Mentype® **Chimera®**

Manual

The new standard for chimerism analysis

In-Vitro-Diagnostics

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Made in Germany

Bio type®
Diagnostic GmbH

Biotype Diagnostic GmbH develops, produces and markets their PCR-based rapid Mentype® Detection Kits. Our products provide customers with fast and reliable testing methods for professional medical diagnostics.

Our Mentype® Test Kits guarantee highest quality standards for clinical research and diagnostics.

For information and enquiries about the Mentype® **Chimera**® PCR Amplification Kit, please do not hesitate to get in touch or visit www.biotype.de/en/home.html.

Product description

Mentype® **Chimera**® is a multiplex-PCR application specifically developed for chimerism monitoring after blood stem cell and bone marrow transplantation, respectively. The assay was validated by chimerism analysis of over 200 HLA-matched related donor-recipient-pairs and its suitability was confirmed in a comparative clinical evaluation study. Ever since the assays is successfully used in routine diagnostics. Genetic markers that are addressed by Mentype® **Chimera**® are distributed over 12 chromosomes and represent highly polymorphic short tandem repeats (STRs) with a very high rate of heterozygosity and a balanced allelic distribution. Together, this significantly increases the chance to identify informative loci for donor-recipient discrimination and provides reliability and robustness of chimerism analyses.

One PCR reaction simultaneously amplifies the autosomal loci **D2S1360**, **D3S1744**, **D4S2366**, **D5S2500**, **D6S474**, **D7S1517**, **D8S1132**, **D10S2325**, **D12S391**, **D18S51**, **D21S2055**, **SE33 (ACTBP2)**, and the gender-specific locus **Amelogenin**. . One primer for each locus is fluorescence-labelled with **6-FAM**, **BTG**, or **BTY**.

The detection limit of the Mentype® **Chimera**® PCR amplification kit is **200 pg genomic DNA**. The optimal range under standard conditions is **0.2-1.0 ng DNA**.

The test kit is validated using the GeneAmp® PCR System 9700 Aluminium, Eppendorf Mastercycler ep-S, Biometra T1, ABI PRISM® 310 Genetic Analyzer and ABI PRISM® 3130 Genetic Analyzer applying the POP-4® polymer. Development, manufacture and distribution of Biotype® products are certified according to DIN EN ISO 9001:2008.

Content

1. Description of Mentype® Chimera®

Table 1. Locus-specific information of Mentype® **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 STR loci with respective repeat motifs and alleles that are concordant with the guidelines for the use of microsatellite markers of the International Society for Forensic Genetics (ISFG; Bär *et al.*, 1997). The nomenclature for STR loci D8S1132 and D12S391 is in accordance with Hering and 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 Wiegand and 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 Mentype® **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

Kit content

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

Nuclease-free water	3.0 ml
Reaction mix **A**	500 µl
Primer mix	250 µl
Multi Taq2 DNA polymerase	40 µl
Control DNA XY5 (2 ng/µl)	10 µl
DNA Size Standard 550 (BTO)	50 µl
Allelic ladder	25 µl

Ordering information

Mentype® **Chimera**®	25 reactions	Cat. No.	45-13210-0025
Mentype® **Chimera**®	100 reactions	Cat. No.	45-13210-0100
Mentype® **Chimera**®	400 reactions	Cat. No.	45-13210-0400
Mentype® **Chimera**®	1000 reactions	Cat. No.	45-13210-1000

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 PCR reagents. The expiry date is indicated on the kit cover.

Additionally required reagents

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

Reagent	Supplier	Order number
Hi-Di™ Formamide, 25 ml	Life Technologies Corporation	4311320
Matrix Standards BT5 single-capillary instruments (5x25 µl)	Biotype Diagnostic GmbH	00-10411-0025
Matrix Standards BT5 multi-capillary instruments (25 µl)	Biotype Diagnostic GmbH	00-10421-0025
Matrix Standards BT5 multi-capillary instruments (50 µl)	Biotype Diagnostic GmbH	00-10421-0050

Warnings and safety instructions

The PCR Amplification Kit contains the following potentially hazardous chemicals:

Kit component	Chemical	Hazards
Reaction mix	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.

Quality assurance

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

Trademarks and Patents

Protocols for PCR amplification, electrophoresis, and analysis

2. PCR amplification

2.1 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
Nuclease-free water	16.1 µl
Reaction mix **A***	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 volume of DNA applied to the assay depends on its concentration. For reference samples 1 µl is mostly sufficient. For critical patient samples the amount of template can be increased appropriately. 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. 0.1 x 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 applied, 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 Control DNA XY5 to 0.5 ng/µl.
Instead of template DNA, pipette 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 that contains the PCR master mix.

