Mentype® **Nonaplex I**

PCR Amplification Kit

Instructions for use

The Mentype® **Nonaplex I** PCR Amplification Kit is a multiplex application for the Short Tandem Repeat (STR) loci, which belong to the German Forensic DNA Database and are recommended by EDNAP. In one PCR reaction, the eight polymorphic STR loci **D3S1358**, **D8S1179**, **D18S51**, **D21S11**, **FGA (FIBRA)**, **SE33 (ACTBP2)**, **TH01 (TC11)**, and **vWA** as well as the gender-specific **Amelogenin** are amplified simultaneously.

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Biotype GmbH develops, produces and distributes PCR-based applications for medical diagnostics.

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Our Mentype® test kits guarantee the highest quality standards for clinics and research.

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Warnings and safety instructions

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.

Content

1. Product description

The Mentype® **Nonaplex I** PCR Amplification Kit is a multiplex application for the Short Tandem Repeat (STR) loci, which belong to the German Forensic DNA Database and are recommended by EDNAP. In one PCR reaction, the eight polymorphic STR loci **D3S1358**, **D8S1179**, **D18S51**, **D21S11**, **FGA (FIBRA)**, **SE33 (ACTBP2)**, **TH01 (TC11)**, and **vWA** as well as the gender-specific **Amelogenin** are amplified simultaneously.

This application was developed for fast and reliable DNA genotyping of blood, buccal swabs and forensic stains. The primers are fluorescence-labelled with **6-FAM** (Amelogenin, D3S1358, TH01, and SE33), **HEX** (vWA, FGA, and D18S51) or **NED** (D8S1179 and D21S11). For genotyping of rare FGA alleles (42.2-51.2), we recommend to use Mentype® **Nonaplex**^{QS}.

The detection limit of Mentype® **Nonaplex I** PCR Amplification Kit is less than **200 pg genomic DNA**. However, it is recommended to use **0.5-1.0 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 Mentype® Nonaplex I

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

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

Table 2. Chromosomal mapping of Mentype® Nonaplex I

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

Content

Mentype® **Nonaplex I** PCR Amplification Kit (100 Reactions)

Nuclease-free water	3.0 mL
Reaction mix **B**	500 µL
Primer mix	250 µL
DNA polymerase	40 µL
Control DNA XY1 (2 ng/µL)	10 µL
DNA Size Standard 550 (ROX)	50 µL
Allelic ladder	10 µL

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 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	Thermo Fisher Scientific Inc.	4311320
Matrix Standards DS-30 for ABI PRISM® 310 Genetic Analyzer	Thermo Fisher Scientific Inc.	401546 and 402996 (NED)
Matrix Standards DS-30 for ABI PRISM® multi-capillary instruments	Thermo Fisher Scientific Inc.	4345827

2. Protocols for amplification and electrophoresis

2.1 PCR amplification

Master mix preparation

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

Component	Volume
Nuklease-free water	16.1 µL
Reaction mix **B***	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 XY1** 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 XY1 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 containing the PCR master mix.

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	
58°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	
58°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.

2.2 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

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 Life Technologies GmbH.

Colour	**Matrix standard**	**Order number**
Blue (B)	6-FAM	Life Technologies GmbH, 4345827
Green (G)	HEX	Life Technologies GmbH, 4345827
Yellow (Y)	NED	Life Technologies GmbH, 4345827
Red (R)	ROX	Life Technologies GmbH, 4345827

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	12.0 µL
	Matrix standard **6-FAM**	1.0 µL
Matrix sample 2	Hi-Di™ Formamide	12.0 µL
	Matrix standard **HEX**	1.0 µL
Matrix sample 3	Hi-Di™ Formamide	12.0 µL
	Matrix standard **NED**	1.0 µL
Matrix sample 4	Hi-Di™ Formamide	12.0 µL
	Matrix standard **ROX**	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).

