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SeqStudio™ Genetic Analyzer Instructions for Use (Protocol Sheet)

For the preparation of SeqStudio™ Genetic Analyzer to perform electrophoresis using Investigator STR Assays

QIAGEN

General information

This guide aims to aid with preparation of the SeqStudio Genetic analyser to perform electrophoresis with QIAGEN's STR assays. It shows how to install/remove cartridges, load CE plates and cathode buffer, perform matrix standard calibration, and perform electrophoresis of PCR products.

The SeqStudio is a compact genetic analyser, with 4 capillaries. It differs from traditional sequencers where the capillaries, anode buffer, and POP1 polymer are all contained in disposable cartridge which can be removed and re-installed with ease and replaced once the polymer or buffer is used or expired.

Important notes

- The default injection settings described in this guide provide the best results in terms of base pair separation and peak morphology. Some optimization maybe required if running samples that contain suboptimal templates. The run parameters are fully modifiable and injection times and voltages can be increased, if required.
- The files created by SeqStudio can only be analyzed in GeneMapper ID-X version 1.6 or later. Earlier versions of this software are not able to analyze the data. Data can be analyzed using GeneMarker HID without adjusting or amending the panels or bins.

Cartridge installation

Procedure

1. Open the instrument door.
2. To prepare the cartridge for installation, remove the capillary protection pod and capillary detection cover.
3. Slide the cartridge into place along with the cartridge holders. There should be a click as indicator that the cartridge is securely placed.

Creating the BT5/6 Dye Set

Procedure

1. From the Home screen, press **Settings**.
2. Go to **Maintenance and Service** > **Calibration** > **Dye Calibration**.
3. In the Dye Calibration window, press **Custom dye**.
4. In the Dye Set window, press **Add** and select these options to use as template:
   - **AnyDye**: Select this option as dye set.
   - **Matrix standard**: Select this option as chemistry standard.
5. To select the applicable dye according to matrix type (BT5/6), select the dye color, then check the **Dye Selection** box. To arrange the order of the dye colors, select the color, then press **Move Up** or **Move down** until the desired order is achieved. Click **Next**. (Figures 1 and 2)

Figure 1. Dye color arrangement for BT5

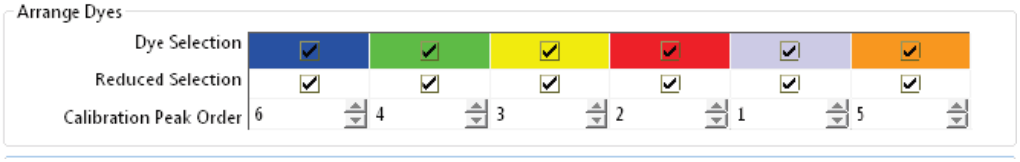

Figure 2. Dye color arrangement for BT6.

Note: If the **Dye Selection** box is checked, the color is added to the dye set. To remove the dye color, select the color and clear the **Dye Selection** box.

6. In the Dye Set Parameter window, enter the following information in these fields:

- **Matrix condition number upper limit** : Set the parameter to **13.5**.
- **Locate start point after scan**: Set the parameter to **1000**.
- **Locate start point before scan**: Set the parameter to **5000**.
- **Limit scans to**: Set the parameter to **2750**.
- **Sensitivity**: Set the parameter to **0.4**.
- **Minimum Quality Score**: Set parameter to **0.95**.

7. Click **Next**.
8. In the Name Your New Dye Set window, specify the dye set name (e.g. BT6) and press **Done**.

Preparing the calibration plate

Procedure

1. Defrost an aliquot of Hi-Di Formamide along with BT5/6 matrix as applicable.
2. Create a master mix as defined in Table 1.

   Note: If you are using the 5 dye chemistry, substitute **Matrix Standard** BT6 for BT5.

Table 1. Master mix

Component	Volume
Hi-Di Formamide	90 µl
Matrix Standard BT6 multi cap.	10 µl

3. Pipette 20 µl of master mix into 4 consecutive vertical wells of a 96-well reaction plate, e.g. A1-D1.

   Important: The minimum volume for SeqStudio that can be used is 12 µl. Any volume below 12 µl runs the risk of not contacting the capillaries and might prevent injection and migration from occuring.

