Animaltype **Pig** PCR Amplification Kit

Features and Benefits

Animaltype **Pig** is the sole commercially available test kit for fast and reliable genotyping of pigs. It includes eleven tetranucleotide markers (see table 1). For the most important breeding stocks German Large White (LW), German Landrace (GL), and "Piétrain" (Pi) the combined Power of Exclusion (cPE) of these STR-Systems is equivalent or even better then the 15 dinucleotide STRs recommended for Germany or Austria (see table 2).

Table 1. Animaltype Pig Tetranucleotide STR Markers

Locus*	Chromosomal Mapping
387A12F	12p14-15
S0655	7p11
SBH1	1p13
SBH2	3p16-17
SBH4	6q35
SBH10	9p11-13
SBH13	13q46-47
SBH18	16q23
SBH19	17q12-14
SBH20	18q13-23
SBH22	Xp24
SBH23	X, Y (gonosomal)

* Manuscripts for publication of Loci SBH1-23 in preparation; for 387A12F see Kiuchi et al. (2002); for S0655 see Renard et al. (2001)

Table 2. Combined Power of Exclusion of STR Markers

German Large White (LW), German Landrace (GL), and "Piétrain" (Pi)

Multiplex	cPE total	cPE GL	cPE LW	cPE Pi
11-4- STRs Biotype	0,9992	0,9997	0,9987	0,9998
15-2- STRs*	0,9965	0,9965	0,9988	0,9978

* Nechtelberger et al. (2001)

Animaltype **Pig** offers basically and essentially technical advantages:
Genotyping of tetranucleotide marker with the Animaltype **Pig** PCR Amplification Kit guaranties definite peaks (see Fig.1). Moreover, the test kit includes primers for amplification of the gender-specific marker Amelogenin (SBH23). Finally, Animaltype **Pig** is provided with an Allelic Ladder that allows standardisation of different laboratories.

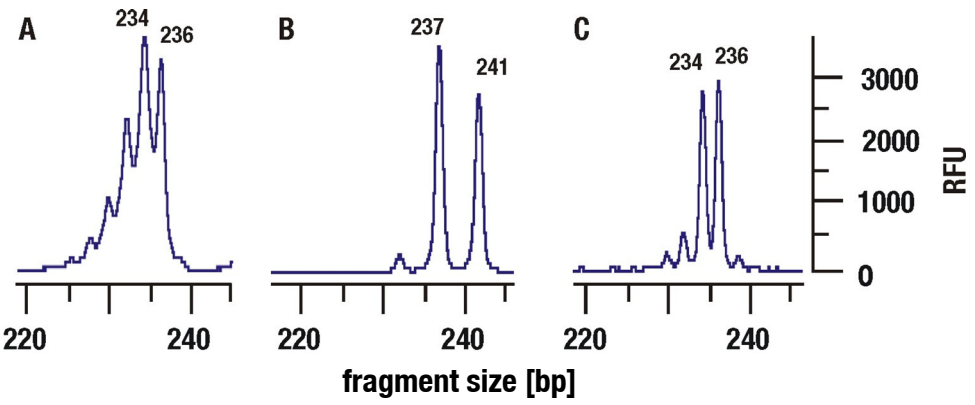

Fig. 1 The electropherogram displays the advantages of tetranucleotide-STRs in comparison to dinucleotide STRs. **A**: S0005 (Dinucleotide STR); **B**: SBH13 (Tetranucleotide STR); **C**: SBH7 (Tetranucleotide STR with 2-bp insertion; not included in the test kit). RFU: relative fluorescence units

Product Description

Animaltype **Pig** PCR Amplification Kit is a multiplex-application for kinship testing and determination of the gender. In one PCR reaction, the eleven polymorphic tetranucleotide Short Tandem Repeats loci **387A12F**, **S0655**, **SBH1**, **SBH2**, **SBH4**, **SBH10**, **SBH13**, **SBH18**, **SBH19**, **SBH20**, and **SBH22** as well as the gender-specific marker **SBH23** are amplified simultaneously.

Preferentially, the test kit is employed for fast and reliable DNA genotyping of blood or tissue samples (especially ear cartilage). One primer for each locus is fluorescence-labelled with **6-FAM** (SBH2, SBH18, SBH4, S0655), **HEX** (SBH23, SBH20, SBH1, SBH10) or **NED** (SBH13, 387A12F, SBH22, SBH19) whereas a well-balanced intensity of all signals was elaborated for the primer mix.

The detection limit of Animaltype **Pig** PCR Amplification Kit is less about **1 ng genomic DNA**. The use of **1-10 ng DNA** is recommended.

Validation and evaluation of the test kit have been performed for the GeneAmp® 9700 thermal cycler, ABI PRISM® 310 Genetic Analyzer, and ABI PRISM® 3100/3130 Genetic Analyzer.

Application

- Proof of origin according to the EU-Directive
- Kinship testing in context with control of breeding
- Status of inbreeding for herd book populations

Table 3. Locus-specific Information of Animaltype Pig

Locus	GenBank® Accession	Repeat Motif of the Reference Allele	Reference Allele	Allele Range
387A12F	AB059041	$[TTCT]_2$ CT $[TTCT]_{19}$	21	9-21
S0655	AJ251829	$[GGAA]_{12}$	12	5-22
SBH1	submitted	$[CTTT]_{13}$	13	7-18
SBH2	submitted	$[AGAA]_{24}$ AA [AGAA]	25	6-34
SBH4	submitted	$[GAAA]_2$ GGAA $[GAAA]_2$ A $[GAAG]_7$ [GAAA] [GAAG] [AAAG] $[AGAG]_5$ $[AAAG]_6$ AA $[AAAG]_4$ A $[AAAG]_3$ AA $[AAAG]_4$ A $[AAAG]_{21}$ AG $[AAAG]_3$ AGAG $[AAAG]_2$	64	47.3-66.1
SBH10	submitted	$[TAGA]_{15}$ $[CAGA]_{12}$ $[TAGA]_7$ TACA [TAGA] TACA [TAGA] TACA $[TAGA]_2$ TACA $[TAGA]_2$ TACA $[TAGA]_2$ CAAA	48	31-50
SBH13	submitted	$[TATC]_{15}$	15	8-18
SBH18	submitted	$[AGGA]_{15}$	15	9-23
SBH19	submitted	$[GTCT]_4$ $[ATCT]_{10}$	14	10-16
SBH20	submitted	$[CTTT]_{14}$ CTTC $[CTTT]_2$ CTTC $[CTTT]_2$ CTTC $[CTTT]_3$	24	19-49
SBH22	submitted	$[ATAG]_6$ ATG $[ATAG]_{11}$ ATG $[ATAG]_3$	20	18-28
SBH23 Y	submitted	-		
SBH23 X	submitted	-		

The repeat motifs shown in Table 3 are concordant with the International Society for Forensic Genetics (ISFG) guidelines for the use of microsatellite markers (Bär et al., 1997).

