Aurora 0.7-50kb DNA from Water Protocol

106-0013-BA-D

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Introduction

The **Aurora 0.7-50kb DNA from Water Protocol** extracts and purifies DNA from contaminated water samples containing inhibitors of enzyme activity. Water samples are filtered through a nitrocellulose filter. The captured cells are lysed chemically and enzymatically and DNA is purified with the Aurora. This protocol is capable of recovering hundreds of nanograms of bacterial DNA from 100 ml of water, but DNA yield will vary depending on the source of the water and the composition of organisms in the sample. The protocol provides purified DNA up to 50 kb in length that is suitable for PCR, library construction, deep sequencing for metagenomic analysis, and other enzymatic processes requiring pure, concentrated DNA samples.

Input Sample Specifications:

Volume of Water Sample: Up to 100 ml

DNA Capacity: Up to 100 µg DNA

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

Example results

To demonstrate the ability of the Aurora to provide highly purified high molecular weight DNA from contaminated water samples, genomic DNA from organisms in 100 ml water samples from a pond in Vancouver, BC was prepared using the **Aurora 0.7-50kb DNA from Water Protocol**.

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. To demonstrate the range of molecular weight recovery, a 5 µl undiluted and purified sample was run on a pulsed field gel electrophoresis apparatus.

Two samples were prepared with the **Aurora 0.7-50kb DNA from Water Protocol** and the final bacterial DNA yield for both samples was estimated to be 40 ng. 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). Both samples amplified in PCR after a two-fold dilution.

Figure 1 shows the pulsed field gel run with equal volumes of undiluted sample output in each well. The **Aurora 0.7-50kb DNA from Water Protocol** purified DNA fragments up to 48.5 kb.

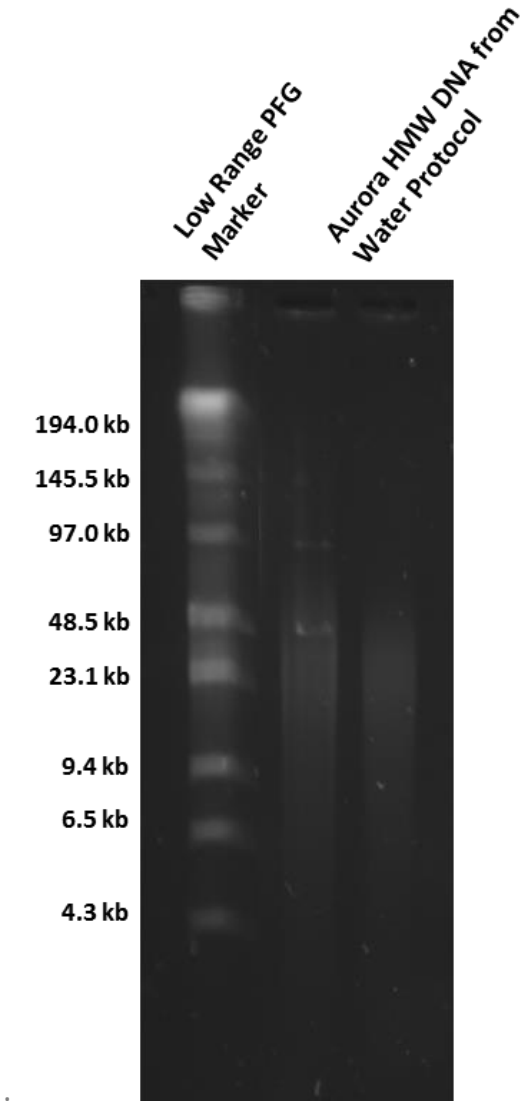

Figure 1. Image of a pulsed field gel run with a sample purified using the Aurora 0.7-50kb DNA from Water Protocol. Samples were separated by pulsed field gel electrophoresis with the following parameters: 1% SeaKem Gold Agarose, 1x TBE gel. 1x TBE Running Buffer. Initial Switch Time: 1 s, Final Switch Time: 12 s. Run Time: 9.5 h Angle: 120° Field: 12 V/cm. The gel is post-stained in 1x SYBR Green, 1x TBE Buffer and imaged under UV transillumination.

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.

Materials required

1. **DNA extraction buffer**

The following table makes enough buffer to process 3 L of water. The extraction buffer can be prepared in advance and stored at room temperature for up to 6 months. 1.5 ml of extraction buffer is required per 100 ml of sample.

Reagent	Volume (ml)	Final Concentration
1M Tris-HCl, pH 8.0	2.5	50 mM
0.5M disodium EDTA, pH 8.0	5	50 mM
Nuclease free dH2O	42.5	-
Total volume	50	

Note: Add lysozyme to 1 mg/ml immediately prior to use.

