



FRAGMENT ANALYSIS USING
ABI 3730/3730XL

HANDBOOK

Contents

1. INTRODUCTION	3
2. DEV-5 SPECTRAL CALIBRATION	3
2.1. Preparing the DEV-5 Spectral Calibration standards.....	3
2.2. Creating a Spectral Instrument Protocol	4
2.3. Creating a new Plate.....	5
2.4. Performing the DEV-5 Spectral Calibration run	6
2.5. Reviewing the DEV-5 Spectral Calibration run	7
3. CREATING ANALYSIS SETTINGS	8
3.1. Creating a Run Module	8
3.2. Creating an Instrument Protocol	9
4. SETTING UP A RUN.....	10
4.1. Selecting the Active Spectral Calibration for the Any5Dye dye set	10
4.2. Creating a Plate	11
4.3. Starting the Sample Run	13
5. REVISION HISTORY	13

Update service

Sign up for the handbook update service to receive notifications via e-mail whenever there is a new version available.

Visit www.devyser.com/ifu-subscription to sign up

1. INTRODUCTION

The **Devyser CFTR 68** kit uses the **G5 dye set**. The ABI® 3730 and 3730xL Genetic Analyzers must be calibrated for the G5 dye set prior to running the Devyser CFTR 68 kit. The spectral calibration is performed using **ABI DS-33 Matrix standard kit** (Thermo Fisher Scientific, Cat. no. 4345833) according to the manufacturer instructions. Go directly to section 3 of this Handbook.

All other Devyser kits use the **DEV-5 dye set**. The ABI 3730 and 3730xL Genetic Analyzers must be calibrated for the DEV-5 dye set prior to running these Devyser kits. The spectral calibration is performed using the **DEV-5 MultiCap** calibration standards (Art. No. 8-A401), according to section 2 of this Handbook.

2. DEV-5 SPECTRAL CALIBRATION

The instructions written below describe how to perform a spectral calibration for the DEV-5 dye set and apply to all Devyser kits except for the Devyser CFTR 68 kit. If using the Devyser 68 kit, go directly to section 3.

Before starting the calibration run on an ABI 3730/3730xL Genetic Analyzer, ensure that the instrument contains fresh buffers in the reservoirs.

2.1. Preparing the DEV-5 Spectral Calibration standards

■ If using an ABI 3730 Genetic Analyzer (48 capillaries)

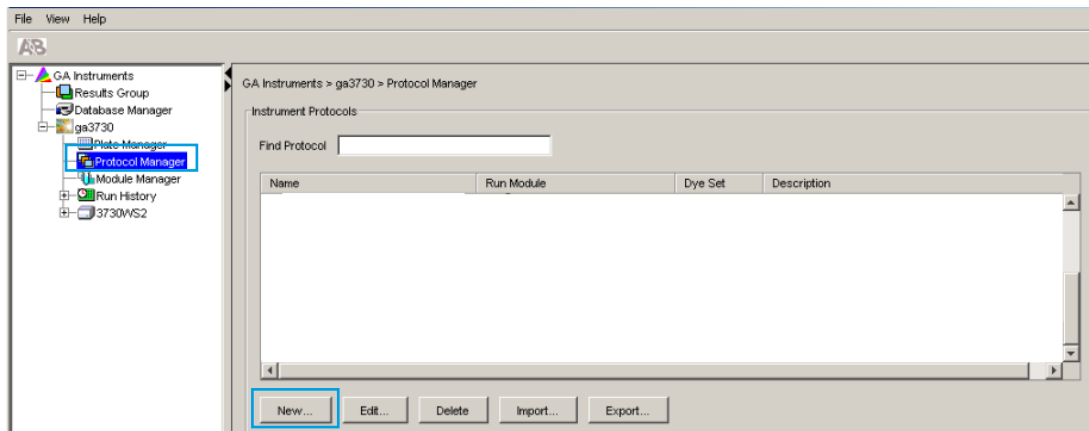
1. Thoroughly vortex the DEV-5 tube and spin the tube briefly
2. Dilute DEV-5 in Hi-Di Formamide by combining 5 µL DEV-5 with 495 µL Hi-Di Formamide
3. Vortex for 15 seconds and spin the tube briefly
4. Dispense 10 µL/well into 48 wells, alternating rows, of a 96-well microtiter plate
5. Seal with a septa and spin the plate briefly to ensure that the reagents are at the bottom of the wells and that no bubbles are present
6. Assemble the plate and load it on the ABI 3730 instrument (refer to the instrument's user guide for further details)

