

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

IDENTIFILER ANALYSIS ON THE ABI 3130XL GENETIC ANALYZER

DATE EFFECTIVE
06-20-2016

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NUCLEAR DNA TECHNICAL LEADER

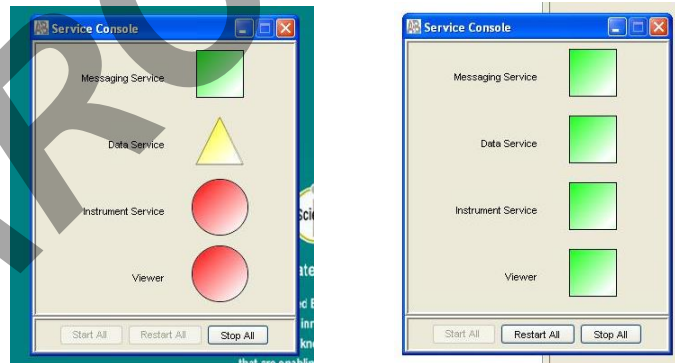
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Identifiler Analysis on the ABI 3130xl Genetic Analyzer

A. Setting Up A 3130xl Run

1. Go to the computer attached to the instrument.
2. If needed, press “CTRL-ALT-DEL” to login.
3. User should be “Administrator”, password should be left blank.
4. Click OK.
5. Open the 3130xl Data Collection v3.0 software by double clicking on the desktop Icon or select **Start > All Programs > AppliedBiosystems > Data Collection > Run 3130xl Data Collection v3.0** to display the **Service Console**.

By default, all applications are off, indicated by the red circles. As each application activates, the red circles (off) change to yellow triangles (activating), eventually progressing to green squares (on) when they are fully functional.



Once all applications are running, the **Foundation Data Collection** window will be displayed at which time the **Service Console** window may be minimized.

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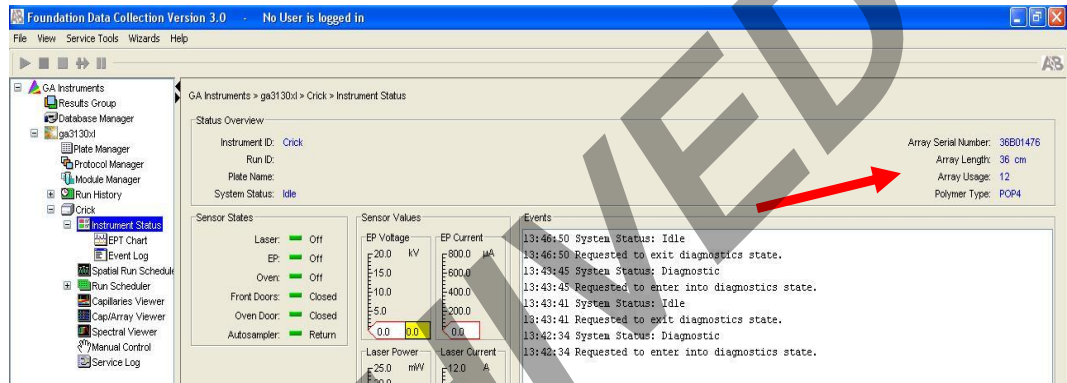
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6. Check the number of injections on the capillary in the 3130xl usage log and in the **Foundation Data Collection** window by clicking on the **ga3130xl > instrument name > Instrument Status**. If the numbers are not the same, update the usage log. If the number is ≥ 140 , notify QA. Proceed only if the number of injections that will be running plus the usage number is ≤ 150 .



7. Check the usage log to see when the POP4 was last changed. If it is >7 days, proceed with POP4 change (See Part K. of this section) and then return to Step 9. The POP4 does not need to be changed if it is the 7th day.
8. Check the level of POP4 in the bottle to ensure there is enough for the run ($\sim 450 \mu\text{L}$ for 6 injections). A full piston chamber is approximately 600ul. If not enough, proceed with POP4 change (See Part K. of this section) and then return to Step 9.

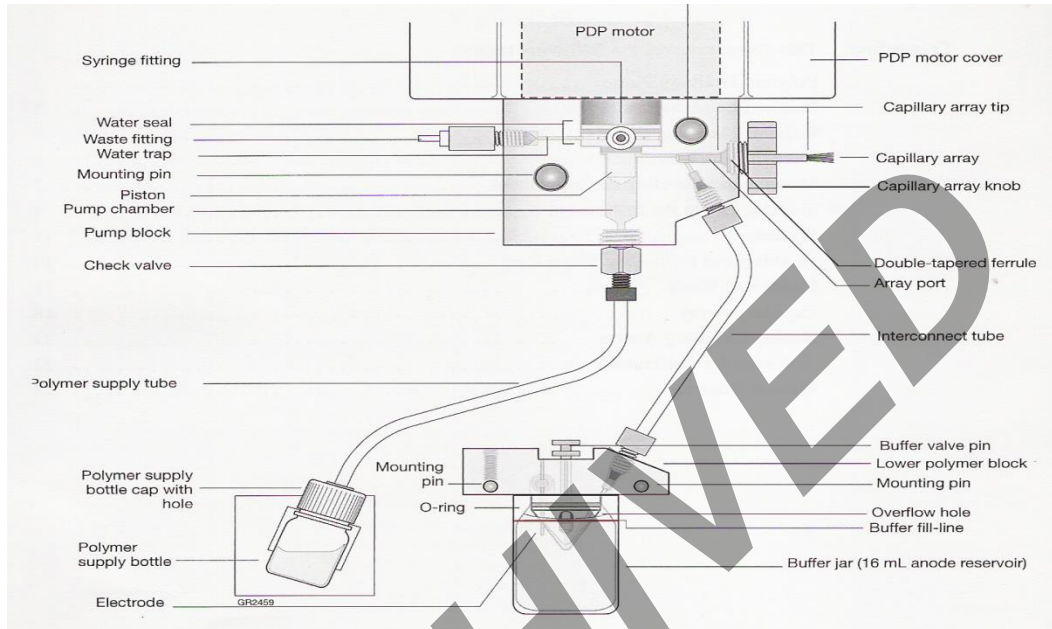
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9. If it is the first run of the day on the instrument, proceed with steps 10-18. If a run has already been performed on the instrument that day and the “buffer changed” column displays that day’s date, skip to Part B of this section.
10. Close the instrument doors and press the tray button on the outside of the instrument to bring the autosampler to the forward position.
11. Wait until the autosampler has stopped moving and the light on the instrument turns green, and then open the instrument doors.
12. Remove the three plastic reservoirs in front of the sample tray and anode jar from the base of the lower pump block and dispose of the fluids.
13. Rinse, dry thoroughly, and then fill the “water” and “waste” reservoirs to the line with deionized water such as INVITROGEN®.
14. Make a batch of 1X buffer (45 ml deionized® water, 5 ml 10X buffer) in a 50 mL conical tube. Record the lot number of the buffer, date of make, and your initials on the side of the tube. Rinse and fill the “buffer” reservoir and anode jar with 1X buffer to the lines.

