

Investigator[®] Argus X-12 QS Handbook

For multiplex amplification
of 12 STR loci of the X-
chromosome, plus
Amelogenin and D21S11

Contents

Kit Contents	4
Storage	5
Intended Use	5
Safety Information	6
Quality Control	6
Introduction	7
Equipment and Reagents to Be Supplied by User	11
Protocol: PCR Amplification	13
Protocol: Electrophoresis Using the ABI PRISM 310 Genetic Analyzer	16
Matrix generation	16
Sample preparation	20
Setting up the GeneScan Software	21
Analysis parameters	22
Protocol: Electrophoresis Using the ABI PRISM 3100-Avant/3100 Genetic Analyzer	23
Spectral calibration/matrix generation	23
Sample preparation	27
Setting up the GeneScan Software	28
Analysis parameters	31
Protocol: Electrophoresis Using the Applied Biosystems 3130/3130xl Genetic Analyzer ..	32
Spectral calibration/matrix generation	32
Checking the matrix	36
Sample preparation	37

Setting up the Data Collection Software	38
Analysis parameters/analysis method	42
Protocol: Electrophoresis Using the Applied Biosystems 3500/3500xL Genetic Analyzer..	43
Spectral calibration/matrix generation.....	44
Analysis parameters/analysis method.....	57
Protocol: Analysis	58
Analysis software	58
Controls	59
Alleles.....	60
Quality Sensor	63
Characteristics and Use of X-chromosomal STRs	64
Calculation of population-genetic data.....	64
Variant alleles and mutations	68
Troubleshooting Guide	70
References	73
Ordering Information	76

Kit Contents

Investigator Argus X-12 QS Kit	(25)	(100)
Catalog no.	383223	383225
Number of 25 µl reactions	25	100
Fast Reaction Mix 2.0*	750 µl	750 µl
Primer Mix Argus X-12 QS	63 µl	250 µl
Nuclease-free water	1.9 ml	1.9 ml
Control DNA 9947A	200 µl	200 µl
DNA size standard 550 (BTO)	13 µl	50 µl
Allelic ladder Argus X-12 QS	25 µl	25 µl
Quick Start Protocol	1	1

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

Storage

The Investigator Argus X-12 QS Kit is shipped on dry ice. It should be stored immediately upon receipt at -30°C to -15°C in a constant-temperature freezer. Avoid repeated thawing and freezing. The Primer Mix and allelic ladder must be stored protected from the light. DNA samples and post-PCR reagents (allelic ladder and DNA size standard) should be stored separately from the PCR reagents. Under these conditions, the components are stable until the expiration date indicated on the kit.

Once opened, the Investigator Argus X-12 QS Kit can be stored at $2-8^{\circ}\text{C}$ for a maximum of 3 months or re-frozen and stored at -30°C to -15°C in a constant-temperature freezer for longer periods.

Intended Use

The Investigator Argus X-12 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.



CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Investigator Argus X-12 QS Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The Investigator Argus X-12 QS Kit is a multiplex application for 12 X-chromosomal short tandem repeat (STR) loci. The kit supplements the Investigator Argus Y-12 QS Kit for kinship and paternity testing, especially in complicated deficiency cases.

The Investigator Argus X-12 QS Kit contains primers for Amelogenin (AM) for gender determination, DXS7132, DXS7423, DXS8378, DXS10074, DXS10079, DXS10101, DXS10103, DXS10134, DXS10135, DXS10146, DXS10148 and HPRTB. The markers are clustered into 4 linkage groups (3 markers per group; Table 1), and thus each set of 3 markers is handled as a haplotype for genotyping (Figure 1, next page). Furthermore, the autosomal STR marker D21S11 is included as a consistent marker for common database kits and prevents sample mixup.

The Investigator Argus X-12 QS Kit Primer Mix contains an internal PCR control (Quality Sensor QS1) to provide helpful information about the efficiency of the PCR. The Quality Sensor is amplified simultaneously with the polymorphic STR markers.

The Investigator Argus X-12 QS Kit is specifically for rapid and reliable generation of DNA profiles from blood, buccal swabs and forensic stains. The kit utilizes QIAGEN's fast-cycling PCR technology, allowing amplification in around 80 minutes. It provides highly robust results with inhibitor-resistant chemistry. The primers are fluorescence-labeled with the following dyes:

- **6-FAM™**: QS1, Amelogenin, DXS10103, DXS8378, DXS10101, DXS10134
- **BTG**: DXS10074, DXS7132, DXS10135
- **BTY**: DXS7423, DXS10146, DXS10079
- **BTR**: HPRTB, DXS10148, D21S11

Table 1. Linkage groups

Linkage group	
1 (Xp22)	DXS8378 – DXS10135 – DXS10148
2 (Xp11)	DXS7132 – DXS10074 – DXS10079
3 (Xp26)	HPRTB – DXS10101 – DXS10103
4 (Xp28)	DXS7423 – DXS10134 – DXS10146

The optimal amount of DNA under standard conditions is 0.2–0.5 ng. Internal validations demonstrated reliable results with <0.1 ng DNA.

The Investigator Argus X-12 QS Kit was validated using the GeneAmp® PCR System 9700 (with Gold-plated 96-Well Silver Block) and the Applied Biosystems® 3500™ Genetic Analyzer.

The Forensic ChrX Research Group initiated the online data base ChrX-STR.org (<http://www.chrx-str.org>) that calculates population-genetic data on basis of X-chromosomal allele frequencies (1).

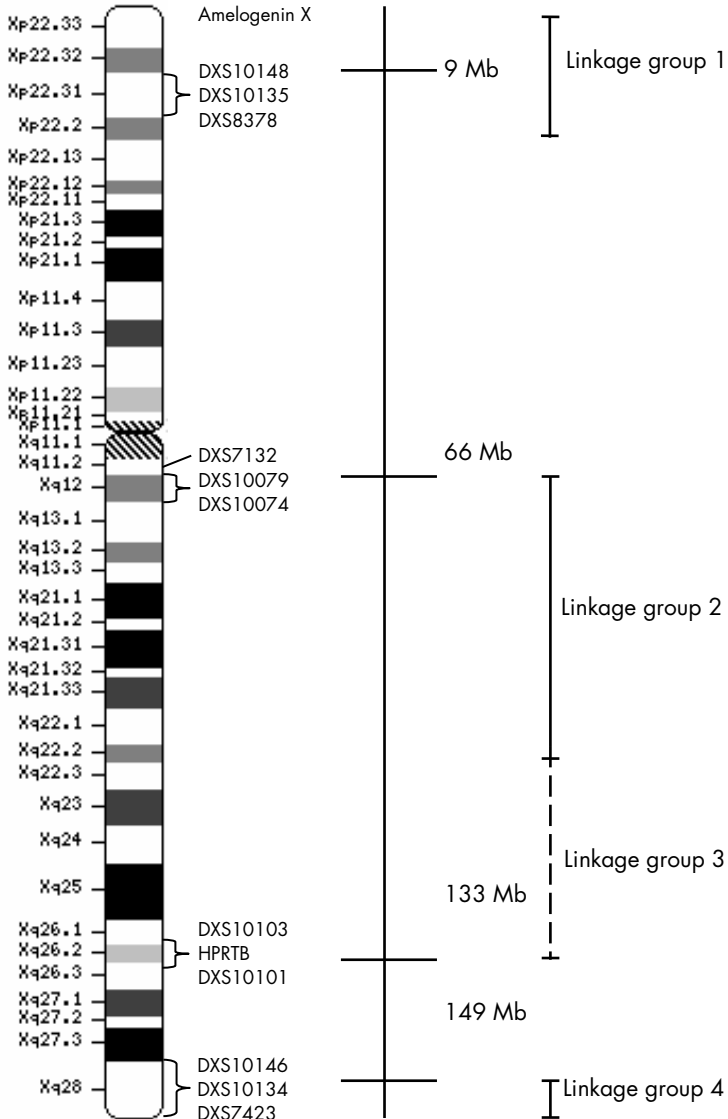


Figure 1. The ideogram of the X-chromosome describes the physical localization of the STR loci that can be analyzed using the Investigator Argus X-12 QS Kit. Distances from the p-telomere are shown in Mb (www.ncbi.nlm.nih.gov/genome/guide/human as of 11/2014).

Table 2 and Table 3 show the STR loci with their chromosomal mapping, repeat motifs and alleles that are concordant with the International Society for Forensic Genetics (ISFG) guidelines for the use of microsatellite markers (2).

Table 2. Locus-specific information of the Investigator Argus X-12 QS Kit

Locus	GenBank® Accession number	Repeat motif of the reference allele	Chromosomal mapping
Amelogenin X	M55418	–	Xp22.1-22.3
Amelogenin Y	M55419	–	Yp11.2
DXS7132	G08111	[TCTA] ₁₃	Xq11.2
DXS7423	AC109994	[TCCA] ₃ TCTGTCT [TCCA] ₁₂	Xq28
DXS8378	G08098	[CTAT] ₁₂	Xp22.31
DXS10074	AL356358	[AAGA] ₁₄	Xq12
DXS10079	AL049564	[AGAG] ₃ TGAAAGAG [AGAA] ₁₇ AGAG [AGAA] ₃	Xq12
DXS10101	AC004383	[AAAG] ₃ GAAAGAAG [GAAA] ₃ A [GAAA] ₄ AAGA [AAAG] ₅ AAAAAGAA [AAAG] ₁₃ AA	Xq26.2
DXS10103	BV680555	[TAGA] ₂ CTGA [CAGA] ₁₁ [TAGA] ₄ [TAGA]	Xq26.2
DXS10134	AL034384	[GAAA] ₃ GAGA [GAAA] ₄ AA [GAAA] GAGA [GAAA] ₄ GAGA [GACAGA] ₃ [GAAA] GTAA [GAAA] ₃ AAA [GAAA] ₄ AAA [GAAA] ₁₅	Xq28
DXS10135	AC003684	[AAGA] ₃ GAAAG [GAAA] ₂₀	Xp22.31
DXS10146	AL034384	[TTCC] ₃ T [TTCC] ₃ TTCC CTCCCTCC [TTCC] [TTCC] TTCTTTTC [TTCC] ₂ TTCTT [CTTT] ₂ CTTC [CTTT] ₁₀ T [CTTT] ₂	Xq28
DXS10148	AC003684	[GGAA] ₄ [AAGA] ₁₂ [AAAG] ₄ N ₈ [AAGG] ₂	Xp22.31
HPRTB	M26434	[AGAT] ₁₂ *	Xq26.2
D21S11	AP000433	[TCTA] ₄ [TCTG] ₆ [TCTA] ₃ TA [TCTA] ₃ TCA [TCTA] ₂ TCCATA [TCTA] ₁₁	21q21.1

* [AGAT] is the most common repeat structure; for variations see the NIST and reference 3.

