COrDIS Plus

Multiplex analysis of 19 STR loci plus Amelogenin

User manual

Table of Content

1 Product information

1.1 Product Description

CO_rDIS Plus is a short tandem repeat (STR) multiplex assay with 19 STR markers and Amelogenin for gender determination. It combines all 13 CODIS loci (D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX, and VWA), 5 new core loci recommended for extension of European national databases (D1S1656, D2S441, D10S11248, D12S391, and D22S1405), 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 440 bp, taking in account all known alleles so far. PCR-products are labelled with blue, green, yellow and red fluorescent colours by using a sizing standard labelled in a fifth, orange colour.

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

The kit is provided as lyophilised in 0.2 ml PCR-reaction tubes or plates and can be stored protected from the light at room temperature for at least one year without any loss of sensitivity. The reaction components should be activated simply by addition of 5 µl of activator solution to each tube. The final reaction volume is 25 µl, so that theoretically up to 20 µl of DNA solution can be added per reaction. Due to its extremely high tolerance to inhibiting substances such high amounts of DNA can be amplified successfully.

CO_rDIS Plus has the potential to be applied for national database DNA-typing in countries that are using CODIS (Combined DNA Index System) markers, as well as in all European countries using ESS (European Standard Set) markers, including those with SE33 as database marker. Due to the highest discrimination power among all commercial kits available it is ideally suited for forensic applications, paternity or parentage testing 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® Accsession	GenBank® Allele	Chromos. Localization	Repeating Unit of GenBank Allel
D1S1656	NC_000001.9	17	1q42	$[TAGA]_{16}[TGA][TAGA][TAGG]_1[TG]_5$
D2S441	AL079112	12	2p14	$[TCTA]_{12}$
D3S1358	NT_005997	18	3p21.31	TCTA $[TCTG]_2$ $[TCTA]_{15}$
D5S818	AC008512	11	5q23.2	$[AGAT]_{11}$
D7S820	AC004848	13	7q21.11	$[GATA]_{13}$
D8S1179	AF216671	13	8q24.13	$[TCTA]_3$
D10S1248	AL391869	13	10q26.3	$[GGAA]_{13}$
D12S391	G08921	19.3	12p13.2	$[AGAT]_5$ GAT $[AGAT]_7$ $[AGAC]_6$ AGAT
D13S317	AL353628	11	13q31.1	$[TATC]_{11}$
D16S539	AC024591	11	16q24.1	$[GATA]_{11}$
D18S51	AP001534	18	18q21.33	$[AGAA]_{18}$
D21S11	AP000433	29	21q21.1	$[TCTA]_4$ $[TCTG]_6$ $[TCTA]_3$ TA $[TCTA]_3$ TCA $[TCTA]_2$ TCCATA $[TCTA]_{11}$
D22S1045	AL022314	17	22q12.3	$[ATT]_{14}$ ACT $[ATT]_2$
CSF1PO	X14720	12	5q33.1	$[AGAT]_{12}$
FGA	M64982	21	4q31.3	$[TTTC]_3$ TTTTTTCT $[CTTT]_{13}$ CTCC $[TTCC]_2$
SE33	V00481	26.2	6q14	$[AAAG]_8$ AA $[AAAG]_{17}$
TH01	D00269	9	11p15.5	$[TCAT]_9$
TPOX	M68651	11	2p25.3	$[AATG]_{11}$
VWA	M25858	18	12p13.31	TCTA $[TCTG]_4$ $[TCTA]_{13}$
Amelogenin X	M55418	X	Xp22.1-22.3	
Amelogenin Y	M55419	Y	Yp11.2	

In **Table 1** the information regarding STR-loci of the COrDIS Plus kit is summarized. 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.

1.2 Ordering Information and Kit Components

This kit can be obtained either as strips (8 reactions) with single caps or micro plates (96 reactions).

