






# DHARMAPLEX9™

## 8 STR loci plus Amelogenin

**REF** : KDMH01

Ver 1.0

**RUO**

<b>RUO</b>	<b>For Research Use Only</b>	<b>REF</b>	<b>Catalog Number</b>
	<b>Store At</b>	<b>LOT</b>	<b>Batch Code</b>
	<b>Manufactured By</b>		<b>Biological Risk</b>
	<b>Expiry Date</b>		<b>Consult Operating Instructions</b>

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**Introduction:**

**DharmaPlex9** is a short tandem repeat (STR) multiplex assay with 8 STR markers and Amelogenin for gender determination. It combines 5 CODIS loci (FGA, D16S539, D8S1179, CSF1PO and D5S818 and), 2 out of the 5 new core loci recommended for extension of European national databases (D10S11248 and D12S391), as well as the most powerful in discrimination STR-locus SE33. PCR primers have been designed to fit into a single amplification reaction with PCR products less than 250 bp (except SE33), taking in account all known alleles so far. Accordingly, PCR-products are labelled with blue, green, yellow and red fluorescent colors by using a sizing standard labeled in a fifth, orange color.

Full STR-profiles can be generated, when at least 200 pg of non-degraded genomic DNA are used for PCR.

The kit is provided in a lyophilized format in 0.2 ml PCR-reaction plates and can be stored protected from light at room temperature for at least six months without loss of sensitivity. The reaction components are activated simply by addition of appropriate amount of activator solution to each tube. Depending on the type of kit the final reaction volume is either 12.5 ul or 25 ul, so that up to 10 ul or 20 ul of DNA can be added per reaction. Due to its extremely high tolerance to inhibiting substances such high amounts of DNA can be amplified successfully.

**DharmaPlex9** can be used for screening purposes as well as additional kit to **DharmaPlex11** or **DharmaPlex20** kits for analysis of highly degraded samples due to the high discrimination power for forensic applications, paternity and complex relationship testing. The development and validation of this kit was performed with GeneAmp®9700 Thermocycler and ABI PRISM® 3130/3130XL Genetic Analyzer.

Table 1. Information on STR loci

Marker	GenBank® Accession	GenBank® Allele	Chromosomal Localization	Repeating Unit of GenBank Allele
D5S818	AC008512	11	5q23.2	[AGAT] <sub>11</sub>
D8S1179	AF216671	13	8q24.13	[TCTA] <sub>3</sub>
D10S1248	AL391869	13	10q26.3	[GGAA] <sub>13</sub>
D12S391	G08921	19.3	12p13.2	[AGAT] <sub>5</sub> GAT[AGAT] <sub>7</sub> [AGAC] <sub>6</sub>
D16S539	AC024591	11	16q24.1	AGAT
CSF1PO	X14720	12	5q33.1	[GATA] <sub>11</sub>
FGA	M64982	21	4q31.3	[AGAT] <sub>12</sub>
SE33	V00481	26.2	6q14	[TTTC] <sub>3</sub> TTTTTTCT[CTTT] <sub>13</sub> CTCC[TTCC] <sub>2</sub>
Amelogenin X	M55418	X	Xp22.1-22.3	[AAAG] <sub>8</sub> AA [AAAG] <sub>17</sub>
Amelogenin Y	M55419	Y	Yp11.2	

**Table 1** indicates the summarized information regarding STR-loci of this kit. The Repeating Unit designation of the reference sequences was performed according to the recommendations of the International Society for Forensic Genetics (ISFG) [Bär et al, 1997]. Amelogenin is not an STR, but displays specific PCR products for X- and Y-chromosomes.

**Materials Provided:**

This kit is provided as micro plates (96 reactions) in two different formats of final PCR-volumes: **12.5 ul** and **25 ul**.

