



GT HapScreen[®] HBB

Product User Manual

CAT# GT-11201

GT HapScreen[®] HBB Kit is an innovative approach which aids more accurate prenatal diagnosis for beta-thalassemia plus screening for aneuploidies of chromosomes 21, 18, 13, X and Y

Produced by

GENETEK BIOPHARMA GmbH

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1. GT HapScreen® HBB Overview

- Aids carrier detection and prenatal diagnosis through segregation and linkage analysis via haplotyping
- Rules out/in sample contamination
- Rules out/in sample mix-up
- Rules out/in maternal cell contamination
- Ascertains sample authenticity
- Determines sex of sample tested
- Screens common chromosomal aneuploidy
- It detects uniparental disomies
- Can confirm de-novo or inherited mutation
- It can be used on extracted DNA from blood, amniotic fluid, CVS
- Five beta-globin gene specific markers, seven autosomal chromosome specific markers and two sex chromosome specific markers in total 14 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's for research use only**

1.1. Intended Use

The GT HapScreen® HBB kit is a screening kit to aid carrier detection and/or prenatal diagnosis of beta-thalassemia, Sickle Cell disease as well as other beta-globin linked hemoglobinopathies. It also simultaneously screens fetal sample for chromosome 13, 18, 21, X and Y aneuploidies. Through these features it has many other benefits described briefly above.

1.2. GT HapScreen® HBB Markers

The GT HapScreen® HBB kit is developed to aid carrier detection and/or prenatal diagnosis beta-Thalassemia. The kit contains multiplex primers for STR markers flanking the beta-globin gene as well as other STR markers on chromosomes 21, 18, 13, X and Y to act as QF PCR technique to screen for aneuploidies for the above chromosomes.

Kit components include multiplex PCR primers which are designed from bet-globin gene. This gene is screened for mutation in carrier detection and prenatal diagnosis of beta-Thalassemia as well as Sickle Cell disease and other beta-globin linked hemoglobinopathies. STR markers designed from 5 regions that covers the upstream and downstream of the beta-globin gene.

This kit is optimised to use DNA samples purified from blood, amniotic fluid, and chorionic villus (CVS). Along with beta-thalassemia detection, the GTHapScreen® HBB 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.

Table 1: Markers used in the GT HapScreen® HBB kit

No.	Marker	Chromosome	Size Range	Location
1	AMXY	XY	103-117	Xp22.2
				Yp11.2
2	D13S325	13	125-190	13q14.11
3	D18S390	18	210-250	18q22.3
4	D21S1446	21	285-328	21q22.3
5	D13S252	13	405-455	13q12.2
6	D11HBBS3.3	11	148-200	11p15.4
7	D11HBBS11.2	11	215-275	11p15.4
8	D21S1414	21	350-430	21q21.1
9	D11HBBSU6.1	11	100-187	11p15.4
10	D18S1002	18	230-310	18q11.2
11	D21S1411	21	346-460	21q22.3
12	D11HBBSU11	11	100-222	11p15.4
13	DXTATC13.3	X	235-271	Xq13.1
14	D11HBBSU2.9	11	309-360	11p15.4

- Since beta-globin gene specific markers are unique and they do not have internationally agreed name or nomenclature; we have created a naming strategy. The nomenclature used for beta-globin specific STR markers are as follow: D stands for DNA, 11 for chromosome 11, HBB is used to designate disease name, S is for single copy sequence and I is for Internal marker (if there is one), U is for upstream and D for downstream of the gene. Numbers are distance from either side of the gene in 10⁵ bp (i.e. SD11.2 means roughly 1120 kb distance from 3' end of the gene).
- Size ranges 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.

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

Fluorescent based STR kits can amplify a small amount of DNA. So care should be taken not to contaminate the working area. Primer Mix, PCR Mix and GT HSTaq DNA polymerase should be stored in a separate place (Pre-PCR freezer). GT500 Size Standard, GTM5 v2 Matrix Standard are amplicons and should be stored in post-PCR area (freezer). In each run, negative control should be added to determine possible and source of contamination. We recommend that DNA from each personnel working in the lab be profiled so in case of contamination, the source can be determined and precautionary measures can be taken.

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 (H2O)	

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

Not provided with GT HapScreen® HBB (but are needed)

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

2.2.3. PCR Amplification by GT HapScreen® HBB

- DNA can be extracted from blood. This kit also works for blood samples on filter paper such as DNA Banking Card (DBC™). For instruction on direct PCR method please contact us by email (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.

2.2.4. GT HapScreen® HBB components

Table 4: PCR reaction set-up

Component	Volume for 1 reaction[μl]
GT QCW (H ₂ O)	10
PCR Mix	7
Primer Mix	1
GT HSTaq	1

2.2.5. GT HapScreen® HBB protocol

- Bring reagents to room temperature.
- Vortex Primer Mix and PCR 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 μL 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 μl of sterile Direct Q dd H₂O 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	∞
27-30 cycles					

- After completion of PCR, 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 QC DM102 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.
- Do not use too much DNA, as non-specific bands may appear.

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® HBB Kit is validated using 50 cm capillary array and POP7 as well as on 36 cm array and POP4 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.
- An increase or decrease in the injection time or voltage may result to run product through the capillary.
- 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® HBB 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 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.

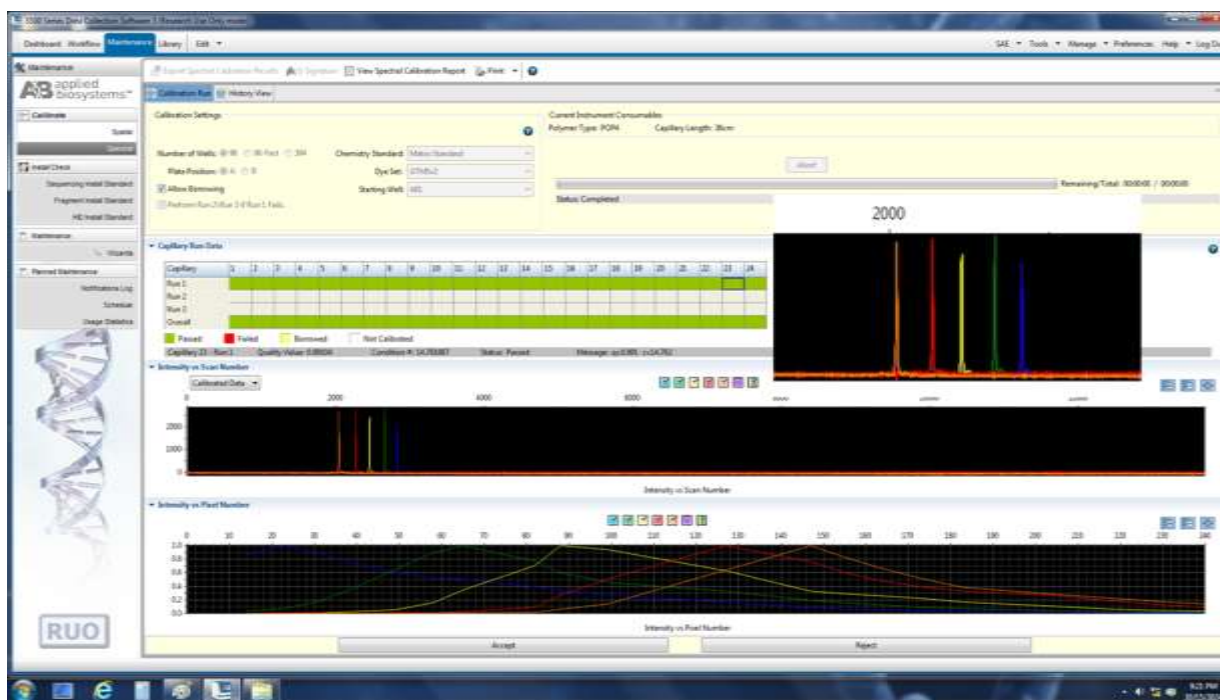


Figure 3. An example of a successful spectral calibration with GT 5-dye system on Applied Biosystems Genetic Analyzer 3500xL

- 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.

