Aurora 200-1100 kb DNA from Agarose Plugs Protocol

September 2016

106-0019-CC-D

http://www.borealgenomics.com

support@borealgenomics.com

+1 (604) 822-4111

Table of Contents

Introduction

This protocol is for purifying ultra-high molecular weight (UHMW) 200 – 1,100 kb DNA from agarose plugs or slices. DNA is purified directly from agarose and delivered into solution, and is then ready to be used in downstream applications such as long-read single-molecule sequencing, optical mapping, large insert cloning, metagenomic library construction, or functional genomics studies.

The input sample can be any free form of high molecular weight DNA in agarose, for example, pulsed field agarose gel slices or agarose gel plugs containing lysed bacterial cells or lysed nuclei. DNA in the sample must be free to migrate in response to electric fields. Each cartridge can be loaded with up to four gel plugs containing a combined 40 µg of DNA. The protocol yields highly purified, UHMW DNA into 80-100 µl of buffer containing polyethylene glycol (PEG). The concentration of DNA in the output will vary depending on the type, integrity and amount of input DNA. Maximum molecular weight and the amount of DNA recovered may potentially be increased if Aurora run times are extended. The presence of shorter molecules will not impair the yield of UHMW DNA.

This protocol can deliver very pure UHMW DNA over 1 Mbp in length in buffer with minimal handling required. Although longer molecules can potentially be recovered if the run time is increased, an upper limit will exist beyond which DNA molecules will not migrate efficiently through the agarose gel matrix. If a significant fraction of the DNA in the sample is expected to be larger than 1 Mbp in length, measures to reduce the molecular weight of DNA in the sample may need to be considered in an attempt to increase DNA mobility and recovery (ex. a partial restriction enzyme digest). This protocol could also be modified to recover smaller DNA molecules from agarose plugs in significantly less time. Please contact Boreal Genomics for more information about customizing this protocol. The purified DNA recovered from this protocol is suitable for downstream applications.

Example results

The following results demonstrate the Aurora's ability to purify UHMW DNA from agarose gel into buffer. Four gel plugs containing free DNA, each approximately 3x3x3 mm and mass of 150 mg, were cast into one Aurora Reusable Cartridge according to the protocol outlined below. Yeast Chromosome PFG Marker (NEB catalogue # N0345S) was used in one experiment, while a second experiment used gel plugs containing bacterial genomic DNA prepared by casting and lysing overnight cultures of *E.coli* DH05α cells in agarose gel according to the method of Liles *et al*. (2009). Pulsed field gel electrophoresis (PFGE) revealed that DNA molecules up to at least 1.1 Mbp in length were recovered (Figure 1).

