DEVYSER
RESULTS FOR LIFE

FRAGMENT ANALYSIS USING ABI 3730/3730XL

HANDBOOK

Contents

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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)

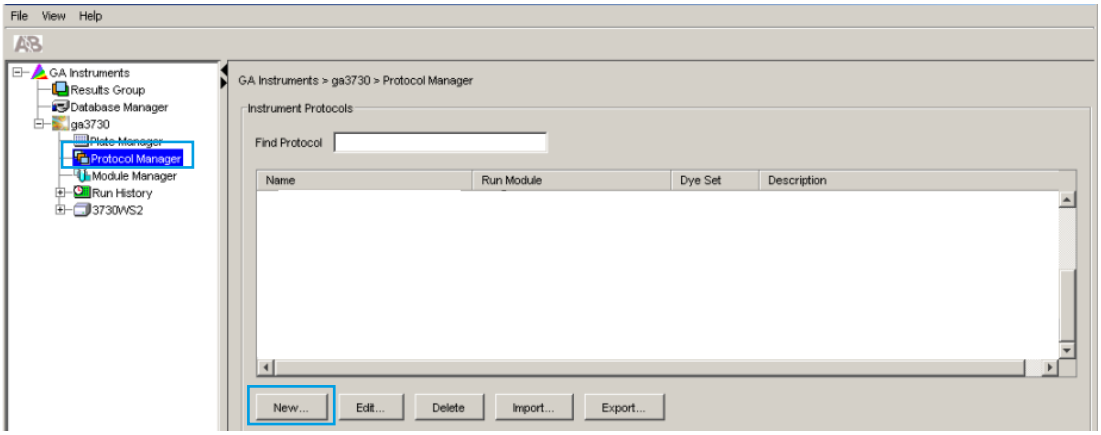
1. Thoroughly vortex the DEV-5 tube and spin the tube briefly
2. Dilute DEV-5 in Hi-Di Formamide by combining 5 µL DEV-5 with 495 µL Hi-Di Formamide
3. Vortex for 15 seconds and spin the tube briefly
4. Dispense 10 µL/well into 48 wells, alternating rows, of a 96-well microtiter plate
5. Seal with a 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)

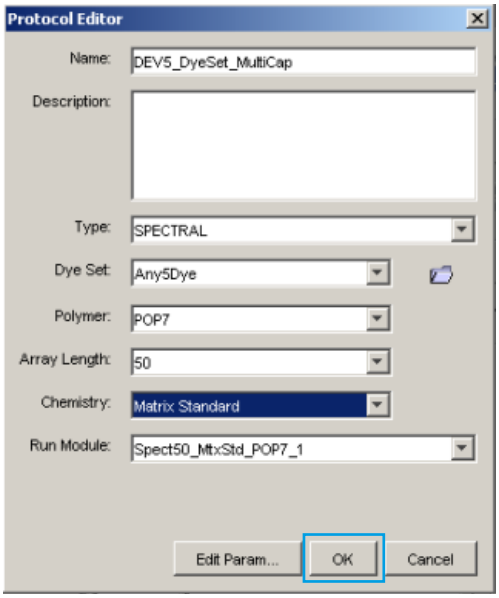
1. Thoroughly vortex the DEV-5 tube and spin the tube briefly
2. Dilute DEV-5 in Hi-Di Formamide by combining 10 µL DEV-5 with 990 µL Hi-Di Formamide
3. Vortex for 15 seconds and spin the tube briefly
4. Dispense 10 µL/well into 96 consecutive wells of a 96-well microtiter plate
5. Seal with a septa 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**

