



# Beckman Coulter GenomeLab™ GeXP Genetic Analysis System

Bergen County Technical Schools  
**Stem Cell Lab**





## **Beckman Coulter GenomeLab™ GeXP Genetic Analysis System Information Sheet**

The Beckman Coulter GenomeLab™ GeXP Genetic Analysis System is a fully automated, versatile platform capable of a magnitude of genetic analysis applications ranging from quantitative multiplexed gene expression to fragment analysis and sequencing. The hardware is used to carry out the task of sample handling as well as the tasks associated with the separation and detection phases of electrophoresis. The software provides the user with an interface for manual or automatic control of the system, and with data capture and basic data analysis capabilities.

The instrument incorporates two plate holders and can accept two 96 well plates at one time, giving the user the ability to sequence the DNA of up to 192 samples at one time. For sequencing purposes, the instrument is able to sequence the bases of DNA strands ranging from less than 200 base pairs to over 700 base pairs. This DNA can exist in the form of plasmids, raw PCR products, and purified PCR products. The method used for sequencing is Capillary Electrophoresis using Dye Terminator Cycle Sequencing.

After DNA has been sequenced, the software offers the user a wide range of options, including the ability to analyze the raw sequence data as well as the edited and corrected data, the ability to compare a sequence to a reference sequence, and the ability to export the sequence data to formats such as .fasta and .tat (sequence text files) and .scf (standard chromatograph file), which can then be used with third party software for further analysis.

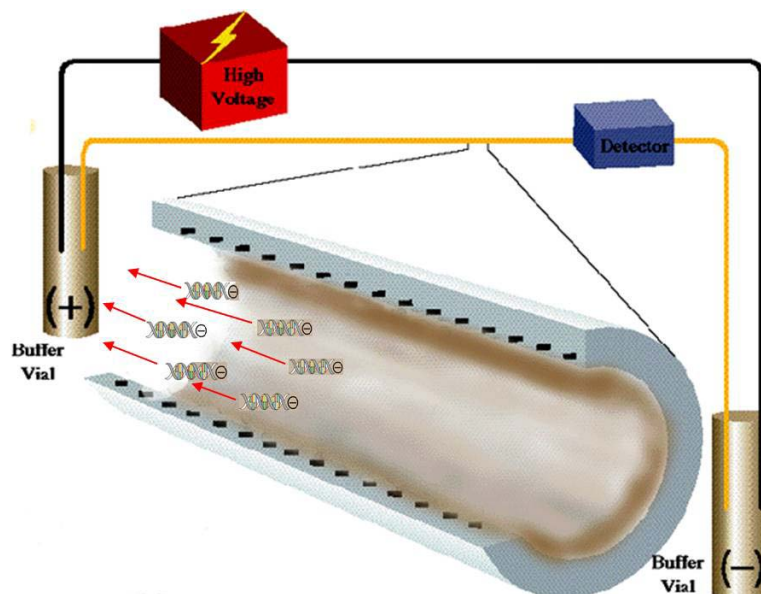
### **Dye Terminator Cycle Sequencing and Capillary Electrophoresis**

The goal of dye terminator cycle sequencing is to synthesize a set of DNA fragments in which each base pair is represented by a fragment of a given length and

labeled with a fluorescent dye. To do this, a polymerase chain reaction (PCR) is run on the DNA strand, which contains primers for the creation of new fragments, and Dideoxynucleotide Triphosphates (ddNTP) containing a fluorescent dye corresponding to each of the four nucleic acids. During the PCR cycle, new strands of DNA are created from existing template strands. When a ddNTP is inserted in a sequence, it stops the elongation of that strand at that base. The insertion of ddNTP's into the sequence is random, and in theory should result in the creation of different length fragments of DNA that contain a ddNTP in every possible position of the original DNA strand.

These DNA fragments are loaded into the instrument and are sequenced using capillary electrophoresis. The instrument uses 8 glass capillaries filled with a polyacrylamide gel to separate the DNA fragments. An electric field is applied to the electrolyte buffer that contains the DNA. The DNA is predominantly negatively charged. This causes the fragments to move through the gel towards the positive charge in the field (see image).

The fragments are separated by size, with the smaller DNA fragments migrating faster through the gel in the capillary than the larger fragments. As the fragment passes through the detection window of the instrument, the color of the fluorescent dye indicates the identity of the base at that position. The detection of these separated, dye-labeled fragments is done by using diode laser-induced fluorescence.





