

Alaska Scientific Crime Detection Laboratory
Forensic Biology Database Procedures Manual

Issued: 9/3/2013
Effective: 9/3/2013

Version: FBDB 2013 R0
Status: Archived

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Section 1 QIASymphony SP DNA Extraction

References:

- QIASymphony® SP/AS User Manual - Operating the QIASymphony SP, Software version 3.5, 10/2010.
- QIASymphony® SP/AS User Manual - General Description, 10/2010.
- QIASymphony® DNA Investigator Handbook, 10/2008.

1.1 Sample Preparation and Digestion

- Place Buffer ATL in a 56°C incubator until all precipitates dissolve. (May require occasional gentle agitation.)
- Prepare a master mix of Buffer ATL and Proteinase K as follows:
 - Obtain a 50ml conical vial
 - Pipette the following into the vial:
 - (Number of samples + 6) x 475µl Buffer ATL
 - (Number of samples + 6) x 25µl Proteinase K
- Prepare sample tubes by labeling ninety-six 1.5mL tubes.

Note: Sample tubes 1, 25, 49, & 73 will be used as place holders for the allelic ladder, DO NOT place samples in these tubes.

- Aliquot 500µl of the Buffer ATL / Proteinase K master mix into each of the labeled 1.5mL sample tubes.
- For serrated buccal swabs, cut a portion of the swab into the appropriate tube. For cotton swabs, cut a portion of the swab tip into the appropriate tube. If two swabs were collected, the entire cotton tip of one swab may be snapped off and used. Dried blood stain cards are sampled with a 3mm hole punch (1-3 punches). The hole punch is cleaned by punching a clean piece of filter paper a few times.
- Each batch of extractions must include a minimum of five randomly placed internal control samples that have been previously typed.
- Vortex the samples for at least 10 seconds.
- Incubate the samples at 56°C (the acceptable range is 50°C - 60°C) for a minimum of 60 minutes or overnight.
- Centrifuge the samples briefly to remove any condensation from the lid.
- Transfer 200µl (or as much as possible up to 200µl) of the liquid from the sample tube into a new 2.0mL Qiagen tube.
- Place the tube in the appropriate slot of the tube carrier destined for the QIASymphony.

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1.2 DNA Isolation and Purification

- Ensure that the power for the QIASymphony has been switched on. The power switch can be located on the left front of the instrument and is designated as a blue **ⓧ** button. The initialization of the instrument will take a few minutes.
- Using the touchscreen, login to the QIASymphony. Select “**AK Crime Lab**” and enter the password “**anchorage1**”. Press “**OK**”.
- In order to facilitate setting up a run, the QIASymphony SP wizard is a step-by-step guide to setting up a run. The wizard will take you through:
 - Loading the “Waste” drawer
 - Loading the “Eluate” drawer
 - Loading the “Reagent & Consumables” drawer
 - Loading the “Sample” drawer
 - Defining a batch/run

Note: It is possible to set up a run on the QIASymphony without the wizard.

- Press “**Wizard**” on the right hand of the “Sample Preparation” screen for step-by-step instructions.
- The “Wizard/ACS and Number of Samples” screen appears.

Note: When this screen is open, all drawers are locked. An error message will appear when “Wizard” is pressed in the following situations:

- If a plate carrier is already loaded in the “Sample” drawer
- If a plate carrier has not been loaded, but there are batches that are already defined to be loaded using the plate carrier
- 4 batches have already been queued

1.2.1 Wizard/ACS and Number of Samples

- Under “Available assay control sets” press “**Investigator**” to view options.
Select: **REF**
200 1.5mL Tubes
- Press “→” to move assay over to the Selected “assay control sets/number of samples”.
- Use the “+” and “-” buttons to adjust the number of samples that will be processed. (89 samples, 4 blanks for allelic ladders, and 1 reagent blank for a total of 94 samples. Positive and negative amplification controls will be added at the amplification step.) Alternatively, press directly on the number and adjust the number using the virtual keypad. Press “**OK**”.
- Press “**Next**” to continue to “Load Waste Drawer”.

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Option: Continuous Sample Loading

Alternatively to preparing and loading all 94 samples at once, samples can be loaded and batches queued in sets of 24. One run is processed after the other. Continuous loading is possible for up to 96 samples, provided that the consumables drawer is fully loaded before commencing the first set of samples.

Note: Before starting a run with continuous loading, ensure the “Reagent Drawer” contains all the reagents and consumables necessary to run a full set of 94 samples.

To set up the batch, start the **Wizard** and continue with batch setup as described in Section 1.2. Under **Wizard/ACS and Number of Samples** screen enter the number of samples you wish to initially load (i.e. initially, if you will only be loading one tube carrier, enter 24. The remaining tube carriers will be loaded and assigned when they are ready to go on the instrument).

The Wizard screens for loading the **Reagents and Consumables** drawer will be skipped. Continue sample loading and assigning assay control sets as described in Section 1.2.8 **Wizard/Load Sample Tubes**.

1.2.2 Wizard/Load Waste Drawer

The “Wizard/Load Waste Drawer” screen summarizes the items that need to be loaded into the “Waste” drawer.

Note: When the “Wizard/Load Waste Drawer” screen is open, it is only possible to open the “Waste” drawer. All other drawers are locked.

- Open the “Waste” drawer.
- Load “Waste” drawer as shown on the touch screen.
 - Ensure the liquid waste container on the right side of the drawer has ample room for liquid waste. If necessary, empty and dispose of according to laboratory guidelines.
 - Ensure tip disposal chute is attached and that there is ample room in the tip disposal bag in the container located under the QIASymphony in the cabinet.
 - Insert partial or empty unit boxes into all four slots ensuring there is an empty unit box in slot 4 (slot closest to you).

Note: If the unit box contains a spacer, make sure to remove this. Do not empty partially filled unit boxes. Partially filled unit boxes will be detected during the inventory scan and can be used until they are full. It is recommended to move any partial boxes to the slot closest to the rear of the instrument. An empty unit box must be placed into

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slot 4. During initialization the handler goes down into the unit box in position 4. If it is not empty, the handler will crash.

- After loading the “Waste” drawer, close the drawer and press “**Next**”.
- The QIASymphony will now do an inventory scan of the “Waste” drawer.
- When the inventory scan is complete, the “Wizard/Elution Slot/Configure Racks” screen appears.

1.2.3 Wizard/Elution Slot/Configure Racks

- Open the “Eluate” drawer.
- Highlight “Slot 1”
- Under “Available rack types” on the right of the screen highlight “Deep Well” and scroll down to
QIA#19585
***S-Block96**
Press to select.
- On the right of the screen select “**EDIT ID**”. Type in batch name i.e. DB12-1030KAH. Press “**OK**”.
- Place the S-Block deep well plate onto the metal transfer bracket in “slot 1” with well A1 in the upper left corner.
- Close the “Eluate” drawer and press “**Next**”. The QIASymphony will now perform an inventory scan of the “Eluate” drawer.

1.2.4 Wizard/Load Reagents

- Prepare the reagents:
 - Ensure the magnetic particles are fully re-suspended by removing the magnetic-particle trough from the reagent cartridge frame and vortex vigorously for at least 3 minutes. Replace the magnetic particles in the reagent cartridge frame.
 - Place the reagent cartridge into the grey holder. **Note:** cRNA is not utilized for the Database protocol and does not need to be added to the reagent cartridge holder.
 - Before using a reagent cartridge for the first time, place the piercing lid on top of the reagent cartridge so that the side with the opening fits against the magnetic particle trough. Gently push the piercing lid downward until it presses into place. **Note:** The piercing lid is sharp and care must be taken to ensure the lid is placed onto the reagent cartridge in the correct orientation.
 - **REMOVE THE MAGNETIC-PARTICLE TROUGH FOIL.**
- Open the “Reagents and Consumables” drawer.

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- Load the reagents by sliding the reagent holder with magnetic beads facing out towards analyst into rear position first. You may add a second set of reagents if necessary.
- Press “**Next**” to continue to the “Wizard/Load Consumables” screen.

1.2.5 Wizard/Load Consumables

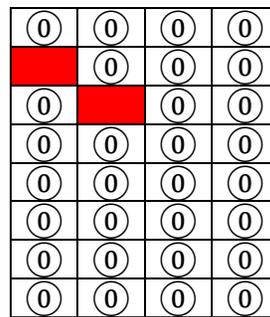
- Uncap and load at least 60 sample prep cartridges in the last three slots as shown on the screen. If there is a partial unit box, ensure it is loaded closest to the rear of the instrument. **Note:** Make sure that the sample prep cartridges are seated properly in the unit box and are not jammed.
- Uncap and load a full box of twelve 8-Rod covers in the slot closest to you.

Note: Do not refill partially used unit boxes. The number of sample prep cartridges or 8-Rod Covers is detected during the inventory scan. Additionally, do not throw empty unit boxes away. Empty unit boxes will be used in the “Waste” drawer for collection of used sample prep cartridges and 8-Rod covers.

- Ensure there are enough tips available (8 blue racks and 10 black racks recommended) and reload if necessary. **Note:** it is ok if tips are missing within a tip rack as long as either the top left or the bottom right tip is missing. Do not refill partially used tip racks.



OK



Not OK

- Close the “Reagents and Consumables” drawer and press “**Next**”. The instrument will perform an inventory scan of the reagents and consumables drawer. **Note:** This scan will take approximately 5 – 10 minutes.

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1.2.6 Wizard/Sample Loading Summary

This screen summarizes the number of samples and internal controls that are required for each sample rack. Press **“Next”** to continue to the “Wizard/Select Sample Carrier” screen.

1.2.7 Wizard/Select Sample Carrier

- In this screen select **“Use tube carrier”** to load samples using the tube carrier.
- Press **“Next”** to continue to the “Wizard/Load Sample Tubes” screen.

1.2.8 Wizard/Load Sample Tubes

This screen indicates which samples should be loaded into which sample slots.

