Project Title

Plant-ProChip 2.0: High throughput transformation of plant protoplasts

Report Title

Optimization of droplet sorting for high throughput transformation of plant protoplasts

Summary

A current limitation for plant synthetic biology involves high-throughput screening of genetic parts in plants. Current approaches require testing circuits in individual plants, through transient or stable transgenics. Applying these techniques to entire libraries is not feasible at a laboratory scale.

In the first stage of the project we aimed to develop a high-throughput screen for the analysis of promoter sequences in plant protoplasts. As a result, we successfully isolated, encapsulated and analysed protoplasts from the model species, *Marchantia polymorpha* and *Arabidopsis thaliana* using a PDMS microfluidic device. Despite of this, there are considerable limitations in terms of protoplast transformation for making these assays high-throughput.

The aim of this project is to use microfluidics to develop both transient and stable protoplast transformation protocols at a high-throughput scale. Encapsulated protoplasts will be transformed by PEG transformation and screened for reporter activity. The transformed cells will be sorted and plated onto regeneration media for whole plants regeneration.

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.

Report and outcomes

In the last stage of the project we isolated and encapsulated protoplasts from different species. However, to succeed in transforming protoplast “on chip” we had to optimize the sorting of transformed protoplasts. During the last months, we have been optimizing this aspect of the project as well as fine tuning our method of protoplast isolation.

We decided to use *Marchantia* polymorpha protoplasts (Figure 1 & 2) as the model species for this part of the project. In our hands, Marchantia gives the highest yields as well as the maximum protoplast integrity when compared with other systems such as Arabidopsis or tobacco.

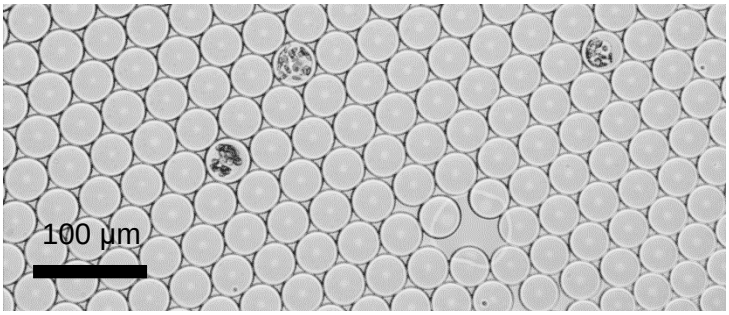

Figure 1 Marchantia polymorpha protoplasts encapsulated in microdroplets.

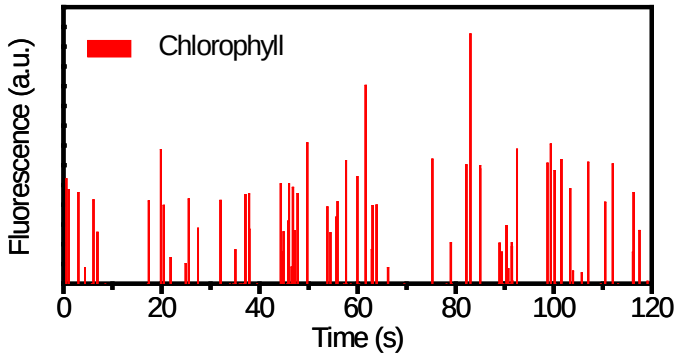

Figure 2 The number of Marchantia protoplasts in microdroplets have been counted by fluorescence-based microdroplets counting system. Each peak represents the chlorophyll fluorescence of one Marchantia protoplast.

Protoplast transformation requires an accurate sorting step in which transformed cells can be selected for further applications such as whole plant regeneration. In this case, we used transgenic *Marchantia polymorpha* plants carrying the pCRB mpt0 plasmid that contains a constitutive promoter expressing a yellow fluorescent protein (YFP). Protoplasts were isolated from this line and the activity of YFP was assessed (Figure 3).

