High molecular weight DNA extraction after Bolger et al.

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Abstract

Extraction of high molecular weight DNA after Bolger et al.

Carried out for *eucalyptus grandis.*

DNA fragments were ranging up to 270 kb on a Pulsfield Electrophoresis Gel

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Guidelines

Modified from Section 1.3 of Supplementary materials from Anthony Bolger et al., The genome of the stress-tolerant wild tomato species *Solanum pennellii*, Nature genetics, Vol 46,1034-1038 (2014) doi:10.1038/ng.3046

Link: http://www.nature.com/ng/journal/v46/n9/full/ng.3046.html#supplementary

When citing please make sure to mention the original Bolger et al. protocol as described above.

Thank you very much Benjamin Schwessinger and Jasmine Saban for carrying out the extraction with me.

Reagents required:

Extraction Buffer (pH 6.0):

1 M 2-methyl-2,4-pentanediol

10 mM PIPES (1,4-Piperazinediethanesulfonic acid sodium salt)

10 mM MgCl2

4% (w/v) PVP-10

10 mM sodium metabisulfite

25 mM 2-mercaptoethanol

0.5% (w/v) sodium diethyldithiocarbamate

200 mM L-lysine

6 mM ethylene glycol tetraacetic acid (EGTA),

Nuclear Buffer (pH 7.0):

0.5 M 2-methly-2,4-pentanediol

10 mM PIPES

10 mM MgCl2

0.5% (v/v) Triton X-100

10 mM sodium metabisulfite

60 mM 2-mercaptoethanol

200 mM L-lysine

6 mM EGTA, pH 7.

TE Buffer (pH 7.0):

10 mM Tris

1 mM EDTA

Others solutions:

Triton X-100

20% (w/v) SDS (Sodium dodecyl sulfate)

5 M sodium perchlorate

Phenol/Chloroform/Isoamylalcohol (25:24:1)

Chloroform/Isoamylalcohol (24:1)

100% Ethanol

70% Ethanol (fresh)

TE buffer

Protocol

Before starting

Step 1.

Prepare extraction buffer, nuclear buffer and TE buffer to cool it down to 4 °C

Extraction

Step 2.

Harvest 50 g of young leaves into 1 L ice cold TE buffer (pH 7)

Extraction

Step 3.

Transfer leaves into pre-chilled Waring blender and add 600 mL lysis buffer

*steps 3-6 were carried out in 4 °C temperature room

Extraction

Step 4.

Homogenise in 15 s bursts for a total of 1 min and 15 s

(homogenising times may vary due to plant material, shorter times could lead to more full nuclei but lower yield)

DURATION
00:01:15

Extraction

Step 5.

Transfer plant material to Miracloth (filtrationmaterial for homogenates) and squeeze through 4 layers and further gravity filter through 8 layers of Miracloth

Extraction

Step 6.

Add 10% (v/v) Triton X-100 to the homogenate to a final concentration of 0.5% (v/v)

Extraction

Step 7.

Incubate mixture on an ice bath with gentle rocking for 30 min

DURATION
00:30:00

Extraction

Step 8.

Centrifuge at 600 g and 4 °C for 20 minutes

DURATION
00:20:00

Extraction

Step 9.

Discard supernatant

Extraction

Step 10.

Gently resuspend the pellet using a normal painters brush (not used and optimaly sterilised before), soaked in freshly made pre-chilled nuclear buffer

Extraction

Step 11.

Add sufficient nuclear buffer to bring the volume up to 10 mL

Extraction

Step 12.

Centrifuge at 600 g and 4 °C for 20 minutes

DURATION
00:20:00

Extraction

Step 13.

Repeat washing steps 9-12 twice more (until the pellet becomes grey-white and no traces of green are visible anymore)

Extraction

Step 14.

Gently resuspend the pellet using a brush soaked in nuclear buffer and add sufficent nuclear buffer to bring volume up to 5 mL

Extraction

Step 15.

Add 20% (w/v) SDS to a final concentration of 2% (w/v) (to lyse the nuclei) and gently invert the tube a few times

Extraction

Step 16.

Incubate at 60 °C for 10 mins and then let cool down on bench to RT

DURATION
00:10:00

Extraction

Step 17.

