Ultra HMW-DNA extraction for optical mapping or PFGE

Category: Experimental Procedures
Author: **Eveline Verhulst**
Version: draft

Labels:

Procedure 1: Nuclei isolation from insect tissue using a Dounce Homogenizer

Step 1

1. Add 2 x 20 larvae (N. vitripennis, stored in fridge) to 800 ul Galbraith buffer (add 50µl RNAse A for each 1ml buffer) in a 2 ml Dounce Homogenizer (Nutacon B.V.) on ice and pulverize by stroking 15 times with an A pestle.
2. Incubate homogenate on ice for 5 minutes to allow any remaining non-homogenized material to settle at the bottom of the tissue grinder.
3. Aliquot 400 µl (i.e. 10 mg equivalent) of tissue homogenate per 1.5 ml microcentrifuge tube while avoiding settled material.
4. Add 400 µl of ice cold 96-100% Ethanol to each microcentrifuge tube, cap and gently invert 5 times to mix. Repeat steps 1-3 for additional tissues to be processed.
5. Incubate for 60 minutes on ice.
6. Spin at 1,500 x g for 5 minutes at 4°C. Pipet off and discard the supernatant.
7. To each pellet, add 0,5 ml of Galbraith buffer and 0.1% Triton X-100. Resuspend gently with a regular pipette tip. Spin again at 1,500 x g for 5 minutes at 4°C. Pipet off and discard the supernatant. Dissolve in 0,5ml of Galbraith.
8. Pipet through 40µM cellstrainer and collect in tube.
9. Spin at 1,500 x g for 5 minutes at 4°C. Pipet off the top part of the supernatant and discard the rest of the supernatant.
10. Pellet supernatant by adding extra Trition X-100 (1µl) and centrifuge for 30min at 3000 x g (4°C)
11. Now I have 3 types of material:
    1. Top of supernatant
    2. Pellet after top of supernatant
    3. Pellet (tube 1 and 2)
12. Store on ice. Continue with CHEF.

Galbraith buffer

- 21 mM $MgCl_2$- 30 mM tri-Sodium citrate dihydrate
- 20 mM MOPS
- 0.1% Triton X-100
- 1 mg/l RNase A
- pH 7.2

Procedure 2: Embedding nuclei in agarose plug

Introduction

Saphyr Genome mapping and Pulsed-Field Gel Electrophoresis (PFGE, allows the separation of DNA ranging in size from a few kilobase pairs to 10 megabase pairs) require intact whole chromosomes. Because of the large size of these molecules, simple pipetting mechanically shears the DNA resulting in unacceptable quality for PFGE separations and Saphyr mapping. This has necessitated procedures for lysis of whole cells embedded in agarose, allowing purification of chromosome-sized DNA without shearing. The CHEF Genomic DNA Plug Kits are designed to produce 100 sample plugs of agarose embedded DNA with the quality necessary for PFGE separations. All kits consist of a core module, which contains all the buffers and proteinase solutions. This core module is the only component supplied with the mammalian DNA kit. Each of the disposable plug molds provided contains 50 wells which are 1.5 mm thick and 5 mm wide and hold 85 µl of volume. It is also possible to use the 10 well reusable sample plug mold (170-3622). Each of the wells in the reusable mold are 1 cm wide and 1.5 cm thick and hold 300 µl of volume.

Reagent and equipement needed

- Sterile transfer pipettes
- 2 and 50 ml sterile plastic tubes
- CHEF Mammalian Genomic DNA Plug kit from Biorad (# 170-3591)
- Water bath (37 °C / 50 °C)
- Microcentrifuge
- Shaking device (NO vortex!!)
- 2 % EDTA
- Stocksolution of 100 mM PMSF in 100% 2-propanol (see step 17)
- Microwave

Step 1

Prepare a Nuclei solution using a Dounce Homogenizer (see procedure 1: Nuclei isolation from insect tissue using a Dounce Homogenizer)

Step 2

Place an amount of Cell Suspension Buffer in 2 x 2 ml tubes in water bath of 50 °C. See the table for the amounts.

Step 3

Melt the 2% CleanCut agarose solution using a microwave and equilibrate the solution to 50 °C in a water bath. Be careful, it's only a small bottle, so it boils quickly.

Step 4

Pipette the liquid agarose from the bottle into the warmed Cell Suspension Buffer and mix well.