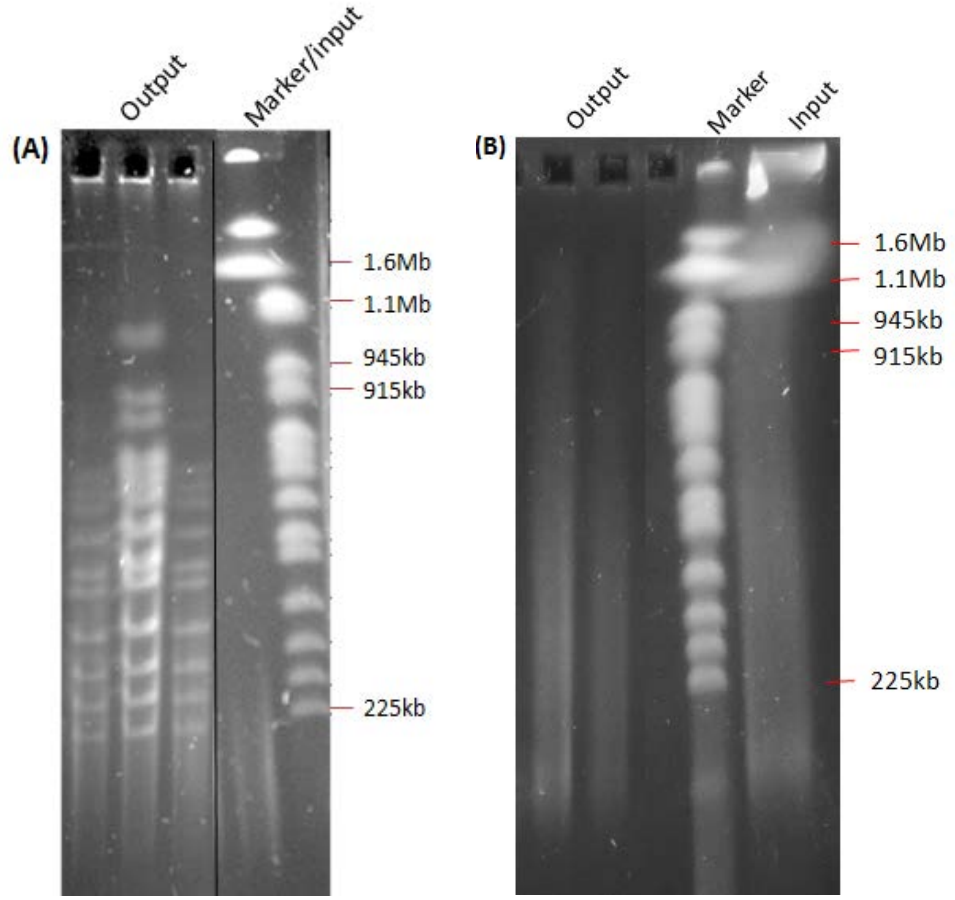

Figure 1. Fluorescent Pulsed Field Gel image of DNA recovered from (A) Yeast Chromosome PFG Marker or (B) lysed E.*coli* cells purified with the Aurora 200-1100kb DNA from Agarose Plugs Protocol, run alongside a Yeast PFG Chromosome Marker. Approximately the same amount of Input DNA shown on the PFGs was used as the input sample in each Aurora run. To load the entire Aurora output, the sample was distributed among three and two wells in (A) and (B) respectively. PFGE used the following parameters: 1% SeaKem Gold Agarose, 1x TBE gel, 1x TBE Running Buffer. Initial Switch Time: 50 s, Final Switch Time: 90 s, Run Time: 19 h, Angle: 120°, Field: 6 V/cm. The gel is post-stained in 1x Gel Green, 1x TBE Buffer and imaged under UV transillumination.

Safety guidelines

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

Preparing the sample

Minimize sample mixing and liquid handling to avoid fragmenting the DNA. The molecular weight distribution of the DNA will vary from sample to sample.

Materials required

The following table makes enough reagents for one run of the **Aurora 200-1100 kb DNA from Agarose Plugs Protocol**. Volumes can be scaled up as required.

Reagent	Total Volume Required (ml)
2x TBE	50
6% Polyethylene glycol (MW 20,000), prepared in 2x TBE [Sigma Aldrich CAS # 25322-68-3]	60
Molten 0.7% Lonza SeaKem® LE Agarose, prepared in 2x TBE [Lonza part # 50001]	5
50 mM $MgCl_2$	30

Equipment

- Horizontal shaker
- 50 ml Falcon centrifuge tubes
- Sterile wide bore 200 µL pipette tips
- Thin spatula
- Scalpel or razor blade
- Aurora instrument
- Aurora reusable cartridge

Preparing the sample for Aurora purification

1. Cut the gel plug containing crude DNA into 5 mm wide pieces. Up to four gel plugs of this size (approx. 150 mg each) may be run in the same cartridge. The total DNA input should not exceed 40 µg.
2. In order to equilibrate the gel plugs with the Aurora concentration gel, soak all gel plugs in 2x TBE for at least 1 h with gentle agitation on a horizontal shaker. This incubation can be extended to overnight if desired.

Gel casting and cartridge assembly

1. Refer to the "Cleaning Cartridges and Accessories" section in the **Aurora Reusable Cartridge Handling Manual (106-0014)** for detailed instructions on how to clean and decontaminate cartridges prior to each run.
2. Each cartridge requires approximately 5 ml of molten 0.7% agarose. Refer to the "Preparing Agarose" section in the **Aurora Reusable Cartridge Handling Manual (106-0014)** for instructions on how to prepare the agarose, but *please note* that for this Aurora 200-1100 kb DNA from Agarose Plugs Protocol, **0.7% Agarose should be prepared in 2x TBE** (instead of a 1% agarose

gel in 0.25x TBE as described in the **Aurora Reusable Cartridge Handling Manual (106-0014)**. Please adjust the amount of reagents used accordingly.

3. Remove the electrode plate from the cartridge and set it aside.
4. Use a thin spatula to insert gel plugs into the Aurora Reusable Cartridge and position the plugs at the locations indicated in Figure 2. Up to four gel plugs can be inserted into the concentration gel area from the opening of the A, B, C or D chambers. Only place an agarose plug near buffer chamber A if you are inserting 4 plugs. The agarose plugs should be placed approximately 2.5 mm from the edge of the concentration gel. Once the plugs are in place, be careful not to move the cartridge around and disturb the position of the plug.

