



# *GT HapScreen<sup>®</sup> PAH*

## **Product User Manual**

**CAT# GT-11202**

A combined Haplotyping and QF-PCR Kit

To aid prenatal diagnosis and carrier screening for Phenylketonuria and detecting aneuploidies of chromosomes 21, 18, 13, X and Y

Produced by

**GENETEK BIOPHARMA GmbH**

# Contents

1. GT HapScreen® PAH Overview.....	4
1.1. Intended Use.....	4
1.2. GT HapScreen® PAH Kit & Markers.....	4
1.3. Five-dye fragment analysis.....	6
2. PCR.....	7
2.1. Storage Condition.....	7
2.2. Materials and equipment.....	7
2.2.1. Laboratory condition.....	7
2.2.2. Material required for Fragment Analysis.....	7
2.2.3. QF-PCR Amplification by GT HapScreen® PAH.....	8
2.2.4. GT HapScreen® PAH components.....	8
2.2.5. GT HapScreen® PAH protocol.....	8
3. Capillary electrophoresis.....	10
3.1. Instrument Preparation Applied Biosystems® 3500/3500xL Genetic Analyzer (before the first use of GT HapScreen® PAH Kit).....	10
3.1.1. Create a new Instrument Protocol.....	12
3.1.2. Create a New Size Standard for the QC protocol.....	12
3.1.3. Create a QC protocol.....	13
3.1.4. Create a new Assay.....	14
3.1.5. Create a new File Name Conventions.....	15
3.1.6. Create a new Result Group.....	16
3.1.7. Create a New Plate.....	17
3.1.8. Select “Assign Plate Contents”.....	18
3.2. Instrument Preparation Applied Biosystems® 3130/3130xl Genetic Analyzer (before the first use of GT HapScreen® PAH Kit).....	20
3.2.1. Create a Run Module.....	21
3.2.2. Create an Instrument Protocol.....	22
3.2.3. Set up a Plate for run.....	23
3.2.4. Fill out the New Plate Dialog.....	24
3.2.5. Fill out the GeneMapper Plate Editor.....	25
3.3. Sample preparation for capillary electrophoresis (3500 Series and 3130 Series instruments).....	28
4. Result analysis and Interpretation.....	29

4.1. Software for sample analysis..... 29

4.2. General guideline for the analysis of GT HapScreen® PAH results..... 29

4.2.1. Criteria for Interpretations..... 29

5. Example case study ..... 30

5.1. Case study 1 – sample mix-up ..... 30

5.2. Case study 2 – uniparental isodisomy ..... 39

6. Troubleshooting ..... 45

7. Limitations and Disclaimer..... 48

8. General Safety Warnings ..... 49

9. Symbols used on labels and packaging..... 50

10. Further Reading ..... 51

## 1. GT HapScreen® PAH Overview

- Aids prenatal diagnosis and carrier for Phenylketonuria disease as well as screening aneuploidies for chromosomes 21, 18, 13, X and Y in a single reaction
- It can be used on extracted DNA from blood, amniotic fluid, CVS
- Contains seven PAH gene linked STR markers, seven autosomal chromosomes specific markers and three sex chromosome specific markers, in total 16 markers amplification in a single reaction
- Analyzed using 5-dyes capillary electrophoresis system. Compatible with the Compact Spectrum CE System from Promega and Applied Biosystems™ 3130/xl, 3500, 3500/xl, SeqStudio platforms.
- It detects sample cross contamination
- It detects maternal cell contamination
- Aids gonadal mosaicism
- It authenticates samples used
- It adds confirmation to mutations screening results
- It detects chromosomal aneuploidies for chr. 21, 18, 13, X and Y at screening level.
- Detects sex of fetus sample

### 1.1. Intended Use

The GT HapScreen® PAH kit is a screening kit to aid prenatal diagnosis and carrier for Phenylketonuria disease, simultaneously with the detection of chromosome 13, 18, 21, X and Y aneuploidies. It's for research use only.

### 1.2. GT HapScreen® PAH Kit & Markers

The GT HapScreen® PAH kit is developed to aid prenatal diagnosis and carrier detection of Phenylketonuria disease. The kit functions on the principle based on segregation analysis using linkage principle by drawing haplotype using STR markers linked to the PAH gene on chromosome 12. The kit also contains STR markers on chromosomes 21, 18, 13, X and Y to act as a QF PCR technique for the detection of common aneuploidies for these chromosomes. Use of STR markers makes prenatal diagnosis more accurate and reliable with many advantages outlined below.

The haplotype part of the kit components includes multiplex PCR primers which are designed from PAH gene. Mutation in PAH gene is responsible for Phenylketonuria disorder. STR markers flanking the PAH gene, have been carefully selected and intensively checked to have high heterozygosity. Primers have been designed from seven regions that covers the upstream and downstream of the PAH gene.

This kit is optimised to use DNA samples purified from blood, amniotic fluid, and chorionic villus (CVS).

Along with Phenylketonuria detection, the GT HapScreen® PAH kit includes autosomal STR markers for chromosome 21, 18, 13, X and Y.

STR loci (short tandem repeat) consist of short and repetitive sequence elements, 3 - 7 base pairs in length. These tandem repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers which can be detected by PCR using their flanking sequences for primer selection. The STR loci alleles are differentiated by the number of copies of the repeat sequence contained within the amplified region (locus) and can be distinguished from each other using fluorescence detection after electrophoretic separation. Genetek has many kits which use STR methodology either as linkage analysis or heterogeneity as in our forensic kits (for more information please visit our website at [www.genetek-biopharma.com](http://www.genetek-biopharma.com)).

**Table 1: Markers used in GT HapScreen® PAH**

No.	Marker	Chromosome	Size	Location
1	AMXY	X, Y	103-117	Xp22.2
				Yp11.2
2	D13S325	13	125-190	Xq28
3	D12PAHSD3	12	200-260	12q23.2
4	D21S1446	21	285-328	21q22.2
5	D12PAHSU3.7	12	438-490	12q23.2
6	DXS7132	X	115-150	Xq12
7	D18S391	18	160-200	18p11.31
8	SRY	Y	208-215	Yp11.31
9	D12PAHSU17.6	12	220-280	12q23.2
10	D12PAHSU3.3	12	281-330	12q23.2
11	D21S1414	21	350-430	21q21.1
12	D12PAHSD4.8	12	150-190	12q23.2
13	D18S1002	18	250-310	18q11.2
14	D21S1411	21	335-460	21q21.1
15	D12PAHSD4.2	12	130-170	12q23.2
16	D12PAHSI1	12	200-260	12q23.2

- Since PAH gene specific markers are unique and they do not have internationally agreed name, we have created a naming strategy. The nomenclature used for PAH specific STR markers are as follow: D stands for DNA, 12 for chromosome 12, PAH is gene name, S is for single copy sequence and U for upstream and D for downstream of the gene. Numbers are distance from either side of the gene in 10<sup>5</sup> bp (i.e. D4.8 means roughly 480 kb distance from end of the gene).
- These sizes are obtained using the ABI Genetic Analyzer 3500xL and the GT500 internal size standard. Out of range can be observed in different populations. Validate the result for your instrument.

### 1.3. Five-dye fragment analysis

ABI 3130, 3130xl, and 3500 and 3500xL (Applied Biosystems®) are recommended for the 5-Dye capillary electrophoresis of amplified products.

Table 2: The fluorescent dyes used in GT HapScreen® PAH kit

Name	6-FAM	GT2907	GT2712	GT1803	GT500
------	-------	--------	--------	--------	-------

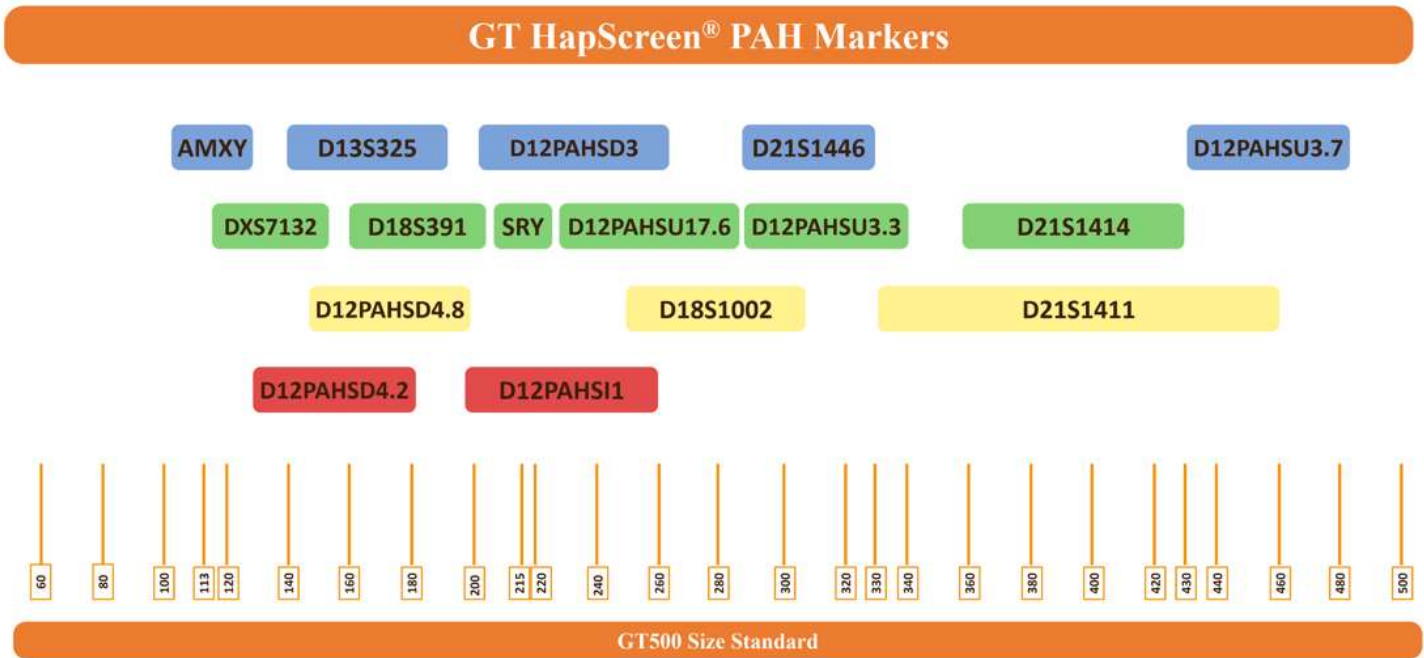


Figure 1. Diagram shows distribution and placement of GT HapScreen® PAH Kit markers with GT500 size standard.

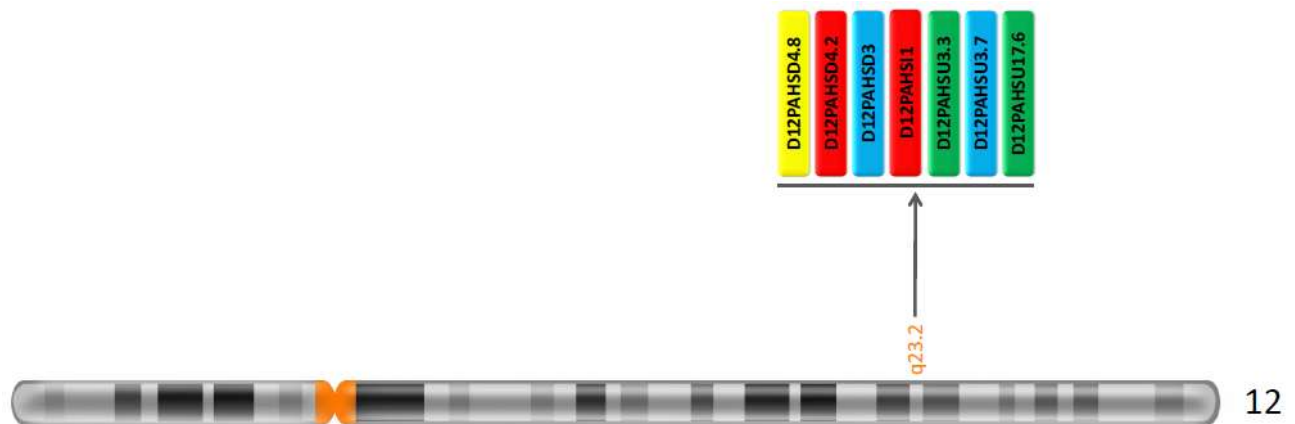


Figure 2. Diagram showing the distribution and placement of GT HapScreen® PAH markers on human chromosome 12

## 2. PCR

### 2.1. Storage Condition

- Store at -20 °C
- Keep the primer mix in a dark place (because of fluorescently labelled primers)
- Avoid frequent freeze and thaw (Store the materials in small aliquots)
- Low-quality result may be obtained after the expiration (12 months)






### 2.2. Materials and equipment

#### 2.2.1. Laboratory condition

GT Hapscreen kits can amplify a small amount of DNA. Primer Mix, PCR Mix, dH<sub>2</sub>O, control DNA and GT HSTaq DNA polymerase (in Box A) should be stored in a separate lab (Pre-PCR area). GT500 Size Standard and GTM5 v2 matrix standard (in Box B) are amplicons and should be stored in post-PCR area freezer. In each run, negative control should be added to determine possible contamination.

#### 2.2.2. Material required for Fragment Analysis

**Table 3: Provided with the Kit in Box A and Box B. They should be kept separately. Box A in one freezer and Box B is another freezer (PCR product)**

BOX-A		
	Tube Label	Tube cap colour
1	PCR Mix	
2	Primer Mix	
3	GT HSTaq	
4	GT QCDM102(Control DNA-50ng/μl)	
5	GT QCW (H <sub>2</sub> O)	

BOX-B		
	Tube Label	Tube cap colour
1	GT500 Size Standard	
2	GTM5 v2 (Optional)	

**Not provided with GT HapScreen<sup>®</sup> PAH (but are needed)**

- Reagents and equipment for DNA extraction
- Equipment and consumable for amplification (i.e. Thermal Cycler, Micropipette, Filter Tips, etc.)
- Applied Biosystems<sup>®</sup> Genetic Analyzer (ABI 3130/xI or 3500/xL) with Data Collection software for 5-dye system detection
- Applied Biosystems<sup>®</sup> Genetic Analyzer (ABI 3130/xI or 3500/xL) relevant Performance optimized polymers (i.e. POP-4, POP-6 or POP-7) and Capillary Array or equivalent
- Applied Biosystems<sup>®</sup> Hi-Di<sup>™</sup> Formamide or equivalent
- GTM5 v2 - Matrix Standard for Spectral calibration (GT- 41103)

### 2.2.3. QF-PCR Amplification by GT HapScreen® PAH

- DNA can be extracted from blood, amniotic fluid, CVS and tissue samples. This kit also works for blood samples on filter paper such as DNA Banking Card as well as amniotic fluid cells collected on DNA Banking Card. For instruction of direct PCR method please contact us by email ([support@genetek.de](mailto:support@genetek.de)).
- 5-10 ng DNA can be used as a template.
- For optimizing and getting the best results, internal validation for each laboratory is recommended.
- We recommend making a test with the control DNA provided with the kit if you are using the kit for the first time or face problem and want to check the quality of your extracted DNA.

### 2.2.4. GT HapScreen® PAH components

Table 4: PCR reaction set-up

Component	Volume for 1 reaction[ $\mu$ l]
GT QCW (H <sub>2</sub> O)	10
PCR Mix	7
Primer Mix	1
GT HSTaq	1

### 2.2.5. GT HapScreen® PAH protocol

- Bring reagents to room temperature.
- Vortex GT Primer Mix and PCR reaction mix, then spin down briefly to remove all residues from the lid. Gently mix the enzyme by inverting or pipetting.
- Prepare a Master Mix calculating number of samples and controls by following the recipe given above. Every preparation can be done at room temperature (no cold condition is required during preparation).
- Vortex Master Mix briefly.
- Transfer 19  $\mu$ L of Master Mix to each 0.2 ml PCR tube for each sample you want to analyse.
- Add 1 of sample DNA (1-5 ng per reaction) into each PCR tube. Make one positive control PCR tube using the DNA provided in the kit and also for negative control add 1  $\mu$ l of sterile Direct Q dd H<sub>2</sub>O instead of DNA.
- Vortex and spin down each PCR tube. Make sure that no drops are left at the tube wall or lid.
- Place tubes into thermal cycler.
- Use the following PCR program for the amplification of all markers.

