DEVYSER

RESULTS FOR LIFE

FRAGMENT ANALYSIS USING ABI PRISM 310

HANDBOOK

Contents

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1. INTRODUCTION

The Devyser CFTR 68 kit uses the G5 dye set. The ABI PRISM® 310 Genetic Analyzer must be calibrated for the G5 dye set prior to running the Devyser CFTR 68 kit. The spectral calibration is performed using DS-33 Matrix Standard Set for the 310 Genetic Analyzer (Thermo Fisher Scientific, Cat. no. 4345833) according to the manufacturer instructions (see chapter 7 of the ABI PRISM® 310 Genetic Analyzer User Guide[1]).

All other Devyser kits use the DEV-5 dye set. The ABI PRISM 310 Genetic Analyzer 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 the instructions below.

2. DEV-5 SPECTRAL CALIBRATION

The ABI PRISM 310 Genetic Analyzers must be calibrated for the DEV-5 Dye Set prior to running the Devyser kits. The spectral calibration is performed using the **DEV-5 SingleCap** (Art. No. #8-A400) calibration standards. The **DEV-5 SingleCap** contains five different calibration standards (BLUE, GREEN, YELLOW, RED and ORANGE) that must be prepared and run separately.

1. Thoroughly vortex each calibration standard and spin the tubes briefly
2. Dispense 15 µL Hi-Di Formamide into five separate 0,5 mL Genetic Analyzer Sample tubes
3. Add 1,5 µL of calibration standard into a dedicated tube (one tube/calibration standard) containing 15 µL Hi-Di Formamide
4. Vortex for 15 seconds and spin the tubes briefly
5. Use the GS STR POP4 (1 mL) G5.md5 module file for detection of the DEV-5 Dye Set

NOTE! Do not use the G5v2.md5 module file.

NOTE! When using a 2.5 mL syringe, the run module should be GS STR POP4 (2.5mL) G5.md5. If using POP6 polymer, change the run module accordingly.

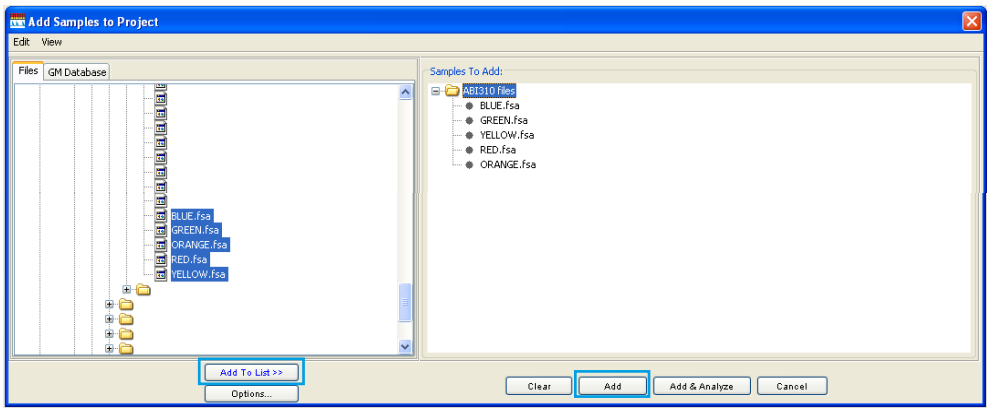
3. CREATING A DEV-5 MATRIX FILE

The data files generated after the spectral calibration runs are used to create a DEV-5 Matrix file in GeneMapper®. A Matrix file contains information that corrects for the spectral overlap between different dyes.

