Aurora 0.7-50 kb DNA from Soil with Enchanced Contaminant Rejection Protocol

106-0015-BA-D

http://www.borealgenomics.com

support@borealgenomics.com

+1 (604) 822-4111

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Introduction

This protocol is for extracting and purifying DNA from soil without the use of mechanical lysis steps to recover and improve yields of genomic DNA up to 50 kb. Output from this procedure may be used in a variety of downstream applications, such as generating genomic libraries, where longer DNA fragments are preferred.

The protocol uses a detergent-based lysis and nucleic acid precipitation adapted from the method described by Zhou *et al*.,[1] to obtain a crude DNA sample from a soil. The crude DNA sample is then further purified by the Aurora.

This protocol will accept up to 1 g of soil per 5 ml, single-sample Aurora cartridge.

Example Results

To demonstrate the protocol, genomic DNA was extracted and purified from two challenging soil samples intended for fosmid library construction. Two replicates from each soil sample of 1 g each were processed through a lysis procedure adapted from Zhou *et al.*[1] and resuspended in 0.2 ml of dH_2O, producing a crude DNA extract that was further purified using the **Aurora 0.7-50 kb DNA from Soil with Enchanced Contaminant Rejection Protocol** . The purified outputs were extracted in 60 µl of buffer and analyzed by quantitative PCR (qPCR) for yield and purity; additionally, they were run on a pulse-field gel (PFG) to assess molecular weight.

A dilution series of each purified sample was prepared and analyzed by qPCR with universal bacterial 16S rDNA primers and probes described by Nadkarni *et al*.[4] Twenty-five microlitre reaction volumes consisting of 5 µl template and 20 µl master mix were used (Roche Applied Science FastStart Universal Probe Master Mix, catalog no. #04913957001), with final concentrations of the forward and reverse primers and probe all 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. C_T values were converted to mass equivalents against a standard curve prepared from *E. coli* genomic DNA.

Figure 1 is a fluorescent gel image that illustrates the length distribution of DNA fragments that were obtained from the Aurora outputs relative to the Low Range PFG Marker located in the first well (NEB, N0350S). It demonstrates that the **Aurora 0.7-50 kb DNA from Soil with Enchanced Contaminant Rejection Protocol** is capable of obtaining DNA fragments as large as 40kb from samples #1 and #2. In addition, the outputs were amplifiable using the 16s qPCR assay, indicating improved purity over other methods (data not shown). Recently, Engel *et al.* (2012), have also demonstrated that the combination of high yield, high purity and high molecular weight DNA recovered with this technology in comparison to others, make the Aurora an ideal candidate for downstream applications including sequencing, large-insert cloning and PCR.

