

# Mentype<sup>®</sup> **Pentaplex ESS** PCR Amplification Kit

## Product description

The Mentype<sup>®</sup> **Pentaplex ESS** PCR Amplification Kit was developed for multiplex amplification of new miniSTR markers and Amelogenin which are recommended by ENFSI and EDNAP (Gill et al., 2006). In one PCR reaction, the five polymorphic STR loci **D1S1656**, **D2S441**, **D10S1248**, **D12S391** and **D22S1045** as well as the gender-specific **Amelogenin** are amplified simultaneously. The primers are fluorescence-labelled with **6-FAM**, **HEX** or **NED**.

These low molecular weight STRs have substantial benefit to detect degraded samples so that the maximal amplicon size was restricted to 225 bp within the Mentype<sup>®</sup> **Pentaplex ESS**. In combination with existing STR markers in use, the new markers improve the discrimination power which in turn is important to improve the power of national DNA databases.

The detection limit of Mentype<sup>®</sup> **Pentaplex ESS** PCR Amplification Kit is about **100 pg genomic DNA**. However, it is recommended to use **0.2-1.0 ng DNA**.

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

**Tabelle 1. Locus-specific information of Mentype<sup>®</sup> Pentaplex ESS**

| <b>Locus</b> | <b>GenBank<sup>®</sup> accession</b> | <b>Repeat motif of the reference allele</b>                           | <b>Reference allele</b> | <b>Allele range</b> |
|--------------|--------------------------------------|---|-------------------------|---------------------|
| Amelogenin X | M55418                               |   |                         |                     |
| Amelogenin Y | M55419                               |   |                         |                     |
| D1S1656      | NC_000001.9                          | [TAGA] <sub>16</sub> [TGA][TAGA][TAGG] <sub>1</sub> [TG] <sub>5</sub> | 17                      | 9-21                |
| D2S441       | AL079112                             | [TCTA] <sub>12</sub>  | 12                      | 8-19                |
| D10S1248     | AL391869                             | [GGAA] <sub>13</sub>  | 13                      | 8-21                |
| D12S391      | G08921                               | [AGAT] <sub>5</sub> GAT [AGAT] <sub>7</sub> [AGAC] <sub>6</sub> AGAT  | 19.3                    | 13-28               |
| D22S1045     | AL022314                             | [ATT] <sub>14</sub> ACT [ATT] <sub>2</sub>                            | 17                      | 7-20                |

Table 1 shows the STR loci with their repeat motifs and alleles that are concordant with the International Society for Forensic Genetics (ISFG) guidelines for the use of microsatellite markers (Bär et al., 1997). The nomenclature for STR Loci D10S1248 and D22S1045 is in accordance with Hill et al. (2008). Locus D22S1045 includes a trinucleotide STR motive that might result in a special stutter performance (see chapter 5). Allele ranges include all known alleles of the National Institute of Standards and Technology (NIST as at 10/2009) and of the current literature.

## Tabelle 2. Chromosomal mapping of Mentype<sup>®</sup> Pentaplex ESS

| Locus        | Chromosomal mapping |
|--------------|---------------------|
| Amelogenin X | Xp22.1-22.3         |
| Amelogenin Y | Yp11.2              |
| D1S1656      | 1q42                |
| D2S441       | 2p14                |
| D10S1248     | 10q26.3             |
| D12S391      | 12p13.2             |
| D22S1045     | 22q12.3             |

## Content

### Mentype<sup>®</sup> Pentaplex ESS PCR Amplification Kit (100 Reactions)

|                             |        |
|-----------------------------|--------|
| Nuclease-free water         | 3.0 mL |
| Reaction mix <b>A</b>       | 500 µL |
| Primer mix                  | 250 µL |
| DNA polymerase              | 40 µL  |
| Control DNA XY13 (2 ng/µL)  | 10 µL  |
| DNA size standard 550 (ROX) | 50 µL  |
| Allelic ladder              | 10 µL  |

## Ordering information

|                                    |               |          |               |
|------------------------------------|---------------|----------|---------------|
| Mentype <sup>®</sup> Pentaplex ESS | 25 reactions  | Cat. No. | 41-06210-0025 |
| Mentype <sup>®</sup> Pentaplex ESS | 100 reactions | Cat. No. | 41-06210-0100 |
| Mentype <sup>®</sup> Pentaplex ESS | 400 reactions | Cat. No. | 41-06210-0400 |

## Storage

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

## Quality assurance

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

## Additionally required reagents

Additional reagents are needed in order to use the Biotype<sup>®</sup> PCR Amplification Kit:

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

## Trademarks and patents

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6-FAM, HEX, NED, ROX, POP-4 and Hi-Di are trademarks of Applied Biosystems Inc. GeneAmp<sup>®</sup> 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<sup>®</sup> is a trademark of National Institute of Health.