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.

- Hi-Di™ Formamide, 25 ml (Applied Biosystems, cat. no. 4311320)
- Matrix Standards BT5 for single-capillary instruments, e.g., ABI PRISM® 310 Genetic Analyzer (QIAGEN, cat. no. 386113)
- Matrix Standards BT5 for multi-capillary instruments, e.g., ABI PRISM 3100 and Applied Biosystems 3130 and 3500 Genetic Analyzers (QIAGEN, cat. nos. 386123 or 386125)
- Pipets and pipet tips
- One of the following DNA analyzers*:
 - ABI PRISM 310 Genetic Analyzer
 - ABI PRISM 3100-*Avanti*™/3100 Genetic Analyzer
 - Applied Biosystems 3130
 - Applied Biosystems 3500 Genetic Analyzer
 - Applied Biosystems 3500xL Genetic Analyzer
- One of the following PCR thermal cyclers:
 - GeneAmp PCR System 9700
 - Applied Biosystems Veriti® Thermal Cycler
 - Bio-Rad® PTC-200
 - Eppendorf® Mastercycler® ep
 - PCR tubes or plates

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

-
- Microcentrifuge for PCR tubes or plates

Validity 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 X-12 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.
- 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 with PCR components, vortex, and then centrifuge briefly to collect 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 3.
As some loss of reagents can occur during transfers, prepare the mix with additional reactions included. Also include positive and negative control reactions.
The master mix contains all of the components needed for PCR except the template (sample) DNA and nuclease-free water.
3. Mix the master mix thoroughly, centrifuge briefly, and 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., 500 pg).

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

Table 3. Master Mix setup

Component	Volume per reaction
Fast Reaction Mix 2.0	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 μl

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 4.

Note: If using the GeneAmp PCR System 9700 with an Aluminum block, use "Std Mode", or with a Silver 96-Well Block or Gold-plated Silver 96-Well Block, use "Max Mode". Do not use "9600 Emulation Mode".

Table 4. Standard cycling protocol recommended for all DNA samples

Component	Time	Number of cycles
98°C*	60 s	3 cycles
61°C	100 s	
72°C	5 s	
96°C	10 s	27 cycles
61°C	100 s	
72°C	5 s	
68°C	2 min	
10°C	∞	–

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

Protocol: Electrophoresis Using the ABI PRISM 310 Genetic Analyzer

For general instructions on instrument setup, matrix generation, and application of the GeneScan® or GeneMapper ID Software, refer to the *ABI PRISM 310 Genetic Analyzer User's Manual*. Electrophoresis using the GeneScan Software is described below.

The virtual filter set G5 is used for combined application of the 5 fluorescent labels 6-FAM, BTG, BTY, BTR and BTO. This matrix standard is known as BT5.

The materials required for electrophoresis are listed in Table 5.

Table 5. Materials required for electrophoresis

Material	Specifications
Capillary	47 cm/50 µm (green)
Polymer	POP-4™ for ABI PRISM 310 Genetic Analyzer
Buffer	10x Genetic Analyzer Buffer with EDTA

Matrix generation

Before conducting DNA fragment size analysis with the filter set G5, a matrix with the 5 fluorescent labels 6-FAM, BTG, BTY, BTR and BTO must be generated (Table 6).

Table 6. The 5 fluorescent labels of BT5

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

Five electrophoresis runs should be conducted, one for each fluorescent label, under the same conditions as for the samples and allelic ladders of the Investigator Argus X-12 QS Kit, to generate suitable matrix files (Table 7).

Table 7. Matrix setup for a single capillary instrument (ABI PRISM 310 Genetic Analyzer)

Matrix sample	Component	Volume
Matrix sample 1	Hi-Di Formamide	12.0 µl
	Matrix standard 6-FAM	1.0 µl
Matrix sample 2	Hi-Di Formamide	12.0 µl
	Matrix standard BTG	1.0 µl
Matrix sample 3	Hi-Di Formamide	12.0 µl
	Matrix standard BTY	1.0 µl
Matrix sample 4	Hi-Di Formamide	12.0 µl
	Matrix standard BTR	1.0 µl
Matrix sample 5	Hi-Di Formamide	12.0 µl
	Matrix standard BTO	1.0 µl

1. Denature for 3 min at 95°C.
2. Snap freeze by placing the plate on ice for 3 min.
3. Alternatively, the thermal cycler set to 4°C may be used to cool the plate.
4. Load the samples on the tray.

5. Create a Sample Sheet and enter the sample designation. Table 8 shows the injection list for matrix generation.

Table 8. Injection list for matrix generation

Parameter	Settings
Module file	GS STR POP-4 (1 ml) G5
Matrix file	None
Size standard	None*
Injection time (s)	5
Injection voltage (kV)	15
Run voltage (kV)	15
Run temperature (°C)	60
Run time (min)	24

* Always prepare matrix standards without DNA Size Standard (BTO).

Analysis of the matrix samples

1. Run the GeneScan Software.
2. Select "New" from the File menu, then select "Project".
3. Open the folder of the current run and select "Add Sample Files".
4. Select a matrix sample in the "Sample File" column.
5. Click "Sample" and then "Raw Data".
6. Check the matrix samples for a flat baseline. As shown in the figure (page 19), there should be at least 5 peaks with peak heights of 1000–4000 RFU for each matrix sample.

Note: The optimal range is 2000–4000 RFU.

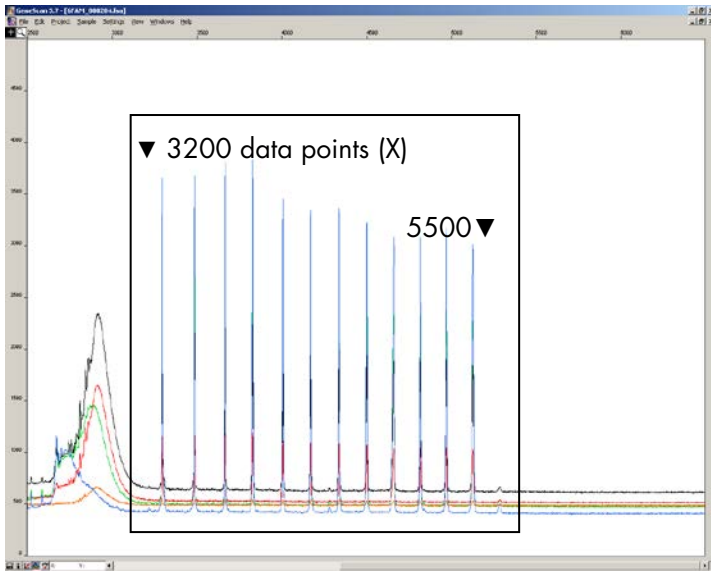


Figure 2. Electropherogram with raw data of the matrix standard 6-FAM.

7. Select an analysis range with a flat baseline and re-inject the matrix sample, if necessary.
8. Record start and end value (data points) of the analysis range; e.g., start value 3200, end value 5500.
9. Calculate the difference between the end and start values; e.g., $5500 - 3200 = 2300$ data points.

Generation of a matrix

1. Select "New" in the File menu, and then select "Matrix".
2. Import the matrix samples for all dyes (B, G, Y, R and O).
3. Enter a "Start At" value, e.g., 3200.
4. Under "Points", enter the calculated difference between end and start values, e.g., 2300.
5. Click "OK" to calculate the new matrix.

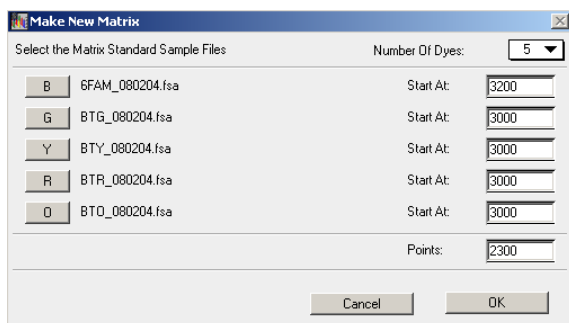


Figure 3. Matrix sample selection.

6. Select "Save as" in the File menu to save the new matrix in the matrix folder.

Checking the matrix

1. To check the new matrix with current samples, select "New" in the File menu, then select "Project".
2. Open the folder of the respective run and select "Add Sample Files".
3. Select the sample(s) in the Sample File column.
4. Click "Sample" and then "Install New Matrix" to open the matrix folder and select the new matrix.
5. Re-analyze the samples.

Note: There should be no pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix.

Sample preparation

1. Set up a mixture of formamide and DNA size standard according to Table 9.

Table 9. Setup of formamide and DNA size standard mixture

Component	Volume per sample
Hi-Di Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl

2. Aliquot 12 µl of the mixture to a tube for each sample to be analyzed.
3. Add 1 µl PCR product or allelic ladder (diluted, if necessary).
4. Denature for 3 min at 95°C.
5. Snap freeze by placing the plate on ice for 3 min.
6. Alternatively, the thermal cycler set to 4°C may be used to cool the plate.
7. Load the samples on the tray.

Setting up the GeneScan Software

Create a Sample Sheet and enter sample designation (see Table 10).

Table 10. Injection list for the ABI PRISM 310 Genetic Analyzer

Component	Settings
Module file	GS STR POP-4 (1 ml) G5
Matrix file	E.g., Matrix BT5
Size standard	E.g., SST-BTO_60-500bp
Injection time (s)	7*
Injection voltage (kV)	15
Run voltage (kV)	15
Run temperature (°C)	60
Run time (min)	22 [†]

* Deviating from standard settings, the injection time may range between 1 and 10 s, depending on the type of sample. If samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content, an injection time of up to 10 s may be necessary.

[†] The run time for the Investigator Argus X-12 QS Kit was modified in order to enable the analysis of fragments with lengths of up to 400 bp.

Analysis parameters

Table 11 lists the recommended analysis parameters.

Table 11. Recommended analysis parameters for the ABI PRISM 310 Genetic Analyzer

Parameter	Settings
Analysis range	Start: 2000 Stop: 10,000
Data processing	Baseline: Checked Multi-component: Checked Smooth options: Light
Peak detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynomial Degree: 3 Peak Window Size: 11 pts [†]
Size call range	Min: 60 Max: 550
Size calling method	Local Southern Method
Split peak correction	None

* The peak amplitude threshold (cut-off value) corresponds to the minimum peak height that will be detected by the GeneScan or GeneMapper *ID* Software. Thresholds are usually 50–200 RFU and should be determined individually by the laboratory. **Recommendation:** The minimal peak height should be 3 times as high as the background noise of the baseline.

