Aurora 0.3-10kb DNA from Soil Protocol

106-0009-BB-D

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Introduction

This protocol is for extracting and purifying DNA from soil samples containing humic acids and other inhibitors of enzyme activity. Output from the MO BIO PowerMax® Soil DNA Isolation Kit is desalted with an Amicon® Ultra-15 centrifugal column and then purified with the Aurora. At least 1 g of soil can be processed per **Aurora Reusable Cartridge**, and larger amounts of soil can be accommodated by one cartridge as long as the conductivity limits below are observed. This protocol is appropriate for recovering highly purified DNA 0.3-10 kb in length that is suitable for PCR amplification, library construction, DNA sequencing or metagenomic analyses.

Input Sample Specifications:

Soil: 1 g or more

Volume: Up to 5 ml

Conductivity: ≤100 µS/cm

Comments: The conductivity of the sample must be ≤100 µS/cm after desalting and bringing the volume back up to 5 ml in 0.0125x TBE. This conductivity is comparable to that of 0.2x TE or 0.1x TBE. Using deionized water or very weak buffer solutions to resuspend or elute samples can help to reduce the conductivity. The conductivity of a sample will vary for different soil samples. Greater than 1 g of soil can be processed in one **Aurora Reusable Cartridge** as long as the sample conductivity is ≤100 µS/cm.

Example results

To demonstrate the ability of the Aurora to provide highly purified bacterial DNA from soil samples, DNA from a 6 g soil sample from Vancouver, BC was prepared using two MO BIO PowerMax® DNA Isolation Kit (catalogue no. 12988) columns. The 5 ml outputs from each column were pooled (10 ml) and then diluted to 30 ml with dH_2O so that the resulting DNA concentration would be equivalent to that expected for 1 g of soil being processed through one PowerMax® column with a 5 ml output volume. A 100 µl aliquot was taken for qPCR analysis in order to quantify the DNA concentration in the PowerMax® output, and a 10 ml aliquot was used for further purification using the **Aurora 0.3-10kb DNA from Soil Protocol**.

The 10 ml aliquot was diluted to 15 ml with dH_2O and then desalted by centrifugation in an Amicon® Ultra-15, 30 kDa centrifugal filter (catalogue no. UFC903096) at 4,000 *g* for 10 min, reducing the sample volume to 250 µl. This sample was then diluted back up to 10 ml with 0.0125x TBE and divided into two 5 ml replicates, each of which were loaded into a 5 ml cartridge and purified using the **Aurora 0.3-10kb DNA from Soil Protocol**, which provided DNA in 60 µl output volumes. Quantitative PCR (qPCR) was used to quantify the DNA concentration in each sample.

A dilution series of each of the PowerMax® and Aurora outputs 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 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 the 5 µl of PCR input for both the PowerMax® output and the average of the outputs from two replicates of the **Aurora 0.3-10kb DNA from Soil 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. Despite containing more template DNA, PCR amplification was inhibited in undiluted samples. The best PCR amplification occurred at a 1:10 dilution using the **Aurora 0.3-10kb DNA from Soil Protocol**. Increasingly dilute samples showed further reductions in inhibition for both the PowerMax® and Aurora outputs, but also decreased PCR yield due to the decreased amount of template DNA in the PCR reactions (data not shown).