3.1. Importing spectral calibration data files

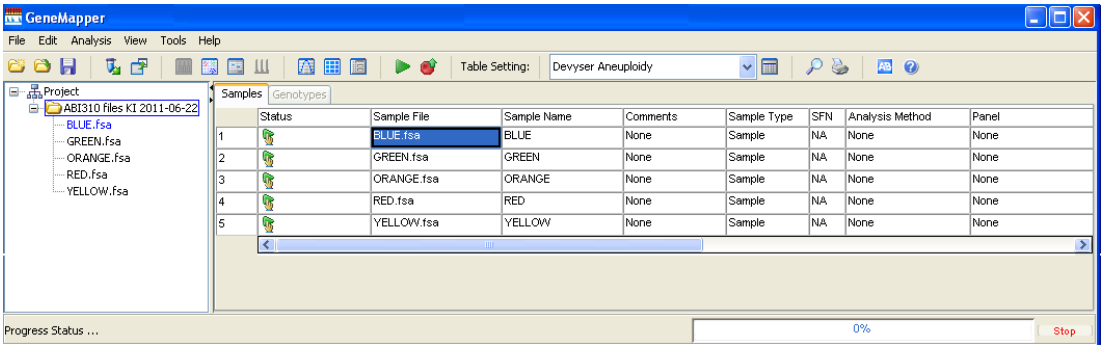
1. Open GeneMapper, import data files (.fsa) by selecting **File** and **Add Samples to Project**
2. Browse to locate the desired run folder using the folder tree in the left navigation window
3. Select the files generated during the DEV-5 SingleCap spectral calibration run (one file for each dye) and click **Add to List >>**

4. The added files are now displayed in the **Samples to Add** window. Click **Add** to import the files into a new project

