April 2010

Investigator Decaplex SE Handbook

For multiplex amplification of the ten loci from the SGM Plus standard, plus SE33 and Amelogenin

For molecular biology applications in forensic, human identity, and paternity testing

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Contents

Kit Contents

Investigator Decaplex SE Kit	**(100)**	**(400)**
Catalog no.	**381025**	**381027**
Number of preps	**100**	**400**
Reaction Mix A	500 µl	2 x 1000 µl
Primer Mix Decaplex SE	250 µl	4 x 250 µl
Multi Taq2 DNA Polymerase	100 U	400 U
Control DNA XY13	10 µl	10 µl
DNA size standard 550 (BTO)	50 µl	200 µl
Allelic ladder Decaplex SE	25 µl	4 x 25 µl
Nuclease-free water	2 x 1.9 ml	5 x 1.9 ml
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Storage

All components of the Investigator Decaplex SE 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.

Product Use Limitations

The Investigator Decaplex SE Kit is intended for molecular biology applications in forensic, human identity, and paternity testing. This product is neither intended for the diagnosis, prevention or treatment of a disease, nor has it been validated for such use.

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.

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the Investigator Decaplex SE Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Investigator Decaplex SE Kits is tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

CAUTION: Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Material Safety Data Sheets (MSDSs) or OSHA*, ACGIH†, or COSHH‡ documents. Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

The Investigator Decaplex SE Kit serves as a multiplex application for special questions in forensic case work. The ten STR markers known as "second generation multiplex plus" loci plus SE33 and Amelogenin are amplified simultaneously in a single PCR. The primers are fluorescence-labeled with 6-FAM (Amelogenin, TH01, D3S1358, vWA, D21S11), BTG (D16S539, D19S433, D8S1179, D2S1338), BTY (D18S51, FGA), or BTR (SE33).

The Investigator Decaplex SE Kit was developed specifically for fast and reliable generation of DNA profiles from blood, buccal swabs and forensic stains. Furthermore, the kit is equipped with a primer-set which covers all known mutations in the primer binding site of the SE33 locus, according to Hering et al. (2002), Heinrich et al. (2004).

The detection limit of the Investigator Decaplex SE Kit is 100 pg genomic DNA. The optimal range under standard conditions is 0.2-0.5 ng DNA. However, internal validations demonstrated reliable results with <0.1 ng DNA.

The Investigator Decaplex SE Kit was validated and evaluated using the GeneAmp® 9700 thermal cycler (in standard mode), ABI PRISM® 310 Genetic Analyzer, and ABI PRISM 3100/3130 Genetic Analyzer.

Tables 1 and 2 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 (Bär et al., 1997). 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 1. Locus-specific information of the Investigator Decaplex SE Kit.

Locus	GenBank® accession number	Repeat motif of the reference allele	Reference allele	Allele range
Amelogenin X	M55418			
Amelogenin Y	M55419			
D2S1338	G08202	$[TGCC]_6[TTCC]_{11}$	17	10-31
D3S1358	11449919	TCTA $[TCTG]_2$ $[TCTA]_{15}$	18	8-26
D8S1179	G08710	$[TCTA]_{12}$	12	6-21.2
D16S539	G07925	$[GATA]_{11}$	11	4-19
D18S51	L18333	$[AGAA]_{13}$	13	5.3-42
D19S433	G08036	AAGG [AAAG] AAGG TAGG $[AAGG]_{11}$	15	5.2-20
D21S11	AP000433	$[TCTA]_4$ $[TCTG]_6$ $[TCTA]_3$ TA $[TCTA]_3$ TCA $[TCTA]_2$ TCCATA $[TCTA]_{11}$	29	12, 20-46
FGA (FIBRA)	M64982	$[TTTC]_3$ TTTTTTCT $[CTTT]_{13}$ CTCC $[TTCC]_2$	21	12.2-51.2
SE33 (ACTBP2)	NG000840	$[AAAG]_9$ AA $[AAAG]_{16}$	25.2	3-50
TH01 (TC11)	D00269	$[TCAT]_9$	9	3-14
vWA	M25858	TCTA $[TCTG]_4$ $[TCTA]_{13}$	18	10-26

Table 2. Chromosomal mapping of the Investigator Decaplex SE Kit.

Locus	Chromosomal mapping
Amelogenin X	Xp22.1-22.3
Amelogenin Y	Yp11.2
D2S1338	2q35
D3S1358	3p25.3
D8S1179	8q23.1-23.2
D16S539	16q24.1
D18S51	18q21.3
D19S433	19q12
D21S11	21q21.1
FGA (FIBRA)	4q28.2
SE33	6q14.2
TH01 (TC11)	11p15.5
vWA	12p13.31

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 material safety data sheets (MSDSs), available from the product supplier.

- Hi-Di™ Formamide, 25 ml (Applied Biosystems, cat no. 4311320)
- Matrix Standard BT5 for either single-capillary or multi-capillary instruments (see ordering information)
- Pipets and pipet tips
- One of the following DNA analyzers:
  ABI PRISM 310 Genetic Analyzer
  ABI PRISM 3100-Avant/3100 Genetic Analyzer
  ABI PRISM 3130/3130x1 Genetic Analyzer
  ABI PRISM 3730 DNA Analyzer
- PCR thermocycler

Protocol: PCR amplification

Master mix preparation

The table below shows the volumes of all PCR reagents per 25 µl reaction volume, including a sample volume of 1.0 µl (template DNA). The number of reactions to be set up shall be determined taking into account positive and negative control reactions. Add one or two reactions to this number to compensate the pipetting error.

Component	Volume
Nuclease-free water	16.1 µl
Reaction mix A*	5.0 µl
Primer mix	2.5 µl
Multi Taq2 DNA Polymerase	0.4 µl
Volume of master mix	24.0 µl

* contains Mg2+, dNTPs, BSA

All components should be mixed (vortex) and centrifuged for about 10 s before preparing the master mix. The DNA volume applied to the assay depends on its concentration. A volume of up to 5 µl may be necessary for DNA trace templates. Larger volumes can be used successfully, however we do not recommend DNA volumes greater than 5 µl because potential PCR inhibitors in the sample may interfere with the process. Fill up the final reaction volume to 25 µl with nuclease-free water.