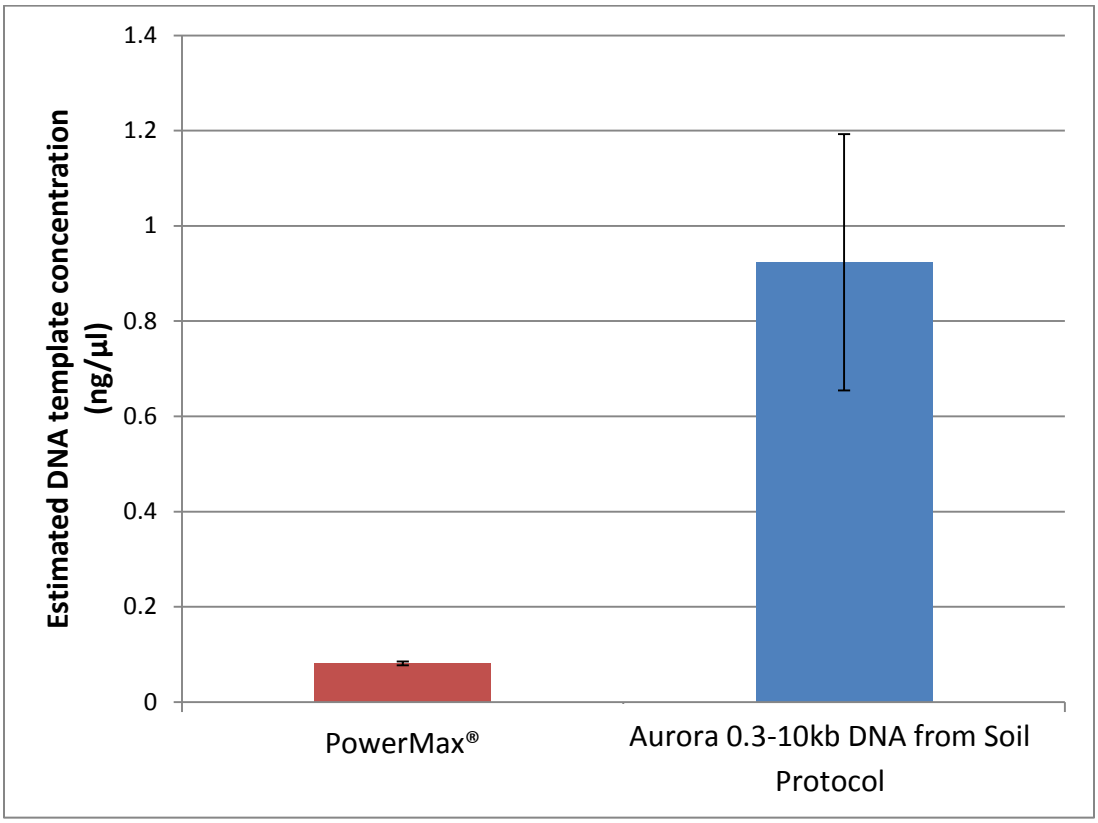

Figure 1. Estimated DNA template concentration in 5µl of PCR input after diluting the PowerMax® and Aurora outputs 1:10 with dH_2O. The average of the estimates for two replicates of the Aurora 0.3-10kb DNA from Soil Protocol is shown. These estimates assume that the average genome size and 16S copy number for 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. 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.

Preparing the sample

Initial DNA extraction with the PowerMax® DNA Soil Isolation Kit

Process your soil sample following the instructions in the MO BIO PowerMax® Soil DNA Isolation Kit (catalogue no. 12988). Although the PowerMax® kit accepts up to 10 g of soil input, the **Aurora Reusable Cartridge** has a maximum capacity of 100 µg of DNA, so consider reducing the input amount of soil to improve extraction efficiency and contaminant rejection achieved by the Aurora.

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

Dilute the PowerMax® output to 15 ml (12 ml if using a fixed angle rotor) with dH_2O. Add the diluted sample to an Amicon® Ultra-15, 30 kDa centrifugal filter (catalogue no. UFC903096) and centrifuge at 4,000 *g* for 10 min, reducing the sample volume to 250 µl. Dilute the sample back up to 15 ml with dH_2O and centrifuge again at 4,000 *g* for 10 min. Dilute the final extracted product to 5 ml with a low conductivity buffer like 0.01x TBE, 0.01x TE, or dH2O . 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 protocol

Please refer to the **Aurora Reusable Cartridge Handling Manual (106-0014)** for detailed instructions on how to prepare your **Aurora Reusable Cartridge (210-0004-AA-D)**, load your sample into the cartridge, prepare the Aurora instrument and run the Aurora protocol. Please select the **106-0009-BB-D AURORA 0.3-10KB DNA FROM SOIL PROTOCOL.SP** 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 **106-0009-BB-D AURORA 0.3-10KB DNA FROM SOIL 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

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