Table 5: PCR program

Initial step	Cycling			Final Extension	Storing in Cycler
	Denaturation	Annealing	Extension		
95 °C	95 °C	63 °C	70 °C	70 °C	4 °C
20 min	1 min	90 sec	2 min	17-20 min	$\infty$
27-30 cycles					

- Store the PCR products at 2-6°C until analysis with Genetic Analyzer.

#### Notes:

- PCR product is persistent for about 24h at room temperature. It is better to keep it in a refrigerator and in dark.
- If the time between amplification and capillary electrophoresis is more than one week, the quality of results may be reduced.
- A positive control DNA (sample with known genotype) and a negative control should be run in each multiplex PCR. We recommend using GT QCDM102 as a positive control especially early on during testing our kit or setup.
- According to the quality or quantity of DNA template, you may require changing the number of cycles in PCR program.

**Attention:**

After PCR is complete, tubes should never be opened in the PCR setup area (pre-PCR area) or near the kit components.

### 3. Capillary electrophoresis

- ABI 3130/xl and 3500/xL (Applied Biosystems®) are recommended for 5-dye capillary electrophoresis of the amplified PCR products.
- Please make sure your ABI Data Collection software supports 5-dye fragment analysis (according to the instrument user manual).
- GT HapScreen® PAH Kit is validated using 50 cm capillary array and POP7 using ABI 3500xL (Applied Biosystems®).
- For more details and optimization, follow the user guide on *DNA Fragment Analysis by Capillary Electrophoresis by Applied Biosystems®*.

#### Notes:

- Injection time or voltage can be adjusted according to the amount of PCR product.
- PCR products can be injected into the capillary more than one time or the results can be re-analysed.

#### 3.1. Instrument Preparation Applied Biosystems® 3500/3500xL Genetic Analyzer (before the first use of GT HapScreen® PAH Kit)

Make sure that maintenance and installation of capillary array, buffers and polymer are done according to Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide.

#### Attention:

Spectral Calibration must be made for the first time use or if capillary array has been changed, using GTM5 v2 Matrix Standard, the machine must be calibrated with GTM5 v2 Matrix Standard. Please find detailed protocol for spectral calibration with GTM5 v2 Matrix Standard here <https://www.genetek-biopharma.com/> or contact us at [support@genetek.de](mailto:support@genetek.de).

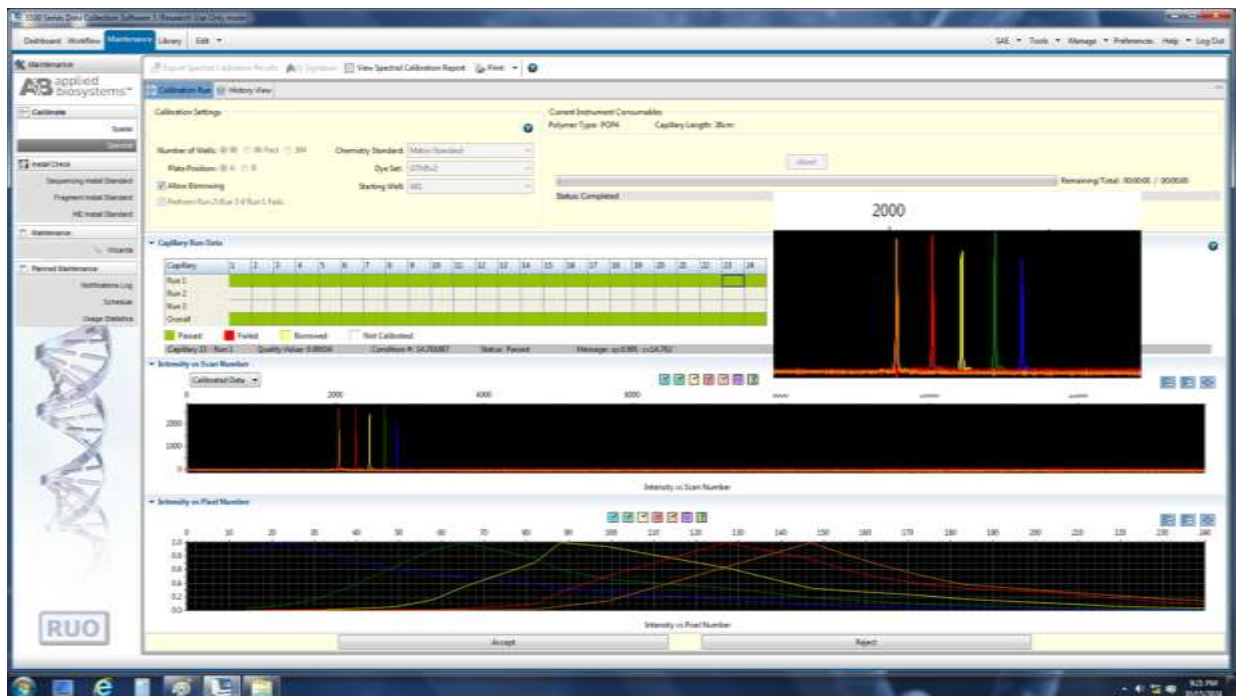


Figure 3. An example of a successful spectral calibration with GT 5 Dye system on Applied Biosystems® Genetic Analyzer 3500xL

- The Dashboard screen (Figure 4) is launched when 3500 Data Collection Software is opened. Click the Refresh button to make sure that all the information on the Dashboard is up to date. Make sure that the Maintenance and Consumables notifications are acceptable.
- Adjust the oven temperature to 60° C, then click “Start Pre-Heat” button. You may proceed for the first injection only after Oven Temperature and Detection Cell Temperature numbers turn green.



Figure 4. Dashboard of Applied Biosystems® 3500 Data Collection software

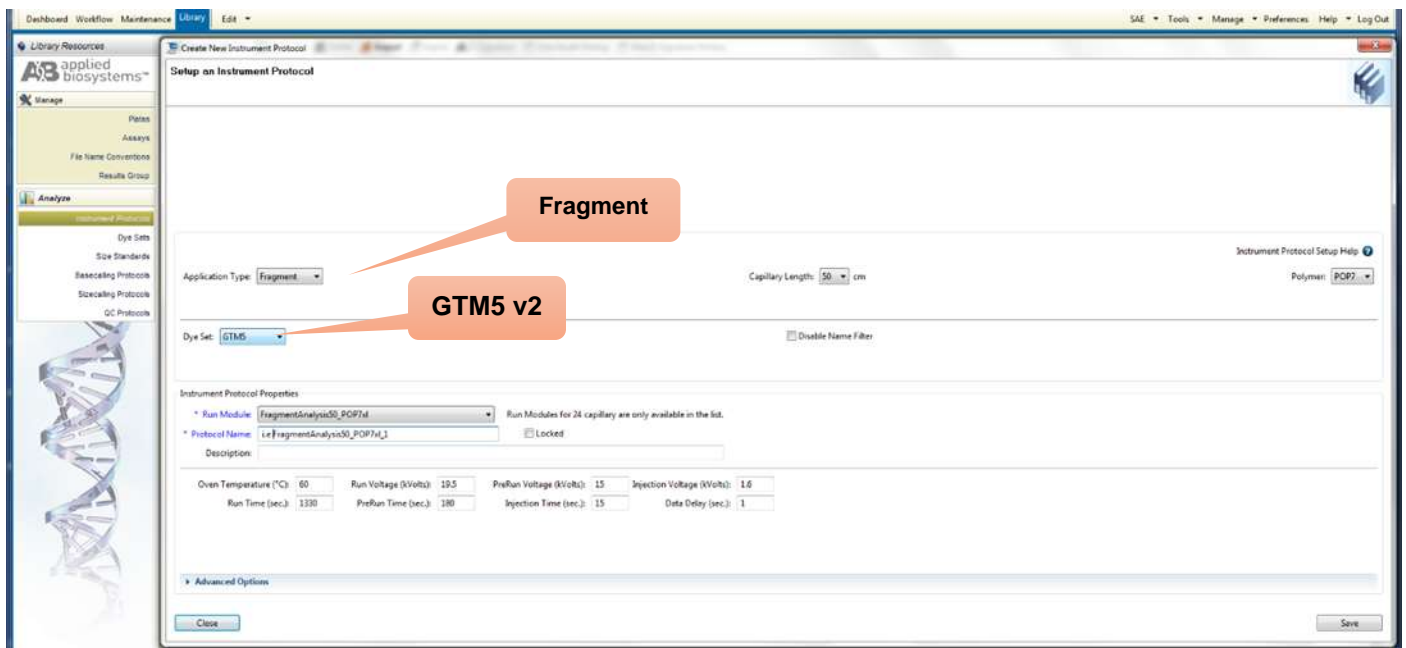


Figure 5. Screenshot for the “Create New Instrument Protocol” window on Applied Biosystems® 3500 Data Collection software

- User can apply settings as shown in the Figure 5. Make sure that you select GTM5 v2 as a *Dye Set* (same name as was used to perform the GTM5 v2 spectral calibration).

Onset of first analysis of GT HapScreen® PAH system, the user must create an Instrument Protocol, Size Standard, QC Protocol, Assay, File Name Convention and Results Group.

### 3.1.1. Create a new Instrument Protocol

- a) navigate to the *Library*
- b) select “Instrument Protocols”
- c) select “Create” (Figure 5)

Data Collection Software will store this information (until there is a change in the physical properties of the instrument), and it can be used for consequent runs.

Alternatively, individual lab should validate and define the settings according to their results. For more detailed information, refer to the Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide.

### 3.1.2. Create a New Size Standard for the QC protocol

- a) navigate to the *Library*
- b) select “Size Standards”
- c) select “Create” (Figure 6)

The Data Collection Software will store this information (until there is a change in the physical properties of the instrument), and it can be used for subsequent runs.

- d) name the Size Standard as “GT500” and as Dye Colour select “Orange”

The fragments size in the GT500 size standard are 60, 80, 100, 113, 120, 140, 160, 180, 200, 215, 220, 240, 260, 280, 300, 320, 330, 340, 360, 380, 400, 420, 430, 440, 460, 480 and 500.

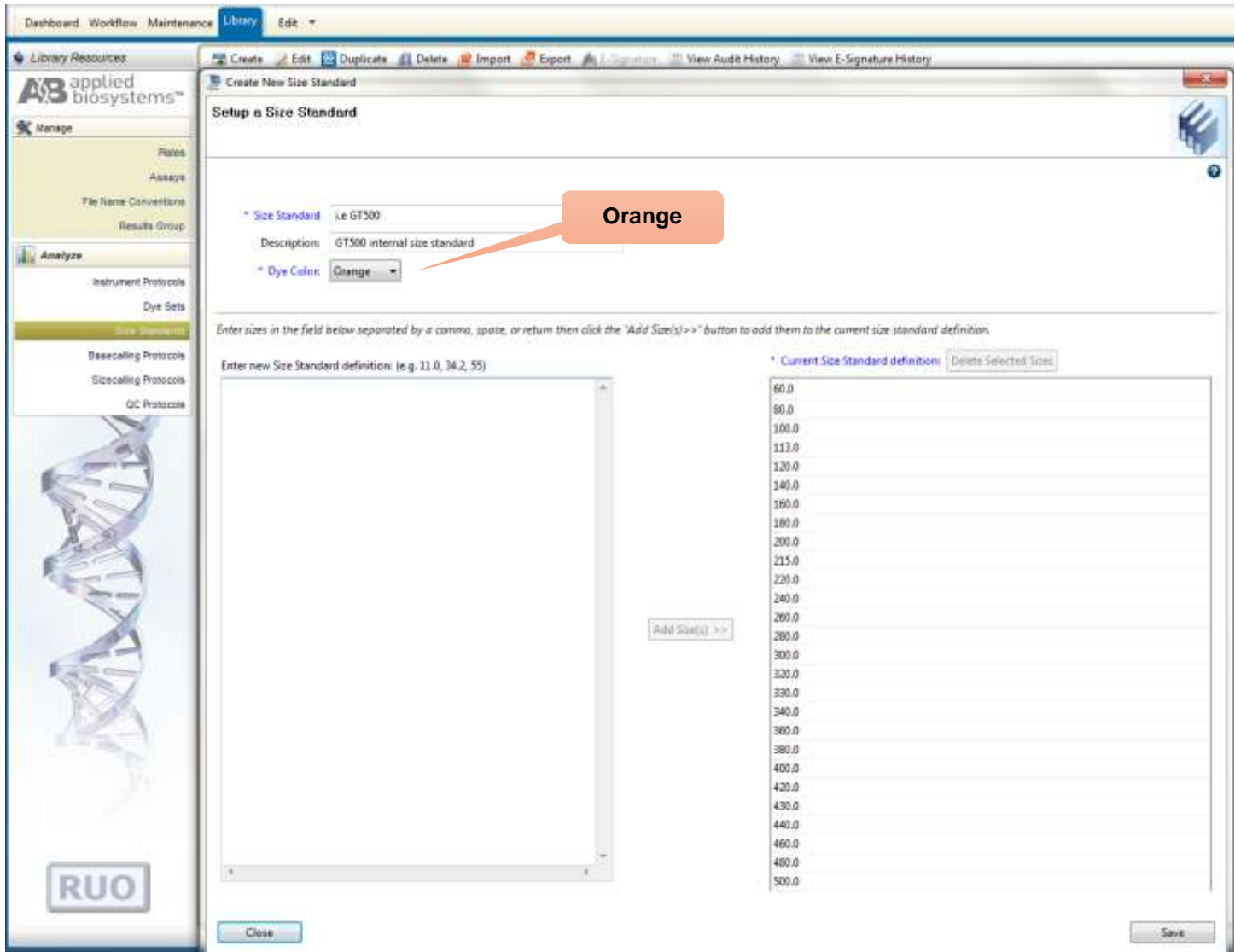


Figure 6. Screenshot for the “Create New Size Standard” window on Applied Biosystems® 3500 Data Collection software

### 3.1.3. Create a QC protocol

- a) navigate to the *Library*
- b) select “QC Protocols”
- c) select “Create” (Figure 7)

The Data Collection Software will store this information (until there is a change in the physical properties of the instrument), and it can be used for subsequent runs.

- d) name the protocol as “*i.e. GT500*” and select the *Size Standard* “GT500”

Users can select settings as shown in the Figure 6 or alternatively may define these settings based on internal validation condition for GT HapScreen® PAH on the Applied Biosystems® 3500/3500xL Genetic Analyzer.