Add 5 M sodium perchlorate to a final concentration of 1 M

Extraction

Step 18.

Centrifuge at 400 g and 22 °C for 20 mins

DURATION
00:20:00

Extraction

Step 19.

Gently remove supernatant to a new tube using a wide bore pipette tip

*use wide bore pipette tips for the rest of the protocol

Extraction

Step 20.

Add an equal volume of phenol/chloroform/isoamylalcohol (25:24:1) to the tube

Extraction

Step 21.

Mix on a rotor for 10 mins

DURATION
00:10:00

Extraction

Step 22.

Centrifuge at 3000 g and 22 oC for 10 mins

DURATION
00:10:00

Extraction

Step 23.

Transfer the upper phase to a new tube and discard lower phase

Extraction

Step 24.

Repeat steps 20-23

Extraction

Step 25.

Repeat steps 20-23 twice more but only using chlorofom/isoamylalcohol (24:1)

Extraction

Step 26.

Precipitate DNA by adding 2x volume of ice cold ethanol and gently invert several times

Extraction

Step 27.

Transfer the precipitated DNA to a new tube using a tweezer or glass rod and wash once more with 10 mL 70 % (v/v) Ethanol

Extraction

Step 28.

Transfer the pellet to an 1.5 mL Eppendorf tube and allow the Ethanol to evaporate for approx. 5 mins (depends on the size of the pellet, but if the pellet dries out completely it might be difficult to dissolve the DNA again)

DURATION
00:05:00

Extraction

Step 29.

Dissolve the DNA in TE buffer or nuclease free water, invert a few times and leave at RT over night for the DNA to dissolve completely

Measurements

Step 30.

Meassure dsDNA concentration using Qubit and meassure absorbance using Nanodrop

(because of the high concentration you might want to make e.g. 1:10 dilutions)

Measurements

Step 31.

Run a 0.8 % TBE Agarose Gel with 200 ng dsDNA and lambda-Hind-III ladder as control.

Measurements

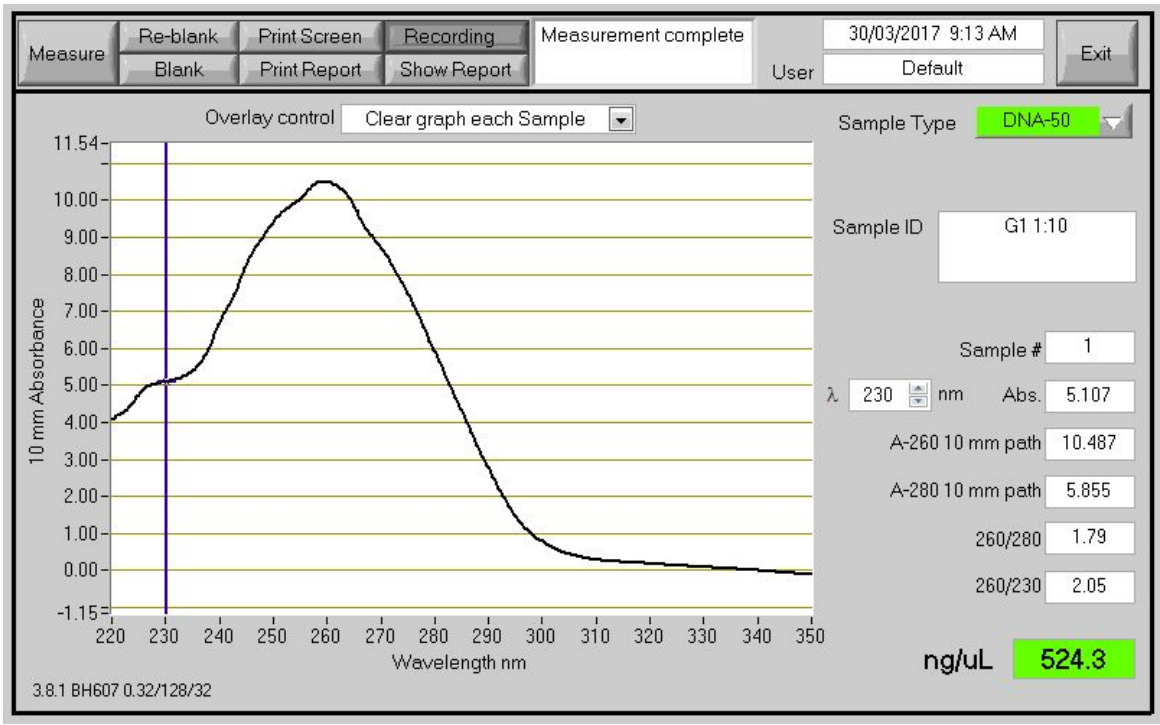
Step 32.

