



# ***Matrix Standard GTM6 v2***

**For Spectral Calibration of CE Instruments**

**Product User Manual**

**CAT# GT- 41104**

Produced by

**GENETEK BIOPHARMA GmbH**

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## 1. GTM6 v2 Overview

Genetic Analyzer such as the Compact Spectrum CE System from Promega and Applied Biosystems™ 3500, 3500/xl, SeqStudio Genetic Analyzer platforms, require accurate spectral calibration to evaluate profiles generated with multiple dye (fluorescence labels) channels. The GTM6 v2 Matrix Standard is produced by Genetek and consists of six DNA fragments labelled with six different fluorescent dyes (6-FAM, GT2907, GT1803, GT2712, GT2107 and GT600) which are used in our 6-dye kits. Once the spectral calibration is done using GTM6 v2 Matrix Standard, the file is applied during sample detection to calculate the spectral overlap and thus create individual colour signal.

GTM6 v2 Matrix Standard has been developed for use with GT 6-dye systems such as GT Detector Globe (a Kit for Human Identification) and can be used with the Compact Spectrum CE System from Promega and Applied Biosystems™ 3500, 3500/xl, SeqStudio Genetic Analyzer platforms with Data Collection Software v3.0 (ThermoFisher Scientific, TF). For instruction on how to operate your Genetic Analyzer, please refer to User Manual provided by the manufacturer (i.e., ThermoFisher Scientific).

Name	6-Fam	GT2907	GT2712	GT1803	GT2107	GT600
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Figure 1- GTM6 v2 Matrix Standard consists of 6 fluorescent labeled DNA fragment.

A spectral calibration is mandatory for each instrument, **new matrix should be run after the laser or CCD camera** has been realigned/replaced, or changing capillary array length or polymer type. It is recommended to generate a new matrix in case of change in physical location of the instrument or if you begin to see a decrease in spectral separation (pull-up and/ or pull-down peaks) in the raw or analysed data. Every lab should define the occurrence of matrix generation for their instrument.

### 2.1 Intended Use

The GTM6 v2 Matrix Standard is developed to perform spectral calibration when GT 6-dye system is used on Applied Biosystems Genetic Analyzers 3500, It is not for medical diagnostic use.

### 2.2 Product components

Table 1: Cat#41104 product components

Product	Size	Cat. #
GTM6 v2 matrix Standard	48 runs or 2X 24 or 6X 8 capillaries calibrations	GT- 41104-48

### 2.3 Storage conditions

GTM6 v2 is a PCR product and it is recommended to be kept at 2°C to 8°C for better preservation. The GTM6 v2 is light sensitive; please keep the it in the given amber tube only. We recommend storing the GTM6 v2 in a post-amplification (post-PCR) fridge.

## 2. Capillary Electrophoresis

ABI 3500/xL and SeqStudio (Applied Biosystems®) Genetic Analyzers are recommended for 6-dye capillary electrophoresis of the amplified PCR products. Please make sure your ABI Data Collection software supports 6-dye fragment analysis (according to the instrument user manual).

Spectral Calibration must be made using GTM6 v2 Matrix Standard, the machine must be calibrated with GTM6 v2 Matrix Standard before using the kit. Please find detailed protocol for spectral calibration with GTM6 v2 Matrix Standard here - [www.genetek-biopharma.com](http://www.genetek-biopharma.com) or contact us at [support@genetek.de](mailto:support@genetek.de).

## 3. Instrument preparation and spectral calibration using the Applied Biosystems® 3500 Genetic Analyzers

### 3.1. Required Materials (not supplied with the GTM6 v2 Matrix Standard)

- HiDi™ Formamide (TF) or equivalent
- MicroAmp® optical 96-well plate and septa
- Aerosol-resistant pipette tips
- Thermoshaker
- Centrifuge usable with 96-well plates
- 3500 capillary array
- Polymer for 3500
- Anode buffer container (TF)
- Cathode Buffer Container (TF)
- 10X Genetic Analyzer buffer with EDTA

### 3.2. Instrument Preparation using POP7 and 50 cm Capillary Assay

For instrument set up using POP7 and 50 cm capillary array for 6 dyes system using GTM6 v2 matrix standard, please follow steps in the following pages. If you enquire more information, please contact us: [support@genetek.de](mailto:support@genetek.de)

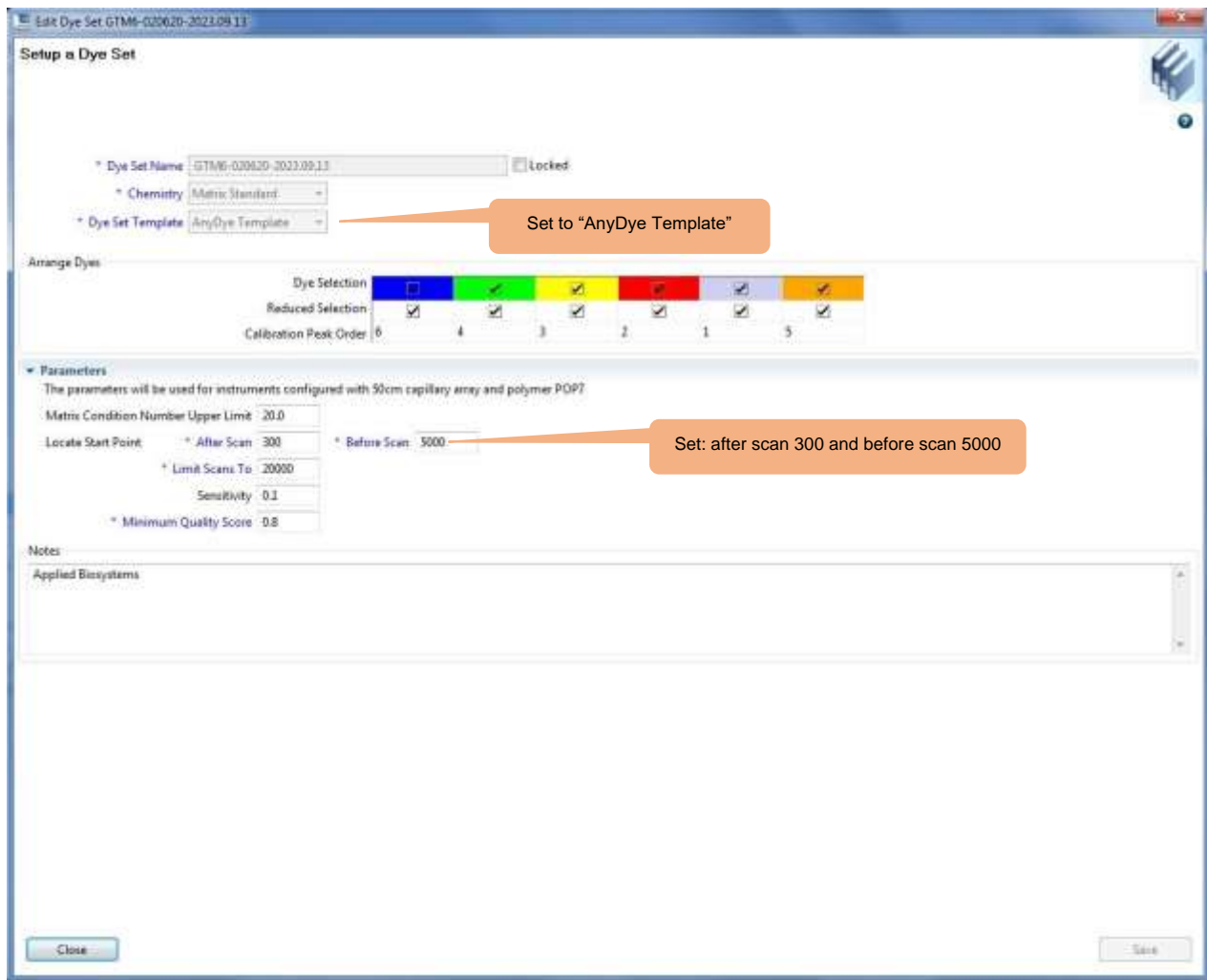
1. We recommend using fresh polymer for spectral calibration with new matrix standard to get the best results. Set the oven temperature to 60°C and then select the **“Start Pre-Heat”** button for at least 20 minutes prior to the first injection to preheat the oven.
2. The Dashboard screen (See Figure 2- Dashboard of Applied Biosystems® 3500 Genetic Analyzer) 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.
3. 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 2- Dashboard of Applied Biosystems® 3500 Genetic Analyzer

