

Forensic Biology Operating Procedures
QIASymphony SP Module – Database Sample DNA Extraction
QIAgility – Sample Set-up for PCR Amplification

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Section 1 Chemicals and Reagents

If the laboratory changes the preparation/verification procedures for a particular chemical/reagent, this change will be reflected in the chemical/reagent log and/or the verification paperwork.

Similarly, variations in vendor supplied materials (changes instituted by the vendor and outside of laboratory control) will be assessed to determine if the change adversely affects the laboratory analysis in which the reagent/chemical is used. This assessment will also be documented in the verification paperwork.

Such changes/modifications will be incorporated into this manual at the time of the next revision.

Please refer to Forensic Biology Work Instructions Manual for Reagent Verification information.

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Section 2 DNA Extraction – Database Samples

Qiagen QIASymphony SP

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.

Note: All required reagents are provided as listed in QIASymphony® DNA Investigator Handbook.

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.

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- 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.

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

“Wizard/ACS and Number of Samples”

- Under “Available assay control sets” press “**Investigator**” to view choices.
Select:
REF
200 1.5mL Tubes
- Press “→” to move assay over to the Selected “assay control sets/number of samples”.

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- 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”.

“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 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.

“Wizard/Elution Slot/Configure Racks”

- Open the “Eluate” drawer.
- Highlight “Slot 1”

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- 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.

“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.
- 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.

“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.

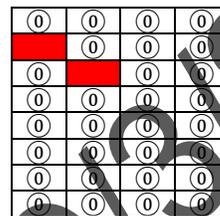
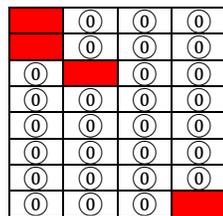
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Note: Do not refill partially used unit boxes. The number of sample prep cartridges or 8-Rod Covers is detected during the inventory scan. Additionally, do not throw empty unit boxes away. Empty unit boxes will be used in the “Waste” drawer for collection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



- 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.

“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.

“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.

“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.

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- 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.

“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.

“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.

“Wizard/Elution Slot & Volume”

- Select the elution volume by selecting **“200”**.
- Select the appropriate slot to assign to the batch.

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- 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 QIASymphony.
- Run will take approximately 3 hours.

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

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

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 2 “**DNA Isolation and Purification**”. 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 2 “**Wizard/Load Sample Tubes**”

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End of Batch Processing, Unloading the QIASymphony SP

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.
- 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.

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.

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.

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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.

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Maintenance

The table below describes the types and frequencies of maintenance required and the personnel who will routinely perform the maintenance.

Type of task	Frequency	Personnel
Daily maintenance	At the end of each day the instrument is in use	DNA analyst
Weekly maintenance	Once per week, if the instrument is in use, after the daily maintenance	Laboratory technician
Monthly maintenance	Once per month, if the instrument is in use, after the daily and weekly maintenance	Laboratory technician
Annual preventive maintenance and servicing	Once per year	QIAGEN Field Service Specialists only

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.

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- 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”**.
- 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.

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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.

Weekly Maintenance Procedure

- Delete result files older than 10 days:
 - Press **“File Transfer”** in the **“Main Menu”**.
 - Select the **“In-/Output Files”** tab.
 - Press **“Delete Old Files”** in the command bar of the screen. A message appears asking if you want to delete files. Press **“Yes”** to delete the old files. After the files have been successfully deleted, a message will appear confirming the deletion. Press **“OK”** to confirm the message.
- Clean the touchscreen by wiping with ethanol. Then wipe with a cloth moistened with water and dry with paper towels.
- Clean the QIASymphony SP hood by wiping the surface with a soft lint-free cloth moistened with deionized water. Then wipe dry with a dry soft lint-free cloth or paper towel. **Important:** Do not use ethanol only use distilled water.
- Check the tightness of the tip adapter O-ring
 - In the **“Main Menu”** screen, press **“Service SP”**.
 - Select the service script **“CheckPipettingChannelORing.lua”**.
 - Press **“Start”** to start the tightness test.
 - Follow the instructions in the messages displayed on the touchscreen. When instructed to do so by the software, place an empty tip-rack containing 4 test tips into the tip rack slot given in the software message (see picture below).

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Note: Do not start an inventory scan during the protocol run. When the inventory scan message appears, press **“No, nothing changed”**.

- If after running the tightness test for a particular tip adapter, a message is displayed with “Failed”, the O-ring must be changed. If one tip-adapter fails the tightness test, we recommend changing all 4 O-rings at the same time.
- Replace the tip-adapter O-ring if necessary
 - Reference 9.5 Maintenance of the tip adapter O-ring in the QIASymphony® SP/AS User Manual — General Description for instructions.
- Document completion of weekly maintenance on the QIASymphony® SP Maintenance Log.

Monthly Maintenance Procedure

- Replace the tip-adapter O-rings
 - Reference Section 9.5 Maintenance of the tip adapter O-ring in the QIASymphony® SP/AS User Manual - General Description for instructions.
- Document completion of monthly maintenance on the QIASymphony® SP Maintenance Log.