**Kit Components (12 x 8 reactions)**

12 strips, each with 8 x 0.2 ml reaction tubes, each with single cap

Activation Solution (blue lid)	1.0 ml
Deionized Water (white lid)	2.0 ml
Allelic Ladder, dried (red lid)	1 tube (20 Applications)
Control DNA, dried (green lid)	1 tube (20 Applications)
Sizing Standard S450, dried (orange label)	1 tube (120 Applications)

**Storage Conditions**

The kit contains all chemical components necessary for successful PCR, **including polymerase**, and is supplied in dry form. Therefore, the kit is shipped at ambient temperature. Labelled primers, Size Standard S450 and Allelic Ladder are light sensitive and must be stored protected from light. Customers can store the kit for a few months at room temperature without significant loss of activity. For long-term storage the reaction tubes should be placed in a refrigerator at 4°C - 8°C. Do not freeze !. This can damage dried polymerase.



**Water** and **Activation solution** should be stored refrigerated at 4°C - 8°C. For **Control-DNA** storage at 4°C - 8°C or long-term storage at -20°C is recommended after dissolving.



Immediately after delivery the tube with **Allelic Ladder** should be separated from other kit components and stored protected from light in a separate place at room temperature (post- amplification area of your lab). Do not freeze! Once the Allelic Ladder has been dissolved, it should be stored in the dark refrigerated at 4°C - 8°C.



**Safety Precautions**

Some of the reagents of the Kit contain NaN<sub>3</sub> and are potentially hazardous and should be handled accordingly. Always wear gloves and avoid inhalation and skin contact.



**Quality Assurance**

The high quality of all kit components was verified and controlled during manufacturing. The final dry kit was regularly tested over time to ensure high sensitivity for at least six months. If there are any questions regarding our quality assurance program, don't hesitate to contact us.

**Supplemental Material**

Supplemental Material, not provided with the Kit, but supplied by KRISHGEN

Matrix Standard MXS_5 for ABI 3130 and ABI 3130xl	MXS_3130
Matrix Standard Set MXS_1 for ABI 310	please inquire
Genotyper™ Macro or GeneMapper™ Macro	please inquire

**Supplemental Material, not provided with the Kit (to be supplied by the user)**

Chemical	Company	Cat Number
Hi-Di™ Formamide	Applied Biosystems	(P/N 4311320)
10 x Genetic Analyzer Buffer	Applied Biosystems	(P/N 402824)
Polymer (POP-4)	Applied Biosystems	(P/N 402838)

**Solubilization of Dried Components**

**Allelic Ladder**

Immediately after delivery the tube with allelic ladder should be separated from other kit components and stored protected from light in a separate place (post-amplification area of your lab). Do not freeze!

To get a working solution, add to the tube with dried allelic ladder (yellow cap) 20 ul of deionized water provided with this kit, mix thoroughly and spin down for a few seconds. Once the allelic ladder is suspended it should be stored in dark at 4 °C. Do not freeze repeatedly.

For electrophoresis 1 ul of ladder has to be added to appropriate formamide/sizing standard mixture.

**Control-DNA**

Add 20 ul of deionized water provided with this kit to the tube with dried control DNA (green cap), mix thoroughly and spin down for a few seconds. For PCR reaction setup 1 ul of control DNA has to be added to the reaction tube. This amount corresponds to 500 pg of DNA. Alternatively, for convenience the control DNA can be resuspended in more than 20 ul of distilled water. In this case the volume of DNA to be added to the reaction tube has to be increased to achieve a final concentration of 500 pg DNA.

Once the control DNA is suspended it should be stored in dark at 4°C – 8°C. Do not freeze repeatedly.

**The Sizing Standard S450**

The sizing standard S450 provided with DharmaPlex9 Kit is a dried mix of different fragments labelled with orange fluorescent dye. It is ideally suited for analysis of DharmaPlex9 when using 5-dye-matrix generated with matrix standard MXS-5.

The sizing standard S450 contains 24 DNA fragments of 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 230, 240, 260, 280, 300, 320, 340, 360, 380, 390, 400, 420, 440 and 450 bases in length.

