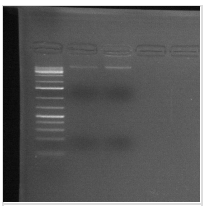

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Extraction of High Molecular Weight DNA from Aureococcus anophagefferens Virus

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High molecular weight DNA has become a necessary resource with the advancement of long-read sequencing from Nanopore ONT and PacBio SMRT. Often times excessive treatment with phenol-chloroform and prolonged periods of centrifugation results in sheared DNA below 10 kilobases. DNA extracted from viruses is notably less stable than that of cellular organisms, and is often heavily sheared upon extraction. Likewise, kits designed specifically for viral DNA extraction tend to return very low yields of DNA. This protocol seeks to subvert the shearing of typical DNA extractions by combining the process with pulsed-field gel electrophoresis while also maintaining high yields.

Alex Truchon, Eric Gann, Steven W Wilhelm 2022. Extraction of High Molecular Weight DNA from Aureococcus anophagefferens Virus. **protocols.io** https://protocols.io/view/extraction-of-high-molecular-weight-dna-from-aureo-byjtpunn

Nucleocytoviricota, virus, DNA extraction, high molecular weight, long-read sequencing

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Low melting agarose gels are extremely fragile and take a long time to cast. Casting and moving the gel inside a 4° C walk-in refrigerator can ease the process.

30 kDA Pelicon Filter
Tangential Flow Filtration Device
10% Triton-X
Tris-HCl
DNAse I
Proteinase K
500 mM EDTA
Molecular grade agarose
Low-melting temperature agarose
BioRad CHEF Mapper Plug Molds (Catalog No. 1703713)

While working with phenol and chloroform, ensure that all skin is properly covered with the appropriate lab safety attire. Any work with these chemicals should be performed in a chemical fume hood as to avoid inhalation.

Viral Particle Preparation and Lysis

1 Infect **1 L** of *Aureococcus anophagefferens* CCMP1984 with **10 mL** of Aureococcus anophagefferens Virus (AaV) lysate.

2 1w 3d
Incubate cultures at 19 degrees C on a 14:10 light-dark cycle until complete lysis of the culture (~10 days).

3 3h
Concentrate the lysed culture to a final volume of 50 – 100 mL final volume *via* tangential flow filtration using a 30 kDa filter.

4 10m
Centrifuge concentrated lysate for **00:10:00** at 3,500 xg at 4° C to pellet cellular organisms in lysate.

5 Remove supernatant and discard pellet.

6 Add 10% Triton-X to supernatant to a final concentration of 1%.

7 1h 15m
Centrifuge 50 mL lysate at a speed of 24,000 xG at 4° C for **01:15:00** to pellet viruses. Discard supernatant.

8 Resuspend the pelleted viruses in a **500 µL** of 50 mM Tris-HCl, pH 7.5, 10 mM MgCl2.

9 Add **9 µL** DNAse I (2.0 mg/mL) and mix gently.

10 Incubate at room temperature for **01:00:00** . 1h

11 Add **6 µL** of 500 mM EDTA (pH 8.0). Mix gently.

12 While incubating, prepare **100 mL** 2% molecular grade agarose solution by microwaving agarose/water mixture .

12.1 Do not allow to solidify: hold in a water bath set to 50° C.

13 Add 2% agarose solution to concentrated lysate at a 1:1 ratio for a final concentration of 1% agarose. Store in water bath until ready to cast.

14 Cast agarose—lysate mixture in BioRad CHEF Mapper Plug Molds (Catalog No. 1703713) and allow to solidify at room temperature.

15 Prepare viral lysis buffer.

15.1 Add **750 µL** 20% SDS, 500 µL 0.5 M EDTA, and 200 µL 3 M sodium acetate to a conical tube.

15.2 Bring to **50 mL** with MilliQ water.

15.3 Filter sterilize through a 0.22 µM syringe filter.

16 After agarose has solidified punch out of molds and add two per Eppendorf tube with **1 mL** lysis buffer.

17 Add proteinase-K to a final concentration of 1 mg/ml to each tube.

18 Incubate over night at 37° C under gentle rotation (~ 2-3 xG or 100 rpm) in a shaking incubator.

19 Cast a 2% low-melting agarose gel and allow at least **01:00:00** for gel to solidify. 1h

20 Decant lysis buffer from overnight incubations of plugs.

21 Prepare TNE wash buffer (10 mM Tris-Base, 200 mM NaCL, 0.5 mM EDTA). Filter sterilize through a 0.22 µM syringe filter.

22 Wash with **1 mL** TNE buffer by resuspending and shaking at room temperature for **00:10:00** . 10m

23 Pour off TNE buffer.

24 Repeat steps 22-23 once.

25 Carefully insert plugs into individual wells of the low melting agarose gel using a sterile razor blade or pipette tip.

26 Seal the wells by pouring liquid 2% agarose over the wells and allow poured agarose to solidify.

27 Run gel at approximately 120 V for **01:30:00** to **02:00:00** or until a high molecular weight band of DNA can be visualized. 3h 30m

High Molecular Weight DNA Extraction 4h 40m

28 Excise high molecular weight bands from the agarose under UV light, being careful to remove as little excess agarose as possible. Place excised pieces of gel in Eppendorf tubes.

29 Melt agarose in a water bath set to 80° C Bring volume in tube up to **500 µL** with MilliQ water. The melted agarose volume should never exceed 500 µL.

30 Once entirely melted (after at least ten minutes), add **1 mL** basic phenol to the tube and invert to mix. 10m

31 Place phenol—agarose mixture at -80° C for **00:10:00** 10m

32 Centrifuge the mixture for **00:05:00** at maximum speed on a microcentrifuge at room temperature. 5m

32.1 If phenol-agarose has frozen, allow sample to thaw before centrifugation.

33 Carefully remove upper aqueous layer and place in a clean **2 mL** Eppendorf tube. Discard the lower phenol layer.

34 Add **500 µL** phenol and **500 µL** chloroform to the aqueous layer. Centrifuge at max speed for **00:05:00** at room temperature. Pipette aqueous layer into a new 2 mL Eppendorf tube. 5m

35 Repeat step 33-34 until there is no remaining protein debris between the aqueous and the phenol layer.

36 Add **1 mL** chloroform to the aqueous layer. Centrifuge at full speed for **00:05:00** . Pipette aqueous layer into a clean 2 mL Eppendorf tube. 5m

37 Repeat step 36 once. 5m

38 Precipitate the DNA by adding 3 M sodium acetate to a final concentration of 0.1 M (~67 µL in 2 mL) and fill to **2 mL** with 100% EtOH.

39 Precipitate DNA at -80°C for at least **02:00:00** or overnight. 2h

40 Centrifuge samples at maximum speed at 4° C for **01:00:00** . 1h

41 Remove supernatant and resuspend DNA pellet in **1 mL** 70% EtOH.

42 Centrifuge samples at maximum speed at 4° C for **01:00:00** . 1h

43 Remove supernatant. Allow residual EtOH to evaporate on a heat block set to 37° C.

44 Resuspend DNA pellet in MilliQ water.

45 Quantify extracted DNA on Nanodrop or Qubit per manufacturer’s specifications before sequencing.