Aurora Reusable Cartridge Handling Manual

106-0014-FA-D

http://www.borealgenomics.com

support@borealgenomics.com

+1 (604) 822-4111

Table of Contents

Introduction

This manual describes the proper method of preparing a reusable cartridge for use with the Aurora, loading the sample in the cartridge, extracting the purified DNA from the cartridge once the Aurora purification is complete, and cleaning the cartridge. Details on preparing a variety of sample types prior to loading them into the Aurora cartridge can be found in separate Aurora protocol documents on the Boreal Genomics website (http://www.borealgenomics.com/customer-service/).

Cartridge compatibility

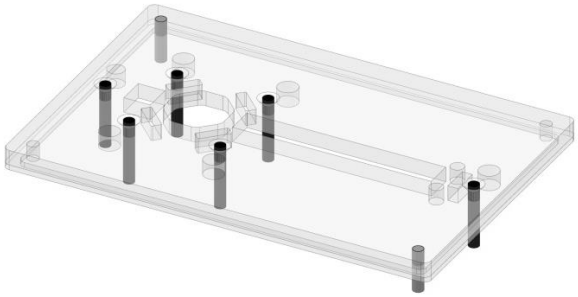
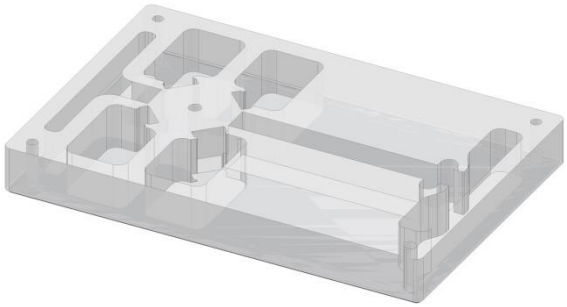
The procedures described in this manual apply to Aurora Reusable Cartridge (211-0004-AA-D).

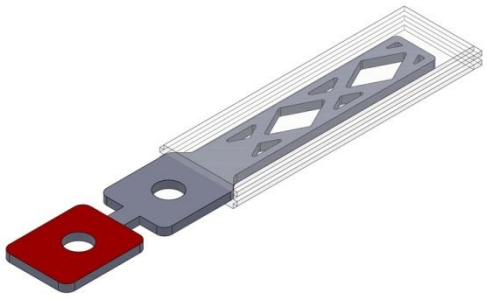
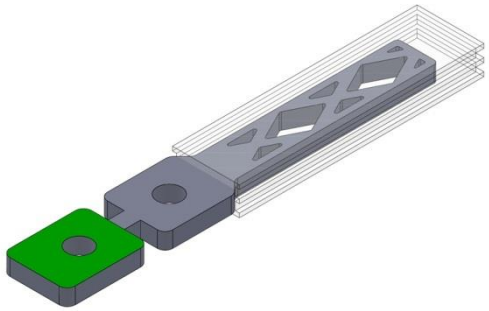
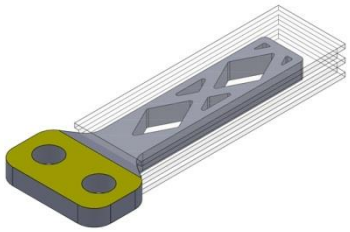
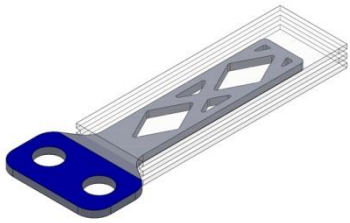
Safety guidelines

Cartridges are made from non-hazardous plastics, graphite electrodes and aluminum. Appropriate precautions should be taken if hazardous samples are used with the cartridges. Wear gloves during all stages of the protocol. Avoid skin contact with all reagents. Refer to safety guidelines in Boreal Genomics Protocol documents for further information.

Pack list

One reusable cartridge kit contains the following items and quantities:

1x	Reusable electrode plate (top) • With 6 graphite electrodes pre-inserted • With 2 alignment pins pre-inserted	
1x	Reusable cartridge (bottom)	
8x	Spare graphite electrodes	
1x	Extraction well post	
2x	Cleaning brushes	
1x	Sheets PCR tape (MicroAmp Optical Adhesive Film)	No image

4x	Type-A gel dams • A-type gel dams are coloured RED • Note: A-type dams are thinner than B-type dams	
2x	Type-B gel dams • B-type gel dams are coloured GREEN • Note: B-type dams are thicker than A-type dams	
1x	Type-C gel dams • C-type gel dams are coloured YELLOW • Note: C-type dams are thicker than D-type dams	
1x	Type-D gel dams • D-type gel dams are coloured BLUE • Note: D-type dams are thinner than C-type dams	

Reagents required

The following materials are required for each Aurora Reusable Cartridge. These values assume you are making a 1% agarose gel with 0.25x TBE running buffer. Scale these values up to prepare enough reagents for multiple cartridges. Molten agarose can be incubated in a water bath for up to three days prior to use.

Reagent	Amount required per cartridge
Agarose powder (Lonza SeaKem® LE; Lonza Cat # 50001)	0.05 g
10x TBE (Ambion Cat # AM9863)	1.25 ml
Nuclease-free water	48.75 ml

Procedure overview

- **Prepare buffer**
- **Prepare agarose**
- **Prepare the sample**
- **Prepare the cartridge**
- **Cast agarose gel**
- **Fill buffer chambers**
- **Load the sample**
- **Prepare the Aurora**
- **Begin the run**
- **Sample extraction**

Preparing buffer

This section describes the steps and materials required to prepare buffer for use with the reusable cartridge.

