Aurora 0.3-10kb DNA from Water Protocol

106-0007-BB-D

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Introduction

The **Aurora 0.3-10kb DNA from Water Protocol** is for extracting and purifying DNA from contaminated water samples containing inhibitors of enzyme activity. Water is first filtered through a nitrocellulose filter, processed with the MO BIO PowerWater® DNA Isolation Kit, and then futher purified by the Aurora to remove contaminating inhibitors. DNA extraction is highly efficient but yield will vary depending on the source of the water and the type and number of organisms in the sample. The protocol provides high purity DNA that is suitable for PCR, library construction, deep sequencing for metagenomic analysis, and other enzymatic processes requiring concentrated samples.

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 water samples, genomic DNA from organisms in eight 100 ml water samples from Trout Lake in Vancouver, BC was prepared using the MO BIO PowerWater® DNA Isolation Kit (catalogue no. 14900). Three samples were held for comparison, two samples were further purified using the MO BIO PowerClean® DNA Clean-Up Kit, and three samples were further purified using the **Aurora 0.3-10kb DNA from Water Protocol**. Both MO BIO protocols provide DNA in 100 µl, while the Aurora delivers a 50 µl DNA sample.

A dilution series of each DNA sample was prepared with nuclease-free water 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 100 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 concentration of template DNA in PowerWater® output, PowerWater® output processed through the MO BIO PowerClean® kit, and PowerWater® output following clean-up with the **Aurora 0.3-10kb DNA from Water Protocol**. Samples prepared with the **Aurora 0.3-10kb DNA from Water Protocol** gave the best PCR amplification in all cases, for both diluted and undiluted samples (Figure 1; data not shown for dilutions beyond 1:5). PCR amplification was inhibited in undiluted samples to varying degrees in all three treatments due to contamination with PCR inhibitors. Both the Aurora and PowerClean® reduced PCR inhibition relative to PowerWater® alone, but the resulting loss of DNA in the PowerClean® procedure was substantial. In contrast to the other undiluted samples, the

undiluted **Aurora 0.3-10kb DNA from Water Protocol** samples amplified very well, making dilution prior to PCR unnecessary.

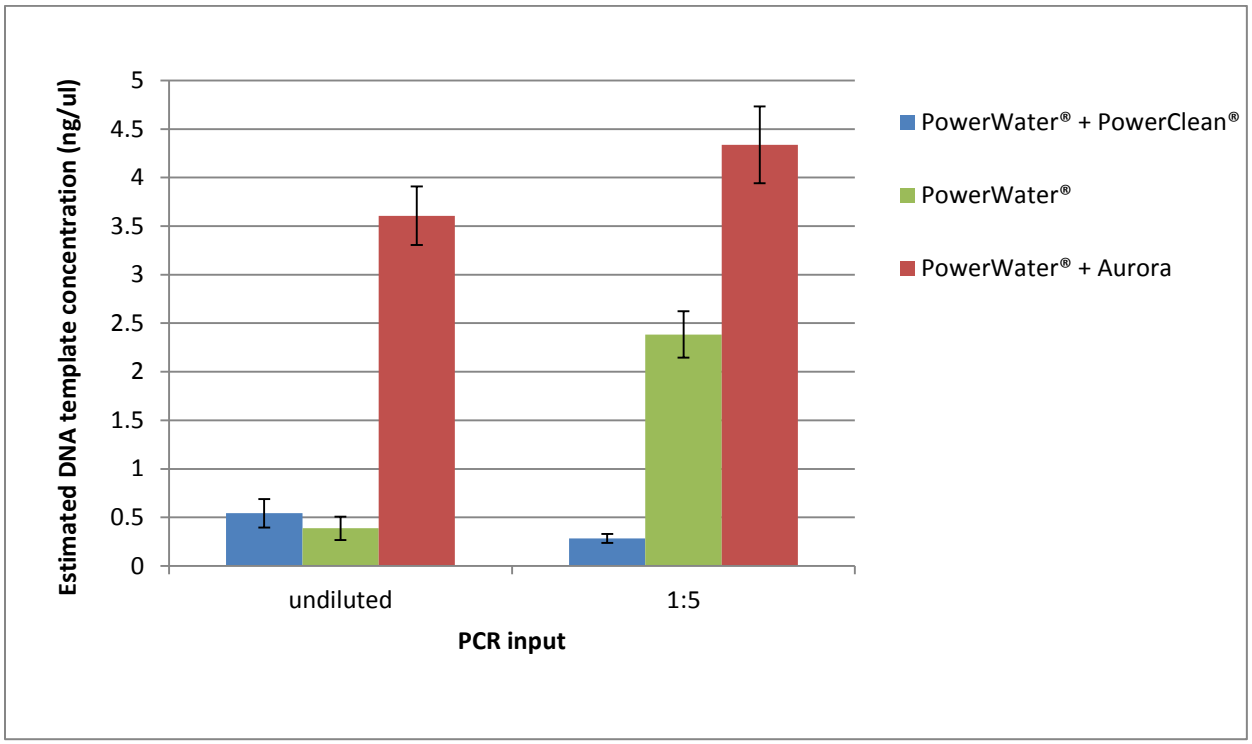

Figure 1. Estimated concentration of DNA template produced by each purification scheme. These estimates assume that the average genome size and 16S copy number of bacteria in the water sample are equivalent to those of *E.coli* (~5 Mbp and n=7, respectively).

Safety guidelines

Wear gloves during all stages of the protocol. Avoid skin contact with all reagents.

Preparing the sample

Initial DNA extraction using the MO BIO PowerWater® DNA Isolation Kit

Filter water through a Millipore 0.22 µm nitrocellulose filter (catalogue no. GSWP047S0), use sterile forceps to place the filter into a MO BIO PowerWater® Bead Tube, and follow the MO BIO PowerWater® DNA Isolation Kit instructions to process your water sample. The volume of water filtered will depend on the source of the water, its turbidity and the number and type of organisms in the sample. To take advantage of the input capacity of the Aurora and its ability to deliver a highly concentrated, purified DNA sample, the final elution from the PowerWater® Spin Filter can be performed multiple times in order to maximize DNA recovery. If necessary, you can minimize the sample conductivity by performing the final MO BIO elution step using nuclease-free dH_2O instead of the recommended Solution PW6.

Final Sample dilution

Pool all eluates from the PowerWater® Spin Filter and then dilute the pooled sample to a final volume of 5 ml with 0.0125x TBE, 0.01x TE, or nuclease-free deionized water. Invert gently until evenly mixed. At this point the sample conductivity must be ≤100 µS/cm prior to loading the sample into the Aurora cartridge. 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 the **106-0007-BB-D Aurora LMW DNA WATER PROTOCOL.SP** file when asked to select the protocol **.sp** file appropriate for your Aurora run.

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 to elute samples from the PowerWater® columns in nuclease-free water or 0.1x TE buffer, or to desalt the sample by diluting it to 15 ml with dH_2O, running the sample through an Amicon Ultra-15, 30 KDa centrifugal filter (catalogue no. UFC903096) at 4,000 *g*, and then diluting the remaining 250 µl sample back up to 5 ml with 0.01x TBE prior to loading the sample into the Aurora cartridge.

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 **106-0007-BB-D Aurora LMW DNA WATER 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