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

Note: For information on the use of the recommended Template Files (as analysis parameters), refer to the appropriate Investigator Template Files User Guide (GeneMapper *ID* or GeneMapper *ID-X*).

Protocol: Electrophoresis Using the ABI PRISM 3100-Avant/3100 Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, application of the ABI PRISM 3100 Data Collection Software version 1.01 or 1.1 and the GeneScan Software, refer to the *ABI PRISM 3100-Avant/3100 Genetic Analyzer User's Manual*.

The system with 4 capillaries is the ABI PRISM 3100-Avant Genetic Analyzer, and the system with 16 capillaries is the ABI PRISM 3100 Genetic Analyzer.

The virtual filter set G5 is used for combined application of the 5 fluorescent labels 6-FAM, BTG, BTY, BTR and BTO. This matrix standard is known as BT5.

The materials required for electrophoresis are listed in Table 12.

Table 32. Materials required for electrophoresis

Material	Specifications
Capillary	36 cm Capillary Array for ABI PRISM 3100-Avant/3100 Genetic Analyzer
Polymer	POP-4 Polymer for ABI PRISM 3100-Avant/3100 Genetic Analyzer
Buffer	10x Genetic Analyzer Buffer with EDTA

Spectral calibration/matrix generation

Proper spectral calibration is critical for evaluation of multicolor systems with the ABI PRISM 3100-Avant/3100 Genetic Analyzer and should be done before conducting fragment length analysis. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:

- Preparation of the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Entering the plate composition
- Performing a spectral calibration run and checking the matrix

Preparing the spectral calibration standards

Example for 4 capillaries (ABI PRISM 3100-Avant Genetic Analyzer)

1. Set up a mixture of formamide and Matrix Standard BT5 according to Table 13.

Table 13. Setup of formamide and Matrix Standard BT5 mixture for 4 capillaries

Component	Volume
Hi-Di Formamide	60 µl
Matrix Standard BT5 multi cap.	5 µl

2. Load 12 µl of the mixture to a 96-well plate; e.g., position A1–D1.
3. Denature for 3 min at 95°C.
4. Snap freeze by placing the plate on ice for 3 min.
Alternatively, the thermal cycler set to 4°C may be used to cool the plate.

Example for 16 capillaries (ABI PRISM 3100 Genetic Analyzer)

1. Set up a mixture of formamide and Matrix Standard BT5 according to Table 14.

Table 14. Setup of formamide and Matrix Standard BT5 mixture for 16 capillaries

Component	Volume
Hi-Di Formamide	204 µl
Matrix Standard BT5 multi cap.	17 µl

2. Load 12 µl of the mixture to a 96-well plate; e.g., position A1–H1 and A2–H2.
3. Denature for 3 min at 95°C.
4. Snap freeze by placing the plate on ice for 3 min.
Alternatively, the thermal cycler set to 4°C may be used to cool the plate.

Performing a spectral calibration run

The parameter file for DyeSetG5 must be modified once to achieve successful calibration with the Data Collection Software version 1.0.1 or 1.1.

Spectral parameter

1. To change settings in the parameter file, go to the following path:
D:\AppliedBio\Support Files\Data Collection SupportFiles\CalibrationData\Spectral Calibration\ParamFiles
2. Select “MtxSTD{Genescan_SetG5}” to open the PAR file.
3. Change “Condition Bounds Range” to [1.0, 20.0].
4. If the calibration was unsuccessful, also change Sensitivity to 0.1 and Quality to 0.8.
5. Select “Save As” in the “File” menu and save the parameter file under a new name; e.g., MtxStd{Genescan_SetG5_BT5}.par.
Note: Always use this parameter file for spectral calibration runs using QIAGEN Matrix Standard BT5.

Plate Editor for spectral calibration

1. Place the 96-well plate on the autosampler tray.

2. Run the ABI PRISM 3100 Data Collection Software.
3. In "Plate View", click "New" to open the Plate Editor dialog box.
4. Enter a name for the plate.
5. Select a Spectral Calibration.
6. Select "96-Well" as plate type, and click "Finish".

Table 15. Plate Editor for spectral calibration

Parameter	Settings
Sample name	Enter name for the matrix samples
Dye set	G5
Spectral run module	Default (e.g., Spect36_POP4)
Spectral parameters	MtxStd{GeneScan_SetG5_BT5}.par (parameters created before)

7. Click the column header to select the entire column, and select "Fill Down" from the Edit menu to apply the information to the selected samples. Confirm by clicking "OK".
8. Link the reaction plate on the autosampler tray with the created plate ID and start the run.
9. Upon completion of the run, check in the Spectral Calibration Result dialog box that all capillaries have successfully passed calibration (label A).

If individual capillaries are labeled X, refer to the *ABI PRISM 3100 Genetic Analyzer User's Manual*.

10. Click "OK" to confirm completion of the run.

Checking the matrix

1. Select "Display Spectral Calibration" from the "Tools" menu, then "Dye Set" and "G5" to review the spectral calibration profile for each capillary.
2. The quality value (Q value) must be greater than 0.95 and the condition number (C value) must be between 1 and 20. Both values must be within the pre-determined range.

3. Check for a flat baseline in the matrix samples. There should be five peaks with heights of 1000–5000 RFU in each matrix sample.
Note: The optimal range is 2000–4000 RFU.
4. Check the new matrix with the current samples. There should be no pull-up peaks between the dye panels (B, G, Y, R and O) with the new matrix.
5. If the calibration failed, follow instructions in the section “Spectral parameter” on page 25.
6. If all capillaries have passed the calibration, the last calibration file for Dye Set G5 must be activated manually. Go to “Tools” and click “Set Active Spectral Calibration”.
7. Rename the calibration file under “Set Matrix Name” (e.g., BT5_Date of calibration).

Sample preparation

1. Set up a mixture of formamide and DNA size standard according to Table 16.

Table 46. Setup of formamide and DNA size standard mixture

Component	Volume per sample
Hi-Di Formamide	12 µl
DNA Size Standard 550 (BTO)	0.5 µl

2. Aliquot 12 µl of the mixture to a tube for each sample to be analyzed.
3. Add 1 µl PCR product or allelic ladder (diluted, if necessary).
4. Denature for 3 min at 95°C.
5. Snap freeze by placing the plate on ice for 3 min.
Alternatively, the thermal cycler set to 4°C may be used to cool the plate.
6. Load the samples on the tray.

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted onto the plate of multi-capillary analyzers. If fewer samples are analyzed, the empty positions must be filled with 12 μ l Hi-Di Formamide.

To ensure a reliable allelic assignment on multi-capillary analyzers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Ensure ambient conditions are kept as recommended by the instrument manufacturer.

Setting up the GeneScan Software

1. Edit the default run module in Dye Set G5 once for the first run. Select "Module Editor" to open the dialog box.
2. Select the appropriate Run Module as template from the GeneScan table (see Table 17).
3. Modify the Injection Voltage to 2.5 kV and the Injection Time to 30 s.
4. Click "Save As" and enter the name of the new module (e.g., 2.5kV_30s_400bp). Confirm by clicking "OK".
5. Click "Close" to exit the Run Module Editor.

Table 17. Run Module 2.5kV_30s_400bp for the ABI PRISM 3100-Avant/3100 Genetic Analyzer

Parameter	Settings
Run temperature (°C)	Default
Cap fill volume	Default
Maximum current (A)	Default
Current tolerance (A)	Default
Run current (A)	Default
Voltage tolerance (kV)	Default
Pre-Run voltage (kV)	Default
Pre-Run time (s)	Default
Injection voltage (kV)	2.5
Injection time (s)	30*
Run voltage (kV)	13
Number of steps	Default
Voltage step interval	Default
Data delay time (s)	Default
Run time (min)	22 [†]

* Deviating from the standard settings, the injection time may range between 1 and 35 s depending on the type of sample. If samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content, an injection time of up to 35 s may be necessary.

[†] The run time for Investigator Argus X-12 QS Kit was modified to enable analysis of fragments with lengths of up to 400 bp.

Starting the run

1. Place the prepared 96-well plate on the autosampler tray.
2. Run the ABI PRISM 3100 Data Collection Software.
3. In "Plate View", click "New" to open the Plate Editor dialog box.
4. Enter a name for the plate.
5. Select "GeneScan" as the application type.
6. Select "96-Well" as plate type, and click "Finish".

Table 18. Settings in Plate Editor

Parameter	Settings
Sample name	Enter name for the matrix samples
Dyes	O
Color info	Ladder or sample
Project name	E.g., 3100_Project1
Dye set	G5
Run module*	2.5kV_30s_400bp
Analysis module 1	DefaultAnalysis.gsp

* See Table 17, "Run Module 2.5kV_30s_400bp for the ABI PRISM 3100-Avant/3100 Genetic Analyzer".

7. Complete the table in the "Plate Editor" and click "OK".
8. Click the column header to highlight the entire column and select "Fill Down" from the "Edit" menu to apply the information to the selected samples.
9. Link the reaction plate on the autosampler tray to the created plate ID and start the run.
10. Upon completion of the run, view the data as Color Data in the Array View of the 3100 Data Collection Software or as Analyzed Sample Files under
D:/AppliedBio/3100/DataExtractor/ExtractRuns.

Analysis parameters

Table 19 lists the recommended analysis parameters.

Table 19. Recommended analysis parameters for the ABI PRISM 3100-Avant/3100 Genetic Analyzer

Parameter	Settings
Analysis range	Start: 2000 Stop: 10,000
Data processing	Baseline: Checked Multi-component: Checked Smooth options: Light
Peak detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynomial Degree: 3 Peak Window Size: 11 pts [†]
Size call range	Min: 60 Max: 550
Size calling method	Local Southern Method
Split peak correction	None

* The peak amplitude threshold (cut-off value) corresponds to the minimum peak height that will be detected by the GeneScan or GeneMapper ID Software. Thresholds are usually 50–200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three-times higher than the background noise of the baseline.

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

Note: For information on the use of the recommended Template Files (as analysis parameters), refer to the appropriate Investigator Template Files User Guide (GeneMapper ID or GeneMapper ID-X).

Protocol: Electrophoresis Using the Applied Biosystems 3130/3130xl Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration or application of the ABI PRISM Data Collection Software version 3.0 and the GeneMapper *ID* Software, refer to the *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide*.

