Humantype **Chimera**® PCR Amplification Kit

Product description

The Humantype **Chimera**® PCR Amplification Kit is a multiplex application for specific forensic demands. The Short Tandem Repeat (STR) loci allow a reliable differentiation of forensic samples which derive from related individuals. Additionally, chimerism monitoring after bone marrow transplantation is also possible which was validated with most of the markers by monitoring chimerism over 200 HLA-matched related donor-recipient-pairs.

Humantype **Chimera**® allows co-amplification of the twelve polymorphic autosomal loci **D2S1360**, **D3S1744**, **D4S2366**, **D5S2500**, **D6S474**, **D7S1517**, **D8S1132**, **D10S2325**, **D12S391**, **D18S51**, **D21S2055**, **SE33 (ACTBP2)**, and the gender-specific **Amelogenin** locus. One primer for each locus is fluorescence-labelled with **6-FAM**, **HEX**, or **NED**. All important population-genetic data can be calculated with the GenoProof® software.

The detection limit of Humantype **Chimera**® PCR Amplification Kit is about **200 pg genomic DNA**. However, it is recommended to use **0.2-1.0 ng DNA**.

The test kit was validated and evaluated using the GeneAmp® 9700 thermal cycler, ABI PRISM® 310 Genetic Analyzer, and ABI PRISM® 3100/3130 Genetic Analyzer.

Table 1. Locus-specific information of Humantype Chimera®

Locus	GenBank® accession	Repeat motif of the reference allele	Reference allele	Allele range
Amelogenin X	M55418			
Amelogenin Y	M55419			
D2S1360	G08130	$[TATC]_9$ $[TGTC]_9$ $[TATC]_5$	23	19-32
D3S1744	G08246	$[TCTA]_2$ TA$[TCTA]_{12}$ TCA $[TCTA]_2$	16	13-22
D4S2366	G08339	$[ATAG]_9$ ATTG $[ATAG]_2$	12	9-15
D5S2500	G08468	$[ATAG]_{12}$	12	9-18
D6S474	G08540	$[TAGA]_5$ TGA $[TAGA]_{12}$	17	11-20
D7S1517	G18365	$[GAAA]_{11}$ CAAA $[GAAA]_2$ CAAA $[GAAA]_2$	17	14-31
D8S1132	G08685	$[TCTA]_9$ TCA $[TCTA]_9$ TCTGTCTA	20	12.1-27
D10S2325	G08790	$[TCTTA]_{12}$	12	6-23
D12S391	G08921	$[AGAT]_5$ GAT $[AGAT]_7$ $[AGAC]_6$ AGAT	19.3	13-28
D18S51	L18333	$[AGAA]_{13}$	13	5.3-42
D21S2055	G27274	$[CTAT]_2$ CTAA $[CTAT]_9$ CTA $[CTAT]_3$ TAT $[CTAT]_3$ TAT $[CTAT]_4$ CAT$[CTAT]_2$	24	16.1-39
SE33 (ACTBP2)	NG000840	$[AAAG]_9$ AA $[AAAG]_{16}$	25.2	3-50

Table 1 shows the STR loci with their 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). The nomenclature for STR loci D8S1132 and D12S391 is in accordance with S Hering and E Müller (2001), for loci D4S2366 und D6S474 with Becker et al. (2007), for locus D10S2325 with Wiegand et al. (1999) and the nomenclature for locus D7S1517 is in accordance with P Wiegand and M Klintschar (2002). Allele ranges include all known alleles of the National Institute of Standards and Technology (NIST as at 12/2008) and the current literature.

Table 2. Chromosomal mapping for Humantype Chimera®

Locus	Chromosomal mapping
Amelogenin X	Xp22.1-22.3
Amelogenin Y	Yp11.2
D2S1360	2p24-p22
D3S1744	3p24
D4S2366	4p16-15.2
D5S2500	5q11.2
D6S474	6q21-22
D7S1517	7q31.33
D8S1132	8q23.1
D10S2325	10p12
D12S391	12p13.2
D18S51	18q21.3
D21S2055	21q22
SE33	6q14.2

Content

Humantype **Chimera**® PCR Amplification Kit (100 Reactions)

Nuclease-free water	3.0 mL
Reaction mix **D**	500 µL
Primer mix	250 µL
DNA polymerase	40 µL
Control DNA XY5 (2 ng/µL)	10 µL
DNA Size Standard 550 (ROX)	50 µL
Allelic ladder	10 µL

Ordering information

Humantype **Chimera**®	25 reactions	Cat. No.	31-13110- 0025
Humantype **Chimera**®	100 reactions	Cat. No.	31-13110- 0100
Humantype **Chimera**®	400 reactions	Cat. No.	31-13110- 0400

Storage

Store all components at -20°C and avoid repeated thawing and freezing. Primer mix and allelic ladder must be stored protected from light. The DNA samples and post-PCR reagents (allelic ladder and DNA size standard) should be stored separately from the PCR reagents. The expiry date is indicated on the kit cover.

Quality assurance

All kit components undergo an intensive quality assurance process at Biotype Diagnostic GmbH. The quality of the test kits is permanently monitored in order to ensure unrestricted usability. Please contact us if you have any questions regarding quality assurance.

