DNA EXTRACTION/PURIFICATION ON THE QIAsymphony® SP - Reference Samples

Background

The QIAsymphony® SP with the QIAsymphony® DNA Investigator Kit reproducibly automates purification of genomic DNA from 1-192 reference samples encountered in forensic applications. Samples, reagents and consumables, eluates and waste are separated in different drawers. Reagents for purification of DNA are contained in an eight trough reagent cartridge. Each trough contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Partially used reagent cartridges can be sealed with the Reuse Seal Strips for later reuse up to two weeks after opening.

The magnetic-particle technology is identical to that currently used at the Marshall University Forensic Science Center (MUFSC) with the EZ1® Advanced XL, and can provide a higher yield when the Carrier RNA, included in the kit, is utilized. The QIAsymphony® SP performs all steps of the sample purification, post-digestion, and can accommodate an input volume of 200µL for reference sample extractions.

Magnetic-particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. DNA is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted in Buffer ATE or Molecular Biology Grade water (MBG H_2O). Purification is efficient, and purified DNA is suitable for use in downstream analyses, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

Summary of Procedure

The reference extraction must be performed using blood card punches (1-3), Bode Collector punches (1-3), swab cuttings, or whole swabs as outlined in the QIAsymphony® SP Extraction- Reference Samples worksheet. The QIAsymphony® SP is utilized in conjunction with QIAsymphony® DNA Investigator Kit available from Qiagen®, which includes all required reagents with the exception of Top Elute for the extraction of 192 samples. Consumables are sold separately by Qiagen®.

Sample Handling

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- **Safety Manual**, *Universal Precautions*
- **Quality Assurance Manual**, *General Sample Control and Forensic Sample Preservation Policy*
- **Analytical Procedures Manual**, *Forensic Evidence Handling*

After purification is complete, samples are securely stored at 2-8°C.

Warnings and Precautions

Lysis buffer contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures and wear gloves when handling. This reagent is **not** compatible with disinfecting agents which contain bleach.

Reagent cartridges contain ethanol, isopropanol and guanidine thiocyanate. These substances should be considered flammable, harmful, and irritants.

Improper use of the QIAsymphony® SP may cause personal injuries and/or damage to the instrument. The instrument must only be operated by qualified personnel who have been appropriately trained.

All users MUST read and be familiar with all safety precautions outlined in the first chapter of the QIAsymphony® SP/AS User Manual-General Description.

Damage to the instrument caused by failure to follow these guidelines may result in void of the warranty.

Reagents and Materials

QIAsymphony® DNA Investigator Kit (192 reactions*)
- 2 Reagent cartridges
- 2 Enzyme racks
- 2 Piercing lids
- 20mL Buffer ATE
- 20mL Buffer AVE
- 2 X 50mL Buffer ATL
- 3 X 1.4mL Proteinase K
- 310µg Carrier RNA
- 2 Reuse Seal Strips
- 1 Trough cover (for magnetic particles)
- 1 Handbook

60mL Top Elute Fluid
MBG H_2O 200uL
Disposable filter tips (32 tips per tip rack / 4 tip racks per sealed blister pack)
1500uL Disposable filter tips (32 tips per tip rack / 4 tip racks per sealed blister pack)
Sample prep cartridges (28 cartridges pre-racked per unit box)
8-rod covers (12 covers pre-racked per unit box)
Qiagen S-blocks (96 well tray lysates)
Aluminum seal plate covers
Qiagen EMT elution trays and septa strips (96 well elutions)
Qiagen skirted 2mL screw top tubes (single tube lysates)
Qiagen 2mL screw top tubes (single tube elutions)
QIAsymphony® SP sample tube carriers
QIAsymphony® SP sample tube carrier adaptors
QIAsymphony® SP sample plate carrier
QIAsymphony® SP elution racks
QIAsymphony® SP Accessory Trough
Universal pipette tips
1mL-5mL pipette tips
0.2mL-1mL pipette tips
Thermo mixer-96 well plate and/or single tube
Incubation oven
Centrifuge – 96 well plate and/or single tube
Spin Baskets
2mL Dolphin tubes
15mL conical tubes
50mL reagent boats
Gloves
Lab Coat
Mask
Permanent Marker/tough tag labels
Kimwipes
Vortex
Micro-centrifuge
Protective Eyewear

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. The QIAsymphony® DNA Investigator Kit reagent cartridges must be used ***within 2 weeks of opening*** **or after 15 hours of uncapped open time**, due to the reagent concentration changes that occur with evaporation during storage. Record in the **Reagent Log**.

Quality Control

Use of the **QIAsymphony® 96-Wel Reference Sample DNA Purification** worksheet or **QIAsymphony® Reference Sample DNA Purification Tube-to-Tube** worksheet is required for documentation. All information must be completed.

Positive Control

Extraction Positive Control (TF- Optional): Positive control(s) may be processed and run with each set of extractions and used to ensure the extraction, amplification and typing procedures are working as expected.

Negative Control

Extraction Negative Control: Reagent negative control(s) are processed and run with each set of extractions and consists of all reagents used in the procedure but contains no DNA sample.

