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Investigator[®] Argus Y-28 QS Handbook

For multiplex amplification of 27
Y-chromosomal STR loci with Quality Sensor

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Kit Contents

Investigator Argus Y-28 QS Kit	(100)	(400)
Catalog no.	383625	383627
Number of 25 µl reactions	100	400
Fast Reaction Mix 3.0*	750 µl	4 x 750 µl
Nuclease-free water	1.9 ml	4 x 1.9 ml
Primer Mix Argus Y-28 QS	250 µl	4 x 250 µl
Control DNA 9948 (0.1 ng/µl)	40 µl	40 µl
Allelic ladder Argus Y-28 QS	25 µl	3 x 25 µl
DNA size standard 24plex (BTO)	55 µl	220 µl

* Contains DNA Polymerase, dNTPs, MgCl₂, and bovine serum albumin (BSA).

Shipping and Storage

The Investigator Argus Y-28 QS Kit is shipped on dry ice. It should be stored immediately upon receipt at -30 to -15°C in a constant-temperature freezer. Avoid repeated thawing and freezing. The primer mix and allelic ladder must be stored protected from light. DNA samples and post-PCR reagents (allelic ladder and DNA size standard) should be stored separately from PCR reagents. Under these conditions, the components are stable until the expiration date indicated on the kit.

Once opened, the Investigator Argus Y-28 QS Kit should be stored at 2 – 8°C for a maximum of 6 months.

Intended Use

The Investigator Argus Y-28 QS Kit is intended for molecular biology applications in forensic, human identity, and paternity testing. This product is 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 the Investigator Argus Y-28 QS Kit is tested against predetermined specifications to ensure consistent product quality. Investigator Argus Y-28 QS Kits meet ISO 18385 requirements.

Introduction

The Investigator Argus Y-28 QS Kit is a multiplex application for Y-chromosomal short tandem repeat (STR) loci. The PCR simultaneously amplifies the 27 Y-STR markers listed below.

The Investigator Argus Y-28 QS Kit Primer Mix contains two innovative internal PCR controls (Quality Sensor QS1 and QS2) to provide helpful information about the efficiency of the PCR and the presence of PCR inhibitors. The quality sensors are amplified simultaneously with the polymorphic STR markers.

The Investigator Argus Y-28 QS Kit was developed specifically for fast and reliable generation of male DNA profiles from mixtures of male and female DNA so that separation of sperm from female cells or differential lysis is not required. With inclusion of 6 rapidly mutating markers (DYS449, DYS481, DYS570, DYS576, DYS518, and DYS627), the kit also supports the resolution of paternal lineages and discrimination between closely related males.

The kit utilizes QIAGEN's fast cycling PCR technology. It provides highly robust results with inhibitor-resistant chemistry. The primers are fluorescence-labeled with these dyes:

- 6-FAM™: DYS389-I, DYS391, DYS389-II, DYS533, DYS390, DYS627
- BTG: DYS458, DYS393, DYS19, DYS437, DYS449
- BTY: DYS460, DYS576, YGATAH4, DYS481, DYS448, DYS518
- BTR2: DYS439, DYS549, DYS438, DYS456, DYS643
- BTP: QS1, DYS570, DYS635, DYS385, DYS392, QS2

The optimal amount of DNA under standard conditions is 0.5 ng. Internal validations demonstrated reliable results with <0.1 ng DNA.

The Investigator Argus Y-28 QS Kit was validated using the QIAamplifier®, Applied Biosystems® Veriti® Thermal Cycler, and Applied Biosystems 3500 and 3500XL Genetic Analyzers.

For information about known microvariants not contained in the Investigator Argus Y-28 QS allelic ladder, see the National Institute of Standards and Technology (NIST) website (strbase.nist.gov/) and the Y Chromosome Haplotype Reference Database at www.yhrd.org.

Table 1. Locus-specific information of the Investigator Argus Y-28 QS Kit

Locus	GenBank® accession number	Repeat motif of the reference allele
DYS19	AC017019	TAGA
DYS385*	AC022486	GAAA
DYS389-I	AC004617	[TCTG][TCTA]
DYS389-II	AC004617	[TCTG][TCTA]
DYS390	AC011289	[TCTG][TCTA]
DYS391	AC011302	TCTA
DYS392	AC011745	TAT
DYS393	AC006152	AGAT
DYS437	AC002992	TCTA
DYS438	AC002531	TTTTC
DYS439	AC002992	GATA
DYS448	AC025227	AGAGAT
DYS449	AC051663	TTTC
DYS456	AC010106	AGAT
DYS458	AC010902	GAAA
DYS460	AC009235	ATAG
DYS481	NC000024	CTT
DYS518	NC000024	AAAG
DYS533	AC053516	ATCT
DYS549	AC010133	GATA
DYS570	AC012068	TTTC
DYS576	AC010104	AAAG
DYS627	NC000024	AAAG
DYS635	AC004772	TSTA compound
DYS643	AC007007	CTTTT
YGATAH4	AC011751	TAGA

* duplicated locus

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.

All protocols

- Hi-Di™ Formamide, 25 ml (Applied Biosystems, cat. no. 4311320)
- Matrix Standard BT6 (cat. no. 386224) for multicapillary instruments, e.g., 3500 Genetic Analyzers
- One of the following DNA analyzers:
 - Applied Biosystems 3500 Genetic Analyzer
 - Applied Biosystems 3500xL Genetic Analyzer
- One of the following PCR thermal cyclers: *
 - QIAGEN QIAamplifier
 - Applied Biosystems Veriti Thermal Cycler
 - GeneAmp® PCR System 9700
 - Bio-Rad® PTC-200
 - Biometra® UNO-Thermoblock
 - Eppendorf® Mastercycler® ep
- PCR tubes or plates
- Pipettes and pipette tips
- Microcentrifuge for PCR tubes or plates

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Validity of analysis software for human identification products

Investigator Human Identification PCR Kits require calibration with an allelic ladder. Therefore, the software used must be compatible with human identification products for forensic applications. We recommend GeneMapper® *ID-X* Software. The Investigator template files facilitate data analysis and are valid with this software.

Protocol: PCR Amplification

This protocol is for PCR amplification of STR loci from forensic samples using the Investigator Argus Y-28 QS Kit.

Important points before starting

- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR analysis).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination risks.
- The recommended amount of DNA under standard conditions is 0.5 ng. Internal validations demonstrated robust and balanced results with 0.2–2 ng DNA and reliable results with <0.1 ng DNA.

Things to do before starting

- Before opening the tubes containing PCR components, vortex and then centrifuge briefly to collect the contents at the bottom of the tubes.

Procedure

1. Thaw PCR components and template nucleic acid.

Mix thoroughly. Centrifuge briefly before use.

2. Prepare a Master Mix according to Table 2.

The Master Mix contains all of the components needed for PCR except the template (sample) DNA and nuclease-free water.

As some loss of reagents can occur during transfers, prepare the mix with additional reactions included. Also include positive and negative control reactions.

3. Vortex the Master Mix thoroughly, centrifuge briefly, and then dispense appropriate volumes into PCR tubes or the wells of a PCR plate.

4. Add template DNA and nuclease-free water to the Master Mix to give a final sample volume of 25 μ l.

5. Prepare positive and negative controls.

Positive control: Use 5 μ l of the control DNA (i.e., 0.5 ng).

Negative control: Use nuclease-free water instead of template DNA in the reaction.

