

# Investigator<sup>®</sup> 24plex QS/GO! Protocol: BT6 Dye Set for ABI 3130/3130xl

This protocol describes the procedures for using Investigator 24plex QS Kits and Investigator 24plex GO! Kits on the Applied Biosystems 3130/3130xl Genetic Analyzer. For detailed instructions on instrument setup, spectral calibration or application of the ABI PRISM<sup>®</sup> Data Collection Software version 3.0 and the GeneMapper<sup>®</sup> ID Software, refer to the Applied Biosystems<sup>®</sup> 3130/3130xl Genetic Analyzers Getting Started Guide.

Important points before starting:

- The Investigator 24plex QS and GO! Kits use 6 fluorescence labels (6-FAM<sup>™</sup>, BTG, BTY, BTR2, BTP and BTO). Therefore, only the 6-dye upgraded Applied Biosystems 3130/3130xl Genetic Analyzer can be used with this protocol.
- The virtual filter set J6 on the upgraded 3130/3130xl Genetic Analyzer cannot be used for combined application of the 6 fluorescent labels. For the Investigator 24plex QS and GO! Kits, a BT6 dye set has to be imported in the ABI PRISM Data Collection Software version 3.0. The new BT6 dye set will exist in parallel to the J6 dye set, and both can be selected independently. The matrix standard is known as BT6.
- The BT6 dye set data file and support for the import can be requested from your local QIAGEN HID Application Specialist.
- For sample preparation there are no changes compared to standard protocol; the mixture of formamide and DNA standard is prepared the same.

The materials required for electrophoresis are listed in Table 1.

**Table 1. Materials required for electrophoresis.**

<b>Material</b>	<b>Specifications</b>
Capillary	36 cm Capillary Array for Applied Biosystems 3130/3130xl Genetic Analyzer
Polymer	POP-4 Polymer for Applied Biosystems 3130/3130xl Genetic Analyzer
Buffer	10x Genetic Analyzer Buffer with EDTA

## Spectral calibration/matrix generation

Before conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the 6 fluorescent labels 6-FAM, BTG, BTY, BTR, BTP and BTO for each analyzer. The calibration procedure creates a matrix that is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration is comprised of the following steps:

- Preparing the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Creating the instrument protocol for spectral calibration (Protocol Manager)
- Defining the plate composition in the plate editor (Plate Manager)
- Performing a spectral calibration run and checking the matrix

## Preparing the spectral calibration standards

### Example for 4 capillaries (Applied Biosystems 3130 Genetic Analyzer)

1. Prepare a mixture of formamide and Matrix Standard BT6 according to Table 2.

**Table 2. Setup of formamide and Matrix Standard BT6 mixture for 4 capillaries**

Component	Volume
Hi-Di™ Formamide	60 µl
Matrix Standard BT6	5 µl

2. Load 12 µl of the mixture into the 96-well plate (e.g., into positions A1–D1).
3. Denature for 3 min at 95°C.
4. Snap chill the mixtures by placing the plate on ice for 3 min.  
Alternatively, use the thermal cycler set to 4°C to cool the plate.

### Example for 16 capillaries (Applied Biosystems 3130xl Genetic Analyzer)

1. Set up a mixture of formamide and Matrix Standard BT6 according to Table 3.

**Table 3. Setup of formamide and Matrix Standard BT6 mixture for 16 capillaries**

Component	Volume
Hi-Di Formamide	204 µl
Matrix Standard BT6	17 µl

2. Load 12 µl of the mixture into the 96-well plate (e.g., positions A1–H1 and A2–H2).
3. Denature for 3 min at 95°C.
4. Snap chill the mixtures by placing the plate on ice for 3 min.  
Alternatively, use the thermal cycler set to 4°C to cool the plate.

## Performing the spectral calibration run

1. Place the 96-well plate onto the autosampler tray.
2. In the **Protocol Manager** of the Data Collection Software, open the **Instrument Protocol** window.
3. Click **New** to open the **Protocol Editor** dialog box.
4. Complete the dialog box with information from Table 4 and click **OK**.

**Table 4. Instrument protocol for spectral calibration**

Protocol Editor	Settings
Name	User (e.g., Spectral36_POP4_BT6)
Type	SPECTRAL
Dye Set	BT6
Polymer	User (e.g., POP4)*
Array Length	User (e.g., 36cm)*
Chemistry	Matrix Standard
Run Module	Default (e.g., Spect36_POP4_1)*

\* Depends on the type of polymer and length of capillary used.

