



GT YDetector 29

Product User Manual

CAT# GT-12301

Human Identification Kit for
Multiplex Amplification of
Y Chromosome STR loci

Produced by

GENETEK BIOPHARMA GmbH

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1. GT YDetector 29 Overview

- Easy to use multiplex PCR - amplification of **28 markers (29 loci)** in a single reaction
- 27 STR specific to Y chromosome loci and the Amelogenin marker
- For forensic applications and in sex assault cases
- Optimized to use in male-male and/or male-female sample mixtures
- Can be used with extracted DNA from blood, hair, bone, etc. or direct PCR using DNA banking card(DBC™)
- 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 YDetector 29 Kit is intended for molecular biology applications in forensic, human identification in sexual assault and kinship issues. This product is not intended for the diagnosis, prevention, or treatment of any disease.

1.2 GT YDetector 29 Markers

STR loci (short tandem repeat) consist of short and repetitive sequence elements, usually 2 - 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 primers from their flanking sequences. The STR loci alleles are differentiated by the number of copies of the repeat sequence contained within the amplified region (locus) and are distinguished from each other using fluorescence detection after capillary electrophoretic separation.

The GT YDetector 29 multiplex system was designed to achieve high power of discrimination without upgrading detection system to 6-dye channels. GT YDetector 29 Kit comprises primers in a multiplex which amplify 28 markers (DYS458, DYS392, DYS448, DYS643, DYS576, DYS460, DYS627, DYS437, DYS393, DYS389I, DYS439, DYS533, DYS389II, DYF387S1, DYS518, DYS391, DYS456, YGATAH4, DYS390, DYS438, DYS481, DYS385 a/b, DYS570, DYS635, DYS19, DYS549, DYS449 and AMXY).

GT YDetector 29 Kit comprises all of the European and U.S. core Y-STR loci and additional 16 markers to obtain high discrimination power.

Table 1: Markers in GT YDetector 29 Kit

No.	Marker	Size Range	Chr. Location
1	DYS458	117-157	Yp11.2
2	DYS392	175-214	Yq11.223
3	DYS448	225-270	Yq11.223
4	DYS643	289-327	Yq11.221
5	DYS576	334-387	Yp11.2
6	DYS460	388-417	Yq11.222
7	DYS627	430-475	Yp11.2
8	DYS437	96-119	Yq11.21
9	DYS393	125-161	Yp11.2
10	DYS389I	174-209	Yq11.21
11	DYS439	215-240	Yq11.21
12	DYS533	260-284	Yq11.221
13	DYS389II	290-330	Yq11.21
14	DYF387S1	343-391	Yq11.23
15	DYS518	392-450	Yq11.221
16	DYS391	100-135	Yq11.21
17	DYS456	160-200	Yp11.2
18	YGATAH4	210-240	Yq11.221
19	DYS390	252-286	Yq11.221
20	DYS438	293-332	Yq11.21
21	DYS481	345-386	Yp11.2
22	DYS385 a, b	390-467	Yq11.222
23	AMXY	102-117	Xp22.2 Yp11.2
24	DYS570	122-180	Yp11.2
25	DYS635	212-250	Yq11.21
26	DYS19	275-310	Yp11.2
27	DYS549	315-355	Yq11.222
28	DYS449	383-450	Yp11.2

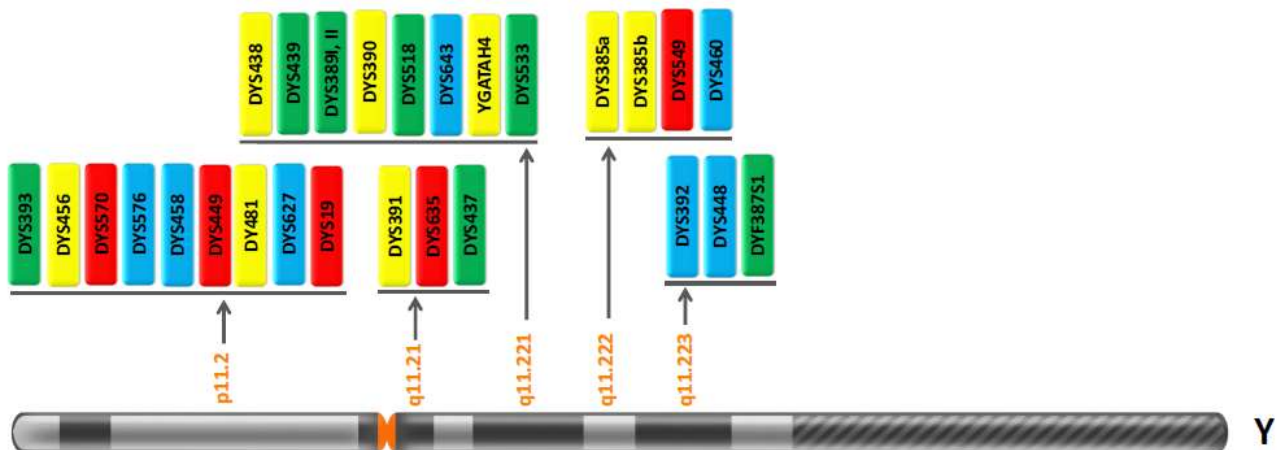


Figure 1. Diagram showing the distribution and placement of GT YDetector 29 kit markers on human Y chromosome

1.3 Five-dye fragment analysis

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

Table 2. The fluorescent dyes used in GT YDetector 29 kit

Name	6-FAM	GT2907	GT2712	GT1803	GT500
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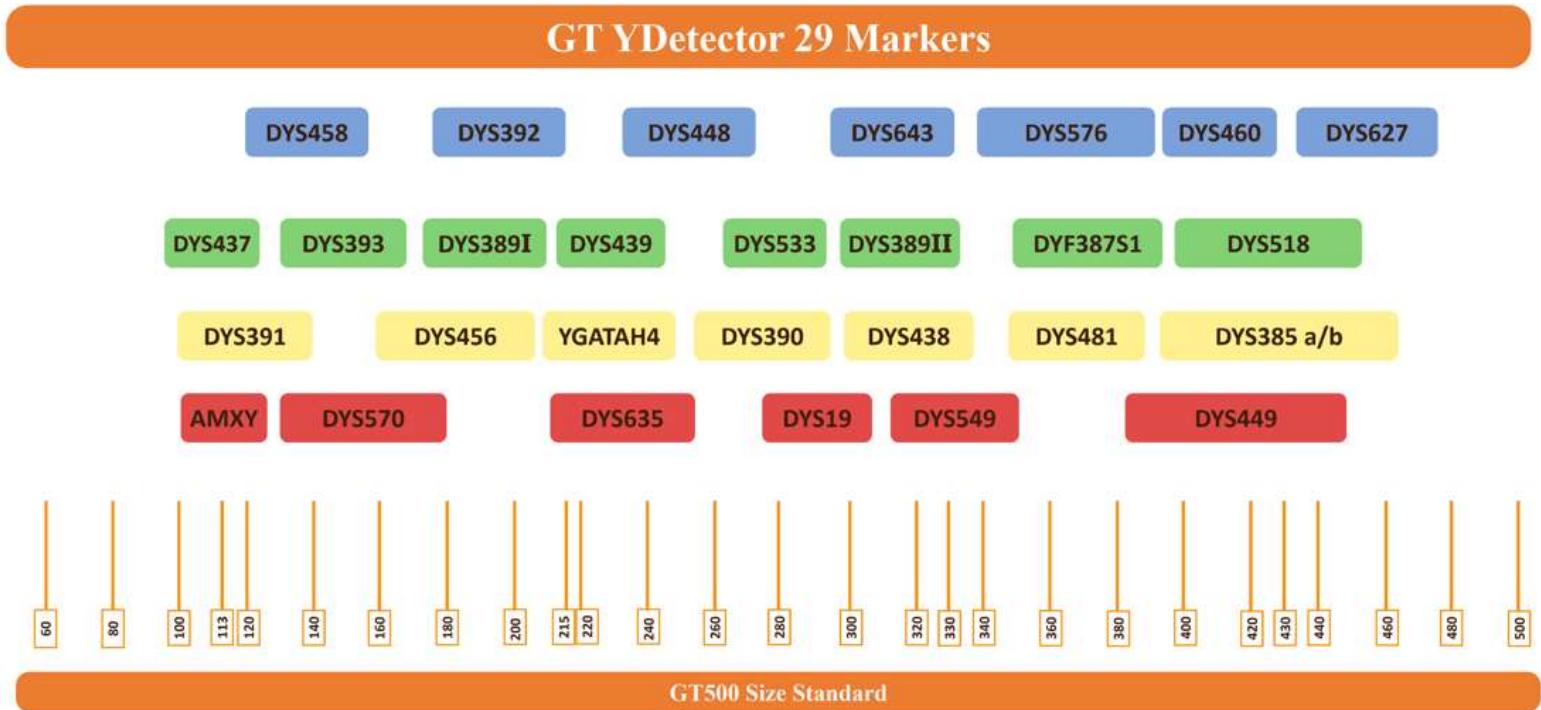


Figure 2. Diagram shows distribution and placement of GT YDetector 29 Kit markers with GT500 Size Standard

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 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 (Pre-PCR area). GT500 Size Standard, GTM5 v2 Matrix Standard and GT YDetector 29 Allelic ladder are amplicons and should be stored in post-PCR area. 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			BOX-B		
	Tube Label	Tube cap colour		Tube Label	Tube cap color
1	PCR Mix		1	GT500 Size Standard	
2	Primer Mix		2	GT YDetector 29 AL	
3	GT HSTaq		3	GTM5 v2 (Optional)	
4	GT QCDM102 (Control DNA-50ng/µl)				
5	GT QCW (H2O)				

Not provided with GTYDetector 29 (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 (POP-7) and Capillary Array or equivalent
- Applied Biosystems Hi-Di™ Formamide or equivalent
- GTM5 v2 Matrix Standard for Spectral calibration (GT- 41103) (can be obtained from Genetek Biopharma).