■ If using an ABI 3730xL Genetic Analyzer (96 capillaries)

1. Thoroughly vortex the DEV-5 tube and spin the tube briefly
2. Dilute DEV-5 in Hi-Di Formamide by combining 10 µL DEV-5 with 990 µL Hi-Di Formamide
3. Vortex for 15 seconds and spin the tube briefly
4. Dispense 10 µL/well into 96 consecutive wells of a 96-well microtiter plate
5. Seal with a septa and spin the plate briefly to ensure that the reagents are at the bottom of the wells and that no bubbles are present
6. Assemble the plate and load it on the ABI 3730xL instrument (refer to the instrument's user guide for further details)

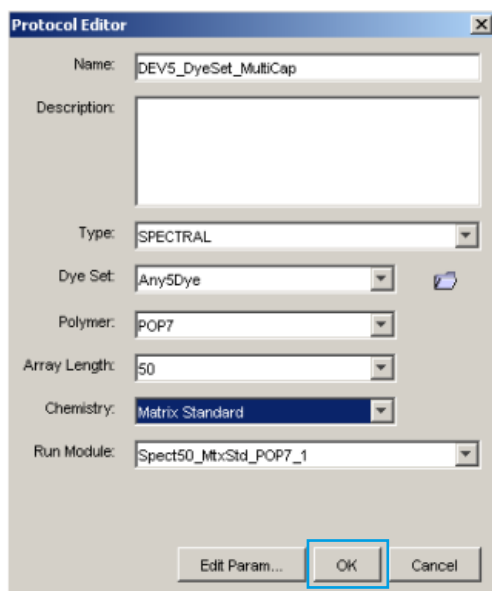
2.2. Creating a Spectral Instrument Protocol

1. In the main window, click **Protocol Manager** and then click **New**



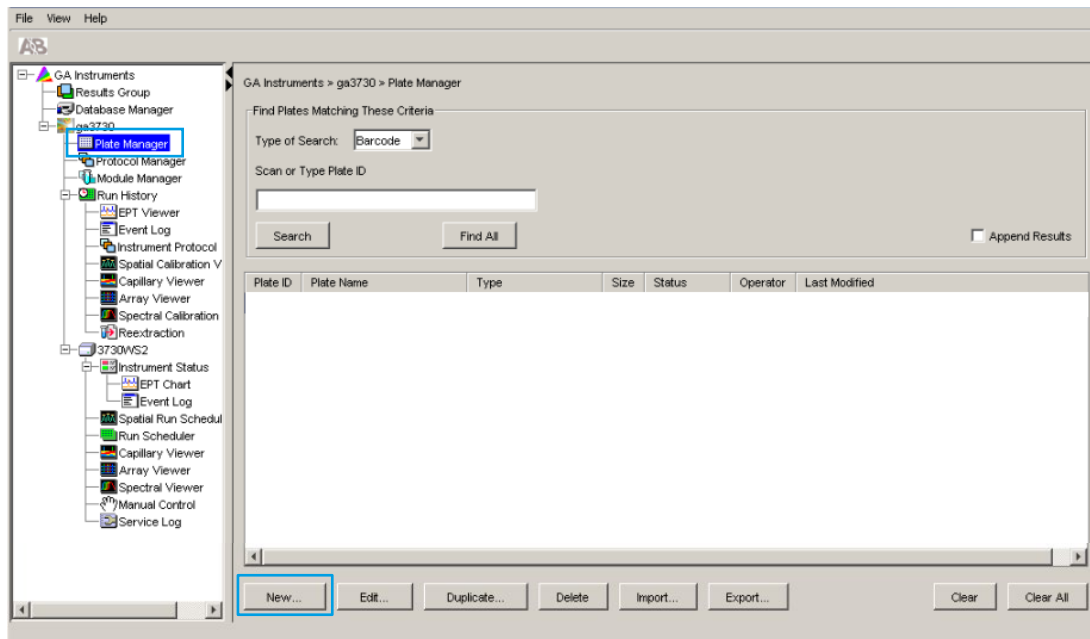
2. Fill out the **Protocol Editor** window:

- a. **Name:** Enter a Protocol name (e.g. DEV5_DyeSet_MultiCap)
- b. **Type:** SPECTRAL
- c. **Dye Set:** Any5Dye, or G5 if using the Devyser CFTR 68 kit
- d. **Polymer:** POP7
- e. **Array Length:** Enter the capillary array length used on the instrument
- f. **Chemistry:** Matrix Standard
- g. **Run Module:** Select the default Run Module for the array, chemistry and polymer used (e.g. Spect50_MtxStd_POP7_1)
- h. Click **OK**

