Mentype® **Argus X-8** PCR Amplification Kit

Product description

Repetitive sequences of the non-coding areas on human chromosomes - also called Short Tandem Repeats (STR) - are used in kinship testing and forensic science.
The majority of the STRs are located on the autosomes, i.e. on the 22 chromosome pairs which are equivalent in both sexes.

Autosomal markers are helpful in solving most of the forensic tasks in DNA analysis. However, some of them need the implementation of STRs on the sex chromosomes (gonosomes). Gonosomal STR markers are helpful in the investigation of relationships among individuals of different generations, especially when key persons of the pedigree are missing. Furthermore the use of gonosomal STRs in the analysis of DNA traces in forensic purposes is strongly rising.

The Mentype® **Argus X-8** contains the primers of **Amelogenin (AM)** for gender-determination, **DXS7132**, **DXS7423**, **DXS8378**, **DXS10074**, **DXS10101**, **DXS10134**, **DXS10135**, and **HPRTB**. Two markers belong to one of the four coupling groups of the X-chromosome (Fig. 1), so that two markers of each group have to be handled as haplotype for genotyping. The primers are fluorescence-labelled with **6-FAM** or **HEX**.

The detection limit of Mentype® **Argus X-8** PCR Amplification Kit is about **100 pg genomic DNA**. However, it is recommended to use **0.1-1.0 ng DNA**.

The Mentype® **Argus X-8** supplements the Mentype® **Argus Y-MH**^{QS} for kinship and paternity testing especially in complicated deficiency cases whereas all important population-genetic data can be calculated with the GenoProof® Software.

The Forensic ChrX Research Group initiated the online data base **ChrX-STR.org** (http://www.chrx-str.org) that calculates population-genetic data on basis of X-chromosmal allele frequencies (Szibor et al., 2006).

Generation of DNA profiles using Mentype® **Argus X-8** conforms to the guidelines of:
- the European DNA Profiling Group (EDNAP), www.isfg.org/EDNAP
- the International Society for Forensic Genetics (ISFG), www.isfg.org
- the Scientific Working Group on DNA Analysis Methods (SWGDAM), www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm

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.

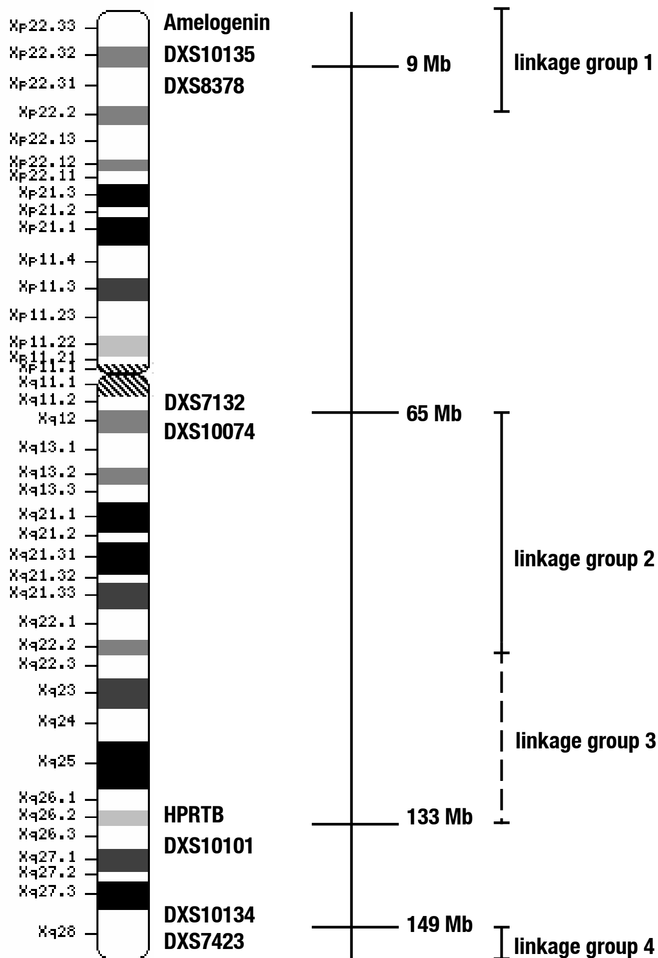

Fig. 1 The Ideogram of the X-chromosome describes the physical localisation of the forensic STRs, which can be analysed with the Mentype® **Argus X-8**. Distances from the p-telomere are shown in Mb (http://www.ncbi.nlm.nih.gov/genome/guide/human as at 10/2005). The markers are linked as follows: DXS8378 and DXS10135, DXS7132 and DXS10074, HPRTB and DXS10101 as well as DXS7423 and DXS10134. Also Amelogenin X and DXS8378 or DXS10135 are in tight neighbourhood, but without relevance for investigations of transmission because Amelogenin X and Amelogenin Y do not show any length polymorphisms and are not responsible for calculation of the likelihood.

Table 1. Locus-specific information of Mentype® Argus X-8

Locus	GenBank® Accession	Repeat motif of the reference allele	Reference allele	Allele range
Amelogenin X	M55418			
Amelogenin Y	M55419			
DXS7132	G08111	$[TCTA]_{13}$	13	8-19
DXS7423	AC109994	$[TCCA]_3$ TCTGTCCT $[TCCA]_{12}$	15	8-19
DXS8378	G08098	$[CTAT]_{12}$	12	7-15
DXS10074	AL356358	$[AAGA]_{14}$	14	4-21
DXS10101	AC004383	$[AAAG]_3$ GAAAGAAG $[GAAA]_3$ A $[GAAA]_4$ AAGA $[AAAG]_5$ AAAAAGAA $[AAAG]_{13}$ AA	28.2	24-36
DXS10134	AL034384	$[GAAA]_3$ GAGA $[GAAA]_4$ AA [GAAA] GAGA $[GAAA]_4$ GAGA $[GACAGA]_3$ [GAAA] GTAA $[GAAA]_3$ AAA $[GAAA]_4$ AAA $[GAAA]_{15}$	35	28-44.3
DXS10135	AC003684	$[AAGA]_3$ GAAAG $[GAAA]_{20}$	23	13-39.2
HPRTB	M26434	$[AGAT]_{12}$	12	7-19

*[AGAT] is the common repeat structure, for variations see NIST and Szibor et al. 2009.