- Open “Sample” drawer by pulling the door toward you.
- Five slots are available. The first four slots can accommodate tube carriers containing sample tubes; the fifth slot (slot A) accommodates a tube carrier containing internal control and is not used for the database protocol.
- Each slot is marked with a line. The status of each slot is shown by LEDs. The LEDs may be illuminated in green, orange, or red.
 - Green – slot is free and ready for loading.
 - Orange – tube carrier is loaded.
 - Red – slot is currently locked.
- Gently load the carrier in the slot illuminated in green to the solid line and wait for the bar code reader to move forward. Once the bar code reader is in position, the slot unlocks and the green LED starts to flash. Slide the carrier into the QIASymphony.
- Upon successful loading, the sample slot on the screen will change from orange to green, loaded will change from no to yes, and the slot illumination will change to orange.
- Press **“Next”** to continue to the “Wizard/Batch X/Define Samples” screen.

Note: Additional tube carriers will be loaded and assigned later.

1.2.9 Wizard/Batch X/Define Samples

Note: All tubes will be assigned a sample ID including the blanks. There is no need to change this to align with the database worksheets.

- Press **“Next”** to continue with the batch definition process and the “Wizard/Batch X/Select Assay Control Sets” screen.

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1.2.10 Wizard/Batch X/Select Assay Control Sets

To process samples, Assay Control Sets must be assigned.

- Press “**Select All**” to highlight all the samples in the tube carrier.
- Under “Application/ACS” under “Investigator” select:
REF
200 1.5mL Tubes
- Press “**Next**” to continue with the “Wizard/Elution Slot & Volume” screen.

1.2.11 Wizard/Elution Slot & Volume

- Select the elution volume by selecting “**200**”.
- Select the appropriate slot to assign to the batch.
- Next to Batch you will now see a “①” showing that the first set of samples has been assigned to the batch.
- Select “**Queue**”.

Repeat the above steps beginning with the “Wizard/Load Sample Tubes” to load samples and assign Assay Control Sets to the remaining 3 tube carriers.

- Verify that you have “①②③④” next to Batch.
- Press “**Finish**”. As soon as a batch is queued, the “Run” button appears.
- Close the “Sample” door.
- Press “**Run**” to start the QAsymphony.
- Run will take approximately 3 hours.

Note: Reference the QAsymphony® SP Recovery Procedure for DNA Applications in the event the QAsymphony protocol is interrupted or aborted during sample processing.

Note: For troubleshooting, reference section 10 in the QAsymphony® SP/AS User Manual General Description for further information.

1.3 End of Batch Processing, Unloading the QAsymphony SP

1.3.1 Unloading the “Eluate” Drawer

- Open the “Eluate” drawer.
- The “Eluate Drawer/Elution Slot” screen appears.
- Select the elution slot from which the elution rack will be removed.
- The “Eluate Drawer/Elution Slot/Change Rack X” screen appears.

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- Press the **“Remove”** button in the **“Configure”** tab to remove the elution rack. A message appears asking whether you want to remove the elution rack from the selected slot. Press **“Yes”** to continue.
- Remove the S-Block containing the eluates from the elution slot/metal transfer bracket. Cover with a plastic seal and store at 4°C or proceed to amplification preparation using Qiagen’s QIAgility.
- Close the **“Eluate”** drawer.
- The **“Eluate Drawer/Elution Slot/Configure Rack X”** screen appears.
- Press the **“OK”** button.
- The QIASymphony SP performs an inventory scan of the **“Eluate”** drawer. Afterwards the **“Sample Preparation/Overview”** screen is displayed.

1.3.2 Unloading Reagents and Consumables

- Remove the reagent cartridge by opening the **“Reagents & Consumables”** drawer and sliding the reagent cartridge out. Reseal the troughs using the reuse seal strips provided in the QIASymphony kit. Remove the reagent cartridge from the holder and discard the reagent cartridge.
- Remove empty tip racks.
- Remove empty unit boxes left in the **“Reagents and Consumables”** drawer and save for collection of used sample prep cartridges and 8-Rod Covers in the **“Waste”** drawer.
- Close the **“Reagents & Consumables”** drawer.
- An inventory scan will be performed prior to starting a new run; therefore, it is not necessary to do a scan of the **“Reagents & Consumables”** drawer.

1.3.3 Unloading Samples

- Press the **“S”** button at the bottom of the touchscreen. The **“Sample Preparation/Define Sample Rack Type”** screen appears. Select the sample slot of the rack to be removed. Press the **“Remove”** button. The sample rack is removed from inventory and can now be removed from the **“Sample”** drawer.

1.3.4 Unloading Waste

- Empty the liquid waste container if necessary according to laboratory guidelines.
- Dispose of any unit boxes that are full.
- Dispose of tip waste if necessary.

1.4 Maintenance

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Daily maintenance is performed at the end of each day the instrument is in use and is described below. Additional maintenance procedures are detailed in the Forensic Biology General Lab Maintenance manual. All maintenance is recorded on the QIASymphony SP Maintenance Log provided at the back of Forensic Biology General Lab Maintenance manual.

1.4.1 Daily Maintenance Procedure

After performing the last run of the day, perform the daily maintenance procedure, described below.

- Remove all removable objects (plate carriers, adapters, inserts, liquid waste station, tip park station, tip disposal chute, liquid waste bottle, waste bag holder, reagent box holder) from the drawers.
- Wipe the drawers, the removed objects, and the lysis station with ethanol. Then wipe with a cloth moistened with water and dry with paper towels. Return the objects to the drawers.

Note: There are spikes below the piercing device in the “Reagents and Consumables” drawer that ensure that the reagent cartridge is correctly positioned. Take care when cleaning the “Reagents and Consumables” drawer.

- Clean the robotic gripper
 - Wipe the robotic gripper with a lint-free cloth moistened with ethanol.
Important: Only wipe the weight. Do not wipe the rods otherwise the ball mechanism may become jammed.
 - Wipe with a lint-free cloth moistened with water and dry with paper towels.
- Clean the pipetting system tip guards
 - Open the “**Main Menu**” and press “**Maintenance SP**”.
 - Move the robotic arm to the cleaning position by pressing “**Tip guards**”.
 - Remove all 4 tip guards by pushing each tip guard upward until it clicks out of place and can be removed.
 - Rinse with ethanol.
 - Rinse with water and wipe dry with paper towels.
- Check the magnetic-head guards. Clean if necessary:
 - Open the “**Maintenance SP**” menu and run the service protocol “**Magnetic head guards**”.
 - Gently raise the catches to release the magnetic-head guards.
 - Wipe the magnetic-head guards with ethanol.
 - Wipe with a lint-free cloth moistened with water and wipe dry with paper towels. Replace the magnetic head guards.
- Open the “**Maintenance SP**” menu and run the service protocol “**Open Magnetic head guards**”.

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- Empty Liquid waste container.
 - Remove the liquid waste container from the “Waste” drawer.
 - Empty the liquid waste container according to laboratory guidelines.
 - Rinse the liquid waste container with ethanol.
 - Rinse the liquid waste container with deionized water.
 - Replace the liquid waste container in the “Waste” drawer.
- UV decontamination of the worktable
 - Before starting the UV irradiation procedure ensure that all samples, eluates, reagents, and consumables have been removed from the worktable. Close all drawers and the hood.
 - Enter the “Maintenance” Screen. Press “**Main Menu**” then press “**Maintenance SP**” in the “Main Menu” screen.
 - To start the UV cleanup procedure, press the “**Start UV light**” button. Enter the duration of the decontamination in minutes (minimum one hour).
 - A message appears asking you to check whether all plastic ware and consumables have been removed from the worktable. Press “**OK**” to start the UV irradiation procedure. Confirm that all removable objects have been removed from the worktable by pressing “**OK**”. The UV lamp then starts and the robotic arm sweeps over the worktable surface for the set irradiation time.

Note: To stop the UV irradiation procedure before the defined period of time has elapsed, press “**Cancel**”. The procedure will stop as soon as the robotic arm completes the current movement.

- Document completion of daily maintenance on the QIASymphony® SP Maintenance Log.
- You may now switch off the QIASymphony SP instrument.

1.5 QIASymphony Recovery Procedure

References:

- *QIASymphony® SP/AS User Manual - Operating the QIASymphony SP, Software version 3.5, 10/2010.*
- *QIASymphony® SP/AS User Manual - General Description, 10/2010.*
- *QIASymphony® DNA Investigator Handbook, 10/2008.*
- *QIASymphony SP recovery procedure for DNA investigator applications, 10/2010.*

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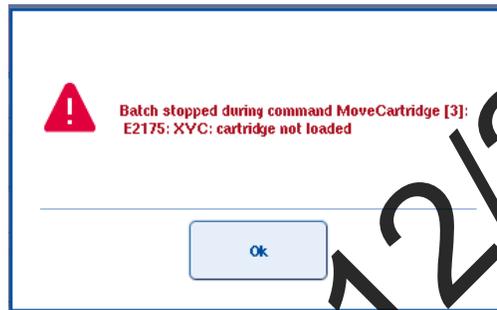
Issued: 9/3/2013
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The purpose of this procedure is to provide a method of recovery for total genomic DNA from database samples and/or reference samples that have encountered an error and the batch processing has stopped during extraction utilizing the QIASymphony SP.

1.5.1 General Recovery Procedures

- When an error occurs, a box similar to the one below should appear on the screen.



- Note and record the error message, specifically the [#] and the E####.
- Based on the [#], use the following procedure to determine how to remove and recover the samples.
- After all samples are recovered and before a run can resume, the QIASymphony must be restarted.

Note: If no error code is given, or the error code is “No Specific Error Code”, then process the samples based on the scenarios listed below under “Command Error Code and Recovery Procedure.”

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1.5.2 Automatic Cleanup

- Cleanup must be run immediately after a crash or error; you cannot continue a run without running cleanup. After a cleanup finishes and samples are removed, the machine must be restarted and all reagents, samples, and trash must be rescanned. If the instrument lost power during a run, cleanup will not work because the instrument has been powered off. In this situation, manual cleanup must be performed.
- Open the "Maintenance" menu and select "Automatic Cleanup". A message will pop up confirming that a forensic protocol had been run. Select "Yes". Follow the prompts regarding the locations of items on the tray and conveyer belt.
- After the automatic cleanup finishes, a new window pops up and asks which forensic recovery protocol to run. Based on the error codes or scenarios listed below under "Command Error Code and Recovery Procedure", select the appropriate recovery method.