## Beckman Coulter GenomeLab™ GeXP Genetic Analysis System Quick Start Guide – Sequencing

### Preparing Samples for DNA Sequencing

#### *Preparing the DNA Sequencing Reaction*

Before any samples can be sequenced, they must be prepared for Dye Terminator Cycle Sequencing using the Beckman Coulter Quick Start Kit. Until samples are fully prepared and ready to be sequenced, the instrument does not need to be turned on.

1. Plasmid or PCR DNA samples can be run for sequencing. These samples should be prepared ahead of time, and should be run on a gel to determine the concentration of DNA in the sample.



**Note:** The instrument contains 8 capillaries. Therefore, a minimum of 8 samples must be run for every round of sequencing. If the user has less than 8 samples, duplicates of samples can be run, or a buffer can be added to blank wells prior to the run.



2. Take the **Quick Start Kit** from -20°C freezer and place the **Master Mix Tube** on ice.

The following steps should be repeated in a tube for each of the samples being prepared.

3. In a 0.2mL PCR tube, add **0.5 – 10.0µL** of the sample template DNA (depending on the concentration determined from the gel) and add distilled H<sub>2</sub>O to bring the final volume in the tube to **10µL**.
  - a. For **ONLY Plasmid DNA samples**, a preheat step is necessary. Place the tube containing the sample in the thermal cycler and program the instrument to preheat at 96°C for 2 minutes, followed by a 4°C hold. This protocol is pre-programmed in the thermal cycler as *96C2min*.

4. Add a total of **2.0µL** of the user's primers to the tube.
5. Add **8.0µL** of **DTCS Quick Start Master Mix** to the tube. The final volume should be **20µL** per sample.
6. Mix the sample thoroughly and then quickly centrifuge the samples.
  - a. Place the rotor for the centrifuge in the -20°C freezer after this step.

### *Thermal Cycling Program*

1. Place the tubes in the thermal cycler.
2. Set the thermal cycler to run the following program: 96°C for 20 Seconds, 50°C for 20 seconds, and 60°C for 4 minutes. Repeat for 30 cycles, and follow the run with a 4°C hold.
3. This cycle is pre-programmed in the thermal cycler as *30dtcs*.

### *Ethanol Precipitation*

1. Prepare a labeled, sterile microfuge tube for each sample (up to 8).
2. In a fresh tube, prepare the fresh Stop Solution / Glycogen mixture as follows: **20µL** of **3M Sodium Acetate (pH 5.2)**, **20µL** of **100mM Na<sub>2</sub>-EDTA (pH 8.0)**, and **10µL** of **20mg/mL glycogen** (from the Quick Start Kit).
3. To each of the labeled tubes, add **5µL** of the **Stop Solution / Glycogen** mixture.
4. Transfer the **20µL** from the thermal cycler to the appropriate tube containing the stop solution. Mix thoroughly using a vortex.
5. Add **60µL** of **cold 95% ethanol** from the -20°C freezer and mix thoroughly using a vortex.

6. Immediately centrifuge at **14,000 RPM** at **4°C** for **15 minutes**. The rotor can be removed from the freezer to spin at 4°C. After completed, carefully remove and discard the supernatant. *DO NOT TOUCH THE PELLETT.*
7. Rinse the pellet **2 times** with **200µL** of **cold 70% ethanol** from the -20°C freezer.
8. Each time, centrifuge immediately without mixing at **14,000 RPM** for **2 minutes** at **4°C**. Carefully remove and discard the supernatant after each spin.
9. Open the tubes and vacuum dry for **10 minutes**, or place inside a hood in the lab until no ethanol remains in the tube (may take 20 – 30 minutes).

**Note:** At this point in the procedure, the instrument can be turned on and all protocol information can be set up in the software, as explained in the following sections.

10. Centrifuge the tubes for **1 minute** at **14,000 RPM** to ensure all DNA in the tube is at the bottom of the wells.
11. Resuspend the sample in **40µL** of the **Sample Loading Solution (SLS)** (from the kit). Mix thoroughly and allow to sit at room temperature for **10 minutes**.
12. At this point, the instrument should be ready for sequencing. Transfer the **40µL** of each sample into the appropriate wells of the sample plate. (Add 40µL SLS buffer to any empty wells in the row).
13. Add **1 drop** of **mineral oil** to each well that contains a sample.

