NCSBI Forensic Biology Section	DNA SOP	Effective Date: September 5, 2006
Title: **Preparing and Running Samples on the 3100 Genetic Analyzer**		Revision 03

****Note: Whenever the instrument doors are closed, WAIT for the autosampler to move and return to the position under the capillary array BEFORE clicking anything on the computer screen.**

1. Checking and Refilling Fluids

1.1. Determine if the polymer needs to be added to the instrument before proceeding with instrument preparation.

1.2. If there is sufficient polymer for the run(s) and polymer is less than one (1) week old and sufficient in quantity to complete the run, then ensure no bubbles are present in the lines and proceed with instrument preparation.

1.3. If polymer is more than one (1) week old or insufficient in quantity to complete run, then fill the syringes and the upper polymer block with polymer by following the **Change Polymer Wizard**.

1.4. Replace the respective solutions in the in the anode buffer reservoir (shot glass), the cathode buffer reservoir and the water reservoir **daily, or before each batch run**.

1.5. To prepare 50 ml of 1X Genetic Analyzer Buffer: Add 5.0 ml of 10X Genetic Analyzer Buffer to a 50 ml conical tube, then add 45 ml of deionized water. Mix well.

2. Filling the Water and Cathode Buffer Reservoirs:

NOTE: Wear gloves when performing the following procedure.

2.1. Close the instrument doors. WAIT for the autosampler to return to the capillary array.

2.2. Press the tray button outside the instrument to bring the autosampler to the forward position.

2.3. Wait until the autosampler has stopped moving before opening the instrument doors.

2.4. Remove the reservoirs from the instrument.

2.5. Dispose of fluids and rinse reservoirs with warm deionized water. Perform a second rinse using deionized water on the water reservoirs and buffer on the cathode buffer reservoir.

2.6. Fill the cathode buffer reservoir with 1X Genetic Analyzer Buffer to the fill line.

2.7. Fill the water reservoirs with deionized water to the fill line.

2.8. At least once a week, place a clean septa strip on each reservoir, and dry the outside of the reservoir using a Kim-wipe. Be sure the septa strip fits snugly and flush on the tops of the reservoirs

2.9. Place reservoirs into the correct positions on the autosampler (two water reservoirs in the back positions, the buffer reservoir in the front left position, and position 3 is empty).

3. Change the Anode Buffer Reservoir (Shot Glass)

3.1. Remove the anode buffer reservoir by firmly pulling down and twisting slowly.

3.2. Discard the used buffer.
3.3. Clean and rinse with warm deionized water then rinse with deionized water, then with buffer.
3.4. Fill the reservoir to the fill line with the freshly made 1X Genetic Analyzer Buffer. The meniscus should line up with the fill line.
3.5. Put the anode buffer reservoir on the instrument.

4. Preparing the Formamide : Size Standard Mixture

NOTE: A run corresponds to a defined set of 16 well on a 96-well reaction plate.

4.1. Prepare the master mix for a 16 well run:

Reagent	Volume
GeneScan-500 Liz Size Standard	Approx. 7 µl
Hi-Di Formamide	174 µl

4.2. Vortex the tube to mix, then spin briefly in a microcentrifuge.

4.3. Load the samples and allelic ladder

4.3.1. Fill out the Sample Tray Worksheet with the location of ladders and samples for the 96-well tray.

4.3.2. Controls Required for Runs:

4.3.2.1. One Ladder must be included in each run. A “run” is defined as the set of 16 samples that is processed through the 3100 at one time. It is acceptable to load more than one ladder or 9947A per 16 sample run. In the case where two ladders or two positive amplification controls are loaded on the same run, it is acceptable for the analyst to choose the best sample to add to the project and not analyze the other one.

4.3.2.2. If a sample (or samples) for a case must be re-injected, then the ladder, 9947A, and the sample(s) must be re-injected and re-analyzed.

4.3.2.3. If a sample (or samples) for a case must be injected for 22 seconds, then the ladder, negative amplification control, 9947A, samples and corresponding negative extraction control must be re-injected and re-analyzed.

4.3.3. Dispense **9 µl** of the formamide:size standard Master Mix into each well on the 96- well tray that will be contain a sample.

Note: Dispense 9-10 µl of the Master Mix to each blank well per run.

4.3.4. Based on the Worksheet load approximately **1.5 µl** allelic ladder and **1 µl** of sample into its appropriate well. Note: Ladder volume may be adjusted depending on lot variation.

4.3.5. Cover the reaction plate with the 96-well septa.

4.3.6. Briefly spin the reaction plate in a centrifuge to ensure the contents of each well are mixed and collected at the bottom.

4.3.7. Denature the reaction plate in a thermocycler at 95^{o}C for 3 minutes.

4.4. Place the reaction plate immediately on an ice block for approximately 3 minutes or allow the thermal cycler to ramp down to 4^{o}C and let the plate remain there for approximately 3 minutes.

