



GT MD1 Detector

Product User Manual

CAT# GT-11601

CTG repeat expansion detection kit for Myotonic Dystrophy
type 1 Disease

Produced by

GENETEK BIOPHARMA GmbH

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1. GT MD1 Detector Overview

- Accurate detection of CTG repeat in exon 1 of the DMPK gene responsible for Myotonic dystrophy type 1 (MD1) Disease.
- Built in panel makes repeat interpretation an easy task
- Can be used on extracted DNA from blood or blood on filter papers such as DNA banking card (GT DBC™) or diluted blood using GT BDB (Blood Diluting Buffer).
- Fragment analysis using capillary electrophoresis incorporating size standard gives precise size for each allele
- 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.

1.1. Intended Use

The GT MD1 Detector kit is intended to be used to determine the CTG repeat on noncoding region of the DMPK gene for Myotonic dystrophy type 1 Disease detection and interpretation. It can show size of the expansion and allele location on the panel showing severity of the disease.

1.2. Introduction to Huntington Disease and the GT MD1 Detector kit usage

Myotonic dystrophy type 1 (DM1) is a multisystem disorder that affects skeletal and smooth muscle. It also affects many other organs such as the eye, heart, endocrine system, and central nervous system. The clinical manifestation differs in different individual due to extend of CTG expansion from mild to severe. Three somewhat overlapping phenotypes are recognized: presymptomatic to mild, classic, and congenital.

DM1 is caused by expansion of a CTG trinucleotide repeat in the noncoding region of the DMPK gene.

The GT DM1 Detector kit is designed to amplify a 152 bp region on the DMPK gene; though the actual size depends on the number of CTG repeat within the amplified region. The amplified fragment is then subject to capillary electrophoresis using any of Applied Biosystems® 3130/3130xL and 3500/3500xL Genetic Analyzers, or similar CE apparatus. Number of CTG repeats can be quickly determined by using the bins and panel provided with the kit. Individual with repeat number less than 37 are normal; 37-49 are premutation; 50-150 are symptomatic and more than 150 are more severely affected.

Table 1. Fragment sizes used for designing panel for the GT MD1 Detector Kit

No.	Panel	Size Range	Disease severity
1	5-35 CTG	(124-215)	Normal
2	36-50 CTG	(218-260)	Presymptomatic
3	51-150 CTG	(263-559)	Classical
4	More than 150 CTG	(560-600)	Juvenile-Congenital

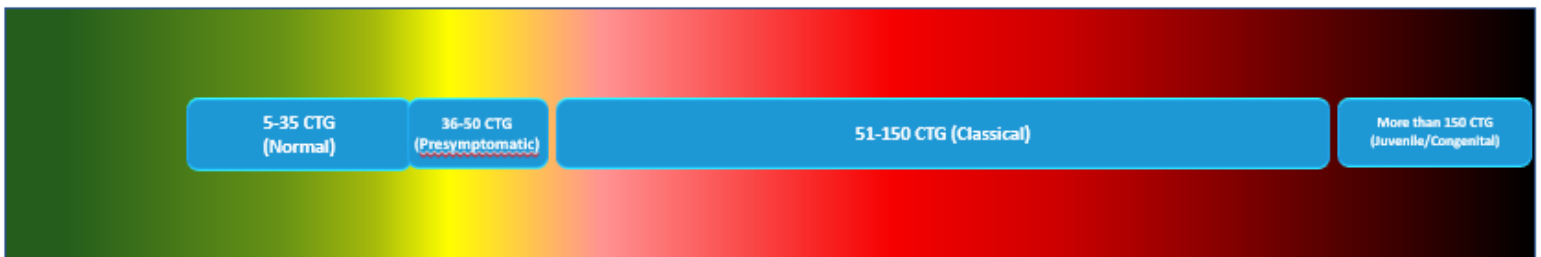
1.3. Five-dye fragment analysis

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

Table 2: The fluorescent dyes used in GT MD1 Detector kit

Name	6-FAM	GTE600
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GT MD1 Detector Panel



GTE600 Size Standard

Figure 1. Diagram shows panel with different repeat numbers and also gradient from normal (dark green to juvenile form (dark red). The lower panel shows repeat numbers and severity or type of disease using the GT MD1 Detector Kit analyzed with GTE600 Size Standard.

2. PCR

2.1. Storage Condition

- Store at -20 °C
- Keep the primer mix in a dark place (because of fluorescently labeled primers)
- Avoid frequent freeze and thaw (store the materials in small aliquots)
- Low quality result may be obtained after the expiration date (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 lab at -20 (Pre-PCR area). GTE600 Size Standard, GTM5 v2 Matrix Standard are amplicons and should be stored in post-PCR area at 2-8°C. 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 in another freezer (PCR product)

BOX-A			BOX-B		
	Tube Label	Tube cap color		Tube Label	Tube cap colour
1	PCR Mix		1	GTE600 Size Standard	
2	Primer Mix		2	GTM5 v2 (Optional)	
3	GT HSTaq				
4	GT QCDM102 (Control DNA-50ng/µl)				
5	GT QCDDMD (Control DNA-50ng/µl)				
	GT QCW (H2O)				

Not provided with GT MD1 Detector (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 Biosystems 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) (is supplied with the kit and also can be obtained from Genetek Biopharma)

2.2.3. PCR amplification by GT MD1 Detector

- 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 MD1 Detector components

Component	Volume for 1 reaction[μ l]
GT QCW (H ₂ O)	10
PCR Mix	10
Primer Mix	1
GT HSTaq	0.5

2.2.5. GT MD1 Detector 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).
- Mix by pipetting or Vortex Master Mix briefly.
- Transfer 21.5 μ L of Master Mix into each 0.2 ml PCR tube for each sample.
- 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 μ l 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	72 °C	72 °C	4 °C
20 min	1 min	70 sec	80 sec	10 min	∞
30 Cycles					

- After completion of PCR, store the PCR products at 2-6°C until analyzing in a 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 for running on the Genetic Analyzer at later days.
- 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 with each multiplex PCR. We recommend using GT QCDM102 and GTQCDMD as a Quality Control especially early on during testing our kit or setup. The result for this control DNA can be found from Genetek website and also in our latest user manual.
- According to the quality or quantity of DNA template, you may require changing the number of cycles in PCR program or the amount of DNA used.

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®) Genetic Analyzers 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 MD1 Detector 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-analyzed.

3.1. Instrument Preparation Applied Biosystems® 3500/3500xL Genetic Analyzer (before the first use of GT MD1 Detector 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 only (which is provided with the kit). Since the dyes used in this kit is unique, the machine must be calibrated with GTM5 v2 Matrix Standard before using the kit. Please find detailed protocol for spectral calibration with GTM5 v2 Matrix Standard contacting us at support@genetek.de or by logging into your personal account with [Genetek](#).

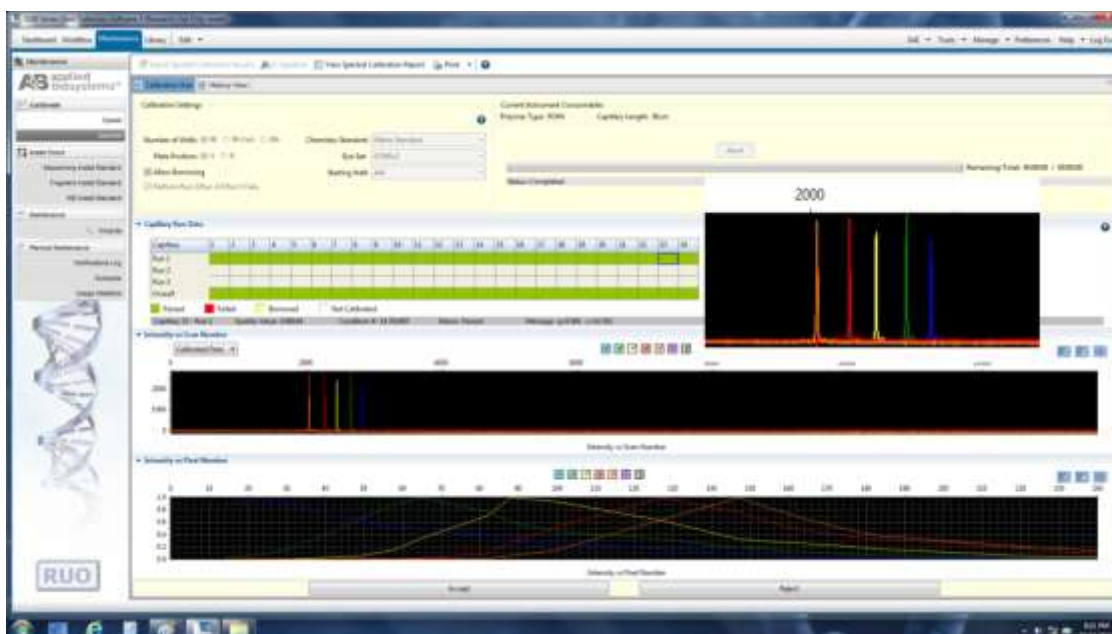


Figure 2. An example of a successful spectral calibration with GT 5-dye system on Applied Biosystems® Genetic Analyzer 3500xL

- 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.

