Aurora DNA From Small Volume Lysates Protocol

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Introduction

This protocol is for extracting and purifying DNA from small, low abundance samples, including cultures, filter rinsates, swabs and similar sample types. The Aurora has demonstrated exemplary performance compared to precipitation or solid phase based purification methods when purifying small quantities of DNA. Output from this procedure may be used in a variety of downstream applications including PCR, permitting the identification of low abundance targets.

This protocol uses a detergent and enzyme-based lysis procedure. No phenol or chloroform are required. The lysate is mechanically sheared with a syringe and then purified using the Aurora instrument.

This protocol allows for a lysis volume of 0.1 ml per single 5 ml-sample Aurora cartridge.

Example results

To demonstrate the protocol, purified genomic DNA from a *Escherichia coli* DH10B™ culture was prepared by both the **Aurora DNA From Small Volume Lysates Protocol** and from QIAGEN Genomic-tip 20/G® columns for the purpose of comparison.

Escherichia coli DH10B™ (Invitrogen catalog # 18290-15) was inoculated in 1 ml of LB Broth (Sigma catalog #L7275) and grown overnight in a shaking incubator at 37 °C. 400 µl of culture (OD_{600} of 2.5) was serially diluted 10-fold to obtain 400 nl of culture. The diluted culture was then purified with the **Aurora DNA From Small Volume Lysates Protocol** (106-0006) or QIAGEN Genomic-tip 20/G® columns (catalog #10223, 19060). Each method was repeated twice.

A dilution series of each purified sample was prepared and analyzed by quantitative PCR (qPCR) with the primers targeting the *E. coli* uidA gene sequence (Forward: 5'- GCC CAA CCT TTC GGT ATA AAG AC -3', Reverse: 5'- GTT CGC CGA TGC AGA TAT TCG T -3', Probe: 5'- FAM -TTC GCG CTG ATA CCA GAC 3'- MGB). 5 µl of each diluted sample was loaded into a 25 µl reaction containing Roche Applied Science FastStart Universal Probe Master Mix (catalog # 04913957001) to 1x. Forward and reverse primers were each used at 625 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 DNA recovered (in ng *E. coli* genome equivalents) by each extraction method. The **Aurora DNA From Small Volume Lysates Protocol** gives significantly higher DNA yield than the QIAGEN Genomic-tip 20/G® columns. Undiluted samples amplified successfully and showed no qPCR inhibition.

