# Investigator® Argus X-12 Handbook

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

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# Contents

# Kit Contents

| Investigator Argus X-12 Kit | (25) | (100) |
|---|---|---|
| Catalog no. | 383213 | 383215 |
| Number of 25 $\mu$l reactions | 25 | 100 |
| Reaction Mix A | 125 $\mu$l | 500 $\mu$l |
| Primer Mix Argus X-12 | 63 $\mu$l | 250 $\mu$l |
| Multi Taq2 DNA Polymerase | 38 U | 150 U |
| Control DNA 9947A | 200 $\mu$l | 200 $\mu$l |
| DNA size standard 550 (BTO) | 13 $\mu$l | 50 $\mu$l |
| Allelic ladder Argus X-12 | 25 $\mu$l | 25 $\mu$l |
| Nuclease-free water | 1.9 ml | 2 x 1.9 ml |
| Quick-Start Protocol | 1 | 1 |

# Storage

All components of the Investigator Argus X-12 Kit should be stored at –20°C. Avoid repeated thawing and freezing. 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.

# Intended Use

The Investigator Argus X-12 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.

**24-hour emergency information**

Chemical emergency or accident assistance is available 24 hours a day from:

CHEMTREC

**USA & Canada**: Tel: 1-800-424-9300

**Outside USA & Canada**:  Tel: +1-703-527-3887 (collect calls accepted)

# Introduction

The Investigator Argus X-12 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. Furthermore, the use of gonosomal STRs for the analysis of DNA traces in the forensic field is increasing. This kit is highly suited for analysis of forensic stains, such as female traces on a male background.

The Investigator Argus X-12 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).

The primers are fluorescence-labeled with one of the following dyes:

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

**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 Kit was validated using the GeneAmp® PCR System 9700 (with Gold-plated 96-Well Silver Block), ABI PRISM® 310, ABI PRISM 3100, Applied Biosystems 3130, and Applied Biosystems 3500 Genetic Analyzers.

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).
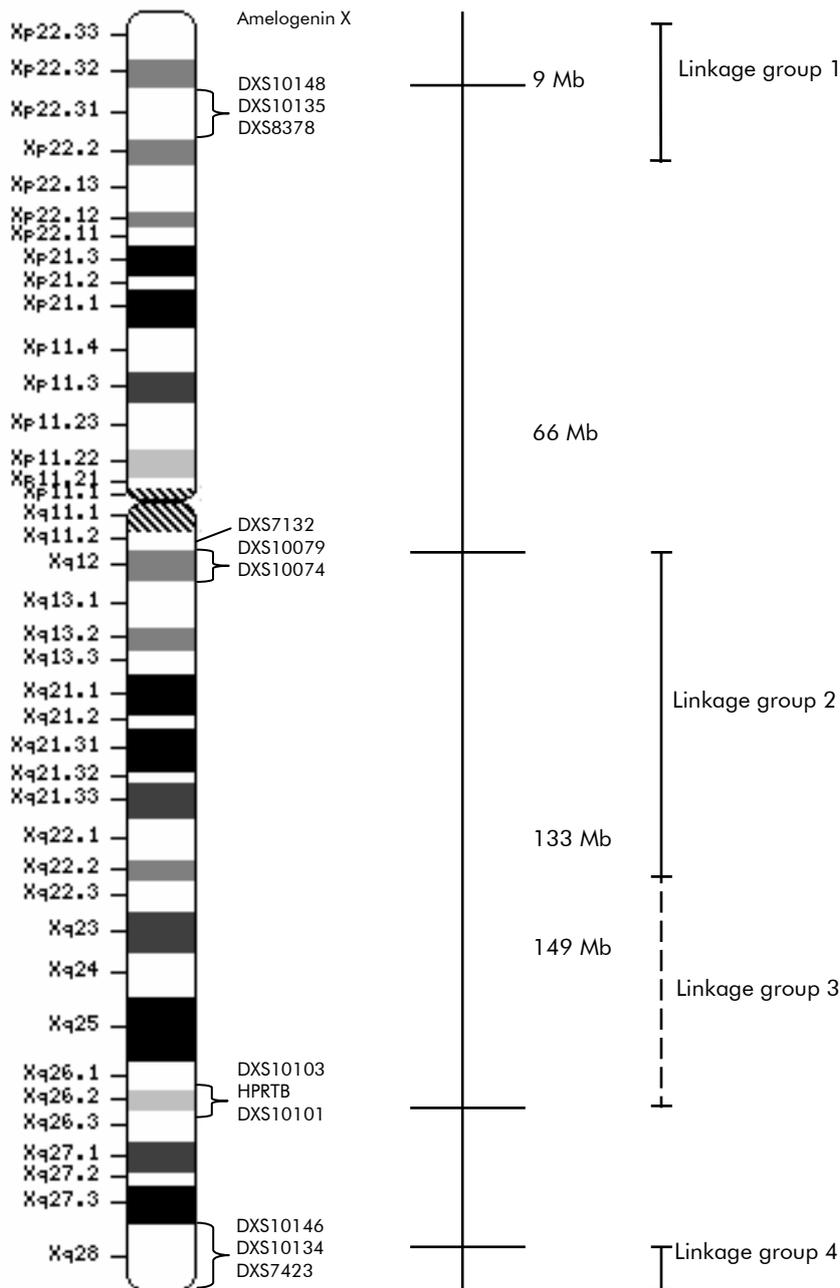
**Figure 1. The ideogram of the X-chromosome describes the physical localization of the STR loci that can be analyzed using the Investigator Argus X-12 Kit**. Distances from the p-telomere are shown in Mb (www.ncbi.nlm.nih.gov/genome/guide/human as of 10/2009).

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). Allele ranges include all known alleles of the National Institute of Standards and Technology (NIST as of 12/2008) and of the current literature.

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

| Locus | GenBank® Accession number | Repeat motif of the reference allele | Ref. allele | Allele range |
|---|---|---|---|---|
| Amelogenin X | M55418 | – | – | – |
| Amelogenin Y | M55419 | – | – | – |
| DXS7132 | G08111 | $[TCTA]_{13}$ | 13 | 8–20 |
| DXS7423 | AC109994 | $[TCCA]_3$ TCTGTCCT $[TCCA]_{12}$ | 15 | 8–19 |
| DXS8378 | G08098 | $[CTAT]_{12}$ | 12 | 7–15 |
| DXS10074 | AL356358 | $[AAGA]_{14}$ | 14 | 4–21 |
| DXS10079 | AL049564 | $[AGAG]_3$ TGAAAGAG $[AGAA]_{17}$ AGAG $[AGAA]_3$ | 21 | 14–25 |
| DXS10101 | AC004383 | $[AAAG]_3$ GAAAGAAG $[GAAA]_3$ A $[GAAA]_4$ AAGA $[AAAG]_5$ AAAAAGAA $[AAAG]_{13}$ AA | 28.2 | 24–38 |
| DXS10103 | BV680555 | $[TAGA]_2$ CTGA $[CAGA][TAGA]^{11}[CAGA]^4[TAGA]$ | 19 | 15–21 |
| DXS10134 | AL034384 | $[GAAA]_3$ GAGA $[GAAA]_4$ AA $[GAAA]$ GAGA $[GAAA]_4$ GAGA $[GACAGA]_3$ $[GAAA]$ GTAA $[GAAA]_3$ AAA $[GAAA]_4$ AAA $[GAAA]_{15}$ | 35 | 28–46.1 |
| DXS10135 | AC003684 | $[AAGA]_3$ GAAAG $[GAAA]_{20}$ | 23 | 13–39.2 |
| DXS10146 | AL034384 | $[TTCC]_3$ T $[TTCC]_3$ TTTC CTCCCTTCC $[TTCC]$ $[TCCC]$ TTCTTCTTTC $[TTCC]_2$ TTTCTT $[CTTT]_2$ CTTC $[CTTT]_{10}$ T $[CTTT]_2$ | 26 | 24–46.2 |
| DXS10148 | AC003684 | $[GGAA]_4[AAGA]_{12}[AAAG]_4$ $N_8$ $[AAGG]_2$ | 22 | 13.3–38.1 |
| HPRTB | M26434 | $[AGAT]_{12}$* | 12 | 6–19 |

