OpenPlant Handbook 2017

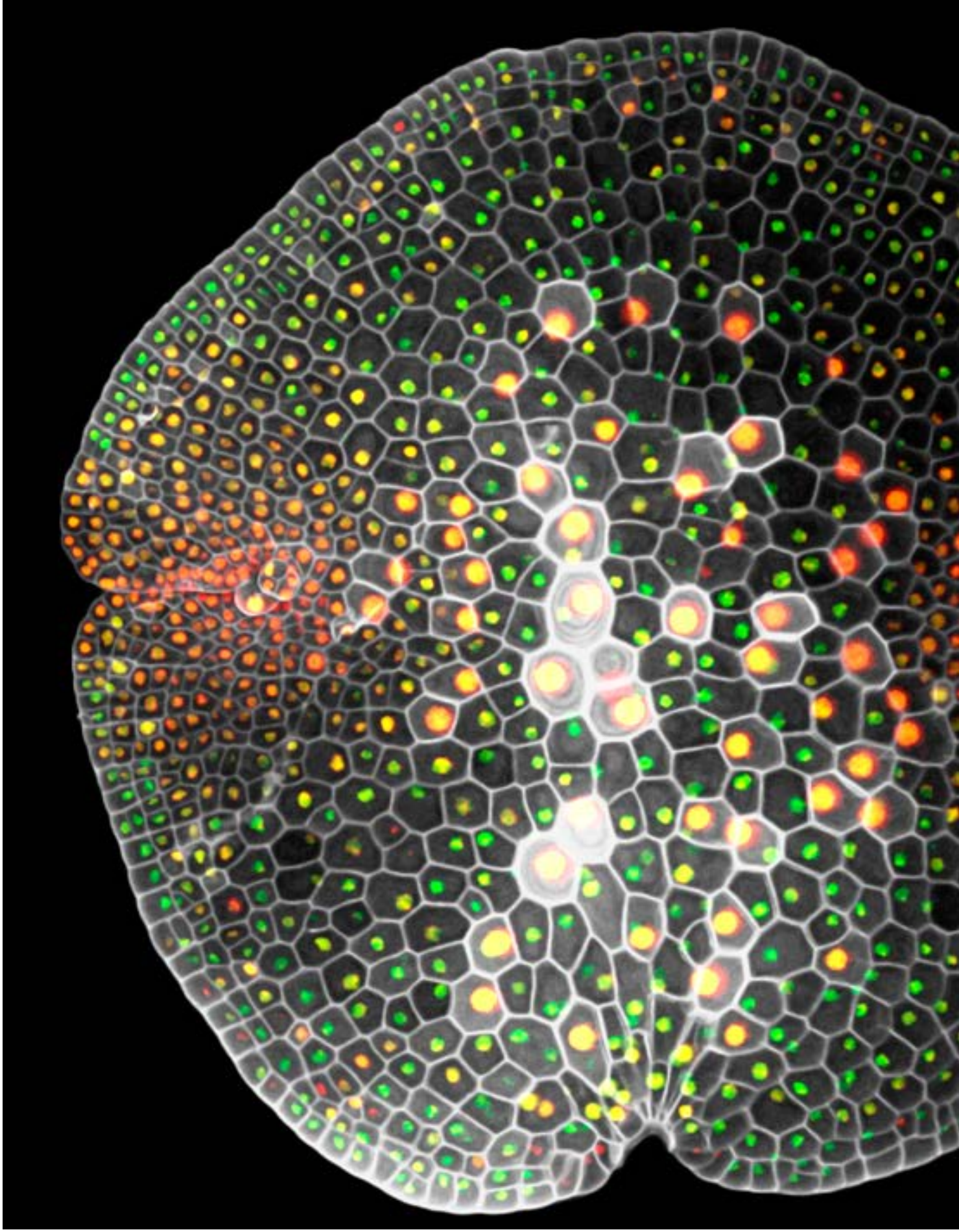
BBSRC Bioscience for the Future

EPSRC Pioneering research and skills

UNIVERSITY OF CAMBRIDGE

John Innes Centre

Earlham Institute



Multi-spectral fluorescence image of a Marchantia polymorpha gemma (clonal propagule) expressing MpEF1a:mTurquoise2-N7 and MpAGL:Venus-N7 fluorescent reporter genes with propidium iodide-stained cell walls. Image captured by Bernardo Pollak using confocal laser scanning microscopy in the Haseloff laboratory at the University of Cambridge.
(Synthetic Botany, Boehm et al. CSH Perspectives in Biology, 2017, doi: 10.1101/cshperspect.a023887)