Table 1 shows the STR loci with their repeat motifs and alleles that are concordant with the International Society for Forensic Genetics (ISFG) guidelines for the use of microsatellite markers (Bär et al., 1997). The most frequent alleles for European populations are included in the allelic ladder. The nomenclature for X-STR Loci DXS7132, DXS7423 and DXS8378 is in accordance with Szibor et al. (2003a). The nomenclature of DXS10074, DXS10101, DXS10134 und DXS10135 is in accordance with Becker et al. (2008). 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® Argus X-8

Locus	Chromosomal mapping
Amelogenin X	Xp22.1-22.3
Amelogenin Y	Yp11.2
DXS7132	Xq11.2
DXS7423	Xq28
DXS8378	Xp22.31
DXS10074	Xq12
DXS10101	Xq26.2
DXS10134	Xq28
DXS10135	Xp22.31
HPRTB	Xq26.2

Content

Mentype® **Argus X-8** PCR Amplification Kit (100 Reactions)

Nuclease-free water	3.0 mL
Reaction mix **A**	500 µL
Primer mix	250 µL
Control DNA XY1 (2 ng/µL)	10 µL
Control DNA XX28 (2 ng/µL)	10 µL
DNA Size Standard 550 (ROX)	50 µL
Allelic ladder	10 µL

Ordering information

Mentype® **Argus X-8**	25	Reactions	Cat. No.	43-09110-0025
Mentype® **Argus X-8**	100	Reactions	Cat. No.	43-09110-0100
Mentype® **Argus X-8**	400	Reactions	Cat. No.	43-09110-0400

Mentype® **Argus X-8** PCR Amplification Kits are distributed exclusively via Promega Corporation. For ordering, please contact Promega´s local branch offices or distributors. Requests and sales within Germany, Austria and Switzerland are handled directly via the Biotype AG.

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 contents of Biotype® test kits undergo an intensive quality assurance process at Biotype AG. 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.

Additionally required reagents

Additional reagents are needed in order to be able to use the Biotype®
PCR Amplification Kit. The use of the following products is strongly recommended:

Reagent	Supplier	Order number
JumpStart™ Taq DNA Polymerase hot start, 2.5 U/µL, 50 U or 250 U	Sigma-Aldrich	D4184
Hi-Di™ Formamide, 25 mL	Applied Biosystems	4311320
Matrix Standards DS-30 for ABI PRISM® 310 Genetic Analyzer	Applied Biosystems	401546 and 402996 (NED)
Matrix Standards DS-30 for ABI PRISM® 3100/3130/3730	Applied Biosystems	4345827

Trademarks and patents

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

Warnings and safety instructions

The PCR Amplification Kit contains the following potentially hazardous chemicals:

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

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

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Protocols for PCR amplification, electrophoresis, and analysis

1. PCR amplification

1.1 Master mix preparation

The table below shows the volumes of all PCR reagents per 25 µL reaction volume, including a sample volume of 3.0 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.

	Number of PCR samples			
Volume in [µL]	**1**	**10**	**25**	**100**
Nuclease-free Water	14.1	141.0	352.5	1410.0
Reaction mix **A***	5.0	50.0	125.0	500.0
Primer mix	2.5	25.0	62.5	250.0
Taq DNA Polymerase (hot start, 2.5 U/µL)	0.4	4.0	10.0	40.0
Volume of master mix	22.0	220.0	550.0	2200.0

* contains Mg^{2+}, dNTP Mix, 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 **0.2 ng Control DNA XX28** 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 to 0.2 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.

1.2 PCR amplification parameter

Perform a “hot start” PCR in order to activate the Taq 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. 34 cycles are recommended optionally in order to achieve optimal signal intensities for stains with small amounts of genomic DNA.

Standard method

Recommended for all DNA samples

Temperature	Time	
94°C	4 min (hot start for activation of the JumpStart™ Taq 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 JumpStart™ Taq DNA Polymerase)	
94°C	30 s	
58°C	120 s	**34 cycles**
72°C	75 s	
68°C	60 min	
10°C	∞	hold

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

2. Electrophoresis using the ABI PRISM® 310 Genetic Analyzer

For general instructions on instrument setup, matrix generation and application of the GeneScan® or GeneMapper™ ID software, refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Electrophoresis by using the GeneScan® software is described below.

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

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

2.1 Matrix generation

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

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

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

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

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

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

Injection list for matrix generation

Injection list	
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)