Procedure

When a new kit is opened:

- Log the Opened Date and Use By Date
- Dissolve the lyophilized carrier RNA in 1.6mL Buffer ATE (provided in the QIAsymphony® DNA Investigator Kit).
- Transfer 400uL to each of the 2 empty tubes within the included enzyme rack
- Add an additional 1.2mL Buffer ATE to each tube and mix by pipetting up and down several times.
- Store the stock carrier RNA at -20°C for use with the second reagent cartridge/enzyme rack in the kit. Store the enzyme rack at 2-8°C (will be stable for 4 weeks).
- Remove the magnetic-particle trough from the reagent cartridge frame, vortex for at least 3 minutes, and replace it in the reagent cartridge frame before the first use.

Before starting:

- Complete the sample orientation portion of the QIAsymphony® SP Extraction- Reference Samples worksheet.
- Switch on the QIAsymphony® SP by pushing the blue button on lower left corner. It may take up to 15 minutes to boot up.
- Heat Buffer ATL at 70°C until all precipitate dissolves.
- Permanently label the sample tubes/trays and elution tubes/trays with tray/case ID, date and initials.
- Ensure that the magnetic particles are fully re-suspended.
- Place the enzyme rack with the diluted carrier RNA into the reagent cartridge holder.
- If desired elution reagent is MBG H_2O, add appropriate volume of MBG H_2O to accessory trough and place in tip rack position 5 or 12.
- Remove the lids of both carrier RNA tubes and store in the dedicated slots of the gray reagent cartridge holder.
  Before using a reagent cartridge for the first time, place the piercing lid on top of the reagent cartridge (it will be forced down and locked in place by the QIAsymphony® SP during purification).

Caution: The piercing lid is sharp. Take care when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

- Before using a reagent cartridge for the first time, check that Buffer QSL3 does not contain a precipitate. If necessary, remove the trough containing Buffer QSLE from the reagent cartridge and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the trough in the correct position. If the reagent cartridge is already pierced, make sure that the troughs are securely sealed with Reuse Seal Strips and incubate the complete reagent cartridge for 30 minutes at 37°C with occasional shaking in a water bath.

1. Select Log-in on the QIAsymphony® SP touchscreen then choose **Supervisor** and enter the password.

2. Prepare the deck of the QIAsymphony® SP and perform a complete scan, following the instructions detailed on the touchscreen and pages 12-22 of the Qiagen QIAsymphony® DNA Investigator Handbook. Make sure you load a sufficient amount of reagent and consumables on the deck for your run. The R+C tab of the touchscreen will show a post-scan count of all reagents and consumables on the deck.

   Note: If insufficient reagents are loaded on the instrument, the R+C tab on the touchscreen will flash yellow and indicate which reagents are insufficient/missing. Any reagent placed in the accessory trough will be referred to as EtOH.

   - Reagent cartridges: 96 samples
   - Sample prep cartridges: 15 cartridges for every 24 samples to be purified
   - 8-rod covers: 3 covers for every 24 samples to be purified
   - 200uL Disposable filter tips: 4 tips for every 24 samples to be purified
   - 1500uL Disposable filter tips: 80 tips for every 24 samples to be purified

3. If eluting in single tubes, place labeled 2mL screw cap tubes in correct position of elution rack. Sample carrier positions are numbered 1-24. Elution tube racks have 6 columns (1-6) and 4 rows (A-D). Lysates in positions 1-4 will be eluted into positions A1-D1 of the elution tube rack; lysates in positions 5-8 of the sample carrier will be eluted into positions A2-D2 of the elution rack. A witness must check the setup of the elution tubes against the worksheet and initial the appropriate space on the worksheet.

4. If eluting in a 96 well tray, remove the bottom of a Qiagen 96 well EMT plate and place on the appropriate elution rack. The Qiagen EMT elution plate base must be labeled with a tough tag on the base prior to the plate being put on the instrument. Label must include: Extraction ID, date, and initials

   Note: The bottom MUST be removed from Qiagen EMT elution plates prior to placing on elution rack.

5. Open the elution drawer and place the elution rack(s) on the deck. Close the drawer and select the rack position on the touchscreen, then select “add tray”. Another scan will be performed on the elution drawer only.

6. Select Rack ID to assign a name to the purification batch.

7. Select the desired elution volume.

8. Wells should not be skipped. However, if necessary, Do NOT enter a value in the “reserved wells” field unless you want to SKIP elution wells. Positions in the elution rack can be skipped by entering a value in the “reserved wells” field. This would be useful if your lysates are in columns 1-6 of the S-block, but you want to elute in columns 7-12 of the elution tray

   Note: The QIAsymphony SP Result File must be printed post extraction. This file must be checked to ensure the Input [well] position corresponds with the Output [well] position (see below).

9. For each sample to be purified, add a portion of the substrate to either a sample tube or the appropriate well of a Qiagen S-block tray. A witness must observe the placement for CODIS punches and initial the appropriate space on the worksheet.

Note: ALL substrates except for 1.2mm Harris punches MUST be incubated in single tubes. The lysate may be added to the appropriate well of the S-block tray after substrate removal, if desired.