Table 2. Reaction setup

Component	Volume per reaction
Fast reaction mix 3.0	7.5 μ l
Primer mix	2.5 μ l
Nuclease-free water (added in step 4)	Variable
Template DNA (added in step 4)	Variable
Total volume	25 μ l

6. If template DNA was pipetted onto the rim or lid of the PCR tube, then centrifuge briefly to collect the contents at the bottom of the tubes.

7. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 3.

8. After the cycling protocol is completed, store samples at -30 to -15°C protected from the light, or proceed directly to electrophoresis.

Table 3. Standard cycling protocol recommended for all DNA samples

Temperature	Time	Number of cycles
96°C*	12 min	–
96°C	10 s	30 cycles
61.5°C	1 min 25 s	
72°C	5 s	
68°C	5 min	–
60°C	5 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Table 4. Optional cycling protocol, recommended for stains containing small amounts (<100 pg) of DNA

Temperature	Time	Number of cycles
96°C*	12 min	–
96°C	10 s	
61.5°C	1 min 25 s	31 cycles
72°C	5 s	
68°C	5 min	–
60°C	5 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Protocol: Electrophoresis Using the Applied Biosystems 3500/3500xL Genetic Analyzer

The Investigator Argus Y-28 QS Kit is intended for use on the 3500/3500xL Genetic Analyzer, which requires the following software:

- 3500 Data Collection Software

Note: The user must be logged on to the PC as local administrator or with equivalent access rights to allow data to be written to the appropriate files.

For detailed instructions on instrument setup, spectral calibration, or application of the Applied Biosystems 3500 Series Data Collection Software and the GeneMapper ID-X Software, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide* (tools.thermofisher.com/content/sfs/manuals/4401661.pdf).

The system with 8 capillaries is the Applied Biosystems 3500 Genetic Analyzer. The system with 24 capillaries is the Applied Biosystems 3500xL Genetic Analyzer.

The virtual filter set AnyDye is used for combined application of the 6 fluorescent labels 6 FAM, BTG, BTY, BTR2, BTP, and BTO. This matrix standard is BT6.

The materials required for electrophoresis are given in Table 5.

Table 5. Materials required for electrophoresis

Material	Specifications
Capillary	36 cm array for Applied Biosystems 3500/3500xL Genetic Analyzer
Polymer	POP-4® for Applied Biosystems 3500/3500xL Genetic Analyzer
Buffer	Anode buffer container (ABC) 3500 series Cathode buffer container (CBC) 3500 series

Spectral calibration/matrix generation

Before conducting DNA fragment size analysis, perform a spectral calibration with the 6 fluorescent labels 6-FAM, BTG, BTY, BTR2, BTP, and BTO for each analyzer (Table 6). The calibration procedure creates a matrix that is used to correct the overlapping of the fluorescence emission spectra of the dyes.

Important: Spectral calibration must be performed for each new capillary array. It comprises the following steps:

- Preparation of the instrument
- Preparation of the standard calibration plate
- Plate assembly and loading into the instrument
- Software setup of dye set BT6
- Performing a spectral calibration run
- Checking the matrix

Preparation of the instrument

Before the spectral calibration process, ensure that the spatial calibration has been performed. This process is described in detail in the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

Table 6. The 6 fluorescent labels of BT6

Color	Matrix standard
Blue (B)	6-FAM
Green (G)	BTG
Yellow (Y)	BTY
Red (R)	BTR2
Purple (P)	BTP
Orange (O)	BTO

Preparation of the standard calibration plate for 8 capillaries (Applied Biosystems 3500 Genetic Analyzer)

1. Before opening the tubes, vortex and then centrifuge briefly to collect the contents at the bottom of the tubes.
2. Set up a mixture of formamide and Matrix Standard BT6 according to Table 7.

Table 7. Setup of formamide and Matrix Standard BT6 mixture for 8 capillaries

Component	Volume
Hi-Di Formamide	90 μ l
Matrix Standard BT6 multi cap.	10 μ l

3. Vortex and then centrifuge the mixture briefly.
4. Load 10 μ l of the mixture into each of the 8 wells in a 96-well plate at positions A1–H1.
5. Denature for 3 min at 95°C.
6. Snap freeze by placing the plate on ice for 3 min.
A thermal cycler set to 4°C may be used to cool the plate instead.

Preparation of the standard calibration plate for 24 capillaries (Applied Biosystems 3500xL Genetic Analyzer)

7. Before opening the tubes, vortex and then centrifuge briefly to collect the contents at the bottom of the tubes.
8. Set up a mixture of formamide and Matrix Standard BT6 according to Table 8.

Table 8. Setup of formamide and Matrix Standard BT6 mixture for 24 capillaries

Component	Volume
Hi-Di Formamide	225 μ l
Matrix Standard BT6 multi cap.	25 μ l

9. Vortex and then centrifuge the mixture briefly.
10. Load 10 μ l of the mixture into each of the 24 wells in a 96-well plate at positions A1–H1, A2–H2, and A3–H3.
11. Denature for 3 min at 95°C.

12. Snap freeze by placing the plate on ice for 3 min.

A thermal cycler set to 4°C may be used to cool the plate instead.

Plate assembly and loading the plate in the instrument

The necessary steps are described in detail in the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

Software setup of dye set BT6

Prior to the spectral calibration, a dye set for the Matrix Standard BT6 must be set up.

1. To create a new dye set, select "Library". Under "Analyze" go to "Dye Sets" and click "Create".
2. Input the "Dye Set Name", for example, "BT6".
3. Under "Chemistry" select "Matrix Standard", and in "Dye Set Template" select "AnyDye Template".
4. In "Calibration Peak Order", arrange the colors as follows: 6 (blue), 5 (orange), 4 (green), 3 (yellow), 2 (red), and 1 (purple).

Note: This is the correct instrument setting of the peak order, although the peak order of the Matrix Standard BT6 is different.

5. Alter the "Parameters" settings as follows:
Matrix Condition Number Upper Limit: 13.5
Locate Start Point After Scan: 1000
Locate Start Point Before Scan: 5000
Limit Scans To: 2750
Sensitivity: 0.4
Minimum Quality Score: 0.95
6. Click "Save" to confirm the changes.

Create New Dye Set

Setup a Dye Set

* Dye Set Name: BT6

* Chemistry: Matrix Standard

* Dye Set Template: AnyDye Template

Arrange Dyes

Dye Selection	<input checked="" type="checkbox"/>					
Reduced Selection	<input checked="" type="checkbox"/>					
Calibration Peak Order	6	4	3	2	1	5

Parameters

The parameters will be used for instruments configured with 36cm capillary array and polymer POP4

Matrix Condition Number Upper Limit: 13.5

Locate Start Point: * After Scan: 1000, * Before Scan: 5000

* Limit Scans To: 2750

Sensitivity: 0.4

* Minimum Quality Score: 0.95

Notes

Close Save

Figure 1. Setup of dye set BT6.

Performing a spectral calibration run

Once the multiwell plates containing the spectral calibration mixture are placed in the autosampler tray, the spectral calibration process can be started.

1. To access the Spectral Calibration screen, select "Maintenance" on the dashboard of the 3500 Series Data Collection Software.
2. To set up a calibration run, go to "Calibrate", followed by "Spectral" and select "Calibration Run".
3. The number of wells in the spectral calibration plate and the position in the instrument must be specified.
4. Under "Chemistry Standard" select "Matrix Standard", and as a "Dye Set" select the previously created BT6 (see "Software setup of dye set BT6").
5. **Optional:** Enable "Allow Borrowing".
6. Click "Start Run".