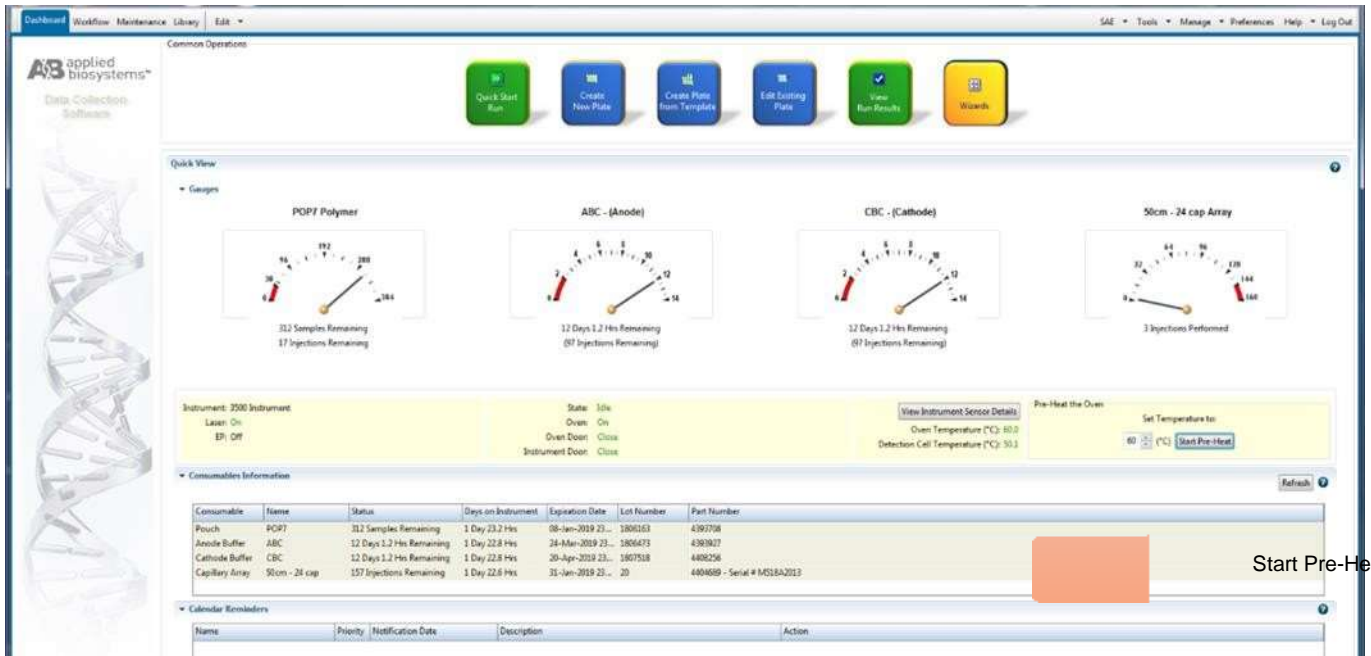


Figure 3. Dashboard of Applied Biosystems® 3500 Data Collection software

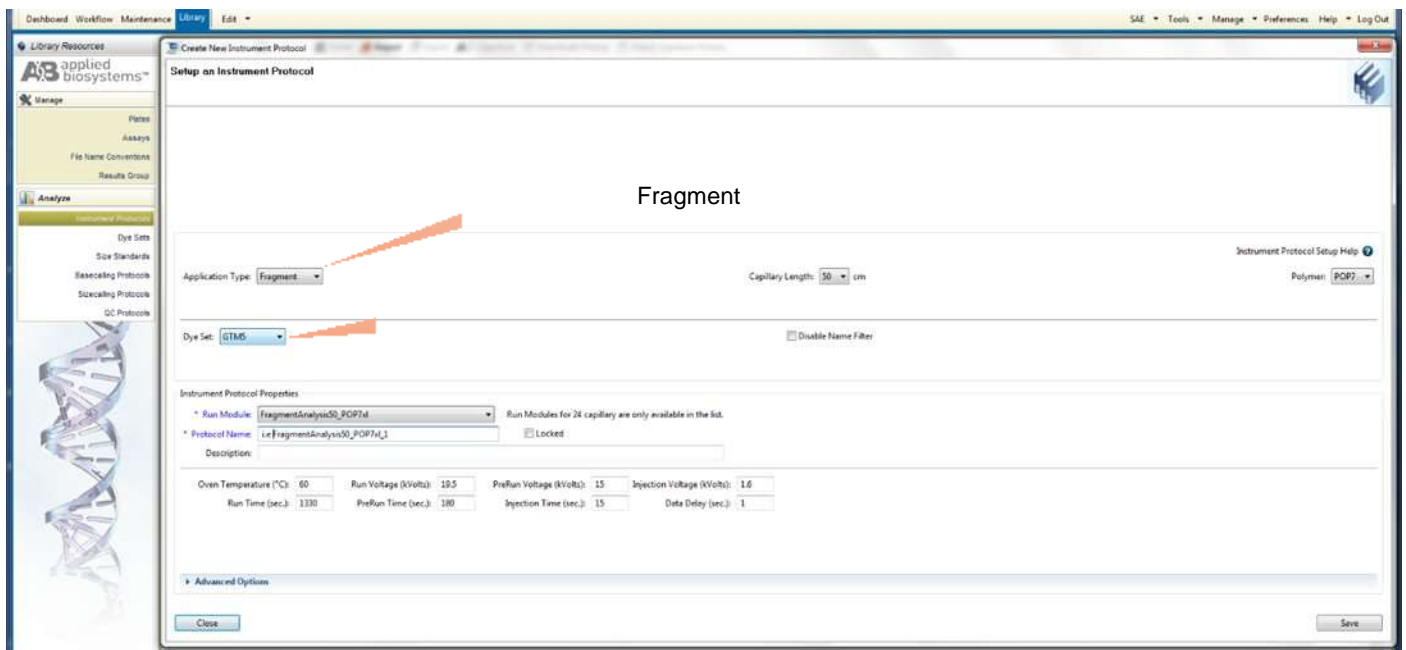


Figure 4. Screenshot for the “Create New Instrument Protocol” window on Applied Biosystems® 3500 Data Collection software

- User can apply settings as shown in the Figure 4. 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 MD1 Detector 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 4)

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 5)

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 “GTE600” and as Dye Color select “Orange”

The fragments size in the GTE600 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, 500, 520, 540, 560, 570, 580, 600 and 630 and they should be added until 630 is added as the last number as shown in figure below in the “Enter new Size Standard definition” box and the clicking “Add Size(s)”, then save the settings.

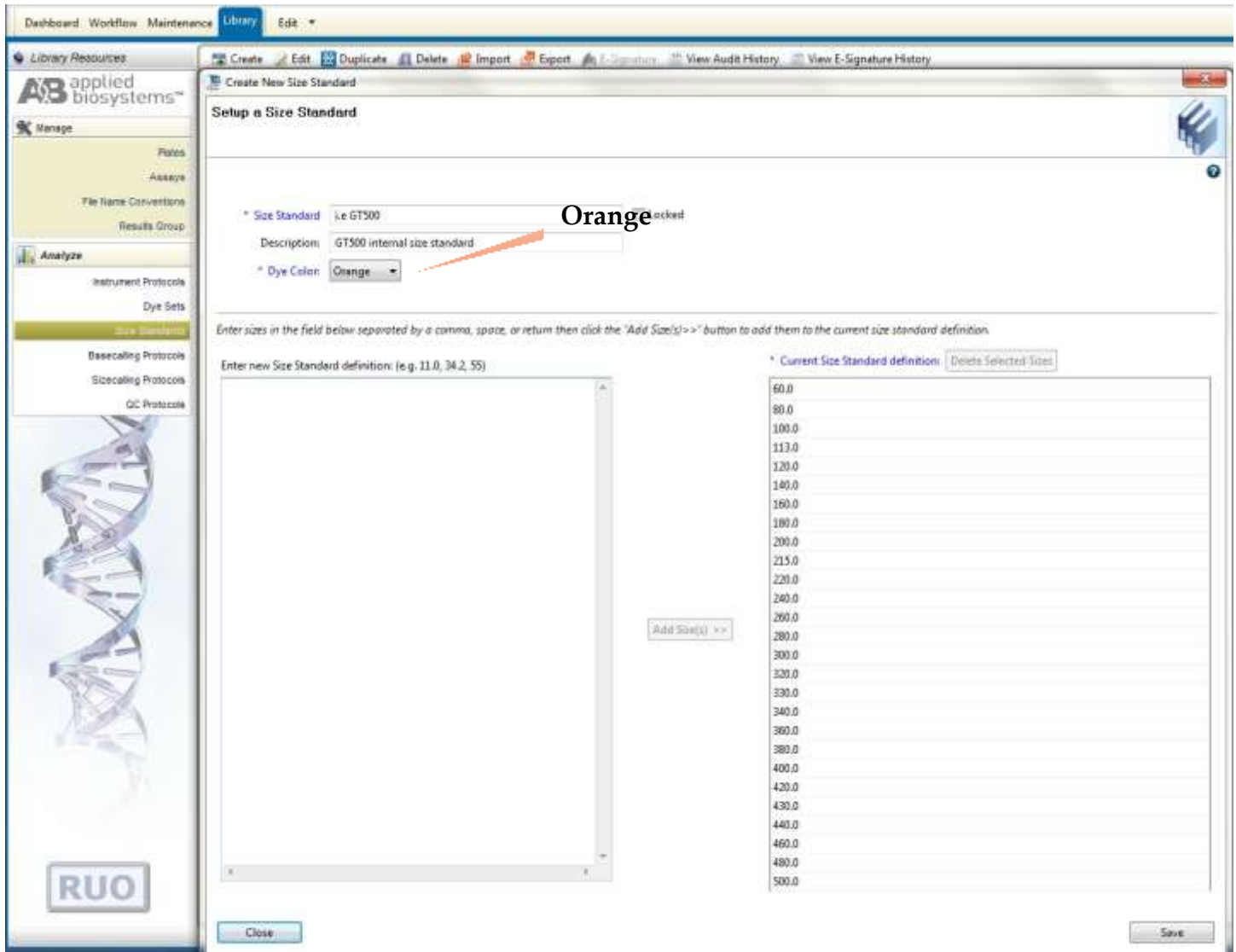


Figure 5. Screenshot for the “Create New Size Standard” window on Applied Biosystems® 3500 Data Collection software

3.1.3. Create a QC protocol

- a) Navigate to the *Library*
- b) Select “QC Protocols”
- c) Select “Create” (Figure 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 protocol as “i.e. GTE600” and select the *Size Standard* “GTE600”

Users can select settings as shown in the Figure 6 or alternatively may define these settings based on internal validation condition for GT MD1 Detector on the Applied Biosystems® 3500/3500xL Genetic Analyzer.