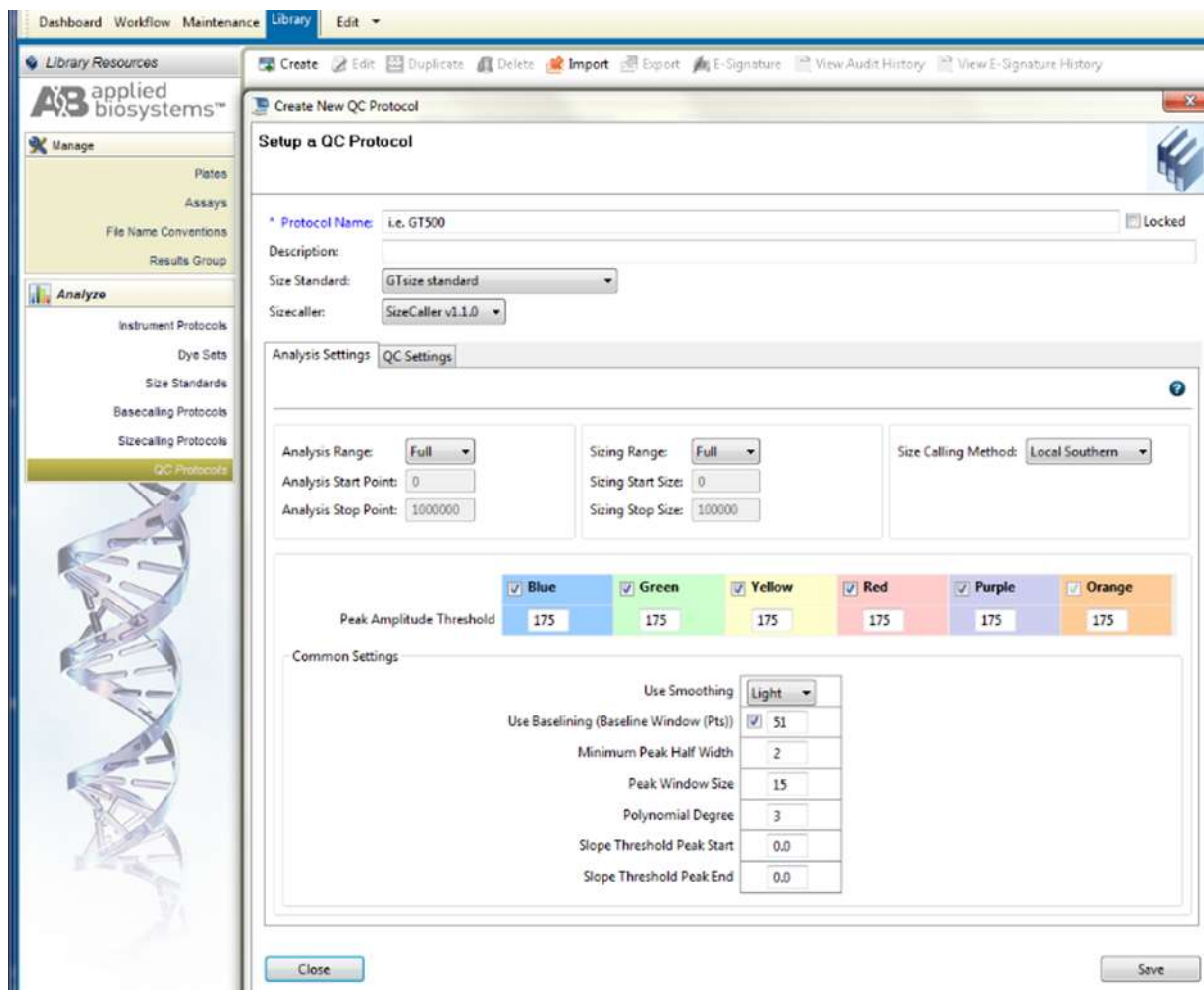


Figure 7. Screenshot for the “Create New QC Protocol” window on Applied Biosystems® 3500 Data Collection software

### 3.1.4. Create a new Assay

- a) navigate to the *Library*
- b) select “Assays”
- c) select “Create” (Figure 8)

Data Collection Software will store this information (until there is a change in the physical properties of the instrument), and it can be used for subsequent runs.

- d) in the *Create New Assay* window, as shown in Figure 7, choose the *Instrument Protocol* created in Step 3.1.1 and the *QC Protocol* created in Step 3.1.3
- e) give a name to the assay
- f) choose the application type “*Fragment Analysis*”

Any named sample on the plate must have an Assay assigned to it.

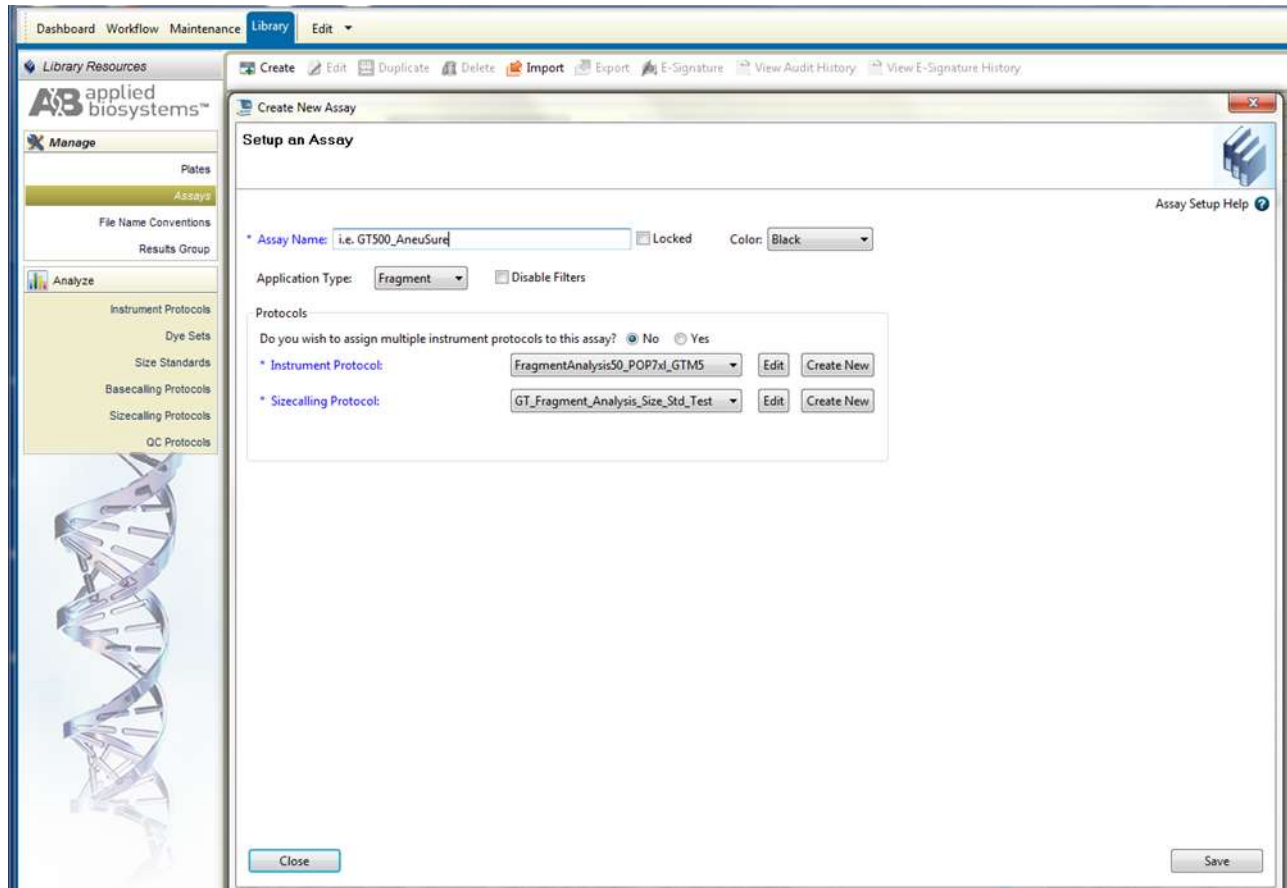


Figure 8. Screenshot for the “Create New Assay” window on Applied Biosystems® 3500 Data Collection software

### 3.1.5. Create a new File Name Conventions

- a) navigate to the *Library*
- b) select “*File Name Conventions*”
- c) select “*Create*” (Figure 9)

Data Collection Software will store this information (until there is a change in the physical properties of the instrument), and it can be used for subsequent runs.

- d) choose the *File Name Attributes* according to your lab practices

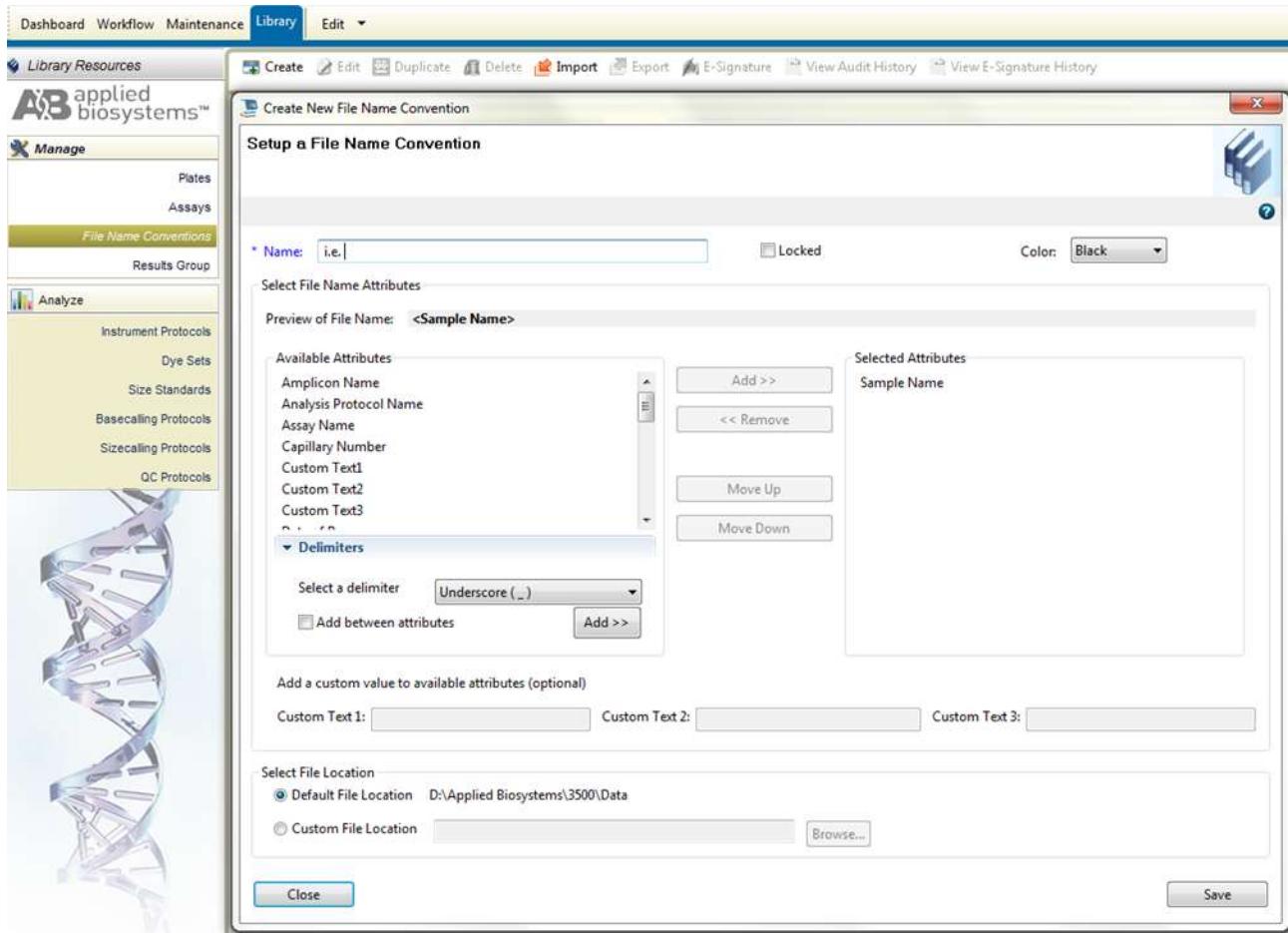


Figure 9. Screenshot for the “Create New File Name Convention” window on Applied Biosystems® 3500 Data Collection software

### 3.1.6. Create a new Result Group

- a) navigate to the *Library*
- b) select “Results Group”
- c) select “Create” (Figure 10)

Data Collection Software will store this information (until there is a change in the physical properties of the instrument) and it can be used for subsequent runs.

- d) choose the *Results Group Attributes* according to your lab practices

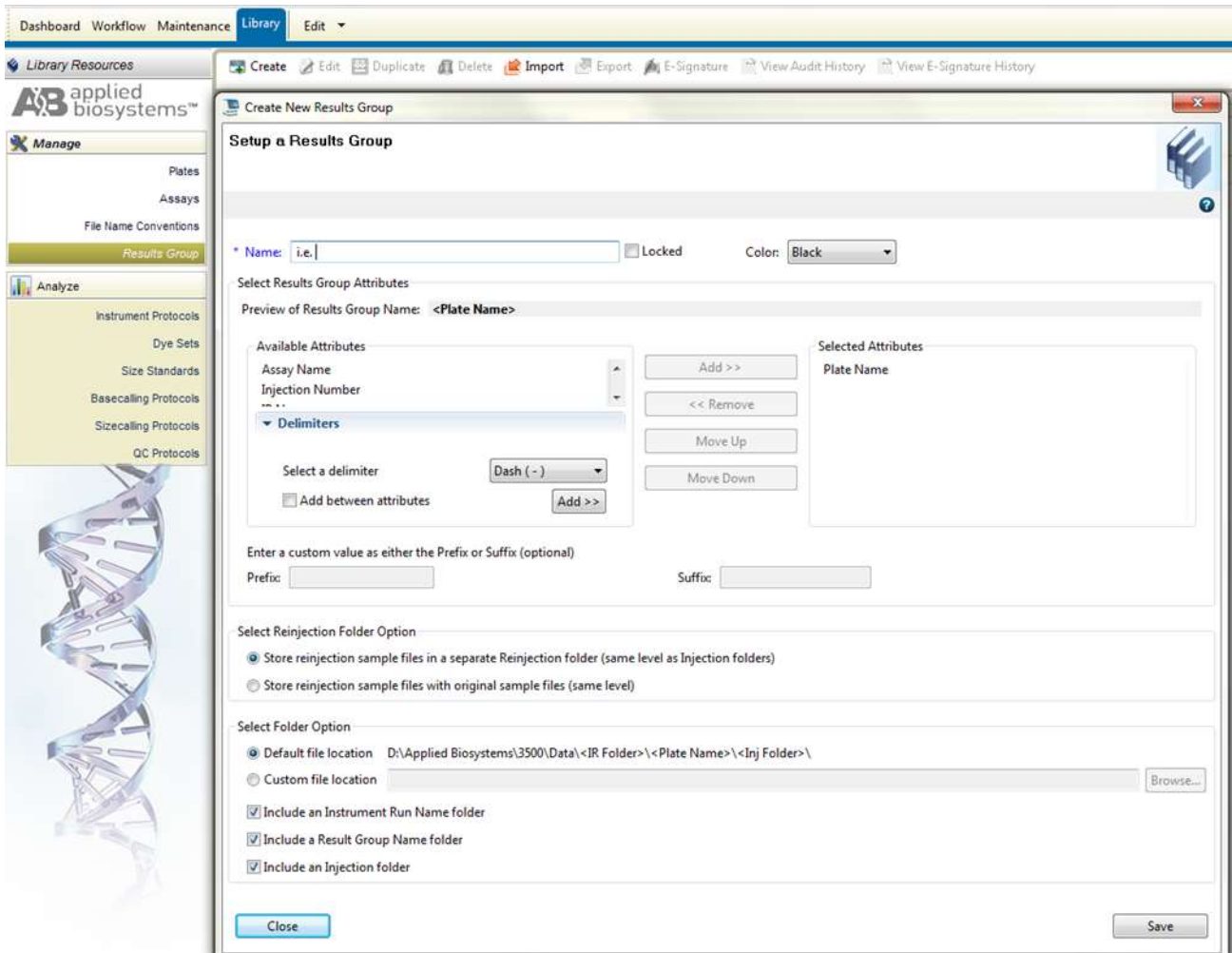


Figure 10. Screenshot for the “Create New Result Group” window on Applied Biosystems® 3500 Data Collection software

### 3.1.7. Create a New Plate

- a) navigate to the *Library*
- b) from the manage menu select “Plates”
- c) select “Create” (Figure 11)
- d) define a name for the plate
- f) choose plate type “Fragment Analysis” from the drop-down menu



Figure 11. Screenshot for the “Defining plate properties” window on Applied Biosystems® 3500 Data Collection software