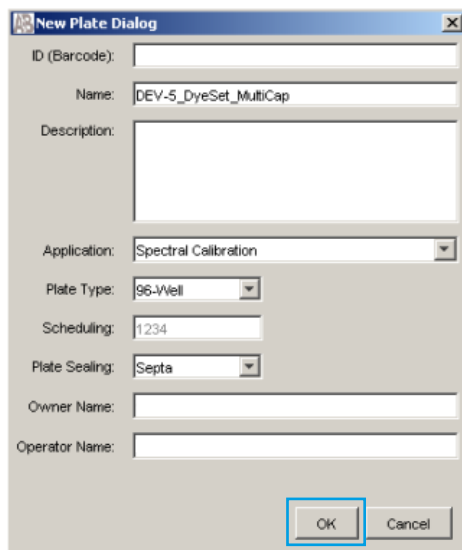


2.3. Creating a new Plate

1. In the main window, click **Plate Manager** and then click **New**

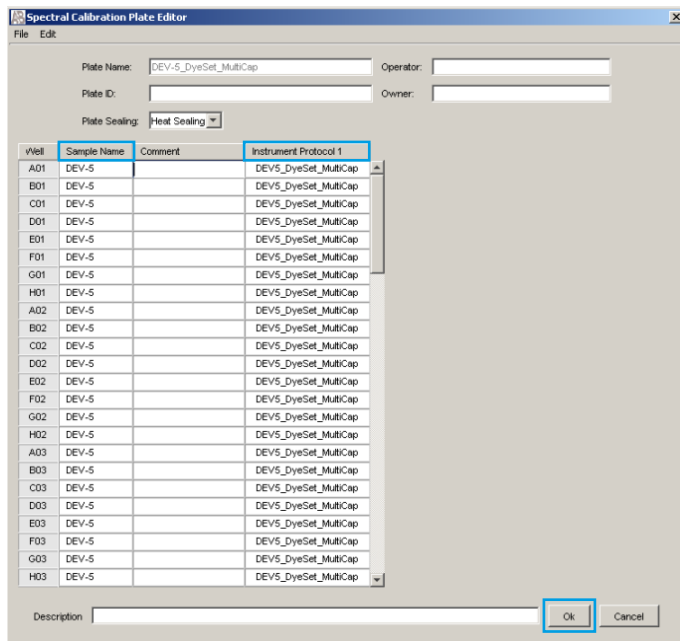


2. Fill out the **New Plate Dialog** window:
 - a. **ID (Barcode):** Scan the plate barcode
 - b. **Name:** Enter a Plate name (e.g. DEV-5_DyeSet_MultiCap)
 - c. **Application:** Spectral Calibration
 - d. **Plate Type:** 96-Well
 - e. **Plate Sealing:** Select Heat Sealing or Septa
 - f. **Owner Name:** Enter the name of the owner
 - g. **Operator Name:** Enter the name of the operator
 - h. Click **OK**