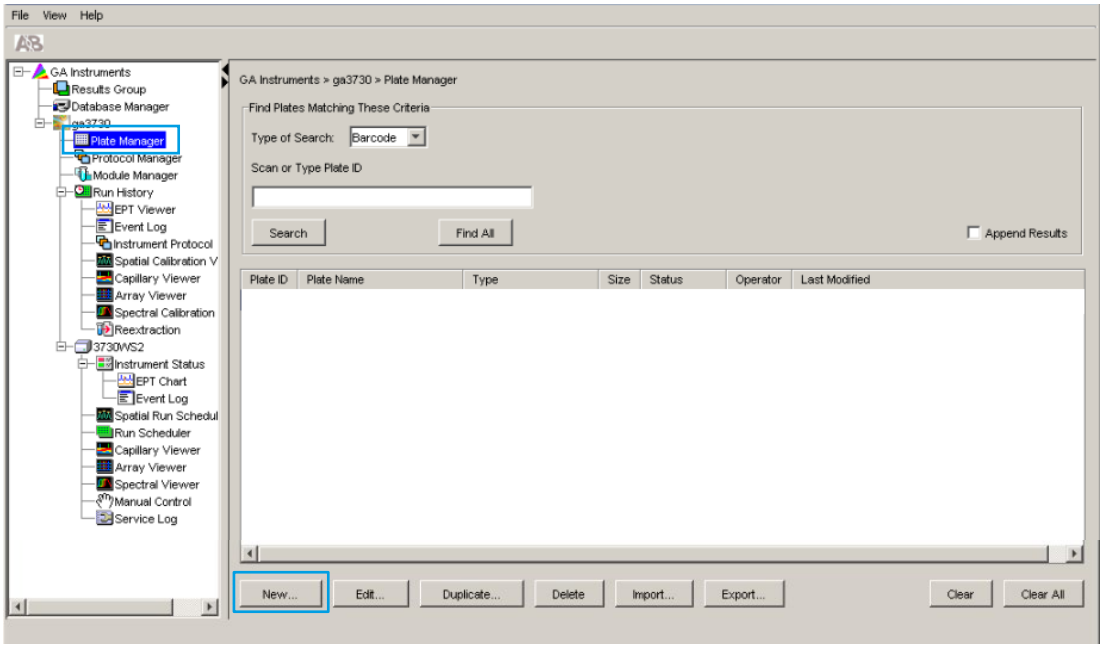


2. Fill out the **Protocol Editor** window:
   a. **Name**: Enter a Protocol name (e.g. DEV5_DyeSet_MultiCap)
   b. **Type**: SPECTRAL
   c. **Dye Set**: Any5Dye, or G5 if using the Devyser CFTR 68 kit
   d. **Polymer**: POP7
   e. **Array Length**: Enter the capillary array length used on the instrument
   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**

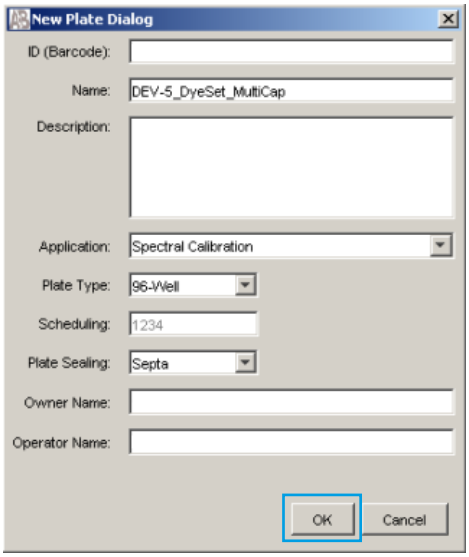


2.3. Creating a new Plate

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

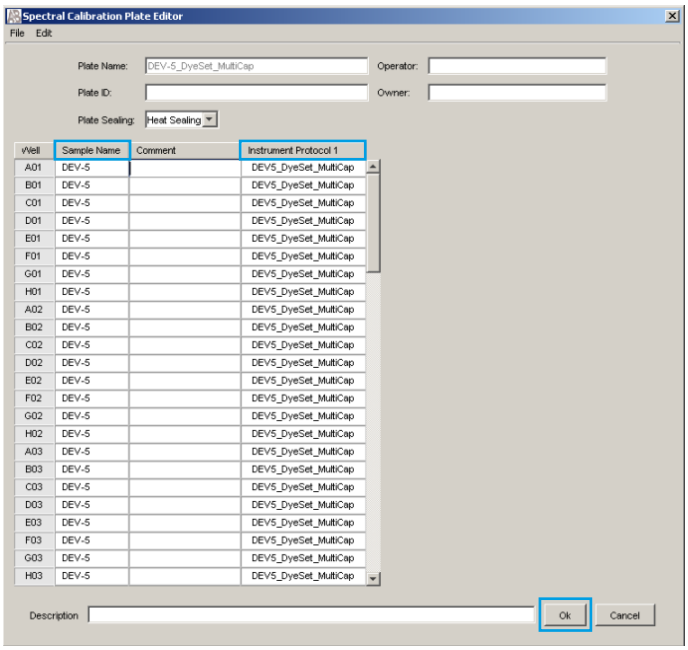


2. Fill out the **New Plate Dialog** window:
   a. **ID (Barcode)**: Scan the plate barcode
   b. **Name**: Enter a Plate name (e.g. DEV-5_DyeSet_MultiCap)
   c. **Application**: Spectral Calibration
   d. **Plate Type**: 96-Well
   e. **Plate Sealing**: Select Heat Sealing or Septa
   f. **Owner Name**: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



