Aurora 0.3-50kb DNA From Soil Protocol

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Introduction

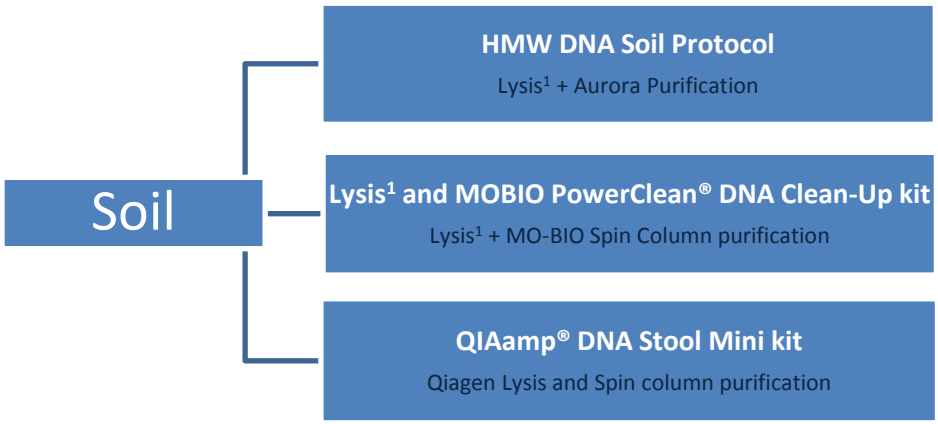
This protocol is for extracting and purifying DNA from soil without the use of mechanical lysis steps to recover genomic DNA from 300 bp to 50 kb in length. Output from this procedure may be used in a variety of downstream applications, such as generating genomic libraries, where longer DNA fragments 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 soil. The crude DNA sample is then further purified using the Boreal Genomics Aurora instrument.

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

Example Results

To demonstrate the protocol, purified genomic DNA from organisms in a sample of farm soil from Vancouver, BC was prepared by three different methods.



10 g of soil was processed through a lysis procedure adapted from Zhou *et al.*[1] and resuspended in 1.5 ml of 0.2x TE buffer, producing a crude DNA extract that was further purified with the Aurora or the MO-BIO PowerClean® DNA Clean-Up Kit (catalog no. 12877). For the HMW DNA Soil Protocol, 150 µl of DNA extract (equivalent to 1 g of soil) was diluted to 5 ml with 0.01x TBE and purified using the Aurora. For the MO-BIO PowerClean® kit, 75 µl of DNA extract (equivalent to 0.5 g of soil) was diluted to 100 µl with nuclease-free dH2O and purified following the manufacturer's recommendations. Soil samples of 0.2 g each were additionally extracted using the QIAamp® DNA Stool Mini Kit (catalog no. 51504) that provides both lysis and purification without additional modifications. Each clean-up method and the QIAamp® method were repeated three times.

The amount of soil processed varies for each purification method due to their different capacities. The QIAamp® DNA Stool Mini Kit can process a maximum of 0.2 g of material per column and was chosen for this comparison due to its non-mechanical lysis that retains longer DNA fragments and its usage in

extracting DNA from soil samples[2,3]. The MO-BIO PowerClean® DNA Clean-Up kit is limited in capacity due to filters being fouled, so the input was reduced to lysate from 0.5 g of soil. The Aurora DNA Clean-Up Protocol can purify lysate from up to 1 g of soil, limited by the lysate's conductivity.

A dilution series of each purified sample was prepared and analyzed by quantitative PCR (qPCR) with the universal bacterial 16S rDNA primers and probes described by Nadkarni *et al*.[4] 5 µl of each diluted sample was loaded into a 25 µl reaction containing Roche Applied Science FastStart Universal Probe Master Mix (catalog 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. C_T values were convered to mass equivalents against a standard curve prepared from *E. coli* genomic DNA.