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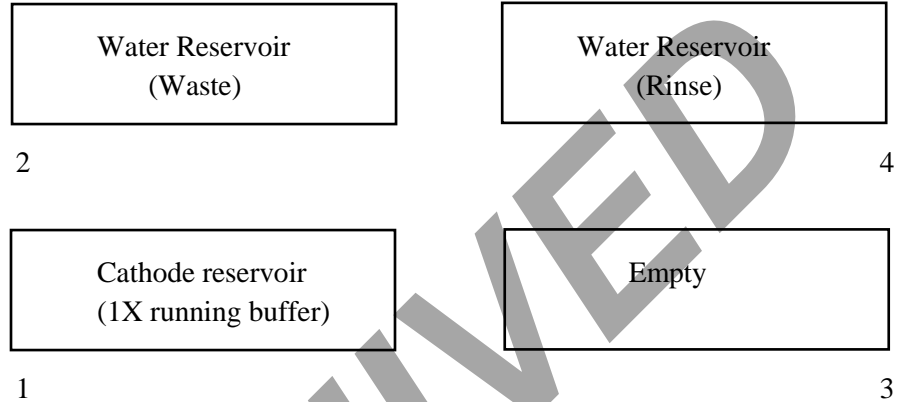
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15. Dry the outside and inside rim of the reservoirs/septa and outside of the anode jar using a lint free wipe and replace the septa strip snugly onto each reservoir.

16. Place the reservoirs in the instrument in their respective positions, as shown below:



17. Place the anode jar at the base of the lower pump block.

18. Close the instrument doors.

19. Record lot numbers for POP4 and buffer.

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B. Creating a Test Batch

3130xl Test Batch Creation for High Copy DNA Testing

Sample names and run names cannot be longer than 50 characters, and must be in correct 3130 format: -_().{}[]+^ only.

Allelic Ladder(s) must be individually added to the test batch. If there are two or more injections of Identifiler samples, Allelic Ladder should be positioned as the first sample of that injection during the plate loading step.

Ensure that the correct System is in the “Sys” column

Amplification System/Cycle	Specification	Run Module Code	Parameters
Identifiler 28	Normal	I	1 kV for 22 sec
	High	IR	5 kV for 20 sec

Name the test batch as follows: *Instrument name & date_Run folders* for example: Athena042407_70-76.

If samples on the test batch are being rerun, confirm that dilution (if applicable), suffix, comments, or any other necessary information is present.

For rerun normal samples, fill up the end of the injection with any normal reruns before starting a new injection.

Rerun high samples should have a separate injection from samples run under normal conditions.

Using the LIMS drive, drag-and-drop the plate record from the LIMS Share folder to the instrument’s plate record folder.

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3130x/ Test Batches For High Sensitivity Testing

The negative controls may be set up in a separate injection from the samples, and injected using “high” run parameters so that they only need to be run once.

For ID31, samples with less than 20 pg ampmed may be injected high immediately to reduce the number of reruns necessary.

For ID28, samples with less than 200 pg ampmed may be injected at rerun parameters immediately as well.

Allelic Ladders and Positive Controls will occupy the first, second, ninth and tenth wells of each injection. It is mandatory that there be a ladder and Positive Control included with each injection set for Identifiler.

1. In the “Sys.” column, confirm that the appropriate letter for the correct run or rerun **module code** is present:

Table 5: Identifiler Injection Parameters for the High Sensitivity Testing

Amplification Cycle	Specification	Run Module Code	Parameters
Identifiler 31	Low	L	1 kV for 22 sec
	Normal	N	3 kV for 20 sec
	High	H	6 kV for 30 sec
Identifiler 28	Normal	I	1 kV for 22 sec
	High	IR	5 kV for 20 sec

2. Proofread documentation, make corrections and re-save as necessary.

IMPORTANT: Remember that all names must consist of letters, numbers, and only the following characters: - _ . () { } [] + ^ (no spaces).

