Aurora Forensic Sample Clean-up Protocol

106-0008-BA-D

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Table of Contents

Introduction

This protocol is for removing inhibitors that remain in DNA samples after purification by other methods, which is necessary in order to obtain a Short Tandem Repeat (STR) profile for forensic studies. The protocol works with input from phenol-chloroform preparations, commercial silica-based spin columns, and other preparative methods that yield DNA with relatively low conductivity (see input specifications below).

This protocol is appropriate for recovering DNA molecules 0.3-50 kb in length. The presence of shorter or longer molecules will not impair yield of 0.3-50 kb fragments.

Input Sample Specifications:

Volume: 5 ml

Conductivity: ≤100 µS/cm when diluted to 5 ml

The conductivity of the sample after dilution to 5 ml must be ≤100 µS/cm, which is similar in conductivity to 0.2x TE or 0.1x TBE. Use deionized water or very weak buffer solutions when resuspending or eluting a sample for use with this protocol. DNA in the sample must be free to migrate in response to electric fields.

Example results

To demonstrate the ability of the Aurora to provide highly purified DNA from an inhibited sample, genomic DNA from an object from a criminal investigation was analyzed. Investigators suspected that this object was used as a weapon in a murder and thus two profiles were expected to emerge, those from the victim and the perpetrator. In this case, phenol:chloroform (PC) extraction was used to obtain DNA from the object, followed by a second PC extraction and dilution in an attempt to remove more inhibition, after which the **Aurora Forensic Sample Clean-up Protocol** was used.

To ensure maximum DNA recovery and avoid losses through swabbing, the tip of the suspected weapon, where touch-DNA was believed to have been, was submersed in lysis buffer (10 mM Tris-HCl pH=8.0, 10 mM EDTA, 50 mM NaCl, 2% SDS and Proteinase K, 0.4 mg/ml). To avoid cross-contamination from the blood to touch-DNA as much as possible, a volume was chosen to cover the end opposite to the victim's blood (Q1-1, Figure 1). However, as a precaution, a second volume of lysis buffer was chosen to cover a larger surface area in case more touch DNA could be retrieved; the second extraction (Q1-2) was performed after the lysate for Q1-1 had been collected. Blood Internal Standards, BIS (20 µl) and a blank were also prepared and extracted in the same manner as positive and negative controls, respectively.

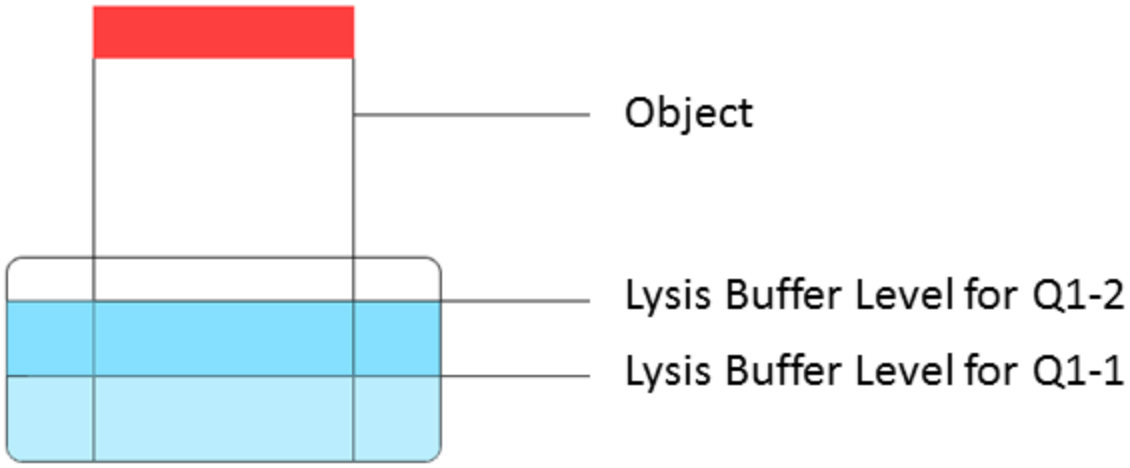


Figure 1. Illustration of the object processed twice with two different volumes.