Additional required reagents

Additional reagents are needed in order to use the Biotype® PCR Amplification Kit:

Reagent	Supplier	Order number
Hi-Di™ Formamide, 25 mL	Applied Biosystems	4311320
Matrix Standards DS-30 for ABI PRISM® 310 Genetic Analyzer	Applied Biosystems	401546 and 402996 (NED)
Matrix Standards DS-30 for ABI PRISM® multi-capillary instruments	Applied Biosystems	4345827

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GenoProof® is a registered trademark of Qualitype AG.
ABI PRISM®, GeneScan®, Genotyper®, GeneMapper™ and Applied Biosystems are registered trademarks of Applied Biosystems Inc. or its subsidiaries in the U.S. and certain other countries.
6-FAM, HEX, NED, ROX, POP-4 and Hi-Di are trademarks of Applied Biosystems Inc.
GeneAmp® is a registered trademark of Roche Molecular Systems.
The PCR is covered by patents. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche).
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Warnings and safety instructions

The PCR Amplification Kit contains the following potentially hazardous chemicals:

Kit component	Chemical	Hazards
Primer mix, reaction mix and allelic ladder	Sodium azide NaN_3	toxic if swallowed, develops toxic gases when it gets in contact with acids

Observe the Material Safety Data Sheets (MSDS) for all Biotype® products, which are available on request. Please contact the respective manufacturers for copies of the MSDS for any additionally needed reagents.

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Protocols for PCR amplification, electrophoresis, and analysis

1. PCR amplification

1.1 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
Nuklease-free water	16.1 µL
Reaction mix **D***	5.0 µL
Primer mix	2.5 µL
Multi Taq2 DNA Polymerase (hot start, 2.5 U/µL)	0.4 µL
Volume of master mix	24.0 µL

* contains Mg^{2+}, 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. DNA volumes of more than 5 µL are not recommended, because potential PCR inhibitors 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 **0.5 ng Control DNA XY5** 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 XY5 to 0.5 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 which contains 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.

1.2 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 from 30 to 34.

Standard method

Recommended for all DNA samples

Temperature	Time	
94°C	4 min (hot start for activation of the Multi Taq2 DNA Polymerase)	
94°C	30 s	
60°C	120 s	**30 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 for activation of the Multi Taq2 DNA Polymerase)	
94°C	30 s	
60°C	120 s	**34 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.

2. 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 D** shall be used for combined application of the four fluorescent labels **6-FAM**, **HEX**, **NED**, and **ROX** (also called **DS-30**). Generally, Filter Sets A and F are suitable, too.

Material

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

2.1 Matrix generation

Prior to conducting DNA fragment size analysis with the filter set D, a matrix with the four fluorescent labels 6-FAM, HEX, NED, and ROX must be generated. The suitable matrix standard **DS-30** is available from Applied Biosystems.

Colour	Matrix standard	Order number
Blue (B)	6-FAM	Applied Biosystems, 401546
Green (G)	HEX	Applied Biosystems, 401546
Yellow (Y)	NED	Applied Biosystems, 402996
Red (R)	ROX	Applied Biosystems, 401546

Four electrophoresis runs shall be conducted, one for each fluorescent label, 6-FAM, HEX, NED, and ROX, under the same conditions as for the samples and allelic ladders of the Biotype® test kit to generate suitable matrix files.

Matrix sample	Component	Volume
Matrix sample 1	Hi-Di™ Formamide Matrix standard **6-FAM**	12.0 µL 1.0 µL
Matrix sample 2	Hi-Di™ Formamide Matrix standard **HEX**	12.0 µL 1.0 µL
Matrix sample 3	Hi-Di™ Formamide Matrix standard **NED**	12.0 µL 1.0 µL
Matrix sample 4	Hi-Di™ Formamide Matrix standard **ROX**	12.0 µL 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) **D**
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 (ROX)**

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)