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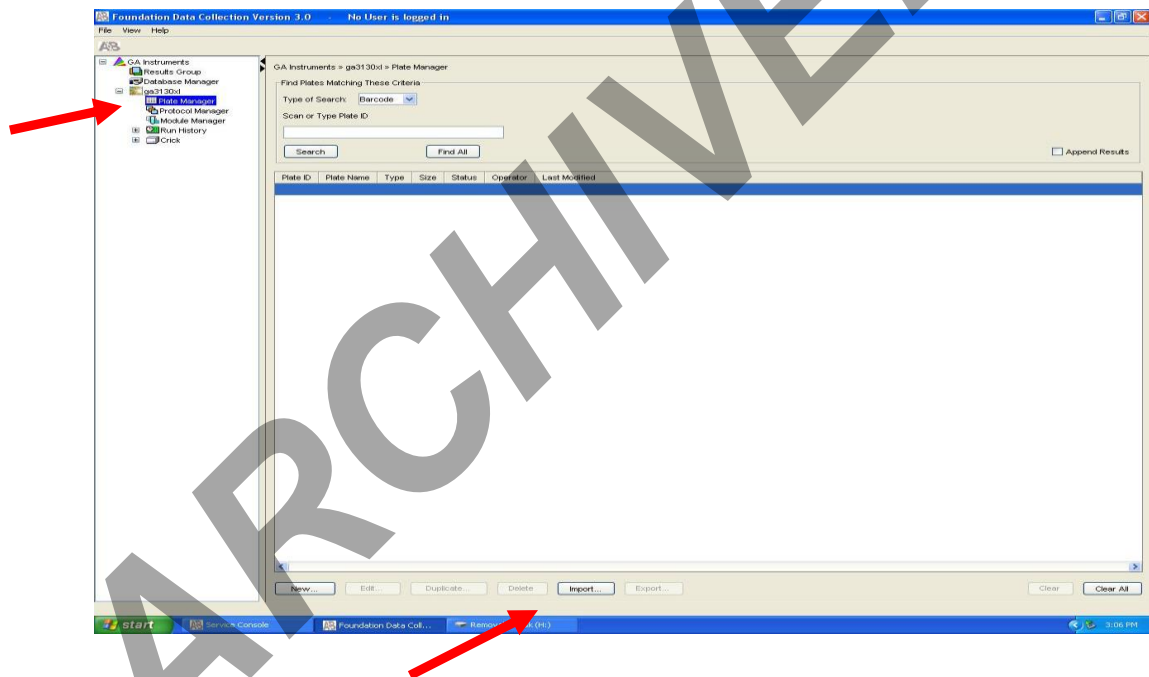
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C. Foundation Data Collection (Importing Plate Record)

1. Maximize the Foundation Data Collection window.
2. Click + to expand subfolders in the left tree pane of “ga 3130xl”.
3. Click on “Plate Manager”.
4. In the Plate Manager window click on “Import...”



5. Browse for the plate record in **D:\AppliedBiosystems\Plate Records**. Double click on the file or highlight it and click **Open**.
6. A window will prompt the user that the plate record was successfully imported. Click **OK**.

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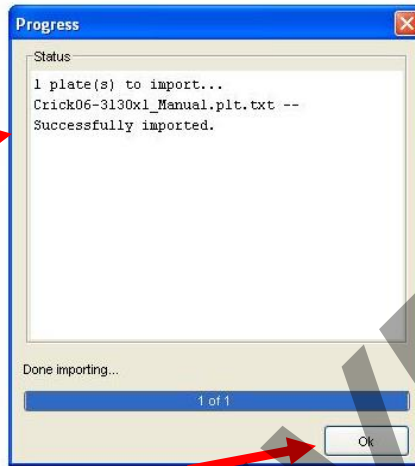
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If the Plate Record will not import, a window will prompt the user where changes are needed. Go back to edit the documentation and resave the corrected Plate Record and Sample Sheet with the same file name.

D. Preparing and Running the DNA Samples

1. Retrieve amplified samples from the thermal cycler or refrigerator. If needed, retrieve a passing positive control from a previous passing run.
2. If condensation is seen in the caps of the tubes, centrifuge tubes briefly.

Mastermix and Sample Addition for High Copy DNA Testing:

1. Arrange amplified samples in a 96-well rack according to how they will be loaded into the 96-well reaction plate. Sample order is as follows: A1, B1, C1...G1, H1, A2, B2, C2...G2, H2, A3, B3, etc. Thus the plate is loaded in a columnar manner where the first injection corresponds to wells A1-H2, the second A3-H4 and so on.
2. Have another analyst **witness** the tube setup by comparing the tube labels and positions indicated on the Load Plate Screen in the LIMS system with the tube labels and positions of the tubes themselves.

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3. Mastermix preparation:
 - a. Prepare one mastermix for all samples, negative and positive controls, and allelic ladders as specified in Table 7.
(26.625 μ L of HIDI + 0.375 μ L of LIZ per sample)

TABLE 7: Identifiler 28

# Samples + 2	HiDi Form (26.6 μ L per sample)	LIZ500 Std (0.375 μ L per sample)
16	480 uL	7 uL
32	906 uL	13 uL
48	1332 uL	19 uL
64	1758 uL	25 uL
80	2184 uL	31 uL
96	2610 uL	37 uL
112	3036 uL	43 uL
128	3462 uL	49 uL

NOTE: HiDi Formamide must not be re-frozen.

- b. Obtain a reaction plate and label the side with a sharpie. Place the plate in an amplification tray or the plate base.
- c. Aliquot **27 μ L** of **mastermix** to each well.
- d. If an injection has less than 16 samples, add at least 12 μ L of either dH₂O, formamide, HiDi, buffer or mastermix to all unused wells within that injection.

Adding Samples:

- a. For sample sets being run at normal parameters: Aliquot **1 μ L** of **allelic ladder**.
For sample sets being run at high parameters: Aliquot **.7 μ L** of **allelic ladder**.