Figure 1 shows the total mass of DNA (in ng *E. coli* equivalents) for each method, normalized to DNA output per gram of soil. The HMW DNA Soil Protocol (SCODA) gives the highest DNA yield of the 3 methods compared. Data shows amplification at 1000-fold sample dilution prior to PCR. Sample dilutions at 1:1, 1:10 and 1:100 do not completely amplify due to PCR inhibition.

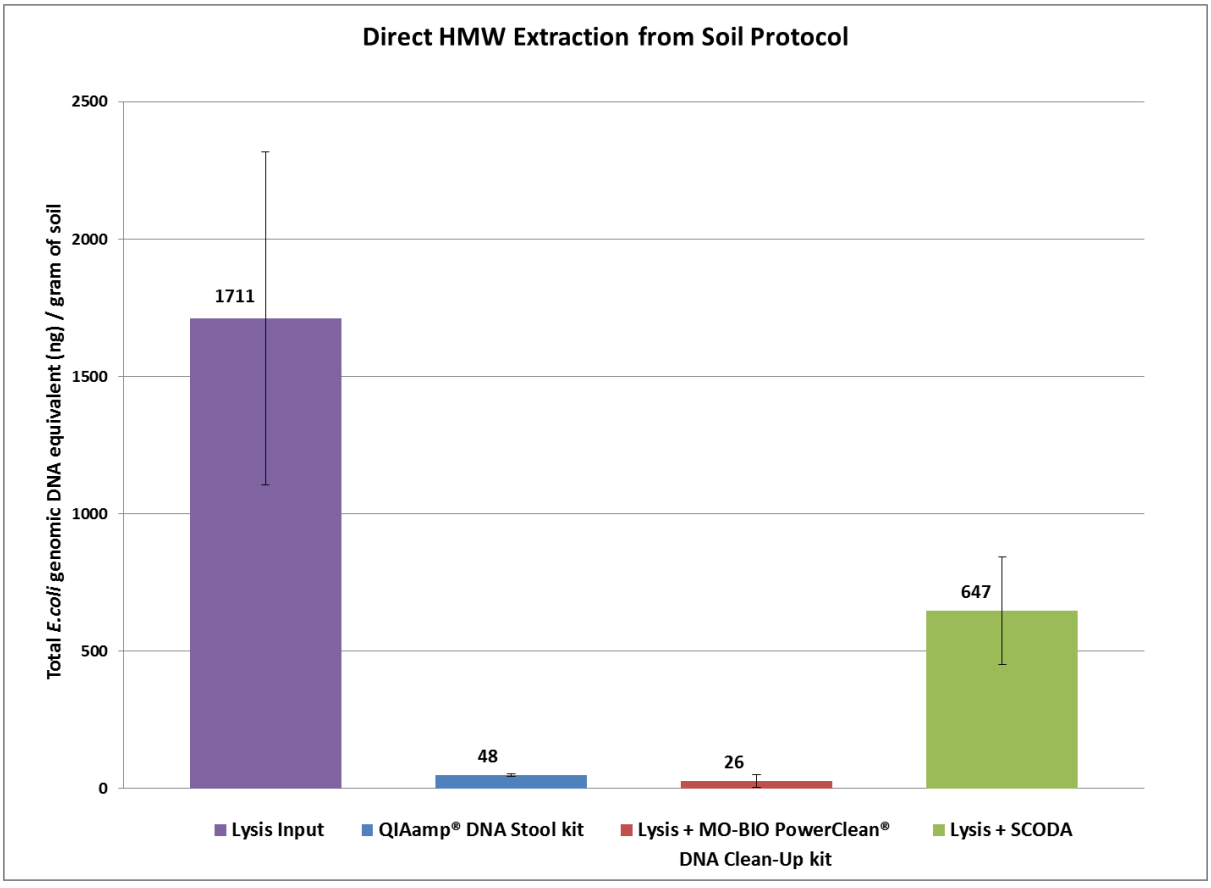


Figure 1. Normalized DNA recovery for each purification method (scaled to account for dilution). Error bars represent standard deviation of PCR replicates (n=2). Data values are calculated according to the following formula: [DNA in PCR]x(volume of protocol output)x(dilution factor)x(sample-size scale factor)

Figure 2 shows a fluorescent gel image presenting the relative length of DNA fragments post-purification from the 3 methods being compared. From this image, it is not possible to clearly define the length of the DNA fragments purified via the QIAamp® DNA Stool Mini Kit as the DNA concentration of the sample was too low. The HMW DNA Soil Protocol yields DNA fragments at least 23.1 kb long, while the majority of DNA fragments purified from the lysis[1] combined with the MO-BIO PowerClean® kit are less than 23.1 kb long.