Materials

- TBE buffer stock
    - Boreal Genomics recommends Ambion 10x TBE (Ambion catalogue number AM9863)
- Nuclease-free water
- Optional: Polystyrene cell culture flask
    - Boreal Genomics recommends Costar 225 cm^2 Cell culture flask (Corning catalogue number #3000)

Time required

Approximately 5 min hands-on time.

Notes

- For sensitive or low copy DNA samples, it is recommended that all handling steps be carried out in a biosafety laminar flow hood and that all glassware be autoclaved to avoid any exogenous DNA contamination.
- Boreal Genomics protocols typically require 0.25x TBE buffer. It is possible to prepare reusable cartridges with different buffer strengths; consult Boreal Genomics for further details.
- It is recommended that buffer be prepared in bulk in advance.
- Buffer is used both to prepare agarose gel, and to fill the buffer chambers of the reusable cartridges. Each reusable cartridge run requires a total of about 50 ml of buffer.

Procedure

- Dilute 10x TBE in nuclease-free water to a concentration of 0.25x to the desired final volume. A minimum of 50 ml of buffer is required for each cartridge; however more can be made in advanced for multiple preparations.
- *Optional*: to further sterilize the buffer, fill a cell culture flask up to a quarter of its capacity (ex. 125 ml of a 500 ml flask) and place the flask containing buffer under UV light on a rotating platform overnight. It is essential that the flask is transparent to UV so that the UV can penetrate the buffer for efficient irradiation.

Preparing agarose

This section describes the steps and materials required to prepare agarose gel for use with the reusable cartridge.

Materials

- Agarose powder
    - Boreal Genomics recommends Lonza SeaKem® LE (Lonza catalogue number 50001)
- TBE buffer prepared in previous step

Time required

Approximately 15 min hands-on time, plus 1 h hands-off time.

Procedure

Prepare a 1% w/v agarose gel by adding 0.05 g agarose and 5 ml 0.25x TBE (made in the previous step) in an Erlenmeyer flask. It is recommended that you scale these numbers up to make extra agarose to have on hand in case gels need to be recast for any reason (such as small leaks, etc.), or if you intend to run multiple cartridges.

- Weigh the flask containing the agarose solution before heating and record the weight.
- To minimize evaporation, cover the flask loosely with a lab tissue and heat the solution in a microwave oven until the gel has completely dissolved without boiling over.
- Weigh the flask after heating and add nuclease-free water to replace water lost to evaporation, bringing it back up to its original weight.

- Transfer the agarose to a conical tube with a cap to avoid further evaporation, and incubate the molten agarose at 90-95°C for 1 hour. This leads to improved DNA recovery and it is recommended that this step is not omitted.
- After 1 hour, transfer the agarose to a water bath at 60-70°C. Allow the temperature to equilibrate and leave the agarose in the water bath until use.

Notes

- Aurora protocols usually require 1% agarose in 0.25x TBE. It is possible to prepare reusable cartridges with different agarose percentages; consult Boreal Genomics for further details.
- Molten agarose should not be stored for more than 3 days before use.
  - Discard any agarose which has been stored for more than 3 days.
  - Using agarose made on the same day results in optimal gel characteristics.

Preparing the sample

Please see your Aurora protocol documentation for details on the method of preparing your sample prior to loading it into the Aurora cartridge.

Preparing the cartridge

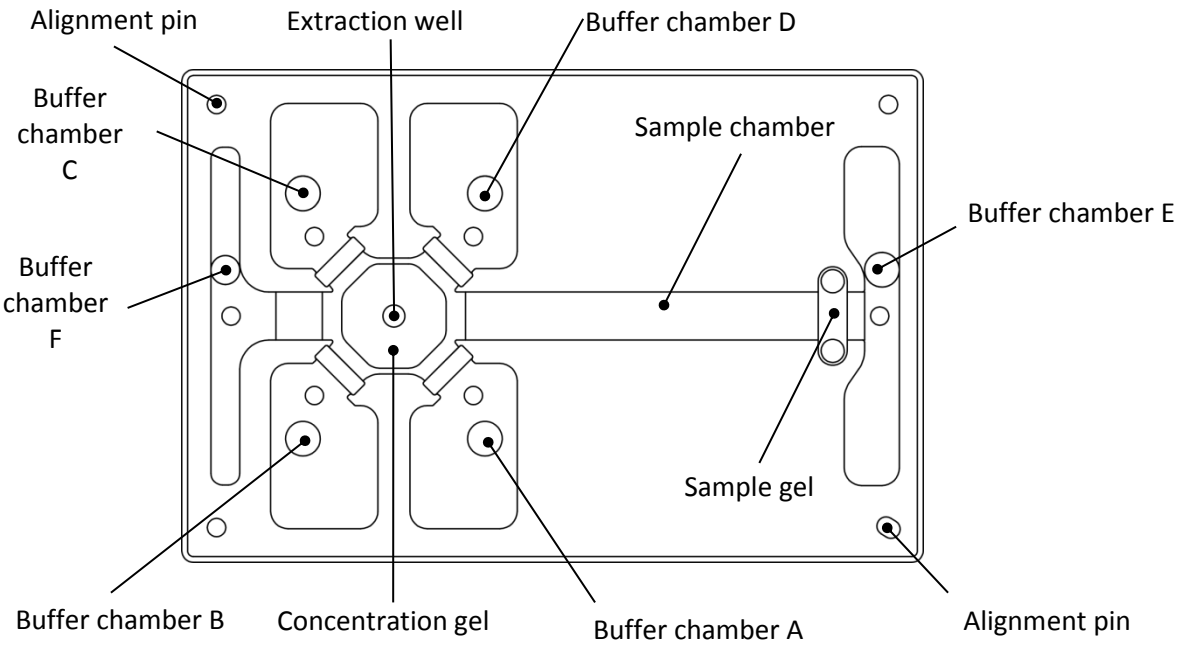


Figure 1: Top view of the cartridge

Inspecting the cartridge

Before beginning cartridge preparation, check that:

- The cartridge and accessories have been cleaned. Refer to the section titled “Cleaning Cartridges and Accessories” for instructions.
- The graphite electrodes are not chipped or cracked.
- The cartridge is not damaged or cracked.