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- b. For sample sets being run at normal parameters: Aliquot **3 μ L** of the **positive control**.
For sample sets being run at high parameters: Aliquot **.5 μ L** of the **positive control** or **1 μ L of a $\frac{1}{2}$ dilution** (2ul positive control in 2ul of water).
- c. Aliquot **3 μ L** of **each sample** and **negative control**.
- d. When adding PCR product, make sure to pipette the solution directly into the mastermix and gently flush the pipette tip up and down a few times to mix it.
- e. Skip to Part E (Denature/Chill) of this section.

Mastermix and Sample Addition for Identifiler 28 for plates that may need to be reinjected under high parameters such as High Sensitivity testing:

1. Arrange amplified samples in a 96-well rack according to how they will be loaded into the 96-well reaction plate. Sample order is as follows: A1, B1, C1...G1, H1, A2, B2, C2...G2, H2, A3, B3, etc. Thus the plate is loaded in a columnar manner where the first injection corresponds to wells A1-H2, the second A3-H4 and so on.
2. Have another analyst **witness** the tube setup by comparing the tube labels and positions indicated on the Load Plate Screen in the LIMS system with the tube labels and positions of the tubes themselves.
3. Obtain a reaction plate and label the side with a sharpie. Place the plate in an amplification tray or the plate base.

NOTE: HiDi Formamide cannot be re-frozen.

Mastermix for 28 Cycles:

- a. Prepare one mastermix for all samples, negative and positive controls, allelic ladders as specified in Table 8
 - i. Add 26.625 μ L of HIDI per sample
 - ii. Add 0.375 μ L of LIZ per sample
 - iii. Aliquot **27 μ L** of **mastermix** to each well

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- b. If an injection has less than 16 samples, add 12ul of either dH₂O, buffer or formamide/LIZ mix to all unused wells within that injection.

Add samples to the plate, adhering to the following guidelines:

NOTE: Multichannel pipettes may be used to load samples. If pipetting from a 96 well PCR plate, pierce the seal.

5. Adding Samples for 28 Cycles:

- a. Aliquot **3 µL** of **each sample** and **negative control** and the **positive control**.
- b. Aliquot **0.5 µL** of **positive control** or **1 µL** of **1/2 dilution** (4 µL positive control in 4uL of water) into the wells labeled “PEH”. This is the positive for the “high” injection parameters.
- c. Aliquot **0.7 µL** of **allelic ladder**. If a full plate will be used, mix 6 µL of ladder with 2.4 µL of water and aliquot 1 µL per ladder well.
- d. Alternatively, **1 µL** and **0.5 µL** of **allelic ladder** can be used for the normal and the rerun parameters for each injection to account for differences in lots of allelic ladder.
 - i. For a full plate, add 3.5 µL of ladder to 3.5 µL of water, mix, and and aliquot 1 µL of this dilution.
 - ii. For a half plate, add 2 µL of ladder to 2 µL of water, mix and aliquot 1 µL of this dilution.
 - iii. A P2 pipet must be used to make 0.7 and 0.5 µL aliquots to avoid making dilutions and to conserve ladder.
- e. Skip to Part E (Denature/Chill) of this section.

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TABLE 8: Identifiler 28 Samples

Injection Parameters	Samples and negs	LIZ	HIDI	Allelic Ladder	Positive Control
I	3 µL	0.375 µL	26.6 µL	1.0 µL or (0.7 µL)*	3 µL
IR	3 µL	0.375 µL	26.6 µL	0.5 µL or (0.7 µL)*	0.5 µL

* Two amounts of allelic ladder, 1 µL and 0.5 µL, may be used for the normal and the rerun parameters to account for differences in lots of ladder rather than 0.7 µL, which is satisfactory for both parameters in most situations.

Mastermix and Sample Addition for Identifiler 31 for High Sensitivity Testing

1. Prepare pooled samples: **IDENTIFILER 31 ONLY**
 - a. Centrifuge all tubes at full speed briefly.
 - b. Label one 0.2 mL PCR tube with the sample name and “abc” to represent the pooled sample injection for the corresponding sample set.
 - c. Take 5 µL of each sample replicate, after mixing by pipetting up and down, and place each aliquot into the “abc” labeled tube.
 - d. Place each pooled sample directly next to the third amplification replicate labeled “c” of each sample set.
2. Arrange amplified samples in a 96-well rack according to how they will be loaded into the 96-well reaction plate. Sample order is as follows: A1, B1, C1..., A2, B2, C2...etc. Thus the plate is loaded in a columnar manner where the first injection corresponds to wells A1-H2, the second A3-H4 and so on.
3. **Witness step.** Have another analyst witness the tube set-up by comparing the tube labels and positions indicated on the Load Plate screen in LIMS with the tube labels and positions of the tubes themselves.
4. Obtain a reaction plate and label the side with a sharpie. Place the plate in an amplification tray or the plate base.

NOTE: HiDi Formamide must not be re-frozen.

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5. Mastermix for 31 CYCLES:

- a. Prepare the following **mastermix** for **samples**, and **negative controls** as specified in Table 8
 - i. 44.6 μL of HIDI per sample
 - ii. 0.375 μL of LIZ per sample
 - iii. Aliquot **45 μL** of **mastermix** to each **sample** and **negative control** well
 - b. Prepare a separate **mastermix** for **allelic ladders** and **positive controls**
 - i. Add 14.6 μL of HIDI to each AL and PE
 - ii. Add 0.375 μL of LIZ per AL and PE
 - iii. Aliquot **15 μL** of **mastermix** to each **Allelic Ladder** and **Positive Control** well
6. If an injection has less than 16 samples, add 12ul of either dH₂O, buffer or formamide/LIZ mix to all unused wells within that injection.

7. Add samples to the plate, adhering to the following guidelines:

NOTE: Multichannel pipettes may be used to load samples. If pipetting from a 96 well PCR plate, pierce the seal.