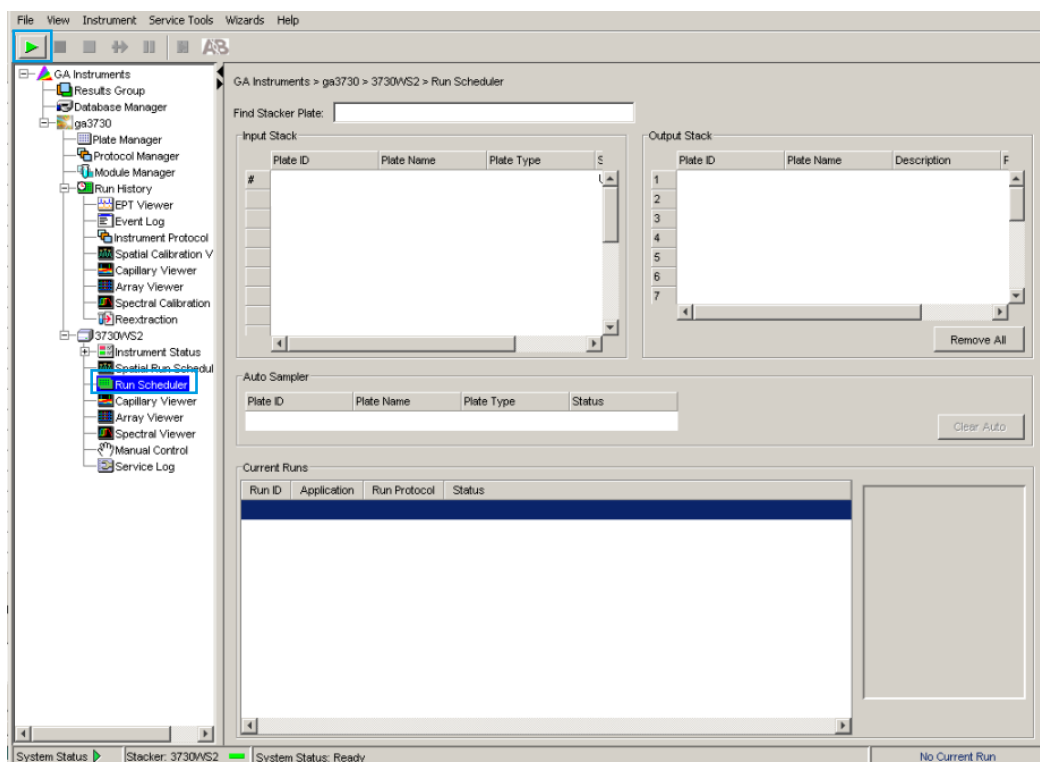
3. Fill out the newly opened **Spectral Calibration Plate Editor** window:
 - a. **Sample Name:** Enter a Spectral Calibration name for all 48 (ABI 3730) or 96 (ABI 3730xL) wells (e.g. DEV-5 or G5)

- b. **Instrument Protocol 1:** Select the Instrument Protocol created in section 2.2 for all 48 (ABI 3730) or 96 (ABI 3730xL) wells
- c. Click **OK**



2.4. Performing the DEV-5 Spectral Calibration run

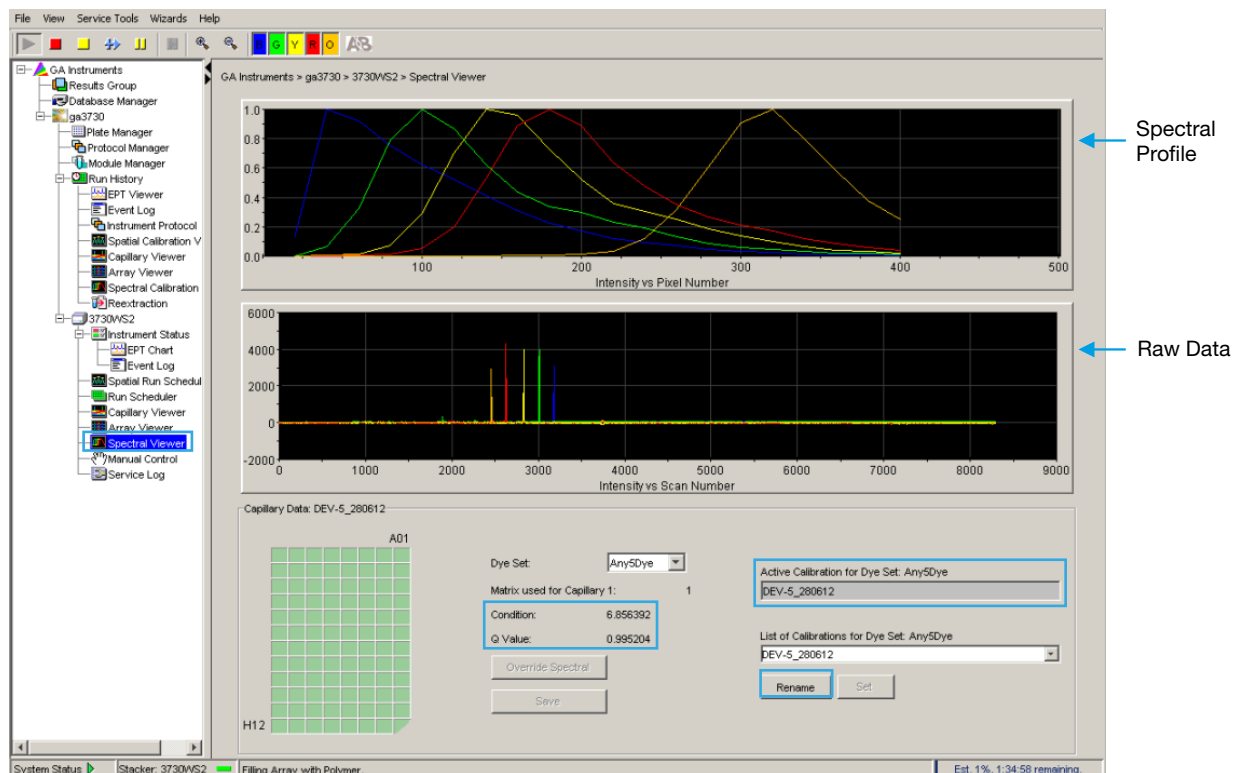
1. In the main window, click **Run Scheduler**
2. Click **Find All** and select the **Plate** created in section 2.3 (status pending)
3. Link the plate by clicking on the yellow plate position indicator. The plate color will change from yellow to green when it is successfully linked
4. In the toolbar, click on the **green arrow** to start the run of the pending plate