2. **General Reagents:**

Reagent	Volume (ml) required per gram of soil
20 mg/ml proteinase K, pH 8.0	0.1
20% sodium dodecyl sulfate (SDS)	0.1

3. **Equipment and materials required:**

- Millipore 0.22 µm Nitrocellulose filter (part number GSWP047S0)
- Axygen Scientific 4 ml screw cap tubes (part number SCT-5ML-S)
- Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-30 membrane (part number UFC903008)
- 1.5 ml microcentrifuge tubes
- Centrifuge with swing bucket or rotor accommodating 50 ml centrifuge tubes
- Microcentrifuge
- 65 °C water bath
- 50 ml centrifuge tubes
- Sterile wide bore 1 ml pipette tips (or cut off the ends of regular 1 ml tips and autoclave)
- Aurora instrument
- Aurora Reusable Cartridge (part number 211-0004-AA-D)

Preparing the sample

Lysis

1. Run up to 100 ml of sample through a Millipore 0.22 µm nitrocellulose filter. Use sterile forceps to roll the filter into a 4 ml screw cap tube.
2. Add 0.9 ml DNA extraction buffer with freshly added lysozyme to each tube. Vortex the sample tubes at maximum speed for 5 s and repeat with tubes inverted.
3. Rotate the tubes horizontally around the long axis for 1 h at 37 °C.
4. Add 0.1 ml of 20 mg/ml proteinase K and 0.1 ml of 20 % SDS. Invert the tube 10 times to mix.
5. Incubate the samples upright at 65 °C for 20 min.

6. Rotate the tubes horizontally, along the long axis, at 65 °C for 100 min.
7. Collect the supernatant from each tube with a wide bore pipette tip and transfer to a 1.5 ml microcentrifuge tube.
8. Rinse the filter with 200 µl of DNA Extraction buffer and vortex at maximum speed for 5 s. Collect the rinse buffer with a wide bore pipette tip and add to the lysate.
9. Centrifuge the lysate at 13,000 *g* for 1 min and transfer the supernatant to a 50 ml centrifuge tube.
10. Dilute the extracted supernatant to 30 ml with nuclease free dH_2O and invert at least 10 times to mix.

Desalting with an Amicon® Ultra-15, 30kDA centrifugal filter

1. Add 15 ml (12 ml if using a fixed angle rotor) of the diluted sample to an Amicon® Ultra-15 30 kDa centrifugal filter and centrifuge at 4,000 rpm for 15 min. Increase the centrifugation time if the sample volume remaining is greater than 500 µl. Discard the flow through.
2. Repeat step 1 until all the diluted sample has passed through the filter.
3. Dilute the sample in the filter back up to 15 ml with dH_2O and centrifuge again at 4,000 rpm for 15 min. Discard the flow through.
4. Extract the remaining sample from the filter into a 15 ml centrifuge tube. Rinse the filter with 800 µl of dH_2O by rocking the filter back and forth a few times. Collect the rinsate and pool the extracted samples. At this point, either proceed to the Aurora purification step or store the sample at -20 °C until ready for use.

Final Sample Dilution

1. Dilute the extracted product to 5 ml with a low conductivity buffer such as 0.01x TBE, 0.01x TE, or nuclease-free deionized water. Invert gently two or three times. Do not vortex the sample in order to avoid shearing the DNA. The final sample conductivity must be ≤100 µS/cm. Running more conductive samples will decrease yield. If necessary you may simply repeat the dilution and centrifugation step to reduce the conductivity.

Loading your sample and running the Aurora

1. Follow the directions in the **Aurora Reusable Cartridge Handling Manual** (**106-0014**) to prepare your **Aurora Reusable Cartridge** (**211-0004-AA-D**), load your sample into the cartridge, prepare the Aurora instrument and run the **AURORA 0.7-50KB DNA FROM WATER PROTOCOL** when asked to select the protocol **.sp** file for the run.
2. During the Aurora run, the instrument will pause the run at two points - 1 h and 2 h into the wash step. During each pause, remove 2 ml of buffer from Buffer Chamber F, close the cartridge drawer and hit the resume button to continue the run.
3. Once the Aurora run is complete, carefully peel off the clear film over the extraction well and extract the buffer. The purified DNA is now ready for use.

Troubleshooting

Please see the Aurora user manual for more information about troubleshooting instrument 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, repeat the desalting step with a Amicon® Ultra-15, 30kDA centrifugal filter. Dilute the sample to 15 ml with dH_2O and centrifuge at 4,000 rpm for 15 min. Extract the remaining sample and prepare the sample for Aurora purification.

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 **106-0013-BA-D AURORA 0.7-50KB DNA FROM 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 (with pauses at 1h, 2h to remove 2 ml of buffer from the F well)
Wash strength	70%

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