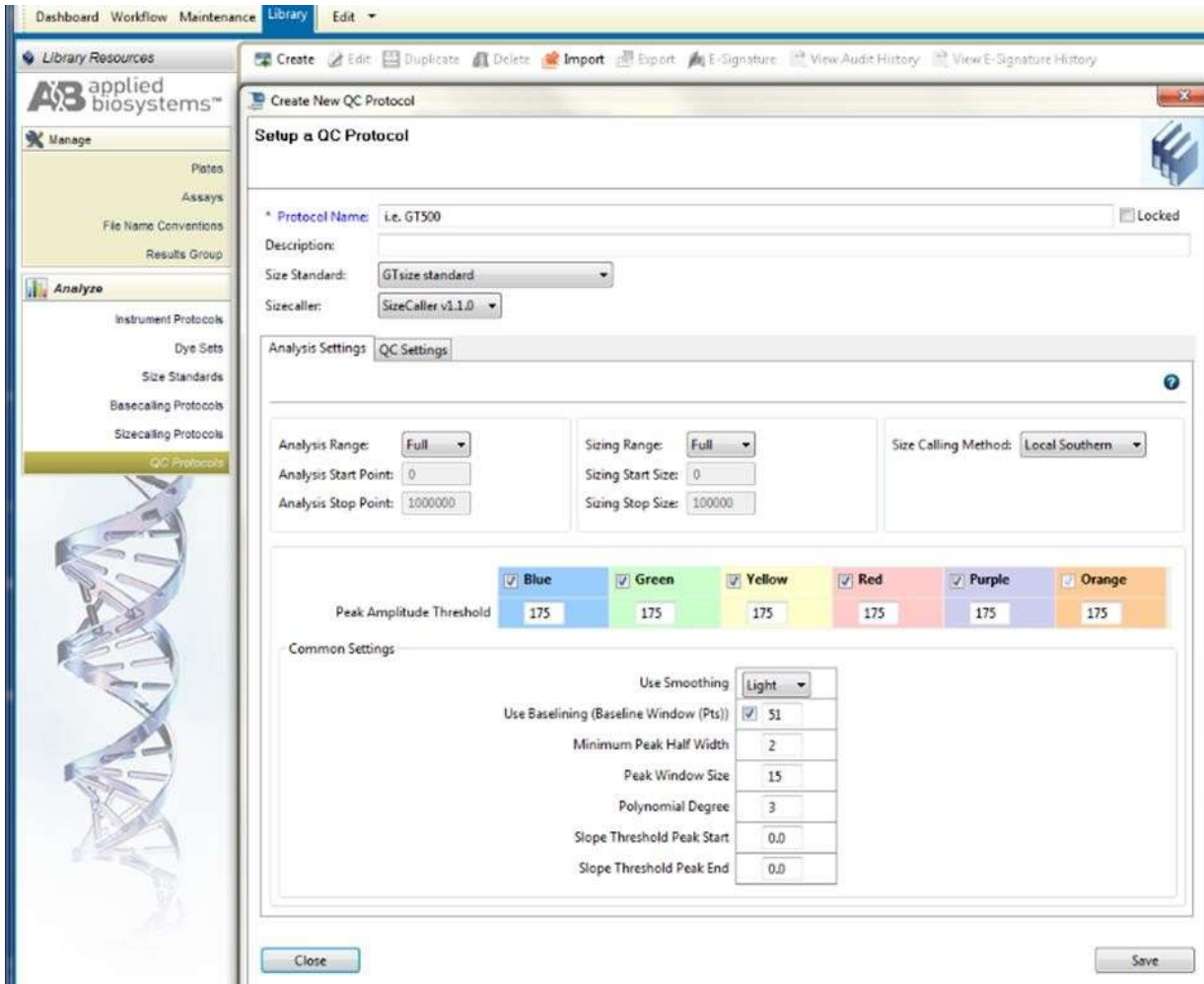


Figure 6. Screenshot for the “Create New QC Protocol” window on Applied Biosystems 3500 Data Collection software

3.1.4. Create a new Assay

- a) Navigate to the *Library*
- b) Select “Assays”
- c) Select “Create” (Figure 7)

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.

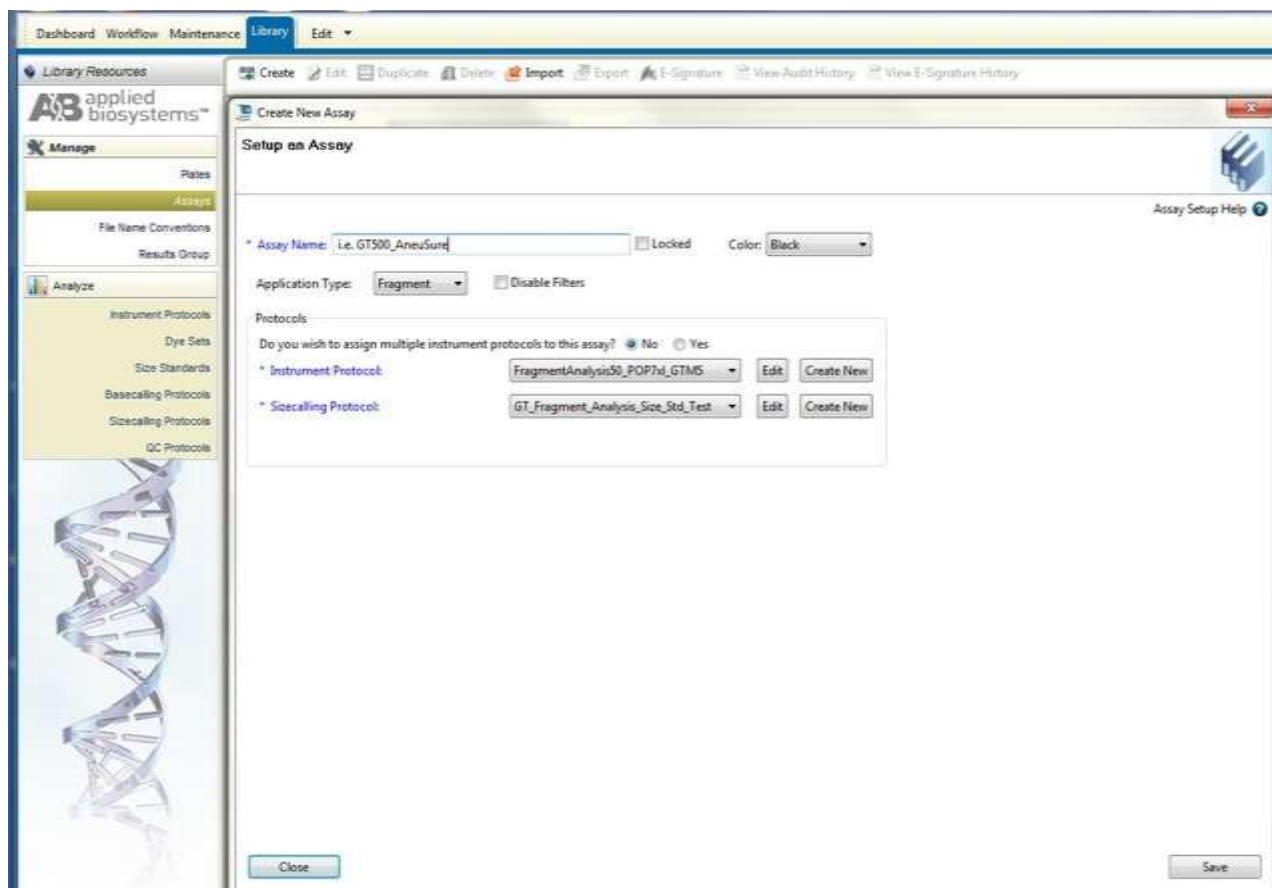


Figure 7. Screenshot for the “Create New Assay” window on Applied Biosystems 3500 Data Collection software

3.1.5. Create a new File Name Conventions

- a) Navigate to the *Library*
- b) Select “*File Name Conventions*”
- c) Select “*Create*” (Figure 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) Choose the *File Name Attributes* according to your lab practices