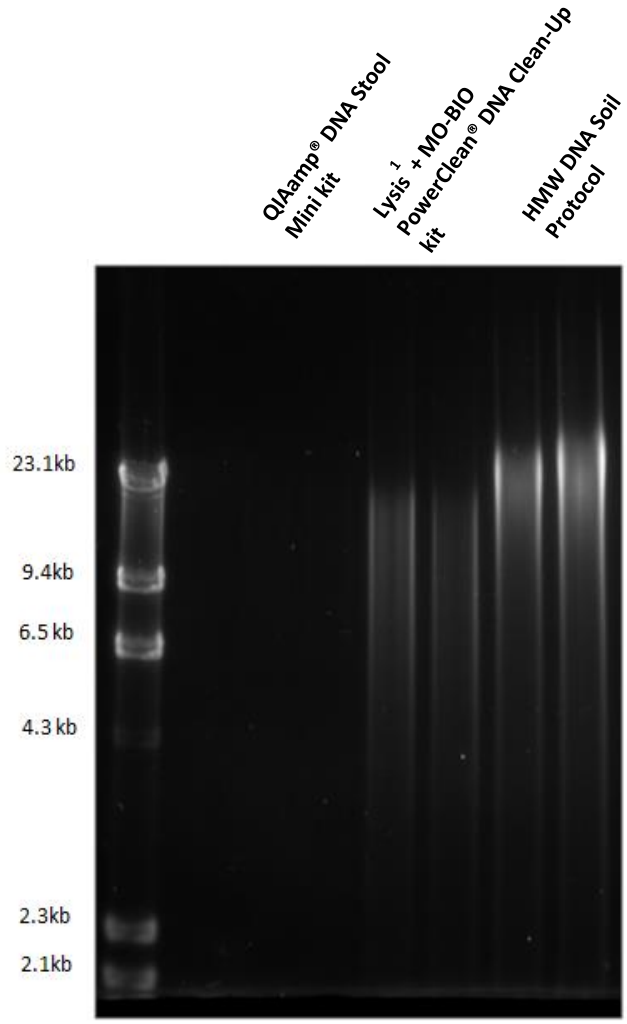


Figure 2. Fluorescent Pulsed Field Gel Image of post-purification outputs from QIAamp® DNA Stool Mini Kit (0.2 g input), Zhou *et al*. lysis + MO-BIO PowerClean® kit (0.5 g input) and HMW DNA Soil Protocol (1 g input). Samples are separated by pulsed field gel electrophoresis run with the following parameters: 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.

These results demonstrate that the HMW DNA Soil Protocol reduces contamination while increasing DNA yield and length compared to commercially available kits for DNA extraction and purification from soil.

Aurora 0.3-50 kb DNA From Soil Protocol

The extraction process takes about 5 - 6 h and the Aurora DNA Clean-Up Protocol takes 4 h. It is safe to pause after the extraction process, storing the crude lysate 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.

Hexadecyltrimethyammonium 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 (w/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
- 5 ml disposable Aurora cartridge (part number 210-0001-AAD)

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 30min 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 10 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 (w/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 layer (top) using a wide bore tip into a new 15 ml centrifuge tube, avoiding 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 16,000 *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 16,000 *g* for 10min. 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 dH2O 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.

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 **106-0005-CA-D AURORA 0.3-50KB DNA FROM SOIL 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 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 .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	600 V
Injection charge	5000 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 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.