Content

Animaltype **Pig** PCR Amplification Kit (100 Reactions)

Nuclease-free Water	3.0 mL
Reaction Mix **D**	500 µL
Primer Mix	250 µL
DNA polymerase	40 µL
Control DNA DL157	10 µL
DNA Size Standard 550	25 µL
Allelic Ladder	10 µL

Ordering Information

Animaltype **Pig**	25	Reactions	Cat. No.	11-12110-0025
Animaltype **Pig**	100	Reactions	Cat. No.	11-12110-0100
Animaltype **Pig**	400	Reactions	Cat. No.	11-12110-0400
Animaltype **Pig**	1000	Reactions	Cat. No.	11-12110-1000

Storage

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

Quality Assurance

The content of Biotype® test kits is subjected to an intensive quality assurance of the Biotype Diagnostic GmbH. The quality of test kits is controlled continuously in order to document the unrestricted usability. For questions regarding the quality assurance, please feel free to contact us.

Additional Required Reagents

In order to use the Biotype® PCR Amplification Kit additional reagents are needed. We strongly recommend the application of the following products:

Reagent	Supplier	Order Number
Hi-Di™ Formamide, 25 mL	Applied Biosystems	4311320
Matrix Standards (DS-30) for ABI PRISM® 310 Genetic Analyzer and ABI PRISM® 377 DNA Sequencer	Applied Biosystems	401546 and 402996 (NED)
Matrix Standards (DS-30) for ABI PRISM® 3100/3130 and ABI PRISM® 3700/3730	Applied Biosystems	4345827
Qiagen DNeasy® Blood & Tissue Kit, 50 Preparations	Qiagen	69504

Trademarks and Patents

ABI PRISM®, GeneScan®, Genotyper®, GeneMapper™ and Applied Biosystems are registered trademarks of Applied Biosystems Inc.
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 under patent law. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche).
DyeEx™ and DNeasy® are registered trademarks of Qiagen.

Warning and Safety Instructions

The PCR Amplification Kit contains the following potentially hazardous chemical:

Kit Component	Chemical	Danger
Primer Mix, Reaction Mix and Allelic Ladder	sodium azide NaN_3	toxic if swallowed, develops toxic gases when it gets in contact with acids

For the Material Safety Data Sheet (MSDS) of all Biotype® products, please contact us. For MSDS of additional reagents to be needed, please contact the corresponding manufactures.

Content

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 DNA sample volume of 3.0 µL (template DNA). Determine the number of reactions to be set up, positive control and negative control reactions should be included. Add one or two reactions to this number to compensate the pipetting error.

	Number of PCR-Reactions			
Volume in [µL]	**1**	**10**	**25**	**100**
Nuclease-free Water	14.1	141.0	352.5	1410.0
Reaction Mix **D***	5.0	50.0	125.0	500.0
Primer Mix	2.5	25.0	62.5	250.0
Multi Taq2 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 preparation of the Master Mix. The DNA volume applied to the assay depends on its concentration. An increase of DNA volume to more than 5 µL is not recommended, because potential PCR inhibitors may interfere. Adjust the final reaction volume with Nuclease-free Water to 25 µL.

Generally, store DNA templates 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 with **30 PCR cycles** and **1-10 ng Control DNA DL157** in a reaction volume of 25 µL. If more DNA template is introduced, higher peaks will be expected for small PCR fragments and relatively low peaks for larger fragments. Reduce the amount of template DNA to correct this imbalance.

Positive Control

For the positive amplification control, dilute the Control DNA DL 157 to 1-10 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 Master Mix Preparation

In order to activate the Multi Taq2 DNA Polymerase and to prevent the formation of non-specific amplification products, perform a “hot start“ PCR reaction.

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	20 s	
60°C	40 s	30 Cycles
72°C	30 s	
70°C	60 min	
10°C	∞	hold

2. Electrophoresis using the ABI PRISM® 377 DNA Sequencer

For general instructions on instrument setup, matrix generation and application of the GeneScan® analysis Software, please read the *ABI PRISM® 377 DNA Sequencer User's Manual*. Electrophoresis by using the GeneScan® Software is described below.

For the combined application of the four fluorescent dyes **6-FAM**, **HEX**, **NED**, and **ROX** (also called **DS-30**) the use of the virtual **Filter Set D** is allocated. Generally, the Filter Sets A and F are suitable, too. Prior to any analysis of DNA fragment size a matrix with the appropriate four fluorescent dyes has to be generated for the instrument. Appropriate matrix standards can be purchased from Applied Biosystems.