### **Turning on the Instrument and Software for Sequencing**

1. Press the **Power Button** located on the front of the instrument.
2. There are two computers associated with the instrument. Start the computer on the **Right Side** for sequencing.
3. **Double Click** the **GenomeLab System Icon** on the *Desktop* to start the software.

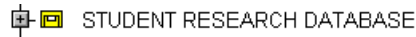


## Setting up a User in the Database

Each user should set up their own folder in the database where all of their sequencing data and protocols will be stored.

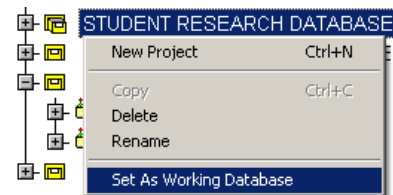


1. On the *Software Main Menu Screen*, select the **Database Icon**.
  - a. A database for student research has already been created. It is labeled *Student Research Database*.



2. Make sure that the database is **Bold**. If it is not, right click the database and select **Set as Working Database**.

- a. If you have already set up a project within the database for the user, continue to the next section.



3. **Right click** on the database and select **New Project**. “New Project” will appear under the database.



4. Select the new project, right click, and select **Rename**.



5. Enter a new name for the project and press **Enter**.

- a. The new project should be named with the **User’s name**.

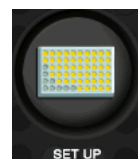
- i. The individual runs that are stored in the project folders will contain more detail about the type of run’s being performed.

6. Once the new project is named in the database, exit the Database Manager by clicking the **(X)** at the top right of the screen.



## Setting Up a Sample Plate

1. On the *Software Main Menu Screen*, select the **Setup Icon**.



2. On the screen that pops-up, select the **Create a New Sample Plate Button** and click **OK**.

A rectangular button with a circular icon on the left and the text "Create a new sample plate" on the right.

3. On the new screen that appears, choose the column (1 – 12) that will contain the 8 samples for the sequencing run.

4. **Click** a box in the column, and enter the sample's name in the **Sample Name Text Box** at the top of the screen. Press **Enter**, and repeat for all 8 boxes in the column.

	1
A	sample1.A01
B	sample1.B01
C	sample1.C01
D	sample1.D01
-	

- a. The boxes will turn **Green** when information is entered. This tells the user the information in those boxes is not saved yet.

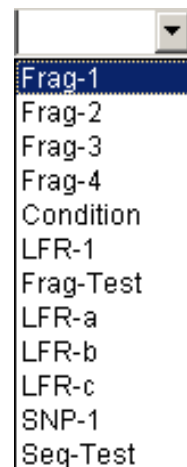
5. Select a **Method** from the *Drop-down Menu* below the column. All samples in the column must be run using the same method.

- a. **Long Fragment Read 1 (LFR-1)** is an 80 minute run used for samples of an unknown length, or for columns with different length samples.

- b. **LFR-a** is a 110 minute run best suited for sequencing DNA >600bp.

- c. **LFR-b** is a 60 minute run best suited for sequencing DNA 400-600bp.

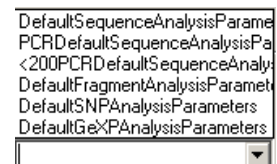
- d. **LFR-c** is a 45 minute run best suited for small PCR products (200–300bp).



6. Select the **Analysis Tab** in the box at the bottom of the screen.

A rectangular button with the text "Analysis" inside.

7. **Check** the box that says **Perform Analysis**. Highlight the boxes in the column that are using a specific analysis protocol (i.e. all <200bp PCR products), and select the appropriate **Analysis Option**.

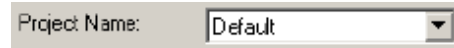
A checkbox with the text "Perform Analysis" to its right.A list of analysis options: DefaultSequenceAnalysisParameters, PCRDefaultSequenceAnalysisParameters, <200PCRDefaultSequenceAnalysisParameters, DefaultFragmentAnalysisParameters, DefaultSNPAnalysisParameters, and DefaultGeXPAnalysisParameters. Below the list is a small downward arrow.

8. Save the plate by going to File → **Save As** on the *Toolbar*.



a. Enter a name for the plate. This should include the User's name, project, and date. *Ex. UserX\_ProjectY\_mm-dd-yy.*

b. Select the **Project Name** that was previously created from the *Project Name Drop-down Menu* at the bottom of the screen.



c. Click **OK** to save the plate.

d. All of the boxes on the screen should now turn **Blue**. This means that they are saved.