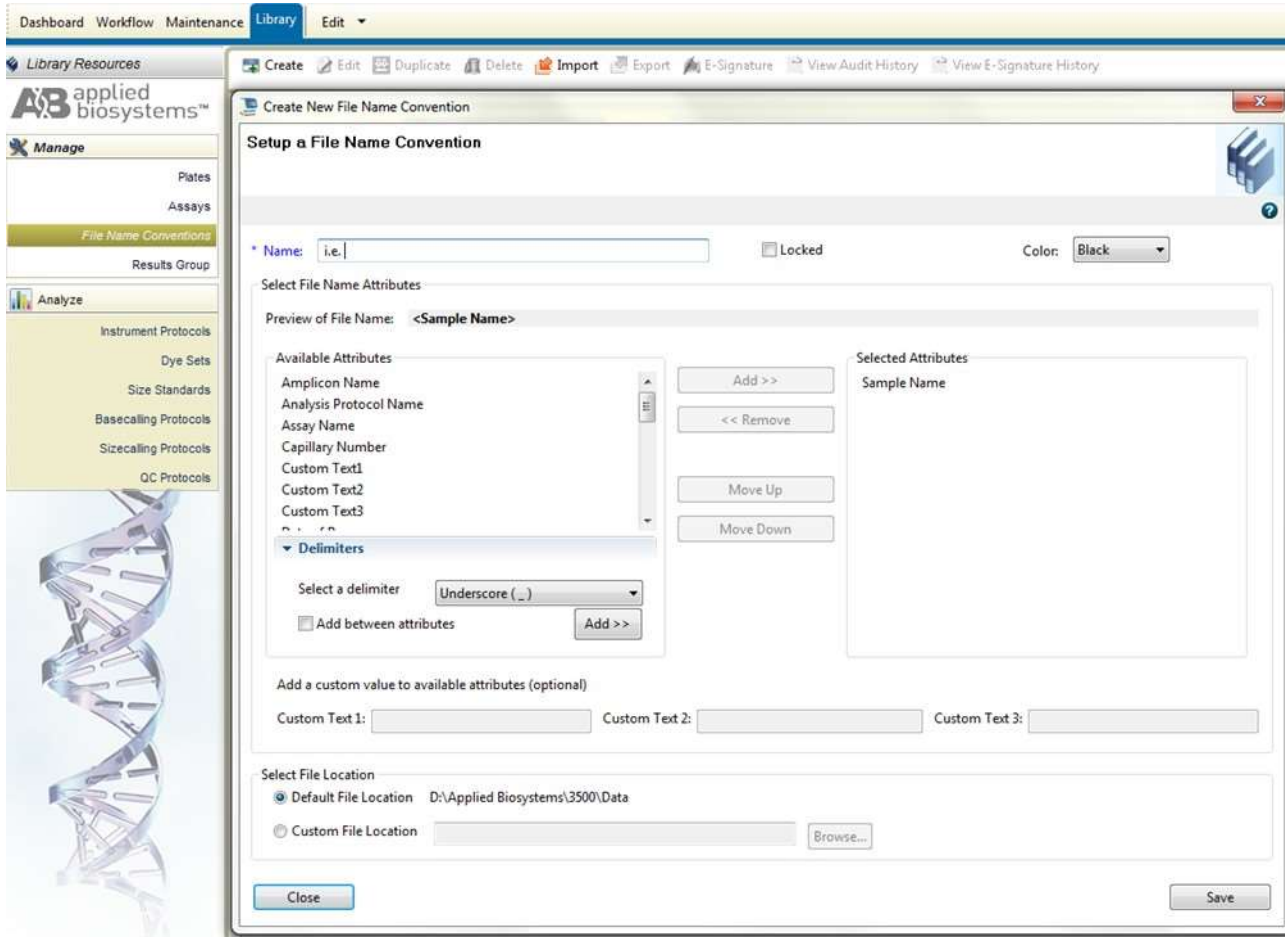


Figure 8. Screenshot for the “Create New File Name Convention” window on Applied Biosystems 3500 Data Collection software

3.1.6. Create a new Result Group

- a) Navigate to the *Library*
- b) Select “Results Group”
- c) Select “Create” (Figure 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 *Results Group Attributes* according to your lab practices

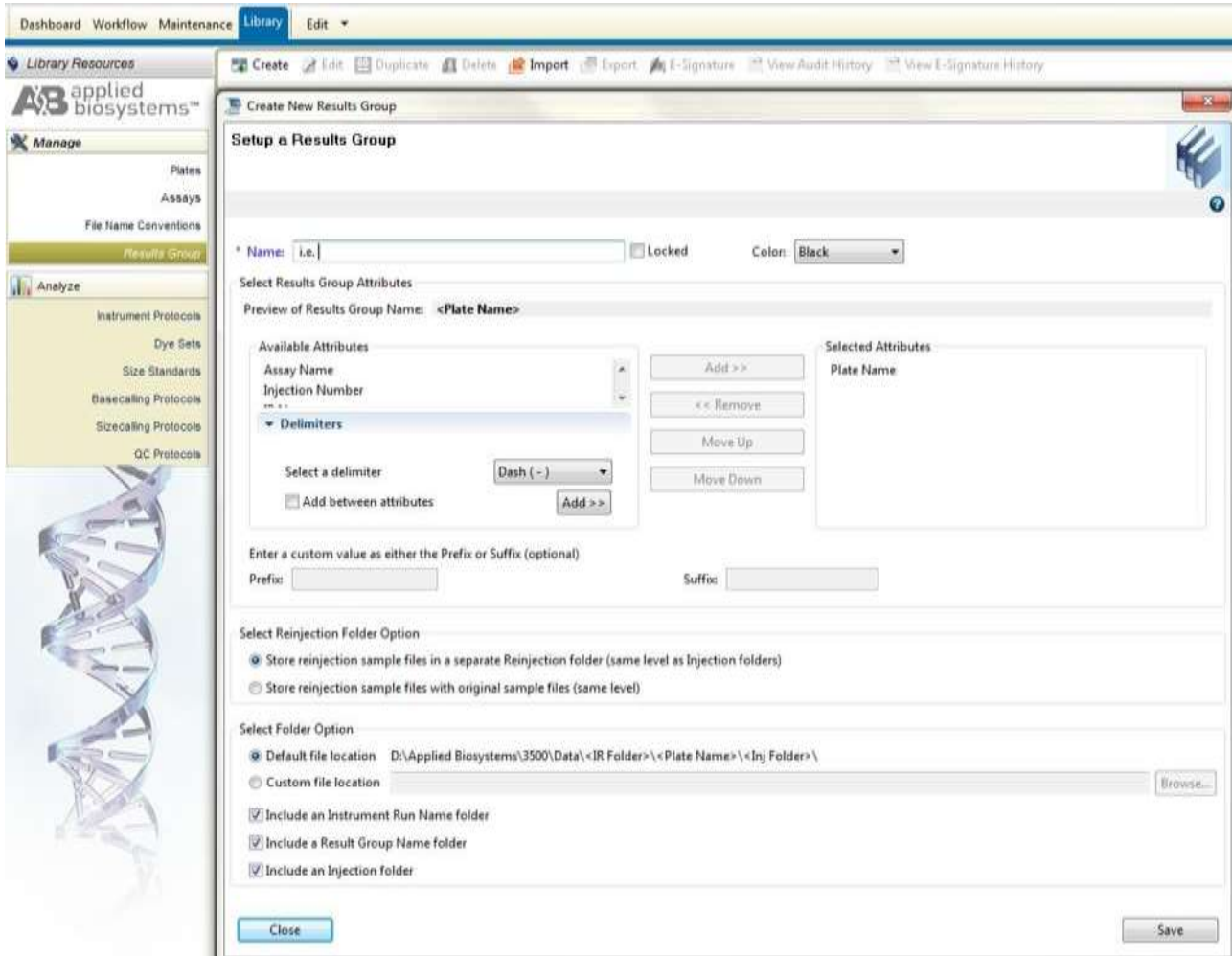


Figure 9. Screenshot for the “Create New Result Group” window on Applied Biosystems 3500 Data Collection software

3.1.7. Create a New Plate

- a) Navigate to the *Library*
- b) From the manage menu select “Plates”
- c) Select “Create” (Figure 10)
- d) Define a name for the plate
- f) Choose plate type “*Fragment Analysis*” from the drop-down menu



Figure 10. Screenshot for the “Defining plate properties” window on Applied Biosystems 3500 Data Collection software

3.1.8. Select “Assign Plate Contents”

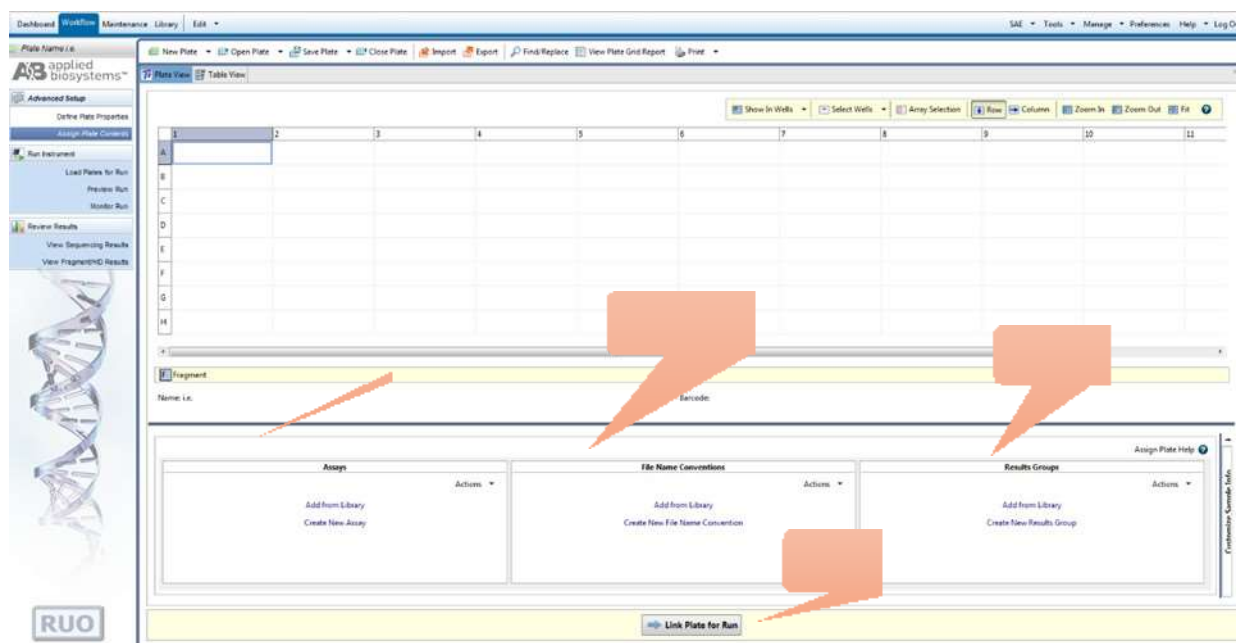


Figure 11. Screenshot for the “Assign Plate Contents” window on Applied Biosystems 3500 Data Collection software

- Define sample names to wells.
- In the *Assign Plate Window* (Figure 11), 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 12). Select “Start Run” after loading the plate.

