Mentype® **Nonaplex I**

Handbook

RUO For research use only. Not for use in diagnostic procedures.

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BIOTYPE

Change Notice

Please note the following changes from the previous version of the Handbook:

Document number	Change	Date
NONAHB01v1en	Update to installation procedure for Matrix DS30, ABI 3130 discontinued	01.04.2026

A printed version of this handbook can be provided free of charge within 7 days.

If you have any further questions, please feel free to contact us

at +49 351 8838 400 or

support@biotype.de

Contents

Product Description

The Mentype® Nonaplex I PCR Amplification Kit is a multiplex assay for the short tandem repeat (STR) loci to be recorded in the German Forensic DNA Analysis Database (DAD). In a single PCR reaction, the eight polymorphic STR loci D3S1358, D8S1179, D18S51, D21S11, FGA (FIBRA), SE33 (ACTBP2), TH01 (TC11), and vWA, as well as the sex marker amelogenin, are amplified simultaneously.

The test kit was specifically developed for the rapid and reliable generation of DNA results from blood samples or buccal swabs from reference individuals, as well as from trace evidence. The primers are labeled with the fluorescent dyes 6-FAM (amelogenin, D3S1358, TH01, and SE33), HEX (vWA, FGA, and D18S51), or NED (D8S1179 and D21S11). When compiling the primer mixture, particular attention was paid to ensuring a balanced signal intensity across the individual DNA systems.

The limit of detection for the Mentype® Nonaplex I test kit is less than 200 pg of genomic DNA. We recommend using 0.5 – 1.0 ng of DNA.

Validation and evaluation of the test kit were performed on the GeneAmp® 9700 Thermocycler, Applied Biosystems™ 310 Genetic Analyzer, Applied Biosystems™ 3100/3130 Genetic Analyzer, and Applied Biosystems™ 3500 Genetic Analyzer.

The test kit is intended for research use only; use for diagnostic purposes is not permitted.

The test kit may only be used by professional users who are trained in molecular biology techniques in general and the handling of Genetic Analyzers of Thermo Fisher Scientific (Applied Biosystems division).

Table 1 Locus-specific information for Mentype® Nonaplex I

Locus	GenBank® Accession	Repeat motif	Locus	GenBank® Accession	Repeat motif	Locus
Amelogenin X	M55418				Amelogenin X	M55418
Amelogenin Y	M55419				Amelogenin Y	M55419
D3S1358	11449919	TCTA $[TCTG]_2$ $[TCTA]_{15}$	18	8-26	D3S1358	11449919

Locus	GenBank® Accession	Repeat motif	Locus	GenBank® Accession	Repeat motif	Locus
D8S1179	G08710	$[TCTA]_{12}$	12	6-21.2	D8S1179	G08710
D18S51	L18333	$[AGAA]_{13}$	13	5.3-42	D18S51	L18333
D21S11	AP000433	$[TCTA]_4$ $[TCTG]_6$ $[TCTA]_3$ TA $[TCTA]_3$ TCA $[TCTA]_2$ TCCATA $[TCTA]_{11}$	29	12-46	D21S11	AP000433
FGA (FIBRA)	M64982	$[TTTC]_3$ TTTTTT CT $[CTTT]_{13}$ CTCC $[TTCC]_2$	21	12.2–51.2	FGA (FIBRA)	M64982
SE33 (ACTBP2)	NG000840	$[AAAG]_9$ AA $[AAAG]_{16}$	25.2	3-50	SE33 (ACTBP2)	NG000840
TH01 (TC11)	D00269	$[TCAT]_9$	9	3-14	TH01 (TC11)	D00269
vWA	M25858	TCTA $[TCTG]_4$ $[TCTA]_{13}$	18	10-26	vWA	M25858

Table1 shows the STR loci with their repeat motifs and alleles. The nomenclature follows the guidelines of the International Society for Forensic Genetics (ISFG), Bär et al. (1997). The specified allele range takes into account the known alleles of the National Institute of Standards and Technology (NIST, as of 12/2008) as well as the current literature.