The system with 4 capillaries is the Applied Biosystems 3130 Genetic Analyzer, and the system with 16 capillaries is the Applied Biosystems 3130xl Genetic Analyzer.

The virtual filter set Any5Dye is used for combined application of the 5 fluorescent labels 6-FAM, BTG, BTY, BTR and BTO. This matrix standard is known as BT5.

The materials required for electrophoresis are given in Table 20.

Table 20. Materials needed for electrophoresis

Material	Specifications
Capillary	36 cm Capillary Array for Applied Biosystems 3130/3130xl Genetic Analyzer
Polymer	POP-4 Polymer for Applied Biosystems 3130/3130xl Genetic Analyzer
Buffer	10x Genetic Analyzer Buffer with EDTA

Spectral calibration/matrix generation

Before conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the 5 fluorescent labels 6-FAM, BTG, BTY, BTR and BTO for each analyzer. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration is comprised of the following steps:

- Preparation of the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Creating the instrument protocol for spectral calibration (Protocol Manager)
- Defining the plate composition in the plate editor (Plate Manager)
- Performing a spectral calibration run and checking the matrix

Preparing the spectral calibration standards

Example for 4 capillaries (Applied Biosystems 3130 Genetic Analyzer)

1. Set up a mixture of formamide and Matrix Standard BT5 according to Table 21.

Table 21. Setup of formamide and Matrix Standard BT5 mixture for 4 capillaries

Component	Volume
Hi-Di Formamide	60 µl
Matrix Standard BT5 multi cap.	5 µl

2. Load 12 µl of the mixture to a 96-well plate, e.g., positions A1–D1.
3. Denature for 3 min at 95°C.
4. Snap freeze by placing the plate on ice for 3 min.
Alternatively, the thermal cycler set to 4°C may be used to cool the plate.

Example for 16 capillaries (Applied Biosystems 3130xl Genetic Analyzer)

1. Set up a mixture of formamide and Matrix Standard BT5 according to Table 22.

Table 22. Setup of formamide and Matrix Standard BT5 mixture for 16 capillaries

Component	Volume
Hi-Di Formamide	204 μ l
Matrix Standard BT5 multi cap.	17 μ l

2. Load 12 μ l of the mixture to a 96-well plate, e.g., position A1–H1 and A2–H2.
3. Denature for 3 min at 95°C.
4. Snap freeze by placing the plate on ice for 3 min.
Alternatively, the thermal cycler set to 4°C may be used to cool the plate.

Performing spectral calibration run

1. Place the 96-well plate on the autosampler tray.
2. In the Protocol Manager of the Data Collection Software, open the Instrument Protocol window.
3. Click “New” to open the Protocol Editor dialog box.
4. Complete the dialog box with information from Table 23 and click “OK”.

Table 23. Instrument protocol for spectral calibration

Protocol Editor	Settings
Name	User (e.g., Spectral36_POP4_BT5)
Type	SPECTRAL
Dye set	Any5Dye
Polymer	User (e.g., POP4)*
Array length	User (e.g., 36cm)*
Chemistry	Matrix Standard
Run module	Default (e.g., Spect36_POP4_1)*

* Depends on the type of polymer and length of capillary used.

5. Click “New” in the Plate Manager of the Data Collection Software to open the “New Plate” dialog box.
6. Enter information from Table 24 and click “OK”. A new table in the Plate Editor opens automatically (Table 25).

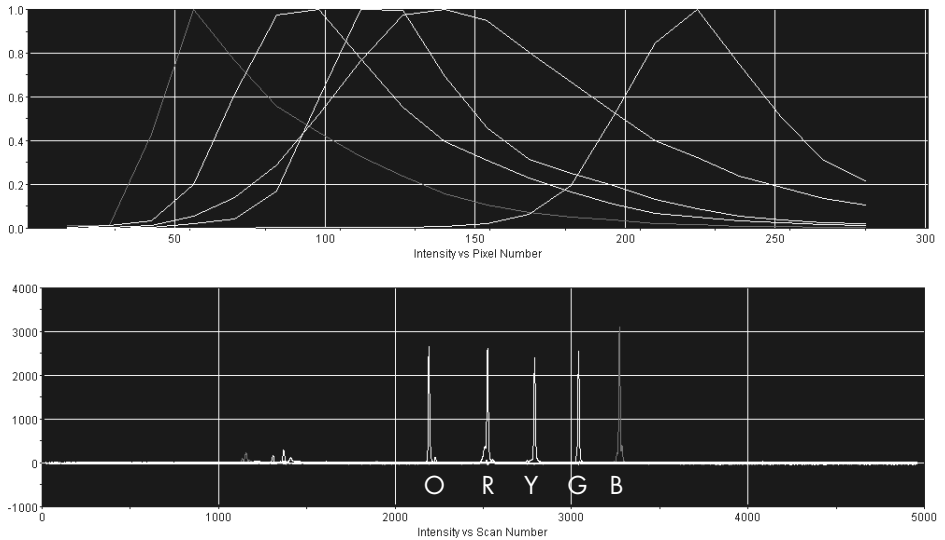
Table 54. Plate Editor for spectral calibration (I)

New plate dialog	Settings
Name	E.g., Spectral_BT5_date
Application	Spectral Calibration
Plate type	96-well
Owner name/ Operator name	–

Table 25. Plate Editor for spectral calibration (II)

Parameter	Settings
Sample name	Enter name for the matrix samples
Priority	E.g., 100
Instrument protocol 1	Spectral36_POP4_BT5 (setting described before)

7. Click the column header to select the entire column, and select “Fill Down” from the “Edit” menu to apply the information to the selected samples. Confirm by clicking “OK”.
8. Link the reaction plate on the autosampler tray with the created plate ID (position A or B) and start the run.



Capillary Data: Mon May 25 09:21:23 CEST 2009

A01

Dye Set:	Any5Dye	Active Calibration for Dye Set: Any5Dye
Matrix used for Capillary 4:	4	Mon May 25 09:21:23 CEST 2009
Condition:	6.937256	
Q Value:	0.976649	List of Calibrations for Dye Set: Any5Dye
		Mon May 25 09:21:23 CEST 2009

Figure 4. Electropherogram of spectral calibration with matrix standard BT5 on an Applied Biosystems 3130 Genetic Analyzer.

Checking the matrix

1. 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 20.
2. Check for a flat baseline in the matrix samples. As shown in the figure on the previous page, there should be 5 peaks with peak heights of about 1000–5000 RFU in each matrix sample.

Note: The optimal range is 2000–4000 RFU.

3. Check the new matrix with the current samples. There should be no pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix.
4. If calibration failed, use the optimized values of the Matrix Standard BT5 and repeat the calibration run.
5. If all capillaries have passed the test, the last calibration file for the Dye Set Any5Dye is activated automatically in the Spectral Viewer. Rename the calibration file (e.g., BT5_Date of calibration).

Sample preparation

1. Set up a mixture of formamide and DNA size standard according to Table 26.

Table 26. Setup of formamide and DNA size standard mixture

Component	Volume per sample
Hi-Di Formamide	12 μ l
DNA Size Standard 550 (BTO)	0.5 μ l

2. Aliquot 12 μ l of the mixture to a tube for each sample to be analyzed.
3. Add 1 μ l PCR product or allelic ladder (diluted, if necessary).
4. Denature for 3 min at 95°C.
5. Snap freeze by placing the plate on ice for 3 min.

Alternatively, the thermal cycler set to 4°C may be used to cool the plate.

6. Load the samples on the tray.

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted onto the plate of multi-capillary analyzers. If fewer samples are analyzed, the empty positions must be filled with 12 μ l Hi-Di Formamide.

To ensure a reliable allelic assignment on multi-capillary analyzers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Ensure ambient conditions are kept as recommended by the instrument manufacturer.

Setting up the Data Collection Software

1. Edit the Run Module once for the first run. In the Module Manager of the Data Collection Software, click "New" to open the "Run Module Editor" dialog box.

Note: Modify the Run Module Default settings from "HIDFragmentAnalysis36_POP4_1" to those shown in Table 27.

2. Modify the Injection Voltage to 2.5 kV and the Injection Time to 30 s (Table 27).
3. Click "Save As", enter a name for the new Run Module (e.g., 2.5kV_30s_400bp), and confirm by clicking "OK".
4. Click "Close" to exit the Run Module Editor.

Table 27. Run Module 2.5kV_30s_400bp for Applied Biosystems 3130/3130xl Genetic Analyzer

Parameter	Settings
Oven temperature (°C)	Default
Poly fill volume	Default
Current stability (µA)	Default
Pre-run voltage (kV)	Default
Pre-run time (s)	Default
Injection voltage (kV)	2.5
Injection time (s)	30*
Voltage number of ateps	Default
Voltage step interval	Default
Data delay time (s)	Default
Run voltage (kV)	Default
Run time (s)	1440†

* Deviating from the standard settings, the injection time may range between 1 and 35 s depending on the type of sample. If samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content, an injection time of up to 35 s may be necessary.

† The run time for Investigator Argus X-12 QS was modified in to enable analysis of fragments with lengths of up to 400 bp.

Starting the run

1. Place the prepared 96-well plate on the autosampler tray.
2. Open the Protocol Manager of the Data Collection Software.
3. Click "New" in the Instrument Protocol window to open the Protocol Editor dialog box and enter the information in Table 28.
4. Click "OK" to exit the Protocol Editor.

Table 28. Settings in Instrument Protocol

Protocol Editor	Settings
Name	Run36_POP4_BT5_X12QS
Type	REGULAR
Run module	2.5kV_30s_400bp*
Dye Set	Any5Dye

* See Table 27, "Run Module 1.2kV_30s_400bp for Applied Biosystems 3130/3130xl Genetic Analyzer".

5. Before each run, it is necessary to create a plate definition. In the Plate Manager of the Data Collection Software, click "New" to open the "New Plate" dialog box.
6. Enter the information in Table 29.

Table 29. GeneMapper Plate Editor (I)

Protocol Editor	Settings
Name	E.g., Plate_BT5_Date
Application	Select GeneMapper Application
Plate type	96-Well
Owner name/ Operator name	–

7. Click "OK" and a new table in the Plate Editor opens automatically (Table 30).
8. Click the column header to select the entire column. Select "Fill Down" from the "Edit" menu to apply the information to all selected samples. Click "OK".
9. In the Run Scheduler, click "Find All", and select "Link" to link the reaction plate on the autosampler tray to the newly created plate record (position A or B).