1.5.3 Command Error Code and Recovery Procedure

For instruction on how to transfer samples back to their original tubes refer to the section "Returning samples to their original tubes" below.

- [1-13] *Samples have not been pipetted and are in the sample tubes.*
- [14] *Some samples have not been pipetted and some samples have been transferred to the sample prep cartridge.*
 - Remove the sample prep cartridge from the left side of the magnetic head and transfer the samples from the sample prep cartridge back to their original tubes.
- [15] *All samples have been pipetted.*
 - Remove the sample prep cartridge from the left side of the magnetic head and transfer the samples from the sample prep cartridge back to their original tubes.
- [16] *Samples are in prep cartridges and are either accessible or under the magnetic head.*
 - Remove any accessible sample prep cartridges from the left side of the magnetic head.
 - If any sample prep cartridges are under the magnetic head, perform "Recovery Forensic 3", to access the cartridges and then remove them.
 - Transfer samples back to their original tubes.

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- *[17-55] Samples are on rod covers and a sample prep cartridge is available below them.*
 - Remove the sample prep cartridges from the left/right side of the magnetic head.
 - Perform “Recovery Forensic 1”, to eject the 8-Rod Cover into the sample prep cartridges.
 - Remove the 8-Rod Cover/sample prep cartridge assembly from the left side of the magnetic head.
 - Transfer the sample back to their original tubes.
- *[56] Cartridges on the right side are accessible, but some cartridges are still under the magnetic head.*
 - Remove all accessible sample prep cartridges from the right side of the magnetic head and save them for manual processing of the samples.
 - If any sample prep cartridges are under the magnetic head, perform “Recovery Forensic 3”, to access the cartridges and then remove them.
 - Transfer samples back to their original tubes.
- *[57] Sample prep cartridges are accessible and contain the eluates.*
 - Remove the accessible sample prep cartridges from the right side of the magnetic head. Transfer the samples back to their original tubes.
 - Samples can be transferred to the elution plate.
- *[58-63] No sample recovery required.*
 - Eluates have already been transferred to the elution plate.

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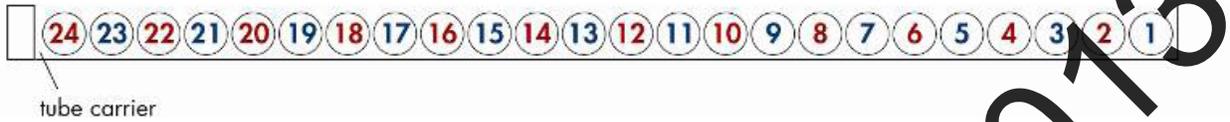
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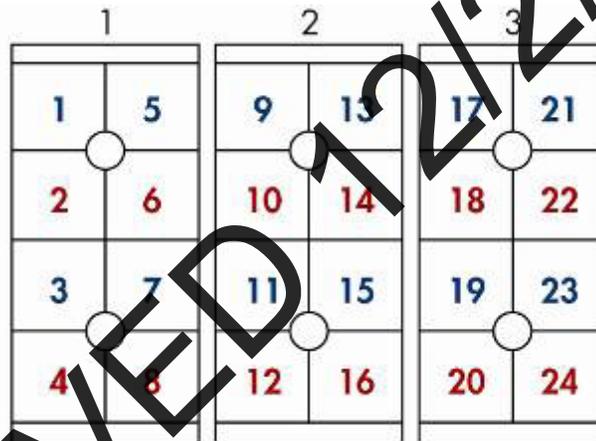
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1.5.4 Returning samples to their original tubes

- Tube carrier racks are numbered 1-24



- Sample prep cartridges contain samples in the pattern displayed below



- As the sample prep cartridges are removed from the instrument, it may be helpful to number the outside to indicate which cartridge was removed and to distinguish the top from the bottom.
- Pipette smoothly and minimize bubbles during the transfer procedure.
- If the sample contains greater than 500µl of volume then spin down the sample once it has been returned to its original tube and remove the supernatant volume in excess of 500µl. Cap the tube and vortex thoroughly to return the beads to suspension.

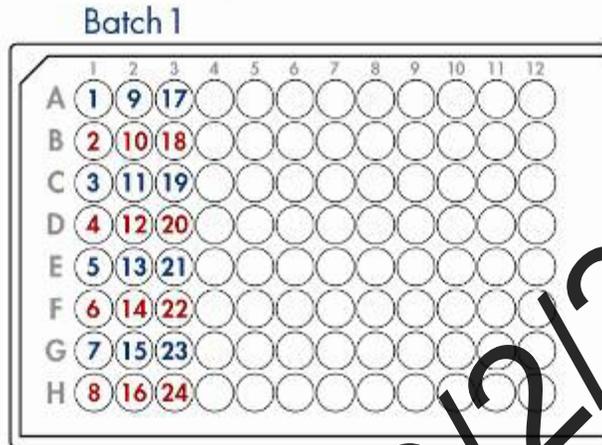
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- Elution plates contain samples in the pattern displayed below



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Section 2 QIAgility Plate Set-up and DNA Amplification

References:

- QIAgility® User Manual, 11/2011
- PowerPlex® 16 System Technical Manual

Note: Quantification prior to amplification is optional for these samples.

2.1 Amplification Cycling Parameters

- Ensure AB GeneAmp® PCR System 9700 thermal cycler has been turned on to allow the instrument time to properly warm up.
- Confirm the cycling parameters for **user: alaska, program: pp16-32cyc**

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 100% to 94°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 10 cycles, then:

ramp 100% to 90°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 22 cycles, then:

60°C for 30 minutes
4°C hold

2.2 Amplification Plate Set-up

- If samples have been stored at 2-8°C prior to loading on the QIAgility, allow the samples to warm to room temperature and spin briefly using a centrifuge.
- Transfer the amplification reagents to the designated PCR set-up area.

Note: Do not expose reagents to light for extended periods of time.

- Ensure that all kit components have thawed completely before use. Vortex reagents and centrifuge briefly to ensure uniform mixing and collection of tube contents.

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Note: Centrifugation of the primer mix should be minimal to avoid primers collecting at the bottom of the tube; however, it is important to remove all bubbles prior to placing on the QIAgility.

2.2.1 Reagent Preparation

- Prepare the following sized tubes labeled A-E as follows:
 - A) Using a 5ml tube, pipette 1720 μ l of water into this tube.
(Water used to dilute samples)
 - B) Using a 1.5ml tube, pipette 20 μ l of working (diluted) positive control into this tube.
 - C) Using a 1.5ml tube, pipette 34.2 μ l of water into this tube.
(Water used for negative control)
 - D) Using a 1.5ml tube, prepare a PCR master mix by adding the following volumes of reagents:
 - 282.5 μ l Gold ST^{*}R 10x Buffer (2.5 μ l per sample)
 - 282.5 μ l PowerPlex[®] 16 10x Primer Pair Mix (2.5 μ l per sample)
 - 90.4 μ l AmpliTaq Gold[™] DNA Polymerase (0.8 μ l per sample)
 - E) Using a 1.5ml tube, pipette 29.2 μ l of water into this tube.
(Water used for positive control)
- Vortex and spin all tubes briefly ensuring all bubbles have been removed.

Note: All volumes include additional reagents and/or water necessary to run the protocol.

2.2.2 QIAgility Set-up

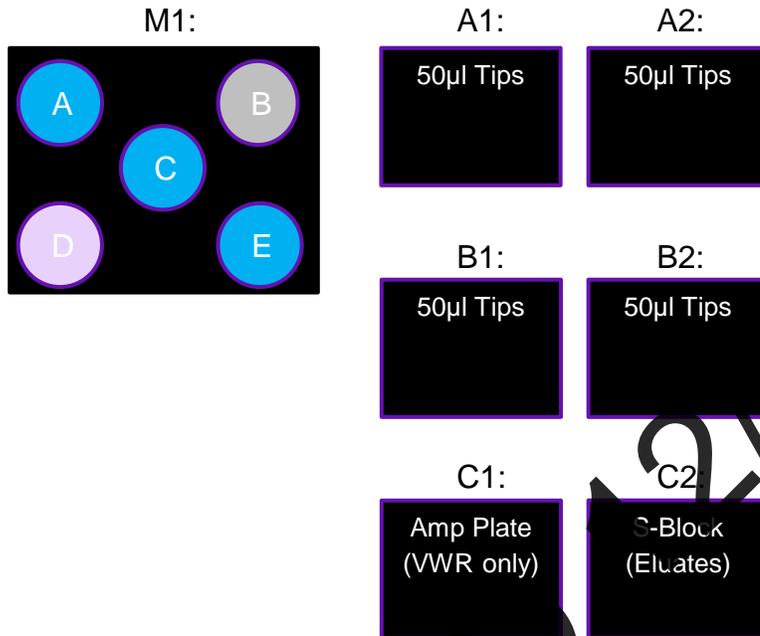
- Obtain a VWR 96-well Reaction Plate. (Note: the use of an alternate plate has not been calibrated for this protocol.)
- Ensure that the power for the QIAgility has been switched on. The power switch can be located on the rear left of the instrument. A blue light on the front of the instrument will indicate the instrument is powered on.
- Click on the QIAgility icon to launch the software; it may take a minute to initialize.
- Navigate to the database amplification protocol by clicking **Recent** → **Browse** → **Alaska** → **Database Norm and amp in SBlocks_1 μ l**.

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- Open the QIAgility and load the deck as follows:



Note: When loading tips, there is only one correct orientation. Ensure tips are loaded correctly.

- To reset the tips, hover over the block of tips to reset. Using the mouse, right click and select “Set all tips on current plate to available”.

Note: Tips designated in blue are available and white are unavailable.

- Ensure tip waste box is attached and there is ample room for waste.
- Click the green arrow at the top of the screen. The “Save As” window appears. Enter the batch name under “File name:” and click “Save”.
- The “Checklist” screen appears. Review the messages and follow the prompts.