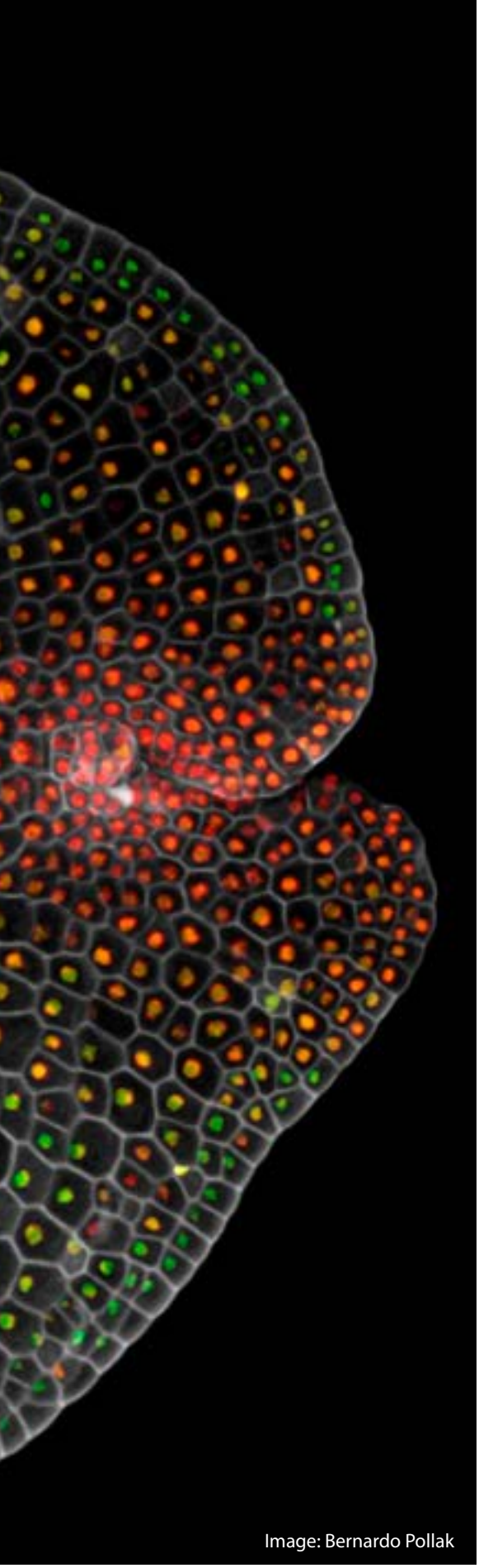


Image: Bernardo Pollak

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OpenPlant

sharing tools for a sustainable future

Synthetic Biology offers the prospect of reprogrammed biological systems for improved and sustainable bioproduction. While early efforts in the field have been directed at microbes, the engineering of plant systems provides even greater potential benefits. In contrast to microbes, plants are already globally cultivated at extremely low cost, harvested on the giga-tonne scale, and routinely used to produce the widest range of biostuffs, from fibres, wood, oils, sugar, fine chemicals, drugs to food. Plants are genetically facile, and GM plants are currently grown on the >100 million hectare scale. Plant systems are ripe for synthetic biology, and any improvement in the ability to reprogram metabolic pathways or plant architecture will have far-reaching consequences.

What is OpenPlant?

Institutional strengths in plant sciences

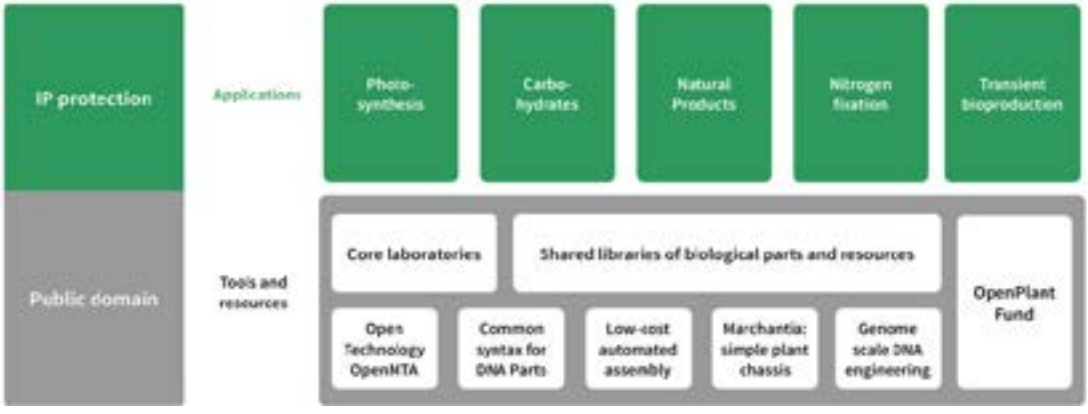
We believe that there is a crucial need to accelerate the development and open sharing of new tools and methods for plant synthetic biology. OpenPlant is a joint initiative between the University of Cambridge, the John Innes Centre and the Earlham Institute, funded by the BBSRC and EPSRC as part of the UK Synthetic Biology for Growth programme. The initiative promotes (i) interdisciplinary exchange, (ii) open technologies and (iii) responsible innovation for improvement of sustainable agriculture and conservation.