Generally, DNA templates shall be stored in nuclease-free water or in diluted TE buffer (10 mM Tris HCl, pH 8.0 and 1 mM EDTA), e.g. 0.1x TE buffer.

The primer mixes are adjusted for balanced peak heights at 30 PCR cycles and 0.35 ng Control DNA XY13 in a reaction volume of 25 µl. If more DNA template is introduced, higher peaks can be expected for small PCR fragments and relatively low peaks for large fragments. Reduce the amount of DNA template to correct this imbalance.

Positive control

For the positive amplification control, dilute the Control DNA XY13 to 0.35 ng in the appropriate volume. Instead of the template DNA pipette the diluted Control DNA into a reaction tube containing the PCR master mix.

Negative control

For the negative amplification control, pipette nuclease-free water instead of template DNA into a reaction tube containing the PCR master mix.

Template DNA

Sometimes, the measured value of the DNA concentration varies depending on the quantification method used, so that it may be necessary to adjust the optimal DNA amount.

PCR amplification parameter

Perform a "hot start" PCR in order to activate the Multi Taq2 DNA Polymerase and to prevent the formation of non-specific amplification products.

The number of cycles depends on the amount of DNA. 30 cycles are recommended for all samples. For critical stains (< 100 pg DNA), it is recommended to increase the number of PCR cycles to 32 cycles, i.e. from 25 to 27 in the second PCR step.

Standard method: Recommended for all DNA samples

Temperature	Time	
94°C	4 min (hot start to activate the Multi Taq2 DNA Polymerase)	
96°C	30 s	
62°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	∞	hold

Optional: Recommended for stains with small amounts of DNA

Temperature	Time	
94°C	4 min (hot start to activate the Multi Taq2 DNA Polymerase)	
96°C	30 s	
62°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	∞	hold

Too small amounts of DNA may result in allelic dropouts and imbalances of the peaks. Furthermore, unspecific amplification products could appear. With increasing numbers of cycles, there is the risk of cross contamination caused by minimal amounts of impurities.

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 shall be used for combined application of the five fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO (the matrix standard will be called BT5 hereinafter).

Material

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

Matrix generation

Prior to conducting DNA fragment size analysis with the filter set G5, a matrix with the five fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO must be generated.

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

Five electrophoresis runs shall be conducted, one for each fluorescent label, 6-FAM, BTG, BTY, BTR, and BTO, under the same conditions as for the samples and allelic ladders of the Investigator Decaplex SE Kit to generate suitable matrix files.

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

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

- Create a Sample Sheet and enter sample designation

Injection list for matrix generation

Parameter	Set up
Module File	GS STR POP-4 (1 ml) G5
Matrix File	NONE
Size Standard*	NONE
Injection [s]	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]	24

* prepare matrix standards always without DNA Size Standard (BTO)

Analysis of the matrix samples

- Run the GeneScan software
- File → New → Project (open folder of current run) → Add Sample Files
- Select a matrix sample in the Sample File column
- Sample → Raw Data

- Check the matrix samples regarding a flat baseline. As shown in the figure below, there should be at least five peaks with peak heights about 1000-4000 (Y-axis) for each matrix sample (optimal range: 2000-4000)

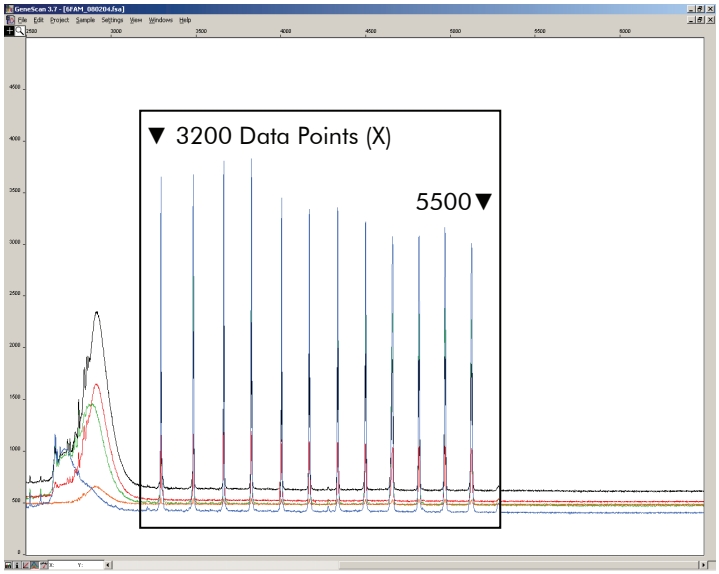

Figure 1. Electropherogram with raw data of the matrix standard 6-FAM

- Select analysis range with flat baseline and re-inject the matrix sample if necessary
- Note down start and end value (data points) of the analysis range, e.g. start value 3200, end value 5500
- Calculate the difference, e.g. 5500-3200 = 2300 data points

Generation of a new matrix

- File → New → Matrix

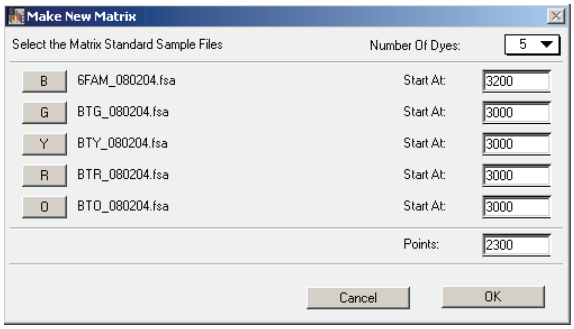

Figure 2. Matrix sample selection

- Import matrix samples for all dyes (B, G, Y, R, O)
- Enter a Start At value, e.g. 3200
- Enter the calculated difference under Points, e.g. 2300
- Click on OK to calculate the new matrix

