Aurora High Capacity 20-50 kb DNA From Soil Protocol

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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 by non-mechanical lysis steps to recover genomic DNA up to 50 kb in length. While DNA fragments as small as 1 kb may be recovered, a large amount of the purified DNA will be between 20-50 kb in length. Output from this procedure is therefore suitable for a variety of downstream applications requiring relatively high molecular weight DNA, including the generation of fosmid libraries and other forms of large-insert library construction.

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

This protocol makes use of the Aurora Reusable Cartridge and 1% agarose gels made in 1x TBE buffer, with 1x TBE running buffer. The protocol will accept up to 5 g of soil per run, and is based on a scaled up version of the **Aurora HMW DNA Soil Protocol** (106-0005) with several modifications, including changes to the SCODA conditions in the .sp file.

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The extraction process takes about 5 - 6 h and the Aurora DNA Clean-Up Protocol takes 4-6 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 5 grams 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 5 g of soil sample into a 50 ml centrifuge tube.

(2) Add 13.5 ml of DNA extraction buffer and 250 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 1.5 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 9.0 ml of DNA extraction buffer and 1.0 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 25 ml total).

(8) Add an equal volume (about 25 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 1,500 *g*. Extract the aqueous (top) layer using a wide bore tip into a new 50 ml centrifuge tube, taking care to avoid the interface.

(11) Add 0.6 x volume of isopropanol (15 ml), invert 5 times to mix, and allow to incubate at room temperature for 1 h.

(12) Centrifuge the tube at 6,000 *g* for 30 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 25 ml of 70% ethanol and invert 10 times to wash the pellet.

(14) Centrifuge the tube at 6,000 *g* for 30 min. Remove all the supernatant and allow to air dry for 10 min at 37 °C.

(15) Gently resuspend the nucleic acid pellet in 1 ml of nuclease free dH_2O or dilute TE buffer (0.2x) with a wide bore tip. If you find the pellet difficult to resuspend, incubate the tube at 65 °C to encourage the DNA to enter into solution. 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 1000 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, but use 1x TBE instead of 0.25x TBE to prepare the 1% agarose gel and for the running buffer.
2. Load the diluted 5 ml sample into the sample chamber, and run the **106-0021-BB-D Aurora High Capacity 20-50 kb DNA From Soil Protocol.sp** file.
3. You may not need to run the entire 3 h Wash Block. If you wish, you can pause the protocol during the Wash Block, open the Cartridge Drawer and visually inspect the concentration gel to roughly determine whether contaminants (ex. brown humic acids)have been cleared from the gel. If you are satisfied, you can then advance to the Focus Block of the protocol; otherwise, resume the Wash Block.
4. 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 1000 µS/cm.

Immediate Remedy: The run can continue, but yield will be decreased. In general, the higher the conductivity over 1000 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 **106-0021-BB-D Aurora High Capacity 20-50 kb 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.

Gel Boat

Running buffer	1x TBE
Sample volume	5 ml
Expected sample conductivity	≤1000 µS/cm

Injection

Injection voltage	400 V
Injection charge	5000 mC

Expected current	18-26 mA
Expected average power	10-16 W
Expected voltage drop across the gel	≤10%

Wash (6 Channel)

SCODA field strength	25 V/cm
SCODA cycle period	16 s
Duration	3.0 h (can be shortened)
Wash strength	40%

Expected current	20-30 mA
Expected power	4-6 W

Focus

SCODA field strength	25 V/cm
SCODA cycle period	16 s
Duration	3.0 h

Expected current	30-45 mA
Expected power	6-10 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.