### 3.1.8. Select “Assign Plate Contents”

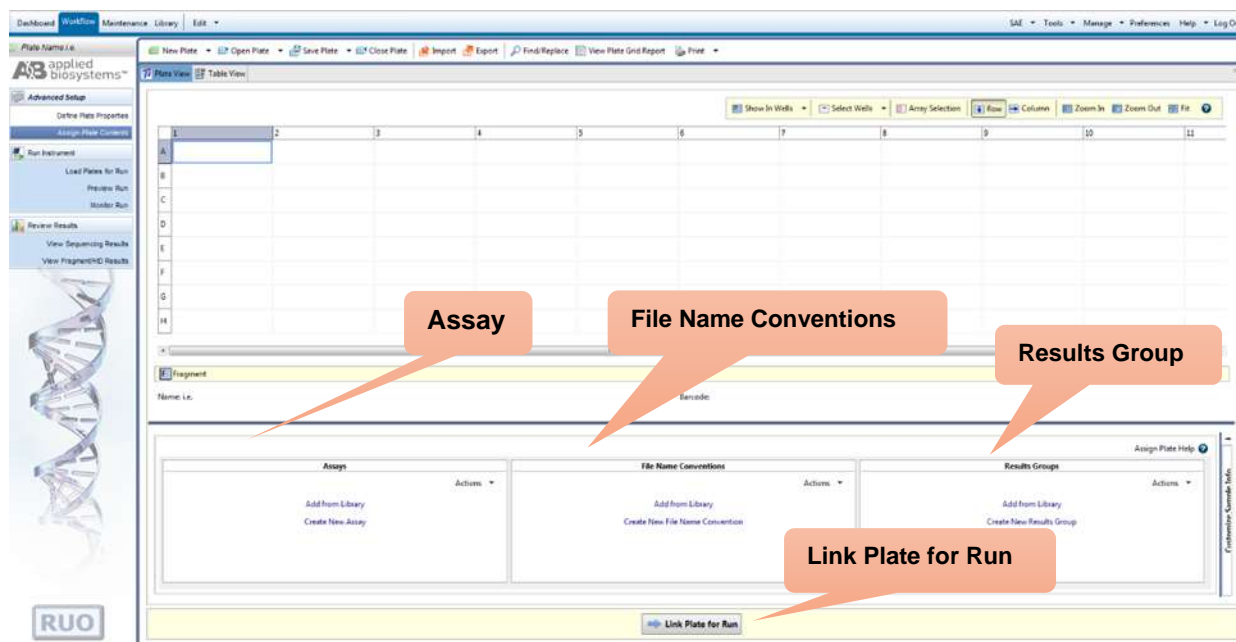


Figure 12. Screenshot for the “Assign Plate Contents” window on Applied Biosystems® 3500 Data Collection software

- Define sample names to wells.
- In the *Assign Plate Window* (Figure 12), in the bottom left corner, in a box “Assay”, click *Add from Library* option to select the Assay created in Step 3.1.4. Click on the Add to Plate button and close the window.
- In the *Assign Plate Window*, in the bottom middle, in the box “File Name Conventions”, click *Add from Library* option to select the File Name Convention created in Step 3.1.5. Click on the Add to Plate button and close the window.
- In the *Assign Plate Window*, in the bottom right, in the box “Results Groups”, click *Add from Library* option to select the Results Group created in Step 3.1.6. Click on the Add to Plate button and close the window.
- Select the sample wells, then select the boxes in the Assay, File Name Convention and Results Groups that relevant to those samples.
- Select “Link Plate for Run”. It will lead to open *Load Plate* window. Select “Yes”.
- In the Run Information window, give a Run name (Figure 13). Select “Start Run” after loading the plate.

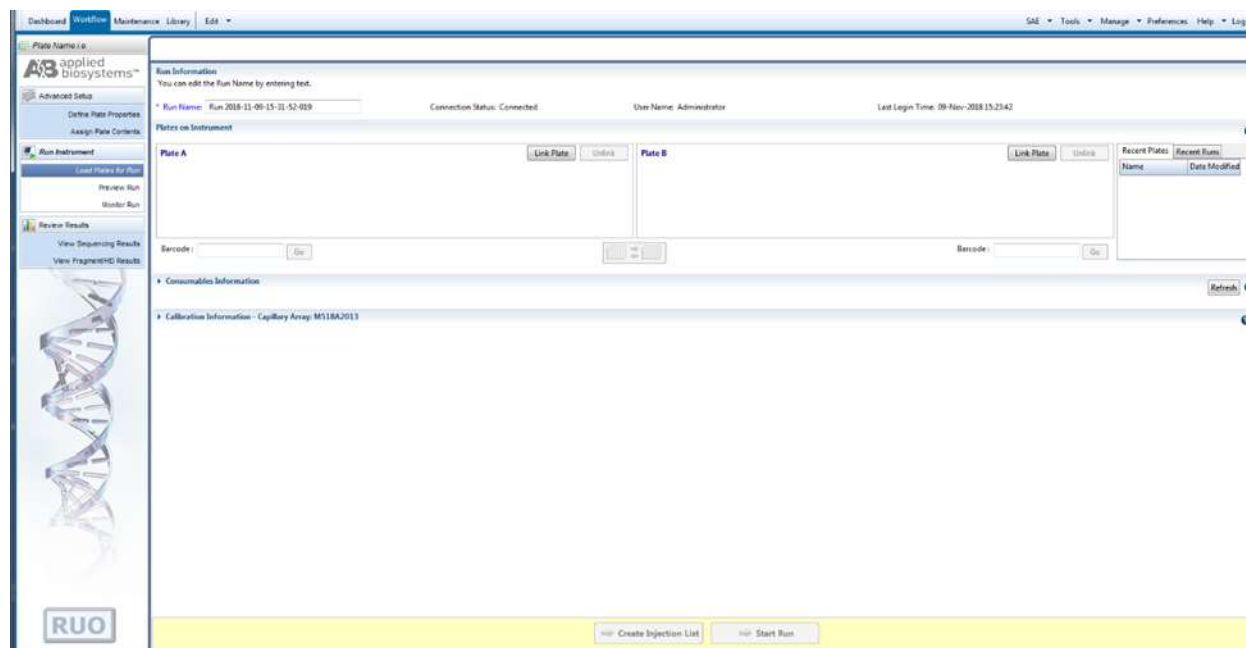


Figure 13. Screenshot for the “Run Information” window on Applied Biosystems® 3500 Data Collection software

### 3.2. Instrument Preparation Applied Biosystems® 3130/3130xl Genetic Analyzer (before the first use of GT HapScreen® PAH Kit)

Make sure that maintenance and installation of capillary array, buffers and polymer are done according to Applied Biosystems® 3130/3130xl Genetic Analyzer User Guide. Ensure that a spectral calibration is performed with GTM5 v2 Matrix Standard as mentioned above in this instruction in Capillary electrophoresis section. Before starting the electrophoresis for fragment analysis on the ABI Genetic Analyzer the following settings need to be set up in the instrument's Data Collection Software; **Run Module, Instrument Protocol** and **Plate**. The instructions below are from an ABI 3130xl Genetic Analyzer with GT HapScreen® PAH as an example (Dye set: Any5Dye, GTM5 v2). The procedure is however similar to the other instruments. For further details, refer to the User Guide for the instrument used.

**Attention:**

Spectral Calibration must be made using GTM5 v2 Matrix Standard, the machine must be calibrated with GTM5 v2 Matrix Standard. Please find detailed protocol for spectral calibration with GTM5 v2 Matrix Standard here - CAT# 41103 or contact us at [support@genetek.de](mailto:support@genetek.de).

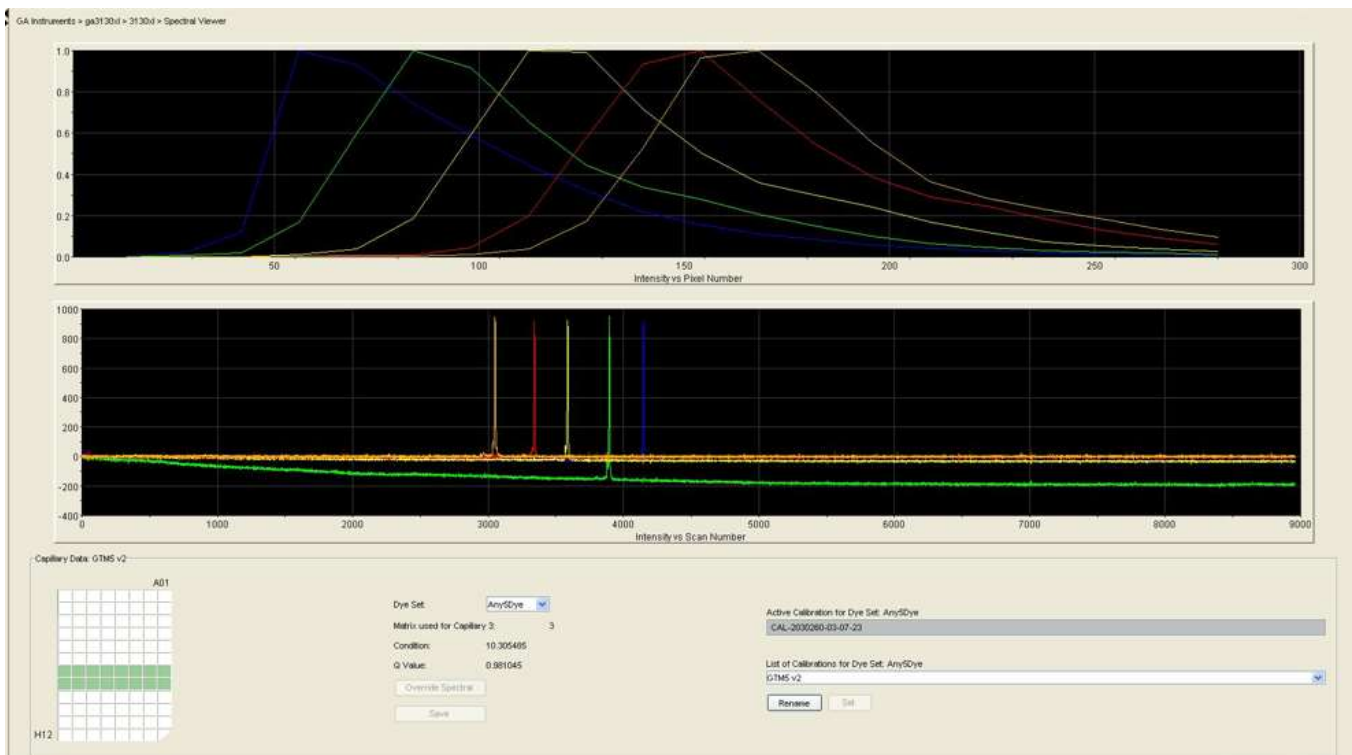


Figure 14. An example of a successful spectral calibration with GT 5 Dye system on Applied Biosystems® Genetic Analyzer 3130xl

### 3.2.1. Create a Run Module

In the left navigation window select Module Manager and New. Fill out the Run Module Editor according to the kit instructions for use (IFU).

- a) Name: Enter a name of the Run Module (GT HapScreen® PAH)
- b) Type: Regular
- c) Template: FragmentAnalysis50\_POP7 (default template for the capillary array and polymer used)
- d) Click OK

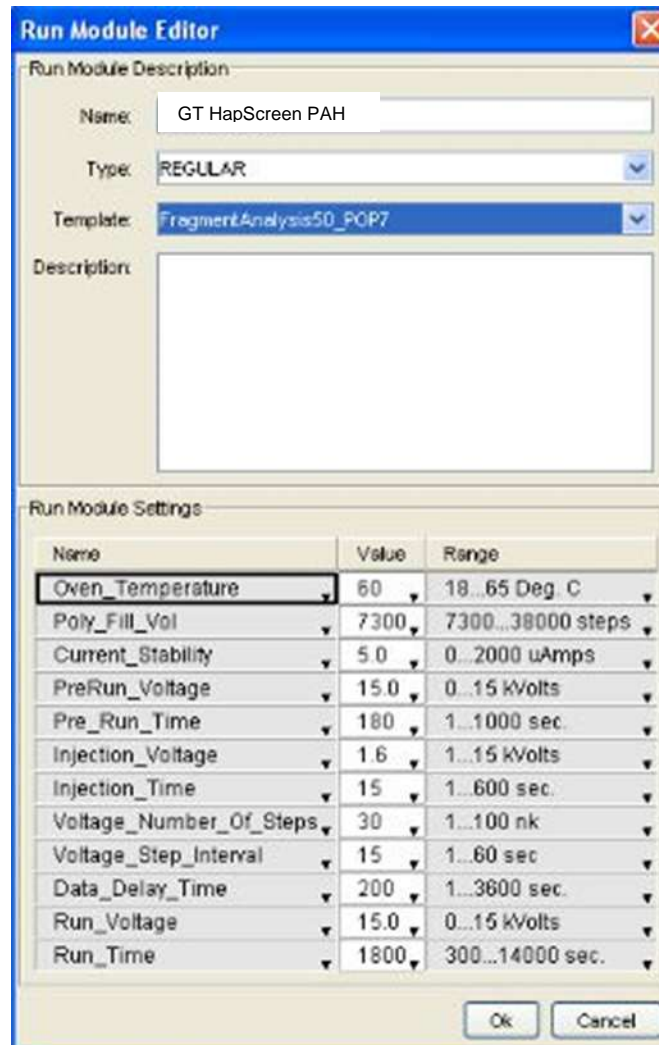
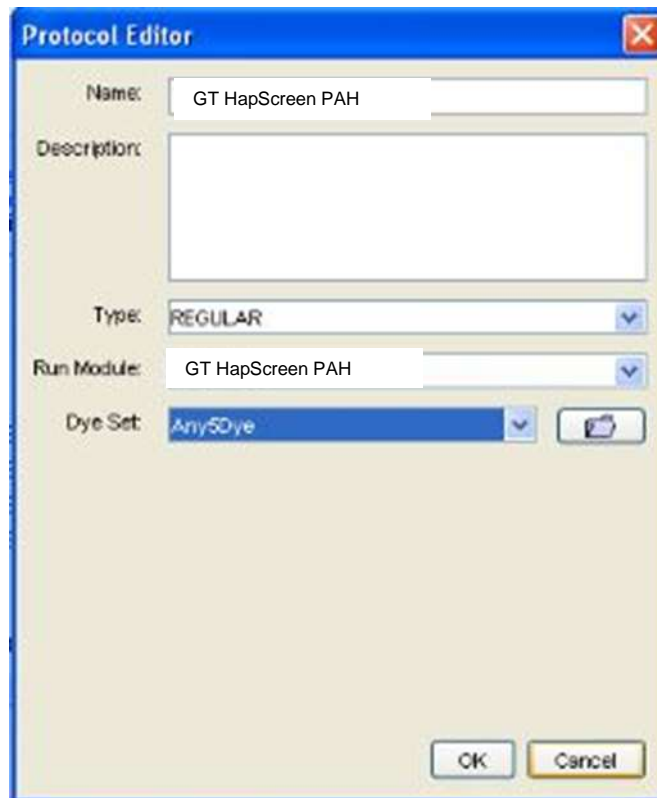


Figure 15. Screenshot for the “Module Manager” window on Applied Biosystems® 3130 Data Collection software

### 3.2.2. Create an Instrument Protocol

From the left navigation window select Protocol Manager and New.

- a) Fill out the Protocol Editor
- b) Name: Enter a name of the Run Module (GT HapScreen® PAH)
- c) Type: Regular
- d) Run Module: Select the Run Module created (GT HapScreen® PAH)
- e) Dye Set: Any5Dye
- f) Click OK



**Figure 16.** Screenshot for the “Create New Instrument Protocol” window on Applied Biosystems® 3130 Data Collection software

### 3.2.3. Set up a Plate for run

- From the left navigation window select Plate Manager and New.