Template DNA

Sometimes, measured DNA concentration varies depending on the quantification method used. It might thus be necessary to adjust the optimal DNA amount.

2.2 PCR amplification parameter

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

Number of PCR cycles depend on the amount of DNA applied. 30 PCR cycles are recommended for all samples. In case of critical samples (< 100 pg DNA), the number of PCR cycles can be increase from 30 to 32.

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 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**	**32 cycles**
72 °C	75 s	
68 °C	60 min	
10 °C	∞	hold

Note: If thermal cyclers with rapid heating and cooling steps (> 2 °C/s) are used, ramping should be adjusted to 2 °C/s in order to provide an optimal kit balance.

Very small amounts of DNA may result in statistical dropouts and imbalances of the peaks. Increasing numbers of PCR cycles raise the risk of cross contamination caused by minimal amounts of impurities. Furthermore, unspecific amplification products could appear.

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3. 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

3.1 Matrix generation

Prior to conducting DNA fragment size analysis with the filter set G5, a matrix with 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**, use the same conditions as for samples and allelic ladders of the Biotype® test 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 and place samples on the autosampler tray

- Create a **Sample Sheet**, choose **5 Dyes** and enter a 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 for a flat baseline. As shown in the figure below there should be at least five peaks with peak heights about 1000-4000 RFU (Y-axis) for each matrix sample (optimal range: 2000-4000 RFU)

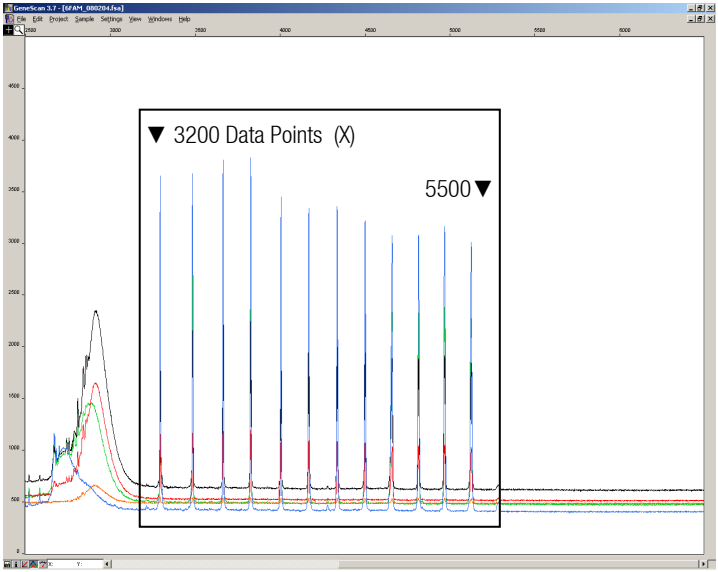

Fig. 1 Electropherogram with raw data of the matrix standard 6-FAM

- Select an 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**

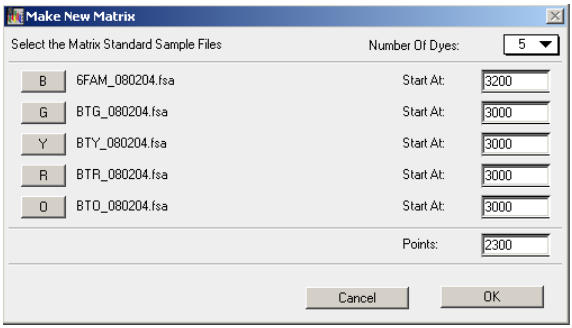

Fig. 2 Matrix sample selection

- 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

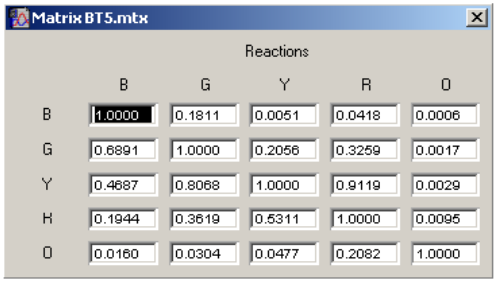

Fig. 3 New matrix BT5

- 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-analyse your samples

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

3.2 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
- Cool down to 4 °C and place samples on the autosampler 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

3.3 Setting up the Data Collection Software

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

Injection list

Parameter	Set up
Module File	GS STR POP-4 (1 ml) **G5**
Matrix File	e.g. Matrix BT5
Size Standard	e.g. SST-BTO_60-500bp
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 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low cDNA content or critical patient samples an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **Chimera**® was modified in order to analyse fragments with lengths of up to **500 bp**.