2.2.3 PCR Amplification by GT YDetector 29

- DNA can be extracted from blood, bone, teeth, saliva, samples. This kit also works on blood/saliva samples on filter paper such as DNA Banking Card (DBC™). For instruction of direct PCR method please consult us by email (support@genetek.de)
- 1-5 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 YDetector 29 components

Table 4: PCR reaction set-up

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

2.2.5. GT YDetector 29 protocol

- Bring the 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 following the recipe given above. Every preparation can be done at room temperature (no cold condition is required during preparation).
- Pipet and Vortex Master Mix briefly.
- Transfer 19 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	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 with each multiplex PCR. We recommend using GT QCDM102 as a positive control especially early on during the testing of 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 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 YDetector 29 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 for Applied Biosystems® 3500/3500xL Genetic Analyzer (before the first use of GT YDetector 29 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 before using the kit. Please find detailed protocol for spectral calibration with GTM5 v2 Matrix Standard here - CAT# 41103 or contact us at support@genetek.de.

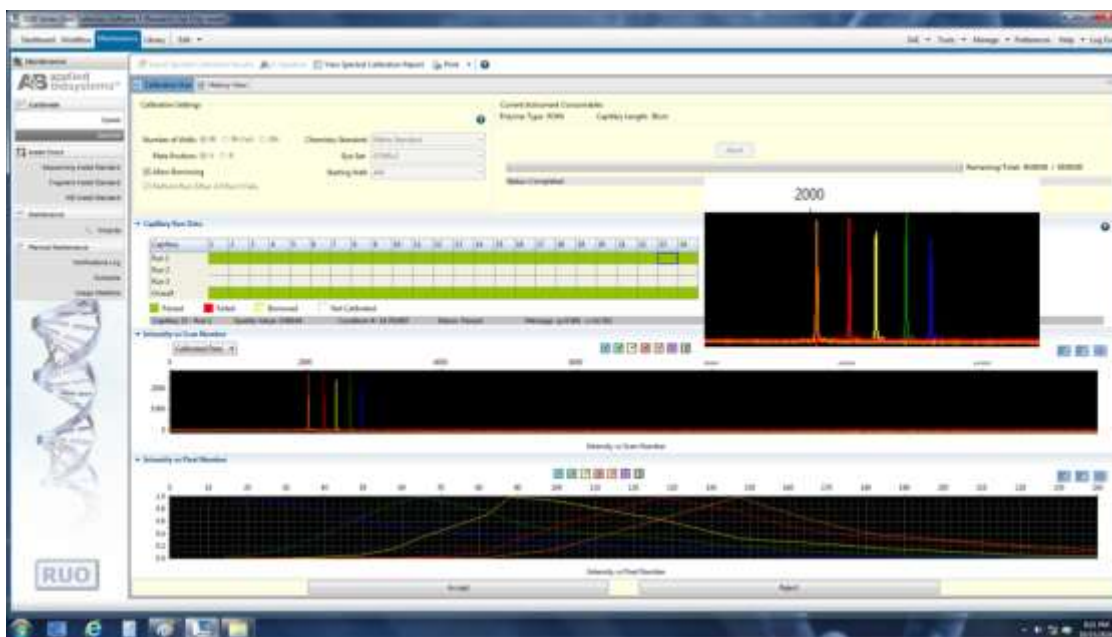


Figure 3. An example of a successful spectral calibration with GT 5-dye system on Applied Biosystems Genetic Analyzer 3500xL

- 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

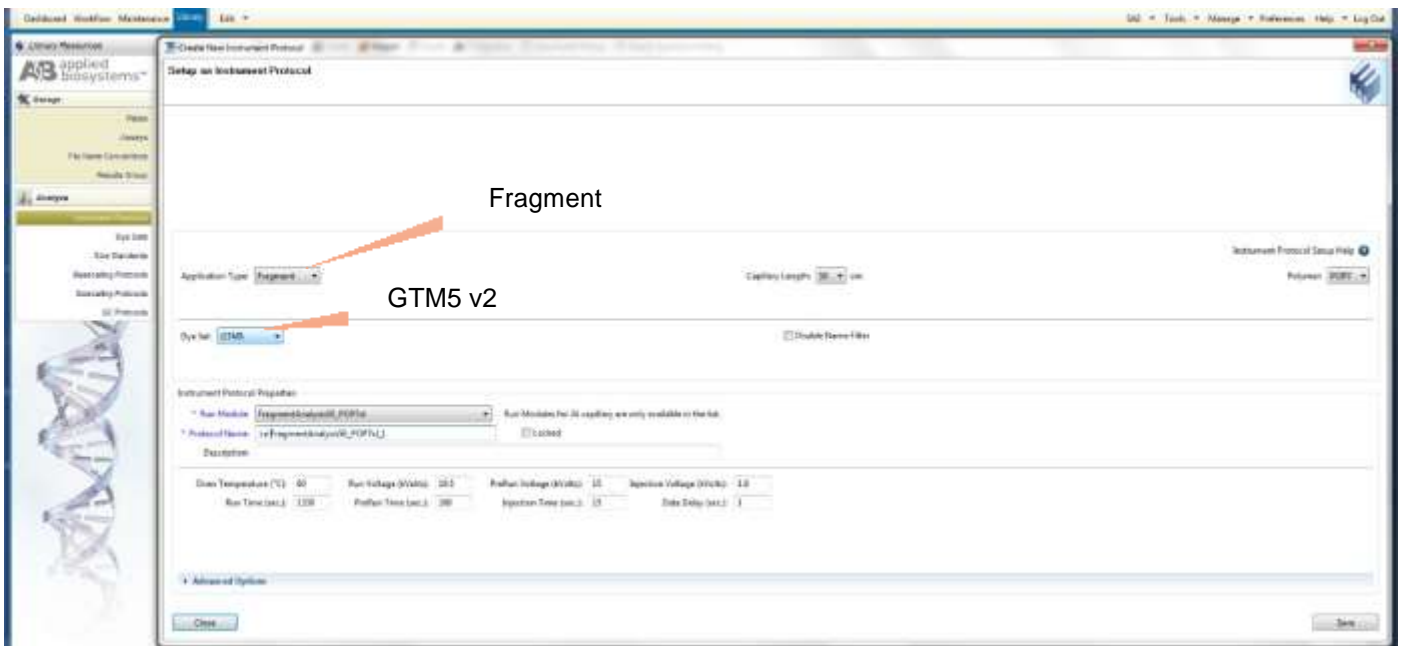


Figure 5. Screenshot for the “Create New Instrument Protocol” window on Applied Biosystems 3500 Data Collection software

- 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 YDetector 29 system, 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 Analyzers 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)

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.

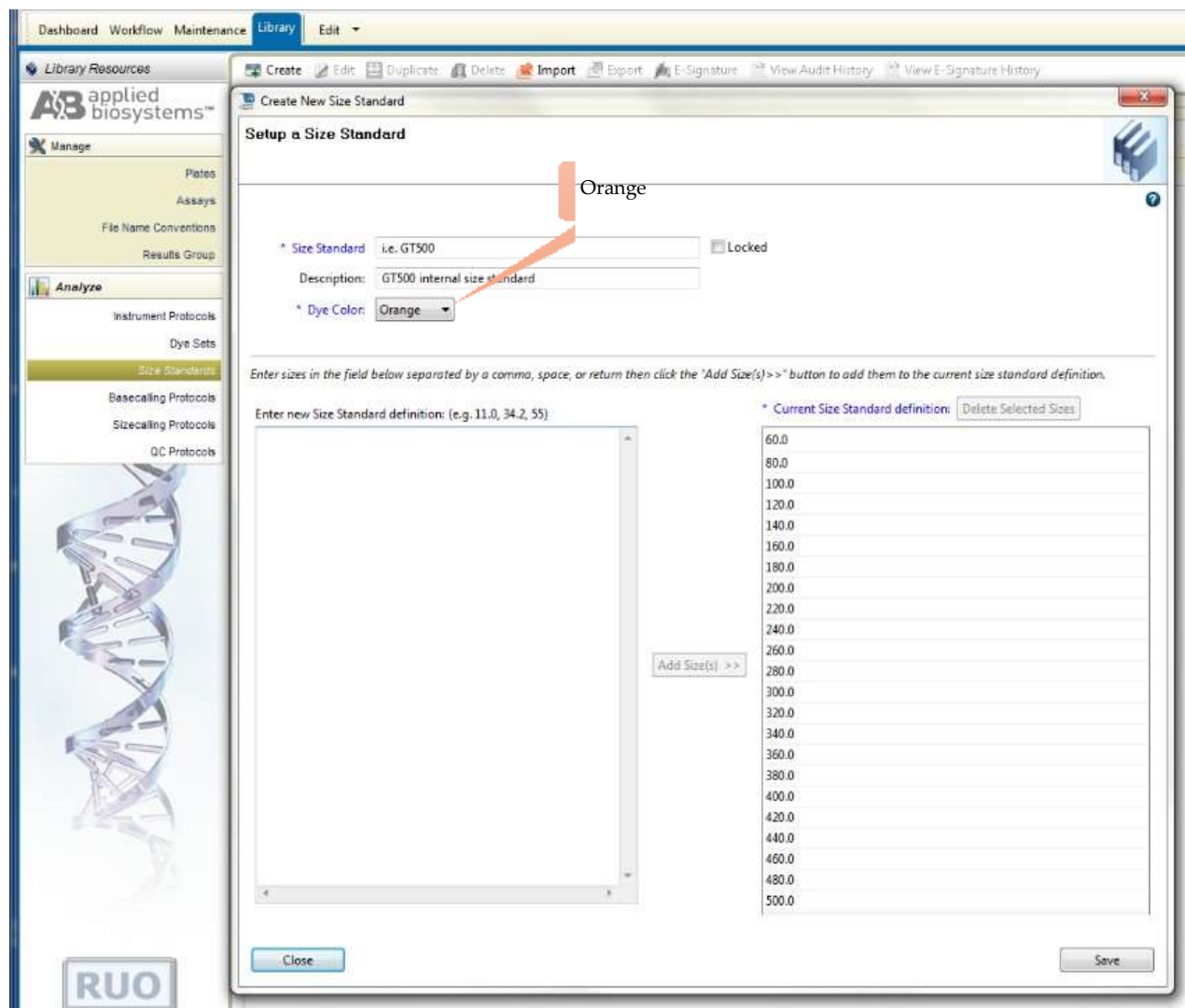


Figure 6. Screenshot for the “Create New Size Standard” window on Applied Biosystems 3500 Data Collection software.