Checking the matrix

Click a capillary in the table to display the results for each capillary below the run results table ("Capillary", "Quality value", and "Condition number").

- The quality value (Q value) of each capillary must be greater than 0.95 and the condition number range (C value) must be between 1 and 13.5.
- Check the matrix samples for a flat baseline. As shown in Figure 2, there should be 6 peaks with peak heights of about 1000–6000 RFU for each matrix sample.

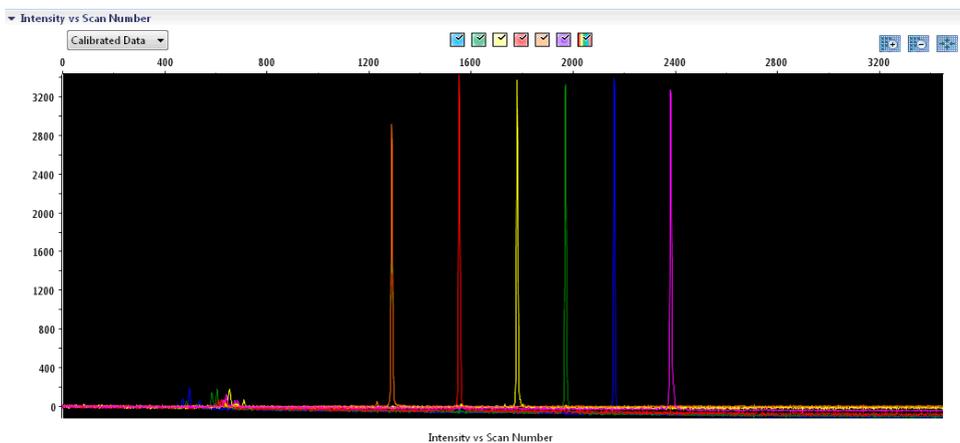


Figure 2. Electropherogram of spectral calibration of the Matrix Standard BT6 on an Applied Biosystems 3500 Genetic Analyzer.

When a spectral calibration is successfully completed, the “Overall” row displays green results (Figure 3). If the “Overall” row displays red results, refer to the “Spectral calibration troubleshooting” section of the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

▼ Capillary Run Data								
Capillary	1	2	3	4	5	6	7	8
Run 1	Passed							
Run 2	Not Calibrated							
Run 3	Not Calibrated							
Overall	Passed							

■ Passed
 ■ Failed
 ■ Borrowed
 ■ Not Calibrated

Figure 3. Example of successful spectral calibration of the Matrix Standard BT6 for all capillaries on an Applied Biosystems 3500 Genetic Analyzer.

For each capillary, select and display the spectral and raw data. Check that the data meet the following criteria:

- The order of the peaks in the spectral profile from left to right should read orange–red–yellow–green–blue–purple.

- No extraneous peaks should appear in the raw data profile.
- Peak morphology in the spectral profile should show no gross overlaps, dips, or other irregularities. Separate and distinct peaks should be visible.

If the data for all capillaries meet the criteria above, click "Accept". If any capillary data do not meet the criteria above, click "Reject" and refer to the "Spectral calibration troubleshooting" section of the *Applied Biosystems 3500/3500xl Genetic Analyzers User Guide*.

Sample preparation

7. Before opening the tubes, vortex and then centrifuge briefly to collect the contents at the bottom of the tubes.
8. Set up a mixture of formamide and DNA size standard according to Table 9.
9. Vortex and then centrifuge the mixture briefly.
10. Aliquot 12 µl of the mixture to a tube for each sample you plan to analyze.
11. Add 1 µl PCR product or allelic ladder (diluted, if necessary).
12. Denature for 3 min at 95°C.
13. Snap freeze by placing the plate on ice for 3 min.
14. A thermal cycler set to 4°C may be used to cool the plate instead.
15. Load the samples on the tray.

Table 9. Setup of formamide and DNA size standard mixture

Component	Volume per sample
Hi-Di Formamide	12.0 µl
DNA Size Standard (BTO)	0.5 µl

Note: Since injections take place simultaneously on all capillaries, a minimum of 1 entire column (8-sample protocol) or 3 entire columns (24-sample protocol) must be pipetted onto the plate of multicapillary analyzers. If fewer samples are analyzed, the empty positions must be filled with 12 µl Hi-Di Formamide.

To ensure a reliable allelic assignment on multicapillary analyzers, inject one allelic ladder for each set of 24 samples:

- 8-capillary instruments: One allelic ladder per 3 injections
- 24-capillary instruments: One allelic ladder per injection

Important: The actual room temperature may influence the performance of PCR products on multicapillary instruments, so shoulder peaks or split peaks can occur, especially at lower temperatures. **Ensure that the ambient conditions are maintained as recommended by the instrument manufacturer.** Also, ensure buffers are equilibrated to ambient conditions.

Setting up a run

If you are using the Investigator Argus Y-28 QS Kit for the first time on an Applied Biosystems 3500 Genetic Analyzer, you will first need to set up a number of protocols:

- Instrument protocol
- Size standard
- QC protocol
- Assay

All protocols can be set up via the Dashboard of the 3500 Series Data Collection Software.

Instrument protocol

1. To set up the instrument protocol, select "Library", and then under "Analyze" go to "Instrument Protocols" and click "Create".

Note: Modify the "Run Module" default settings from "HID36_POP4" as shown in Table 10.

2. The parameters from Table 10 must be entered or selected.
3. Click "Save" to confirm the changes.

Table 10. Instrument protocol parameters for Applied Biosystems 3500/3500xL Genetic Analyzer

Parameter	3500 setting	3500xL setting
Application type	HID	HID
Capillary length	36 cm	36 cm
Polymer	POP4	POP4
Dye set	e.g., BT6	e.g., BT6
Run module	HID36_POP4	HID36_POP4
Protocol name	e.g., Investigator Argus Y-28	e.g., Investigator Argus Y-28
Oven temperature (°C)	Default (60)	Default (60)
Run voltage (kV)	13.0	13.0
PreRun voltage (kV)	Default (15)	Default (15)
Injection voltage (kV)	1.2	1.6
Run time (s)	1550	1550
PreRun time (s)	Default (180)	Default (180)
Injection time (s)	30.0*	27.0*
Data delay (s)	Default (1)	Default (1)
Advanced options	Default	Default

* Deviating from the settings above, the injection time can be varied depending on the type of samples and PCR cycle number used. The injection times designate maximum injection times at the given voltages. If samples with very high signal intensities are recorded, a shorter injection time may be selected to reduce risk of pull-up peaks.

Size standard

- To set up the size standard, select “Library”, and then under “Analyze” go to “Size Standards” and click “Create”.
- The parameters in Table 11 must be entered or selected.

DNA Size Standard 24plex (BTO) should be used with the following lengths of fragments:

- DNA Size Standard 24plex (BTO): 60, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 250, 260, 280, 300, 320, 340, 360, 380, 400, 425, 450, 475, 500, 525, and 550 bp

Table 11. Size standard parameters

Parameter	Setting
Size standard	e.g., SST-BTO_60-500bp
Dye color	Orange

6. Alternatively, import the DNA size standard parameters using the recommended Investigator template files (Table 16).
7. Click "Save" to confirm the changes.

QC protocol

8. To set up the QC protocol, select "Library", and then under "Analyze" go to "QC Protocols" and click "Create".
9. The parameters in Table 12 must be entered or selected.

