DEVYSER
RESULTS FOR LIFE

FRAGMENT ANALYSIS USING ABI® 3500/3500XL

HANDBOOK

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1. INTRODUCTION

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The **Devyser CFTR 68** kit uses the **G5 dye set**. The ABI® 3500 and 3500xL 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 3500 and 3500xL 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 following instructions 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.

2.1 Setting up the DEV-5 Dye Set in the Data Collection Software

Before starting the DEV-5 spectral calibration run on an ABI 3500/3500xL Genetic Analyzer, the DEV-5 Dye Set must be set up in the instruments' Data Collection Software using one of the two options below:

- Creating the DEV-5 Dye Set manually (see **Protocol 1**)
- Importing the DEV-5 Dye Set using the **DEV-5.zip** file (see **Protocol 2**). The DEV-5.zip file can be downloaded from www.devyser.com/ifu using the download code printed on the kit label

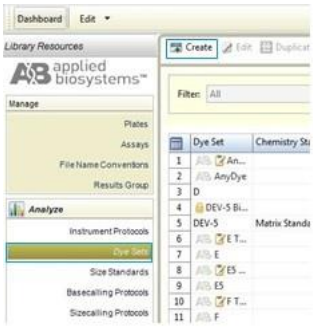
NOTE! If using an IVD-labeled Data Collection Software, contact Devyser support techsupport@devyser.com

2.1.1 Protocol 1. Creating the Dev-5 Dye Set in the Data Collection Software

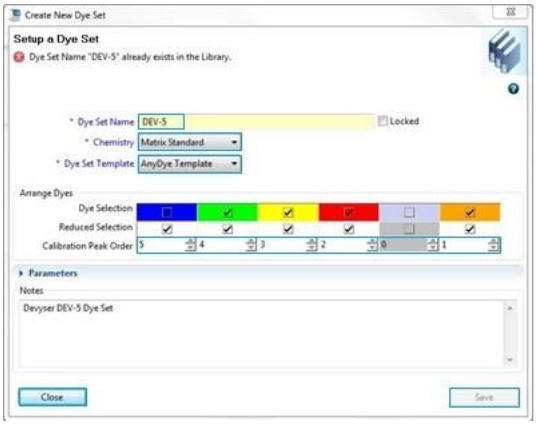
1. In the **Dashboard**, click **Library**

2. Click **Dye Sets** in the **Analyze** menu and then click **Create**

3. Fill out the **Create New Dye Set** window:
   A. **Dye Set Name**: Enter the name of the Dye Set, i.e. DEV-5
   B. **Chemistry**: Matrix Standard
   C. **Dye Set Template**: AnyDye Template
   D. Deselect the **Purple** dye and make sure that the other dyes are selected
   E. Arrange the dyes from left to right in the following order:
   BLUE: 5, GREEN: 4, YELLOW: 3, RED: 2, ORANGE: 1



   F. **Notes**: Enter a note about the Dye Set (optional)
   G. Click **Save** and **Close**.

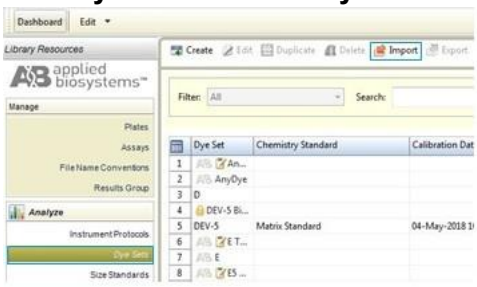


2.1.2 Protocol 2. Importing the Dev-5 Dye Set in the Data Collection Software

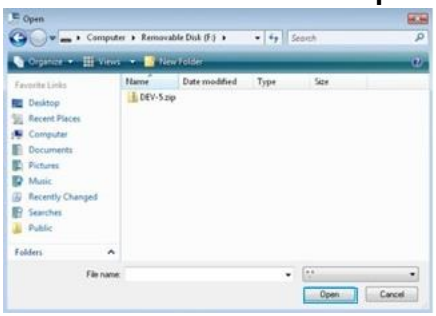
NOTE! If using an IVD-labeled Data Collection Software, contact Devyser support at techsupport@devyser.com

1. In the **Dashboard**, click **Library**

2. Click **Dye Sets** in the **Analyze** menu and then click **Import**



3. Browse to locate the **Dev-5.zip file** and click **Open**



4. Click **OK**

2.2 Verifying consumable status

Before starting the DEV-5 spectral calibration run on the ABI 3500 or 3500xL Genetic Analyzers, check the consumable status and the maintenance notifications. Refer to the instruments' user guide if consumables need to be replaced.