Table 2 Chromosomal mapping for 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

Supplied Materials

The following reagents for performing the Mentype® Nonaplex I kit are included in the kit:

Component	Cap Color		Volume per package size	
			100 reactions	400 reactions
Nuclease-Free Water	Light blue		2 x 1.5 mL	6 x 1.5 mL
Reaction Mix B	Violet		500 µL	2 x 1.0 mL
Mentype® Nonaplex I Primer Mix	Red		250 µL	4 x 250 µL
Multi Taq 2 DNA Polymerase	White		40 µL	160 µL
Control DNA XY 82 (2 ng/µL)	White		10 µL	10 µL
DNA Size Standard 550 (ROX)	Orange		50 µL	200 µL
Mentype® Nonaplex I Allelic Ladder	Green		10 µL	4 x 10 µL

NOTE

Please note that the package size indicates the number of tests, without taking into account the number of required controls or the excess volume needed for pipetting.

Reagent Storage and Handling

The kit is shipped on dry ice. The kit components should arrive in a frozen state, with the exception of the Multi Taq 2 DNA polymerase. This is stored in a buffer that prevents the reagent from freezing.

Please check the kit for completeness upon receipt. Do not use kits that have thawed upon arrival. If one or more components are not frozen, or if the tubes or packaging have been damaged during transport, performance cannot be guaranteed.

Store all components protected from light at -25 °C to -15 °C. In particular, the Mentype® Nonaplex I Primer Mix, DNA Size Standard 550 (ROX), and Mentype® Nonaplex I Allelic Ladder must be stored protected from light.

To avoid contamination, we recommend storing the pre-PCR templates (DNA samples, Control DNA XY82) and the post-PCR components (DNA Size Standard 550 (ROX) and Mentype® Nonaplex I Allelic Ladder) separately from the PCR reagents (Nuclease-Free Water, Multi Taq 2 DNA Polymerase, Reaction Mix A, Mentype® Nonaplex I Primer Mix) and to use them separately.

The shelf life of the kit is indicated on the label of the kit box. Do not exceed the maximum number of 20 freeze-thaw cycles.

Required materials not included

General Laboratory Equipment

- Benchtop centrifuge with a rotor for 2 mL reaction tubes
- Centrifuge with a rotor for microtiter plates
- Vortex mixer

- Calibrated, adjustable pipettes with aerosol-tight filter tips
- Suitable 200 µL 96-well reaction plates (depending on the manufacturer) with suitable film, PCR quality
- Suitable racks for 2 mL reaction tubes
- Cooling rack for 2 mL reaction tubes
- Powder-free disposable gloves
- NanoDrop™ spectrophotometer or Qubit fluorometer
- PCR workstation or laminar flow hood

Reagents, kits, and consumables

Table 3 Required but not supplied reagents

Reagent	Supplier	Order number
Hi-Di™ Formamide, 25 mL	Thermo Fisher Scientific Inc.	4311320
Matrix Standard DS-30 for Applied Biosystems™ multi-capillary instruments	Thermo Fisher Scientific Inc.	4345827

Warnings and Safety Instructions

- Read the handbook carefully before using the product.
- Read the Safety Data Sheets (SDS) and Non-Hazardous Statements (NHS) for all BIOTYPE products. We will send these to you upon request or they are available for download on our website (https://www.biotype.de/sicherheitsdatenblatter). For products that do not contain any substances of very high concern or are not subject to other restrictions under Regulation 1272/2008 (CLP) and therefore do not require an SDS, BIOTYPE will provide the SDS upon request.
- Please contact the respective manufacturers to obtain copies of the safety data sheets for any additional reagents required.
- Kit components from different kit batches must not be mixed.
- Aliquoting kit components into other reaction vessels is not permitted.
- Use of this product is restricted to professional laboratory users trained in molecular genetic techniques, multiplex PCR, and the operation of Thermo Fisher Scientific genetic analyzers.

- Before first use, check the product and its components for: Integrity
- Completeness in terms of quantity, type, and contents (see the “Supplied Materials” section)
- Correct labeling
- Condition upon arrival (all components frozen, except Multi Taq 2 DNA Polymerase)

- Samples should always be treated as infectious and/or biohazardous and in accordance with safe laboratory procedures and good laboratory practice.
- Do not use a kit that has passed its expiration date.
- Dispose of the samples and kit waste in accordance with local safety regulations.

Protocols for amplification and electrophoresis

PCR amplification

Master Mix Preparation

The following table shows the volumes of PCR reagents used for a 1.0 µL sample volume (template DNA) in a 25 µL reaction volume. When determining the number of PCR reactions, include the positive and negative controls. Add one or two additional reactions to this number to account for pipetting errors.