Interdisciplinary exchange

The UK provides an ideal hub for interdisciplinary exchange between foundational sciences like botany, agronomy, physics, chemistry, computer sciences and engineering. This exchange drives innovation for the engineering of biological systems. OpenPlant promotes and funds the development of novel foundational technologies, the creation of international standards for plant synthetic biology, and open tools for trait development. We believe that advances in plant synthetic biology will provide a key to securing and sustaining future food and materials production, and that there should be worldwide open access to these benefits.

Open technologies for innovation

Current IP practices and restrictive licensing threaten to restrict innovation as the scale of DNA systems increases. We believe that the field needs to explore new "two-tier" intellectual property models that will protect investment in applications, while promote sharing of DNA components and freedom-to-operate for innovators in business and social enterprises. We are building new frameworks and collaborations for open innovation in plant synthetic biology.



Overview of the OpenPlant work programme showing development of new plant traits (upper tier) and low level public domain tools and resources (lower tier).

Marchantia polymorpha plants

Responsible innovation for sustainable agriculture and conservation

Past experiences with GM technologies have shown that they cannot be developed in isolation from social, ethical and environmental considerations, and OpenPlant supports work on the wider implications of the technology at local and global scales, including discussions on the potential impact of Synthetic Biology on environmental conservation and sustainable human practices. These bring together a wide range of engineers, scientists and policy developers to explore the implications for adopting new technologies and different models for sustainable agriculture, bioproduction and land use.

OpenPlant supports the standardisation and global sharing of open technologies for plant synthetic biology. We aim to catalyse responsible innovation for sustainable agriculture and conservation. Further, OpenPlant provides new tools and a point of exchange for young scientists and entrepreneurs in the UK.

OpenPlant objectives

The OpenPlant initiative has been funded with three main aims:

1. To create a hub for interdisciplinary exchange between Cambridge and Norwich, between the fundamental and applied sciences, that will underpin advances in UK agriculture and bioproduction.
2. To establish systems for the open exchange of new plant tools and DNA components that will promote commercial innovation and international scientific exchange.
3. To explore the wider implications of the technology at local and global scales. This will bring together a wide range of engineers, scientists and policy developers to explore new technologies and possible models for sustainable agriculture, bioproduction and land use.