Follow the steps below to create the new dye set and perform a spectral calibration for the Genetek 6-dye system:

1. To create the new dye set, navigate the library, highlight “Dye Sets” and select “Create”
2. The Create a New Dye Set window will appear as shown in **Error! Reference source not found..**
3. Name the Dye Set (i.e., GTM6 v2, select “Matrix Standard” for the Chemistry and select ‘AnyDye Template” for the Dye Set Template.



**Figure 3-** Screenshot for “Create New Dye Set Window” on Applied Biosystems® 3500 Genetic Analyzer

1. Change the parameters under the “Parameter Settings” as shown in table 2 and press “Save”.

**Table 2: Parameters for spectral calibration with GTM6 v2 Matrix Standard using POP7 and 50 cm capillary assay.**

Condition # upper limit	20
After Scan	300
Before Scan	5000
Limit Scans to	20000
Sensitivity	0.1
Minimum Quality Score	0.8

2. To perform spectral calibration, go to the Maintenance tab, select “Spectral”, and under the Calibration Run tab choose “Matrix Standard” from the Chemistry Standard drop-down menu and from the Dye Set drop-down menu select the newly created **Genetek Dye Set** (i.e., GTM6 v2).
3. Fill the “Starting Well” position and keep the other settings as shown in Figure 4. Then select “Start Run”.

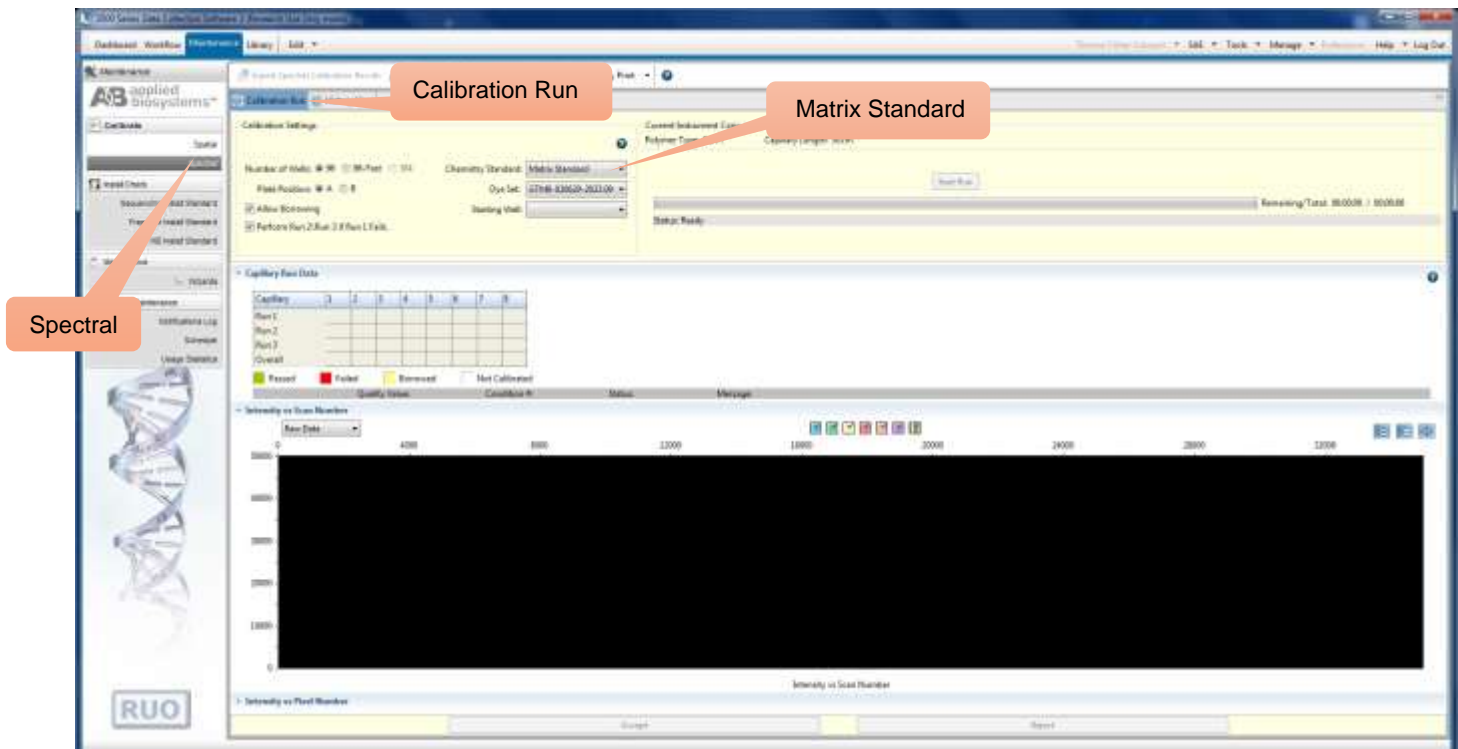


Figure 4- Screenshot for “Create New Dye Set Window” on Applied Biosystems® 3500 Genetic Analyzer

4. When the spectral calibration status is completed, Accept or Reject it according to quality criteria recommended in the 3500 series [Data Collection Software User Manual](#).
5. After the acceptance of the calibration, Matrix calibration data will be stored in the data collection software until there is a change in the capillary array or the software itself. Calibration history can be viewed for quality parameters from the history tab as shown in Figure 5.