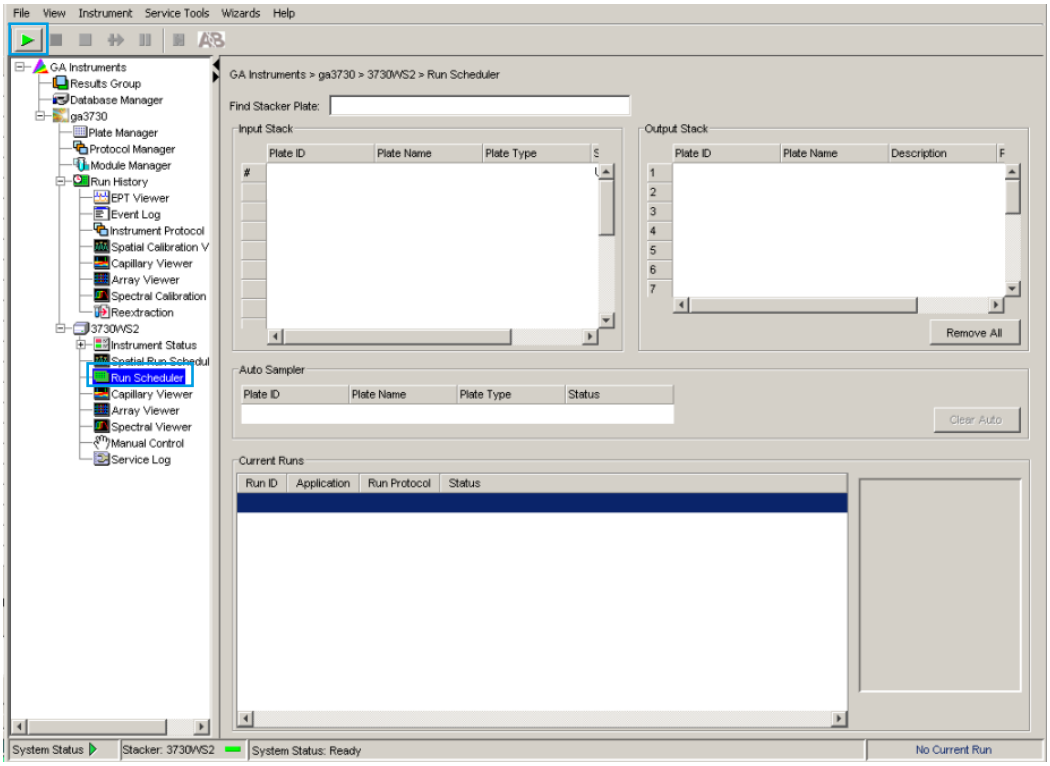
3. Fill out the newly opened **Spectral Calibration Plate Editor** window:
   a. **Sample Name**: Enter a Spectral Calibration name for all 48 (ABI 3730) or 96 (ABI 3730xL) wells (e.g. DEV-5 or G5)