Image: Fernan Federici

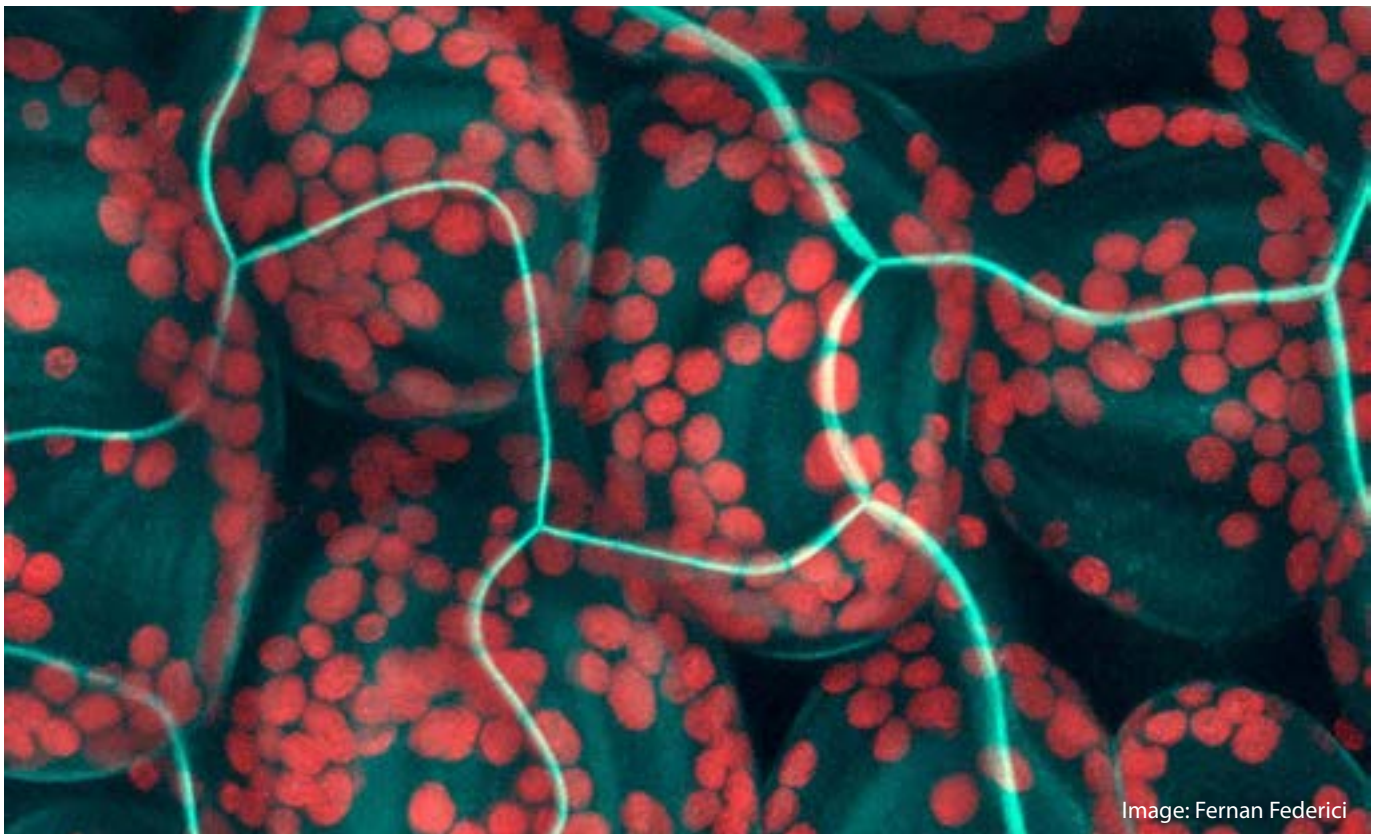


Image: Fernan Federici

OpenPlant Work Programme

The OpenPlant initiative supports two tiers of activities.

Current agricultural practices and cultivation of trees, crops and pastures are responsible for major pressures on natural environments and land use globally. The OpenPlant initiative brings together an exceptional collection of scientists, whose skill sets range from biophysics, chemistry and DNA assembly to crop physiology and agronomy. In addition, we have recruited experts involved in conservation, entrepreneurship, law, policy development and the social sciences in Cambridge and elsewhere in the UK – who have demonstrated an interest in tackling the technical aspects of surveying future technologies. An overarching aim of the project is to provide a map of feasible technical approaches to improving bioproduction and agriculture – including studies of possible economic models, opportunities and social implications for different scenarios and current practices.

First, we are developing open technologies that will underpin systematic approaches to bioengineering of plants. These include:

Workpackage A: Development of the lower plant Marchantia as a simple and facile chassis for Synthetic Biology, to enable high throughput screening and analysis at the cellular scale.

Workpackage B: A common syntax for plant DNA parts and assembly of genetic circuits. Establishment of a moderated archive for publication of DNA part descriptions.

Workpackage C: New DNA parts for the control and quantitative imaging of genetic circuits.

Workpackage D: Techniques for routine genome-scale engineering in plants.

Workpackage E: Software tools with improved performance for DNA part catalogues, automated assembly, modelling of synthetic gene circuits and cellular morphogenesis.

Second, the development of new tools is contributing to the engineering of new traits in plants:

Workpackage F: Altered photosynthesis and leaf structure.

Workpackage G: Changes in plant carbohydrate content.

Workpackage H: Engineered pathways for the metabolic engineering of natural products.

Workpackage I: New forms of symbiosis and nitrogen fixation for crop plants.

Workpackage J: Methods for high level production of biomolecules by transient expression.

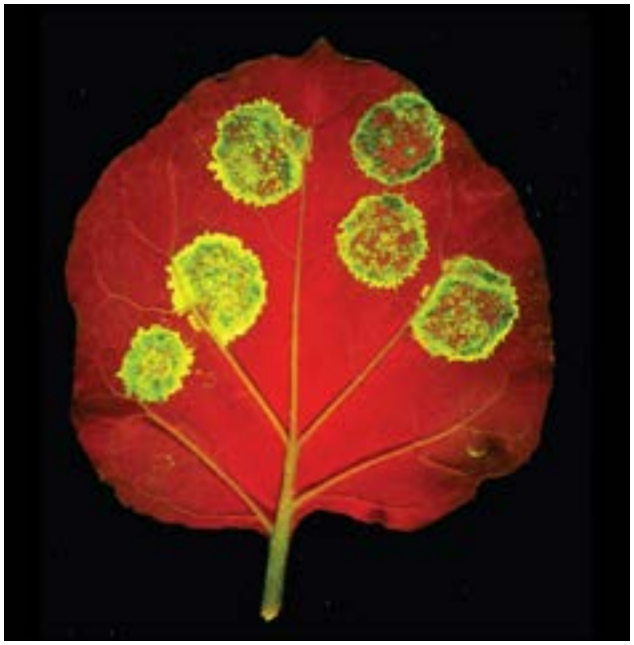
Workpackage K: Annual funding round to support small-scale interdisciplinary grants.