Following an overnight incubation at 56 ^{o}C for each extraction, the object was removed and the collected Q1-1 and Q1-2 lysates were diluted by a factor of 10 with Filtered Autoclaved Water (FAD H_2O). The diluted supernatants were then concentrated to 500 µl in an Amicon Ultra-15, 30 KDa centrifugal filter (catalogue no. UFC903096) at 4,000 *g*.

The supernatants were each transferred to a 2 ml heavy phase-lock gel (Inter-Medico, 5P2302830) where 400 µl of phenol: chloroform: isoamyl alcohol (25:24:1) was added and centrifuged at 13,000 rpm for 5 min. A final extraction step with N-butanol, in place of phenol:chloroform, was performed using the same centrifugal conditions as above. The supernatants were then concentrated and washed twice with TE buffer in a Vivacon-500 ETO centrifugal filter (Vivaproducts, VN01H42ETO) before being eluted in 50 µl of the same buffer.

SCODA and Speed-Vac Purification

The samples eluted from PC extraction (Round 2) were each diluted to 5 ml with FAD H_2O in the sample chamber of a 210-0001-AA-D cartridge, the cartridges were loaded onto the Aurora, and the **Aurora Forensic Sample Clean-up Protocol** was run. In each case a 60 µl output was collected and further concentrated by speed-vac (Vacufuge™, Eppendorf) at room temperature. This was only possible because the **Aurora Forensic Sample Clean-up Protocol** enables successful PCR amplification from very highly concentrated DNA samples due to its thorough removal of PCR inhibitors. Finally, the lyophilized samples were re-suspended in 15 µl TE buffer for quantification and STR amplification.

Quantification and STR Profiling

Quantification and STR profiling of DNA was performed using the Applied Biosystems Quantifiler® Human DNA Quantification Kit (catalogue no. 4343895) and the AmpFlSTR® Profiler Plus™ PCR Kit (catalogue no. 4303326), respectively. 25 µl PCR reactions were carried out for both assays and amplified according to manufacturer specifications.

STR products were separated and visualized on an ABI Prism™ 310 Genetic Analyzer (catalogue no. 310-00-100/120/W) with POP-4™ polymer (catalogue no. 402838). Electrokinetic injection and electrophoresis run times were set to 5 s and 24 min respectively, allowing for the resolution of the

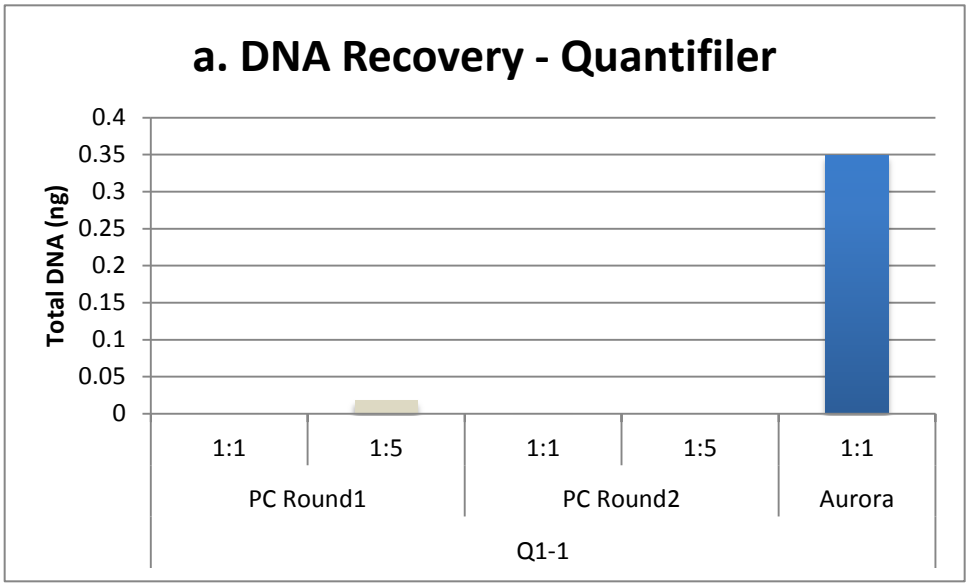
400 bp peak of the GeneScan™ 500 ROX™ sizing standard (catalogue no. 401734). Data Collection Software v3.1 and Genemapper ID v3.2 (Applied Biosystems) were used for data collection and analysis.