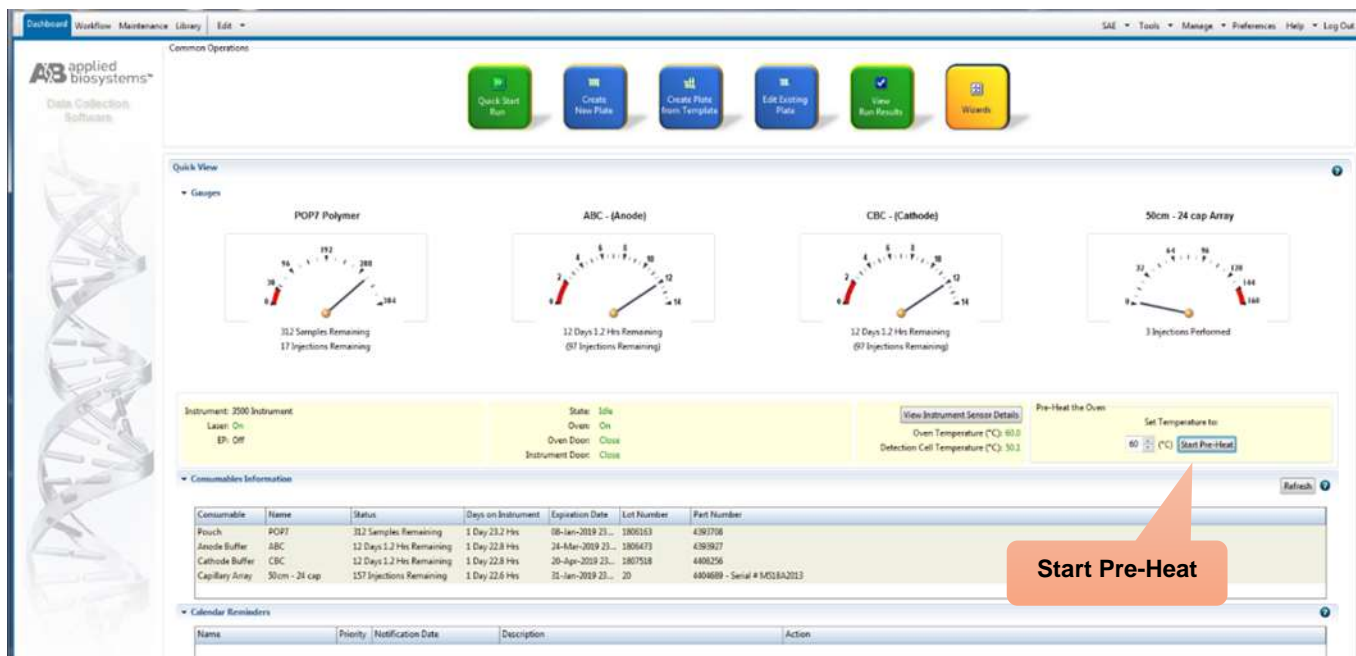


Figure 4. Dashboard of Applied Biosystems 3500 Data Collection software

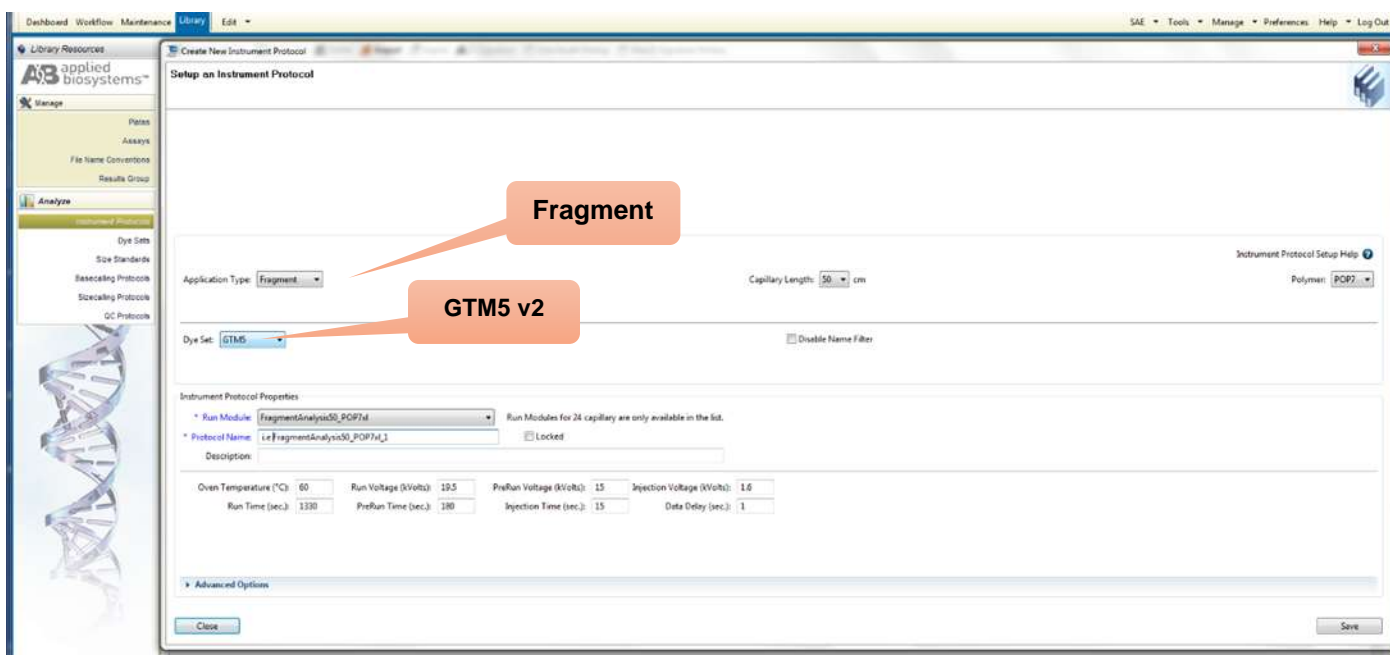


Figure 5. Screenshot for the “Create New Instrument Protocol” window on Applied Biosystems 3500 Data Collection software

- 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 HBB 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.

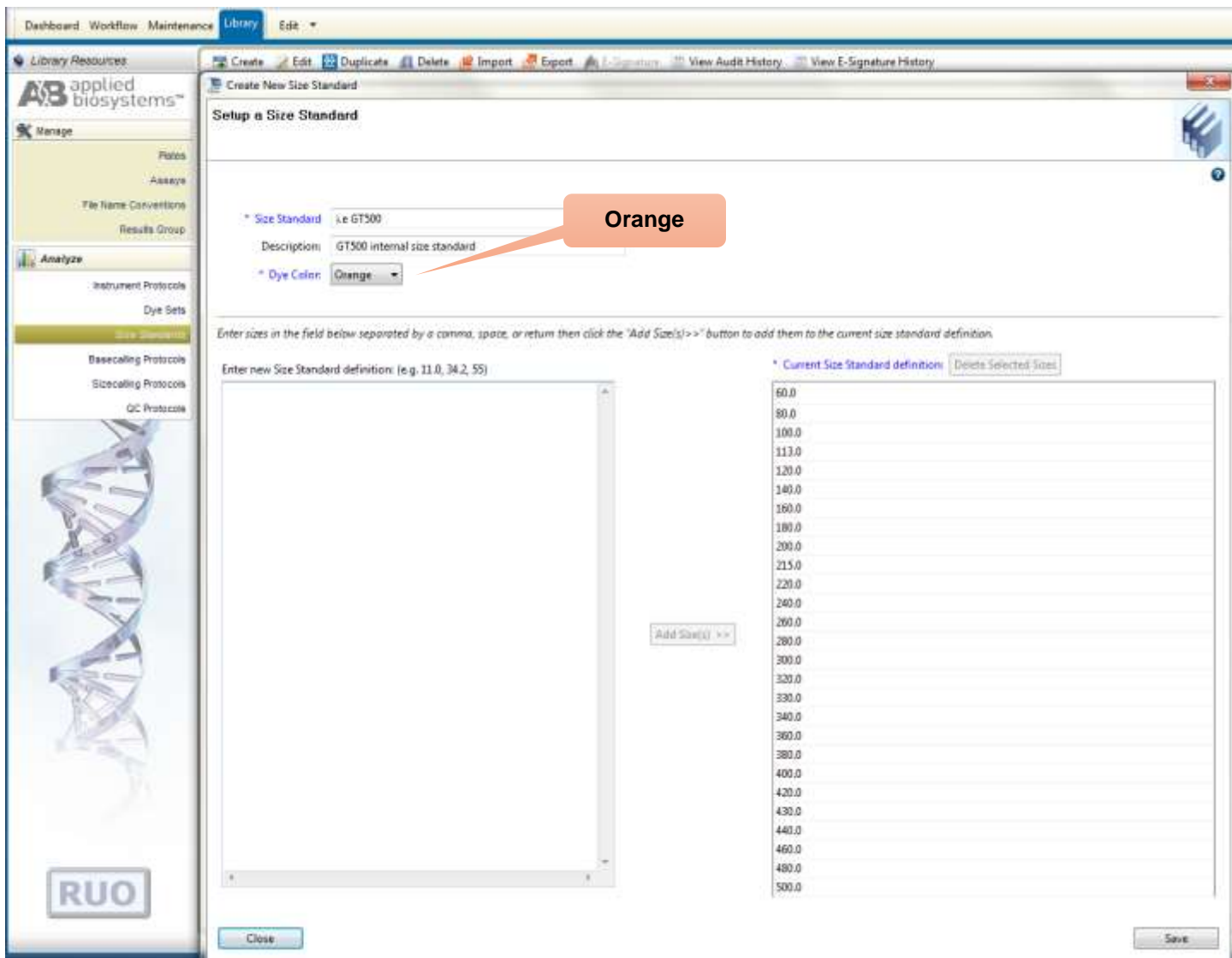


Figure 6. Screenshot for the “Create New Size Standard” window on Applied Biosystems 3500 Data Collection software

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® HBB on the Applied Biosystems® 3500/3500xL Genetic Analyzer.

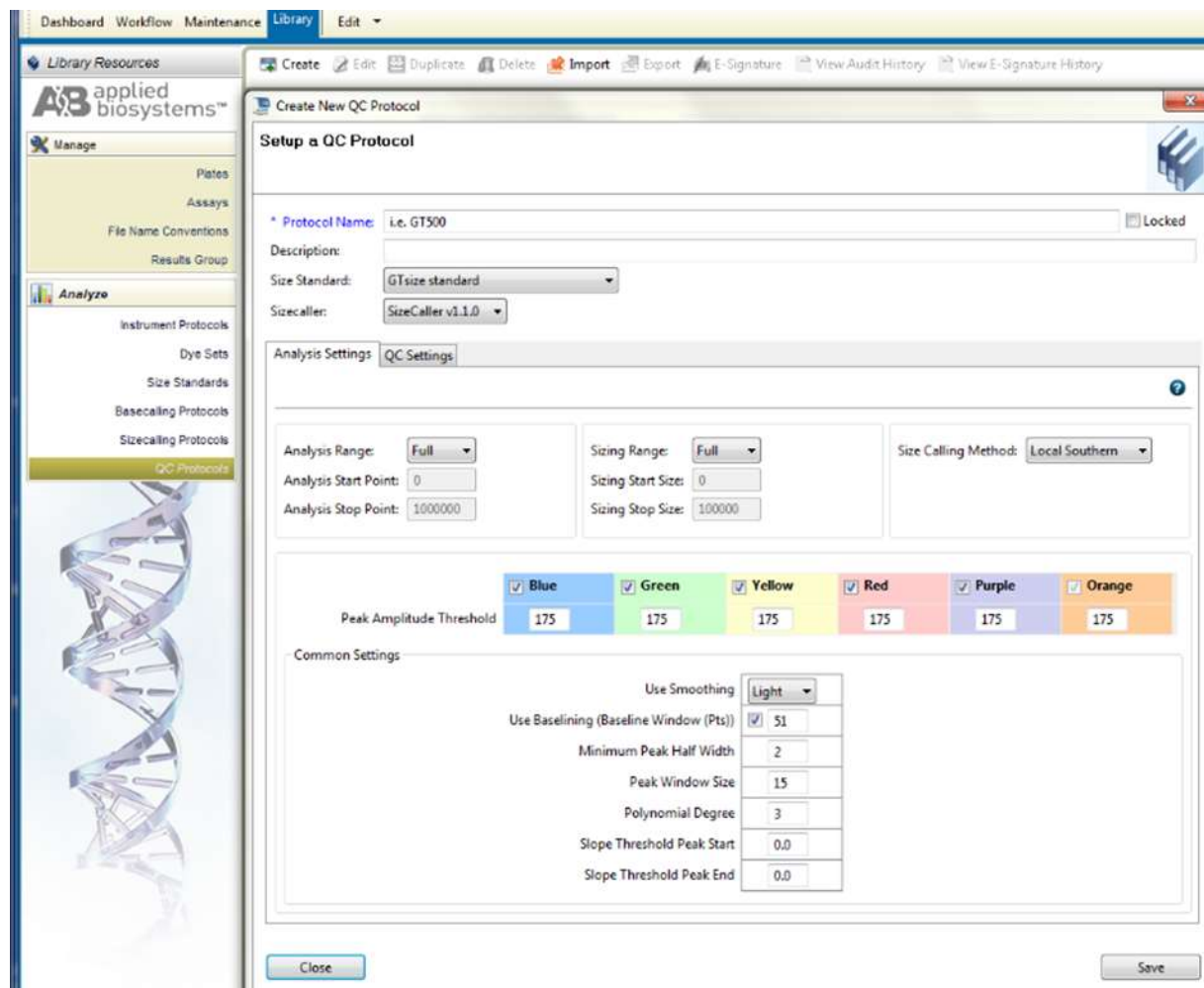


Figure 7. Screenshot for the “Create New QC Protocol” window on Applied Biosystems 3500 Data Collection software

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 8, 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.