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

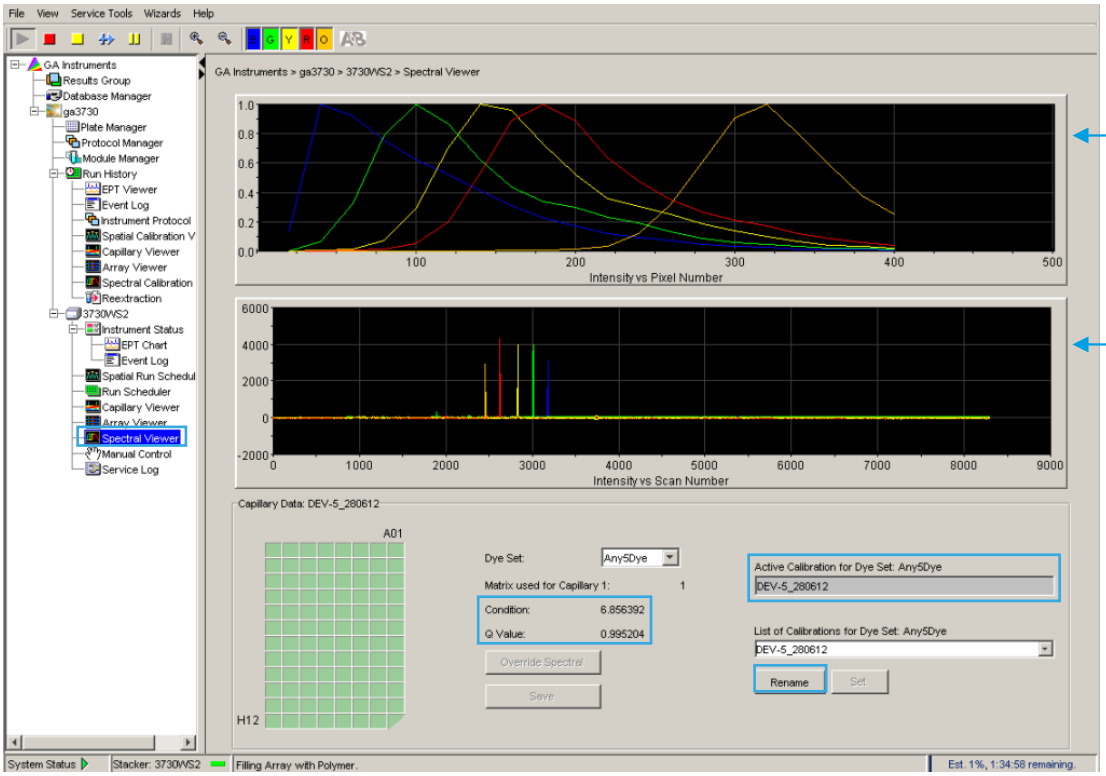
1. In the main window, click **Run Scheduler**
2. Click **Find All** and select the **Plate** created in section 2.3 (status pending)
3. Link the plate by clicking on the yellow plate position indicator. The plate color will change from yellow to green when it is successfully linked
4. In the toolbar,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

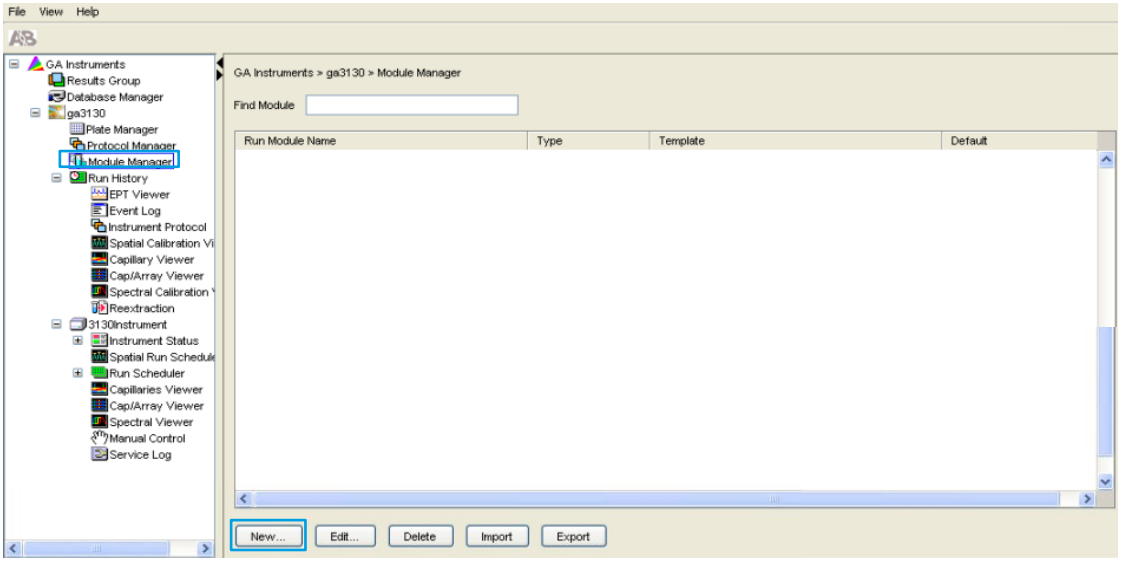
Before starting a fragment analysis run on the ABI 3730/3730xL Genetic Analyzer, the following settings need to be set up in the instruments' Data Collection Software:

- 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.