3.4 Analysis parameter / analysis method

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 (cut-off value) corresponds to the minimum peak height that will be detected by the GeneScan® or GeneMapper® ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times 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. 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 1.0.1 or 1.1 and the GeneScan® software, refer to the *ABI PRISM® 3100-Avant/3100 Genetic Analyzer User's Manual*. For systems with Data Collection Software 2.0 or 3.0 refer to chapter 5.

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 BT0** (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

4.1 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 with the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BT0**. 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 standard

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 and place samples on the autosampler tray

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 and place samples on the autosampler tray

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]
- 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 Biotype® matrix standard **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* (enter the name for spectral run module)
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 the 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 labelled **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 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- 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)
- If calibration was not successful, try to re-inject the samples with higher injection voltage or injection time. The editing of the Spectral Run Module will be necessary. You can re-inject the same samples up to three times. Otherwise use more matrix standard for spectral calibration
- 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

4.2 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 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 analysed, the empty positions must be filled with 12 µl Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analyzers, several allelic 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. Optimal will be a stable room temperature > 22 °C.

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

4.3 Setting up the Data Collection 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]**	**26**

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content or critical patient samples an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **Chimera**® was modified in order 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
- 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 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

4.4 Analysis parameter / analysis method

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 (cut-off value) corresponds to the minimum peak height that will be detected by the GeneScan or GeneMapper® ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times 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.

5. 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/ID-X 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

5.1 Spectral calibration / matrix generation

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

Spectral calibration comprises the following steps:
- Preparation of spectral calibration standards
- Loading 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 and place samples on the autosampler tray

Example for 16 capillaries/ABI 3130xl

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 and place samples on the autosampler tray

Performing a spectral calibration run

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

Instrument Protocol for spectral calibration

Protocol Editor	**Set up**
Name	*User* (enter name)
Type	SPECTRAL
Dye Set	**Any5Dye**
Polymer*	*User* (POP4)
Array Length*	*User* (36cm)
Chemistry	Matrix Standard
Run Module*	*Default* (enter a name for the run module)

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

- Click on **OK** to leave the **Protocol Editor** dialog box
- In the **Plate Manager** of the Data Collection Software, click on **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** will open automatically

Plate Editor for spectral calibration (II)

Parameter	**Set up**
Sample Name	Enter 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 to the newly created plate record (position A or B) and start the run

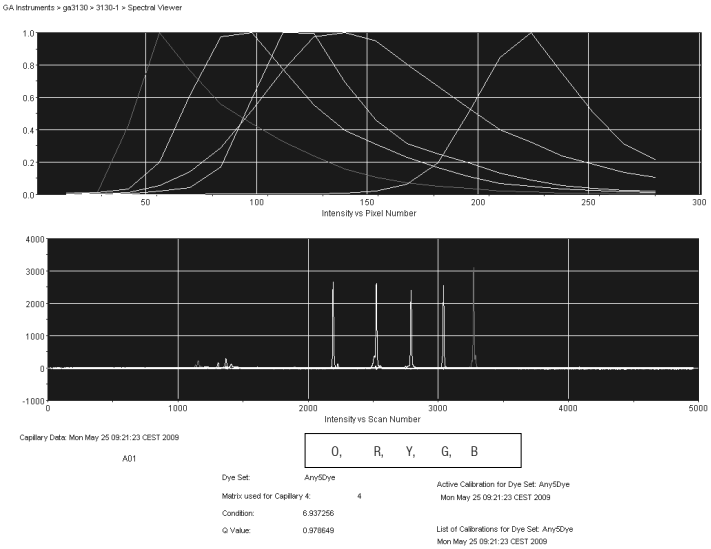

Fig. 4 Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3130

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 five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- 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
- If calibration was not successful, try to re-inject the samples with higher injection voltage or injection time. Editing of the Spectral Run Module will be necessary. You can re-inject the same samples up to three times. Otherwise use more matrix standard for spectral calibration

- 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

5.2 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 and place samples on the autosampler 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 analysed, 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. Optimal will be a stable room temperature > 22 °C.

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

5.3 Setting up the Data Collection 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]**	**1560**

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content or critical patient samples an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **Chimera**® was modified in order 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	enter a name
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

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 on **OK**. A new table in the **Plate Editor** will open automatically

Plate Editor (II)

Parameter	**Set up**
Sample Name	Enter a name for the samples
Priority	e.g. 100 (Default)
Sample Type	Sample or allelic ladder
Size Standard	e.g. SST-BTO_60-500bp
Panel	e.g. Chimera_Panels_v1
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_26min (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 to 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**

5.4 Analysis parameter / analysis method

The recommended analysis parameters 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 (cut-off value) corresponds to the minimum peak height that will be detected by the GeneMapper® ID/ID-X software. The thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times 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.