Materials required

- Gel dams
- Extraction well post

Time required

Approximately 5 minutes of hands-on time.

Procedure

Begin by removing the electrode plate from the cartridge, and set it aside. The following steps should **not** be performed with the electrode plate installed.

Refer to Figure 2 and Figure 3 for insertion locations for each type of gel casting accessory.

Note that it is important to press the gel casting accessories down fully so that they create a good seal with the floor of the cartridge. A good seal is essential to prevent leaks.

- Insert the extraction well post into the hole in the middle of the concentration gel region. The post can be tight to insert. Make sure the post goes all the way through the gel cavity and touches the floor of the cartridge.
    - A common mistake is to not insert the post far enough, which will result in a poorly formed extraction well.
- Insert 4 Type-A gel dams into the grooves surrounding the gel octagon.
- Insert 2 Type-B gel dams
    - On the F buffer chamber side, insert the gel dam about 4.5 mm away from the octagon wall at the location marked by etched lines on the reusable cartridge. This gap will be used to cast the concentration gel.
    - In the sample chamber, insert a Type B gel dam flush against the octagon wall
- Insert the Type-C gel dam to fit in the sample chamber on the left side of the sample gel cavity.
- Insert the Type-D gel dam to fit between the short walls on the right side of the sample gel cavity.
- The cartridge should now resemble Figure 3. Ensure that the stainless steel tube and all the gel dams are vertical and in contact with the bottom surface of the cartridge.

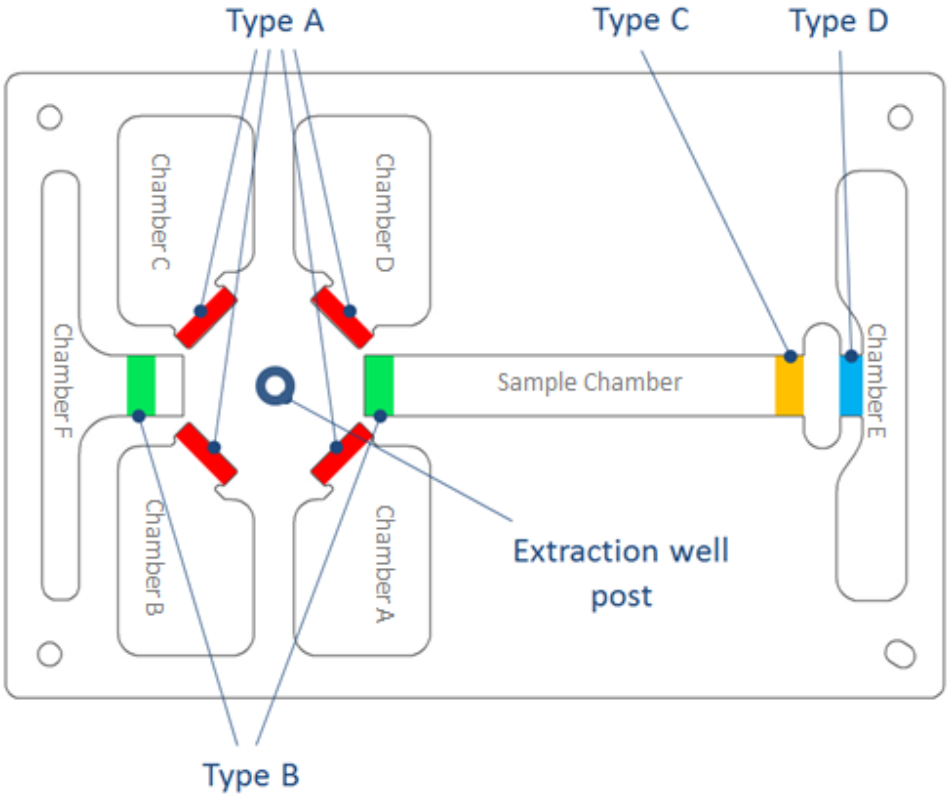


Figure 2: Cartridge showing locations of gel dams (diagram)