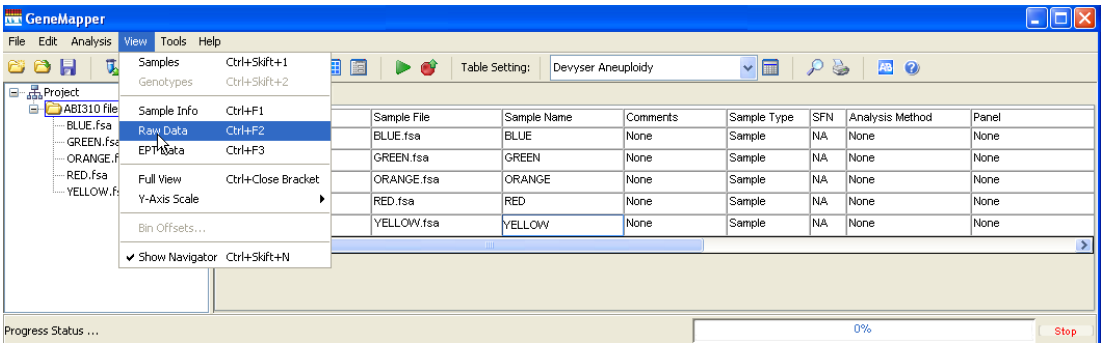


3.2. Viewing the DEV-5 Matrix Raw Data

1. In the left navigation window, click on the folder containing the sample files and select a matrix sample file (.fsa)

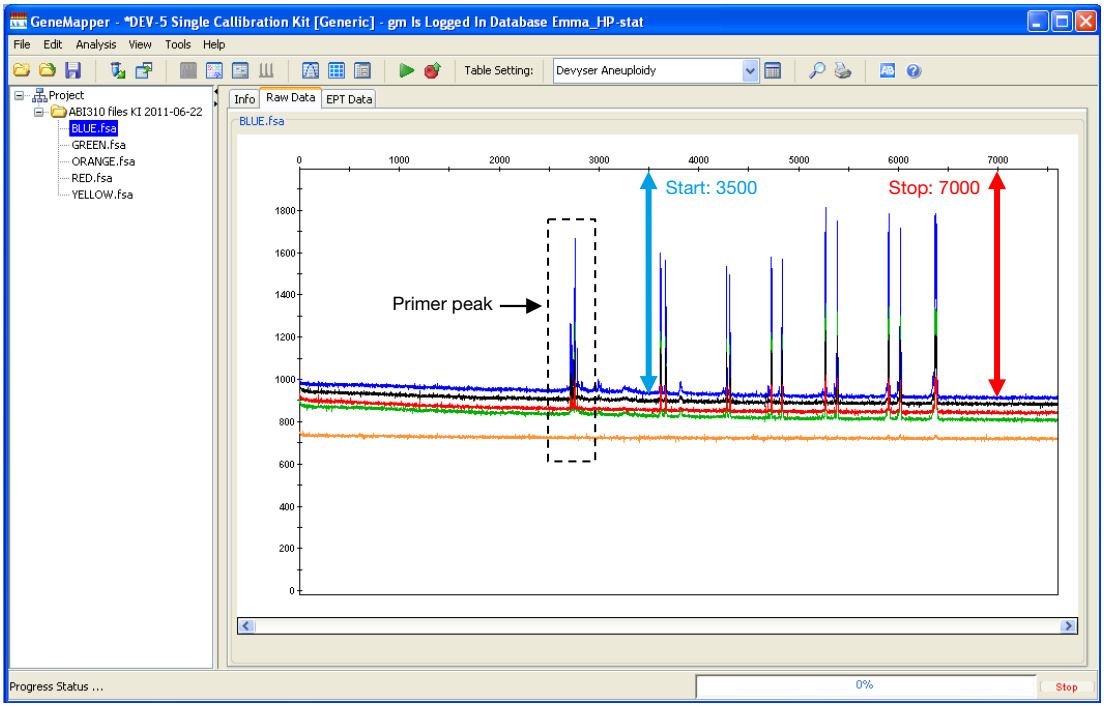


3. Select **View** and then **Raw Data**



4. Select the **Raw Data** tab and move the cursor to record the Start and Stop data points values in a flat part of the baseline (indicated by the blue and red arrows in the picture below)

5. Record the distance from the Start point to the Stop point (3500 in the example below)

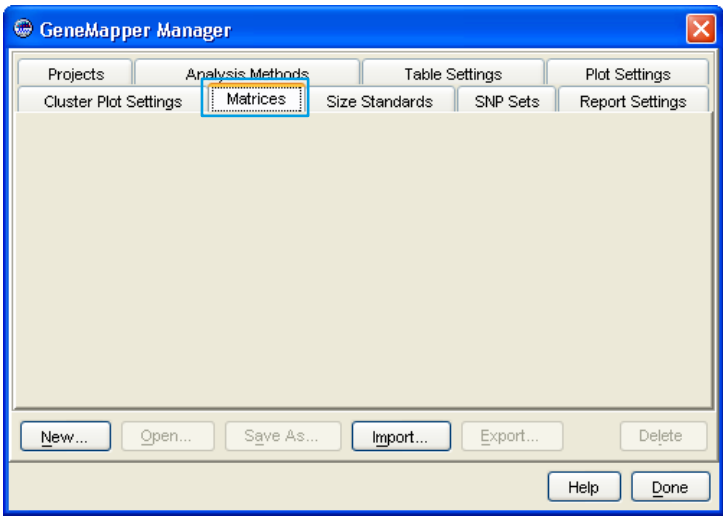


6. Repeat steps 4 and 5 for each (BLUE, GREEN, YELLOW, RED and ORANGE)
7. After reviewing the five files, return to the Samples view by selecting **View** and **Samples**

