GeneProof
Molecular diagnostics for your routine

GeneProof OmniPlex QF PCR Kit

Ref. Nr.
OMNI/ 050
OMNI/ 100
OMNI/DEMO

User Manual
Using Genetic Analyzer ABI 3130

RUO **Research use only**

The kit is designed for professional use in specialized clinical and research laboratories.

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1 Product Information

1.1 Examination Purpose

Aneuploidy examination by means of the QF-PCR method is designed for quick diagnostics of the 13, 18, 21 autosomal chromosomes aneuploidy and X and Y sexual chromosomes aneuploidy by means of analyzing specific STR loci. It is based on the PCR method and an automatic genetic analyzer is used to visualize the fragments.
The OmniPlex QF-PCR Kit has been validated for the ABI PRISM 3130 (Applied Biosystems) genetic analyzer.

Product	Number of react.	Product code	Product description
OmniPlex QF-PCR Kit	50	OMNI/50	A test for a routine *in vitro* detection of the three most common types of autosomal trisomy: Chromosome 13 trisomy (Patau syndrome), Chromosome 18 trisomy (Edwards syndrome) and Chromosome 21 trisomy (Down syndrome). The kit contains also markers for the X and Y chromosomes and the TAF9L marker for sex determination.
	100	OMNI/100	
OmniPlex 13plus QF-PCR Kit	25	OMNI13/025	A complementary test containing the same and several additional markers, such as the OmniPlex test for chromosome 13. It is designed specifically for situations when the result of the original examination by means of the OmniPlex kit was not sufficiently informative and for the verification of pathology findings.
OmniPlex 18plus QF-PCR Kit	25	OMNI18/025	A complementary test containing the same and several additional markers, such as the OmniPlex test for chromosome 18. It is designed specifically for situations when the result of the original examination by means of the OmniPlex kit was not sufficiently informative and for the verification of pathology findings.
OmniPlex 21plus QF-PCR Kit	25	OMNI21/025	A complementary test containing the same and several additional markers, such as the OmniPlex test for chromosome 21. It is designed specifically for situations when the result of the original examination by means of the OmniPlex kit was not sufficiently informative and for the verification of pathology findings.
OmniPlex XYplus QF-PCR Kit	25	OMNIXY/025	A complementary test containing the same and several additional markers, such as the OmniPlex test for chromosomes X and Y. It is designed specifically for situations when the result of the original examination by means of the OmniPlex kit was not sufficiently informative and for the verification of pathology findings.

1.2 Kit Composition

Cat. No.	OMNI/DEMO 25 reactions	OMNI/050 50 reactions	OMNI/100 100 reactions
MasterMix OmniPlex	1 x 200 µl	2 x 200 µl	4 x 200 µl

2 Storage and Transportation Conditions

Transport the kits at temperatures ranging from -20°C to -80°C. The kit remains stable for 6 months from the date of manufacturing at the temperature of -20°C. Repeated freezing and thawing of the MasterMix may result in lower detection quality. The manufacturer therefore recommends:

- Dissolve and thoroughly mix the OmniPlex QF-PCR Kit
- Distribute the prepared MasterMix by 8µl aliquots into 0.2mL PCR micro-tubes
- Keep the micro-tubes from -20°C to -80°C

3 Additional Required Material and Equipment

3.1 Devices

- **Laminar box**, MSC 12, Trigon-Plus s. r. o.
- **Vortex shaker**, Yellow line TTS2, BioTech a. s.
- **Mini-centrifuge**, Labnet force 14, BioTech a. s.
- **PCR thermocycler**, GeneAmp PCR System 9700 or Veriti™ 96-Well Thermal Cycler, Applied Biosystems; Peltier Thermal Cycler PTC-2000, MJ Research, Inc.
- 3130 Genetic Analyzer with relevant software, Applied Biosystems
    - 3130 Data Collection Software v3.0
    - GeneMapper® Software v 4.0
- Cooler-freezer

3.2 Accessories

- Automatic micropipettes (10 µl, 100 µl, 1000 µl)
- Laboratory timer
- Micropipette stands

3.3 Consumables

- Single-use sterile tips for 10µl, 100µl, 1000µl pipettes, filter-tipped
- 0.2 ml, 0.5 ml and 1.5 ml micro-tubes
- Single-use protective latex gloves (no talcum powder)
- 96-well plates - MicroAmp™ Optical 96-Well Reaction Plate (Applied Biosystems)
- Rubber septa – 96-Well Plate Septa (Applied Biosystems)
- Lower plate retainer – 96-Well Base Plate (Applied Biosystems)
- Upper plate retainer – 96-Well Plate Retainer (Applied Biosystems)

3.4 Reagents and Agents

- 10x Running Buffer with EDTA (Applied Biosystems)
- GeneScan™ -500 LIZ™ Size Standard (Applied Biosystems), GeneTrace 500 LIZ (Carolina Biosystems s.r.o.)
- Hi-Di™ Formamide (Applied Biosystems)
- GeneScan™ Install Standard DS-33 (set G5) (Applied Biosystems)
- 3130 POP-7™ Performance Optimized Polymer (Applied Biosystems)
- Aqua pro injectione
- Chromasolv® Plus water, for HPLC (Sigma)
- TE (Fluka)

4 Method Principles

This examination is designed for quick discovering of chromosome 13, 18, 21 aneuploidy and sexual chromosome aneuploidy (these aneuploidies represent over 80% of all significant chromosomal aberrations) related to serious genetic impairments such as Down syndrome, Edwards syndrome, Patau syndrome, Turner syndrome, Klinefelter syndrome, etc.
The method uses multiplex QF-PCR (*Quantitative Fluorescent Polymerase Chain Reaction*) to amplify a large number of fluorescence-marked DNA fragments of specific STR markers at chromosomes under review during a single reaction.
After amplification the DNA fragments are separated, detected and analyzed using capillary electrophoresis and the relevant software of the genetic analyzer. The individual STR marker fragments are specific due to their lengths and types of fluoresce marking.
Each fluorescence-marked DNA fragment displays as a peak with specific height/surface area, which is in direct proportion to the DNA amount in the primary sample. In case of diploidy in a heterozygote the signal of the two alleles (peaks) of the particular STR marker is in the ratio of 1:1; in case of a homozygote there is one peak with a double signal. In case of trisomy there is detected a signal in the ratio of approximately 2:1 or 1:2, or 3 peaks in the ratio of approximately 1:1:1.

Samples indicated for a chromosome aneuploidy examination by the QF-PCR method will be analyzed by means of the **OmniPlex** basic set upon a physician's request. In case of insufficient informative contents of the acquired results after the marker analysis by means of this basic set it is possible to use a **superstructure set** for the specific chromosome (OmniPlex 13plus, 18plus, 21plus and XYplus QF-PCR Kit) for which a valid result had not been obtained (i.e. at least 2 informative markers).
A QF-PCR method examination can be summarized in three steps:

1. DNA isolation from the examined material
2. Multiplex PCR amplifying specific STR loci
3. Separation of the amplified DNA fragments and their evaluation by means of a genetic analyzer.

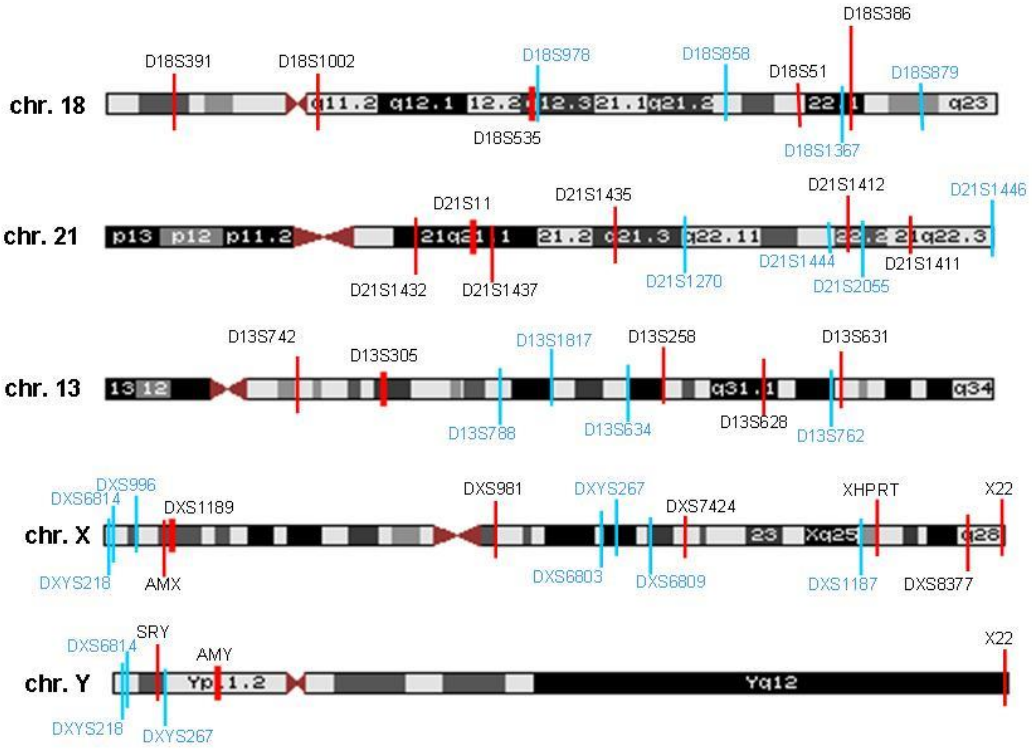


Fig.1 Diagram of STR marker localizations at chromosomes using the GeneProof a.s. kits

Note: Red-marked loci are common for both the OmniPlex QF-PCR Kit and the relevant superstructure kit. Blue-marked loci are included only in the OmniPlex 21plus, 18plus, 13plus and OmniPlex XYplus QF-PCR superstructure kits.