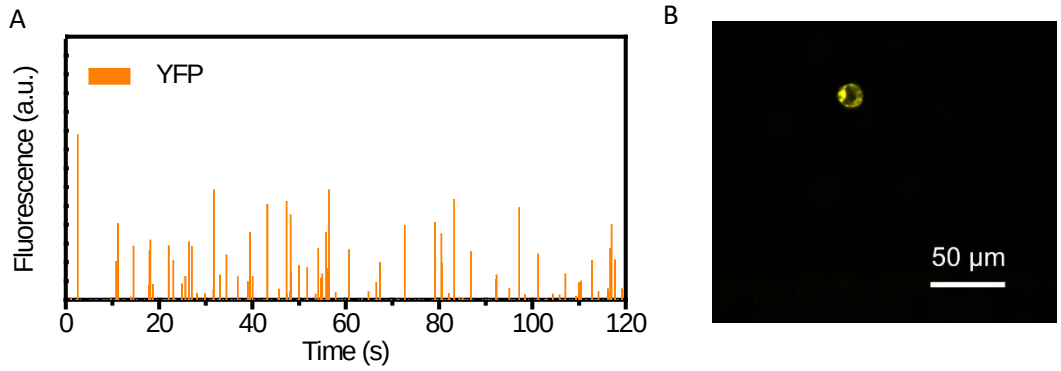

Figure 3 A) Quantification of YFP in encapsulated marchantia protoplasts. Each peak represents the activity of an individual protoplast. B) Isolated protoplast expressing YFP.

We now have an assay to evaluate transgenic (YFP) and a non-transgenic (Chlorophyll) cells that can be used for sorting plant protoplasts. The next step was to sort WT and transgenic Marchantia lines. In a first attempt, we manage to sort chlorophyll positive droplets (Figure 4). However, most of the droplets contained damaged or ruptured protoplasts.

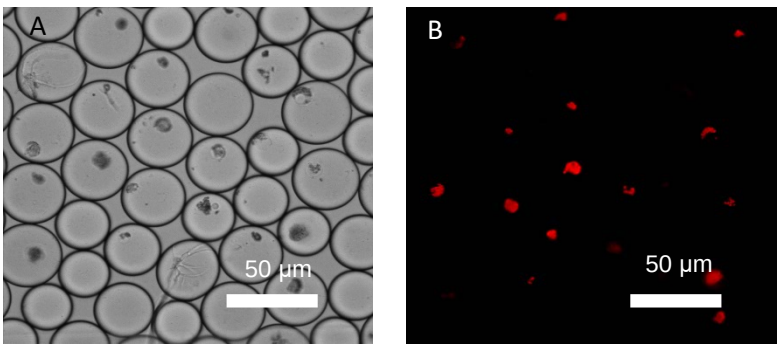

Figure 4 Sorted encapsulated protoplasts from Marchantia polymorpha visualized under A) light microscopy and B) fluorescent microscopy.

After modifying the isolation of protoplast so most of the cells will retain their integrity, the transgenic lines expressing YFP were encapsulated and sorted using the YFP channel (Figure 5, Supplementary file 1).

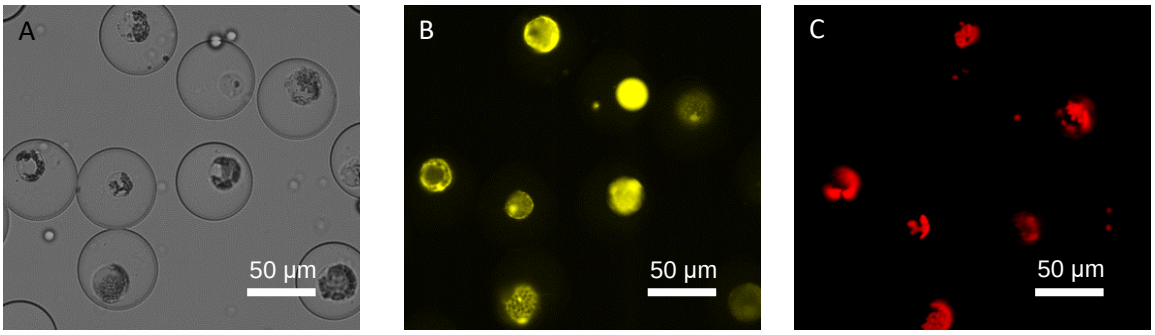

Figure 5 Marchantia polymorpha protoplasts sorted using a microfluidic device and visualised under A) light microscopy, and a fluorescent microscope using B) a YFP channel (491 nm) and C) a chlorophyll channel (630 nm).

Based on these results, we now have a minimal pipeline for protoplast isolation and sorting in Marchantia. In the next phase of the project, we want to assess the activity of different genetic circuits as well as use our chip to optimize protoplast transformation "*in chip*".

Expenditure

Please see Supplementary file 2

Follow on Plans

At the moment, we are finishing some experiments and writing up a manuscript with the aim of publication. We would like to use the extra £1000 to cover the expenses related to this. In addition, we would like to use the remaining funding to optimize the transformation module in our system. We hope we could get a prototype chip for protoplast transformation in the next two months and do the corresponding tests before the end of the year.