Although quantification suggested that sufficient amounts of DNA were obtained for Q1-2 for PCR amplification, no profile was obtained (data not shown). The amplicon size in the Human Quantifiler Kit is 62 bp, while those from Profiler Plus range from 100-350 bp, thus the Quantifiler is not always a good indicator of suitability for STR amplification. It is probable that DNA from Q1-2 was too degraded or fragmented for STR amplification. It is also worth noting that because the Q1-2 lysis area included the area previously sampled for Q1-1, the concentration of DNA recovered from Q1-2 might be expected to be less than that for Q1-1; however, the distribution of touch-DNA on the surface of the object remains unknown.

Figure 2a shows that amplification of Q1-1 was either below the standard curve or non-existent in undiluted and 1:5 diluted PC extracts, but was successful with undiluted output from **the Aurora Forensic Sample Clean-up Protocol**. These results are consistent with the presence of PCR inhibitors in the PC extracts. Figure 1b shows that PCR amplification of the internal positive control (IPC) was completely inhibited in the undiluted Q1-1 sample even after two rounds of PC extraction, and Q1-1 at a dilution of 1:5 amplified over 5 cycles later than the DNA standards, resulting in failed STR amplification. Although IPC amplification for the 1:5 dilution of the second PC extract was uninhibited, there was insufficient DNA present for STR amplification, indicating the necessity of removing inhibitors without further diluting the sample.

When the same PC extract from Round 2 was further purified using the **Aurora Forensic Sample Clean-up Protocol**, amplification of the undiluted sample was successful even after subsequent concentration by speed-vac. (Note that analysis of the output from Aurora for Q1-1 at a 1:5 dilution was not performed due to limited amounts of material available for STR amplification). Visually, the brown pigmentation seen in Q1-1 after PC extraction, and the fact that organic matter covered the suspected weapon, suggests that the contaminant responsible for PCR inhibition may be humic acid.

Figure 3 is an electropherogram of the mixed, partial profile that was obtained from sample Q1-1 using the **Aurora Forensic Sample Clean-up Protocol**. Amplification was unsuccessful for 1:5 and 1:10 dilutions of the Q1-1 PC extracts. These extracts were likely too inhibited, and/or contained insufficient DNA for STR amplification, consistent with results from qPCR. The STR profiles for the Q1-1, BIS and negative control extracts were compared, confirming that no cross-contamination had occurred (data not shown).