Table 30. GeneMapper Plate Editor (II)

Parameter	Settings
Sample name	Enter the name for the samples
Priority	E.g., 100 (Default)
Sample type	Sample or Allelic Ladder
Size standard	E.g., SST-BTO_60-400bp
Panel	E.g., Argus_X12_QS_Panels
Analysis method	E.g., Analysis_HID_3130
Snp set	–
User-defined 1-3	–
Results group 1	(Select results group)
Instrument protocol 1	Run36_POP4_BT5_X12QS (setting described before)

10. Start the run.

11. During the run, view Error Status in the Event Log or examine the quality of the raw data for each capillary in the Capillaries Viewer or the Cap/Array Viewer.

12. View data as an overview in Run History or Cap/Array Viewer of the Data Collection Software.

Run data are saved in the Run Folder of the previously chosen Result Group.

Analysis parameters/analysis method

Table 31 lists the recommended analysis parameters in the worksheet Peak Detector.

Table 31. Recommended settings for the Applied Biosystems 3130/3130xI Genetic Analyzer

Parameter	Settings
Peak detection algorithm	Advanced
Ranges	Analysis: Partial Range Start Point: 2000; Stop Point: 10,000 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:* O:* Min. Peak Half Width: 2 pts Polynomial Degree: 3 Peak Window Size: 11 pts [†] Slope Thresholds: 0.0

* The peak amplitude threshold (cut-off value) corresponds to the minimum peak height that will be detected by the GeneMapper *ID* or *ID-X* Software. The thresholds are usually 50–200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three-times higher than the background noise of the baseline.

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

Note: For information on the use of the recommended Template Files (as analysis parameters), refer to the appropriate Investigator Template Files User Guide (GeneMapper *ID* or GeneMapper *ID-X*).

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

The Investigator Argus X-12 QS Kit is validated for use on the 3500/3500xL Genetic Analyzer, which requires the following software:

- 3500 Data Collection Software v1 or v2
- HID Updater 3500 Data Collection v2.0

Note: The user must be logged on to the PC as a 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 v1 or v2 and the GeneMapper *ID-X* Software v1.2, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

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 5 fluorescent labels 6-FAM, BTG, BTY, BTR and BTO. This matrix standard is known as BT5.

The materials required for electrophoresis are given in Table 32.

Table 32. 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 5 fluorescent labels 6-FAM, BTG, BTY, BTR and BTO for each analyzer (Table 33). 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 dye set BT5
- 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 33. The 5 fluorescent labels of BT5

Color	Matrix standard
Blue (B)	6-FAM
Green (G)	BTG
Yellow (Y)	BTY
Red (R)	BTR
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 BT5 according Table 34.

Table 34. Setup of formamide and Matrix Standard BT5 mixture for 8 capillaries

Component	Volume
Hi-Di Formamide	90 µl
Matrix Standard BT5 multi cap.	10 µl

3. Vortex and then centrifuge the mixture briefly.
4. Load 10 µl 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.
7. 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)

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 BT5 according to Table 35.

Table 35. Setup of formamide and Matrix Standard BT5 mixture for 24 capillaries

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

3. Vortex and then centrifuge the mixture briefly.
4. 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.
5. Denature for 3 min at 95°C.
6. Snap freeze by placing the plate on ice for 3 min.
7. 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 BT5

Prior to the spectral calibration, a dye set for the Matrix Standard BT5 must be set up.

1. To create a new dye set, select "Library". Under "Analyze" go to "Dye Sets" and click "Create".
2. Enter a "Dye Set Name", for example, BT5.

3. Under “Chemistry” select “Matrix Standard” and as a “dye set template” select “AnyDye Template”
4. Disable “Purple” in the field “Arrange Dyes”. Ensure that all other colors are enabled.
5. Under “Calibration Peak Order” the colors need to be arranged as follows: 5 – blue, 4 – green, 3 – yellow, 2 – red, and 1 – orange.
6. Do not alter the “Parameter” settings.
7. Click “Save” to confirm the changes.

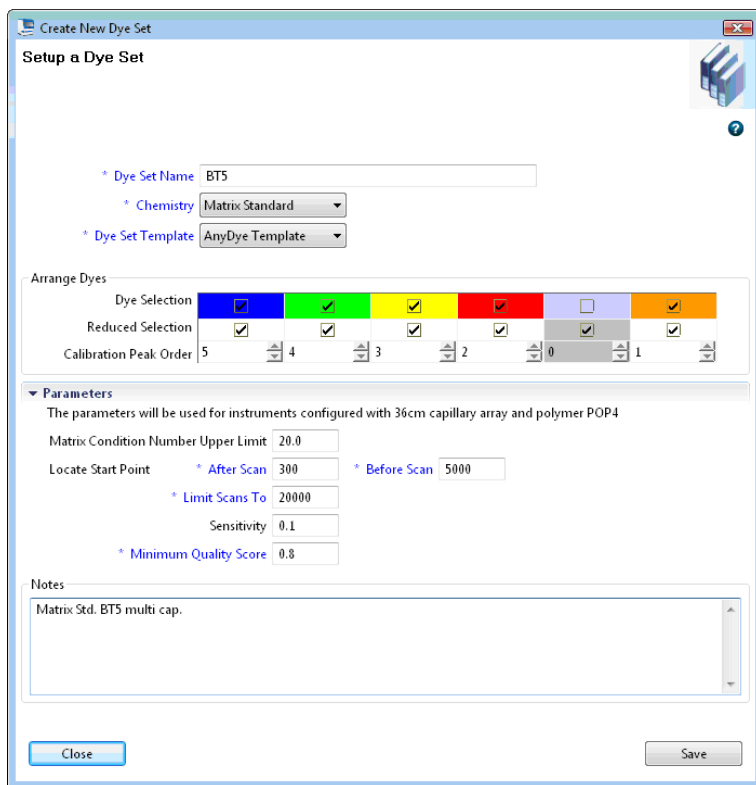


Figure 5. Setup of dye set BT5.

Performing a spectral calibration run

Once the multi-well 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, for example, the previously created BT5 (from step 2).
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 0.8 and the number range (C value) must be between 1 and 20.
- Check the matrix samples for a flat baseline. As shown in Figure 6, there should be 5 peaks with peak heights of about 1000–5000 RFU for each matrix sample (**Note:** The optimal range is 2000–4000 RFU).

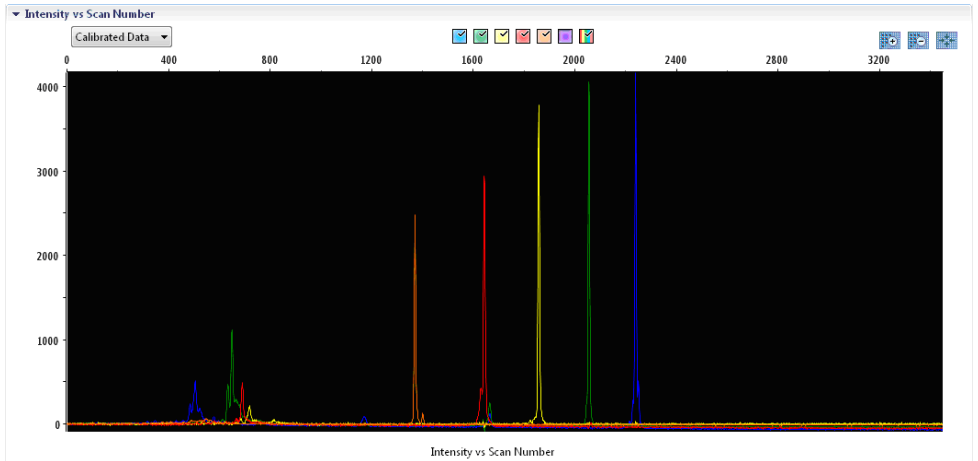


Figure 6. Electropherogram of spectral calibration of the matrix standard BT5 on an Applied Biosystems 3500 Genetic Analyzer. When a spectral calibration is successfully completed, the “Overall” row displays green results. If the “Overall” row displays red results, refer to the “Spectral calibration 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	Passed	Passed	Passed	Passed	Passed	Passed	Passed
Run 2	Not Calibrated	Not Calibrated	Not Calibrated	Not Calibrated	Not Calibrated	Not Calibrated	Not Calibrated	Not Calibrated
Run 3	Not Calibrated	Not Calibrated	Not Calibrated	Not Calibrated	Not Calibrated	Not Calibrated	Not Calibrated	Not Calibrated
Overall	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed

Passed
 Failed
 Borrowed
 Not Calibrated

Figure 7. Example of successful spectral calibration of the matrix standard BT5 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 read orange-red-yellow-green-blue
- 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

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 DNA size standard according to Table 36.
3. Vortex and then centrifuge the mixture briefly.
4. Aliquot 12 µl of the mixture to a tube for each sample to be analyzed.
5. Add 1 µl PCR product or allelic ladder (diluted, if necessary).
6. Denature for 3 min at 95°C.
7. Snap freeze by placing the plate on ice for 3 min.
8. A thermal cycler set to 4°C may be used to cool the plate instead.
9. Load the samples on the tray.

Table 36. Setup of formamide and DNA size standard mixture

Component	Volume per sample
Hi-Di Formamide	12.0 µl
DNA Size Standard 550 (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 multi-capillary analyzers. If fewer samples are analyzed, the empty positions must be filled with 12 μ l Hi-Di Formamide.

To ensure a reliable allelic assignment on multi-capillary 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 multi-capillary 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 X-12 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", under "Analyze" go to "Instrument Protocols" and click "Create".

Note: Modify the Run Module Default settings from "HID36_POP4" as shown in Table 37 (Applied Biosystems 3500) or Table 38 (Applied Biosystems 3500xl).

2. The parameters from Table 37 or Table 38 must be entered or selected.
3. Click "Save" to confirm the changes.

Table 37. Instrument Protocol parameters for Applied Biosystems 3500 Genetic Analyzer

Parameter	Setting
Application type	HID
Capillary length	36 cm
Polymer	POP4
Dye set	E.g., BT5
Run module	HID36_POP4
Protocol name	E.g., Investigator Argus X-12 QS
Oven temperature (°C)	Default (60)
Run voltage (kV)	13.0
PreRun voltage (kV)	Default (15)
Injection voltage (kV)	1.2
Run time (s)	1300
Pre-run time (s)	Default (180)
Injection time (s)	30.0*
Data delay (s)	Default (1)
Advanced options	Default

* When deviating from the standard settings, the injection time may range between 1 and 35 s depending on the type of sample. If samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content, an injection time of up to 35 s may be necessary.

