**High Molecular Weight DNA extraction for Chromium 10X *de novo* sequencing (used for *Lymantria monacha*):**

*Protocol written by Carl Yung*

**Equipment required:**

**170-3591 CHEF Mammalian Genomic DNA Plug Kit, contains** Cell Suspension Buffer, 12 ml; >600 U/ml Proteinase K, 1.3 ml; Proteinase K Reaction Buffer, 30 ml; 2% CleanCut™ Agarose, 12 ml; 10x Wash Buffer, 60 ml, 50 well disposable plug mold, 2; Screened Cap, 1

CHEF Genomic DNA Plug Kit

(Bio-Rad170-3591)

Liquid nitrogen.

0.1M DTT (invitrogen 707265ML)

UltraPure™ DNase/RNase-Free Distilled Water (Thermo Fisher 10977035).

Dialysis tubing cellulose membrane avg. flat width 10 mm (0.4 in.) (Sigma-Aldrich D9277-100FT).

Dialysis tubing closures blue, size 50 mm (Sigma-Aldrich Z371092-10EA).

1.5ml Eppendorf® LoBind Genomic Microcentrifuge Tubes (VWR 80077-230).

2.0ml Eppendorf® LoBind Genomic Microcentrifuge Tubes (VWR 80077-234).

Ethidium bromide solution (Sigma-Aldrich E1510-10ML)

Sterile Gel electrophoresis bath.

Pestle and mortar or Tenbroeck tissue grinder should be soaked in bleach rinsed in RO water and oven dried. Prior to use could be baked @200⁰C four hours so is DNase and RNase free.

Micro spatula autoclaved and baked @200⁰C four hours so is DNase and RNase free.

**Before extraction prepare solutions:**

0.5M EDTA pH 8.0 autoclaved.

100ml 50xTAE (24.2g Tris base, 5.71ml glacial acetic acid, 10ml 0.5M EDTA>) filter sterilized.

Dialysis tubing is boiled in 10mM EDTA pH 8.0 and kept at 4⁰C.

Dialysis tubing closures are autoclaved.

CHEF Genomic Plug kit 2% clean cut agarose is first heated in a water bath at 50⁰C until liquid and aliquoted into Eppendorf lobind safe lock 1.5ml microfuge tubes (150µl).

**Method**

Place an aliquot of CHEF Genomic Plug kit 2% clean cut agarose into 50⁰C water bath.

Grind tissue in Tenbroeck tissue grinder in liquid nitrogen.

Transfer sample powder using a sterilised micro spatula into a 1.5ml Lobind safe lock tube resuspend in 130µl CHEF Cell Suspension Buffer add 1.3µl 0.1M DTT and warmup to 50⁰C in water bath (should take about 2 minutes)

Add 70µl 2% agarose from the water bath. Transfer with 200µl wide orifice filter tips and pipette mix slowly.

Once in suspension transfer with a fresh 200µl wide orifice filter tips 100µl sample, agarose and DTT mix in to a prepared plug mould. Aspirate the mix slowly down one side of the mould so air does not get trapped in the mould. Should be enough to make 2. Set the gels at 4⁰C (in the fridge) for 15 minutes.

In a 2.0ml microfuge tube prepare 1250µl Proteinase K Reaction Buffer and 50µl Proteinase K. Add the agarose gels of samples into the Proteinase K Reaction Buffer mixture. Place on 50⁰C water bath overnight.

Next day prepare wash buffer: each plug is washed 3 times and stored in the fourth, each wash is 1ml. 2 plugs 4 washes is 8ml total volume

800µl 10x Wash Buffer

7.2ml UltraPure™ DNase/RNase-Free Distilled Water

80µl 0.1M DTT

Using pipette aspirate the Proteinase K Reaction Buffer mixture, leaving the gel plug behind. Transfer 1 of the two plugs into a fresh 2.0ml microfuge tube with a sterile micro spatula. In each tube add 1ml of the wash buffer and mix on a rocker for 1 hour. Repeat the washes until wash 3 has finished before aspirating the spent wash totally (using a 10µl filter tip) before adding the final 1ml of wash buffer to store.

Store the plugs at 4 °C in 1x Wash Buffer. The plugs should be stable for 3 months.

Make enough 0.5X TAE with ultrapure water for gel electrophoresis tank and 50ml falcon tube.

In a 2.0ml microfuge tube add 1000µl 0.5X TAE and 1µl Ethidium bromide. Place one of the gel plugs into the TAE ethidium bromide mix and stain for 10 minutes.

After staining place plug into 10cm section of dialysis tubing clipped at one end with 500µl 0.5X TAE inside using a micro spatula. Seal up the tube with a second clip expelling any air bubble.

Place in the gel electrophoresis tank and mark specifically the direction the current is running (from negative to positive). Apply 5V/cm and time the run, periodically checking the progress under UV.

The DNA will migrate out of the gel towards the positive electrode. It will be retained by the dialysis tubing.

Once the majority had run out of the gel and at the side of the dialysis tubing. Reverse the polarity and run for 1 minute so the DNA is free off the dialysis tubing.



Initial plug electroelution mid-run reverse run 1 min

Undo one clip and using the UV light slowly draw up the DNA and into a 1.5ml lobind micro tube.

Run on 0.5% agarose gel 20V overnight (16hours).

Run electroelution of the remainder plug WITHOUT ethidium bromide submitted the DNA in 0.5X TAE.

All λ ladders were heated to 60⁰C for 5 minutes and then into ice before the gel. λ DNA is 48,502 base pairs in length



λhindIII ladder