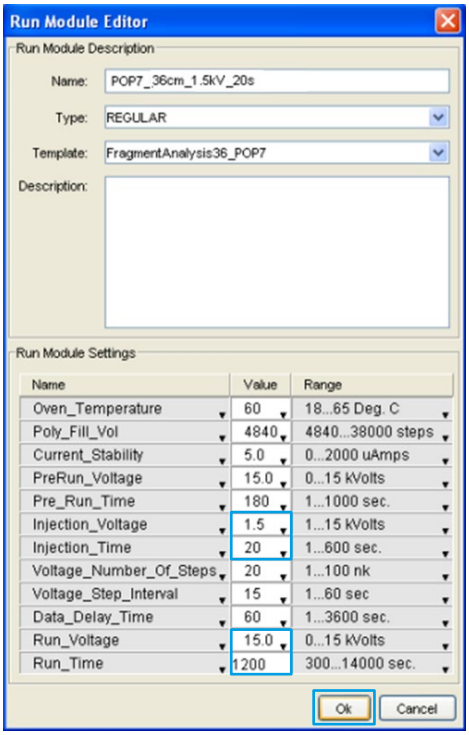
3.1. Creating a Run Module

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



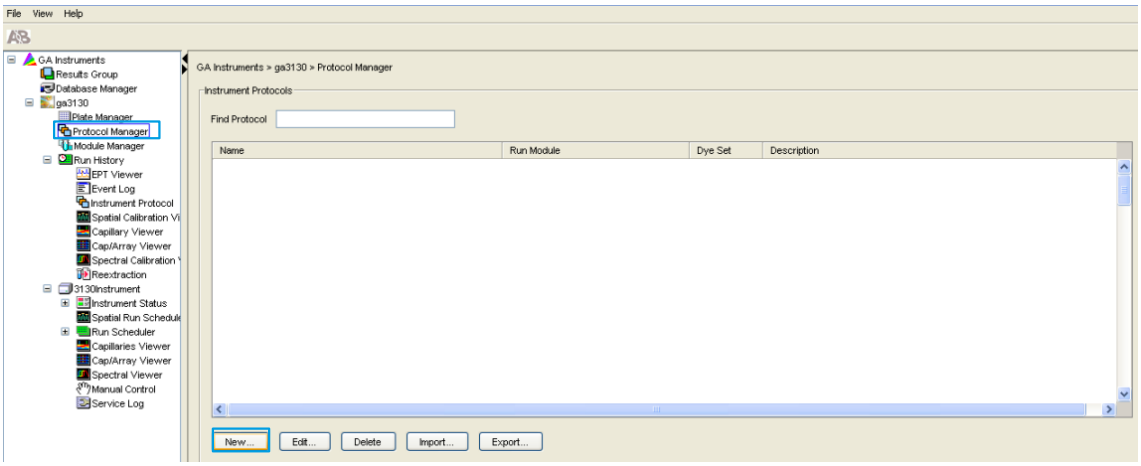
2. Fill out the **Run Module Editor** window:
   a. **Name**: Enter a Run Module name (e.g. POP7_36cm_1.6kV_15s)
   b. **Type**: Regular
   c. **Template**: Select the default template for the capillary array and polymer used (e.g. FragmentAnalysis36_POP7)
   d. **Run Module Settings**: Enter the Injection 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