If available run a Pulse Field Gel Electrophoresis with 200 ng dsDNA and a MidRange PFG Marker II ladder as control.

Results

Step 33.

Nanodrop results:

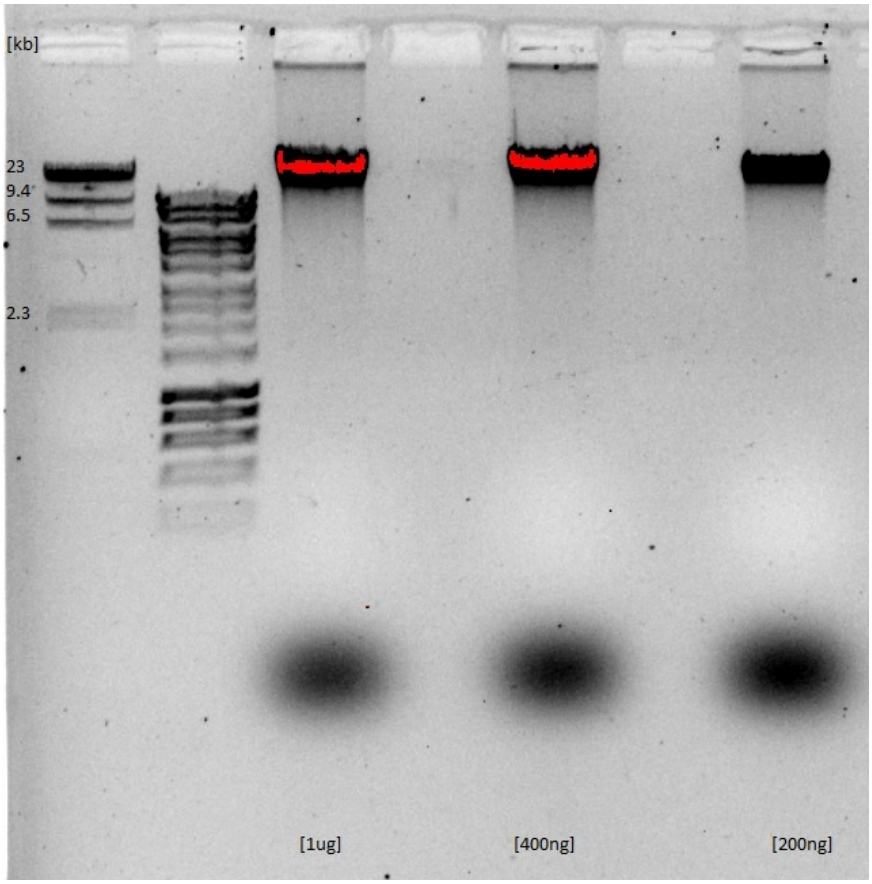


Qubit to Nanodrop ratio: 0.7

Results

Step 34.

