Pulse Field Gel Electrophoresis (PFGE) Protocol for separation of Chlorella chromosomal DNA and Chlorella virus DNA

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Abstract

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Guidelines

Buffers and Solutions

Suspension Buffer (SB):

25 mM Tris pH 7.5

1 M Sorbitol

25 mM EDTA pH 8.0

Digestion Buffer (DB):

250 mM EDTA pH 9.5

1% N-Lauroylsarcosine

1 × TAE Buffer:

40 mM Tris

20 mM acetic acid

1 mM EDTA

Formulation of MBBM (Modified Bold's Basal Medium)

Stock Solutions:

1. 25.0 g $NaNO_3$ /1L d-H_2O
2. 2.5 g $CaCl_2 \cdot 2H_2O$ /1L d-H_2O
3. 7.5 g $MgSO_4 \cdot 7H_2O$ /1L d-H_2O
4. 7.5 g K_2HPO_4 /1L d-H_2O
5. 17.5 g KH_2PO_4 /1L d-H_2O
6. 2.5 g NaCl /1L d-H_2O
7. 50.0 g disodium EDTA; 31.0 g KOH /1L d-H_2O
8. 4.98 g $FeSO_4 \cdot 7H_2O$ / 1L acidified H_2O (Acidified H_2O is 999.0 mL d-H_2O + 1.0 mL concentrated H_2SO_4)
9. 11.42 g H_3BO_3 /1L d-H_2O
10. 8.82 g $ZnSO_4 \cdot 7H_2O$; 1.44 g $MnCl_2 \cdot 4H_2O$; 0.71 g MoO_3; 1.57 g $CuSO_4 \cdot 5H_2O$; 0.49 g $CoNO_3 \cdot 6H_2O$ /1L d-H_2O

MBBM preparation:

to 950 mL of d-H_2O add:

10.0 mL of stock solutions 1; 2; 3; 4; 5 and 6

1.0 mL of stock solutions 7; 8 and 9

1.0 mL of stock solution 10

1.0 g of bacto-peptone

5.0 g of sucrose

Formulation of FES Medium (for Chlorella Pbi growth)

Stock Solutions:

1). 10.0 gm $MgSO_4 \cdot 7H_2O$/1L d-H_2O

2). 1.0 gm KNO_3/1L d-H_2O

3). 1.0 gm K_2HPO_4 /1L d-H_2O

4). 50.0 g disodium EDTA; 31.0 g KOH /1L d-H_2O

5). 4.98 g $FeSO_4 \cdot 7H_2O$ / 1L acidified H_2O (Acidified H_2O is 999.0 mL d-H_2O + 1.0 mL concentrated H_2SO_4)

6). 11.42 g H_3BO_3 /1L d-H_2O

7). 8.82 g $ZnSO_4 \cdot 7H_2O$; 1.44 g $MnCl_2 \cdot 4H_2O$; 0.71 g MoO_3; 1.57 g $CuSO_4 \cdot 5H_2O$;

0.49 g $CoNO_3 \cdot 6H_2O$ /1L d-H_2O

FES preparation:

to 950 mL of d-H_2O add:

20.0 mL of stock solutions 1, 2, and 3.

1.0 mL of stock solutions 4, 5 and 6.

2.0 mL of stock solution 7

1.0 g of bacto-peptone

2.0 gm of Oxoid Lab-Lemco Powder

5.0 g of sucrose

Protocol

Step 1.
Harvest Chlorella NC64A (or Pbi) cells from 4 day old cultures (1.2 - 2.0 x 10^7 cells/ml) by centrifugation at 4000 x g for 5 minutes.

DURATION
00:05:00

Step 2.
Re-suspend in MBBM (NC64A cells) or FES (Pbi cells) at concentration of 8.6 x 10^7 cells/mL.

Step 3.
Add chlorella virus to a multiplicity of infection (MOI) = 10 plaque-forming units (pfu)/cell.

Step 4.
Add 3.1 ml of 37% formaldehyde into 40 ml centrifuge tubes and place them on ice.

Step 5.
Sample infected chlorella cells (25 mL) into prepared centrifuge tubes with formaldehyde (the final formaldehyde concentration is 4%) and place on ice.

Step 6.
Centrifuge at 4000 x g for 5 minutes.

DURATION
00:05:00

Step 7.

Wash samples by re-suspending them in 10 mL of MBBM amended with 50 mM EDTA and following centrifugation at 4000 x g for 5 minutes. Repeat wash step 3 times.

Step 8.
Re-suspend washed infected cells in 0.5 mL of SB.

Step 9.
Add to the cells 0.5 mL of 2% low melting point agarose (BioRad) in SB (kept at 45°C), mix well (work quickly, try not to generate any air bubbles), and pour the mix into BioRad plug molds.

Step 10.
Place plug molds in refrigerator for 15 minutes to solidify.

DURATION
00:15:00

Step 11.
Carefully remove agarose blocks from mold and place them into 2 mL of DB amended with 1mg/mL Proteinase K.

Step 12.
Incubate agarose blocks for 24 hrs at 50°C

Step 13.
After incubation, wash agarose blocks for 30 minutes with DB 4 times. Cut blocks in small pieces to fit gel wells.

DURATION
00:30:00

Step 14.
Prepare 1% agarose gel (PFGE grade) in 1× TAE buffer using BioRad casting stand.

Step 15.
Load agarose blocks into gel wells and seal them with melted (45°C) 1% low melting point agarose in running buffer.

Step 16.
The chromosomes of *Hansenula wingei* (1.05-3.13 Mbp), cat#170-3667; Schizosaccharomyces (3.5-5.7 Mbp), cat#170-3633 (Bio-Rad, Hercules, CA, USA), and Yeast Chromosome PFG Marker (225-1,900 Kbp), cat#N0345S (New England Biolabs, Beverly, MA, USA) are used as molecular weight markers.

Step 17.
Separate chromosomal DNA in CHEF-DR (BioRad, Hercules, CA) electrophoresis unit with 1X TAE running buffer. Run electrophoresis at 3 V/cm (100 V) with pulse time ramping from 250 to 900 seconds for 60 hrs. Change buffer every 24 hrs.

DURATION
00:00:00

Step 18.
Stain gel with 0.5 mg/L ethidium bromide for 20.

NOTES
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Alternatively, Sybr-Gold (Molecular Probes, Eugene, OR).