5 Safety Information

Be very careful when handling the clinical material – incorrect handling could result in contamination and the impairment of the MasterMix. The manufacturer is not responsible for the kit impairment due to incorrect handling or storaging.
Use suitable protection equipment (rubber gloves) when working with biological material.

PCR reactions should be prepared in laminar boxes that have to be carefully decontaminated by UV radiation before and after work. Exposure to large doses of UV radiation could damage your eyes or skin and therefore you should avoid any unnecessary exposure.
The genetic analyzer optical system includes an argon laser, which could, when used in an unauthorized way, seriously damage your eyes. Principles of safe use of the device are described in the user manual supplied with the genetic analyzer.
Be careful when working with chemicals supplied with the ABI PRISM 3130 (POP-7 polymer, 10x Running buffer) analyzer and DMSO - these are irritable agents. Formamide, used to insert samples into the analyzer plate, is considered a potential teratogen. Use protective gloves when handling these chemicals.
The kit should be disposed of after use according to the current legal regulations considering the fact that the kit doesn't contain any dangerous, infectious or toxic components that would be subject to special safety regulations and the packaging materials are made of paper and polypropylene (for detailed information see the safety sheets, MSDS).
All reagents should be used for *in vitro* diagnostics only. It is unconditionally necessary to exactly follow all the steps listed in the following chapter (User Manual).
It is necessary to observe the expiration date specified on the product's package for the PCR Kit's correct operation.

6 User Manual

6.1 Sampling and Sample Storage

Amniotic fluid and the cells retrieved from this fluid (the so-called amniocytes) are the primary materials for a prenatal molecular-genetic examination. Since this is an invasive examination, with a risk of the surgery complications about 0.5%, the performance of this examination (amniocentesis) has to be explicitly indicated. The amniotic fluid contains cutaneous and mucous cells from the fetus surface. Sampling is most often performed between weeks 16 and 18 of pregnancy; it can be performed since week 15, though.
Chorionic villus sampling (CVS) is performed in those cases, when the examination has to be started before week 15 of pregnancy due to the indication severity; the surgery risk is about 1%. If the CVS sample is the source material for DNA isolation, it is necessary to supply this sample into the MGL laboratory cleared of all blood clots that have stuck to it during the sampling procedure. The clotted blood is of the mother's origin and therefore the isolated DNA would be a mix of the fetus' and mother's DNA and this would make the examination impossible.
If the material is sampled later in pregnancy, it is necessary to remember that pregnancy interruption due to fetus damage is allowed only until the 24th week of pregnancy, with certain exceptions.
Examined material:

- Native amniotic fluid
- Cultured amniocytes
- Buccal swab
- Peripheral blood
- Chorionic villi
- Fetus tissue after SAB

- Cordocentesis

The extracted samples must be preserved in a cold environment (4°C) and immediately transported into the laboratory.
Freeze the samples for long-term preservation.

6.2 Preparatory Works

6.2.1 Matrix Installation

Installation of the G5 color matrix (GeneScan®Install Standard DS-33 (set G5)) into the ABI 3130 device should be performed by the service engineer during the sequencer installation.

6.2.2 Creation of a Device Protocol for the OmniPlex QF-PCR Fragmentation Analysis

a. Start the 3130 Data Collection v3.0 program
b. Open Protocol Manager in the ga3130 file
c. Select **New**
d. This will open the **Protocol Editor**.
e. Create a new protocol, MODULG5-16SEC-POP7 for example, in the table.
f. Fill in data according to Figure 2.

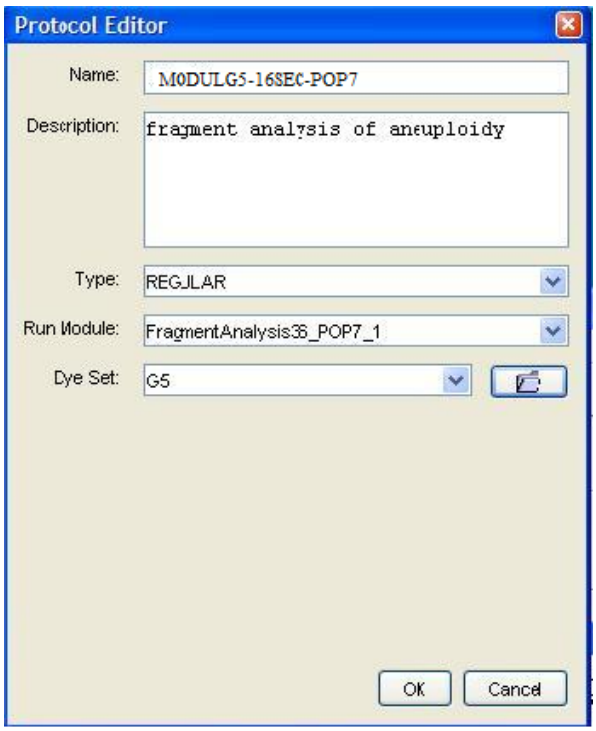


Fig.2 Create a new device protocol for fragmentation analysis using the OmniPlex QF-PCR Kit

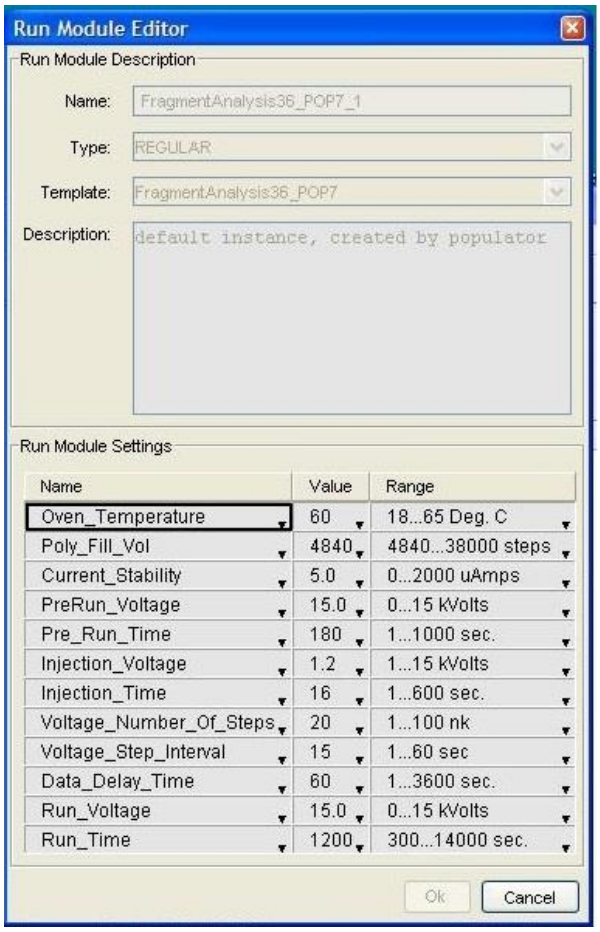


Fig.3 Run Module settings: FragmentAnalysis36_POP7_1 for fragmentation analysis using the OmniPlex QF-PCR Kit

6.2.3 Creation of a Panel for the Studied Markers

Follow the instructions described in the software documentation for the GeneMapper® v 4.0 application to create marker panels defining the detection ranges for the alleles of the markers under examination. These are used to assign the analyzed PCR products to the appropriate STR markers.

6.3 DNA Isolation

It is recommended to process the DNA from the amniotic fluid using the QIAamp®DNA Blood Mini Kit (Qiagen; Cat. No. 51106); from tissue (CVS) using the QIAamp DNA Mini Kit (Qiagen; Cat. No. 51306); from blood (in case of postnatal testing, comparative analysis with the mother, cordocentesis, etc.) using the Nucleic Acid Isolation Kit I (Roche) in the Magna Pure Compact device or manually using the QIAamp®DNA Blood Mini Kit (Qiagen; Cat. No. 51106). Recommended DNA insert ranges from 5 to 10 ng.

6.4 Polymerase Chain Reaction (PCR) Performance

1. Add **8 µl of MasterMix** and **2 µl of the isolated DNA** (5-20 ng/µl) into a 0.2 ml PCR tube. The final reaction mix volume should be 10 µl.
2. Close the tubes, centrifuge shortly, insert them into a thermocycler and amplify according to the following profile:

Amplification program:

	Initial denaturation	95°C/15 min.
	Denaturation	94°C/30 sec.
	Annealing	59°C/60 sec.
	Extension	72°C/60 sec.
	Number of cycles	25
	Final extension I	72°C/ 10 min.
	Final extension II	60°C/ 30 min.
	Cooling	4°C/ until the program manual interruption

3. After the end of the PCR reaction, and before the capillary electrophoresis itself, it is possible to keep the micro-tubes with the PCR products in a dark refrigerator at 2-8°C.

Note: To minimize the risk of the PCR mix contamination the complete preparation is performed in a laminar box using rubber gloves (no talcum powder) and sterile micropipette tips. The laminar box must be decontaminated by UV light before the PCR mix pipetting. It is recommended to verify any possible contamination of the PCR mix components using the so-called negative sample, which contains all the PCR components except for the template DNA.

6.5 Fragmentation Analysis

A fragmentation analysis makes it possible to separate the amplified DNA fragments (PCR products) according to their base pair (bp) lengths in the capillary system of the ABI PRISM 3130 genetic analyzer. Process the acquired data with the GeneMapper® v4.0 application.