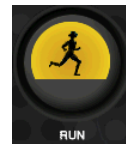
	1
A	sample1_A01
B	sample1_B01
C	sample1_C01

9. Press the **(X) Button** at the top of the screen to exit the plate setup.

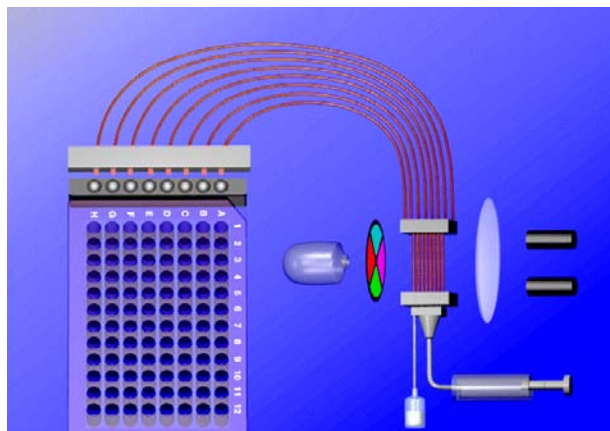


### Running a Sample Plate and Installing the Gel and Capillaries

1. On the **Software Main Menu Screen**, select the **Run Button**.



2. On the screen that appears, select the **Direct Control Tab**. This shows a cartoon view of the instrument and its parts. From here, the gel and capillaries can be accessed.



Note: Students should **NOT** install the gel or capillaries. This should only be performed by the instructor. Have an instructor install the gel and capillaries at this point, and students can then proceed to the section *Setting up the Run*.

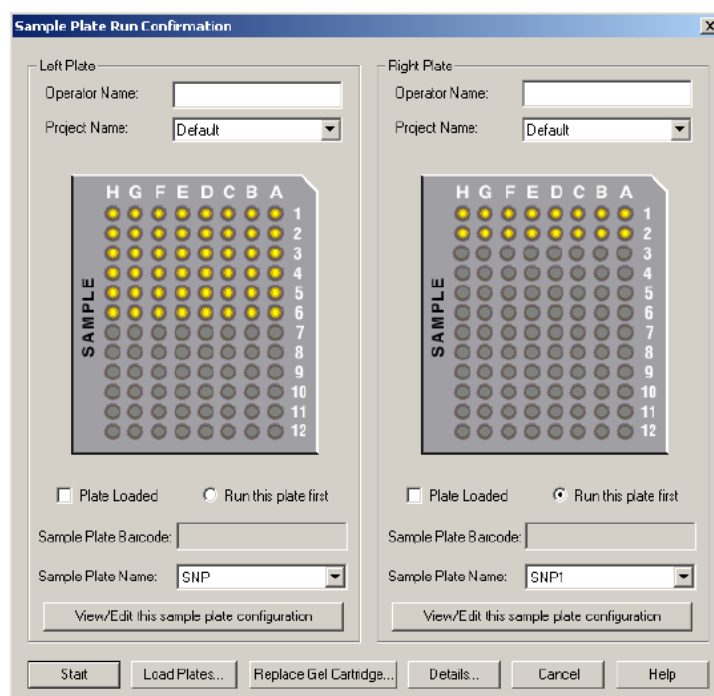


## Setting up the Run

1. Click the **Run Sample Plate Button** located at the top of the *Run Screen*.



2. In the **Sample Plate Run Configuration Screen**:

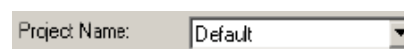


- a. Determine if the samples will be loaded in the **Left or Right Plate Carrier**. Either is fine to use.

- b. Enter the **Operator's Name**.



- c. Select the **Project Name** previously setup in the *Drop-down Menu*.



- d. Select the rows that contain sample by clicking on the corresponding wells in the picture.

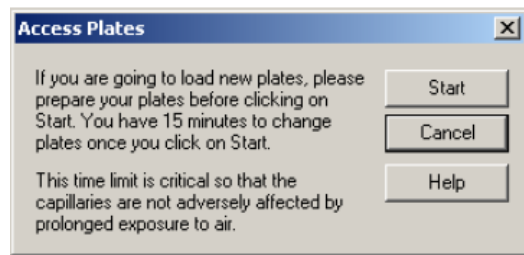


- i. Wells will turn yellow once selected.