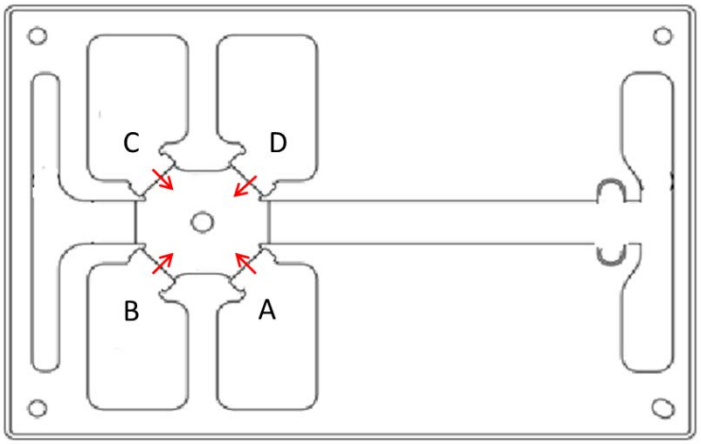

Figure 2. Diagram showing an Aurora Reusable Cartridge and the location where gel plugs are inserted prior to concentration gel casting. One, two, three or four gel plugs of up to 200 mg each can be cast per cartridge. Prior to casting the concentration gel, gel plugs containing free DNA are inserted through the opening of the four buffer chambers as indicated by the arrows A, B, C and D. If fewer than four plugs are being cast, do not place a gel plug near buffer chamber A. Position the gel plugs about 2.5 mm from the edge of the region occupied by the concentration gel.

5. Follow the instructions in the **Aurora Reusable Cartridge Handling Manual (106-0014)**, sections "Prepare the cartridge" and "Casting gel". Note that once the molten agarose is added to the area where the concentration gel will form, the gel plugs may shift position slightly. This is acceptable as long as the concentration gel solidifies with the gel plugs contained within it.

   Optional: If you wish to purify the DNA into an agarose plug instead of into buffer, simply do not insert the metal post in the hole for the extraction well. When filling the concentration gel with agarose, add just enough agarose to completely fill the octagon shaped area. Do not overfill and allow agarose into the cylindrical space normally occupied by the metal extraction well post.

   Alternatively, if you wish to collect the DNA in Low Melting Point (LMP) agarose, you can first cast the concentration gel with the metal post in place, and subsequently fill the extraction well with 0.7% LMP agarose in 2x TBE, however be aware that the melting process may cause some DNA fragmentation.

6. Follow the instructions in the "Filling buffer" section of the **Aurora Reusable Cartridge Handling Manual (106-0014)** with the modification that the extraction well buffer is 6% PEG (MW 20,000), prepared in 2x TBE. (**Note:** If PEG is omitted from this step, addition of $MgCl_2$ to the extraction well at the end of the run is not required; however, DNA fragmentation may occur when aspirating the purified DNA sample from the cartridge. Exercise extreme care when aspirating the purified sample.)
7. Fill the sample chamber with 5 ml of 2x TBE. Although no DNA is injected from the sample chamber in this protocol, buffer must be in the sample chamber in order for the Aurora to perform routine checks to ensure proper electrode contact.

Run the Aurora

1. Prepare the Aurora and chiller as described in the **Aurora User Manual (BG-2002-07-004)**.
2. As described in the "Operating the Aurora" section of the Aurora User Manual, load and run the **106-0019-CC-D Aurora 200-1100 kb DNA from Agarose Plugs Protocol.sp** file. Create an experiment folder for the run to save the logs. Once the run is started, it will take 30 h to complete.

   Note: The duration of Focus Blocks 3-6 should not be altered, but the duration of Focus Blocks 1 and 2 can be adjusted. Run time can be reduced if very high molecular weight molecules are not desired or expected. Maximum molecular weight and the amount of DNA recovered will both increase with longer run times. If a longer run time is desired, in an attempt to increase yield or maximize the length of DNA that is recovered, extend the duration of Focus Block 2.

Post run processing

If you are collecting DNA in buffer:

1. After the run is complete, promptly remove the adhesive film from the extraction well. Gently top up the extraction well with 50 mM $MgCl_2$ by slowly dispensing the liquid against the side of the extraction well. This should require approximately 20 µl of $MgCl_2$. Do not mix or disturb the 60 µl of buffer containing purified DNA in the extraction well. Allow the mixture to rest at room temperature for 5-15 minutes to equilibrate and give time for the large DNA molecules to condense in solution.
2. Using a wide bore pipette tip, very gently extract the contents of the extraction well (approximately 80 µl) and transfer to a wide-bottomed storage tube such as 50 ml Falcon tube. Do this as slowly as possible (taking *at least* 30 seconds) to avoid fragmenting the DNA. Large DNA molecules are susceptible to mechanical shearing forces and need to be stored, handled and transported with great care to minimize fragmentation. The purified DNA is now in solution and ready for other downstream applications.