6.5.1 Preparation of a Sample for Fragmentation Analysis

a) Prepare a mix of the Hi-Di™ Formamide and the GeneScan™-500 LIZ™ size standard in the ratio of 1:0.02.
b) For each sample under examination pipette 10 µl of this mix into the individual wells of the 96-well plate, add 2 µl of the amplified sample and carefully mix the contents with the pipette.
c) Perform the PCR product denaturation by heating the plate to 94°C/3 min. and then 4°C/30 sec.
d) Centrifuge the plate at 1,000xg for 10 seconds to remove all bubbles; you can also remove the bubbles by the pipette.
e) Cover the plate with the rubber septum, insert it into the lower plate retainer, cover it with the upper plate retainer and then insert it into the genetic analyzer.

6.5.2 Fragmentation Analysis Start

1. Switch on the devices in the following sequence:
   A Computer
   B ABI PRISM 3130 genetic analyzer
   C 3130 Data Collection v3.0 application
2. Insert the plate with the samples into the analyzer.
3. In the **Foundation Data Collection** window select **GA Instruments** in the navigator menu and then select **ga3130** and **Plate Manager** in the submenus.
4. Click **New** in the **Plate Manager** box to open the **New Plate Dialog** table for the new plate.
5. Use the **New Plate Dialog** to enter basic information about the new plate (see **Fig. 4**) and then click **OK**.
6. In **GeneMapper Plate Editor** enter the information about the sample positions at the plate and also the parameters for the analysis (see an example in **Fig. 5**) and click **OK**.
7. Select **ga3130** in the main navigator menu and then select **Run Scheduler** in the **GA3130** submenu.
8. You can use the **Find All** button in the **Plate View** box to generate a list of all the registered plates.
9. Click to select a plate with the requested name and then click the image of the plate to link the plate record with the current plate in the device - the plate image will change its color from yellow to green to indicate the success of this operation.
10. Make sure in the **Run View** menu that "**Validated**" is assigned to the individual runs in the "**Status**" column; click the green arrow symbol in the upper bar to start the analysis. A simultaneous analysis of 4 samples (i.e. 1 run) takes approximately 35 minutes.
11. When the capillary electrophoresis has finished the data will be stored in the predefined folder of disk E: in the .fsa format; this location will then be used for the subsequent data analysis in the GeneMapper® v4.0 software.

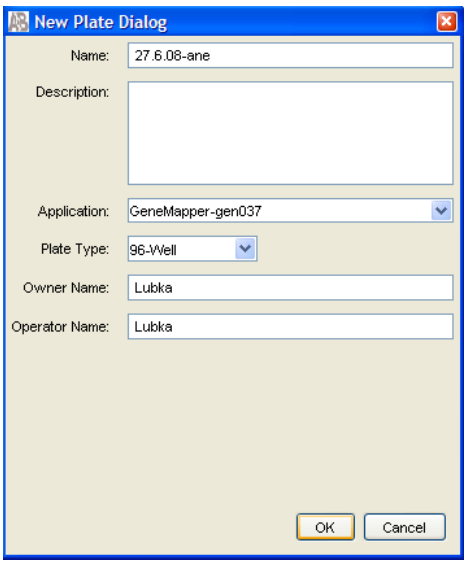
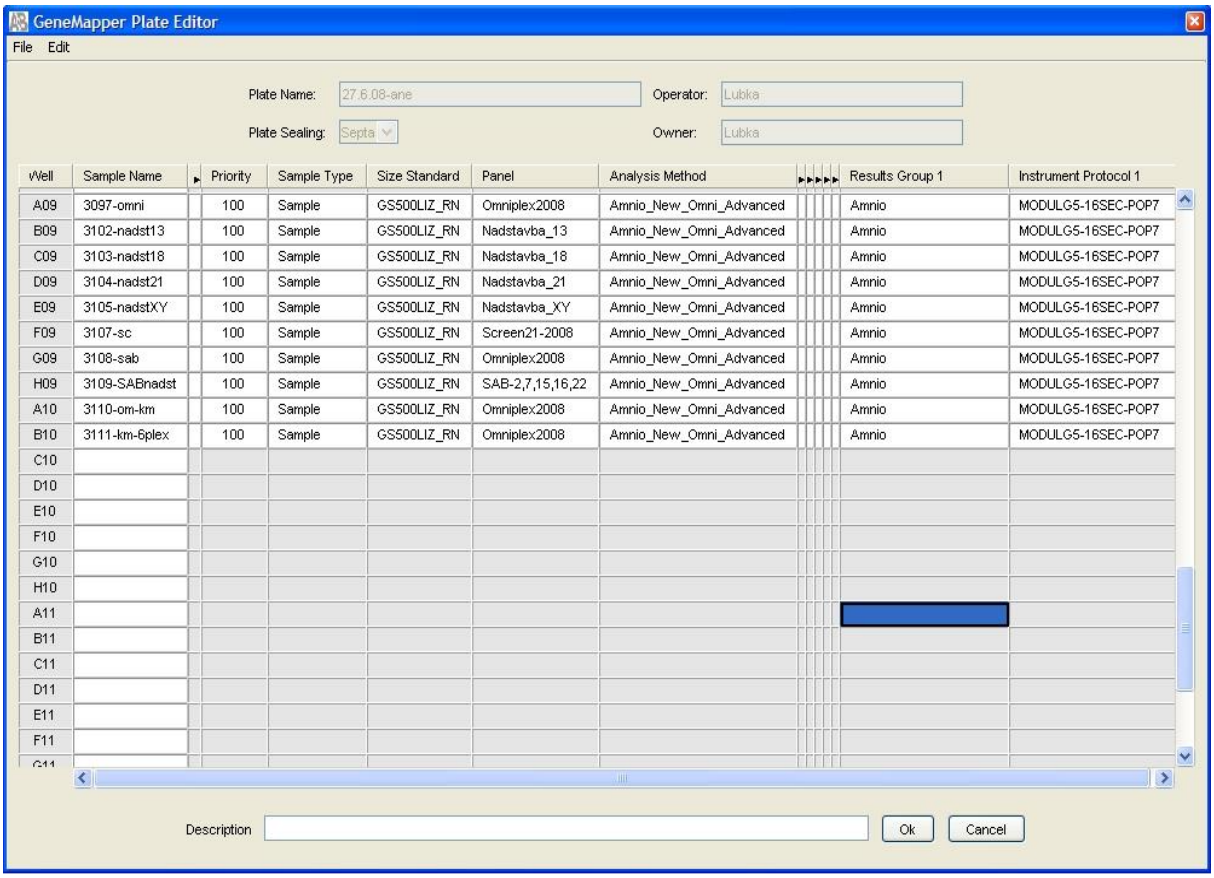


Fig. 4 New Plate Dialog table



Well	Sample Name	Priority	Sample Type	Size Standard	Panel	Analysis Method	Results Group 1	Instrument Protocol 1
A09	3097-omni	100	Sample	GS500LIZ_RN	Omniplex2008	Amnio_New_Omni_Advanced	Amnio	MODULG5-16SEC-POP7
B09	3102-nadst13	100	Sample	GS500LIZ_RN	Nadstavba_13	Amnio_New_Omni_Advanced	Amnio	MODULG5-16SEC-POP7
C09	3103-nadst18	100	Sample	GS500LIZ_RN	Nadstavba_18	Amnio_New_Omni_Advanced	Amnio	MODULG5-16SEC-POP7
D09	3104-nadst21	100	Sample	GS500LIZ_RN	Nadstavba_21	Amnio_New_Omni_Advanced	Amnio	MODULG5-16SEC-POP7
E09	3105-nadstXY	100	Sample	GS500LIZ_RN	Nadstavba_XY	Amnio_New_Omni_Advanced	Amnio	MODULG5-16SEC-POP7
F09	3107-sc	100	Sample	GS500LIZ_RN	Screen21-2008	Amnio_New_Omni_Advanced	Amnio	MODULG5-16SEC-POP7
G09	3108-sab	100	Sample	GS500LIZ_RN	Omniplex2008	Amnio_New_Omni_Advanced	Amnio	MODULG5-16SEC-POP7
H09	3109-SABnadst	100	Sample	GS500LIZ_RN	SAB-2,7,15,16,22	Amnio_New_Omni_Advanced	Amnio	MODULG5-16SEC-POP7
A10	3110-om-km	100	Sample	GS500LIZ_RN	Omniplex2008	Amnio_New_Omni_Advanced	Amnio	MODULG5-16SEC-POP7
B10	3111-km-6plex	100	Sample	GS500LIZ_RN	Omniplex2008	Amnio_New_Omni_Advanced	Amnio	MODULG5-16SEC-POP7
C10								
D10								
E10								
F10								
G10								
H10								
A11								
B11								
C11								
D11								
E11								
F11								