1. In the **Dashboard**, click **Refresh** to update the consumable status
2. Click **Start Pre-Heat** to pre-heat the oven

2.3 Preparing the DEV-5 Spectral Calibration standards

If using an ABI 3500 Genetic Analyzer (8 capillaries)

1. Thoroughly vortex the DEV-5 tube and spin the tube briefly
2. Dilute DEV-5 in Hi-Di Formamide by combining 5 uL DEV-5 with 95 µL Hi-Di Formamide
3. Vortex for 15 seconds and spin the tube briefly
4. Dispense 10 µL/well into the first row of a 96-well microtiter plate (wells A1 to H1)
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 3500 instrument (refer to the instruments' user guide for further details)

NOTE! Using the correct wells (A1 to H1) is very important because the software uses predetermined well positions for the spectral calibration.

If using an ABI 3500xL Genetic Analyzer (24 capillaries)

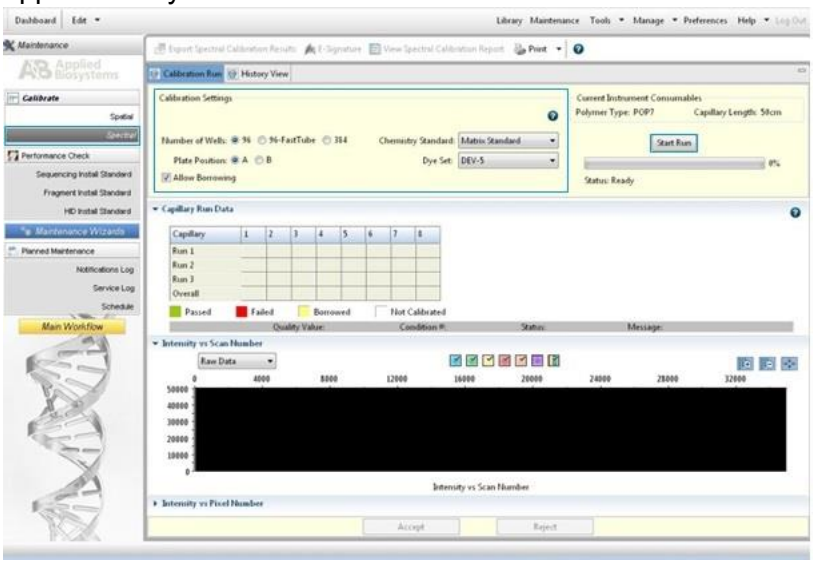
1. Thoroughly vortex the DEV-5 tube and spin the tube briefly
2. Dilute DEV-5 in Hi-Di Formamide by combining 14 uL DEV-5 with 266 µL Hi-Di Formamide
3. Vortex for 15 seconds and spin the tube briefly
4. Dispense 10 µL/well into the first row of a 96-well microtiter plate (wells A1 to H3)
5. Seal with 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 3500xL instrument (refer to the instruments' user guide for further details)

NOTE! Using the correct wells (A1 to H3) is very important because the software uses predetermined well positions for the spectral calibration.

