ABI 3730xl DNA Analyzer Operation & Maintenance

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SUMMARY

This protocol outlines the procedure of operating and maintaining the 3730 machine. Note: Most information on this protocol was obtained from the Applied Biosystems 3730 DNA Analyzer Installation Manuel and the Wizards.

MATERIALS & REAGENTS

Materials/Reagents/Equipment ***Vendor*** ***Catalog Number***

DISPOSABLES

50-cm 96 Capillary Array	Applied Biosystems	4331246
Half Skirted 96 well plate	ISC BioExpress	T-3060-1
96 Well Septa	Applied Biosystems	4315933
96 Well Septa Plate Bases	Applied Biosystems	4334873
96 Well Septa Plate Retainers	Applied Biosystems	4334869
36-cm 48 Capillary Array	Applied Biosystems	4331247

REAGENTS

Hi-Di Formamide (25mL)	Applied Biosystems	4311320
3730 POP-7 Ploymer (30mL)	Applied Biosystems	4332241
3730 Buffer (10X) with EDTA (500 mL)	Applied Biosystems	4335613
GeneScan DS-33 Installation Standard	Applied Biosystems	4330397
GeneScan 500 LIZ Size Standard	Applied Biosystems	4322682

EQUIPMENT

3730xl DNA Analyzer	Applied Biosystems	
Centrifuge	Beckman Coulter	
Mini-Vortexer	VWR Scientific Products	
Select HeatBlock	VWR Scientific Products	

PROCEDURE

Daily Tasks

1. Before each run ensure that there are adequate levels of buffer and water in the reservoirs.
2. Before each run ensure that the plate assemblies were put together properly.

IMPORTANT! The holes in the plate retainer must be aligned with the holes in the septa or the capillary tips will be damaged.

3. Before each run ensure that the plate assembles are positioned on the plate deck properly. Plates should sit snugly on the deck.
   IMPORTANT! Never use warped plates.
4. Before each run check the level of buffer in the buffer jar and ensure that the drain hole is not occluded.
5. Every 24 hours replace the water and 1X running buffer reservoirs on the instrument.
6. Check for bubbles in the polymer block, interconnecting tubing, polymer cap tubing and polymer block channels, and syringe. Remove all bubbles. For opaque tubing, manually flush polymer with syringe.
7. Check the loading-end header to ensure the capillary tips are not crushed or damaged.
8. Check the level of polymer in the bottle to ensure sufficient volume for runs.
9. Check the polymer block to ensure it fits securely on the instrument.
10. Clean the instrument surfaces.
11. Check for dried polymer around the polymer block and clean as necessary.
12. Check for leaks around the syringe, array knob, interconnecting tube nut, and check valve. Also ensure that the buffer jar drain hole is not occluded.

Weekly Maintenance

1. Monday mornings it is necessary to clean the polymer blocks to ensure that the capillary is properly maintained and free of dried polymer residue. Please refer to the Polymer Block Cleaning Kit Protocol in the CGF protocol book for cleaning instructions.
2. Clean the syringe.
3. Clean the water and buffer reservoirs with warm water.
4. Clean the complete polymer path including the upper and lower polymer blocks.
5. Replace the polymer in the bottle, syringe, upper polymer, and capillary array.
6. Check the storage condition of the used arrays in the refrigerator.

Capillary Array Maintenance

1. Wear gloves and handle the capillary array gently.
2. Do not touch the detection cell. If it is dirty, clean the detection cell with lens paper.
3. Keep the ends of the capillary wet at all times.
4. Always loosen the capillary array knob before pulling

Installing and Removing Capillary Array

1. A capillary array should last about 300-1000 runs. The following problems may indicate that a new capillary array is required: poor resolution, decreased signal intensity, poor sizing precision, and poor allele calling.