Component	Volume
Nuclease-Free Water	16.1 µL
Reaction Mix B*	5.0 µL
Mentype® Nonaplex I Primer Mix	2.5 µL
Multi Taq 2 DNA Polymerase	0.4 µL
Volume of the Master Mix	**24.0 µL**

* contains Mg^{2+}, dNTPs, BSA

All reagents should be mixed (vortexed) and briefly centrifuged (approx. 10 s) before preparing the Master Mix.

The amount of DNA to be used depends on its concentration. For reference samples, 1 µL is usually sufficient. For trace samples, 5 µL may be necessary. Increasing the DNA amount beyond 5 µL is not recommended, as any PCR inhibitors present may not be sufficiently diluted. The amount of nuclease-free water must be adjusted accordingly so that the total volume of the PCR reaction mixture is always 25 µL.

Store your DNA samples 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 mixtures are designed such that balanced peak heights are achieved with 0.5 ng of Control DNA XY82 in a reaction volume of 25 µL over 30 PCR cycles. If more template DNA is used, very high peaks are to be expected for small PCR fragments and relatively low peaks for larger PCR fragments. Reduce the amount of DNA to correct this imbalance.

Positive Control (PC)

For the positive control, dilute Control DNA XY82 to 0.5 ng in the appropriate volume with Nuclease-Free Water. Pipette the diluted Control DNA into the reaction tubes containing the provided PCR Master Mix in place of the template DNA.

Negative control (NTC)

As a negative control, pipette Nuclease-Free Water into the reaction tubes containing the provided PCR Master Mix instead of the template DNA.

PCR Amplification Parameters

Program the PCR cycler with the following protocol and set the heating and cooling rates (ramping) to 1.5 °C/s. Perform a "Hot Start" PCR to activate the polymerase and prevent the formation of non-specific amplification products.

The number of cycles depends on the amount of DNA. For all samples, 30 PCR cycles are recommended. For critical trace samples (< 100 pg DNA), 34 cycles are optionally recommended to achieve optimal signal intensities.

Table 4 Standard method, recommended for all DNA samples

Temperature	Time*	
94°C	4 min (hot start for activation of Multi Taq2 DNA polymerase)	
94°C	30 s	
58°C	**120 s**	**30 cycles**
72°C	75 s	
68°C	60 min	
10°C	∞	until the end

*Heating and cooling rates (ramping) should be set to 1.5 °C/s

Table 5 Optional settings, recommended for trace samples with low DNA quantities

Temperature	Time*	
94°C	4 min (hot start for activation of Multi Taq2 DNA polymerase)	
94°C	30 s	
58°C	**120 s**	**34 cycles**
72°C	75 s	
68°C	60 min	
10°C	∞	until the end

*Heating and cooling rates (ramping) should be set to 1.5 °C/s

Due to insufficient DNA quantities, statistical dropouts (allelic dropouts) and unbalanced peak heights may occur. Additionally, the likelihood of nonspecific amplification products increases. As the number of cycles increases, cross-contamination may also occur due to minimal amounts of foreign DNA.

Electrophoresis on the Applied Biosystems™ 3500/3500XL Genetic Analyzer

General instructions regarding the analyzer, spectral calibration, and the use of the Applied Biosystems 3500 Series Data Collection Software and the

GeneMapper™ ID/ID-X Software can be found in the corresponding Applied Biosystems™ 3500/3500XL Genetic Analyzer User Guide.

For the combined use of the five fluorescent dyes 6-FAM, HEX, NED, and ROX, the virtual filter set F is intended for use (the matrix standard is referred to below as DS-30).

Material	
Capillary	36 cm Capillary Array for 3500/3500xL
Polymer	POP-4® Polymer for 3500/3500xL

Spectral Calibration/Matrix Preparation

Before performing fragment length analysis, spectral calibration must first be performed using the DS-30 matrix standard for the respective analyzer. This creates a matrix that corrects for the overlap of the color-specific fluorescence emission spectra.

Please strictly follow the instructions provided by the matrix standard manufacturer. Adherence to these guidelines is crucial for creating a reliable matrix and, consequently, for the precise quantification and qualitative analysis of your samples. Deviations can lead to incorrect measurement results.