2.4 Performing the DEV-5 Spectral Calibration run

1. In the **Dashboard**, click **Maintenance**

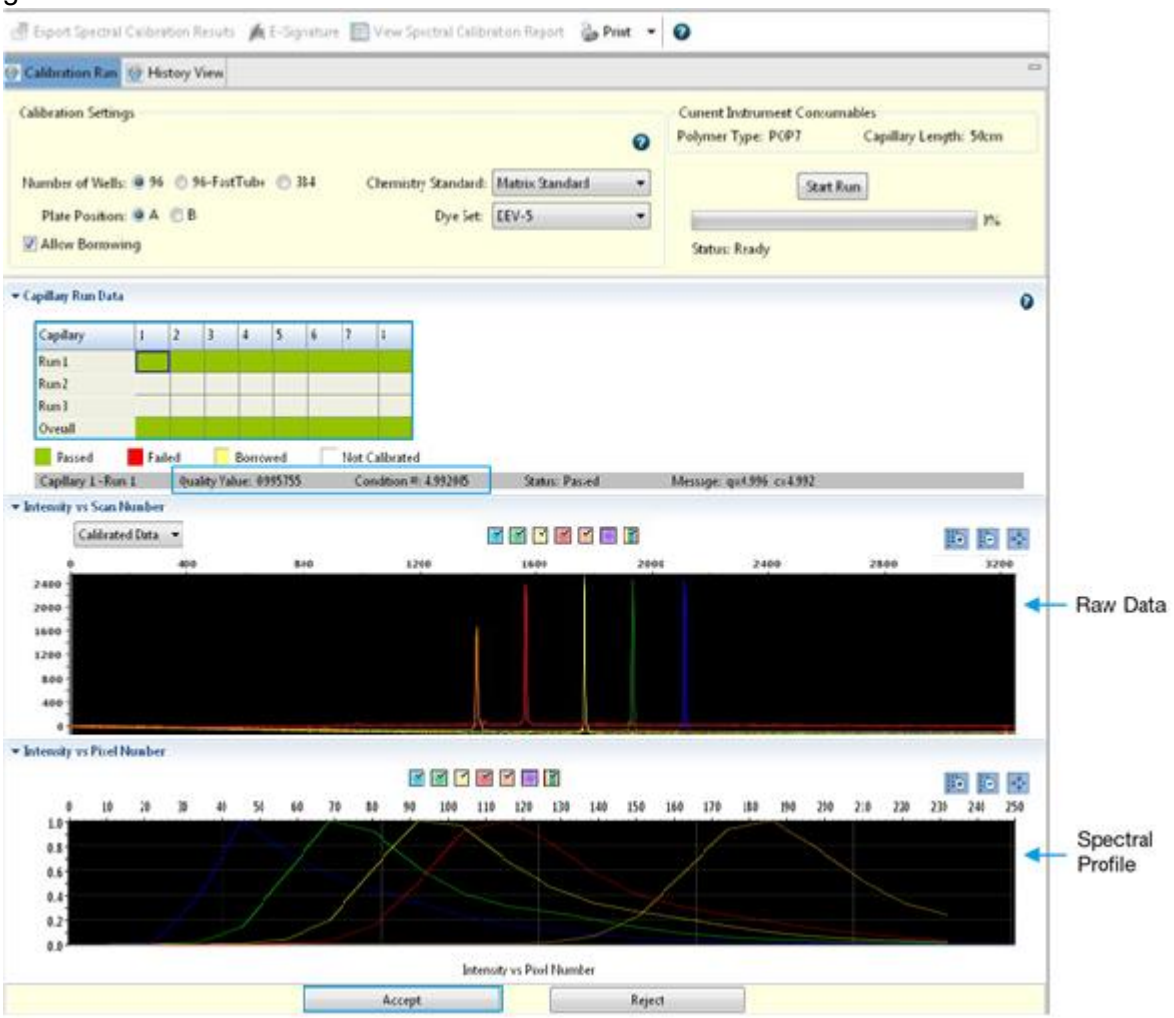
2. Click **Spectral** in the **Calibrate** menu
3. Fill out the **Calibration Settings** window:
   A. **Number of Wells**: Select the number of wells for the microtiter plate used
   B. **Plate Position**: Select the plate position into which the plate has been loaded (A or B)
   C. **Chemistry Standard**: Matrix Standard
   D. **Dye Set**: DEV-5 (previously created or imported in section 2.1)
   E. Make sure that the **Allow Borrowing** box is selected
   F. Click **Start Run** and then **Yes** in the newly opened window. The run duration is approximately one hour



2.5 Reviewing the DEV-5 Spectral Calibration run

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

1. Review by clicking on each capillary in the **Capillary Run Data** table
2. Visually inspect and evaluate the **Raw Data** and spectral profile for each capillary
3. In the **Raw Data** window, verify that the order of the peaks from left to right is: ORANGE, RED, YELLOW, GREEN, BLUE
4. In the **Spectral Profile** window, verify that the order of the peaks from left to right is: BLUE, GREEN, YELLOW, RED, ORANGE
5. Verify that the peaks are distinct and regular
6. Each capillary should have a **Quality Value** > 0,8 and a **Condition #** ranging from 1 to 20
7. 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
8. Click **Accept** to approve the spectral calibration. A spectral calibration report is automatically generated