8. Adding Samples for Identifiler 31:

- a. Aliquot **5 μL** of **each sample (including pooled)** and **negative control**.
- b. Aliquot **1 μL** of a **1/10 dilution** of **positive control** into each well labeled “PE”. (Make the 1/10 dilution by mixing 2 μL of Positive Control with 18 μL water). This is the positive for the “normal” injection parameters.
- c. Aliquot **1 μL** of a **1/20 dilution** of **positive control** into each well labeled “PEH”. (Make the 1/20 dilution by mixing 2 μL of Positive Control with 38 μL water). This is the positive control for the “high” injection parameters.
- d. Aliquot **0.5 μL** of **allelic ladder** into each well labeled “AL”. Alternatively, make a 1/2 dilution of ladder and aliquot 1 μL per “AL” well. Make this dilution by mixing 2 μL ladder with 2 μL of water for 1-2 injections, 3 μL ladder with 3 μL of water for 3-4 injections or 4 μL ladder with 4 μL water for 5-6 injections. This is the allelic ladder for the “normal” injection parameters.
- e. Aliquot **0.3 μL** of **allelic ladder** into each well labeled “ALH”. Alternatively, make a 3/10 dilution of ladder and aliquot 1 μL per “ALH” well. Make this

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dilution by mixing 1 μL of ladder with 2.3 μL of water for 1-2 injections, 2 μL of ladder and 4.6 μL of water for 3-4 injections, or 3 μL of ladder with 6.9 μL water for 5-6 injections. This is the allelic ladder for “high” injection parameters.

TABLE 9: 31 Cycle Samples for High Sensitivity

Injection Parameters	Samples and negs	LIZ for samples and negs	HIDI for samples and negs	Allelic Ladder	Positive Control	LIZ for ALs And PEs	HIDI for ALs And PEs
L	5 μL	0.375 μL	44.6 μL	0.5 μL	1 μL of 1/10 dil	0.375 μL	14.6 μL
N	5 μL	0.375 μL	44.6 μL	0.5 μL	1 μL of 1/10 dil	0.375 μL	14.6 μL
H	5 μL	0.375 μL	44.6 μL	0.3 μL	1 μL of 1/20 dil	0.375 μL	14.6 μL

9. Proceed to Part E (Denature/Chill) in this section.

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E. Denature/Chill - For All Systems After Sample Addition

1. Once all of the samples have been added to the plate, place a new 96-well septa over the reaction plate and firmly press the septa into place.
2. Spin plate in centrifuge at 1000 RPM for one minute.
3. For Denature/Chill:
 - a. 9700 Thermal Cycler
 - i. Place the plate on a 9700 thermal cycler (Make sure to keep the thermal cycler lid off of the sample tray).
 - ii. Select the “denature/chill” program.
 - iii. Make sure the volume is set to 30 μ L for Identifiler 28, and 50 μ L for Identifiler 31. If more than one system is loaded on the same plate, use the higher value.
 - iv. Press **Run** on the thermal cycler. The program will denature samples at 95°C for 5 minutes followed by a chill at 4°C (the plate should be left to chill for at least 5 min).
 - v. While the denature/chill is occurring, the oven may be turned on.
 - b. Heat Block
 - i. Place the plate on a 95°C heat block for 5 minutes.
 - ii. Place the plate on a 4°C heat block for 5 minutes.

F. Turning the Oven on and Setting the Temperature

1. In the tree pane of the Data Collection v3.0 software click on **GA Instrument > ga3130xl > instrument name > Manual Control**
2. Under Manual Control “**Send Defined Command For:**” click on **Oven**.
3. Under “**Command Name**” click on “**Turn On/Off oven**”.
4. Click on the “**Send Command**” button.

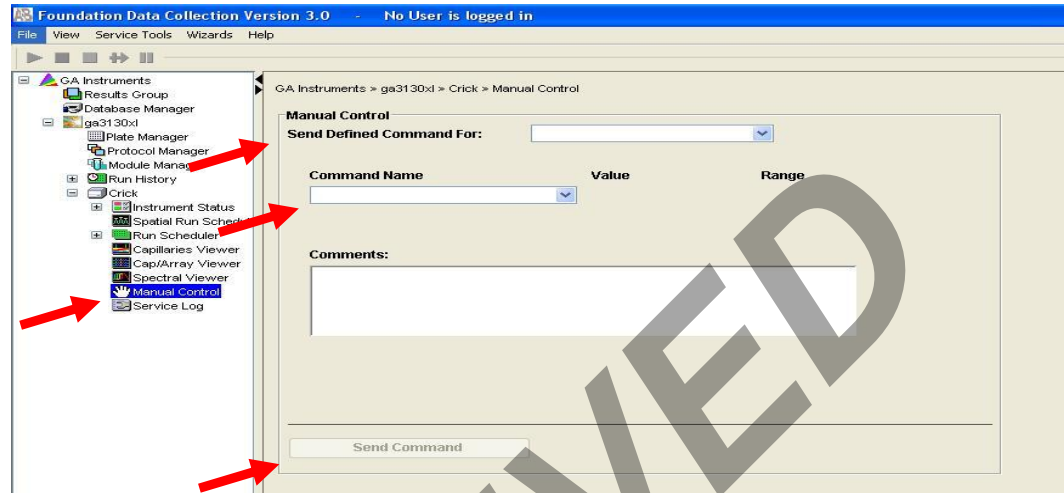
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5. Under “**Command Name**” click on “**Set oven temperature**” and Under “**Value**” set it to **60**.
6. Click on the “**Send Command**” button.
7. Once denatured, spin the plate in centrifuge at 1000 RPM for one minute before placing the reaction plate into the plate base. Secure the plate base and reaction plate with the plate retainer.