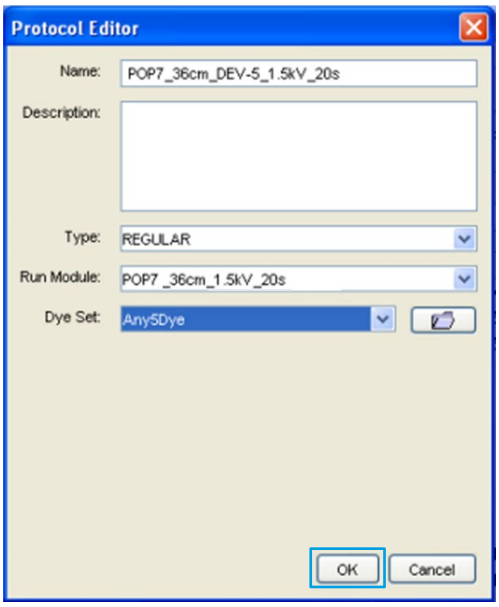


3.2. Creating an Instrument Protocol

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**



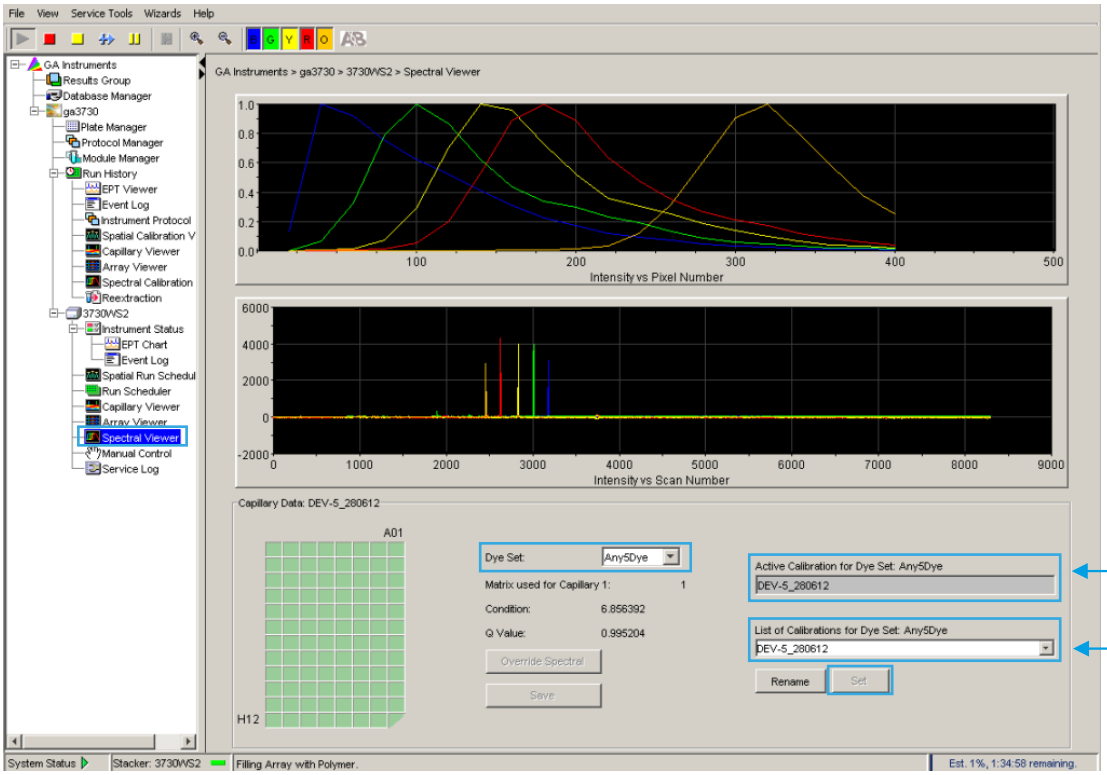
4. SETTING UP A RUN

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.