Table 12. QC protocol parameters

Parameter	Setting
Protocol name	e.g., BTO_550
Size standard	SST-BTO_60-500bp
Sizecaller	SizeCaller v1.1.0

10. Go to "Analysis Settings", followed by "Peak Amplitude Threshold", and ensure that all colors are enabled.
Check the recommended analysis settings in Table 13. All other settings should remain as "Default".
11. Click "Save" to confirm the changes.

Assay

12. To set up an assay, go to "Library", and then under "Manage" go to "Assays" and click "Create".
13. To analyze Investigator Argus Y-28 fragments, select the parameters in Table 13.
14. Click "Save" to confirm the changes.

Table 13. Assay parameters

Parameter	Setting
Assay name	e.g., Investigator Argus Y-28
Color	Default
Application type	HID
Instrument protocol	e.g., Investigator Argus Y-28
QC protocols	e.g., BTO_550

Starting the run

1. In the dashboard, click “Create New Plate”.
2. Go to “Setup”, followed by “Define Plate Properties”, and select “Plate Details”. Select or enter the parameters in Table 14.

Table 14. Plate properties

Property	Setting
Name	e.g., Investigator Argus Y-28
Number of wells	96
Plate type	HID
Capillary length	36 cm
Polymer	POP4

3. Click “Assign Plate Contents” to implement the changes.
4. Enter the designated sample name in each well containing a sample or allelic ladder.
This will identify the well positions of each sample for the data collection and processing.
5. Under “Assay”, choose the correct assay for the analysis. If you followed the steps under “Setting up a run”, click “Add from Library” and select “Investigator Argus Y-28” from “as Instrument Protocol”. All named wells on the plate must have an assigned assay.
6. Repeat for “File name conventions” and “Results group”.
7. Select the wells for which to specify an assay. Tick the boxes that are next to “the name of the Assay”, “File name conventions”, and “Results group” to assign those to the selected wells.

- If not already done, load the assembled plate to the instrument and close the instrument door to reinitialize the instrument. Click "Link Plate for Run". In the next screen, enter the desired run name and click "Start Run".

Analysis parameters/analysis method

Table 15 lists the recommended analysis parameters in the worksheet Peak Detector.

Table 15. Recommended settings for the Applied Biosystems 3500/3500xL Genetic Analyzer

Parameter	Settings
Peak detection algorithm	Advanced
Ranges	Analysis: Partial Range Start Point: 1000; Stop Point: 20,000 Sizing: All Sizes
Smoothing and baselining	Smoothing: Light Baseline Window: 51 pts
Size calling method	Local Southern Method
Peak detection	Peak Amplitude Thresholds B:* Y:* G:* R:* P:* O:* Min. Peak Half Width: 2 pts Polynomial Degree: 3 Peak Window Size: 11 pts [†] Slope Thresholds: 0.0

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneMapper *1D-X* Software. The thresholds are usually 50–200 RFU and should be determined individually by the laboratory.

Recommendation: The minimal peak height should be 3 times higher than the background noise of the baseline.

[†] Only the setting for Peak Window Size is different from the Applied Biosystems defaults for HID analysis.

Protocol: Analysis

For general instructions on automatic sample analysis, refer to the appropriate user guides for the GeneMapper ID-X Software.

Finding the exact lengths of the amplified products depends on the device type, the conditions of electrophoresis, and the DNA size standard used. Due to the complexity of some loci, size determination should be based on evenly distributed references. DNA Size Standard 24plex (BTO) should be used with the following fragment lengths:

DNA Size Standard 24plex (BTO): 60, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 250, 260, 280, 300, 320, 340, 360, 380, 400, 425, 450, 475, 500, 525, and 550 bp

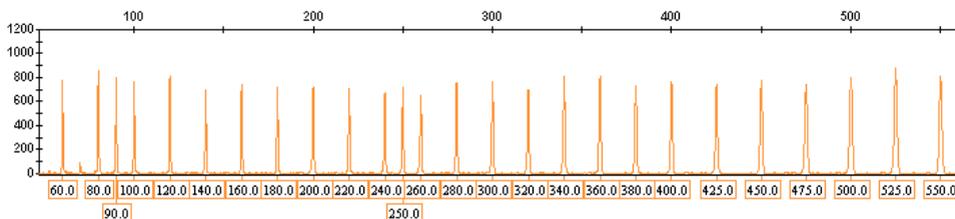


Figure 4. Electropherogram of the DNA Size Standard 24plex (BTO). Fragments lengths in bp.

Analysis software

Allele allocation should be carried out using suitable analysis software (e.g., GeneMapper ID-X Software) in combination with the Investigator template files, which are available for download from www.qiagen.com.

Table 16. Recommended Investigator template files for GeneMapper *ID-X*

File type	File name
Panels*	Argus Y-28_Panels
BinSets*	Argus Y-28_Bins
Stutter	Argus Y-28_Stutter
Size standard	SST-BTO_60–500bp
Analysis method	Analysis_HID_3500_50rfu Analysis_HID_3500_200rfu
Plot settings	Plots_6dyes

* Panels and bin sets must always be used; other template files are optional.

Controls

The alleles listed in Table 17 represent the Control DNA 9948 (included in the Investigator Argus Y-28 QS Kit).

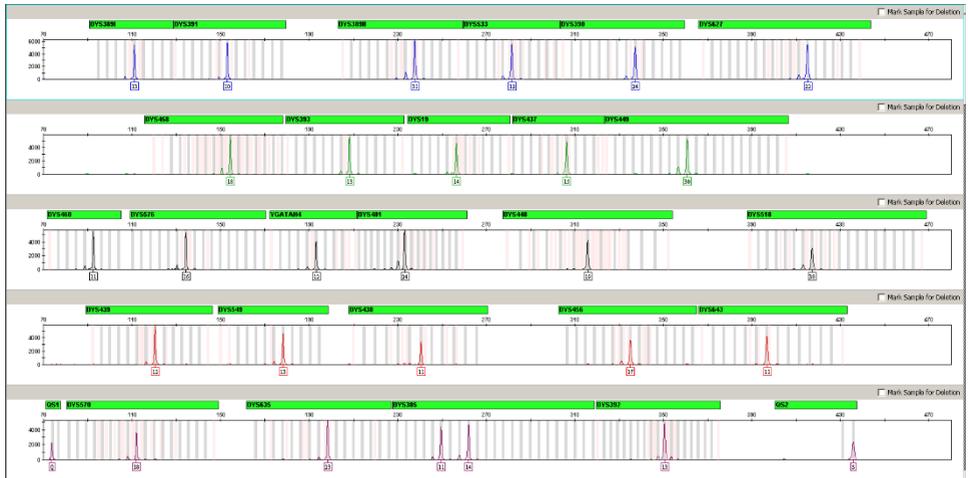


Figure 5. Electropherogram of the positive control DNA 9948 analyzed on an Applied Biosystems 3500xL Genetic Analyzer.

Special features

In general, the electropherogram displays a single peak for each Y-STR locus. However, locus DYS385 produces two peaks of different lengths or of the same length. These two fragments originate from duplicated and inversed copies of one Y-chromosomal locus. The primers provided in the Investigator Argus Y-28 QS Kit simultaneously co-amplify the two homologous loci. For separate amplification, see reference 5. If more than one peak is obtained in the electropherogram for one or several markers, this does not necessarily suggest mixed samples. Duplications or triplications of STR markers also result in such an effect and have already been observed for DYS385 and DYS19 (7). Rarely, single systems can also fail because of Y-chromosomal deletions as known in azoospermic patients, which has already been described for DYS385 and DYS392 (8).