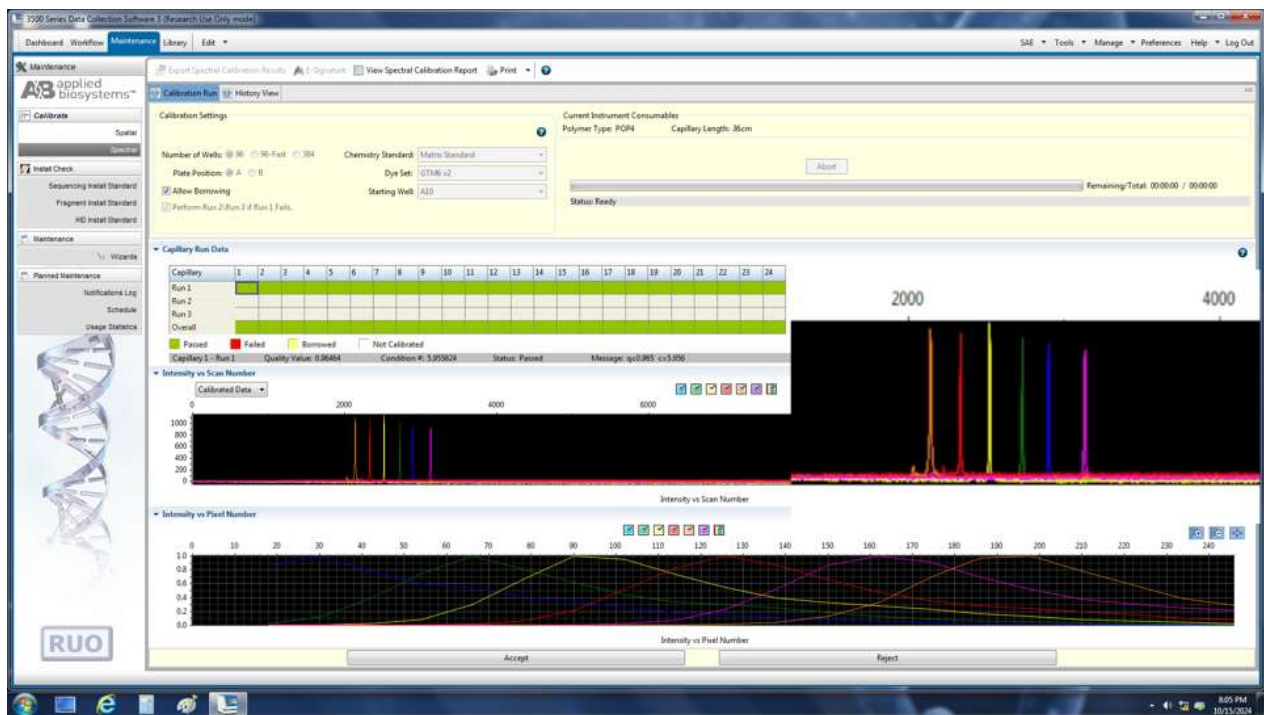
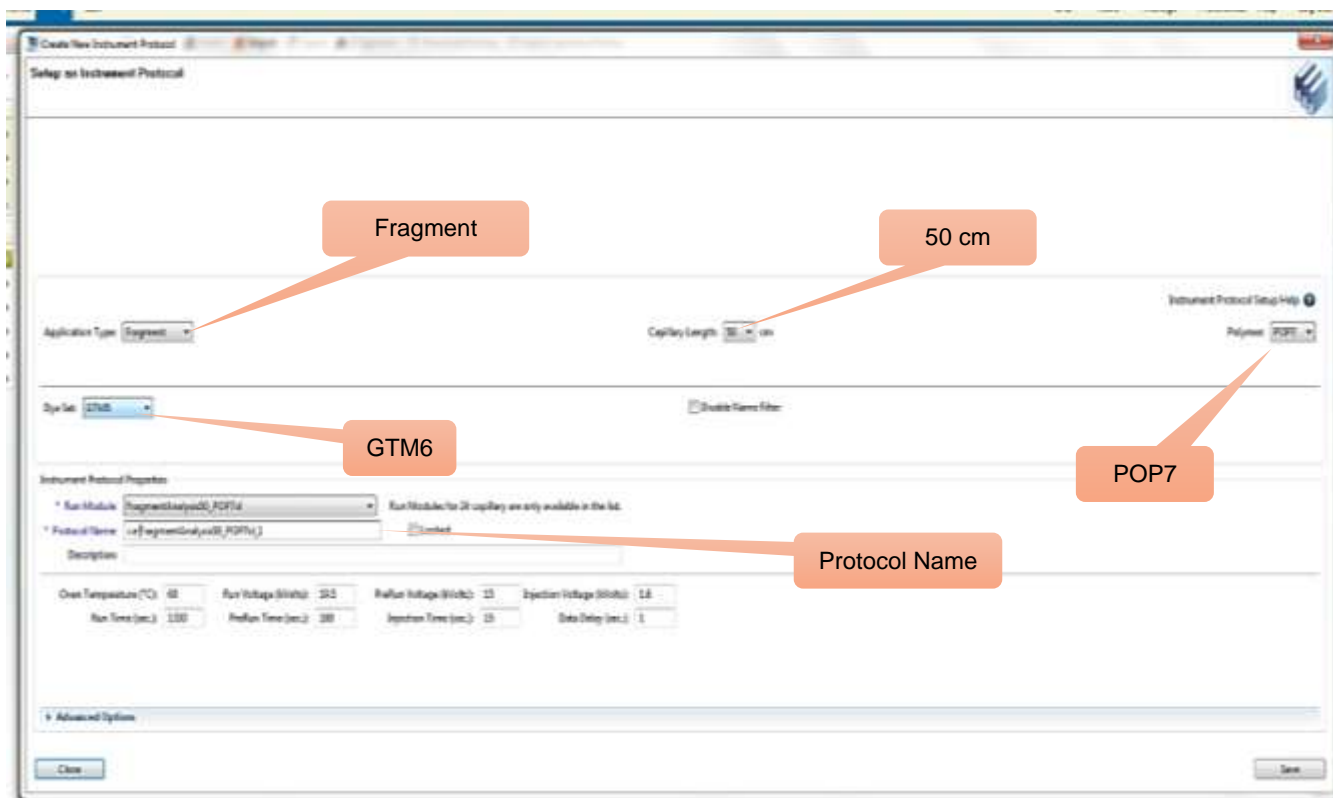


Figure 5- Spectral calibration data history view for the GTM6 v2 Matrix Standard on the Applied Biosystems® 3500xL Genetic Analyzer using POP-7® polymer and Data Collection Software, Version 3.0.