Table 38. Instrument Protocol parameters for Applied Biosystems 3500xL Genetic Analyzer

Parameter	Setting
Application type	HID
Capillary length	36 cm
Polymer	POP4
Dye sset	E.g., BT5
Run module	HID36_POP4
Protocol name	E.g., Investigator Argus X-12 QS
Oven temperature (°C)	Default (60)
Run voltage (kV)	13.0
Pre-run voltage (kV)	Default (15)
Injection voltage (kV)	1.6
Run time (s)	1300
Pre-run time (s)	Default (180)
Injection time (s)	27.0*
Data delay (s)	Default (1)
Advanced options	Default

* When deviating from the standard settings, the injection time may range between 1 and 30 s depending on the type of sample. If samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content, an injection time of up to 35 s may be necessary.

Size Standard

1. To set up the Size Standard, select "Library", under "Analyze" go to "Size Standards", and click "Create".
2. The parameters in Table 39 must be entered or selected.

The DNA Size Standard 550 (BTO) should be used with the following lengths of fragments: 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.

3. Alternatively, import the DNA Size Standard 550 (BTO) parameters using the recommended Investigator template file "SST-BTO_60-500bp" (Table 39).

4. Click "Save" to confirm the changes.

Table 39. Size standard parameters

Parameter	Setting
Size Standard	E.g., SST-BTO_60-500bp
Dye Color	Orange

QC Protocol

1. To set up the QC Protocol, select "Library", under "Analyze" go to "QC Protocols", and click "Create".
2. The parameters in Table 40 must be entered or selected.

Table 40. QC Protocol parameters

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

3. Go to "Analysis Settings", followed by "Peak Amplitude Threshold" and disable "Purple". Ensure that all other colors are enabled.
Check the recommended analysis settings in Table 43. All other settings should remain as "Default".
4. Click "Save" to confirm the changes.

Assay

1. To set up an Assay, go to "Library", under "Manage" go to "Assays", and click "Create".

- To analyze Investigator Argus X-12 QS fragments, the parameters in Table 41 must be selected.
- Click "Save" to confirm the changes.

Table 41. Assay parameters

Parameter	Setting
Assay name	E.g., Investigator Argus X-12 QS
Color	Default
Application type	HID
Instrument protocol	E.g., Investigator Argus X-12 QS
QC protocols	E.g., BTO_550

Starting the run

- In the Dashboard, click "Create New Plate".
- Go to "Setup", followed by "Define Plate Properties" and select "Plate Details". Select or enter the parameters in Table 42.

Table 42. Plate properties

Property	Setting
Name	E.g., Investigator Argus X-12 QS
Number of wells	96
Plate type	HID
Capillary length	36 cm
Polymer	POP4

- Click "Assign Plate Contents" to confirm the changes.
- 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 the Run”, click “Add from Library” and select Investigator Argus X-12 QS from step 11. 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. Check the boxes next to the names of “Assay”, “File name conventions” and “Results group” to assign those to the selected wells.
 8. If not already done, load the assembled plate to the instrument and close the instrument door to re-initialize the instrument. Then click “Link Plate for Run”. In the next screen, enter the desired Run Name and click “Start Run”.

Analysis parameters/analysis method

Table 43 lists the recommended analysis parameters in the worksheet Peak Detector.

Table 43. 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:* O:* Min. Peak Half Width: 2 pts Polynomial Degree: 3 Peak Window Size: 11 pts [†] Slope Thresholds: 0.0

* The peak amplitude threshold (cut-off value) corresponds to the minimum peak height that will be detected by the GeneMapper *ID-X* Software. The thresholds are usually 50–200 RFU and should be determined individually by the laboratory. **Recommendation:** The minimal peak height should be three times higher than the background noise of the baseline.

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

Protocol: Analysis

For general instructions on automatic sample analysis, refer to the appropriate User Guides for GeneMapper *ID* or GeneMapper *ID-X* Software.

Finding the exact lengths of the amplified products depends on the device type, the conditions of electrophoresis, as well as the DNA size standard used. Due to the complexity of some loci, determining the size should be based on evenly distributed references. The DNA Size Standard 550 (BTO) should be used with the following lengths of fragments: 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.

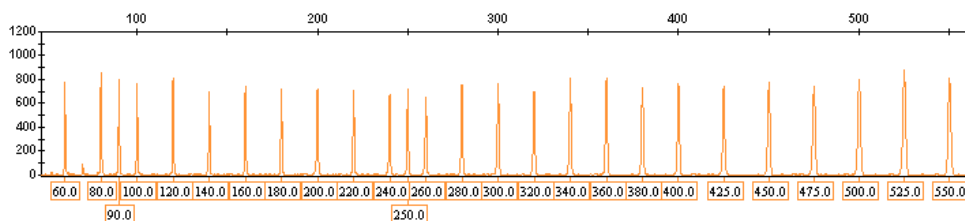


Figure 8. Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp.

Analysis software

Allele allocation should be carried out with suitable analysis software, for example, GeneMapper *ID* or GeneMapper *ID-X* Software, in combination with the Investigator Template Files available as a download from www.qiagen.com, see Table 44 and Table 45.

Table 44. Recommended Investigator Template Files for GeneMapper ID

File type	File name
Panels	Argus_X12_QS_Panels
BinSets	Argus_X12_QS_Bins
Size standard	SST-BTO_60–500bp
Analysis method	Analysis_HID_3130_50rfu Analysis_HID_3130_200rfu Analysis_HID_310_50rfu Analysis_HID_310_200rfu
Plot settings	Plots_5dyes

Panels and BinSets must always be used; other template files are optional.

Table 45. Recommended Investigator Template Files for GeneMapper ID-X

File type	File name
Panels	Argus_X12_QS_Panels_x
BinSets	Argus_X12_QS_Bins_x
Stutter	Argus_X12_QS_Stutter_x
Size standard	SST-BTO_60–500bp
Analysis method	Analysis_HID_3130_50rfu Analysis_HID_3130_200rfu Analysis_HID_3500_50rfu Analysis_HID_3500_200rfu
Plot Settings	Plots_5dyes

Panels and BinSets must always be used; other template files are optional.

Controls

The alleles listed in Table 46 represent the Control DNA 9947A (included in the Investigator Argus X-12 QS Kit) and DNA from other commercially available standard cell lines.

Table 46. Allele assignment of the Investigator Argus X-12 QS Kit

Locus	CCR 9947A	CCR 9948	CCR 3657
Amelogenin	X/X	X / Y	X / Y
DXS7132	12/12	13	12
DXS7423	14/15	14	13
DXS8378	10/11	11	12
DXS10074	16/19	18	7
DXS10079	20/23	19	19
DXS10101	30/31	32	29.2
DXS10103	17/17	18	20
DXS10134	35 /36	34	34
DXS10135	21.1/27	22	25
DXS10146	28/28	29	27
DXS10148	22.1/23.1	23	23.1
HPRTB	14/14	14	13
D21S11	30/30	29/30	28/29

For further confirmation, the table displays the alleles of the reference DNA purchased from Coriell Cell Repositories (CCR), as well as 3 reference DNAs purchased from CCR up to the standard of Szibor et al. (4).

Alleles

Table 47 shows the alleles of the allelic ladder. All analyses have been performed using an Applied Biosystems 3500 Genetic Analyzer with POP-4 polymer (Figure 9 and Figure 10). 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: 65–400 bp

- Vertical: Depending on signal intensity

Table 47. Allelic ladder fragments included in the Investigator Argus X-12 QS Kit

Locus	Dye label	Repeat numbers of allelic ladder
QS1	6-FAM	S, Q
Amelogenin	6-FAM	X, Y
DXS10103	6-FAM	15, 16, 17, 18, 19, 20, 21, 22
DXS8378	6-FAM	8, 9, 10, 11, 12, 13, 14, 15
DXS10101	6-FAM	21, 24, 24.2, 25, 25.2, 26, 26.2, 27, 27.2, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35
DXS10134	6-FAM	28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 38.3, 39.3, 40.3, 41.3, 42.3, 43.3, 44.3
DXS10074	BTG	4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 16.2, 17, 18, 19, 20, 21
DXS7132	BTG	10, 11, 12, 13, 14, 15, 16, 17
DXS10135	BTG	12.1, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37.2, 39.2
DXS7423	BTY	12, 13, 14, 15, 16, 17, 18
DXS10146	BTY	21, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 32.2, 33, 33.2, 34, 34.2, 35.2, 39.2, 40.2, 41.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2
DXS10079	BTY	14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 27
HPRTB	BTR	8, 9, 10, 11, 12, 13, 14, 15, 16, 17
DXS10148	BTR	13.3, , 16, 17, 18, 19, 20, 21, 22.1, 23.1, 24.1, 25.1, 26.1, 27.1, 28.1, 29.1, 30.1, 31, 33.1, 38.1
D21S11	BTR	24, 24.2, 25, 26, 26.2, 27, 28, 28.2, 29, 29.2, 30, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 36, 36.2, 37

For information about known microvariants not contained in the Investigator Argus X-12 QS Allelic Ladder, see the National Institute of Standards and Technology (NIST) web site (www.cstl.nist.gov/biotech/strbase/).

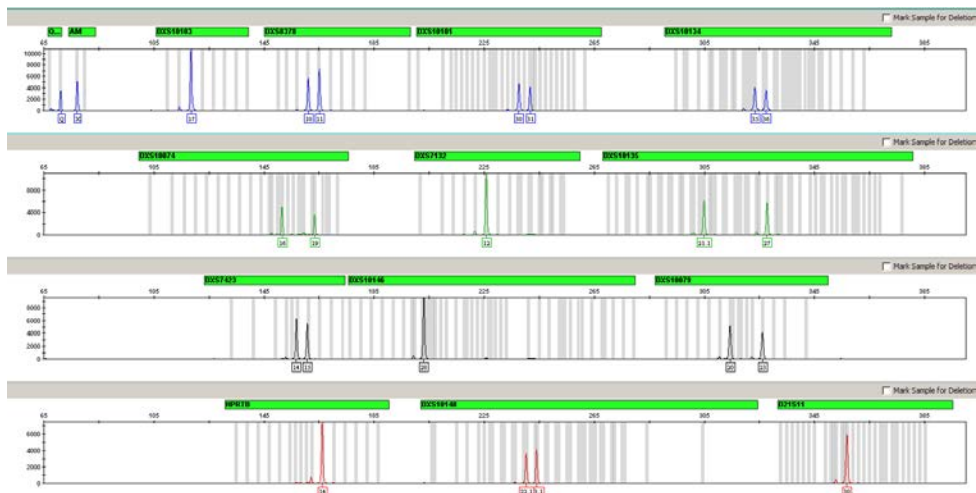


Figure 9. Electropherogram of the Investigator Argus X-12 QS Kit using 500 pg Control DNA 9947A. Analysis was performed on an Applied Biosystems 3500 Genetic Analyzer. Allele assignment was performed using GeneMapper ID-X Software.