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## Warnings and safety instructions

The PCR Amplification Kit contains the following potentially hazardous chemicals:

| Kit Component                                  | Chemical                      | Hazards  |
|--|-------------------------------|--|
| Primer mix, reaction mix<br>and allelic ladder | Sodium azide NaN <sub>3</sub> | toxic if swallowed, develops toxic gases<br>when it gets in contact with acids |

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

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## Protocols for PCR amplification, electrophoresis, and analysis

### 1. PCR amplification

#### 1.1 Master mix preparation

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

| Component                                       | Volume  |
|---|---------|
| Nuklease-free water                             | 16.1 µL |
| Reaction mix <b>A</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<sup>2+</sup>, 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 **0.2 ng Control DNA XY13** 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 XY13 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.

#### Template DNA

Sometimes, the measured value of the DNA concentration varies depending on the quantification method used, so that it may be necessary to adjust the optimal DNA amount.

## 1.2 PCR amplification parameter

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

The number of cycles depends on the amount of DNA. 30 cycles are recommended for all samples. For critical stains (< 100 pg DNA), it is recommended to increase the number of PCR cycles from 30 to 32.

### Standard Method

Recommended for all DNA samples

| Temperature | Time  |                  |
|-------------|---|------------------|
| 94°C        | 4 min (hot start for activation of the Multi Taq2 DNA Polymerase) |                  |
| 94°C        | 30 s  |                  |
| 60°C        | 120 s   | <b>30 cycles</b> |
| 72°C        | 75 s  |                  |
| 68°C        | 60 min  |                  |
| 10°C        | ∞   | hold             |

### Optional

Recommended for stains with small amounts of DNA

| Temperature | Time  |                  |
|-------------|---|------------------|
| 94°C        | 4 min (hot start for activation of the Multi Taq2 DNA Polymerase) |                  |
| 94°C        | 30 s  |                  |
| 60°C        | 120 s   | <b>32 cycles</b> |
| 72°C        | 75 s  |                  |
| 68°C        | 60 min  |                  |
| 10°C        | ∞   | hold             |

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

## 2. Electrophoresis using the ABI PRISM® 310 Genetic Analyzer

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

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

### Material

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

### 2.1 Matrix generation

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

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

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

| Matrix sample   | Component                    | Volume  |
|-----------------|------------------------------|---------|
| Matrix sample 1 | Hi-Di™ Formamide             | 12.0 µL |
|                 | Matrix standard <b>6-FAM</b> | 1.0 µL  |
| Matrix sample 2 | Hi-Di™ Formamide             | 12.0 µL |
|                 | Matrix standard <b>HEX</b>   | 1.0 µL  |
| Matrix sample 3 | Hi-Di™ Formamide             | 12.0 µL |
|                 | Matrix standard <b>NED</b>   | 1.0 µL  |
| Matrix sample 4 | Hi-Di™ Formamide             | 12.0 µL |
|                 | Matrix standard <b>ROX</b>   | 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