2.5. Reviewing the DEV-5 Spectral Calibration run

Review the Raw Data and Spectral Profile for each capillary after completion of the DEV-5 spectral calibration run.

1. In the main window, click **Spectral Viewer**
2. Review by clicking on each capillary in the plate diagram
3. Visually inspect and evaluate the **Spectral Profile** and **Raw Data** for each capillary
4. In the **Spectral Profile** window, verify that the order of the peaks from left to right is: **BLUE, GREEN, YELLOW, RED, ORANGE**
5. In the **Raw Data** window, verify that the order of the peaks from left to right is: **ORANGE, RED, YELLOW, GREEN, BLUE**
6. Verify that the peaks are distinct and regular
7. Verify that the peak heights are between 1000 and 30 000 relative fluorescent units (rfu)
8. Each capillary should have a **Quality Value** > 0,8 and a **Condition #** ranging from 1 to 20
9. A green capillary indicates that the capillary has passed the spectral calibration. A red capillary indicates that the capillary has failed the spectral calibration. A failed capillary is automatically assigned the spectral profile of its nearest passing capillary
10. When the calibration is approved, click **Rename** and enter an appropriate name for the calibration (e.g DEV-5_DDMMYY). The calibration will then automatically be set as the active calibration in the **Active Calibration** drop list



3. CREATING ANALYSIS SETTINGS

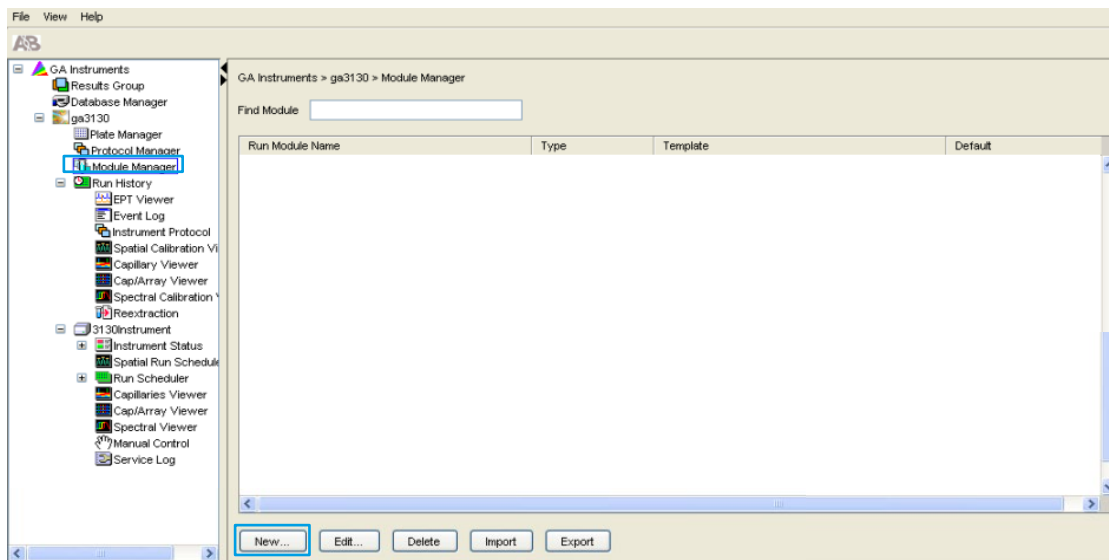
Before starting a fragment analysis run on the ABI 3730/3730xL Genetic Analyzer, the following settings need to be set up in the instruments' Data Collection Software:

- Run Module
- Instrument Protocol

The instructions below are from an ABI 3130 Genetic Analyzer, but the procedure is similar for ABI 3730/3730xL Genetic Analyzers. Refer to the instruments' user guide for further details.