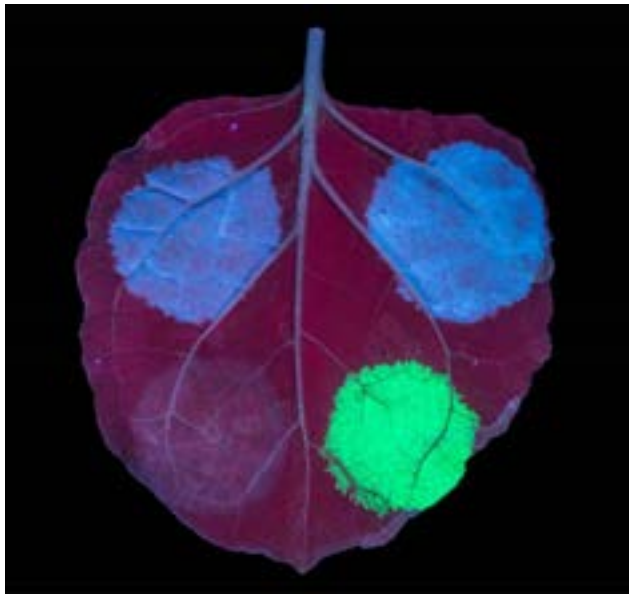
Workpackage L: Outreach activities, training and tools for open exchange of DNA parts and other reagents in biotechnology.

Year 1 progress

The OpenPlant initiative started in September 2014. Over the first year of the research programme we made notable progress in: (i) standards and infrastructure for DNA assembly in plants, (ii) development of a framework and methods for more open sharing of DNA parts, (iii) substantial advances in the development of Marchantia as a simple model for plant synthetic biology, (iv) development of improved capacity for automated metabolic analysis and (v) translation of these approaches to the HyperTrans system for rapid testing of metabolic circuits in plants.

Top: Nicotiana benthamiana leaf showing the hypersensitive response for defence against pathogens. Marina Pais (TSL) & Andrew Davis (JIC). Bottom: *Nicotiana benthamiana* leaf producing components of avenacin, a fluorescent antimicrobial produced by oat roots. Aymeric Leveau (JIC).





Foundational work

- Refurbishment and equipping of new OpenPlant laboratories in Cambridge and Norwich.
- Establishment of a common genetic syntax for exchange of DNA parts for plants, extensible to all eukaryotes (Patron et al., 2015; RFC106).
- Drafting of an Open Materials Transfer Agreement, a simple, standardised legal tool to enable sharing of materials and associated data on a more open basis.
- Implementation of a "single-click" OSX-installable version of the JBEIR-ICE open source DNA registry and DNA manipulation software.
- Development of routine methods for transformation and gene editing in *Marchantia polymorpha*.
- Characterisation of miR1157 and miR1162 precursors for use as synthetic gene regulators in *Chlamydomonas reinhardtii*.

Trait Development

- Development of *Marchantia paleacea* as a new system for engineering actinomycorrhizal associations.
- Generation of draft genome and transcriptome maps for *M. polymorpha* and *M. paleacea*.
- Generation of system for transient induction of gene expression in tomato fruit, based on the HyperTrans vectors.
- Refactoring and use of the HyperTrans system for rapid testing of DNA circuits for terpene synthesis in *Nicotiana benthamiana*.
- New vectors created for fine tuning of protein levels using the HyperTrans system (Meshcheriakova et al., 2015)

Outreach and Responsible Innovation

- Funding of 16 mini-grants that incorporate broad interdisciplinarity and collaboration between Cambridge and Norwich - including hardware, wetware development and support for collaboration between OpenPlant and African scientists.
- Support for a joint Cambridge-JIC iGEM2015 team in the Hardware Track.
- Support for a Synbio Beta Activate event in Cambridge, to promote entrepreneurial interactions.
- Organisation of the OpenPlant Forum and international exchange.
- Delivery of two summer schools on Plant Synthetic Biology and CRISPR Technology in Plants, co-sponsored by ERA-SynBio and Plant Methods/GarNET, respectively.
- Delivery of three Science, Art and Writing educational workshops, and two school outreach events.
- Delivery of international workshops on IP, MTAs and Innovation.