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

**Table 3. Chromosomal mapping of Investigator Argus X-12 Kit**

| Locus | Chromosomal mapping |
|---|---|
| Amelogenin X | Xp22.1-22.3 |
| Amelogenin Y | Yp11.2 |
| DXS7132 | Xq11.2 |
| DXS7423 | Xq28 |
| DXS8378 | Xp22.31 |
| DXS10074 | Xq12 |
| DXS10079 | Xq12 |
| DXS10101 | Xq26.2 |
| DXS10103 | Xq26.2 |
| DXS10134 | Xq28 |
| DXS10135 | Xp22.31 |
| DXS10146 | Xq28 |
| DXS10148 | Xp22.31 |
| HPRTB | Xq26.2 |

# 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-*Avant*™/3100 Genetic Analyzer
  Applied Biosystems 3130/3130*xl* Genetic Analyzer
  Applied Biosystems 3500/3500xL Genetic Analyzer

- One of the following PCR thermal cyclers:
  QIAGEN Rotor-Gene® Q
  GeneAmp PCR System 9700
  Bio-Rad PTC-200
  Techne TC-512
  Biometra T1
  Eppendorf® Mastercycler® ep

- 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 (HID) products for forensic applications. We recommend Investigator IDproof Software, Investigator IDproof Mixture Software, GeneMapper® *ID*, GeneMapper *ID-X*, or Genotyper® Software. The Investigator Template Files facilitate data analysis and are compatible with the software mentioned above.

# Protocol: PCR Amplification

This protocol is for PCR amplification of STR loci from forensic samples using the Investigator Argus X-12 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.

**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 before use.

2. **Prepare a master mix according to Table 4.**

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

   Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. This should include positive and negative control reactions.

3. **Mix the master mix thoroughly, 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 μl.**

5. **Prepare positive and negative controls.**

   Positive control: Use 5 μl of the Control DNA.

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

**Table 4. Reaction setup**

| Component | Volume per reaction |
|---|---|
| Reaction Mix A* | 5.0 µl |
| Primer Mix | 2.5 µl |
| Multi Taq2 DNA Polymerase | 0.6 µl |
| Nuclease-free water (added in step 4) | Variable |
| Template DNA (added in step 4) | Variable |
| **Total volume** | **25 µl** |

* Contains dNTP mix, $MgCl_2$, and bovine serum albumin (BSA).

6. **Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 5.**

    For stains containing small amounts of DNA (<100 pg/25 µl reaction), we recommend using the cycling conditions outlined in Table 6.

    **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 5. Standard cycling protocol, recommended for all DNA samples**

| Temperature | Time | Number of cycles |
|---|---|---|
| 94°C* | 4 min | – |
| 96°C | 30 s | |
| 63°C | 120 s | 5 cycles |
| 72°C | 75 s | |
| 94°C | 30 s | |
| 60°C | 120 s | 25 cycles |
| 72°C | 75 s | |
| 68°C | 60 min | – |
| 10°C | ∞ | – |

* Hot-start to activate DNA polymerase.

**Table 6. Optional cycling protocol, recommended for stains containing small amounts (<100 pg) of DNA**

| Temperature | Time | Number of cycles |
|---|---|---|
| 94°C* | 4 min | – |
| 96°C | 30 s | |
| 63°C | 120 s | 5 cycles |
| 72°C | 75 s | |
| 94°C | 30 s | |
| 60°C | 120 s | 27 cycles |
| 72°C | 75 s | |
| 68°C | 60 min | – |
| 10°C | ∞ | – |

\* Hot-start to activate DNA polymerase.

7. After the cycling protocol is completed, store samples at –20°C protected from the light, or proceed directly with 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 given in Table 7.

**Table 7. 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 8).

**Table 8. The fluorescent labels of BT5**

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

1.  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 Kit, in order to generate suitable matrix files (Table 9).

Table 9. Matrix setup for ABI single capillary instrument (ABI PRISM 310 Genetic Analyzer)

| Matrix sample | Component | Volume |
|---|---|---|
| Matrix sample 1 | Hi-Di Formamide | 12.0 $\mu$l |
| | Matrix standard 6-FAM | 1.0 $\mu$l |
| Matrix sample 2 | Hi-Di Formamide | 12.0 $\mu$l |
| | Matrix standard BTG | 1.0 $\mu$l |
| Matrix sample 3 | Hi-Di Formamide | 12.0 $\mu$l |
| | Matrix standard BTY | 1.0 $\mu$l |
| Matrix sample 4 | Hi-Di Formamide | 12.0 $\mu$l |
| | Matrix standard BTR | 1.0 $\mu$l |
| Matrix sample 5 | Hi-Di Formamide | 12.0 $\mu$l |
| | Matrix standard BTO | 1.0 $\mu$l |

2.  Denature for 3 min at 95°C.
3.  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.
4.  Load the samples on the tray.
5.  Create a Sample Sheet and enter the sample designation. Table 10 shows the injection list for matrix generation.

**Table 10. 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, and 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 (next page), 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.
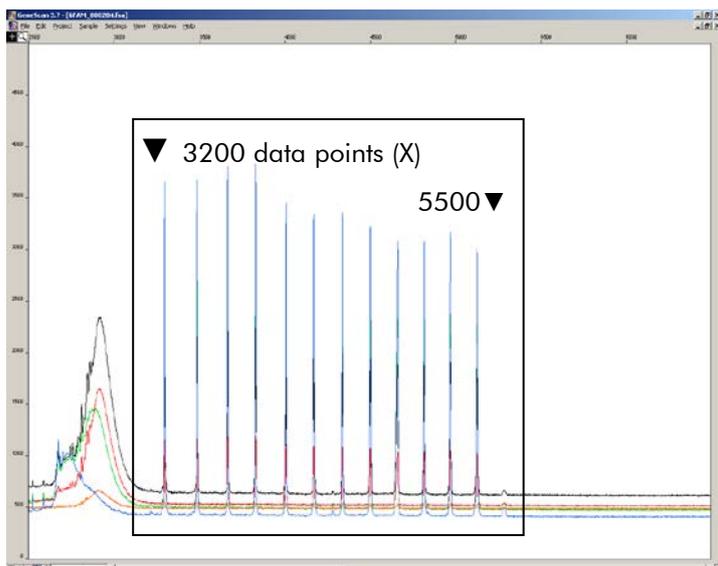
**Electropherogram with raw data of the matrix standard 6-FAM.**

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.
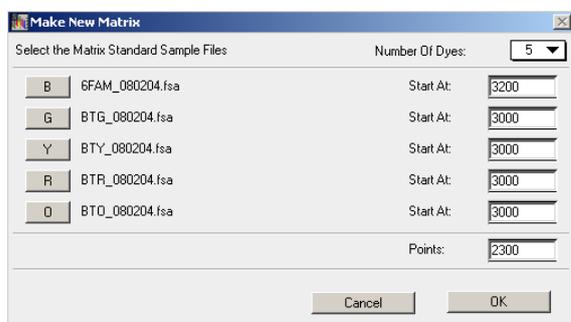


**Matrix sample selection.**

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



New matrix BT5.

