Aurora 0.7-10kb DNA Direct from Soil Protocol

106-0012-BA-D

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Introduction

This protocol is for direct purification of DNA from soil samples. It may be used to remove humic acids and other inhibitors of enzyme activity that are not cleared by traditional DNA purification methods such as spin columns and alcohol based precipitation. Microbes in soil are chemically, enzymatically and mechanically disrupted with detergent-based lysis, proteinase-K and bead beating. The lysed sample is desalted with a centrifugal filter before DNA is purified and concentrated with the Aurora. Up to 0.25 g of soil can be processed per Aurora cartridge. This protocol is appropriate for recovering highly purified DNA from 0.7-10 kb in length that is suitable for PCR amplification, library construction, DNA sequencing or metagenomic analyses.

Input Sample Specifications:

Amount of Soil: Up to 0.25g of soil

DNA Capacity: Up to 100 µg of DNA

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

Safety guidelines

Wear gloves during all stages of the protocol. Avoid skin contact with all reagents. Cartridges are made from non-hazardous plastics, metal, 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 extraction buffer to process 10 g of soil. The extraction buffer can be prepared in advance and stored at room temperature for up to 6 months. 1.2 ml of extraction buffer is required per 0.25 g soil sample.

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 required per 0.25 g of soil
20 mg/ml proteinase K, pH 8.0	12.5 µl
20% sodium dodecyl sulfate (SDS)	120 µl
Nuclease free dH_2O	80 ml

3. **Equipment and materials required:**

- Horizontal shaking incubator set to 37 °C
- Centrifuge with swing bucket or rotor to accommodate 50 ml centrifuge tubes
- Microcentrifuge
- 65 °C water bath
- 15 ml and 50 ml centrifuge tubes
- Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-30 membrane (part number UFC903008)
- Aurora instrument
- Aurora Reusable Cartridge (211-0004-AA-D)
- 2 ml screw cap microcentrifuge tubes
- 1.5 ml microcentrifuge tubes
- 0.1 mm Zirconia/Silica beads (Biospec, part number 11079101z)
- Bead beater – (MPBIO Fastprep, adaptor part number 116002512)

Preparing the sample

Heat the required amount of DNA extraction buffer (1.2 ml x number of samples) to 37 °C before use.

Weigh out 1 g of the Zirconia/Silica beads into a 2 ml screw cap microcentrifuge tube per sample.

Lysis

1. Weigh out up to 0.25 g of soil into a 2 ml screw cap microcentrifuge tube containing Zirconia/Silica beads.
2. Add 1.08 ml of preheated DNA extraction buffer to the tube and close securely.
3. Tape the tubes horizontally in a shaking incubator set at 37 °C, for 10 min at 225 rpm.
4. Add 12.5 µl of 20 mg/ml proteinase K and 120 µl of 20 % SDS to each tube.
5. Vortex briefly to mix the extraction buffer and the sample.
6. Mechanically disrupt the samples with a bead beater. Fastprep-24 from MP Biosciences with five 60 second cycles at 6.0 m/s with a 1 minute pause step between each cycle is recommended. Incubate the tubes in a 65 °C heat block for 10 min.
7. Centrifuge the tubes at 16,000 *g* for 5 min.
8. After centrifugation, there might be a floating layer of precipitate on top of the supernatant. Avoid this precipitate and the soil pellet while extracting the supernatant (1 ml) to a 1.5 ml microcentrifuge tube. If small amounts of precipitate and particulate are transferred, repeat the centrifugation step and transfer the supernatant to a new 1.5 ml microcentrifuge tube.

9. Split the supernatant, avoiding all particulate, between two 50 ml centrifuge tubes.
10. Fill both centrifuge tubes to 30 ml with nuclease free dH_2O each, for a total of 60 ml per sample.
11. Mix the sample between both tubes by pouring the liquid from one falcon tube to the other at least 10 times. To prevent spilling any sample, do not overfill tubes.

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 three more times 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. Remove the remaining sample from the filter to a 15 ml centrifuge tube. Rinse the filter with 800ul 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 to mix. 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 0.7-10KB DNA DIRECT FROM SOIL 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 >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 **106-0012-BA-D 0.7-10KB DNA DIRECT 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 (with pauses at 1h, 2h to remove 2 ml of buffer from Buffer Chamber F)
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