2.1 Polyacrylamide-Gel (5%)

Composition	**Amount/Volume**
Urea	21.0 g
30% Acrylamide / bisacrylamide solution (29:1)	8.4 mL
10x TBE buffer	6.0 mL
Water	20.0 mL
Filtrate and degas solution	
10% Ammonium persulfate	350 µL
TEMED	15 µL

Use glass plates with a spacing of 36 cm

2.2 Sample Preparation

Composition	**Volume**
Hi-Di™ Formamide / Blue Dextran	1.8 µL
DNA Size Standard 550 (ROX)	0.2 µL
PCR product (diluted if necessary) or Allelic Ladder	1.0 µL

- 3 min denaturation at 95°C
- cooling at 4°C
- apply 1.5 µL sample to the gel

2.3 Setting for GeneScan® Software

Plate Check Module **D**
PreRun Module "GS PR 36D - 1200"
Run Module "GS Run 36D - 1200"
Matrix **D** (6-FAM, HEX, NED, ROX)
Standard SST550 (ROX)

Programming of the Run Module for Biotype® Test Kits (6-FAM / HEX / NED / ROX)

- Open program "377 Collection"
- Open File - New - GeneScan Run
- Select the module "GS Run 36D -1200" in the run window
- Click at the sheet symbol in the run window
- Use the following settings:

Parameter	**Setting**
Voltage	3000 V
Current	50.0 mA
Power	150 W
Collection time	**3.0 h**
Gel temperature	51°C
Laser power	40.0 mW

Save the module in the field „Save Copy“

3. Electrophoresis using the ABI PRISM® 310 Genetic Analyzer

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

For the combined application of the four fluorescent labels **6-FAM**, **HEX**, **NED**, and **ROX** (also called **DS-30**) the use of the virtual **Filter Set D** is allocated. Generally, Filter Sets A and F are suitable, too.

Material

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

3.1 Matrix Generation

Prior to any analysis of DNA fragment size a matrix with the appropriate four fluorescent labels has to be generated. Suitable matrix standards can be purchased from Applied Biosystems.

Dye Color	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

To generate useful matrix files, it is necessary to perform four electrophoresis runs with the matrix samples (PCR fragments labelled with 6-FAM, HEX, NED and ROX). The runs have to be done under the same conditions like for the samples and the Allelic Ladders of the Biotype® test kit.

Matrix sample	Composition	Volume
Matrix sample 1	Hi-Di™ Formamide	12.5 µL
	Matrix Standard **6-FAM**	1.0 µL
Matrix sample 2	Hi-Di™ Formamide	12.5 µL
	Matrix Standard **HEX**	1.0 µL
Matrix sample 3	Hi-Di™ Formamide	12.5 µL
	Matrix Standard **NED**	1.0 µL
Matrix sample 4	Hi-Di™ Formamide	12.5 µL
	Matrix Standard **ROX**	1.0 µL

- 3 min denaturation at 95°C
- cooling at 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

- Open GeneScan® or GeneMapper™ ID Software
- FILE → NEW → PROJECT (open current run folder) → ADD SAMPLE FILES
- Click a single matrix sample in the column SAMPLE FILE
- Mark SAMPLE → RAW DATA
- Review the matrix samples for a flat baseline beyond the primer peak. There should be at least five peaks with peak heights about 400-4000 (Y) in every matrix sample (optimal range: 1000-3000), as shown in Fig. 2

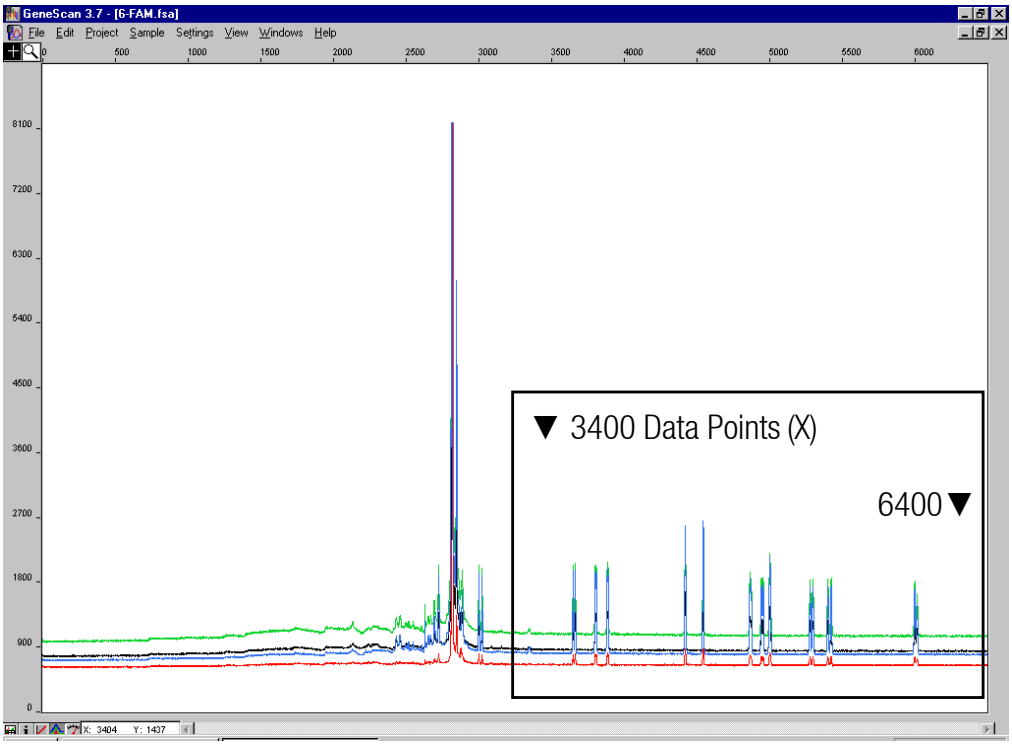

Fig. 2 Electropherogram with Raw Data of the Matrix Standard 6-FAM

- Select analysis range with flat baseline
- Re-inject the matrix sample if necessary
- Note start and end value (Data Points) of the analysis range, e. g. start value 3400, end value 6400
- Calculate the difference value, e. g. 6400-3400 = 3000 Data Points