Checking the matrix

1. To check the new matrix with current samples, select "New" in the "File" menu, and 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 11.

Table 11. 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.**

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

6. **Load the samples on the tray.**

## Setting up the GeneScan Software

Create a Sample Sheet and enter sample designation.

**Table 12. 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_60-400bp |
| Injection Time (s) | 5* |
| Injection Voltage (kV) | 15 |
| Run Voltage (kV) | 15 |
| Run Temperature (°C) | 60 |
| Run Time (min) | 26[†] |

* 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 up to 10 s may be necessary.

[†] The run time for Investigator Argus X-12 was modified in order to be able to analyze fragments with lengths of up to 400 bp.

## Analysis parameters

Table 13 lists the recommended analysis parameters.

**Table 13. Recommended analysis parameters for the ABI PRISM 310 Genetic Analyzer**

| Parameter | Settings |
| --- | --- |
| Analysis Range | Start: 2000<br>Stop: 10,000 |
| Data Processing | Baseline: Checked<br>Multi-component: Checked<br>Smooth options: Light |
| Peak Detection | Peak Amplitude Thresholds<br>B:*   Y:*<br>G:*   R:*<br>O:*<br>Min. Peak Half Width: 2 pts<br>Polynomial Degree: 3<br>Peak Window Size: 11 pts[†] |
| Size Call Range | Min: 60<br>Max: 550 |
| Size Calling Method | Local Southern Method |
| Split Peak Correction | None |

\* The peak amplitude threshold (cutoff 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 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 (Genotyper, 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 given in Table 14.

**Table 14. 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 15.

**Table 15. Setup of formamide and Matrix Standard BT5 mixture for 4 capillaries**

| Component | Volume |
|---|---|
| Hi-Di Formamide | 60 $\mu$l |
| Matrix Standard BT5 multi cap. | 5 $\mu$l |

2. Load 12 $\mu$l of the mixture to 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 16.

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

| Component | Volume |
|---|---|
| Hi-Di Formamide | 204 $\mu$l |
| Matrix Standard BT5 multi cap. | 17 $\mu$l |

2. Load 12 $\mu$l of the mixture to 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 17. 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 23.

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 18.

**Table 18. Setup of formamide and DNA size standard mixture**

| Component | Volume per sample |
|---|---|
| Hi-Di Formamide | 12 $\mu$l |
| DNA Size Standard 550 (BTO) | 0.5 $\mu$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 GeneScan Software

7. **Edit the default run module in Dye Set G5 once for the first run. Select "Module Editor" to open the dialog box.**

8. **Select the appropriate Run Module as template from the GeneScan table (see Table 19).**

9. **Modify the Injection Voltage to 3 kV and the Injection Time to 10 s.**

10. **Click "Save As" and enter the name of the new module (e.g., 3kV_10s_400bp). Confirm by clicking "OK".**

11. **Click "Close" to exit the Run Module Editor.**

**Table 19. Run Module 3kV_10s_400bp for the ABI PRISM 3100-**
***Avant*/3100 Genetic Analyzer**

| Parameter | Setting |
|---|---|
| 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) | 3.0 |
| Injection Time (s) | 10* |
| Run Voltage (kV) | Default |
| Number of Steps | Default |
| Voltage Step Interval | Default |
| Data Delay Time (s) | Default |
| Run Time (min) | 20[†] |

\* Deviating from the standard settings, the injection time may range between 1 and 20 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 20 s may be necessary.

[†] The run time for Investigator Argus X-12 was modified in order to be able to analyze 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 20. 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* | 3kV_10s_400bp |
| Analysis Module 1 | DefaultAnalysis.gsp |

* See Table 19, "Run Module 3kV_10s_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 21 lists the recommended analysis parameters.

**Table 21. 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 (cutoff 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 (Genotyper, GeneMapper *ID*, or GeneMapper *ID-X*).

# Protocol: Electrophoresis Using the Applied Biosystems 3130/3130*xl* 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 3130/3130*xl *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 3130*xl* 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 22.

**Table 22. Materials needed for electrophoresis**

| Material | Specifications |
|----------|----------------|
| Capillary | 36 cm Capillary Array for Applied Biosystems 3130/3130*xl* Genetic Analyzer |
| Polymer | POP-4 Polymer for Applied Biosystems 3130/3130*xl* 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 23.

Table 23. Setup of formamide and Matrix Standard BT5 mixture for 4 capillaries

| Component | Volume |
|---|---|
| Hi-Di Formamide | 60 $\mu$l |
| Matrix Standard BT5 multi cap. | 5 $\mu$l |

2. Load 12 $\mu$l of the mixture to 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 3130*xl* Genetic Analyzer)

1. Set up a mixture of formamide and Matrix Standard BT5 according to Table 23.

Table 24. Setup of formamide and Matrix Standard BT5 mixture for 16 capillaries

| Component | Volume |
|---|---|
| Hi-Di Formamide | 204 $\mu$l |
| Matrix Standard BT5 multi cap. | 17 $\mu$l |

2. Load 12 $\mu$l of the mixture to 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 25 and click "OK".

**Table 25. 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 26 and click "OK". A new table in the Plate Editor opens automatically (Table 27).

