

# Q Protocol

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## Guidelines for the QIAgility® forensic workflow using Investigator® kits

This document describes how to use the QIAgility to set up assays for STR analysis, and how to prepare daughter plates for subsequent sequence analysis by capillary electrophoresis. The 3 QIAgility Investigator Q Protocols are for use with Investigator Kits for amplification of autosomal and gonosomal loci. The Investigator CE-Loading Q Protocol is intended to be run after the others, enabling transfer of sample information along the workflow. The Q Protocols are templates. The user can adjust them for any special requirements.

**Note:** The Investigator Autosomal STR Q Protocol can be used with all Investigator STR kits for amplification of autosomal loci. This protocol is also suitable for setup of the Investigator Argus X-12 Kit for amplification of gonosomal loci. The Investigator Argus Y-12 QS Kit is supplied with 2 DNA controls, male and female. When both of these controls are used, the Investigator Argus Y-12 QS Q Protocol should be used. When just one of these controls is used, the Investigator Autosomal STR Q Protocol should be used instead.

**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) with software version 4.1.3.5 or later
- GeneAmp® PCR System 9700 (Applied Biosystems)\*
- DNA analyzer, e.g., ABI PRISM® 310, ABI PRISM 3100-*Avant*™/3100, Applied Biosystems® 3130/3130xl, or 3500™/3500xL Genetic Analyzer (Applied Biosystems)\*

### Kits and consumables

- Investigator Kits for amplification of autosomal and gonosomal loci (various cat. nos., see [www.qiagen.com](http://www.qiagen.com))

**Note:** Q protocols described in this document are not suitable for use with the Investigator DIPplex, Investigator IDplex Plus, Investigator ESSplex Plus, or Investigator ESSplex SE Plus Kits.

\* For more information, visit [www.appliedbiosystems.com](http://www.appliedbiosystems.com).



- Hi-Di™ Formamide (cat. no. 4311320, 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)
- 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)†

## Q protocols

- Amplification of autosomal STR loci using Investigator Human Identification PCR Kits
  - Investigator Argus Y-12 QS Kit
  - CE loading with Investigator Human Identification PCR Kits
- Note:** For detailed information about using and installing Q Protocols, see “Guidelines for using Q Protocols” and “Guidelines for installing Q Protocols”.

## Software patch

To install the software patch, double-click on the \*.zip file. Follow the instructions.

- QIAgility\_4-12-9\_ROSYS-51\_ABgene-vertical-additionalSegmentations.zip

## Procedure

### Preparation

1. **Download the required Q Protocols from the “User Support” tab of [www.qiagen.com/goto/QIAgility](http://www.qiagen.com/goto/QIAgility) and install them.**
2. **Download the required software patch from the same website and install it.**
3. **Select the correct Q Protocol for the kit that you are using.**
4. **The Investigator Autosomal STR Q Protocol can be used with all autosomal Investigator STR Kits for amplification of autosomal loci, and with the Investigator Argus X-12 Kit for amplification of gonosomal loci.**

**Note:** The Investigator Argus Y-12 QS Kit is supplied with 2 DNA controls. When both of these controls are used, the Investigator Argus Y-12 QS Q protocol should be used. When just one of these controls is used, the Investigator Autosomal STR Q Protocol should be used instead.

\* For more information, visit [www.appliedbiosystems.com](http://www.appliedbiosystems.com).

† For more information, visit [www.thermoscientificbio.com](http://www.thermoscientificbio.com).

## Running the Investigator Autosomal STR Q Protocol

1. **Open the Investigator Autosomal STR Q Protocol in the QIAgility software.**

2. **Import sample names, IDs, and concentrations from a file (\*.txt or \*.csv format).**

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:** Samples with a concentration greater than 20 ng/ $\mu$ l must be manually diluted.

- Filter samples in the first sample bank, “Fillup (0–0.3)”, by selecting “Use selected wells INSIDE range”
- Filter samples in the second sample bank, “1. Dilution (0.3–20)”, by selecting “Use selected wells OUTSIDE range”
- Close the window.