### 3.2.1. Create a New Instrument Protocol

3. Navigate to the Library.
4. Select "Instrument Protocols".
5. Select "Create" (Figure 6).
6. The Instrument Protocol Properties for POP7 is shown in Table 3.



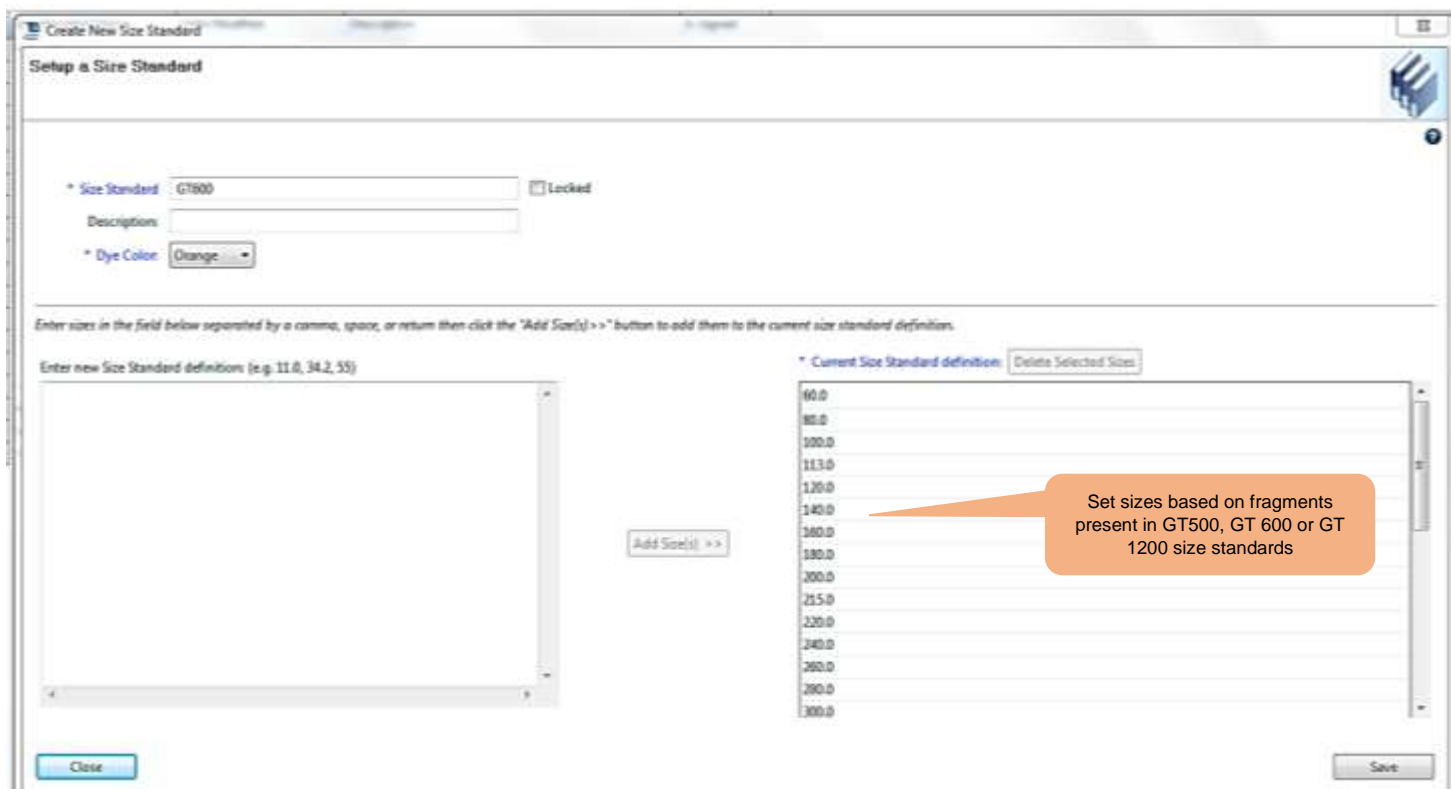
**Figure 6-** Screenshot for the "Create New Instrument Protocol" window on Applied Biosystems 3500 Data Collection software

**Table 3: Parameters for spectral calibration with GTM6 v2 Matrix Standard using POP7**

Parameters	POP7 and 50 cm Capillary Array
Oven Temperature (°C)	60
Run Time (sec.)	1330
Run Voltage (kVolts)	19.5
PreRun Time (sec.)	180
PreRun Voltage (kVolts)	15
Injection Time (sec.)	15
Injection Voltage (kVolts)	1.6
Date Delay (sec.)	1

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

1. Navigate to the Library.
2. Select "Size Standards".
3. Select "Create" (Figure 7).
4. Name the Size Standard as "GT600" and as Dye Color select "Orange".
5. The fragments size in the GT600 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 and 600.



**Figure 7-** Screenshot for the "Create New Size Standard" window on Applied Biosystems 3500 Data Collection software

### 3.2.3. Create a QC protocol

1. Navigate to the Library.
2. Select "QC Protocol".
3. Select "Create" (Figure 8).
4. Name the Protocol as "i.e., *GT600*" and select the *Size Standard* "GT600"

The screenshot shows the 'Create New QC Protocol' window with the following settings:

- Protocol Name:** GT600 (with a 'Locked' checkbox)
- Description:** (empty text field)
- Size Standard:** GT600 (dropdown menu)
- Sizecaller:** SizeCaller v1.1.0 (dropdown menu)
- Analysis Settings:** QC Settings (selected tab)
- Analysis Range:** Full (dropdown menu)
- Analysis Start Point:** 0 (text input)
- Analysis Stop Point:** 1000000 (text input)
- Sizing Range:** Partial (dropdown menu)
- Sizing Start Size:** 60 (text input)
- Sizing Stop Size:** 600 (text input)
- Size Calling Method:** Local Southern (dropdown menu)
- Peak Amplitude Threshold:** A table with columns for Blue, Green, Yellow, Red, Purple, and Orange, each with a checked checkbox and a value of 175.
- Common Settings:**
  - Use Smoothing: Light (dropdown menu)
  - Use Baseline (Baseline Window (Pts)): [checked] 51 (text input)
  - Minimum Peak Half Width: 2 (text input)
  - Peak Window Size: 15 (text input)
  - Polynomial Degree: 3 (text input)
  - Slope Threshold Peak Start: 0.0 (text input)
  - Slope Threshold Peak End: 0.0 (text input)

Buttons for 'Close' and 'Save' are located at the bottom of the window.