Table 17. Allele assignment of the Investigator Argus Y-28 QS Kit

Locus	DNA 9948
DYS19	14
DYS385	11/14
DYS389-I	13
DYS389-II	31
DYS390	24
DYS391	10
DYS392	13
DYS393	13
DYS437	15
DYS438	11
DYS439	12
DYS448	19
DYS456	17
DYS458	18
DYS449	30
DYS460	11
DYS481	24
DYS518	38
DYS533	12
DYS549	13
DYS570	18
DYS576	16
DYS627	22
DYS635	23
DYS643	11
YGATAH4	12

Quality sensor

The Investigator Argus Y-28 QS Kit contains two internal PCR controls (quality sensors QS1 and QS2), which provide helpful information about the PCR amplification efficiency in general and about the presence of PCR inhibitors. The internal quality sensors are enclosed in the primer mix and are amplified simultaneously with the polymorphic STR markers. The quality sensors are labeled with BTP and appear at fragment sizes of 74 bp (QS1) and 435 bp (QS2).

To address the issue of sequence similarity and the possibility of nonspecific binding, a synthetic internal control DNA template was designed using a random algorithm. The template sequence differs from all known DNA sequences and, in particular, bears no similarity to human DNA. The chance of nonspecific binding in the context of a multiplex PCR amplification reaction is therefore very low.

In general, the successful amplification of the small quality sensor (QS1) indicates that the PCR was set up and conducted correctly, regardless of whether DNA was present or absent in the sample. If no quality sensor is detected in the analysis of the amplification products, this means that either the pipetting during PCR setup or the PCR itself was performed incorrectly. The user could repeat the experiment for improved results.

Sensitivity experiments have revealed that the internal controls have no effect on the performance of the PCR. The amplification of low DNA template amounts showed similar results for primer mixes with or without the quality sensors.

In addition, the analysis of the 2 internal control fragments, QS1 and QS2, and of the STR target amplification products allows for the differential identification of the presence of inhibitors or the presence of DNA degradation in an amplification reaction.

In the case of sample degradation, the amplification of smaller target fragments is more efficient than the amplification of larger target fragments. However, degradation of the target template does not hamper amplification of the internal control fragments from the internal

control template. Thus, an equal ratio of QS1 and QS2, together with a ratio in favor of small STR target products, suggests the presence of sample degradation.

If inhibitors such as hematin and humic acid are present in the sample, amplification is less efficient and larger DNA fragments are amplified less than smaller ones. If the analysis of the amplification products indicates an inefficient amplification of the larger STR target sequences and the larger quality sensor (QS2) fragment, but the smaller quality sensor (QS1) is amplified successfully, the sample is likely to have been contaminated with inhibitors. This means that a shift of the ratio in favor of the small quality sensor (QS1) suggests the presence of inhibitors.

Analysis of the presence of the two quality sensors allows the user to differentially identify the presence of PCR inhibitors or the occurrence of degradation in the forensic sample. This gives the user helpful information for data interpretation and for planning the next steps. Table 18 summarizes the possible profile appearances and their meanings.

Table 18. Profile appearances and their meanings

Allele peaks	QS1	QS2	Interpretation
Present	Present	Present	Successful profile
Absent	Present	Present	No DNA
Absent	Absent	Absent	Failed PCR
Ski-slope profile	Present	Drop/Absent	Inhibitors present
Ski-slope profile	Present	Present	Degraded DNA

Note: The peak heights of QS1 and QS2 may vary slightly between different experiments. A slight peak height scattering is usual and is not dependent on inhibitor influence. During the validation, the analyst should evaluate the usual variation spectrum in relation to their certain samples type, and should define a regular peak height range for both QS.

A drop of the QS2 signal below 20% of the QS1 signal indicates inhibition of the PCR reaction.

Alleles

Table 19 shows the alleles of the allelic ladder. All analyses were performed using POP-4 polymer (Table 19 and Figure 6). Different analysis instruments, DNA size standards, or polymers may result in different fragment lengths. In addition, a visual alignment with the allelic ladder is recommended.

Scaling

- Horizontal: 70–470 bp
- Vertical: Depending on signal intensity

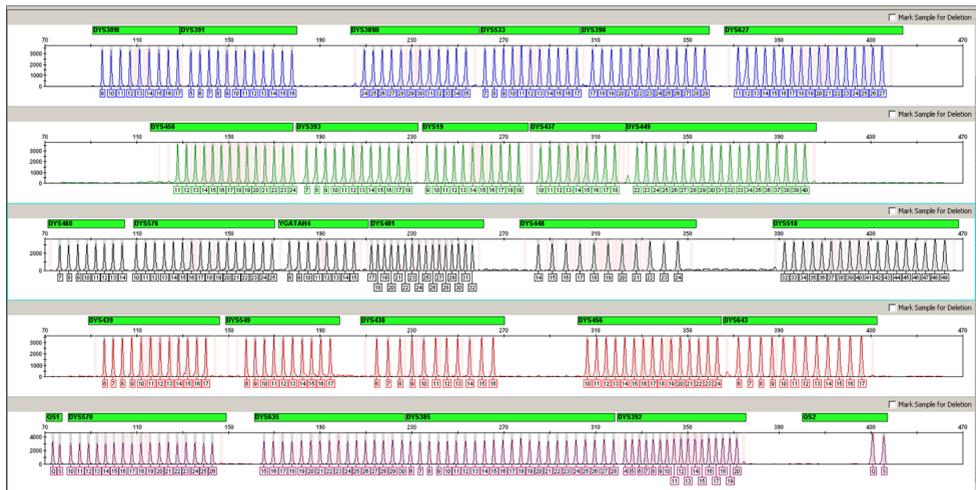


Figure 6. Electropherogram of the allelic ladder Argus Y-28 QS analyzed on an Applied Biosystems 3500xL Genetic Analyzer. The allelic ladder contains 2 alleles for each quality sensor (QS1 and QS2). This allows automated calling of the QS peaks for sample analysis.

Table 19. Allelic ladder fragments.

Locus	Dye label	Repeat numbers of allelic ladder
DYS389-I	6-FAM	9, 10, 11, 12, 13, 14, 15, 16, 17
DYS391	6-FAM	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16
DYS389-II	6-FAM	24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35
DYS533	6-FAM	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17
DYS390	6-FAM	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29
DYS627	6-FAM	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27
DYS458	BTG	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
DYS393	BTG	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18
DYS19	BTG	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19
DYS437	BTG	10, 11, 12, 13, 14, 15, 16, 17, 18
DYS449	BTG	22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40
DYS460	BTY	7, 8, 9, 10, 11, 12, 13, 14
DYS576	BTY	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
YGATAH4	BTY	8, 9, 10, 11, 12, 13, 14, 15
DYS481	BTY	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32
DYS448	BTY	14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
DYS518	BTY	32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49
DYS439	BTR2	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17
DYS549	BTR2	8, 9, 10, 11, 12, 13, 14, 15, 16, 17
DYS438	BTR2	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16
DYS456	BTR2	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
DYS643	BTR2	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17
QS1	BTP	Q, S
DYS570	BTP	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26
DYS635	BTP	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30
DYS385	BTP	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28
DYS392	BTP	4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20
QS2	BTP	Q, S

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

Unbalanced profiles, low signals

- | | |
|--|--|
| a) Incorrect volume of fast reaction mix or primer mix | Check reaction setup and repeat amplification. |
| b) Master Mix not vortexed before distribution | Vortex Master Mix thoroughly and centrifuge briefly. |

Artefacts in profiles

- | | |
|--|---|
| a) Excessive amount of female background DNA | Male/female mixtures containing more than 1 µg of total human DNA may lead to unspecific amplification from autosomal and X-chromosomes (See Figure 7 for an example). |
| b) Wrong annealing conditions | In case of frequent female artefacts below 1 µg background, the annealing conditions should be checked. Ensure the protocol uses 61.5°C annealing temperature for any sample with female background. Check the PCR cyclers for correct performance. Use thin walled PCR tubes or plates to ensure efficient temperature transfer. Alternatively, increase annealing temperature to 62–63°C. |

Reduced peak heights of QS1 and/or QS2

A slight peak height scattering of the quality sensors is normal and is not dependent on inhibitor influence.