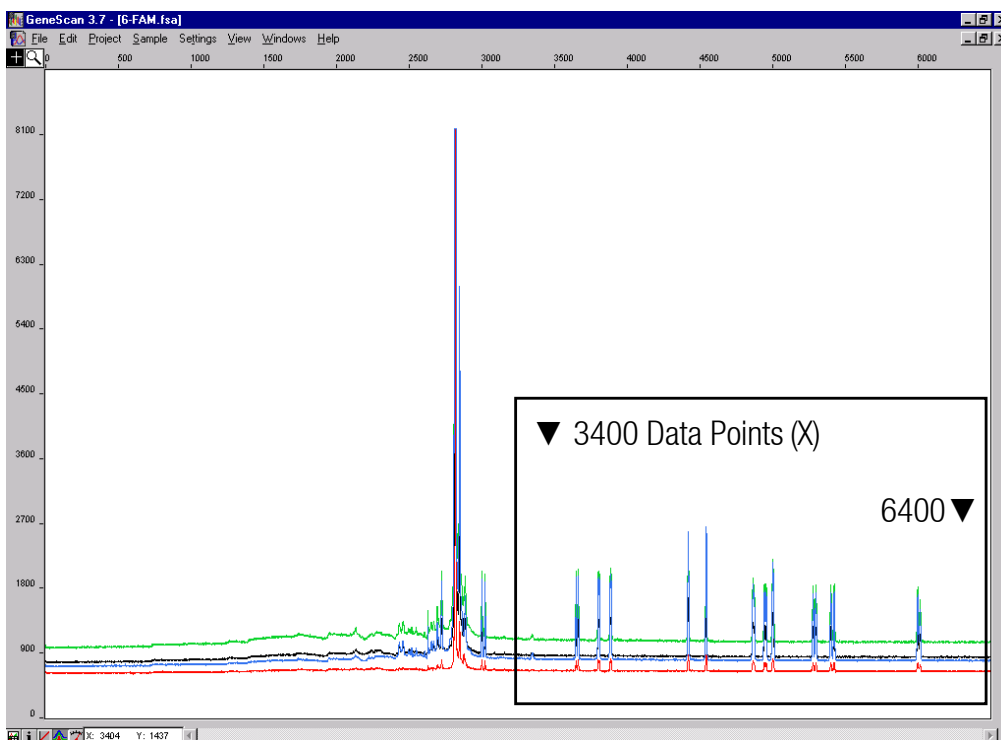
## Injection list for matrix generation

| Parameter      | Set up                |
|----------------|-----------------------|
| Module File    | GS STR POP-4 (1 mL) D |
| Matrix File    | <b>NONE</b>           |
| Size Standard* | <b>NONE</b>           |
| 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)

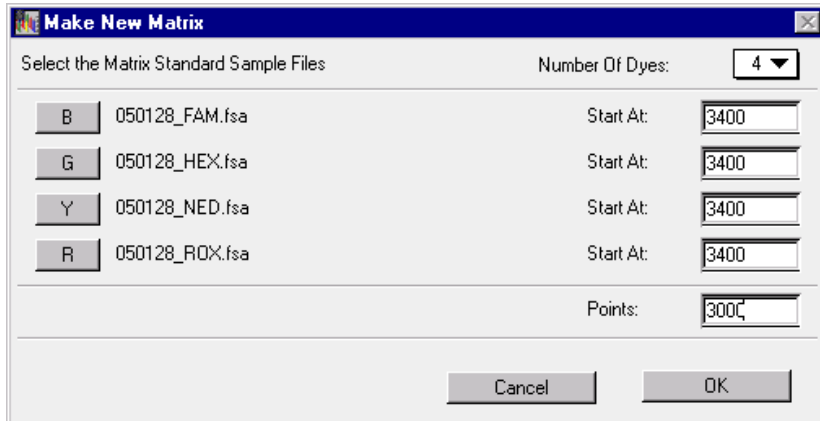


**Fig. 1** Electropherogram with raw data of the matrix standard 6-FAM

- 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

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

**Fig. 3** New matrix DS-30

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

## Matrix check

Check the new matrix with current samples.

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

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

## 2.2 Sample preparation

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

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

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

## Signal intensities

Options to increase the signal intensity:

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

## 2.3 Setting up the GeneScan® software

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

## Injection list

| Parameter        | Set up                       |
|------------------|------------------------------|
| Module File      | GS STR POP-4 (1 mL) <b>D</b> |
| Matrix File      | e.g. Matrix DS-30            |
| Size Standard    | e.g. SST-ROX_50-240bp        |
| Injection [s]*   | 5                            |
| Injection [kV]   | 15.0                         |
| Run [kV]         | 15.0                         |
| Run [°C]         | 60                           |
| Run Time [min]** | <b>20</b>                    |

\* 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® **Pentaplex ESS** was modified in order to analyse fragments with lengths of up to **240 bp**.

## 2.4 Analysis parameter

The recommended analysis parameters are:

|                       |  |
|-----------------------|--|
| Analysis Range        | Start: 2000<br>Stop: 10000   |
| Data Processing       | Baseline: Checked<br>Multicomponent: Checked<br>Smooth Options: Light  |
| Peak Detection        | Peak Amplitude Thresholds<br>B:* Y:*<br>G:* R:*<br>Min. Peak Half Width: 2 pts<br>Polynomial Degree: 3<br>Peak Window Size: 11 pts** |
| Size Call Range       | Min: 50<br>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<sup>®</sup> or GeneMapper<sup>™</sup> ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

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

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

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

The system with 4 capillaries is named ABI 3130 (former ABI 3100-Avant), and the system with 16 capillaries is named ABI 3130xl (former ABI 3100).

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

#### Material

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

#### 3.1 Spectral calibration / matrix generation

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

Spectral calibration comprises the following steps:

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

## Setting up the spectral calibration standards

Example for 4 capillaries/ABI 3130

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

- Load 12 µL of the mix to a 96-well reaction plate, e.g. position **A1-D1**
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Example for 16 capillaries/ABI 3130xl

| Component             | Volume   |
|-----------------------|----------|
| Hi-Di™ Formamide      | 190.0 µL |
| Matrix standard DS-30 | 10.0 µL  |

- Load 12 µL of the mix to a 96-well reaction plate, e.g. position **A1-H1** and **A2-H2**
- Denaturation for 3 min at 95°C
- Cool down to 4°C