Note: A “Pre-Run Report” may be previewed by clicking “Pre-Run Report”. Click “Close” to return to the “Checklist” screen.

- Click “OK”.
- The run will begin and will take approximately 1 hour to process. Upon completion, the samples will be ready for amplification using an AB GeneAmp 9700 thermal cycler.

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- Remove the S-Block containing the eluates and cover with a plastic seal. Store at 4°C. All extracts will be disposed of upon completion of the reviews and upload of the batch.
- Unload the deck of the QIAgility by removing and discarding empty tip racks and tubes located in the M1 block.

2.3 Amplification

- Cover the amp plate with amp tape and transfer to the PCR room and place directly into the thermal cycler.
- Store amplified products at 2-8°C. All amplified products will be disposed of upon completion of the reviews and upload of the batch.

2.2.3 QIAgility Maintenance

Daily maintenance is performed at the end of each day the instrument is in use and is described below. Additional maintenance procedures are detailed in the Forensic Biology General Lab Maintenance manual. All maintenance is recorded on the QIAgility Maintenance Log provided at the back of the Forensic Biology General Lab Maintenance manual.

2.2.3.1 Daily Maintenance Procedure

Daily maintenance is required after each run on the QIAgility and at the end of the day.

- Wipe down the deck with ethanol.
- Wipe with water and wipe dry with paper towels.

Note: Do not wipe the rails. These rails support the pipetting head and allow it to slide backwards and forwards easily. Wiping the rails will remove the grease and make them more susceptible to rust.

- You may now run another protocol or switch off the QIAgility instrument.

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Section 3 Capillary Electrophoresis on the AB 3500xl Genetic Analyzer

References: *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide*

3.1 Start the Software and Instrument

- Turn the computer on and log into the computer
- Turn on the 3500xl Genetic Analyzer. Wait for the green status light to turn on.
- Ensure both the Daemon and Service Monitor has started by observing a green checkmark icon in the lower right hand corner. This indicates that all 3500 services have loaded. This may take several minutes.
- Launch the Data Collection Software:
Start > Programs > Applied Biosystems > 3500
- Log in to the 3500 Series Data Collection Software

3.2 Preparing the Instrument

- Navigate to the Dashboard of the software
- Check consumables by clicking **Refresh** to update consumable status.
- Refer to the **Forensic Biology General Lab Maintenance** manual if any maintenance or instrument preparations are required prior to running samples on the instrument.
- Click **Start Pre-heat** to warm the oven
- Check the pump assembly for bubbles and run the Remove Bubble wizard if necessary.

3.3 Sample Preparation for the 3500xl

- Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:
 - # of samples x 0.5µl ILS 600
 - # of samples x 9.5µl of Hi-Di Formamide

Note: Prepare enough for a few extra reactions to allow for loss during pipetting. This is calculated automatically on the Database Batch Worksheet.

Vortex the master mix and spin briefly. Transfer 10µl of master mix to appropriate sample wells of a 96 well plate.

- Add 10µl of Hi-Di Formamide to unused wells of a set of 24 (i.e. A1-H3).
- Add 1µl of allelic ladder and up to 2µl of each amplified sample to the appropriate wells. Reagent and amplification blank volumes must correspond to the largest volume of amplified sample added to the plate. When all samples have been added, cover with plate septa.
- Briefly centrifuge the 96 well plate to remove any bubbles. Denature samples at 95°C for 3 minutes and then snap chill for 3 minutes.

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- Place the 96-well plate into the plate base provided with the instrument and snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.

3.4 Creating a Plate Record

3.4.1 Create a Plate Record from the Data Collection Software

- In the dashboard click **Create New Plate**
- In the Define Plate Properties screen:
 - Enter a plate name (i.e. DB10-1222AB_KAL) this may vary depending on analyst
 - Select 96 for the number of wells
 - Select HID for plate type
 - Select 36cm for capillary length
 - Choose POP4 for polymer
 - Owner, barcode and description are optional fields.
- Click **Save Plate**
- Click **Assign Plate Contents**
- Using either plate view or table view enter sample names in the corresponding wells.
- Under the heading Assays, File Name Conventions, and Results Groups click **Add from Library** select one of the following Assays:
 - PPlax16 15sec
- Select a naming convention, and a results group of your choice. Click **add to plate** and then click **close**.
- Select the wells for which to specify the assay file name conventions and results groups and enable the checkbox next to the name to assign it to the selected wells.
- Click **Save Plate** and save.

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3.4.2 Create a Plate Record from an Export File

- Open a plate record template export file (i.e. .xls exported from instrument).
- Copy and paste sample name info into the export file. (Rows not in use may be deleted but do not delete any columns) Save the record.
- Using the 3500xl Data Collection Software, click on **Create New Plate** on the dashboard.
- In the Define Plate Properties screen:
 - Enter a plate name (i.e. DB10-1222AB_KAL)
 - Select 96 for the number of wells
 - Select HID for plate type
 - Select 36cm for capillary length
 - Choose POP4 for polymer
 - Owner, barcode and description are optional fields.
- Click **Save Plate**.
- Click **Assign Plate Contents**.
- Click **Import** at the top of the screen and navigate to the saved plate record (make sure .xls files are searched).
- Click **Save Plate** and save.

3.5 Linking a Plate and Starting a Run

- Click **Link Plate for Run**. A message will appear saying Plate loaded successfully. Click **OK**.
- Ensure plate is linked to proper position on 3500xl.
- Select **Create Injection List**.
- Click **Start Run**.

Note: Ensure all prompts have cleared and the run begins before walking away.

3.6 Viewing Data During a Run

Refer to Applied Biosystems 3500/3500xl Genetic Analyzer User Guide

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Section 4 Data Analysis with GeneMapper™ ID-X

*References: GeneMapper™ ID-X Software User Guide
GeneMapper™ ID-X Software Tutorial
GeneMapper™ ID-X Software User Bulletin*

4.1 Logging in to GeneMapper™ ID-X

- Open GeneMapper™ ID-X
- Select the User Name and Database Host from the drop-down list and enter the appropriate password.
- Click OK.
- The main project window will open.

4.2 Creating a Project

- To add samples from the collection software at a workstation, go to the edit menu and select **Add Samples to Project**. Navigate to **My Computer > Uncontrolled Documents > Section Shares > DNA Share > CE Data** on the laboratory "I Drive" as per current designation(s).

Note: Location is dependent on network mapping and may vary slightly.

- Select the raw data folder to be imported or select individual samples and click **Add to list**.
- When all samples have been selected, click **Add**.
- Select the appropriate Sample Type, Analysis Method (refer to section 1.2.1), Panel (Promega Bins_IDX_alpha) and Size Standard (ILS_80-550_Adv) for each sample and click the green arrow on the tool bar to analyze the samples.
- The user will be prompted to name and save the project to the appropriate Security Group (GeneMapper ID-X Security Group). Name the project with the Batch name. Click OK.
 - Example: DB13-0319A_KAH

- Analysis is complete when the green arrows in the Status column on the left are gone.

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Issued: 9/3/2013
Effective: 9/3/2013

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4.2.1 Analysis Methods

A range of Analysis Methods are available to analyze database samples. Options for Analysis Methods vary by RFU and analysis range. These methods shall not be modified. New methods shall only be created or modified with permission from the DNA Technical Manager.

- 250RFU Database-2400
- 250RFU Database-2800
- 250RFU Database-3200

- 700RFU Database-2400
- 700RFU Database-2800
- 700RFU Database-3200

- Blank Database Global

The settings for the Analysis Methods are viewed by selecting GeneMapper ID-X Manager under the Tools drop-down menu, clicking on the Analysis Methods tab, then double clicking to select a particular Analysis Method.

Based on the laboratory's internal validation, allelic ladders will typically need to be analyzed at 250RFU to achieve complete allele calling. The most efficient analysis and review will generally be achieved by first analyzing all samples at 250RFU. Samples with baseline artifacts can be identified and re-analyzed at 700RFU.

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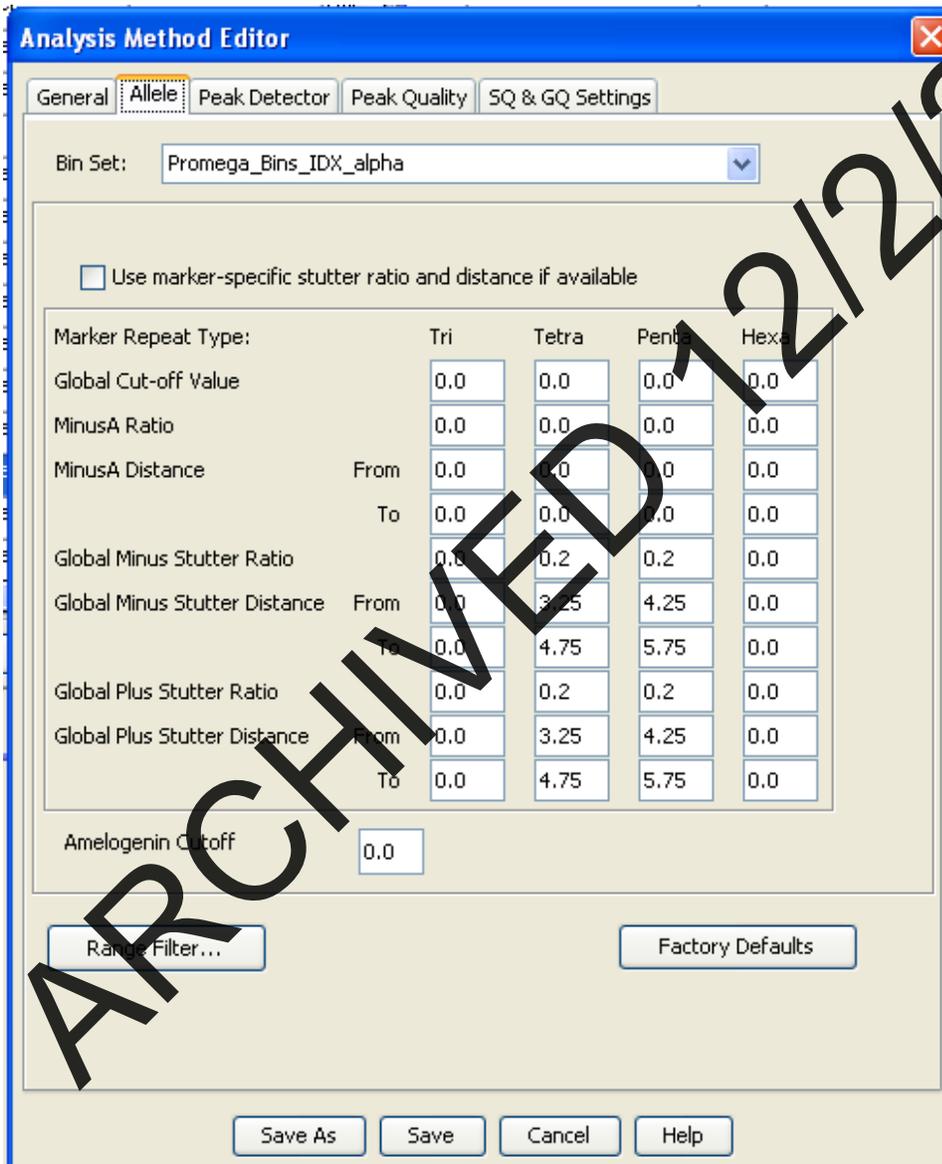
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 Effective: 9/3/2013