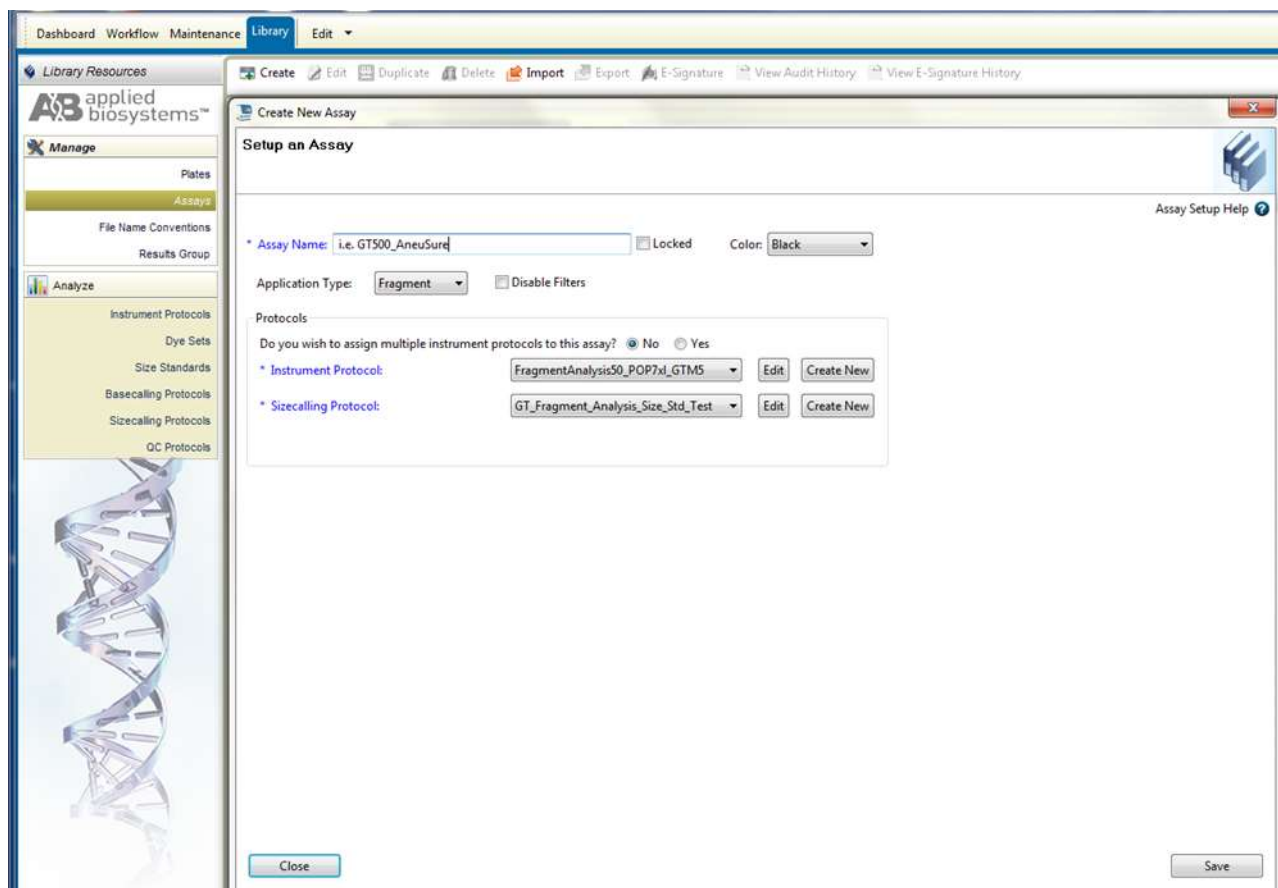


Figure 8. Screenshot for the “Create New Assay” window on Applied Biosystems 3500 Data Collection software

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

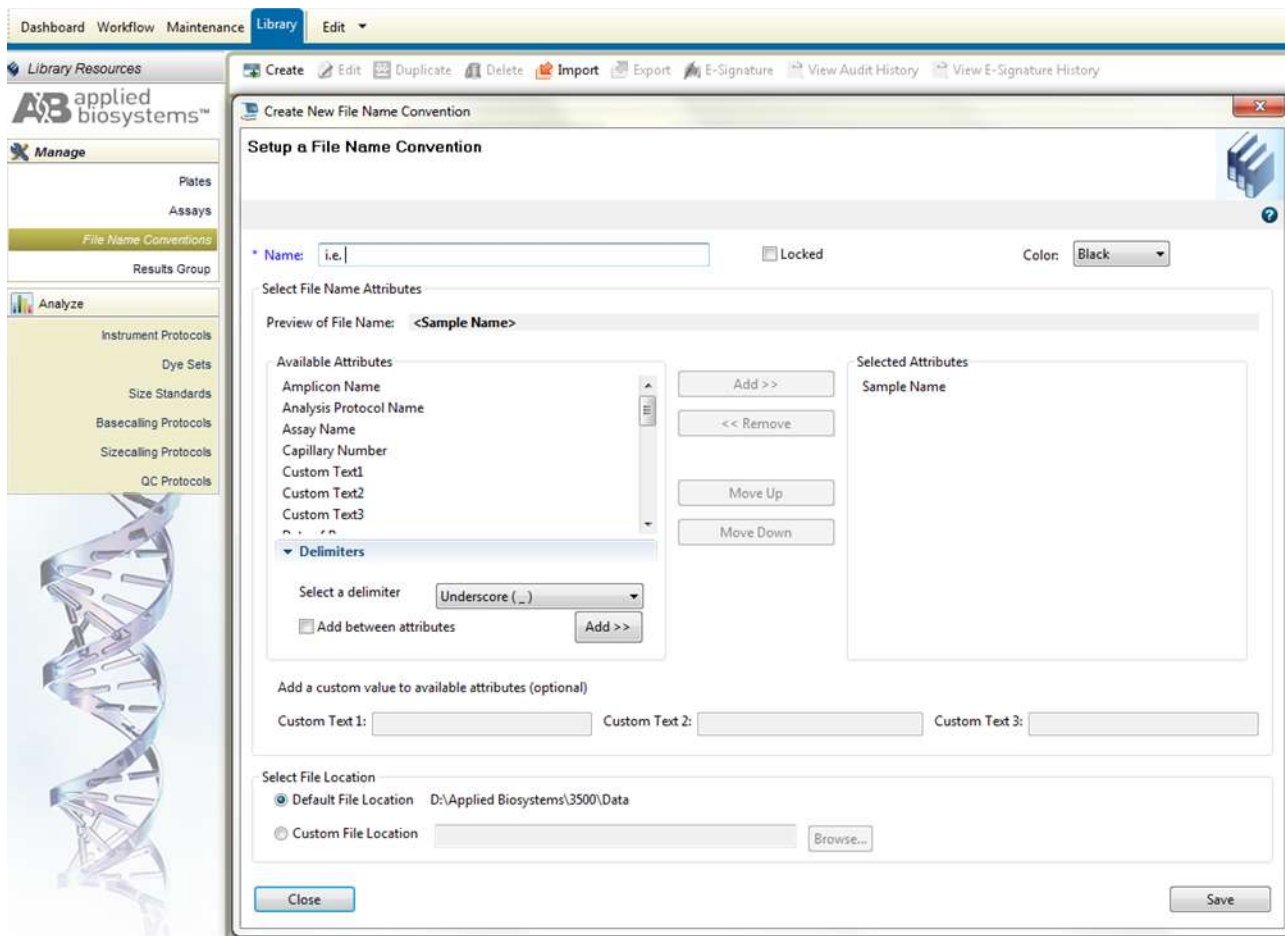


Figure 9. Screenshot for the “Create New File Name Convention” window on Applied Biosystems 3500 Data Collection software

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

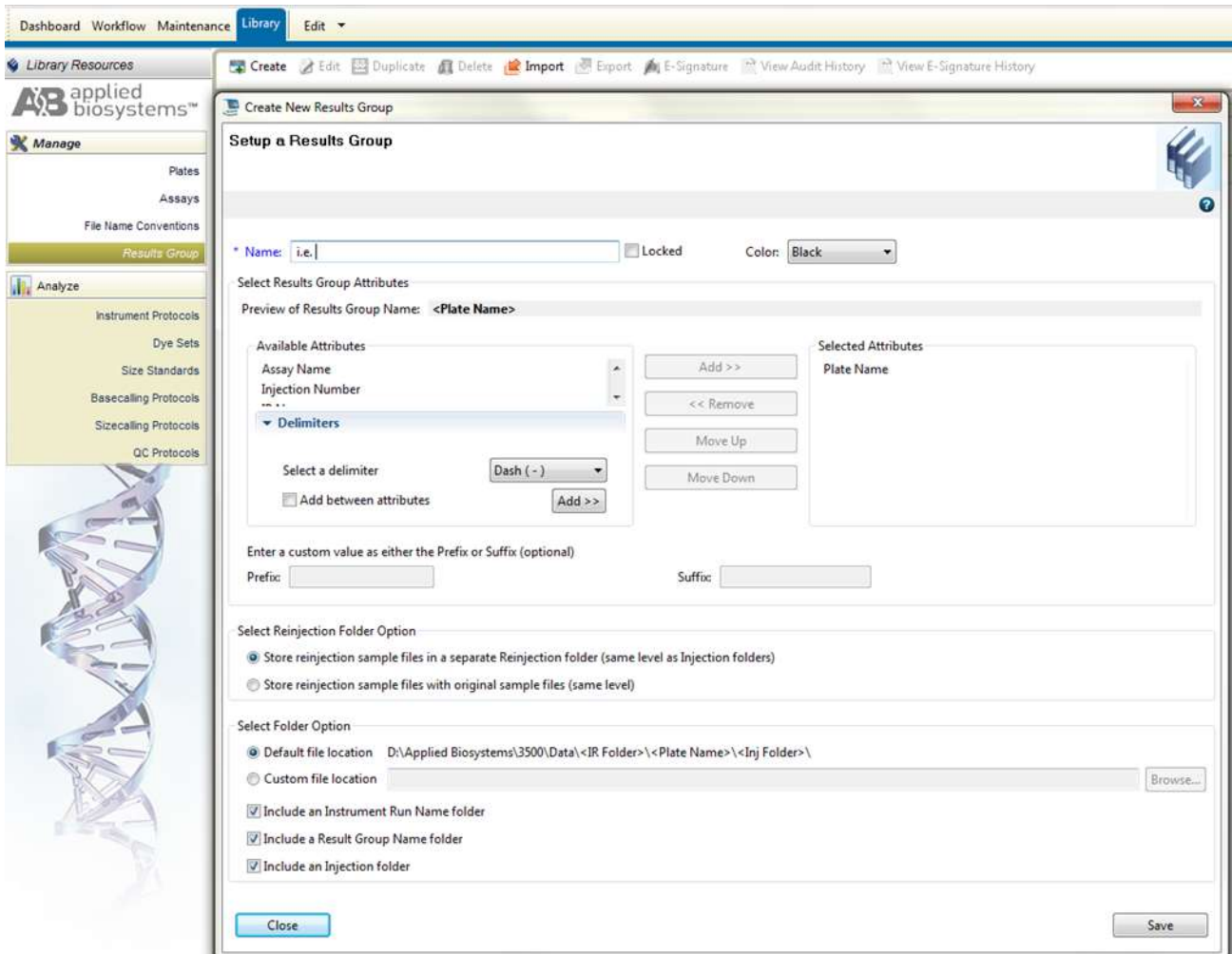


Figure 10. Screenshot for the “Create New Result Group” window on Applied Biosystems 3500 Data Collection software