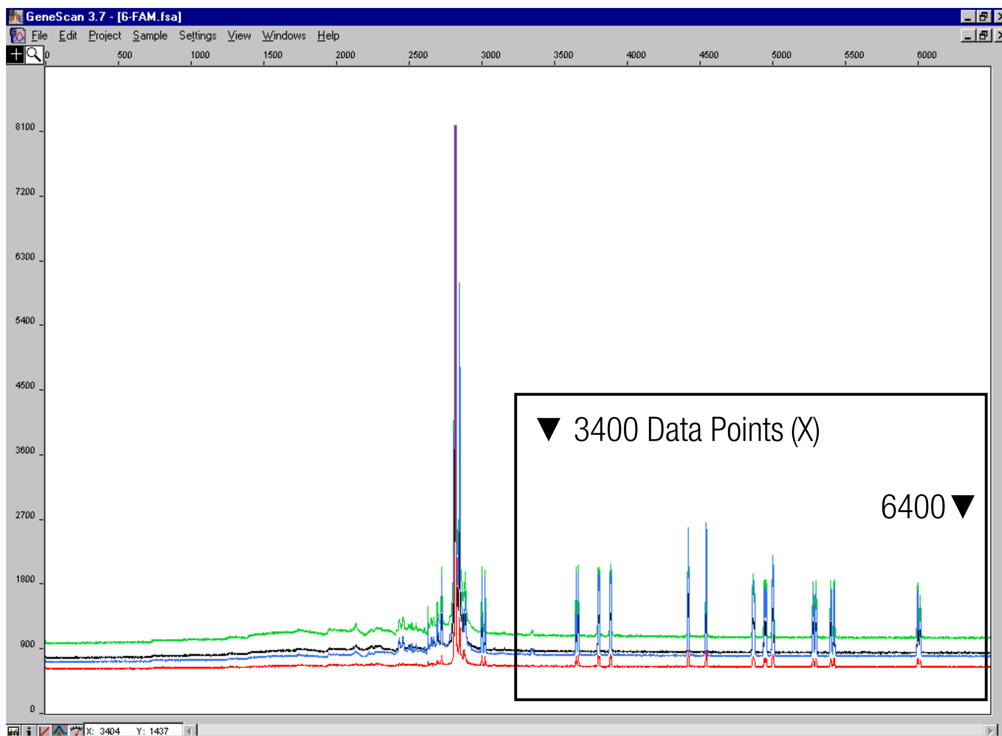

Fig. 1 Electropherogram with raw data of the matrix standard 6-FAM

- 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 3400, end value 6400
- Calculate the difference, e.g. 6400-3400 = 3000 data points

Generation of a new matrix

- **File** → **New** → **Matrix**

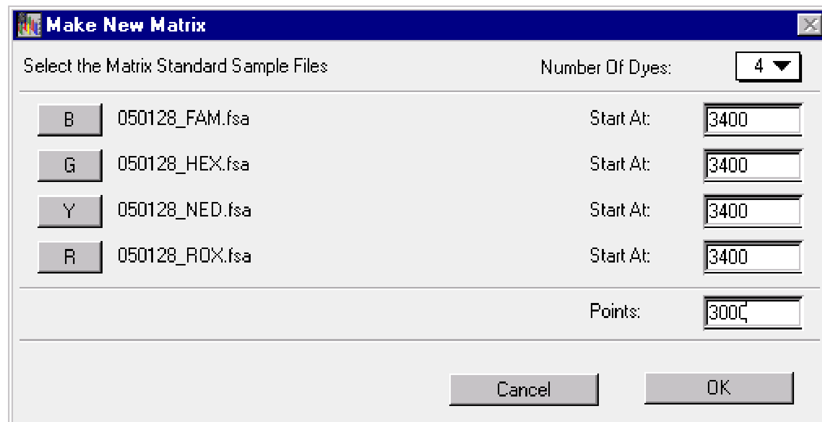

Fig. 2 Matrix samples selection

- Import matrix samples for all dyes (B, G, Y, R)
- Enter a **Start At** value, e.g. 3400
- Enter the calculated difference under **Points**, e.g. 3000
- Click on **OK** to calculate the new matrix

Matrix Biotype D.mtx

Reactions

	B	G	Y	R
B	1.0000	0.4164	0.0415	0.0012
G	0.8472	1.0000	0.6863	0.0107
Y	0.4509	0.4886	1.0000	0.0456
R	0.1273	0.1792	0.4964	1.0000

Fig. 3 New matrix DS-30

- Save the matrix in the matrix folder: **File** → **Save as**, e.g. Matrix DS-30

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-analyse your samples

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

2.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (ROX)	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 (ROX) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

2.3 Setting up the GeneScan® software

- Create a **Sample Sheet** and enter sample designation

Injection list

Parameter	Set up
Module File	GS STR POP-4 (1 mL) **D**
Matrix File	e.g. Matrix DS-30
Size Standard	e.g. SST-ROX_50-500bp
Injection [s]*	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]**	**28**

* Deviating from the standard settings, the injection time may range between 1 and 10 s depending on the type of sample. If blood 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 Humantype **Chimera**® was modified in order to analyse fragments with lengths of up to **500 bp**.

2.4 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:* Min. Peak Half Width: 2 pts Polynorminal Degree: 3 Peak Window Size: 11 pts**
Size Call Range	Min: 50 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, minimise the Peak Window Size further.

3. 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 (former ABI 3100-Avant), and the system with 16 capillaries is named ABI 3130xl (former ABI 3100).

The virtual **filter set D** shall be used for combined application of the four fluorescent labels **6-FAM**, **HEX**, **NED**, and **ROX** (also called **DS-30**).