During the validation, the analyst should evaluate the usual variation spectrum in relation to specific sample types and define a regular peak height range for both QSs. A drop of the QS2 signal below 20% of the QS1 signal indicates inhibition of the PCR reaction. Smaller reduction of QS2 vs QS1 are indicating mild inhibition, if the ratio is clearly different from the one seen in positive and negative controls of the same run.

Comments and suggestions

Dominance of quality sensor peaks

QS1 and QS2 peaks are too dominant.

If samples of low peak height are analyzed without fixed scaling, alleles in the purple channel may be displayed too small. Using GeneMapper *ID-X* Software, choose a new setting for "all-dye range" under "Display Settings" to zoom in. The range should be between the QS1 and QS2.

Important: In addition, adjust in the "Analysis Method Editor", under "peak detector", the size-calling (Ranges; Sizing) to 75 → 450.

Sample preparation

Sample signal intensity must be increased.

Reduce the volume of the DNA Size Standard (BTO) to peak heights of about 500 RFU.

Purify the PCR products before starting the analysis. We recommend the MinElute® PCR Purification Kit (cat. nos. 28004 and 28006) for rapid and effective purification.

Spectral calibration is not appropriate

There are pull-up peaks between the dye panels (B, G, Y, R, P, O) with the current matrix/spectral calibration.

Pull-up peaks should not exceed 5% of the causing peak, if the profile is still in the linear detection range (e.g., up to 15.000 RFU on 3500 Genetic Analyzers). Repeat the spectral calibration.

Many peaks are labeled as off-ladder (OL) alleles in the samples

a) DNA Size Standard 24plex (BTO) was not defined or identified correctly.

Click the orange "Size Match Editor" icon in the upper toolbar of the GeneMapper *ID* or GeneMapper *ID-X* Software. Mark the orange fragments of all samples.

Always use DNA Size Standard 24plex BTO for Investigator Human Identification PCR Kits.

b) Signal intensities are too high. If the peak heights of the samples are outside the linear detection range using Applied Biosystems 3500/3500xL Genetic Analyzers, the occurrence of stutters, split peaks, and artifacts may increase.

Reduce the injection time. Reduce the amount of the PCR amplification product for analysis; or reduce the quantity of DNA for PCR.

c) Bubbles in the capillary lead to pull up peaks in all color panels ("spikes"), resulting in allele misnomer.

Repeat electrophoresis to confirm results. Check the maximum number of injections recommended by the instrument manufacturer. Set up a new capillary array, if necessary.

Comments and suggestions

- | | | |
|----|--|--|
| d) | Differences in the run performance among the capillaries of a multi-capillary analyzer may result in allelic assignment shift. | For reliable allelic assignment on multi-capillary analyzers, each third row should have an allelic ladder. |
| e) | Low room temperature or low CE buffer temperature may result in fragment migration shifts or OL peaks. | Ensure that ambient conditions are maintained as recommended by the instrument manufacturer. Ensure that buffers are equilibrated to ambient conditions. Preheating of the CE instrument (~30 min) is recommended. Temperature control is particularly important for Applied Biosystems 3130 Genetic Analyzers. Migration issues occurring in the first injection of the day or after changing buffers indicate too low temperature. |

Allelic ladder issues

- | | | |
|----|--|---|
| a) | An additional signal can be identified as peak of the allelic ladder because of dysfunctions during the electrophoresis. If peaks of the allelic ladder are miscalled, the ladder cannot be used for the analysis. | Use a different injection/file of the allelic ladder and check the data of the analyzed sizes from the size standard (in bp) of the allelic ladder. Always use DNA Size Standard 24plex (BTO) for Investigator Human Identification PCR Kits. |
| b) | Peaks of the allelic ladder are below the peak detection value (50–200 RFU) of the analysis method used, and thus are not identified. | Check the amount of allelic ladder used. Check the correctness of the injection parameters. Alternatively, the allelic ladder data can be analyzed with a lower peak detection value in the analysis software. |
| c) | One peak of the allelic ladder is not identified because it is outside the expected size range of the software (in bp). | Compare the length of the fragments (in bp) of the first allele in one color of the allelic ladder with the corresponding value in the categories. Then, compare it with the other alleles. |
| d) | Point alleles are not separated. | Point alleles are alleles with at least 1 bp difference to the next integer allele. Check the settings of the analysis method and ensure the Peak Window Size value is set to 11 points. Check the Size Standard for the occurrence of broad peaks, indicating poor size resolution. In case of poor resolution, check the usage of the array, polymer, and buffers, and replace if needed. Alternatively, reduce the injection time. |
| e) | Allelic ladder signals too high. | Due to the high number of peaks, allelic ladders are more prone to pull-up and signal saturation issues. Signal Off Scale (SOS) flags in GMID/X indicate overall signal intensity is too high. Affected ladders can show imbalanced profiles, with lower peaks in areas of overlapping alleles in multiple colors. Reduce the amount of allelic ladder, or the injection time. |

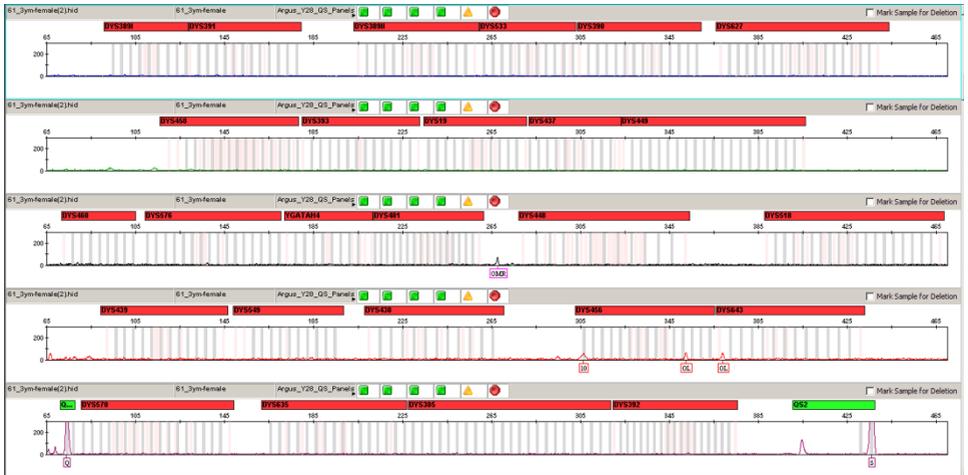


Figure 7. Example of typical amplification artefacts caused by excessive female background DNA. 3 µg female DNA was amplified at an annealing temperature of 61°C to induce unspecific amplification. Most frequent artefact peaks occur in the BTY channel between the markers DYS481 and DYS448, and in the BTRII channel in the marker areas of DYS456 and DYS643.