Fig. 5 GeneMapper Plate Editor Table

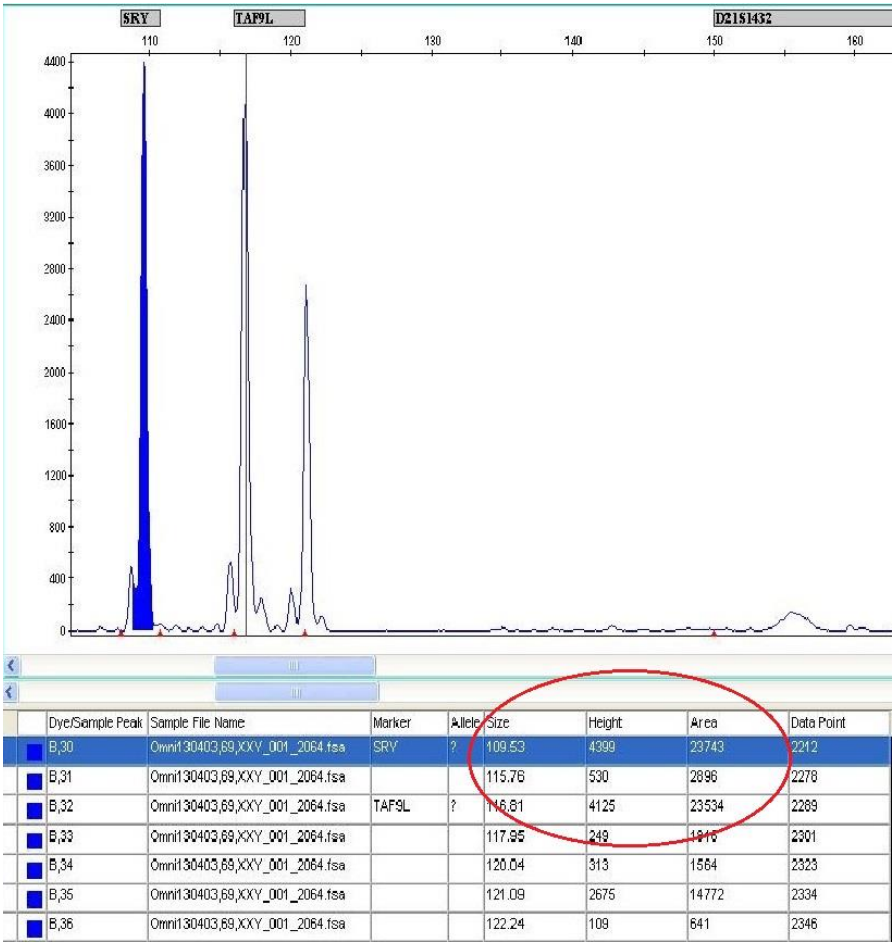
For a professional guidebook for clinical cytogeneticists and clinical molecular geneticists describing the aneuploidy diagnostics using the QF-PCR method see **www.cmgs.org.uk**

7 Calculation and Expression of Results

7.1 Result Analysis

Chromosome - specific short repeating DNA sequences (STR) are amplified by means of the Polymerase Chain Reaction (PCR). Using the visualization of the fluorescence-marked primers it is possible to quantify the PCR products by means of an automatic genetic analyzer. Quantification is based on a calculation of the height/area ratio of the allele peaks in the particular STR. Individuals differ by the allele lengths depending on the number of the particular STR repetitions.

Size is the length of the amplicons defined in base pairs (bp). **Area** and **Height** are absolute values of the measured fluorescence activity, i.e. of the PCR product amount (Fig.6).



Dye/Sample Peak	Sample File Name	Marker	Allele	Size	Height	Area	Data Point
B,30	Omni130403,69,XXY_001_2064.fsa	SRY	?	109.53	4399	23743	2212
B,31	Omni130403,69,XXY_001_2064.fsa			115.76	530	2896	2278
B,32	Omni130403,69,XXY_001_2064.fsa	TAF9L	?	116.81	4125	23534	2289
B,33	Omni130403,69,XXY_001_2064.fsa			117.95	249	1316	2301
B,34	Omni130403,69,XXY_001_2064.fsa			120.04	313	1564	2323
B,35	Omni130403,69,XXY_001_2064.fsa			121.09	2675	14772	2334
B,36	Omni130403,69,XXY_001_2064.fsa			122.24	109	641	2346

Fig. 6 Fragmentation analysis output from the GeneMapper v4.0 software - identification of the amplification products

Homozygous individuals feature alleles with the same length and therefore they feature just one peak. In individuals homozygous for STR, which are specific for the X chromosome, it is impossible to distinguish samples with the QF-PCR homozygous result of a normal XX woman from samples with a single X chromosome - the Turner syndrome, for example. Incorporation of additional chromosome X STR markers (especially of the TAF9L quantitative

marker) into the analysis significantly reduces the probability of homozygosity, yet it cannot eliminate it altogether.

DNA amplified from a normal heterozygous individual, who has alleles with distinct lengths for a specific STR sequence, will feature two peaks with the same height/area (1:1 ratio) and a distinct length (in bp) within the particular size range of the STR marker - see Fig.7 for an example.

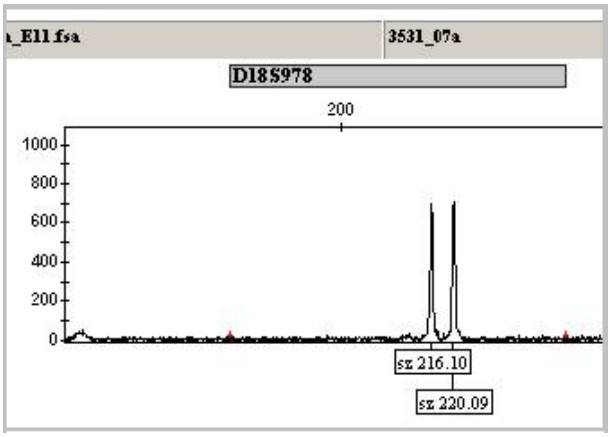


Fig. 7 Two alleles (= two peaks) of a heterozygous individual in the 1:1 ratio

Diallelic marker analysis is performed by means of the peak height/area ratio calculation (peak1/peak2), where peak1 represents height/area of the shorter fragment and peak2 represents height/area of the longer fragment - see **Table 1**.

Table 1: Ratio criteria for diallelic markers

Ratio	1:2	Inconclusive	1:1	Inconclusive	2:1
Peak distance <24 bp	<0.65	0.65-0.74	0.75-1.44	1.45-1.80	>1.80
Peak distance ≥24 bp	<0.65	0.65-0.74	0.75-1.54	1.55-1.80	>1.80

Fluorescence criteria - an acceptable range for the peak heights - between 50 and 8000 relative fluorescence units. Peaks beyond this range shouldn't be assessed.

DNA amplified from trisomic individuals features either another peak (three different alleles) with the same height/area (1:1:1 ratio) or two peaks (two different alleles) where one has twice the height/area of the other (1:2 or 2:1 ratio). See **Fig.8** for an example.

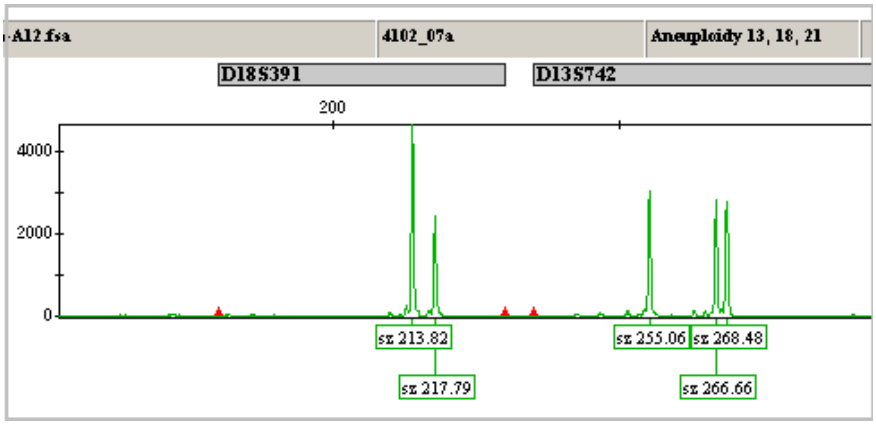


Fig. 8: An example of two trisomic markers with peaks in the 2:1 and 1:1:1 ratios.

The calculation of the tri-allelic marker ratio starts with the calculation of height/area of the shortest fragment (peak1), i.e. peak1/peak2/peak3 - see **Table 2**.

Table 2 Ratio criteria of tri-allelic (trisomic) markers

Ratio	Inconclusive	1:1:1	Inconclusive
Peak distance <24 bp	<0.74	0.75-1.44	>1.45
Peak distance ≥24 bp	<0.74	0.75-1.54	>1.55

Fluorescence criteria - an acceptable range for the peak heights - between 50 and 8000 relative fluorescence units. Peaks beyond this range shouldn't be assessed.

7.2 Result Evaluation

- **Diallelic profile**

The result shall be evaluated as normal, if at least two informative markers match the diallelic genotype; the other markers are non-informative. The diallelic profile is specified using markers with two peaks of similar area/height, whose ratio is evaluated as 1:1.

- **Tri-allelic profile**

The result shall be evaluated as abnormal (trisomic), if at least two informative markers match the tri-allelic genotype; the other markers are non-informative. The tri-allelic profile is specified using:

Markers with two peaks of different area/height, whose ratio is evaluated as 2:1 or 1:2.

Three peaks of similar area/height, where the peak ratio is evaluated as 1:1:1.

- **Non-informative result**

  Homozygous (monoallelic) markers are indicated as non-informative.

- **Sexual chromosomes analysis**

It is necessary to evaluate all the informative markers of the sexual chromosomes together.

Non-polymorphic X and Y markers

AMEL marker (AMLX/AMLY) visualizes non-polymorphic sections of the X chromosome (104 bp) and of the Y chromosome (110 bp). This marker makes it possible to detect the presence or absence of the Y chromosome and it can also establish a proportion of the X sequences to the Y sequences.

TAF9L marker is an immutable paralogous marker with sequences at chromosomes 3 and X. The specific peak for chromosome 3 (116 bp, represents 2 copies of chromosome 3) can therefore be used as a reference peak for establishing the number of present copies of the X chromosome (121 bp peak). An analysis of this marker, in combination with the Amelogenin marker and the other markers of the sexual chromosomes, is especially useful when diagnosing aneuploidy of sexual chromosomes, the Turner syndrome for instance. Their ratio should be between 0.8 and 1.4 in a healthy woman. The markers shall yield a ratio ≥1.8 in a healthy man or in case of the X chromosome monosomy.