6. Electrophoresis using the ABI PRISM® 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 named AB 3500 and the system with 24 capillaries is named AB 3500xL.

The virtual **filter set Any5Dye** shall be used for the combined application of 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 3500/3500xL
Polymer	POP-4® Polymer for 3500/3500xL
Buffer	10x Genetic Analyzer Buffer with EDTA for 3500/3500xL

6.1 Spectral calibration / matrix generation

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

Spectral calibration comprises the following steps:
- Preparation of spectral calibration standards
- Loading the standards to the multi-well reaction plate (one sample per capillary)
- Preparation of instrument and creating a Dye Set BT5
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 8 capillaries/ABI 3500

Component	Volume
Hi-Di™ Formamide	108.0 µl
Matrix standard **BT5**	9.0 µl

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

Example for 24 capillaries/ABI 3500xL

Component	Volume
Hi-Di™ Formamide	300.0 µl
Matrix standard **BT5**	25.0 µl

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-H1**, **A2-H2 and A3-H3***
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

* When using a 384-well plate, load 10 µl of the mixtures to columns 1, 3, and 5 in rows A, C, E, G, I, K, M, and O.

Performing a spectral calibration run

- Place the multi-well plate on the autosampler tray
- Now prepare the instrument and specific spectral calibration run settings

Preparation of the instrument

Before starting the spectral calibration process ensure that the spatial calibration has been performed. This process is necessary if a new capillary array was installed before and is described in detail in the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

Preparation of dye set BT5

Prior to the spectral calibration, a dye set for the matrix standard BT5 needs to be setup.

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.

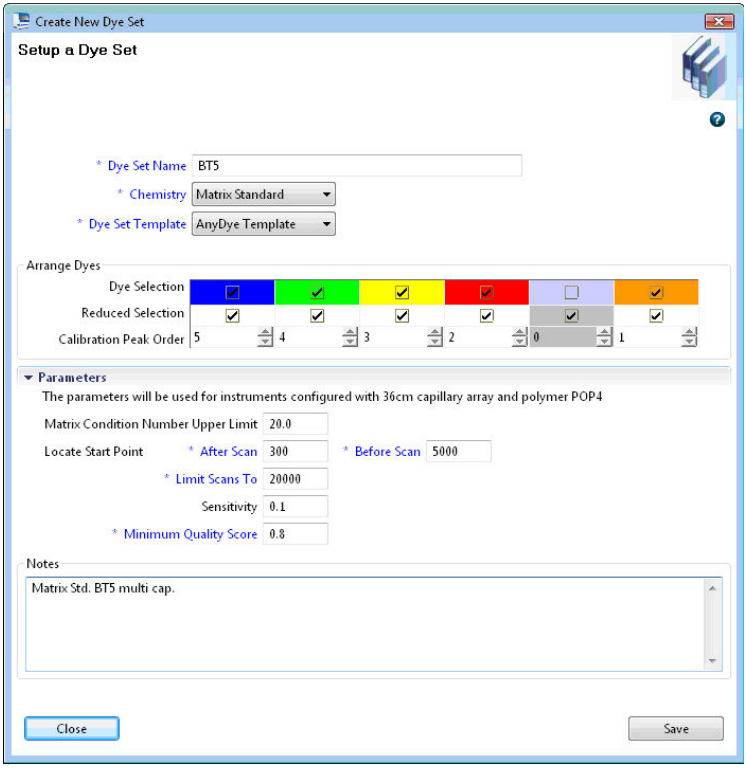

Fig. 5 Setup for dye set BT5

- In the **Protocol Manager** of the Data Collection Software click on **New** in **Instrument Protocol** to open the **Protocol Editor** dialog box

Performing a spectral calibration run

Once the multi-well plates containing the spectral calibration mixture is 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**.