**Figure 8-** Screenshot for the "Create New QC Protocol" window on Applied Biosystems 3500 Data Collection software

### 3.2.4. Create a New Assay

4. Navigate to the Library.
5. Select “Assays”.
6. Select “Create” (Figure 9).

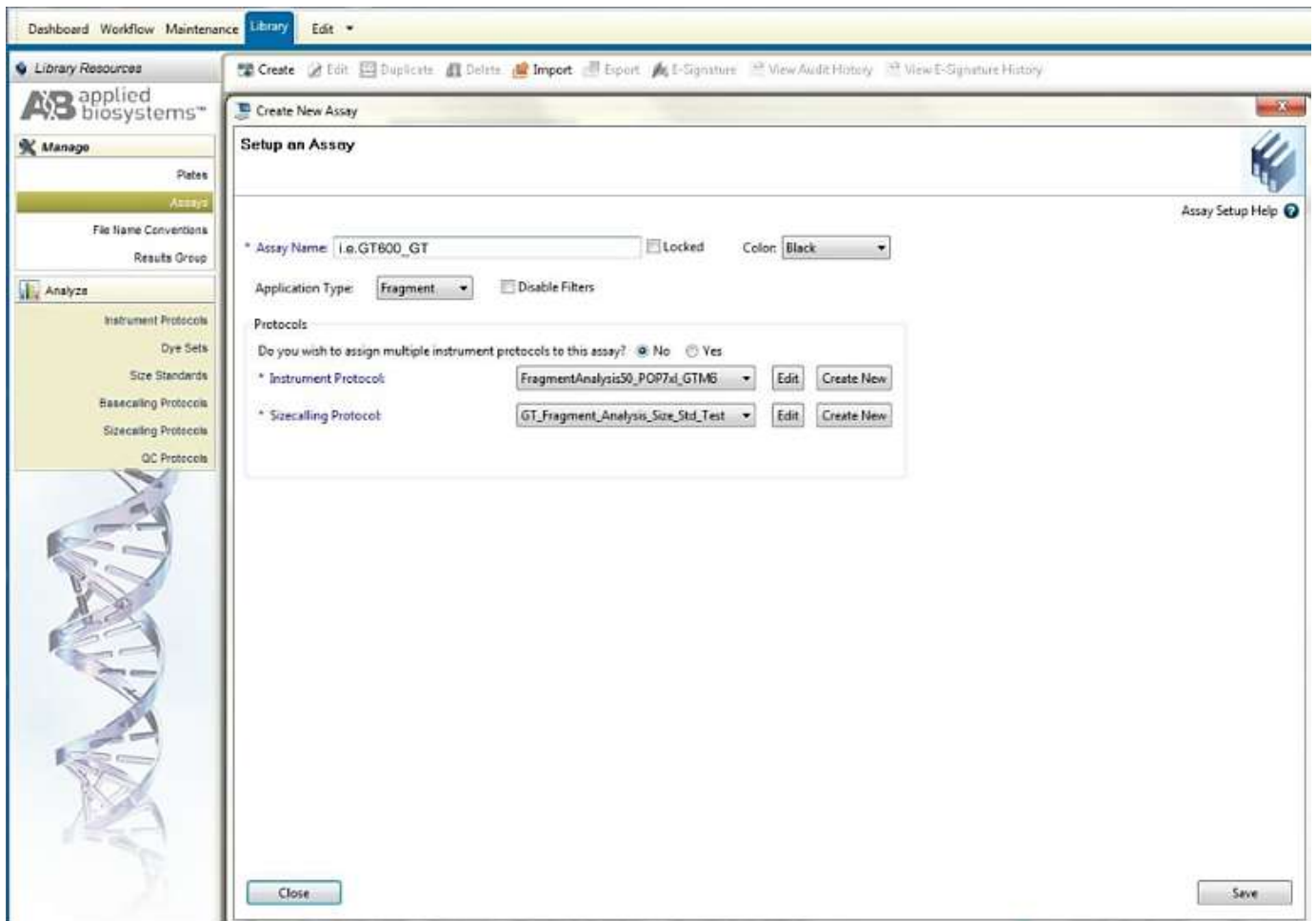


Figure 9- Screenshot for the “Create New Assay” window on Applied Biosystems 3500 Data Collection software.

### 3.5.5. Create a new File Name Conventions

1. Navigate to the Library
2. Select “File Name Conventions”
3. Select “Create” (Figure 10)
4. Choose the File Name Attributes according to your lab practices

