Quick analytical system for plastid genome modifications

Mario Juhas
Email Address: mj417@cam.ac.uk

The Idea

The efficiently organized plastid genome is easily amenable to genetic manipulation and serves as a blueprint for the design of minimal genomes. Plastids are also frequently used as models in a number of bottom-up and top-down synthetic biology approaches and applications. New reliable tools are therefore needed to investigate the plastid genome. Pulsed-Field Gel Electrophoresis (PFGE) allowing separation of the high molecular weight DNA molecules could be exploited for the quick analysis of the plastid genome modifications. The pipetting mechanically shears the high molecular weight DNA molecules and is unacceptable for PFGE separations. This has necessitated procedures for lysis of whole cells embedded in agarose, allowing purification of chromosome-sized DNA without shearing. Although kits for the production of sample plugs of agarose embedded mammalian, bacterial and yeast genomic DNA are commercially available, there are no reliable kits for the production of sample plugs of the plant/plastid genomic DNA with the quality necessary for PFGE separations. Furthermore, there is only limited information available about PFGE settings and conditions for plastid DNA separation. This project aims to develop a quick PFGE-based analytical system for plastid genome modifications. This includes development of the kit and protocol for the reliable PFGE of plastid DNA. Alternative methods for plastid DNA analysis, such as the Phi29 polymerase based approach for the amplification of plastid genome sequences out of crude chloroplast extracts will be also explored.

Who We Are

Dr. Mario Juhas, Postdoctoral research associate, Department of Pathology of the University of Cambridge, Email: mj417@cam.ac.uk, synthetic biologists/molecular geneticist with relevant expertise in PFGE and high molecular weight DNA analysis
There are no other official members yet; however, the project will benefit from the collaboration with the Jim Haseloff's lab at the Department of Plant Sciences of the University of Cambridge with expertise in the plastid DNA extraction and analysis
Collaborators from Norwich might join the project later

Implementation

The main goal, to develop a quick analytical system for plastid genome modifications fits the remit of the OpenPlant that encompasses development of open technologies for plant synthetic biology. The project fits with the existing work in our laboratories. The PFGE expertise that has been successfully employed for the separation of the high molecular weight DNA of bacteria (BACs) and yeast in our laboratory at the Department of Pathology (Cambridge) will be used for the development of the reliable protocol for the analysis of plastid DNA modifications. This will benefit from the plastid DNA extraction and modification expertise of Jim Haseloff's laboratory at the Department of Plant Biology (Cambridge). It will lead to tangible, publicly documented and open outcomes, including educational resource (PFGE and alternative protocols for plastid DNA analysis), synthesis

and sharing of useful DNA parts (kit for reliable sample plugs of agarose embedded plastid DNA for PFGE) and publication in a peer-reviewed journal.

Benefits and outcomes

The main goal is to develop a quick and reliable analytical system for the plastid genome modifications. This is important for the synthetic biology community as plastids are one of the key synthetic biology 'workhorses'.
This encompasses three key aims:
1. Development of the kit for reliable sample plugs of agarose embedded plastid DNA for PFGE
2. Development of the PFGE and alternative (such as Phi29 polymerase-based) protocols for plastid DNA analysis
3. Dissemination of the achieved outcomes (protocols will be open and publicly available on the website provided by the OpenPlant Fund and in a peer-reviewed journal)
The main methods used will be:
PFGE (this method will be adapted for the quick analysis of the plastid genome modifications)
Phi29 polymerase-based amplification of plastid genome sequences out of crude chloroplast extracts

Budget

Reagents and materials for PFGE, PFGE agarose, standards, buffers, gel and gel plug forms and combs (£2000)
Reagents and materials for Phi29 polymerase based method (£250)
Reagents and materials for plastid DNA extraction (£250)
Publication costs (£1500)
Additional funding is available from the EPSRC grant to meet the aims of the project