**Table 26. 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 27. 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.**
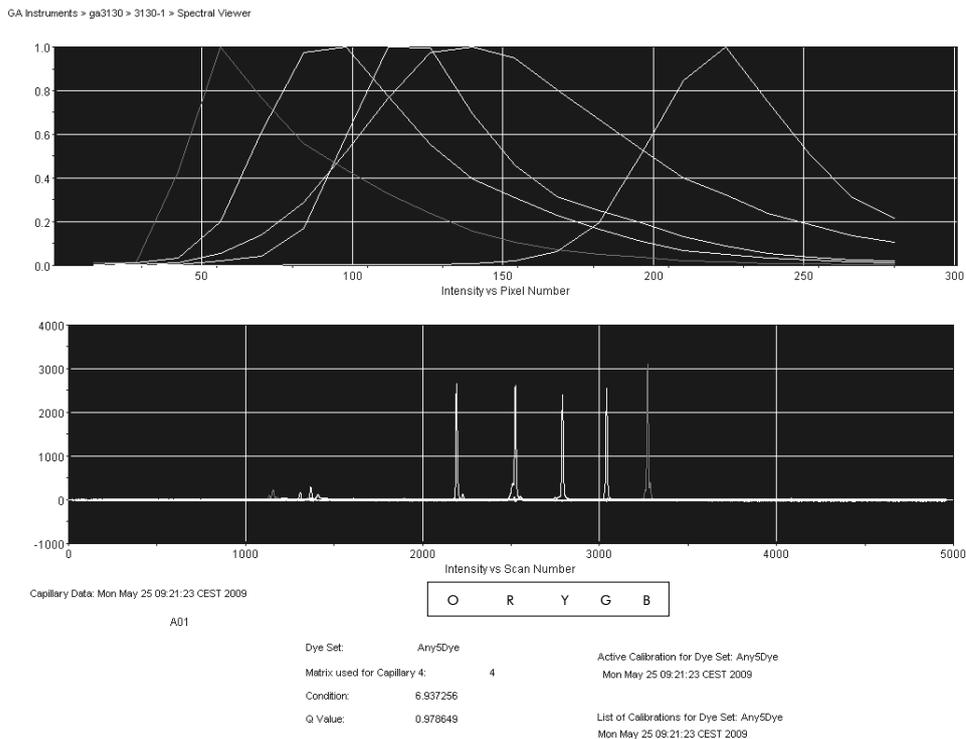


**Electropherogram of spectral calibration with matrix standard BT5 on an Applied Biosystems 3130 Genetic Analyzer.**

### 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 28.

**Table 28. Setup of formamide and DNA size standard mixture**

| Component | Volume per sample |
|---|---|
| Hi-Di Formamide | 12 $\mu$l |
| DNA Size Standard 550 (BTO) | 0.5 $\mu$l |

2. Aliquot 12 $\mu$l of the mixture to a tube for each sample to be analyzed.

3. Add 1 $\mu$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 $\mu$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

7. **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 29 .

8. **Modify the Injection Voltage to 3 kV and the Injection Time to 10 s (Table 29).**

9. **Click "Save As", enter a name for the new Run Module (e.g., 3kV_10s_400bp), and confirm by clicking "OK".**

10. **Click "Close" to exit the Run Module Editor.**

**Table 29. Run Module 3kV_10s_400bp for Applied Biosystems 3130/3130*xl* 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) | 3.0 |
| Injection Time (s) | 10* |
| Voltage Number of Steps | Default |
| Voltage Step Interval | Default |
| Data Delay Time (s) | Default |
| Run Voltage (kV) | Default |
| Run Time (s) | 1200[†] |

* Deviating from the standard settings, the injection time may range between 1 and 20 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 20 s may be necessary.

[†] The run time for Investigator Argus X-12 was modified in order to be able to analyze 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 30.
4. Click "OK" to exit the Protocol Editor.

**Table 30. Settings in Instrument Protocol**

| Protocol Editor | Settings |
|---|---|
| Name | Run36_POP4_BT5_20min |
| Type | REGULAR |
| Run Module | 3kV_10s_400bp* |
| Dye Set | Any5Dye |

\* See Table 29, "Run Module 3kV_10s_400bp for Applied Biosystems 3130/3130*xl* 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 31.

**Table 31. 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 32).

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 32. 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_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_20min (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 33 lists the recommended analysis parameters in the worksheet Peak Detector.

**Table 33. Recommended settings for the Applied Biosystems 3130/3130*xl* Genetic Analyzer**

| Parameter | Settings |
|---|---|
| Peak Detection Algorithm | Advanced |
| Ranges | Analysis: Partial Range<br>Start Point: 2000; Stop Point: 10,000<br>Sizing: All Sizes |
| Smoothing and Baselining | Smoothing: Light<br>Baseline Window: 51 pts |
| Size Calling Method | Local Southern Method |
| Peak Detection | Peak Amplitude Thresholds<br>B:*    Y:*<br>G:*    R:*<br>O:*<br>Min. Peak Half Width: 2 pts<br>Polynomial Degree: 3<br>Peak Window Size: 11 pts†<br>Slope Thresholds: 0.0 |

\* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneMapper *ID* 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 (Genotyper, GeneMapper *ID*, or GeneMapper *ID-X*).

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

For detailed instructions on instrument setup, spectral calibration, or application of the Applied Biosystems 3500 Series Data Collection Software version 1.0 and the GeneMapper *ID-X* Software version 1.2, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

The system with 8 capillaries is the Applied Biosystems 3500 Genetic Analyzer and 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 31.

**Table 34. 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, it is necessary to perform a spectral calibration with the 5 fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO for each analyzer (Table 35). The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

**IMPORTANT**: Spectral calibration must be performed for each new capillary array.

Spectral calibration is comprised of the following steps:

- Preparation of the instrument
- Preparation of dye set BT5
- Preparation of the standard calibration plate
- Plate assembly and loading the plate in the instrument

■ 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*.

## Preparation of dye set BT5

**Table 35. 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 |

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

1. To create a new dye set, go to "Library" and select "Analyze", followed by "Dye Sets" and click "Create".
2. Enter a "Dye Set Name", e.g., BT5.
3. Select "Matrix Standard" as a chemistry and "AnyDye Template" as a dye set 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.
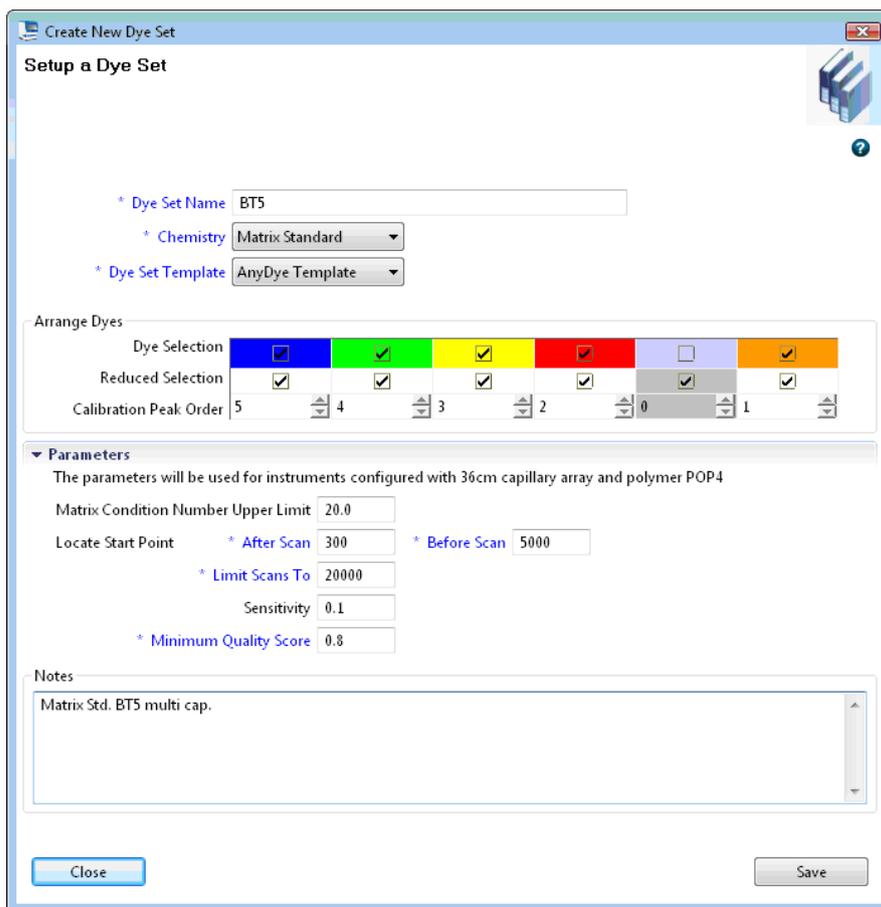
**Setup of dye set BT5.**