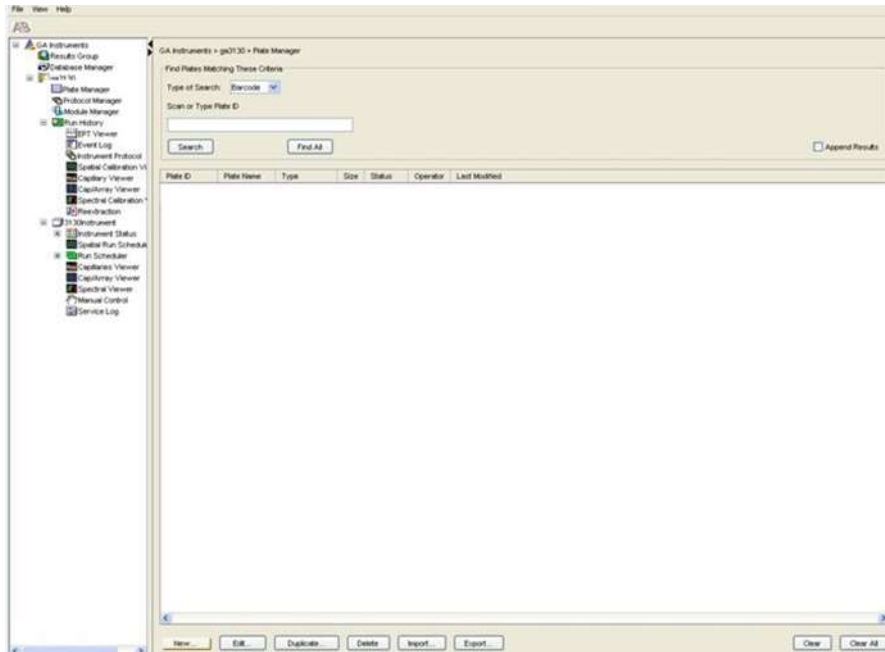
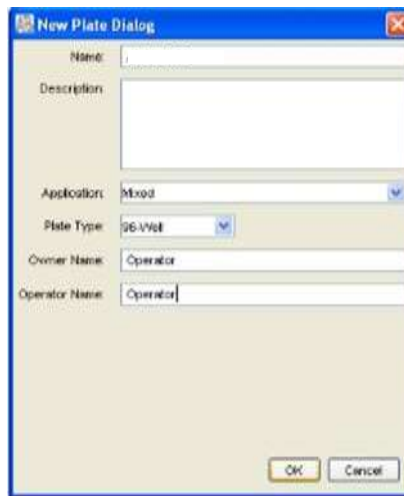


Figure 17. Screenshot for the “Plate Manager” window on Applied Biosystems® 3130 Data Collection software

### 3.2.4. Fill out the New Plate Dialog

- a) Name: Enter a name of the plate
- b) Application: GeneMapper-Generic (used if data is analysed on a separate computer)
- c) Plate type: 96-Well
- d) Owner Name: enter the name of the owner
- e) Operator Name: enter the name of the operator
- f) Click OK



**Figure 18.** Screenshot for the “*New Plate Dialog*” window on Applied Biosystems® 3130 Data Collection software

### 3.2.5. Fill out the GeneMapper Plate Editor

- a) Sample name: Enter the sample names
- b) Comment: optional
- c) Instrument Protocol 1: Select the instrument protocol that you created before
- d) Click OK

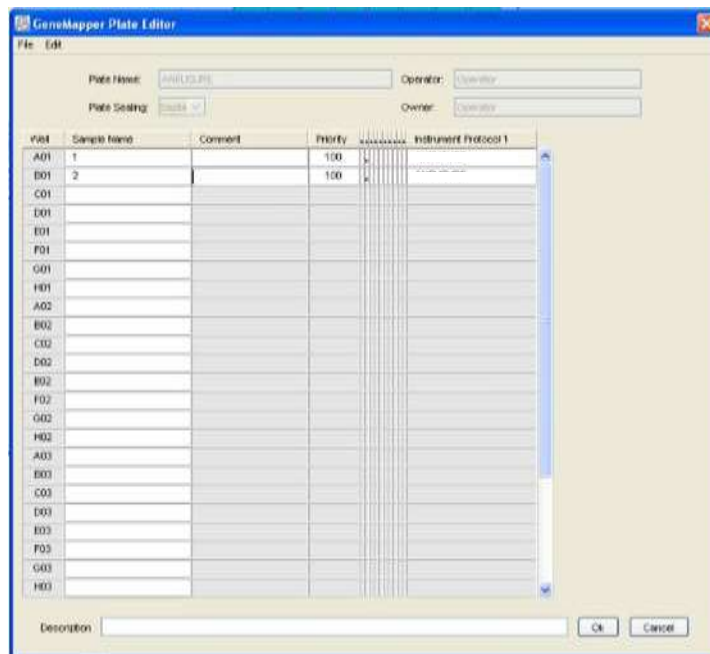
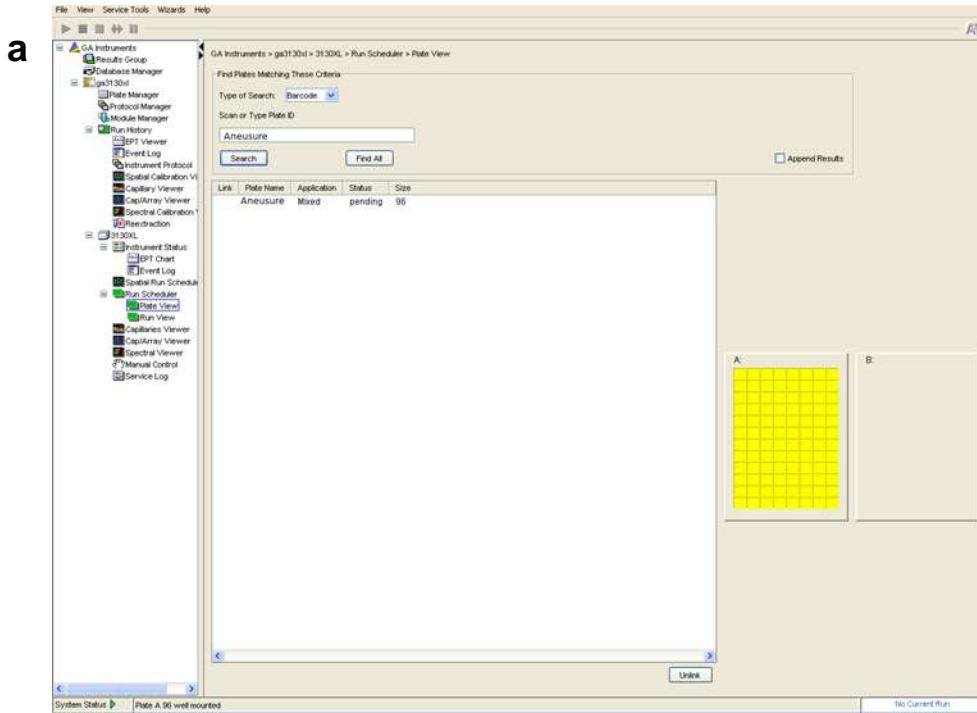


Figure 19. Screenshot for the “GeneMapper Plate Editor” window on Applied Biosystems® 3130 Data Collection software

- From the left navigation window, select Run Scheduler, search for GT HapScreen® PAH (plate name).



- Select the plate created in Step 3 (status pending). Link the plate by clicking on the yellow plate position indicator, which will turn green when linked. Start the run on the green arrow.

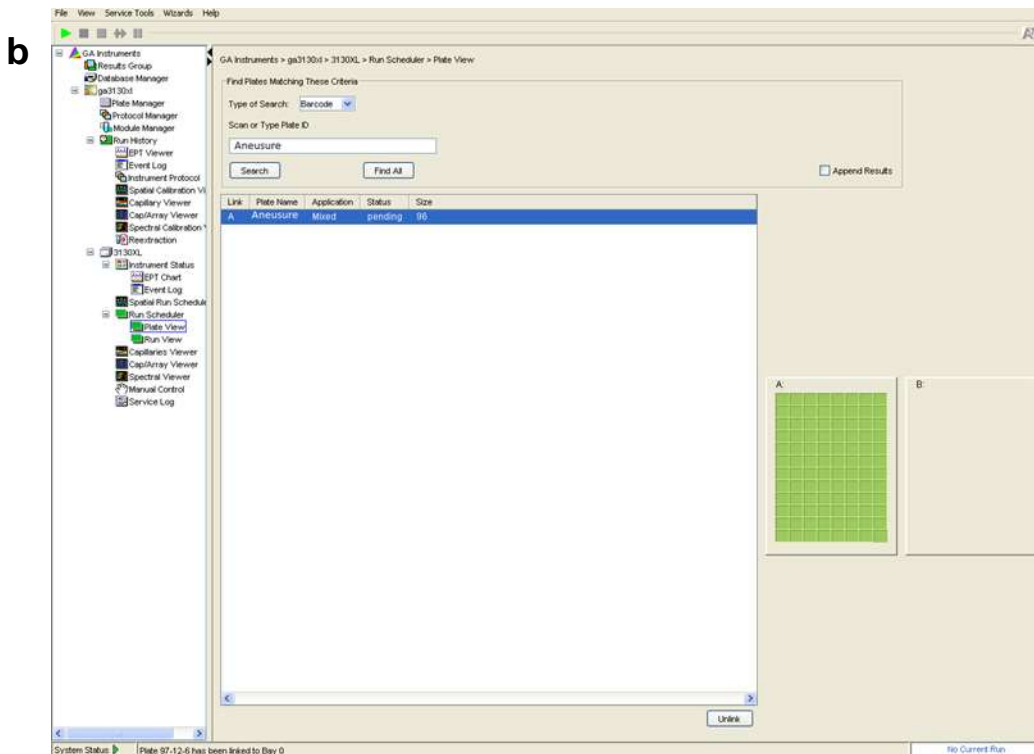


Figure 20 a & b. “Plate view” window on Applied Biosystems® 3130 Data Collection software.

- The Process Plates dialog box appears. Click OK to start processing the plate.



**Figure 21.** "Process Plates dialog" window on Applied Biosystems® 3130 Data Collection software.

### 3.3. Sample preparation for capillary electrophoresis (3500 Series and 3130 Series instruments)

**Please note:** The Size Standard used in the GT HapScreen® PAH kit is GT500.

- Vortex and spin 9.5 µL (x number of samples) Hi-Di™ Formamide and 0.5 µL GT500 (x number of samples) in a 1.5 mL tube. For every 8 samples prepare 10 since there may be pipetting error. The amounts below are for 10 injections.
- Pipette 10 µL of the prepared size standard mix to required number of well and add 1 µL PCR product to it and use pipet to mix. Cover the wells with appropriate septa.
- Denature the PCR product by heating the plate in a thermal cycler. Set the cycler as:
  - 95°C for 5 minutes
  - 4°C for 30 seconds
- Place the PCR products on ice (or cool box at -20) for 3 minutes
- Centrifuge the plate at 1,000xg for 10 seconds to remove any bubbles in the wells.
- Place the plate in the Genetic Analyzer and start run.

**Please note:** Detection limits for each instrument is different; hence, injection time, injection voltage or the amount of sample mixed with loading mix (Hi-Di™ Formamide and GT500 internal size standard) may need to be adjusted. Use the Module Manager in the data collection software to modify the injection time or voltage in the run module according to your lab validation (as mentioned in the instrument preparation above).

## 4. Result analysis and Interpretation

### 4.1. Software for sample analysis

- For GT HapScreen® PAH, the Applied Biosystems® fragment analysis software compatible with your genetic Analyzer is recommended. This kit is compatible with GeneMapper software. Analysis method depends on the software.

Each diagnostic lab should have individual interpretation and reporting procedure and criteria. To develop such procedure, use of “**Best Practice Guidelines for internal Quality Control in Genetic Laboratories**”, “**Practice guidelines for the Testing for maternal cell contamination in prenatal samples for molecular studies**” and “**QF-PCR for the diagnosis of aneuploidy best practice guideline**” is recommended. You can download it from:

[https://www.cytogenetics.org.uk/prof\\_standards/professional\\_standards.html](https://www.cytogenetics.org.uk/prof_standards/professional_standards.html).

### 4.2. General guideline for the analysis of GT HapScreen® PAH results

PCR products are observed with 5-dye system on an electropherograms in the GeneMapper® software. For the analysis, import GT HapScreen® PAH panels. It can be downloaded from our website: [www.genetek-biopharma.com](http://www.genetek-biopharma.com).

For detailed procedure on fragment analysis on GeneMapper® software please refer to the GeneMapper® *user guide*.

#### 4.2.1. Criteria for Interpretations

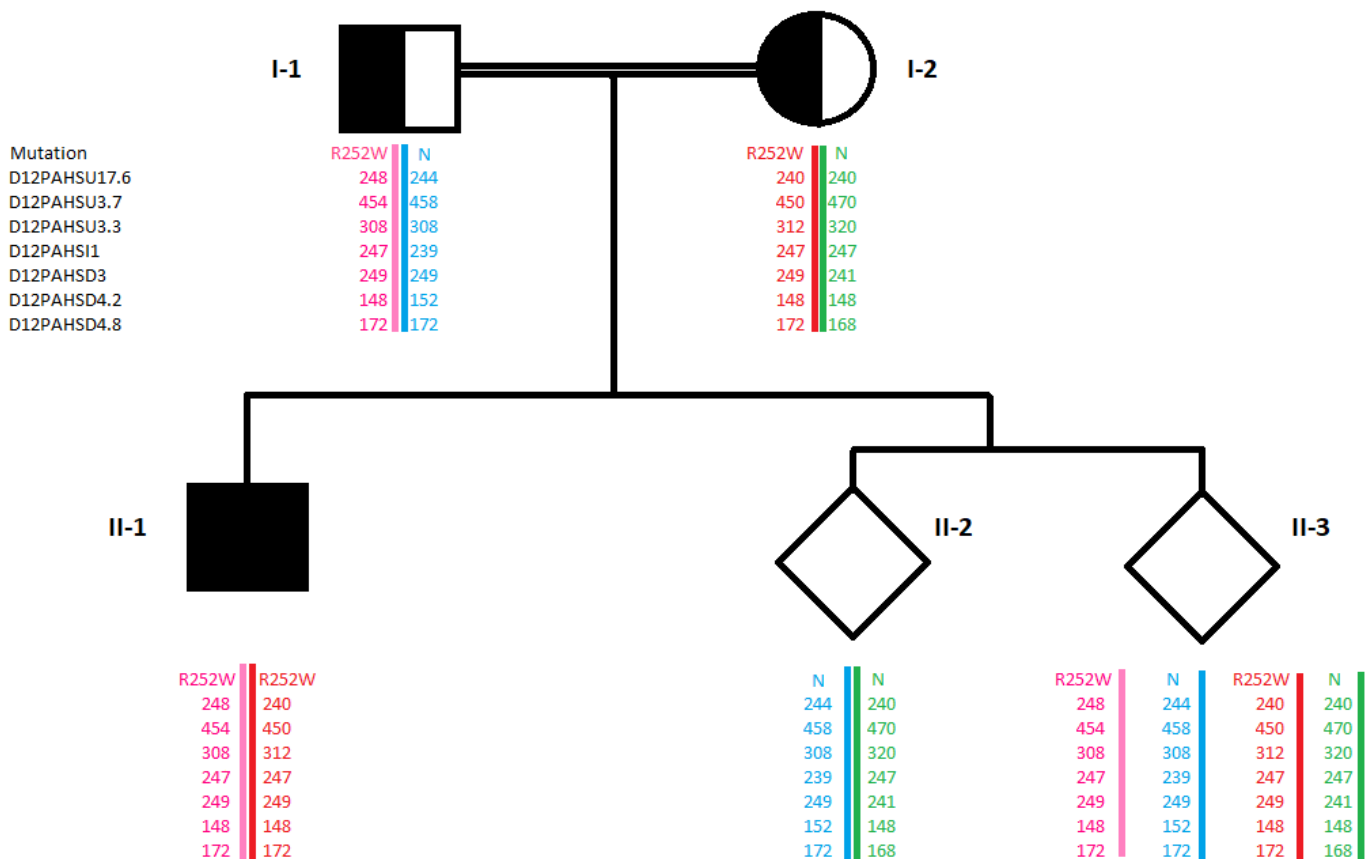
- “Size” shows the fragment size. The size may differ between individuals but are usually constant within a person and his/her parents if all samples are analysed in one run. With QF-PCR one is not concerned with size unless maternal cell contamination or samples mix-up or similar issues are involved.
- The area under each peak in electropherogram represents the amount of amplified PCR product.
- The height of each peak represents the activity of each fluorescent component which shows the quantity of the fluorescent compartment of each marker.
- These results are shown as electropherograms in the analysis software. Height and the area related to each peak are observable in this software.
- Negative control should not show any peculiar fragment of length between 100 to 500 bp.
- Quality control DNA (if used) should show expected results as shown here – see Example profile GT QC DM102.
- There should not be excessive bleed-through between dye colours or “Pull-up” effect in the electropherograms.
- Successful amplification must result into at least one peak for each marker (except for Y chromosome markers which would be absent in normal female sample)