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Section 3 Database Sample DNA Amplification Set-up using QIAgility

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

Technical references:

- QIAgility® User Manual, 11/2011
- PowerPlex® 16 System Technical Manual, 05/2008

Cycling parameters for database samples (program pp16-32cyc) are as follows:

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

Before setting up the QIAgility:

- Ensure AB GeneAmp® PCR System 9700 thermal cycler has been turned on to ensure the instrument has time to properly warm up.
- If samples have been stored at 4°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.

- 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.

- 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**.

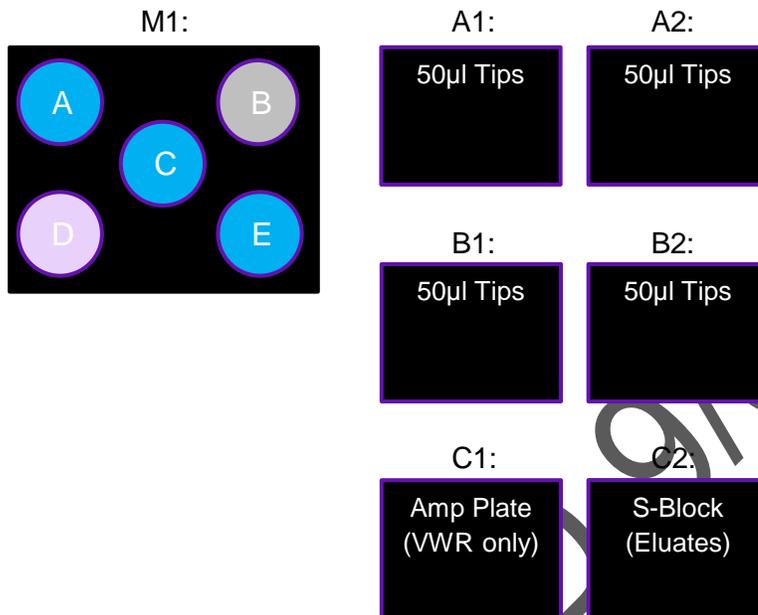
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Setting up the QIAgility

- 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”**.

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- 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.
- 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.
- 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.
- Unload the deck of the QIAgility by removing and discarding empty tip racks and tubes located in the M1 block.

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Maintenance

The table below describes the types and frequencies of maintenance required and the personnel required to carry out the maintenance.

Type of task	Frequency	Personnel
Daily maintenance	At the end of each run and at the end of each day the instrument is in use	DNA analyst
Weekly maintenance	Once per week, if the instrument is in use, after the regular and daily maintenance	Laboratory technician

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.

Weekly Maintenance Procedure

- Remove all loading blocks and the tip ejector chute from the worktable.

Note: Reference Section 8.2.2 of the QIAgility® User Manual for instructions on how to remove and replace the tip ejector chute.

- Rinse the blocks and the tip ejector chute with ethanol and rinse with de-ionized water.
- Dry with a soft paper towel.
- Replace the blocks and the tip ejector chute.

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- UV decontaminate the worktable for a minimum of 30 minutes ensuring all samples, reagents, and consumables have been removed.
 - Click the light bulb button on the top bar of the software screen.
 - Use the arrows to adjust the time.
 - Press “**Start**”. An alert window appears. Verify all conditions are met and press “**Yes**” to start.
- Document the completion of the weekly maintenance on the QIAgility® Maintenance Log.
- Switch off the QIAgility instrument.

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DNA Critical Reagent Verification Form
(to be completed as required for QIASymphony SP operation)

Analyst:

Date:

Lot #

Expiration Date

QIASymphony Kits (for Database samples)

QIASymphony Reagent Cartridges

Buffer ATL

Proteinase K

Buffer ATL (if purchased separately)

Proteinase K (if purchased separately)

Note: Verification of reagents will be performed similar to EZ1 reagent cartridges. Please see FBWI 2012 (active version).

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QIASymphony® SP Maintenance Log for Calendar Year _____
Alaska State Tag # _____
S/N: _____

Day of	Task Completed (analyst initial in box)	Comments
	<input type="checkbox"/> QIASymphony maintenance completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> QIASymphony maintenance completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> QIASymphony maintenance completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> QIASymphony maintenance completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> QIASymphony maintenance completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> QIASymphony maintenance completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	

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QIAgility® Maintenance Log for Calendar Year _____
Alaska State Tag # _____
S/N: _____

Day of	Task Completed (analyst initial in box)	Comments
	<input type="checkbox"/> QIAgility maintenance completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> service call	
	<input type="checkbox"/> QIAgility maintenance completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> service call	
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Appendix A Revision History