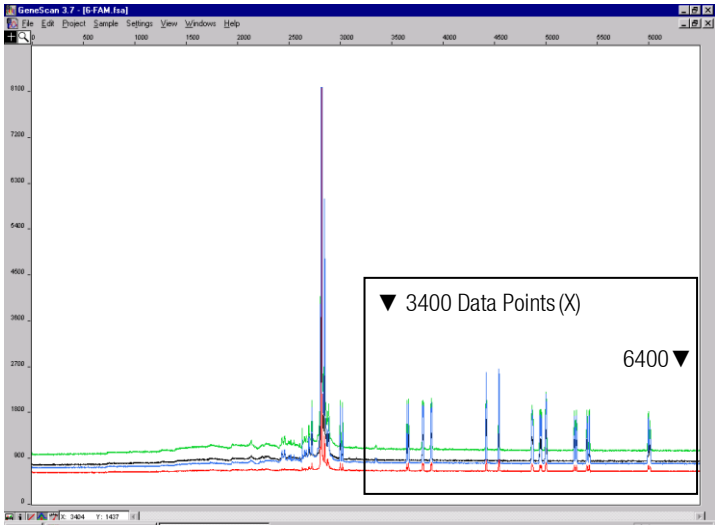

Fig. 1 Electropherogram with raw data of the matrix standard 6-FAM

- 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**

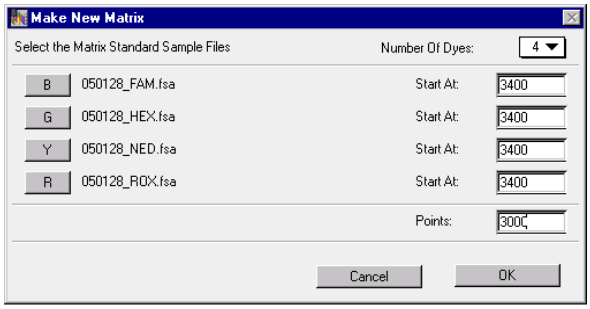

Fig. 2 Matrix sample selection

- 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

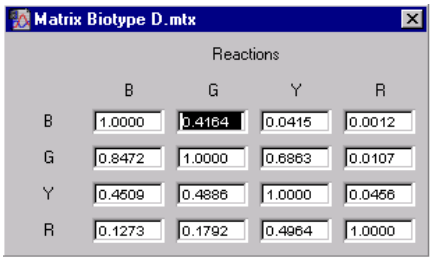

Fig. 3 New matrix DS-30

- 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.

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

Setting up the GeneScan® software

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

Injection list

Component	**Set up**
Module File	GS STR POP-4 (1 mL) **D**
Matrix File	e.g. Matrix DS-30
Size Standard	e.g. SST_ROX_50-400bp
Injection [s]*	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]**	**26**

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

** Depending on the analysis conditions the run time for Mentype® **Nonaplex I** was modified in order to analyse fragments with lengths of up to **400 bp**.

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.

2.3 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

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

- Add 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

- Add 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_POP4_DS30 (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.

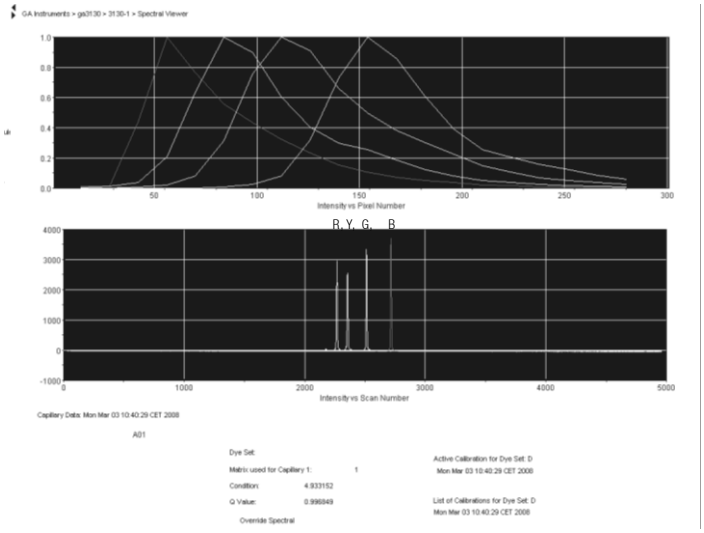

Fig. 4 Electropherogram of spectral calibration with matrix standard DS-30 on an ABI 3130

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.