- 8 x 0.2 ml strips, each reaction tube with single cap

Product		Size	Order-Number
CO_rDIS Plus	**25 µl format**	12 x 8 reactions (96 rxn)	C20-108S

- 96 x 0.2 ml plate

Product		Size	Order-Number
CO_rDIS Plus	**25 µl format**	1 x 96 reactions (96 rxn)	C20-196P

Kit Components (e.g. 12 x 8 reactions)

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

Activation Solution (blue lid)	0.5 ml
Deionised Water (white lid)	2x1.7 ml
Allelic Ladder, dried (red lid)	1 tube (20 Applications)
Control DNA, dried (green lid)	1 tube (40 Applications)
Sizing Standard S450, dried (orange label)	1 tube (120 Applications)

1.3 Storage Conditions

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

Water and **Activator** 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 the light in a separate place at room temperature (post-amplification area of your lab). 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_3 and are potentially hazardous and should be handled accordingly. Always wear gloves and avoid inhalation and skin contact.

Quality Assurance

The 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 18 months. If there are any questions regarding our quality assurance program, don`t hesitate to contact us.

1.4 Supplemental Material

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

Matrix Standard MXS_5+ for ABI 3130 and ABI 3500	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	Order 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)

2 Solubilization of dried Components

2.1 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).

To get a working solution, add 20 µl of deionised water provided with this kit to the tube with dried allelic ladder (yellow cap), mix thoroughly and spin down for a few seconds. Once the allelic ladder is suspended it should be stored in the dark at 4 °C. Do not freeze repeatedly.
For electrophoresis 1 µl of ladder has to be added to appropriate formamide/sizing standard mixture.

2.2 Control-DNA

Add 20 µl of deionised 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 µl 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 µl 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 the dark at 4°C – 8°C.

2.3 The Sizing Standard S450

The sizing standard S450 provided with COrDIS Plus kit is a dried mix of different fragments labelled with orange fluorescent dye. It is ideally suited for analysis of COrDIS Plus 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, 400, 420, 440 and 450 bases in length.

Before use add 120 µl of deionised water to the yellow 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. For analysis on sequencers add 1 µl of S450 to each well (containing formamide and PCR products as well).

3 PCR Amplification

3.1 Reaction setup

The final reaction volume is 25 µl. Before addition, 5x concentrated **Activator** solution should be mixed thoroughly. Add 5 µl of the **Activator** to each reaction tube and incubate at room temperature for 2 minutes. After this, add **DNA** and compensate the remaining volume difference to 25 µl 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 0.5 – 2 ng per reaction.** The **maximum volume of DNA** to be added is 20 µl.

Kit Component	Volume per reaction
Activator Solution	5 µl
Template DNA (0.2 – 2 ng)	up to 20 µl
Deionized Water to a final volume of	25 µl

Please keep in mind, that when adding more than 10 µl 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 µl 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 µl of control DNA provided by the kit) and one **negative controls** (deionized water instead of DNA).

3.2 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 59°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 (< 100 pg of DNA) 2 more PCR-cycles can be added. Generally, we do not recommend more than 34 cycles in total. It should be taken in mind that allelic drop out and heterozygotic disbalances become more frequent in this case.

PCR parameters:

94°C	3 min	initial denaturation
98°C	30 s	
59°C	120 s	4 cycles
72°C*	90 s	
94°C	30 s	
59°C	120 s	6 cycles
72°C*	90 s	
90°C	30 s	
59°C	120 s	20 cycles
72°C*	75 s	
68°C	5 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 week, 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 COrDIS Plus 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 µl of deionised water to the tube containing dried MXS_5 (purple label) and incubate at room temperature for 5 min. Then, mix the solution thoroughly and spin briefly in a microcentrifuge. The dissolved solution can be stored refrigerated in the dark and is stable for at least 6 months at 5°C-8°C.