## Preparation of the standard calibration plate

**Example for 8 capillaries (Applied Biosystems 3500 Genetic Analyzer)**

1. Set up a mixture of formamide and Matrix Standard BT5 according to Table 36.

**Table 36. Setup of formamide and Matrix Standard BT5 mixture for 8 capillaries**

| Component | Volume |
|---|---|
| Hi-Di Formamide | 90 $\mu$l |
| Matrix Standard BT5 multi cap. | 10 $\mu$l |

2. Load 10 *μ*l of the mixture to a 96-well plate, e.g., positions A1–H1.

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 24 capillaries (Applied Biosystems 3500xL Genetic Analyzer)**

1. Set up a mixture of formamide and Matrix Standard BT5 according to Table 37.

**Table 37. Setup of formamide and Matrix Standard BT5 mixture for 24 capillaries**

| Component | Volume |
|---|---|
| Hi-Di Formamide | 225 *μ*l |
| Matrix Standard BT5 multi cap. | 25 *μ*l |

2. Load 10 *μ*l of the mixture to a 96-well plate, e.g., positions A1–H1, A2–H2, and A3–H3.

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.


**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*.


**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. The number of wells in the spectral calibration plate and their location in the instrument must be specified.

3. Select "Matrix Standard" as a chemistry standard and "BT5" for dye set.

4. (Optional) Enable "Allow Borrowing".

5. Click "Start Run".

## Checking the matrix

Click a capillary in the table in order to display the results for each capillary (spectral data, Quality value, and Condition Number) below the run results table.

- ■ 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 the figure, 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).
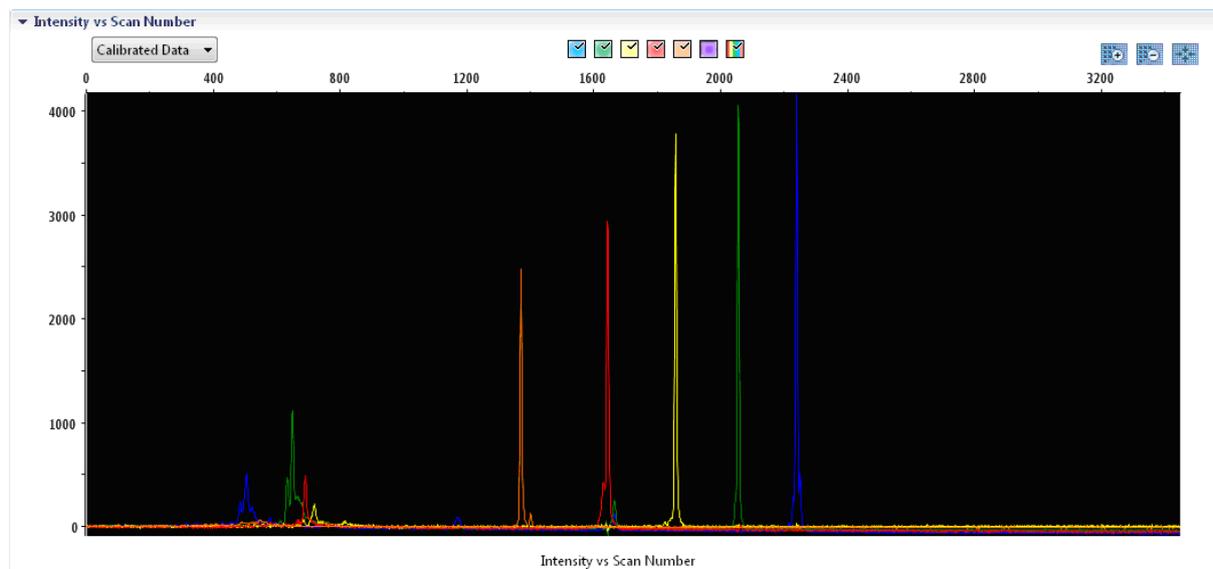


**Electropherogram of spectral calibration of the matrix standard BT5 on an Applied Biosystems 3500 Genetic Analyzer.**

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*.



**Example for a 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 appear in the raw data profile

■ Peak morphology in the spectral profile shows 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 Results". If any capillary data does not meet the criteria above, click "Reject Results", and refer to the "spectral calibration troubleshooting" section of the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

### Sample preparation

1. **Set up a mixture of formamide and DNA size standard according to Table 38.**

**Table 38. Setup of formamide and DNA size standard mixture**

| Component | Volume per sample |
|---|---|
| Hi-Di Formamide | 12 $\mu$l |
| DNA Size Standard 550 (BTO) | 0.5 $\mu$l |

2. **Aliquot 12 $\mu$l of the mixture to a tube for each sample to be analyzed.**
3. **Add 1 $\mu$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, 8 or 24 samples must be pipetted onto the plate of multi-capillary analyzers. If fewer samples are analyzed, the empty positions must be filled with 12 $\mu$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 1 injection

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 a run**

If you are using the Investigator Argus X-12 Kit for the first time on an Applied Biosystems 3500 Genetic Analyzer, you will first need to setup 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.

1. **To set up the Instrument Protocol, go to "Library" and select "Analyze", followed by "Instrument Protocols" and click "Create".**
   **Note**: Modify the Run Module Default settings from "HID36_POP4" as shown in Table 39.

2. **The parameters in Table 39 must be entered or selected.**

**Table 39. Instrument Protocol parameters for Applied Biosystems 3500/3500xL 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 |
| Oven Temperature (°C) | Default |
| Run Voltage (kV) | Default |
| PreRun Voltage (kV) | Default |
| Injection Voltage (kV) | 3.0 |
| Run Time (s) | 1300 |
| PreRun Time (s) | Default |
| Injection Time (s) | 8.0* |
| Data Delay (s) | Default |
| Advanced Options | Default |

\* Deviating from the standard settings, the injection time may range between 1 and 20 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 20 s may be necessary.

3. **Click "Save" to confirm the changes.**
4. **To set up the Size Standard, go to "Library", select "Analyze", followed by "Size Standards", and click "Create".**
5. **The parameters in Table 40 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.

**Table 40. Size standard parameters**

| Parameter | Setting |
|---|---|
| Size Standard | e.g., SST-BTO_60-550 |
| Dye Color | Orange |

6. Click "Save" to confirm the changes.
7. To set up the QC Protocol, go to "Library" and select "Analyze", followed by "QC Protocols", and click "Create".
8. The parameters in Table 41 must be entered or selected.

**Table 41. QC Protocol parameters**

| Parameter | Setting |
|---|---|
| Protocol Name | e.g., BTO_550 |
| Size Standard | SST-BTO_60-550 (from step 4) |
| Sizecaller | SizeCaller v1.1.0 |

9. 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 44. All other settings should remain as "Default".