3.1.3 Create a QC protocol

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

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 *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 YDetector 29 on the Applied Biosystems® 3500/3500xL Genetic Analyzer.

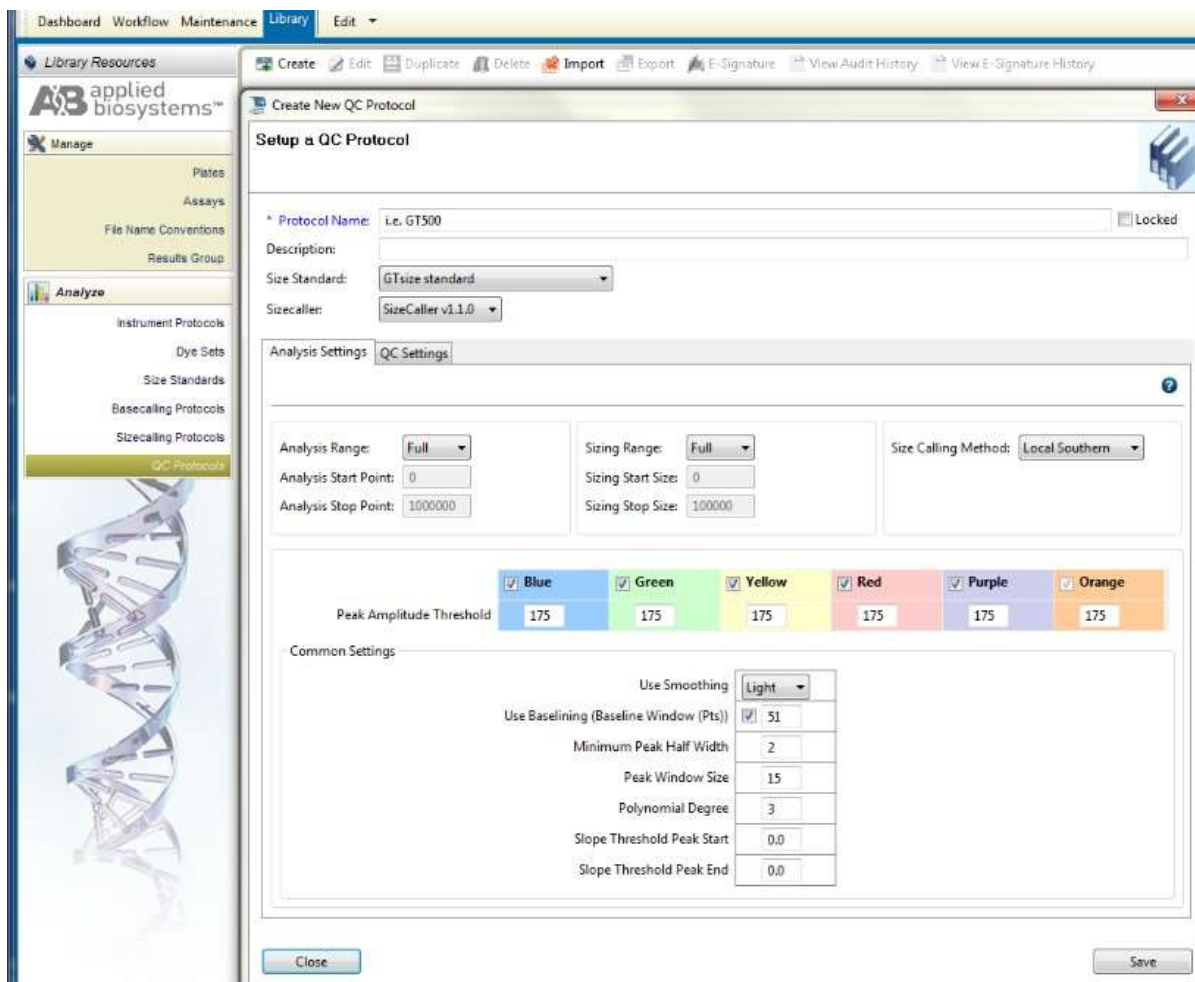


Figure 7. Screenshot for the “Create New QC Protocol” window on Applied Biosystems 3500 Data Collection software.

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.

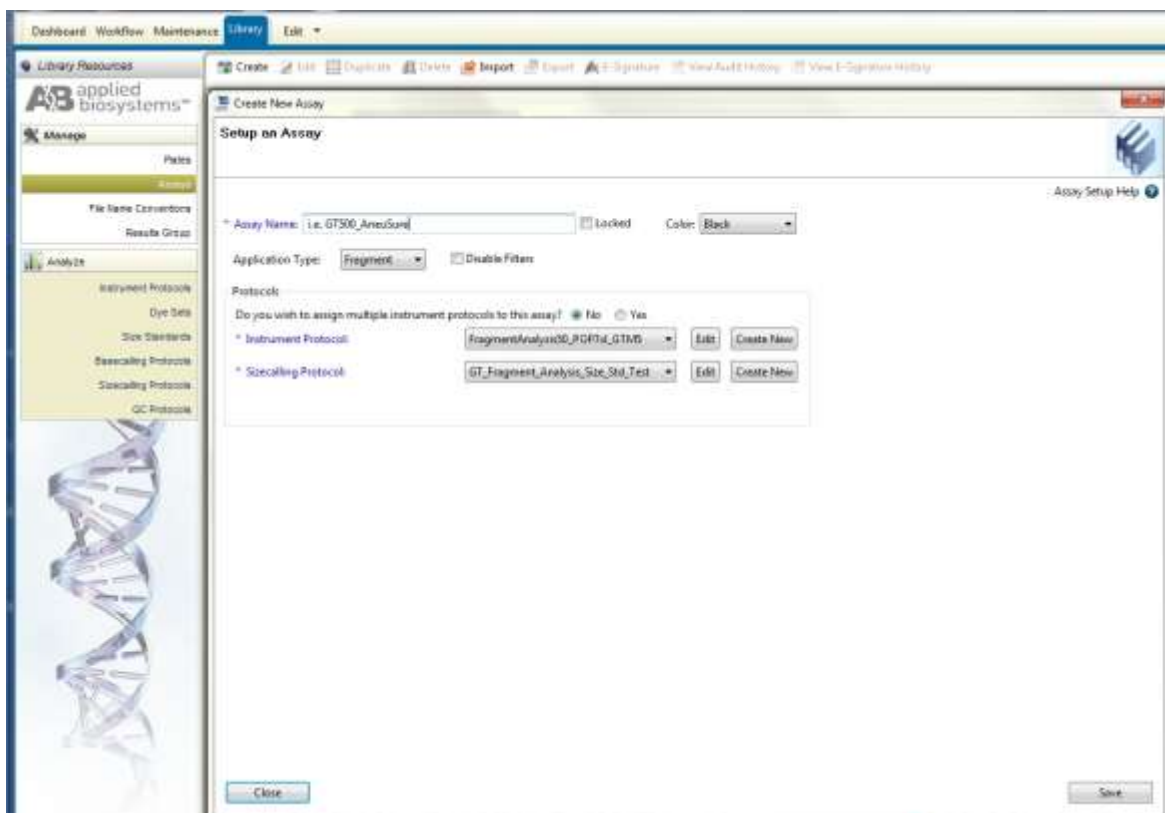


Figure 8. Screenshot for the “Create New Assay” window on Applied Biosystems 3500 Data Collection software.

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.