Before use add 120 ul of deionized water to the orange labelled tube and mix thoroughly after 5 minutes incubation. The diluted size standard can be stored refrigerated at 4°C – 8°C for several weeks. Do not freeze repeatedly. For analysis on sequencers add 1 ul of S450 to each well (containing formamide and PCR products as well).

**PCR Amplification**

**Reaction setup**

Depending on the kit format the final reaction volume is either 12.5 ul or 25 ul. Before addition, 5 x concentrated Activation Solution should be mixed thoroughly. Depending on kit format add 2.5 ul or 5 ul of the liquid to each reaction tube and incubate at room temperature for 2 minutes. After this, add DNA and compensate the remaining volume difference to 12.5 ul or 25 ul respectively, by addition of appropriate volume of deionized water.

The amount of DNA to be added depends on its concentration. For routine applications optimal final DNA concentration is 0.2 – 1 ng per reaction. The maximum volume of DNA to be added is 10 ul (12.5 ul format) or 20 ul (25 ul format).

Kit Component per tube	12.5 ul- format	25 ul- format
Activator Solution	2.5 ul	5 ul
Template DNA (0.2 – 2 ng)	up to 10 ul	up to 20 ul
Deionized Water to a final volume of	12.5 ul	25 ul

Please keep in mind, that when adding more than 5 ul of DNA-solution the possible presence of residual inhibiting substances can lead to negative effects on PCR-sensitivity. On the other hand, the kit has a very high tolerance to many inhibiting substances. Therefore, usually higher amounts of DNA can be applied without problems when the extracted DNA is present in a buffer with low EDTA content (eg. TE buffer). Otherwise, due to chelation by EDTA available magnesium concentration of the reaction mix can become suboptimal.

Mix the final reaction volume 5 – 8 times thoroughly with a 20 ul pipette tip until the suspension becomes completely clear and spin down for a few seconds. Since the reaction components are to be dissolved immediately before amplification proper mixing is crucial for optimal performance.

For every reaction setup should be run in parallel additionally one positive (1 ul of control DNA provided by the kit) and one negative controls (deionized water instead of DNA).

**Amplification Parameters**

The following PCR-parameters are recommended as standard procedure for all DNA probes. A ramp of 0,3°C/s should be used during heating from 57°C to 72°C. Due to the high complexity of amplifying 18 primer pairs simultaneously in one reaction this ramping is crucial for optimal performance! If ramping speed can not be programmed directly, we recommend check correct ramping time by using a timer.

Under low-copy-conditions (< 80 pg of DNA) 2 more PCR-cycles can be added or the ramping speed could be further reduced up to 0.3°C/s. Generally, we do not recommend more than 34 cycles in total. It should be taken in mind that allelic drop out and heterozygotic unbalances become more frequent in this case.

PCR Parameters:

94°C	3 min	initial denaturation
98°C	30 s	
57°C	120 s	4 cycles
72°C*	90 s	
94°C	30 s	
57°C	120 s	6 cycles
72°C*	90 s	
90°C	30 s	
57°C	120 s	20 cycles
72°C*	75 s	
68°C	30 min 15°C	to the end

After completion of PCR store the amplification products refrigerated at 4°C – 8°C and protected from light. If the amplified samples are to be stored for more than a few days, freezing at -20°C is recommended.



**4. Electrophoresis on ABI PRISM 3130/3130xl Genetic Analyzer**

For running the analyzer, spectral calibration procedure, proper use of ABI PRISM Data Collection Software as well as GeneMapper Software, please follow the instructions given at manual ABI PRISM 3130/3130XL Getting Started Guide. (When using a different DNA- analyzer please refer to the corresponding application guide of the manufacturer.)

For detection of the 5 dyes of this kit use dye set “any5 dyes” Material

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

**4.1 Generation of a Matrix / Spectral Calibration**

The use of DharmaPlex9 needs the application of a 5-colour spectral matrix. The correct matrix can be generated using Matrix Standard MXS\_5 (not provided with the kit, order number: MXS\_5). It contains a mixture of 5 different fragments each labelled with a different single dye. These labels are corresponding to the dyes of the STR amplification products and the sizing standard S450 as well.