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

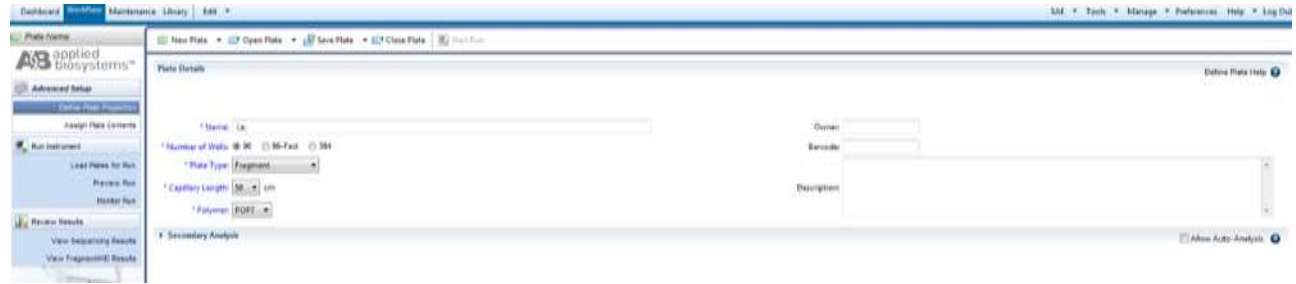


Figure 11. Screenshot for the “Defining plate properties” window on Applied Biosystems 3500 Data Collection software

3.1.8. Select “Assign Plate Contents”

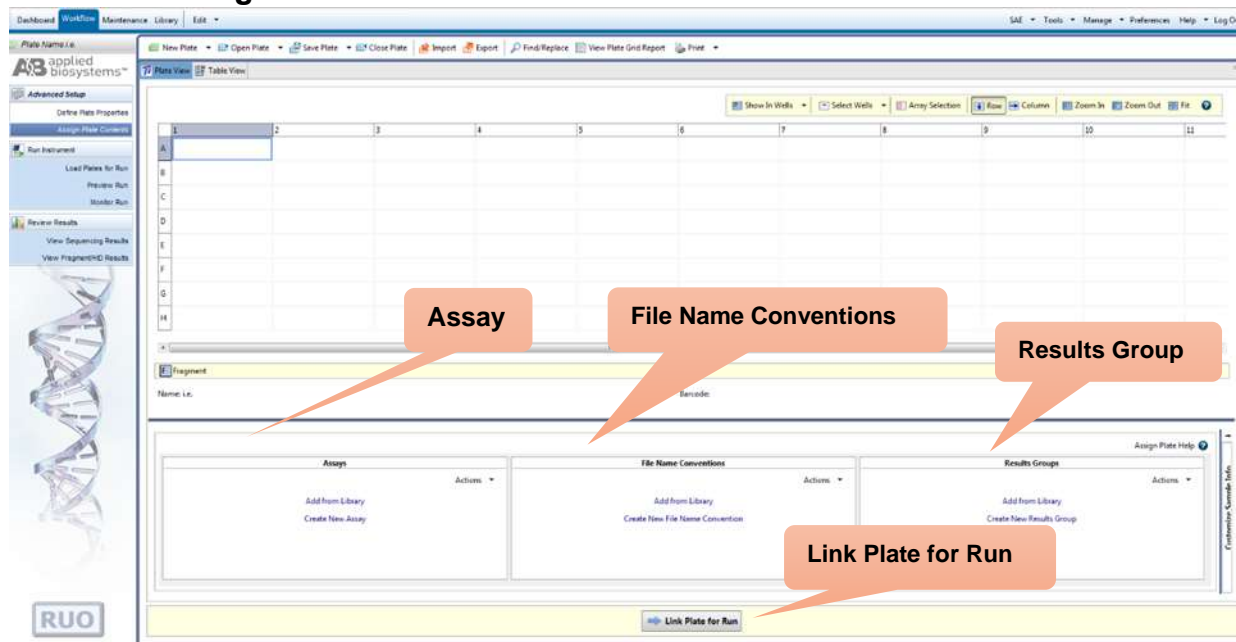


Figure 12. Screenshot for the “Assign Plate Contents” window on Applied Biosystems 3500 Data Collection software

- 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.

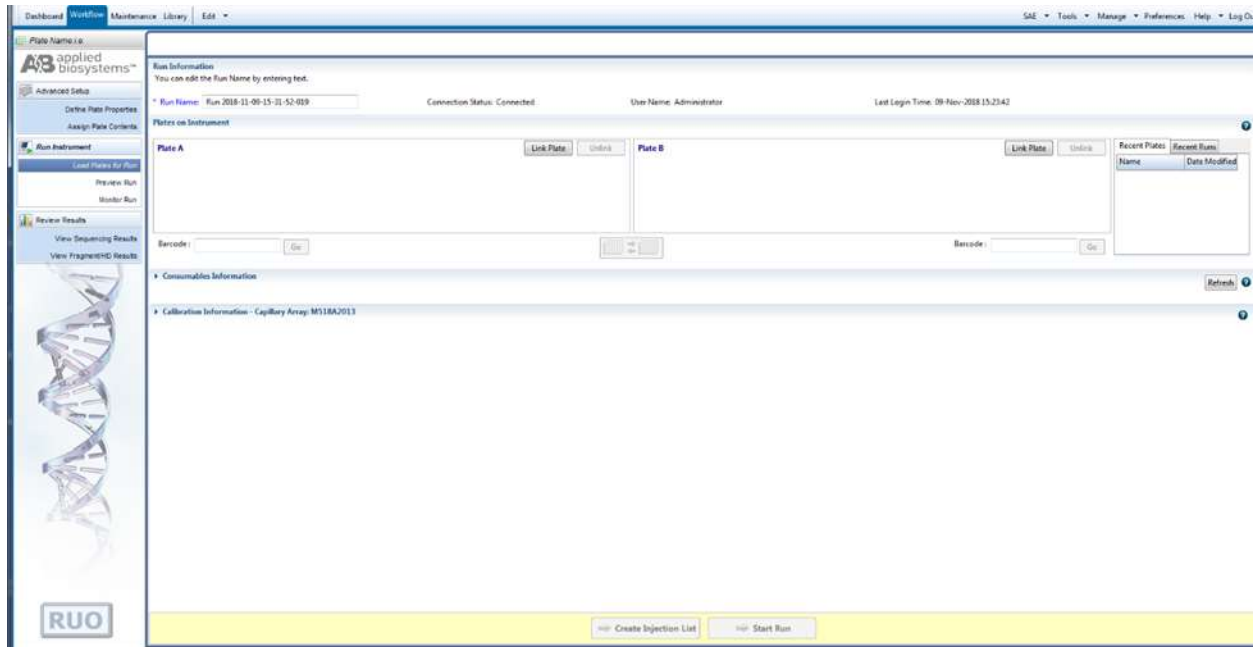


Figure 13. Screenshot for the “Run Information” window on Applied Biosystems 3500 Data Collection software

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

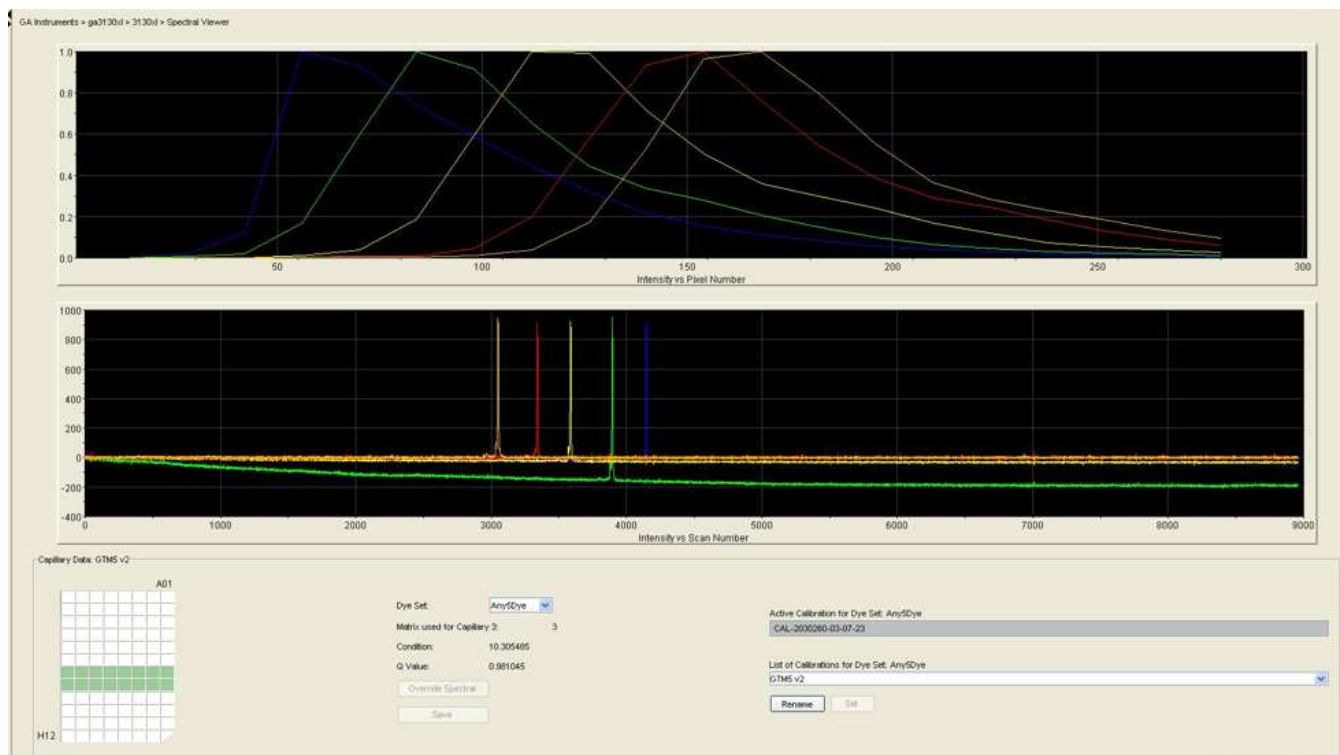


Figure 14. An example of a successful spectral calibration with GT 5-dye system on Applied Biosystems Genetic Analyzer 3130xl