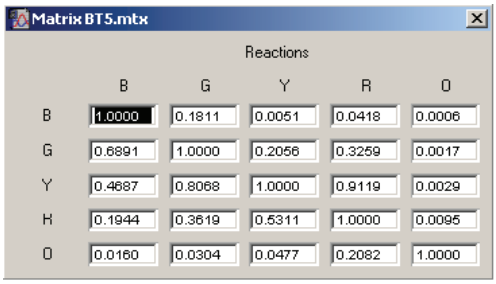

Figure 3. New matrix BT5

- Save the matrix in the matrix folder: File → Save as, e.g. Matrix BT5

Matrix check

Check the new matrix with current samples.
- File → New → Project (open folder of the respective run) → Add Sample Files
- Select sample(s) in the Sample File column
- Sample → Install New Matrix (open matrix folder and select new matrix)
- Re-analyze your samples

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

Sample preparation

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

prepare 12 µl of the mix (formamide + DNA size standard) for all samples

add 1 µl PCR product (diluted if necessary) or allelic ladder

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

Signal intensities

Options to increase the signal intensity:
- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

Setting up the GeneScan software

- Create a Sample Sheet and enter sample designation

Injection list

Component	Set up
Module File	GS STR POP-4 (1 ml) G5
Matrix File	e.g. Matrix BT5
Size Standard	e.g. SST-BTO_60-475bp
Injection [s]*	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°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.

† Depending on the analysis conditions, the run time for Investigator Decaplex SE was modified in order to be able to analyze fragments with lengths of up to 475bp.

Analysis parameter

The recommended analysis parameters are:

Analysis Range	Start: 2000 Stop: 10000
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal 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 as high as the background noise of the baseline.

† Point alleles (i.e. alleles with at least 1 bp difference to the next integer allele) may occasionally not be distinguished. For improved peak detection, minimize the Peak Window Size further.

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 named ABI 3100-Avant, and the system with 16 capillaries is named ABI 3100.

The virtual filter set G5 shall be used for combined application of the five fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO (the matrix standard will be called BT5 hereinafter).

Material

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

Spectral calibration / matrix generation

Proper spectral calibration is critical to evaluate multicolour systems with the ABI PRISM 3100-Avant/3100 Genetic Analyzer and shall be done prior to 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

Setting up the spectral calibration standards

Example for 4 capillaries/ABI 3100-Avant

Component	**Volume**
Hi-Di™ Formamide	60.0 µl
Matrix standard BT5	5.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position A1-D1
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Example for 16 capillaries/ABI 3100

Component	Volume
Hi-Di™ Formamide	204.0 µl
Matrix standard BT5	17.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position A1-H1 and A2-H2
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Performing a spectral calibration run

First of all, 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

To change settings in the parameter file go to the following path:
D:\AppliedBio\Support Files\Data Collection Support Files\CalibrationData\Spectral Calibration\ParamFiles

- Select MtxStd{Genescan_SetG5} to open the PAR-file
- Change Condition Bounds Range to [1.0; 20.0]. If calibration was not successful, also change Sensitivity to 0.1 and Quality to 0.8 in a second step
- Select File → Save As to save the parameter file under a new name, e.g. MtxStd{Genescan_SetG5_BT5}.par

Always use this parameter file for spectral calibration runs using QIAGEN matrix standards BT5.

Plate Editor for spectral calibration (I)

- Place the 96-well plate on the autosampler tray
- Run the ABI PRISM 3100 Data Collection software
- In Plate View click New to open the Plate Editor dialog box
- Enter a name of the plate
- Select Spectral Calibration
- Select 96-Well as plate type and click on Finish

Plate Editor for spectral calibration (II)

Parameter	**Set up**
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)

- Click into the column header to select the entire column, select Edit → Fill Down to apply the information of the selected samples and confirm with OK
- Link your reaction plate on the autosampler tray with the created plate ID and start run
- On completion of the run check in the Spectral Calibration Result dialog box if all capillaries have successfully passed calibration (label A). If individual capillaries are labeled X, refer to *ABI PRISM Genetic Analyzer User's Manual*.
- Click on OK to confirm completion of the run

Matrix check

- Select Tools → Display Spectral Calibration → Dye Set → G5 to review the spectral calibration profile for each capillary
- 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 previously determined range
- Check the matrix samples for a flat baseline. There should be five peaks with peak heights of about 1000-5000 (Y-axis) in each matrix sample (optimal range: 2000-4000)
- Check the new matrix with your current samples. There should be no pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix
- If calibration was not successful, try to change the Sensitivity and Quality values in the parameter file as described above
- If all capillaries have passed the calibration, the last calibration file for Dye Set G5 must be activated manually under Tools → Set Active Spectral Calibration. Rename the calibration file under Set Matrix Name (e.g. BT5_Date of calibration)

Sample preparation

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

Prepare 12 µl of the mix (formamide + DNA size standard) for all samples
Add 1 µl PCR product (diluted if necessary) or allelic ladder

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

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

To ensure a reliable allelic assignment on 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. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer.

Signal intensities

Options to increase the signal intensity:
- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

Settings up the GeneScan software

Edit the default run module in Dye Set G5 once for the first run.
- Select Module Editor to open the dialog box
- Select the appropriate Run Module as template from the GeneScan table
- Modify the Injection Voltage to 3 kV and the Injection Time to 10 s

Run Module 3kV_10s_500bp

Parameter	**Set up**
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]†	24

* 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.

† Depending on the analysis conditions the run time for the Investigator Decaplex SE Kit was modified in order to be able to analyze fragments with lengths of up to 475 bp.

