Aurora 0.3-50kb DNA from Stool Protocol

106-0011

http://www.borealgenomics.com

support@borealgenomics.com

+1 (604) 822-4111

Table of Contents

Introduction

This protocol is for extracting and purifying DNA from stool samples without the use of mechanical lysis steps to recover genomic DNA up to 50 kb in length. Output from this procedure may be used in a variety of downstream applications, including the generation of genomic libraries, sequencing and metagenomics analysis, 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 extract from a stool sample. The crude extract is then purified using the Boreal Genomics Aurora instrument.

Input Sample Specifications:

Amount of sample: Up to 0.2 g of stool

Conductivity: <200 µS/cm when diluted to 5 ml

The conductivity of the sample after dilution to 5 ml must be ≤200 µS/cm, which is similar in conductivity to 0.2x TE or 0.1x TBE. Use deionized water or very weak buffer solutions when resuspending or eluting a sample for use with this protocol. DNA in the sample must be free to migrate in response to electric fields.

Example results

To demonstrate the protocol, 0.2 g of rat feces obtained from the Centre for Disease Modeling at University of Britisih Columbia, Vancouver B.C., was processed through a lysis procedure adapted from Zhou *et al.*[1]. DNA was resuspended in 200 µl of dH_2O and further purified with the Aurora. Separately, the same amount of rat feces was processed through the QIAamp® DNA Stool Mini Kit (catalog no. 51504) following manufacturer's recommendations. Both methods were repeated three times.

A dilution series of each purified DNA sample was prepared and analyzed by quantitative PCR (qPCR) with the universal bacterial 16S rDNA primers and probes described by Nadkarni *et al*.[2] 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 converted to mass equivalents against a standard curve prepared from *E. coli* genomic DNA.

Figure 1 shows the total mass of bacterial DNA (in µg *E. coli* genome equivalents) recovered for each method. The **Aurora 0.3-50kb DNA from Stool Protocol** yielded more DNA than the QIAamp method. The yield was averaged from 1:100 and 1:1000 dilutions in qPCR because the large amount of DNA in the Aurora purified samples inhibited PCR reactions at lower dilutions.