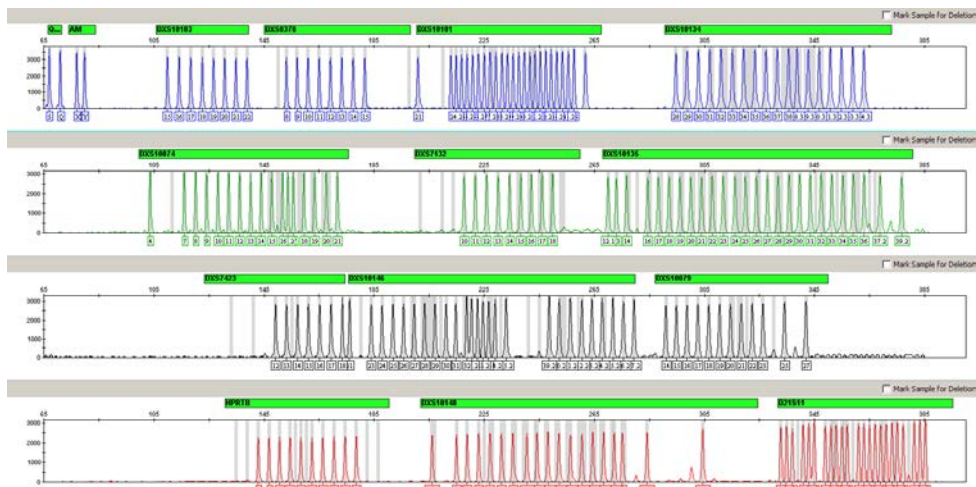


Figure 10. Electropherogram of the allelic ladder Argus X-12 QS analyzed on an Applied Biosystems 3500 Genetic Analyzer. Allele assignment was performed using GeneMapper ID-X Software.

Quality Sensor

The Investigator Argus X-12 QS Kit contains an internal PCR controls (Quality Sensor QS1), which provides helpful information about the PCR amplification efficiency.

To address the issue of sequence similarity and the possibility of non-specific 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 non-specific 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 pipetting of the PCR setup or the PCR itself were performed incorrectly. This indicates that the user could repeat the experiment with more care around the setup in the hope of getting results.

Sensitivity experiments 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.

Characteristics and Use of X-chromosomal STRs

In principle, the guidelines of the ISFG (www.isfg.org) are also valid for working with X-chromosomal STRs. However, the following points should be noted:

- Typing of X-chromosomal markers is only permissible when the phenotypic and genetic sex is identical.
- The genetic sex can easily be determined by analysis of the Amelogenin locus.
- If the phenotypic sex differs from the genotypic sex, results from ChrX-typing may not be used.
- Following national laws, this information must be held in the strictest confidential and should only be forwarded to the analyzed person on request.
- Generation of X-chromosomal profiles for kinship testing should be taken into account whenever autosomal markers do not give reliable or unequivocal results, notably in deficiency cases. In some instances, for example, for complex kinship and paternity testing, markers located on the sex chromosomes may be more informative than their autosomal counterparts, and the use of sex-chromosomal markers may therefore substantially improve the efficiency of a kinship test. This is because the mean exclusion chance (MEC) of a X-chromosomal marker (see below for calculation) is consistently larger than that of an autosomal marker with the same allele frequencies.

Calculation of population-genetic data

The Forensic ChrX Research Group initiated the online database “ChrX-STR.org” (www.chrx-str.org) that calculates population genetics data on the basis of X-chromosomal allele frequencies (1).

The formula for calculating polymorphism information content (PIC) was published by Botstein et al. (5), and the formula for expected heterozygosity (HET) by Nei and Roychoudhury (6). Both can be used for autosomal or X-chromosomal markers.

Krüger et al. (7) introduced the formula for the MEC — the MECKrüger, which was developed for either autosomal marker or for trios. With exception of deficiency cases, MECKrüger is not suitable for X-chromosomal markers. Here, the paternal grandmother can be analyzed instead of the putative father. Kishida et al. (8) devised the MECKishida for X-chromosomal markers as regards trios including a daughter. In comparison with MECKrüger, MECKishida is more complex highlighting the fact that in trios involving a daughter X-STRs are more efficient than autosomal markers.

Finally, Desmarais et al. (9) introduced formulae for the MEC of ChrX markers in trios involving daughters, as well as in father–daughter duos without information about the maternal genotype. MECDesmarais is equivalent to MECKishida whereas MECDesmarais Duo can also be used for maternity testing of mother–son duos. The formula for calculation of the power of discrimination was published by Desmarais et al. (9).

$$PIC = 1 - \sum_{i=1}^n f_i^2 - 2 \sum_{i=1}^{n-1} \sum_{j=i+1}^n f_i^2 f_j^2$$

$$HET = \frac{n}{n-1} \left(1 - \sum_{j=1}^K f_j^2 \right)$$

$$MECKrüger = \sum_i f_i^3 (1 - f_i)^2 + \sum_i f_i (1 - f_i)^3 + \sum_{i < j} f_i f_j (f_i + f_j) (1 - f_i - f_j)^2$$

$$MECKishida = \sum_i f_i^3 (1 - f_i) + \sum_i f_i (1 - f_i)^2 + \sum_{i < j} f_i f_j (f_i + f_j) (1 - f_i - f_j)$$

$$\text{MECDesmarais} = 1 - \sum_i f_{i2} + \sum_i f_{i4} - (\sum_{i<j} f_{i2}^2)$$

$$\text{MECDesmarais Duo} = 1 - 2\sum_i f_{i2} + \sum_i f_{i3}$$

$$\text{PDfemale} = 1 - 2(\sum_i f_{i2})^2 + \sum_i f_{i4}$$

$$\text{PDmale} = 1 - \sum_i f_{i2}$$

Linkage groups

In contrast to earlier findings (10), recent studies (11, 12) showed that markers of linkage groups 3 (HPRTB, DXS10101) and 4 (DXS10134, DXS7423) cannot be regarded as unlinked. The possibility of linkage between these markers should be considered in calculation and interpretation of relationship tests in deficiency cases. If necessary, both probabilities should be calculated from the linkage groups 1, 2 and 3 and linkage groups 1, 2 and 4. For use and calculation of X-chromosomal markers see reference 13 and <http://linkage.rockefeller.edu>.

Kinship tests and paternity cases with ChrX STRs

Note: X-chromosomal markers are particularly informative for inferring parent–offspring relationships that involve at least one female, i.e., mother–daughter, mother–son, and father–daughter duos. For paternity cases only, testing between father and daughter is possible, since fathers pass on only their X-chromosome to their daughter, whereas sons inherit the X-chromosome from their mother.

Deficiency cases

If the putative father (PV) descends from the putative grandmother (PGM) without any doubt, his X-chromosomal characteristics originate from the PGM. Therefore, she (PGM) can be analyzed X-chromosomally instead. The calculation of the PGM-likelihood with X-chromosomal markers (PGM-daughter) may be performed like the calculation with autosomal

markers (PV-daughter). Here, the MEC values according to the formula of Krüger et al., (7) are applied, whereas only unlinked X-STR markers of the Investigator Argus X-12 QS Kit can be used. For markers which are in coupling equilibrium, haplotype frequencies must be involved for calculation.

Siblings and distant relationships

The X-chromosomal analysis (i.e., without parental samples) cannot be used as relationship test in the following cases:

- Different putative fathers may be the father of the child
- Half-brothers with same father and different mothers
- Brothers and half-brothers with same mother (statistically, they have 50% of the characteristics in common, thus the test is comparable to a test with autosomal markers)

Whenever distant relationship should be analyzed, genotyping of X-chromosomal markers is very useful, because the usage of coupled markers allows ChrX-haplotyping. Three markers of each linkage group from the Investigator Argus X-12 QS Kit have to be handled as haplotype for genotyping. For further details, see reference 10, or for the calculation, see the online data base "ChrX-STR.org" (www.chrx-str.org).

Use of X-chromosomal markers for forensic traces

In most cases, the use of autosomal markers for profiling is more reliable since all autosomal loci have two alleles. Whenever female DNA traces require analysis in a male background, e.g., female traces under the fingernails of male, X-chromosomal markers are advantageous. The likelihood that one allele of the trace is covered by the male background is only half in comparison to autosomal tests.

Note: International DNA databases do not usually include markers of the X-chromosome.

Variant alleles and mutations

Additional allelic ladder alleles as well as numerous virtual alleles have been added according to published data to improve interpretation. However, due to the highly polymorphic structure of some markers further rare alleles not covered in the template files to date may occur.

Sequence variants of DXS10101, DXS10146 and DXS10148 have been reported to occur at an elevated frequency in African populations (Elakkary, et. al.). The corresponding mutations affected primer binding and have been addressed by introducing additional SNP primers to the Argus X-12 QS primer mix compared to earlier X-chromosomal commercially available STR Kits for human identification and paternity analysis.

For marker DXS10135 off-ladder calls have been observed with typing of rare 24.1 alleles. Due to repeat sequence variations of this allele, represented by a virtual BIN in the template files, shows a sizing difference of only app. 0.7 bp compared to the frequent allele 24 represented in the ladder. Due to slight variations in migration during capillary electrophoresis, allele 24.1 eventually falls off-ladder. We recommend repeating capillary electrophoresis running allelic ladders next to the sample in question. Running a mixture of the sample and the allelic ladder can provide further confirmation if the allele in the sample is distinguishable as extra peak next to allelic ladder allele 24.

Similarly, off-ladder calls of rare allele 39.1 of marker DXS10146 have been observed. Again the allele might shows a slightly different electrophoretic mobility compared to adjacent 35.2 and 39.2 alleles of the ladder. This leads to a size difference <1 bp between the 39.1 variant and 39.2.

For marker DXS10148 sequence variants within the STR flanking regions have been found. According to the current marker nomenclature an 8 bp (AAGGAAAG)₂ motif is considered to be constant (14). However, single and triplicate (AAGGAAAG) motifs have been identified as well (15). In that these cases the 8 bp deletion or insertion shifts the allele repeat number by minus or plus 2. Within data created with the Investigator Argus X-12 QS Kit this mutation does not lead to inconsistencies, but care must be taken when confirming results by sequencing.