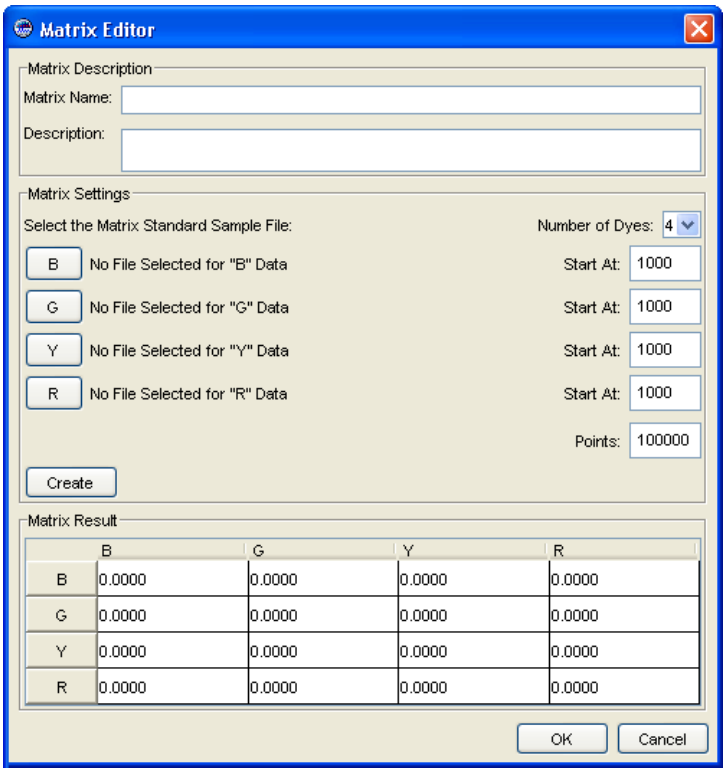
NOTE! Do not include the primer peak in the data point range when selecting the start point. Make sure to include at least five fragments (peaks) to create a good matrix.

3.3. Creating the DEV-5 Matrix file

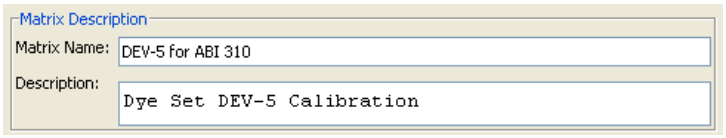
1. Select **Tools** and **GeneMapper Manager**
2. Select the **Matrices** tab and then click **New**



3. The **Matrix Editor** window opens

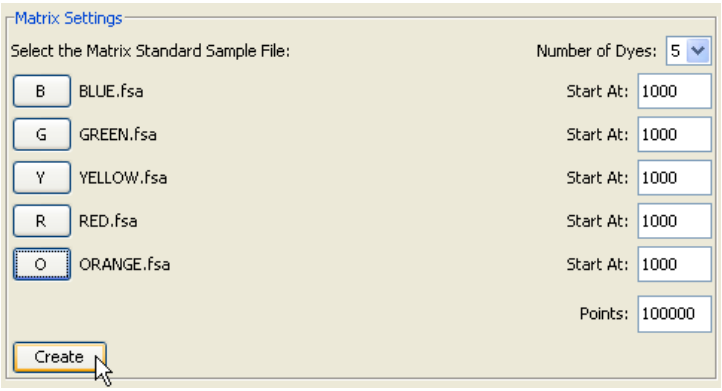


4. In the **Matrix Description** box, fill out the **Matrix Name** and **Description** as shown below:

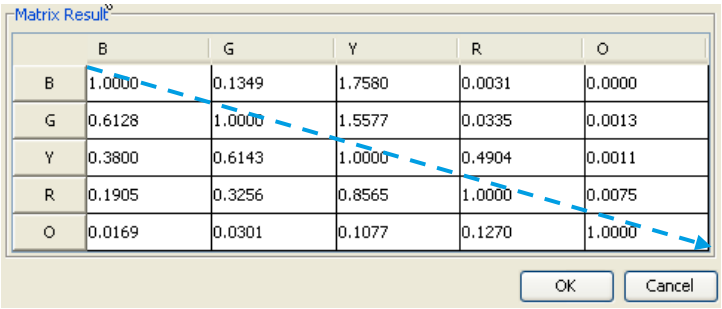


5. In the **Matrix Settings** box:
   a. Select “5” in the **Number of Dyes** drop-down menu
   b. Click on **B** and browse to locate and select the .fsa file corresponding to the BLUE dye and then click **Open**
   c. In the **Start At** field, enter the start data point value for the BLUE dye determined in section 3.2
   d. Repeat steps b and c for the four other dyes (GREEN, YELLOW, RED and ORANGE)
   e. In the **Points** field, keep the default data points value
   f. Click **Create** to create a matrix