## Performing a 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            | <i>User</i> (e.g. Spectral36_POP4_DS30) |
| Type            | SPECTRAL                                |
| Dye Set         | D                                       |
| Polymer*        | <i>User</i> (e.g. POP4)                 |
| Array Length*   | <i>User</i> (e.g. 36cm)                 |
| Chemistry       | Matrix Standard                         |
| Run Module*     | <i>Default</i> (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)

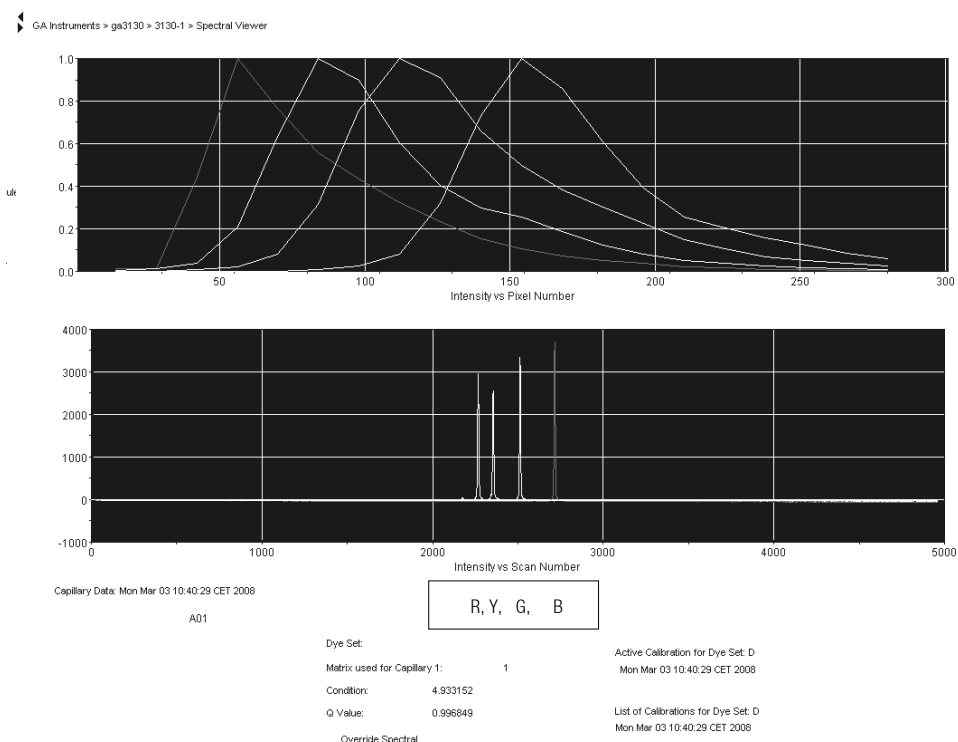
| New Plate Dialog           | Set up                   |
|----------------------------|--------------------------|
| Name                       | e.g. Spectral_DS-30_date |
| Application                | Spectral Calibration     |
| Plate Type                 | 96-Well                  |
| Owner Name / 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** Electroferogram with raw data of the matrix standard DS-30 on an ABI 3130

## Matrix check

- The quality value (**Q value**) of each capillary must be greater than 0.95 and the condition number range (**C value**) must be between 1 and 20.
- Check the matrix samples for a flat baseline. As shown in the figure above, there should be four peaks with peak heights of about 1000-5000 (Y-axis) in each matrix sample (optimal range: 2000-4000)
- Check the new matrix with your current samples. There should be **no** pull-up peaks between the dye panels (B, G, Y, R) with the new matrix
- If calibration was not successful, use the optimised values and repeat the calibration run
- If all capillaries have passed the test, the last calibration file for the **Dye Set D** is activated automatically in the **Spectral Viewer**. **Rename** the calibration file (e.g. DS-30\_Date of calibration) using the respective button

### 3.2 Sample preparation

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

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

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

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analysers. If fewer samples are analysed, the empty positions must be filled with 12 µL Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analysers, 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

### 3.3 Setting up the GeneMapper™ ID software

Edit the Run Module as follows for the first run:

- In the **Module Manager** of the Data Collection software click on **New** to open the **Run Module Editor** dialog box

#### Run Module 3kV\_10s\_260bp

| Parameter               | Set up         |
|-------------------------|----------------|
| Oven Temperature [°C]   | <i>Default</i> |
| Poly Fill Volume        | <i>Default</i> |
| Current Stability [µA]  | <i>Default</i> |
| PreRun Voltage [kV]     | <i>Default</i> |
| PreRun Time [s]         | <i>Default</i> |
| Injection Voltage [kV]  | <b>3.0</b>     |
| Injection Time [s]*     | <b>10</b>      |
| Voltage Number of Steps | <i>Default</i> |
| Voltage Step Interval   | <i>Default</i> |
| Data Delay Time [s]     | <i>Default</i> |
| Run Voltage [kV]        | <i>Default</i> |
| Run Time [s]**          | <b>900</b>     |