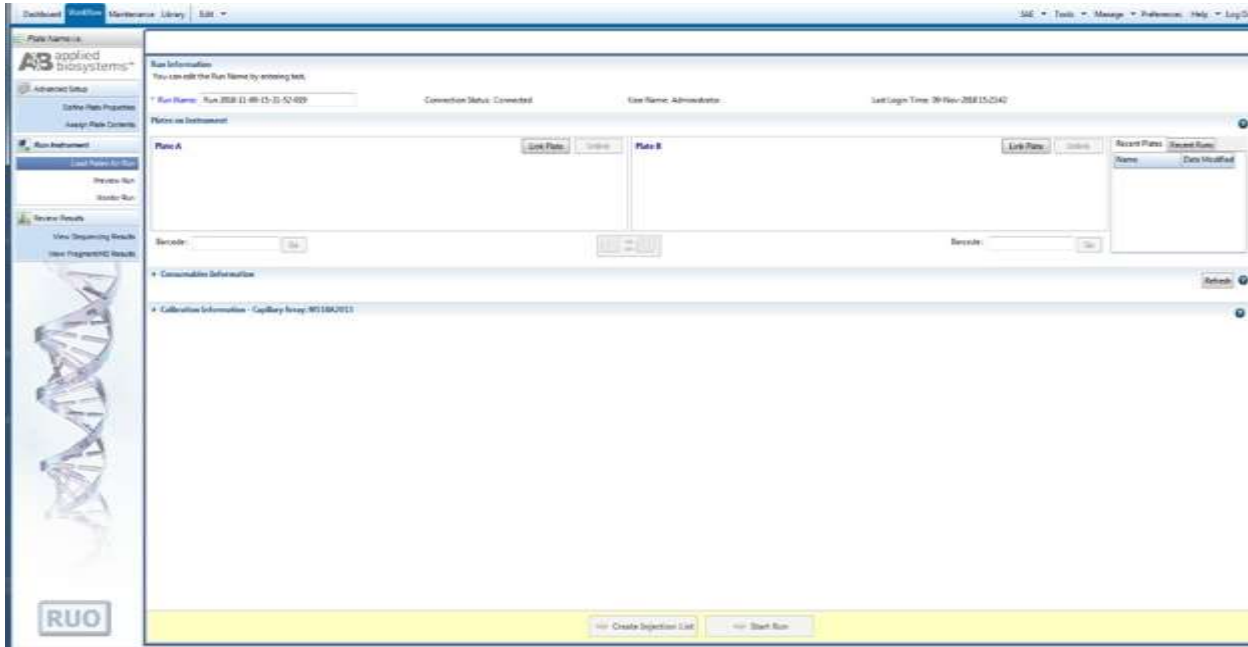


Figure 12. Screenshot for the “Run Information” window on Applied Biosystems® 3500 Data Collection software

3.2. Instrument Preparation Applied Biosystems® 3130/3130xl Genetic Analyzer (before the first use of GT MD1 Detector 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 MD1 Detector 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 by contacting us at support@genetek.de or by logging into your personal account with Genetek.

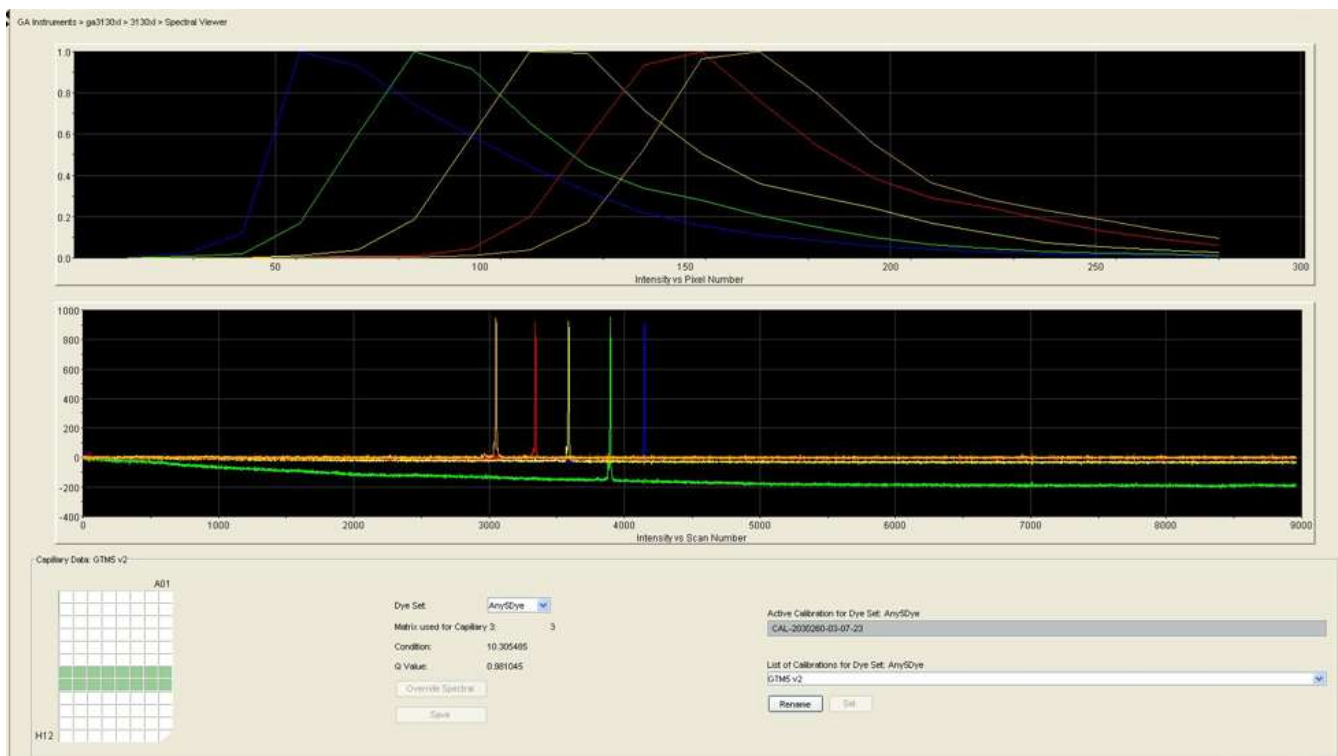


Figure 13. An example of a successful spectral calibration with GT 5-dye system on Applied Biosystems Genetic Analyzer 3130xl

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 MD1 Detector)
- b) Type: Regular
- c) Template: FragmentAnalysis50_POP7 (default template for the capillary array and polymer used)
- d) Click OK

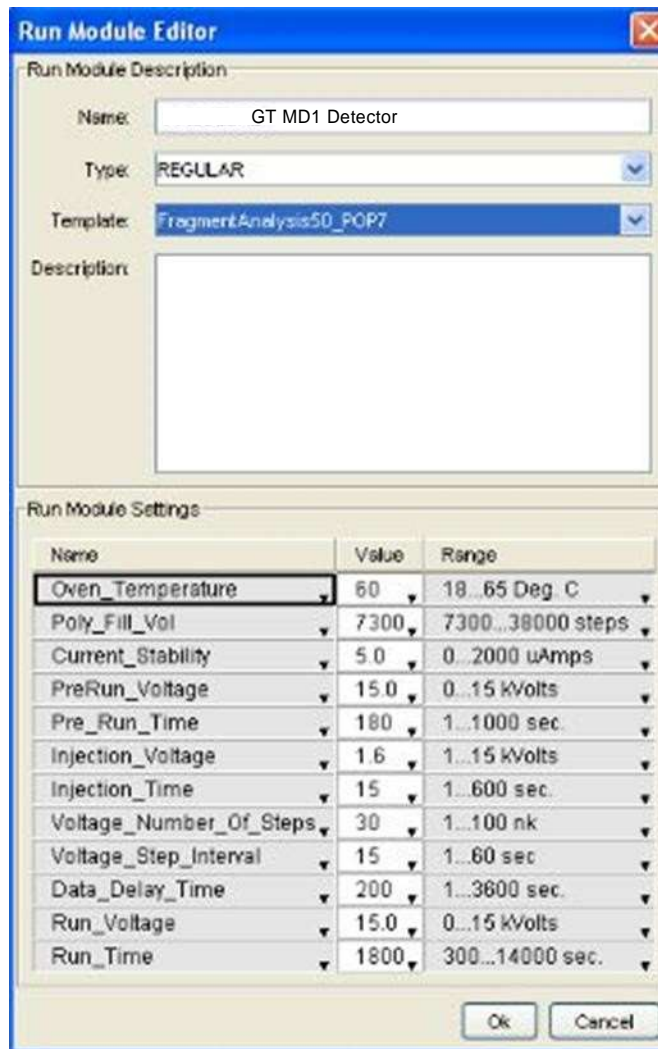


Figure 14. Screenshot for the “Module Manager” window on Applied Biosystems 3130 Data Collection software

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 MD1 Detector)
- c) Type: Regular
- d) Run Module: Select the Run Module created (GT MD1 Detector)
- e) Dye Set: Any5Dye
- f) Click OK



The screenshot shows a 'Protocol Editor' dialog box with the following fields and values:

- Name: GT MD1 Detector
- Description: (Empty)
- Type: REGULAR
- Run Module: GT MD1 Detector
- Dye Set: Any5Dye

Buttons: OK, Cancel

Figure 15. 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.