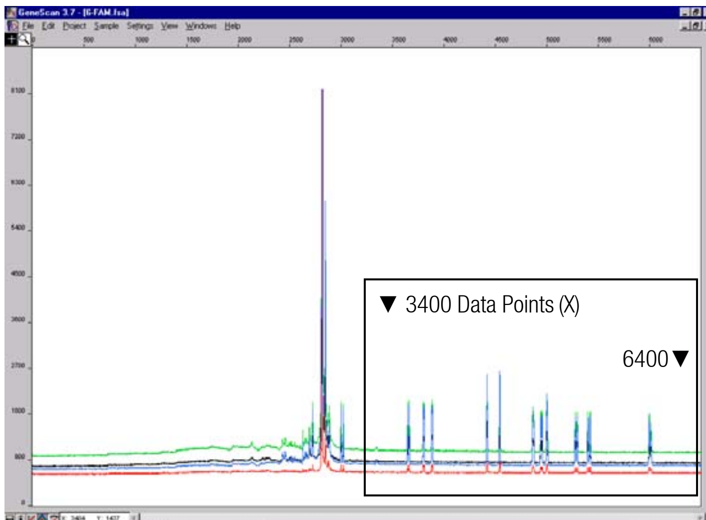

Fig. 2 Electropherogram with raw data of the matrix standard 6-FAM

- 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**

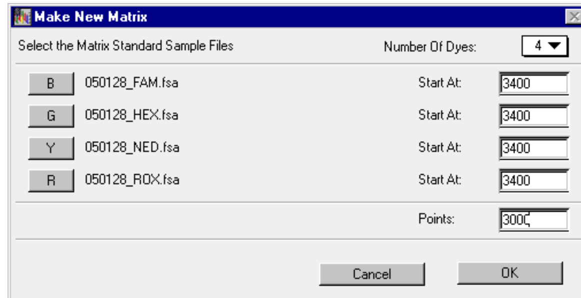

Fig. 3 Matrix sample selection

- 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

Matrix Biotype D.mtx

Reactions

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

Fig. 4 New matrix DS-30

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

Matrix check

Check the new matrix with current samples.

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

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

2.2 Sample preparation

Composition	Volume
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (ROX)	0.5 µL

prepare 12 µL of the mix (formamide + DNA size standard) for all samples
add 1 µL PCR product (diluted if necessary) or allelic ladder

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

Signal intensities

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

2.3 Setting up the GeneScan® software

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

Injection list

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 blood samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content an injection time up to 10 s may be necessary.

** Depending on the analysis conditions the run time for Mentype® **Argus X-8** was modified in order to analyse fragments with lengths of up to **400 bp**.

2.4 Analysis parameter

The recommended analysis parameters are:

Analysis Range	Start: 2000 Stop: 10000
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* Min. Peak Half Width: 2 pts Polynorminal Degree: 3 Peak Window Size: 11 pts**
Size Call Range	Min: 50 Max: 550
Size Calling Method	Local Southern Method
Split Peak Correction	None

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneScan® or GeneMapper™ ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

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

3. Electrophoresis using the ABI PRISM® 3130/3130xl Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, or application of the ABI PRISM® Data Collection software version 3.0 and the GeneMapper™ ID software, refer to the *ABI PRISM ® 3130/3130xl Genetic Analyzers Getting Started Guide*. Electrophoresis on an ABI PRISM® 3130 Genetic Analyser by using the GeneMapper™ ID software is described below.

The system with 4 capillaries is named ABI 3130 (former ABI 31300-Avant), and the system with 16 capillaries is named ABI 3130xl (former ABI 31000).

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

Material

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

3.1 Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the four fluorescent labels 6-FAM, HEX, NED, and ROX for each analyzer. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Creating the instrument protocol for spectral calibration (Protocol Manager)
- Defining the plate composition in the plate editor (Plate Manager)
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 4 capillaries/ABI 3130

Composition	Volume
Hi-Di™ Formamide	47.5 µL
Matrix standard DS-30	2.5 µL

- Denaturation for 3 min at 95°C
- Cool down to 4°
- For analysis, load 10 µL of the matrix standard into a 96-well reaction plate, well **A1-D1**

Example for 16 capillaries/ABI 3130xl

Composition	Volume
Hi-Di™ Formamide	190 µL
Matrix standard DS-30	10.0 µL

- Denaturation for 3 min at 95°C
- Cool down to 4°
- For analysis, load 10 µL of the matrix standard into a 96-well reaction plate, well **A1-H1** and **A2-H2**

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	
Name	e.g. Spectral36_POP4_DS30
Type	SPECTRAL
Dye Set	D
Polymer	POP4
Array Length	36
Chemistry	Matrix Standard
Run Module	Spect36_POP4_1

- 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	
Name	e.g. Spectral_DS-30
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)

Column	
Sample Name	Type name for the matrix samples
Priority	e.g. 100
Instrument Protocol 1	Spectral36_POP4_DS30 (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

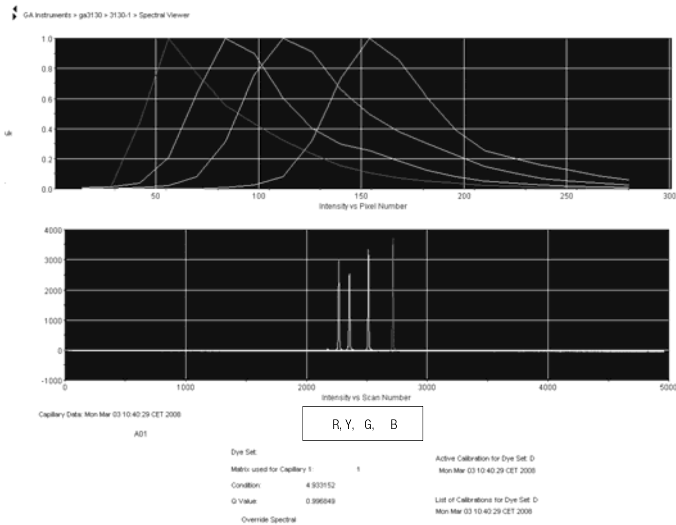

Fig. 5 Electropherogram of spectral calibration with matrix standard for DS-30

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 Fig. 5, there should be four peaks with peak heights of about 1000-5000 (Y-axis) in each matrix sample (optimal range: 2000-4000)
- Check the new matrix with your current samples. There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix
- If calibration was not successful, use the optimised values and repeat the calibration run
- If all capillaries have passed the test, the last calibration file for the **Dye Set D** is activated automatically in the **Spectral Viewer**. **Rename** the calibration file (e.g. DS-30_Date of calibration) using the respective button

3.2 Sample preparation

Composition	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

Because injections take place simultaneously on all capillaries, four samples must be pipetted when using 4-capillary analysers. If less than four samples are analysed, fill up the empty positions on the plate with 12 µL Hi-Di™ Formamide.

One allelic ladder should be run per capillary in order to ensure reliable allelic assignment on 4-capillary analysers.