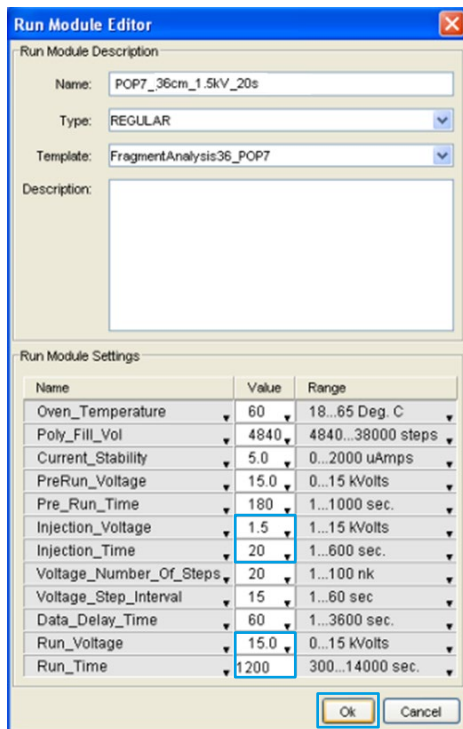
3.1. Creating a Run Module

1. In the main window, click **Module Manager** and **New**



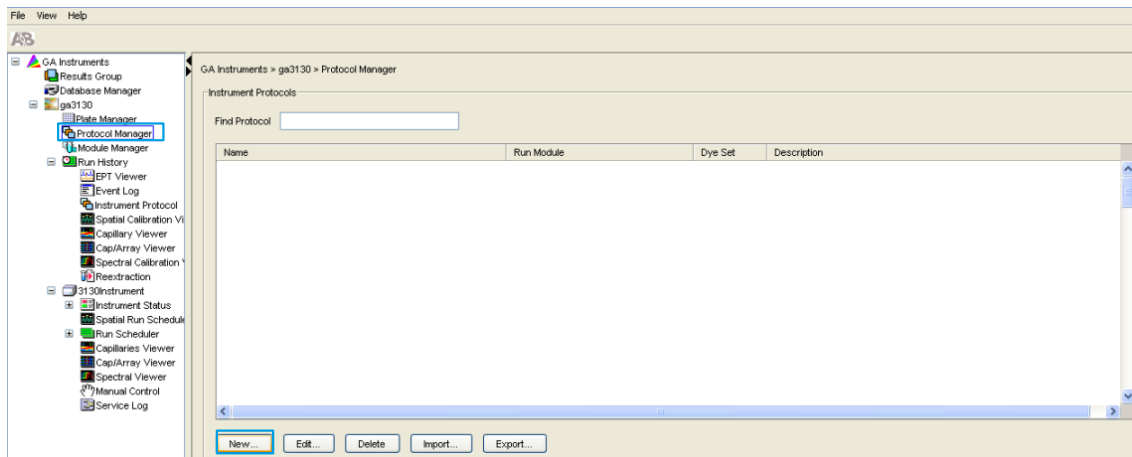
2. Fill out the **Run Module Editor** window:
 - a. **Name:** Enter a Run Module name (e.g. POP7_36cm_1.6kV_15s)
 - b. **Type:** Regular
 - c. **Template:** Select the default template for the capillary array and polymer used (e.g. FragmentAnalysis36_POP7)
 - d. **Run Module Settings:** Enter the Injection Voltage, Injection Time and Run Time as indicated in the Devyser kit IFU/handbook
 - e. Click **OK**

NOTE! The injection time/voltage can be decreased or increased by creating an additional Run Module and Instrument Protocol if the peak signals are too high or too low. The increase/decrease in injection time/voltage is typically proportional to the measured signal intensity (peak area and peak height). Increasing the injection time >30 seconds might however decrease the peak resolution. A too high injection voltage can also cause sloping and broadening of longer length fragments