For preparation of a Matrix Standard MXS\_5 working solution add 50 ul of deionized water to the tube containing dried MXS\_5 (green label) and incubate at room temperature for 5 min. Then, mix the solution thoroughly and spin briefly in a micro-centrifuge. The dissolved solution can be stored refrigerated in the dark and is stable for at least 6 months at 5°C-8°C. Do not freeze repeatedly.

Matrix Standard Setup for Spectral Calibration (ABI 3130 / 4 capillaries)

Hi-Di Formamide	47.5 ul
Dissolved MXS-5	2.5 ul

Denaturation at 95°C for 4 min  
Cool at 10°C for 3 min  
Add 10 ul of the mixture per well to a 96-well microplate at positions A01-D01 Spin briefly the microplate to remove all bubbles from the bottom of the wells.

Matrix Standard Setup for Spectral Calibration (ABI 3130XL / 16 capillaries)

Hi-Di Formamide	190 ul
Dissolved MXS-5	10 ul

Denaturation at 95°C for 4 min  
Cool at 10°C for 3 min  
Add 10 ul of the mixture per well to a 96-well microplate at positions A01-H01 and A02-H02 Spin briefly the microplate to remove all bubbles from the bottom of the wells..

**Spectral calibration**

After correct plate assembly place the 96-well microplate with denatured matrix standards onto autosampler.

**Step A - Creating a Spectral Instrument Protocol**

Open Protocol Manager of the Data Collection Software  
Go to Instrument Protocol and click New for opening of the Protocol Editor  
Select the following parameters (Instrument Protocol): Protocol Editor

Name	e.g. Spectral36_POP4_MXS5
Type	SPECTRAL
Dye Set	any5
Polymer	POP4
Array Length	36
Chemistry	Matrix Standard
Run Module	Spect36_POP4_1 Click OK and close Protocol Editor

**Step B - Creating the Plate Record**

Go to Plate Manager of the Data Collection Software and click New, the Plate Dialog window opens

Plate Editor for Spectral Calibration New Plate Dialog

Name	e.g. Spectral_any5_MXS_date
Application	Spectral Calibration
Plate Type	96-Well
Owner Name / Operator Name	...

Click OK, a new table of the Plate Editor opens

Plate Editor

Add to position A01:

Sample Name	Name of Matrix Probes
Priority	e.g. 100
Instrument Protocol	Spectral36_POP4_MXS5 (as specified earlier)

Highlight the entire well A01 and select in the Edit window Fill Down Special. The software fills the appropriate number of wells for a single run e.g. A01 to A04 (ABI 3130 / 4 capillaries) or A01 to H02 (ABI 3130XL / 16 capillaries).

Click OK to finish and to leave Plate Editor

**Step C - Running Spectral Calibration**

Go to Run Scheduler – Plate View and click Find All. Select the appropriate Plate Name (e.g. Spectral\_any5\_MXS\_date). Link this plate and start the run.

**Step D – Evaluation of the Spectral Matrix**

After completing Spectral Run view the pass or fail status of each capillary. Open Instrument Status and go to Event Log. In the Event Messages the status of each capillary is displayed.

Each capillary should have a Q-value not less than 0.95. The peak height should be at least 1.000 rfu, but less than 5.000 rfu (optimal range is between 2000 and 4000 rfu).

Additionally, at the Spectral Viewer the spectral calibration profile for each capillary can be evaluated. Correct calibration must be achieved for at least 3 out of 4 capillaries (or 12 out of 16 capillaries, respectively). When using MXS5 as the matrix standard at the spectral calibration profile the correct peak order is blue-green-yellow-red-orange, beginning from the left.

Finally, save the run data by clicking Rename and rename the run, eg. Any5\_MXS\_date and click OK. You should be aware that for each filter set always the latest calibration run is active. If you wish to use another matrix, it must be activated before.