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4.2.1.1 Allele Tab Settings

The Allele Tab Settings are viewed by opening a selected analysis method. These settings are consistent within all database methods.

All Analysis Methods



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4.2.1.2 Peak Detector Tab Settings

The Analysis Range and/or the Peak Amplitude Thresholds are the only settings that vary between the alternative Analysis Methods. The Analysis Range is selected to ensure capture of the 80bp ILS peak for all allelic ladders, positive controls, and questioned and known samples in the project. The Analysis Range is selected to capture the primer peaks for reagent blanks, negative amplification controls, and samples that do not yield at least a partial profile. Peak Amplitude Thresholds (for blue, green and yellow) of 250RFU and 700RFU are approved for database analyses. For each RFU threshold, there are methods with analysis start points of 2400, 2800 and 3200. All other settings are the same among the methods.

Example: 700RFU Database-2800 Analysis Method

Analysis Method Editor

General | Allele | **Peak Detector** | Peak Quality | SQ & GQ Settings

Peak Detection Algorithm: Advanced

Ranges

Analysis: Partial Range | Sizing: Partial Sizes
Start Pt: 2800 | Start Size: 80
Stop Pt: 14000 | Stop Size: 600

Smoothing and Baseline

Smoothing: None Light Heavy
Baseline Window: 51 pts

Size Calling Method

2nd Order Least Squares
 3rd Order Least Squares
 Cubic Spline Interpolation
 Local Southern Method
 Global Southern Method

Peak Detection

Peak Amplitude Thresholds:
B: 700 | R: 100
G: 700 | Y: 500
O: 500

Min. Peak Half Width: 2 pts
Polynomial Degree: 3
Peak Window Size: 15 pts

Slope Threshold

Peak Start: 0.0
Peak End: 0.0

Normalization

Use Normalization, if applicable

Factory Defaults

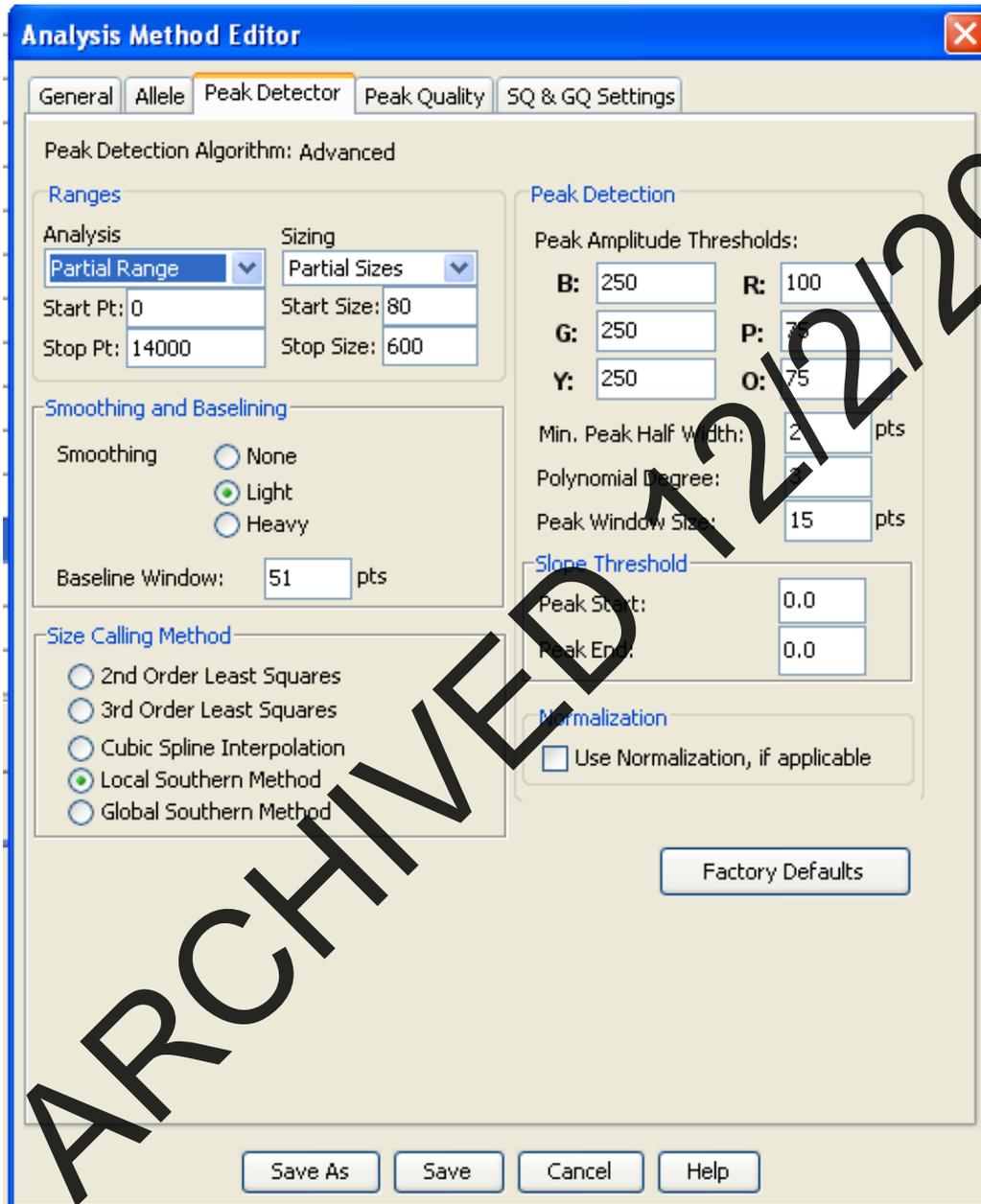
Save As | Save | Cancel | Help

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Issued: 9/3/2013
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Blank Database Global Analysis Method



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Issued: 9/3/2013
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4.2.1.3 Peak Quality Tab Settings

These settings do not vary among the methods, and they are not relevant in analysis of any samples where the sample type is set to Negative Control.

700RFU Analysis Methods

Analysis Method Editor

General | Allele | Peak Detector | **Peak Quality** | SQ & GQ Settings

Min/Max Peak Height (LPH/MPH)

Homozygous min peak height	1400.0
Heterozygous min peak height	1050.0
Max Peak Height (MPH)	30000.0

Peak Height Ratio (PHR)

Min peak height ratio	0.5
-----------------------	-----

Broad Peak (BD)

Max peak width (basepairs)	1.5
----------------------------	-----

Allele Number (AN)

Max expected alleles	2
----------------------	---

Allelic Ladder Spikes

Spike Detection	Enable
Cutoff Value	0.2

Factory Defaults

Save As | Save | Cancel | Help

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Issued: 9/3/2013
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250RFU Analysis Methods

Analysis Method Editor

General | Allele | Peak Detector | **Peak Quality** | SQ & GQ Settings

Min/Max Peak Height (LPH/MPH)

Homozygous min peak height	500.0
Heterozygous min peak height	375.0
Max Peak Height (MPH)	30000.0

Peak Height Ratio (PHR)

Min peak height ratio	0.5
-----------------------	-----

Broad Peak (BD)

Max peak width (basepairs)	1.5
----------------------------	-----

Allele Number (AN)

Max expected alleles	7
----------------------	---

Allelic Ladder Spike

Spike Detection	Enable
Cut-off Value	0.2

Factory Defaults

Save As | Save | Cancel | Help

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Issued: 9/3/2013
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4.2.1.4 SQ and GQ Tab Settings

These settings do not vary among any of the analysis methods, and they are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.

All Analysis Methods



Issued: 9/3/2013
Effective: 9/3/2013

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4.3 Viewing the Data

- The ILS is viewed by highlighting all samples and clicking on the Size Match Editor, the icon with the red peaks on the toolbar.
- To view each sample, highlight the sample and click on the icon with the colored peaks to display plots. (This can also be done by View > display plots or Ctrl + L).
- Electropherograms can be printed from the sample's plot window. Various plots have been created for different sample types. Commonly used plot displays include: Traditional Genotype Plot, Casework Blank, Casework artifacts and Casework zoom view.
- To view information on a sample's injection time and other run information, highlight the sample of interest and click **View** on the toolbar > Sample Info (Ctrl + F1).
- To view raw data for a sample, click **View > Raw Data**.
- To view allele calls by sample and locus click the Genotypes tab from the main project window.

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Effective: 9/3/13

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Section 5 STR Data Interpretation

Interpretation of database samples is based on the guidelines contained in this section, as well as the qualified DNA analyst's training and experience. In all cases, interpretations must be independently reviewed and verified based on established peer review procedures. The DNA Technical Manager may authorize the interpretation/reporting of data outside of the criteria defined herein and will document this approval by initialing and dating comments on the relevant paperwork.