If you are collecting DNA in an agarose plug:

1. Insert the metal extraction post into the extraction well, pressing down until the post is in contact with the bottom of the cartridge. Using a 1 ml pipette and tip, first expel the air from the 1 ml tip and keeping the pipette in the expelled position, insert the tip firmly into the extraction post and release the pipette to draw up air and induce the sample to move into the extraction post. Expel the plug from the extraction post into a microcentrifuge tube. The expected mass of the resulting gel plug is approximately 60 mg.

Troubleshooting

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

1. **Failure Mode: There is evidence of DNA degradation.**

   Solution: There are many possible causes of DNA damage. Steps must be taken to maintain the integrity of the DNA in the input sample. Avoid handling procedures that could mechanically or chemically fragment the DNA. Extreme care must be taken in post-run sample handling and storage of DNA in order to maintain the integrity of DNA molecules. When extracting the purified sample, release pressure on the pipette plunger as slowly as possible to minimize the rate of fluid flow into the pipette tip.

2. **Failure Mode: Long fragments are not recovered.**

   Solution: This issue shares some of the causes of Failure Mode 1, and could be due to degradation of DNA in the input sample. Careful post-run handling and storage of DNA is crucial to retain high molecular weight DNA integrity. DNA should be gently pipetted using wide bore pipettes, and mechanical shearing of DNA should be avoided. Extending the duration of Focus Block 2 can increase the recovery of high molecular weight molecules. Also, it is possible that the large globular DNA fragments are unevenly distributed in the output sample. One way of mitigating this is to heat the DNA to 37°C to homogenize the solution prior to downstream quantification and use.

3. **Failure Mode: DNA recovery is low.**

   Solution: If the input sample did not contain sufficient DNA, try processing more of the sample. Yield may be increased with longer run times. Extend the duration of Focus Block 2 if you wish to increase the run time. It is also possible to recover a purified sample, refill the extraction well with fresh buffer, and resume the protocol to recover additional DNA for a second output sample.

   Yield measurements can vary greatly depending on the position of the pipette tip when sampling the output DNA, since very large DNA molecules may not be homogenously dispersed in the output volume. Measurements taken from different positions in the output sample

volume may differ greatly in the apparent DNA concentration. Gently heating the sample to 37 °C can help to homogenize the sample without greatly fragmenting the DNA.

Another possible cause of low DNA recovery is that DNA in the agarose plugs is too large to migrate efficiently through the agarose gel matrix. Consider methods of slightly reducing the molecular weight distribution of DNA in the input sample. One possibility is to perform a partial (single-cut) restriction enzyme digest with an enzyme such as MboI. This step can be performed after cells or nuclei are embedded in the agarose and lysed, protecting the free DNA from liquid shearing forces and reducing the likelihood of a restriction enzyme cutting multiple sites on the same molecule.

It is important to extract the sample promptly after the Aurora run has completed, so that time is not given for DNA to diffuse back into the gel from the extraction well. If the output sample is not extracted promptly, re-run Focus Blocks 3-6 before extracting the sample.

Ordering and support

For support for Aurora protocols or cartridges, or to order additional cartridges, please contact support@borealgenomics.com.

References

Liles et al. 2009. Isolation and Cloning of High-Molecular-Weight Metagenomic DNA from Soil Microorganisms. Cold Spring Harbor Protocols; 2009: pdb.prot5271

SCODA conditions

These conditions are pre-programmed in the **106-0019-CC-D Aurora 200-1100 kb DNA from Agarose Plugs Protocol.sp** file that accompanies this protocol 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	2x TBE
Input Sample	1-4 150 mg agarose gel plugs

Focus 1

SCODA field strength	8 V/cm
SCODA cycle period	20 min
Duration	20 h

Focus 2

SCODA field strength	8 V/cm
SCODA cycle period	20 min
Duration	7 h

Focus 3

SCODA field strength	8 V/cm
SCODA cycle period	8 min
Duration	2 h

Focus 4

SCODA field strength	8 V/cm
SCODA cycle period	2 min
Duration	40 min

Focus 5

SCODA field strength	8 V/cm
SCODA cycle period	20 sec
Duration	10 min

Focus 6

SCODA field strength	8 V/cm
SCODA cycle period	4 sec
Duration	2 min

Expected current	10-20 mA
Expected power	0.3-1 W