\* 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® **Pentaplex ESS** was modified in order to be able to analyse fragments with lengths of up to **240 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV\_10s\_260bp) 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_15min |
| Type            | REGULAR                     |
| Run Module*     | 3kV_10s_260bp               |
| 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)

| <b>New Plate Dialog</b>    | <b>Set up</b>                 |
|----------------------------|-------------------------------|
| 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 **Plate Editor** opens automatically

### GeneMapper™ Plate Editor (II)

| <b>Column</b>         | <b>Set up</b>                                |
|-----------------------|--|
| Sample Name           | Type name for the samples                    |
| Priority              | e.g. 100 (Default)                           |
| Sample Type           | Sample or Allelic Ladder                     |
| Size Standard         | e.g. SST-ROX_50-240bp                        |
| Panel                 | e.g. Biotype_Panels_v3a (choose test kit)    |
| Analysis Method       | e.g. Analysis_HID_3130                       |
| Snp Set               | -  |
| User-defined 1-3      | -  |
| Results Group 1       | (select results group)                       |
| Instrument Protocol 1 | Run36_POP4_DS-30 (setting described earlier) |

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

### 3.4 Analysis parameter / analysis method

The recommended settings in the worksheet Peak Detector are:

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

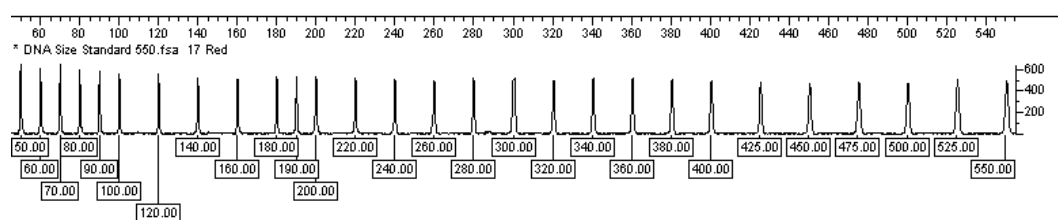
\* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneMapper™ ID software. The thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high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

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

#### 4. Analysis

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

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



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

**Note:** The basic template files for the DNA Size Standard 550 (ROX) has to be adjusted to 240 bp within the GeneMapper™ ID software. The new template could be saved as e.g. SST-ROX\_50-240bp and used for further analyses.

#### 4.1 Biotype<sup>®</sup> template files

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

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

|                 |  |                    |
|-----------------|--|--------------------|
| Panels          | Biotype_Panels_v3a/v3X (choose kit)  | or higher versions |
| BinSets         | Biotype_Bins_v3a/v3X   | or higher versions |
| Size Standard   | SST-ROX_50-500bp (adjust to 240bp adjustment described earlier)                            |                    |
| Analysis Method | Analysis_HID_310<br>Analysis_HID_3130<br>Analysis_HID_310_50rfu<br>Analysis_HID_3130_50rfu |                    |
| Plot Settings   | Plots_4dyes  |                    |
| Table Settings  | Table for 2 Alleles<br>Table for 10 Alleles  |                    |

Panels and BinSets always have to be used whereas the other template files are optional.

Additional Biotype<sup>®</sup> 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<sup>®</sup> template files for Genotyper<sup>®</sup> software are:

|                  |                    |
|------------------|--------------------|
| Pentaplex_ESS_v1 | 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 XY13 of the test kit and other commercially available DNA from standard cell lines represent the following alleles:

**Table 3. Allele assignment of Mentype® Pentaplex ESS**

| <b>Locus</b> | <b>Control<br/>DNA XY13</b> | <b>ATCC<br/>K-562</b> | <b>CCR<br/>9947A</b> | <b>CCR<br/>9948</b> | <b>CCR<br/>3657</b> |
|--------------|-----------------------------|-----------------------|----------------------|---------------------|---------------------|
| Amelogenin   | X/Y                         | X/X                   | X/X                  | X/Y                 | X/Y                 |
| D1S1656      | 16 / 17.3                   | 15 / 16               | 18.3 / 18.3          | 14 / 17             | 13 / 18.3           |
| D2S441       | 10 / 11                     | 10 / 14               | 10 / 14              | 11 / 12             | 14 / 14             |
| D10S1248     | 14 / 15                     | 12 / 12               | 13 / 15              | 12 / 15             | 14 / 16             |
| D12S391      | 16 / 18                     | 23 / 23               | 18 / 20              | 18 / 24             | 18 / 19             |
| D22S1045     | 17 / 18                     | 16 / 16               | 11 / 14              | 16 / 18             | 11 / 17             |

For further confirmation, the table above displays the alleles of the reference DNA purchased from ATCC (<http://atcc.org/Products/PurifiedDNA.cfm#celllines>) as well as three reference DNA purchased from Coriell Cell Repositories (CCR; <http://locus.umdj.edu/nigms/>) that is up to standard of Szibor et al. (2003).

