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| ATF-LS-FB15 Applied Biosystems™ 3130 Genetic Analyzer | Published Online: March 2018 |
| Authority: Technical Leader | |
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1. Scope

This protocol is used to analyze the amplified DNA product on the Applied Biosystems™ (AB) 3130 Genetic Analyzer. The DNA fragments amplified with the AB Identifiler™ kit are separated according to size by capillary electrophoresis using the AB 3130 Genetic Analyzer, a multi-capillary instrument. An internal lane standard (ILS) is injected along with the amplified DNA fragments. The ILS fragments are then used to determine the base pair size for each amplified DNA fragment. The use of ILS provides consistency in sizing between samples.

The amplified fragments are detected by laser excitation and the subsequent emission spectra are captured by a charge-coupled device (CCD) camera that displays the signals as peaks. The resulting data is graphically displayed as colored peaks noted by height in relative fluorescent units (RFUs) and time (scan number). This display is called an electropherogram.

The reference allelic ladders for each of the STR loci and reference fragments for amelogenin are also subjected to capillary electrophoresis. These allelic ladders contain the more common alleles in the general population for each locus. Using the ladders, the alleles present in known and questioned DNA specimens may be determined.

2. References

The procedures described here are derived from a variety of sources. Portions of the protocol come

directly from some of the references cited below:

- 2.1. Applied Biosystems™ 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide, 2004
- 2.2. Applied Biosystems™ 3130/3130xl Genetic Analyzers Getting Started Guide, 2004.
- 2.3. Applied Biosystems™ 3130/3130xl Genetic Analyzers Using Data Collection v3.0 User Bulletin, 2005.
- 2.4. Prince George's County (MD) Police, Forensic Services Division, Serology / DNA Laboratory Short Tandem Repeat (STR) Analysis Protocol, 2006.
- 2.5. J.M. Butler, DNA separation Methods, DNA detection methods, Instrumentation for STR typing, and STR genotyping issues, in: Forensic DNA Typing, second ed., Elsevier Academic Press, Burlington, 2005, pp 313-388.
- 2.6. J.M. Butler, B.R. McCord, J.M. Jung, R.O. Allen, Rapid analysis of the short tandem repeat HUMTH01 by capillary electrophoresis, *BioTechniques* 17 (1994) 1062-1070.
- 2.7. M.M. Holland, L.A. Turni, S.A. Del Rio, M.A. Marino, R.S. Lofts, D.L. Fisher, J. Ross, J.W. Schumm, P.E. Williams, Typing human DNA using capillary electrophoresis: comparison of slab gel and capillary formats, in: W. Bär, A. Fiori, U. Rossi (Eds.), *Advances in Forensic Haemogenetics*, volume 5 Springer, Berlin, 1994, pp. 156-159.
- 2.8. T.R. Moretti, A.L. Baumstark, D.A. Defenbaugh, K.M. Keys, A.L. Brown, and B. Budowle, Validation of STR typing by capillary electrophoresis, *J. Forensic Sci.* 46 (2001) 661-676.
- 2.9. J.M. Butler, E. Buel, F. Crivellente, and B.R. McCord, Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis, *Electrophoresis.* 25 (2004) 1397-1412.
- 2.10. A.R. Isenberg, R.O. Allen, K.M. Keys, J.B. Smerick, B. Budowle, and B.R. McCord, Analysis of two multiplexed short tandem repeat systems using capillary electrophoresis with multiwavelength fluorescence detection, *Electrophoresis.* 19 (1998) 94-100.