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

Signal intensities

Options to increase the signal intensity:

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

3.3 Setting up the GeneMapper™ ID software

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

Run Module 20min_400bp

Parameter	Value
Oven Temperature [°C]	60
Poly Fill Volume	4840
Current Stability [µA]	5
PreRun Voltage [kV]	15
PreRun Time [s]	180
Injection Voltage [kV]	**3**
Injection Time [s]*	**5**
Voltage Number of Steps	40
Voltage Step Interval	15
Data Delay Time [s]	1
Run Voltage [kV]	15.0
Run Time [s]**	**1200**

* 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® **Argus X-** 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. 20min_400bp) 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	
Name	e.g. Run36_POP4_DS-30
Type	REGULAR
Run Module*	HIDFragmentAnalysis36_POP4_1
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 **Plate Dialog** box

GeneMapper™ Plate Editor (I)

New Plate Dialog	
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)

Column	
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_ v2 (choose test kit)
Analysis Method	e.g. Analysis_HID_3130
Snp Set	-
User-defined 1-3	-
Results Group 1	(select results group)
Instrument Protocol 1	Run36_POP4_DS-30 (setting described earlier)

- Click into the column header to select the entire column, select **Edit** → **Fill Down** to apply the information to all selected samples and click on **OK**
- In the **Run Scheduler,** click on **Find All**, select **Link** to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run
- During the run, view **Error Status** in the **Event Log** or examine the quality of the raw data for each capillary in the **Capillaries Viewer** or the **Cap/Array Viewer**
- View data as overview in **Run History** or **Cap/Array Viewer** of the Data Collection software. Run data are saved in the **Run Folder** of the previously chosen **Result Group**

3.4 Analysis parameter / analysis method

The recommended settings in the worksheet Peak Detector are:

Peak Detection Algorithm	Advanced
Ranges	Analysis: Partial Range Start Pt: 2000; Stop Pt: 10000 Sizing: All Sizes
Smoothing and Baselining	Smoothing: Light Baseline Window: 51 pts
Size Calling Method	Local Southern Method
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 11 pts** Slope Thresholds: 0.0

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneMapper™ ID software. The thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

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

4. Analysis

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

Finding the exact lengths of the amplified products depends on the device type, the conditions of electrophoresis, as well as the DNA size standard used. Due to the complexity of some loci, determining the size should be based on evenly distributed references. The DNA Size Standard 550 (ROX) shall thus be used with the following lengths of fragments: **50**, **60**, **70**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **190**, **200**, **220**, **240**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525** and **550 bp.**

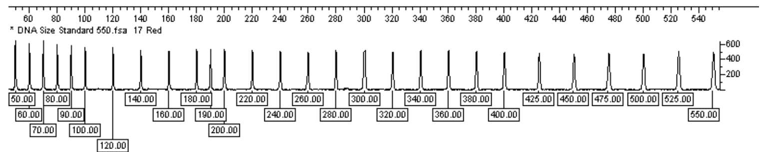

Fig. 6 Electropherogram of the DNA Size Standard 550 (ROX), fragments with lengths in bp

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.

4.1 Biotype® template files

Allele allocation should be carried out with a suitable analysis software, e.g. GeneMapper™ ID or Genotyper® software in combination with the Mentype® **Argus X-8** template files from Biotype AG. Template files are available from our homepage or as CD-ROM on request.

Recommended Biotype® templates for GeneMapper™ ID software are:

Panels	Biotype_Panels_v2 (choose kit)	or higher versions
BinSets	Biotype_Bins_v2	or higher versions
Size Standard	SST-BTO_50-500bp (adjust up to 400bp, adjustment described earlier)	
Analysis Method	Analysis_HID_310	
	Analysis_HID_3130	
Plot Settings	Plots_Blue	
	Plots_Green	
	Plots_Yellow	
	Plots_Red	
	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.

Recommended Biotype® template files for Genotyper® software are:

Argus X8_v1a	or higher versions

General procedure for the analysis

1. Check the DNA size standard
2. Check the allelic ladder
3. Check the positive control
4. Check the negative control
5. Analyse and interpret the sample data

4.2 Controls

The control DNA XY1 and XX28 of the test kit, and other commercially available DNA from standard cell lines, represent the following alleles:

Table 3. Allele assignment of Mentype® Argus X-8

Locus	Control DNA XY1	Control DNA XX28	ATCC K-562	CCR 9947A	CCR 9948	CCR 3657
Amelogenin	X / Y	X / X	X / X	X / X	X / Y	X / Y
DXS7132	13	13 / 14	13 / 13	12 / 12	13	12
DXS7423	15	14 / 15	17 / 17	14 / 15	14	13
DXS8378	12	10 / 12	10 / 10	10 / 11	11	12
DXS10074	16	18 / 20	17 / 17	16 / 19	18	7
DXS10101	32	28.2 / 28.2	31 / 31	30 / 31	32	29.2
DXS10134	38	36 / 38.3	32 / 32	35 / 36	34	34
DXS10135	24	16 / 30	27 / 27	21.1 / 27	22	25
HPRTB	16	12 / 13	13 / 13	14 / 14	14	13

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. (2003c).