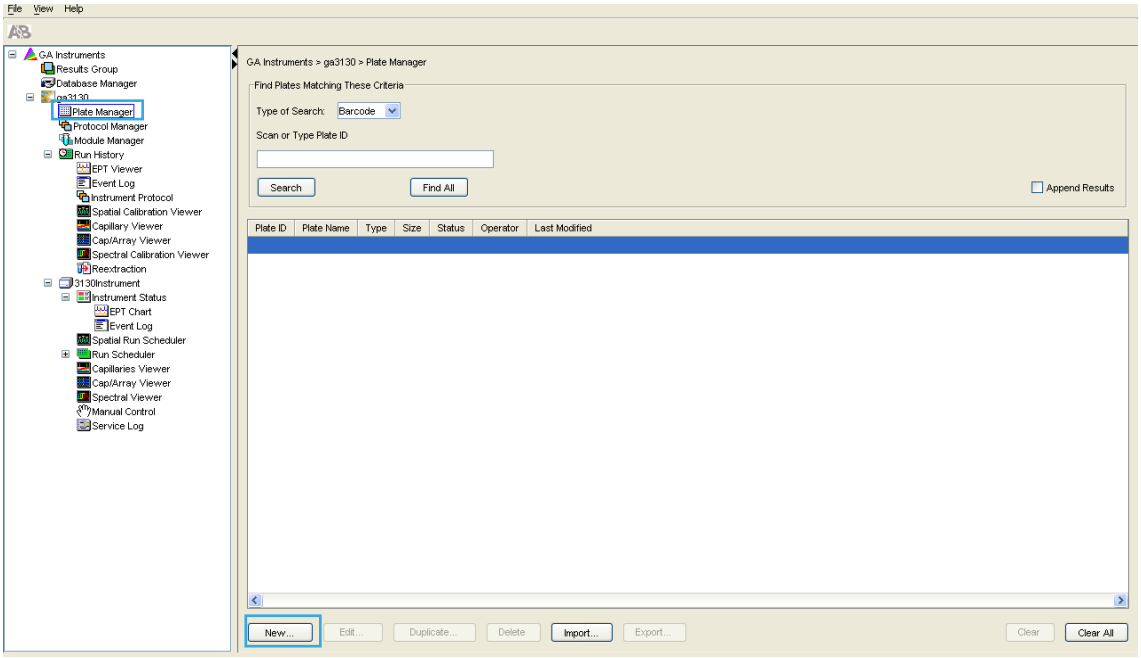
The most recent spectral calibration is automatically chosen as the active calibration for the “Any5Dye” dye set. Prior to creating a run plate, ensure that the desired calibration is set as active (e.g DEV-5 calibration or other calibration):

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**

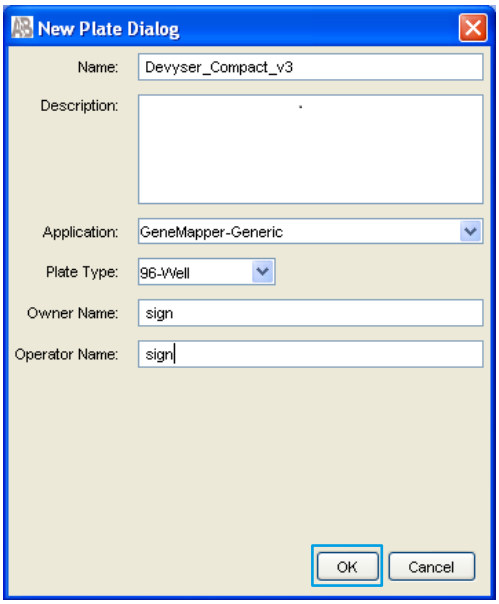


4.2. Creating a Plate

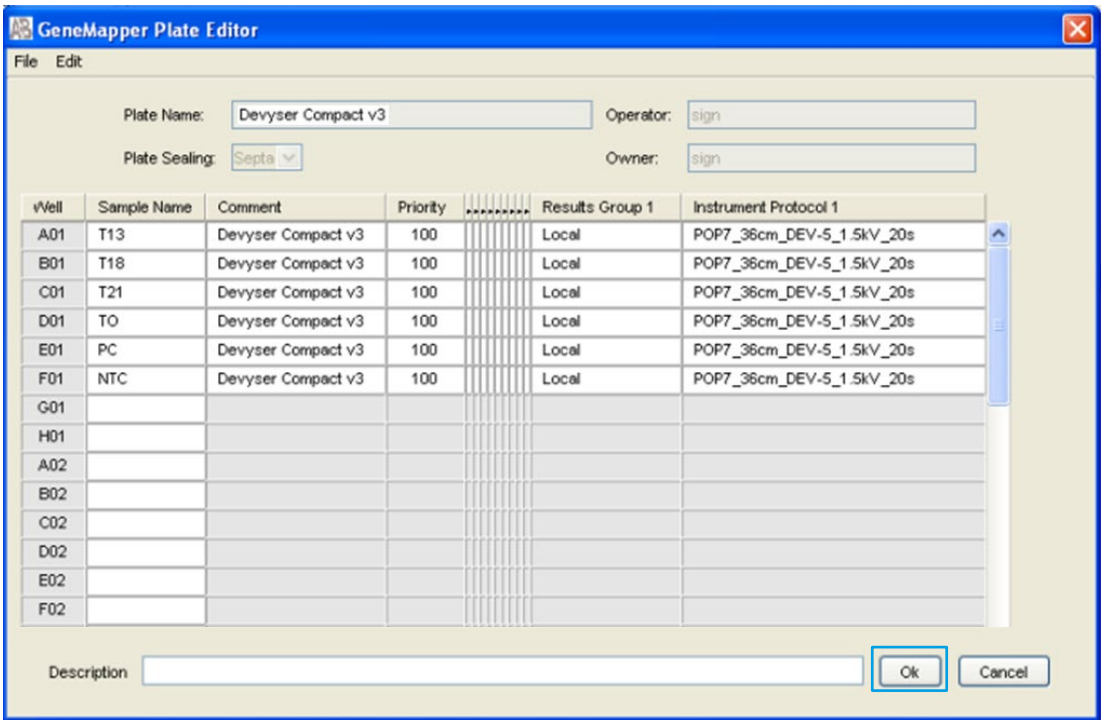
1. In the main window, click **Plate Manager** and then click **New**