3. Equipment

- 3.1. Gloves
- 3.2. Lab coat
- 3.3. Eye protection
- 3.4. Deionized water
- 3.5. Computer with data collection software
- 3.6. AB 3130 Genetic analyzer
- 3.7. AB 10X Buffer with EDTA
- 3.8. AB 1X Buffer with EDTA
- 3.9. AB Performance-optimized polymer 4 (POP4)
- 3.10. AB Hi-Di Formamide
- 3.11. AB Identifiler™ allelic ladder
- 3.12. AB GS500 LIZ sizing standard
- 3.13. MicroAmp™ Optical Reaction Plate
- 3.14. MicroAmp™ Splash Free Support Base
- 3.15. AB 96-well plate base

- 3.16. AB 96-well plate retainer
- 3.17. Buffer/water/waste reservoirs
- 3.18. Plate and reservoir septa
- 3.19. 10% bleach solution
- 3.20. 70% ethanol
- 3.21. 1.5 mL microcentrifuge tubes
- 3.22. 50 mL conical tubes
- 3.23. Pipette
- 3.24. Disposable aerosol-resistant pipette tips
- 3.25. Centrifuge
- 3.26. Heat block
- 3.27. Cold block
- 3.28. Vortexer

4. Safety/Quality Assurance

- 4.1. Disposable gloves shall be worn when handling reagents and amplified DNA.
- 4.2. Lab coat and eye protection must be worn at all times while performing this procedure.
- 4.3. Sample setup must be performed on bench paper, tissue wipes (e.g. Kimwipes), or clean laboratory bench surfaces (10% bleach solution followed by 70% ethanol).
- 4.4. Exercise caution when opening tubes.
- 4.5. Only one tube of amplified DNA shall be open at a time.
- 4.6. Use aerosol-resistant pipette tips when transferring liquids containing DNA.
- 4.7. Change pipette tips after transferring any liquids potentially containing DNA.
- 4.8. Record the lot number of each reagent used in case notes. Do not use the reagents after the expiration date.
- 4.9. Check the AB 3130 Instrument Log to ensure that 1X buffer and water in the reservoirs (buffer, water, waste) have not been sitting on the instrument for more than two days. Replace reservoir septa when refilling reservoirs.

NOTE: When refilling reservoirs, the septa may be rotated 180 degrees and reused one time since only four capillaries are being used.

- 4.10. Check the AB 3130 Instrument Log to ensure that POP4 polymer has not been on the instrument for more than two weeks.
- 4.11. Clean plate base and retainer with water between runs.
- 4.12. Use new plate septa with each set of runs.
- 4.13. At least one positive amplification control (9947A) must be included with each sample run.
- 4.14. It is good practice to include multiple wells with allelic ladder and positive control to account for variations in electrophoresis conditions.
- 4.15. Buffer jar, reservoirs, plate base, and plate retainer shall be cleaned with tap water followed by deionized water. Do not clean with bleach.

- 4.16. Minor deviations from the protocol may be made at the analyst's discretion based on the analyst's training and experience and should be indicated in the analyst's notes. Significant deviations from the protocol must be approved by the DNA Technical Leader.
- 4.17. The spatial calibration determines the position on the CCD camera where the signal emitted by each capillary is expected to fall. A new spatial calibration is required when the capillary array is replaced, temporarily removed from the instrument detection block, or the instrument is moved.
- 4.18. The spectral calibration accounts for the overlapping emission spectra of the five dyes detected by the instrument. If optical components of the instrument (laser, CCD camera, capillary array) have been realigned/replaced, a new spectral calibration is required. Additionally, if there is a noticeable degradation in spectral separation (increased pull-up and/or pull-down peaks) in the electropherograms, a new spectral calibration may be needed.
- 4.19. For instructions of performing spatial and spectral calibrations, refer to Applied Biosystems™ 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide.