Polymorphic STR markers

DXYS218, DXYS267, X22 and DX[Y]S6814 are polymorphic STR markers that are present on both the X and Y chromosomes. They establish the total number of sexual chromosomes. It is impossible to distinguish which alleles come from the X and which from the Y chromosome.

X-specific markers

Informative X-specific markers DXS1189, DXS7424, DXS8377, DXS981, DXS1187, DXS6809, DXS6803, DXS996 and XHPRT express the number of the X chromosomes.

Y-specific marker

Y-specific marker, SRY, produces one peak for healthy men, it is not amplified for women.

7.3 Results Interpretation

The examination result in the form of a table listing the numbers of the individual alleles of the examined STR markers along with a short comment and an image of the resulting electrophoretograms shall be delivered by the laboratory to the physician of the genetic department, who will compile a final report. Only the physician shall always perform the results interpretation.

A recommendation for a follow-up cytogenetic analysis should be an essential part of a laboratory report on a QF-PCR examination.

8 Quality Control

8.1 Internal Control

Negative sample

Together with each analysis of a sample we also examine a negative sample, which contains all the PCR mix components except for the template DNA. This negative sample verifies any possible contamination of the PCR mix components. The negative sample should not demonstrate in the fragmentation analysis any peaks within the range of the monitored STR markers higher then 50 relative fluorescence units (RFU) .

NOTE: Contamination of chemicals and the environment can be prevented by careful observation of the disinfection program principles, regular decontamination of the laminar box and observation of the PCR product handling principles - you must not work with the PCR product in the same room where you prepare the PCR reactions.

Analysis control using mother's peripheral blood

Together with each examined CVS sample, blood-contaminated amniotic fluid sample or aborted fetus sample we recommend analyzing a DNA sample from the mother's peripheral blood to verify any possible sample contamination by the mother's biological material.

Verification of the result correctness by repeating analysis in case of pathology detection

If chromosome aneuploidy is detected, the sample (except for DNA samples from aborted fetus tissues) should be subjected to another STR analysis by means of the QF-PCR method; use the superstructure set of markers for the chromosome featuring the pathological profile for this validation. If polyploidy is detected, validate the result by repeating the STR analysis with the OmniPlex set.

Feedback check can be performed by comparing the results with the cytogenetic examination results

Fetus chromosome aneuploidy QF-PCR examination result must always be validated by an independent result from a cytogenetic examination by a cultivation method.

8.2 External Control

Interlaboratory quality control for the examinations of the most frequent aneuploidies by means of the QF-PCR method is currently provided by the Department of Medical Genetics of GENNET. s.r.o., Kostelní 9, Praha 7, www.gennet.cz. These tests are performed once a year in cooperation with the Czech Accreditation Institute (CAI) as a part of the National Program of Proficiency Testing (PT).

9 Dealing with Abnormal and Inconclusive Results

A result is deemed **abnormal** if at least two markers at the same chromosome feature fully informative pathological detection; the remaining markers can be non-informative. We recommend validating an abnormal detection using the superstructure kit for the particular chromosome.

A result is deemed **inconclusive** if the height/area ratio of a peak oscillates at the boundary between the normal and the abnormal detection. These cases can be resolved using a superstructure kit for the particular chromosome.

9.1 Inconclusive Results

There can be several reasons for a situation when a marker features inconclusive results or fails to be detected:

a) Chromosomal mosaicism
b) Stutter peak
c) -/+ A peak (peak arm)
d) Color signal overlaps between channels
e) Electrophoretic spikes
f) Asymmetric amplification
g) DNA contamination: the other genotype, PCR amplicons
h) Polymorphism in the area of the primer annealing
i) Too high or too low DNA concentration
j) DNA used for the PCR is degraded

If characteristics for both normal and abnormal alleles are detected in a single chromosome, it is advisable to perform additional testing to discover the cause of this situation.
If the electrophoretograms are of low quality (significantly overlapping signals between the channels or electrophoretic spikes), the respective data shouldn't be analyzed. You can repeatedly insert the PCR product into an analyzer and repeat the analysis.
Fluorescence criteria - an acceptable range for the peak heights - between 50 and 8000 relative fluorescence units. Peaks beyond this range shouldn’t be assessed.

9.2 PCR Artefacts

"Stutter peaks" are detected as individual peaks, which are one or several repetitions smaller then the current STR allele. Height/area of a typical stutter peak is smaller then 15% of the appropriate STR peak (Fig.9).

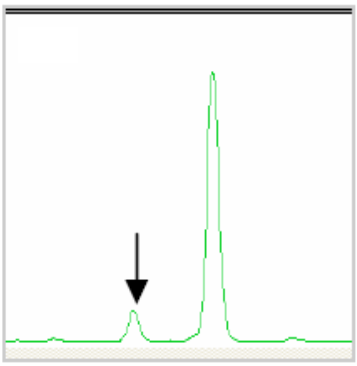


Fig. 9. A stutter peak is marked with an arrow

"-A peaks" are detected as individual peaks, which are one pair of bases shorter then the full-length PCR product (+A peak). -A peaks can be included in the ratio calculations. -A peaks are determined by the used DNA polymerase, which tends to add a nucleotide with an adenine base to the end of each PCR product. This feature can be minimized by including the step of heating to 60°C for 30 to 60 minutes at the end of the PCR program.

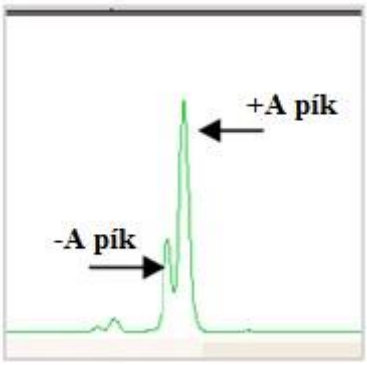


Fig. 10 –A and +A peaks are marked with arrows

9.3 Electrophoretic and Detection Artefacts

Signal overlapping between the individual detection channels can occur in the course of the detection. The resulting overlapping peaks must be excluded from the analysis (Fig.11).

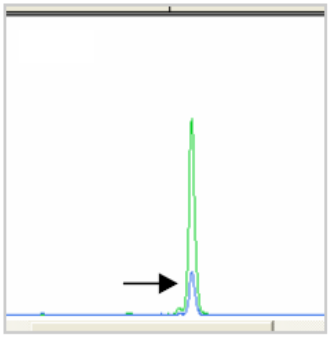


Fig. 11 The peak overlapping from the green into the blue channel is marked by an arrow

Electrophoretic spikes can appear during the detection as sharp peaks in several color channels. The peaks resulting from these electrophoretic spikes must be excluded from the analysis (Fig.12).

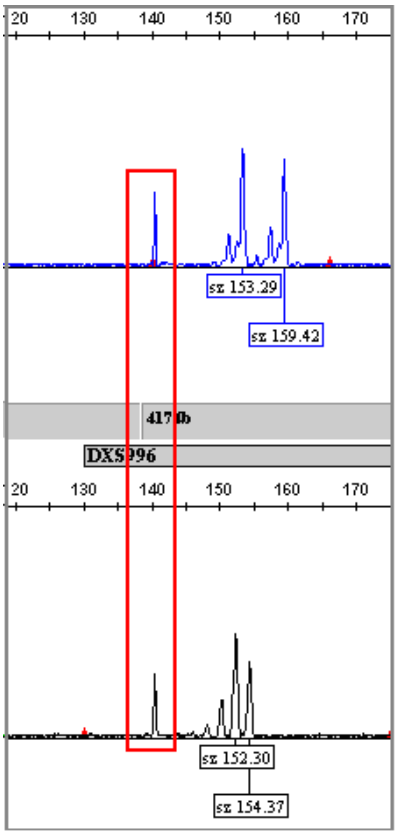


Fig. 12 Electrophoretic spikes are marked by red frames

10 Customer Support

GeneProof PCR detection kit customers are provided with free customer service support.

This support includes the following:

- Provision of free demonstration kits and personnel training;
- Introduction of routine diagnostics and help with standard operating procedures application at the customer's site;
- Help when dealing with any matters related to molecular diagnostics at the customer's site;
- Quick solution of problems related to the supplied products – service guaranteed within 24 hours from the time of announcement;
- Cooperation in research projects using GeneProof products;
- Consultations concerning technological and clinical interpretations.

Customer Service

E-mail: support@geneproof.com
Phone: +420 543 211 679

11 Used Symbol List

Symbol	Definition
	Catalogue Number
	Batch Number
	Manufacturer
	Use Before
	Temperature Limits

Aneuploidy	A numerical deviation in chromosomes, which is not a multiple of the haploid count
Marker	An area specified on a chromosome
PCR product	A specific DNA section multiplied by means of PCR
PCR Master Mix	A mix of chemicals for a PCR reaction before distribution into the individual PCR tubes, i.e. before adding the DNA sample
Primer	A short section of a single-stranded DNA used to initiate the synthesis of a complementary DNA section using the DNA
Primermix	A mix of F (forward) and R (reverse) primers all STR loci under examination
QF-PCR	Quantitative Fluorescence Polymerase Chain Reaction; provides for quantification of the acquired PCR products; PCR products are detected by means of fluorescence markings.
Multiplex QF-PCR	Multiple QF-PCR - multiplication of several PCR products in a single PCR reaction in the presence of several primer pairs
bp	Base pairs
CVS	Chorionic biopsy sample
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
SAB	Spontaneous abortion
TE	Stabilization buffer with the final concentration of 10mM Tris,
STR	„Short tandem repeat“ - repeating of several (mostly 2 - 5) nucleotides several times over

12 Ordering Information

Product Name	Catalogue Number	Technology	Packaging
GeneProof OmniPlex QF PCR Kit	OMNI/050	real-time PCR	50 reactions
GeneProof OmniPlex QF PCR Kit	OMNI/100	real-time PCR	100 reactions

13 Contact Information

Company Address

GeneProof a.s.
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619 00 Brno, Czech Republic
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Mail orders to:

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Customer Service

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ANNEX A

Markers of the OmniPlex QF-PCR Kit, GeneProof a.s.