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 at 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 and/or protocols in this handbook or sample and assay technologies (for contact information, visit www.qiagen.com).

Comments and suggestions

Sample preparation

Sample signal intensity must be increased

Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 RFU.

Purify the PCR products before starting the analysis. We recommend the MinElute® PCR Purification Kit for rapid and effective purification.

Matrix/spectral calibration is not appropriate

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

This matrix cannot be used for the analysis. Repeat the matrix generation/spectral calibration. Be sure to carefully follow the correct protocol for the specific analysis instrument.

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

- a) DNA Size Standard 550 (BTO) was not defined or identified correctly

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

Always use the DNA Size Standard 550 included in Investigator Human Identification PCR Kits.

Comments and suggestions

- | | | |
|----|---|--|
| b) | Signal intensities are too high. If the peak heights of the samples are outside the linear detection range*, stutters, split peaks and artifacts may be increased | Reduce the injection time in increments to a minimum of 1 s, 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") that result in allele misnomer | Repeat electrophoresis to confirm results. |
| 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, a number of allelic ladders should be run. |

Injection/file of the allelic ladder is not appropriate

- | | | |
|----|---|---|
| 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 the DNA Size Standard 550 included in Investigator Human Identification PCR Kits. |
| b) | One peak of the allelic ladder is below the peak detection value (50–200 RFU) of the analysis method used, and thus, is not identified | The allelic ladder must be loaded onto the analysis instrument at a higher concentration than samples to be analyzed.
Alternatively, allelic ladder data can be analyzed with a lower peak detection value in Analysis Software. |

* >4000 RFU for the ABI PRISM 310 Genetic Analyzer; >5000 RFU for the ABI PRISM 3100 and Applied Biosystems 3130; >20000 RFU for the 3500 Genetic Analyzers.

Comments and suggestions

- | | |
|---|--|
| 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 found | Point alleles are i.e., alleles with at least 1 bp difference to the next integer allele. Check the settings of the analysis method. Lower the Peak Window Size value to 11 points. |

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, contact QIAGEN Technical Services.

Cited references

1. Szibor, R., Hering, S., and Edelman, J. (2006) A new Web site compiling forensic chromosome X research is now online. *Int. J. Legal Med.* **120**, 252.
2. Bär, W., et al. (1997) DNA recommendations. Further report of the DNA commission of the ISFH regarding the use of short tandem repeat systems. *Forensic Sci. Int.* **87**, 181.
3. Szibor, R., Edelman, J., Hering, S., Gomes, I., and Gusmao, L. (2009) Nomenclature discrepancies in the HPRTB short tandem repeat. *Int. J. Legal Med.* **123**, 185.
4. Szibor, R., et al. (2003) Cell line DNA typing in forensic genetics – the necessity of reliable standards. *Forensic Sci. Int.* **138**, 37.
5. Botstein, D., White, R.I., Skolnick, M., and Davis, R.W. (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **32**, 314.
6. Nei, M., and Roychoudhury, A.K. (1974) Sampling variances of heterozygosity and genetic distance. *Genet.* **76**, 379.
7. Krüger, J., Fuhrmann, W., Lichte, K.H., and Steffens, C. (1968) Zur Verwendung der sauren Erythrocytenphosphatase bei der Vaterschaftsbegutachtung. [Article in German] *Dtsch. Z. Gerichtl. Med.* **64**, 127.
8. Kishida, T., Wang, W., Fukuda, M., and Tamaki, Y. (1997) Duplex PCR of the Y-27H39 and HPRT loci with reference to Japanese population data on the HPRT locus. *Jpn. J. Legal Med.* **51**, 67.

9. Desmarais, D., Zhong, Y., Chakraborty, R., Perreault, C., and Busque, L. (1998) Development of a highly polymorphic STR marker for identity testing purposes at the human androgen receptor gene (HUMARA). *J. Forensic Sci.* **43**, 1046.
10. Szibor, R., Krawczak, M., Hering, S., Edelmann, J., Kuhlisch, E., and Krause, D. (2003) Use of X-linked markers for forensic purposes. *Int. J. Legal Med.* **117**, 67.
11. Tillmar, A., Mostad, P., Egeland, T., Lindblom, B., Holmlund, G., Montelius, K. (2008) Analysis of linkage and linkage disequilibrium for eight X-STR markers. *Forensic Sci. Int. Genet.* **3**, 37.
12. Machado, F.B., and Medina-Acosta, E. (2009) Genetic map of human X-linked microsatellites used in forensic practice. *Forensic Sci. Int. Genet.* **3**, 202.
13. Krawczak, M. (2007) Kinship testing with X-chromosomal markers: Mathematical and statistical issues. *Forensic Sci. Int. Genet.* **1**, 111.
14. Hundertmark, T., Hering, S., Edelmann, J., Augustin, C., Plate, I., Szibor, R. (2008) The STR cluster DXS10148-DXS8378-DXS10135 provides a powerful tool for X-chromosomal haplotyping at Xp22. *Int. J. Legal Med.* **122**, 489-92.
15. Gomes, I., Brehm, A., Gusmão, L., Schneider, P.M. (2015) Allele nomenclature implications of new sequence variants detected at DXS10148, DXS10074 and DXS10134 loci. *Forensic Sci Int. Genet.* (in publication).

General references

Becker, D., et al. (2008) Population genetic evaluation of eight X-chromosomal short tandem repeat loci using Mentype Argus X-8 PCR amplification kit. *Forensic Sci. Int. Genet.* **2**, 69.

Edelmann, J., et al. (2001) 16 X chromosome STR loci frequency data from a German population. *Forensic Sci. Int.* **124**, 215.

Edelmann, J., Hering, S., Augustin, C., and Szibor, R. (2007) Characterisation of STR markers DXS10146, DXS10134 and DXS10147 located within a 79.1 kb region at Xp28. *Forensic Sci. Int. Genet.* (DOI 10.1016/j.fsigen.2007.08.001).

Gomes, I., et al. (2007) Genetic analysis of three US population groups using an X-chromosomal STR decaplex. *Int. J. Legal Med.* **121**, 198.

Hering S., et al. (2006) DXS10079, DXS10074 and DXS10075 are STRs located within a 280 kb region of Xq12 and provide stable haplotypes useful for solving of complex kinship cases. *Int. J. Legal Med.* **120**, 337.

Mertens, G., et al. (1999) Mutation of the repeat number of the HPRTB locus and structure of rare intermediate alleles. *Int. J. Legal Med.* **112**,192.

Szibor, R., Edelmann, J., and Zarrabeitia, M.T. (2003b) Sequence structure and population data of the X-linked markers DXS7423 and DXS8377 – clarification of conflicting statements published by two working groups. *Forensic Sci. Int.* **134**, 72.

Ordering Information

Product	Contents	Cat. no.
Investigator Argus X-12 QS Kit (25)	Primer mix, Reaction Mix, DNA Polymerase, Control DNA, Allelic Ladder Argus X-12 QS, DNA Size Standard and Nuclease-Free Water	383223
Investigator Argus X-12 QS Kit (100)	Primer mix, Reaction Mix, DNA Polymerase, Control DNA, Allelic Ladder Argus X-12 QS, DNA Size Standard and Nuclease-Free Water	383225
Related products		
Investigator Human Identification PCR Kits		
Investigator Argus Y-12 QS Kit (100)	Primer mix, Reaction Mix, DNA Polymerase, Control DNA, Allelic Ladder Argus Y-12 QS, DNA Size Standard and Nuclease-Free Water	383615
Investigator 24plex QS Kit (100) *	Primer Mix, Fast Reaction Mix including <i>Taq</i> DNA Polymerase, Control DNA, Allelic Ladder 24plex, DNA Size Standard and Nuclease-Free Water	382415
Investigator 24plex GO! Kit (200) *	Primer Mix, Fast Reaction Mix 2.0 including <i>Taq</i> DNA Polymerase, Control DNA, Allelic Ladder 24plex, DNA Size Standard and Nuclease-Free Water	382426

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
Investigator ESSplex SE QS Kit (100) *	Primer mix, Fast Reaction Mix 2.0 including HotStarTaq® Plus DNA Polymerase, Control DNA, Allelic Ladder ESSplex SE QS, DNA Size Standard 550 (BTO) and Nuclease-Free Water	381575
Investigator Quantification Kits		
Investigator Quantiplex™ Kit (200)	Primer Mix IC FQ, Reaction Mix FQ, Control DNA Z1 and Dilution Buffer	387016
Investigator Quantiplex HYres Kit (200)	Primer Mix IC YQ, Reaction Mix YQ, Control DNA Z1 and Dilution Buffer	387116
Investigator Human Identification PCR Kit Accessories		
DNA Size Standard 550 (BTO) (100)	DNA Size Standard 550 (BTO) for 100 reactions	386015
Matrix Standard BT5 single cap. (5 x 25)	Matrix Standard 6-FAM, BTG, BTY, BTR and BTO	386113
Matrix Standard BT5 multi cap. (25)	Matrix Standard 6-FAM, BTG, BTY, BTR and BTO	386123
Matrix Standard BT5 multi cap. (50)	Matrix Standard 6-FAM, BTG, BTY, BTR and BTO	386125
DNA extraction and purification		
QIAamp® DNA Investigator Kit (50)	50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers and Collection Tubes (2 ml)	56504

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
EZ1® DNA Investigator Kit (48)	Reagent Cartridges, Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 ml), Elution Tubes (1.5 ml), Buffer G2, Proteinase K and Carrier RNA	952034
MinElute PCR Purification Kit (50) *	50 MinElute Spin Columns, Buffers and Collection Tubes (2 ml)	28004

* Larger kit sizes available; please inquire.

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.

Trademarks: QIAGEN[®], Sample to Insight[®], QIAamp[®], EZ1[®], HotStarTaq[®], Investigator[®], MinElute[®], QuantiPlex[™] (QIAGEN Group); Bio-Rad[®] (Bio-Rad Laboratories, Inc.); 3500[™], ABI PRISM[®], Applied Biosystems[®], Avant[™], 6-FAM[™], GeneAmp[®], GeneMapper[®], GeneScan[®], Hi-Di[™], POP-4[™], Veriti[®] (Life Technologies Corporation); Eppendorf[®], Mastercycler[®] (Eppendorf AG); GenBank[®] (US Department of Health and Human Services). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

Limited License Agreement for Investigator Argus X-12 QS Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.qiagen.com.

1095535 HB-1976-001 06/2015 © 2015

Notes

Notes

Notes

www.qiagen.com