- Click **New** in the **Plate Manager** of the Data Collection Software to open the **New Plate** Dialog box.
- Enter information from Table 5 and click **OK**. A new table in the **Plate Editor** opens automatically (Table 6).

**Table 5. Plate Editor for spectral calibration (I)**

New plate dialog	Settings
Name	e.g., Spectral_BT6_date
Application	Spectral Calibration
Plate Type	96-well
Owner Name/Operator Name	...

**Table 6. Plate Editor for spectral calibration (II)**

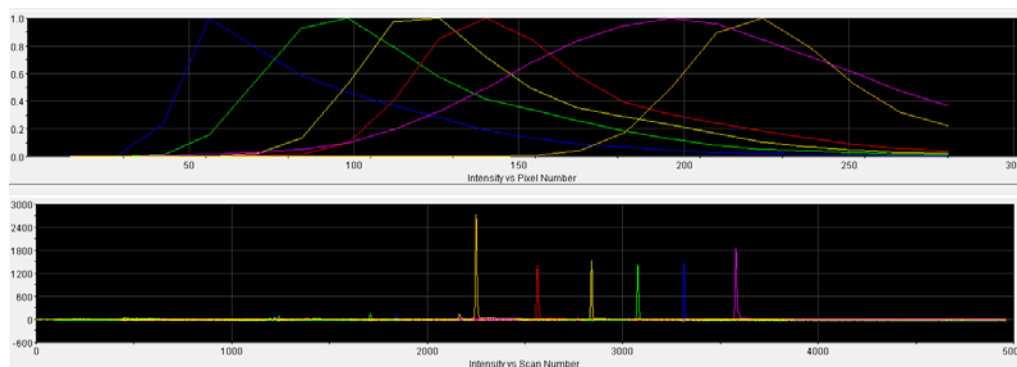
New plate dialog	Settings
Sample Name	Enter name for the matrix samples
Priority	e.g., 100
Instrument Protocol 1	Spectral36_POP4_BT6 (setting described before)

- Click the column header to select the entire column, and select **Fill Down** from the **Edit** menu to apply the information to the selected samples. Confirm by clicking **OK**.
- Link the reaction plate on the autosampler tray with the newly created plate ID (position A or B) and start the run.

## Checking the matrix

1. The quality value (Q value) of each capillary must be greater than 0.95, and the condition number range (C value) must be between 1 and 20.
2. Check for a flat baseline in the matrix samples. As shown in Figure 1, there should be 6 peaks with peak heights of about 1000–7000 RFU in each matrix sample. The optimal range is 2000–4000 RFU, and the peak intensity should be similar for all dyes other than orange.

**Note:** Orange will come across as the highest peak.



**Figure 1.** Electropherogram of spectral calibration with matrix standard BT6 on an Applied Biosystems 3130 Genetic Analyzer.

3. Check the new matrix with the current samples. There should be no pull-up peaks between the dye panels (B, G, Y, R, P, O) with the new matrix.
4. If calibration failed, repeat the calibration run.
5. If all capillaries have passed the test, the last calibration file for the Dye Set BT6 is activated automatically in the **Spectral Viewer**. Rename the calibration file (e.g., BT6\_Date of calibration).

## Sample preparation

1. Set up a mixture of formamide and DNA size standard according to Table 7.

**Table 7. Setup of formamide and DNA size standard mixture**

Component	Volume
Hi-Di Formamide	12.0 $\mu$ l
DNA Size Standard 550 (BTO)	0.5 $\mu$ l

2. Aliquot 12  $\mu$ l of the mixture to a tube for each sample to be analyzed.
3. Add 1  $\mu$ l PCR product or allelic ladder (diluted, if necessary).

4. Denature for 3 min at 95°C.
5. Snap chill by placing the plate on ice for 3 min.  
Alternatively, use the thermal cycler set to 4°C to cool the tubes.
6. Load the samples on the tray.

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted onto the plate of multi-capillary analyzers. If fewer samples are analyzed, the empty positions must be filled with 12 µl Hi-Di Formamide.

To ensure a reliable allelic assignment on multi-capillary analyzers, several ladders should be run. Room temperature may influence the performance of PCR products on multi-capillary instruments, such that shoulder peaks or split peaks may occur at low temperatures. Ensure ambient conditions are kept, as recommended by the instrument manufacturer.

## Setting up the Data Collection Software

1. Edit the Run Module once for the first run. In the **Module Manager** of the Data Collection Software, click **New** to open the **Run Module Editor** dialog box.  
**Note:** Modify the Run Module Default settings from “HIDFragmentAnalysis36\_POP4\_1” to those shown in Table 8.
2. Modify the Injection Voltage to 2.5 kV, the Injection Time to 30 s, the Run Voltage to 13 kV and the Run Time to 1800 s (Table 8).