- Click on Save As, enter the name of the new module (e.g. 3kV_10s_500bp) and confirm with OK
- Click on Close to exit the Run Module Editor

Starting the run

- Place the prepared 96-well plate on the autosampler tray
- Run the ABI PRISM 3100 Data Collection software
- In Plate View click on New to open the Plate Editor dialog box
- Enter a name of the plate
- Select GeneScan
- Select 96-Well as plate type and click on Finish

Plate Editor

Parameter	**Set up**
Sample Name	Enter name for the matrix samples
Dyes	O
Colour Info	Ladder or sample
Project Name	e.g. 3100_Project1
Dye Set	G5
Run Module*	3kV_10s_500bp
Analysis Module 1	DefaultAnalysis.gsp

* parameter see above

- Complete the table in the Plate Editor and click on OK
- Click into the column header to select the entire column and select Edit → Fill Down to apply the information of the selected samples
- Link your reaction plate on the autosampler tray with the created plate ID and start the run
- On completion of the run, view data as Color Data in Array View of the 3100 Data Collection software or as Analyzed Sample Files under D:/AppliedBio/3100/DataExtractor/ExtractRuns

Analysis parameter

The recommended analysis parameters are:

Analysis Range	Start: 2000 Stop: 10000
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal 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 as high as the background noise of the baseline.

† Point alleles (i.e. alleles with at least 1 bp difference to the next integer allele) may occasionally not be distinguished. For improved peak detection, minimize the Peak Window Size further.

Protocol: Electrophoresis using the ABI PRISM 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 *ABI PRISM 3130/3130xl Genetic Analyzers Getting Started Guide*.

The system with 4 capillaries is named ABI 3130, and the system with 16 capillaries is named ABI 3130xl.

The virtual filter set Any5Dye shall be used for the combined application of the five fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO (the matrix standard will be called BT5 hereinafter).

Material

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

Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the five 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 comprises the following steps:
- Preparation 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

Setting up the spectral calibration standards

Example for 4 capillaries/ABI 3130

Component	**Volume**
Hi-Di™ Formamide	60.0 µl
Matrix standard BT5	5.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position A1-D1
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Example for 16 capillaries/ABI 3130xl

Component	Volume
Hi-Di™ Formamide	204.0 µl
Matrix standard DS-30	17.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position A1-H1 and A2-H2
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Performing spectral calibration run

- Place the 96-well plate on the autosampler tray
- In the Protocol Manager of the Data Collection software click New the window Instrument Protocol to open the Protocol Editor dialog box

Instrument Protocol for spectral calibration

Protocol Editor	Set up
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

- Select OK to complete the Protocol Editor dialog box
- In the Plate Manager of the Data Collection software click New to open the New Plate Dialog box

Plate Editor for spectral calibration (I)

New Plate Dialog	Set up
Name	e.g. Spectral_BT5_date
Application	Spectral Calibration
Plate Type	96-Well
Owner Name / Operator Name	…

- Click on OK. A new table in the Plate Editor opens automatically

Plate Editor for spectral calibration (II)

Parameter	Set up
Sample Name	Type name for the matrix samples
Priority	e.g. 100
Instrument Protocol 1	Spectral36_POP4_BT5 (setting described before)

- Click into the column header to select the entire column, select Edit → Fill Down to apply the information to all selected samples, and click on OK
- In the Run Scheduler click on Find All, select Link to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run

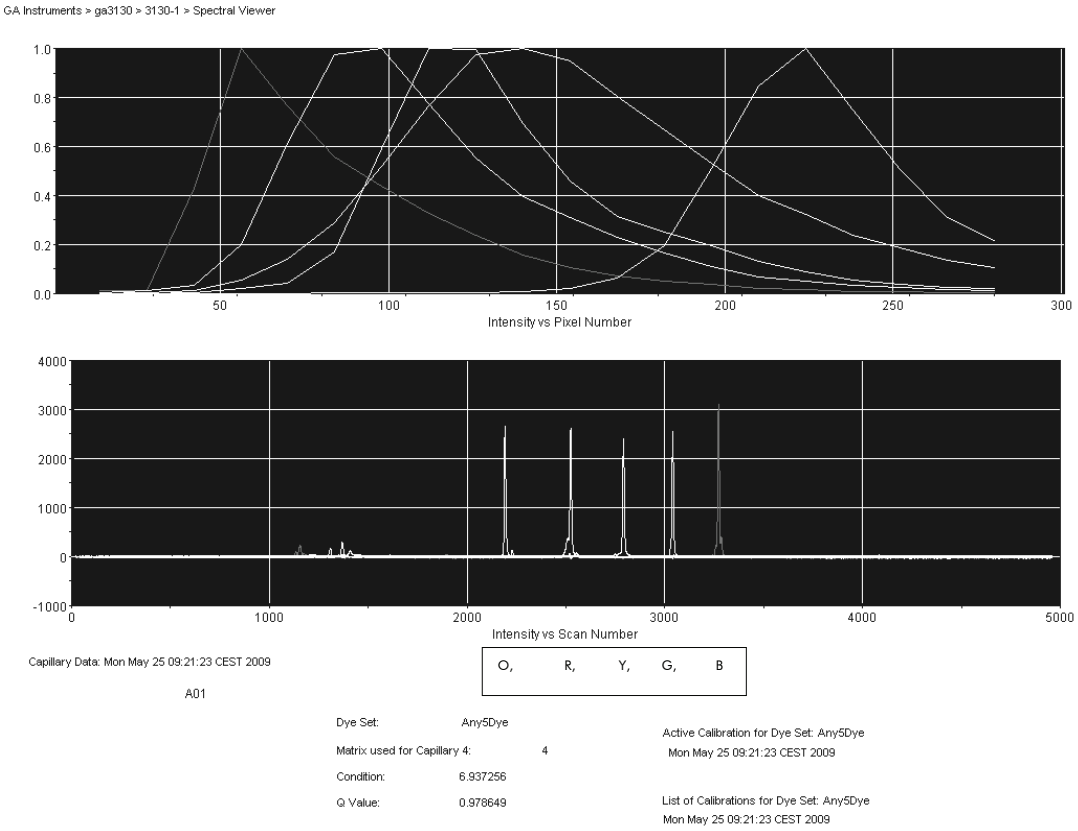

Figure 4. Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3130

Matrix check

- 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.
- Check the matrix samples for a flat baseline. As shown in the figure above, there should be four peaks with peak heights of about 1000-5000 (Y-axis) in each matrix sample (optimal range: 2000-4000)

- Check the new matrix with your current samples. There should be no pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix
- If calibration was not successful, use the optimised values and repeat the calibration run
- 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) using the respective button

Sample preparation

Component	**Volume**
Hi-Di™ Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl
prepare 12 µl of the mix (formamide + DNA size standard) for all samples	
add 1 µl PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

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

To ensure a reliable allelic assignment on 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. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer.