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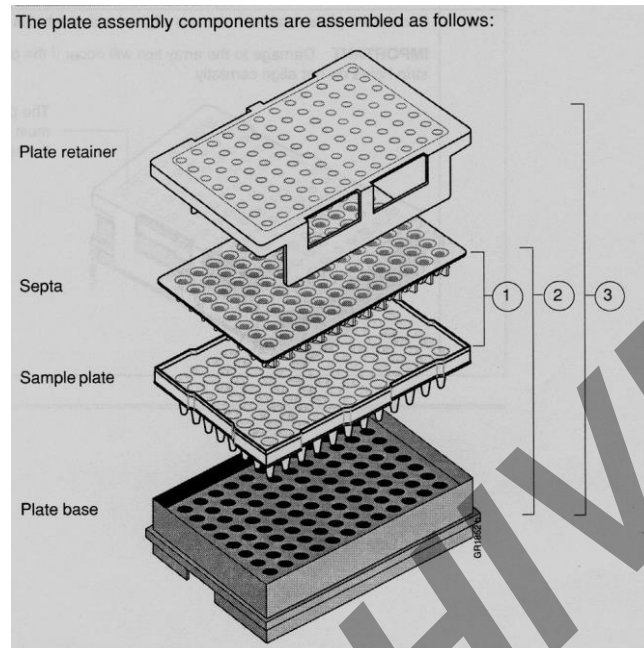
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G. Placing the Plate onto the Autosampler (Linking and Unlinking Plate)

1. In the tree pane of the Foundation Data Collection v3.0 software click on **GA Instrument > ga3130xl > instrument name > Run Scheduler > Plate View**
2. Push the tray button on the bottom left of the machine and wait for the autosampler to move forward and stop at the forward position.
3. Open the doors and place the tray onto the autosampler in the correct tray position, A or B. There is only one orientation for the plate. (The notched end faces away from the user.)
4. Ensure the plate assembly fits flat in the autosampler.

When the plate is correctly positioned, the plate position indicator on the **Plate View** window changes from gray to yellow. Close the instrument doors and allow the autosampler to move back to the home position.

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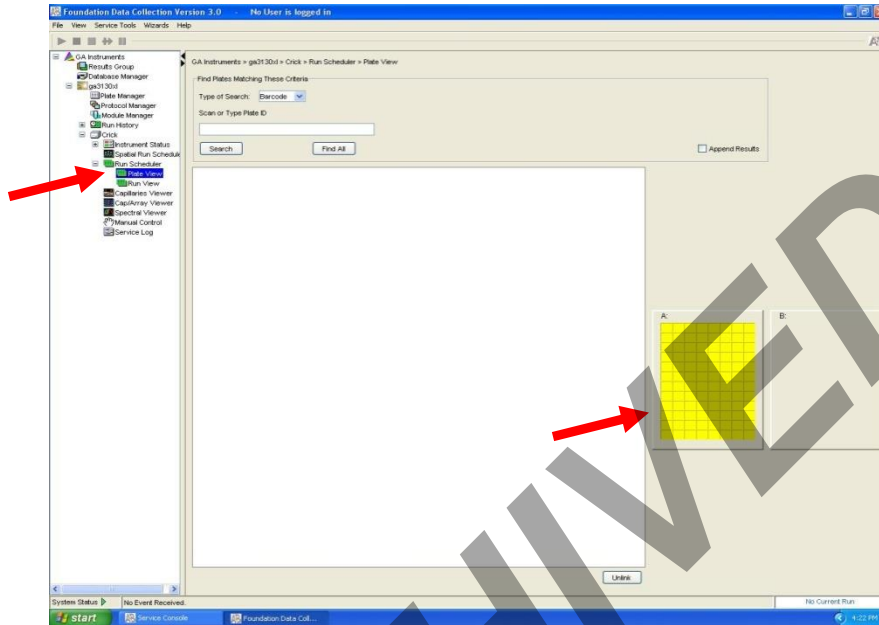
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Linking/Unlinking the Plate Record to Plate

5. Type the exact plate name in the Plate ID window and click **“Search.”** Or, click the **“Find All”** button and select the desired plate record.

NOTE: If the plate name is not typed in correctly, your plate will not be found. Instead, a prompt to create a new plate will appear. Click **“No”** and retype the plate name correctly.

Click the plate position indicator corresponding to the plate position in the instrument. The plate position (A or B) displays in the link column.

If two plates are being run, the order in which they are run is based on the order in which the plates were linked.

6. The plate position indicator changes from yellow to green when linked correctly and the green run button becomes active.

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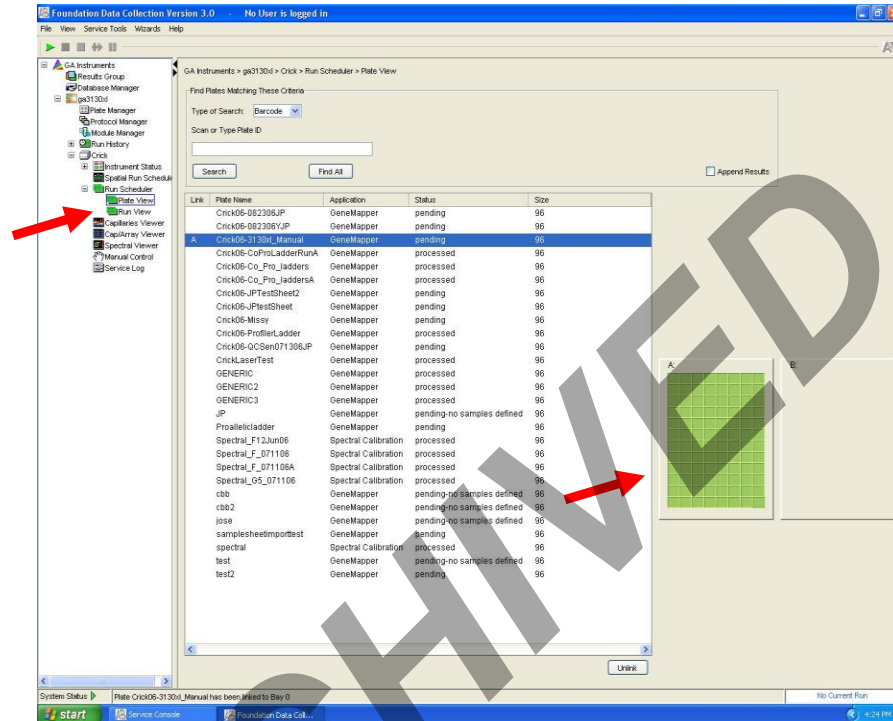
FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

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7. To unlink a plate record just click the plate record to be unlinked and click “Unlink”.