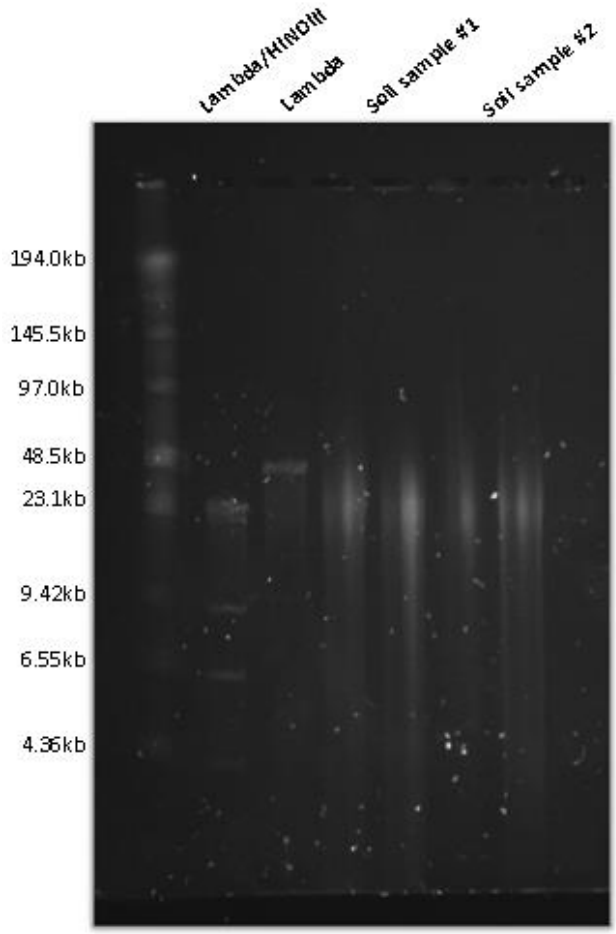

Figure 1. Fluorescent Pulse Field Gel Image of gDNA purified from Soil sample #1 and Soil sample #2 using the Aurora 0.7-50kb DNA from Soil with Enhanced Contaminant Rejection Protocol. Ten microlitres of the Aurora output were loaded for each well. Samples were separated using the following parameters: 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.

Preparing the sample

The extraction process takes about 5 - 6 h and the **Aurora 0.7-50 kb DNA from Soil with Enchanced Contaminant Rejection Protocol**. It is safe to pause after the extraction process, storing the crude DNA sample at -20 °C.

Safety guidelines

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

Chloroform:isoamyl alcohol is toxic and should be handled in a fume hood and disposed of accordingly.

Hexadecyltrimethylammonium bromide (CTAB) is an irritant; avoid inhalation.

Materials required

1. **DNA extraction buffer**

The following table makes enough buffer to work with 10 g of soil. The volumes can be scaled up or down as required. The extraction buffer can be prepared in advance and stored at room temperature for up to 6 months. 5 ml of extraction buffer is required per gram of soil.

Reagent	Volume (ml)	Final Concentration
1M Tris-HCl, pH 8.0	5	100 mM
0.5M disodium EDTA, pH 8.0	10	100 mM
1M sodium phosphate, pH 8.0	5	100 mM
5M NaCl	15	1.5 M
10% CTAB	5	1%
Nuclease free dH2O	10	-
Total volume	50	

2. **General Reagents:**

Reagent	Volume (ml) required per gram of soil
20 mg/ml proteinase K, pH 8.0	0.05
20% sodium dodecyl sulfate (SDS)	2
24:1 (v/v) chloroform: isoamyl alcohol	5
isopropanol	5
70% ethanol	5

3. **Equipment and materials required:**

- Horizontal shaking incubator
- Tabletop centrifuge
- Microcentrifuge
- 65 °C water bath
- 15 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)

Lysis protocol[1]

(1) Weigh out up to 1 g of soil sample into a 15 ml centrifuge tube.

(2) Add 2.7 ml of DNA extraction buffer and 50 µl of 20 mg/ml proteinase K. Secure the tubes on a horizontal shaking incubator and shake at 225 rpm for 30 min at 37 °C. This step aids in breaking up the soil particles and homogenizing the sample.

(3) Remove the tube from the shaking apparatus. Add 0.3 ml of 20% SDS and invert the tube gently 5 times to mix.

(4) Incubate the sample in a 65 °C water bath for 2 h, mixing by gentle inversion every 20 min.

(5) Centrifuge the tube at room temperature for 10 min at 6,000 *g*. Extract all the supernatant with a sterile wide bore tip and store at 4 °C for 30 min while continuing the extraction. Wide bore tips are used to reduce the possibility of shearing the DNA through pipetting.

(6) Add 1.8 ml of DNA extraction buffer and 0.2 ml of 20% SDS to the remaining pellet, vortexing on the highest setting for 10 s and incubating in a 65 °C water bath for 30 min.

(7) Repeat step 5 and pool the supernatant with the previously extracted supernatant (about 5 ml total).

(8) Add an equal volume (about 5 ml) of 24:1 (v/v) chloroform:isoamyl alcohol to the pooled supernatant.

(9) Mix the two phases by gently inverting the tubes at least 10 times. Do not shake vigorously or vortex the tube as the DNA will be sheared.

(10) Centrifuge the tube at room temperature for 10 min at 6,000 *g*. Extract the aqueous (top) layer using a wide bore tip into a new 15 ml centrifuge tube, taking care to avoid the interface.