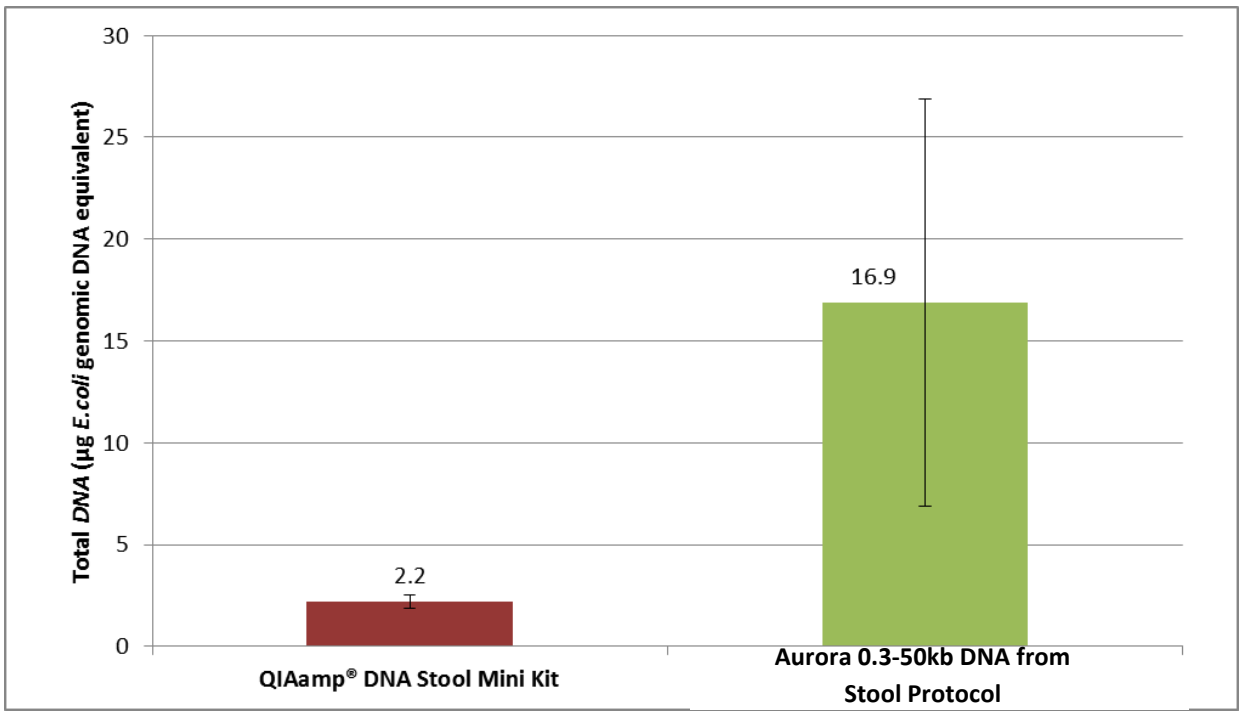

Figure 1. Average DNA recovered from 0.2 g of rat stool by each purification method. Error bars represent the standard deviation of PCR replicates (n=2) for 1:100 and 1:1,000 dilutions of each of 3 biological replicates. Data values are calculated according to the following formula: [DNA in PCR]x(volume of protocol output)x(dilution factor)

Figure 2 shows a fluorescent gel image presenting the length of DNA fragments produced by both methods. The maximum length of the DNA fragments purified via the QIAamp® DNA Stool Mini Kit is less than 23.1 kb, whereas the **Aurora 0.3-50kb DNA from Stool Protocol** yields higher molecular weight DNA.

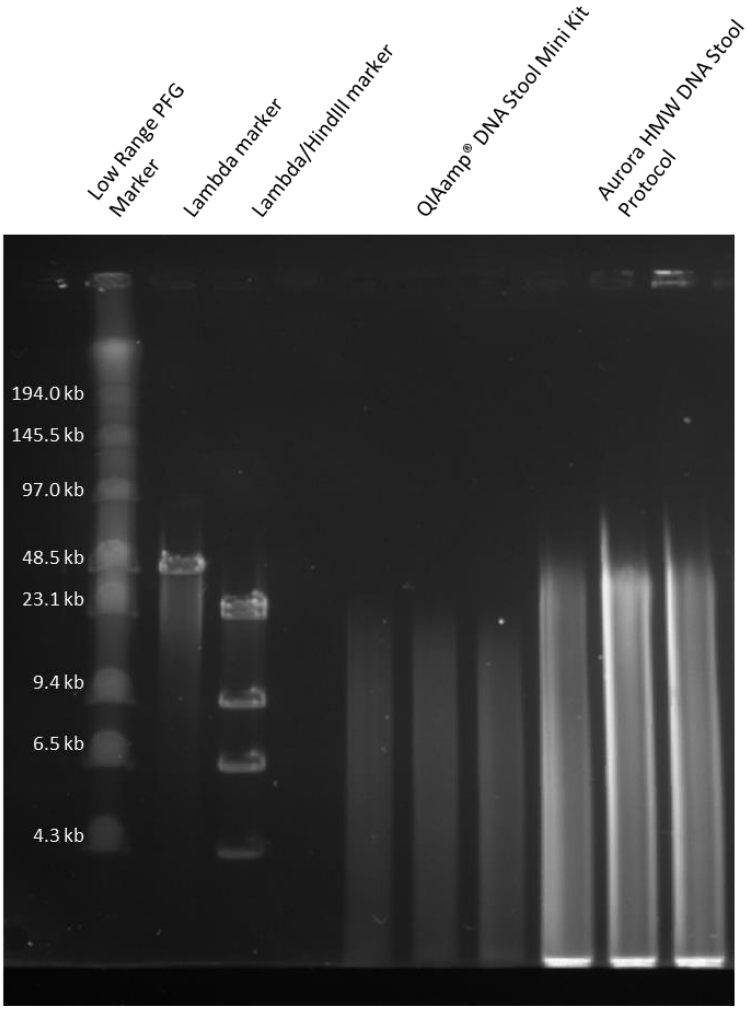

Figure 2. Fluorescent Pulsed Field Gel Image of purified DNA from QIAamp® DNA Stool Mini Kit and the Aurora 0.3-50kb DNA from Stool Protocol. Samples are separated by pulsed field gel electrophoresis run with the following parameters: 1% SeaKem Gold Agarose, 1x TBE gel, 1x TBE Running Buffer. Initial 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 **Aurora 0.3-50kb DNA from Stool Protocol** can provide improved DNA yield and higher molecular weight DNA than commercially available kits for DNA extraction and purification from stool.