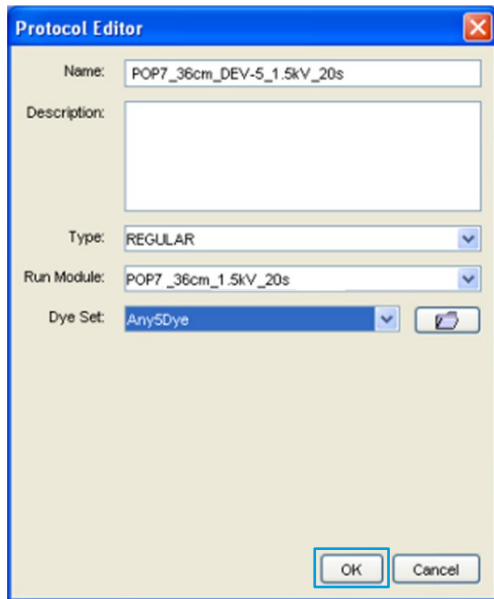
3.2. Creating an Instrument Protocol

1. In the main window, click **Protocol Manager** and **New**



2. Fill out the **Protocol Editor** window:

- a. **Name:** Enter a Protocol name (e.g. POP7_36cm_DEV-5_1.6kV_15s)
- b. **Type:** Regular
- c. **Run Module:** Select the Run Module created in section 3.1
- d. **Dye Set:** Any5Dye, or G5 if using the Devyser CFTR 68 kit
- e. Click **OK**



4. SETTING UP A RUN

4.1. Selecting the Active Spectral Calibration for the Any5Dye dye set

If several spectral calibrations for different dye sets are saved under the “Any5Dye” dye set (e.g calibration for the DEV-5 dye set and calibration for another dye set), see instructions below, otherwise go directly to section 4.2.

The most recent spectral calibration is automatically chosen as the active calibration for the “Any5Dye” dye set. Prior to creating a run plate, ensure that the desired calibration is set as active (e.g DEV-5 calibration or other calibration):

1. In the main window, click **Spectral Viewer**
2. Select Any5Dye in the **Dye Set** drop list. The most recent spectral calibration is automatically chosen as the **Active Calibration** (e.g DEV-5_DDMMYY)
3. To set another calibration as the active calibration, select the desired calibration in the **List of Calibrations** drop list
4. Click **Set**. The newly selected calibration is now set as the **Active Calibration**

The screenshot shows the 'Spectral Viewer' window in the software. The top plot, 'Intensity vs Pixel Number', shows several overlapping peaks. The bottom plot, 'Intensity vs Scan Number', shows a series of vertical spikes. Below the plots, the 'Capillary Data' section for 'DEV-5_280612' is visible, including a grid for 'A01' and 'H12', and fields for 'Dye Set' (Any5Dye), 'Matrix used for Capillary 1' (1), 'Condition' (6.856392), and 'Q Value' (0.995204). Two blue boxes highlight the 'Active Calibration for Dye Set: Any5Dye' field (containing 'DEV-5_280612') and the 'List of Calibrations for Dye Set: Any5Dye' dropdown menu (also containing 'DEV-5_280612'). Blue arrows point from text labels to these elements.

Current active calibration

Drop list of other calibrations available for the selected dye set

4.2. Creating a Plate

1. In the main window, click **Plate Manager** and then click **New**

The screenshot shows the 'Plate Manager' window. The 'Find Plates Matching These Criteria' section has 'Type of Search' set to 'Barcode'. Below this is a search input field and 'Search' and 'Find All' buttons. A table with columns 'Plate ID', 'Plate Name', 'Type', 'Size', 'Status', 'Operator', and 'Last Modified' is shown, but it is currently empty. At the bottom, the 'New...' button is highlighted with a blue box.

2. Fill out the **New Plate Dialog** window
 - a. **Name:** Enter Plate name
 - b. **Application:** GeneMapper-Generic (used if data is analyzed on a separate computer)
 - c. **Plate type:** 96-Well
 - d. **Owner Name:** Enter owner name
 - e. **Operator Name:** Enter the operator name
 - f. Click **OK**

New Plate Dialog

Name: Devyser_Compact_v3

Description:

Application: GeneMapper-Generic

Plate Type: 96-Well

Owner Name: sign

Operator Name: sign

OK Cancel

3. Fill out the newly opened **GeneMapper Plate Editor** window:
 - a. **Sample name:** Enter the sample names
 - b. **Comment:** Optional
 - c. **Result Group 1:** Location where the raw data files (.fsa files) will be saved
 - d. **Instrument Protocol 1:** Select the Instrument Protocol created in section 3.2
 - e. Click **OK**