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

Hi-Di™ Formamide	40 µl
Dissolved MXS-5	4 µl

Add 10 µl 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-DiTM Formamide	160 µl
Dissolved MXS-5	16 µl

Add 10 µl 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 MXS-5+ 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.

4.2 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 Paremeters 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	**1600**

Click **Save As** and select an appropriate name for this module (eg. COrDIS-Plus_450bp). After this click **OK** and leave the module by clicking **Close**.

4.3 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*	COrDIS-Plus_450bp
Dye Set	Any5Dye

*parameters see at 3.2

Click **OK** to leave Protocol Editor.

4.4 Sample Preparation and Loading

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

Components per analysis tube	Amount
Hi-Di™ Formamide	10.0 µl
Size Standard S450	1.0 µl

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 10 µl of the cocktail to each well of the test plate. Then, add 1 µl 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.

No temperature denaturation is needed for COrDIS Plus. Centrifuge the plate briefly to remove any remaining air bubbles. Place the sample plate onto analysis tray and start the run.

4.5 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. COrDIS_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 Table Settings

Sample Name	Name of the sample
Priority	usually 100 (lower numbers will be analyzed first)
Sample Type	Sample / Allelic Ladder / Positive Control / Neg. Control
Size Standard	S450
Panel	COrDIS_Panels
Analysis Method	e.g. Analysis_HID_3130
User-defined 1-3	
SNP Set	
Results Group	(choose appropriate Results Group)
Instrument Protocol	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.

4.6 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 10 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.

5 Data Analysis

5.1 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, 400, 420, 440 and 450 bases in length.

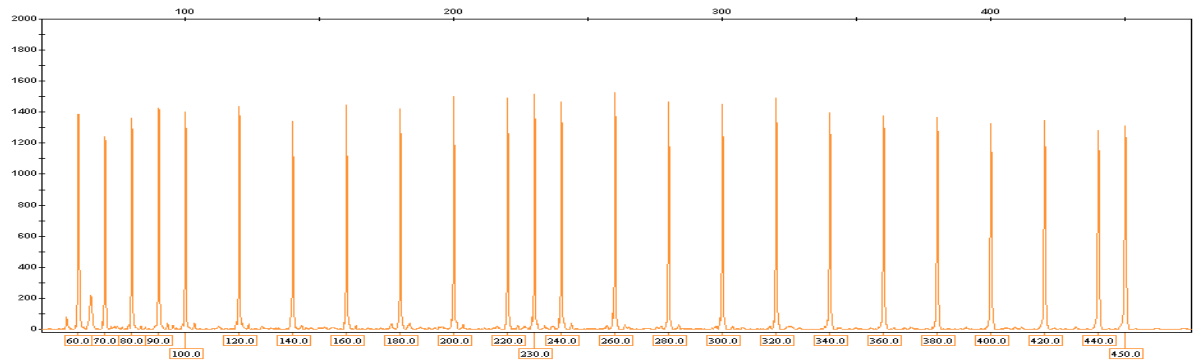

Figure 1 Electropherogram of S450, Size distribution of the labelled fragments

5.2 Allele and size ranges of STR markers

Marker	Allele Range	Marker Size Range (bp)	MK1 DNA Alleles	Label Colour
Amelogenin X	X	81	X	blue
Amelogenin Y	Y	84	Y	blue
D3S1358	8 – 21	93 - 147	15/17	blue
TH01	3 - 14	152 - 196	6/9.3	blue
D12S391	13 - 28	204 - 264	21/23	blue
D1S1656	9 - 21	275 - 324	14/17.3	blue
D10S1248	8 - 21	338 - 390	15/15	blue
D22S1045	8 – 19	400 – 440	15/15	blue
D2S441	8 - 19	84 - 134	14/14	green
D7S820	5 - 16	137 - 181	10/12	green
D13S317	5 - 17	188 - 236	11/11	green
FGA	12.2 – 51.2	238 - 391	20/22.2	green
TPOX	4 - 16	66 - 113	8/9	yellow
D18S51	7 - 27	124 - 200	14/16	yellow
D16S539	4 - 16	208 - 256	12/13	yellow
D8S1179	7 - 20	262 - 314	10/10	yellow
CSF1PO	5 - 16	317 - 361	9/11	yellow
D5S818	6 - 18	369 - 413	9/12	yellow
VWA	10 - 25	94 - 155	16/18	red
D21S11	24 – 41.2	158 - 228	30.2/30.2	red
SE33	4.2 – 50.2	233 - 403	24.2/29.2	red

