Aurora DNA Clean-up Protocol

106-0001-CA-D

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Table of Contents

Introduction

This protocol is for removing contaminants from DNA that remain after purification by other methods, including solid phase extraction. The protocol works with input from phenol-chloroform preparations, commercial silica-based spin columns, and other preparative methods that yield DNA in small volumes with relatively low conductivity (see input specifications below). The protocol recovers 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. This protocol provides purified DNA that is suitable for downstream processes including PCR, library construction and DNA sequencing.

Input Sample Specifications:

Volume: Up to 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.

Example results

To demonstrate the ability of the Aurora to provide highly purified DNA from contaminated samples, genomic DNA from organisms in nine 0.25 g soil samples from Vancouver, BC was prepared using the MO BIO PowerSoil® DNA Isolation Kit (catalogue no. 12888). Three samples were held for analysis with no additional purification, three samples were further purified using the MO BIO PowerClean® DNA Clean-Up Kit, and three samples were further purified using this protocol.

To take advantage of the greater input capacity of the Aurora and its ability to deliver a highly concentrated DNA sample, the final elution from the PowerSoil® column for samples prepared for Aurora purification was performed with 500 µl of nuclease-free water (instead of the recommended 100 µl of MO BIO C6 Elution Buffer) and then further diluted to 5 ml in 0.01x TBE. Both MO BIO protocols provide DNA in 100 µl, while the Aurora delivers a 50 µl DNA sample.

A dilution series of each sample was prepared and analyzed by qPCR with the 16S rDNA primers and probes described by Nadkarni *et al.* (Microbiology 148:257, 2002). 5 µl of each diluted sample was used as the template in a 25 µl reaction containing Roche Applied Science FastStart Universal Probe Master Mix (catalogue no. 04913957001) to 1x. Forward and reverse primers were each used at 200 nM and probe was used at 100 nM. Amplification reaction conditions were 50 °C for 2 min, 95 °C for 10 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The relative amount of DNA present was estimated by comparison to a standard curve prepared from *E. coli* genomic DNA.

Figure 1 shows the estimated amount of template DNA per 5 µl PCR input for PowerSoil® output, PowerSoil® output processed through the MO BIO PowerClean® kit, and PowerSoil® output following clean-up using the Aurora DNA Clean-up Protocol. Because this estimate is constrained by the presence

of PCR inhibitors in the sample, it can be viewed as a measure of the clearance of PCR inhibition after the removal of inhibitors while minimizing the concomitant loss of DNA. The estimated amount of DNA template in each PCR reaction is shown. Despite containing the most template DNA, PCR amplification was inhibited in undiluted samples for all three treatments due to the presence of PCR inhibitors. The best PCR amplification occurred at a 1:5 dilution using the Aurora DNA Clean-up Protocol, which gave the greatest reduction in PCR inhibition. Increasingly dilute samples showed further reductions in inhibition but also decreased PCR yield due to the decreased amount of template DNA in the PCR reactions.

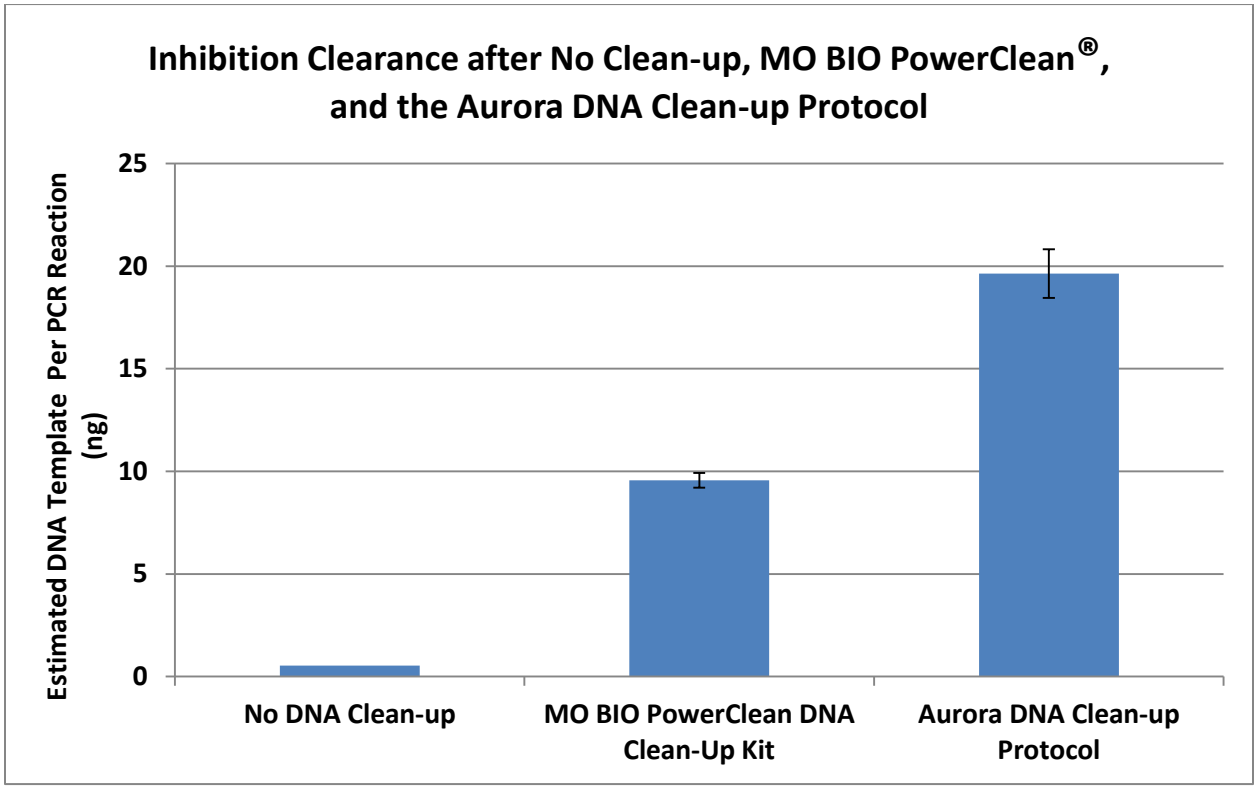

Figure 1. Estimated mass of DNA template per PCR reaction. These estimates assume that the average genome size and 16s copy number of bacteria in the soil sample are equivalent to those of *E.coli* (~5 Mbp and n=7, respectively).

Safety guidelines

Wear gloves during all stages of the protocol. Avoid skin contact with all reagents. Appropriate precautions should be taken if hazardous samples are used with the cartridges.

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.

SCODA works best with low-conductivity samples. To maximize SCODA yield, take steps to keep conductivity of the SCODA 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.

Final Sample Dilution

Dilute the DNA extract to 5 ml with a low conductivity buffer such as 0.01x TBE, 0.01x TE, or nuclease-free deionized water. Invert gently until evenly mixed. Do not vortex as the DNA may shear. Final sample conductivity must be less than or equal to 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 prepare your Aurora Reusable Cartridge (**211-0004-AA-D**), load your sample into the cartridge, prepare the Aurora instrument and run the Aurora protocol. Please select **106-0001-CA-D AURORA DNA CLEAN-UP PROTOCOL** 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 ≤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. This protocol uses cartridge part number **211-0004-AA-D**.

SCODA conditions

These conditions are pre-programmed in the **106-0001-CA-D AURORA DNA 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