H. Viewing the Run Schedule

1. In the tree pane of the Foundation Data Collection software, click **GA Instruments** > **ga3130xl** > **instrument name** > **Run Scheduler** > **Run View**.
2. The **RunID** column indicates the folder number(s) associated with each injection (e.g. Run_Einstein_2011-03-10-0018 or Run_Venus_2006-07-13_0018-0019). Note: This RunID may not be indicative of the Run Collection folder depending on results group used.
3. Click on the run file to see the Plate Map or grid diagram of the plate on the right. Check if the blue highlighted boxes correspond to the correct placement of the samples in the injections.

NOTE: Before starting a run, check for air bubbles in the polymer blocks. If present, click on the **Wizards** tool box on the top and select “**Bubble Remove**”

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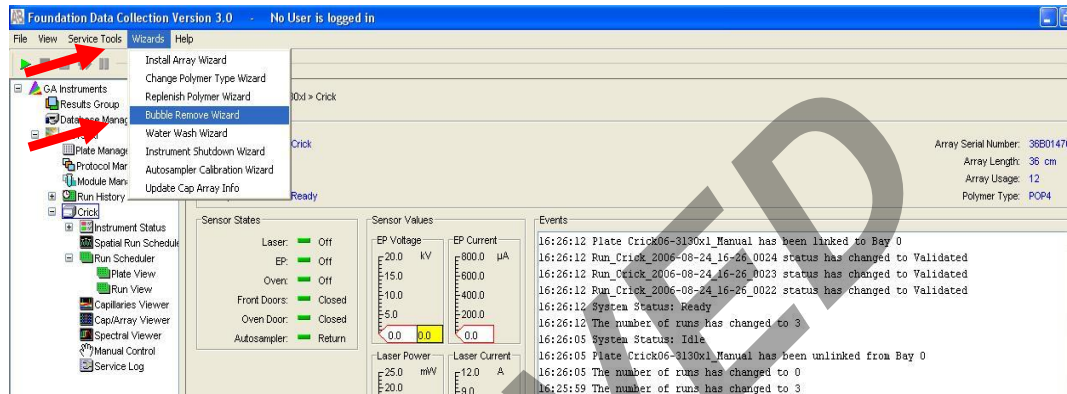
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Wizard". Follow the wizard until all bubbles are removed.



4. Click on green **Run** button in the tool bar when you are ready to start the run. When the **Processing Plate** dialog box opens (You are about to start processing plates...), click **OK**.
5. To check the progress of a run, click on the **Capillary Viewer** or **Cap/Array Viewer** in the tree pane of the Foundation Data Collection software. The **Capillary Viewer** will show you the raw data of the capillaries you select to view whereas the **Cap/Array Viewer** will show the raw data of all 16 capillaries at once.

IMPORTANT: Always exit from the **Capillary Viewer** and **Cap/Array Viewer** windows. During a run, do not leave these pages open for extended periods. Leave the **Instrument Status** window open.

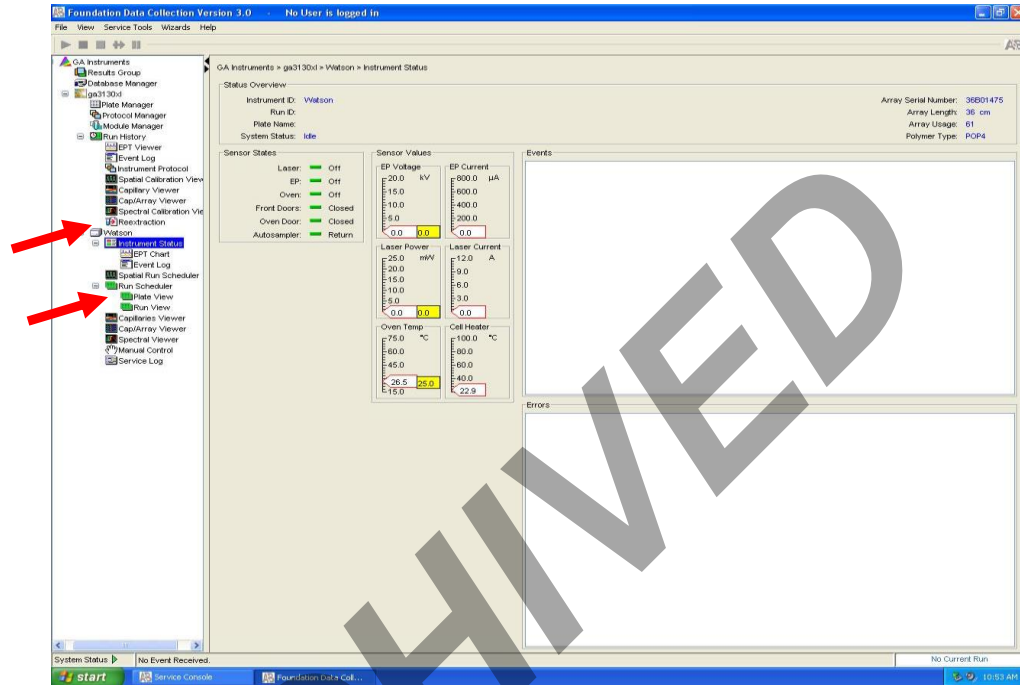
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The visible settings should be: EP voltage 15kV

EP current (no set value)

Laser Power Prerun 15 mW

Laser Power During run 15mW

Laser Current (no set value)

Oven temperature 60°C

Expected values are:

EP current constant around 120 to 160µA

Laser current: 5.0A ± 1.0

It is good practice to monitor the initial injections in order to detect problems.