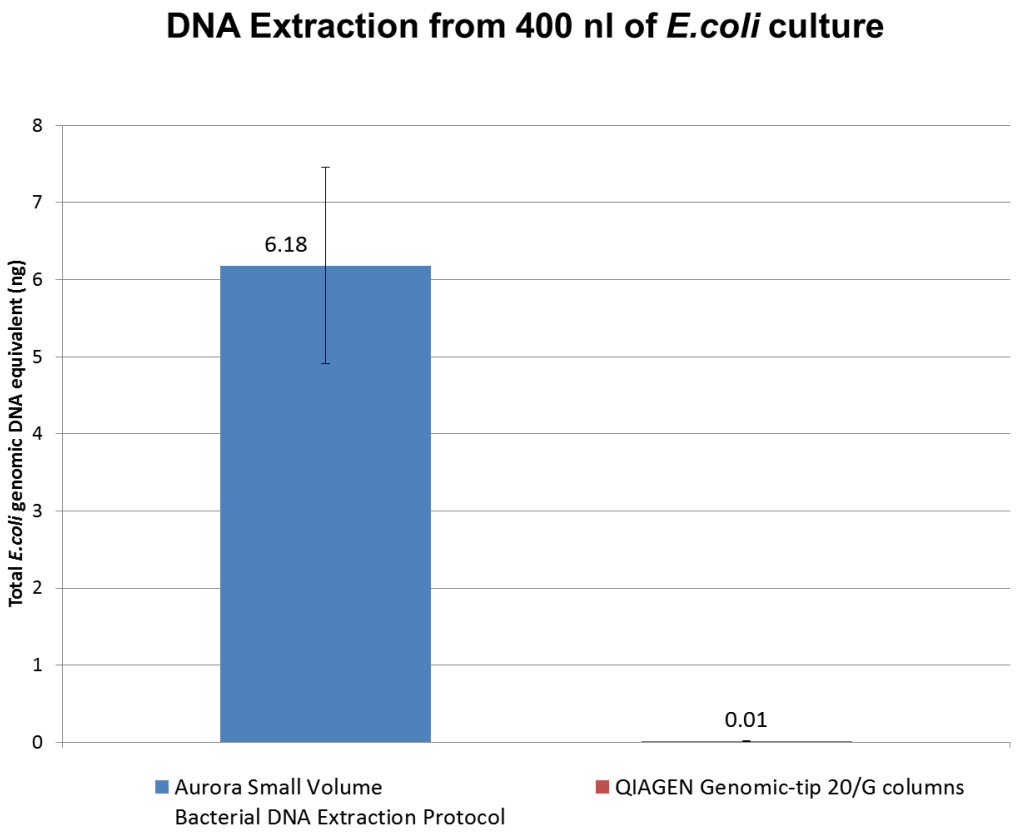

Figure 1. Average DNA recovery for each purification method. Error bars represent standard deviation among PCR replicates (n=2) and technical replicates (n=2). Data values are calculated according to the following formula: [DNA in PCR]x(volume of protocol output)

Aurora DNA from Small Volume Lysates Protocol

Cell lysis takes about 45 min and the **Aurora DNA From Small Volume Lysates Protocol** takes 4 h. If desired, crude lysate may be stored at -20 °C prior to Aurora purification.

Safety guidelines

Gloves should be worn during all stages of the protocol. Avoid skin contact with all reagents.

Materials required

1. **Lysis Buffer**

The amounts given below make enough buffer to process 100 samples. The volumes can be scaled up or down as required. The lysis buffer can be prepared in advance and stored at room temperature for up to 6 months. 0.1 ml of lysis buffer is required per sample.

Reagent	Volume (ml)	Final Concentration
1M Tris-HCl, pH 8.0	0.5	50 mM
0.5M disodium EDTA, pH 8.0	1	50 mM
20% sodium dodecyl sulfate (SDS)	0.25	0.5 %
Nuclease free dH_2O	8.25	-
Total volume	10	

2. **Other Reagents**

Reagent	Volume required per sample
20 mg/ml proteinase K, pH 8.0	2 µl
50 mg/ml lysozyme, pH 8.0	5 µl
20 mg/ml RNAse A	1 µl
0.0125x TBE	4.9 ml

3. **Equipment and materials required:**

- 37 °C Heat Block
- Microcentrifuge
- 21G2 needle
- 1 ml syringe
- Aurora instrument
- Aurora Reusable Cartridge (211-0004-AA-D)
- Clear adhesive film

Lysis protocol

(1) If working from a culture, spin down cells at 5,000 *g* for 5 min. Carefully pipette out and discard the supernatant.

(2) Add 0.1 ml of lysis buffer and the specified amounts of proteinase K, lysozyme and RNAse A. Use a pipette to resuspend the pelleted sample in the lysis buffer thoroughly.

(3) Incubate in a heat block at 37 °C for 30 min. The incubation can be extended as necessary.

(4) Add 400 µl of 0.0125x TBE to the lysate. At this point, either proceed to the Aurora purification or store 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. Select the **106-0006-BA-D AURORA DNA FROM SMALL VOLUME LYSATES 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 400 µS/cm.

Immediate Remedy: The run can continue, but yield will be decreased. In general, the higher the conductivity over 400 µS/cm, the lower the yield.

Solution: To solve this problem, run a reduced amount of sample such that the sample conductivity is within the set limit.

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 DNA. Adding 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 step.

Yield may be reduced if the sample conductivity is too high. See troubleshooting Error 1, as well as troubleshooting 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 **.sp** file that accompanies this protocol guide and are provided for reference. 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	≤400 µS/cm

Injection

Injection voltage	500 V
Injection charge	8800 mC

Expected current	9-13 mA
Expected average power	8-12 W
Expected voltage drop across the gel	≤23%

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