3. CREATING ANALYSIS SETTINGS

Before starting a fragment analysis run on an ABI 3500/3500xL Genetic Analyzer, the following settings need to be set up in the instruments' Data Collection Software (refer to the instruments' user guide for further details):

- Size Standard (optional)
- Sizecalling Protocol (optional)
- Instrument Protocol
- Assay

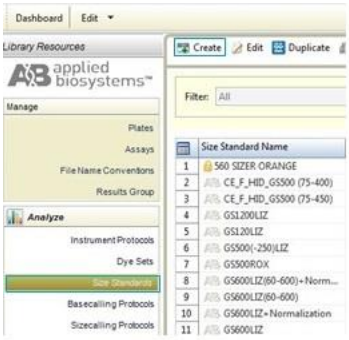
3.1 Creating a Size Standard

A Size Standard defines the sizes of known fragments and is used to determine the sizing of unknown samples.

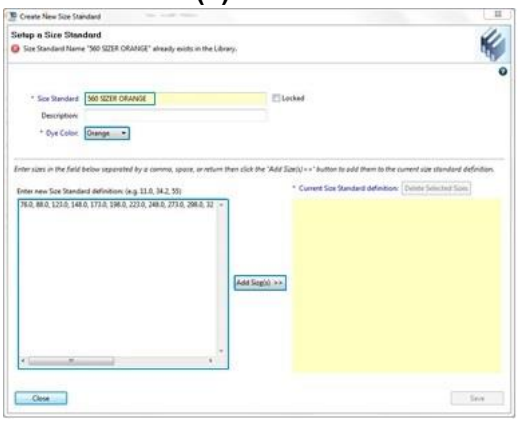
NOTE! This step is only performed if using the 560 SIZER ORANGE as size standard.

1. In the **Dashboard**, click **Library**

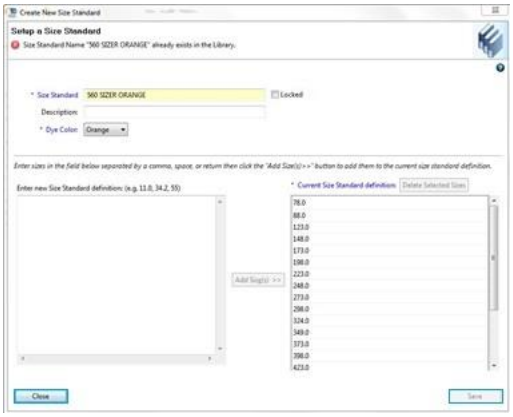
2. Click **Size Standards** in the **Analyze** menu and then click **Create**



3. Fill out the **Create New Size Standard** window:
   A. **Size Standard**: 560 SIZER ORANGE
   B. **Dye Color**: Orange
   C. Manually enter the size of each fragment as follows: 73.0, 88.0, 123.0, 148.0, 173.0, 198.0, 223.0, 248.0, 273.0, 298.0, 324.0, 349.0, 373.0, 398.0, 423.0, 448.0, 470.0, 495.0, 520.0, 545.0, 555.0
   D. Click **Add Size(s) >>**



4. Click **Save** and **Close**

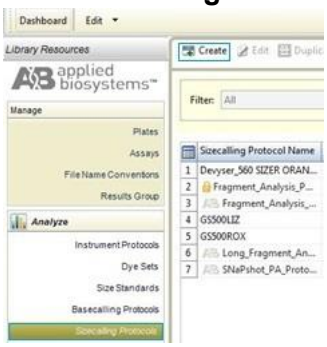


3.2 Creating a Sizecalling Protocol

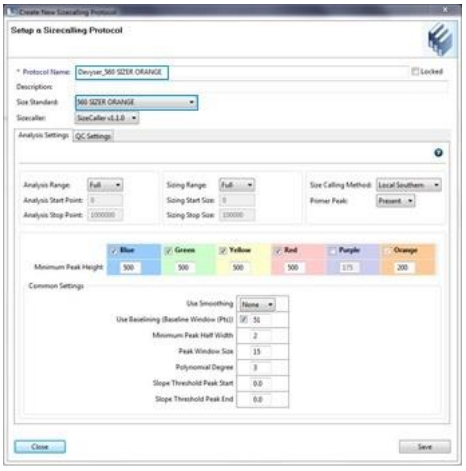
A Sizecalling Protocol defines peak detection, sizing and quality values.

