Aurora 200-1100 kb DNA from Soil Protocol

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Introduction

This protocol is for purifying ultra-high molecular weight (UHMW) 200-1,100 kb DNA from cells in soil. The DNA can be used for a variety of purposes such as cloning into large-insert vectors, library construction and studying complete and intact functional pathways. The Aurora is ideally suited to purifying extremely long DNA from lysed, contaminated samples in an agarose matrix, avoiding the shearing forces associated with liquid phase extractions.

Approximately 10^9 to 10^{10} cells are cast into agarose plugs, gently lysed (based on a modification of the protocol by Liles *et al*., Cold Spring Harbor Protocols, 2009), and subsequently purified using the Aurora. Yields will vary depending on the microbial load and composition, and DNA integrity may vary due to handling and storage conditions. The maximum molecular weight and concentration of DNA in the output may potentially be increased further by extending the Aurora run time. DNA can be purified into either buffer or an agarose plug.

Example results

To demonstrate the Aurora's ability to isolate high purity, UHMW bacterial DNA from soil samples, genomic DNA from microbes in a 30 g soil sample was prepared using the method of Liles *et al*. (Cold Spring Harbor Protocols, 2009) with modifications detailed below. 0.15 g of the post-lysis agarose plug (containing cells from an equivalent of 1.5 g of soil) was then loaded into the Aurora Reusable Cartridge and purified by the **Aurora 200-1,100 kb DNA from Soil Protocol**. Two replicates were run through the entire protocol. The molecular weight distribution of the purified DNA was determined by pulsed field gel electrophoresis (PFGE) and the purity of the DNA was determined by quantitative PCR (qPCR) analysis.

A dilution series of the purified DNA was prepared and analyzed by qPCR with the 16S rDNA primers and probes described by Nadkarni *et al.* (Microbiology 148:257, 2002). One replicate was diluted with 2 volumes of dH_2O and heated at 65 °C to melt. 5 µl of each diluted sample was used as the template in a 25 µl reaction containing Roche Applied Science FastStart® Universal Probe Master Mix (catalogue no. 04913957001). Forward and reverse primers were each used at 200 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. The relative amount of DNA present was estimated by comparison to a standard curve prepared from *Escherichia coli* genomic DNA.

The Aurora recovered 0.35 µg of bacterial DNA per gram of soil. The purified DNA amplified without additional dilution, in contrast to the DNA in the post-lysis agarose plug that would only amplify after a 1:1,000 dilution due to the presence of PCR inhibitors in the sample. In Figure 1, a sample of the post-lysis agarose plug and the DNA purified by the **Aurora 200-1,100 kb DNA from Soil Protocol** are compared on a pulsed field gel. The Aurora was able to purify DNA fragments over 900 kb in length in this experiment.

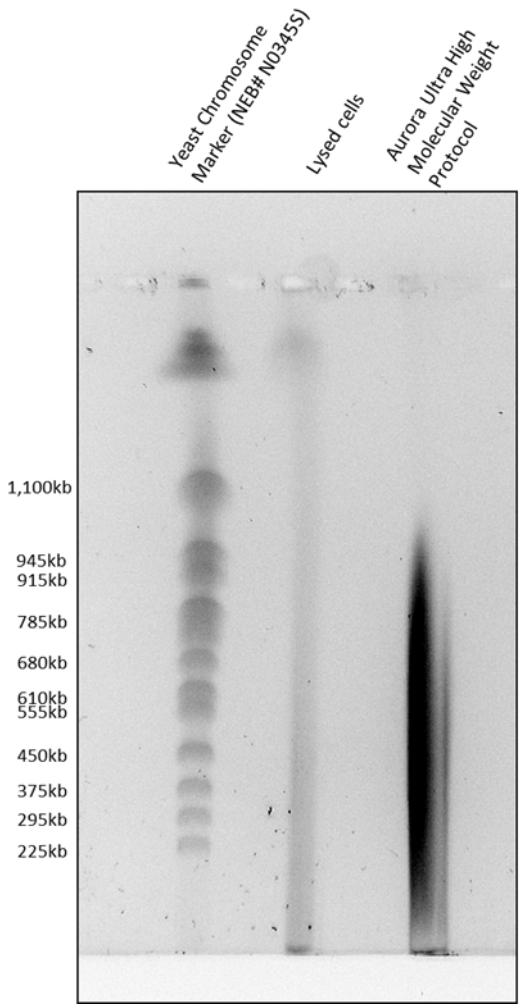

Figure 1. Fluorescent Pulsed Field Gel image of DNA fragments extracted from bacterial cells from soil with the Aurora 200-1100 kb DNA from Soil Protocol. Samples are separated by pulsed field gel electrophoresis run with the following parameters: 1% SeaKem Gold Agarose, 1x TBE gel, 1x TBE Running Buffer. 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 SYBR Green, 1x TBE Buffer and imaged under UV transillumination.