## 4.3 Lengths of fragments and alleles

**Table 4** to **Table 6** show the fragment lengths of individual alleles that refer to the DNA Size Standard 550 (ROX). All analyses have been performed on an ABI PRISM® 310/3130 Genetic Analyzer with POP-4 polymer. Different analysis instruments, DNA size standards or polymers may result in different fragment lengths. In addition, a visual alignment with the allelic ladder is recommended.

### Scaling

Horizontal: 75-245 bp

Vertical: Depending on signal intensity

Figure 6

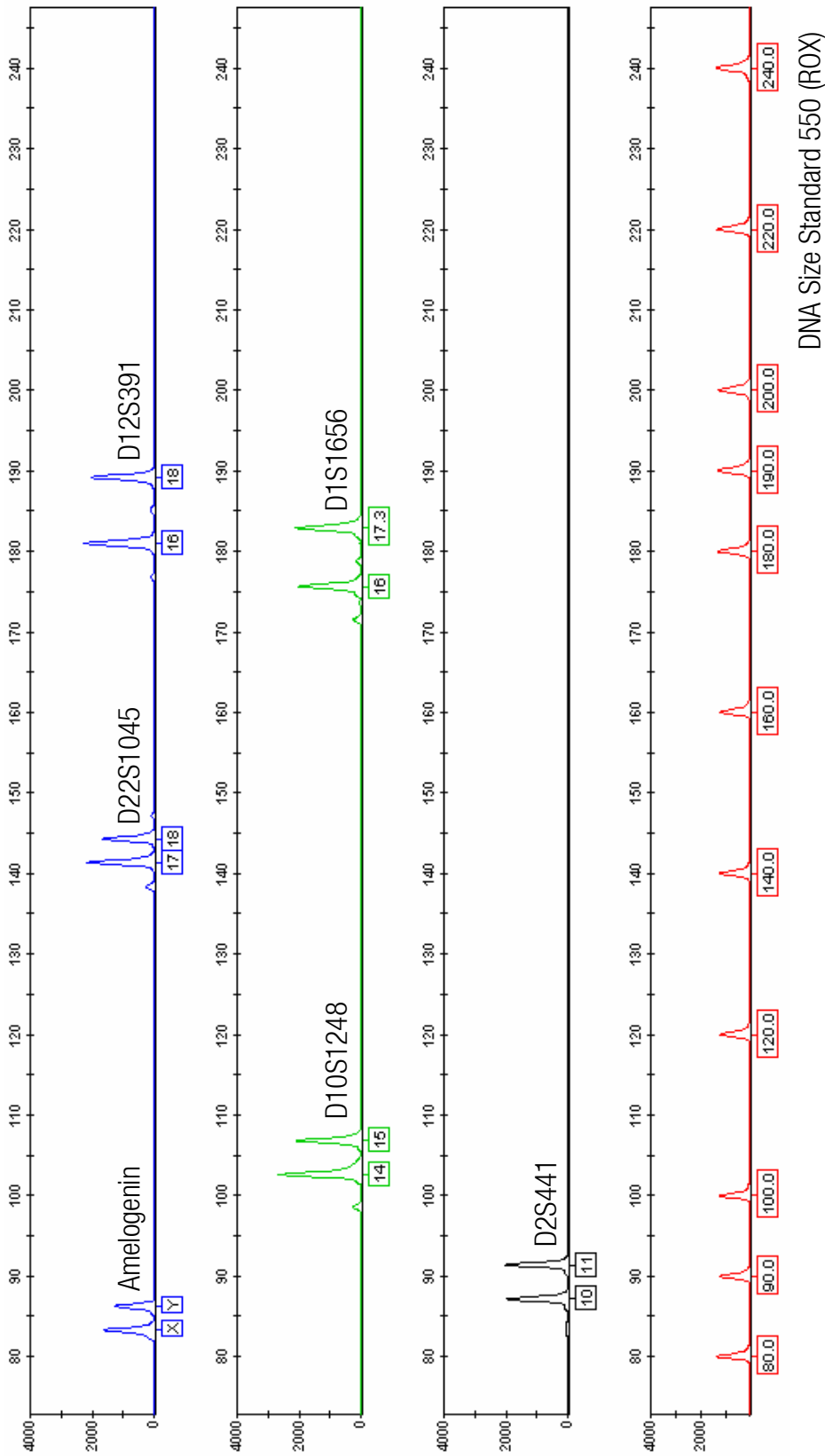