Material

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

3.1 Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the four fluorescent labels 6-FAM, HEX, NED, and ROX 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	47.5 µL
Matrix standard DS-30	2.5 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	190.0 µL
Matrix standard DS-30	10.0 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_DS30)
Type	SPECTRAL
Dye Set	D
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_DS-30_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_POP4DS30 (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

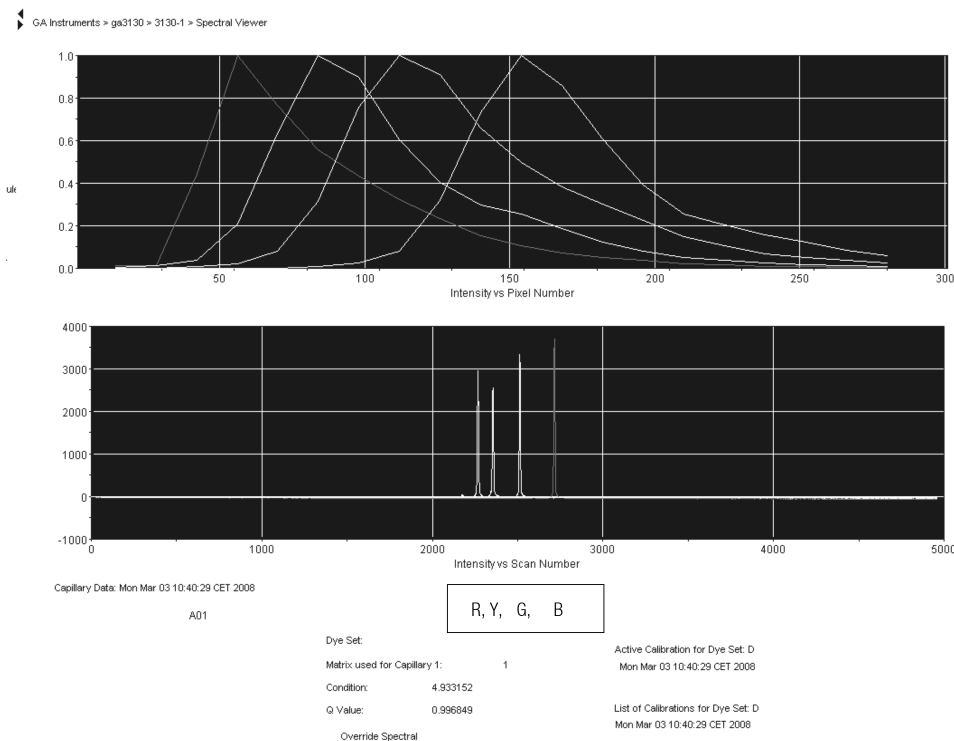

Fig. 4 Electropherogram of spectral calibration with matrix standard DS-30 on an ABI 3130

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) 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 D** is activated automatically in the **Spectral Viewer**. **Rename** the calibration file (e.g. DS-30_Date of calibration) using the respective button

3.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (ROX)	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 analysers. If fewer samples are analysed, the empty positions must be filled with 12 µL Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analysers, a number of allelic ladders should be run, independently from sample number.

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 (ROX) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

3.3 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

Parameter	**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 Humantype **Chimera**® was modified in order to be able to analyse fragments with lengths of up to **500 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	e.g. Run36_POP4_DS-30_24min
Type	REGULAR
Run Module*	3kV_10s_500bp
Dye Set	D

* 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_DS-30_Date
Application	select GeneMapper Application
Plate Type	96-Well
Owner Name / Operator Name	…

- Click **OK**. A new table in the **Pate 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-ROX_50-500bp
Panel	e.g. Biotype_Panels_v3a (choose test kit)
Analysis Method	e.g. Analysis_HID_3130
Snp Set	-
User-defined 1-3	-
Results Group 1	(select results group)
Instrument Protocol 1	Run36_POP4_DS-30 (setting described earlier)

- 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**

3.4 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:* 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, minimise the Peak Window Size further.

4. 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 (ROX) shall thus be used with the following lengths of fragments: **50**, **60**, **70**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **190**, **200**, **220**, **240**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550 bp**.

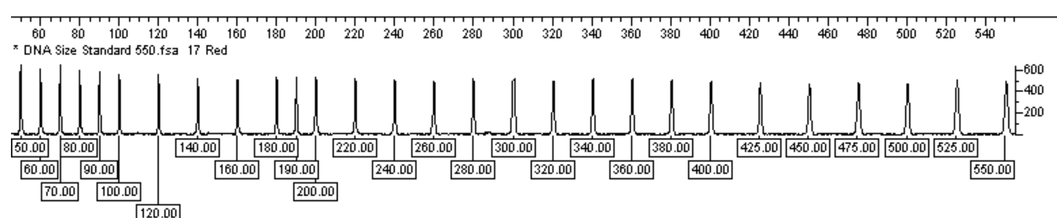

Fig. 5 Electropherogram of the DNA Size Standard 550 (ROX), fragments with lengths in bp

4.1 Biotype® template files

Allele allocation should be carried out with a suitable analysis software, e.g. GeneMapper™ ID or Genotyper® software in combination with the Humantype **Chimera**® template files from Biotype. Template files are available from our homepage or as CD-ROM on request.