Signal intensities

Options to increase the signal intensity:
- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

Setting up the GeneMapper ID software

Edit the Run Module as follows for the first run:
- In the Module Manager of the Data Collection software click on New to open the Run Module Editor dialog box

Run Module 3kV_10s_500bp

Run Modul Editor	**Set up**
Oven Temperature [°C]	*Default*
Poly Fill Volume	*Default*
Current Stability [µA]	*Default*
PreRun Voltage [kV]	*Default*
PreRun 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]†	1440

* 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.

† Depending on the analysis conditions the run time for Investigator Decaplex SE was modified in order to be able to analyze fragments with lengths of up to 475 bp.

- Click on Save As, enter the name of the new module (e.g. 3kV_10s_500bp) and confirm with OK
- Click on Close to exit the Run Module Editor

Starting the run

- Place the prepared 96-well plate on the autosampler tray
- In the Protocol Manager of the Data Collection software, click on New in the Instrument Protocol window to open the Protocol Editor dialog box

Instrument Protocol

Protocol Editor	**Set up**
Name	Run36_POP4_BT5_24min
Type	REGULAR
Run Module*	3kV_10s_500bp
Dye Set	Any5Dye

* parameter see above

- Click on OK to exit the Protocol Editor

Prior to each run, it is necessary to create a plate definition as follows:
- In the Plate Manager of the Data Collection software click on New to open the New Plate Dialog box

GeneMapper Plate Editor (I)

New Plate Dialog	**Set up**
Name	e.g. Plate_BT5_Date
Application	select GeneMapper Application
Plate Type	96-Well
Owner Name / Operator Name	…

- Click OK. A new table in the Plate Editor opens automatically

GeneMapper Plate Editor (II)

Parameter	**Set up**
Sample Name	Type name for the samples
Priority	e.g. 100 (Default)
Sample Type	Sample or Allelic Ladder
Size Standard	e.g. SST-BTO_60-475bp
Panel	e.g. Decaplex_SE_Panels_v0
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_24min (setting described before)

- Click into the column header to select the entire column, select Edit → Fill Down to apply the information to all selected samples and click on OK
- In the Run Scheduler, click on Find All, select Link to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run
- 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
- View data as 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 parameter / analysis method

The recommended settings in the worksheet Peak Detector are:

Peak Detection Algorithm	Advanced
Ranges	Analysis: Partial Range Start Pt: 2000; Stop Pt: 10000 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 Polynominal Degree: 3 Peak Window Size: 11 pts† Slope Thresholds: 0.0

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneMapper 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 as high as the background noise of the baseline.

† Point alleles (i.e. alleles with at least 1 bp difference to the next integer allele) may occasionally not be distinguished. For improved peak detection, minimize the Peak Window Size further.

Protocol: Analysis

For general instructions on automatic sample analysing, refer to the *GeneScan* or *GeneMapper ID Software User's Manual*.

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) shall thus 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.

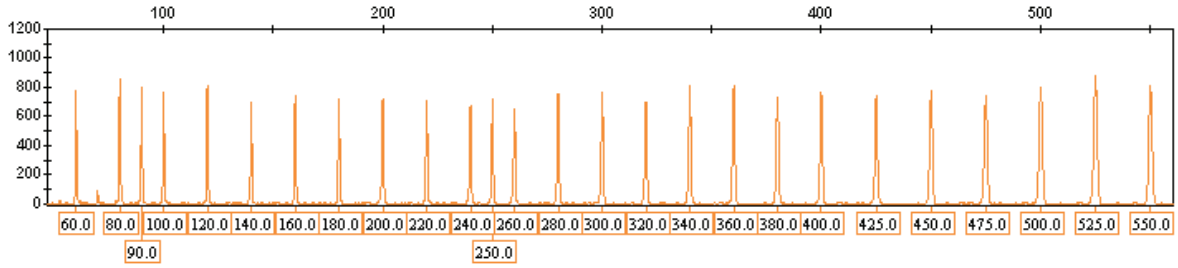

Figure 5. Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

Note: The basic template file for the DNA Size Standard 550 (BTO) has to be adjusted to 475 bp within the GeneMapper ID software. The new template could be saved as e.g. SST550_60-475bp and used for further analyzes.

Investigator Template Files

Allele allocation should be carried out with a suitable analysis software, e.g. GeneMapper ID or Genotyper® software in combination with the Investigator Decaplex SE Template Files from QIAGEN. Template files are available from our homepage or as CD-ROM on request.

Recommended Investigator Template Files for GeneMapper ID software are:

Panels	Decaplex_SE_Panels_v0 or higher versions
BinSets	Decaplex_SE_Bins_v0 or higher versions
Size Standard	SST-BTO_60-500bp (adjust up to 475bp, adjustment described before)
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 always have to be used whereas the other template files are optional.

Additional Investigator Template Files for GeneMapper ID-X Software:

Stutter* QIAGEN_Stutter_v3 or higher version

* When loading the above mentioned panels, the stutter settings will not be accepted. Thus, the stutter data has to be imported separately.