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® HBB)
- b) Type: Regular
- c) Template: FragmentAnalysis50_POP7 (default template for the capillary array and polymer used)
- d) Click OK

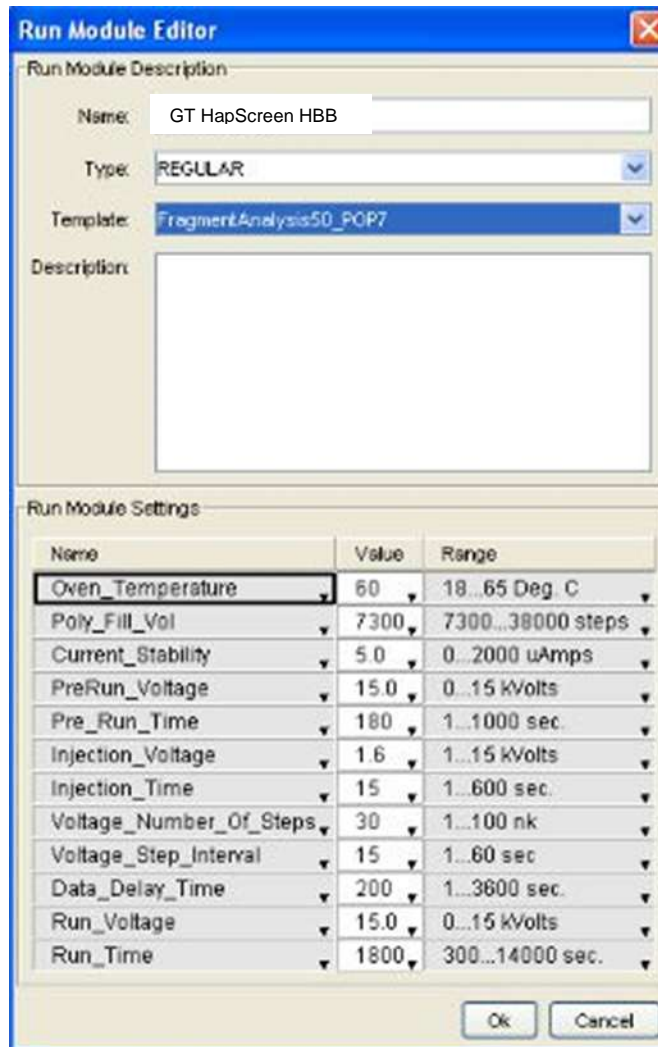


Figure 15. Screenshot for the “Module Manager” window on Applied Biosystems 3130 Data Collection software

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® HBB)
- c) Type: Regular
- d) Run Module: Select the Run Module created (GT HapScreen® HBB)
- e) Dye Set: Any5Dye
- f) Click OK

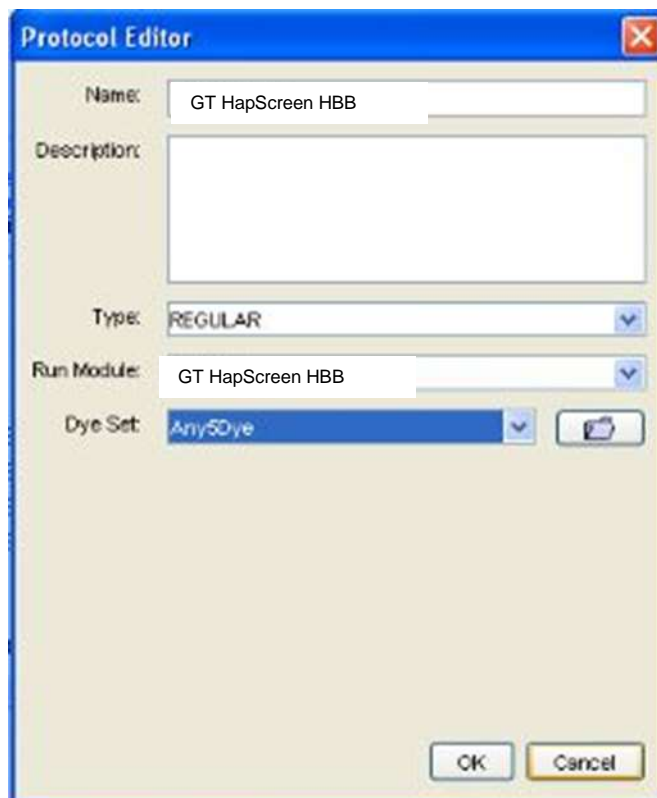


Figure 16. Screenshot for the “Create New Instrument Protocol” window on Applied Biosystems 3130 Data Collection software

3.2.3. Set up a Plate for run

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

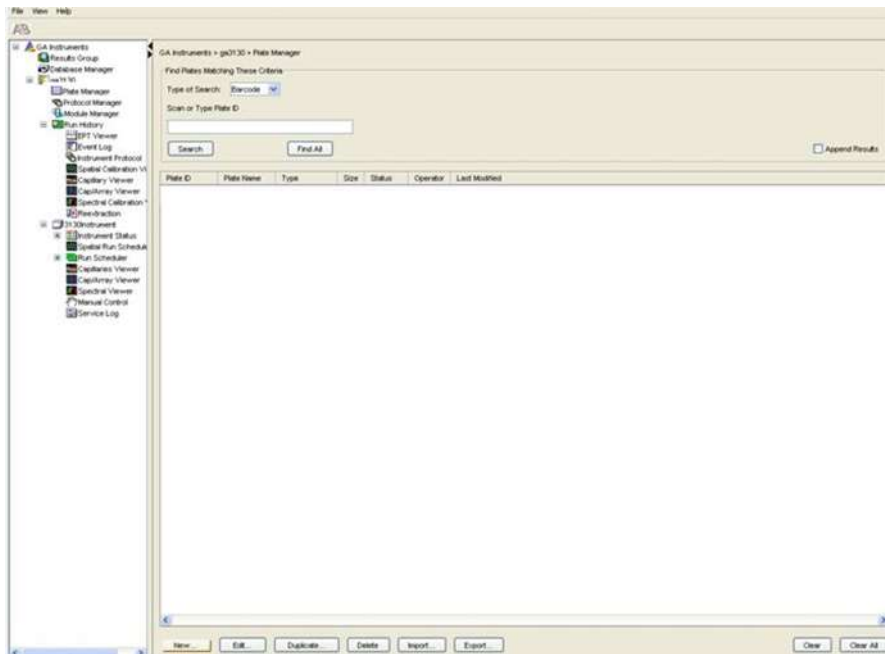


Figure 17. Screenshot for the “Plate Manager” window on Applied Biosystems 3130 Data Collection software

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

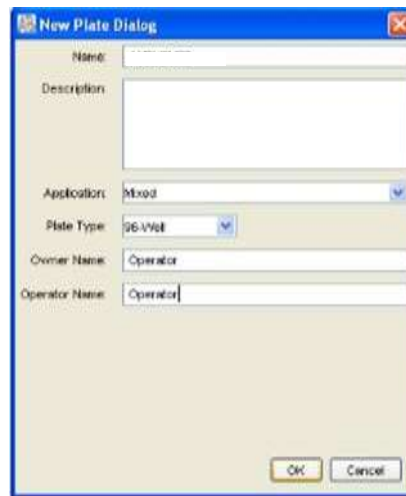


Figure 18. Screenshot for the “*New Plate Dialog*” window on Applied Biosystems 3130 Data Collection software

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

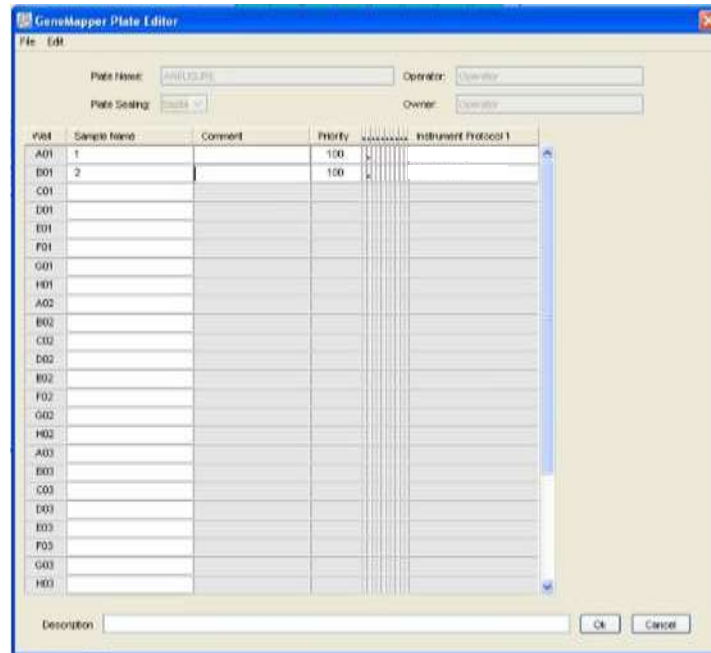
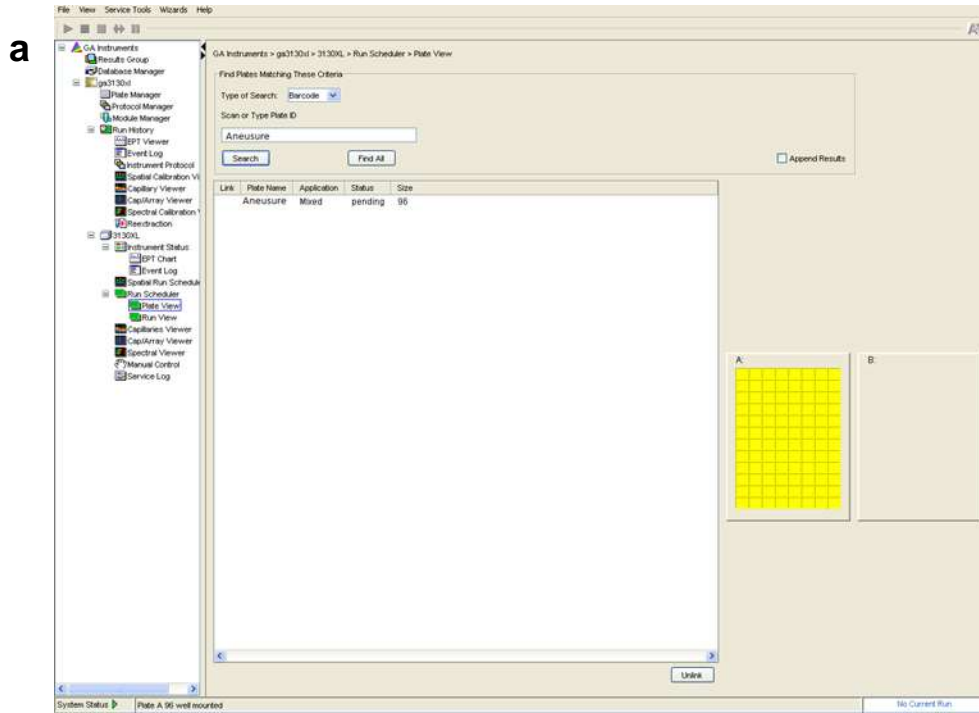


Figure 19. Screenshot for the “GeneMapper Plate Editor” window on Applied Biosystems 3130 Data Collection software

- From the left navigation window, select Run Scheduler, search for GT HapScreen® HBB (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.