Recommended Biotype® templates for GeneMapper™ ID/ID-X software are:

Panels	Biotype_Panels_ v3a/v3X (choose kit)	or higher versions
BinSets	Biotype_Bins_ v3a/v3X	or higher versions
Size Standard	SST-ROX_50-500bp	
Analysis Method	Analysis_HID_310	
	Analysis_HID_3130	
	Analysis_HID_310_50rfu	
	Analysis_HID_3130_50rfu	
Plot Settings	Plots_4dyes	
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 Biotype® templates for GeneMapper™ ID-X Software:

Stutter*	Biotype_Stutter_v3X	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 Biotype® template files for Genotyper® software are:

Humantype Chimera_v1e 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. Analyse and interpret the sample data

4.2 Controls

The Control DNA XY5 of the test kit and other commercially available DNA from standard cell lines represent the following alleles:

Table 3. Allele assignment of Humantype Chimera®

Locus	Control DNA XY5	ATCC K-562	CCR 9947A	CCR 9948	CCR 3657
Amelogenin	X/Y	X/X	X/X	X/Y	X/Y
D2S1360	22/25	20/28	23/24	22/25	22/23
D3S1744	17/18	18/18	17/17	18/18	14/17
D4S2366	9/12	13/13	11/13	9/14	9/14
D5S2500	10/11	15/15	15/16	11/15	11/16
D6S474	15/16	14/17	13/17	16/16	15/16
D7S1517	22/27	21/24/25	19/25	20/22	24/25
D8S1132	18/20	20/24	19/21	20/24	17/18
D10S2325	13/14	7/13	9/10	8/14	9/14
D12S391	17/19	23/23	18/20	18/24	18/19
D18S51	13/15	15/16	15/19	15/18	12/20
D21S2055	25/27	28/35	19.1/26	19.1/26	19.1/25
SE33	15/21.2	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).

4.3 Lengths of fragments and alleles

Table 4 to **Table 6** show the fragment lengths of individual alleles that refer to the DNA Size Standard 550 (ROX). All analyses 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: 75-505 bp
Vertical: Depending on signal intensity

Figure 6

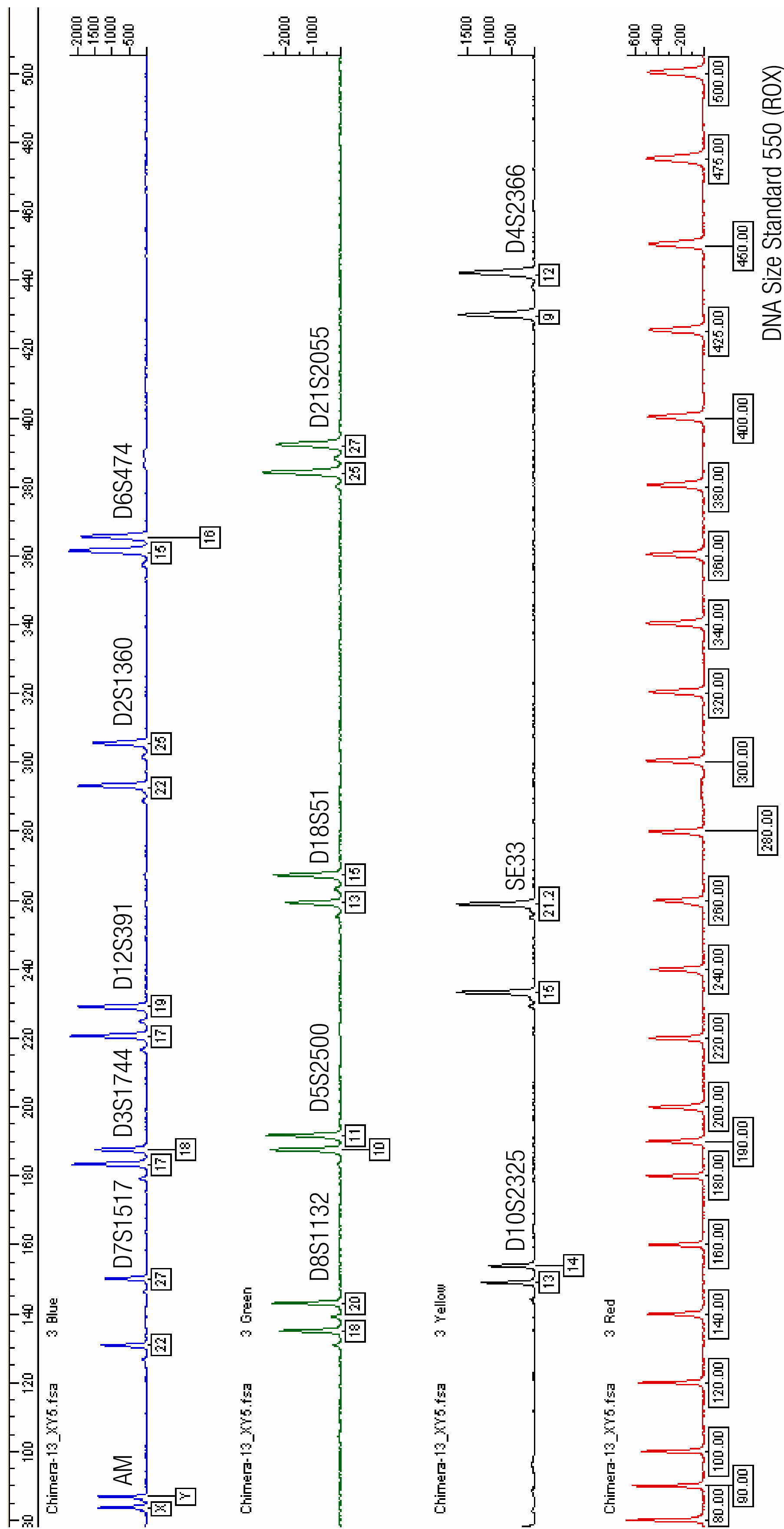

Fig. 6 Electropherogram of the Humantype **Chimera**® using 500 pg Control DNA XY5. Aanalysis was performed on an ABI PRISM® 310 Genetic Analyzer with the DNA Size Standard 550 (ROX). Allele assignment was performed using the Genotyper® software and the Humantype **Chimera**® template file.