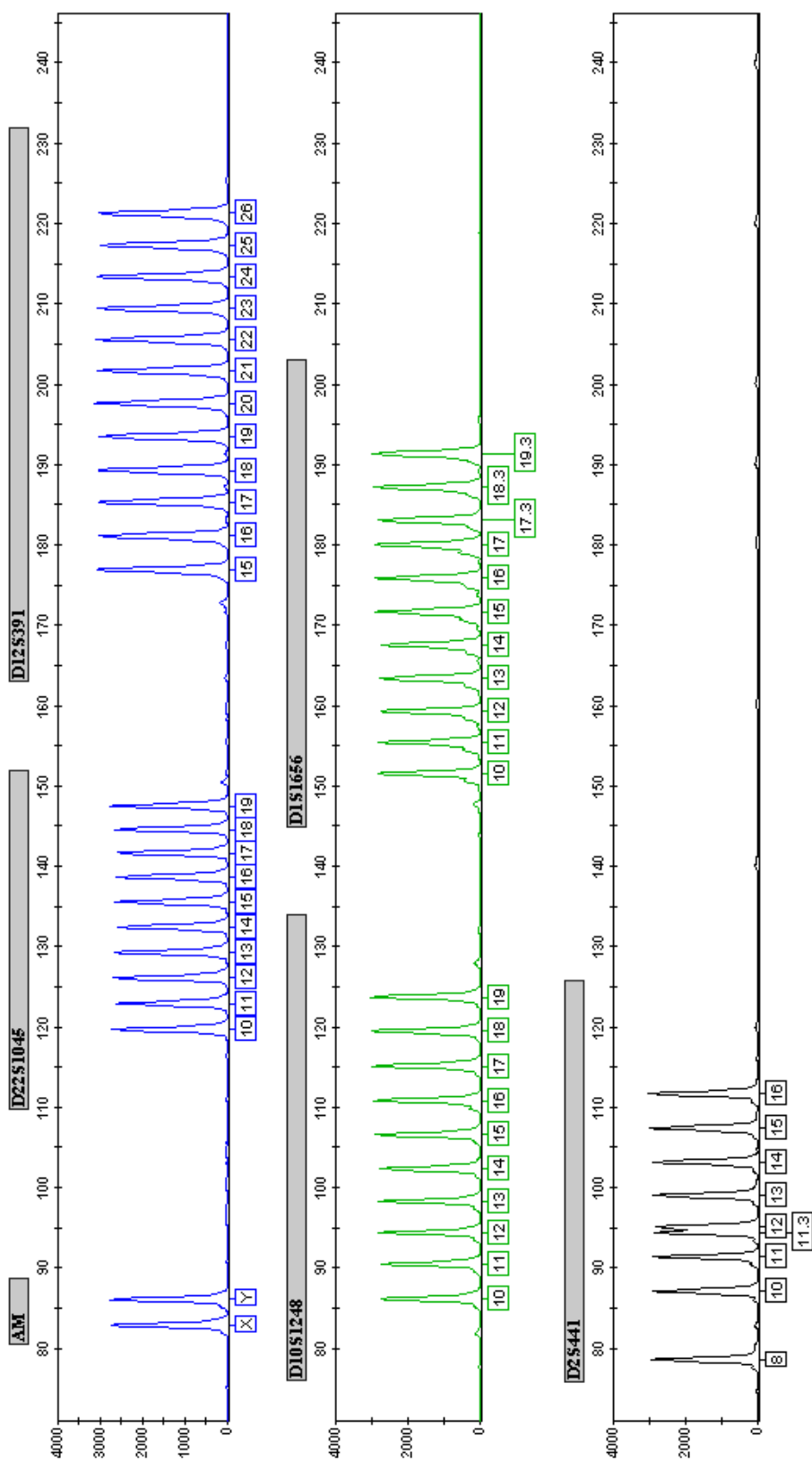


Fig. 6 Electropherogram of the Menteype® Pentaplex ESS using 200 pg Control DNA XY13. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (ROX). Allele assignment was performed using the GeneMapper™ ID Software and the Menteype® Pentaplex ESS template file.

Figure 7



**Fig. 7** Electropherogram of the allelic ladder Mentype® Pentaplex ESS analysed on an ABI PRISM® 3130 Genetic Analyzer. Allele assignment was performed using the GeneMapper™ Software and the Mentype® Pentaplex ESS template files.