2012 R0 Page	2013 R0 page	Location	Revision made
1	1	DOCUMENT STRUCTURE	<p>ADDED “- DNA Extraction-Database Samples” after “Section 2 QIASymphony SP”</p> <p>Under Section 2 - REMOVED “DNA Extraction” and ADDED “Sample Preparation and Digestion”, “DNA Isolation and Purification”, “Continuous Sample Loading”, and “End of Batch Processing”</p> <p>ADDED “- DNA Amplification-Database Samples” after “Section 3 QIAgility”</p> <p>ADDED “Appendix A Revision History”</p>
3	NA	Section 2 Qiagen QIASymphony SP	REMOVED the entire section covering DNA extraction using Promega Slicprep™ 96 device and Slicprep™ protocol reference
4-5	3-4	Section 2 Sample Preparation and Digestion	CHANGED master mix preparation volumes and sample preparation instruction to reflect tube preparation rather than Slicprep preparation
7	4	Section 2 “Wizard/ACS and Number of Samples”	REPLACED “REF 200 SlicPrepV1” with “REF 200 1.5mL Tubes”
7	5	Section 2 “Wizard/Load Waste Drawer”	REMOVED “)” and ADDED “(slot closest to you)” after sentence “Insert partial or empty unit boxes into all four slots ensuring there is an empty unit box in slot 4”

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8	6	Section 2 "Wizard/Elution Slot/Configure Racks"	ADDED "S-Block" to the sentence "Place the deep well plate onto the metal transfer bracket in 'slot 1' with well A1 in the upper left corner."
9	6	Section 2 "Wizard/Load Reagents"	CHANGED "Remove the magnetic particle trough foil" to all caps and bold font
9	6	Section 2 "Wizard/Load Consumables"	ADDED "at least 60" to the sentence "Uncap and load sample prep cartridges in the last three slots as shown on the screen." ADDED "a full box of twelve" to the sentence "Uncap and load 8-Rod covers in the slot closest to you."
10	7	Section 2 "Wizard/Select Sample Carrier"	REPLACED all instances of "plate carrier" with "tube carrier" REPLACED "Wizard/Select Sample Carrier" with "Wizard/Load Sample Tubes"
11	7-8	Section 2 "Wizard/Load Sample Plate Carrier"	REPLACED "Wizard/Load Sample Plate Carrier" with "Wizard/Load Sample Tubes" REMOVED instructions on how to load a plate carrier and ADDED instructions on how to load tube carriers
11	NA	Section 2 "Wizard/Define Sample Rack Type" and "Wizard/Define Sample Rack Area"	REMOVED the sections "Wizard/Define Sample Rack Type" and "Wizard/Define Sample Rack Area"
12	8	Section 2 "Wizard/BatchX/Define Samples"	REPLACED "wells" with "tubes"

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12	8	Section 2 “Wizard/Batch X/Select Assay Control Sets”	REPLACED “quadrant” with “tube carrier” REPLACED “200 Slicprep V1” with “200 1.5mL Tubes”
12	8-9	Section 2 “Wizard/Elution Slot & Volume	REPLACED “quadrant” with “first set” in the sentence “Next to batch you will now see a ①” showing that the first quadrant of samples has been assigned to the batch.” REPLACED the paragraph beginning with “Repeat the above 4 steps” with “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. ADDED bullet point “Close the ‘Sample’ door”
NA	9	Section 2 Continuous Sample Loading	ADDED section “Continuous Sample Loading”
14	10	Section 2 Unloading Reagents and Consumables	ADDED “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.”
16	14	Section 2 Daily Maintenance Procedure	MOVED “You may now switch off the QIASymphony SP instrument” to the end of the section

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21	17	Section 3 Database Sample DNA Amplification Set-up using QIAgility	<p>REMOVED “not required” from the sentence “Quantification prior to amplification is not required or optional for these samples.”</p> <p>ADDED “however, it is important to remove all bubbles prior to placing on the QIAgility.” To the sentence “Centrifugation of the primer mix should be minimal to avoid primers collection at the bottom of the tube.”</p> <p>CHANGED master mix preparation volumes</p> <p>ADDED bullet point “vortex and spin all tubes briefly ensuring all bubbles have been removed.”</p> <p>REPLACED “an Applied Biosystems MicroAmp® Optical” with “a VWR” in the sentence Obtain an Applied Biosystems MicroAmp® Optical 96 well Reaction Plate.</p>
22	18	Section 3 Setting up the QIAgility	REPLACED “AB” with “VWR” in the C1 block
23	19	Section 3 Setting up the QIAgility	<p>ADDED “Cover the amp plate with amp tape and” to the sentence “transfer to the PCR room and place directly into the thermal cycler.”</p> <p>ADDED bullet point “Unload the deck of the QIAgility by removing and discarding empty tip racks and tubes located in the M1 block.”</p>
24	20	Section 3 Daily Maintenance Procedure	ADDED “the deck” to the sentence “Wipe down with ethanol.”

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25	20-21	Section 3 Weekly Maintenance Procedure	<p>ADDED “the blocks and” to the sentence “Replace the tip ejector chute”</p> <p>REPLACED “ one hour” with “30 minutes” in the sentence “UV decontaminate the worktable for a minimum of one hour ensuring all, samples, reagents, and consumables have been removed.”</p> <p>REMOVED bullet point “Return all components t the work table and close the hood”</p>
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