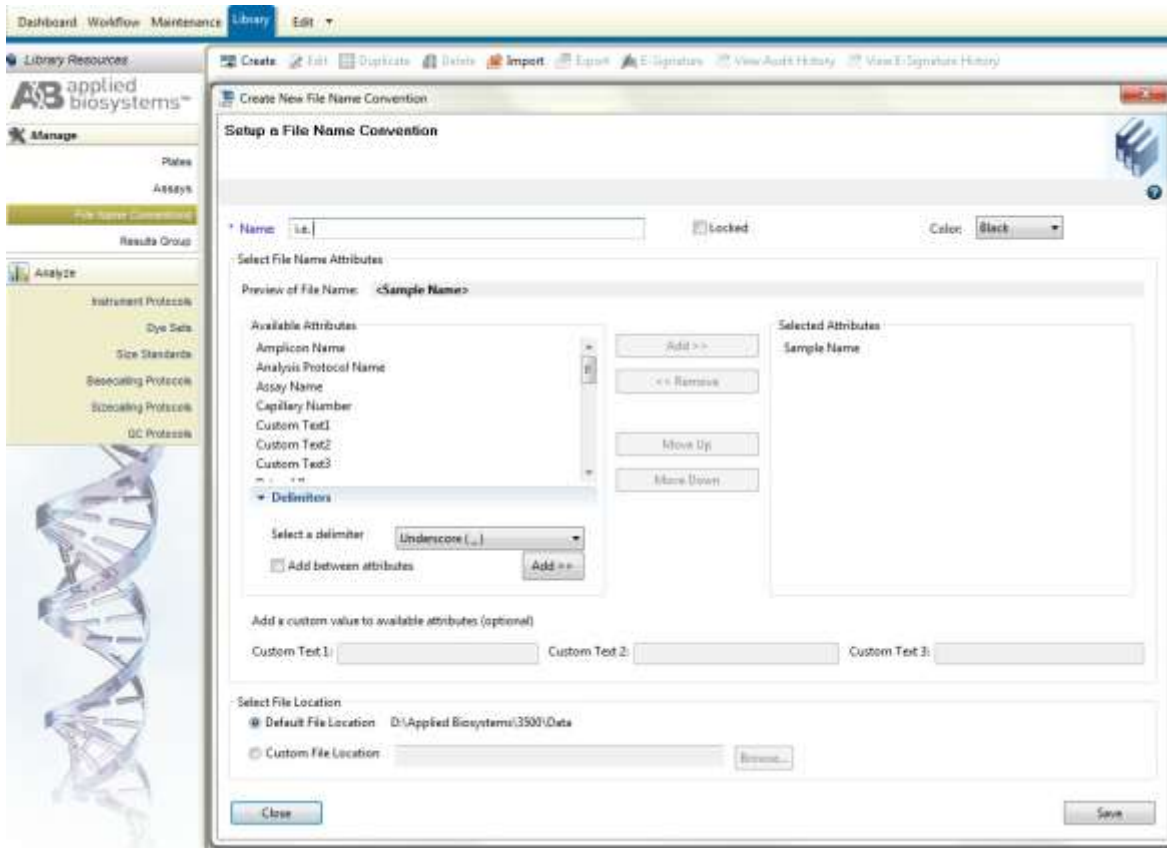


Figure 9. Screenshot for the “Create New File Name Convention” window on Applied Biosystems 3500 Data Collection software.

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.

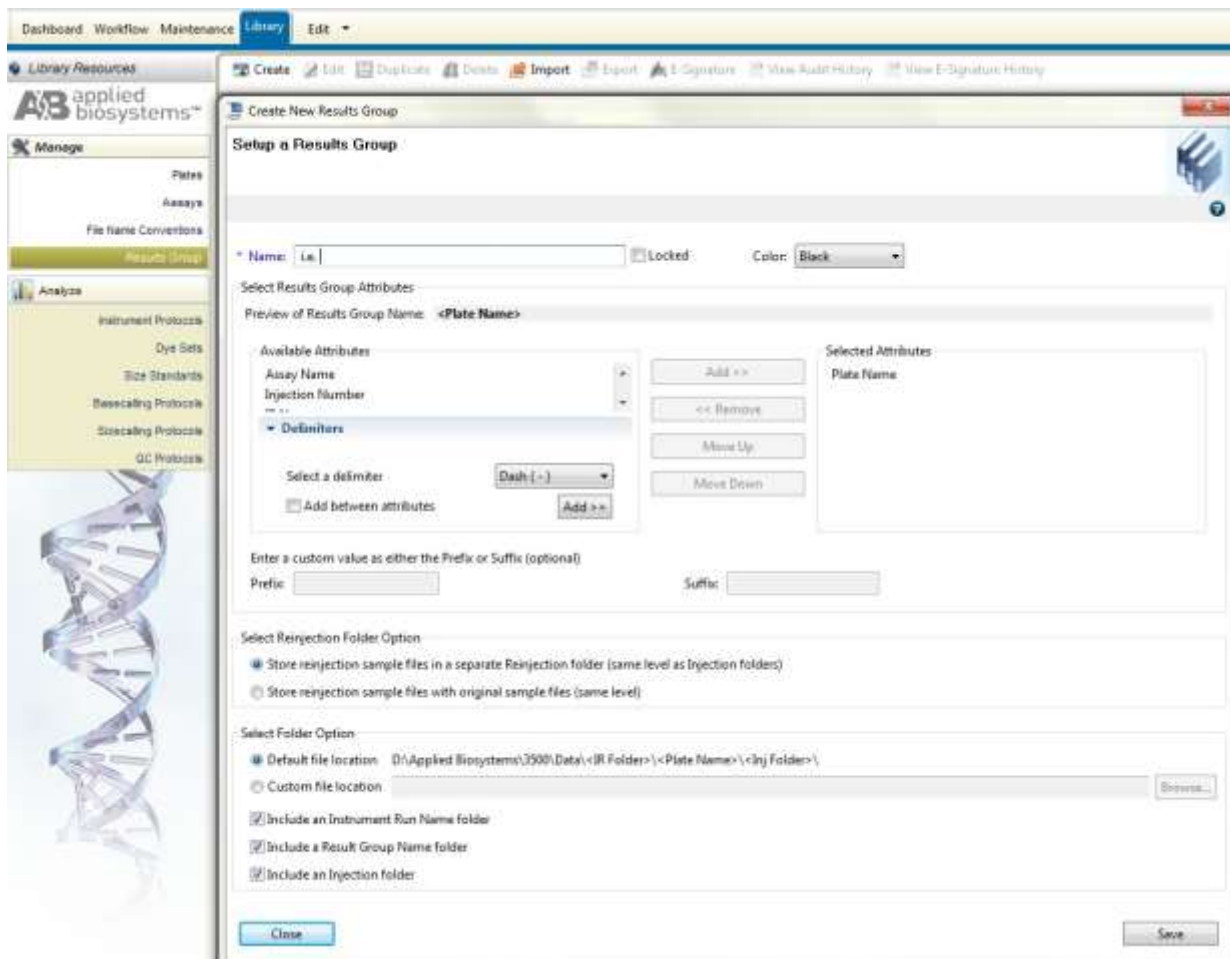


Figure 10. Screenshot for the “Create New Result Group” window on Applied Biosystems 3500 Data Collection software.

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
- e) 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”

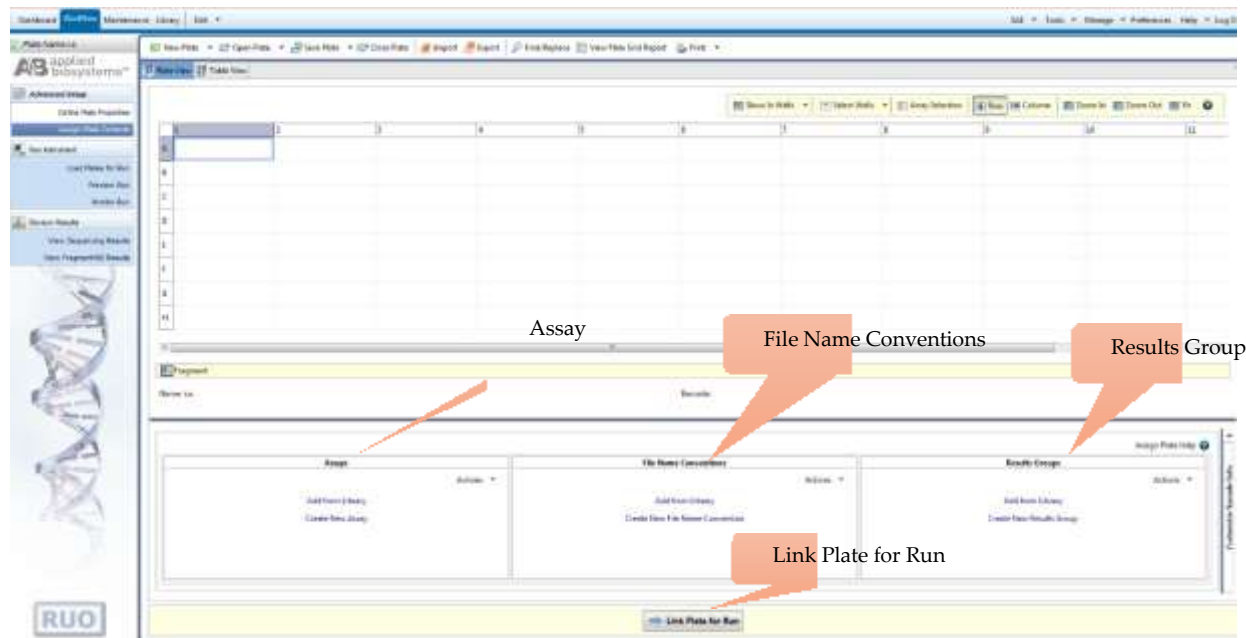


Figure 12. Screenshot for the “Assign Plate Contents” window on Applied Biosystems 3500 Data Collection software.

- Define sample names to wells.
- In the *Assign Plate Window*, 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 are 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.