**Run Conditions**

Before performing first run a Run Module has to be edited. Go to Module Manager of Data Collection Software and click New. The Run Module Editor will be opened. Make sure that the following Run Parameters are set:

Run Module Parameter	Setting
Oven Temperature	60
Poly Fill Volume	4840
Current Stability	5
PreRun Voltage	15
PreRun Time	180
Injection Voltage	3
Injection Time	5
Voltage Number of Steps	40
Voltage Step Interval	15
Data Delay Time	1
Run Voltage	15.0
Run Time	1440

Click Save As and select an appropriate name for this module (eg. C-18\_450bp). After this click OK and leave the module by clicking Close.

**Create an Instrument Protocol**

Go to Protocol Manager of the Data Collection Software. At the Instrument Protocol window click New to open the Protocol Editor. The following parameters have to be set:

Protocol Editor  
 Name Run36\_POP4\_MXS5  
 Type REGULAR  
 Run Module\* C-18\_450bp  
 Dye Set Any5Dye

\*parameters see at 3.2

Click OK to leave Protocol Editor.

**Sample Preparation and Loading**

For sample preparation prepare a run-mix by combining Hi-Di™ Formamide and Sizing Standard S450 (dissolved as described in 2.3) as follows:

Components per analysis tube	Amount
Hi-Di Formamide	12.0 ul
Size Standard S450	1.0 ul

Keep in mind that always a complete number of 4 or 16 wells have to be filled with formamide. Do not forget to add at least one well with Allelic Ladder.

After mixing add 13 ul of the cocktail to each well of the test plate. Then, add 1 ul of PCR- product or Allelic Ladder. Finally, cover the plate with a septa pad, according to the user’s manual for the ABI PRISM Genetic Analyzer.

The samples must be denatured for 3 min at 95°C, cooled down and hold at 10°C for 3 min. Perform this denaturation step immediately prior to loading the analysis instrument. After cooling centrifuge the plate briefly to remove any remaining air bubbles. Place the sample plate onto analysis tray and start the run.

**Starting Run**

For correct instrument preparation and starting run, please refer to the instrument user’s manual. For optimal performance the correct setup of Matrix (see section 4.1), Run Module (see section 4.2), and Instrument Protocol (see section 4.3) must be used.

**Step A Open Plate Editor**

Before starting a run each samples to be analyzed must be assigned to a position at an analysis plate. Go to Plate Manager of the Data Collection Software and click New. A new Plate Dialog opens:

New Plate Dialog  
 Name e.g. DharmaPlex9\_date  
 Application choose GeneMapper Application  
 Plate Type 96-Well  
 Owner Name / Operator Name

Click OK and a new Table at the Plate Editor opens.



**Step B**

Sample Name  
 Priority  
 Sample Type  
 Size Standard  
 Panel  
 Analysis Method  
 SNP Set  
 Results Group  
 Instrument Protocol

**Table Settings**

Name of the sample  
 usually 100 (lower numbers will be analyzed first)  
 Sample / Allelic Ladder / Positive Control / Neg. Control  
 S450  
 DharmaPlex9\_Panels  
 e.g. Analysis\_HID\_3130 User-defined 1-3  
  
 (choose appropriate Results Group)  
 Run36\_POP4\_MXS5

The easiest way is to type all Sample Names first. Then, add to the first sample the data for table settings shown. Then, go to the first sample and highlight the entire well beginning at priority to the right and go down to the last sample while holding the mouse pressed. Select in the Edit window Fill Down. The software fills the appropriate settings to all highlighted positions. After this change at the correct positions the Sample Type to Allelic Ladder / Positive Control / Negative Control.

**Step C Starting Run and Run Information**

Go to Run Schedule and click to Find All. Find the plate, click the plate name and go to link. After this the run can be started.

The run can be viewed during electrophoresis at Capillaries Viewer or Cap/Array Viewer. In Event Log information about errors can be viewed (Error Status).

An overview about all relevant run data is presented at Run History of the Data Collection Software. The run sample data are located at the corresponding Run Folder in the Results Group that was chosen in Step B of section 4.5.