The minimum peak height acceptable for database sample STR loci alleles is 250RFU. The threshold may be raised to a maximum of 700RFU for samples with an elevated baseline. Lower threshold limits for hit confirmations or ILS (Internal Lane Standard) may be authorized by the DNA Technical Manager.

The following abbreviations are commonly used in bench notes and on other documents generated during DNA analysis.

DNA Abbreviations and Definitions	
BL	Noisy baseline
PHR	Peak Height Ratio
PU	Put-Up
DO	Complete Drop-Out at a Locus
PDO	Partial Drop Out at a Locus
D5Mut	D5 Mutation
OL	Off Ladder
OMR	Outside Marker Range
OS	Off Scale/Saturation
NP	No genetic profile obtained
NR	Data not reported
NS	Data not used for statistics
A/TA	Artifact (i.e. stutter) or true allele
IF	Instrument failure
ILS	ILS failure
DBRF	Data below reporting threshold
ART	Artifact
TRI	Tri-allele
()	Minor component allele
[]	Major/Minor components not separated

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Issued: 9/3/13
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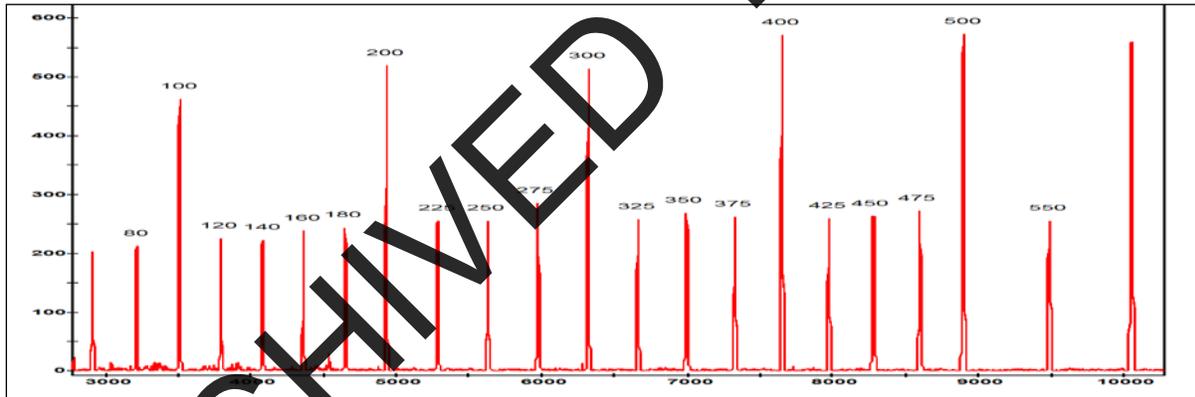
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5.1 Interpretation of Batch Controls

Prior to assessment of data, the analyst must first examine the batch controls to ensure that the extraction, amplification and genetic analysis processes are functioning correctly. These include: Internal Lane Standard (ILS), allelic ladder(s), internal control specimen (ICS), positive and negative amplification controls, and extraction reagent blanks. All or part of an extraction, amplification and/or run may need to be repeated depending on the results of the batch controls. Any issues raised by the performance of a batch control must be addressed prior to the release of affected casework samples. Unresolved failure of a batch control requires the Technical Manager, or designated individual, to approve a course of action.

5.1.1 Internal Lane Standard (ILS)

The Promega PowerPlex16® System uses ILS 600. The analyst should verify that all peaks from 80-550 base pairs are present and labeled as shown.



The peak heights for the ILS peaks must be at least 100RFU (relative fluorescence units) to be identified and labeled by the GeneMapper™ ID-X software with a database method.

Failure of an ILS can often be resolved by re-prepping and/or re-injecting the affected sample(s). If the ILS continues to fail after re-prepping and re-injecting, the analyst should consult the Technical manager for a course of action.

Refer to the GeneMapper™ ID-X Software User Guide for details on manually labeling the size standard.

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5.1.2 Allelic Ladder

The GeneMapper™ ID-X software uses the allele calls of the ladder to assign allele calls to all the other samples in the project. The analyst should verify that all peaks from the allelic ladder(s) are present and labeled as shown.

The allelic ladder contains the most common alleles determined for each of the 13 FBI STR core loci, the Penta D and Penta E loci, and Amelogenin. In addition, alleles not labeled in the allelic ladder (virtual alleles), may be detected and labeled in some of the samples analyzed.

At least one allelic ladder must be included in a GeneMapper™ ID-X project with the sample type designated as “allelic ladder”. All the peaks in the allelic ladder must be labeled correctly for the software to assign the correct allele calls to samples in the run. When more than one ladder in a project has the sample type designated as “allelic ladder”, the software will average the allelic ladders to make the allele calls for the samples in the project.

Artifacts such as spikes or “pull-up” peaks may be present that are labeled as off-ladder (OL) peaks in the allelic ladder. If all the true allele peaks are labeled correctly, such OL peaks will not affect the sample allele calls. If a spurious peak (not a true allelic peak) is labeled as a true allele (i.e. it falls within an allele size range and is designated and labeled as an allele) at any particular locus, the remaining allele designations / labels at the locus shift and will impact the correct allele call / labeling of the samples in the project. Allelic ladders with such artifacts cannot be used to analyze data in the project. In such instances, re-injection is usually appropriate.

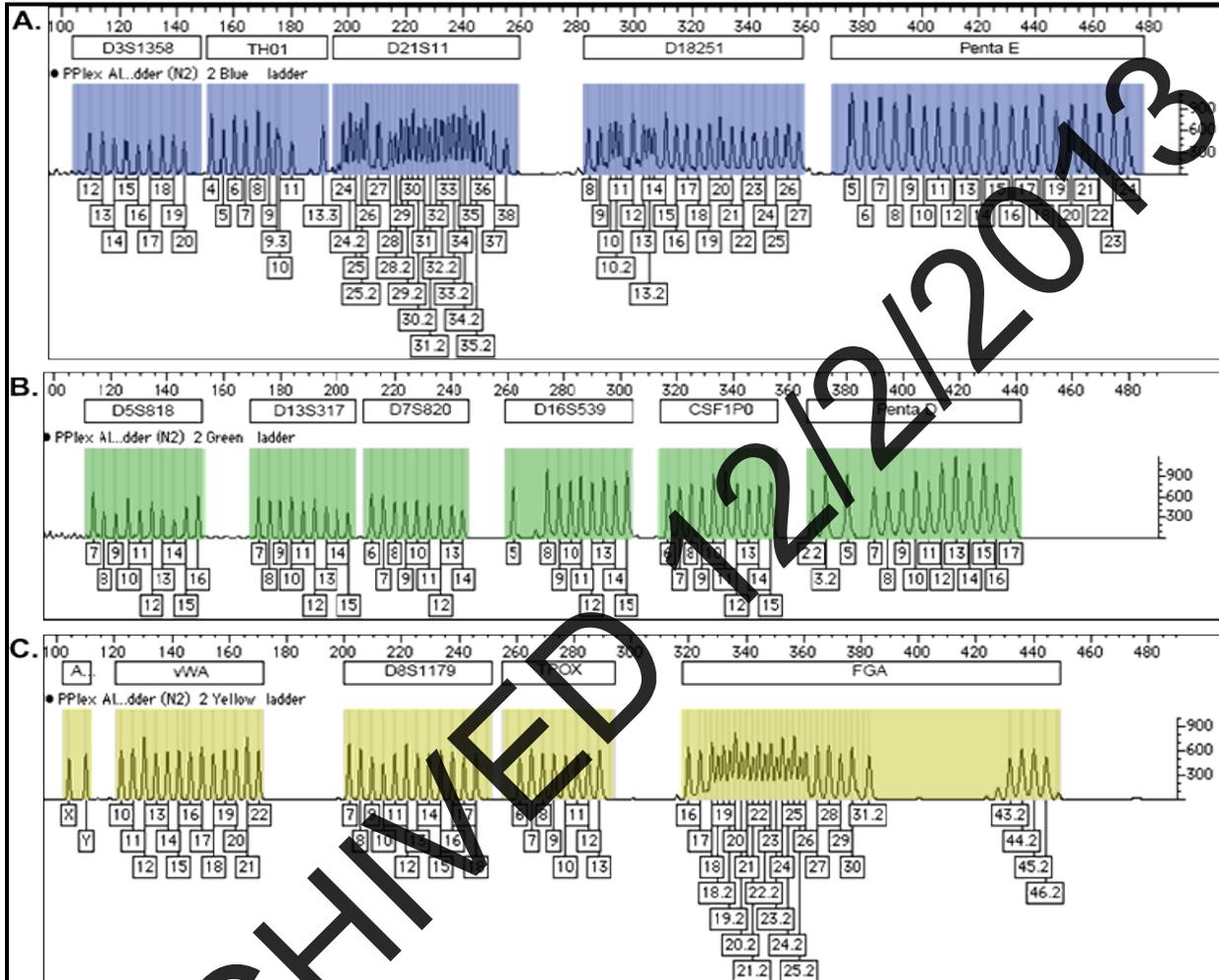
Additional information about the allelic ladder can be found in the Promega PowerPlex16® System Technical Manual.

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PowerPlex16® Allelic Ladder



5.1.3 Positive Amplification Control

A positive control is defined as a single source sample whose genetic profile was previously determined and from which a full profile was developed; it is used to evaluate the performance of the amplification and typing procedures. The standard DNA template 2800M is currently used as the positive amplification control for the Promega PowerPlex16® System.

It may be necessary to re-prep and/or re-inject the positive control if the cause of failure appears to be a failed electrophoretic injection. As long as the re-prepped and/or re-injected positive control types successfully, the positive control is considered successful.