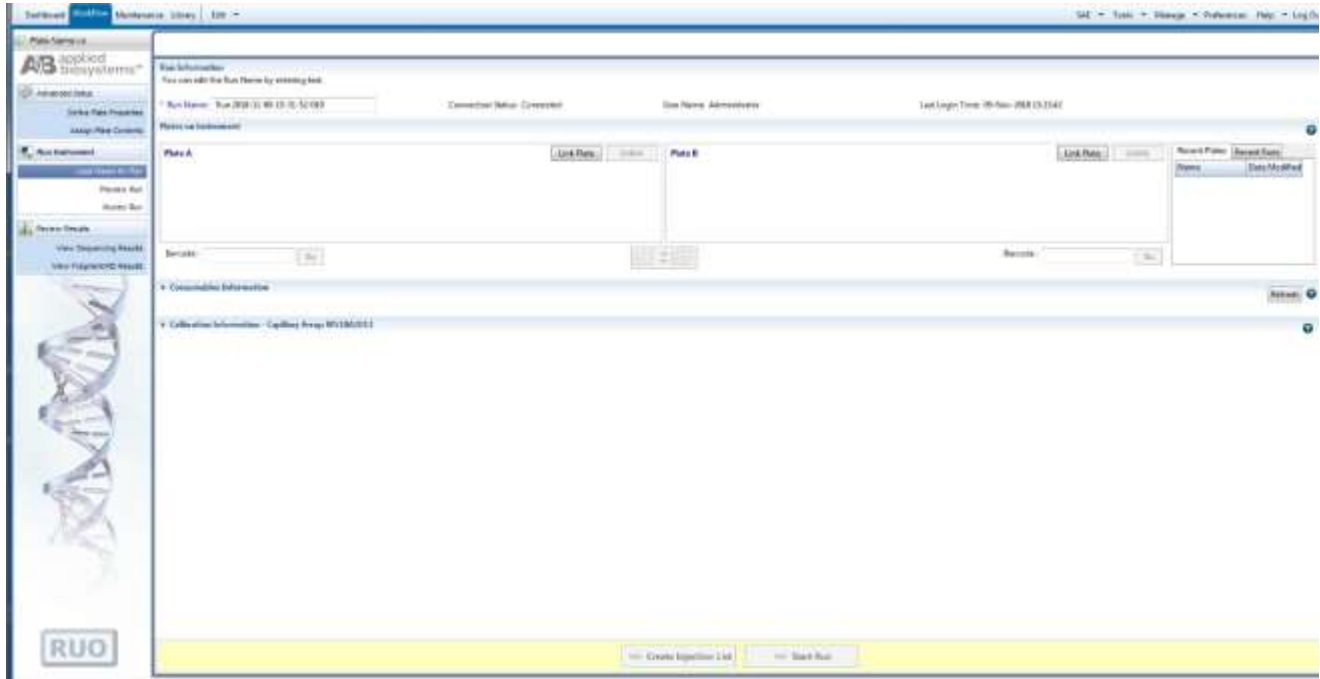


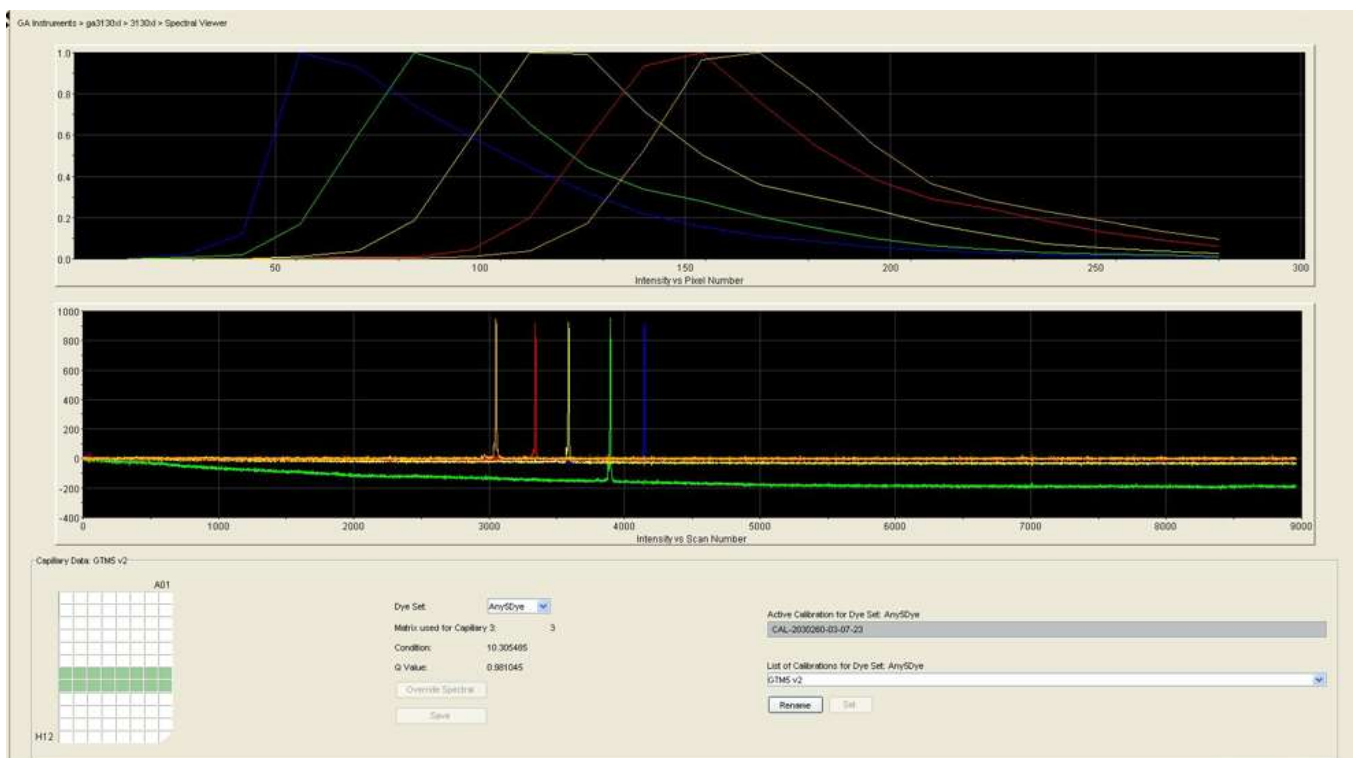
Figure 13. Screenshot for the “Run Information” window on Applied Biosystems 3500 Data Collection software.

3.2 Instrument Preparation for Applied Biosystems® 3130/3130xl Genetic Analyzer (before the first use of GT YDetector 29 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 YDetector 29 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 v 2 Matrix Standard. Please find detailed protocol for spectral calibration with GTM5 v2 Matrix Standar here - CAT# 41103 or contact us at support@genetek.de



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 YDetector 29).
- b) Type: Regular
- c) Template: FragmentAnalysis50_POP7 (default template for the capillary array and polymer used).
- d) Click OK.

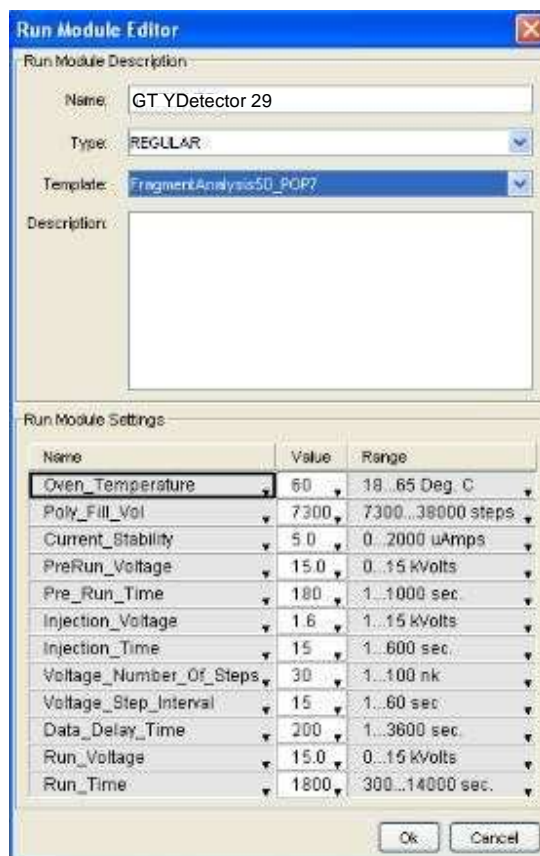


Figure 15. Screenshot for the “Module Manager” window on Applied Biosystems 3130 Data Collection software.

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 YDetector 29)
- c) type: Regular
- d) run Module: Select the Run Module created (GT YDetector 29)
- e) dye Set: Any5Dye
- f) click OK.

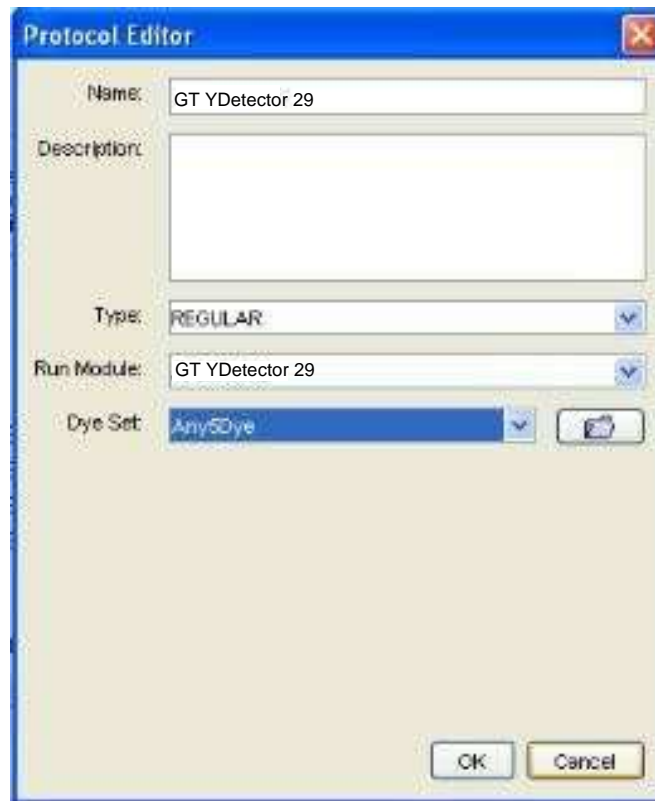


Figure 16. Screenshot for the “Create New Instrument Protocol” window on Applied Biosystems 3130 Data Collection software.

3.2.3 Set up a Plate for run

From the left navigation window select Plate Manager and New.