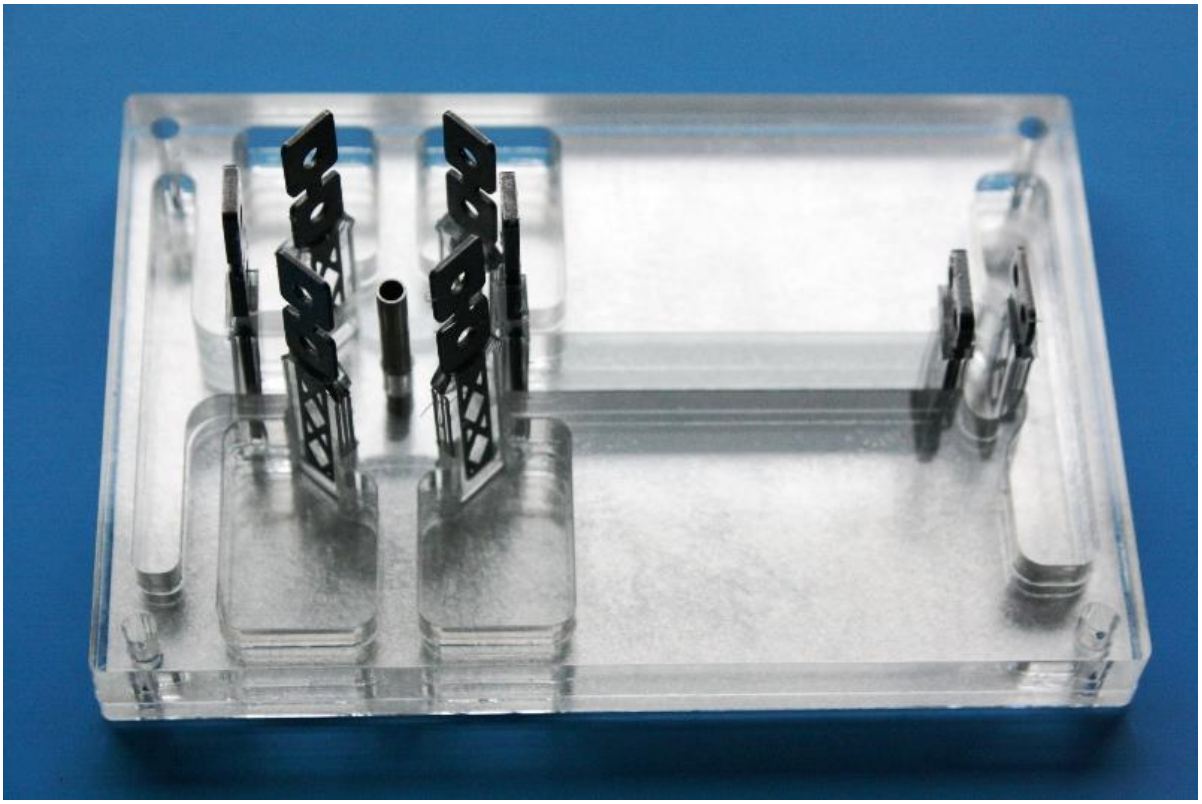


Figure 3: Cartridge with gel dams inserted (photograph)

Casting agarose gel

Materials required

- Molten agarose/TBE solution at the desired buffer concentration
- P1000 or larger pipette

Time required

Approximately 10 min hands-on time, plus 20 min hands-off time.

Procedure

To cast the concentration gel, use the pipette to transfer molten agarose into the 4.5 mm gap between the left Type B dam and concentration gel area until the whole concentration gel area is filled with gel (see Figure 4) and there are no voids present in the concentration gel. To facilitate casting and avoid air bubbles, it may be possible to tilt the cartridge to the right and not fully expel the agarose from the pipette tip at one time.

- The 4.5 mm gap should be filled with gel also until the agarose is flush with the top of cartridge.

- Slightly more than 2 ml of agarose is required for this step, but may vary based on gel dam placement and any leakage that occurs. Add enough agarose so that the gel fills all corners of the gel volume.
- Immediately check for any air bubbles or leaks. If either is present, make any necessary gel dam adjustments and top off any lost agarose. The gel will begin to set within minutes so ensure that this is done quickly.

To cast the sample gel, pipette molten agarose into the sample gel region between the Type C and Type D gel dams on the far right side of the cartridge.

- Use enough agarose to make the gel flush with the top of the cartridge – typically slightly more than 1 ml. Perform this step with the cartridge on a flat surface
- If any of the agarose leaks underneath a dam, re-adjust the dam and top up with agarose.