3. Click the button at the bottom of the box that says **Load Plates**.



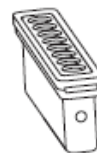
- a. In the dialog box that appears, click **Start**. Wait for the instrument to say it is OK to load the plates. The user will have 15 minutes to complete all sample loading.



4. Lift the **Sample Access Cover** and load the samples that were prepared earlier (40µL SLS and DNA with 1 drop of Mineral Oil in each of the 8 sample wells) into either the left or right plate carriage (depending on which was selected).
5. **Load 250 - 300µL** of buffer into **Buffer Plate** wells that correspond to the location of the samples in the sample plate. Place the **Buffer Evaporation Cover** back over the buffer plate.



6. Empty the **Wetting Tray**, and refill it  $\frac{3}{4}$  with D.I. water. Place it back into the instrument.



7. On the screen, select the buttons next to **Plate Loaded** and **Immerse Capillaires** under the left or right plate depending on which side the samples were loaded into.



8. Click the **Load Button**.



9. On the screen, click the **Start Button** to start the sequencing.



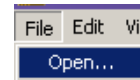
## Analyzing the Results of the Sequencing

After the sequencing protocol has been run:

1. Click the **Sequencing Button** on the *Software Main Menu Screen*.



2. In the *Sequence Analysis Screen*, click **File → Open**.



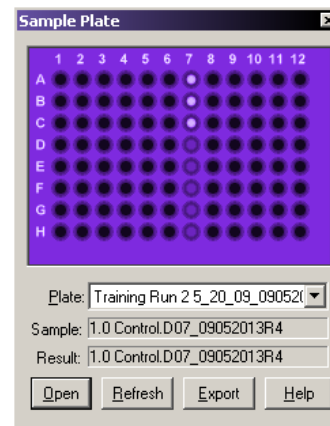
- a. Select the correct **Project Name** from the *Drop-down Menu* at the bottom of the dialog box.




- b. Select the correct run data from the list in the dialog box. Click **OK**.

Name	Date-Time
Sequencing test 5-19-09...	05/19/09 1...
Training Run 2 5_20_09...	05/20/09 1...

3. In the screen that pops up, select the wells in the plate that contained the samples that you would like to view.



- a. The wells that have sample data are filled in **Purple**. When selected for viewing (by clicking

on each),  the wells will turn **Yellow**.

4. Click **Open**.
5. A screen will open for each of the wells that were selected.
6. The user can look at multiple features in this screen by selecting the **Icons** on the left side of the data, including:

- the **Analyzed Data** as a **Chromatograph**,
- the bases viewed as **Text**,
- the **Raw Data**,
- the **Current** and **Voltage** from throughout the run,
- Comparing** one sequence to another,
- and the **Call Scores** for each base.



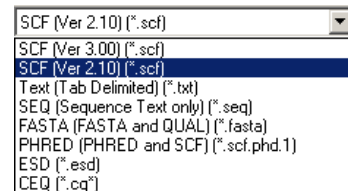
## Exporting Data

### To Export Results:

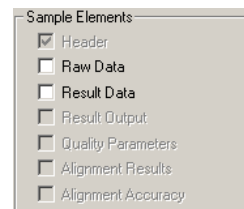
1. Select **File** → **Export**.



2. Choose the type of file you would like to export the data as (.txt, .seq, or .fasta for text, .scf for a chromatograph).

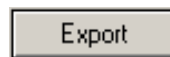


3. Choose the **Sample Elements** to be exported in the file.  
This is located on the left side of the window.



4. Select where the exported data should be saved to.

5. Click **Export**.



6. These files can then be saved to a USB drive and analyzed on a different computer.

## Shutting Down the Instrument

1. Follow the procedure for **Removing the Gel Cartridge and Capillary Array** listed above.
2. At the same time as the capillary array is being removed from the instrument, also remove the sample plate.