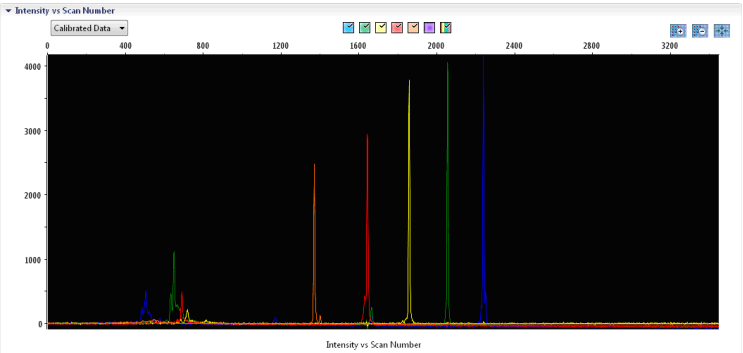

Fig. 6 Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3500

Matrix check

- The quality value (**Q value**) of each capillary must be greater than 0.8 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 five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- A successful calibration will be displayed in green in **Overall** and for each capillary
- If all capillaries have passed the test, **Accept Results**
- If calibration failed, **Reject Results** and refer to **spectral calibration troubleshooting** of Applied Biosystems 3500/3500xL Genetic Analyzer User Guides

6.2 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 and place samples on the autosampler tray

Since injections take place simultaneously on all capillaries, 8 or 24 samples must be pipetted on the plate of multi-capillary analyzers. If fewer samples are analysed empty positions need to 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. Optimal will be a stable room temperature > 22 °C.

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

6.3 Setting up a run

For the first run using the Mentype® **Chimera**® Kit you will need to setup a number of protocols within the 3500 Series Data Collection Software.

Create Instrument protocol

- Go to **Library** and select **Analyze / Instrument protocol** and click **Create**
- Change the parameters according the table below

Instrument protocol for Mentype® Chimera®

Parameter	**Set up**
Application Type	HID / Microsatellite
Capillary Length	*Default*
Polymer	*Default*
Dye Set	BT5
Run Module	*Default*
Protocol Name	e.g. Mentype Chimera
Oven Temperature [°C]	*Default*
Run Voltage [kV]	*Default*
Injection Voltage [kV]	**3.0**
Run Time [s]**	**1560**
PreRun Time [s]	*Default*
Injection Time [s]*	**10**
Data Delay Time [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 reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content or critical patient samples an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions, the run time for Mentype® **Chimera**® was modified in order to analyse fragments with lengths of up to **500 bp**.

- Click on **Save** to confirm the settings

Create Size Standard

- Go to **Library** and select **Analyze / Size Standards** and click **Create**
- Change the parameters according the table below

Parameter	Set up
Size Standard	BTO_550
Dye Color	Orange

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.

- Click on **Save** to confirm the settings

Create QC (Size Calling) Protocol

- Go to **Library** and select **Analyze / QC (Size Calling)** and click **Create**
- Change the parameters according the table below

Parameter	Set up
Protocol Name	enter a name
Size Standard	BTO_550 (from above)
Sizecaller	Size Caller v.1.1.0

- Go to **Analysis Settings / Peak Amplitude Treshold** and **disable purple**. All other colours should be enabled
- Keep all other settings as Default
- Click on **Save** to confirm the settings

Create an Assay

- Go to **Library** and select **Manage / Assays** and click **Create**
- Change the parameters according the table below

Parameter	Set up
Assay Name	e.g. Mentype Chimera
Color	Default
Application Type	HID
Instrument Protocol	e.g. Mentype Chimera
QC Protocols	e.g. BTO_550
Genemapper Protocol	could be defined

- Click on **Save** to confirm the settings

Starting the run

- Place the prepared multi-well plate on the autosampler tray
- In the Dashboard of the Data Collection Software, click **Create New Plate**
- Go **to Define Plate Properties** and select **Plate Details**
- Change the parameters according the table below

Plate Details

Property	**Set up**
Name	e.g. Mentype Chimera
Number of Wells	96 or 384
Plate Type*	HID
Capillary Length	**36cm**
Polymer	**POP4**

- Click **Assign Plate Contents** to confirm the settings
- Define well position of each sample or ladder for data collection and processing by entering sample names
- Assign an **Assay** (required) a **File Name Conventions** and a **Result Group** to all named wells in the plate
- Click **Link the plate for Run** and enter Run Name
- Click **Start Run**

7. Analysis

For general instructions on automatic sample analysis refer to the *GeneScan®* or *GeneMapper® ID or GeneMapper® ID-X Software User's Manual.*

Note: Within the Mentype® **Chimera**® the red panel should be faded out.

Finding the exact lengths of 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 STR loci, size-determination 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.

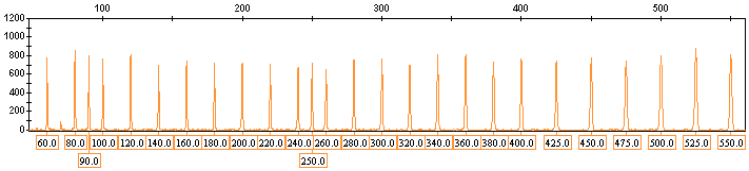

Fig. 7 Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

Note: The provided template files for the DNA size standard SST-BTO_60-500bp can be applied for the evaluation and analysis of the Mentype® **Chimera**® using the GeneMapper® ID or ID-X Software.