4.3 Lengths of fragment and alleles

Table 4 and **Table 5** 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: 85-405 bp
Vertical: Depending on signal intensity

Figure 7

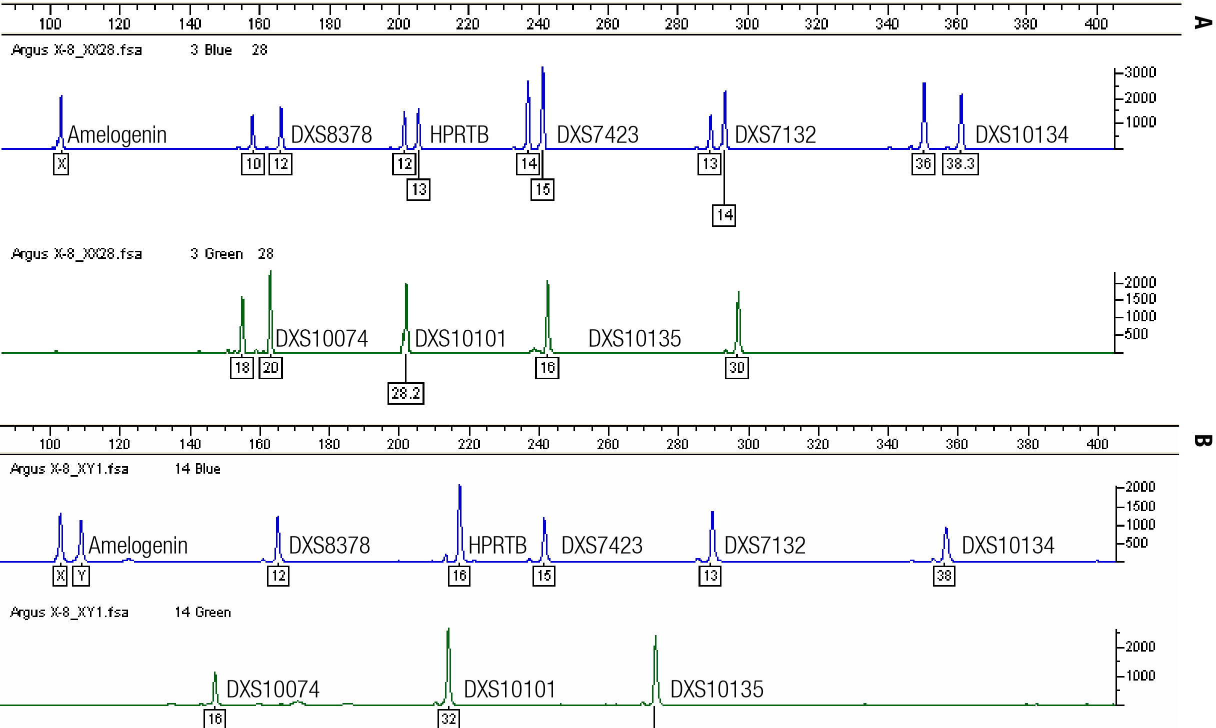

Fig. 7 Electropherogram of the Mentype® **Argus X-8** using 200 pg Control DNA XX28 (**A**) and 100 pg Control DNA XY1 (**B**) analysed on an ABI PRISM® 310 Genetic Analyzer. Allele assignment was performed using the Genotyper® software and the Mentype® **Argus X-8** template file.

Figure 8

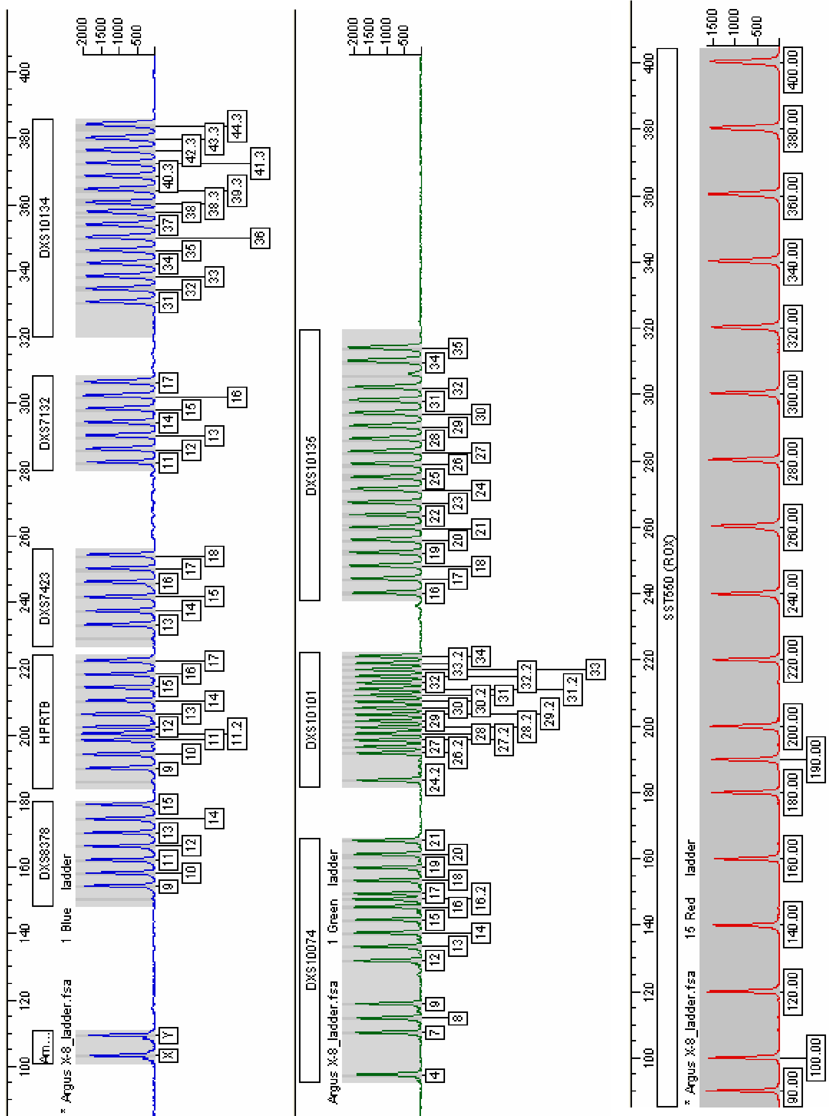

Fig. 8 Electropherogram of the allelic ladder Mentype® **Argus X-8** analysed on an ABI PRISM® 310 Genetic Analyzer with the DNA Size Standard 550 (ROX). Allele assignment was performed using the Genotyper® software and the Mentype® **Argus X-8** template files.