Figure 7

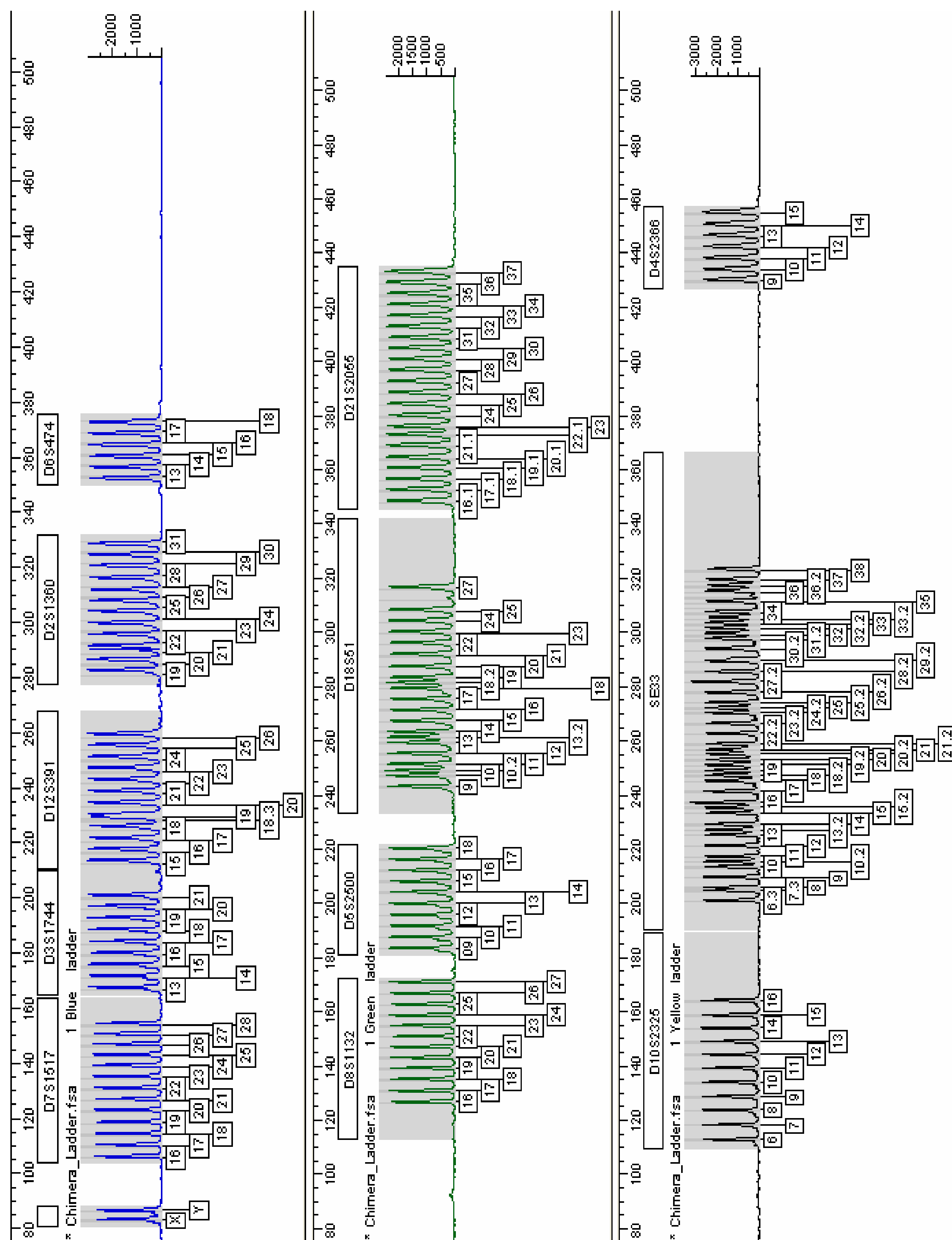

Fig. 7 Electropherogram of the allelic ladder Humantype **Chimera**® analysed on an ABI PRISM® 310 Genetic Analyzer. Allele assignment was performed using the Genotyper® software and the Humantype **Chimera**® template files.