Table 1 Markers in the OmniPlex QF-PCR Kit, GeneProof a.s.

Marker	Location	Observed Heterozygosity in Czech population	Allele Size Range	Marker Dye Color
SRY	Yp11.31	-	108-111 bp	blue
X/TAF9L	Xq21.1/3p24.2	-	116-121 bp	blue
D21S1432	21q21.1	0.709	150-180 bp	blue
D18S51	18q21.33	0.827	195-252 bp	blue
XHPRT	Xq26.2	0.765	260-300 bp	blue
DXS981	Xq13.1	0.83	340-375 bp	blue
D21S1435	21q21.3	0.755	386-440 bp	blue
D21S1437	21q21.1	0.818	445-496 bp	blue
D18S391	18p11.31	0.636	130-162 bp	green
DXS7424	Xq22.1	0.845	180-215 bp	green
D21S11	21q21.1	0.9	218-268 bp	green
D21S1412	21q22.2	0.9	270-335 bp	green
D21S1411	21q22.3	0.909	345-405 bp	green
D13S305	13q13.3	0.809	430-475 bp	green
D18S1002	18q11.2	0.827	103-135 bp	yellow
D18S535	18q12.3	0.782	170-210 bp	yellow
DXS1189	Xp22.2	0.816	255-296 bp	yellow
D18S386	18q22.1	0.936	307-378 bp	yellow
D13S742	13q12.12	0.891	390-442 bp	yellow
D13S628	13q31.1	0.782	443-490 bp	yellow
AMX/AMY	Xp22.2/Yp11.2	-	107-116 bp	red
D13S631	13q32.1	0.827	155-195 bp	red
X22	Xq28/Yq12	0.818	200-260 bp	red
D13S258	13q21.33-q22.1	0.873	275-350 bp	red
DXS8377	Xq28	0.966	389-455 bp	red

Table 2 Markers in the OmniPlex 21plus QF-PCR Kit, GeneProof a.s.

Marker	Location	Observed Heterozygosity in Czech population	Allele Size Range	Marker Dye Color
D21S11	21q21.1	0.9	219-270 bp	green
D21S1411	21q22.3	0.909	348-398 bp	green
D21S1412	21q22.2	0.9	273-329 bp	green
D21S1432	21q21.1	0.709	152-177 bp	blue
D21S1435	21q21.3	0.755	389-430 bp	blue
D21S1437	21q21.1	0.818	449-478 bp	blue
D21S1446	21q22.3	0.769	280-320 bp	red
D21S1270	21q22.11		251-295 bp	yellow
D21S2055	21q22.1		350-425 bp	yellow
D21S1444	21q22.13		230-2710 bp	red

Table 3 Markers in the OmniPlex 18plus QF-PCR Kit, GeneProof a.s.

Marker	Location	Observed Heterozygosity in Czech population	Allele Size Range	Marker Dye Color
D18S1002	18q11.2	0.827	104-134 bp	yellow
D18S386	18q22.1	0.936	309-370 bp	yellow
D18S391	18p11.31	0.636	138-156 bp	green
D18S51	18q21.33	0.827	195-245 bp	blue
D18S535	18q12.3	0.782	180-207 bp	yellow
D18S879	18q22.3		230-270 bp	green
D18S978	18q12.3		200-240 bp	red
D18S858	18q21.31		300-340 bp	red
D18S1367	18q22.1		145-185 bp	blue

Table 4 Markers in the OmniPlex 13plus QF-PCR Kit, GeneProof a.s.

Marker	Location	Observed Heterozygosity in Czech population	Allele Size Range	Marker Dye Color
D13S258	13q21.33-q22.1	0.873	280-340 bp	Red
D13S305	13q13.3	0.809	435-475 bp	Green
D13S628	13q31.1	0.782	445-485 bp	Yellow
D13S631	13q32.1	0.827	164-185 bp	Red
D13S742	13q12.12	0.891	390-435 bp	Yellow
D13S634	13q21.33	0.836	380-440 bp	blue
D13S762	13q31.3		180-220 bp	Blue
D13S788	13q14.3		248-295 bp	Green
D13S1817	13q21.1		195-240 bp	Yellow

Table 5 Markers in the OmniPlex XYplus QF-PCR Kit, GeneProof a.s.

Marker	Location	Observed Heterozygosity in Czech population	Allele Size Range	Marker Dye Color
AMX/AMY	Xp22.2/Yp11.2	-	107-116 bp	red
DXS1189	Xp22.2	0.816	258-287 bp	yellow
DXS7424	Xq22.1	0.845	186-210 bp	Green
DXS8377	Xq28	0.966	390-447 bp	red
DXS981	Xq13.1	0.83	349-365 bp	blue
SRY	Yp11.31	-	109-111 bp	blue
X22	Xq28/Yq12	0.818	207-250 bp	red
XHPRT	Xq26.2	0.765	269-293 bp	blue
X/TAF9L	Xq21.1/3p24.2	-	116-121 bp	blue
DXYS218	Xp22.32/Yp11.3		260-300 bp	Green
DX[Y]S6814	Xp22.33/Yp11.32		130-180 bp	blue
DXYS267	Xq21.31/Yp11.31		230-270 bp	blue
DXS1187	Xq26.2		120-165 bp	green
DXS6809	Xq21.33		315-355 bp	red
DXS6803	Xq21.31		360-400 bp	yellow
DXS996	Xp22.3	0.906	130-170 bp	yellow

Dyes of the OmniPlex QF-PCR Kit, GeneProof a.s.

Fluorophore NED™ used in the kit is spectrally distinguished as yellow. Commonly it is displayed as black, though, to assure better readability.

Table 6 Fluorophores (dyes) used for the OmniPlex QF-PCR Kit, GeneProof a.s.

6-FAM™ (blue)	VIC© (green)	NED™ (yellow)	PET© (red)
SRY	D18S391	D18S1002	AMX/AMY
D21S1432	DXS7424	D18S535	D13S631
D18S51	D21S11	DXS1189	X22
XHPRT	D21S1412	D18S386	D13S258
DXS981	D21S1411	D13S742	DXS8377
D21S1435	D13S305	D13S628	
D21S1437			
X/TAF9L			

Table 7 Fluorophores (dyes) used for the OmniPlex 21plus QF-PCR Kit, GeneProof a.s.

6-FAM™ (blue)	VIC© (green)	NED™ (yellow)	PET© (red)
D21S1432	D21S11	D21S1270	D21S1446
D21S1435	D21S1411	D21S2055	D21S1444
D21S1437	D21S1412		

Table 8 Fluorophores (dyes) used for the OmniPlex 18plus QF-PCR Kit, GeneProof a.s.

6-FAM™ (blue)	VIC© (green)	NED™ (yellow)	PET© (red)
D18S51	D18S391	D18S1002	D18S978
D18S1367	D18S8/79	D18S386	D18S858
		D18S535	

Table 9 Fluorophores (dyes) used for the OmniPlex 13plus QF-PCR Kit, GeneProof a.s.

6-FAM™ (blue)	VIC© (green)	NED™ (yellow)	PET© (red)
D13S634	D13S305	D13S628	D13S258
D13S762	D13S788	D13S1817	D13S631
		D13S742	

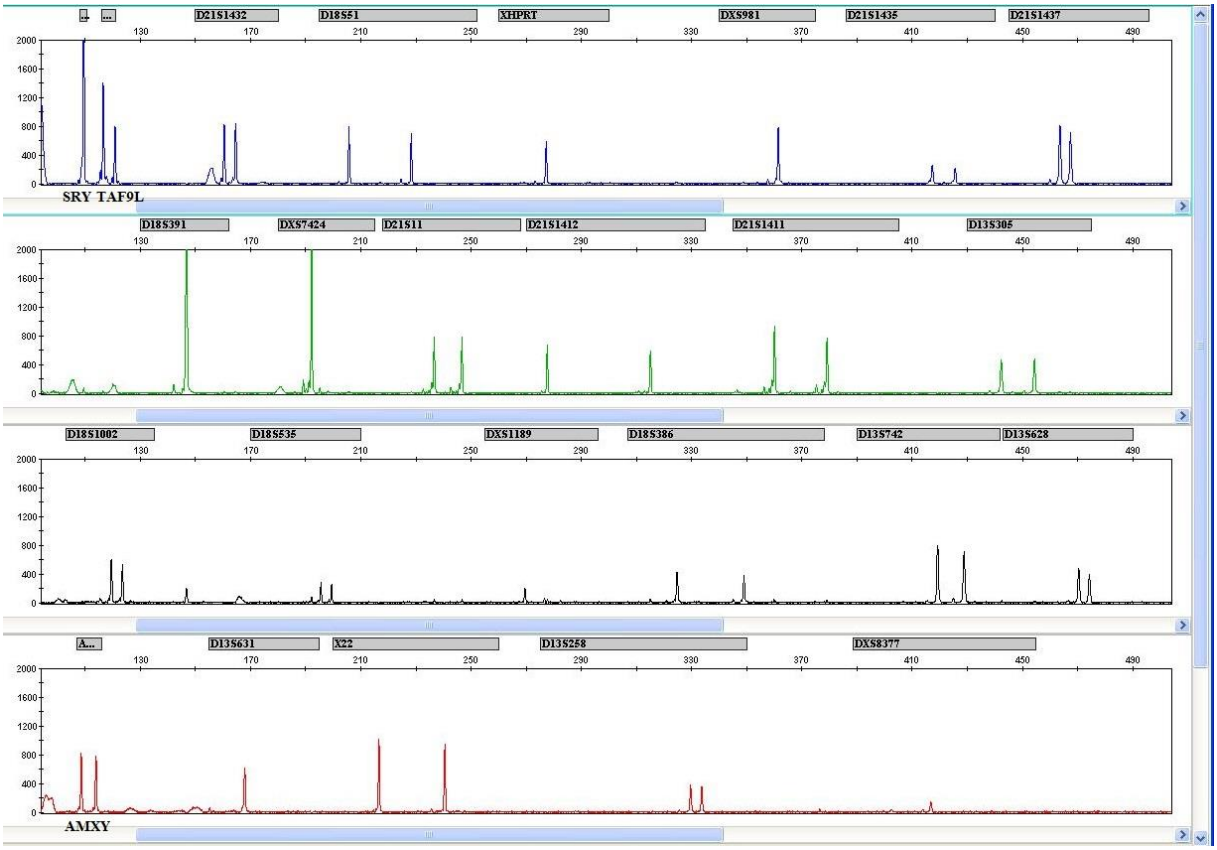
Table 10 Fluorophores (dyes) used for the OmniPlex XYplus QF-PCR Kit, GeneProof a.s.