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Appendix A: Interpretation of Results

Post-PCR analysis and automatic allele assignment with suitable analysis software ensure a precise and reliable discrimination of alleles.

General procedure for the analysis

1. Check the DNA size standard.
2. Check the allelic ladder.
3. Check the positive and negative controls.
4. Analyze and interpret the sample data.

Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range (see “Troubleshooting Guide”) or if an incorrect matrix was applied. They appear at positions of specific peaks in other color channels, typically with lower signal intensities. To prevent pull-up peaks, peak heights should not exceed thresholds.

Stutter peaks

The occurrence of stutter peaks depends on the sequence of the repeat structure and the number of alleles. These peaks should be interpreted using the Investigator Template Files for GeneMapper *1D-X* Software.

Template-independent addition of nucleotides

Because of its terminal transferase activity, the *Taq* DNA Polymerase may cause incomplete adenylation at the 3' end of the amplified DNA fragments. The artifact peak is one base shorter than expected (–1 peaks). All primers included in the Investigator Argus Y-28 QS Kit are designed to minimize these artifacts. The peak height of the artifact correlates with the amount of DNA. Laboratories should define their own limits for analysis of the peaks.

Artifacts

Room temperature may influence the performance of PCR products on multicapillary instruments, so shoulder peaks or split peaks might occur. If shoulder or split peaks appear, we recommend injecting the sample again. Ensure that ambient conditions are maintained as recommended by the instrument manufacturer. Ensure that buffers are equilibrated to the ambient conditions.

Appendix B: Varying PCR Volumes Using Investigator Argus Y-28 QS Kit

The Investigator Argus Y-28 QS Kit can be run with half reaction mix volumes (Fast Reaction Mix + Primer Mix). Note that while we have successfully tested the reduced reaction volume stated here, the highest overall success rates still have to be expected when using the full reaction volumes as recommended in the kit manual.

Appendix C: Direct Amplification of DNA Using the Investigator Argus Y-28 QS Kit

This protocol describes how to perform STR analysis by direct amplification using the Investigator Argus Y-28 QS Kit.

The experimental conditions outlined in this protocol have been found to give the best results. However, depending on the sample material, PCR cycle numbers may be adapted to ensure the highest possible first-round success rates. We recommend running a representative batch of samples to confirm that the cycle numbers given in this protocol are optimal. Increase the cycle number by 1 if the signals in the resulting electropherograms are too low. Decrease the cycle number by 1 if the signals in the resulting electropherograms are too high.

Equipment and reagents to be supplied by the user

For protocols based on blood or buccal cells on paper

- UniCore Punch Kit 1.2 mm (cat. no. WB100028) and Cutting Mat, 6.0" x 8.0" (cat. no. WB100020)
- Investigator STR GO! Punch Buffer (1000) or (200) (cat. no. 386528 or 386526)

For protocols based on buccal swab lysates

- Investigator STR GO! Lysis Buffer (cat. no. 386516)
- 2 ml microcentrifuge tubes
- Shaker for 2 ml microcentrifuge tubes

Protocol: PCR amplification from blood on FTA and other paper

This protocol is for direct PCR amplification of STR loci from punches of blood on FTA and other paper using the Investigator Argus Y-28 QS Kit.

Important points before starting

- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination risks.

Things to do before starting

- Before opening the tubes containing PCR components, vortex and then centrifuge the tubes briefly to collect the contents at the bottom.

Procedure

1. Prepare a master mix according to Table 20 (for full volume PCR) or Table 21 (for reduced volume PCR).

The master mix contains all the components needed for PCR. Prepare a volume of reaction mix 10% greater than what is required for the total number of PCR assays to be performed. This should include both positive and negative control reactions.

Table 20. Recommended master mix setup for full volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	7.5 µl
Primer Mix	2.5 µl
Investigator STR GO! Punch Buffer	2.0 µl
Nuclease-free water	8.0 µl
Total reaction volume per sample	20.0 µl

Table 21. Recommended master mix setup for reduced volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	3.75 μ l
Primer Mix	1.25 μ l
Investigator STR GO! Punch Buffer*	2.0 μ l
Nuclease-free water	3.0 μ l
Total reaction volume per sample	10.0 μ l

* If reduced PCR volumes are used, it is important to always use 2 μ l of the STR GO! Punch Buffer, regardless of the master mix volume. All other reagents should be scaled proportionally. Any changes to the recommended protocol must be validated by the testing laboratory.

2. Vortex the reaction mix thoroughly and dispense the total required reaction volume per sample into PCR tubes or the wells of a PCR plate.
3. Take a 1.2 mm punch from the center of the sample spot with a suitable tool (e.g., Uni Core Punch 1.2 mm).
4. Transfer one 1.2 mm disc to each reaction. Do not mix the reaction after disc transfer.
5. Prepare the positive and negative controls.

Positive control: Use 2 μ l Control DNA (5 ng/ μ l).

Note: If signals are too low or too high in the subsequent PCR, you may need to adapt the amount of control DNA for your laboratory after setting the optimal PCR cycle number. Do not add a blank disc to the positive control well.

Negative control: Do not add template DNA to the negative control. Do not add a blank disc or water to the negative control PCR tube or well.

6. Briefly centrifuge reactions to ensure that discs are fully submerged.
7. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 22.
8. After completion of the protocol, store samples at -30 to -15°C protected from light, or proceed directly with electrophoresis. Add 1 μ l of the PCR product directly to 12 μ l Hi-Di Formamide plus the Size Standard. Start the analyzer run.

Table 22. Recommended cycling protocol for blood on FTA or other paper

Temperature	Time	Number of cycles
96°C*	12 min	–
96°C	10 s	26 cycles
60°C	1 min 25 s	
72°C	5 s	
68°C	5 min	–
60°C	5 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Protocol: PCR amplification from buccal cells on FTA and other paper

This protocol is for direct PCR amplification of STR loci from punches of buccal cells on FTA and other paper using the Investigator Argus Y-28 QS Kit.

Important points before starting

- For buccal cells collected using the Uni Core Punch 1.2 mm, take the punch from a white area. This color indicates successful sample transfer.
- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination risks.

Things to do before starting

- Before opening the tubes containing PCR components, vortex and then centrifuge the tubes briefly to collect the contents at the bottom.

Procedure

1. Prepare a master mix according to Table 23 (for full volume PCR) or Table 24 (for reduced volume PCR).

The master mix contains all the components needed for PCR. Prepare a volume of reaction mix 10% greater than what is required for the total number of PCR assays to be performed. This should include both positive and negative control reactions.

Table 23. Recommended master mix setup for full volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	7.5 µl
Primer Mix	2.5 µl
Investigator STR GO! Punch Buffer	2.0 µl*
Nuclease-free water	8.0 µl
Total reaction volume per sample	20.0 µl

* If reduced PCR volumes are used, it is important to always use 2 µl of the STR GO! Punch Buffer, regardless of the master mix volume. All other reagents should be scaled proportionally. Any changes to the recommended protocol must be validated by the testing laboratory.

Table 24. Recommended master mix setup for reduced volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	3.75 µl
Primer Mix	1.25 µl
Investigator STR GO! Punch Buffer	2.0 µl*
Nuclease-free water	5.0 µl
Total reaction volume per sample	12 µl

* If reduced PCR volumes are used, it is important to always use 2 µl of the STR GO! Punch Buffer, regardless of the master mix volume. All other reagents should be scaled proportionally. Any changes to the recommended protocol must be validated by the testing laboratory.