Table 4. Fragment lengths of the allelic ladder Humantype Chimera® analysed 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**		**D3S1744**	**6-FAM**		**D2S1360**	**6-FAM**	
X	83		13	167		19	281	
Y	86		14	171		20	285	
			15	175		21	289	
D7S1517	**6-FAM**		16	179		22	293	
16	106	14, 15	17	183		23	297	
17	110		18	188		24	301	
18	114		19	192		25	305	
19	118		20	196		26	309	
20	122		21	200	22	27	313	
21	126					28	317	
22	130		**D12S391**	**6-FAM**		29	322	
23	134		15	213		30	326	
24	138		16	217		31	330	32
25	142		17	221	17.3			
26	146		18	225		**D6S474**	**6-FAM**	
27	150		18.3	228		13	353	11, 12
28	154	29	19	229	19.1, 19.3	14	357	
			20	233	20.3	15	361	
			21	237		16	365	
			22	242		17	369	
			23	246		18	373	19
			24	250				
			25	254				
			26	258	27			

Table 5. Fragment lengths of the allelic ladder Humantype Chimera® analysed 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**
D8S1132	**HEX**		**D18S51**	**HEX**		**D21S2055**	**HEX**	
16	127	12.1, 13, 14.3, 15	9	243	7, 8, 9.2	16.1	348	
17	131		10	247		17.1	352	
18	135		10.2	249		18.1	356	
19	139		11	251	11.2	19.1	360	
20	143		12	255	12.2	20.1	364	
21	147		13	259		21.1	368	
22	151		13.2	261		22.1	372	22
23	155		14	263	14.2	23	375	23.1
24	159		15	267		24	379	
25	163		16	271	16.2	25	384	
26	167		17	275	17.2, 17.3	26	388	
27	171		18	279		27	392	
			18.2	281		28	396	
D5S2500	**HEX**		19	283	19.2	29	400	
9	184		20	287		30	404	
10	188		21	291	21.2	31	408	
11	192		22	295		32	412	
12	196		23	299	23.1	33	416	
13	200		24	304		34	421	
14	204		25	308	26	35	425	
15	208		27	316	28, 29	36	429	
16	212					37	433	38, 39
17	216							
18	220							

Table 6. Fragment lengths of the allelic ladder Humantype Chimera® analysed on an ABI PRISM® 310 Genetic Analyzer (yellow panel)

Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**
D10S2325	**NED**		**SE33**	**NED**		**SE33**	**NED**	
6	113		6.3	201	4.2, 5.3	25.2	274	
7	119		7.3	205	7	26.2	278	26
8	124		8	206	8.2	**27.2**‡	**282**	27
9	129	9.4	9	210	9.2	28.2	286	28, 28.3
10	134		10	214		29.2	289	29
11	139	11.4	10.2	216		30.2	293	30
12	144		11	218	11.2	31.2	297	31
13	149		12	222	12.2	32	299	
14	154		13	226		32.2	301	
15	159		13.2	227	13.3	33	303	
16	164	17, 18 ,19	14	229	14.2, 14.3	33.2	305	
			15	233		34	307	34.2
			15.2	235		35	311	35.2
			16‡	**237**	16.2, 16.3	36	315	
			17	241	17.2, 17.3	36.2	317	
			18	245		37	319	37.2
			18.2	247	18.3	38	323	39, 42, 49
			19	249				
			19.2	251		**D4S2366**	**NED**	
			20	253	20.1	9	430	9.2
			20.2	255		10	434	10.2
			21	257		11	438	11.2
			21.2	259	22	12	442	
			22.2	263		13	446	
			23.2	266	23	14	450	
			24.2	270	24	15	454	
			25	272				

* rounded to integer

** The “off-ladder” alleles of Biotype’s DNA pool are allocated with the actual Biotype® template files for GeneMapper™ ID or Genotyper® software. For further alleles see amongst others http://www.cstl.nist.gov/biotech/strbase/str_fact.htm

‡ For better orientation, these alleles are heightened within the allelic ladder.

5. 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 Biotype® primers are designed to minimise 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.

6. Population-genetic data

Most important population-genetic data of the STR markers are listed in **table 7-10**. The formula for calculation of the “Polymorphism Information Content” (PIC) was published by Botstein et al. (1980), the one for the “Expected Heterocygosity” (HET) by Nei and Roychoudhury (1974) and the one for “Power of Discrimination” (PD) by Jones (1972). All formulas are suitable for autosomale markers.

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

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

$$\mathrm{PD} = 1 - \Sigma_i f_i^2$$

All important population-genetic data could be calculated with the GenoProof® Software from Qualitype AG.

Tabelle 7. Population-genetic data

Marker D2S1360		Marker D3S1744		Marker D4S2366	
Allele	**Allele frequency**	**Allele**	**Allele frequency**	**Allele**	**Allele frequency**
19	0.007	13	0.007	9	0.347
20	0.126	14	0.104	10	0.179
21	0.060	15	0.053	11	0.074
22	0.309	16	0.100	12	0.147
23	0.142	17	0.319	13	0.168
24	0.098	18	0.197	14	0.074
25	0.086	19	0.130	15	0.011
26	0.093	20	0.067		
27	0.035	21	0.023		
28	0.023				
29	0.012	PIC	0.790	PIC	0.760
30	0.002	PD	0.943	PD	0.919
31	0.005	HET	0.792	HET	0.795
32	0.002				
PIC	0.820				
PD	0.955				
HET	0.856				