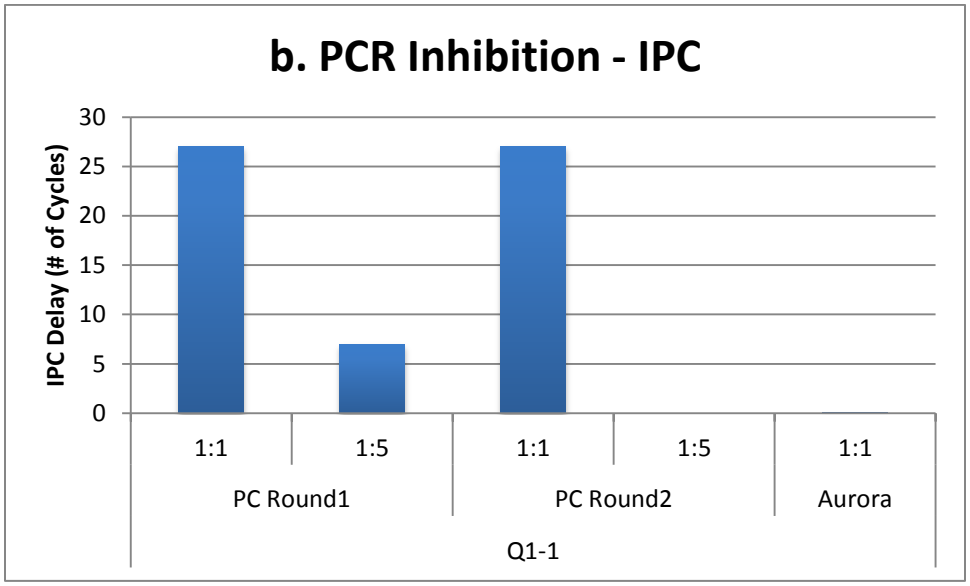


Figure 2. Quantifiler™ Human DNA Quantification Results.
(a) Quantifiler Human DNA quantities of Q1-1 from two phenol: chloroform extracts (PC Round 1 and 2) and the **Aurora Forensic Sample Clean-up Protocol**. Undiluted samples are denoted as 1:1, while 1:5 indicates that the sample was diluted by a factor of 5. The beige bar indicates that DNA amplified below the standard curve. **(b)** PCR inhibition represented as the delay in IPC threshold cycle (C_T) from averaged IPC C_T values of DNA standards.

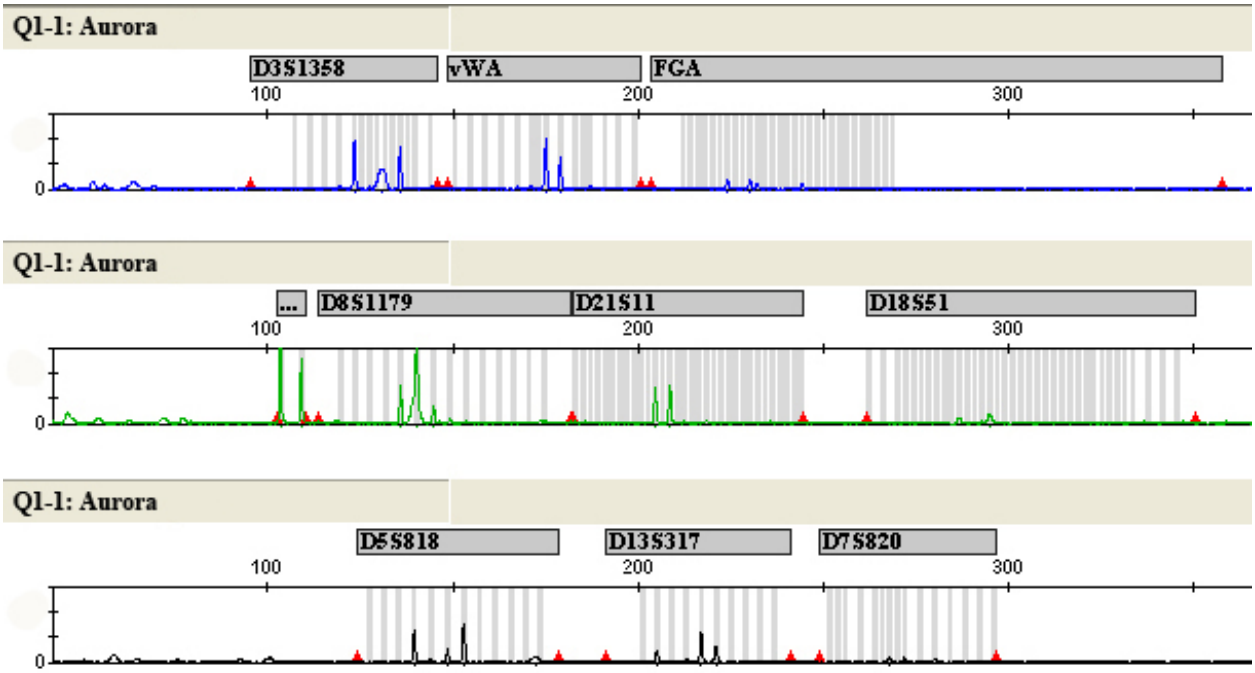


Figure 3. STR profile recovered from the object after the Aurora Forensic Sample Clean-up Protocol. Scale and range of Relative Fluorescent Units (RFUs) and allele calls were removed to protect the identity of those involved in the investigation.

Safety guidelines

Wear gloves during all stages of the protocol. Avoid skin contact with all reagents. Cartridges are made from non-hazardous plastics, metal, TBE buffer and agarose. Appropriate precautions should be taken if hazardous samples are used with the cartridges.

Procedure overview

- **Prepare the sample**
- **Prepare the cartridge**
- **Load the sample**
- **Prepare the Aurora**
- **Begin the run**

Preparing the sample

Process samples using your choice of DNA purification methods, following required safety procedures. Alternatively, if your sample meets input sample specifications, it may be run directly without pre-processing.