(11) Add 0.6 x volume of isopropanol (3 ml), invert 5 times to mix, and allow to incubate at room temperature for 1 h.

(12) Centrifuge the tube at 10,000 *g* for 20 min at room temperature to pellet the nucleic acids. The pellet will likely be dark brown in color. Carefully pour off and discard the supernatant.

(13) Add 5 ml of 70% ethanol and invert 10 times to wash the pellet.

(14) Centrifuge the tube at 10,000 *g* for 20 min. Remove all the supernatant and allow to air dry for 10 min at 37 °C.

(15) Gently resuspend the nucleic acid pellet in 200 µl of nuclease free dH_2O or dilute TE buffer (0.2x) with a wide bore tip. The resuspended sample will likely be dark brown and viscous. At this point, either proceed to the Aurora purification step or store the extract at -20 °C until ready for use.

Final Sample Dilution

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 until evenly mixed. Do not vortex as the DNA may shear. Final sample conductivity must be less than or equal to 200 µS/cm. Running more conductive samples will decrease yield.

Loading your sample and running the Aurora protocol

1. Follow the directions in the **Aurora Reusable Cartridge Handling Manual** (**106-0014**) to prepare the **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 SOIL WITH ENHANCED CONTAMINANT REJECTION PROTOCOL.SP** file 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 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 equal to or less than 200 µS/cm.

Immediate Remedy: The run can continue, but yield will be decreased. In general, the higher the conductivity over 200 µS/cm, the lower the yield.

Solution: To solve this problem, adjust the lysis protocol to reduce the amount of salt in the sample by increasing the number of ethanol wash steps, or by reducing the amount of soil on input.

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 or bound to the DNA of interest. Low conductivity additives (such as proteinase K) or processes (such as heat) 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 step.

If the sample was too high in conductivity, yield can be reduced. See troubleshooting Error 1, as well as troubleshooting in the Aurora user manual for details in resolving this failure mode. If the sample contains contaminants that bind DNA during injection or focusing, yield will also appear to be low. If they are bound during injection, and impede DNA from entering the SCODA gel, dilution into multiple samples to reduce contaminant concentration may help, as may low conductivity additives that reduce DNA-contaminant binding interactions (such as proteinase K or heat). Alternatively, if the contaminant is bound to the DNA but DNA is still able to focus, PCR dilutions after processing will indicate remaining 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 **106-0015-BA-D AURORA 0.7-50KB DNA FROM SOIL WITH ENHANCED CONTAMINANT REJECTION 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.

Gel Boat

Running buffer	0.25x TBE
Sample volume	5 ml
Expected sample conductivity	≤200 µS/cm

Injection

Injection voltage	500 V
Injection charge	5000 mC

Expected current	9-13 mA
Expected average power	4-7 W
Expected voltage drop across the gel	≤20%

Wash (6 Channel)

SCODA field strength	70 V/cm
SCODA cycle period	4 s
Duration	2.5 h (**with pauses after 1h and 2h to remove 2 ml of buffer from the F well**)
Wash strength	70%

Expected current	15-25 mA
Expected power	5-6 W

Focus

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

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

References

1. Zhou, J., Bruns, M.A., and Tiedje, J.M. (1996). DNA Recovery from Soils of Diverse Composition. *Applied and Environmental Microbiology*, **62** (2) : 316-22.
2. Zoll, G., Grote, G., Dierstein, R., and Köhne, S. (2002). Rapid isolation of anthrax DNA from large-volume soil samples using QIAamp® Kits. *Qiagen News*, **1** : 22.
3. Fitzpatrick, K.A., Kersh, G.J., and Massung, R.F. (2010). Practical method for extraction of PCR-quality DNA from environmental soil samples. *Applied and Environmental Microbiology*, **76** (13) : 4571-73.
4. Nadkarni, M.A., Martin, F.E., Jacques, N.A., and Hunter, N. (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology*, **148** : 257-66.
5. Engel, K., Pinnell, L., Cheng, J., Charles, T.C., Neufeld, J.D. (2012). Nonlinear electrophoresis for purification of soil DNA for metagenomics. J. Microbiol. Methods 88(1):35-40.