3. Shut the instrument's power off by pressing the **Power Button** located on the front of the instrument.



4. Close all of the software windows that are open by pressing the **(X) Button** in the top right corner of the screen.



5. On the *Software Main Menu Screen*, click the **Exit Button**.



6. **Make sure the station is neat before leaving the instrument!**



**Beckman Coulter GenomeLab™ GeXP Genetic Analysis System**  
**Safety Sheet**

1. Samples should be handled according to good laboratory procedures and methods in order to prevent accidents.
2. Normal operation of the instrument can involve the use of solvents and reagents that are toxic, flammable, or biologically harmful. Observe all precautionary information printed on the original solution containers.
3. Wear appropriate laboratory attire (safety glasses, gloves, lab coat) when working with potentially hazardous materials.
4. Dispose of all waste solutions according to waste disposal procedures.
5. The instrument uses a high voltage power supply:
  - a. Do not remove any panels or cords from the instrument to avoid electrical shock.
  - b. Do not perform any maintenance on the instrument while the power is on to avoid electrical shock.
6. The instrument has moving parts:
  - a. Do not attempt to remove or exchange sample trays, the gel cartridge, capillaries, or any other parts of the instrument while it is operating.
  - b. Be aware of clothing and body parts around any of the instruments moving parts.
  - c. Most of the instruments moving parts are controlled through the software. Use the software to make changes in the instrument. Never forcefully attempt to add or remove anything, such as the capillaries, to or from the instrument.
7. The instrument uses a "Class 3B" laser, which means that direct viewing of this type of laser is always hazardous. The laser is housed in a sealed container which makes the laser an overall "Class 1" laser, which means it is safe to users under normal operation conditions. Never attempt to remove the laser from the sealed container or open the outer casing.
8. Instruments used in the preparation of samples for sequencing, such as the Thermocycler, can reach temperatures near 100°C. Use caution when working near the instrument to avoid injury.

9. The capillaries are made of glass and are extremely fragile. Use caution when replacing the capillaries in the instrument.
10. Cleaning the workstation around the instrument is necessary. Never attempt to clean any internal spaces of the instrument.
11. Food and drinks should not be placed on or near the instrument.
12. Running unauthorized programs or changing preferences on the instrument's computer is not allowed. Laptops are provided for personal use and internet access. Do not plug memory sticks in the instrument's computer unless instructed to do so by the instructor.





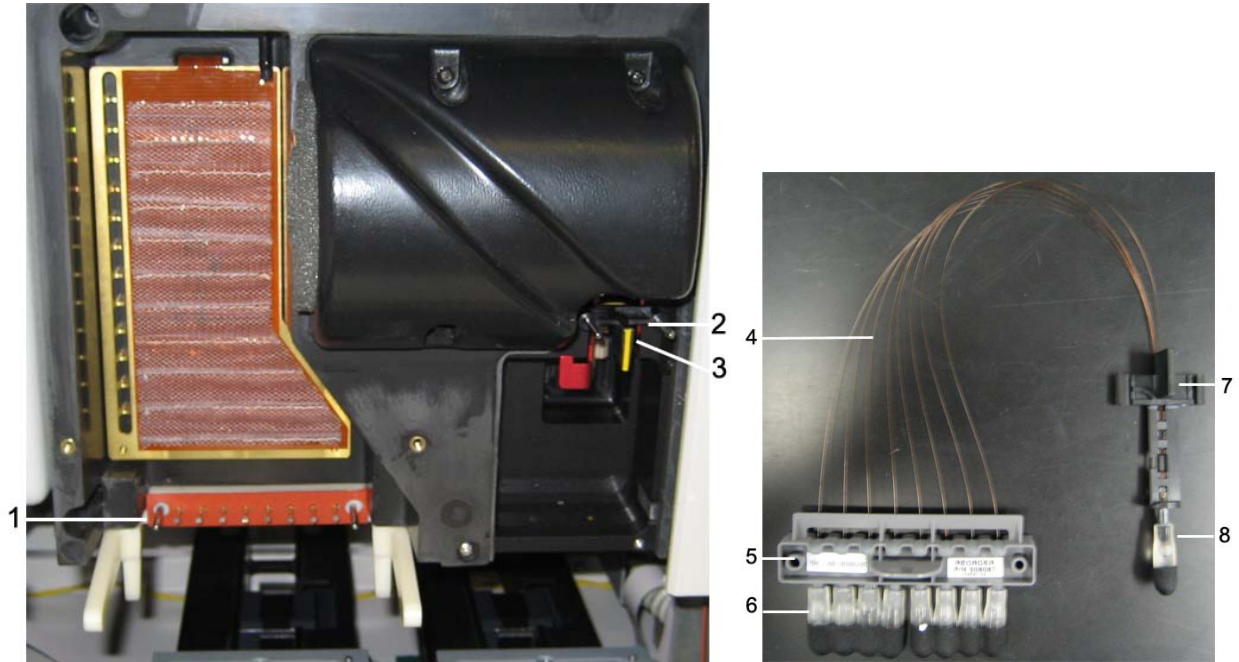
**Beckman Coulter GenomeLab™ GeXP Genetic Analysis System**  
**Instrument Information Sheet**