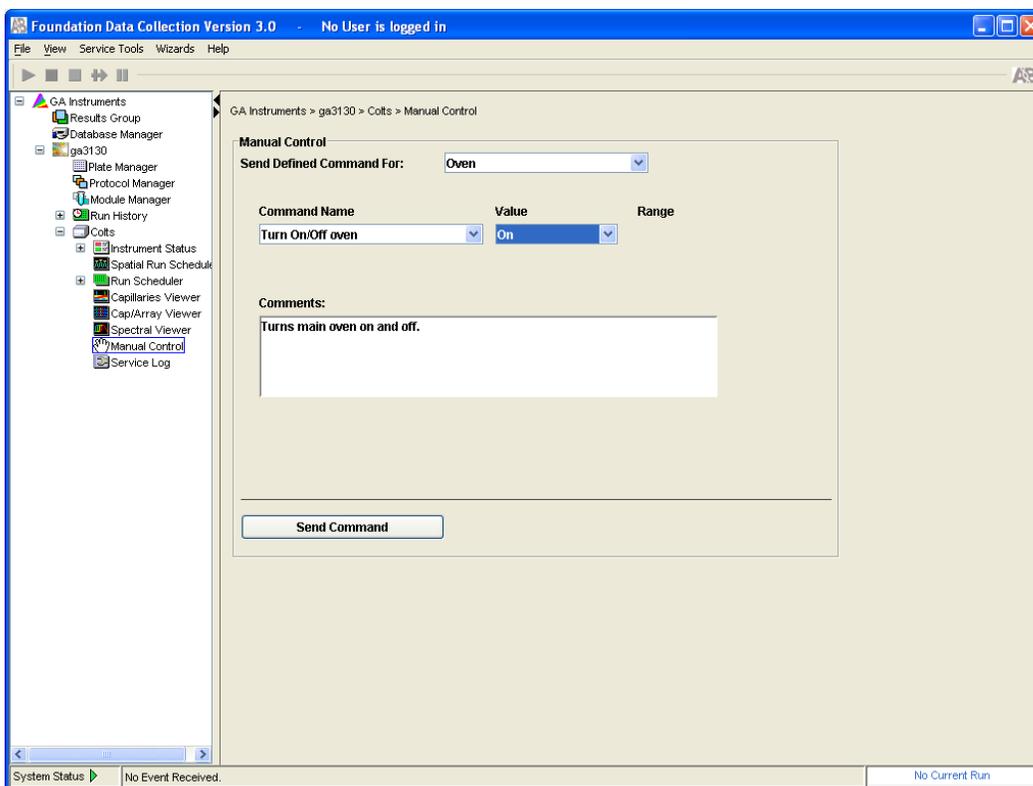
5. Procedure

- 5.1. Remove appropriate number of tubes of formamide from freezer and allow them to thaw completely.

Preparing the Instrument

- 5.2. Turn on computer workstation and log in.
- 5.3. Turn on instrument and wait for green status light to come on and stop flashing.
- 5.4. Launch Data Collection software. Wait for all four status indicator lights in *Service Console* window to turn green (if lights do not turn green, refer to *Applied Biosystems™ 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide*).
- 5.5. Check quality control chart for instrument, and if necessary, press Tray button on the front of the instrument and wait for green status light to stop flashing prior to opening instrument doors and performing necessary maintenance tasks. These tasks include, but are not limited to, installing or replacing the capillary array, replenishing or changing polymer, preparing buffer and filling reservoirs. When possible, follow wizards contained in data collection software (see *Applied Biosystems™ 3130/3130xl Genetic Analyzers Getting Started Guide* or *Applied Biosystems™ 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* for specific instructions).
- 5.6. Check levels and time on instrument of buffer in buffer jar and buffer and water in reservoirs. Ensure that reservoir septa fit snugly on the reservoirs.
- 5.7. Check levels and time on instrument of polymer. If polymer must be added or replaced, ensure that new polymer is warmed to room temperature before using. Use the *Polymer Replenish Wizard* to replenish polymer.
- 5.8. Check for bubbles in the pump block, lower polymer block, interconnect tube, polymer supply tube, and channels. Very small bubbles on top of the array port may be disregarded. Use the *Bubble Remove Wizard* to remove bubbles. If *Bubble Remove Wizard* is used, ensure that sufficient polymer remains for completing the scheduled runs.
- 5.9. Check for leaks around the array knob, the interconnecting tube nuts, and the polymer supply tube nut. Clean dried polymer from nuts and/or knob, if possible.
- 5.10. Shut instrument doors and wait for tray to complete homing procedure.
- 5.11. In the tree pane of the Data Collection software, click *GA Instruments > ga3130 > instrument name > Manual Control*.
- 5.12. In the *Send Defined Command For* drop-down menu, select "Oven". In the *Command Name* dropdown menu, select "Temperature Set", type "60" in the *Value* field, and click *Send Command*.
- 5.13. In the *Command Name* drop-down menu, select "Turn On/Off", select "On", then click *Send Command*.