Recommended Investigator Template Files for Genotyper software are:

Decaplex_SE_v0 or higher versions

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

Controls

The Control DNA XY13 included in the Investigator Decaplex SE Kit and other commercially available DNA from standard cell lines represent the following alleles:

Table 3. Allele assignment of the Investigator Decaplex SE Kit.

Locus	Control DNA XY13	ATCC K-562	CCR 9947A	CCR 9948	CCR 3657
Amelogenin	X / Y	X / X	X / X	X / Y	X / Y
D2S1338	17 / 23	17 / 17	19 / 23	23 / 23	18 / 22
D3S1358	15 / 16	16 / 16	14 / 15	15 / 17	16 / 18
D8S1179	13 / 14	12 / 12	13 / 13	12 / 13	15 / 16
D16S539	11 / 12	11 / 12	11 / 12	11 / 11	13 / 13
D18S51	14 / 15	15 / 16	15 / 19	15 / 18	12 / 20
D19S433	13 / 14	14 / 14.2	14 / 15	13 / 14	13 / 14
D21S11	28.2 / 33.2	29 / 30 / 31	30 / 30	29 / 30	28 / 29
FGA	22 / 23	21 / 24	23 / 24	24 / 26	18 / 23
THO1	6 / 7	9.3 / 9.3	8 / 9.3	6 / 9.3	7 / 9.3
vWA	15 / 16	16 / 16	17 / 18	17 / 17	14 / 19
SE33	14 / 24.2	26.2 / 28.2	19 / 29.2	23.2 / 26.2	22.2 / 27.2

For further confirmation, the table above displays the alleles of the reference DNA purchased from ATCC (http://atcc.org/Produtcs/PurifiedDNA.cfm#celllines) as well as three reference DNA purchased from Coriell Cell Repositories (CCR; http://locus.umdnj.edu/nigms/) that is up to standard of Szibor et al. (2003).

Lengths of fragments and alleles

Tables 4–7 show the fragment lengths of individual alleles that refer to the DNA Size Standard 550 (BTO). All analyzes have been performed on an ABI PRISM 310/3130 Genetic Analyzer with POP-4 polymer. Different analysis instruments, DNA size standards or polymers may result in different fragment lengths. In addition, a visual alignment with the allelic ladder is recommended.

Scaling

Horizontal: 70-475 bp
Vertical: Depending on signal intensity

Table 4. Fragment lengths of the allelic ladder Decaplex SE analyzed on an ABI PRISM 310 Genetic Analyzer (blue panel)

Marker/ allele	Size [bp]*	Further alleles†	Marker/ allele	Size [bp]*	Further alleles†	Marker/ allele	Size [bp]*	Further Alleles†
Amelogenin	6-FAM		D3S1358	6-FAM		D21S11	6-FAM	
X	77		9	147	8	24	330	23.2
Y	80		10	151		24.2	332	
			11	155		25	334	25.2
THO1	6-FAM		12	159		26	338	
4	91	3	13	163		26.2	340	
5	95		14	167		27	342	
6	99	6.3	15	171		28	346	
7	103	7.3	16	175		28.2	348	28.3
8	107	8.3	17	179		29	350	
9	111	9.1	18	183		29.2	352	29.3
9.3	114		19	187		30	354	30.2
10	115		20	191		31	358	
10.3	118	11	21	195		31.2	360	
13	127					32	362	
13.3	130		vWA	6-FAM		32.2	364	
			11	242	10	33	366	33.1
			12	246		33.2	368	
			13	250		34	370	34.1
			14	254		34.2	372	
			15	258		35	374	35.2
			16	262		36	378	
			17	266		36.2	380	
			18	270		37	382	37.2, 38, 38.2, 39
			19	274				
			20	278				
			21	282				
			22	286	23, 24			

Table 5. Fragment lengths of the allelic ladder Decaplex SE analyzed on an ABI PRISM 310 Genetic Analyzer (green panel)

Marker/ allele	Size [bp]*	Further alleles†	Marker/ allele	Size [bp]*	Further alleles†	Marker/ allele	Size [bp]*	Further alleles†
D16S539	BTG		D8S1179	BTG		D2S1338	BTG	
8	100	4, 5, 6, 7	7	287		16	391	10, 12, 14, 15
9	104		8	291		17	395	
10	108		9	295		18	399	
11	112		10	299		19	403	
12	116		11	303		20	407	
13	120		12	307		21	411	
14	124		13	311		22	415	
15	128	16	14	315		23	419	
			15	319		24	423	
D19S433	BTG		16	323		25	427	
6.2	222	5.2	17	327		26	431	
10	236	8, 9	18	331		27	435	
11	240		19	335	20	28	439	
12	244							
12.2	246							
13	248							
13.2	250							
14	252							
14.2	254							
15	256							
15.2	258							
16	260							
16.2	262							
17	264							
17.2	266	18, 18.2						

Table 6. Fragment lengths of the allelic ladder Decaplex SE analyzed on an ABI PRISM 310 Genetic Analyzer (yellow panel)

Marker/ allele	Size [bp]*	Further alleles†	Marker/ allele	Size [bp]*	Further alleles†
D18S51	BTY		FGA	BTY	
8	137	7	14	294	15
9	141	9.2	16	302	16.1
10	145		17	306	
10.2	147		18	310	18.2
11	149	11.2	19	314	19.2
12	152	12.2	20	318	20.2
13	156	13.2	21	322	
14	160	14.2	21.2	324	
15	164		22	326	22.2
16	168	16.2	23	330	
17	172		23.2	332	23.3
17.2	174	17.3	24	334	24.1, 24.2
18	176		25	338	25.2
18.2	178		26	342	26.2
19	180	19.2	27	346	
20	183		28	350	
21	187		29	354	
21.2	189		30	357	30.2
22	191		31.2	363	31, 32.2
23	195	23.1	33	368	33.2
24	199		34	372	
25	203		37.2	386	
26	207		42.2	405	43.2
27	211		44.2	413	
28	215	29	45.2	417	
			47.2	425	48.2
			50.2	437	51.2