NOTE! This step is only performed if using the 560 SIZER ORANGE as size standard.

1. Click **Sizecalling Protocols** in the **Analyze** menu and then click **Create**



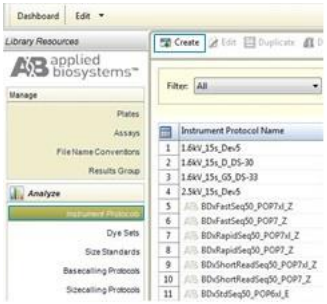
2. Fill out the **Create New Sizecalling Protocol** window:
   A. **Protocol Name**: Devyser_560 SIZER ORANGE
   B. **Size Standard**: 560 SIZER ORANGE
   C. **Minimum Peak Height**: 500 for Blue, Green, Yellow and Red
   D. **Minimum Peak Height**: 200 for Orange
   E. Deselect the **Purple** box
   F. Click **Save** and **Close**



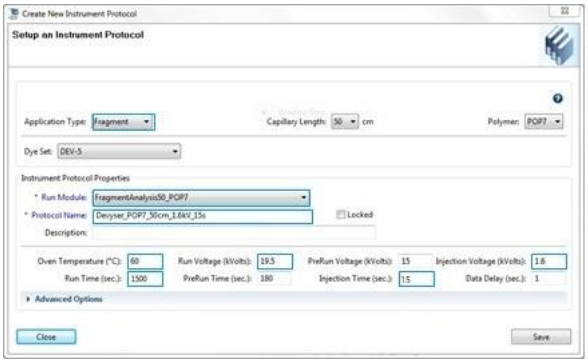
3.3 Creating an Instrument Protocol

An Instrument Protocol contains the parameters that control the instrument during data acquisition.

1. Click **Instrument Protocols** in the **Analyze** menu and then click **Create**



2. Fill out the **Create New Instrument Protocol** window:
   A. **Application Type**: Fragment
   B. **Dye Set**: Dev-5, or G5 if running the Devyser CFTR 68 kit
   C. **Run Module**: FragmentAnalysis50_POP7
   D. **Protocol Name**: Devyser_POP7_50cm_1.6kV_15s
   E. **Oven Temperature (˚C):** 60
   F. **Run Voltage (kVolts)**: 19.5
   G. **Injection Voltage (kVolts)**: 1.6
   H. **Run Time (sec.)**: 1500
   I. **Injection Time (sec.)**: 15
   J. Click **Save** and **Close**



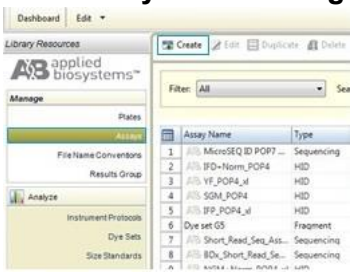
NOTE! The DEV-5 Dye Set was created during the DEV-5 spectral calibration (see section 2).

NOTE! The run time and injection time/voltage can be decreased or increased by creating an additional 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.

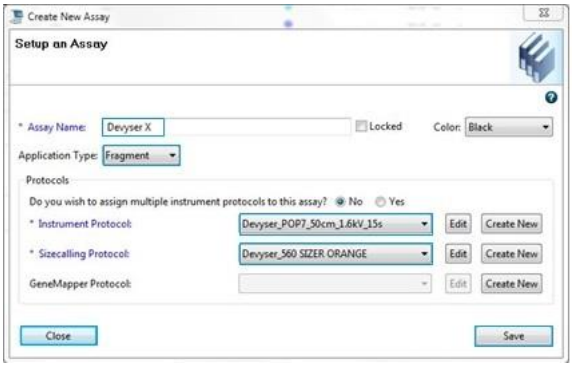
3.4 Creating an Assay

An Assay contains the Instrument Protocol and the Sizecalling Protocol needed to collect data and sizecall a sample.