Table 4. Fragment lengths of the allelic ladder Mentype® Argus X-8 analysed on an ABI PRISM® 310/3130 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**		**DXS7423**	**6-FAM**		**DXS10134**	**6-FAM**	
X	103		13	233	12	31	330	28, 29, 30, 31.1
Y	109		14	237		32	334	32.1
			15	241		33	338	33.1
DXS8378	**6-FAM**		16	245		34	342	
9	154	8	17	249		35	346	35.3
10	158		18	253		36	350	
11	162					37	354	37.2, 37.3
12	166		**DXS7132**	**6-FAM**		38	358	38.2
13	170		11	281	10	38.3	360	
14	174		12	285		39.3	364	39, 39.2
15	178		13	289		40.3	368	40
			14	293		41.3	372	41
HPRTB	**6-FAM**		15	297		42.3	376	
9	190	8	16	301		43.3	380	
10	194		17	305		44.3	384	
11	198							
11.2	200							
12	202							
13	206							
14	210							
15	214							
16	218							
17	222							

Table 5. Fragment lengths of the allelic ladder Mentype® Argus X-8 analysed on an ABI PRISM® 310/3130 Genetic Analyzer (green panel)

Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**
DXS10074	**HEX**		**DXS10101**	**HEX**		**DXS10135**	**HEX**	
4	98		24.2	185	25, 25.2	16	242	14, 16.1
7	110		26.2	193	26	17	246	17.1
8	114		27	195		18	250	18.1
9	119		27.2	197		19	254	19.1
12	132	10, 11	28	199		20	258	20.1
13	136		28.2	201		21	262	21.1
14	140	14.3	29	203		22	266	22.1
15	144		29.2	205		23	269	23.1
16	148		30	207		24	273	24.1, 24.2
16.2	150		30.2	209		25	277	25.1
17	152		31	211		26	281	26.1
18	156		31.2	213		27	285	
19	160	19.3	32	215		28	289	28.1
20	164		32.2	217		29	293	
21	168		33	219		30	296	
			33.2	221		31	300	
			34	223	34.2, 35	32	304	32.1, 33.1
						34	312	34.1
						35	316	35.1, 35.2, 36, 37.2, 39.2

* 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

5. Interpretation of results

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

Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range (>3000 RFU), or if an incorrect matrix was applied. They appear at positions of specific peaks in other colour channels, typically with lower signal intensities. Peak heights should not exceed 3000 RFU in order to prevent pull-up peaks.

Stutter peaks

The occurrence of stutter peaks depends on the sequence of the repeat structure and the number of alleles. n-4 peaks are caused by a loss of a repeat unit during amplification of tetranucleotide STR motives, caused by slippage effects of the Taq DNA Polymerase. Interpretation of those peaks should be done in accordance with the Template Files of the Genotyper® and GeneMapper™ ID software.

Template-independent addition of nucleotides

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

6. Population-genetic data

The most important data for the X-STR marker of the test kit are listed in **table 6-8**. The formula for calculation of the “Polymorphism Information Content” (PIC) was published by Botstein et al. (1980), the one for the “Expected Heterocygosity” (HET) by Nei and Roychoudhury (1974). Both can be used for autosomal or X-chromosomal marker. Krüger et al. (1968) introduced the formula for the “Mean Exclusion Chance“ $MEC_{Krüger}$ which was developed for either autosomal marker or for trios. With exception of deficient cases, $MEC_{Krüger}$ is not suitable for X-chromosomal marker. Here, the paternal grandmother can be analysed instead of the putative father. Kishida et al. (1997) devised the $MEC_{Kishida}$ for X-chromosomal marker in consideration of trios including a daughter. In comparison with $MEC_{Krüger}$, $MEC_{Kishida}$ is more complex which highlights the fact that in trios involving a daughter X-STRs are more efficient then autosomal markers. Finally, Desmarais et al.(1998) introduced formulae for the “Mean Exclusion Chance“ of ChrX markers in trios involving daughters as well as in father-daughter duos without information about the maternal genotype. $MEC_{Desmarais}$ is equivalent to $MEC_{Kishida}$ whereas $MEC_{Desmarais\ Duo}$ can also be used for maternity testing of mother-son duos. The formula for calculation of the “Power of Discrimination“ (PD) erfolgt nach Desmarais et al. (1998).

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

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

for deficiency cases (mother, daughter, putative grandmother):
$MEC_{Krüger} = \Sigma_i f_i^3 (1 - f_i)^2 + \Sigma_i f_i (1 - f_i)^3 + \Sigma_{i<j} f_i f_j (f_i + f_j) (1 - f_i - f_j)^2$

for trios (mother, daughter, putative father):
$MEC_{Kishida} = \Sigma_i f_i^3 (1 - f_i) + \Sigma_i f_i (1 - f_i)^2 + \Sigma_{i<j} f_i f_j (f_i + f_j) (1 - f_i - f_j)$

for trios (mother, daughter, putative father):
$MEC_{Desmarais} = 1 - \Sigma_i f_i^2 + \Sigma_i f_i^4 - (\Sigma_{i<j} f_i^2)^2$

for duos (putative father, daughter) :
$MEC_{Desmarais\ Duo} = 1 - 2\Sigma_i f_i^2 + \Sigma_i f_i^3$

$PD_{female} = 1 - 2(\Sigma_i f_i^2)^2 + \Sigma_i f_i^4$

$PD_{male} = 1 - \Sigma_i f_i^2$

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

Table 6. Population-genetic data

Marker DXS7132	
Allele	**Allele frequency***
11	0.012
12	0.089
13	0.266
14	0.379
15	0.211
16	0.036
17	0.007
PIC	0.687
HET	0.733
PD_{female}	0.884
PD_{male}	0.732
$MEC_{Kishida}$	0.687
$MEC_{Krüger}$	0.497
$MEC_{Desmarais}$	0.687
$MEC_{Desmarais\ Duo}$	0.547