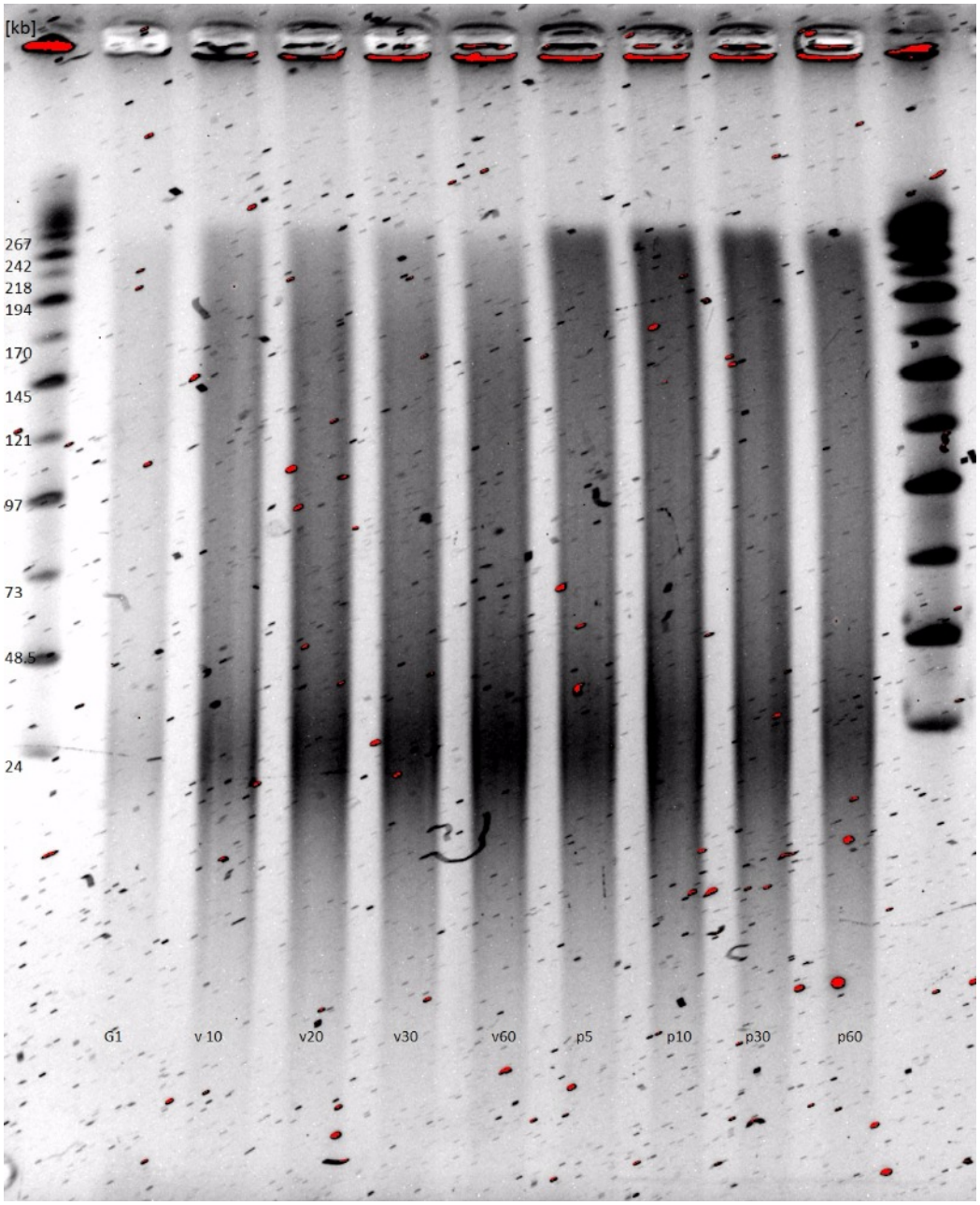
0.8 % TBE Agarose Gel with different amount of DNA but all from the same extraction above



Results

Step 35.

Pulse Field Gel Electrophoresis:



In order to determine the degeree of shearing of the DNA different vortexing times and pipetting times were tested:

G1..original sample from extraction (maybe problems with the concentration here -->repeat the gel)

v10..10 seconds vortexing

v20.. 20 s

v30.. 30 s

v60.. 60 s

p5.. 5 times pipetting up and down

p10.. 10 times

p30.. 30 times

p60.. 60 times

Conclusions:

It looks like that at 267 kb there is a straight cut off due to the fact that the longer DNA couldn't enter the gel, also indicated by the red saturated bits still stuck in the pockets.

As the amount of DNA between 24-48 kb seems to be increasing for bands 2-5, we think that with longer vortexing times more smaller and smaller fragments are produced.

For bands 6-9 we think that fragmets are also sheared through pipetting but that there is a certain cut off due to the pipette tip size and what length of fragmetns fits through.

*pipette tips were cut