Aurora 200 KB DNA CLEAN-UP PROTOCOL

November 2012

106-0018-BB-D

http://www.borealgenomics.com

support@borealgenomics.com

+1 (604) 822-4111

Table of Contents

Introduction

This protocol is for removing contaminants from DNA that remain after purification by other methods, including solid phase extraction. The protocol works with input from phenol-chloroform preparations, commercial silica-based spin columns, and other preparative methods that yield DNA in small volumes with relatively low conductivity (see input specifications below). This protocol is optimized to recover DNA molecules from 300 bp up to 200 kb in length with up to 60% yield. Note that the presence of shorter molecules will not impair this yield. The purified DNA is suitable for downstream processes including PCR, library construction and DNA sequencing.

Input Sample Specifications:

Volume: Up to 5 ml

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

The conductivity of the sample after dilution to 5 ml must be ≤100 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.

Safety guidelines

Wear gloves during all stages of the protocol. Avoid skin contact with all reagents. Appropriate precautions should be taken if hazardous samples are used with the cartridges.

Preparing the sample

Process samples using your choice of DNA purification method, following required safety procedures.

SCODA works best with low-conductivity samples. To maximize SCODA yield, take steps to keep conductivity of the SCODA input as low as possible. This may include eluting in deionized water instead of buffer or repeating wash steps, depending on the purification method. Additionally, it may be possible to improve the yield of silica columns by eluting in larger-than-recommended volumes, for subsequent concentration with SCODA.

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. Avoid vortexing as the DNA may shear. Final sample conductivity must be less than or equal to 100 µS/cm. Running more conductive samples will decrease yield.

Loading your sample and running the Aurora protocol

1. Follow the directions in either the **Aurora Disposable Cartridge Handling Manual** (106-0010), saving the buffer removed from each chamber, or the **Aurora Reusable Cartridge Handling Manual** (106-0014-BA-D) to prepare the Aurora.

2. Load the diluted 5 ml sample into the sample chamber, and run the **106-0018-BB-D Aurora 200 KB DNA CLEAN-UP PROTOCOL.SP** file.
3. **Optional:** After the Injection Block has completed, you can pause the run and modify the duration of the Timed Wait block so that the Final Focus 1 block will complete at a convenient time to promptly extract your DNA sample, and then resume the run as usual.
4. Once the Focus 1 and Final Focus 1 blocks are complete, a wait step will appear to allow for the sample to be extracted. Carefully peel off the clear film over the extraction well and extract the buffer with a pipette.
5. **Optional:** To increase final DNA recovery by up to 10%, refill the extraction well with 60 µl of buffer that was previously saved, reseal the extraction well with tape and continue the protocol with Focus 2 by selecting the play button. The duration of this second focus step is 5 h.
6. When Final Focus 2 is complete, collect the sample as before by first peeling off the tape and extracting with a pipette. This step will increase the overall yield (Total DNA) but will double the effective output volume once the two DNA extractions are combined.

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 ≤100 µS/cm.

Immediate Remedy: The run can continue, but yield will be decreased. In general, yield decreases with increasing conductivity over 100 µS/cm.

Solution: To solve this problem, adjust the DNA extraction protocol to reduce the amount of salt in the sample. Some suggestions are, depending on the method employed, to elute samples from silica column based methods in nuclease-free water or 0.1x TE buffer, resuspend DNA pellets in nuclease free water or 0.1x TE buffer, and to increase the number of ethanol-based washes in precipitation methods.

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. This protocol uses cartridge part number **210-0001-CA-D** or **211-0004-AA-D**.

SCODA conditions

These conditions are pre-programmed in the **106-0018-BB-D AURORA 200KB DNA CLEAN-UP 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	60 V
Injection charge	6250 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	25 V/cm
SCODA cycle period	10 s
Duration	4.4 h
Wash strength	20%

Expected current	20-30 mA
Expected power	7-9 W

Focus 1

SCODA field strength	15 V/cm
SCODA cycle period	40 s
Duration	10 h

Final Focus 1

SCODA field strength	70 V/cm
SCODA cycle period	4s
Duration	10 min

Expected current	30-45 mA
Expected power	9-11 W

Focus 2 (Optional)

SCODA field strength	15 V/cm
SCODA cycle period	40 s
Duration	5 h

Final Focus 2 (Optional)

SCODA field strength	70 V/cm
SCODA cycle period	4s
Duration	10 min

Expected current	30-45 mA
Expected power	9-11 W