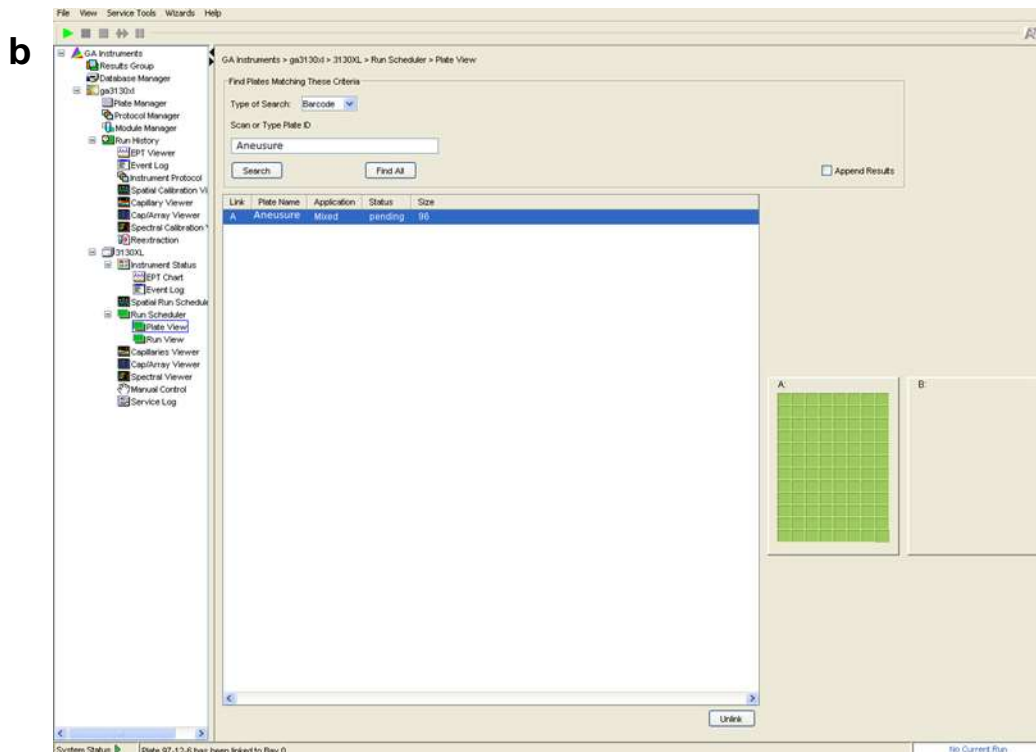


Figure 20 a & b. “Plate view” window on Applied Biosystems 3130 Data Collection software.

- 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® HBB 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 the 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® HBB, 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.

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

PCR products are observed with 5-dye system on an electropherograms in the GeneMapper® software. For the analysis, import GT HapScreen® HBB panels. It can be downloaded from our website: 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. 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

Please refer to **GT HapScreen® User Guide** for how to draw pedigree, haplotype inheritance pattern for GT HapScreen® kits. The guide details how fragment analysis results should be plotted into the pedigree and how allele segregation is drawn.

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® HBB kit is used to screen samples for beta-Thalassemia. GT HapScreen® HBB is developed to make sample analysis accurate and safe. Some of these results are generated and most others are real cases provided to use by customers using GT HapScreen® kits including GT HapScreen® HBB.

5.1. Sample case study 1- ruling out sample mix-up

Lab X gets samples for prenatal screening. These are 3 fetus samples with a family history of beta – Thalassemia. Lab X also collects samples from parents. If now Lab X uses the conventional approach to use just beta-globin gene specific markers and analyses these samples with Capillary Electrophoresis, the resulting linkage map is as shown below –

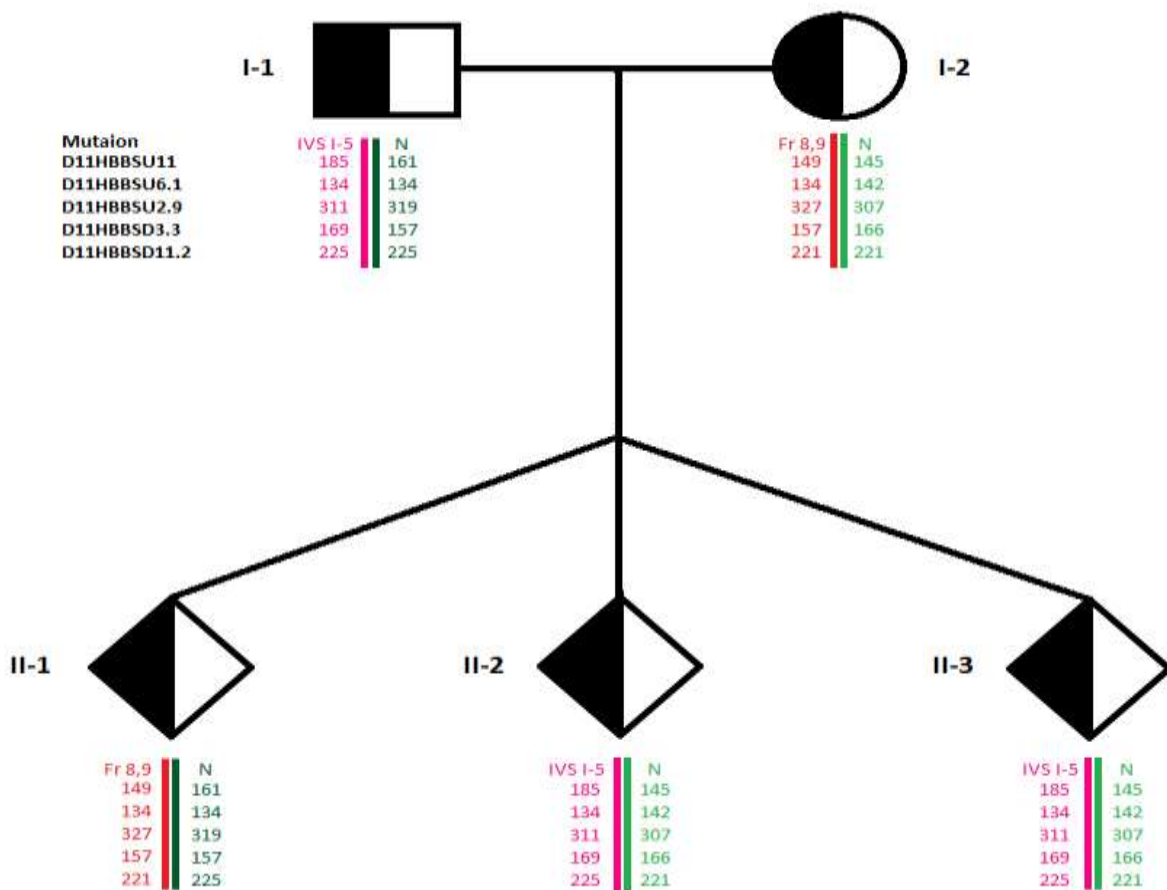


Figure 22: Linkage analysis showing family with mutant alleles for beta-globin gene, mutant alleles in father (I-1) shown in pink and in mother (I-2) in red color. As can be seen II-2 and II-3 have similar haplotype for beta-globin specific markers. Are they identical or they are contaminated?

Lab geneticist can only conclude that one out of three fetus sample will be carrier for beta-Thalassemia. Because linkage map for other two samples showed exactly the same alleles for all the beta-globin gene specific markers. How would user rule out cross-contamination or sample mix-up to avoid false negative/positive in such cases? If Lab X uses GT-HapScreen® HBB kit to analyze these samples, the resulting linkage map will be as in figure 23.

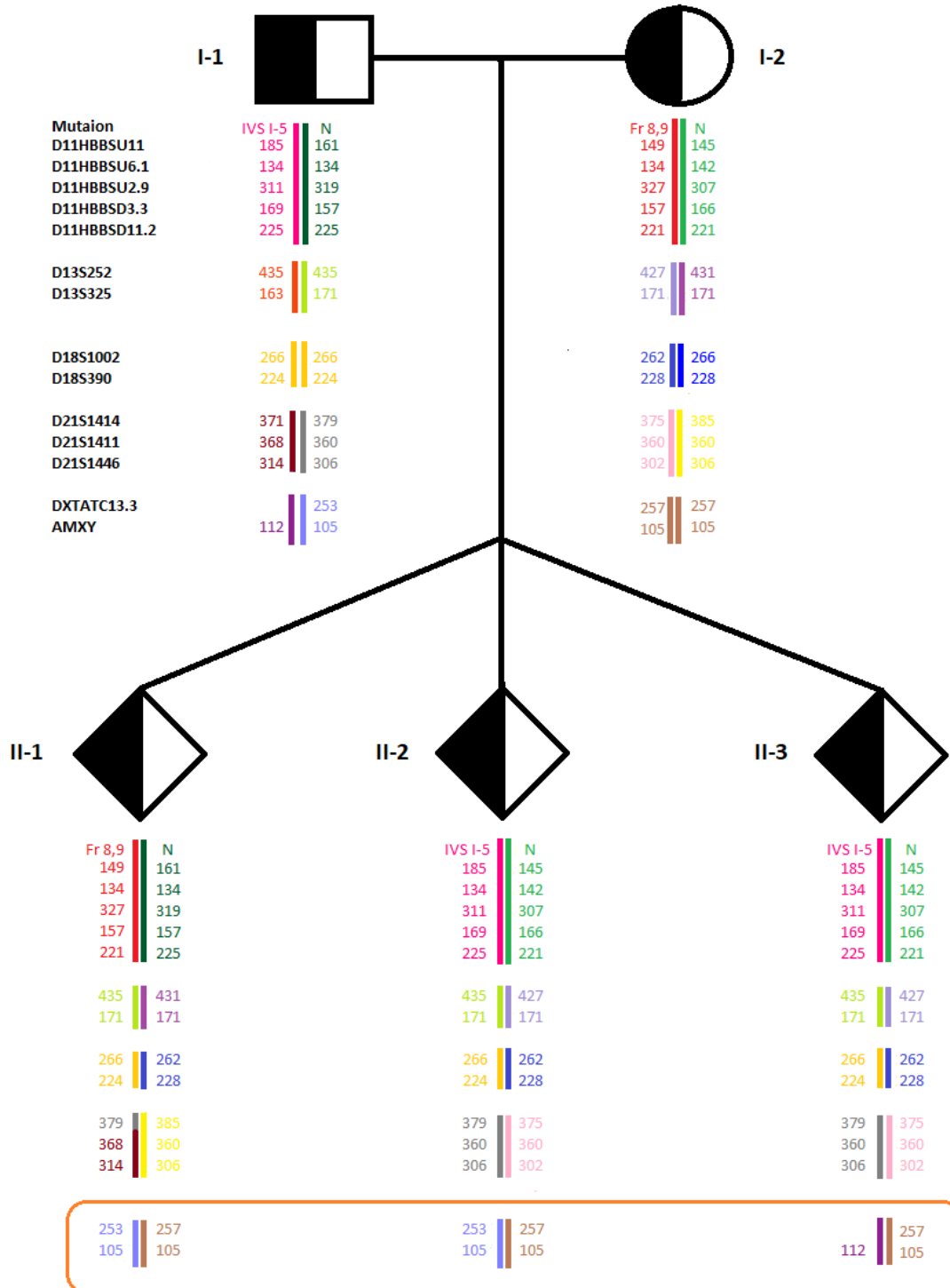


Figure 23: Linkage analysis showing family with mutant alleles for beta-globin gene, mutant alleles in father (I-1) shown in pink and in mother (I-2) in red color, sex chromosome markers are marked.

