sample data" in the *QIAgility User Manual*.

9. **Import this reaction data to the Applied Biosystems 7500 Real-Time PCR System and then start the run.**

   For more details about how to do this, refer to the instrument user manual supplied by Applied Biosystems. Ensure that you are using the correct software version with your 7500 Real-Time PCR System.

10. **When the run is finished, export the data from the 7500 Real-Time PCR System.**

    Note: The format in which the data is exported depends on the software settings.

11. **Manually process the exported data so that it can be imported to the QIAgility.**

    Before importing the data to the QIAgility, ensure that:

    - Data are in columns (table format)
    - There are no exponential numbers
    - There are no empty wells for samples to be processed (e.g., replace "undetermined" with "0")
    - The decimal separator is correct, this depends on the language settings
    - A detector has been selected (analysis data contain data for 3 detectors)
    - Data has been filtered (e.g., by using AutoFilter in Excel for the "Detector" column) and copied to a separate sheet

Set up assays using the AmpFℓSTR Identifiler PCR Amplification Kit

1. **Select the AmpFℓSTR Identifiler PCR V1 Q Protocol in the QIAgility software.**
2. **Import sample names, IDs, and concentrations using the data file that was exported from the 7500 Real-Time PCR System.**

   For more details about importing sample information, see the section "Importing and exporting reaction and sample data" in the *QIAgility User Manual*.

3. **Assign samples to predefined sample banks using the row filter.**

   Note: The first 3 wells of the sample bank (i.e., wells A1, A2, and A3) must be empty to allow for 2 controls and allelic ladder to be added later in the forensic workflow.

   Note: Samples with a concentration greater than 20 µg/ml must be manually diluted.

   - Filter samples in the first sample bank, "Fillup (0–1)", by selecting "Use selected wells INSIDE range"
   - Filter samples in the second sample bank, "1. Dilution (1-20)", by selecting "Use selected wells OUTSIDE range"
   - Close the window, select the normalized samples from the reaction list, and then select the third sample bank, "Apply Filter to Interdilution"

4. **Prepare the master mix, including additional volume to the equivalent of 3 extra reactions.**
5. **Set up the QIAgility worktable and load the same samples as for the Quantifiler ABI DUO protocol, according to the instructions in the pre-run report.**

6. **Start the protocol.**
7. **When the protocol is complete, transfer the assays to the GeneAmp PCR System 9700 for amplification.**

   For more details about operating the GeneAmp PCR System 9700, refer to the user manual supplied with the instrument.
8. **Export the reaction data from the QIAgility as a *.csv file.**

Set up samples for capillary electrophoresis

1. **Select the CE-Loading-Setup V1 Q Protocol in the QIAgility software.**
2. **Import sample names, IDs, and well positions using the *.csv file that was previously exported from the QIAgility.**
3. **Assign samples to a predefined sample bank.**

   Note: If fewer than 32 reactions were processed, the number of wells filled with diluent must be adjusted in the Q Protocol.
4. **Premix Hi-Di Formamide and GeneScan-500 LIZ Size Standard according to the manufacturer's instructions, including additional volume to the equivalent of 3 extra reactions.**
5. **Setup the QIAgility worktable and load the samples (i.e., amplified DNA fragments) according to the pre-run report.**
6. **Start the protocol.**
7. **Export the reaction data to the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).**
8. **Perform analysis by capillary electrophoresis according to the manufacturer's instructions.**

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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