To install a new capillary array:

1. With the instrument door closed, press the tray button to ensure that the buffer tray is in its proper position.
2. Click Wizards>Install Capillary Array Wizard.
3. Follow the directions in the Wizard.
4. Select **Install a new capillary array.**
5. Enter the capillary array serial number.
6. From the **Type** list select 48 or 96.
7. From the **Length** list, select 36 or 50.
8. Click Next and follow the rest of the directions.
9. Prime the blocks and remove bubble twice.
10. Fill array only if it is a new capillary
11. Perform Spatial Calibration. See below.

Removing an Array for Storage

1. With the instrument door closed, press the tray bottom to ensure that the buffer tray is in its proper position.
2. Click Wizards>**Install Capillary Array Wizard.**
3. Follow the wizard's instructions.
4. Flush the capillary array with fresh polymer before replacing it.
5. Clean off any polymer buildup on the instrument, including the capillary electrodes with deionized water and kimwipe tissue.
   Note: When cleaning the capillary electrodes, be careful not to bend them out of position.
6. Clean the detection cell by gently swabbing the surface of the cell with lens paper. Make sure to swab in one direction.
7. Replace the cover over the detection cell.
8. IMPORTANT: DO NOT let the capillaries dry out. Store the capillary array with both ends in fresh 1X running buffer. Fill the buffer container with buffer and shipping vial with fresh buffer and cover tightly with parafilm.
9. Place the capillary in the appropriate storage box.
10. Store in 4 $^{\circ}C$ refrigerator and check the 1X running buffer level in the reservoir and vial weekly.

Performing Spatial Calibration

A spatial calibration maps the pixel positions of the signal from each capillary in the spatial dimension of the CCD camera. A spatial calibration must be performed each time you install or replace a capillary array, temporarily remove the capillary array from the detection block, open the detection block door, and move the instrument.

There are two spatial calibration run modules:

- Spatial calibration with the capillaries filled with polymer first (default module: spatial_fill)
  A spatial calibration with fill is recommended whenever there is old polymer in the capillary array or the calibration is done after a run.
- Spatial calibration without the capillaries filled (default module:spatial_nofill).
  A spatial calibration without fill is recommended whenever there is fresh/new polymer in the capillary array.

1. Expand the view in the tree pane.
   a. Click the + box next to the GA Instruments icon.
   b. Click the + box next to the ga3730 icon.
   c. Click the + box next to the instrument name icon.
2. Click the Spatial Run Scheduler icon.
   The Spatial Run Scheduler view opens.
3. Select the spatial protocol you want to use from the Spatial Protocols drop-down list box.
   - Use the SpatialFill protocol if the:
     Capillaries have no polymer (new capillary array)
     Polymer in the capillaries was used in a run.
   - Use the SpatialNoFill protocol if the capillaries contain fresh polymer.
     Note: You need not fill the capillaries each time you perform a spatial calibration.
4. Click Start. The calibration takes approximately 2 minutes without filling the capillaries and 6 minutes with filling the capillaries.
5. When the spatial is complete the view is updated.
6. Please refer to "Evaluating a Spatial Calibration Profile".

Performing Spectral Calibration

A spectral calibration creates a matrix that corrects for the overlapping fluorescence emission spectra of the dyes.

You must perform a spectral calibration:

- Whenever you use a new dye set on the instrument.
- After the laser or CCD camera has been realigned/replace by a service engineer.

- If you begin to see a decrease in spectral separation (pull-up and/or pull-down peaks)
- If you go from using a 96 capillary array to a 48 capillary array and vice versa.
- Please refer to the Procedure Overview for Spectral Calibration and Evaluating the Spectral Calibration Results.