Mix	1 sample	5 samples	10 samples	25 samples

Buffer	60 µl	300 µl	600 µl	2 x 750 µl
2% agarose	40 µl	200 µl	400 µl	2 x 500 µl

Step 5

Aliquot quickly from the mix 100 µl in 0,5 ml tubes (those tubes don't need to be written) and place the tubes in the water bath at 50 °C.

Step 6

Pipette 3-5 µl cells in a 0,5 ml tube with buffer/agarose-mix.

Step 7

Resuspend the cells with the buffer/agarose-mix by pipetting up and down with a 100 µl pipette. Then pipette carefully the cells/buffer/agarose-mix into a plug-mold.

Step 8

Let the agarose cool down to a plug by placing the plugmolds in the fridge for 10-15 minutes.

Step 9

In the meanwhile, prepare a Proteinase K solution in a 2 ml or 50 ml Greiner-tube:

	1 sample	**5 samples**	**10 samples**	**25 samples**
Proteinase K	10 µl	50 µl	100 µl	250 µl
Prot. K reaction buffer	250 µl	1250 µl	2500 µl	6250 µl
MilliQ	170 µl	850 µl	1700 µl	4250 µl

Step 10

Label 2 ml tubes (with date and sampleID) and aliquot 430 µl Proteinase K solution in each tube.

Step 11

Push out each agarose-plug from its mold, into the corresponding tube.

Step 12

Place the tubes with the plugs in a water bath of 50 °C without agitation, for overnight incubation.

Step 13

NEXT DAY: Dilute an amount of 10x Wash Buffer with MilliQ in a 50 ml tube: 5 ml 10x Wash Buffer + 45 ml Milli Q.

Step 14

Stop the incubation by pipetting off the Proteinase K solution from the tubes with a pasteurpipet. (Leave the agarose plug in the tube.)

Step 15

Add 0.75 ml 1x Wash Buffer to the plug and wash for 1 hour at room temperature, with gentle agitation.

Step 16

Remove the 1x Wash Buffer and wash again with 1 x Wash Buffer for 1 hour at room temperature with gentle agitation.

Step 17

Make a fresh (!) 1 mM PMSF solution in a 50 ml Greiner tube:

	1 sample	**25 samples**
Stock 100 mM PMSF	10 µl	250 µl
1 x wash buffer	990 µl	24750 µl

Step 18

Remove the 1x Wash Buffer and wash the plugs with 1 mM PMSF during 1 hour at room temperature with gentle agitation.

Step 19

Remove the PMSF-solution and add 0.75 ml 1x Wash Buffer, wash 1 hour at room temperature with gentle agitation.

Step 20

Remove the 1x Wash Buffer and add 0.75 ml new 1x Wash Buffer into the tubes.

Step 21

The agarose plugs are now ready for restriction digestion and or Saphyre or PFGE, they are 3 months stable in the fridge!

Procedure 3: Pulse Field Gel Electrophoresis to check integrity of DNA

Step 1

Setup running tray:
- add 2.2L 0.5x TBE to the running tray.
- setup cooling system: optimal running temperature: approx. 14°C.

Step 2

Prepare Agarose gel:
- 0.8%: 0.8 gram in 100ml 0.5x TBE buffer (No EtBr!!!). Boil in microwave.
- Cool and pour agarose in gel tray. Leave some agarose in the Erlenmeyer flask and store at 50°C stove.
- Cut the CHEF plugs in half using a razor blade. Store one half at 4°C.
- Use tweezers to load the other half CHEF plug.
- Top (seal) the plug with left over agar using a pasteur pipet.
- Remove the upper and lower part of the casting tray and put the gel in the running tray.

Step 3

Electrophoresis settings:
- Flow rate: 70
- Volts: 200 (6V/cm)
- Time: 18-19 hours
- Initial switch time: 1 second
- Final switch time: 50 seconds

After running switch power of first!
Run time: approx. 18 hours.

Step 3

Poststaining
• Dilute MIDORIGreen Xtra 1 to 10,000 (10µl in 100ml) in 0.5x TBE buffer.
• Staining solution should be stored in a plastic container at RT in the dark and can be used for up to one week or more.
• Incubate the gel in staining solution for 20 minutes.
Staining time varies with the thickness of the gel and percentage of the agarose

Buffer

5x TBE:
- 54 g of **Tris base** (**CAS#** 77-86-1)
- 27.5 g of **boric acid** (CAS# 10043-35-3)
- 20 ml of 0.5 M **EDTA** (CAS# 60-00-4) (**pH** 8.0)

Adjust pH to 8.3 by HCl.

Attachments

no file attachments