5. Complete the Plate Editor spreadsheet on the 3100.

5.1. Open the 3100 Data Collection Software.

5.2. In the **Plate View** dialog box, click **New** to access the **Plate Editor spreadsheet**. Type the plate name; choose **Genescan** for the Application; then click **Finish**.

5.3. **Plate Editor Spreadsheet**: Complete the "Plate Editor Spreadsheet" for the wells that will be run. For each of the columns, enter the information, click the column header to select the entire column, then select **Edit> Fill down (Ctrl D)** to apply the information to all of the selected samples.

Note: The plate records may be reused by importing data from an existing plate into the current plate.

5.3.1. In the **Sample Name** column, type a name for the samples.

5.3.2. In the **Dyes** column, choose the orange box.

5.3.3. In the **Color Info** column, type the word ladder for each ladder in the **Sample Name** column.

5.3.4. In the **Project Name** column, select **3100_Project1**.

5.3.5. In the **Dye Set** column, select **G5.**

5.3.6. Select a run module.

5.3.7. In the **Run Module 1** column, select **GeneScan 36cm_POP4 Dye SetG5** for a 10 second injection, or **GeneScan 36cm_POP4 Dye SetG5_3Kv_22sec** for a 22 second injection.

5.3.8. In the **Analysis Module 1** column, select the **GS500Analysis.gsp** module as the size standard.

5.4. Double check all entries and click **OK.**

6. After a few seconds, the entry for the Plate Record appears in the Pending Plate Records table on the Plate Setup page.

7. Link the reaction plate and start the run.

7.1. Place the reaction tray containing denatured DNA into the 3100, positioned correctly with the notch in the lower right corner.

7.2. Click the **Plate View** tab on the 3100 Data Collection Software window to go to the **Plate View** page

7.3. On the **Plate View** page, in the **Pending Plate Records** table, click the **Plate Record** for the plate that is being linked.

7.4. Click the plate position indicator that corresponds to the plate you are linking.

7.5. Verify that the plate has been linked.

7.5.1. The **Run Instrument** button on the toolbar is enabled, meaning that the instrument is ready to run.
7.5.2. Plate position indicator for the linked plate becomes green.
7.5.3. Plate record moves from the **Pending Plate Records** table to the **Linked Plate Records** table.
7.5.4. Repeat steps b. through d. above to link a second plate, if applicable.
7.5.5. Click on the **Run View** tab to view the run schedule.

8. To start a run, click on the green Run Instrument button to begin the scheduled runs.

NOTE: If the plate is placed on the 3100, linked, and the green Run Instrument icon is clicked prior to the plate stopping its movement, a fatal error will occur and the 3100 will have to be restarted.

9. Monitoring the run:

9.1. Click the Status View tab to monitor the status of the instrument during the run.
9.2. View the data using the Array View and Capillary View

IMPORTANT!!!! Always EXIT from the Array View and the Capillary View windows. DO NOT leave these windows open for an extended period of time during a run because unrecoverable screen update problems will occur. Leave the Status View window open.

10. Reviewing the Data

10.1. To review the color data after the run has completed, click on the **Array View** page. This is the raw data, multi-component data for a selected capillary.

10.2. To review the sample files open the runs from the following default location: **D:\AppliedBio\3100\DataExtractor\Extracted Runs.**

10.3. After run is completed, analyze data using the Continue analysis with GeneMapper ID SOP.

11. Sequence for Restarting Instrument

11.1. Close 3100 Data Collection Software.
11.2. Turn off 3100.
11.3. Restart computer. Wait for OrbixWeb Daemon and AE Server Software to load.
11.4. Turn on 3100. Allow instrument to cycle until green light comes on.
11.5. Open 3100 Data Collection Software.
11.6.

References:

ABI Prism 3100 Genetic Analyzer. Quick Start Guide for Fragment Analysis. 2001 Applied Biosystems. Part Number 4315832. Rev C. (or most recent revision)

Document Revision History		
Revision Number	Date	Reason
00	5/21/04	New Document
01	12/23/04	No substantive changes; wording changed to clarify certain steps in SOP.
02	12/22/05	2.5 and 3.3 Add warm tap water step to wash; 2.8 Specify that septa strip can be replaced once a week; 2.9 Specify position of reservoirs; 4.1 Change in Liz Volume from 8 to 7 µl; 4.3.2 Added requirements for Ladders and Controls; 8 Note added to prevent a fatal error on the 3100
03	09/05/06	Global change from rinsing with tap water to deionized water; 2.5- clarified 2nd wash; 4.3.3- allows for ladder volume adjustment; Section 11 Added- procedure for restarting computer and instrument.

APPROVAL SIGNATURES	Date
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