Aurora Validation Protocol

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106-0002-EA-D

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Introduction

This validation protocol is intended to confirm the normal operation of the Aurora instrument and provide visual confirmation that DNA will focus normally. The validation run concentrates 200 ng of a DNA ladder in the presence of GelGreen nucleic acid stain for visualization.

The validation run will take four hours to complete, plus the time required to prepare the sample and cartridge.

If instrument troubleshooting is required, Boreal Genomics technical support may ask you to reattempt the validation run. If possible, please retain the images and data logs from your initial run so that they are available for comparison.

Materials provided

The validation kit (part 102-0001) contains:

- 20 µl Gel Green (1,000x) nucleic acid stain in water (Cedarlane Cat# 41005-F, diluted 1:10)
- 200 µl 1 kb ladder, 10 ng/µl (New England Biolabs Cat# N3232, diluted 1:50)
- 1 sheet clear adhesive film
- Material Safety Data Sheets

Store GelGreen, DNA ladder and TBE at 4 °C. Refer to MSDSs for these materials before handling them.

Materials required

This protocol requires either the Aurora Reusable Cartridge (211-0004-AA-D) or the Aurora Disposable Cartridge (210-0001-CA-D). Please refer to the appropriate user manual for the cartridge you are using. Nuclease-free deionized water and a 15 ml centrifuge tube are required but not supplied.

Safety guidelines

Wear gloves during all stages of the protocol. Cartridges are made from non-hazardous plastics, graphite, metal, TBE buffer and agarose. Appropriate precautions should be taken if hazardous samples are used with the cartridges.

Preparing the sample

Prepare a clean 15 ml centrifuge tube with 5 ml nuclease-free deionized water (not provided). Add 200 µl 0.25x TBE, 5 µl Gel Green (1,000x), and 50 µl 1 kb ladder solution to the tube and vortex thoroughly. *Note: You can use 0.25x TBE buffer prepared as described in the **Aurora Reusable Cartridge Handling Manual** (106-0014-BA-D). Alternatively, Aurora Disposable Cartridges (210-0001-CA-D) are shipped with 0.25x TBE in the sample chamber, and this buffer can be used.*

Loading your sample and running the Aurora protocol

Please refer to either the **Aurora Reusable Cartridge Handling Manual** (**106-0014-BA-D**) or the **Aurora Disposable Cartridge Handling Manual** (**106-0010-BA-D**) for detailed instructions on how to load the sample you have prepared into the Aurora cartridge. If you are using and Aurora Reusable Cartridge, you should prepare a 1% agarose gel in 0.25x TBE buffer, and use 0.25x TBE buffer in the buffer chambers exactly as described in the manual. Prepare the Aurora instrument and run the Aurora protocol as described in the **Aurora User Manual (BG-2002-07-004)**. Please select the file **106-0002-EA-D AURORA VALIDATION PROTOCOL.SP** when asked to select the protocol .sp file appropriate for your application.

Example Results

Figure 1 is a series of images taken at various time points during a typical validation run on the Aurora. These images can be used as a guideline to gauge the success of a run.

- Figure 1a is the very start of a run. No fluorescence should be seen in the gel.
- Figure 1b is the end of injection. Faint fluorescence may be seen as the DNA enters the gel from the right.
- Figure 1c is midway through a wash block. DNA is beginning to focus and is located to the right of the extraction well. The fluorescence will increase in brightness during this block of the protocol as the DNA begins to concentrate.
- Figure 1d is the end of a wash / start of a focus block. DNA is tightly focused but has not entered the extraction well. Some condensation may be seen on the PCR tape at this point as a result of heat being generated during a run.
- Figure 1e is midway through a focus block. DNA is in the process of entering the extraction well.
- Figure 1f is at the end of the validation run. At this point, the DNA should be entirely in the extraction well.

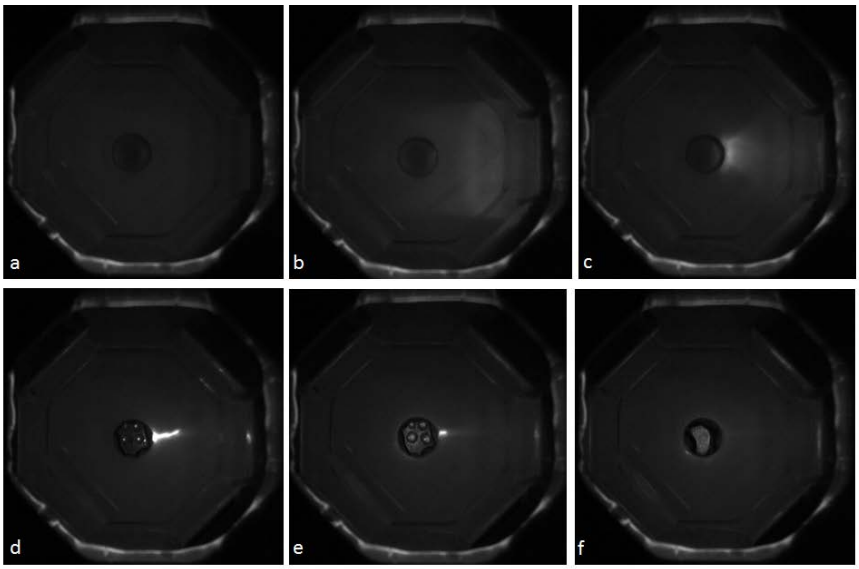

Figure 1 – Time-lapse sequence of a DNA ladder during an Aurora validation run. 1x GelGreen stain is diluted in 5 ml 0.01x TBE and 200 ng of a DNA ladder was loaded into the sample chamber of a disposable cartridge.

Troubleshooting

Please see the Aurora user manual for more information about troubleshooting machine faults.

1 Error: There is still remaining fluorescence in the gel at the end of the validation run.

Check the extraction well to ensure there is sufficient buffer. Excessive evaporation or failure to add sufficient buffer to the extraction well can cause liquid levels to be lower than the gel level, which would prevent DNA from entering the gel.

Solution: If this occurs, top up the extraction well to 60 µl 0.25x TBE and ensure that all of the buffer is at the bottom of the well and that no air bubbles have formed. Seal the extraction well with a new PCR tape and resume the run with a focus block (see SCODA Conditions below).

2 Error: Excessive evaporation can be seen and the concentration gel is bubbling.

This indicates that the gel and cartridge were not properly cooled during the run.

Solution: Ensure that cooling has been enabled on the chiller, and that 1 ml of water was added to the spreader plate. Also take care to clean the spreader plate and the bottom of the cartridge of all particulates and debris, as these can lead to uneven cooling of the cartridge and the gel.

SCODA Conditions

These conditions are pre-programmed in the **106-0002-EA-D AURORA VALIDATION 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

Running buffer	0.25x TBE
Sample volume	5 ml
Expected sample conductivity	≤100 µS/cm

Injection

Injection voltage	600 V
Injection charge	3000 mC

Expected current	3-25 mA
Expected average power	3-12 W
Expected voltage drop across the gel	≤10%

Wash

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 hrs

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