**GeXP Genetic Analysis System Overview:**



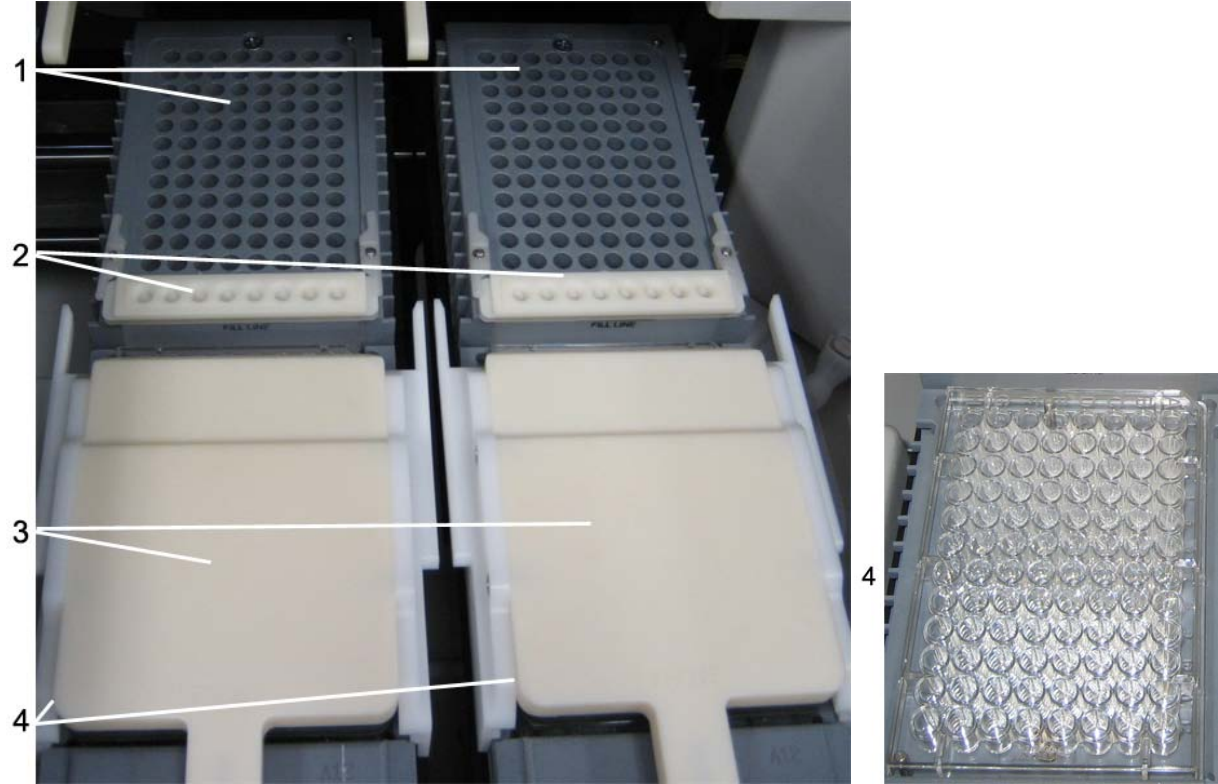
1. Instrument Status Indicators (Power, Laser, and High Voltage)
2. Capillary Access Cover
3. Sample Access Cover
4. Power Button
5. Gel Pump / Gel Cartridge Access Cover