NOTE: The oven will turn itself off after 45 minutes if the run has not been started.



Preparing the Samples

- 5.14. Determine plate configuration including placement of samples, allelic ladders, and controls. Four wells in a column (rows A through D / E through H) are injected simultaneously. For each injection, ensure that all four wells contain either a sample, allelic ladder, control, or formamide blank.
- 5.15. Prepare formamide / GS500 LIZ sizing standard master mix (8.7 μL formamide and 0.3 μL GS500 LIZ sizing standard per sample, adding several extra samples to ensure adequate volume). The volume of GS500 LIZ sizing standard can be reduced to prevent spectral pull-up. Any remaining formamide shall be discarded.
- 5.16. Vortex and briefly spin tube of master mix.
- 5.17. Dispense 9 μL master mix to each well of 96-well optical reaction plate that will contain sample or allelic ladder.
- 5.18. Add 10 μL master mix to each well designated as a formamide blank.
- 5.19. Add 1 μL sample or allelic ladder to the appropriate wells.
- 5.20. Cover plate with new plate septa.
- 5.21. Briefly spin plate on plate centrifuge.
- 5.22. Place plate in heat block ($\sim 95^{\circ}\text{C}$) for 3 minutes.
- 5.23. Place plate in frozen cold block for 3 minutes.
- 5.24. Place optical reaction plate in plate base, ensuring that it is correctly aligned.
- 5.25. Snap plate retainer onto optical reaction plate / plate base assembly, ensuring that the holes in the septa align with those in the plate retainer.

- 5.26. Align and place plate assembly securely on the plate deck of the instrument. Note the notch on the bottom of the plate.

Creating a Plate Record, Linking the Plate, and Starting the Instrument

- 5.27. In the tree pane of the Data Collection software, click *GA Instruments > ga3130 > Plate Manager*

- 5.28. To create a new plate:

- Select *New*.
- In the *New Plate Dialog* window, type in the plate name. The plate name shall include at a minimum the date, analyst's initials, and instrument name.
- For the *Application* drop-down menu select "GeneMapper-instrument name."
- For the *Plate Type* drop-down menu, select "96-Well."
- Type the analyst's initials in the *Owner* and *Operator* fields, then click *Ok*. The *Plate Editor* opens.
- In the *Plate Editor* window, type in the sample name.
- Select the appropriate sample type in the *Sample Type* drop-down menu.
- Select "CE_G5_HID_GS500" in the *Size Standard* drop-down menu .
- Select "AmpFLSTR_Panels_v1 > Identifiler_v1" for the *Panel*.
- Select "G5_AdvancedHID" in the *Analysis Method* drop-down menu.
- Select "HID_Identifiler" in the *Results Group* drop-down menu.
- Select "HID" in the *Instrument Protocol* drop down menu.

NOTE: Only select the Size Standard, Panel, Analysis Method, Results Group, and

Instrument Protocol for the first sample. Typing Alt-D will fill down the information for

four samples. Alternatively, highlight a number of wells and type Ctrl-D. This will fill down

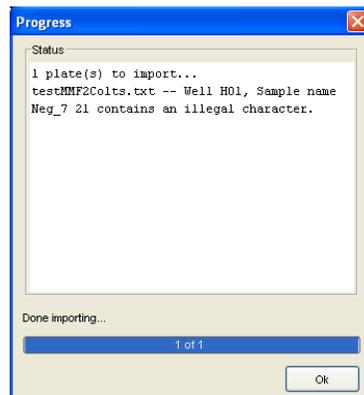
the information for any highlighted wells.