6-FAM™ (blue)	VIC© (green)	NED™ (yellow)	PET© (red)
DXS981	DXS7424	DXS1189	AMX/AMY
SRY	DXYS218	DXS6803	DXS8377
XHPRT	DXS1187	DXS996	X22
DX[Y]S6814			DXS6809
DXYS267			
X/TAF9L			

ANNEX B

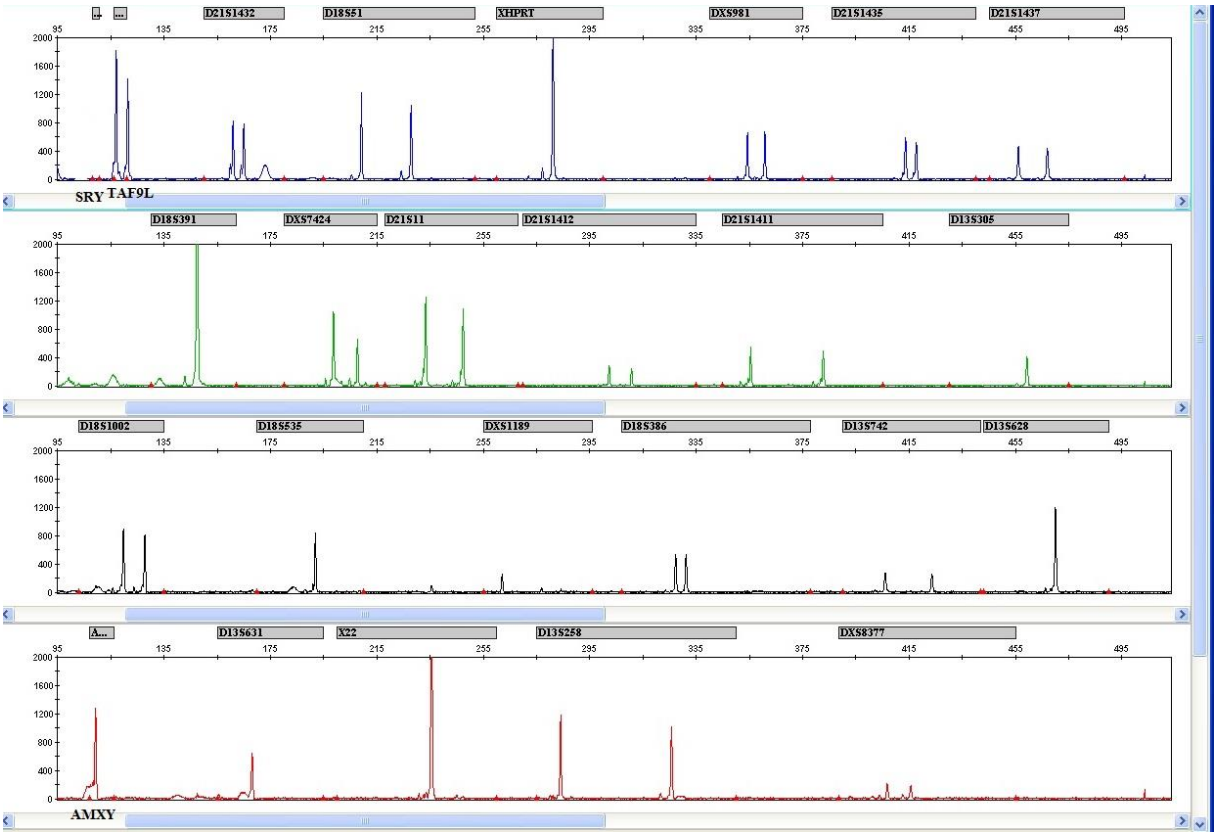
Examples of results

Example 1: Normal detection of male constitution of the XY sexual chromosomes



Both X and Y specific products of the AMXY marker are presented in the ratio of 1:1.
Presence of the Y chromosome is confirmed by the occurrence of the SRY marker product.
Presence of two sexual chromosomes is confirmed by the heterozygous character of the X22 pseudoautosomal marker.
Presence of a single X chromosome is confirmed by the X-specific markers XHPRT, DXS981, DXS7424, DXS1189, DXS8377 and especially by the TAF9L marker (the first peak's height indicates the presence of two autosomes 3, the second one indicates a single X gonosome).
The remaining markers for autosomes appear to be homozygous (D18S391, D13S631) or heterozygous.

Example 2: Normal detection of female constitution of the XY sexual chromosomes



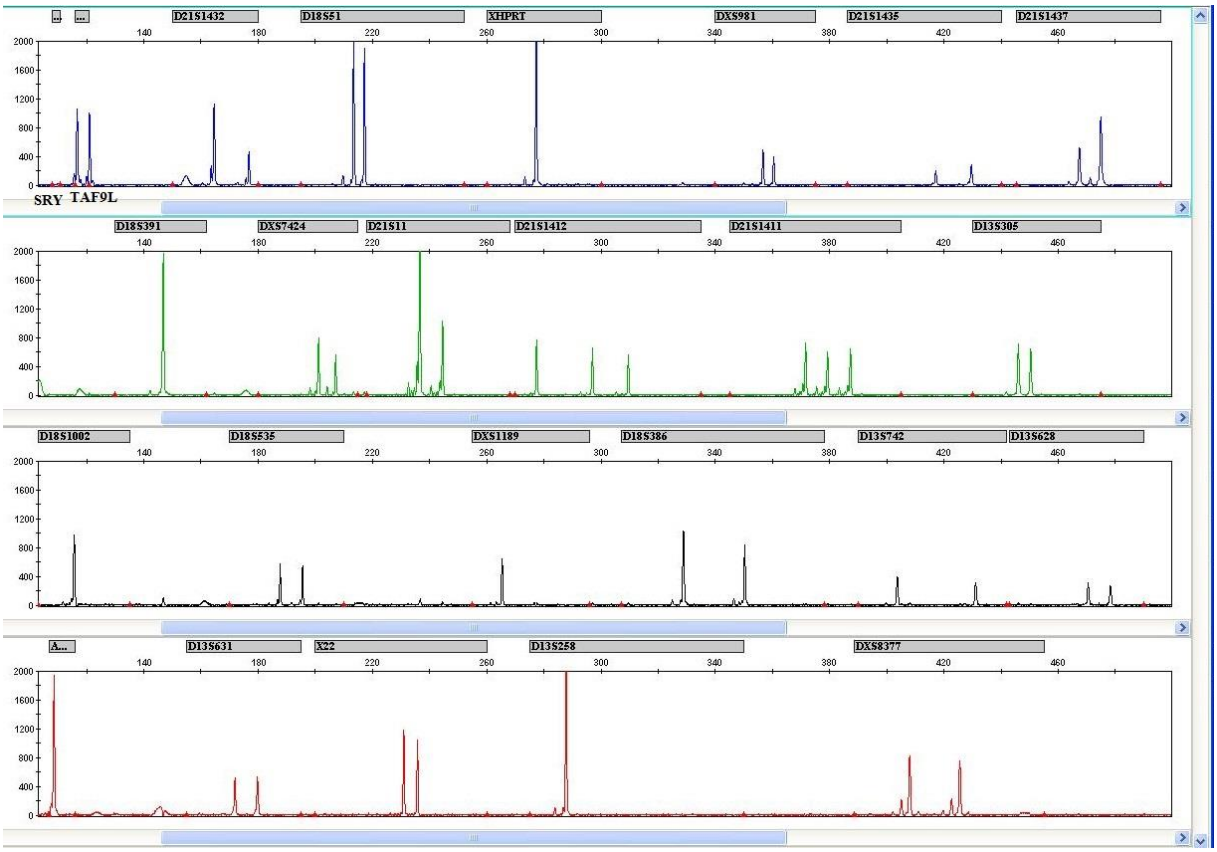
One peak corresponding to the X-specific product of this marker has been identified in the AMXY marker area.