7.1 Biotype® template files

Allele allocation should be carried out with suitable analysis software, e.g. GeneMapper® ID/ID-X or Genotyper software in combination with the Mentype® **Chimera**® template files from Biotype. Biotype® template files are available on our homepage (www.biotype.de) for download or as CD-ROM on request.

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

Panels	Chimera_Panels_v1/v1X	or higher versions
BinSets	Chimera_Bins_v1/v1X	or higher versions
Size Standard	SST-BTO_60-500bp	
Analysis Method	Analysis_HID_310	
	Analysis_HID_3130	
	Analysis_HID_310_50rfu	
	Analysis_HID_3130_50rfu	
Plot Settings	PlotsBT5_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*	Chimera_Stutter_v1X	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:

Mentype_Chimera_v1	or higher versions

Important Note: Import and allele calling with provided template files is only guarantied using GeneMapper® ID/ID-X software. If GeneMapper® software is applied you may experience import problems using some template files. You may have to adjust Panels and Bins with one ore more runs of the allelic ladder on your specific instrument setup. Contact us for support (support@biotype,de).

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

7.2 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 Mentype® **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 alleles of reference DNA purchased from ATCC (http://atcc.org/Produtcs/PurifiedDNA.cfm#celllines), as well as three assignments of reference DNA purchased from Coriell Cell Repositories (CCR; http://locus.umdnj.edu/nigms/) standard of Szibor *et al.* (2003).

7.3 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 (BTO). 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: 70-480 bp
Vertical: Depending on signal intensity

Figure 8

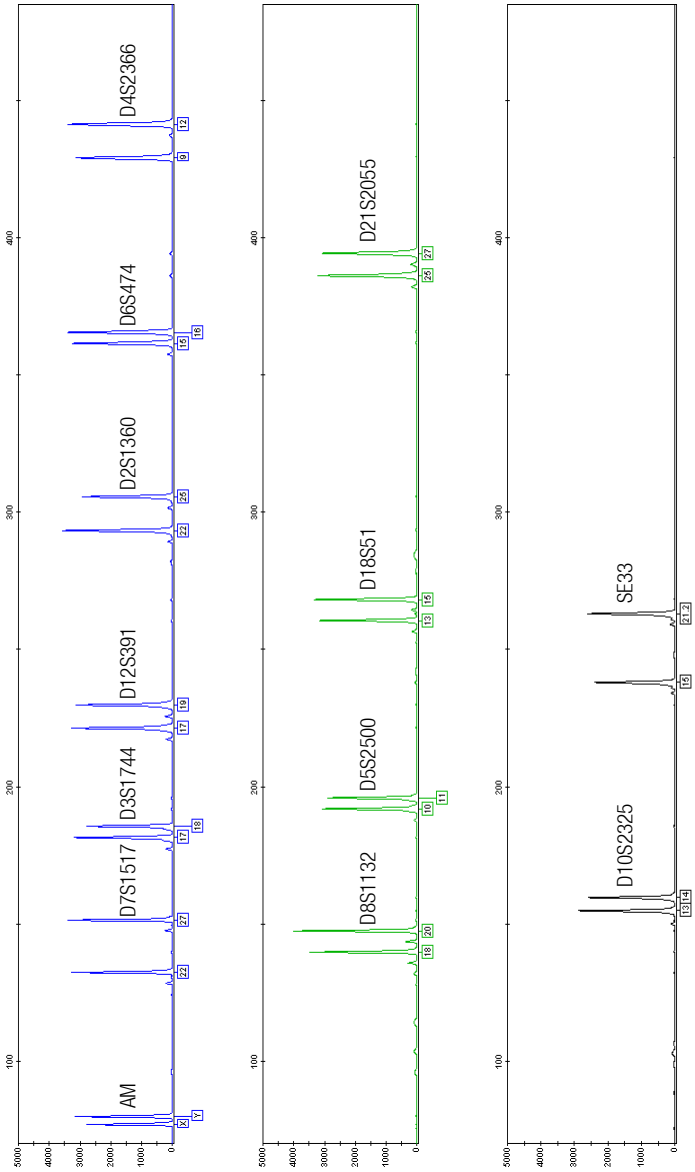

Fig. 8 Electropherogram of the Mentype® **Chimera**® using 500 pg Control DNA XY5. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper® ID Software and the Mentype® **Chimera®** template file.