Generation of a New Matrix

- FILE → NEW → MATRIX, as shown in Fig. 3

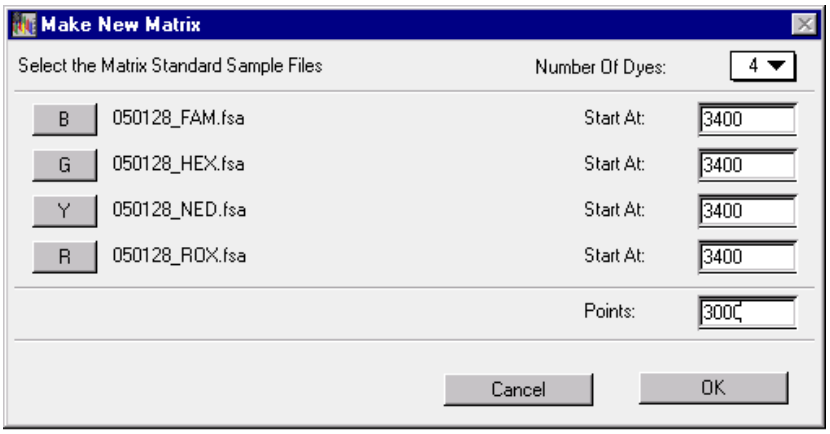

Fig. 3 Select Matrix Samples

- Import matrix samples for all dyes (B, G, Y, R)
- Enter „Start At“ value, e. g. 3400
- Enter difference value at *Points*, e. g. 3000
- Select OK to calculate the new matrix, as shown in Fig. 4

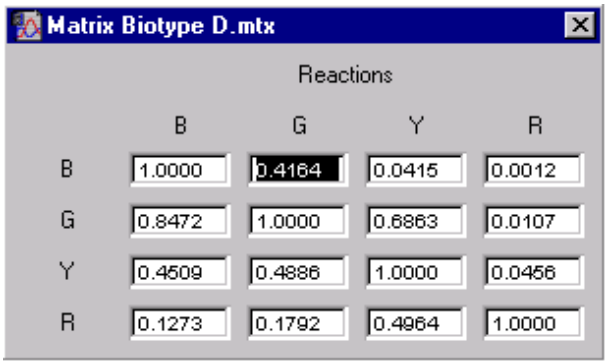

Fig. 4 New Matrix Biotype

- Save in the Matrix Folder: FILE → SAVE AS, e. g. Matrix Biotype

Matrix Check

Please check the new matrix with current samples.
- FILE → NEW → PROJECT (open run folder) → ADD SAMPLE FILES
- Select sample(s) in the column SAMPLE FILE
- SAMPLE → INSTALL NEW MATRIX (open matrix folder and select new matrix)
- Re-Analyse your samples

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

3.2 Sample Preparation

Composition	Volume
Hi-Di™ Formamide	12.3 µL
DNA Size Standard 550 (ROX)	0.2 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	

- 3 min denaturation at 95°C
- cooling at 4°C
- for analysis: load the samples on the tray

Signal Intensities

In order to increase the intensity of signals:
- Reduce the volume of the DNA Size Standard 550 (ROX); the peaks of the Size Standard should be about 500 relative fluorescent units (RFU)
- Purify the PCR products with DyeEx™ 2.0 Spin Kit, Qiagen (63204)

3.3 Setting for GeneScan® Software

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

Injection List

Module File	GS STR POP-4 (1 mL) **D**
Matrix File	z. B. Matrix Biotype
Size Standard	z. B. SST550_50-500bp
Injection [s]*	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]**	**28**

* Apart from standard setting, the injection time may be between 1 and 10 s depending on the type of sample. If blood samples with very high signal intensities are to be 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 Animaltype **Pig** was modified in order to analyze lengths of fragments up to **500 bp**.

3.4 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 from 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 higher then the background noise of the baseline.

** Sometimes, point alleles (i. e. alleles with at least 1 bp difference to the next integer allele) like 387A12F allele 13 and 13.1 or 16 and 16.1 can not be distinguished. For improved peak detection, minimize the Peak Window Size further on.

4. 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 and the GeneMapper™ ID Software, refer to the *ABI PRISM® 3130/3130xl Genetic Analyzers Getting Started Guide*. Electrophoresis by using the GeneMapper™ ID Software is described below.

The 4-Capillary-System is named ABI 3130 (before ABI 3100-Avant), the 16-Capillary-System is named ABI 3130xl (before ABI 3100).

For the combined application of the four fluorescent labels **6-FAM**, **HEX**, **NED**, and **ROX** (also called **DS-30**) the utilization of the **Dye Set D** is allocated.

Material

Capillary	3130 capillary array, 36 cm
Polymer	3130 POP-4 polymer
Buffer	10x Genetic Analyzer Buffer with EDTA

4.1 Spectral Calibration / Matrix Generation

Prior to any analysis of DNA fragment size a spectral calibration with the four florescent labels 6-FAM, HEX, NED and ROX has to be generated for each analyzer. The spectral calibration creates a matrix to correct the overlapping of fluorescence emission spectra of the dyes.

Performing a spectral calibration can be divided into the following tasks:
- Choosing and setting up the Spectral Calibration Standards
- Loading the standards on the 96-well reaction plate (per capillary one sample)
- Creating the instrument protocol for performing spectral calibration (Protocol Manager)
- Define the plate assembly within the plate editor (Plate Manager)
- Performing a spectral calibration run and reviewing data

Setting up the Spectral Calibration Standards

Example for 4 Capillaries/ABI 3130

Composition	**Volume**
Hi-Di™ Formamide	47.5 µL
Matrix Standard	2.5 µL

- 3 min denaturation at 95°C
- cooling at 4°C
- 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	10.0 µL

- 3 min denaturation at 95°C
- cooling at 4°C
- 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

- Insert the 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection Software click **New** in 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_DyeSet D_Date
Application	Spectral Calibration
Plate Type	96-Well
Owner Name / Operator Name	…

- Select **OK** to complete the New Plate dialog box. A new table in the Plate Editor opens automatically
- For further analyses for spectral calibration use with the same plate setting, click **Import**, select the .xml file and click **Open**

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 before)

- For each of the columns, click the column header to select entire column, then select **Edit → Fill Down** to apply the information to all of the selected samples, and enter **OK**
- In **Run Scheduler** click **Find All**, select **link** to link up the reaction plate on the autosampler with the newly created plate record (position A or B) and start the run
- View Pass/Fail Status after the run in the **Event Log** and open the **Spectral Viewer** to review and evaluate the spectral calibration profile for each capillary