   Note: You can reuse 96-well plates and redefine the location of the master mix.
4. Place a plate septa over the plate to seal, and denature the plate for 3 mins at 95°C, then 3 mins on ice (or at 4°C).

Starting the matrix calibration

Procedure

1. From the Home screen, press the **Eject** > **Eject plate**.
2. Place the reaction plate into the plate holder. Ensure that the cathode buffer is installed.

Figure 3. Plate holder and cathode buffer holder. 1 – Plate holder; 2 – Cathode buffer holder.

3. Go to Home screen. Press **Settings** > **Maintenance and Service** > **Calibration** > **Dye Calibration**.
4. Select the wells containing BT5 or BT6 calibration dye, then press **Dye Set**.

   Note: The software automatically highlights the block of 4 wells per injection run.
5. In the Select Dye Set window, select **Matrix Standard**.
6. Select the dye set to be calibrated (BT5 or BT6).
7. Press **Calibrate** to start the matrix calibration.
8. After the run is finished, press **Results** to check if each capillary has passed the dye calibration.

Note: If the custom dye calibration fails, close calibration screen and repeat the calibration.

Creating a new size standard

Procedure

1. From the Home screen, press **Settings** > **Run settings** > **Size standard**.
2. Select a size standard to use as template (e.g. GS1200_LIZ_(60-1000) and press **Copy**.
3. In the Edit Size Standard window, perform these steps:
   - 3a. Enter a new size standard name in the **Name** field. For example, enter SST_BTO_60-500bp.
   - 3b. In the **Dye (Color)** field, select **Orange, if not already selected**.
   - 3c. Select all fragment sizes that are not applicable to the QIAGEN size standard and press **Delete**.

     Note: You can select multiple size standards to delete at once.
   - 3d. To add a fragment size according to the QIAGEN size standard, press **Add and** specify the required fragment size.
   - 3e. After adding all the required fragment sizes, press **Done**.

Performing a CE run

Procedure

1. Prepare a Hi-Di Formamide/size standard as defined in Table 2.

Table 2. Volume required per sample

Component	Volume per sample
Hi-Di Formamide	12.0 µl
DNA size standard 24 plex (BTO)	0.5 µl

2. Pipette 12 µl of master mix into all wells which will contain a sample, and any empty well which will be included in an injection of 4.
3. Add 1 µl of PCR product to each well. Include allelic ladder, as necessary.
4. Place a plate septa over the plate to seal and denature the plate for 3 minutes at 95°C, then 3 minutes on ice (or at 4°C).
5. Go to Home screen. Press **Eject** > **Eject plate**.
6. Insert the reaction plate into the plate holder. Ensure that the cathode buffer is installed.
7. From the Home screen, press **Setup run**.
8. In the Setup run window, press **Create new plate setup**.
9. In the Plate Properties window, go to **Properties** and perform these steps:
   - 9a. Enter the plate name in the **Plate name** field.
   - 9b. Ensure that **Fragment analysis** is selected in the **Application** field.
   - 9c. Select **Instrument** in the **Save location** field.
10. Press the **Plate** field. Highlight all wells containing the samples. Press **Edit**.
11. In the Edit Plate window, perform these steps:
    - 11a. In the **Selected Injections** field, ensure that all wells containing the samples or allelic ladder are selected.
    - 11b. In the **Run module** field, select **FragAnalysis**.
    - 11c. In the **Size Standard** field, select the size standard you created in Creating a new size standard, page 6.
    - 11d. In the **Dye set** field, select the applicable dye set (BT5 or BT6).
    - 11e. Press **Done**.
12. Select **Sample** from the left panel, then enter the name of each sample in the Well ID column, if required.
13. Assign the sample type for each well. Select **Sample**, **Control**, or **Ladder**. Press **Done**.
14. Press **Start run** to start the run.

Exporting files for analysis

Procedure

1. From the Home screen, go to **Settings** > **Run history**.
2. Select the run you want to export, then press **Export**.
3. Ensure that the USB drive is inserted to the instrument. Select USB in the Export File window, then specify the required destination path in the **Destination** field. Press **Export**.

Revision history

Date	Changes
07/2022	Initial release

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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