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

5.3 Amplification of Control DNA

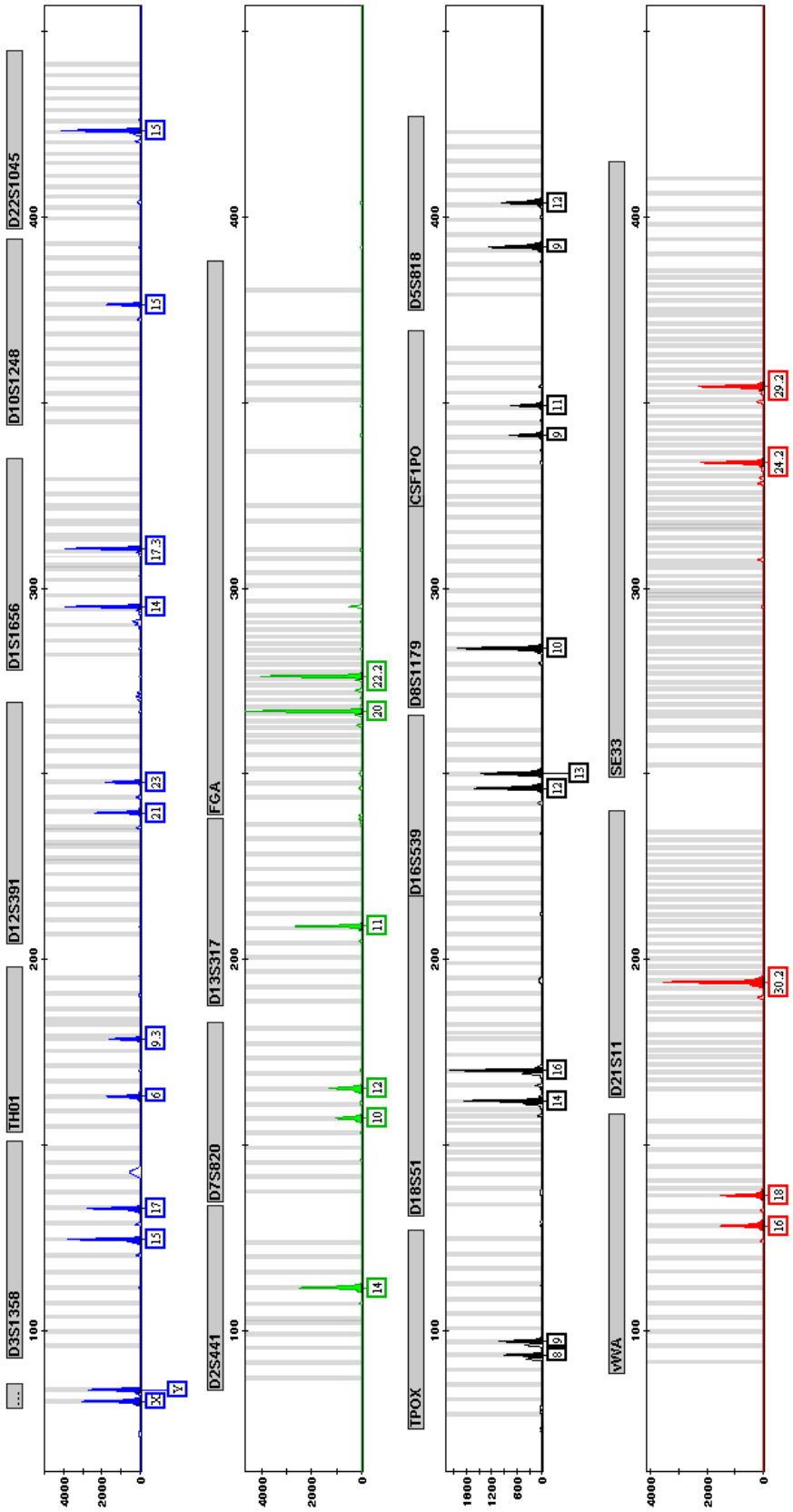

Figure 2 COrDIS Plus. A template of 500 pg Control DNA was amplified. The analysis was performed at ABI PRISM 3130 Genetic Analyzer by using the Size Standard S450. For allele designation the GeneMapper™ ID Software in combination with COrDIS Plus template file were used. The expected alleles of the Control DNA are shown in Table 2 at section 5.2.