**Improving Signal Intensities**

If the STR peaks are too weak there are some after-PCR procedures available for further improvement. Performing an additional run with increased Injection Time (up to 15 sec) or with increased Injection Voltage (up to 10kV) is usually the simplest and fastest way to achieve higher signal intensities (see section 4.2).

A post-PCR clean-up of the amplified products to remove residual primers and salts is another possible method. In this case the necessary amount of Sizing Standard S450 in the formamide mixture also should be reduced.

**Data Analysis**

The Sizing Standard S450

The sizing standard S450 provided is labelled with orange fluorescent dye. It contains 24 DNA fragments of 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 230, 240, 260, 280, 300, 320, 340, 360, 380, 390, 400, 420, 440 and 450 bases in length.

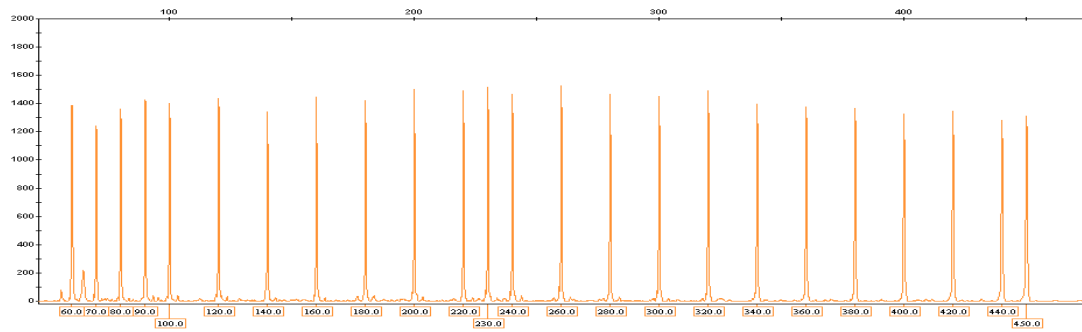


Figure 1 Electropherogram of S450, Size distribution of the labelled fragments

**Allele and Size Ranges of STR markers**

Marker	Allele Range	Marker Size Range	MK-1 Alleles	Label Color
D8S1179	7 - 20	70 - 128	10/10	blue
D5S818	6 - 18	129 - 179	9/12	blue
SE33	4.2 – 50.2	190 - 360	24.2/29.2	blue
D10S1248	8 - 21	72 - 137	15/15	green
D16S539	4 - 16	140 - 198	12/13	green
D12S391	13 - 28	90 - 160	21/23	yellow
CSF1PO	5 - 16	162 - 210	9/11	yellow
Amelogenin X**	X	106	X	red
Amelogenin Y	Y	112	Y	red
FGA	12.2 – 51.2	120 - 280	20/22.2	red

Table 2 The allele range of STRs and the expected size ranges are shown for each dye panel.