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**

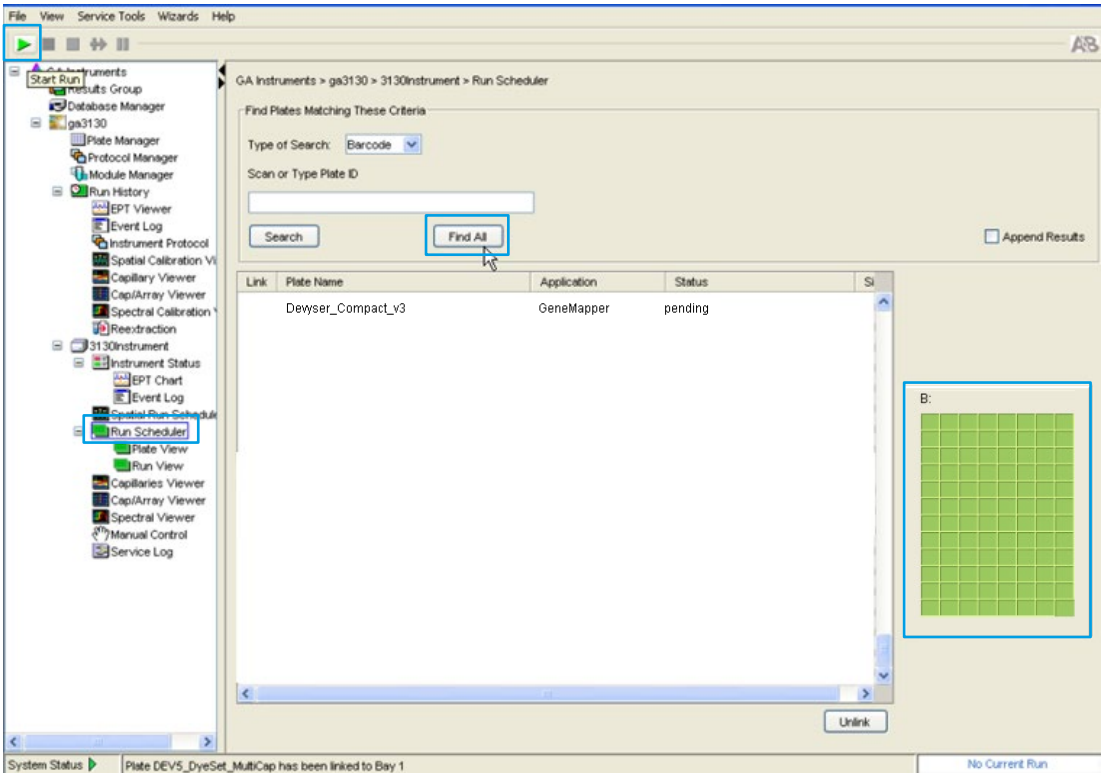


3. Fill out the newly opened **GeneMapper Plate Editor** window:
   a. **Sample name**: Enter the sample names
   b. **Comment**: Optional
   c. **Result Group 1**: Location where the raw data files (.fsa files) will be saved
   d. **Instrument Protocol 1**: Select the Instrument Protocol created in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

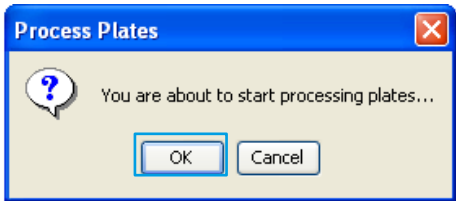


4.3. Starting the Sample Run

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