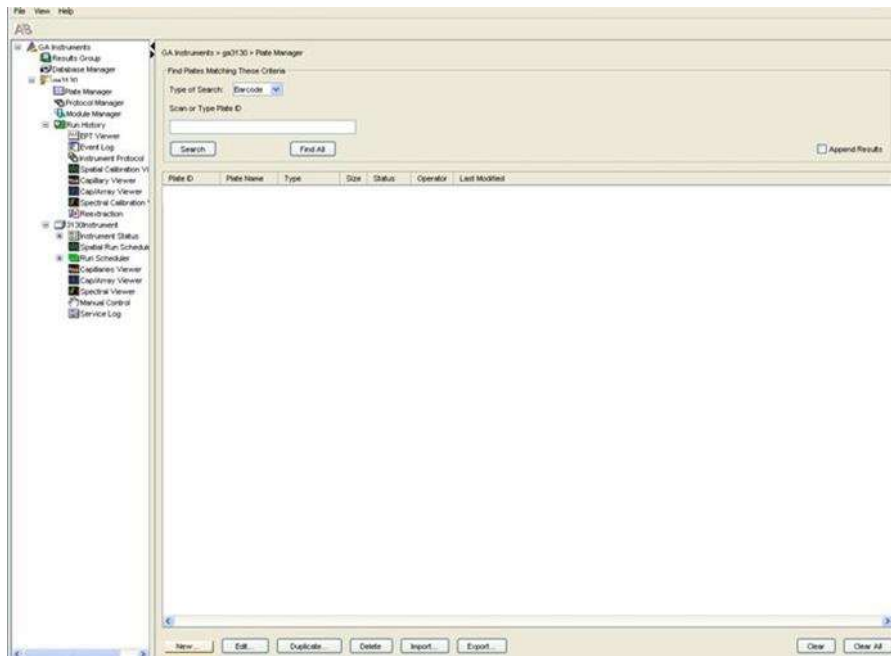


Figure 16. Screenshot for the “Plate Manager” window on Applied Biosystems 3130 Data Collection software

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 analyzed 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 17. 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

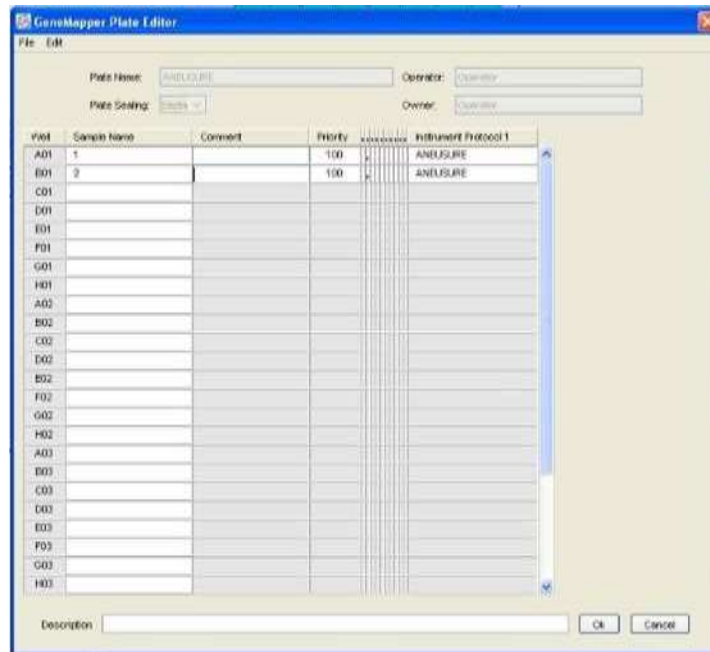
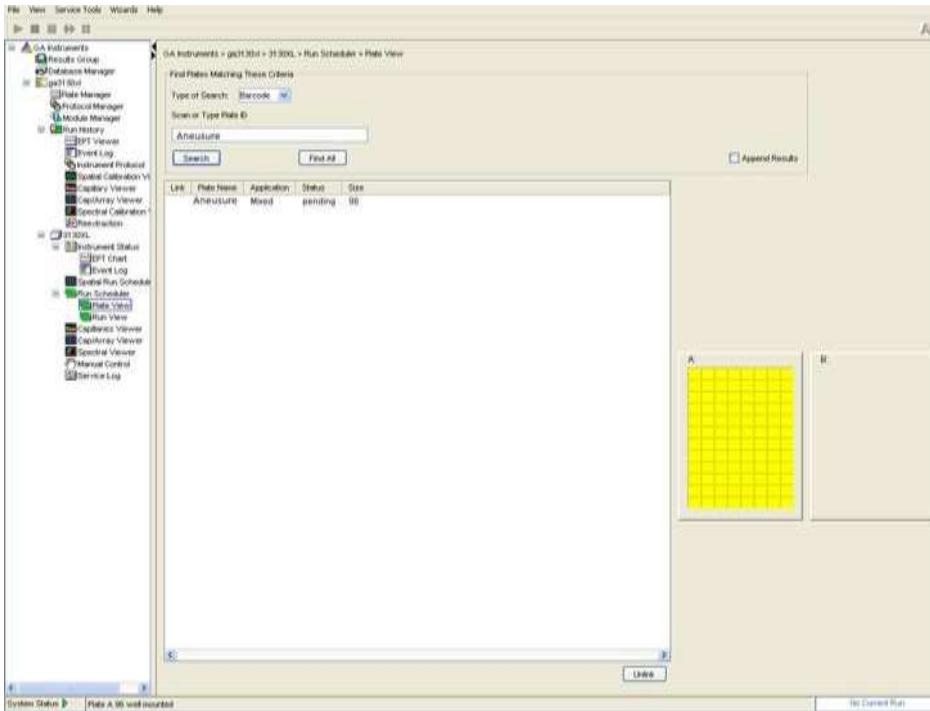


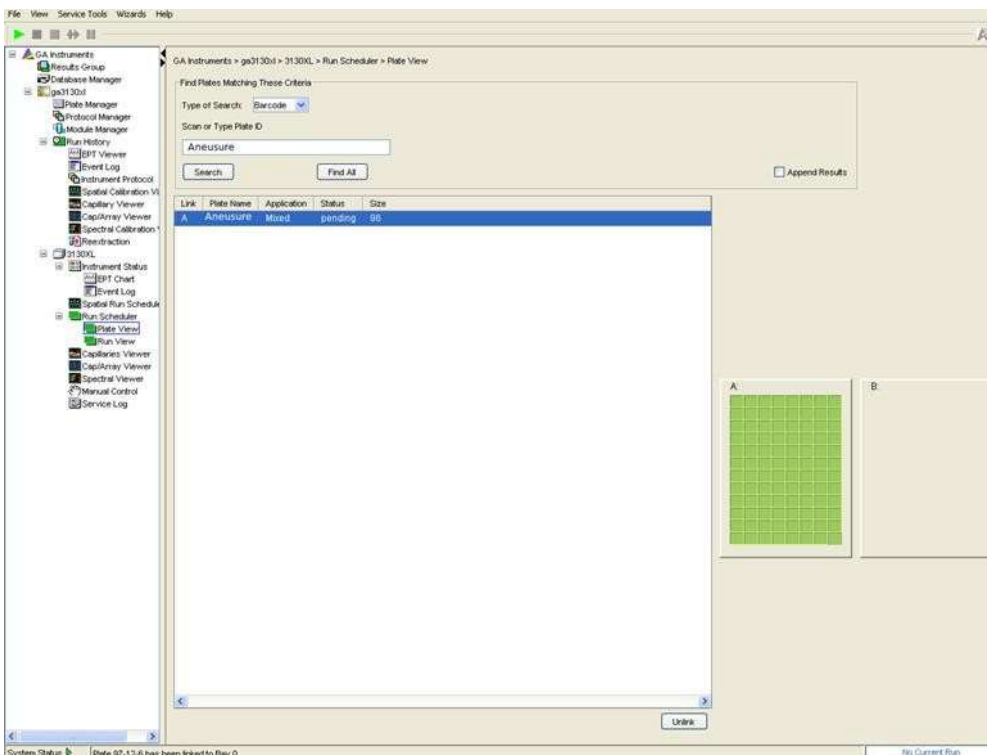
Figure 18. Screenshot for the “GeneMapper Plate Editor” window on Applied Biosystems 3130 Data Collection software

- From the left navigation window, select Run Scheduler, search for GT MD1 Detector (plate name).



a

- 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.



b

Figure 19 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 20. “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 MD1 Detector kit is GTE600.

- Vortex and spin 9.5 μL (x number of samples) Hi-Di™ Formamide and 0.5 μL GTE600 (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:
 - 94°C for 3 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 GTE600 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 MD1 Detector, 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.
- Please consult [best practice guidelines](#) for interpreting and analyzing DM1 results when using any kit including GT MD1 Detector Kit. More information can also be found in [GeneReviews](#) from NCBI.

4.2. General guideline for the analysis of GT MD1 Detector results

GT MD1 Detector PCR products are observed with 5-dye system on an electropherograms in the GeneMapper® software. For the analysis, import GT MD1 Detector panels. It can be downloaded from our website or contact us at support@genetek.de.

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.
- 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 size of between 100 to 630 bp.
- Quality control DNA (if used) should show expected results as shown here – see Examples profile GTQCDM102 and GTQCDMD.
- There should not be excessive bleed-through between dye colors or “Pull-up” effect in the electropherograms.