Table 11

	I/L	IR	N	H
Oven Temp	60°C	60°C	60°C	60°C
Pre-Run Voltage	15.0 kV	15.0 kV	15.0 kV	15.0 kV
Pre-Run Time	180 sec	180 sec	180 sec	180 sec
Injection Voltage	1 kV	5 kV	3 kV	6 kV
Injection Time	22 sec	20 sec	20 sec	30 sec
Run Voltage	15 kV	15 kV	15 kV	15 kV

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Run Time	1500 sec	1500 sec	1500 sec	1500 sec
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Table 12

	M	MR
Oven Temp	60°C	60°C
Pre-Run Voltage	15.0 kV	15.0 kV
Pre-Run Time	180 sec	180 sec
Injection Voltage	3 kV	3 kV
Injection Time	10 sec	20 sec
Run Voltage	15 kV	15 kV
Run Time	1500 sec	1500 sec

I. Collecting Data

When a run is complete, it will automatically be placed in **D:/AppliedBio/Current Runs** folder, labeled with either the *plate name-date* (e.g. Einstein11-025ID-015PPY-2011-03-11) or *instrument name, date and runID* (e.g. Run_Venus_2006-07-13_0018). Proceed to Analysis section of this manual.

J. Re-injecting Plates

1. Plates should be re-injected as soon as possible, preferably the same day.
2. Create a new test batch and plate record using the original documentation as a guide. Select only those samples that need to be rerun by re-assigning "Sys". For example, assign "IR" for an ID28 sample that needs to be re-run high.

NOTE: See Section 7 for information on which controls need to be run.

3. Follow the instructions for creating a test batch. Re-import the plate record.
4. Re-denature/chill the plate (if needed) as described in Part E. If a plate is being re-injected the same day on which it was originally run, it does not require an additional denature/chill step before being rerun.

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K. Water Wash and POP Change

Refer to Section A for schematic of 3130xl while proceeding with the water wash and POP change procedure.

1. Remove a new bottle of POP4 from the refrigerator.
2. Select **Wizards > Water Wash Wizard** and follow the wizard.
3. When the “Fill Array” step has completed, remove the anode buffer jar, empty, and fill with 1x TBE Buffer (~15 mL).
4. Close instrument doors and wait for the steady green light.
5. Click “Finish.”

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TROUBLESHOOTING GUIDE

This section is provided as a guide. The decision on which of the recommended actions is the most promising should be made after consultation with a supervisor.

PROBLEM: Many artifacts in sample.

Possible Cause	Recommended Action
Secondary structure present. Sample not denatured properly.	Clean pump block and change polymer to refresh the urea environment. Denature/chill samples.

PROBLEM: Decreasing peak heights in all samples.

Possible Cause	Recommended Action
Poor quality formamide or sample environment very ionic.	Realiquot samples with fresh HID1.

PROBLEM: Individual injections run at varying speeds. For example, the scan number where the 100 bp size standard appears differs significantly from one injection to another, usually appearing earlier.

Possible Cause	Recommended Action
Warm laboratory temperatures.	Redefine size standard. If this fails, reinject.

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PROBLEM: Loss of resolution of peaks.

Possible Cause	Recommended Action
Loss of resolution of peaks.	Clean pump block and change polymer to refresh the urea environment. Denature chill samples.

PROBLEM: An off ladder peak appears to be a pull up, but it is not exactly the same basepair as the true peak.

Possible Cause	Recommended Action
Matrix over-subtraction. Usually in the green channel, the true peak is overblown and is split. Pull up peaks appear in the blue and the red channels. In the yellow channel, there is a negative peak at the base pairs of the true peak, however immediately to the right and to the left are off ladder peaks.	Remove off ladder peaks as matrix over-subtraction.

PROBLEM: Peaks overblown and running as off ladder alleles.

Possible Cause	Recommended Action
More than the optimum amount of sample amplified.	Rerun samples at lower injection parameters. Or rerun samples with less DNA.

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PROBLEM: Pull up peaks.

Possible Cause	Recommended Action
Colors bleeding into other colors.	Run a spectral.

PROBLEM: Spikes in the electropherogram.

Possible Cause	Recommended Action
Crystals in the polymer solution due to the polymer warming and congealing from fluctuations in the room temperature.	Change the polymer.

PROBLEM: Spikes in electropherogram and artifacts.

Possible Cause	Recommended Action
Arcing: water around the buffer chambers.	Clean chambers; beware of drops accumulating around the septa.

PROBLEM: Split peaks.

Possible Cause	Recommended Action
Lower pump block is in the process of burning out due to the formation of a bubble.	Clean the block.

PROBLEM: Increasing number of spurious alleles.

Possible Cause	Recommended Action
Extraneous DNA in reagents, consumables, or instrument.	Stop laboratory work under advisement of technical leader. Implement a major laboratory clean-up.

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GENERAL PROBLEMS

Problems	Recommended Action
Fatal Errors.	Close collection software. Restart collection software.
3130xl not cooperating. Shutter problems.	Restart Computer and Instrument. Call Service.

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