Table 7. Fragment lengths of the allelic ladder Decaplex SE analyzed on an ABI PRISM 310 Genetic Analyzer (red panel)

Marker/ allele	Size [bp]*	Further alleles†	Marker/ allele	Size [bp]*	Further alleles†
SE33	BTR		SE33	BTR	
3	267		23.2	350	23
4.2	273	5.3	24.2	354	24
6.3	282	7, 7.3, 8, 8.2	25	356	
9	291	9.2	25.2	358	
10	295	10.2	26.2	362	26
11	299	11.2	27.2‡	366	27
12	303	12.2	28.2	370	28, 28.3
13	307		29.2	374	29
13.2	309	13.3	30.2	378	30
14	311	14.2, 14.3	31.2	382	31
15	315	15.2	32	384	
16‡	319	16.2, 16.3	32.2	386	
17	323	17.2, 17.3	33	388	
18	327		33.2	390	
18.2	329	18.3	34	392	34.2
19	331		35	396	35.2
19.2	333		36	401	
20	335	20.1	36.2	403	
20.2	337		37	405	37.2
21	339		38	409	39, 42
21.2	341		49	454	
22.2	345	22			

* rounded to integer

† The "off-ladder" alleles of QIAGEN's DNA pool are allocated with the actual Investigator Template Files for GeneMapper ID or Genotyper software. For further alleles see amongst others http://www.cstl.nist.gov/biotech/strbase/str_fact.htm

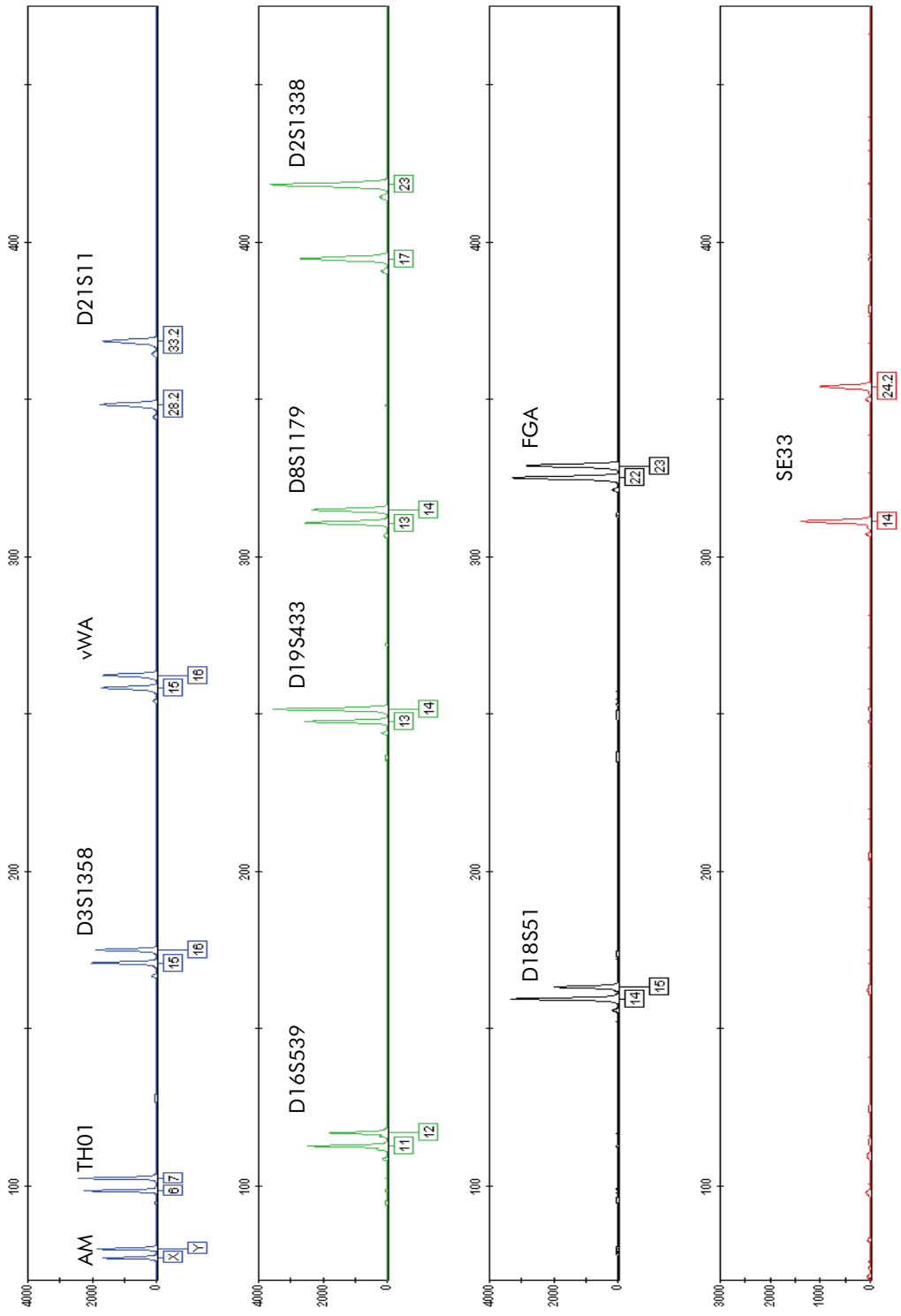

Figure 6. Electropherogram of the Investigator Decaplex SE Kit using 350 pg Control DNA XY13. Analysis was performed on an ABI PRISM 3130 Genetic Analyzer. Allele assignment was performed using the GeneMapper ID software and the Investigator Decaplex SE Template File.