Aurora 200-1100 kb DNA from Soil Protocol

The homogenization and agarose plug preparation takes 1 day to complete. Lysis takes 2 days and the Aurora purification requires 30 h. It is safe to pause between each major step as long as the sample is stored as recommended in the protocol.

The homogenization, preparation and lysis protocol below follows the method of Liles *et al*. (Cold Spring Harbor Protocols, 2009) with slight modifications.

Safety guidelines

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

Materials required

The following buffer volumes are required to process 30 g of soil. Volumes can be scaled up or down as required. All buffers can be prepared in advance and stored at room temperature for up to 6 months.

1. **Crombach Buffer**

Reagent	Volume (ml)	Final Concentration
1M Tris-HCl, pH 8.0	16.5	33 mM
0.5M disodium EDTA, pH 8.0	1	1 mM
Nuclease-free dH2O	482.5	-
Total volume	500	

2. **Microbial Lysis Buffer**

Reagent	Volume (ml)	Final Concentration
1M Tris-HCl, pH 8.0	0.5	10 mM
0.5M disodium EDTA, pH 8.0	20	200 mM
10% w/v sodium deoxycholate, pH 8.0	5	1% w/v
5M NaCl	0.5	50 mM
20% Sarkosyl	2.5	1% w/v
Nuclease-free dH2O	21.5	-
Total volume	50	

Autoclave after preparing as listed in the table. Immediately before use, add 50 mg/ml of lysozyme to a final concentration of 1 mg/ml.

3. **ESP Solution**

Reagent	Volume (ml)	Final Concentration
N-lauroyl sarcosine sodium salt	5 g	1 % w/v
0.5M disodium EDTA, pH 8.0	500	500mM
Total volume	500	

Autoclave after preparing as listed in the table. Immediately before use, add 50 mg/ml of proteinase K to a final concentration of 1 mg/ml.

4. Agarose Plug Storage Buffer

Reagent	Volume (ml)	Final Concentration
1M Tris-HCl, pH 8.0	0.5	10 mM
0.5M disodium EDTA, pH 8.0	5	50 mM
Nuclease-free dH2O	44.5	-
Total volume	50	

5. General Reagents

Reagent	Volume (ml) required per 30 g of soil
50 mg/ml proteinase K, pH 8.0 (Roche, catalog no. 03115879001)	2
50 mg/ml lysozyme, pH 8.0 (Sigma, catalog no. 62970)	0.5
2% sodium hexametaphosphate, cold	200
Sterile water, cold	300
100 mM PMSF	0.2
Molten 0.7% Lonza SeaKem® LE Agarose, prepared in 2x TBE [Lonza part # 50001]	5 ml
TE, pH 8.0	200

6. Equipment and materials required:

- Blender
- Horizontal shaking incubator
- Tabletop centrifuge with adaptors for 50 ml centrifuge tubes or 500 ml centrifuge bottles
- Microcentrifuge
- Scalpel or razor blade
- Thin spatula
- 1 cc syringes
- 50 ml or 500 ml centrifuge tubes
- 15 ml and 50 ml Falcon tubes
- Aurora instrument
- Aurora Reusable Cartridge

Homogenization of soil and extraction of bacterial cells

1. Pass soil samples through a 2 mm sieve. Both fresh and frozen soil can be used in this protocol. Weigh out a 30 g soil sample.
2. Add 100 ml of cold, sterile water to the 30 g sample and homogenize in a blender on low speed 3 times for 1 min each, cooling the blender on ice for 5 min between homogenizations.