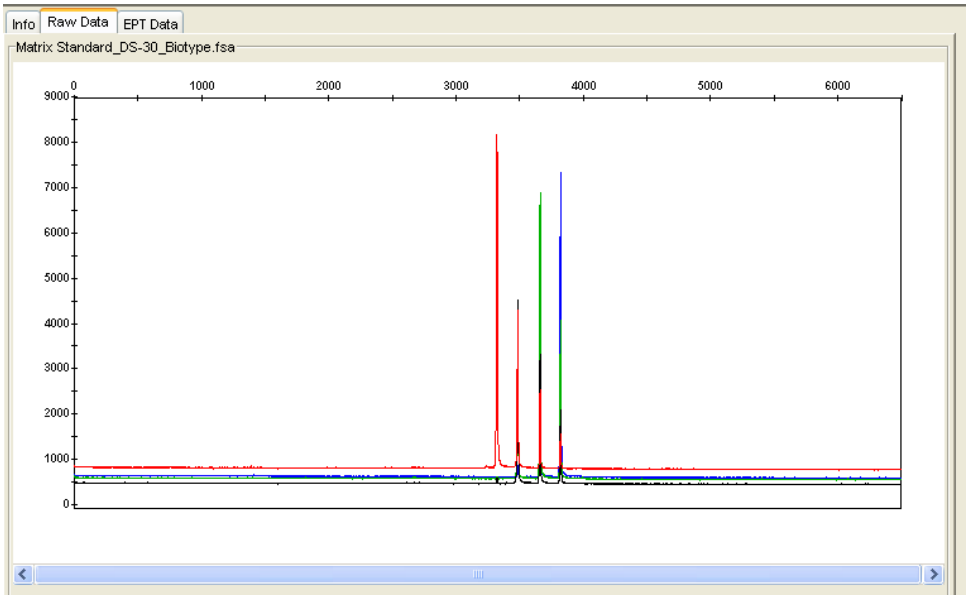

Fig. 5 Electropherogram with Raw Data of the Matrix Standard for DS-30

Note: If all capillaries passed the test, activate this Spectral Calibration file for Dye Set D in the **Spectral Viewer**.

4.2 Sample Preparation

Composition	Volume
Hi-Di™ Formamide	12.3 µL
DNA Size Standard 550 (ROX)	0.2 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	

- 3 min denaturation at 95°C
- cooling at 4°C
- for analysis: load the samples on the tray

Because injections proceed simultaneously for all capillaries, four samples must be pipette for analysis on 4-capillary analysers. If less then four samples are analysed, fill up the empty positions on the plate with 12 µL Hi-Di™ Formamide.

For reliable allelic assignment on 4-capillary analysers, one Allelic Ladder per capillary 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.

4.3 Setting for GeneMapper™ ID Software

Performing Run

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

Instrument Protocol

Protocol Editor

Name	Run36_POP4_DyeSet D
Type	REGULAR
Run Module*	HIDFragmentAnalysis36_POP4_1
Dye Set	D

* for detailed description see **Setting of the Run Module** on the next page

- Select **OK** to complete the Protocol Editor dialog box

Previous to the first run, it is necessary to edit the Run Module as follows:
- In the **Module Manager** of the Data Collection Software click **New** to open the **Run Module Editor** dialog box

Run Module (24min_50-500bp)

Run Modul Editor	**Value**
Oven Temperature [°C]	60
Poly Fill Volume	4840
Current Stability [µA]	5
PreRun Voltage [kV]	15
PreRun Time [s]	180
Injection Voltage [kV]	3.0
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]**	**1440**

* Apart from 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 to be recorded, a shorter injection time may be selected. For samples with low DNA content an injection time up to 20 s may be necessary.

** Depending on the analysis conditions, the Run Time for Animaltype **Pig** was modified in order to analyze lengths of fragments up to **500 bp**.

- Click **Save As** and enter the name of the new module (e. g. 24min_50-500bp) and enter **OK**
- Click **Close** to exit the Module Editor

Previous to every run, it is necessary to compile the plate as follows:
- In the **Plate Manager** of the Data Collection Software click **New** to open the **New Plate Dialog** box

GeneMapper™ Plate Editor (I)

New Plate Dialog	
Name	e. g. Plate_DyeSet D_Date
Application	select GeneMapper Application
Plate Type	96-Well
Owner Name / Operator Name	…

- Select **OK** to complete the New Plate dialog box. A new table in the Plate Editor opens automatically

GeneMapper™ Plate Editor (II)

Column Name	
Sample Name	Type name for the samples
Priority	e. g. 100 (Default)
Sample Type	Sample or Allelic Ladder
Size Standard	e. g. SST550_50-500bp
Panel	e. g. Animaltype_Panels_v1
Analysis Method	e. g. Analysis_HID_3130
Snp Set	-
User-defined 1-3	-
Results Group 1	(select results group)
Instrument Protocol 1	Run36_POP4_DyeSet D (setting typed before)

- For each of the columns, click the column header to select entire column, then select **Edit → Fill Down** to apply the information to all of the selected samples and enter **OK**
- In **Run Scheduler** click **Find All**, select **link** to link up the reaction plate on the autosampler 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 former chosen **Result Group**

4.4 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 from 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 higher then the background noise of the baseline.

** Sometimes, point alleles (i. e. alleles with at least 1 bp difference to the next integer allele) like 387A12F allele 13 and 13.1 or 16 and 16.1 can not be distinguished. For improved peak detection, minimize the Peak Window Size further on.

5. Analysis

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

The determination of the exact lengths of the amplified products depends on the type of device, on the conditions of electrophoresis, as well as on the DNA Size Standard used. Due to the complexity of some STR loci, the size determination should be based on evenly distributed points of references. Thus, please use the DNA Size Standard 550 (ROX) 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.**

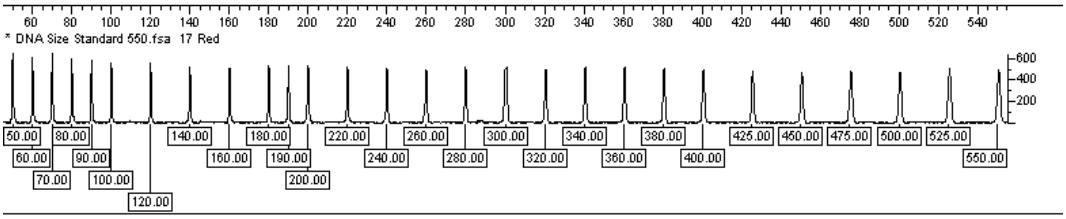

Fig. 6 Electropherogram of the DNA Size Standard 550 (ROX), Lengths of Fragments in bp

Allele designation of analyzed samples should be carried out either manually or with suitable analysis software, e. g. GeneMapper™ ID or Genotyper® Software in combination with the Animaltype **Pig** Template File from Biotype.
Template Files can be received as free downloads from our homepage (www.biotype.de) or as CD-ROM on request.