Sample Preparation

Component	Volume
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (ROX)	0.5 µL
Add 12 µL of the mixture (formamide + DNA Size Standard 550) to all samples	
Add 1 µL PCR product (diluted if necessary) or allele ladder	
- Denature for 3 min at 95 °C	
- Cool to 4 °C and place in the instrument for analysis	

Since injection occurs simultaneously in all capillaries, 8 or 24 samples must always be pipetted onto the plate on the multi-capillary instrument. If fewer

samples are to be measured, the corresponding positions must be filled with 12 µL of Hi-Di™ formamide.

Room temperature can significantly influence the migration behavior of PCR products in multi-capillary instruments and, if the temperature is too low, lead to the occurrence of split peaks. Under certain circumstances, the temperature may also affect the migration speed of the fragments. Please ensure that the operating temperature recommended by the instrument manufacturer is maintained. Stable room temperatures > 22 °C are optimal.

Analysis

General instructions for automatic analysis can be found in the corresponding *GeneMapper™ ID/ID-X Software User's Manual*.

Determining the exact fragment lengths of the amplified products depends on the type of instrument, the electrophoresis conditions, and the DNA length standard used. Due to the complexity of some STR loci, as many evenly distributed reference points as possible should be used for length determination. For this purpose, use the DNA length standard 550 (ROX) with the following fragment lengths: **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**.

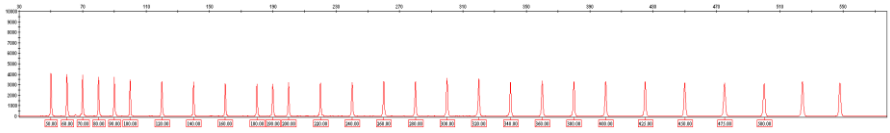


Fig.1 Electropherogram of the DNA Length Standard 550 (ROX), fragment lengths in bp

Note: The template for the DNA Length Standard 550 (ROX) used with GeneMapper™ ID/ID-X software must be adjusted to 400 bp for Mentype® Nonaplex I. The new template can be saved under the name SST-ROX_50-400bp and used for further analyses.

BIOTYPE Analysis Templates

Allele assignment of the separated PCR products (genotyping) can be performed using suitable analysis software, e. g., GeneMapper™ ID or ID-X

software in combination with BIOTYPE's Mentype® Nonaplex I analysis templates. Mentype® analysis templates (template files) for GeneMapper™ ID or ID-X software are available for download on our website (www.biotype.de) or upon request via support@biotype.de.

The recommended BIOTYPE templates for GeneMapper™ ID/ID-X software are:

Template	Template name	
Panels	Nonaplex_I_Panels_v3/v3x	or higher version
BinSets	Nonaplex_I_Bins_v3/v3x	or higher version
Size Standard	SST-ROX_50-500bp (adjust up to 400bp; setting described above)	
Analysis Method	Analysis_HID_Nonal	
	Analysis_HID_Nonal_50rfu	
Plot Settings	Plots_4dyes	
Table Settings	Table for 2 Alleles	
	Table for 10 Alleles	

The panels and bin sets must always be used; the other analysis templates are optional.

Stutter*	Nonaplex_I_Stutter_v3X	or higher version

* When loading the panels listed above, the stutter settings are not accepted; therefore, the stutter file must be imported separately.

General procedure for evaluation:

1. Check the size standard
2. Check the Allelic Ladder
3. Check the positive control
4. Check the negative control (No Template Control)
5. Evaluate sample data

Controls

The control DNA XY82 included in the test kit, as well as commercially available DNAs, represent the following alleles:

Table 6 Allele assignments with Mentype® Nonaplex I

Locus	Control DNA XY82	Control DNA XY1	ATCC K-562	CCR 9947A	CCR 9948	CCR 3657
Amelogenin	X / Y	X / Y	X / X	X / X	X / Y	X / Y
D3S1358	16 / 17	17 / 18	16 / 16	14 / 15	15 / 17	16 / 18
D8S1179	8 / 14	9 / 10	12 / 12	13 / 13	12 / 13	15 / 16
D18S51	13 / 16	12 / 14	15 / 16	15 / 19	15 / 18	12 / 20
D21S11	30 / 31	27 / 28	29 / 30 / 31	30 / 30	29 / 30	28 / 29
FGA	22 / 26	20 / 26	21 / 24	23 / 24	24 / 26	18 / 23
SE33	2.27 / 2.28	17 / 21.2	2.26 / 2.28	19 / 29.2	2.23 / 2.26	2.22 / 2.27
THO1	6 / 9	6 / 9.3	9.3 / 9.3	8 / 9.3	6 / 9.3	7 / 9.3
vWA	15 / 17	15 / 18	16 / 16	17 / 18	17 / 17	14 / 19

The table lists the alleles of reference DNA available from ATCC (http://www.atcc.org/Products/PurifiedDNA.cfm#celllines) and Coriell Cell Repositories (CCR; http://locus.umdnj.edu/nigms). This complies with the requirements of Szibor et al. (2003).