5. Sample cases

Below there are several cases either normal or affected or even at risk. The allele has been obtained by methods given in section.

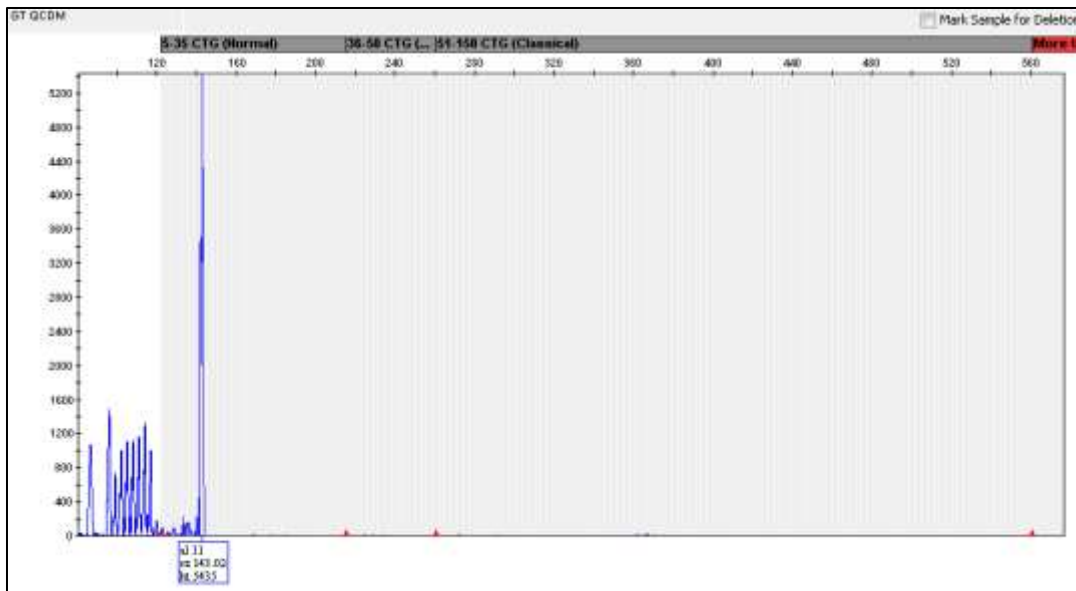


Figure 21. An example of a normal individual (i.e. GTQCDM) with homozygote 11 repeats. The result has been generated with GeneMapper® ID-X 1.5 and 3500xL Genetic Analyzer.

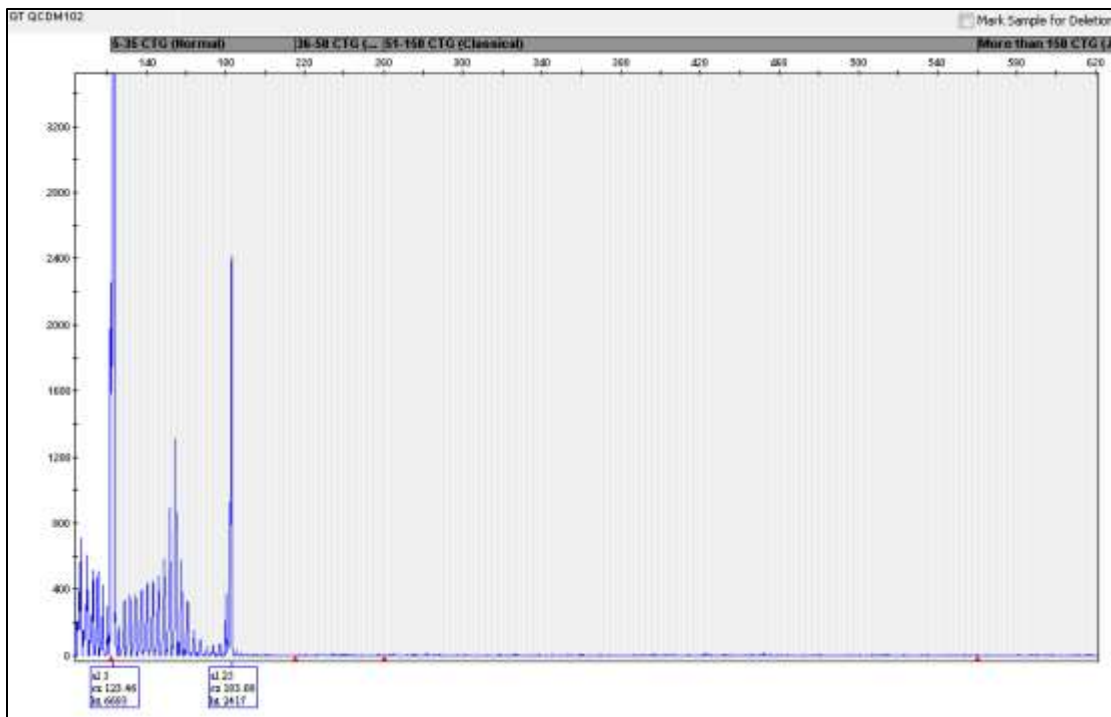


Figure 22. An example of a normal individual (i.e. GTQCDM102) with heterozygote repeats of 5 and 25. The result has been generated with GeneMapper® ID-X 1.5 and 3500xL Genetic Analyzer.

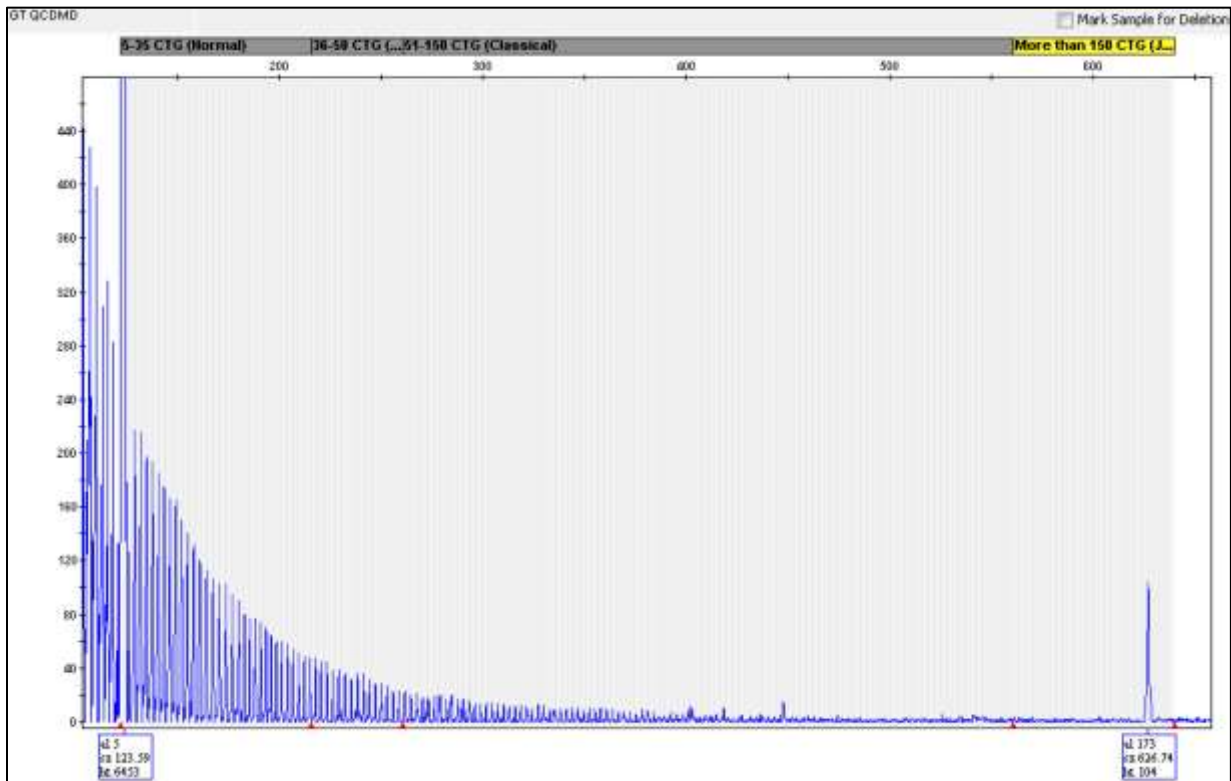


Figure 23. An example of an affected individual (i.e. GTQCDMD) with a normal peak of 5 repeats and an expanded peak of 173 repeats. The data has been generated using GeneMapper® ID-X 1.5 and 3500xL Genetic Analyzer.