As shown in figure 23, presence of Sex Chromosome markers along with beta-globin gene specific markers reveal the differentiation between the sex of these two samples and hence confirm the authenticity of samples. They are not identical though they share the same haplotype for chromosomes 11, 21, 18 and 13.

Alleles used in the linkage analysis depicted in figure 22 are obtained using GT HapScreen® HBB Kit. Fragment analysis was done using GeneMapper® and Genetic Analyzer 3500xL. See below for the data from Paternal (I-1, carrier), Maternal (I-2, carrier), Fetus 1 (II-1, carrier), Fetus 2 (II-2, carrier) and Fetus 3 (II-3, carrier) samples.

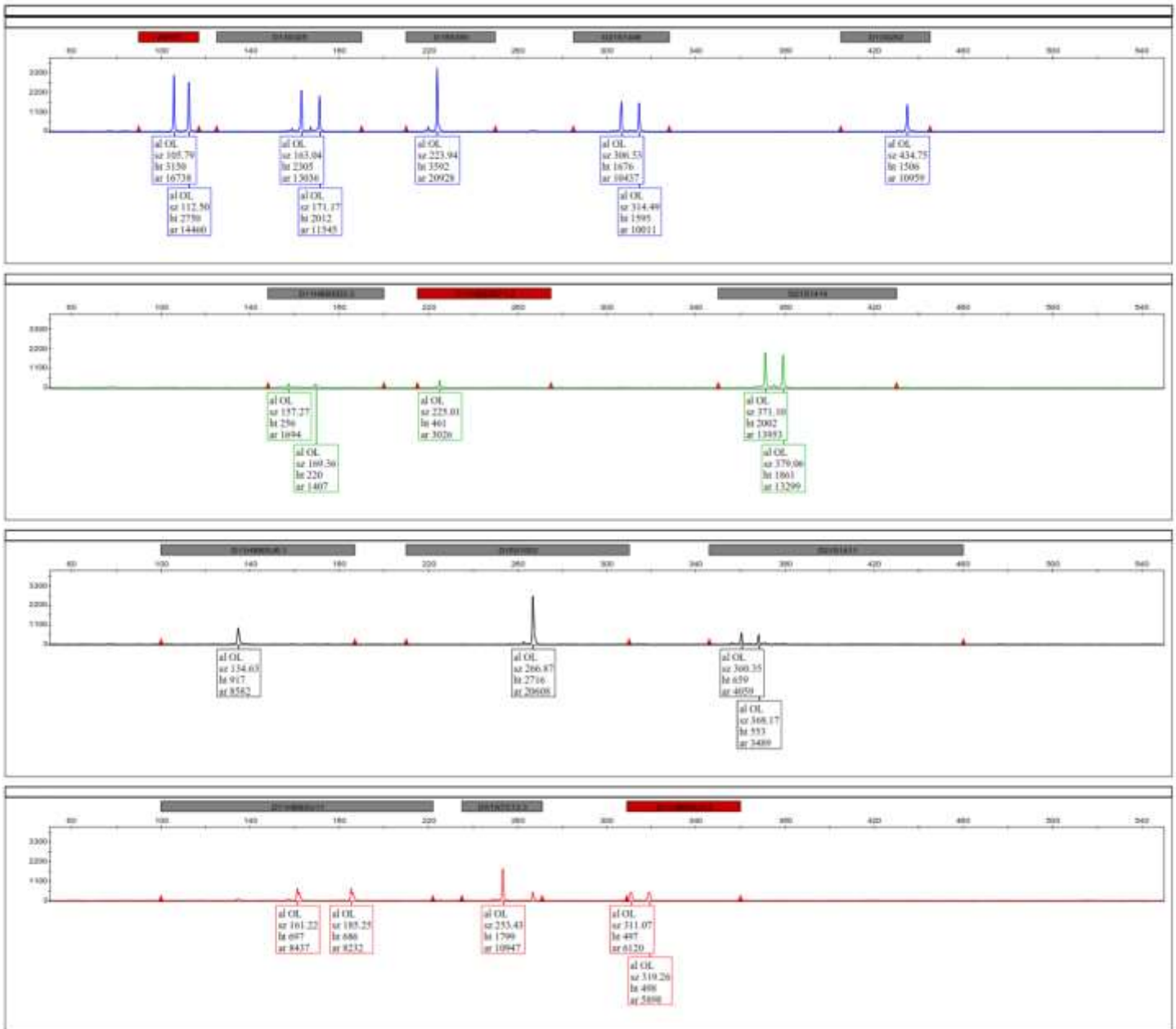


Figure 24. GT HapScreen® HBB profile for Paternal (I-1) sample with the mutant alleles for beta-Thalassemia (beta-globin gene)



Figure 25. GT HapScreen® HBB profile for Maternal (I-2) sample with the mutant alleles for beta-Thalassemia (beta-globin gene)



Figure 26. GT HapScreen® HBB profile for Fetus 1 (II-1) sample with the mutant alleles for beta-Thalassemia (beta-globin gene)



Figure 27. GT HapScreen® HBB profile for Fetus 2 (II_2) sample with the mutant alleles for beta-Thalassemia (beta-globin gene)



Figure 28. GT HapScreen® HBB profile for Fetus 3 (II_3) sample with the mutant alleles for beta-Thalassemia (beta-globin gene)

5.2. Sample case study 2- ruling out maternal contamination and detection of trisomy 21

Lab Y gets samples for prenatal screening, a fetus sample with a family history of beta-Thalassemia. Lab Y also collects samples from parents. If now Lab Y uses the conventional approach to use just beta-globin gene specific markers and analyzes these samples with Capillary Electrophoresis, the resulting pedigree analysis is as shown below:

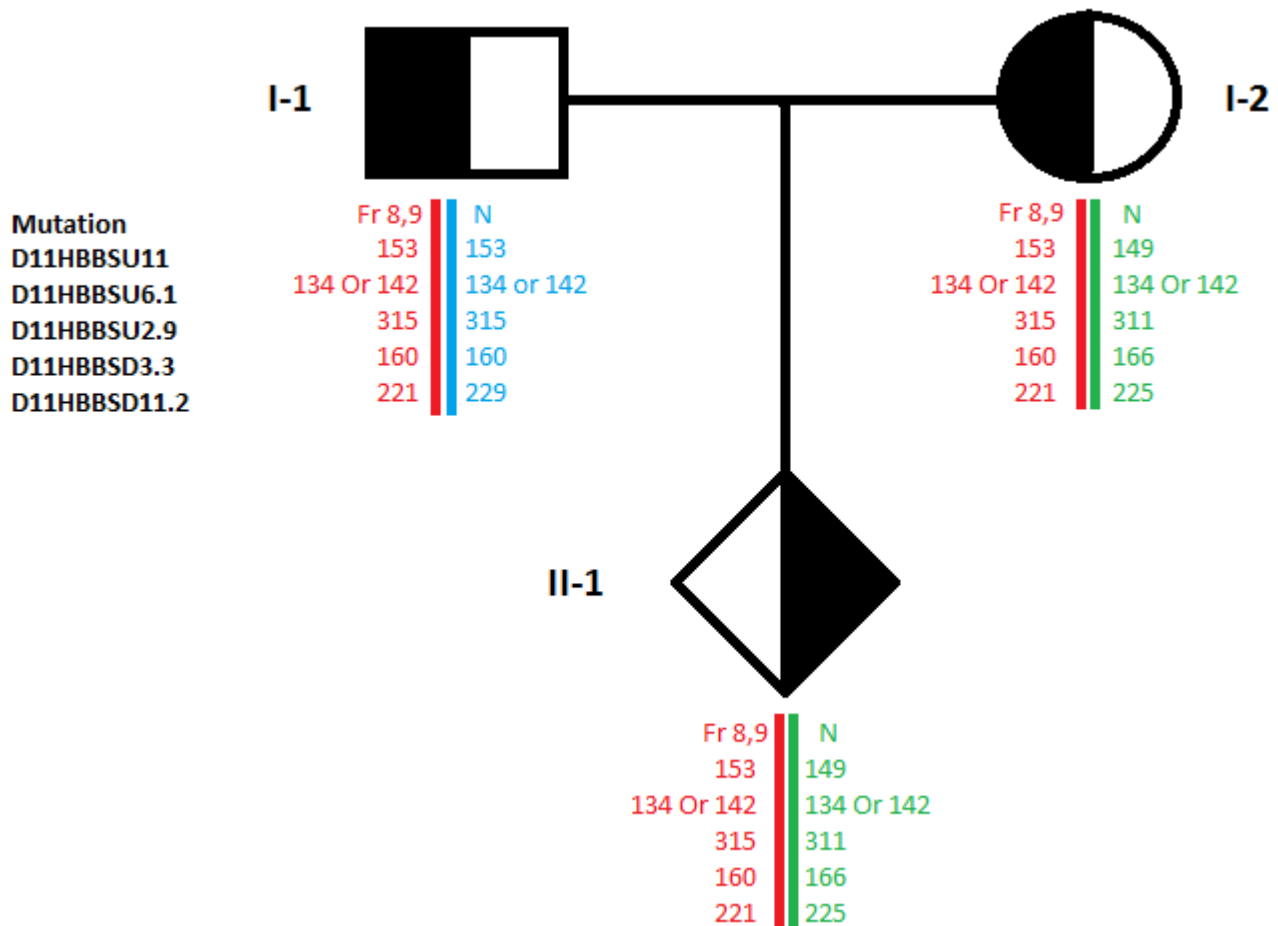


Figure 29: Linkage analysis showing family with mutant alleles for beta-globin gene, mutant alleles shown in red color.