Amplification of Control DNA

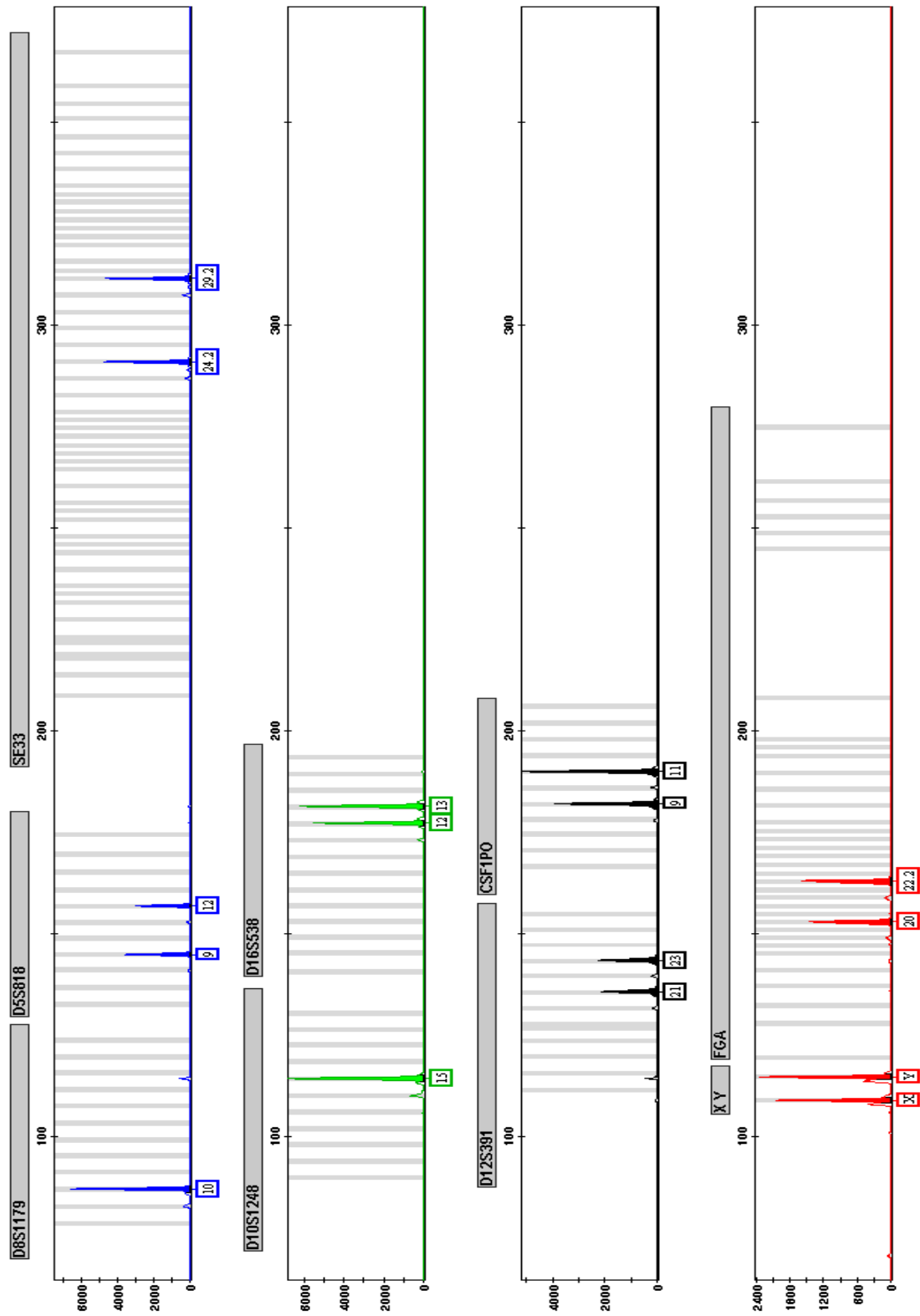


Figure 2 DharmaPlex9. A template of 250 pg Control DNA was amplified. The analysis was performed at ABI PRISM 3130 Genetic Analyzer by using the Size Standard S450. For allele designation the GeneMapper ID Software in combination with DharmaPlex9 template file were used. The expected alleles of the Control DNA are shown in Table 2 at section 5.2.