Aurora DNA purification works best with low-conductivity samples. To maximize DNA yield, take steps to keep conductivity of the Aurora input as low as possible. These may include eluting in deionized water instead of buffer or repeating wash steps, depending on the purification method. Additionally, it may be possible to improve the yield of silica columns by eluting in larger-than-recommended volumes, for subsequent concentration with SCODA.

Dilute the extracted product to 5 ml with a low conductivity buffer like 0.01x TBE, 0.01x TE, or nuclease-free deionized water. Final sample conductivity must be ≤100 µS/cm. Running more conductive samples will decrease yield.

Loading your sample and running the Aurora

Please refer to the **Aurora Reusable Cartridge Handling Manual** (**106-0014**) for detailed instructions on how to load your samples into the **Aurora Reusable Cartridge (211-0004-AA-D)**, prepare the Aurora instrument and run the Aurora protocol. Please select the **106-0008-BA-D FORENSIC DNA CLEAN-UP PROTOCOL** file when asked to select the protocol **.sp** file appropriate for your application.

Troubleshooting

Please see the Aurora user manual for more information about troubleshooting machine faults.

1 Error: The Aurora control software warns that the sample is too conductive.

Running high conductivity samples will result in lower yields and may cause gel damage and other issues during the run. The Aurora instrument will give the warning “Injection Conductivity test failed. Sample conductivity is too high. Injection might fail” for highly conductive samples. Conductivity for a 5 ml sample should be ≤100 µS/cm.

Immediate Remedy: The run can continue, but yield will be decreased. In general, yield decreases with increasing conductivity over 100 µS/cm.

Solution: To solve this problem, adjust the DNA extraction protocol to reduce the amount of salt in the sample. Some suggestions are, depending on the method employed, to elute samples from silica column based methods in nuclease-free water or 0.1x TE buffer, resuspend DNA pellets in nuclease free water or 0.1x TE buffer, and to increase the number of ethanol-based washes in precipitation methods.

2. Failure Mode: PCR reactions remain inhibited even after processing with the Aurora.

Solution: Increasing the time of the wash block may help improve contaminant rejection. Some dilution of the Aurora output may still be necessary for best amplification. This protocol may also be less effective at rejecting contaminants that are complexed with or bound to DNA. The addition of low conductivity additives such as proteinase K prior to injection may help reduce the amount of bound contaminants.

3. Failure Mode: Yield is too low.

Solution: This failure mode can have several causes. In the case where the sample did not contain sufficient DNA, try processing more of the sample through the lysis and desalting steps. If using a silica-based column, eluting off the column with larger-than-recommended volumes can assist in the recovery of small amounts of DNA.

Yield may be reduced if the sample conductivity is too high. See troubleshooting Error 1, as well as the Troubleshooting section in the Aurora user manual for details in resolving this failure mode. Yield may also appear low if the sample contains contaminants that are bound to DNA. Diluting the input sample and performing multiple SCODA runs may help. Post-SCODA, a dilution series of DNA templates will indicate any remaining PCR inhibition. See Failure Mode 2 to address this problem.

Ordering and support

For support for Aurora protocols or cartridges, or to order additional cartridges, please contact support@borealgenomics.com.

SCODA conditions

These conditions are pre-programmed in the **AURORA FORENSIC SAMPLE CLEAN-UP PROTOCOL.SP** file that accompanies this protocol guide and are intended for reference purposes. Note that electric current or power values that slightly exceed these expected values may not indicate a problem.

Cartridge

Running buffer	0.25x TBE
Sample volume	5 ml
Expected sample conductivity	≤100 µS/cm

Injection

Injection voltage	600 V
Injection charge	3000 mC

Expected current	9-13 mA
Expected average power	8-12 W
Expected voltage drop across the gel	≤10%

Wash (6 Channel)

SCODA field strength	70 V/cm
SCODA cycle period	4 s
Duration	2.5 h
Wash strength	20%

Expected current	20-30 mA
Expected power	7-9 W

Focus

SCODA field strength	70 V/cm
SCODA cycle period	4s
Duration	1.5 hrs

Expected current	30-45 mA
Expected power	9-11 W