**Table 4. Fragment lengths of the allelic ladder Mentype<sup>®</sup> Pentaplex ESS analysed on an ABI PRISM<sup>®</sup> 310/3130 Genetic Analyzer (blue panel)**

| Allele/Marker     | Size [bp]*   | Allele/Marker   | Size [bp]*   | Further Alleles** | Allele/Marker  | Size [bp]*   | Further Alleles** |
|-------------------|--------------|-----------------|--------------|-------------------|----------------|--------------|-------------------|
| <b>Amelogenin</b> | <b>6-FAM</b> | <b>D22S1045</b> | <b>6-FAM</b> |                   | <b>D12S391</b> | <b>6-FAM</b> |                   |
| X                 | 88           | 10              | 122          | 7, 8, 9           | 15             | 179          | 14                |
| Y                 | 91           | 11              | 125          |                   | 16             | 183          |                   |
|                   |              | 12              | 128          |                   | 17             | 187          | 17.3              |
|                   |              | 13              | 131          |                   | 18             | 191          | 18.3              |
|                   |              | 14              | 134          |                   | 19             | 195          | 19.1, 19.3        |
|                   |              | 15              | 137          |                   | 20             | 199          | 20.3              |
|                   |              | 16              | 140          |                   | 21             | 203          |                   |
|                   |              | 17              | 143          |                   | 22             | 207          |                   |
|                   |              | 18              | 146          |                   | 23             | 211          |                   |
|                   |              | 19              | 149          | 20                | 24             | 215          |                   |
|                   |              |                 |              |                   | 25             | 219          |                   |
|                   |              |                 |              |                   | 26             | 223          | 27                |

**Table 5. Fragment lengths of the allelic ladder Mentype<sup>®</sup> Pentaplex ESS analysed on an ABI PRISM<sup>®</sup> 310/3130 Genetic Analyzer (green panel)**

| Allele/Marker   | Size [bp]* | Further Alleles** | Allele/Marker  | Size [bp]* | Further Alleles** |
|-----------------|------------|-------------------|----------------|------------|-------------------|
| <b>D10S1248</b> | <b>HEX</b> |                   | <b>D1S1656</b> | <b>HEX</b> |                   |
| 10              | 91         | 8, 9              | 10             | 153        | 9                 |
| 11              | 95         |                   | 11             | 157        |                   |
| 12              | 99         |                   | 12             | 161        |                   |
| 13              | 103        |                   | 13             | 165        |                   |
| 14              | 107        |                   | 14             | 169        | 14.3              |
| 15              | 111        |                   | 15             | 173        | 15.3              |
| 16              | 115        |                   | 16             | 177        | 16.3              |
| 17              | 119        |                   | 17             | 181        | 17.1              |
| 18              | 123        |                   | 17.3           | 184        |                   |
| 19              | 127        |                   | 18.3           | 188        | 18                |
|                 |            |                   | 19.3           | 192        | 19, 20.3          |

**Table 6. Fragment lengths of the allelic ladder Mentype<sup>®</sup> Pentaplex ESS analysed on an ABI PRISM<sup>®</sup> 310/3130 Genetic Analyzer (yellow panel)**

| Allele/Marker | Size [bp]* | Further Alleles** |
|---------------|------------|-------------------|
| <b>D2S441</b> | <b>NED</b> |                   |
| 8             | 82         | 9                 |
| 10            | 90         |                   |
| 11            | 94         |                   |
| 11.3          | 97         |                   |
| 12            | 98         | 12.3              |
| 13            | 102        | 13.1              |
| 14            | 106        |                   |
| 15            | 110        |                   |
| 16            | 114        | 17                |

\* rounded to integer

\*\* The “off-ladder” alleles of Biotype’s DNA pool are allocated with the actual Biotype<sup>®</sup> template files for GeneMapper™ ID or Genotyper<sup>®</sup> software. For further alleles see amongst others [http://www.cstl.nist.gov/biotech/strbase/str\\_fact.htm](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, whereas n-3 peaks appear particularly during amplification of the trinucleotide STR motif D22S1045. Those peaks should be interpreted in accordance with the template files of the Genotyper<sup>®</sup> and GeneMapper<sup>™</sup> 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<sup>®</sup> primers are designed to minimise these artefacts. Artefact formation is further reduced by the final extension step of the PCR protocol at 68°C for 60 minutes. Peak height of the artefact correlates with the amount of DNA. Laboratories should define their own limits for analysis of the peaks.

### Artefacts

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur. If shoulder or split peaks appear, we recommend injecting the sample again.

## 6. 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.

**Gill P, Fereday L, Morling N, Schneider PM (2006)** New multiplexes for Europe-amendments and clarification of strategic development. *Forensic Sci Int.* 2006 Nov 10;163(1-2):155-7. Epub 2006 Jan 19.

**Hill CR, Kline MC, Coble MD, Butler JM (2008)** Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples, *J Forensic Sci*, January 2008, Vol. 53, No. 1: 73-80.

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

**Notes**