Special Features

SBH4

In rare cases deviation of 0.6 bp between signals versus the Allelic Ladder due to single nucleotide polymorphisms (SNPs) within repeating units can be observed. Exact assignment of all alleles according to the Allelic Ladder is achieved by setting the tolerance to +/- 1.0 bp in the analysis software. This point is already fixed in Biotype® Template Files for Genotyper® or GeneMapper™.

SBH1

Sometimes deviation of the signals for allele 14 and 15 versus the Allelic Ladder due to SNPs within repeating units can be observed. Exact assignment of these alleles according to the Allelic Ladder is achieved by setting the tolerance to +/- 2.0 bp in the analysis software. This point is already fixed in Biotype® Template Files for Genotyper® or GeneMapper™.

SBH23 (**Amelogenin**)

Rarly, deviation of + 1.0 bp for the X-specific signal versus the Allelic Ladder due to an insertion of a base can be observed. Exact assignment of the X-specific allele according to the Allelic Ladder is achieved by setting the tolerance to +/- 2.0 bp in the analysis software. This point is already fixed in Biotype® Template Files for Genotyper® or GeneMapper™.

Unspecific amplificates

Two artefact signals were observed in 6% of the DNA samples from Biotype Diagnostic GmbH at position 294 bp in 6-FAM between the allelic ranges of SBH18 and SBH4 and at position 298 bp in HEX within the allelic region of SBH1. These artefacts do not match with signals of the Allelic Ladder or with known alleles from the population study.

5.1 Controls

The Control DNA DL157 of the PCR Amplification Kit represents the following alleles:

Table 4. Allele Determinations of Animaltype Pig

Locus	Control DNA DL157
387A12F	13/21
S0655	9/12
SBH1	14/16
SBH2	22/33
SBH4	56/62
SBH10	48/49
SBH13	13/15
SBH18	12/14
SBH19	14/14
SBH20	32/39
SBH22	20/23
SBH23	Y/X

5.2 Fragment Lengths and Alleles

Table 5 to **table 7** display the values for fragment lengths of individual alleles that refer to the DNA Size Standard 550 (ROX). All analyses have been performed on an ABI PRISM® 310 Genetic Analyzer with POP-4 polymer. Different analysis instruments, DNA Size Standards or polymers might result in different lengths of fragment. These Data could serve as approximate allele sizes for the creation of a new analysis template. In order to balance instrument-specific deviations further fine tuning of the equipment should be done by measuring sample fragments of known lengths. In addition, a visual alignment with the Allelic Ladder is recommended.