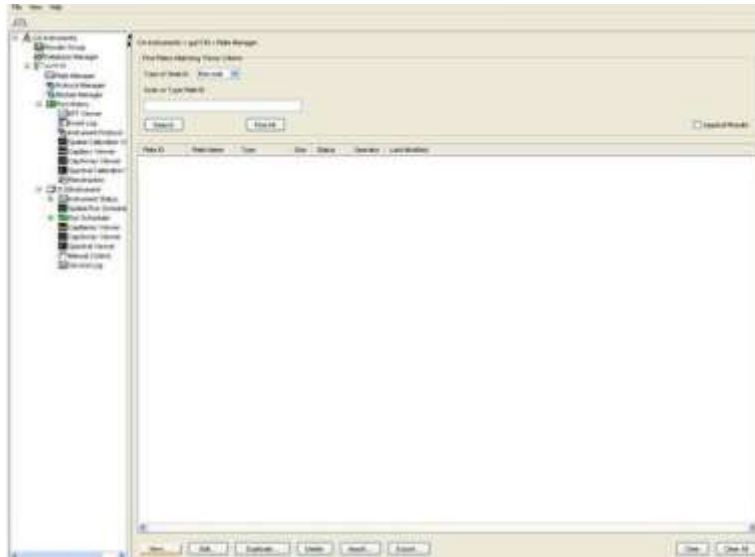


Figure 17. Screenshot for the “Plate Manager” window on Applied Biosystems 3130 Data Collection software.

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 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 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.

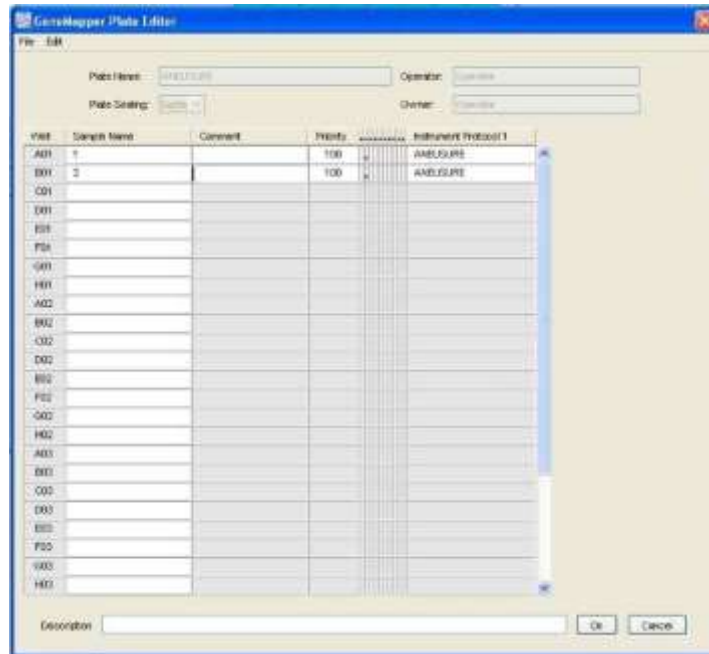
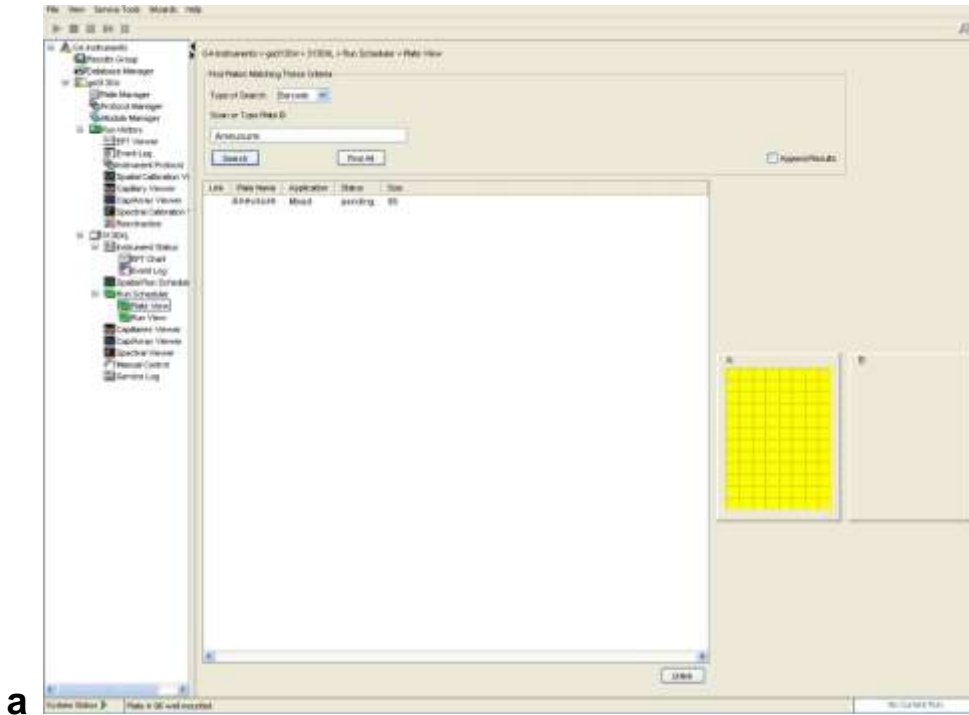


Figure 19. Screenshot for the “GeneMapper Plate Editor” window on Applied Biosystems 3130 Data Collection software.

- From the left navigation window, select Run Scheduler, search for GT YDetector 29 (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.

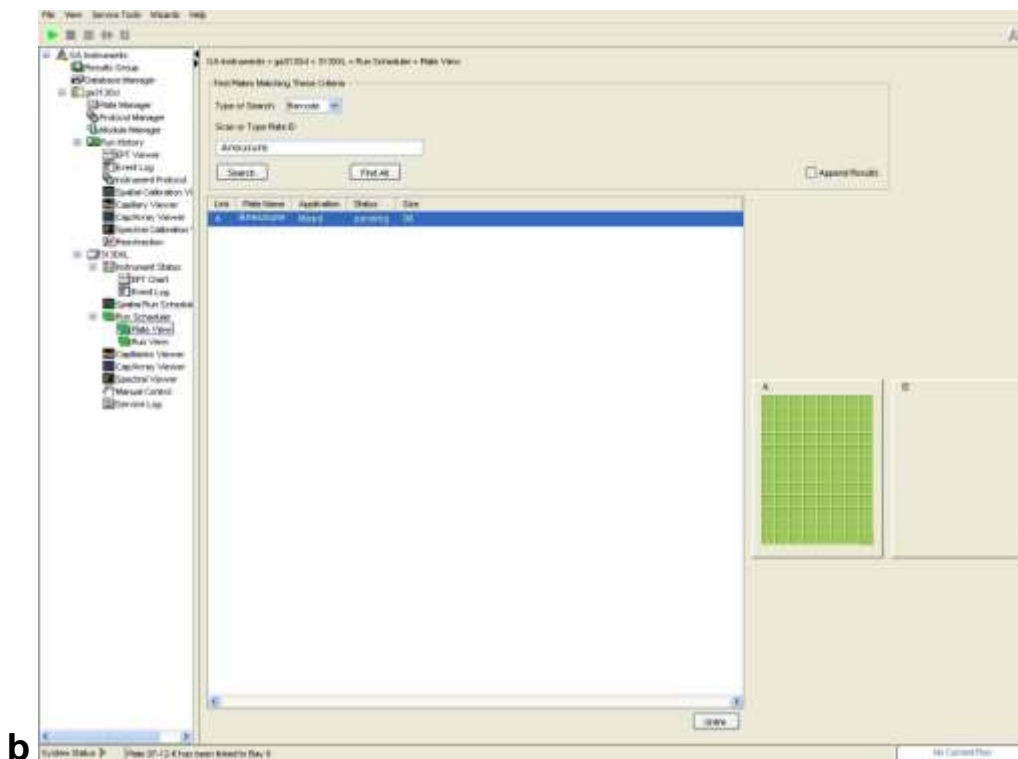


Figure 20 a & b. "Plate view" window on Applied Biosystems 3130 Data Collection software.

- 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 YDetector 29 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 or GTY allelic ladder 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).
- In a multi-capillary Analyzer, injections take place simultaneously on all the capillaries. Therefore, 1 entire column (8 samples) or 3 entire columns (24 samples) must be pipetted onto the plate. If fewer samples are to be analyzed, the empty well positions must be filled with 10µl Hi-Di Formamide.
- To get reliable allele call, inject at least one allelic ladder for each set of 24 samples (one allelic ladder per injection for 24-capillary instrument or one allelic ladder per 3 injections for 8-capillary instrument).
- Actual room temperature may influence the performance of the multi-capillary instrument which could result into shoulder peaks or split peaks, so make sure that the ambient conditions are maintained as described by the instrument manufacturer.

4. Result analysis and Interpretation

4.1. Software for sample analysis

- For GT YDetector 29, the Applied Biosystems fragment analysis software compatible with your genetic Analyzer is recommended. This kit is compatible with GeneMapper® ID-X software. Analysis method depends on the software version.

Each Forensic lab should have individual interpretation and reporting procedure and criteria. To develop such procedure, we recommend Forensic lab to use “SWGDM guidelines for STR detection, interpretation, and contamination prevention”. You can download it from - <https://www.swgdam.org/publications>.

4.2. General guideline for the analysis of GT YDetector 29 results

GT YDetector 29 PCR products are observed with 5-dye system on an electropherograms in the GeneMapper® ID-X software. For the analysis, import GT YDetector 29 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.

