Quick analytical system for plastid genome modifications

Summary
New reliable tools are required to investigate the plastid genome which serves as a blueprint for the design of minimal genomes and is used in a number of synthetic biology applications. Pulsed-Field Gel Electrophoresis (PFGE) allowing separation of the high molecular weight DNA molecules could be used for the quick analysis of the plastid genome modifications. However, there is only limited information available on the preparation of sample plugs of the plastid DNA with the quality necessary for PFGE and on PFGE settings and conditions for plastid DNA separation. We set out to provide the synthetic biology community with a quick PFGE-based analytical system for plastid genome modifications. The project led to a number of educational resources, including protocols for the sample plugs preparation for PFGE of plastid and BAC DNA and for PFGE analysis of plastid and BAC DNA using CHEF-DRII PFGE system. All protocols will be open and publicly available on the website provided by the OpenPlant Fund. OpenPlant Fund will be also acknowledged in the manuscript containing relevant PFGE protocol.

Report and Outcomes
The project led to the development of the protocol for the sample plugs preparation for plastid and BAC DNA for PFGE analysis and for PFGE analysis of plastid and BAC DNA using CHEF-DRII PFGE system (please see below the detailed protocols). We have also tested components of the kit required for the sample plugs preparation for plastid and BAC DNA for PFGE analysis (shown below). These are available from our laboratory. Some of the outcomes from this project have been summarized in the attached manuscript (marioj(2016c)_jmicrobiolmethods.pdf).

Preparation of sample plugs for Pulsed Field Gel Electrophoresis (PFGE) of plastid DNA

1. Mix chloroplast suspension with 1 % low melting point agarose (SeaPlaque GTG LMP agarose, BMA BioWhittaker Molecular Applications resuspended in wash buffer containing 0.35 M sorbitol and 50 mM Tris pH 7.6)
    - alternatively use 2 % ClenCut agarose (Bio-Rad)
    - Mixing ratio 1:1 (chloroplast suspension:agarose)
2. Pipette 90 µl of chloroplast/agarose suspension into plug molds (Bio-Rad) and allow to solidify at 4 °C for 15 minutes
3. Place plugs into lysis buffer (2% sarkosyl, 0.45 M EDTA, 10 µg/ml proteinase K) and incubate with shaking at 50 °C for 30 hours • Exchange buffer 3 times in 30 hours
    - Cover with aluminum foil during all the procedures to avoid light damage to DNA

4. Wash plugs 2 times with TE buffer (10mM Tris pH 8.0, 1mM EDTA) containing 1mM phenylmethylsulfonyl fluoride (Sigma) at 4 °C with shaking

- Each wash for 4-5 hours
- Cover with aluminum foil during all the procedures to avoid light damage to DNA

5. Wash plugs 4 times with TE buffer (10mM Tris pH 8.0, 1mM EDTA) at 4 °C with shaking Each wash 4-5 hours

- Cover with aluminum foil during all the procedures to avoid light damage to DNA

6. Store plugs at 4 °C.

Notes: - Modified protocol from Lilly, JW et al (2001) The Plant Cell and Xing-Wang Deng et al (1989) PNAS

Pulsed Field Gel Electrophoresis (PFGE) of plastid DNA using CHEF-DR II PFGE system

1. Equilibrate plugs at least for 30 mins in electrophoresis buffer (0.5 x TBE buffer) before starting PFGE
2. Pour 100 ml of the gel (1 % Pulsed field certified agarose gel in 0.5 x TBE buffer) in the casting stand and allow to solidify for 45 mins at room temperature
3. Remove comb and casting stand walls
4. Pour 2 liters of 0.5 x TBE buffer into the CHEF-DRII cell
5. Switch on the CHEF-DRII power, then switch on the water pump and the cooling module (Bio-Rad Laboratories)
6. Allow buffer to equilibrate to 14 °C
7. Load the sample plugs to the gel wells
    - Samples should be less than 90 % of the height of the wells
    - Place agarose plugs onto the front walls of wells using a spatula and gently press them to the bottom and against the front walls of the wells
    - Use λ ladder 48.5 kb -1000 kb (Bio-Rad) as the molecular weight marker
8. Fill each sample with 1 % low melting point agarose (SeaPlaque GTG LMP agarose, BMA BioWhittaker Molecular Applications) in 0.5 x TBE buffer and allow to solidify for 15 mins at room temperature
9. Place the gel and platform into the frame of the CHEF-DRII cell so that the bottom of the platform rests at the bottom of the cell
    - Gel should be covered by approximately 2 mm of buffer
10. Enter run parameters into the control unit (Bio-Rad Laboratories)
11. Run the gel with the following electrophoretic parameters: Run time of 22 hours, switch time of 50-90 seconds, voltage gradient of 6V/cm
12. Before removing the gel check that the run is completed (the control unit will display END)
13. Remove the lid from the cell, remove gel with the platform and then slide the gel off the platform into the SYBR®Safe DNA gel dye solution (Invitrogen) in distilled autoclaved water.
    - (100 µl the SYBR®Safe DNA gel dye)
14. Stain for 3-5 hours at 4 °C
15. De-stain the gel in distilled autoclaved water for 2-5 hours at 4 °C.
16. Visualize DNA fragments with Safe Imager Blue-light trans-illuminator.

Notes: - Modified protocol from Xing-Wang Deng et al (1989) PNAS and BioRad CHEF-DRII-PFGE manual
PFGE system used: CHEF-DRII (BioRad Laboratories) CHEF Plastid DNA Plug Kit Chloroplast suspension buffer (0.35 M sorbitol, 50 mM Tris pH7.6, 0.5 % BSA) 2 % ClenCut agarose (Bio-Rad) or low melting point agarose (SeaPlaque GTG) (50 well) disposable plug mold (Bio-Rad) Lysis buffer (2 % sarkosyl, 0.45 M EDTA) Proteinase K (Fermentas) TE buffer (10 mM Tris, pH8.0, 1mM EDTA) TE buffer + phenylmethylsulfonyl fluoride (PMSF) (10 mM Tris, pH8.0, 1mM EDTA, 1mM PMSF)