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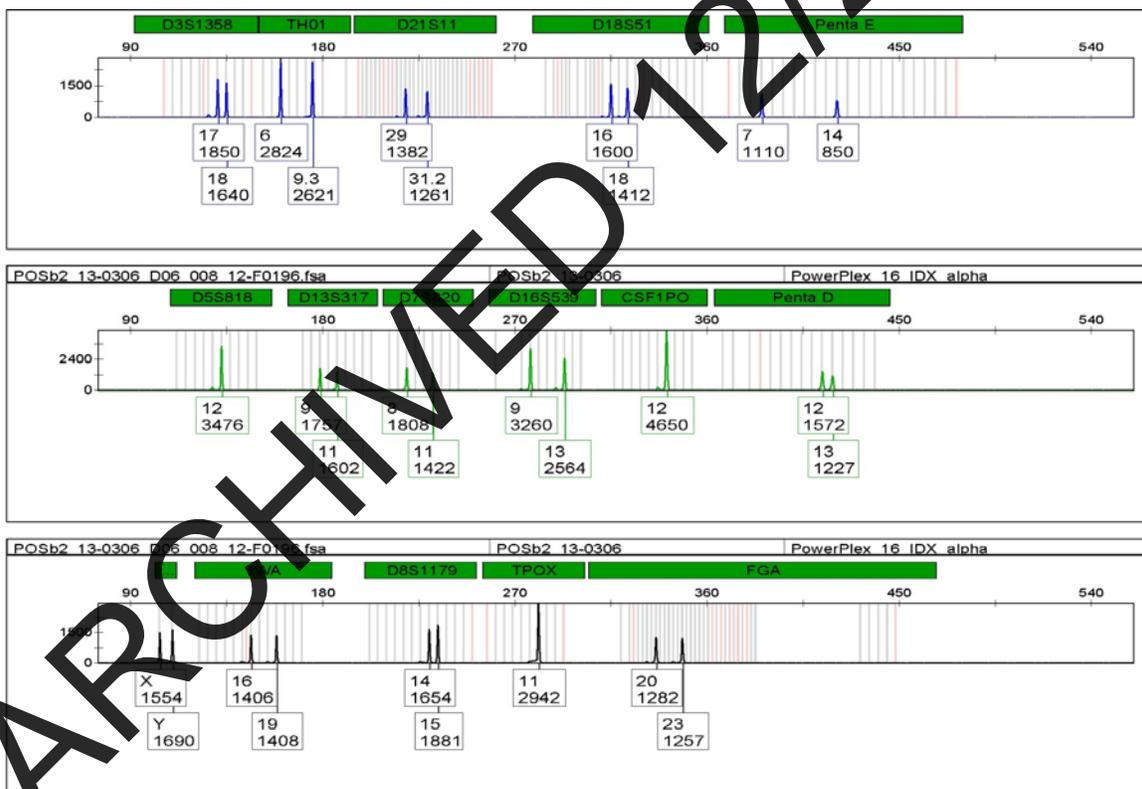
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Generally, approximately 5% of the samples in a database batch will be quality control samples such as a duplicate offender sample or a staff duplicate sample. One of these samples may also serve as the positive amplification control when the kit control fails. Failure to obtain the expected profile for at least one positive control sample requires re-amplification of the entire plate.

When an incorrect STR profile is obtained for one or more of the positive control samples in a batch, other profiles in the batch will not be entered into CODIS until the issue is resolved. The CODIS Administrator, or a designated individual, may approve the entry of select profiles on a case by case basis.

STR profile of 2800M



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5.1.4 Negative Controls

A reagent blank is carried through the entire analytical process as part of each extraction or type of extraction. It contains all the reagents - except DNA template - used during extraction, amplification and typing.

The negative amplification control contains only the PCR master mix (reagents used to prepare the PCR amplification mixture) for each batch of samples and the water used to dilute the DNA samples.

The purpose of the negative controls is to detect DNA contamination that might occur from the reagents, the laboratory environment, between the samples being processed, and/or due to improper handling of the samples by the analyst. The negative controls must be run at the most stringent set of conditions for the batch.

Verification of the presence of amplicon in the negative controls is performed by viewing the presence of unincorporated primer peaks.

The negative controls should not yield any true STR allelic peaks above the reporting threshold. When peaks greater than or equal to the reporting threshold are present in the range between 80-550 base pairs, the analyst will determine if the peaks are artifacts (e.g. spike, pull-up) or true allele peaks.

The presence of peaks above the reporting threshold will not invalidate the sample as long as the peaks can be shown to be artifacts. Artifacts will be documented on the electropherogram or the database batch worksheet.

When probable true allele peaks are detected above the reporting threshold, at several or all STR loci, the analyst will investigate possible causes. The Technical Manager will be consulted to determine the appropriate course of action.

Since the failure of a negative control may indicate a problem at the extraction or amplification level, a Quality Review Form may need to be completed based on the nature of the discrepancy.

Note Although appropriate quality assurance practices are stringently applied and enforced, it is not unexpected that low-levels of adventitious DNA (i.e., levels that result in peak heights for allelic data that are below the Laboratory's reporting threshold) attributable to staff scientists and/or consumable products directly involved in the analytical steps of such a highly sensitive technique may be detected

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5.2 Interpretation of Samples

Typically, each locus is characterized by one or two labeled peaks or alleles of approximately equal intensity.

Allele peak heights should typically fall between 1000 and 6000RFU. Peak heights outside this range may be acceptable; however, it is possible that these samples will require careful interpretation. Alleles with peak heights less than 1000RFU may occasionally exhibit peak height imbalance due to stochastic effects caused by low template copy number.

Batches of database samples are uploaded into SDIS by creating an Export Table for CODIS. Prior to creating this table, the analyst must confirm that only eligible profiles are marked for export (as Convicted Offender, CO Duplicate, Arrestee, Staff Duplicate). This is done by selecting the appropriate Specimen Category while in GeneMapper ID-X. Samples not exported to CODIS are marked "no export".

Peak height ratios (PHR) are calculated by dividing the peak height (in RFUs) of the lower RFU allele by the peak height of the higher RFU allele, and then multiplying this dividend by 100 to express the PHR as a percentage. The GeneMapper™ ID-X software is set to flag samples with an intralocus PHR below the expected minimum of 50%. PHR imbalance (in a single source sample) may occur with low quantities of template DNA, degraded template DNA or in the presence of PCR inhibitors. Severe imbalance may result in allelic dropout, where one or more alleles occur below the detection threshold or are not visible at all.

The presence of more than two peaks or severe PHR imbalance at a locus may be explained by a tri-allele, primer binding site mutation, variant allele, or other biological or procedural artifact. The presence of these at several loci is indicative of a mixed sample.

5.2.1 Tri-alleles

Tri-allele patterns (three alleles at a single locus) are known to occur in single source samples and there are documented instances where different tissues from the same individual may or may not exhibit the tri-allelic pattern.

Samples exhibiting tri-allelic patterns with all alleles of approximately equal intensity, or with the 3rd allele having a peak height greater than or equal to 20% of the neighboring peak, are entered as such in CODIS.

Samples exhibiting possible tri-allelic patterns where the 3rd allele is less than 20% the height of the neighboring peak will be re-amplified to verify the pattern. If the pattern

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reproduces, the 3rd allele is designated as A/TA (artifact/possible true allele) on the batch worksheet and the comments section in CODIS. Only the larger two alleles are entered into CODIS.

5.2.2 Off-Ladder (OL) Alleles

The allelic ladder contains the most commonly observed alleles for the STR loci. True alleles are peaks that are detected and labeled by the GeneMapper™ D-X software with a number and should lie within the range of the alleles in the ladder. Alleles which are not assigned a numeric designation in the standard ladder will be designated as off-ladder (OL) alleles.

Peaks that are labeled as OL (off ladder) at a particular locus have a base size that is different from any of the alleles in the ladder and any of the virtual alleles. These peaks may be true alleles but additional analysis is required to verify this. Before determining whether an OL is a true allele or an artifact, the analyst must consider all other possible causes of OL occurrence.

True OL alleles will exhibit peak morphology similar to the other alleles in the sample and the PHR should be appropriate relative to the other allelic peaks in the sample. When the overall sample quality is poor, the analyst should consider re-amplification prior to designating an OL peak as a true allele.

When a true OL allele lies within the range of alleles for a particular locus, the allele will be reported as a variant of the integer (i.e. X.1, X.2, etc.). The appropriate allele call is determined by simultaneously examining the base sizes for the sample allele peak and the associated allelic ladder peaks in the relevant locus.

When the OL allele lies either above or below the largest or smallest allele in the ladder, the OL allele will be designated as either greater than (>) or less than (<) the allele from the ladder that is closest in proximity to the OL allele.

5.2.3 Low-Level DNA Samples

Peaks may be observed that are below the reporting threshold of 250RFU. When these peaks exhibit peak morphology similar to allelic peaks and fall within an allele bin, the analyst should consider the possibility of allelic dropout. If allelic dropout is suspected in one or more core STR loci, the analyst will make a "best attempt" to re-process the sample in order to generate a complete genetic profile. The repeat analysis may include any or all of the following: re-extraction using more substrate, maximum template input in PCR, and maximum "prep" or amplified sample loading volume for capillary electrophoresis.

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Note: The reagent blank and negative PCR control shall be processed under the most stringent conditions utilized for any associated sample.

If the above “best attempt” also fails to generate a complete genetic profile, a maximum of three core STR loci with elevated stutter and/or stochastic issues (e.g., partial dropout, heterozygous peak height ratio less than 50%) may be permissible in a sample. Samples that do not contain at least partial data at each of the 13 core STR loci are not eligible for upload to NDIS. The known allele 10 variant at the D5 locus is an accepted phenomenon and is not counted towards the maximum three core STR loci with stochastic issues. The presence of this variant, when observed, should be documented on the database batch worksheet.

When incomplete typing results are suspected at one or more STR loci, it is indicated by selecting “Yes” in the “Partial Profile” field in CODIS. The comments field in CODIS may be used to indicate in which loci the dropout is suspected.

Data will not be composited from multiple amplifications/injections of a database sample to obtain a more complete profile.

5.2.4 Artifacts

Artifacts are data peaks resulting from the analytical process and are not attributable to an individual contributor to the sample.