Figure 9

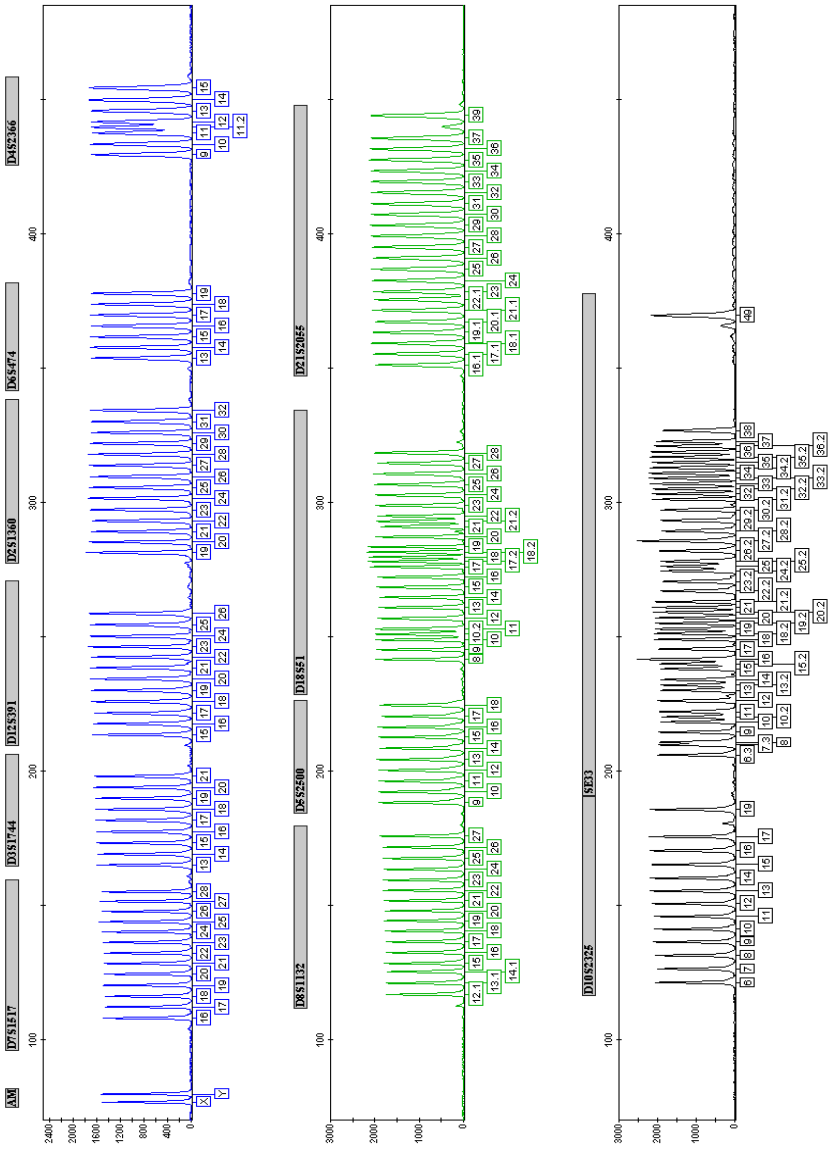

Fig. 9 Electropherogram of the allelic ladder Mentype® **Chimera®.** Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper® ID Software and the Mentype® **Chimera®** template file.

Table 4. Fragment lengths of the Mentype® **Chimera**® allelic ladder analysed on an ABI PRISM® 3130 Genetic Analyzer with POP-4® polymer. (blue panel)

Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**
Amelogenin	**6-FAM**		**D12S391**	**6-FAM**		**D6S474**	**6-FAM**	
X	77		15	213		13	354	11, 12
Y	80		16	217	16.3	14	358	
			17	221	17.3	15	362	
D7S1517	**6-FAM**		18	226	18.3	16	366	
16	108	14, 15	19	230	19.1, 19.3	17	370	
17	112		20	234	20.3	18	374	
18	116		21	238		19	378	
19	120		22	242				
20	124		23	246		**D4S2366**	**6-FAM**	
21	128		24	250		9	429	9.2
22	132		25	254		10	433	10.2
23	136		26	258	27	11	437	
24	140					11.2	440	
25	144		**D2S1360**	**6-FAM**		12	441	
26	148		19	281		13	445	
27	152		20	285		14	449	
28	155	29	21	289		15	454	
			22	293				
D3S1744	**6-FAM**		23	297				
13	165		24	302				
14	169		25	306				
15	173		26	310				
16	177		27	314				
17	182		28	318				
18	186		29	322				
19	190		30	326				
20	194		31	330				
21	198	22	32	334				