## 5. Example case study

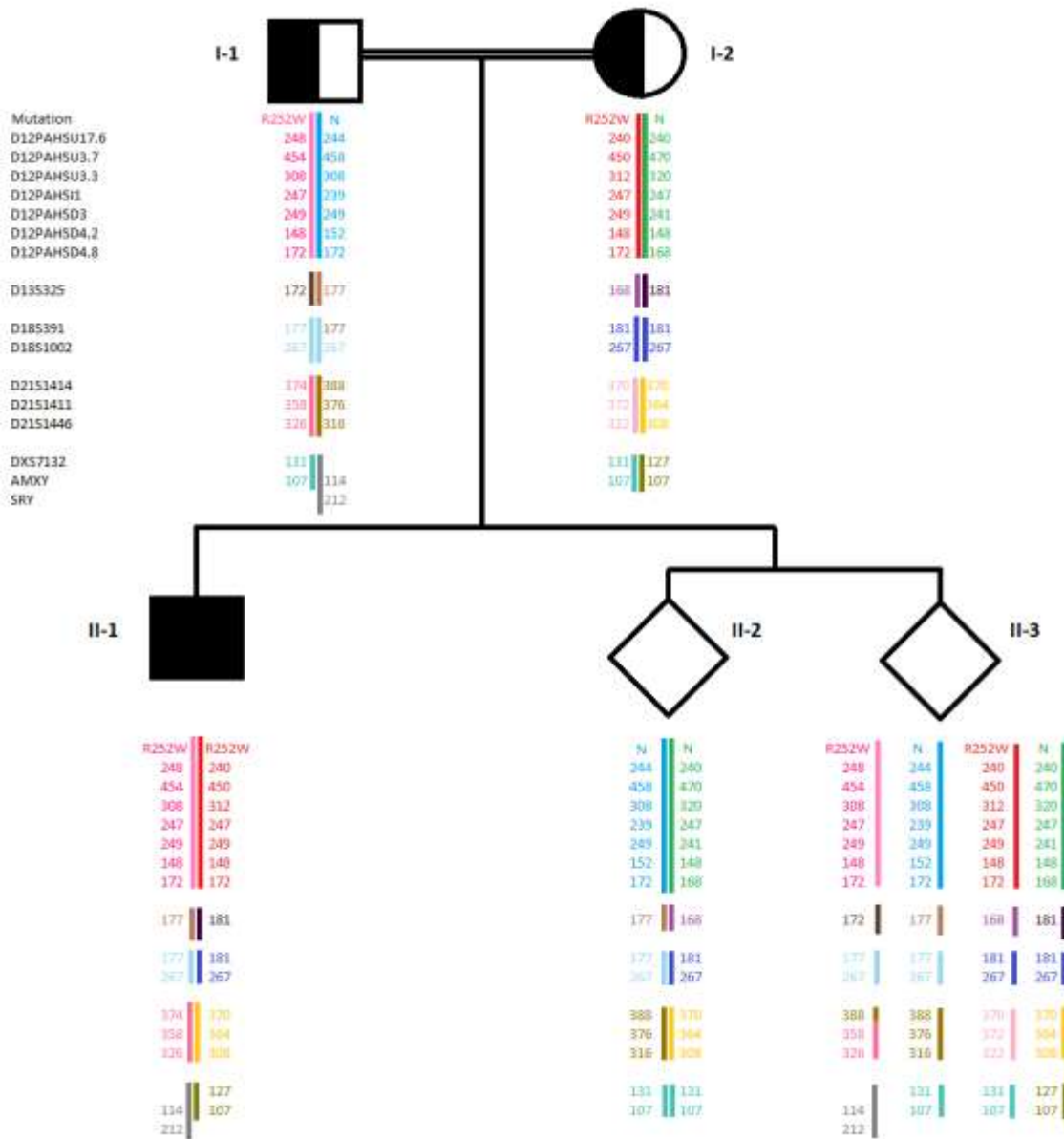
Genetic diagnostic centres and genetic labs must handle sample analysis for genetic diseases very carefully since the fate of the fetus relies solely in their hands. Sometimes due to heavy influx of samples, human errors such as sample mix-up or cross-contamination may result into false negative or false positive. In the following different examples, various advantages user can achieve when GT HapScreen® PAH kit is used to screen samples for Phenylketonuria. GT HapScreen® PAH is developed to make sample analysis accurate and safe.

### 5.1. Case study 1 – sample mix-up

In the following example, the fetus II-2 was normal based on haplotype and mutation. However, fetus II-3 had several peaks for each locus indicating contamination. It can be seen in the **figure 22** that II-3 has both paternal and maternal alleles. Hence, we would rule out maternal cell contamination since the fetus has both maternal & paternal haplotypes. Another suggestion is that this fetus is a mixture of another fetus since he can't have both of maternal and paternal alleles. Uniparental heterodisomy can be ruled out since there should be double uniparental inheritance. But markers for other chromosomes presents in the GT HapScreen® PAH kit would reveal sample mix-up for this case and advocate resampling.



**Figure 22.** Linkage analysis showing fetus III-3 with alleles from the mother and also from father



**Figure 23.** Linkage analysis showing fetus II-3 with alleles from other fetus II-2 Alleles used in the linkage analysis depicted in figure 22 are obtained using GT HapScreen® PAH Kit. Fragment analysis was done using GeneMapper® and Genetic Analyzer 3500xL. See below for the data from I-1, I-2, II-1, II-2 and contaminated II-3 samples.

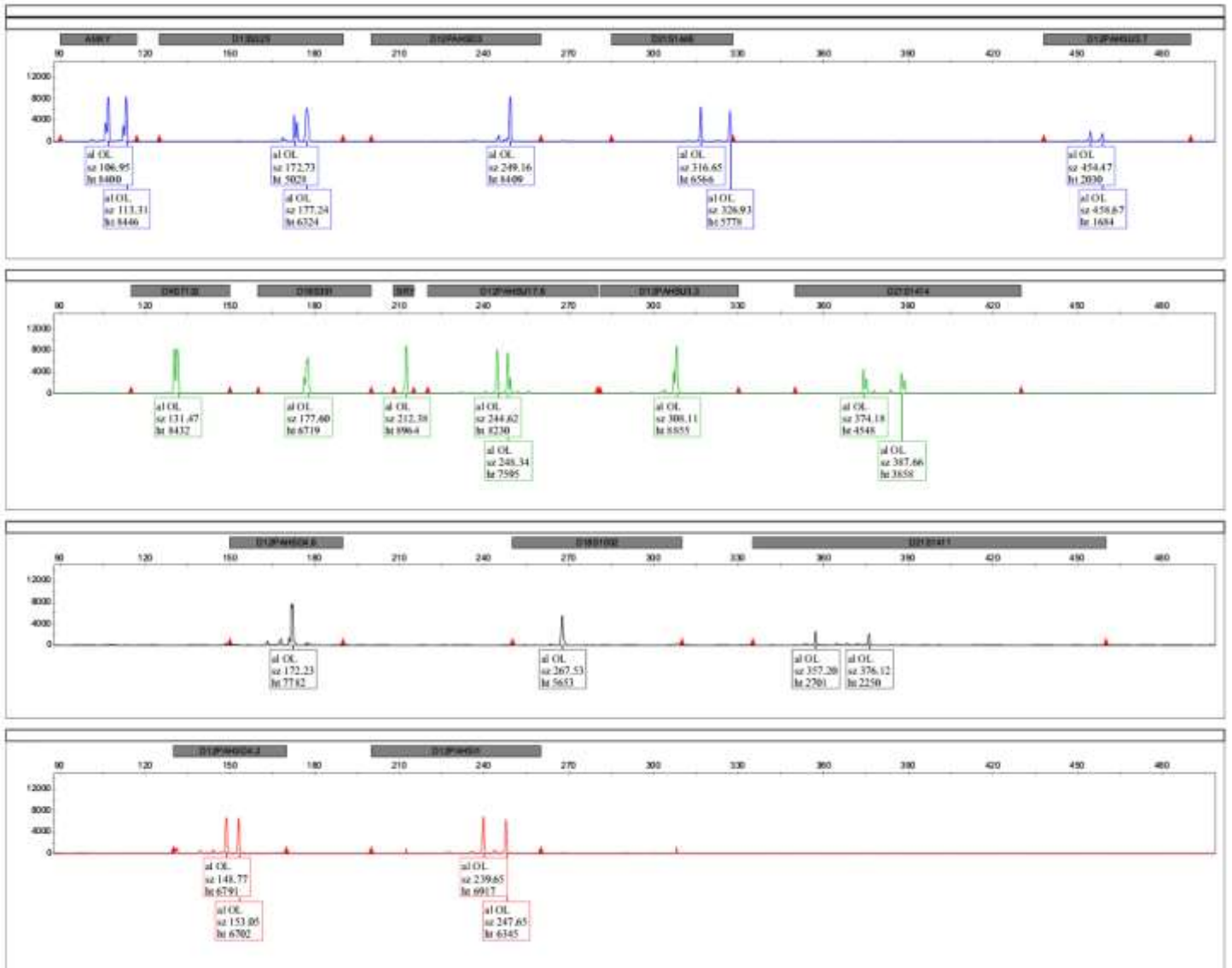


Figure 24. GT HapScreen® PAH profile for I-1 sample with the mutant alleles for PAH gene.



Figure 25. GT HapScreen® PAH profile for I-2 sample with the mutant alleles for PAH gene.

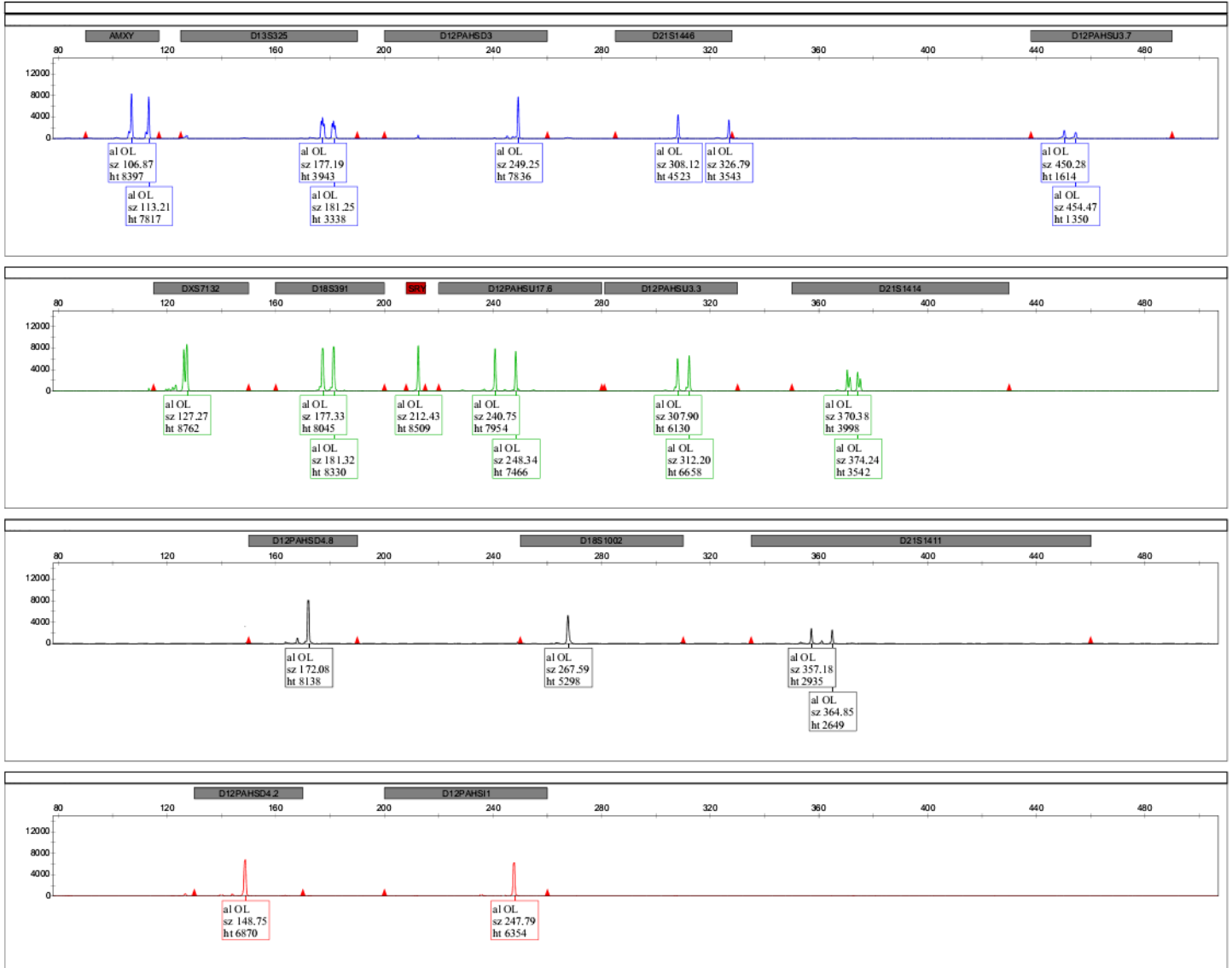


Figure 26. GT HapScreen® PAH profile for II-1 sample with the mutant alleles for PAH gene.

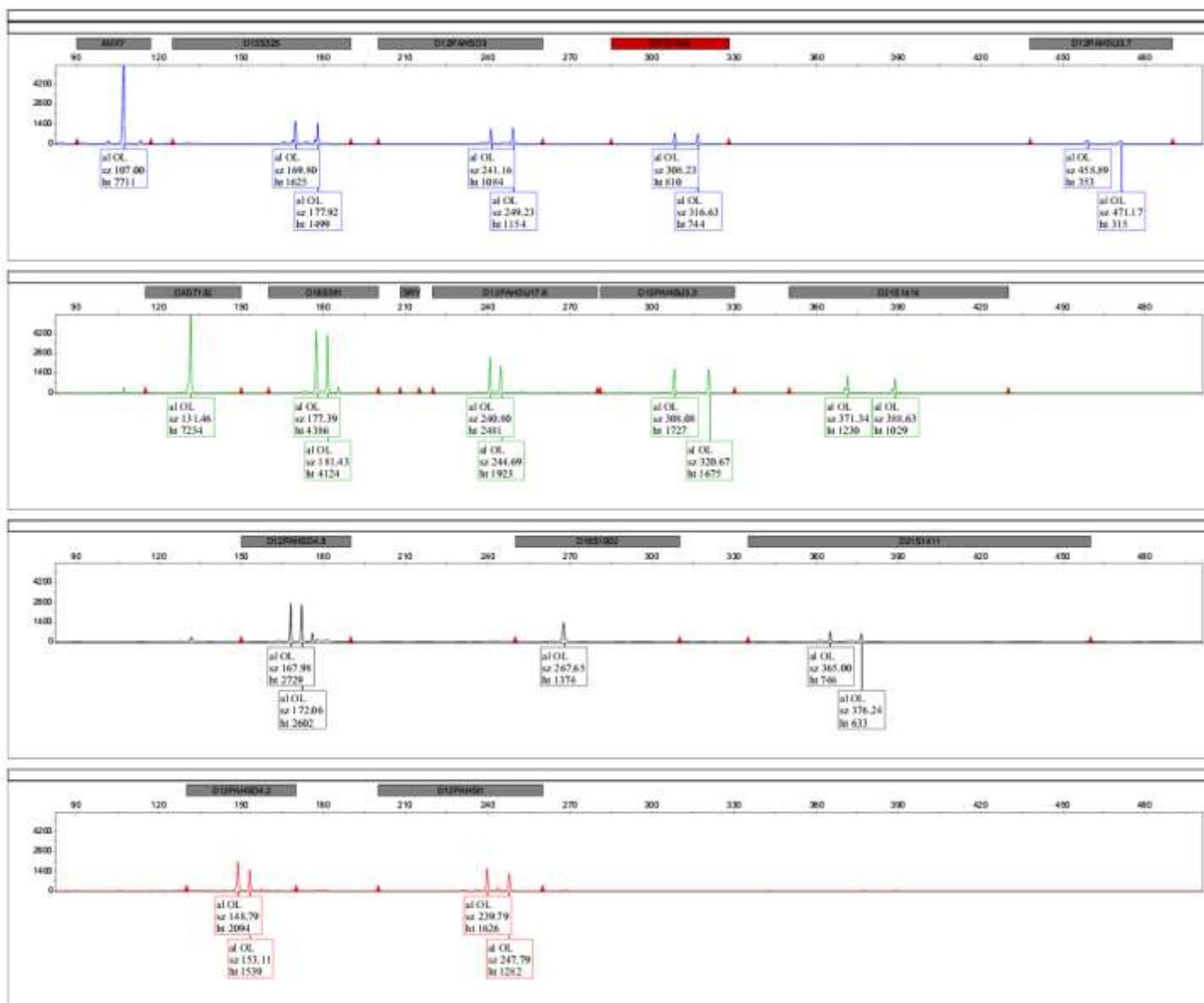


Figure 27. GT HapScreen® PAH profile for II-2 with the non-mutant alleles.