When artifacts are not pervasive throughout the sample and do not interfere with data interpretation, the sample does not require re-analysis. When an artifact is present within the size range of the alleles in a core locus and the presence of the artifact interferes with interpretation of the data in that locus, or when a sample exhibits artifacts in several loci, interpretation of the sample becomes more challenging and it may be necessary to reprocess the sample. Artifacts may be resolved by merely re-injecting a sample, or may require re-amplification or re-extraction to resolve. If the interfering artifact is located in one of the Penta loci, the analyst may choose to enter the profile, omitting all data from the affected Penta locus.

Samples uploaded into CODIS must not contain any labeled artifacts or OL alleles. The analyst may remove the artifact label. Artifact or OL peaks are either deleted or re-labeled by right clicking on the peak and selecting one of the given options. The analyst should note any changes made to the allele calls in the appropriate column on the database batch worksheet.

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5.2.4.1 Incomplete 'A' nucleotide addition (-A or split peaks)

AmpliTaq Gold™, like many other DNA polymerases, catalyzes the addition of a single nucleotide (predominantly adenosine) to the 3' ends of double-stranded PCR products. This non-template addition results in a PCR product that is one base longer than the actual template, and the PCR product with the extra nucleotide is referred to as the "+A" (base peak) form. The final step of the STR amplification process is a terminal extension step to promote complete non-template 'A' nucleotide addition. The "-A" form is the peak that represents the actual template length. The "+A" peak will be the predominant form, with the "-A" peak typically not being detected.

Failure to attain complete terminal nucleotide addition (typically due to an excess of template DNA) results in "split peaks", two peaks above threshold that are one base apart. If the labeled "-A" peaks are not pervasive throughout the sample and the sample appears to otherwise be a single source sample, the peaks may be documented and such on the paperwork and the OL labels removed. When split peaks occur at several loci, re-amplification with less template DNA may be appropriate.

5.2.4.2 Pull-up

Multi-component analysis is the process that separates the four different fluorescent dye colors into distinct spectral components. The four dyes (Fluorescein, JOE, TMR and CXR) emit their maximum fluorescence at different wavelengths, but there is some overlap of the emission spectra. A spectral calibration is performed for a specific dye set to create a matrix that corrects for the spectral overlap.

Pull-up is the result of incomplete separation of the emission spectra and is typically observed as a non-allelic peak at the same base size as a peak in another dye. This typically occurs at high RFU values, when an excess of template DNA has been amplified.

Pull-up may also occur when the matrix file is not current. A new spectral calibration is performed whenever a new capillary array is placed on the instrument or whenever any part of the optics have been realigned and/ or replaced. A new spectral calibration may also need to be performed when an overall decrease in spectral separation is observed.

If the pull-up is prevalent enough to interfere with data interpretation, the sample may need to be re-injected or re-amplified with less DNA template. Otherwise, it can be noted on the batch worksheet and the label(s) manually removed.

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5.2.4.3 Stutter

The PCR amplification of tetranucleotide STR loci typically produces a minor product peak four bases shorter than the corresponding base peak. This artifact is the result of slippage of the Taq polymerase and is referred to as the stutter peak or stutter product. Stutter products four bases longer, or two bases shorter, than the corresponding base peak are less frequently observed. Two base pair stutter products are so rare that peaks in these positions are not filtered out in the analysis parameters. In the pentanucleotide loci, the stutter peak is five bases shorter than the corresponding base peak.

The proportion of stutter product relative to the main allele peak is calculated by dividing the height of the stutter peak by the height of the main allele peak. The stutter percentage is fairly reproducible for a particular locus.

The GeneMapper™ ID-X database analysis methods have a global stutter filter of 20%. Peaks in the stutter position greater than 20% may indicate the presence of DNA from more than one individual. When there is no indication of a mixture, and the data is determined to be elevated stutter, the analyst will document the elevated stutter on the database batch worksheet and manually remove the stutter label.

It is unlikely that the presence of stutter peaks would interfere with the interpretation of database samples and therefore, re-amplification and/or re-injection of the sample may not be required.

5.2.4.4 Spikes

Spikes are non-allele peaks that may arise due to air bubbles, urea crystals or voltage spikes. Spikes are typically quite sharp and easy to distinguish from a true allele, usually appearing in more than one color at the same base size, and not reproducible by re-injection.

Peaks labeled as such by the GeneMapper™ ID software cannot be manually removed. However, they do not interfere with upload to CODIS and no re-analysis is required if the spike does not interfere with interpretation of the sample.

5.2.4.5 Dye Blobs

Dye blobs, like spikes, are non-allele peaks that are easily distinguished from true alleles. Dye blobs may be the result from residual dye molecules left over from the synthesis of the primers or they may be dye molecules that fell off the primer during the amplification.

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If the artifact is labeled but does not interfere with data interpretation, the dye blob(s) shall be documented on the batch worksheet and the label manually removed.

5.2.4.6 Persistent Kit Artifacts

Occasionally, PCR kits may exhibit persistent artifacts that may or may not appear as true alleles. Typically, these artifacts are observed and documented during kit verification process. As with other artifacts, if the artifact does not interfere with data interpretation, it may be documented on the batch worksheet and the label manually removed, if necessary.

ARCHIVED 12/2/2013

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Section 6 Review of Data

Database projects are routinely reviewed electronically, with the technical reviewer looking at the controls/samples in GeneMapper ID-X rather than printed electropherograms. The analyst may choose to submit printed electropherograms to facilitate documentation and review of artifacts, true OL alleles, tri-alleles, etc.

6.1 Review of Offender Database Samples Analyzed In-House

Genetic profiles and all supporting documentation generated in the course of analysis will be subjected to a technical review. The DNA analyst will submit a packet containing the database batch worksheet and any printed electropherograms to another qualified database analyst for technical review. This review will be conducted in accordance with the FBI QAS Guidelines and includes the following, at a minimum, to ensure compliance with the interpretation guidelines contained within this document:

- Review of the ILS for all passing samples
- Review of all allelic ladders designated as such
- Review of all amplification positive controls and quality control samples
- Review of all reagent blanks and negative amplification controls
- Review of all DNA typing data for all passing samples
- Verification of eligibility for all profiles marked for export, to include selection of an appropriate specimen category

The analyst and the reviewer may consult a third qualified DNA analyst, if necessary, when there is a disagreement on how to report a result. If the analysts are unable to come to an agreement, the DNA Technical Manager will be consulted to make the final decision.

A review checklist is used to document completion of the individual components of the technical and administrative review. Upon completion of the technical review, the analyst may import the passing samples to SDIS. The analyst or a CODIS Administrator will then complete an administrative review, to include performing a duplicate offender search in SDIS.

Upon completion of the technical and administrative reviews, NDIS eligible samples are uploaded by a CODIS Administrator. Additional information on CODIS procedures is contained in the CODIS Administrative Manual. Upon completion of the upload, the following paperwork is archived as a .pdf in the LIMS:

- Database batch worksheet
- Electropherograms requiring documented review
- STR Offender Batch Review Checklist
- SDIS Import Reconciliation Report

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6.2 Review of Outsourced Offender Database Samples

The laboratory does not currently use a vendor laboratory for DNA analysis. Should the laboratory enter into a contract for DNA analysis of database samples with a vendor laboratory, a procedure for the in-house review of outsourced samples will be created before analysis begins.

ARCHIVED 12/2/2013

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Issued: 9/3/13
Effective: 9/3/13

Version: FBDB2013 R0
Status: Active

STR Offender Batch Review Checklist

Date: _____
 Technical Review Started: _____
 Administrative Review Started: _____
 Database Batch Number: _____

Analyst:	Tech. Review:	Admin. Review:
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Database batch # and analyst's initials on each page			
Reagent worksheet complete: analysis dates, instruments, # samples, reagent lot # and expiration dates, raw data folder(s) and project name(s)			
Batch worksheet complete: well #s, sample IDs, upload eligibility, specimen category and analysis range.			
Technical Reviewer checked batch worksheet (inc. reagents): initialed and dated each page			
ILS labeled correctly (80-550) for all passing samples			
Allelic Ladder(s): correct allele calls obtained ¹			
Positive control(s): correct DNA profile obtained ¹			
Negative control(s): results are acceptable ¹			
Reagent blank(s): results are acceptable ¹			
Check for consistency between NO EXPORT samples on paperwork and in project			
Confirm appropriateness of NO EXPORT status			
Technical review of passing samples: ≤3 problem core loci, RFU 250/700, appropriate allele edits, OL allele documentation			
Correct specimen categories are assigned			
Confirm specimen category is either convicted offender or arrestee for passing samples (check for agreement between paperwork and project)			
Samples for SDIS only are flagged on paperwork			

¹ includes verification of ILS and controls for re-worked samples

Eligible profiles uploaded into SDIS (in the Offender Index)			
Reconciliation report: #samples uploaded consistent with batch worksheet			
Problem samples corrected and documentation attached			
Duplicate offender search performed and all high stringency matches reconciled			
AK new moderate stringency search performed (only after previous step completed) may be completed by analyst or administrative reviewer			

Performed by CODIS Administrator

Upload to CODIS performed			
Reconciliation report received; consistent with # samples uploaded			

NOTES:

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Effective: 9/3/13

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Appendix A Revision History

Sections of this manual were taken from the current versions of QIA2013 R0, FBP2012 R0, FBWI2012 R0 and RBAIR2013 R0. The revision history contains the substantive changes to the sections contained within this document.

FBDB 2013 R0 Page	Original Manual Page	Location	Revision made
n/a	n/a	Entire manual	Updated references to other discipline manuals. Added missing references to instrument/equipment user manuals. Updated formatting.
1	1	Document Structure	Updated as required.
11-15	n/a	Section 4.5	Added QIA Symphony recovery procedure
22	FBWI2012 R0, page 49	Section 3.4.1	Removed 20 and 24 second injection assays as options.
25	RBAIR2013 R0, page 3	Section 4.2.1	Corrected typographical error in last paragraph, changing 750RFU to 700RFU.
33	FBP2012 R0, page 63	Section 5	Removed the following abbreviations from the table as they are not used or are defined elsewhere: SH, SPK, LV, RBQ, RBE, RBS, RBK, CIDI.
33-44	RBAIR2013 R0, pages 11-20	Section 5	Removed unnecessary non-procedural language to streamline and align with Data Interpretation of casework where appropriate.