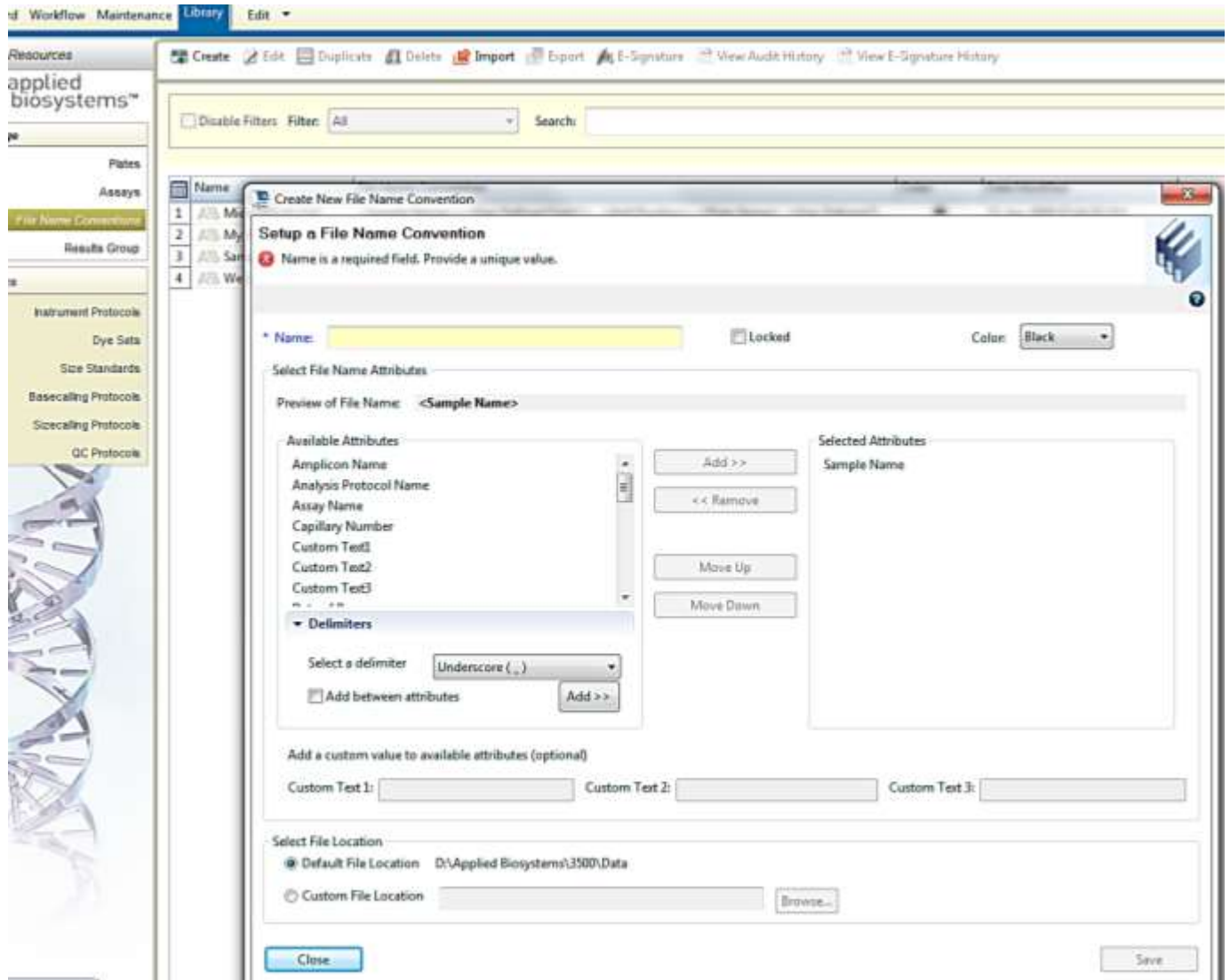


Figure 10- Screenshot for the “Create New File Name Convention” window on Applied Biosystems 3500 Data Collection software.

### 3.5.6. Create a new Result Group

1. Navigate to the Library
2. Select “Results Group”
3. Select “Create” (Figure 11).
4. Choose the Results Group Attributes according to your lab practices.

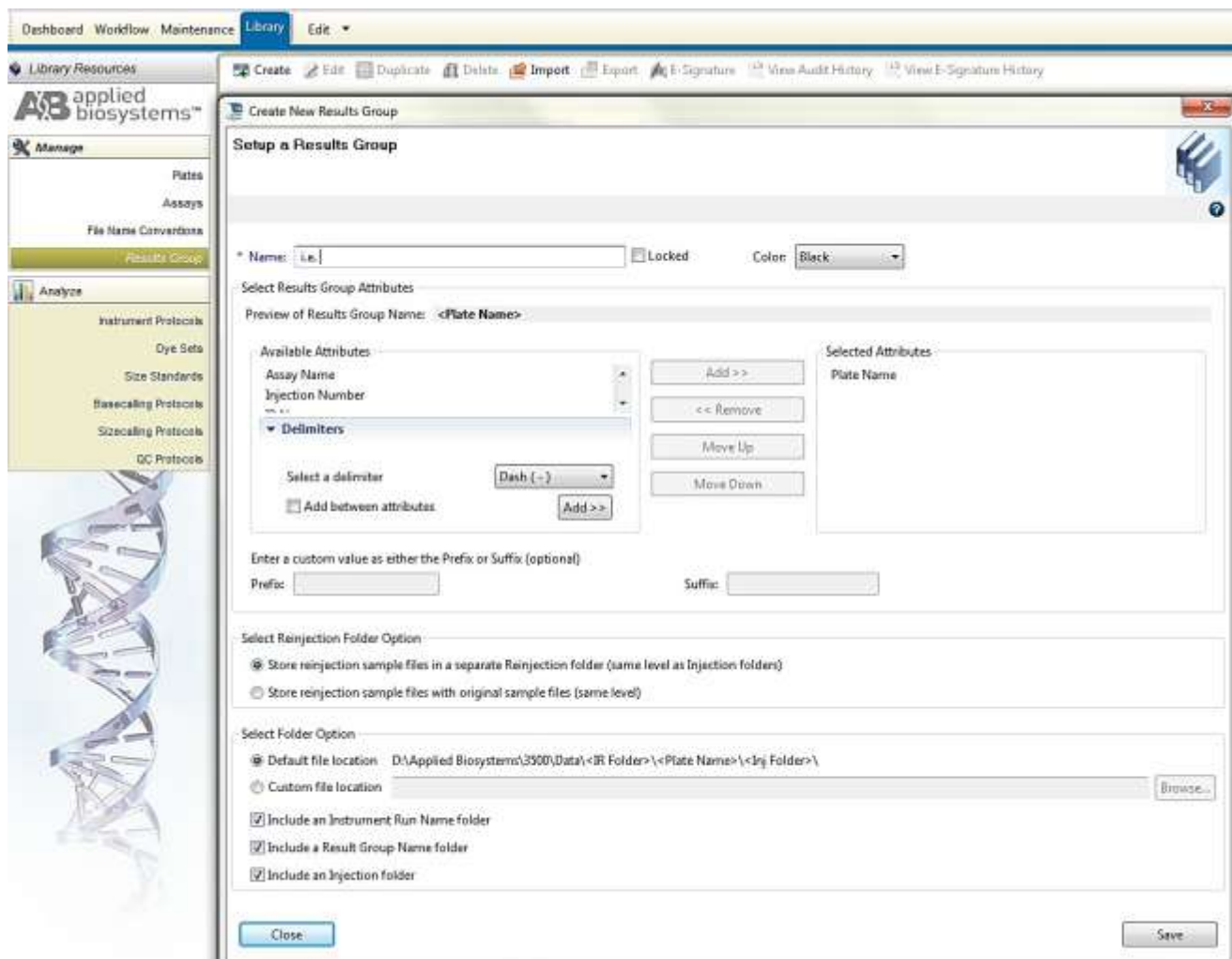


Figure 11- Screenshot for the “Create New Result Group” window on Applied Biosystems 3500 Data Collection software

### 3.5.7. Create a New Plate

1. Navigate to the *Library*
2. From the manage menu select “*Plates*”
3. Select “*Create*” (Figure 12)
4. Define a name for the plate
5. Choose plate type “*Fragment Analysis*” from the drop-down menu.

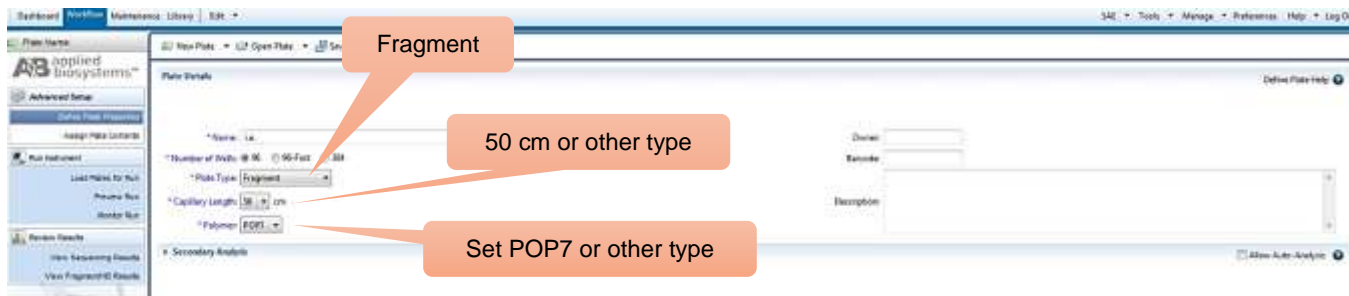


Figure 12- Screenshot for the “Defining plate properties” window on Applied Biosystems 3500 Data Collection software.