Figure 28. GT HapScreen® PAH profile for fetus sample II-3 (contaminated) with alleles from sample II-2.

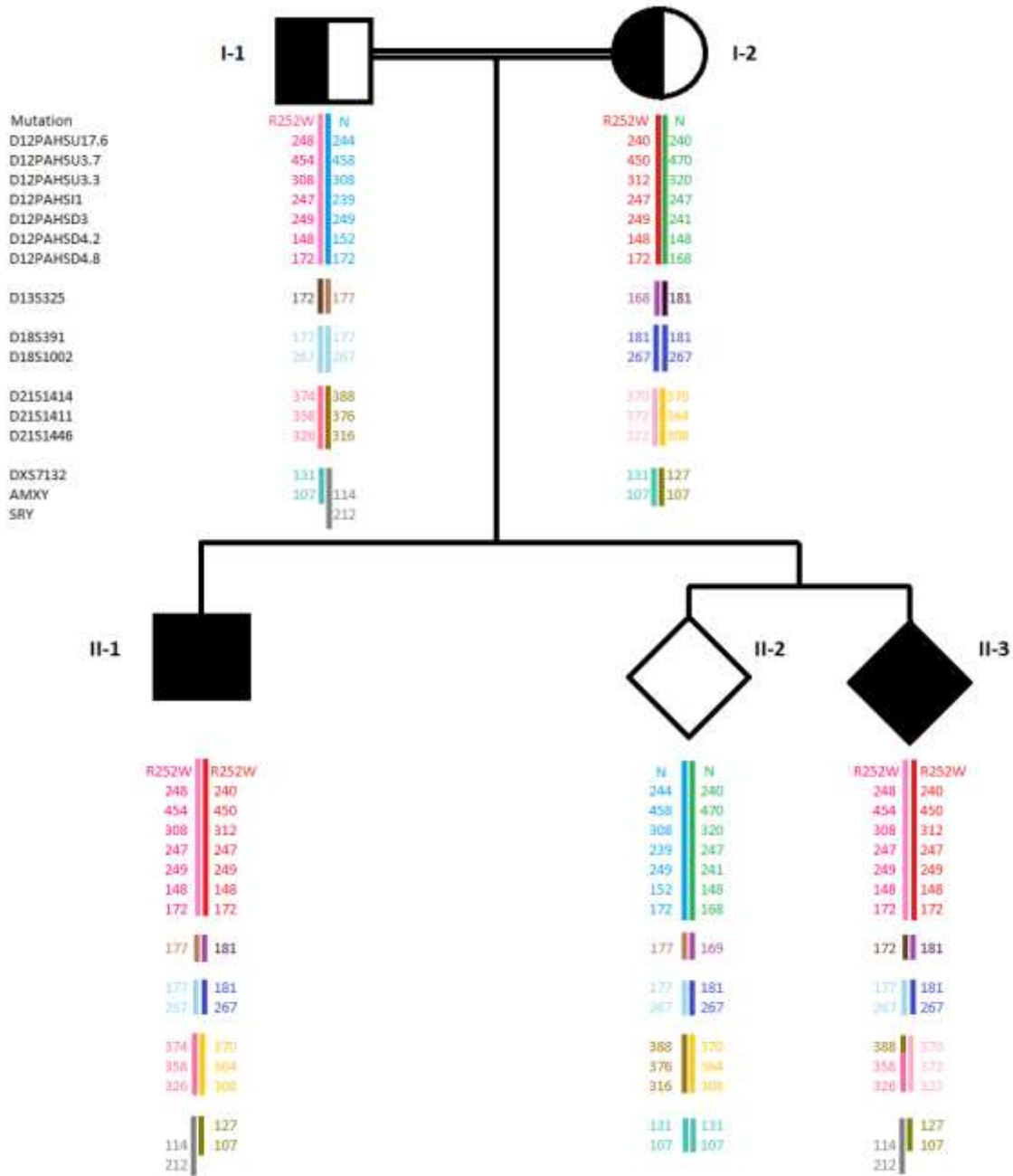


Figure 29. Linkage analysis showing resampled fetus II-3 (re-sampled) with mutant alleles from both parents and its affected with Phenylketonuria disease.

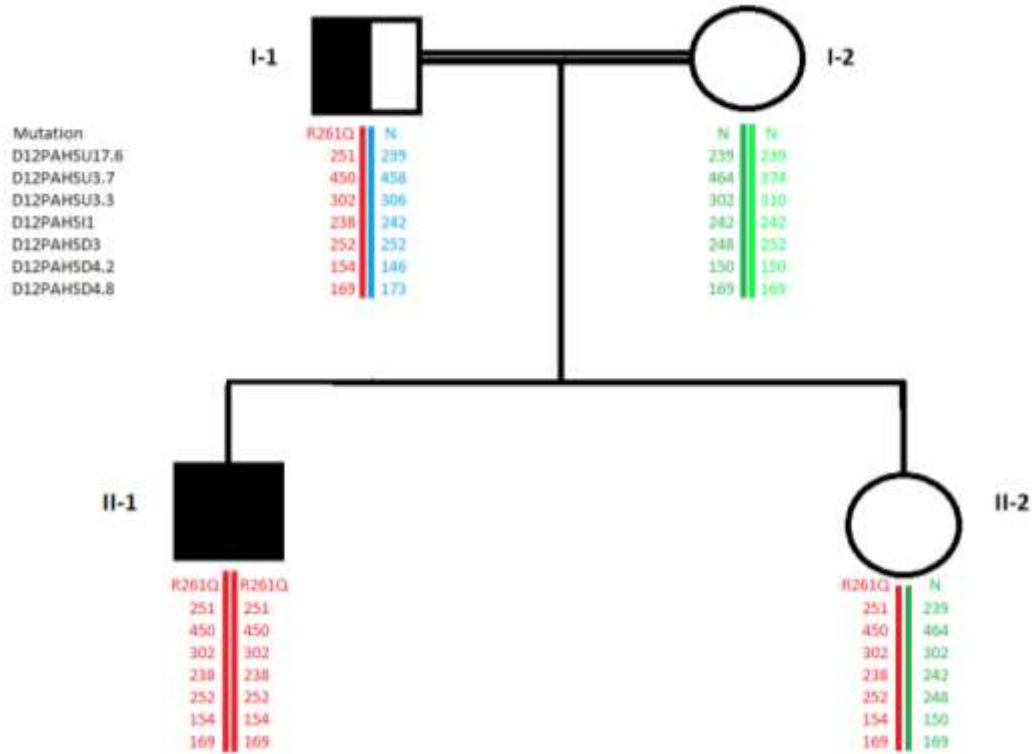


Figure 30. GT HapScreen® PAH profile for fetus sample II-3 (re-sampled) with mutant alleles from both parents and its affected with Phenylketonuria disease.

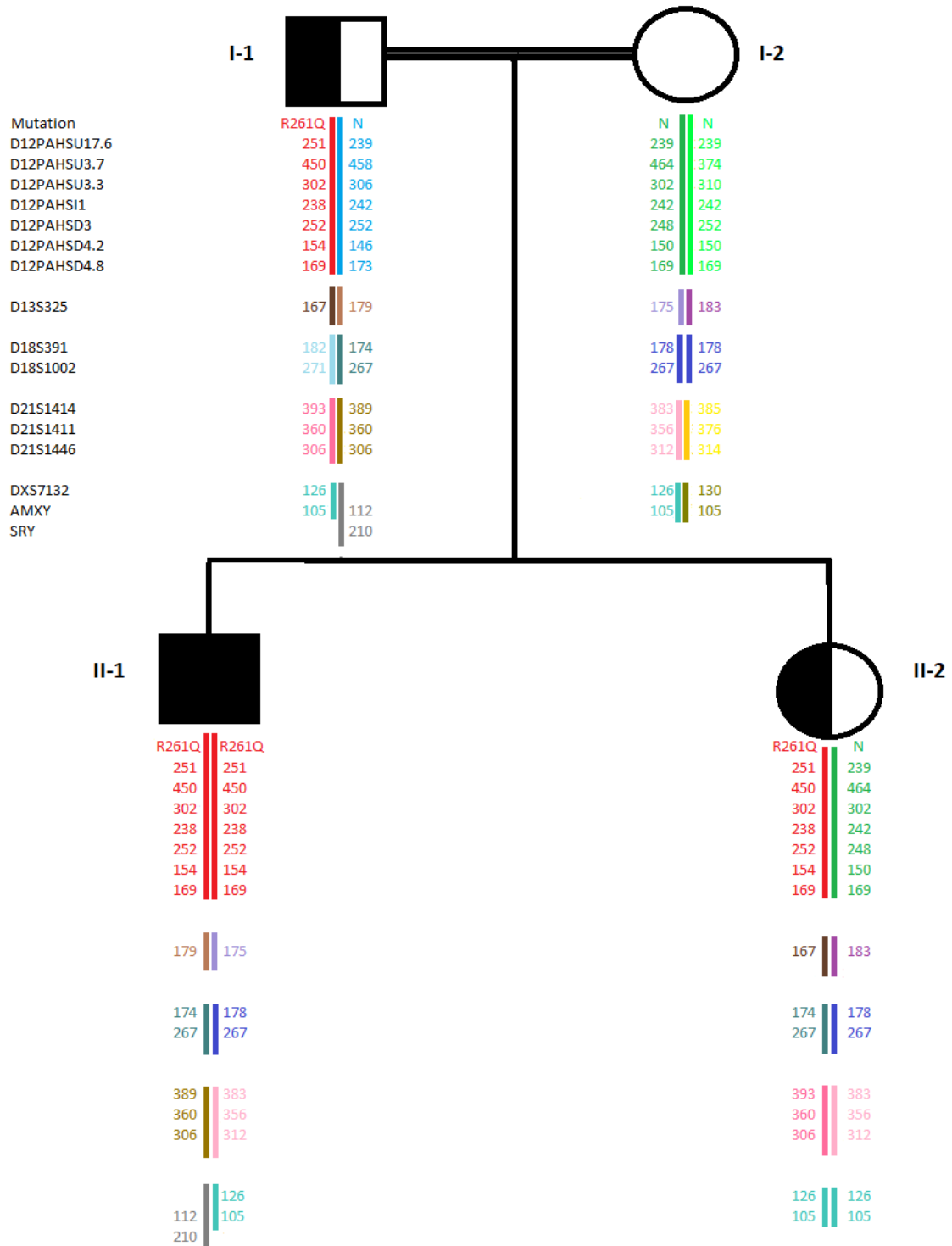
### 5.2. Case study 2 – uniparental isodisomy

In the following example case, the affected child is homozygote for R261Q mutation but only the father has the mutation. One of the possible reasons for such haplotype pattern might be that the mother does not show the mutation due to allele drop out usually seen during mutation screening mostly due to presence of SNP at the primer annealing site.

When GT HapScreen® PAH kit is applied, results showed that the affected child has inherited both of his chromosomes from the father and mother does not share the PAH haplotype with either her husband or the affected child.



**Figure 31.** Analysis with only PAH gene linked markers, shows that sample II-1 is affected with Phenylketonuria and homozygote for R261Q mutation.



**Figure 32.** Analysis with GT HapScreen® PAH shows that sample I-2 does not share same mutant haplotype as I-1 and II-1, sample mix-up can also be ruled out and it is II-1 is case of uniparental isodisomy.

Alleles used in the linkage analysis depicted in figure 31 are obtained using GT HapScreen® PAH Kit. Fragment analysis was done using GeneMapper® and Genetic Analyzer 3500xL. See below for the data from I-1 (carrier), I-2 (normal), II-1 (affected), II-2 (normal).

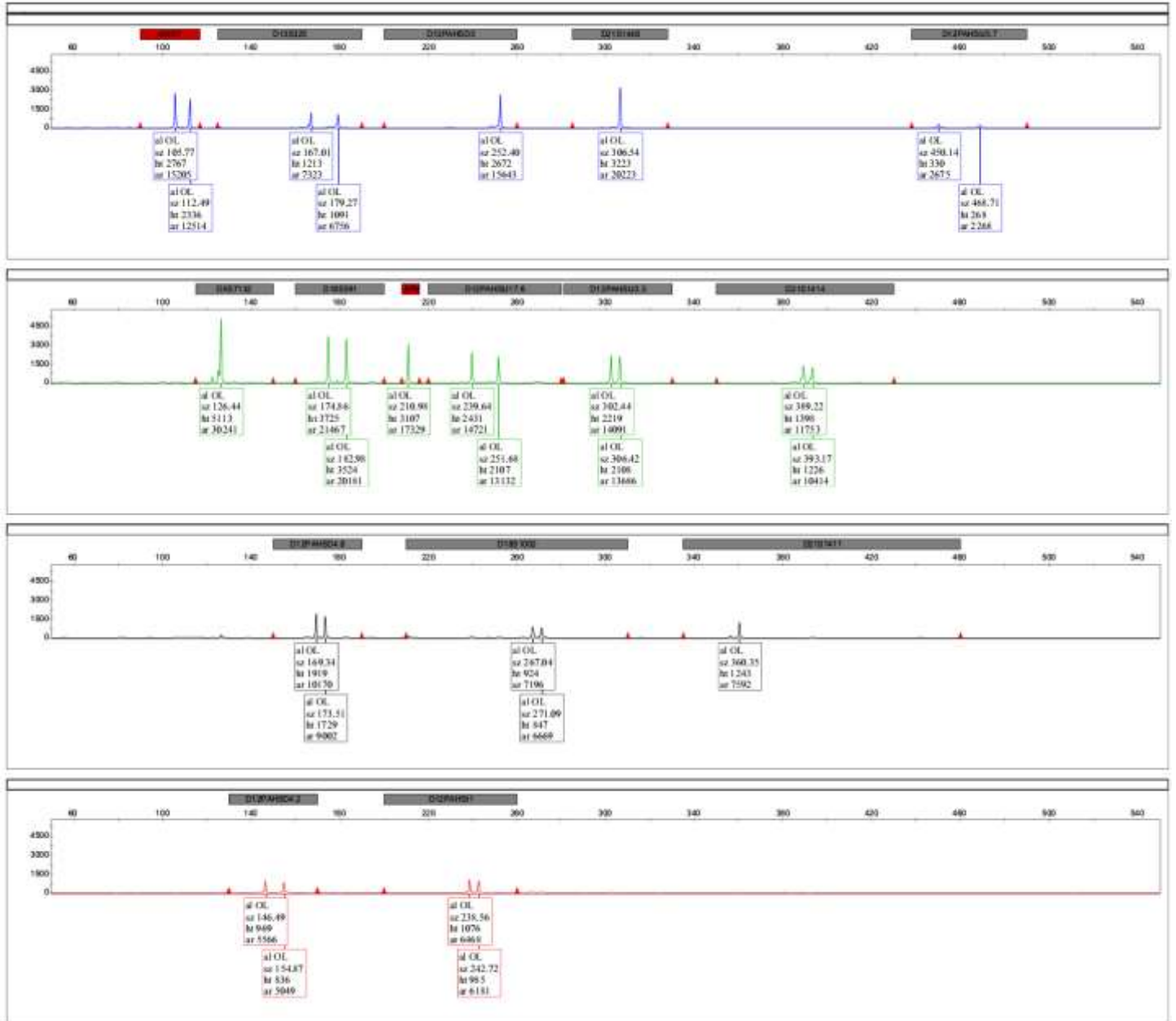


Figure 33. GT HapScreen® PAH profile for I-1 sample with the mutant alleles for PAH gene.