5.4 Allelic Ladder

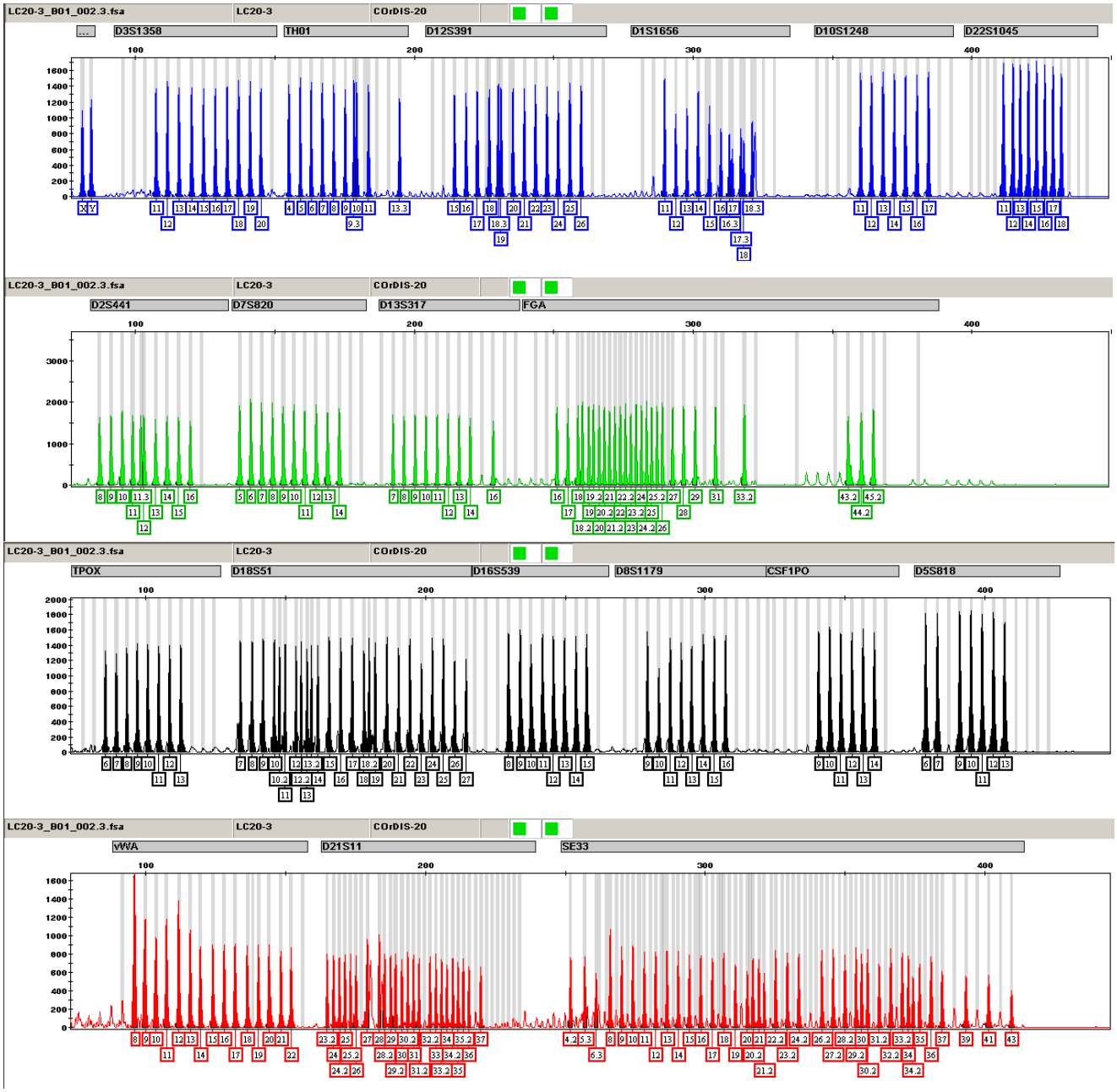

Figure 3 COrDIS Plus Allelic Ladder.
The analysis was performed at ABI PRISM Genetic Analyzer by using the Size Standard S450. For allele designation the GeneMapper™ ID Software in combination with COrDIS Plus template file were used.

6 Results Interpretation – Amplification Artifacts

6.1 Pull-up Peaks

A Pull-up (or bleedthrough) can occur when the matrix can not compensate high amplification signals (higher than 4.000-5.000 rfu). Typically, there are seen peaks exactly at the same size position of the main peak in the neighbour colour panels, but with lower signal intensities comparing to the main peak. Another reason can be the application of a wrong matrix during Data Collection.

6.2 Stutter Peaks and Split Peaks

Occurrence of stutter peaks is typical for STRs, but not for Amelogenin locus. Stutters are observed at one repeat unit shorter than the true peak (n-1) and are slippage artefacts of polymerase during amplification. Usually, for alleles of one particular STR the relative height of stutter peak increases with allelic number.

STR locus SE33 has a typical composed stutter consisting of n-1 stutter (4 base pairs shorter) and a second stutter signal exactly at the middle of distance between n-1 stutter and main peak (2 base pairs shorter than main peak). The height of this intermediate stutter peak is about half of the N-1 stutter.