Year 2 progress

Foundational work

- Commissioning of advanced imaging and robotics equipment at the Cambridge OpenPlant laboratory.
- Completion of the genome sequence and transcript map of the Cam-1 (male) and Cam-2 (female) isolates of *Marchantia polymorpha*. Data will be included in forthcoming publication of genome.
- High resolution map of the time course of gene expression during sporeling germination and chloroplast differentiation.
- Construction of MarpoDB, a gene-o-centric database for mining and describing DNA parts from Marchantia (http://marpodb.io)
- Official acceptance of the common syntax for plant DNA parts as a new standard (Phytobricks) in the iGEM 2016 competition, and introduction of an award for plant synthetic biology.
- Development of Phytobrick and UNS standards for efficient hierarchical assembly of DNA circuits.
- Expansion of a Chlamydomonas DNA toolkit for target gene expression and assay of miRNA-dependent gene silencing.
- Development of a suite of Cas9 variants and toolkit for targeted mutagenesis and gene deletion in multiple plant species.

Trait Development

- Design and synthesis of an artificial protein scaffold library, built to the Phytobrick standard and verified by BiFC.
- Production of cell-specific epitope tags for identifying DNA motifs that drive gene expression in photosynthetic tissues in Arabidopsis.
- Publication of a novel reporter for chloroplast transformation, and identification of transit peptides for chloroplast localisation of nuclear encoded products in Marchantia.
- Identification of a large repertoire of carbohydrate active enzymes in *Euglena gracilis*.
- Transformation of gene editing constructs into potato, to create digestion-resistant starches, and preliminary screening of transformed plantlets.
- Gram-scale production of triterpenes for analysis and assay, using the HyperTrans system.
- Generation of a trichome-specific protein database for enzyme discovery.
- Asteraceae P450 proteins as a toolkit for targeted modification of sesquiterpenes.
- Continued development of the HyperTrans system for use in tomato.
- Screening tomato introgression lines for regulators of monoterpene biosynthesis.
- Yeast one-hybrid analysis for the identification of transcription factors that regulate triterpene metabolic gene clusters.
- Characterisation of the gene targets of AtMYB12 and SlMYB12 in tomato, as an effective way of enhancing phenylpropanoid metabolism, for high levels of resveratrol and genistin production.
- Construction of synthetic wide host range metabolons for ectopic production of dhurrin, a plant defence compound.
- Construction and distribution of HyperTrans DNA vectors that are compatible with the Phytobrick standard.
- Testing of the HyperTrans system in Marchantia and BY2 cells.
- Consultation on the design of the LeafSystems high throughput production facility, due for completion in Q2 2017.

Outreach and Responsible Innovation

- Funding of 14 mini-grants that incorporate broad interdisciplinarity and collaboration between Cambridge and Norwich, and include projects to deliver SynBio training in Africa, increase SynBio capacity in Africa, and produce resources for schools and universities in South America.
- The Cambridge-JIC iGEM2015 team won a gold medal at the international Jamboree, with a project entitled "OpenScope" - a low cost, open source, 3D printed, fully automated microscope powered by a RaspberryPi and customised software.
- Obtained support for a joint Cambridge-JIC iGEM2016 undergraduate team with a plant-based project: chloroplast engineering in Chlamydomonas. Obtained co-sponsorship from Cambridge Consultants and Wellcome Trust/BBSRC/SEB fund.
- Responsible Innovation workshop with Kathy Liddell, Law Faculty, Cambridge
- OpenPlant continues to collaborate with Linda Kahl, Biobricks Foundation, and an international IP working group to implement an Open Materials Transfer Agreement with the aim of improving freedom-to-operate by enabling international exchange of DNA parts. OpenPlant participated in the inaugural meeting of BioNet group at Asilomar and supports the development of an open technology platform for peer-to-peer exchange and provenance tracking of biomaterials (http://www.bionet.io).
- Organised OpenPlant All Hands meeting for scientific exchange, Newmarket.
- Participated in Open Technology for Biology workshop, Chile.
- OpenTechnology Week events in Cambridge, including Technology for the Bottom Billion workshop and Makethon, coordinated with the Centre for Global Equality (http://centreforglobalequality.org).
- Workshops on ethics and openness run at OpenPlant (Mar 2016), outreach with the SAW trust (Mar 2016), and BBSRC Media Training for OpenPlant (Mar 2016).
- Outreach in schools: OpenPlant exhibit on schools day and continuing in family area during Latitude Festival (Jul 2016). Exhibit at 2-day Cambridge Science Festival (Mar 2016). Exhibit at Youth STEMM Awards mid-year conference, John Innes Centre (Jan 2016). SAW workshops in primary schools with scientists from OpenPlant (Mar 2015, Jan 2016). SynBio workshop at Inside Science, event at John Innes Centre for Year 10 pupils interested in science careers (Nov 2015)
- Nineteen post-graduate students are participating in projects funded by the OpenPlant Fund. In addition, three PhD students were recruited directly to OpenPlant (Cambridge) this year.
- Undergraduates have formed a student society for Synthetic Biology at the University of Cambridge (http://cusbs.soc.srcf.net).
- Other students and postdocs at University of Cambridge and John Innes Centre are being recruited to OpenPlant, to share projects, resources and equipment, through the ROC Group - a self organised and highly effective group of junior researchers.