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, several ladders should be run.

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

Signal intensities

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

Setting up the GeneMapper™ ID software

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

Run Module 3kV_10s_450bp

Run Modul Editor	**Set up**
Oven Temperature [°C]	*Default*
Poly Fill Volume	*Default*
Current Stability [µA]	*Default*
PreRun Voltage [kV]	*Default*
PreRun Time [s]	*Default*
Injection Voltage [kV]	**3.0**
Injection Time [s]*	**10**
Voltage Number of Steps	*Default*
Voltage Step Interval	*Default*
Data Delay Time [s]	*Default*
Run Voltage [kV]	*Default*
Run Time [s]**	**1320**

* 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 Mentype® **Nonaplex I** was modified in order to be able to analyse fragments with lengths of up to **400 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_450bp) 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_22min
Type	REGULAR
Run Module*	3kV_10s_450bp
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_50-400bp
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.**

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.

3. 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**.

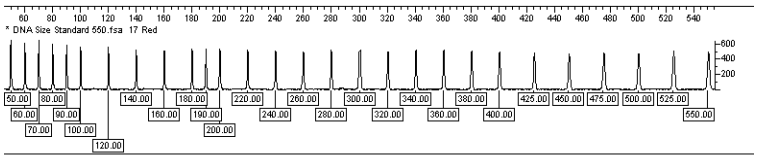

Fig. 5 Electropherogram of the DNA Size Standard 550 (ROX), fragments with lengths in bp

Note: The basic template files for the DNA Size Standard 550 (ROX) has to be adjusted to 400 bp within the GeneMapper™ ID software. The new template could be saved as e.g. SST-ROX_50-400bp and used for further analyses.

4. 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 Mentype® **Nonaplex I** template files from Biotype. Template files are available on 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-BTO_50-500bp (adjust up to 400bp, adjustment described earlier)	
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:

Nonaplex I_v3e	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

5. Controls

The Control DNA XY1 of the test kit and other commercially available DNA from standard cell lines represent the following alleles:

Table 3. Allele assignment of Mentype® Nonaplex I

Locus	Control DNA XY1	ATCC K-562	CCR 9947A	CCR 9948	CCR 3657
Amelogenin	X / Y	X / X	X / X	X / Y	X / Y
D3S1358	17 / 18	16 / 16	14 / 15	15 / 17	16 / 18
D8S1179	9 / 10	12 / 12	13 / 13	12 / 13	15 / 16
D18S51	12 / 14	15 / 16	15 / 19	15 / 18	12 / 20
D21S11	27 / 28	29 / 30 / 31	30 / 30	29 / 30	28 / 29
FGA	20 / 26	21 / 24	23 / 24	24 / 26	18 / 23
SE33	17 / 21.2	26.2 / 28.2	19 / 29.2	23.2 / 26.2	22.2 / 27.2
THO1	6 / 9.3	9.3 / 9.3	8 / 9.3	6 / 9.3	7 / 9.3
vWA	15 / 18	16 / 16	17 / 18	17 / 17	14 / 19

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).