Interpretation of the results

The evaluation described above, which includes automatic allele assignment, ensures accurate and reliable differentiation of the alleles.

Pull-up peaks

Pull-up peaks may occur between color panels if an unsuitable matrix was used for the analysis or if the peak heights lie outside the linear detection range, e. g., greater than 10,000 RFU (Applied Biosystems™ 3500 Genetic Analyzer). These appear at the same position as specific peaks in other color panels (usually with lower signal intensities). To avoid pull-up peaks between color panels, peak heights should therefore not significantly exceed these limits.

Stutter Peaks

The occurrence of stutter peaks depends on the sequence and number of repeat units. In tetranucleotide STR motifs, errors by Taq DNA polymerase during PCR result in n-4 peaks, i. e., the stutter peak is 4 bases shorter than the true allele. Repeat units are skipped within the STR. For peak evaluation, the specifications of the template files for the GeneMapper™ ID or ID-X software apply.

Template-independent nucleotide annealing

Due to its terminal transferase activity, Taq DNA polymerase preferentially appends an adenosine to the 3' end of the amplified DNA fragment. If the PCR system does not have sufficient time for extension or if the primer sequences do not favor extension, this addition does not occur. This artifact is recognizable by the appearance of a fragment shortened by one base (-1 peak). All Biotype primers are designed to minimize this artifact formation. Additionally, the formation of the artifact is reduced by the final extension step in the PCR protocol (68 °C for 60 min). The peak height of the artifact increases with high DNA concentrations. Each analytical laboratory should establish its own threshold values for evaluating the peaks.

Artifacts

Room temperature can significantly influence the run behavior of PCR products on capillary instruments and, if the temperature is too low, lead to the appearance of shoulders or double peaks (split peaks). If these effects are observed, we recommend reinjecting the samples.

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.*

Symbols

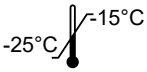
Manufacturer

Batch code

Sufficient for <N> tests

Reference to eIFU

Use by



Temperature limit

Catalogue Number

RUO	For scientific use only. Not for use in diagnostic procedures.
	Protect from light
	Store in a dry place

Other terms used in this manual:

	Useful tips
	Warning: Be sure to follow this instruction!
Text underlined in blue	Links to external content such as websites or email addresses
Text underlined in black	Cross-references in the document for easy navigation

Appendix

Electropherograms of reference samples

The values for fragment lengths of individual alleles listed inTable77 throughTable9 refer to the DNA Size Standard 550 (ROX) and measurements taken on the Applied Biosystems™ 3130 Genetic Analyzer with POP-4 polymer. Different analyzers, DNA length standards, or polymers may result in different fragment lengths. It is recommended to perform a visual comparison with the corresponding allele ladder for each run.

Scaling

Horizontal: 75 – 405 bp

Vertical: based on sample signal intensity

Fig.2 Electropherogram of the Mentype® Nonaplex I using 500 pg of control DNA XY82. The analysis was performed on the Applied Biosystems™ 3500 Genetic Analyzer using the DNA Size Standard 550 (ROX). Allele assignment was performed using Genotyper® software and the Mentype® Nonaplex I template file.