10. Prepare lysis buffer master mix containing 180uL Buffer ATL and 20uL Proteinase K per sample (plus an additional 5%).

11. Vortex master mix and spin briefly. Add 200uL to each tube/well containing substrate or reagent blanks.

12. If incubating in the Qiagen S-block tray, seal with an aluminum cover.

13. Incubate at 56^{o}C for at least 15 minutes on a thermo mixer at 900rpm. Vortex and spin down briefly.

14. Remove all substrates except for 1.2mm punches from lysates. If transferring lysate into another tube or into the S-block, a witness must observe this transfer and initial the appropriate space on the worksheet.

Note: Optional step to dry substrate for maximum yield: Place substrate into Spin basket. Centrifuge at 13200rpm for 5 minutes. Remove Spin basket and place in a new labeled sample tube for storage. Transfer entire lysate to labeled sample tube or appropriate well of S-block for purification on the QIAsymphony.

1. If purifying from single tube lysates, place the sample tubes on a sample tube carrier containing the appropriate sample carrier tube adaptors. A witness must check the setup of the sample tubes against the worksheet and initial the appropriate space on the worksheet. If purifying from a 96 well S-block, place the tray on the sample plate carrier.

15. Open the sample drawer and slide the sample carrier(s) onto the deck. A batch will appear on the touchscreen. Follow the directions on the touchscreen to assign tube type and protocol for each batch of 24 samples to be purified.

16. Select Queue for each batch and then run.

17. When purification is complete, open the elution drawer and remove the elution rack. Cap, remove and store eluates.

18. Select the elution rack position on the touchscreen and select “remove rack”. The instrument will perform a scan to ensure the rack has in fact been removed.

19. Open the waste drawer and empty the unit boxes into the biohazard waste. Check the tip waste container and empty into biohazard sharps as needed. Check the liquid waste bottle and empty into designated container as needed.

20. Open the reagent drawer and remove the Top elute and reagent cartridge from the deck. Cap all troughs and the Top elute and store at room temperature.

21. Discard any empty tip racks into the regular trash.

22. Complete required cleaning/maintenance procedures outlined in the MUFSC Maintenance Procedures.

23. Using a thumb drive collect the *QIAsymphony SP Result File* from the software. This is located in *“Tools”* tab on the main screen. Select “File Transfer” button under the “In-/Output Files” tab, select the “Results File” button and choose “Transfer” button. This will transfer the files to the USB. Using this HTML file, compare the “Input Position” well to the “Output Position” well. This should correspond to the worksheet used to set-up the QIAsymphony and any subsequent worksheet. This HTML file must be printed and included in the case file. This file must be checked to ensure the Input [well] position corresponds with the Output [well] position.

Samples										
Sample ID	Input position	Type	Liquid-level detection	Output position	Assay control set	Reagent rack (beads + reagents)	Reagent rack (enzymes)	IC tube position/Liquid-level detection	Eluate volume	Validity of result
_01_2000219	A:1	S	N	A:1	CW200_ADV_HE CR21308 ID1604 V1	1,BufferBottle-1	1	n/a	30 µl	unclear, 180047
_02_2000219	B:1	S	N	B:1	CW200_ADV_HE CR21308 ID1604 V1	1,BufferBottle-1	1	n/a	30 µl	unclear, 180047
_03_2000219	C:1	S	N	C:1	CW200_ADV_HE CR21308 ID1604 V1	1,BufferBottle-1	1	n/a	30 µl	unclear, 180047
_04_2000219	D:1	S	N	D:1	CW200_ADV_HE CR21308 ID1604 V1	1,BufferBottle-1	1	n/a	30 µl	unclear, 180047
					CW200_ADV_HE					

24. Shut down the instrument by pushing the blue button on lower left corner.

Comments on Sample Storage

DNA samples must be stored separately from reagents and non-amplified DNA must be stored separately from amplified product.

Technical Assistance

For information and assistance regarding the performance or applications, contact the Qiagen Technical Service Department 1-800-362-7737.

References

1. QIAsymphony® SP DNA Investigator Handbook, February 2013. Qiagen
2. QIAGEN® Sample & Assay Technologies QIAsymphony® SP Protocol Sheet
3. QIAsymphony® SP/AS User Manual- General Description version 3.1, May 2013. Qiagen
4. QIAsymphony® SP/AS User Manual- Operating the QIAsymphony® SP, April 2012. Qiagen

Appendix A: Reference Sheet for QIAsymphony

Custom Protocol

CW200ADVHE_CR23153_ID3974	200*µl* lysate with H_2O elution
CW500ADVHE_CR23153_ID3975	500*µl* lysate with H_2O elution
CW200ADVHE_CR22931_ID3666	200*µl* lysate with ATE elution
CW500ADVHE_CR22931_ID3667	500*µl* lysate with ATE elution

Labware

Elution Tray	Deep Well------QIA#19588 *EMTR
Elution Tubes	SAR#72.607 *T1.5 Screw
Lysis Tray- S-Block	Deep Well------QIA#19585 S-Block96
Lysis Tubes	SAR#72.694 T2.0 ScrewSkirt