6. 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: 75-405 bp
Vertical: Depending on signal intensity

Figure 6

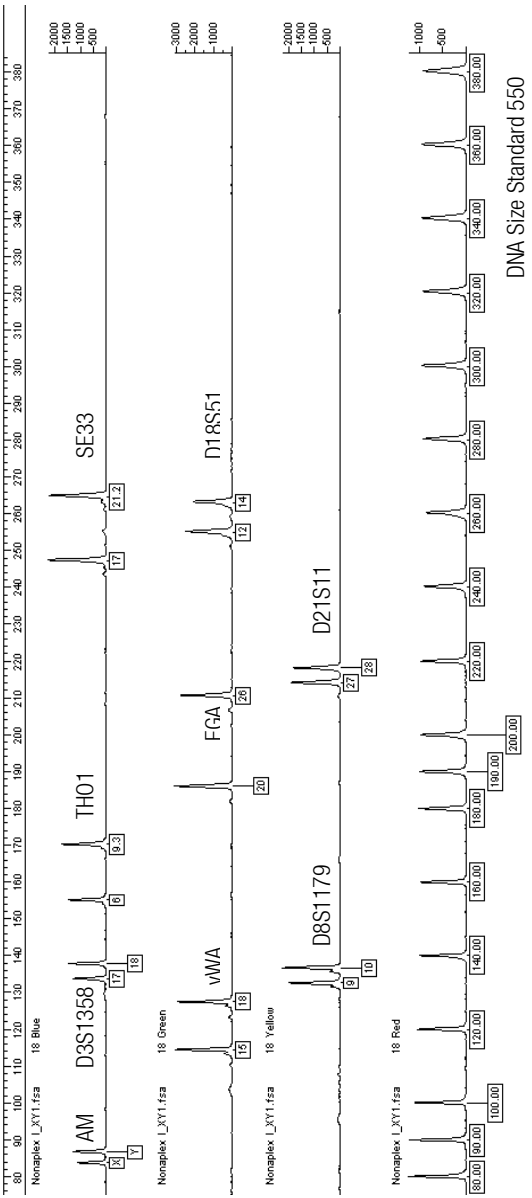

Fig. 6 Electropherogram of the Mentype® **Nonaplex I** using 500 pg Control DNA XY1. Analysis was performed on an ABI PRISM® 310 Genetic Analyzer with the DNA Size Standard 550 (ROX). Allele assignment was performed using the Genotyper® software and the Mentype® **Nonaplex I** template file.