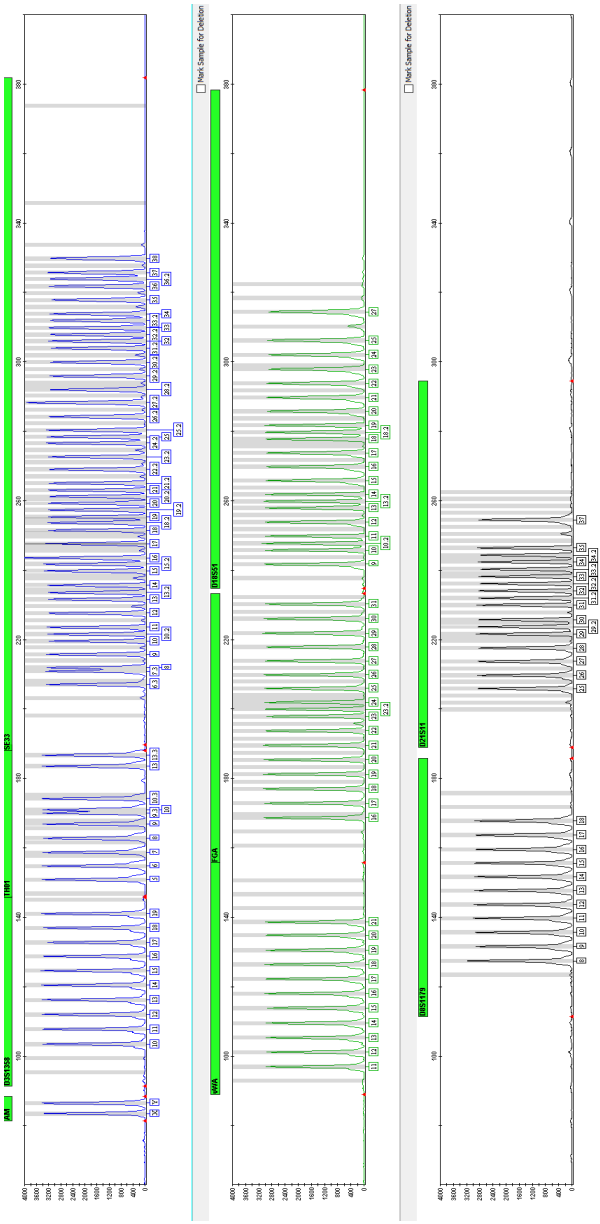


Fig.3 Electropherogram of the Mentype® Nonaplex I Allelic Ladder analyzed on the Applied Biosystems™ Genetic Analyzer. Allele assignment was performed using GeneMapper™ ID-X software and the Mentype® Nonaplex I template file.

Table 7 Fragment lengths of the Mentype®Nonaplex I Allelic Ladder measured on the Applied Biosystems™ 3130 Genetic Analyzer (blue panel)

Marker /Allele	Size [bp]*	Additional Alleles**	Marker /Allele	Size [bp]*	Additional Alleles**	Marker /Allele	Size [bp]*	Additional 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			

‡ These alleles are highlighted for better orientation within the allele ladder.
* rounded to whole numbers

Marker /Allele	Size [bp]*	Additional Alleles**	Marker /Allele	Size [bp]*	Additional Alleles**	Marker /Allele	Size [bp]*	Additional Alleles**

** These “off-ladder” alleles of the BIOTYPE DNA pool are assigned using the current BIOTYPE template files for the GeneMapper™ ID/ID-X software. For additional alleles, see, among others, http://www.cstl.nist.gov/biotech/strbase/str_fact.htm

Table 8 Fragment lengths of the Mentype® Nonaplex I Allelic Ladder measured on the Applied Biosystems™ 3130 Genetic Analyzer (green panel)

Marker/ Allele	Size [bp]*	Additional Alleles**	Marker /Allele	Size [bp]*	Additional Alleles**	Marker /Allele	Size [bp]*	Additional 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 whole numbers

** These “off-ladder” alleles from the BIOTYPE DNA pool are assigned to the current BIOTYPE template files for the GeneMapper™ ID/ID-X software. For additional alleles, see, among others, http://www.cstl.nist.gov/biotech/strbase/str_fact.htm

Table 9 Fragment lengths of the Mentype® Nonaplex I allele ladder measured on the Applied Biosystems™ 3130 Genetic Analyzer (yellow panel)

Marker/Allele	Size [bp]*	Other Alleles**	Marker/Allele	Size [bp]*	Additional Alleles**
D8S1179	**NED**		**D21S11**	**NED**	
8	129	7	25	206	Feb. 23, 24, 24, 25
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 whole numbers

** These “off-ladder” alleles of the BIOTYPE DNA pool are assigned using the current BIOTYPE template files for the GeneMapper™ ID/ID-X software. For additional alleles, see, among others, http://www.cstl.nist.gov/biotech/strbase/str_fact.htm

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