**Table 8. Run Module 2.5kV\_30s\_500bp for the Applied Biosystems 3130/3130xl Genetic Analyzer**

Parameter	Settings
Oven Temperature (°C)	Default
Poly Fill Volume	Default
Current Stability (µA)	Default
Pre-Run Voltage (kV)	Default
Pre-Run Time (s)	Default
Injection Voltage (kV)	2.5
Injection Time (s)	30*
Voltage Number of Steps	Default
Voltage Step Interval	Default
Data Delay Time (s)	Default
Run Voltage (kV)	13 kV
Run Time (s)	1800†

\* Deviating from the standard settings, the injection time may range between 1 and 35 s depending on the type of sample. If samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content, an injection time of up to 35 s may be necessary.

† The run time for Investigator 24plex Kits was modified to enable analysis of fragments with lengths of up to 500 bp.

3. Click **Save As**, enter a name for the new Run Module (e.g., 2.5kV\_30s\_500bp), and confirm by clicking **OK**.
4. Click **Close** to exit the **Run Module Editor**.

## Starting the run

1. Place the prepared 96-well plate on the autosampler tray.
2. Open the **Protocol Manager** of the Data Collection Software.
3. Click **New** in the **Instrument Protocol** window to open the **Protocol Editor** dialog box and enter the information in Table 9.

**Table 9. Settings in Instrument Protocol**

Protocol Editor	Settings
Name	e.g., Run36_POP4_BT6_30min
Type	REGULAR
Run Module	2.5kV_30s_500bp*
Dye Set	BT6

\* See Table 8, "Run Module 2.5kV\_30s\_500bp for the Applied Biosystems 3130/3130xl Genetic Analyzer".

4. Click **OK** to exit the **Protocol Editor**.
5. Before each run, it is necessary to create a plate definition. In the **Plate Manager** of the Data Collection Software, click **New** to open the **New Plate Dialog** box.
6. Enter the information in Table 10.

**Table 10. GeneMapper Plate Editor (I)**

Protocol Editor	Settings
Name	e.g., Plate_BT6_Date
Application	Select GeneMapper Application
Plate type	96-Well
Owner Name/	...

7. Click **OK** and a new table in the **Plate Editor** opens automatically (Table 11).
8. Click the column header to select the entire column. Select **Fill Down** from the **Edit** menu to apply the information to all selected samples. Click **OK**.
9. In the **Run Scheduler**, click **Find All**, and select **Link** to link the reaction plate on the autosampler tray to the newly created plate record (position A or B).

**Table 11. GeneMapper Plate Editor (II)**

Parameter	Settings
Sample Name	Enter the name for the samples
Priority	e.g., 100 (Default)
Sample Type	Sample or Allelic Ladder
Size Standard	e.g., SST-BTO_60-500bp
Panel	e.g., 24plex_QS_Panels
Analysis Method	e.g., Analysis_HID_3130
Snp Set	–
User-defined 1-3	–
Results Group 1	(Select results group)
Instrument Protocol 1	Run36_POP4_BT6_30min (setting described before)

10. Start the run.
11. During the run, view Error Status in the Event Log or examine the quality of the raw data for each capillary in the **Capillaries Viewer** or the **Cap/Array Viewer**.
12. View data as an overview in **Run History** or **Cap/Array Viewer** of the Data Collection Software.  
Run data are saved in the Run Folder of the previously chosen Result Group.



## Analysis parameters/analysis method

Table 12 lists the recommended analysis parameters.

**Table 12. Recommended settings for the Applied Biosystems 3130/3130xl Genetic Analyzer**

Parameter	Settings
Peak Detection Algorithm	Advanced
Ranges	Analysis: Partial Range Start Point: 2000; Stop Point: 10,000 Sizing: All Sizes
Smoothing and Baselineing	Smoothing: Light Baseline Window: 51 pts
Size Calling Method	Local Southern Method
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* P:* O:* Min. Peak Half Width: 2 pts Polynomial Degree: 3 Peak Window Size: 11 pts <sup>†</sup> Slope Thresholds: 0.0

\* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneMapper ID or ID-X Software. The thresholds are usually 50–200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be 3 times higher than the background noise of the baseline.

<sup>†</sup> Only the setting for Peak Window Size is different to defaults from Applied Biosystems for HID analysis.

**Note:** For information on the use of the recommended Template Files (as analysis parameters), refer to the appropriate Investigator Template Files User Guide (GeneMapper ID, or GeneMapper ID-X).

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