Scaling

Horizontal: 75-505 bp
Vertical: Depending on signal intensity

Figure 7

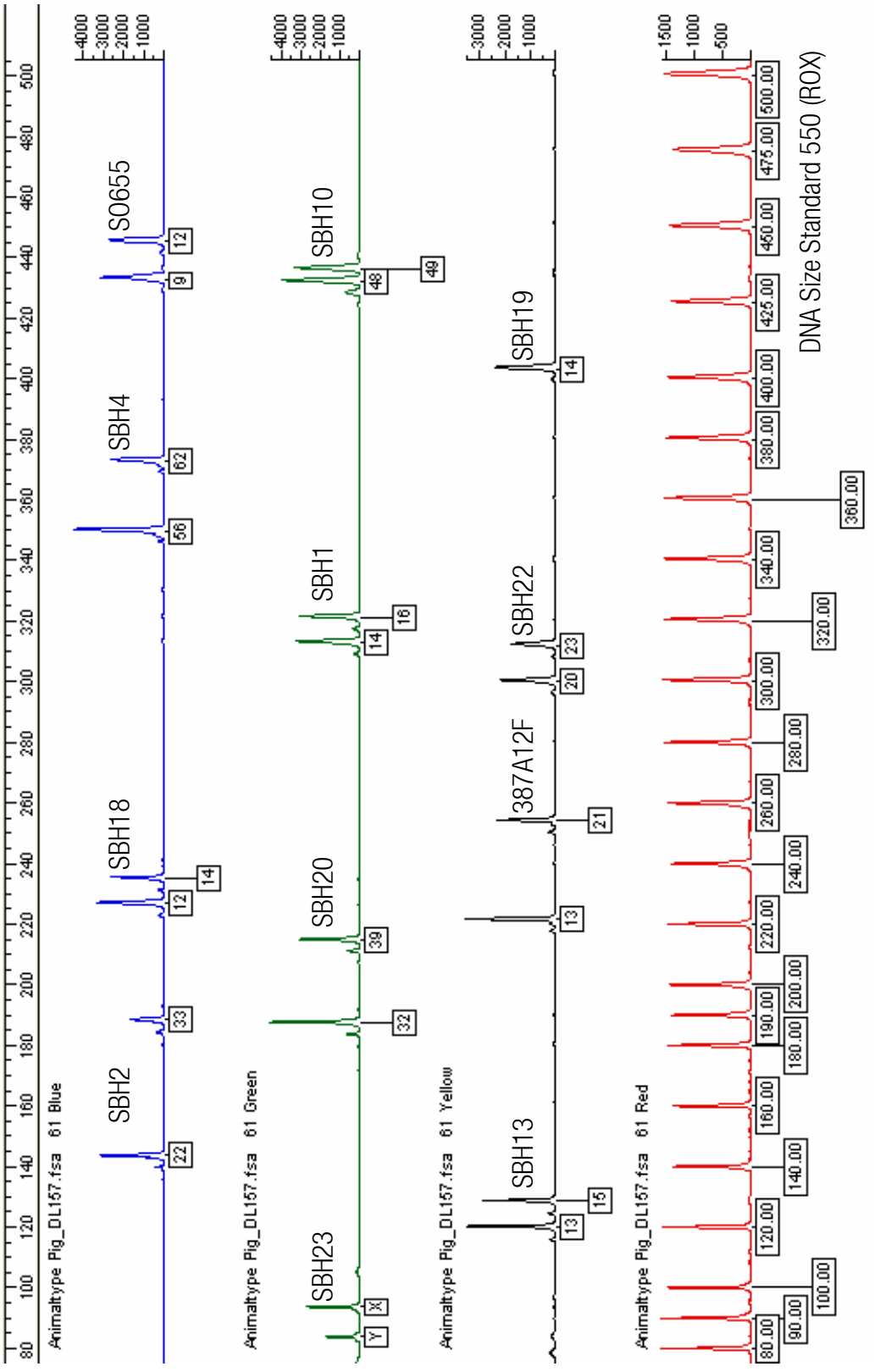

Fig. 7 Electropherogram of the Animaltype **Pig** using 2 ng Control DNA DL157. Analysis was done on an ABI PRISM® 310 Genetic Analyzer with the DNA Size Standard 550 (ROX). Allele assignment was performed using the Genotyper® Software and the Animaltype **Pig** Template File.

Figure 8

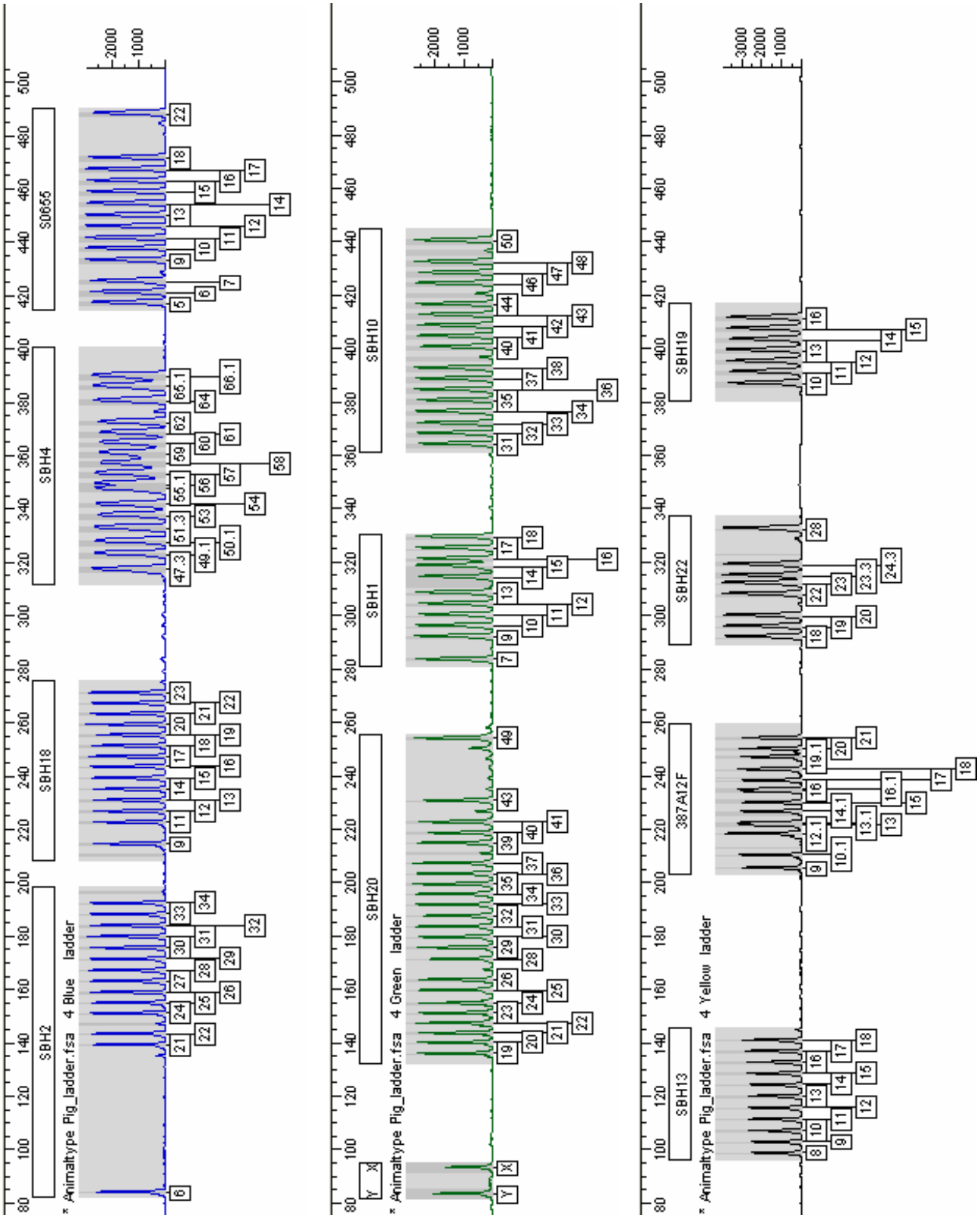

Fig. 8 Electropherogram of the Animaltype **Pig** Allelic Ladder analysed on an ABI PRISM® 310 Genetic Analyzer. Allele assignment was performed using the Genotyper® Software and the Animaltype **Pig** Template File.