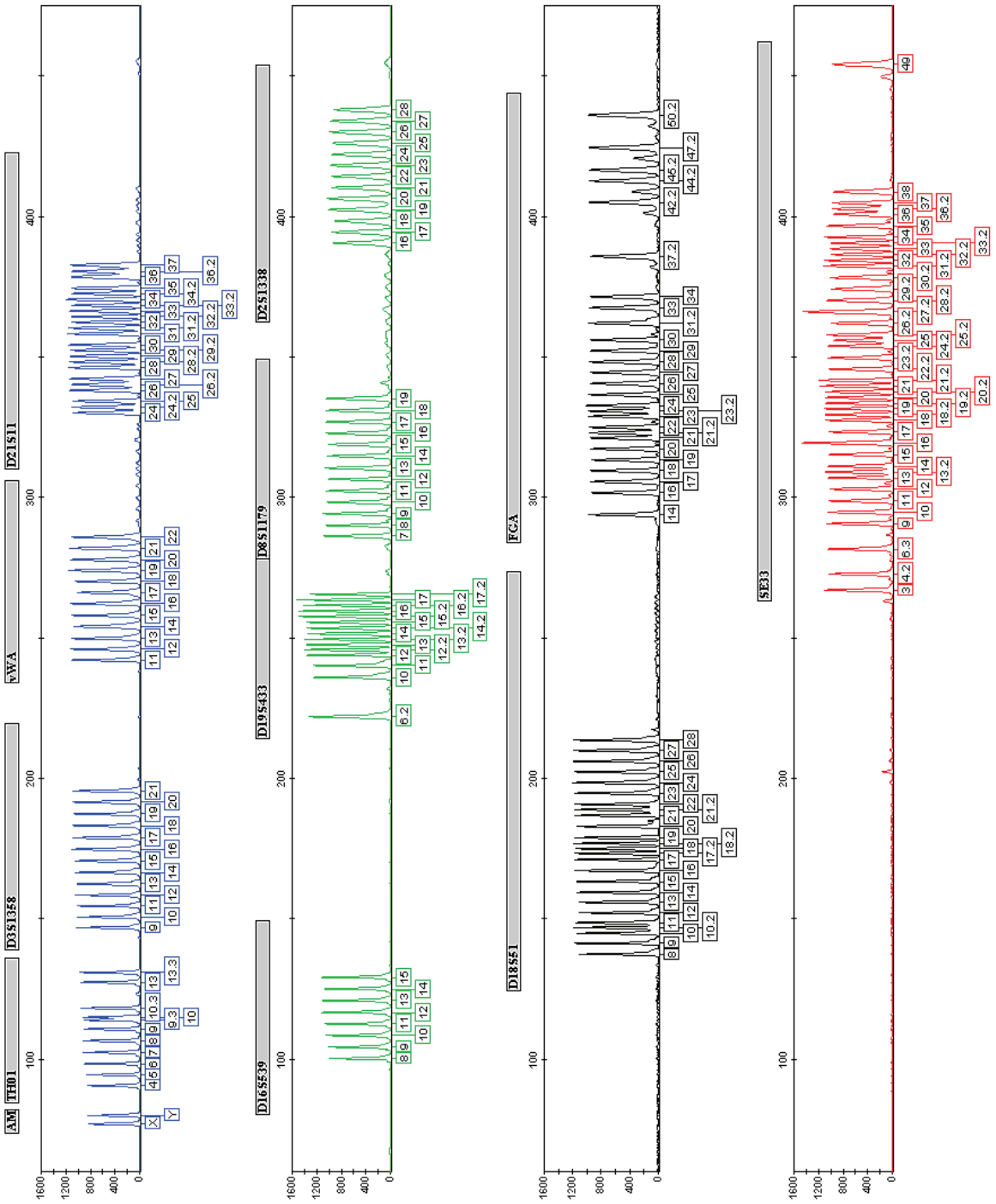

Figure 7. Electropherogram of the allelic ladder Decaplex SE analyzed on an ABI PRISM 310 Genetic Analyzer. Allele assignment was performed using the GeneMapper ID software and the Investigator Decaplex SE Template File.

Protocol: Interpretation of results

As mentioned above, post PCR analysis and automatic allele assignment with suitable analysis software ensure a precise and reliable discrimination of alleles.

Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range (>3000 RFU), or if an incorrect matrix was applied. They appear at positions of specific peaks in other colour 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 motives, caused by slippage effects of the Taq DNA Polymerase. Interpretation of those peaks should be done in accordance with the Template Files of the Genotyper and GeneMapper ID software.

Template-independent addition of nucleotides

Because of its terminal transferase activity, the Taq DNA Polymerase tends to add an adenosine radical at the 3'-end of the amplified DNA fragments. The artefact peak is one base shorter than expected (-1 peaks). All primers included in the Investigator Decaplex SE Kit are designed to minimize these artefacts. Artefact formation is further reduced by the final extension step of the PCR protocol at 68°C for 60 minutes. Peak height of the artefact correlates with the amount of DNA. Laboratories should define their own limits for analysis of the peaks.

Artefacts

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.

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.

Cited references

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Szibor, R., et al. (2003) Cell line DNA typing in forensic genetics – the necessity of reliable standards. Forensic Sci. Int. **138**, 37.

Ordering Information

Product	Contents	Cat. no.
Investigator Decaplex SE Kit (100)	Reaction mix, primer mix, control DNA, Polymerase, DNA size standard, and allelic ladder	381025
Investigator Decaplex SE Kit (400)	Reaction mix, primer mix, control DNA, Polymerase, DNA size standard, and allelic ladder	381027
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

Cross-referenced ordering information

QIAGEN Product	Cat. no.	Former product name	Cat. no.
Investigator Decaplex SE Kit (100)	381025	Mentype® Decaplex SE (100)	41-12110-0100
Investigator Decaplex SE Kit (400)	381027	Mentype Decaplex SE (400)	41-12110-0400

Notes

Notes

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the Investigator Decaplex SE Kit to the following terms:

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Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 86-21-3865-3865 ■ Fax 86-21-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 800-789-544 ■ Fax 02-334304-826 ■ Technical 800-787980

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Singapore ■ Orders 1800-742-4362 ■ Fax 65-6854-8184 ■ Technical 1800-742-4368

Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

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