2. Vortex the reaction mix thoroughly and dispense the total required reaction volume per sample into PCR tubes or the wells of a PCR plate.
3. Take a 1.2 mm punch from the center of the sample spot with a suitable tool (e.g., Uni Core Punch 1.2 mm).
Important: Do not use more than one punch at a time.
4. Transfer one 1.2 mm disc to each reaction. Do not mix the reaction after disc transfer.

5. Prepare the positive and negative controls.

Positive control: Use 1 μl Control DNA (5 ng/ μl).

Note: If signals are too low or too high in the subsequent PCR, you may need to adapt the amount of control DNA for your laboratory after setting the optimal PCR cycle number. Do not add a blank disc to the positive control well.

Negative control: Do not add template DNA to the negative control. Do not add a blank disc or water to the negative control PCR tube or well.

6. Briefly centrifuge reactions to ensure discs are fully submerged.

7. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 25.

8. After completion of the protocol, store samples at -30 to -15°C protected from light, or proceed directly with electrophoresis. Add 1 μl of the PCR product directly to 12 μl Hi-Di Formamide plus the Size Standard. Start the analyzer run.

Table 25. Recommended cycling protocol for buccal cells on FTA or other paper

Temperature	Time	Number of cycles
96°C*	12 min	–
96°C	10 s	
60°C	1 min 25 s	27 cycles
72°C	5 s	
68°C	5 min	–
60°C	5 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Protocol: PCR amplification from buccal swab lysates

This protocol is for direct PCR amplification of STR loci from crude lysates of buccal swabs using the Investigator Argus Y-28 QS Kit.

Important points before starting

- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination risks.

Things to do before starting

- Before opening the tubes containing PCR components, vortex and then centrifuge the tubes briefly to collect the contents at the bottom.

Procedure

1. Place the swab in a 2 ml microcentrifuge tube.
Carefully cut, break off, or eject the end part of the swab.
2. Add 500 μ l STR GO! Lysis Buffer to the sample.
3. Incubate the sample at 95°C for 5 min, shaking at 1200 rpm in a thermomixer.
4. Prepare a master mix according to Table 26 (for full volume PCR) or Table 27 (for reduced volume PCR).

The master mix contains all the components needed for PCR. Prepare a volume of reaction mix 10% greater than what is required for the total number of PCR assays to be performed. This should include both positive and negative control reactions.

Table 26. Recommended master mix setup for full volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	7.5 µl
Primer Mix	2.5 µl
Nuclease-free water	10.0 µl
Total reaction volume per sample	20.0 µl

Table 27. Recommended master mix setup for reduced volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	3.75 µl
Primer Mix	1.25 µl
Nuclease-free water	5.0 µl
Total reaction volume per sample	10.0 µl

- Vortex the reaction mix thoroughly and dispense the total required reaction volume per sample into PCR tubes or the wells of a PCR plate.
- Mix the swab lysate thoroughly. Transfer 2 µl (for full reaction volume) or 1 µl (for reduced reaction volume) of swab lysate directly to each reaction.
- Prepare the positive and negative controls.
Positive control: Use 1 µl Control DNA (5 ng/µl).
Note: If signals are too low or too high in the subsequent PCR, you may need to adapt the amount of control DNA for your laboratory after setting the optimal PCR cycle number.
Negative control: Use a blank swab lysate.
- Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 28.
- After completion of the protocol, store samples at -30 to -15°C protected from light, or proceed directly with electrophoresis. Add 1 µl of the PCR product directly to 12 µl Hi-Di Formamide plus the Size Standard. Start the analyzer run.

Table 28. Recommended cycling protocol for buccal swab lysates

Temperature	Time	Number of cycles
96°C*	12 min	–
96°C	10 s	27 cycles
60°C	1 min 25 s	
72°C	5 s	
68°C	5 min	–
60°C	5 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Appendix D: Fast Amplification of DNA from Reference Samples Using the Investigator Argus Y-28 QS Kit

Protocol: PCR amplification

This protocol is for PCR amplification of STR loci from extracted forensic reference samples using the Investigator Argus Y-28 QS Kit.

Important points before starting

- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR analysis).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination risks.
- The recommended amount of DNA under standard conditions is 0.5 ng to 1 ng. For samples containing less DNA and for mixtures, the standard cycling protocols are recommended.

Things to do before starting

- Before opening the tubes containing PCR components, vortex and then centrifuge briefly to collect the contents at the bottom of the tubes.

Procedure

1. Thaw PCR components and template nucleic acid.
Mix thoroughly. Centrifuge briefly before use.
2. Prepare a Master Mix according to Table 29.

The Master Mix contains all of the components needed for PCR except the template (sample) DNA and nuclease-free water.

As some loss of reagents can occur during transfers, prepare the mix with additional reactions included. Also include positive and negative control reactions.

3. Vortex the Master Mix thoroughly, centrifuge briefly, and then dispense appropriate volumes into PCR tubes or the wells of a PCR plate.
4. Add template DNA and nuclease-free water to the Master Mix to give a final sample volume of 25 μ l.
5. Prepare positive and negative controls.

Positive control: Use 5 μ l of the control DNA (i.e., 0.5 ng).

Negative control: Use nuclease-free water instead of template DNA in the reaction.

Table 29. Reaction setup

Component	Volume per reaction
Fast reaction mix 3.0	7.5 μ l
Primer mix	2.5 μ l
Nuclease-free water (added in step 4)	Variable
Template DNA (added in step 4)	Variable
Total volume	25 μ l

6. If template DNA was pipetted onto the rim or lid of the PCR tube, then centrifuge briefly to collect the contents at the bottom of the tubes.
7. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 30.
8. After the cycling protocol is completed, store samples at -30 to -15°C protected from the light, or proceed directly to electrophoresis.

Table 30. Fast cycling protocol recommended for extracted reference samples

Temperature	Time	Number of cycles
96°C*	10 min	–
96°C	10 s	30 cycles
61.5°C	55 s	
72°C	5 s	
68°C	5 min	–
60°C	5 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Ordering Information

Product	Contents	Cat. no.
Investigator Argus Y-28 QS Kit (100)	Primer mix, fast reaction mix 3.0, control DNA, allelic ladder Argus Y-28, DNA Size Standard, and nuclease-free water	383625
Investigator Argus Y-28 QS Kit (400)	Primer mix, fast reaction mix 3.0, control DNA, allelic ladder Argus Y-28, DNA Size Standard, and nuclease-free water	383627
Related products		
Matrix Standard BT6 (50)	Matrix standard for 6-FAM, BTG, BTY, BTR2, BTP, and BTO, for Applied Biosystems 3500 Genetic Analyzers	386224
Investigator STR GO! Lysis Buffer (200)	Lysis buffer for 200 swab samples	386516
Investigator STR GO! Punch Buffer (200)*	Lysis buffer for 200 samples of epithelial cells or blood on paper	386526
UniCore Punch Kit 1.2 mm	4 pieces	WB100028
Investigator Quantification Kits		
Investigator Quantiplex Pro Kit (200)	For use on Applied Biosystems 7500 Real-Time Systems: Quantiplex Pro Reaction Mix, Quantiplex Pro Primer Mix, Quantiplex Pro Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387216

Product	Contents	Cat. no.
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN RotorGene Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316

* Larger kit sizes are available; please inquire.

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Document Revision History

Date	Changes
06/2021	Initial revision
01/2022	Corrected some STR markers for the 6-FAM and BTR2 dyes listed in the Introduction section (page 6).

Notes

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