4. **Members of the third sample bank, “Normalized samples”, are assigned from the first reaction plate containing the normalized samples of the second sample bank.**

- Go to the reaction list
- Right-click on the “Normalize samples...” command to show the “Create Sample Bank from target wells” menu item
- Select the sample bank “Normalized samples”
- Add selected samples and click “Done”

5. **Prepare the master mix, including additional volume to the equivalent of 4–7 reactions.**

- For 1–11 calculated reactions, add additional volume to the equivalent of 4 reactions
- For 12–20 reactions, add additional volume to the equivalent of 5 reactions
- For 21–30 reactions, add additional volume to the equivalent of 6 reactions
- For 31–35 reactions, add additional volume to the equivalent of 7 reactions

**Note:** Add water to a final volume of 10  $\mu$ l for each reaction. For the Investigator Argus Y-12 QS Kit, the master mix for each reaction can be prepared according to the following example.

Component	Volume ( $\mu$ l)
Reaction Mix A	5
Primer Mix Argus Y-12 QS	2.5
Multi Taq 2 DNA Polymerase	0.6
Nuclease-free water	1.9 (instead of 15.9 $\mu$ l)

6. **Prepare the appropriate volume of Positive Control DNA at the required concentration, as described in the kit handbook and in the table below.**

**Note:** 15  $\mu$ l Positive Control DNA is needed for the procedure, but additional volume is required by the QIAgility. Therefore a volume of 30  $\mu$ l is indicated in the pre-run report.

Positive Control DNA required (ng/ reaction)	Positive Control in 30 $\mu$ l
0.2	0.4
0.25	0.5
0.35	0.7
0.5	1.0

7. Set up the QIAgility worktable according to the instructions in the pre-run report.
8. Start the protocol.
9. When the protocol is complete, export the reaction data (sample names or IDs) as a \*.csv file for the Investigator CE-Loading Q Protocol.
10. Transfer the assays to the GeneAmp PCR System 9700 (Applied Biosystems) for amplification.

For more details about operating the GeneAmp PCR System 9700, refer to the user manual supplied with the instrument.

**Note:** No import or export occurs at this step.

## Running the Investigator Argus Y-12 QS Protocol

The Investigator Argus Y-12 QS Kit is supplied with 2 DNA controls — Control DNA XY1 and Control DNA XX.

1. Prepare the required volume of Control DNA XY1 at the correct concentration, as described in the kit handbook and in the table below.

**Note:** 15  $\mu$ l Control DNA XY1 is needed for the procedure, but additional volume is required by the QIAgility. Therefore a volume of 30  $\mu$ l is indicated in the pre-run report.

Control DNA XY1 required (ng/ reaction)	Control DNA XY1 in 30 $\mu$ l (ng)
0.2	0.4
0.25	0.5
0.35	0.7
0.5	1.0

2. Prepare the required volume of Control DNA XX at the correct concentration (e.g., 5 ng in 30  $\mu$ l).

**3. Open the Investigator Argus Y-12 QS Q Protocol in the QIAgility software.**

**Note:** If only Control DNA XY1 is used, the Investigator Autosomal STR Q Protocol should be selected instead.

**4. Proceed to step 2 of “Investigator Autosomal STR Q Protocol”.**

## Set up samples for capillary electrophoresis

**1. Select the Investigator CE-Loading 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 are processed, the number of wells filled with diluent must be adjusted.

**Note:** The number of wells filled with water must be adjusted if the Investigator Argus Y-12 QS Kit was used.

**Note:** The number of wells filled with diluent can be adjusted if the whole plate is not needed.

**4. Premix Hi-Di Formamide and DNA Size Standard according to the corresponding kit handbook, including additional volume to the equivalent of 5 extra reactions.**

**5. Set up the QIAgility worktable and load the samples (i.e., amplified DNA fragments) according to the pre-run report.**

**6. Start the protocol.**

**7. After the setup protocol is finished, load the samples on a Genetic Analyzer instrument and perform capillary electrophoresis.**

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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

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