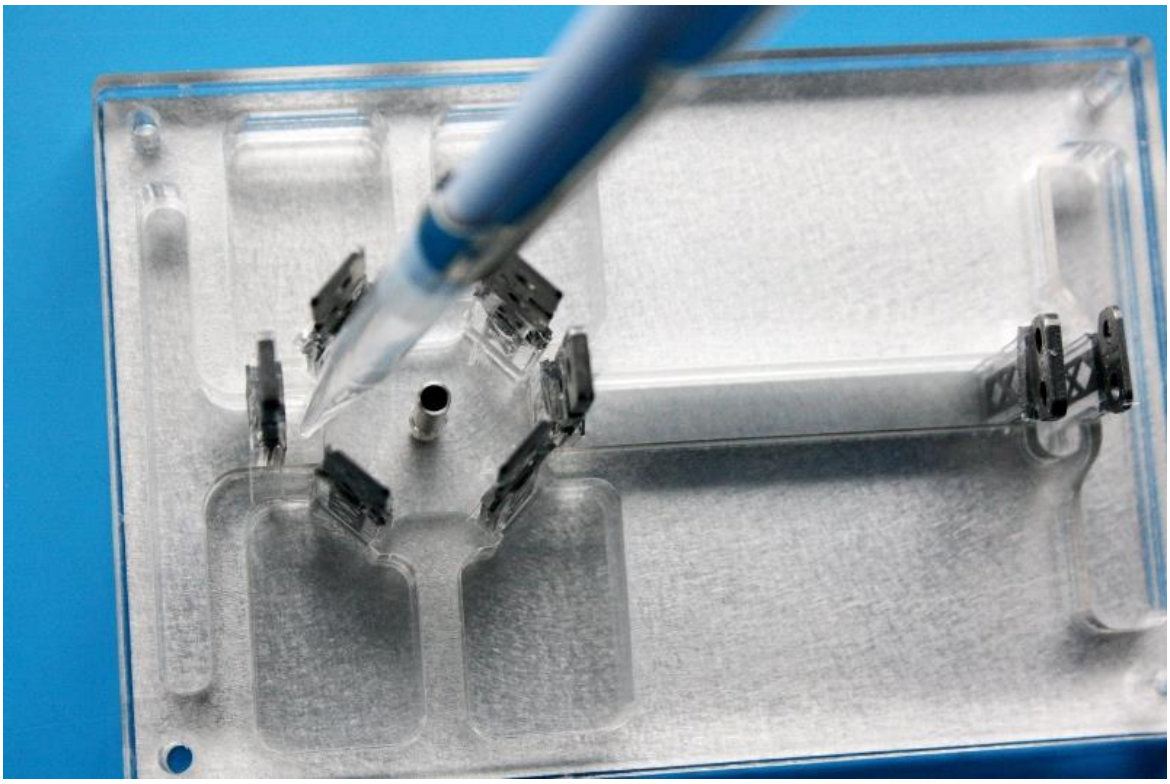


Figure 4: Casting the SCODA gel (photograph)

- Leave the cartridge on a flat surface at room temperature. Visually inspect both gels for the presence of air bubbles. The gel will begin setting immediately, so if air bubbles are present immediately add more molten agarose. Let the gel set for 20 min.
- Carefully remove all the gel dams around the concentration gel by pulling them straight up, while taking extreme care to ensure that the gel is not disturbed or delaminated from the plastic surfaces of the cartridge.

- Carefully remove the gel dams surrounding the sample gel For the sample gel, it is best to pull the dams horizontally away from the gel first, and then pull them up; this is to prevent the gel from being detached from the bottom of the cartridge, which can result in sample loss.
- Use a spatula to remove any loose gel that may have leaked under the dams.
- To remove the extraction well post, use a gentle twisting motion to break the tension between the gel and the post, and then gently pull it straight out of the cartridge. Do not cover the hollow center region of the post with your finger when removing it as this will create a small vacuum and could damage the gel.
- Inspect the well formed by the post – the walls should be straight and clean, and the center of the well should be free of any gel. If there are any gel fragments in this well, use forceps or a bent wire to remove it, taking care to avoid damage to the sides of the well.

Filling buffer chambers

Materials required

- The same buffer that was used to make the gel
- Serological pipette: 5, 10, or 20 ml.

Procedure

On a workbench, prepare the cartridge for running.

- Add 5 ml of buffer to each of the buffer chambers: A, B, C, D, and E, and then add 4 ml of buffer to buffer chamber F (see Figure 1).
- Place the electrode plate on top of the cartridge, so that the 2 plastic alignment pins fit inside the respective hole and slot. The graphite electrodes should not mechanically interfere with the floor of the cartridge, however, it is acceptable if they come very close to the floor.
- Add 60 µl of buffer to the extraction well.
- Cut a piece of PCR tape into a rectangle sized 28 mm x 10 mm. Boreal Genomics recommends MicroAmp Optical Adhesive Film (available LIFE/Invitrogen 4311971).
- Remove the white backing from the piece of PCR tape and place it on top of the extraction well as shown in **Figure 6**. One edge of the film should stick up above the cartridge slightly to allow for easier removal of the adhesive film after the run. With a gloved finger, firmly press down on the film, ensuring a good seal on all edges and taking care to avoid wrinkles and bubbles.
- Align the extraction well sealing tool to the extraction well, as shown in **Figure 7** (left). Firmly press down on the tool until it is fully compressed, as shown in **Figure 7** (right). Release and then press down a second time to ensure a good seal.

Load the cartridge into the Aurora instrument

- Pull gently outward at both left and right sides of the cartridge drawer until the drawer slides out and latches in the open position.

- Examine the cold plate and the metal bottom of the cartridge for debris that may prevent the cartridge from sitting flat on the cold plate. Remove any debris that is found.
- Pipette 1 ml of water onto the center of the cold plate and place the cartridge onto the cold plate oriented so the concentration gel is on the left hand side, as pictured in Figure 5.
- Ensure that the cartridge is sitting flat on the surface of the cold plate and is not resting on top of the cartridge registration features.

These steps ensure good thermal contact between the cartridge and cold plate.