Figure 7

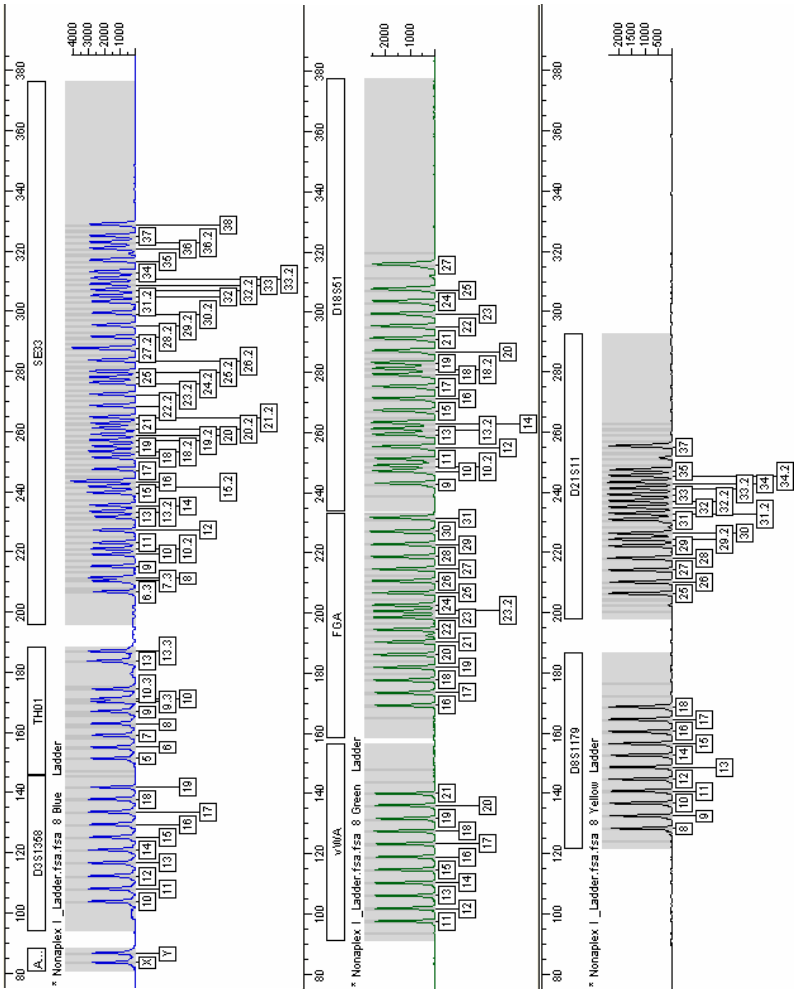

Fig. 7 Electropherogram of the allelic ladder Mentype® **Nonaplex I** analysed on an ABI PRISM® 310 Genetic Analyzer. Allele assignment was performed using the Genotyper® software and the Mentype® **Nonaplex I** template files.

Table 4. Fragment lengths of the allelic ladder Mentype® Nonaplex I 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**		**SE33**	**6-FAM**		**SE33**	**6-FAM**	
X	83		6.3	207	4.2, 5.3	25	278	
Y	86		7.3	211	7	25.2	280	
			8	212	8.2	26.2	283	26
D3S1358	**6-FAM**		9	215	9.2	**27.2‡**	287	27
10	104	8, 9	10	219		28.2	291	28, 28.3
11	108		10.2	221		29.2	295	29
12	112		11	223	11.2	30.2	299	30
13	117		12	227	12.2	31.2	303	
14	121		13	231		32	305	
15	125		13.2	233	13.3	32.2	307	
16	130		14	235	14.2, 14.3	33	309	
17	134		15	239		33.2	311	
18	138		15.2	241		34	313	34.2
19	142	20	**16‡**	243	16.2, 16.3	35	317	35.2
			17	247	17.2, 17.3	36	321	
TH01	**6-FAM**		18	251		36.2	323	
5	152	4	18.2	253	18.3	37	325	37.2,39, 42
6	155	6.3	19	255		38	329	49
7	159	7.3	19.2	257				
8	163	8.3	20	259	20.1			
9	167	9.1	20.2	261				
9.3	170		21	262				
10	171		21.2	264				
10.3	174	11	22.2	268	22			
13	184		23.2	272	23			
13.3	187		24.2	276	24			

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

* 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

Table 5. Fragment lengths of the allelic ladder Mentype® Nonaplex I 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**
vWA	**HEX**		**FGA**	**HEX**		**D18S51**	**HEX**	
11	98	10	16	170	14, 15, 16.1	9	243	8, 9.2
12	102		17	174		10	247	
13	106		18	178	18.2	10.2	249	
14	110		19	182	19.2	11	251	11.2
15	115		20	187	20.2	12	255	12.2
16	119		21	191	21.2	13	259	
17	123		22	195	22.2	13.2	261	
18	128		23	199		14	263	14.2
19	132		23.2	201	23.3	15	267	
20	136		24	203	24.1, 24.2	16	271	16.2
21	140	22, 23, 24	25	207	25.2	17	275	17.2, 17.3
			26	211	26.2	18	279	
			27	215		18.2	281	
			28	219		19	283	19.2
			29	223		20	287	
			30	228	30.2	21	291	21.2
			31	232	31.2	22	295	
						23	299	23.1
						24	303	
						25	308	26
						27	316	28, 29

* 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

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

Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**
D8S1179	**NED**		**D21S11**	**NED**	
8	129	7	25	206	23.2, 24, 24.2, 25.2
9	133		26	210	26.2
10	137		27	214	
11	141		28	218	28.2, 28.3
12	145		29	222	
13	149		29.2	224	29.3
14	153		30	226	30.2
15	157		31	231	
16	161		31.2	233	
17	165		32	235	
18	169	19, 20	32.2	237	
			33	239	33.1
			33.2	241	
			34	243	34.1
			34.2	245	
			35	247	35.2, 36, 36.2
			37	255	37.2, 38, 38.2, 39

* 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

7. 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.

8. 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*.

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.*