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INSTRUMENT STANDARD OPERATING PROCEDURE MANUAL

College of Medicine

SAFETY AND LABORATORY COOMMITTEE, C.O.M, K.F.U.

Prepared by

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Document History

Document Section	Details of Amendments	Date	Modified by (Initials)
SOP	First Draft on SOP for the operation of the 3500 Genetic analyzer instrument.		

1. OBJECTIVE

- ☐ The document describes the operation of the 3500 Genetic analyzer instrument. .

2. Introduction:

The 3500 Genetic Analyzer is a multi-capillary electrophoresis instrument designed to separate amplified DNA product based on size and record the resulting data in a computerized data file capable of being analyzed using specialized software.

Capillary electrophoresis separates DNA fragments based on their size to charge ratio. The cathode is placed into the sample. An electrical pulse activates the migration and separation of the DNA through the capillary. The negatively charged DNA migrates from the cathode to the anode because of the attraction of opposite charges. Smaller DNA fragments migrate faster than larger fragments thus reaching the detector sooner. The DNA fragments have fluorescently-labelled primers attached so that when the DNA goes past the detection window, a narrow beam of light from the laser excites the dyes. The excitation of the dyes give off an emission wavelength which is a longer wavelength of light than the laser's excitation wavelength in all directions. Some of this light passes through a diffraction grating, which then sends the light to the CCD detector. The CCD detector can detect the colour based on the wavelength. Along with an internal size standard and allelic ladder, a software program takes these peaks that are detected and give it a specific allele designation for a given locus. The combinations of all of the fluorescent peaks give rise to an electropherogram.

The 8-capillary 3500 DNA analyzer can be used for traditional DNA sequencing and DNA fragment analysis applications such as microsatellites, AFLP, SNP analysis and mutation detection.

3. RESPONSIBILITIES

- ☐ It is the responsibility of designated personnel in the lab to train new staff and students on this procedure and to ensure adherence to this procedure under supervision.
- ☐ It is the responsibility of designated personnel (staff or Student) to follow the instructions of this procedure under supervision.
- ☐ The head of the physiology department must resolve any problem with the process and difficulties in using this SOP.

4. REFERENCES

Applied Biosystems 3500/3500xL Genetic Analyzer User Guide (Part no. 4401661)

5. DEFINITIONS

ABC: Anode Buffer Container

CBC: Cathode Buffer Container

RFID: Radio Frequency Identification

PDP: Polymer delivery pump

POP: Polymer pouch

HID: Human Identification

6. SAFETY PRECAUTIONS

-Wear personal protective equipment (e.g., lab coat, gloves, eye protection), when carrying out standard operating procedures.
-Read Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

7. Materials Required

1. POP-7™
2. Cathode Buffer Container
3. Anode Buffer Container
4. Hi-Di Formamide
5. 8-capillary Array
6. Conditioning Reagent
7. Septa
8. Plates 96 well

8. PROCEDURE FOR OPERATING 3500 Genetic analyzer

8.1.Turning on the instrument:

8.1.1. Power on the computer and monitor, but do not log in to the Windows® operating system.

8.1.2. Once the computer and monitor are powered on, ensure that the instrument door is closed and power on the instrument by pressing the power on/off button on the front. Wait for the green status light.

8.1.3. Once status light is green, log on to the Windows® operating system.

8.2. **Launching the Applications**:

8.2.1. The Server Monitor should launch automatically. If not, then go to Start → Programs →Applied Biosystems → 3500 →Server Monitor.

8.2.2. Once all green checkmarks are displayed on the Server Monitor, click on the 3500 icon on the desktop. The 3500 Series Data Collection Software splash screen appears and will require a log in. Once the User Name and password are entered click OK.

8.3. **Check System Status in the Dashboard**

8.3.1. Perform appropriate maintenance tasks.

8.3.2. If applicable, click on the checkmark to mark a task complete.

8.3.3. Check Consumables Status

8.3.3.1. Click Refresh to update consumable status.

8.3.3.2. Check the consumables gauges for the number of injections, samples, or days remaining for each. If consumables have expired or if buffer fill level is too low, replenish as directed below. (See also 3500 Genetic Analyzer User Guide).

8.3.4. **Changing the Polymer**

8.3.4.1. Remove the polymer pouch from the refrigerator and allow

to come to room temperature.

8.3.4.2 In the Dashboard, click Wizards, then click Replenish

Polymer and follow the prompts.

8.3.5. **Changing the Anode Buffer Container (ABC)**

8.3.5.1. Remove the ABC from the refrigerator and allow to come

to room temperature.

8.3.5.2. Verify that the seal is intact. Do not use if the buffer level

is too low or the seal has been compromised.