NOTE! If parts of the calibration data need to be excluded because of artifacts or bleed-through, enter the total number of data points to include when calculating the matrix in the **Points** field. Calculate the total number of data points using the Start and Stop data point values recorded in section 3.2 as follows: **Stop point - Start point = Total number of points.**



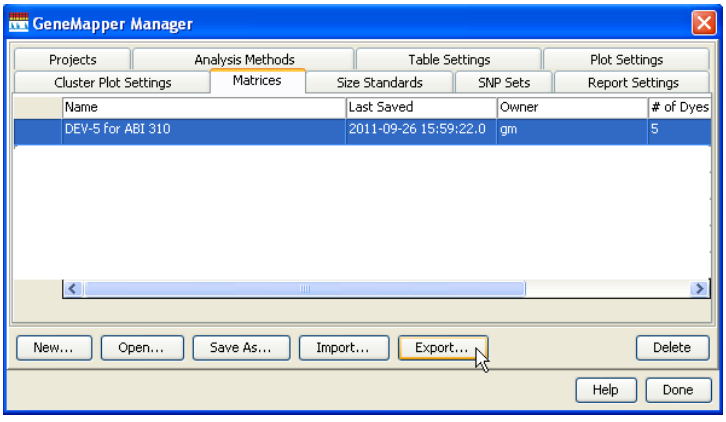
6. Review the matrix values in the **Matrix Result**
7. A successfully created matrix displays the value “1.0000” on the diagonal axis and decreasing values as you move away from the diagonal axis. If the matrix values differ from this pattern, contact Technical Support at Applied Biosystems/Life Technologies
8. Click **OK** and then click **Done**



3.4. Exporting the DEV-5 Matrix file

The DEV-5 Matrix must be exported manually from GeneMapper to the 310 Data Collection Software

1. Close the 310 Data Collection Software
2. In the GeneMapper Software, select **Tools** and then **GeneMapper Manager**
3. Select the **Matrices** tab and the DEV-5 Matrix file created in section 3.3
4. Click **Export**



5. In the **Exporting Matrix** window, navigate to D:\AppliedBio\Shared\Analysis\Sizecaller\Matrix
6. Click **Save**
7. Click **Done** to close the GeneMapper Manager window

4. SETTING UP A RUN

When setting up a run, the DEV-5 Matrix file needs to be selected to interpret the data and correct for the spectral overlap. Refer to the instrument's user guide for details on how to set up a run.

NOTE! When running samples, always use the run module used during the spectral calibration.

5. PERFORMING DATA ANALYSIS

When analyzing data files generated from an ABI 310 Genetic Analyzer, the DEV-5 Matrix file created in section 3.3 must be selected for all the samples in the project.

1. In GeneMapper, open the **Project** that contains the samples to be analyzed
2. In the **Samples** tab, select the **DEV-5 Matrix** file for each sample. Alternatively, choose the **DEV-5 Matrix** file for one sample, select the desired samples and use **Ctrl + D** keys to apply the settings to the selected samples

NOTE! Further instructions on how to analyze data using GeneMapper can be found in the "Data Analysis using GeneMapper Handbook" that can be downloaded from www.devyser.com/ifu using the download code printed on the kit label.

6. REFERENCES

1. ABI PRISM® 310 Genetic Analyzer User Guide. Printed in the USA, 06/2010. Part Number 4317588 Rev. B.

7. REVISION HISTORY

Version	Description
2020-06-15	Editorial changes 1., 5. and 6. New sections
2018-12-10	New