### 3.5.8. Select “Assign Plate Contents”

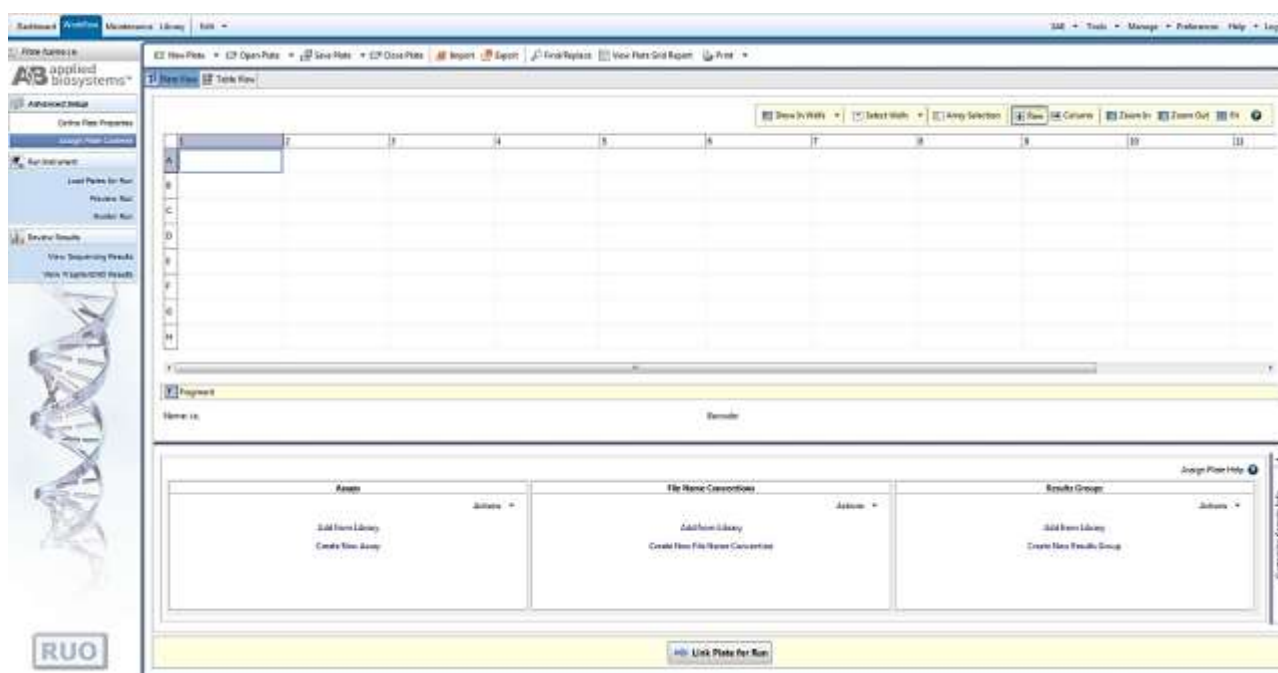


Figure 13- Screenshot for the “Assign Plate Contents” window on Applied Biosystems 3500 Data Collection software.

1. Define sample names to wells.
2. In the Assign Plate Window (Figure 13), in the bottom left corner, in a box “Assay”, click Add from Library option to select the Assay created in Step 3.5.4. Click on the “Add to Plate” button and close the window.

3. 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 3.5.5. Click on the Add to Plate button and close the window.
4. 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 3.5.6. Click on the Add to Plate button and close the window.
5. Select the sample wells, then select the boxes in the Assay, File Name Convention and Results
6. Groups that are relevant to those samples.
7. Select “Link Plate for Run”. It will lead to open Load Plate window. Select “Yes”.
8. In the Run Information window, give a Run name (Figure 14). Select “Start Run” after loading the plate.

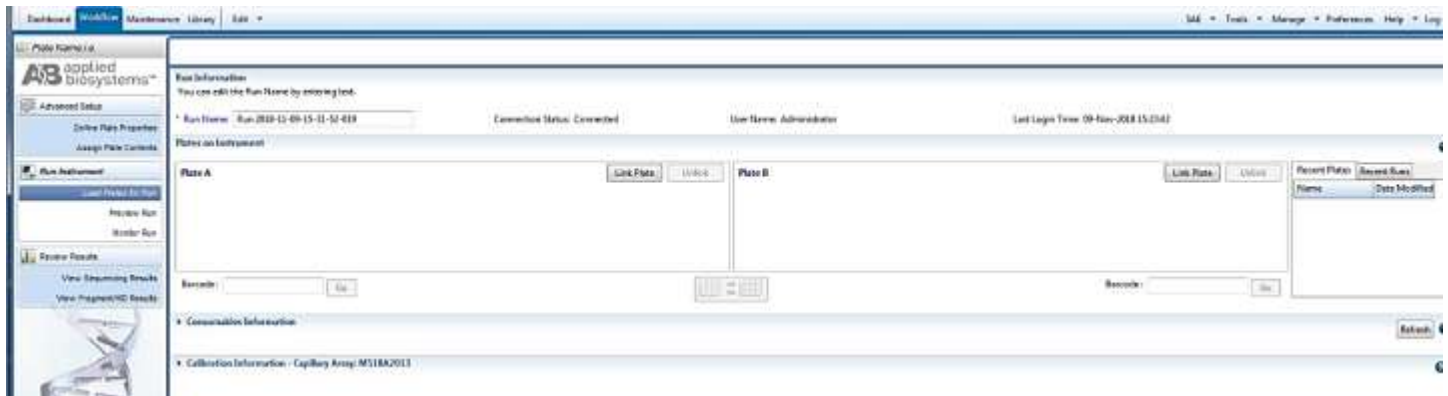


Figure 14- Screenshot for the “Run Information” window on Applied Biosystems 3500 Data Collection software