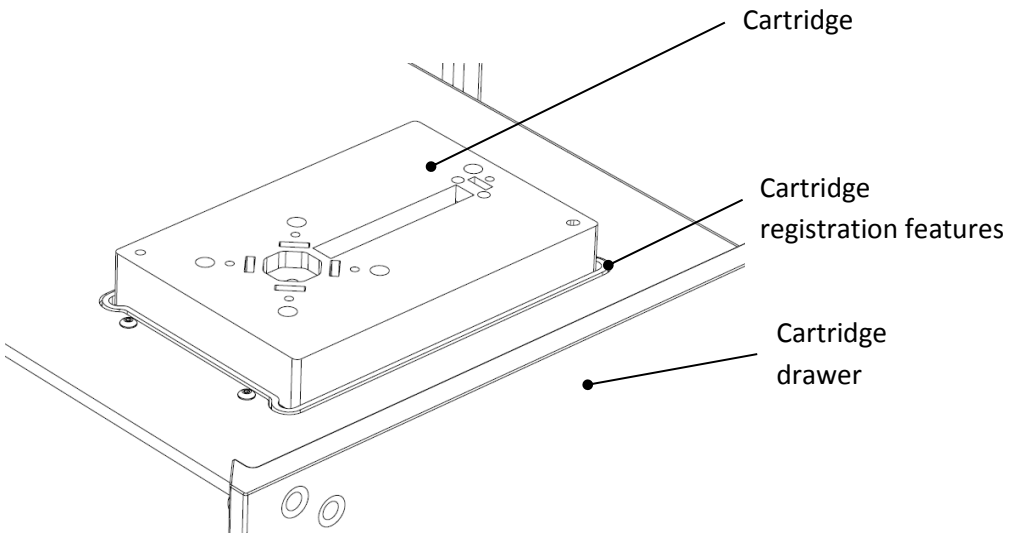


Figure 5: Cartridge oriented correctly on the cold plate

Final cartridge inspection

Inspect the empty sample chamber prior to loading your sample. If any buffer has leaked into the sample from the other reservoirs, it is best to re-cast the gel(s) to avoid loss of sample during the run. For optimal results, the sample should be loaded and the run started as quickly as possible.

Optional:

As an added precaution to reduce the risk of DNA contamination, cut a piece of PCR tape large enough to cover the sample chamber. Remove the white backing from the adhesive film and carefully lay it over the sample chamber from left to right, while leaving a small air gap between the concentration gel and the sample chamber to relieve any pressure during the run. Use a scalpel blade and forceps to cut away and remove any tape that may be covering any of the electrodes. To seal the tape, use a PCR paddle or a flat object to seal the edges, take care to avoid any wrinkles or bubbles.

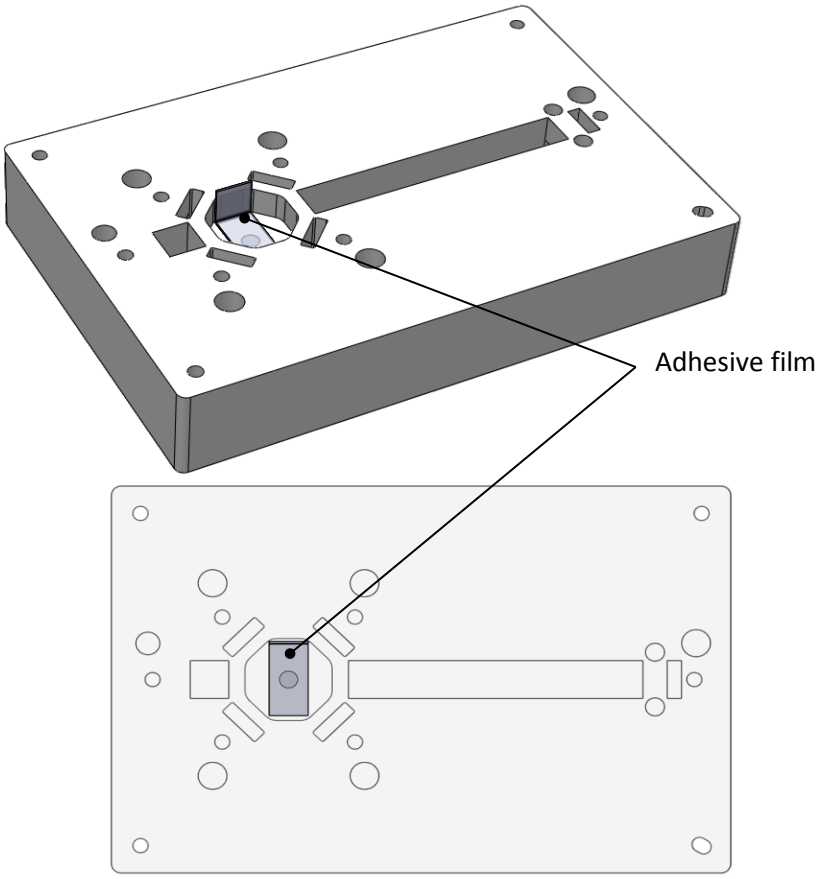


Figure 6: Cartridge with adhesive film over extraction well

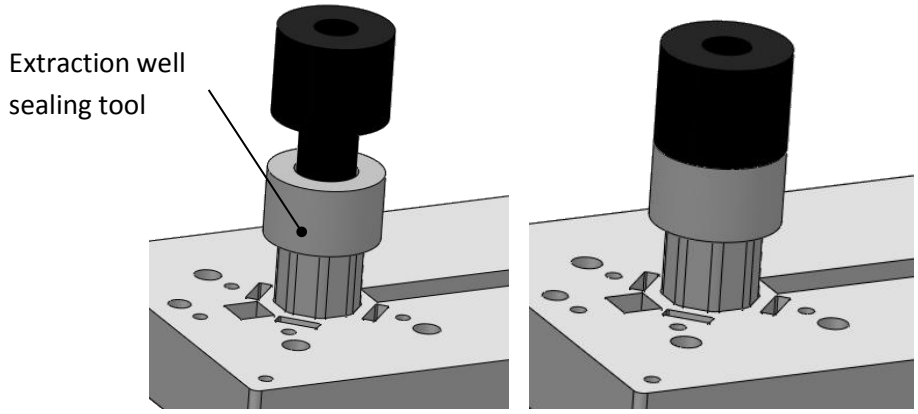


Figure 7: Extraction well sealing tool aligned and uncompressed (left) and fully compressed (right)

Load the sample

Bring the volume of the sample you prepared earlier up to 5 ml with nuclease-free water. Transfer the entire 5 ml sample into the empty sample chamber (see Figure 1). Close the cartridge drawer.