Table 6: Analysis parameters for GeneMapper® ID-X software

Parameter	
Peak detection algorithm	Advanced
Ranges	Analysis – Full Range, All Sizes Start Point – 0, Stop Point – 10000
Smoothing and baselining	Smoothing – Light Baseline Window – 51 pts
Size calling method	Local Southern Method
Peak detection	Minimum Peak Half Width – 2 Polynomial Degree – 3 Peak Window Size – 15

Note – In the Peak detection settings, the peak amplitude threshold (cut-off value) for each dye-channel should be determined by the individual laboratory but one should consider that the minimum peak height should be three-times higher than the background noise of the baseline

Table 7. Template files to be used for analysis

File type	File name
Panels*	GT YDetector
29_panels BinSets*	GT YDetector 29_Bins
Size Standard*	GT500_SS
Analysis method	Analysis_GTYD_3130
	Analysis_GTYD_3500
Plot settings	GTYD_Plot

*User must use Panels, BinSets and Size Standard files provided by GENETEK BIOPHARMA, other files are optional.

Allocation of alleles depends significantly on finding the exact length of the PCR products. The later one depends on the device type, the condition of the electrophoresis and also on the Size Standard. The GT500 Size Standard should be used with following horizontal size distribution: 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 bp.

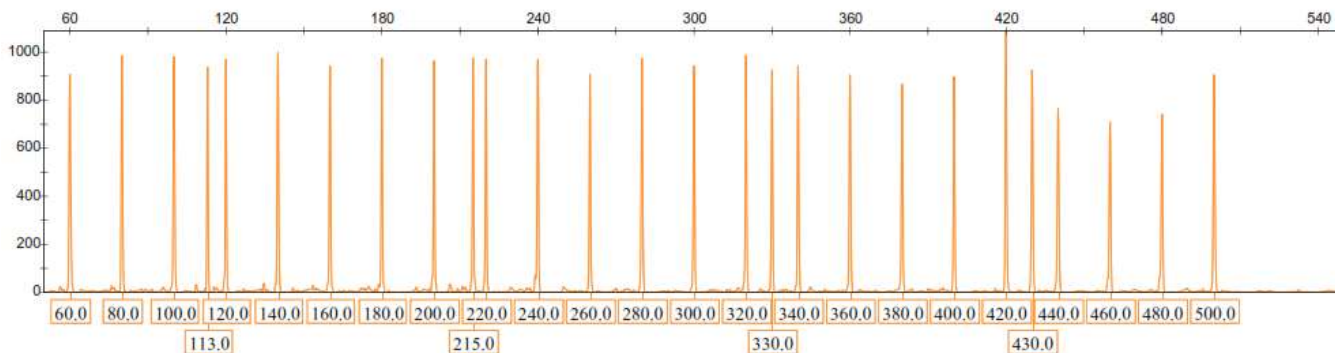


Figure 22. GT500 DNA Size Standard electropherogram on ABI 3500xL Genetic Analyzer

4.3. GT YDetector 29 Allelic Ladder

Allelic ladder development and validation has been performed using Applied Biosystems 3500 Genetic Analyzer with POP 7 polymer. Different analysis instruments, DNA size standard other than GT500 or polymer may result in different fragment lengths. Table 4 shows the alleles of the GTD allelic ladder.

Table 8. Allelic ladder fragments included in the GT YDetector 29 Kit

No.	Markers	Alleles used in GTY 29 allelic ladder	GT QCDM (Control DNA) profile	GT QCDM102 (Control DNA) profile
1	DYS458	14, 15, 16, 17, 17.2, 18, 18.2, 19, 19.2, 20.2, 21.2	17	15
2	DYS392	10, 11, 12, 13, 14, 15, 16	11	11
3	DYS448	18, 19, 20, 21, 22	21	20
4	DYS643	8, 9, 10, 11, 12, 13	10	11
5	DYS576	12, 13, 14, 15, 16, 17, 18, 19, 20, 21	17	19
6	DYS460	9, 10, 11, 12	11	10
7	DYS627	16, 17, 18, 19, 20, 22, 23, 24	17	20
8	DYS437	14, 15, 16	14	14
9	DYS393	11, 12, 13, 14, 15	13	12
10	DYS389I	11, 12, 13, 14	13	13
11	DYS439	10, 11, 12, 13, 14	10	11
12	DYS533	9, 10, 11, 12, 13, 14	13	11
13	DYS389II	28, 29, 30, 32	30	30
14	DYF387S1	34, 35, 36, 37, 38, 39, 40, 42	36/38	40/41
15	DYS518	34, 35, 36, 37, 38, 39, 40, 41, 42, 43	41	38
16	DYS391	9, 10, 11	11	10
17	DYS456	13, 14, 15, 16, 17	16	15
18	YGATAH4	10, 11, 12, 13	13	11
19	DYS390	21, 22, 23, 24, 25	25	23
20	DYS438	9, 10, 11, 12	11	9
21	DYS481	21, 22, 23, 24, 25, 26, 27, 28	23	24
22	DYS385 a/b	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	11/14	13/17
23	AMXY	X, Y	X/Y	X/Y
24	DYS570	14, 15, 16, 17, 18, 19, 20, 21	19	17
25	DYS635	19, 20, 21, 22, 23, 24, 25	24	22
26	DYS19	13, 14, 15, 16, 17	16	15
27	DYS549	10, 11, 12, 13, 14	12	12
28	DYS449	25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36	32	33

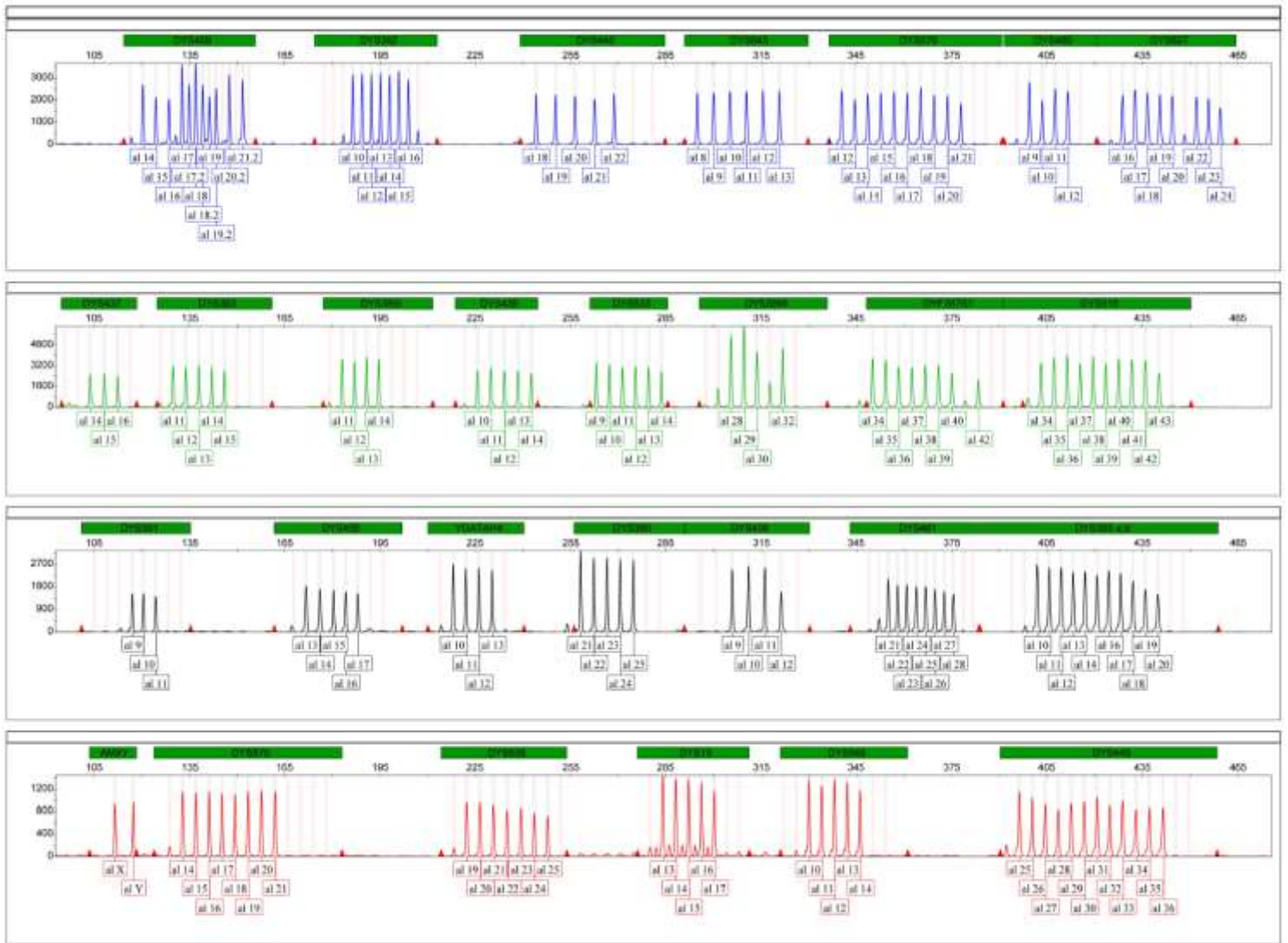


Figure 23. Electropherogram of the GTY allelic ladder. Allele assignment was performed using GeneMapper ID-X software.