Allelic Ladder

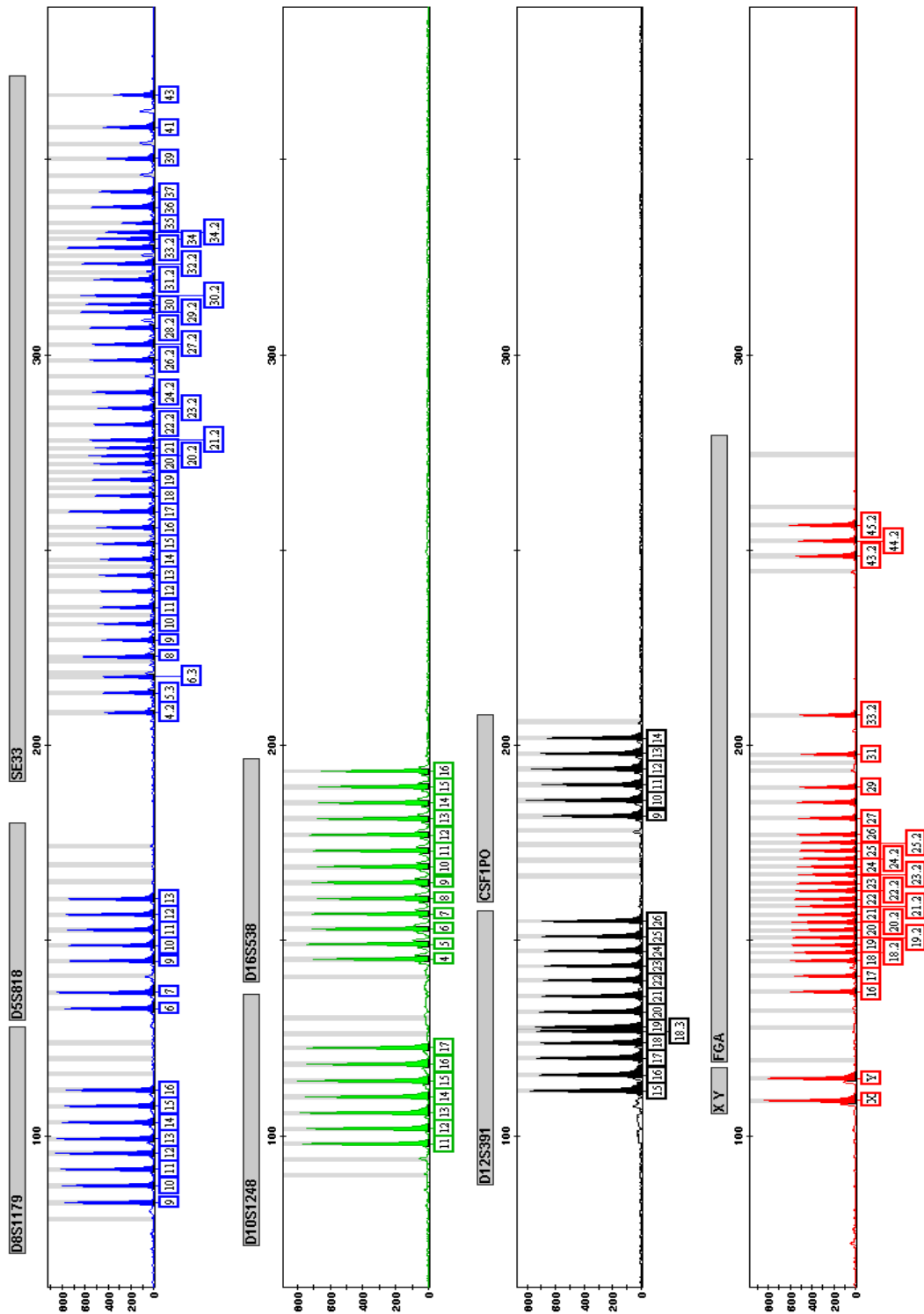


Figure 3 DharmaPlex9 Allelic Ladder.

The analysis was performed at ABI PRISM Genetic Analyzer by using the Size Standard S450. For allele designation the GeneMapper ID Software in combination with DharmaPlex9 template file were used.

**Performance of the Kit:**

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

**LIMITED WARRANTY**

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

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**THANK YOU FOR USING KRISHGEN PRODUCT!****References**

- 1) Bär, W., Brinkmann, B., Budowle, B., Carracedo, A., Gill, P., Lincoln, P., Mayr, W., and 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.
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- 7) <http://www.cstl.nist.gov/biotech/strbase>
- 8) [www.uni-duesseldorf.de/www/MedFak/Serology/database.html](http://www.uni-duesseldorf.de/www/MedFak/Serology/database.html) (population data)

**Trade Marks and Patents**

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