Year 3 progress

Foundational work

- Consolidation of the Phytobrick standard for Type IIS based DNA parts for plants, including acceptance as first accepted standard for eukaryotic DNA parts, and introduction of the Plant Prize in iGEM 2016.
- Design, domestication and synthesis of the first 500 DNA parts for Marchantia, headed by Susana Sauret-Gueto.
- Establishment of the Loop assembly technique by Bernardo Pollak & Fernan Federici, first presented at the iGEM2016 Jamboree.
- Automation of Loop assembly at 500nL scale using Labcyte acoustic focusing and Hamilton robots at Earlham Institute, Cambridge-Earlham Institute collaboration.
- Locke lab developed *S. elongatus* constructs for examining the circadian clock and its outputs at the single cell level, and published frequency doubling of the clock in Molecular Systems Biology.
- Cyanobacterial circadian clock models made available in SBML format.
- Validated the use of fluorescent protein based reporter for evaluating miRNA mediated gene silencing in Chlamydomonas.
- Optimised miRNA abundance, extent of sequence complementarity and target sites for gene silencing in Chlamydomonas.
- Constructing synthetic genetic circuits with miRNA mediated incoherent feed forward loop to confer robust levels of gene expression.
- Gene parts for Chlamydomonas are now constructed according standardised Type IIS common syntax.
- Synthesis of a panel of codon optimised fluorescent reporters spanning the visual spectrum, including five variants of the fluorescent reporters: iRFP670, mCardinal, mPlum, mCerukean, mNeptune, mRaspberry, mTurquoise, mWasabi, eBFP, Sirius and TagCFP, all modified for chloroplast expression in the Ajioka lab.
- The Marchantia chloroplast genome has bee re-annotated. Missing ORFs have been identified, partial annotations have been completed and likely promoter sites identified throughout the chloroplast genome using BPROM.
- CRISPR-Cas9 mediated gene KO to produce "giant chloroplast" phenotypes in Marchantia, (Male, Pollack, Sauret-Gueto, Silvestri in Haseloff lab).
- Established reproducible colonisation of several liverwort species (*Marchantia spp., Lunularia cruciata*) with *Glomeromycota* fungi (*Funnelliformis mossae, Rhizophagus irregularis*) in custom vermiculite system and detection using staining and high resolution confocal fluorescence microscopy, by Philip Carella (PDRA) in the Schornack lab.
- Established constructs for secretion system pathway and tonoplast labelling and have confirmed functionality in *Marchantia polymorpha*.
- Enhancer trap screen underway in Haseloff lab, led by Linda Silvestri, Susana Suaret-Gueto, Marta Tomaselli and Dave Preston, Bernado Pollak.
- *In planta* cytometry techniques developed in Marchantia gemma by Bernardo Pollak and Mihails Delmans.
- Marta Tomaselli (OpenPlant PhD student) develops clearing techniques for image reconstruction of Marchantia air chambers.
- Lukas Mueller (PDRA) building ubiquitin-tagged rapid-turnover fluorescent markers for imaging dynamic genetic responses in Webb-Haseloff labs.
- New synthetic version of the 5′ UTR used in HyperTrans system has been shown to be twice as effective as the original HT sequence, Lomonossoff lab.
- Developed a new vector system (pEFF) which combines the high translational benefits of the CPMV-HT system with the replication ability of potato virus X, in collaboration with the Centre for Bioengineering at the Russian Academy of Sciences. The system will be extremely useful where virus spread throughout a host is desirable.