* according to Edelmann et al., 2001

Marker DXS7423	
Allele	**Allele frequency***
12	0.002
13	0.077
14	0.348
15	0.356
16	0.193
17	0.022
18	0.002
PIC	0.656
HET	0.710
PD_{female}	0.862
PD_{male}	0.709
$MEC_{Kishida}$	0.656
$MEC_{Krüger}$	0.456
$MEC_{Desmarais}$	0.656
$MEC_{Desmarais\ Duo}$	0.512

* according to Szibor et al., 2003a

Marker DXS8378	
Allele	**Allele frequency***
8	0.002
9	0.046
10	0.312
11	0.332
12	0.277
13	0.029
14	0.002
PIC	0.658
HET	0.714
PD_{female}	0.863
PD_{male}	0.713
$MEC_{Kishida}$	0.658
$MEC_{Krüger}$	0.454
$MEC_{Desmarais}$	0.658
$MEC_{Desmarais\ Duo}$	0.514

* according to Edelmann et al., 2001

Table 7. Population-genetic data

Marker DXS10074	
Allele	**Allele frequency***
7	0.066
8	0.135
9	0.014
10	0.001
13	0.005
14	0.009
15	0.070
16	0.218
16.2	0.002
17	0.249
18	0.149
19	0.065
20	0.013
21	0.003
PIC	0.816
HET	0.837
PD_{female}	0.953
PD_{male}	0.836
$MEC_{Kishida}$	0.815
$MEC_{Krüger}$	0.675
$MEC_{Desmarais}$	0.816
$MEC_{Desmarais\ Duo}$	0.705

* according to Hering et al., 2006

Marker DXS10101	
Allele	**Allele frequency***
26.2	0.025
27	0.011
27.2	0.025
28	0.040
28.2	0.148
29	0.011
29.2	0.120
30	0.043
30.2	0.152
31	0.113
31.2	0.106
32	0.101
32.2	0.047
33	0.040
33.2	0.007
34	0.011
PIC	0.889
HET	0.902
PD_{female}	0.981
PD_{male}	0.898
$MEC_{Kishida}$	0.889
$MEC_{Krüger}$	0.793
$MEC_{Desmarais}$	0.889
$MEC_{Desmarais\ Duo}$	0.807

* according to Rodig et al., submitted

Marker DXS10134	
Allele	**Allele frequency***
30	0.001
31	0.002
32	0.008
33	0.054
34	0.107
35	0.209
36	0.222
37	0.168
37.2	0.002
38	0.059
39	0.035
39.3	0.036
40.3	0.044
41.3	0.027
42.3	0.019
43.3	0.006
44.3	0.001
PIC	0.844
HET	0.858
PD_{female}	0.965
PD_{male}	0.859
$MEC_{Kishida}$	0.836
$MEC_{Krüger}$	0.844
$MEC_{Desmarais}$	0.842
$MEC_{Desmarais\ Duo}$	0.741

* according to Edelmann et al., 2007

Table 8. Population-genetic data

Marker DXS10135		Marker HPRTB	
Allele	**Allele frequency***	**Allele**	**Allele frequency***
16	0.004	9	0.010
17	0.028	10	0.019
18	0.040	11	0.116
19	0.068	12	0.276
20	0.072	13	0.322
21	0.112	14	0.169
22	0.108	15	0.076
23	0.084	16	0.012
24	0.108		
25	0.072	PIC	0.737
26	0.100	HET	0.773
27	0.060	PD_{female}	0.913
28	0.048	PD_{male}	0.772
29	0.028	$MEC_{Kishida}$	0.737
30	0.028	$MEC_{Krüger}$	0.562
31	0.028	$MEC_{Desmarais}$	0.737
32	0.003	$MEC_{Desmarais\ Duo}$	0.605
33	0.004		
PIC	0.916		
HET	0.925		
PD_{female}	0.988		
PD_{male}	0.921		
$MEC_{Kishida}$	0.911		
$MEC_{Krüger}$	0.835		
$MEC_{Desmarais}$	0.916		
$MEC_{Desmarais\ Duo}$	0.850		
* according to R. Szibor, pers. communication		* according to Edelmann et al., 2001	

The Forensic ChrX Research Group initiated the online data base “ChrX-STR.org” (http://www.chrx-str.org) that calculates population-genetic data on the basis of X-chromosmal allele frequencies (Szibor et al., 2006).

7. Usage of the X-chromosomal STRs and their characteristics

In principle, the guidelines of the ISFG (www.isfg.org) are valid for working with X-chromosomal STRs, too. However, the following special characteristics should be pointed out: Typing of X-chromosomal markers is only permissible when the phenotypic **and** genetic sex is identical. The genetic sex can easily be determined by the analysis of the Amelogenin locus. If the phenotypic sex differs from the genotypic sex, results from ChrX-typing could not be used. Following the national laws, this information has to be handled strictly confidential and should only be forwarded to the analysed person on request.

Generation of X-chromosomal profiles for kinship testing should be taken into account whenever autosomal markers do not give reliable or unequivocal results, mainly in deficiency cases. In some instances, e.g. for complex kinship and paternity testing, markers located on the sex chromosomes may turn out to be more informative than their autosomal counterparts, and the use of sex-chromosomal markers may therefore substantially improve the efficiency of a kinship test. This arises from the fact that the mean exclusion chance (MEC) of a X-chromosomal marker is consistently larger than that of an autosomal marker with the same allele frequencies.

Linkage groups

In contrast to ealier findings (Szibor 2003b), recent studies showed (Tillmar et al. 2008, Machado 2009) that markers of linkage groups three (HPRTB, DXS10101) and four (DXS10134, DXS7423) cannot be regarded as unlinked. The possibility of linkage between these markers should be considered in calculation and interpretation of relationship tests in deficiency cases. If necessary both probabilities should be calculated from the linkage groups 1,2,3 **and** linkage groups 1,2,4. For use and calculation of X-chromosomal markers see Krawczak M, 2007 and http://linkage.rockefeller.edu.