## Capillary Access Cover Overview:



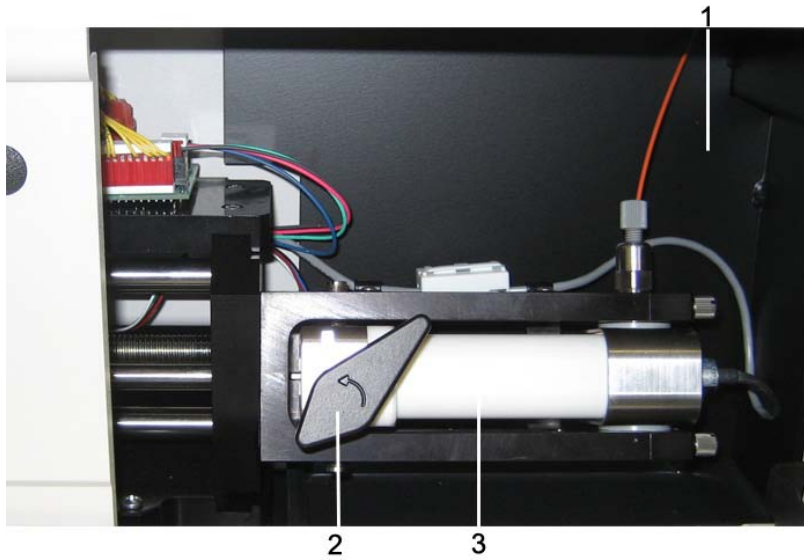
1. Electrode Block Holder
2. Array Fitting Holder
3. Manifold Plug (used when capillary not in instrument)
4. 8 Glass Capillaries
5. Electrode Block
6. Electrode Block inlets
7. Array Fitting
8. Array Fitting outlets

**Sample Access Cover Overview:**



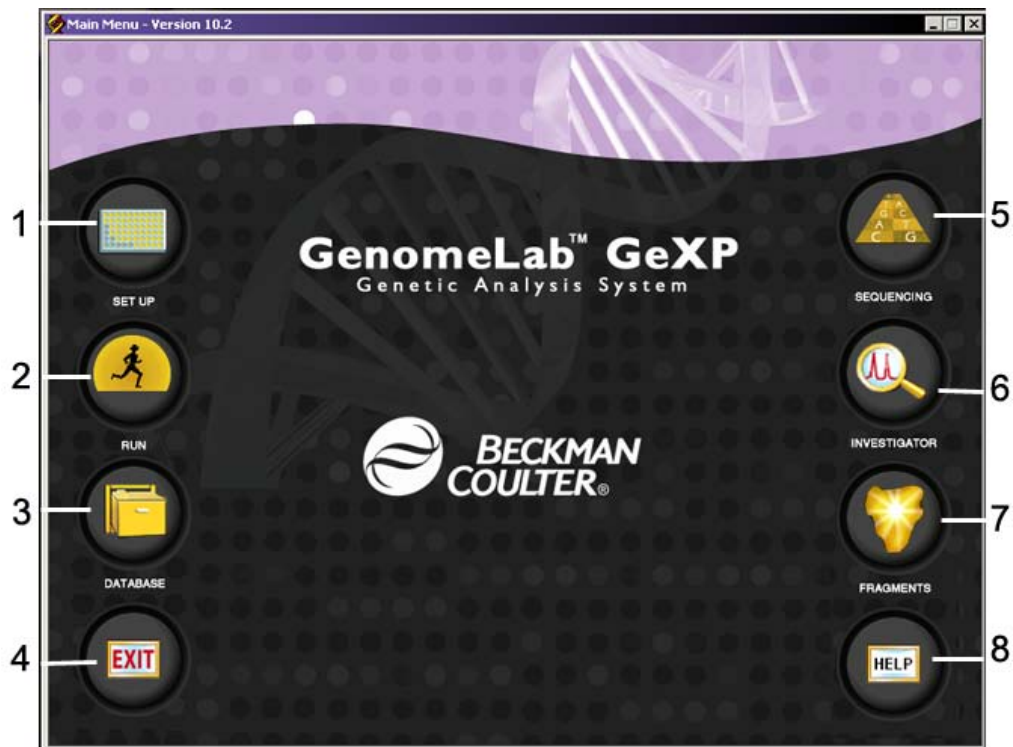
- 1. Sample Plate Holder (2)
- 2. Wetting Tray (2)
- 3. Buffer Evaporation Cover (2)
- 4. Buffer Tray (2)

## Gel Pump / Gel Access Cover Overview:



1. Gel Waste Bottle (behind panel)
2. Gel Cartridge Locking Lever
3. Gel Cartridge Barrel
4. Gel Plug (used when gel is not in the instrument)
5. Gel Cartridge

## GeXP Software Main Screen Overview:



1. **Setup** – Used to setup sample locations in a plate and the run conditions.
2. **Run** – Used to set run parameters for a plate.
3. **Database** – Contains all files associated with runs, plates, protocols, etc.
4. **Exit** – Shuts down the program.
5. **Sequencing** – Used to visualize all run results when a sample is sequenced.
6. **Investigator** – Used to perform specific analysis techniques on samples.
7. **Fragment** – Used to visualize run results when fragment analysis is performed.
8. **Help** – Provides help options for a user.