Table 5. Fragment Lengths of the Allelic Ladder Animaltype Pig analysed on an ABI PRISM® 310 Genetic Analyzer (Blue Panel)

Allele	Size [bp] ABI 310	Further Alleles*	Allele	Size [bp] ABI 310	Remarks	Allele	Size [bp] ABI 310	Further Alleles*
SBH2	**6-FAM**		**SBH4**	**6-FAM**		**S0655**	**6-FAM**	
6	84.0		47.3	317.4	±1.0 bp[b]	5	416.6	
21	139.2		49.1	322.7	±1.0 bp[b]	6	420.6	
22	143.2	23[a]	50.1	327.3	±1.0 bp[b]	7	424.7	
24	151.0		51.3	332.6	±1.0 bp[b]	9	432.5	
25	154.9		53	337.2	±1.0 bp[b]	10	436.8	
26	158.9		54	341.5	±1.0 bp[b]	11	440.8	
27	163.0		55.1	347.6	±1.0 bp[b]	12	445.2	
28	167.1		56	349.1	±1.0 bp[b]	13	449.4	
29	171.3		57	353.0	±1.0 bp[b]	14	453.8	
30	175.5		58	357.0	±1.0 bp[b]	15	458.1	
31	179.7		59	360.7	±1.0 bp[b]	16	462.2	
32	183.9		60	364.6	±1.0 bp[b]	17	466.5	
33	188.0		61	368.4	±1.0 bp[b]	18	470.8	
34	192.2	35[a]	62	372.2	±1.0 bp[b]	22	487.6	
			64	380.2	±1.0 bp[b]			
SBH18	**6-FAM**		65.1	385.8	±1.0 bp[b]			
9	214.4		66.1	389.7	±1.0 bp[b]			
11	222.5							
12	226.6							
13	230.8							
14	235.0							
15	239.2							
16	243.5							
17	247.3							
18	251.3							
19	255.2							
20	259.2							
21	263.2							
22	267.1							
23	271.0							

Table 6. Fragment Lengths of the Allelic Ladder Animaltype Pig analysed on an ABI PRISM® 310 Genetic Analyzer (Green Panel)

Allele	Size [bp] ABI 310	Further Alleles*	Allele	Size [bp] ABI 310	Further Alleles*
SBH23	**HEX**		**SBH1**	**HEX**	
Y	83.4		7	283.8	8[a]
X	93.4	X (94.4)[b]	9	292.0	
			10	296.0	
SBH20	**HEX**		11	300.0	
19	136.0		12	304.2	
20	139.9		13	308.4	
21	143.6		14	312.6	±2.0 bp[b]
22	147.3		15	316.8	±2.0 bp[b]
23	151.1		16	320.9	
24	154.8		17	324.9	
25	159.7		18	329.1	
26	163.6				
28	171.2		**SBH10**	**HEX**	
29	175.5		31	363.5	
30	179.5		32	367.5	
31	183.5		33	371.5	
32	187.5		34	375.7	
33	191.6		35	379.9	
34	195.6		36	383.9	
35	199.4		37	388.0	
36	203.3		38	392.0	39[a]
37	207.3	38[a]	40	400.0	
39	214.8		41	404.0	
40	218.6		42	408.0	
41	222.6		43	412.0	
43	230.6		44	415.8	45[a]
49	254.1		46	423.8	
			47	427.8	
			48	431.8	49[a]
			50	439.8	

Table 7. Fragment lengths of the Allelic Ladder Animaltype Pig analysed on an ABI PRISM® 310 Genetic Analyzer (yellow panel)

Allele	Size [bp] ABI 310	Further Alleles*	Allele	Size [bp] ABI 310	Further Alleles*
SBH13	**NED**		**SBH22**	**NED**	
8	98.7		18	291.7	
9	102.8		19	295.7	
10	107.0		20	299.7	
11	111.2		22	307.7	
12	115.6		23	311.7	
13	119.9		23.3	314.7	24[a]
14	124.2		24.3	318.7	25[a], 25.3[a]
15	128.5		28	332.3	27.3[a]
16	132.7				
17	136.9		**SBH19**	**NED**	
18	140.9		10	386.5	
			11	390.7	
387A12F	**NED**		12	394.7	
9	205.4		13	399.0	
10.1	210.3	11[a]	14	403.0	
12.1	218.3		15	407.0	
13	221.4		16	411.0	
13.1	222.4				
14.1	226.6	14[a]			
15	229.8	15.1[a]			
16	234.0				
16.1	235.0				
17	238.2	17.1[a]			
18	242.2				
19.1	247.2				
20	250.2				
21	254.2				

* **a.** Biotype (DNA pool), **b.** see Special Features (chapter 5)

6. Interpretation of Results

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

Pull-up Peaks

If peak heights are outside the linear detection range (>3000 RFU) or if an incorrect matrix has applied, pull-up peaks can occur at positions of a specific peaks in all colour channels. In order to avoid pull-up peaks, peak heights should not exceed more than 3000 RFU.

Stutter Peaks

Appearance of stutter peaks depends on the sequence of the repeat structure and on the number of alleles. n-4 peaks are due to a loss of a repeat unit during amplification of tetranucleotide STR motives caused by slippage effects of the Multi Taq2 DNA Polymerase. Interpretation of those peaks should be done in accordance with the Template Files of the Genotyper® and GeneMapper™ ID Software.

Template-independent Nucleotide Addition

The terminal transferase activity of Multi Taq2 DNA Polymerase leads to the addition of an adenosine residue preferentially at the 3'-end of amplified DNA fragments. Incomplete extension is responsible for double peaks, resulting from the absence of the terminal adenosine residue. The artefact peak is one base shorter than expected (-1 peaks). All Biotype® primers are designed to minimize these artefacts. In addition, the final extension step of 70°C for 60 minutes is included to the PCR protocol in order to reduce formation of artefacts. Peak height of the artefact correlates with the amount of DNA. Every laboratory should determine their own thresholds for analysis of the peaks.

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. *Int. J. Legal Med. 110: 175-176*.

Kiuchi S, Inage Y, Hiraiwa H, Uenishi H, Yasue H (2002) Assignment of 280 swine genomic inserts including 31 microsatellites from BAC clones to the swine RH map (IMpRH map). *Mamm. Genome 13: 80-88.*

Nechtelberger D, Kaltwasser C, Stur I, Meyer JN, Brem G, Mueller M, Mueller S (2001) DNA microsatellite analysis for parentage control in Austrian pigs. *Anim Biotechnol 12: 141-144.*

Renard C, Vaiman M, Chiannilkulchai N, Cattolico L, Robert C, Chardon P (2001) Sequence of the pig major histocompatibility region containing the classical class I genes. *Immunogenetics 53: 490-500.*

Notes