Kinship tests and paternity cases with ChrX STRs

X-chromosomal markers are particularly informative for inferring parent–offspring relationships that involve at least one female, i.e. mother–daughter, mother–son, and father–daughter duos. For paternity cases only testing between father and daughter is possible, since fathers leave their only X-chromosome to their daughter, and sons get the X-chromosome from their mother.

Deficiency cases

If the putative father (PV) descends from the putative grandmother (PGM) without any doubt, his X-chromosomal characteristics originate from the PGM. Therefore, she (PGM) can be analysed X-chromosomally instead of him (PV). The calculation of the PGM-likelihood with X chromosomal markers (PGM-daughter) may be performed like the calculation with autosomal markers (PV-daughter). Here, the MEC values according to the formula of Krüger et al., 1968 shall be applied, whereas only unlinked X-STR markers of the Mentype **Argus X-8** can be used. For markers which are in coupling equilibrium, haplotype frequencies must be involved for calculation.

Siblings and distant relationships

The X-chromosomal analysis – without parental samples – can **not** be used as relationship test in the following cases:

- Different putative fathers could be father of the child
- Half-brothers with same father and different mothers
- Brothers and half-brothers with same mother (statistically, they have 50% of the characteristics in common, thus the test is comparable to a test with autosomal markers)

Whenever distant relationship should be analysed, genotyping of X-chromosomal markers is very useful, because the usage of coupled markers allows ChrX-haplotyping. Three markers of each linkage group from Mentype® **Argus X-8** have to be handled as haplotype for genotyping. For further details see Szibor et al., 2003b, for calculation see the online data base "ChrX-STR.org" (http://www.chrx-str.org).

Usage of X-chromosomal markers for forensic traces

In most cases the use of autosomal markers for profiling is much better because all autosomal loci have two alleles. Whenever female DNA traces have to be analysed in a male background, e.g. female traces under nails of a man, X-chromosomal markers are advantaged. The likelihood that one allele of the trace is covered by the male background is only half in comparison to autosomal tests. Note: International DNA databases usually do not include markers of the X-chromosome.

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 ISFH regarding the use of short tandem repeat systems. *Forensic Sci Int 87:181-184.*

Becker D, Rodig H, Augustin C, Edelmann J, Götz F, Hering S, Szibor R, Brabetz W (2008) Population genetic evaluation of eight X-chromosomal short tandem repeat loci using Mentype Argus X-8 PCR amplification kit. *Forensic Sci Int: Genetics 2:69-74.*

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

Desmarais D, Zhong Y, Chakraborty R, Perreault C, Busque L (1998) Development of a highly polymorphic STR marker for identity testing purposes at the human androgen receptor gene (HUMARA). *J Forensic Sci 43:1046–1049.*

Edelmann J, Hering S, Michael M, Lessig R, Deichsler D, Meier-Sundhausen G, Roewer L, Plate I, Szibor R (2001) 16 X chromosome STR loci frequency data from a german population. *Forensic Sci Int 124 215-218.*

Edelmann J, Hering S, Augustin C, Szibor R (2007) Characterisation of STR markers DXS10146, DXS10134 and DXS10147 located within a 79.1 kb region at Xp28. *Forensic Sci Int: Genetics (DOI 10.1016/j.fsigen.2007.08.001).*

Hering S, Augustin C, Edelmann J, Heidel M, Dressler J, Rodig H, Kuhlisch E, Szibor R (2006) DXS10079, DXS10074 and DXS10075 are STRs located within a 280 kb region of Xq12 and provide stable haplotypes useful for solving of complex kinship cases. *Int J Legal Med 120(6):337-45.*

Kishida T, Wang W, Fukuda M, Tamaki Y (1997) Duplex PCR of the Y-27H39 and HPRT loci with reference to Japanese population data on the HPRT locus. *Jpn J Legal Med 51: 67–69.*

Krawczak M (2007) Kinship testing with X-chromosomal markers: Mathematical and statistical issues. *Forensic Sci Int: Genetics Jun;1(2):111-4*

Krüger J, Fuhrmann W, Lichte KH, Steffens C (1968) Zur Verwendung der sauren Erythrocytenphosphatase bei der Vaterschaftsbegutachtung. *Dtsch Z Gerichtl Med 64:127–146.*

Machado FB (2009) Genetic map of human X-linked microsatellites used in forensic practice. *Forensic Sci Int: Genetics; doi:10.1016 (paper in press)*

Rodig H, Kloep F, Weißbach L, Augustin C, Edelmann J, Hering S, Szibor R, Götz F, Brabetz W Evaluation of 7 X-chromosomal Short Tandem Repeat loci located within the Xq26 region. *Forensic Sci Int (submitted)*

Szibor R, Edelmann J, Zarrabeitia, MT (2003a) Sequence structure and population data of the X-linked markers DXS7423 and DXS8377 – clarification of conflicting statements published by two working groups. *Forensic Sci Int 134 72-73.*

Szibor R, Krawczak M, Hering S, Edelmann J, Kuhlisch E, Krause D (2003b) Use of X-linked markers for forensic purposes. *Int J Legal Med 117 67-74.*

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

Szibor R, Hering S, Edelmann J (2006) A new Web site compiling forensic chromosome X research is now online. *Int J Legal Med 120(4):252-4.*

Szibor R, Edelmann J, Hering S, Gomes I, Gusmao L (2009) Nomenclatur discrepancies in the HPRTB short tandem repeat. *Int J Legal Med DOI 10.1007/s00414-008-0314-7 (Published online 10 January 2009).*

Tillmar A, Mostad P, Egeland T, Lindblom B, Holmlund G, Montelius K (2008) Analysis of linkage and linkage disequilibrium for eight X-STR markers. *Forensic Sci Int: Genetics Dec;3(1):37-41.*

Notes