No peak has been detected in the Y-specific SRY marker area and therefore the Y chromosome is not present in the sample.

Presence of two X chromosomes is confirmed by the X-specific markers DXS981, DXS7424, DXS8377 and especially by the TAF9L marker (the first peak's height indicates the presence of two autosomes 3, the second one indicates two X gonosomes - they are approximately in the ration of 1:1).

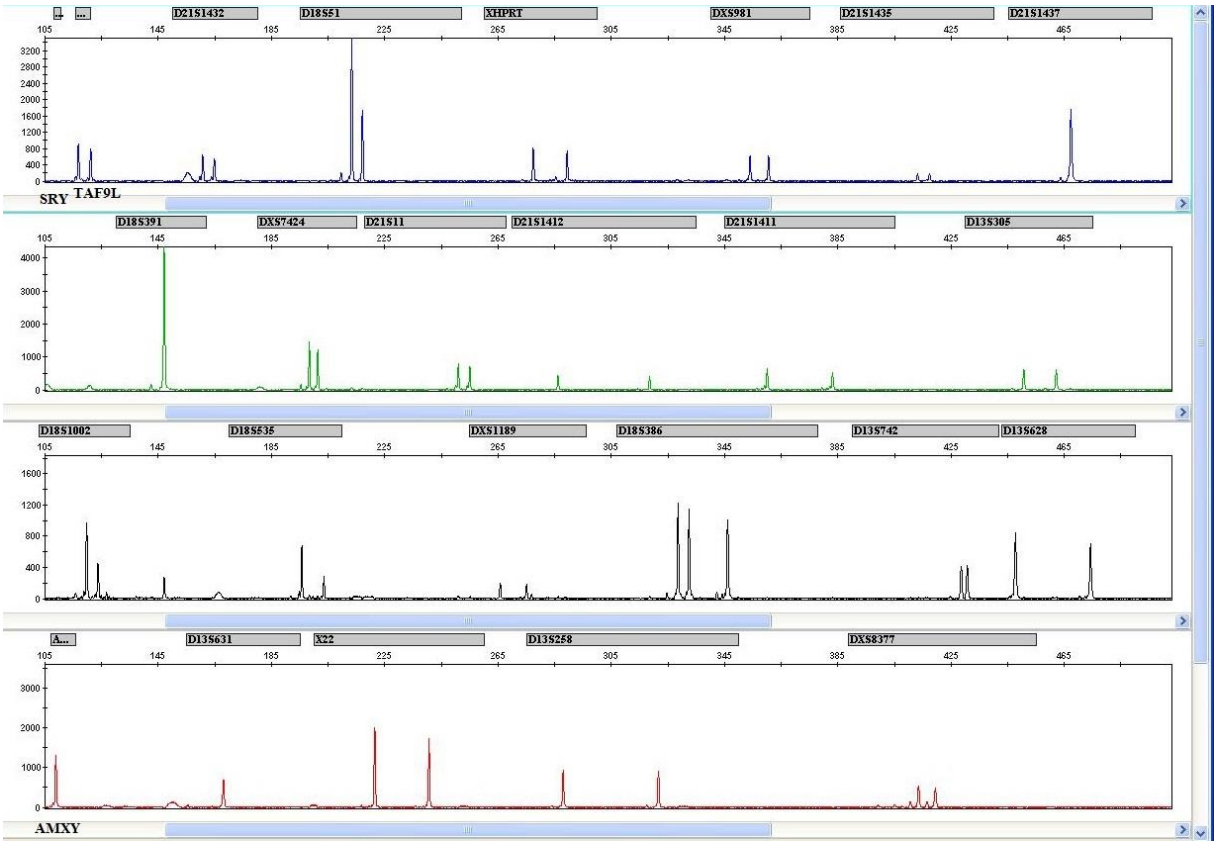
The remaining markers for autosomes appear to be homozygous (D18S391,D13S305, D18S535, D13S628, D13S631) or heterozygous.

Example 3: Detection of the chromosome 21 trisomy (47,XX,+21)



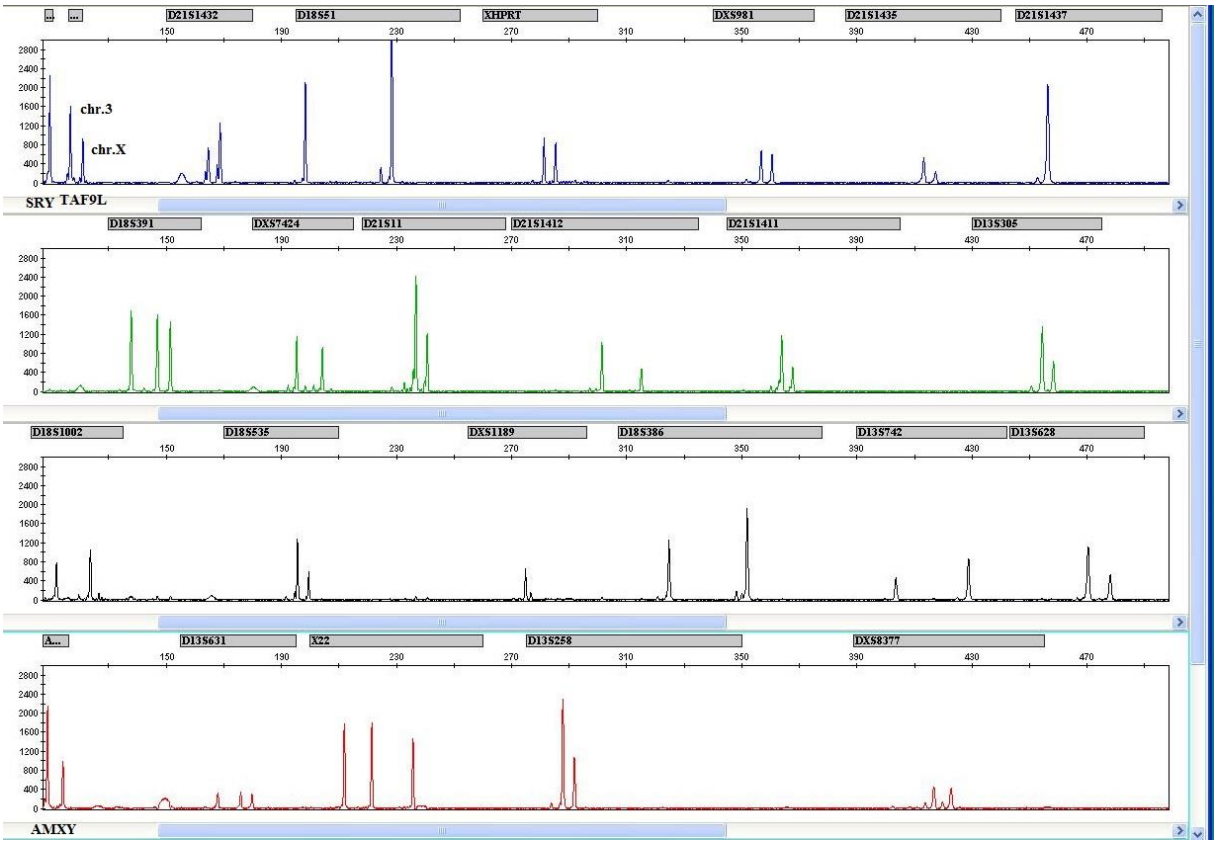
Two markers at chromosome 21 indicate the trisomic tri-allelic profile (D21S1412, D21S1411).
The D21S1432, D21S1437, D21S11 loci indicate the trisomic diallelic profile.
Only a single marker for chromosome 13 is non-informative (D13S258). All other markers are informative for the normal number of chromosomes number 13 (D13S305, D13S742, D13S628, D13S631). Markers for chromosome 18 are similar: D18S51, D18S535, D18S386 informative; D18S391, D18S1002 non-informative.
Constitution of the sexual XX chromosomes is explicitly established by the X-specific product of the AMXY marker, absence of the SRY product and by the heterozygous detection in loci DXS981, DXS7424, X22 and DXS8377. The number of the X chromosomes matches the number of autosomes no. 3 since the ratio of the TAF9L marker peaks is 1:1.

Example 4: Detection of the chromosome 18 trisomy (47,XX,+18)



One marker at chromosome 18 indicates the trisomic tri-allelic profile (D18S386).
The D18S51, D18S1002, D18S535 loci indicate the trisomic diallelic profile 2:1. Locus D18S391 is homozygous, and consequently non-informative.
Markers for chromosomes 13 and 21 are heterozygous, in the ratio of 1:1. Only markers D13S631 and D21S1437 are homozygous, and consequently non-informative.
Constitution of the sexual XX chromosomes is explicitly established by the X-specific product of the AMX marker, absence of the SRY product and heterozygous detection in the XHPRT, DXS981, DXS7424, DXS1189, X22 and DXS8377 loci. There are two X chromosomes in the ratio of 1:1 according to the TAF9L marker.

Example 5: Triploidy detection (69,XXY)



Presence of three sexual chromosomes is detected as a trisomic tri-allelic finding of the X22 pseudosomal marker. The finding of the two X chromosomes has been confirmed by the X-specific markers XHPRT, DXS981 and DXS7424, DXS8377.
The electrophoretogram indicates the presence of the Y-specific product of the SRY marker. The X-specific product of the AMXY marker is double the Y (2:1).
The number of the chromosomes 3 in comparison to the X gonosomes is generally higher as indicated by the TAF9L marker peak ratio.
To specify the number of the Y chromosomes it is recommended to use the OmniPlex XYplus QF-PCR Kit.
All markers feature a trisomic tri-allelic (D18S391, D13S631) or dialellic profile (2:1. 1:2).