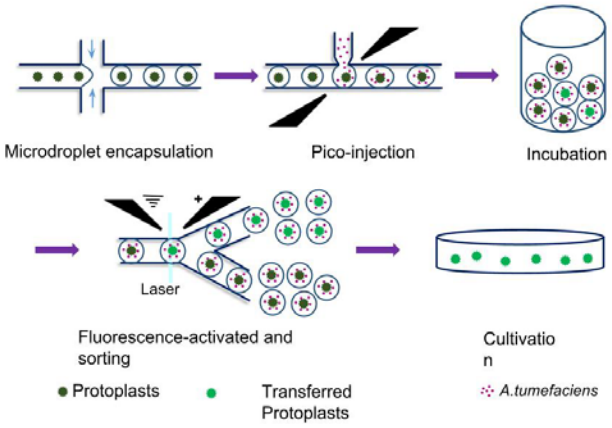
Plant-ProChip 2.0: High throughput transformation of plant protoplasts

Name	Ivan Reyna-Llorens
Email Address	iar28@cam.ac.uk
Proposal Title	Plant-ProChip 2.0: High throughput transformation of plant protoplasts
The Idea	A current limitation for plant synthetic biology involves the high-throughput screening of genetic parts in plant cells. Current validation techniques involve fusing sequences to a reporter and analysing expression in planta, which requires testing each circuit in an individual plant, either through transient biolistic transformation or the generation of stable transgenics (Brown et al. 2011). Applying these techniques to whole regulatory element libraries or to a considerable number of variations of a is not feasible at a laboratory scale. In a previous project funded by the SynbioFund we aimed to develop a high-throughput screen for the analysis of promoter sequences driving the expression of a reporter gene in plant protoplasts. As a result of this project we successfully managed to isolate, encapsulate and analyse protoplasts from the model species, Marchantia polymorpha, Arabidopsis thaliana and Nicotiana benthamiana using a PDMS microfluidic device (Figure 1). Despite this success, we realised that the efficiency of protoplast transformation now limits the assay, preventing them from being truly high-throughput. For instance, current methods for protoplast transformation using polyethylene glycol (PEG) require high amounts of plasmid, implying many resources need to be invested for the purification of a small number of constructs. The aim of this project is to make use of microfluidics to develop both transient and stable protoplast transformation protocols on-chip, at a high throughput scale. In this case wild type protoplasts will be isolated and encapsulated with our current methodologies. Protoplasts will be then transformed “in chipo” either by PEG transformation or by co-incubation with Agrobacterium tumefaciens. Droplets carrying the transformed protoplasts will be incubated and screened for reporter activity. Finally, transformed cells will be sorted and plated onto regeneration media with the aim of regenerating whole plants from single transformed protoplasts. We envisage this system to be applicable to a range of plant species not just for testing DNA parts but to other applications such as the generation of random mutagenesis lines, enhancer trap lines or inserting novel pathways in plants using minimal amount of resources.



Who We Are

Dr. Ivan Reyna-Llorens, Hibberd Group, Dept. Plant Sciences, iar28
Experience with molecular biology, golden-gate cloning, RNA-Seq and DNase-Seq

Dr. Steven Burgess, Hibberd Group, Dept. Plant Sciences, sjb287
Experience in molecular biology, RNA-Seq and DNase-Seq

Dr. Ziyi Yu zy251
Experience in microfluidics and analysis of fluorescent reporters.

Mr Gregory Reeves gr360
Experience in tissue culture and plant transformation.

Mr. Christian R. Boehm, Haseloff Group, Dept. Plant Sciences, crb59
Experience with molecular biology, handling and engineering of Marchantia, ratiometric quantification of gene expression in planta, and microfluidics

Implementation

Stage 1.1 - Development of a PDMS/glass microfluidic device designed for PEG or *A.tumefaciens* transformation of protoplasts.

- Droplet-based microfluidic devices will be used for protoplasts transfection, in which one droplet defines a picoliter compartment that allows single protoplasts encapsulation and expression of genes, using fewer reagents than conventional methods in bulk solution. As each protoplasts is trapped in individual droplets, the transfection efficiency will be evaluated by fluorescence-activated microdroplets detection system, with more than 1,000 potential transformation evens per minute.

Stage 1.2 - Implementing the method for whole plant regeneration of protoplasts in Marchantia, Nicotiana and Rice.

- It has been shown that whole plant regeneration is possible in Tobacco (Takebe et al., 1971) rice (Yamada et al., 1986) and Gracilaria(Huddy et al., 2015). The methods will be implemented in isolated protoplasts from different tissues.

Stage 2 - Transformation, sorting and whole plant regeneration of Marchantia, Nicotiana and Rice protoplasts

- A reporter construct using Golden Gate Cloning, which is routinely employed in the Hibberd Lab. All parts will be compatible with the Plant MoClo system (Engler et al. 2014), and made publically available via Addgene. Vectors will embrace a site for insertion of gene regulatory regions of interest, which will drive a codon optimized mVenus, together with mTurquoise2 constitutively expressed under the control of the UbiQ promoter. In order to test the system, we will clone in the PEPC promoter of maize to drive the expression of various

reporter genes. All the promoter level 0 Golden Gate modules are already available in the Hibberd lab.
- Transformed protoplasts will be sorted and incubated in regeneration media with the aim of obtaining whole plant tissue.

Benefits and outcomes

(1) An open source golden-gate reporter system for analysis DNA parts.
(2) A microfluidic based system for transformation and screening of plant protoplasts. Our approach allows the use of individual cells rather than entire organisms for transformation facilitating screening of large libraries of genetic elements.
(3) A method for whole plant regeneration from single protoplasts. This approach has the potential to generate whole mutant populations or to do stable transformation of complete libraries of DNA parts.
(4) Publication of our method and results firstly in bioRxiv then in an open access journal depending on outcomes.

Outreach
We will continue to publish updates via our blog and twitter to inform the community and conduct our work in an open manner.

Sponsor for the research and cost centre

Prof Julian Hibberd, Department of Plant Sciences, University of Cambridge
jmh65@cam.ac.uk

Budget

Budget

Fabrication of a Microfluidic device

Item	**Total £**
HR Photomask	90
Photopolymer	100
Photopolymer developer (PGMEA)	20
Silicon wafers	30
Sylgard PDMS - 0.5 Kg of polymer + curing agent kit	50
Aquapel, 5mL	20
Tubing, 3 metres - for flow pumping	100
Syringes and needles	50
Surfactant for droplet formation	free
Subtotal	460

Average price per chip and it makes a price **per chip** on average of **25 pence**.

Generation of reporter construct

Item	**Total £**
Promoters modules for Golden gate cloning	Free

Codon optimized reporter gene synthesis (~1500 bp)	270.0
Restriction Enzymes (*BsaI* and *BpiI*)	70.0
T4 DNA ligase	45.0
ATP	30.0
QIAprep Spin Miniprep Kit (50)	50.0
DNeasy Plant Mini Kit (50)	150.0
Sanger sequencing service (~20 rxns)	100.00
SYBR Green Taq ready mix (100 rxns)	120.00
Oligonucleotides	100.00
Subtotal	935.00

Generation of protoplasts & protoplast transformation

Item	**Total £**
Driselase (5g, Sigma)	175.07
Hemicellulase (300KU, Sigma)	81.14
Subtotal	256.21

Contingency budget

Item	**Total £**
Extra sanger sequencing (~20 rxns)	100.00
Gene synthesis of more reporter constructs	270.00