Table 5. Fragment lengths of the Mentype® **Chimera**® allelic ladder analysed on an ABI PRISM® 3130 Genetic Analyzer with POP-4® polymer (green panel)

Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**
D8S1132	**BTG**		**D18S51**	**BTG**		**D21S2055**	**BTG**	
12.1	117	12, 13	8	241	7	16.1	351	
13.1	121		9	245	9.2	17.1	355	
14.1	125	14.3	10	249		18.1	359	
15	128		10.2	251		19.1	363	
16	132		11	253	11.2	20.1	367	
17	136		12	257	12.2	21.1	371	
18	140		13	261	13.2	22.1	375	22
19	144		14	264	14.2	23	378	23.1
20	148		15	268		24	382	
21	151		16	272	16.2	25	386	
22	155		17	276		26	390	
23	159		17.2	278	17.3	27	395	
24	163		18	279		28	399	
25	167		18.2	281		29	403	
26	171		19	283	19.2	30	406	
27	175		20	287		31	411	
			21	291		32	415	
D5S2500	**BTG**		21.2	293		33	419	
9	188		22	295		34	423	
10	192		23	299	23.1	35	427	
11	196		24	302		36	431	
12	200		25	306		37	435	38
13	204		26	310		39	443	
14	208		27	314				
15	212		28	318	29			
16	216							
17	220							
18	224							

Table 6. Fragment lengths of the Mentype® **Chimera**® allelic ladder analysed on an ABI PRISM® 3130 Genetic Analyzer with POP-4® polymer (yellow panel)

Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**
D10S2325	**BTY**		**SE33**	**BTY**		**SE33**	**BTY**	
6	121		6.3	205	4.2, 5.3	25.2	278	
7	126		7.3	209	7	26.2	282	26
8	131		8	210	8.2	**27.2**‡	**285**	27
9	136		9	214	9.2	28.2	289	28, 28.3
10	141		10	218		29.2	293	29
11	145		10.2	220		30.2	297	30
12	150		11	222	11.2	31.2	301	31
13	155		12	226	12.2	32	303	
14	160		13	230		32.2	305	
15	165		13.2	232	13.3	33	307	
16	170		14	234	14.2, 14.3	33.2	309	
17	175	18	15	238		34	311	
19	185		15.2	240		34.2	313	
			16‡	**241**	16.2, 16.3	35	315	
			17	245	17.2, 17.3	35.2	317	
			18	249		36	318	
			18.2	251	18.3	36.2	321	
			19	253		37	322	37.2
			19.2	255		**38**	**326**	**39,42**
			20	257	20.1	49	369	50
			20.2	259				
			21	261				
			21.2	263	22			
			22.2	267				
			23.2	270	23			
			24.2	274	24			
			25	276				

* 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.

8. 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.

An automated calculation of the donor/recipient DNA ratio, as well as standard deviations and detection limits can be obtained directly from raw data of a fragment size analysis using e.g. Chimeris™ **Monitor** Software from Biotype Diagnostic GmbH.

If results that are obtained with Mentype® **Chimera**® should be harmonized to results from cytological analyses, make sure that cytological analyses were performed with at least 500 leucocytes.

Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range, or if an incorrect matrix was applied. They appear at positions of specific peaks in other color channels, typically with lower signal intensities.

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/ID-X software.

Template-independent addition of nucleotides

Because of its terminal transferase activity, the Multi 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 bp 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 min. Peak height of the artefact correlates with the amount of DNA. Laboratories should define their individual limits for analysis of the peaks.

Artefacts

Room temperature may influence the performance of PCR products on multi-capillary instruments, shoulder peaks or split peaks occur. Furthermore, automated assignment could be influenced in some cases. If these effects occur we recommend injecting the sample again at higher room temperature and maybe using more than one allelic ladder sample per run.

Influence of polymers

The Mentype® **Chimera**® kit was validated and certified for the analysis on POP-4® polymer.. The use of other polymers (e.g. POP-7™ or POP-6™) might influence the run behaviour of specific PCR products. Furthermore background noise might increase through different behaviour of free fluorescent dyes.

9. Population-genetic data

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

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

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

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

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.

10. References

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11. Explanation of Symbols

Manufacturer

Date of manufacture

Batch code

Contains sufficient reagents for <N> tests

Consult instructions (handbook) for use

Use by

Temperature limitations

Catalogue number

In-Vitro-Diagnostics

Notes

Notes