Prepare the Aurora

Prepare the Aurora and chiller as described in the user manual. Start the chiller and ensure that a plus or minus symbol appears on the display to indicate that thermal control is operating. If a star appears, press Start again.

Begin the run

From the home screen of the Aurora software, load and run the protocol appropriate for your application (ex. 106-0001-CA-D AURORA DNA CLEAN-UP PROTOCOL) as described in the "Operating the Aurora" section of the Aurora User Manual. Create an experiment folder for the run to save the logs.

The run will typically complete in 2 - 4 hours depending on the protocol that you are running.

Sample extraction

After a run is complete:

- Pull gently outward at both left and right sides of the cartridge drawer until the drawer slides out and latches in the open position.
- Lift the cartridge from the platform, taking care not to spill any liquid, and place the cartridge on a flat work surface.

- Examine the concentration gel and ensure that there are no large bubbles present. Presence of large bubbles indicates that the gel overheated during the run, which will adversely affect the quality of the output.
- Using forceps or gloved fingers, remove the clear film covering the extraction well in the center of the gel.
- Transfer concentrated DNA from this well using a pipette, taking care not to disturb the concentration gel.
- Typically, two pipetting steps are required in order to extract most of the liquid from the extraction well. Be sure to extract any sample that is suspended on the walls of the extraction well.
- The expected output volume is 50-60 µl, but may vary from 40-70 µl depending on sample and run conditions.

Cleaning cartridges and accessories

Important

The user is responsible for the safe use, transport, storage, and disposal of any materials that may be considered a chemical or biological hazard. Refer to accompanying material safety data sheets (MSDS) for safety information and handling instructions, and comply with all federal, state/provincial, municipal/local, and institutional requirements and guidelines for disposal.

Materials required

Boreal Genomics recommends the Ultra-Lūm UVC 508 UV crosslinker, set at the maximum energy setting (999 Joules), for all UV irradiation steps. The total accumulated intensity over 10 min is approximately 4.2 J/cm^2 at this setting.

Important Note Regarding Cartridge Durability

Never expose cartridges to ethanol, which causes cracks in the acrylic. It is important not to soak the cartridges in liquids for extended periods of time as part of the cleaning and decontamination procedure. Do not expose the cartridge to high temperatures and do not use heat as part of the cleaning or drying procedure. Extended soaking periods or exposure to heat can damage the cartridge. Take care not to scratch the plastic when cleaning the cartridges, in particular the thin bottom layer of plastic that is in contact with the aluminum bottom of the cartridge.

Cleaning the cartridge

- Decant and dispose of the buffer from the cartridge accordingly. If the run has not been stained with a hazardous dye, it is okay to dispose of it down a sink with running water.
- Using the cleaning brushes provided, gently break up and remove the sample gel and concentration gel. Use warm running tap water to help remove the concentration gel, if necessary.

Reminder: It is very important to take care not to scratch the bottom layer of plastic in the cartridge. Do not scour or scratch the plastic. Do not use brushes or other cleaning implements with sharp edges or points. The layer of plastic separating the gel and buffer in a cartridge from the aluminum plate on the bottom of the cartridge is very thin, and if it is scratched it may be necessary to replace the cartridge.

- Fill the cartridge with a 10% v/v solution of household bleach (5% sodium hypochlorite) in deionized water and let it soak for 30 min. Do not soak for extended periods of time, as this can damage the cartridge.
- Rinse the cartridge thoroughly with deionized water, and then let the cartridge air dry or use pressurized nitrogen or filtered air.
- *Optional:* Once the cartridge is dry, subject it to UV irradiation for 10 min to further minimize contamination. (For the most demanding applications with very low copy number DNA samples, extend the duration of UV exposure to 30 min for thorough decontamination.)
- **Note:** Repeated exposure of the cartridge to bleach and UV irradiation will eventually lead to degradation of the plastic and the cartridge will have to be replaced. Visible cracks in the plastic, or repeated leaks or instances of Run Screen Error Messages RTE030 or RTE031 may indicate that a cartridge has worn out or been damaged and should be replaced.

Cleaning the casting accessories

- Remove any visible debris or dried gel from the gel dams and extraction well post.
- Soak the gel dams and extraction well post in 10% household bleach for 10-30 min. Do not soak for longer than 30 min.
- Rinse all accessories repeatedly with deionized water, followed by a rinse and 10 min soak in deionized water.
- Let the accessories air dry.
- *Optional:* Once the accessories are dry, expose them to UV irradiation for 10 min on each side.

Cleaning the electrode plate

- Wipe the electrode plate down using 10% household bleach. Take care to avoid snapping the graphite electrodes.
- Rinse the electrode plate thoroughly with deionized water and soak in deionized water for 10-30 min.
- Let the electrode plate air dry or use pressurized nitrogen or air to dry it.
- *Optional:* Expose the electrode plate to UV irradiation for 10 min, electrode side up.

Replacing graphite electrodes

The graphite electrodes on the electrode plate should be replaced if substantial wear or pitting is visible or if the electrodes become chipped or broken. A rough surface finish on the graphite electrodes is normal.

To replace the graphite electrodes:

- Use a small drill bit or similar thin, metal object to force out the graphite electrodes from the electrode plate.
- Wash or wipe the electrode plate clean using deionized water.
- Press fresh graphite electrodes into the appropriate holes in the electrode plate from the bottom side of the electrode plate (the side where the white alignment pins protrude). The electrodes should be pressed into the holes by hand for the first few millimeters at least. Pushing the electrodes down against a flat surface may be required to press them in fully. Push the electrodes in until they are flush with the top surface of the electrode plate.
- Clean off any graphite debris that may have flaked off during the process.