If too high amounts of DNA are used for amplification, additional stutter peaks can be observed at n-2 and/or n+1 positions of the main peak.

High amounts of DNA in reaction setup are also reason for occurrence of Split Peaks. A portion of amplification product is 1 base pair smaller than the main peak. The reason is insufficient adenylation by polymerase so that not all amplification products become an Adenosine added to its 3´-end.

7 References

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.

Coble, M.D. and Butler, J. M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. J Forensic Sci. 50: 43–53.

Gill, P., Fereday, L., Morling, N., Schneider P.M. (2006) The evolution of DNA databases – recommendations for new European loci. Forensic Sci. Int. 156: 242-244.

Gill, P., Fereday, L., Morling, N., and Schneider, P.M. (2006) Letter to the Editor: New multiplexes for Europe – Amendments and clarification of strategic development. Forensic Sci. Int. 163: 155-157.

Hill, C.R., Kline, M.C., Coble, M.D., Butlerm J.M. (2008) Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples. J. Forensic Sci. 53 (1): 73-80.

Huckenbeck, W, Kuntze, K and Scheil, H.-G. (1997) The distribution of the human DNA-PCR polymorphisms. Verlag Dr. Köster, 1. Auflage Berlin, ISBN 3-89574-300-3 (see also web-based references)

Web-based references on STR loci

http://www.cstl.nist.gov/biotech/strbase

www.uni-duesseldorf.de/www/MedFak/Serology/database.html (population data)

Trade Marks and Patents