5.29. To duplicate an existing plate:

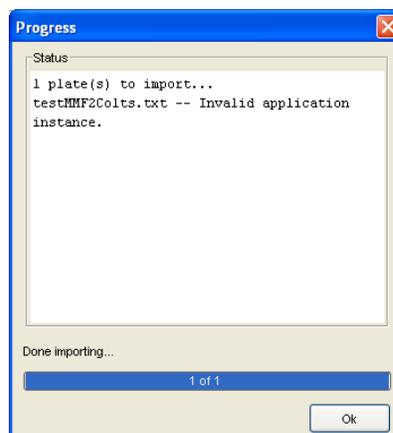
- Click *Find All*.
- Highlight the plate you want to duplicate and click the *Duplicate* button at the bottom of the screen.
- In the *Duplicate Plate* dialog window, type in the new plate name. The plate name shall include at a minimum the date, analyst's initials, and instrument name.
- Type the analyst's initials in the *Owner* and *Operator* fields, then click *Ok*.

5.30. To import a plate:

- Click *Import*.
- In the Open window, find the saved plate that will be imported, highlight the file name, and click the *Open* button.
- A window will appear with the status of the import. For a successful import, click *Ok*.
- Importing problems occur when incorrect characters are used in sample names. In these instances, an error message will appear:



- Click *Ok* and check the sample name of the well listed in the error message. Attempt to import the file again after making corrections. The following characters are NOT allowed to be used in creating sample names: \ / : " < > ? ' | SPACE
- Importing problems occur when a template created for one 3130 is imported to a different 3130. In these instances, an error message will appear:



- Click *Ok*. Check to make sure that the correct template is being used for the desired 3130. If it is the correct 3130, check/change the application instance in the import file. Attempt to import the file again on the correct 3130.
- 5.31. By default, the priority setting for each sample is 100. The priority setting determines the order in which the injections are analyzed by the instrument. Lowering the priority setting (1-99) will result in the injections being processed before those with higher priority settings.
 - 5.32. To inject the same sample(s) multiple times, select *Edit > Add Sample Run* from the menu at the top of the screen. This will create additional *Results Group* and *Instrument Protocol* columns for all of the samples in the run. Be sure to select the appropriate *Results Group* and *Instrument Protocol* settings for only the set of four samples that contains the sample(s) that needs to be injected multiple times. Leave the settings blank for those samples that will be injected only once.
 - 5.33. Click *Ok* to save and close plate record.
 - 5.34. In the tree pane of the Data Collection software, click *GA Instruments > ga3130 > instrument name > Run Scheduler*.
 - 5.35. Highlight the plate name and click on the yellow plate diagram on the right hand side of the screen to “link” the plate. The plate diagram will turn green once it has been linked. A run cannot be started until a plate has been linked.
 - 5.36. In the tree pane of the Data Collection software, click *GA Instruments > ga3130 > instrument name > Run Scheduler > Run View*. Verify that all the necessary injections have been scheduled. When a particular injection is highlighted, the corresponding wells to be injected will be highlighted in the plate diagram.
 - 5.37. After verifying that all settings are correct, click the green triangle icon in the toolbar. Click *Ok* on the pop-up screen to start the run.

Shutting Down the Instrument

- 5.38. In the *Service Console* window, click the *Stop All* button. Wait for all four status indicator lights to turn red. At this point, you can close the *Service Console* window by clicking on the “x” in the upper right hand corner.

NOTE: Do not close the *Service Console* window without first waiting for all four status

indicator lights to turn red.

- 5.39. Press the power button on the 3130 Genetic Analyzer.
- 5.40. Shut down the computer.