Figure 34. GT HapScreen® PAH profile for I-2 sample with no mutant alleles for PAH gene.



Figure 35. GT HapScreen® PAH profile for II-1 sample with homozygote mutant alleles for PAH gene.

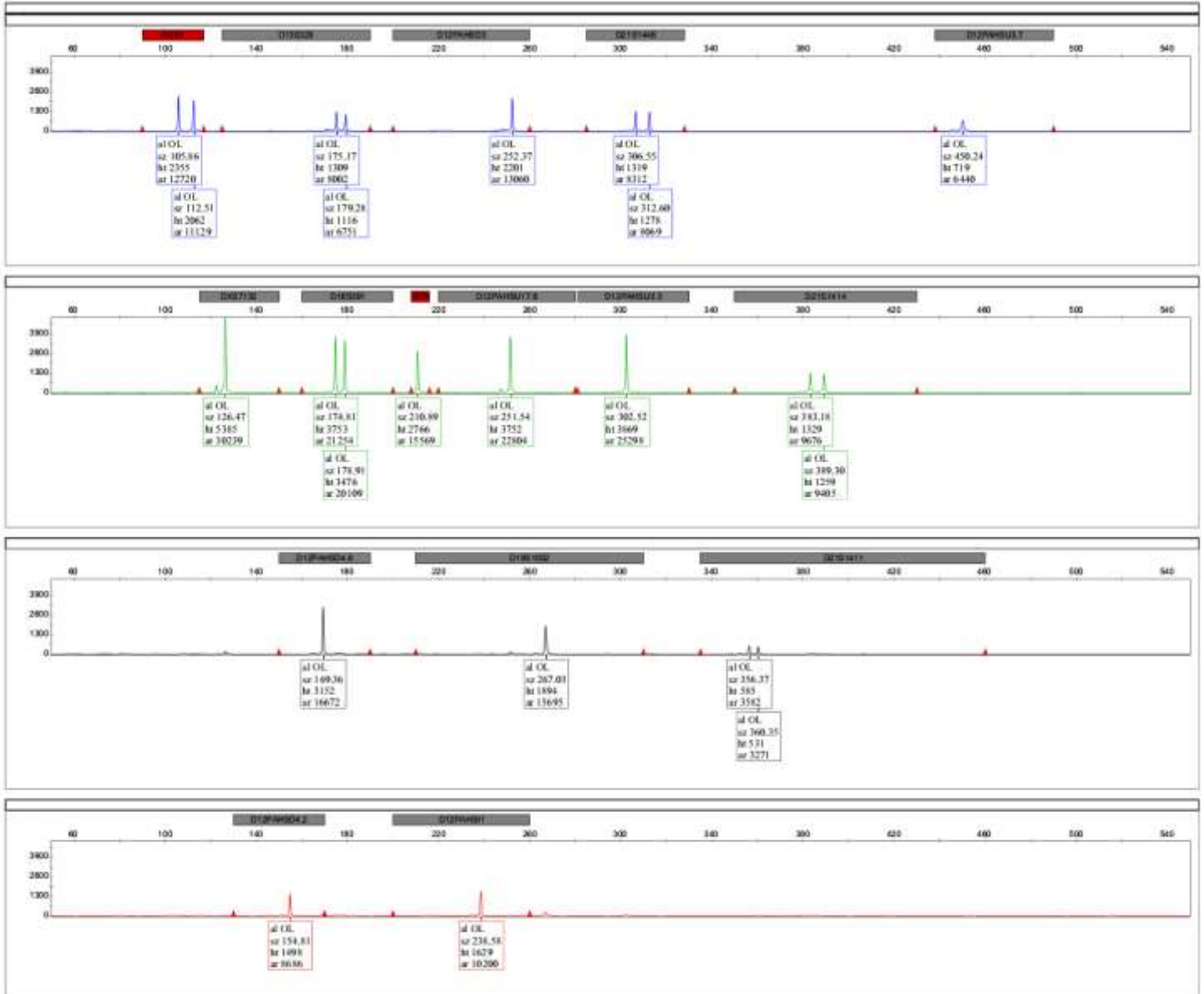
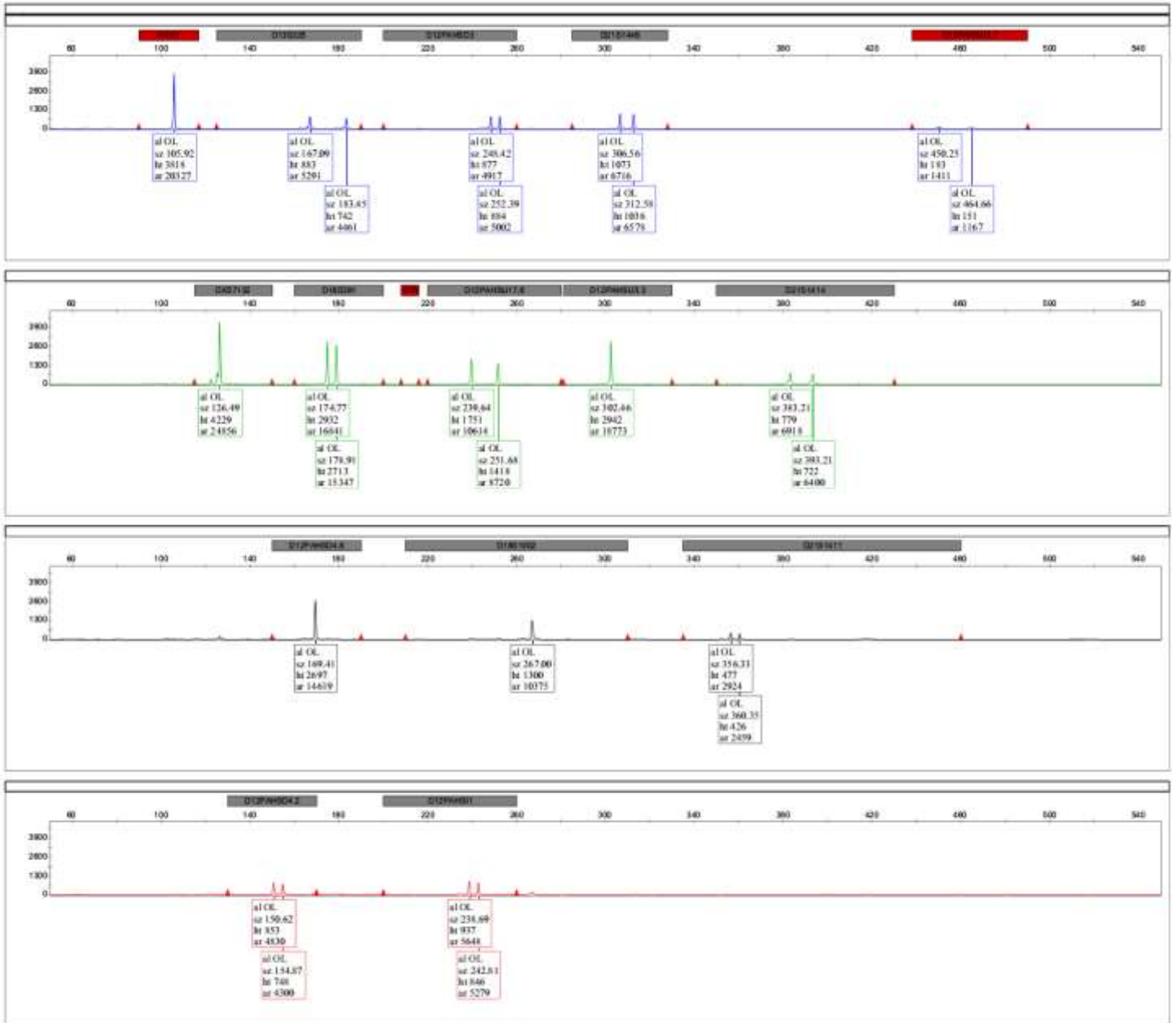


Figure 36. GT HapScreen® PAH profile for II-2 sample with no mutant alleles for PAH gene.



## 6. Troubleshooting

For any technical question or issue (not mentioned here) please contact our customer support here – [support@genetek.de](mailto:support@genetek.de).

Issue Observed	Possible cause and Solution
No peak detection or faint peaks	<p>PCR reaction mix is not well mixed with enzyme and DNA. Vortex or use pipette to mix the PCR reaction mixture after adding DNA.</p>
	<p>An air bubble formation in the reaction tube can cause poor mixing of reaction mixture. Use a pipette to remove the air bubble or centrifuge the reaction mixture before thermal cycling.</p>
	<p>Poor amplification due to improper thermal cycling. GT HapScreen® PAH Kit amplification protocol is validated using Eppendorf Mastercycler® nexus. Individual lab must perform internal validation for different thermal cycler to confirm the cycling protocol.</p>
	<p>Poor capillary electrophoresis injection if faint peaks for GT500 size standard is also observed. Re-inject samples or increase injection time.</p>
	<p>Lower quality formamide was used. Use only the recommended formamide.</p>
	<p>Run quality control GT QC DM102 provided with GT HapScreen® PAH Kit to check efficiency of primer mix and other PCR reagents.</p>
	<p>Inhibition of PCR because of too much template or other impurity in DNA extraction. Check the quality and quantity of extracted DNA. Use only the recommended DNA concentration. Make sure DNA is not degraded.</p>

---

Extra peaks observed in one or more dye channels

Amplification of STRs can result in artifacts that seems as peaks one base smaller than actual peak due to incomplete addition of the 3' "A" residue. To avoid this phenomenon, we recommend:

- a) Make sure to perform complete extension step as described in the protocol.
- b) Decrease the amount of DNA template in the reaction, too much DNA can lead to incomplete adenylation.
- c) Make sure reaction is not over amplified, decrease cycle number. Eventually each lab should perform internal validation for cycling condition.

---

Pull-up or bleed-through because of too high peaks. Make sure that analysis method is performed using GTM5 v2 Dye set Spectral Calibration.

Check if Spectral Calibration results are acceptable. See instructions in instrument preparation in section 3.

---

Samples not denatured completely, perform denaturation step as recommended.

---

Cross contamination with another sample DNA or PCR reagent is contaminated with amplicons. Use aerosol-resistant pipette tips, change gloves for pre- and post- PCR steps.

Store reagents in appropriate (Pre- and Post-) storage space. Do not open pre - PCR reagent tubes in Post-PCR lab.

---

Long-term stored PCR products are used.

---

	<p>Polymer-caused artifacts, check Polymer expiration date and storage time as mentioned in the manufacture guide.</p>
<p>Off-scale peaks</p>	<p>If off-scale peaks after primer peaks are observed –</p> <ul style="list-style-type: none"> <li>• a) Excessive DNA is added as template. Prepare new reaction with diluted DNA to repeat the PCR and capillary electrophoresis.</li> <li>• b) Excessive size standard in sample. Prepare new reaction using less size standard and repeat electrophoresis run.</li> </ul>
<p>No sizing data or size quality fails</p>	<ul style="list-style-type: none"> <li>• a) Incorrect or no size standard is selected in analysis method or protocol editor. Make sure that size standard option is edited with GT500 size standard.</li> <li>• b) Incorrect size standard is used. We recommend using GT500 with GT HapScreen® PAH Kit to obtain optimum results.</li> </ul>

## 7. Limitations and Disclaimer

Any result obtained from GT HapScreen® PAH or any other Kit should be used and interpreted by qualified medical geneticist or trained technicians. GENETEK BIOPHARMA GmbH cannot bear any responsibilities for false use and interpretation being made by any lab. The results obtained by GT HapScreen® PAH or any other Kits should only be used to indicate overall clinical scenario hence GENETEK BIOPHARMA GmbH cannot be responsible for any clinical decisions made by user or client lab.

GT HapScreen® PAH Kit is designed to screen for mutant alleles for PAH gene (only those alleles which are previously reported in patient's family. It's used in de novo cases has to be carefully used since in de novo cases, the mother may be carrier or not or she may be carrier of gonadal mosaicism), 21, 18 and 13 chromosomes specific trisomies and sex-chromosome aneuploidy (aneuploidy markers are included to act as screening and should not be used as diagnosis, confirmation should be carried out using aneuploidy detection methods). It will not detect other chromosome abnormalities or defect. User must carefully inspect any case of Maternal Cell Contamination before interpretation and patient consultation.

Result analysis guideline is generated using set of samples from specific populations. User lab should perform internal validation for any specific population for heterozygosity in each population.

We recommend that individual laboratory perform and develop its own test procedure and interpretation standard operative procedure. Best practice guidelines as mentioned in following section can be used to generate such documents.

**GT HapScreen® PAH Kit is for Research Use Only** and user bears all the responsibility for its use in clinical practice. Please consult best practice guidelines when using any QF-PCR kits including GT HapScreen® PAH.

For latest information, changes, as well as additional material please visit our website or contact us. This manual is intended for general information and should not be used as absolute facts. Its content may change without notice. For latest changes or modifications please contact us.

## 8. General Safety Warnings

- Any procedure should be performed by professional/qualified personal.
- Care should be taken while handling any human origin material, all samples should be considered potentially infectious. Lab technician or person handling the DNA must follow good lab practice and safety guidelines.
- Store all the components as described in the user guide.
- Laboratories should test their own quality check samples for each type of the assay to validate the Kit procedure.







### Chemical safety

- Before handling any chemicals, refer to the Safety Data Sheet provided by the manufacturer and follow relevant precautions.
- Minimize the contact with chemicals. Wear appropriate personal protective lab wear i.e. safety glasses, protective clothing, gloves.
- Check for chemical leaks and spills.
- Comply with local regulation regarding chemical storage, handling and disposal.

### SDSs

- The SDS for each of the Kit component is available online at GENETEK BIOPHARMA GmbH website <https://genetek-biopharma.com/>
- Any request for specific SDS can also be made from [support@genetek.de](mailto:support@genetek.de).

## 9. Symbols used on labels and packaging

Description	Symbol
Read Instructions before Use	
Do not use after the year, month and date mentioned	
Manufacturer name and address	
Storage temperature limit – Upper and Lower	
Manufacturer's Catalogue number	
Manufacturer's Batch code or Lot number	

## 10. Further Reading

1. Best Practice Guidelines for Internal Quality Control in Genetic Laboratories. <http://www.eurogentest.org/index.php?id=700>.
2. DNA Fragment Analysis by Capillary Electrophoresis User Guide by Applied Biosystems® Publication Number 4474504.
3. Mann, K. and Ogilvie, C. M. (2012), QF-PCR: application, overview and review of the literature. *Prenat Diagn*, 32: 309-314. doi:[10.1002/pd.2945](https://doi.org/10.1002/pd.2945)
4. Mattocks CJ, Morris MA, Matthijs G, et al. A standardized framework for the validation and verification of clinical molecular genetic tests. *Eur J Hum Genet*. 2010;18(12):1276-88.