10. Click "Save" to confirm the changes.
11. To set up an Assay, go to "Library" and select "Manage", followed by "Assays", and click "Create".
12. To analyze Investigator Argus X-12 fragments, the parameters in Table 42 must be selected.

Table 42. Assay parameters

| Parameter | Setting |
|---|---|
| Assay Name | e.g., Investigator Argus X-12 |
| Color | Default |
| Application Type | HID |
| Instrument Protocol | e.g., Investigator Argus X-12 (from step 1) |
| QC Protocols | e.g., BTO_550 (from step 7) |

13. Click "Save" to confirm the changes.

Starting the run

1. In the Dashboard, click "Create New Plate".
2. Go to "Define Plate Properties" and select "Plate Details". Select or enter the parameters in Table 43.

Table 43. Plate properties

| Property | Setting |
|---|---|
| Name | e.g., Investigator Argus X-12 |
| Number of Wells | 96 |
| Plate Type | HID |
| Capillary Length | 36 cm |
| Polymer | POP4 |

3. Click "Assign Plate Contents" to confirm 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. Choose the correct Assay for the analysis. If you followed the steps under "Setting up the Run", this would be Investigator Argus X-12 from step 11. All named wells on the plate must have an assigned assay.
6. Select the wells for which to specify an assay. Check the box next to the assay name to assign it to the selected wells.

7. (Optional) Repeat for file name conventions and results group.

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 44 lists the recommended analysis parameters in the worksheet Peak Detector.

**Table 44. Recommended settings for the Applied Biosystems 3500/3500xL**

| Parameter | Settings |
|---|---|
| Peak Detection Algorithm | Advanced |
| Ranges | Analysis: Partial Range <br> Start Point: 1000; Stop Point: 20,000 <br> Sizing: All Sizes |
| Smoothing and Baselining | Smoothing: Light <br> Baseline Window: 51 pts |
| Size Calling Method | Local Southern Method |
| Peak Detection | Peak Amplitude Thresholds <br> B:*  Y:* <br> G:*  R:* <br> O:* <br> Min. Peak Half Width: 2 pts <br> Polynomial Degree: 3 <br> Peak Window Size: 11 pts[†] <br> Slope Thresholds: 0.0 |

\* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneMapper *ID-X* Software version 1.2. 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.

# Protocol: Analysis

For general instructions on automatic sample analysis, refer to the appropriate User and/or Workflow Guides for Investigator IDproof Software, Investigator IDproof Mixture Software, GeneScan, 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.
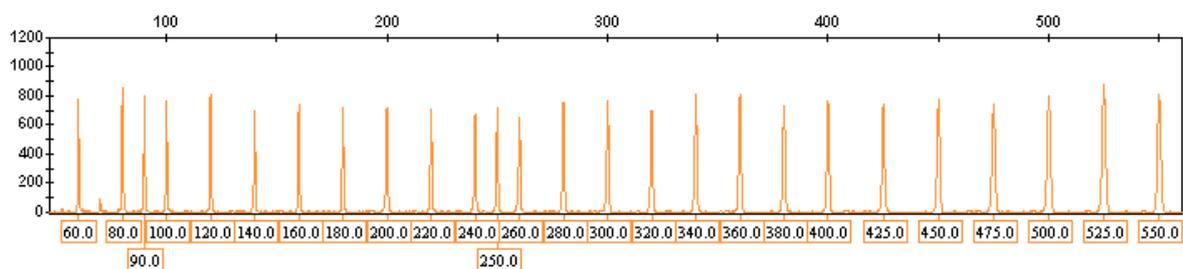


**Figure 2. Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp.**

# Analysis software

Allele allocation should be carried out with suitable analysis software, e.g., QIAGEN Investigator IDproof or IDproof Mixture Software, or Genotyper, GeneMapper *ID*, or GeneMapper *ID-X* Software in combination with the Investigator Template Files available as a download from www.qiagen.com or on a CD-ROM (cat. no. 389900), see Table 45 and Table 46.

The recommended Investigator Template File for Genotyper Software is the Argus X-12.

**Table 45. Recommended Investigator Template Files for GeneMapper *ID***

| File type | File name |
|---|---|
| Panels | Argus_X12_Panels |
| BinSets | Argus_X12_Bins |
| Size standard | SST-BTO_60–500bp |
| Analysis Method | Analysis_HID_310 |
| | Analysis_HID_3130 |
| | Analysis_HID_310_50rfu |
| | Analysis_HID_3130_50rfu |
| Plot Settings | Plots_5dyes |
| Table Settings | Table for 2 alleles |
| | Table for 10 alleles |

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

**Table 46. Recommended Investigator Template Files for GeneMapper *ID-X***

| File type | File name |
|---|---|
| Panels | Argus_X12_Panels_x |
| BinSets | Argus_X12_Bins_x |
| Stutter | Argus_X12_Stutter_x |
| Size standard | SST-BTO_60–500bp |
| Analysis Method | Analysis_HID_310 |
| | Analysis_HID_3130 |
| | Analysis_HID_310_50rfu |
| | Analysis_HID_3130_50rfu |
| | Analysis_HID_3500 |
| Plot Settings | Plots_5dyes |
| Table Settings | 310 Data Analysis/31xx Data Analysis |

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

# Controls

The alleles listed in Table 47 represent the Control DNA 9947A (included in the Investigator Argus X-12 Kit) and DNA from other commercially available standard cell lines.

**Table 47. Allele assignment of the Investigator Argus X-12 Kit**

| Locus | CCR 9947A | CCR 9948 | CCR 3657 | ATCC K-562 |
|---|---|---|---|---|
| Amelogenin | X/X | X / Y | X / Y | X/X |
| DXS7132 | 12/12 | 13 | 12 | 13/13 |
| DXS7423 | 14/15 | 14 | 13 | 17/17 |
| DXS8378 | 10/11 | 11 | 12 | 10/10 |
| DXS10074 | 16/19 | 18 | 7 | 17/17 |
| DXS10079 | 20/23 | 19 | 19 | 17/17 |
| DXS10101 | 30/31 | 32 | 29.2 | 31/31 |
| DXS10103 | 17/17 | 18 | 20 | 17/17 |
| DXS10134 | 35 /36 | 34 | 34 | 32/32 |
| DXS10135 | 21.1/27 | 22 | 25 | 27/27 |
| DXS10146 | 28/28 | 29 | 27 | 29 / 29 |
| DXS10148 | 22.1/23.1 | 23 | 23.1 | 23.1/23.1 |
| HPRTB | 14/14 | 14 | 13 | 13/13 |

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 and ATCC up to the standard of Szibor et al. (4).