1. Click **Assays** in the **Manage** menu and then click **Create**



2. Fill out the **Create New Assay** window:
   A. **Assay Name**: enter the name of the Devyser kit (i.e. Devyser Compact v3)
   B. **Application Type**: Fragment
   C. **Instrument Protocol**: select the Instrument Protocol created in section 3.3
   D. **Sizecalling Protocol**: select the Instrument Protocol created in section 3.2
   NOTE! If using another size standard than the 560 SIZER ORANGE, select the corresponding Sizecalling Protocol.
   E. Click **Save** then **Close**

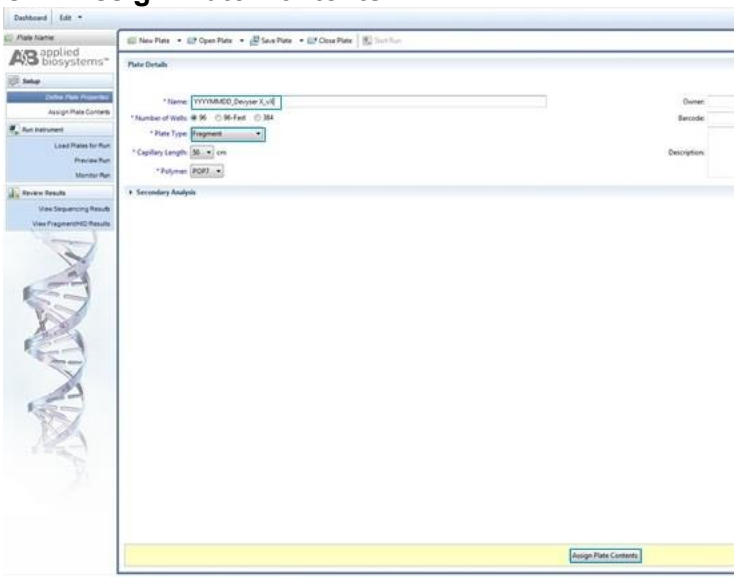


4. SETTING UP A RUN

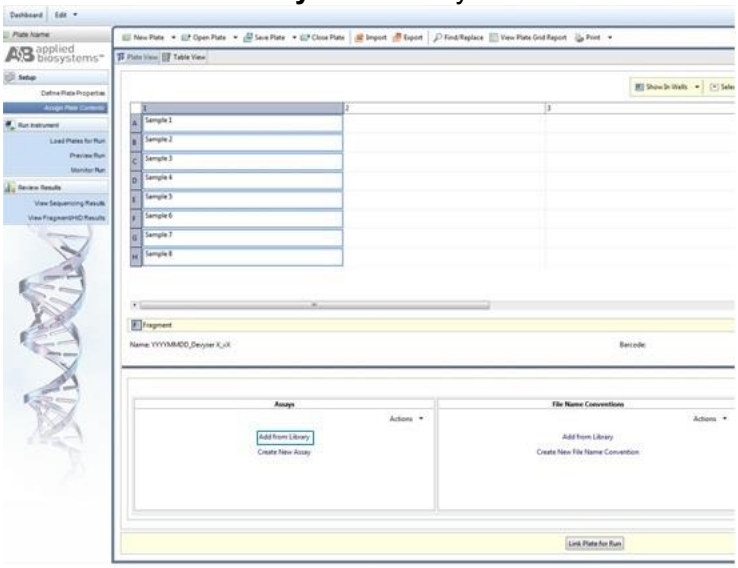
A Plate needs to be created before starting the electrophoresis on an ABI 3500/3500xL Genetic Analyzer. A Plate associates samples with well position. It also defines how samples are analyzed during capillary electrophoresis and how sample files are named and stored after analysis.

1. In the **Dashboard**, click **Create New Plate**

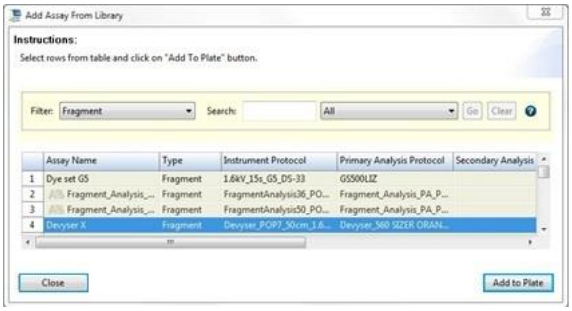
2. Fill out the **Plate Details** window:
   A. **Name**: YYYYMMDD_Devyser X_vX (i.e. 20180301_Devyser Compact_v3)
   B. **Plate Type**: Fragment
   C. Click **Assign Plate Contents**