GeneMapper Plate Editor

File Edit

Plate Name: Devyser Compact v3 Operator: sign

Plate Sealing: Septa Owner: sign

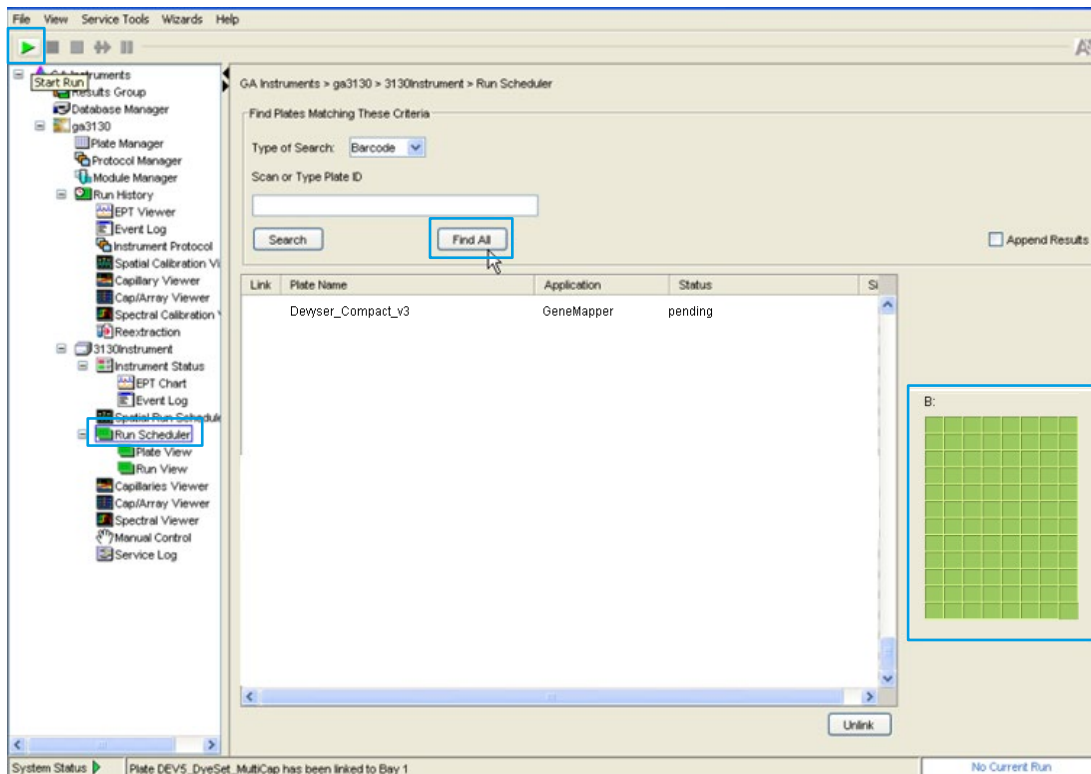
Well	Sample Name	Comment	Priority	Results Group 1	Instrument Protocol 1
A01	T13	Devyser Compact v3	100	Local	POP7_36cm_DEV-5_1.5kV_20s
B01	T18	Devyser Compact v3	100	Local	POP7_36cm_DEV-5_1.5kV_20s
C01	T21	Devyser Compact v3	100	Local	POP7_36cm_DEV-5_1.5kV_20s
D01	TO	Devyser Compact v3	100	Local	POP7_36cm_DEV-5_1.5kV_20s
E01	PC	Devyser Compact v3	100	Local	POP7_36cm_DEV-5_1.5kV_20s
F01	NTC	Devyser Compact v3	100	Local	POP7_36cm_DEV-5_1.5kV_20s
G01					
H01					
A02					
B02					
C02					
D02					
E02					
F02					

Description

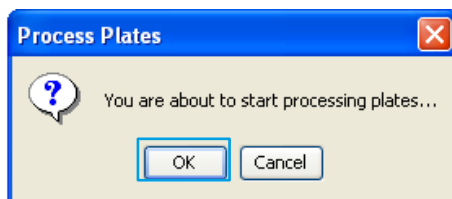
Ok Cancel

4.3. Starting the Sample Run

1. In the main window, click **Run Scheduler**
2. Click **Find All** and select the **Plate** created in section 4.2 (status pending)
3. Link the plate by clicking on the yellow plate position indicator. The plate color will change from yellow to green when it is successfully linked
4. In the toolbar, click on the **green arrow** to start the run



5. Click **OK** to start processing the plate



5. REVISION HISTORY

Version	Description
2020-06-15	Editorial changes 1. New section 2. Introduction text to section 2 edited 2.2 and 3.2. Step 2: "or G5 if running the Devyser CFTR 68 kit" added 2.5. Step 11 removed 4.1. New section 5. New section
2018-12-20	New