8.3.5.3. Invert the ABC, and then tilt it slightly to move most of the

buffer to the larger side of the container. The smaller side

of the container should contain <1 mL of the buffer.

8.3.5.4. Verify that the buffer is at the fill line.

8.3.5.5. Peel off the seal at the top of the ABC and with the radio frequency

identification (RFID) label pointed toward the instrument, place the ABC into the anode-end of the instrument, below the pump. Position the anode in the large chamber of the ABC, then push the ABC up and back to install.

8.3.5.6. Close the instrument door to re-initialize.

8.3.5.7. In the Dashboard, click Refresh, then check the Quick View section for updated status.

8.3.6. Changing the Cathode Buffer Container (CBC)

8.3.6.1. Remove the CBC from the refrigerator and allow to come

to room temperature.

8.3.6.2. Wipe away condensation on the CBC exterior with a lint-free lab cloth.

8.3.6.3. Check that seal is intact. Do not use if buffer level is too

low or seal has been compromised.

8.3.6.4. Tilt the CBC back and forth gently and carefully to ensure

the buffer is evenly distributed across the top of the baffles.

8.3.6.5. When ready to install CBC, place the container on a flat

surface and peel off the seal.

83.6.6. Wipe off any buffer on top of the CBC with a lint-free cloth.

8.3.6.7. Place the appropriate septa on both sides of the CBC.

8.3.6.8. Ensure the instrument door is closed, then click the Tray

button to move the autosampler to the front position.

8.3.6.9.install the CBC on the autosampler. When properly installed, the CBC tabs will click as you snap them into place on the autosampler.

8.3.6.10. Close the instrument door to retract the autosampler.

8.3.6.11. In the Dashboard, click Refresh, then check the Quick

View section for updated status.

8.4. **Prepare the instrument:**

1. In the Dashboard, check consumable status. Ensure that:

• Consumables are not expired

• Adequate buffer levels are at the fill lines.

2. Set the oven temperature, then click Start Pre-heat:

• 60 °C – POP-7™ and POP-4™ polymers

• 50 °C – POP-6™ polymer

OPTIONAL: This is an optional but recommended step. If this step is not done, the instrument run will not begin

until the oven is 60°C.

3. Check the pump assembly for bubbles and run the Remove Bubble wizard if needed.

8.5. **Create a plate:**

A. In the Dashboard, click Create Plate from Template, then select an

appropriate plate template.

B. Click Open to define plate properties.

C. Enter the plate details.

D. Click the Assign Plate Contents button at the bottom of the screen.

8.6 **Assign plate contents:**

A. Enter sample names:

1. Click the Plate Map or Table View tab, then in the plate view, click a well.

2. Type a sample name directly into the field, then press Enter.

B. To change the assay, File Name Conventions and Results Groups,

select the wells you want to change, then select the check box(es) of

interest.

C. (Optional) In the Customize Sample Info pane, select the sample type for either a few or all of the samples on the plate.

D. Save the changes made by selecting Save Plate►Save or Save As.

E. Click Link Plate for Run in the Assign Plates for Run screen or Load Plates for Run in the navigation pane to assign the plate and specify the position of the plate in the autosampler.

8.7. **Load plates for the run:**

A. Inspect the information presented on the screen.

B. After instrument doors are closed, press the Tray button on the front of the 3500. Wait for the autosampler to finish moving to the front and then open the instrument doors.

C. Place the plate assembly on the autosampler. Confirm that the linked plate is in the correct position of the autosampler.

D. Check the septa on the CBC reservoir to be certain that they are flush.

E. Close the doors and allow the autosampler to completely move into the home position before continuing to next step.

F. Click Start Run or review the injection list by clicking Create Injection List in the Load Plates for Run screen or Preview Run in the navigation pane.

H. Click Start Run or review the injection list by clicking Create Injection List in the Load Plates for Run screen or Preview Run in the navigation pane

8.8. **Exporting Data:**

1. In the Dashboard, navigate to the "Workflow" and "Monitor Run" tabs. If there are no reinjections to perform, click Resume Run. If there are reinjections, proceed to Section.
2. Once the run has been completed select "My Computer" on the desktop > "AB SW & DATA (D:)" > "Applied Biosystems" > "3500" > "DATA". Alternatively, click on the data collection shortcut.
3. Locate the project folder, copy and paste the project to the designated storage location.

8.9. Turning off the Instrument

- ☐ Click on [X] in the upper right corner.
- ☐ Go to the 3500 Daemon Page
- ☐ Click on Ctrl + C
- ☐ Write Exit
- ☐ Turn off the PC and the Instrument

9. **Warning**

All specimens should be considered potentially infectious and must be handled with precautions used for Samples.