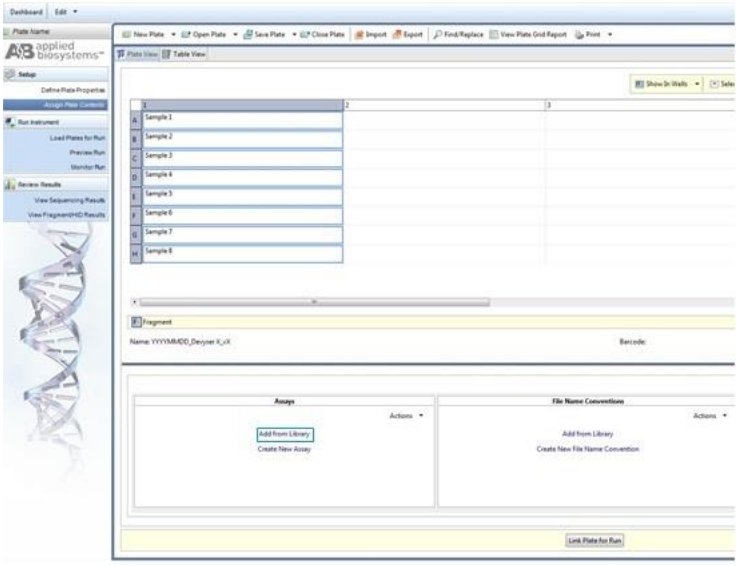
3. In the **Plate View** window:
   A. Enter the sample names at their respective well positions on the plate
   B. Click **Add from Library** in the Assays window



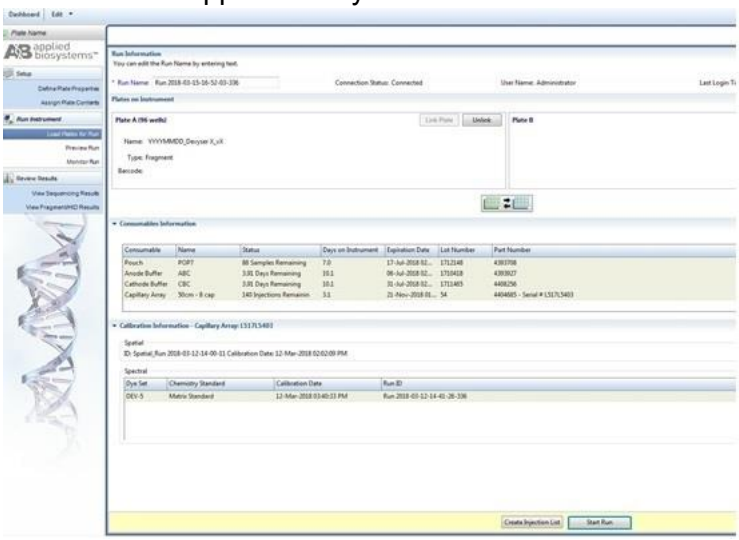
4. In the **Add Assay From Library** window:
   A. Select the **Assay** created in section 3.4
   B. Click **Add to Plate**, then **Close**



5. In the **Plate View** window:
   A. Select the samples to be run and then tick the box next to the assay name in the **Assays** window
   B. Choose the optional File **Name Convention** and **Results Groups**
   C. Click **Link Plate for Run**



6. To start processing the plate, click **Start Run** and then **Yes** in the newly opened window. The run duration is approximately 45 minutes



5. REVISION HISTORY

Version 2020-06-15 Correction 2020-01-19

Editorial changes
3.3 Step 2 I: Changed Injection Time from 8 sec to 15 sec and updated related figure

Version 2020-06-15

Editorial changes
1. New section
2. Introduction to text in section 2 changed
3.3 Step 2: "or G5 if running the Devyser CFTR 68 kit" added
5., 6., New sections

Version 2019-11-11

Editorial changes
1.3. 3500xL protocol: wells changed from H1 to H3
2.1. Size of fragments changed from 78 to 73
4. Revision history added

Version 2018-12-10

New

6. CONTACT INFORMATION

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