### 3.6. Matrix Standard Preparation

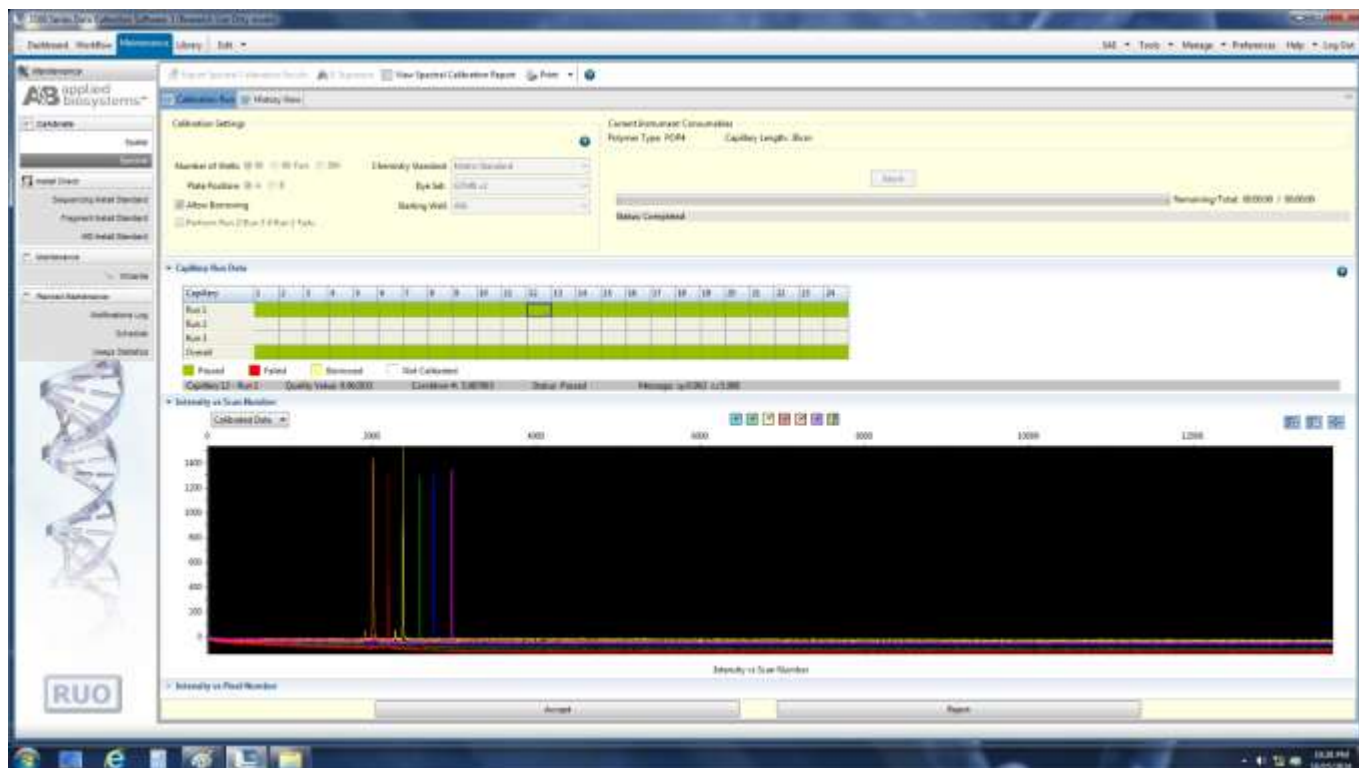
Store GTM6 v2 Matrix Standard at 2 - 8°C.

#### For spectral calibration on Applied Biosystems® 3500:

1. Vortex GTM6 v2 Matrix Standard thoroughly and then quickly spin. Add 195µL of HiDi™ Formamide in 1.5mL tube and then add 5µL of GTM6 v2 Matrix Standard into the tube containing formamide. Vortex for at least 10 seconds. Spin down the tube to make sure that no residues are left at the tube wall or inside the cap.
2. Denature the mix at 95°C for 5 minutes. Immediately after denaturation, put the tube on ice or on to a cooling block at -20°C for 3 minutes.
3. Decide which wells of the 96 well plate you want to use (for example A1 to H1). Add 10µL of GTM6 v2 Matrix Mix with HiDi™ Formamide prepared earlier to each well (8 in total). Place the septa on the plate. Shortly centrifuge the plate to remove bubbles and residues from the tube wall and cap.

### 3.7. Completion of Spectral Calibration

1. When the spectral calibration status is completed, check the quality of the spectral in the “Capillary Run Data” display (see **Error! Reference source not found.**) and select either “Accept” or “Reject” according to quality criteria recommended in the 3500 series [Data Collection Software User Manual](#).
2. GTM6 v2 Matrix Standard should be used when setting up the analysis method for any of the Genetek kit analysis.
3. In case of fewer than recommended number of capillary pass (Completion of Spectral Calibration section), Spectral calibration run must be repeated, (for more information on the Spectral Calibration and how it works please consult the relevant user guide from ThermoFisher under the section “What you see during a spectral calibration” <http://tools.thermofisher.com/content/sfs/manuals/4401661.pdf>).



**Figure 15-** Result view for the GTM6 v2 Matrix Standard on the Applied Biosystems® 3500 Genetic Analyzer using POP-7 TM polymer and Data Collection Software, Version 3.0.

## 4. Troubleshooting

This section provides general troubleshooting's for 3500 series Genetic Analyzer, for more specific information and further details please contact us [support@genetek.de](mailto:support@genetek.de).

Issue observed	Causes and Comments
Fewer than recommended number of capillaries passed the spectral calibration	Use of poor quality Formamide, multiple freeze-thaw cycles can lower the quality of reagents such as Formamide. This can affect the sensitivity and height of peaks. We highly recommend using HiDi™ Formamide or high quality Formamide. The quality of formamide is critical. Use HiDi™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C can cause breakdown of formamide. Poor-quality formamide can contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.
	Too concentrated Matrix standard, it may result in spectral calibration failure due to saturated peaks or dye bleed-through. Use less volume of the GTM6 v2 Matrix and adjust the HiDi™ Formamide volume accordingly.
	Too diluted matrix Standard, it may result in spectral calibration failure due to low peak height. Increase the volume of GTM6 v2 Matrix and adjust the HiDi™ Formamide volume accordingly.
	Restart the CE instrument and the computer. Repeat the spectral calibration.
	Make sure that oven is preheated to 60°C before the spectral calibration.

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All capillaries failed spectral calibration

For best results we recommend using fresh polymer, fresh buffer and a capillary array with fewer than 100 injections.

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## 5. Limitations and Disclaimer

Any result obtained from GTM6 v2 Matrix Standard or any other reagent 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 GTM6 v2 Matrix Standard or any other diagnostic and/or forensic 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.

GTM6 v2 Matrix Standard is developed to use for spectral calibration when GT 6-dye system is used with Applied Biosystems™ 3500/xL series Genetic Analyzer. We recommend that individual laboratory perform and develop its own test procedure and interpretation standard operative procedure. To develop such internal protocol please use Data Collection software user instruction manual from Applied Biosystems™ or contact us – [support@genetek.de](mailto:support@genetek.de).

## 6. General Safety Warnings

6. Any procedure should be performed by professional/qualified personal.
7. 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.
8. Store all the components as described in the user guide.
9. Laboratories should test their own quality check samples for each type of the assay to validate the Kit procedure.






### Chemical safety

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

### SDSs

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

## 7. 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	