## Lengths of fragments and alleles

Table 48 shows the alleles of the allelic ladder. All analyses have been performed using an Applied Biosystems 3500 Genetic Analyzer with POP-4 polymer (Figure 3 and Figure 4, page 55). 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: 75–405 bp

■ Vertical: Depending on signal intensity

**Table 48. Allelic ladder fragments included in the Investigator Argus X-12 Kit**

| Locus | Dye label | Repeat numbers of allelic ladder |
|---|---|---|
| Amelogenin | 6-FAM | X, Y |
| DXS10103 | 6-FAM | 15, 16,17, 18, 19, 20, 21 |
| DXS8378 | 6-FAM | 9, 10, 11, 12, 13, 14, 15 |
| DXS7132 | 6-FAM | 10, 11, 12, 13, 14, 15, 16, 17 |
| 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 |
| DXS10101 | BTG | 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 |
| DXS10135 | BTG | 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 | 13, 14, 15, 16, 17, 18 |
| DXS10146 | BTY | 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 |
| DXS10079 | BTY | 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25 |
| HPRTB | BTR | 9, 10, 11, 12, 13, 14, 15, 16, 17 |
| DXS10148 | BTR | 13.3, 18, 19, 20, 21, 22.1, 23.1, 24.1, 25.1, 26.1, 27.1, 28.1, 29.1, 30.1, 31, 38.1 |

For information about known microvariants not contained in the Investigator Argus X-12 Allelic Ladder, see the National Institute of Standards and Technology (NIST) web site (www.cstl.nist.gov/biotech/strbase/).
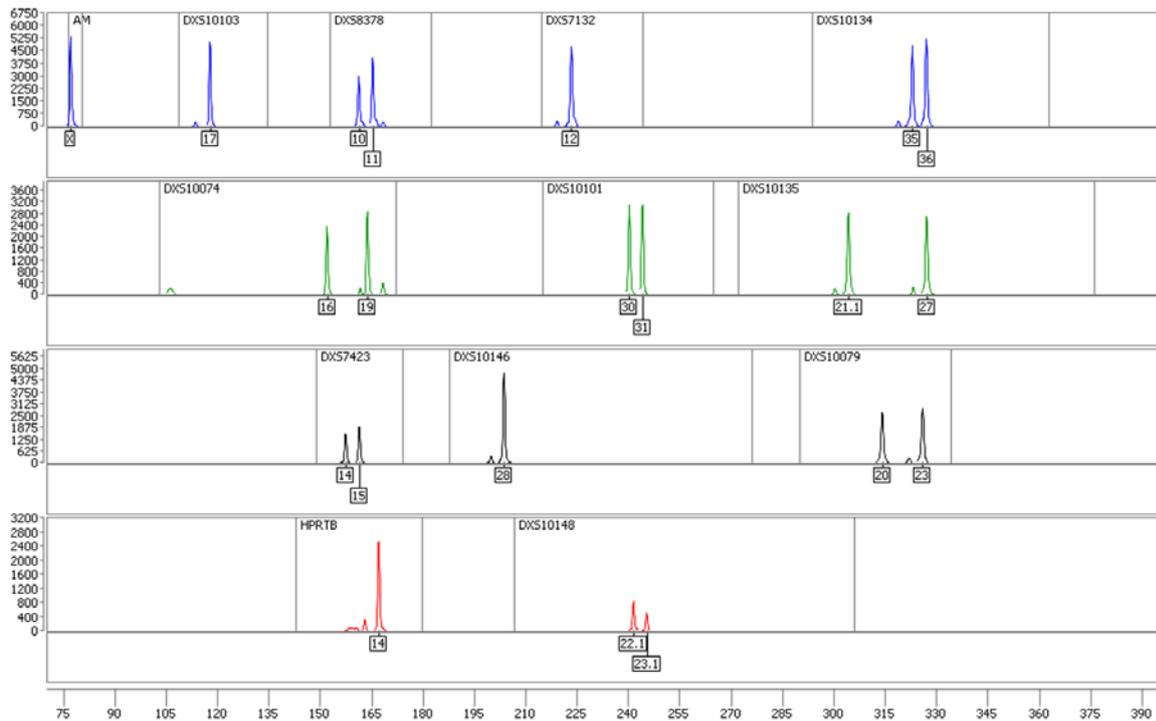
**Figure 3. Electropherogram of the Investigator Argus X-12 Kit using 500 pg Control DNA 9947A**. Analysis was performed on an Applied Biosystems 3500 Genetic Analyzer. Allele assignment was performed using Investigator IDproof Software.
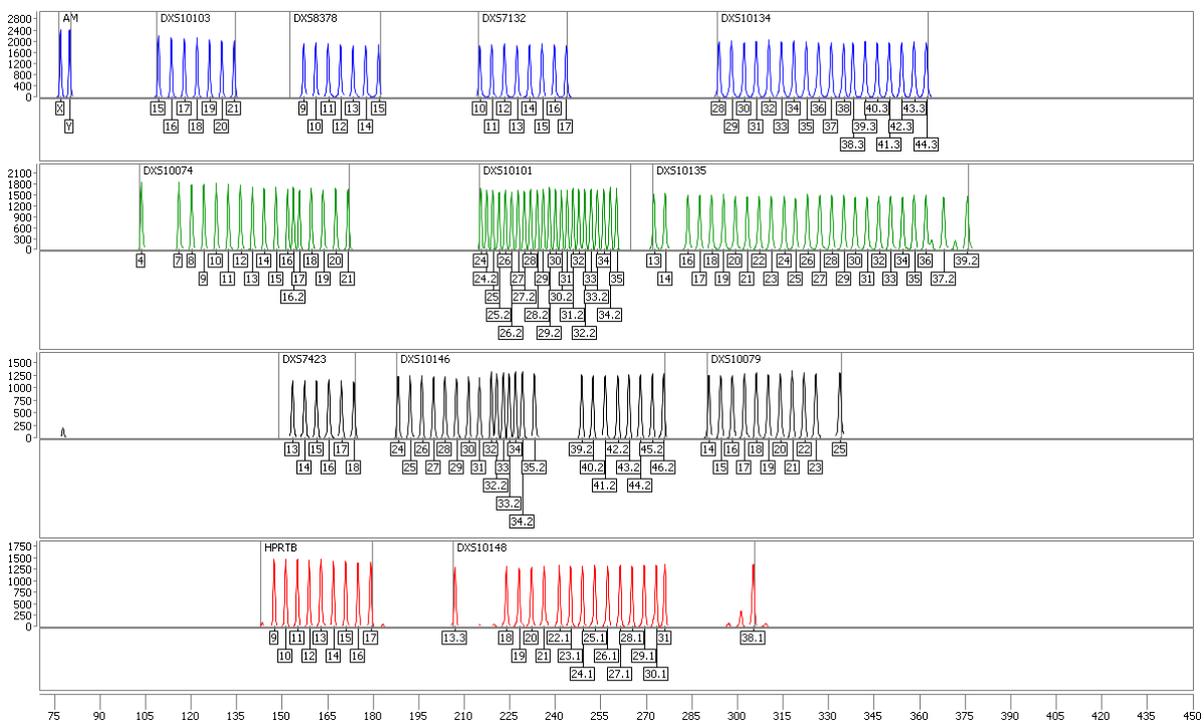


**Figure 4. Electropherogram of the allelic ladder Argus X-12 analyzed on an Applied Biosystems 3500 Genetic Analyzer**. Allele assignment was performed using Investigator IDproof Software.

# 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 control.
4. Check the negative control.
5. 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", page 60), or if an incorrect matrix was applied. They appear at positions of specific peaks in other color channels, typically with lower signal intensities. Peak heights should not exceed 3000 RFU in order to prevent pull-up peaks.