Tabelle 8. Population-genetic data

Marker D5S2500		Marker D6S474		Marker D7S1517	
Allele	**Allele frequency**	**Allele**	**Allele frequency**	**Allele**	**Allele frequency**
9	0.007	13	0.246	16	0.007
10	0.084	14	0.212	17	0.007
11	0.313	15	0.154	18	0.049
12	0.161	16	0.285	19	0.120
13	0.061	17	0.097	20	0.101
14	0.042	18	0.005	21	0.099
15	0.213			22	0.082
16	0.103	PIC	0.740	23	0.077
17	0.009	PD	0.918	24	0.155
18	0.007	HET	0.733	25	0.230
				26	0.054
PIC	0.780			27	0.014
PD	0.938			28	0.005
HET	0.804				
				PIC	0.860
				PD	0.967
				HET	0.826

Tabelle 9. Population-genetic data

Marker D8S1132		Marker D10S2325		Marker D12S391	
Allele	**Allele frequency**	**Allele**	**Allele frequency**	**Allele**	**Allele frequency**
16	0.007	6	0.002	15	0.035
17	0.095	7	0.102	16	0.019
18	0.221	8	0.056	17	0.107
19	0.153	9	0.121	17.3	0.019
20	0.128	10	0.142	18	0.215
21	0.119	11	0.144	18.3	0.007
22	0.133	12	0.193	19	0.121
23	0.077	13	0.133	19.3	0.016
24	0.056	14	0.065	20	0.117
25	0.005	15	0.037	21	0.093
26	0.005	16	0.005	22	0.114
27	0.002			23	0.072
		PIC	0.860	24	0.040
PIC	0.850	PD	0.967	25	0.021
PD	0.964	HET	0.851	26	0.002
HET	0.828				
				PIC	0.870
				PD	0.971
				HET	0.893

Tabelle 10. Population-genetic data

Marker D18S51		Marker D21S2055		Marker SE33 (ACTBP2)	
Allele	**Allele frequency**	**Allele**	**Allele frequency**	**Allele**	**Allele frequency**
10	0.005	16.1	0.056	11	0.002
12	0.103	17.1	0.021	12	0.014
13	0.110	18.1	0.023	13	0.002
14	0.157	19.1	0.274	13.2	0.002
15	0.199	20.1	0.040	14	0.026
16	0.161	21.1	0.019	15	0.049
17	0.112	22.1	0.005	16	0.047
18	0.072	23	0.007	17	0.070
19	0.028	25	0.112	17.3	0.002
20	0.030	26	0.116	18	0.044
21	0.021	27	0.016	18.3	0.002
24	0.002	28	0.007	19	0.082
		29	0.030	19.2	0.009
PIC	0.850	30	0.021	20	0.044
PD	0.964	31	0.023	20.2	0.009
HET	0.902	32	0.026	21	0.035
		33	0.067	21.2	0.019
		34	0.074	22	0.007
		35	0.053	22.2	0.035
		36	0.007	23.2	0.023
		37	0.002	24	0.002
				24.2	0.035
		PIC	0.870	25.2	0.044
		PD	0.971	26.2	0.040
		HET	0.856	27.2	0.084
				28.2	0.084
				29.2	0.051
				30	0.002
				30.2	0.061
				31.2	0.028
				32.2	0.023
				33	0.009
				33.2	0.005
				34	0.002
				36	0.002
				PIC	0.950
				PD	0.990
				HET	0.949

All population-genetic data based on an analysis of ca. 210 unlinked Caucasians performed by Biotype Diagnostic GmbH.

7. References

Bär W, **Brinkmann B**, **Budowle B**, **Carracedo A**, **Gill P**, **Lincoln P**, **Mayr W**, **Olaisen B (1997)** DNA Recommendations. Further report of the DNA commission of the ISFG regarding the use of short tandem repeat systems. *Int J Legal Med 110:175-176.*

Becker D, **Vogelsang D, Brabetz W (2007)** Population data on the seven short tandem repeat loci D4S2366, D6S474, D14S608, D19S246, D20S480, D21S226 and D22S689 in a German population. *Int J Legal Med 121:78-81.*

Botstein D, White RI, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet 32:314–331.*

Hering S, **Müller E (2001)** New allele and mutational events in D12S391 and D8S1132: sequence data from an eastern German population. *Forensic Sci Int 124:187-191.*

Jones DA (1972) Blood samples: Probability of Discrimination. *J Forensic Sci Soc 12:355-359.*

Nei M, Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. *Genetics 76:379–390.*

Szibor R, Edelmann J, Hering S, Plate I, Wittig H, Roewer L, Wiegand P, Calì F, Romano V, Michael M (2003) Cell line DNA typing in forensic genetics – the necessity of reliable standards. *Forensic Sci. Int. 138 37-43.*

Wiegand P, Lareu M. V., Schürenkamp M (1999) D18S535, D1S1656 and D10S2325: three efficient short tandem repeats for forensic genetics. *Int. J. Legal. Med 112:360-363.*

Wiegand P, Klintschar M (2002) Population genetic data, comparison of the repeat structure and mutation events of two short STRs. *Int J Legal Med 116:258-261.*

Notes