Safety guidelines

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.

Preparing the sample

The extraction process takes 5 - 6 h and the Aurora purification takes 4 h. It is safe to pause after the extraction process, storing the crude lysate at -20 °C.

Materials required

1. **DNA extraction buffer**

The following table makes enough buffer to work with 10 g of stool. 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. 1.6 ml of extraction buffer is required per 0.2 g sample of stool.

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 dH_2O	10	-
Total volume	50	

2. **General Reagents:**

Reagent	Volume (ml) required per 0.2 g of stool
20 mg/ml proteinase K, pH 8.0	0.01
20% sodium dodecyl sulfate (SDS)	0.2
24:1 (v/v) chloroform: isoamyl alcohol	2
isopropanol	2
70% ethanol	5

3. **Equipment and materials required:**

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

- 2 ml microcentrifuge 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[1]

Larger amounts of stool can be processed with the method described below by scaling up the buffer volumes accordingly. The amount of sample that can be purified through the Aurora will depend on the conductivity of the DNA extract.

(1) Weigh out up to 0.2 g of stool sample into a 2 ml microcentrifuge tube. Keep the tubes on ice to prevent the sample from thawing out.

(2) Add 0.9 ml of DNA extraction buffer and 10 µl of 20 mg/ml proteinase K. Vortex the tubes on the highest setting for 45 s to 1 min or until the sample is evenly suspended. 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 stool particles and homogenizing the sample.

(3) Remove the tube from the shaking apparatus. Add 0.1 ml of 20% SDS and invert the tube gently 10 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 5 min at 16,000 *g*. Gently extract the supernatant (about 0.6 ml) with a sterile wide bore tip into a 2 ml microcentrifuge tube and store at 4 °C for 30 min while continuing the extraction. Some particulates may be extracted at this step. Wide bore tips are used to reduce the possibility of shearing the DNA through pipetting.

(6) Add 540 µl of DNA extraction buffer and 60 µl of 20% SDS to the remaining pellet. Vortex on the highest setting for 10 s and incubate in a 65 °C water bath for 30 min, inverting the tubes every 10 min.

(7) Repeat step 5 and pool the supernatant (about 0.4 ml) with the previously extracted supernatant (about 1 ml total).

(8) Add an equal volume (about 1 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 25 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 16,000 *g*. Using a wide bore tip, transfer the aqueous (top) layer (0.8 ml) into a new 2 ml microcentrifuge tube, taking care to avoid the interface.

(11) Add 0.6x volume of isopropanol (0.48 ml), invert 10 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 may be brown in color. Carefully pour off and discard the supernatant.

(13) Add 1.5 ml of cold 70% ethanol and invert 10 times to wash the pellet.

(14) Centrifuge the tube at 16,000 *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 (0.2x) with a wide bore tip. Incubate the sample at 37 °C for 15 min if the pellet does not resuspend. The resuspended sample may be 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 DNA extract 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

Please refer to the **Aurora Reusable Cartridge Handling Manual** (**106-0014**) for detailed instructions on how prepare the **Aurora Reusable Cartridge** (**211-0004**), load your samping into the cartridge, prepare the Aurora instrument and run the Aurora protocol. Please select the **106-0011-BA-D AURORA 0.3-50KB DNA FROM STOOL PROTOCOL** when asked to select the protocol 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 as large amounts of DNA can inhibit PCR. 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 **106-0011-BA-D AURORA 0.3-50KB DNA FROM STOOL 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	6-10 mA
Expected average power	5-6 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
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. 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.