Contact Boreal Genomics for replacement graphite electrodes.

Disposal

Dispose of the cartridge and contents following all applicable policies, laws, and regulations.

Aurora cartridges, buffers, and gels are non-hazardous as shipped, however cartridges that have contained hazardous samples (including many nucleic acid stains) may be considered hazardous in your jurisdiction.

Important

The user is responsible for the safe use, transport, storage, and disposal of any materials that may be considered a chemical or biological hazard. Refer to accompanying material safety data sheets (MSDS) for safety information and handling instructions, and comply with all federal, state/provincial, municipal/local, and institutional requirements and guidelines for disposal.

Troubleshooting

Please see the Aurora User Manual for more information about troubleshooting instrument faults. Troubleshooting information related to processing your sample can be found in your protocol documentation.

1. **The Aurora or the camera does not connect to the computer.**

Unplug the instrument's USB cable, wait five seconds, and reconnect the cable.

2. **A cartridge fails a contact test.**

   Before each run, the Aurora checks to make sure the instrument has good electrical contact with the cartridge. Poor contact can be caused by misalignment of the cartridge. Re-seat the cartridge firmly within the registration features on the cold plate. Check that the correct contact plate is installed. If your contact plate has spring pin electrodes, check to ensure that none of the spring pins are jammed in the up position. If you notice stuck pins, see the Aurora manual for cleaning instructions. If your contact plate has coiled spring electrodes, check to make sure there is nothing blocking contact with any of the springs and the graphite electrodes on the cartridge, such as PCR tape or salt deposits.

3. **A "sample current is below minimum current limit" warning appears.**

   The Aurora may not be making electrical contact with the cartridge. Pause the run, open the cartridge drawer, check that the cartridge is firmly seated within the registration features on the cold plate, close the cartridge drawer, and press the play button to resume. If the cartridge fails the contact check, see above.

4. **A temperature controller error appears.**

   Make sure the chiller is on and operating. If you see a ∗ on the display, the chiller is pumping coolant but temperature control is not active. Press Start/Stop on the chiller to enable temperature control.

5. **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. Please refer to the Troubleshooting section in your Protocol document for further instructions on reducing sample conductivity.

6. **Error: The Aurora control software reports Run Screen Error Messages RTE030 or RTE031.**

   These errors may indicate that the Aurora reusable cartridge has been damaged. Inspect the cartridge for cracks or scratches in the bottom layer of plastic. The cartridge can be filled with water and left on top of a paper towel to look for any leaks that may be present. Leaks, scratches or cracks that lead to these errors may require that the cartridge be replaced. Contact Boreal Genomics for assistance.

Ordering and support

For support for Aurora protocols or cartridges, or to order additional cartridges, please contact support@borealgenomics.com. This protocol uses the Aurora Reusable Cartridge (part number 211-0004-AA-D).

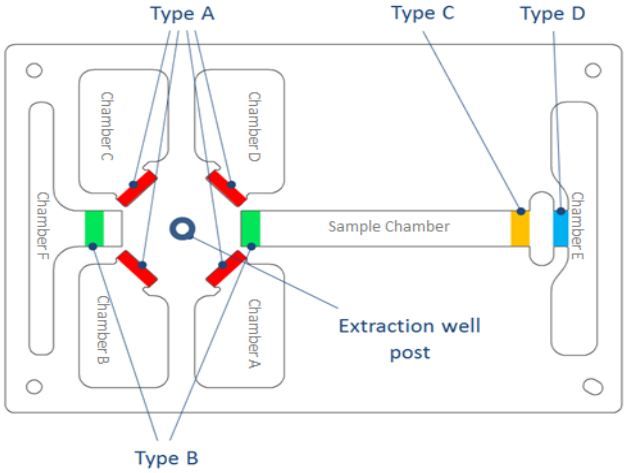
Experienced User Protocol

Please wear gloves at all times.

Materials required:

- 0.2g agarose powder (Lonza SeaKem® LE; Lonza Cat # 50001)
- 60ml 0.25xTBE running buffer
- Nuclease-free water
- Decontaminated reusable cartridge and accessories
- PCR tape and extraction well sealing tool

1. In an Erlenmeyer flask, add 20 ml 0.25xTBE buffer to 0.2g agarose powder to make a 1% gel. Record the weight of the flask and heat the solution in a microwave until the powder has dissolved.
   Note: Only 5 ml is required for each cartridge.
2. Re-weigh the flask and add nuclease-free water to bring the flask back to its original weight.
3. Transfer the agarose to a conical tube with a cap and incubate at 90-95°C for 1 h.
4. After 1 h, transfer the agarose into a 60-70°C water bath and allow the temperature to equilibrate before casting.
5. Insert the gel casting accessories, including the extraction post, into the cartridge as shown below:



6. Using a 1 ml pipette, fill the concentration gel from the gap in Chamber F until the agarose is flush with the top of the cartridge. Next, cast the sample gel between Type C and D dams.
7. Let the gel set for 20 min before carefully removing all the dams.
8. Place the electrode plate on top of the cartridge and add 5 ml 0.25xTBE buffer to Chambers: A, B, C, D, and E, and 4 ml to Chamber F.
9. Add 60 µl of buffer to the extraction well and seal it with PCR tape. Use the extraction well tool to press down on the tape and ensure a good seal.
10. Add the 5ml sample to be concentrated (<100 µs/cm) into the sample chamber.
11. Prepare the Aurora.
12. Add 1 ml of water onto the center of the cold plate and load the cartridge. Select and run the desired Aurora protocol.