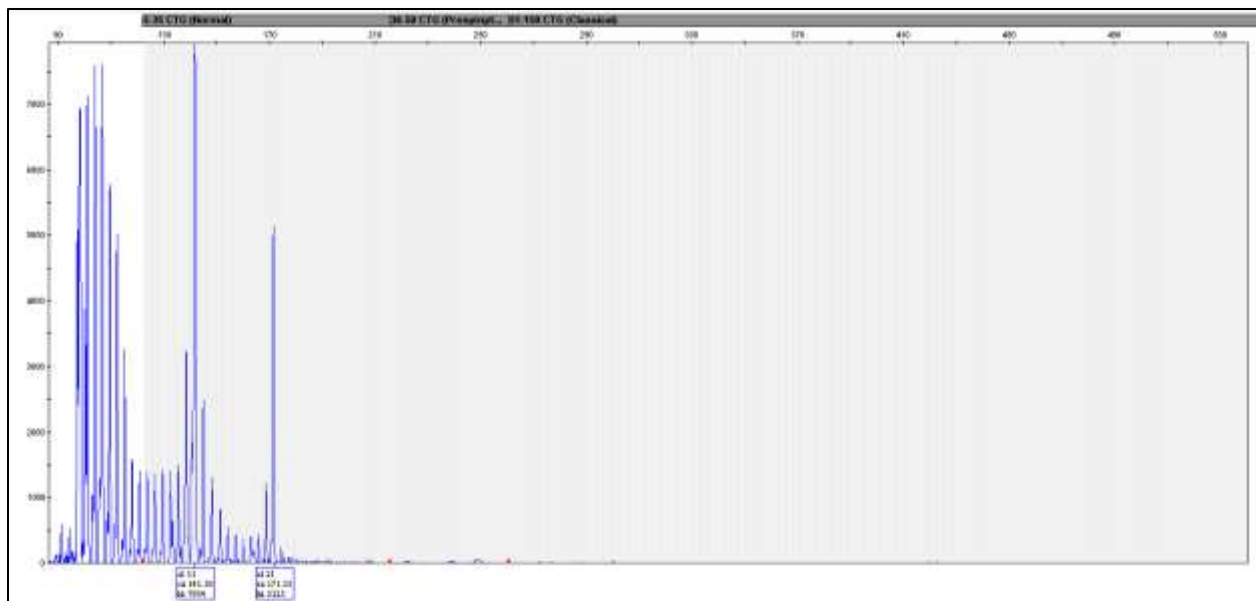


Figure 24. An example of a normal individual with heterozygote repeats of 11 and 21. The result has been generated with GeneMapper® ID-X 1.5 and 3500xL Genetic Analyzer.

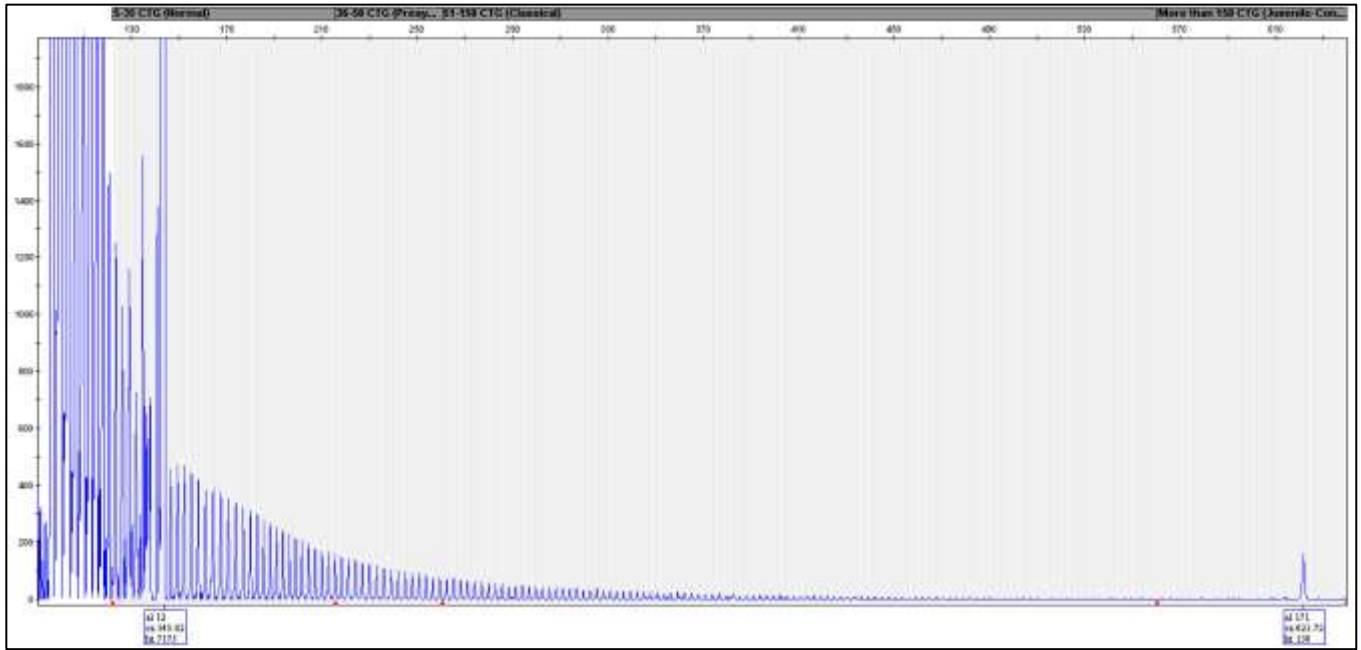


Figure 25. An example of an affected individual with a normal peak of 12 repeats and an expanded peak of 171 repeats. The data has been generated using GeneMapper® ID-X 1.5 and 3500xL Genetic Analyzer.

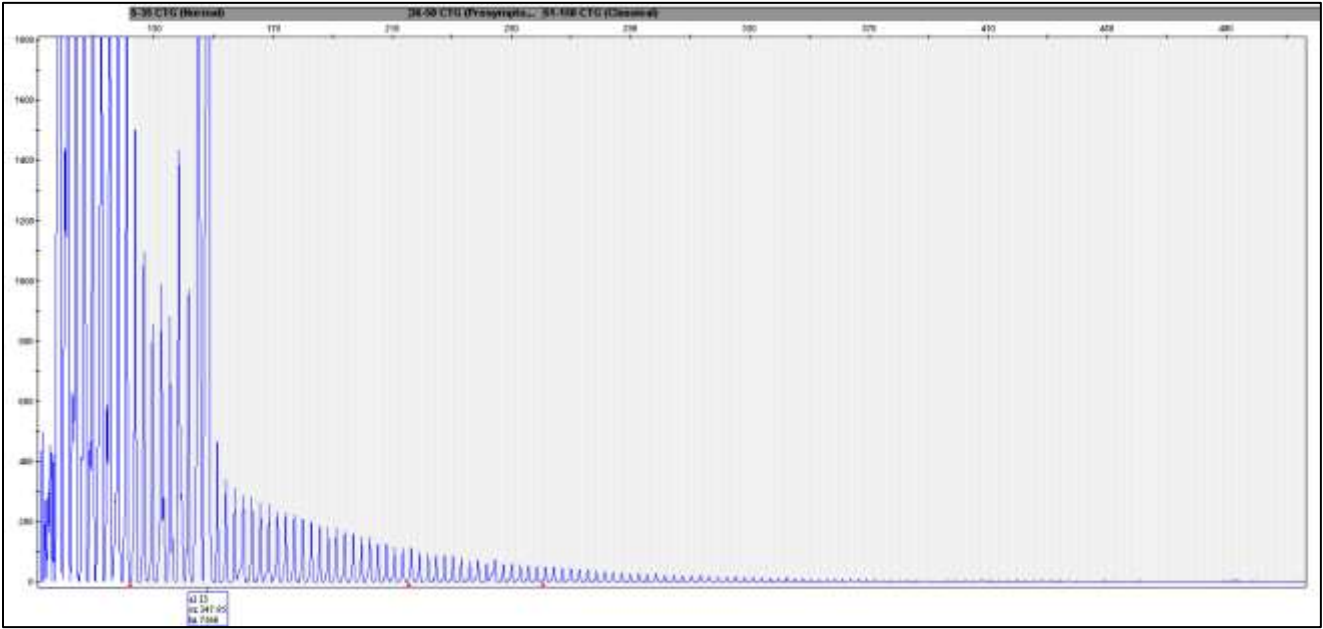


Figure 26. An example of an affected individual with a normal peak of 12 repeats and an expanded peak beyond 200 repeats. Only presence of stutter peaks shows that there is expansion and since the expanded peak can't be seen we assume it be more than 200 repeats. The data has been generated using GeneMapper® ID-X 1.5 and 3500xL Genetic Analyzer.

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 MD1 Detector 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 GTEEx600 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 QCDDM102 and GT QCDDM provided with GT MD1 Detector 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 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"> • 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.
<p>No sizing data or size quality fails</p>	<ul style="list-style-type: none"> • a) Incorrect or no size standard is selected in analysis method or protocol editor. Make sure that size standard option is edited with GTE600 Size Standard. • b) Incorrect size standard is used. We recommend using GTE600 with GT MD1 Detector Kit to obtain optimum results.

7. Limitations and Disclaimer

Any result obtained from GT MD1 Detector or any other diagnostic 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 MD1 Detector or any other diagnostic 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 MD1 Detector Kit is designed to detect the CTG repeat in the MD1 gene. The kit and its associated panel will guide the user to easily determine the number of CTG repeats. The information provided are for guidance and it is expected that the individuals be heterozygote for the mutation, however, in rare cases homozygotes may be observed. Each individual laboratory must interpret the results based on clinical severity and other guidelines.

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 MD1 Detector Kit is for RESEARCH USE ONLY and user must bears all the responsibility for its use in clinical practice. Please consult [best practice guidelines](#) when using any kit including GT MD1 Detector Kit. More information can also be found in [GeneReviews](#) from NCBI.

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

<u>Description</u>	<u>Symbol</u>
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. [Kamsteeg et al.](#); Best practice guidelines and recommendations on the molecular diagnosis of myotonic dystrophy types 1 and 2. Eur J Hum Genet. 2012; 20(12): 1203–1208.
2. DNA Fragment Analysis by Capillary Electrophoresis User Guide by Applied Biosystems® Publication [Number 4474504](#).
<https://www.thermofisher.com/content/dam/LifeTech/global/Forms/PDF/fragment-analysis-chemistry-guide.pdf>
3. [Best Practice Guidelines](#) for Internal Quality Control in Genetic Laboratories by Association for Clinical Genetic Science. <https://www.acgs.uk.com/quality/best-practice-guidelines/>
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.