5. An example for GTYDetector29 result

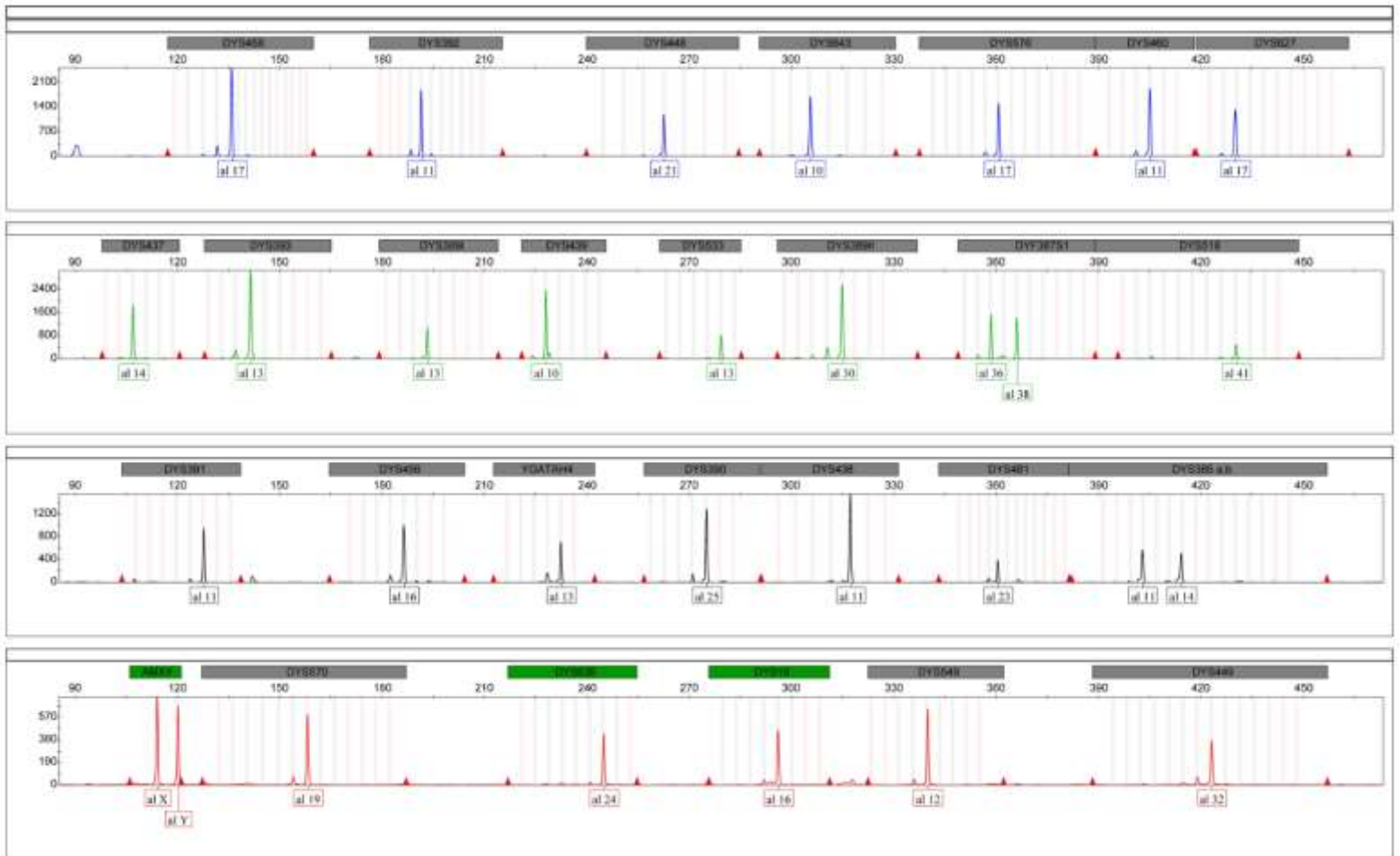


Figure 24. Electropherogram of the GT QCDM (Quality control DNA) showing male DNA profile analysed using GT YDetector 29 Kit. 1ng control DNA used as a template and Analysis was performed on an Applied Biosystems 3500xL Genetic Analyzer. Allele allocation was performed using GeneMapper *ID-X* software.

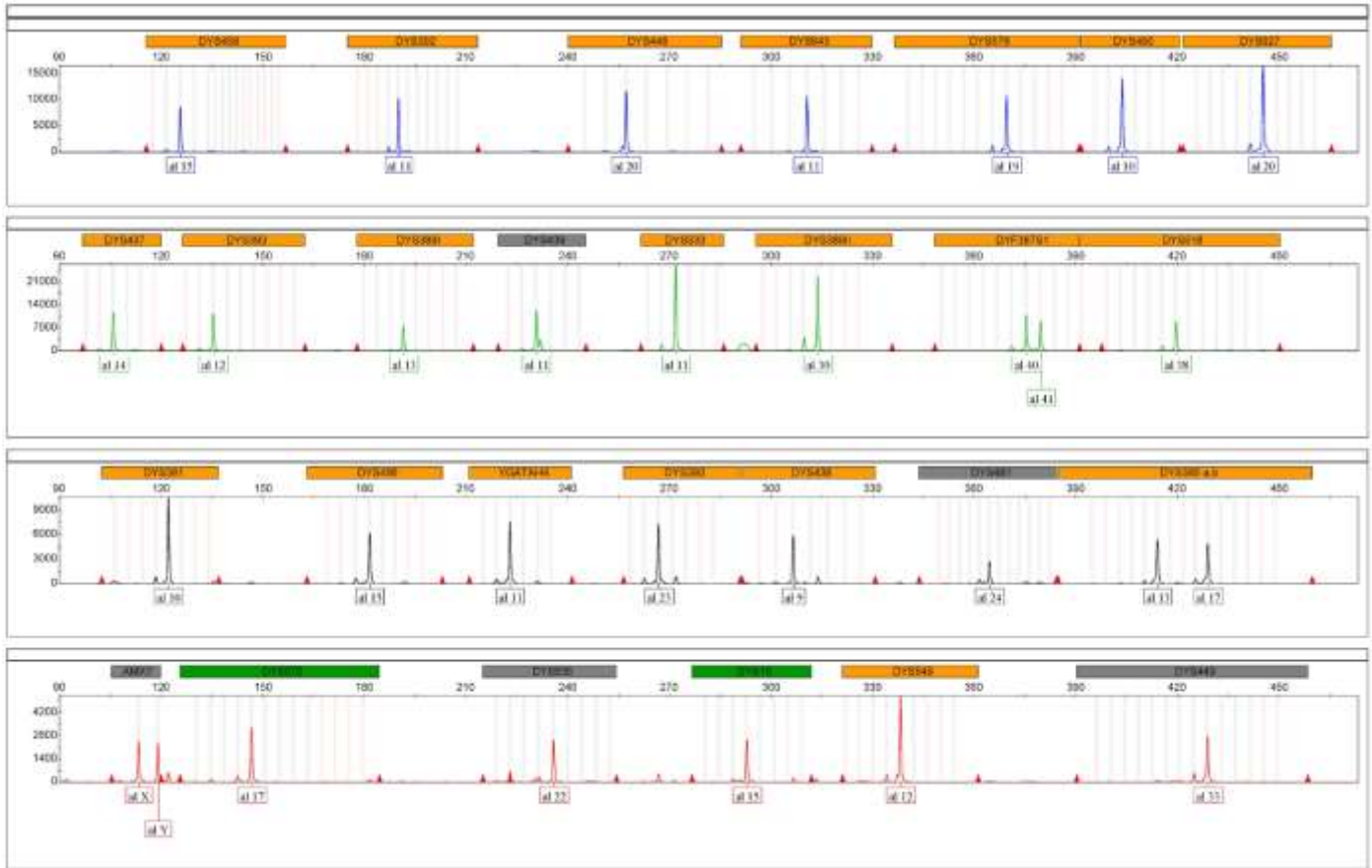


Figure 25. Electropherogram of the GT QCDM102 (Quality control DNA) showing male DNA profile analysed using GT YDetector 29 Kit. 1ng control DNA used as a template and Analysis was performed on an Applied Biosystems 3500xL Genetic Analyzer. Allele allocation was performed using GeneMapper *ID-X* software.

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 YDetector 29 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 QCDDM102 provided with GT YDetector 29 Kit to check efficiency of primer mix and other PCR reagents.</p>
<p>Inhibition of PCR because of too much template or other impurity in DNA extraction. Check the quality and quantity of extracted DNA. Use only the recommended DNA concentration. Make sure DNA is not degraded.</p>	

Extra peaks observed in one or more dye channels

Amplification of STRs can result in artifacts that seems as peaks one base smaller than actual peak due to incomplete addition of the 3' "A" residue.

To avoid this phenomenon, we recommend:

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

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

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

Samples not denatured completely, perform denaturation step as recommended.

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

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

Long-term stored PCR products are used.

	<p>Polymer-caused artifacts, check Polymer expiration date and storage time as mentioned in the manufacture guide.</p>
<p>Off-scale peaks</p>	<p>If off-scale peaks after primer peaks are observed –</p> <ul style="list-style-type: none"> • 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 size standard selected or no size standard 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 GTYDetector 29 Kit to obtain optimum results.

7. Limitations and Disclaimer

Any result obtained from GT YDetector 29 or any other Kit should be used and interpreted by qualified persons. GENETEK BIOPHARMA GmbH cannot bear any responsibilities for false use and interpretation being made by any lab. The results obtained by GT YDetector 29 or any other Forensic Kit should not use for diagnostic purpose and only be used as mentioned in intended use, hence GENETEK BIOPHARMA GmbH cannot be responsible for any clinical decisions made by user or client lab.

GT YDetector 29 Kit is designed to be used for forensic, human identification and paternity testing. It will not detect chromosome abnormalities or defect. User must carefully inspect any case of sample mix-up and cross-contamination before interpretation and 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 YDetector 29 Kit is for Research Use Only and user bears all the responsibility for its use in diagnostics. Please consult best practice guidelines when using any forensic kits including GT YDetector 29 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 technicians or persons 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. DNA Fragment Analysis by Capillary Electrophoresis User Guide by Applied Biosystems® Publication
Number 4474504.
2. Best Practice Guidelines for Internal Quality Control in Genetic Laboratories by Association for Clinical
Genetic Science.