Linkage map for fetus shows exactly the same alleles as Mother's sample for all the beta-globin gene specific markers. The fetal sample may not be fetal and be maternal sample or it may be overloaded with maternal sample as sometimes seen in heavily stained amniotic fluid with maternal blood or it may actually be fetal sample. Lab Y cannot conclude any result and must re-analyze the sample. How would user rule out maternal cell contamination to avoid false negative/positive in such cases? They may either ask for resampling or perform DNA profiling using human identification kits like GT Detector or GT Detector Globe or similar kits. Lab Y can reduce its costs and use the **GT HapScreen® HBB** kit to simultaneously analyze these samples for mutation confirmation, aneuploidy and sample authenticity. The resulting linkage map will be as shown in figure 29.

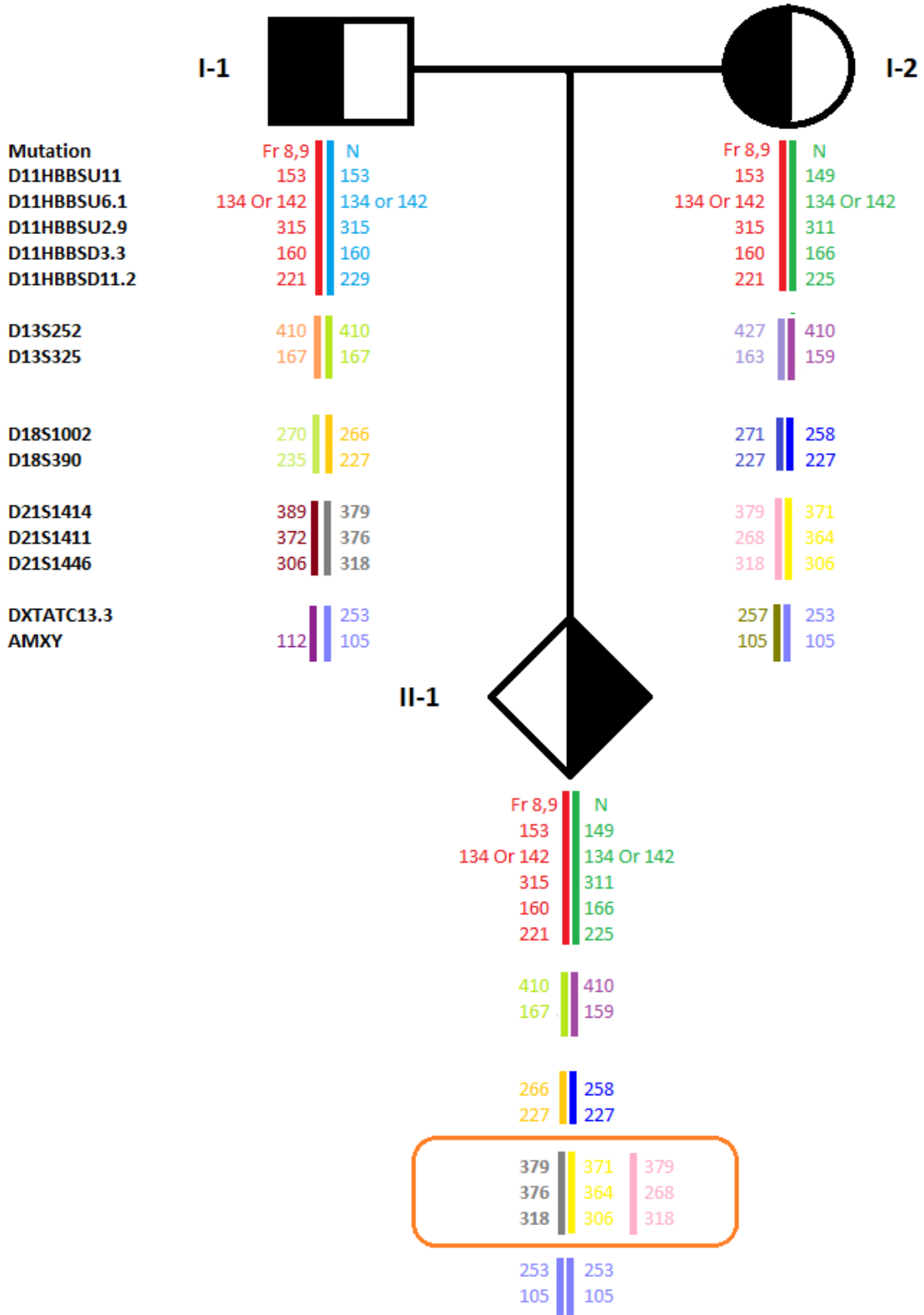


Figure 30: Linkage analysis showing family with mutant alleles for beta-globin gene, mutant alleles shown in red color.

Presence of Autosomal chromosome markers along with beta-globin gene specific markers reveal the differentiation between mother and fetal samples. Hence, confirms authenticity of the fetal sample. **This analysis also reveals that the fetus has Trisomy 21** (orange border in figure 30). Further analysis of this sample using AneuSure kit confirmed +21. This fetus would be regarded as a normal carrier and pregnancy would follow if conventional methods like ARMS or Sanger sequencing were applied.

Alleles used in the linkage analysis depicted in figure 30 were obtained using GT HapScreen® HBB Kit. Fragment analysis was done using GeneMapper® and Genetic Analyzer 3500xL. See below for the data from Paternal (I-1, carrier), Maternal (I-2, carrier), Fetus (II-1, carrier).

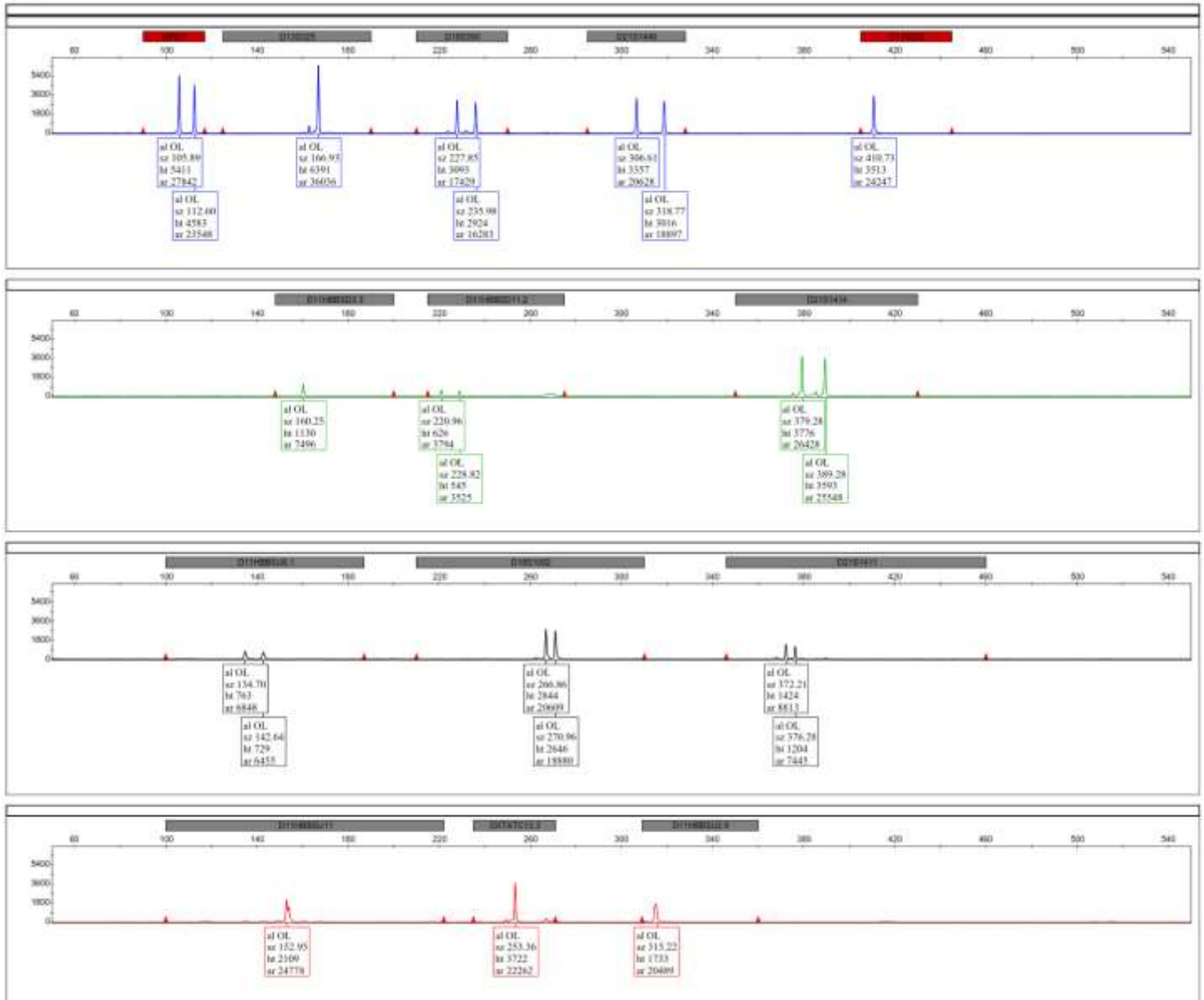


Figure 31. GT HapScreen® HBB profile for Paternal (I-1) sample with the mutant alleles for beta-Thalassemia (beta-globin gene)



Figure 32. GT HapScreen® HBB profile for Maternal (I-2) sample with the mutant alleles for beta-Thalassemia (beta-globin gene)



Figure 33. GT HapScreen® HBB profile for Fetus (II-1) sample with the mutant alleles for beta-globin gene and has trisomy 21

6. Troubleshooting

For any technical question or issue (not mentioned here) please contact our customer support here – 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® HBB 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 QCDM102 provided with GT HapScreen® HBB 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.

Polymer-caused artifacts, check Polymer expiration date and storage time as mentioned in the manufacture guide.

Off-scale peaks

If off-scale peaks after primer peaks are observed –

- a) Excessive DNA is added as template. Prepare new reaction with diluted DNA to repeat the PCR and capillary electrophoresis.
- b) Excessive size standard in sample. Prepare new reaction using less size standard and repeat electrophoresis run.

No sizing data or size quality fails

- 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.
 - b) Incorrect size standard is used. We recommend using GT500 with GT HapScreen® HBB Kit to obtain optimum results.
-

7. Limitations and Disclaimer

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

GT HapScreen® HBB Kit is designed to screen for mutant alleles for beta-globin gene (only those alleles which are previously reported in patient's family), 21, 18 and 13 chromosomes specific trisomies and sex-chromosome aneuploidy. It will not detect other chromosome abnormalities or defect. User must carefully inspect any case of Maternal Cell Contamination and placental mosaicism 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® HBB 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® HBB Kit.

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.

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.