3. Transfer the soil suspension into a 500 ml centrifuge tube or split evenly into 50 ml centrifuge tubes. Rinse the blender vessel with 50 ml of cold sterile water and add the suspensions to the centrifuge tubes.
4. Centrifuge the tubes at 1,000 *g* for 15 min at 5 °C to sediment most of the soil without pelleting bacterial cells.
5. Save the supernatant from all the tubes and store at 4 °C.
6. Transfer the soil pellets back into the blender with 100 ml of cold sterile water and homogenize on low speed for 1 min.
7. Repeat the centrifugation outlined in step 4.
8. Repeat steps 6 and 7. This step can be omitted if the centrifuge does not have the capacity to process such large volumes; however yield may be reduced if a smaller volume is used.
9. Combine the supernatants (300 – 400 ml) and centrifuge at 15,000 *g* for 30 min at 5 °C. Discard the supernatant.
10. Add 200 ml of cold 2% sodium hexametaphosphate to the bacterial pellet and transfer to the blender. Homogenize the pellet at low speed 3 times for 1 min each, cooling the blender on ice for 5 min between homogenizations.
11. Transfer the suspension to centrifuge tubes and centrifuge at 15,000 *g* for 30 min at 5 °C. Discard the supernatant.
12. Resuspend the pellets in 200 ml of Crombach Buffer and centrifuge at 15,000 *g* for 30 min at 5 °C. Discard the supernatant.
13. Repeat step 12.
14. Combine and resuspend the pellets in 5 ml of Crombach Buffer and centrifuge at 15,000 *g* for 30 min at 5 °C. Discard the supernatant.

Preparing the agarose plugs

1. Mix 500 µl of the cell pellets with an equal volume of Molten 1.4% Lonza SeaKem® LE Agarose, prepared in 2x TBE [Lonza part # 50001], to achieve a final agarose percentage of 0.7%.
2. Draw up the agarose – soil suspension in 1 cc syringes and allow to fully solidify at 4 °C for at least 30 min. Cap the end of the syringe to prevent the plugs from drying out.
3. The agarose plugs can be stored at 4 °C for several weeks.

Lysis

1. Remove the plungers from the syringes and slide the plugs out into a 50 ml sterile Falcon tube containing 10 ml of Microbial Lysis Buffer (with lysozyme added) per plug.
2. Incubate the plugs for 3 h at 37 °C with gentle agitation. The lysis can be extended to up to 24 h if desired.
3. Transfer the plugs to a new sterile 50 ml Falcon tube containing 40 ml of ESP solution. Incubate for 16 h at 55 °C.
4. Replace the ESP solution and incubate for an additional 5 h at 55 °C.
5. Remove the ESP solution and wash the agarose plugs 3 times with 10 ml of TE per plug at room temperature.
6. Decant the TE buffer. Replace with 50 ml of TE buffer containing 1 mM PMSF. Incubate the plugs with gentle agitation for 1 h at room temperature.
7. Repeat step 6 of Lysis with fresh TE-PMSF buffer.

8. Remove the TE-PMSF buffer and replace with 10 ml of agarose plug storage buffer per 1 cc plug. Incubate overnight at 4 °C before use. Plugs can be stored in agarose plug storage buffer at 4 °C for several weeks.

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 cartridges prior to setting up 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 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 plugs.

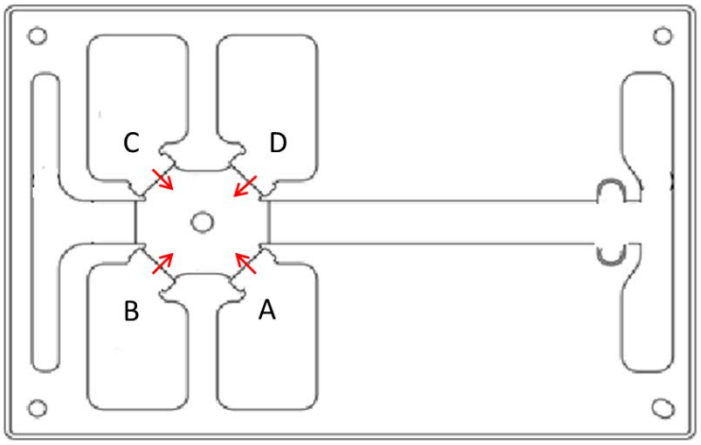

Figure 2. Diagram showing an Aurora Reusable Cartridge and the location where gel plugs are inserted prior to concentration gel casting. One to four gel plugs of up to 200 mg each can be cast per cartridge. Prior to casting the concentration gel, sample gel plugs are inserted through the opening of the 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 **106-0020-DC-D Aurora 200-1100 kb DNA from Soil 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 the 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. The large DNA molecules are susceptible to mechanical shearing forces and need to be stored, handled and transported with care to minimize fragmentation. This purified DNA is 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 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 **106-0020-DC-D Aurora 200-1100 kb DNA from Soil 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	2x TBE
Sample volume	1-4 150 mg agarose plugs

Focus

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