Preparing for Sequencing Run

1. Label the 96 well PCR plates with the appropriate barcode label in the front of the plate.

    a. The barcode is retrieved from the CGF website.
    b. Select **CGF Staff Login** and login using username and password.
    c. Select **Sequencing Jobs in Progress.**
    d. Select the appropriate job from the list.
    e. Check the appropriate boxes in the REACTION PLATE section.
    f. Click on the SELECT button and the appropriate barcodes should be printed out. Place these barcodes on the front side of the plate.
2. After the clean up step, add 12 uL of Hi-Formamide to each well. Cover with the septa.
3. Vortex it for a couple of seconds to ensure mixing.
4. Place the plates in the centrifuge and spin up to 2000 RPM.
5. Place the plates on the heat block for 2 minutes to denature the samples.
6. Then place the plates on ice for 2 minutes.
7. Assemble the plate for 3730 run. Note: The black plate base, sample plate, plate septa, and plate retainer are assembled in this order with the black plate base at the bottom.
8. Centrifuge for a couple of seconds the assembled plates to ensure that all the products are in its proper place.
9. Place the assembled plate in the plate stacker.

To start the computer workstation:

1. Turn on the monitor.
2. Power on the computer.
3. Enter the user name and password.
   Username:3730User
   Password:3730User
4. Turn on the instrument by pressing the on/off power button on the front of the instrument. Ensure the green status light is on and constant before proceeding.
5. If a solid green light does not display, launch the 3730/3730xl software and look at the event log messages.
6. Click on the Data Collection v.1.0 icon on the desktop.
7. When the software is ready the service console will show all green squares.
8. Now go to the window that displays Foundation Data Collection v.1.0 viewer.
9. Click the + to expand subfolders in the left window pane. All application folders-except for Run History-are now visible and ready to access.
10. To schedule a run, click the Run Scheduler icon in the left window pane.
11. Click the mouse in the box the displays Add plates(Scan or type plate ID).
12. Scan the barcode of the desired plate or type in the barcode. Press enter.
13. It will say Plate…. Was not found? Click Yes

ID (Barcode)	Stays the same
Name	Enter the name on the plate i.e. CAST0001_F
Description	Blank
Application	SequencingAnalysis
Plate Type	96 well
Plate Sealing	Septa
Owner Name	Your name
Operator Name	Your name

14. Press Enter

Sample Name	Barcode
Comment	Can be left Blank
Results Group	CGF_1
Instrument Protocol	50
Analysis Protocol	5Primebasecalling

    Highlight all the boxes and press Control D to fill down.
    Press OK and the plate should show up in the Input Stack window.
15. Press the green play button near the top left corner to begin the run.

    Note: Up to 16 plates can be stacked in the plate stacker and entered into the computer ready to be ran.

Preparing for Genotyping Run

To start the computer workstation:

1. Turn on the monitor.
2. Power on the computer.
3. Enter the user name and password.
   Username:3730User
   Password:3730User
4. Turn on the instrument by pressing the on/off power button on the front of the instrument. Ensure the green status light is on and constant before proceeding.
5. If a solid green light does not display, launch the 3730/3730xl software and look at the event log messages.
6. Click on the Data Collection v.1.0 icon on the desktop.
7. When the software is ready the service console will show all green squares.
8. Now go to the window that displays Foundation Data Collection v.1.0 viewer.
9. Click the + to expand subfolders in the left window pane. All application folders-except for Run History-are now visible and ready to access.
10. To schedule a run, click the Run Scheduler icon in the left window pane.

ID Barcode	Barcode
Name	Plate name
Description	N/A
Application	GeneMapper-CGF3730
Plate Type	96-well
Scheduling	N/A
Plate Sealing	Septa
Owner Name	Your name
Operator Name	Your name

11. Click OK button which validates the entries in the fields, creates the new Plate Document, and displays it in the Plate Editor Window.
12. The Plate Editor displays an empty plate record for the selected application.
13. The following table describes the columns inserted in the Plate Record for a GeneMapper software run.

Column	Insert
Sample Name	Barcode
Comment	Optional
Results Group	Genotyping_1
Sample Type	Sample
Size Standard	GS500LIZ_3730
Panel	DS-33
Analysis Method	3730 DS-3730 Install
3 User Defined Columns	Optional
Instrument Protocol	CGF_Genotyping

14. Press Control D to fill down and press OK. The plate should show up in the Input Stack window.
15. Press the green play button near the top left corner to begin the run.