### Stutter peaks

The occurrence of stutter peaks depends on the sequence of the repeat structure and the number of alleles. n –4 peaks are caused by a loss of a repeat unit during amplification of tetranucleotide STR motifs, caused by slippage effects of the *Taq* DNA polymerase. These peaks should be interpreted using the Investigator Template Files for Genotyper, GeneMapper *ID*, and GeneMapper *ID-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 X-12 Kit are designed to minimize these artifacts. Artifact formation is further reduced by the final extension step of the PCR protocol at 68°C for 60 minutes. 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 multi-capillary instruments, so that shoulder peaks or split peaks occur. If shoulder or split peaks appear, we recommend injecting the sample again.

# 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, e.g., 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 $MEC_{Krüger}$, which was developed for either autosomal marker or for trios. With exception of deficiency cases, $MEC_{Krüger}$ 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 $MEC_{Kishida}$ for X-chromosomal markers as regards trios including a daughter. In comparison with $MEC_{Krüger}$, $MEC_{Kishida}$ is more complex highlighting the fact that in trios involving a daughter X-STRs are more efficient then 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. $MEC_{Desmarais}$ is equivalent to $MEC_{Kishida}$ whereas $MEC_{Desmarais\ Duo}$ 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).

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

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

$MEC_{Krüger} = \Sigma_i\, f_i^3\,(1 - f_i)^2 + \Sigma_i\, f_i\,(1 - f_i)^3 + \Sigma_{i<j}\, f_i f_j\,(f_i + f_j)\,(1 - f_i - f_j)^2$

$MEC_{Kishida} = \Sigma_i\, f_i^3\,(1 - f_i) + \Sigma_i\, f_i\,(1 - f_i)^2 + \Sigma_{i<j}\, f_i f_j\,(f_i + f_j)\,(1 - f_i - f_j)$

$MEC_{Desmarais} = 1 - \Sigma_i\, f_i^2 + \Sigma_i\, f_i^4 - (\Sigma_{i<j}\, f_i^2)^2$

$MEC_{Desmarais\ Duo} = 1 - 2\Sigma_i\, f_i^2 + \Sigma_i\, f_i^3$

$PD_{female} = 1 - 2(\Sigma_i\, f_i^2)^2 + \Sigma_i\, f_i^4$

$PD_{male} = 1 - \Sigma_i\, f_i^2$

## 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 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 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.

# 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 protocols in this handbook or sample and assay technologies (for contact information, see back cover or 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 (see Ordering Information). |
| **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 (>4000 RFU/>5000 RFU*), 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.

e) Use of 32-cycle PCR program for small amounts of DNA

Too small amounts of DNA may result in allelic dropouts and imbalances of the peaks. Furthermore, unspecific amplification products may appear. By increasing the number of cycles, there is a risk of cross-contamination due to impurities.

### 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.

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

## Comments and suggestions

| | |
|---|---|
| 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. |
| 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, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

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**General references**

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# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| Investigator Argus X-12 Kit (25) | Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water | 383213 |
| Investigator Argus X-12 Kit (100) | Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water | 383215 |
| **Related products** | | |
| **Investigator Human Identification PCR Kits** | | |
| Investigator Argus Y-12 QS Kit (100)* | Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water | 383615 |
| Investigator ESSplex Plus Kit (100)* | Primer mix, Fast Reaction Mix including HotStarTaq® *Plus* DNA Polymerase, Control DNA, allelic ladder ESSplex Plus, DNA size standard 550 (BTO), and nuclease-free water | 381535 |
| Investigator ESSplex SE Plus Kit (100)* | Primer mix, Fast Reaction Mix including HotStarTaq *Plus* DNA Polymerase, Control DNA, allelic ladder ESSplex SE Plus, DNA size standard 550 (BTO), and nuclease-free water | 381545 |
| Investigator IDplex Plus Kit (100)* | Primer mix, Fast Reaction Mix including HotStarTaq *Plus* DNA Polymerase, Control DNA, allelic ladder IDplex Plus, DNA size standard 550 (BTO), and nuclease-free water | 381625 |
| Investigator HDplex Kit (100) | Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water | 381215 |

* Larger kit sizes available; please inquire.

| Product | Contents | Cat. no. |
|---------|----------|----------|
| Investigator Triplex AFS QS Kit (400) | Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water | 380317 |
| Investigator Triplex DSF Kit (400) | Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water | 380327 |
| Investigator DIPplex Kit (100)* | Primer mix, reaction mix, DNA Polymerase, Control DNA, allelic ladder, DNA size standard, and nuclease-free water | 384015 |
| **Investigator Quantification Kits** | | |
| Investigator Quantiplex Kit (200) | Primer mix IC FQ, reaction mix FQ, Control DNA Z1, dilution buffer | 387016 |
| Investigator Quantiplex HYres Kit | Primer mix IC YQ, reaction mix FQ, Control DNA Z1, 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 |
| Multi Taq2 DNA Polymerase (100) | 100 Units Multi Taq2 DNA Polymerase | 386315 |

* Larger kit sizes available; please inquire.

| Product | Contents | Cat. no. |
|---|---|---|
| **Analysis software** | | |
| Investigator IDproof Software | Software package on CD including installation files for the Desktop, Server and Client versions of IDproof Software | 9020775 |
| Investigator IDproof Demo Key | Free use of the IDproof Desktop version of the software for 30 days after installation | 389001 |
| Investigator IDproof Single Key | Allows the unlimited use of the Desktop version of the software; to be installed on a single workstation with a local database | 389002 |
| Investigator IDproof Server Key | Allows for setup of a server that maintains the database and various workstations to access that database. Must be purchased in conjunction with the Client Key | 389003 |
| Investigator IDproof Client Key | Must be purchased in conjunction with the Server Key | 389004 |
| Investigator IDproof Mixture Software | Software package on CD including installation files for the Desktop, Server and Client versions of IDproof Mixture Software | 9020777 |
| Investigator IDproof Mixture Demo Key | Free use of the IDproof Mixture Desktop version of the software for 30 days after installation | 389401 |
| Investigator IDproof Mixture Single Key | Allows the unlimited use of the Desktop version of the software; to be installed on a single workstation with a local database | 389402 |
| Investigator IDproof Mixture Server Key | Allows for setup of a server that maintains the database and various workstations to access that database. Must be purchased in conjunction with the Client Key | 389403 |

| Product | Contents | Cat. no. |
|---|---|---|
| Investigator IDproof Mixture Client Key | Must be purchased in conjunction with the Server Key | 389404 |
| Investigator Template Files | Template files for Investigator Human Identification PCR Kits for use with GeneMapper *ID*, GeneMapper *ID-X*, and Genotyper Software, plus DIPSorter freeware (CD-ROM) | 389900 |
| **DNA extraction and purification** | | |
| QIAamp® DNA Investigator Kit (50) | 50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml) | 56504 |
| 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, Carrier RNA | 952034 |
| MinElute PCR Purification Kit (50)* | 50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml) | 28004 |

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.

* Larger kit sizes available; please inquire.

**Notes**

**Notes**

**Limited License Agreement for the Investigator Argus X-12 Kit**

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