Trait Development

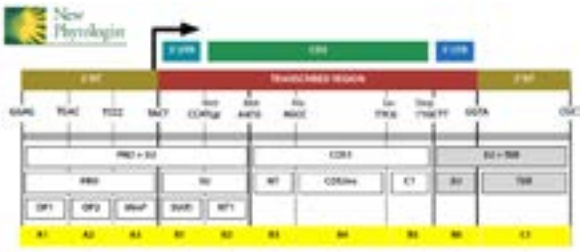
- Hibberd lab has characterised promoter elements that drive specific expression in leaves, and a negative regulator that represses expression in mesophyll and veinal cells.
- Compiled a list of transcription factors that are preferentially expressed in bundle sheath cells of Arabidopsis, including the cognate TF for identifiied promoter element.
- Heterologous xylan arabinosyltransferases have been cloned in Arabidopsis lines using common syntax rules.
- The enzymatic activities of genes are being tested using *N. benthamiana* transient expression with the pEAQ-HT vector system.
- Field lab (JIC) is exploring artificial, *in vitro* metabolic cycles, driving production of glucose-based oligosaccharides from cheap and readily available sucrose by using sucrose phosphorylase and glucan phosphorylases
- Aytug Tuncel (PDRA, Smith lab) is applying and testing the genome editing tools and technologies developed in the Patron lab to generate potatoes that contain digestion-resistant starches with potential nutritional benefits.
- Developed methods to transform and engineer protoplasts directly isolated from potato leaves.
- Transcriptome of *Euglena gracilis* mined for metabolic carbohydrate metabolism and natural product biochemistry, and released in the CAZy database, primary data at EBI.
- Osbourn lab, collaborated with Medema lab (Wageningen) to release plantiSMASH, a customised algorithm for mining for biosynthetic gene clusters in plant genomes (Kautsar et al. 2017).
- Improved agro-infiltration methodology for production of triterpenes using the HyperTrans transient plant expression system (Reed et al., 2017).
- Lichman (PDRA in O'Connor lab) has identified genes that modulate the stereochemistry of the iridoid ring system, with potential for compounds with important agrichemical activity.
- Noam Chayut (PDRA) is working on a project for plant sourced L-DOPA production for Parkinson's treatment, blocking turnover in beetroot to enable low-tech bioproduction.
- Don Nguyen (ex O'Maille lab) has engineered enzymes from the Asteraceae family into yeast to generate oxygenated sesquiterpenes (Nguyen et al., 2016).
- Hans Nützmann (PDRA, Osbourn lab) is using HiC mapping and FISH analysis to investigate the three-dimensional positioning of biosynthetic gene clusters in the genome of *Arabidopsis thaliana*.
- Yeast-one-hybrid assays have been used to identify a candidate transcription factor for a biosynthetic gene cluster from oat (the avenacin cluster).
- Promoters of genes of the oat avenacin cluster retain root-specific expression patterns in a wide range of plant species. Nine promoters have been isolated from the cluster for use as synthetic parts.
- Three of these oat promoters have been used to drive the

expression of a 3 gene pathway for a plant defence compound (dhurrin) from sorghum in Arabidopsis roots.
- Produced synthetic virus-like particles (VLPs) to enable the structure of particles of potato leafroll virus to be solved to near atomic resolution.

Outreach and Responsible Innovation
- Revision and launch of new OpenPlant branding and website (Jan 2017).
- OpenPlant launched new website, www.biomaker.org, to promote SynBio SRI, OpenPlant Fund and Biomaker Challenge projects (Jun 2017).

OpenPlant organised and participated in a wide range of workshops over the last year:
- Developed SAW workshop to enable dissemination and share best practice with other research centres. Delivered two of these workshops for SynthSys and the UK Centre for Mammalian Synthetic Biology (University of Edinburgh), and plan similar collaboration with Warwick Integrative Synthetic Biology Centre.
- Training workshop on the BY2 cell pack system ("cookies"; developed by Fraunhofer Institute, Aachen, Germany), run by the Lomonossoff group (July 2016).
- Norwich Science Festival OpenPlant Exhibit (22nd Oct 2016), and talk 'The green vaccine machine'.
- Nagoya Protocol Workshop, Cambridge (Nov 2016).
- Youth STEMM Award Mid-Year Conference OpenPlant Exhibit (Jan 2017).
- SynBio and Nagoya workshop presentation, HVCfP, London, Jan 2017 (Jan 2017).
- CSER Biorisk workshop (Mar 2017).
- Standards & responsible governance, (PAGIT) project workshop. London, Mar 2017.
- Talk on 'Finding drugs in the garden', 'Just Eat Your Greens - A New Way of Vaccinating?' and ' 20,000 Leagues Under the Microscope: Viruses & Nanomachines' at the second Pint of Science Festival in Norwich.
- Talk and discussion panel at Kew Garden's "State of the World's Plants" Symposium.
- The SAW Trust organised workshops in primary schools inspired by: vaccine work in the Lomonossoff lab, biodiversity, plant evolution and genetics.
- The OpenPlant Fund project "Accessible 3D models of molecules", included a public effort to make the largest virus like particle possible out of paper "protein pieces" decorated by visitors.
- Colette Matthewman and Jenni Rant (SAW) secured funding for a robot, DNA Dave, who saw action at the Cambridge Science Festival along with other OpenPlant exhibits (Mar 2017).
- A public workshop called the Global Garden was run as a collaboration between OpenPlant, the SAW Trust, social scientist Dr Nick Lee (WISB) and the Writers Centre Norwich.
- OpenPlant and SAW are teaming up to deliver a science tent for exploration of "George's Marvelous Medicines" at Boomtown festival this year (Aug 2017).
- The OpenPlant Fund supported £5K mini-projects: 14 in Jul 2016, 10 in Dec 2016, and 12 new proposals to be pitched in Jul 2017.
- OpenPlant Fund project "Big Algae Open Experiment" (http://bigalgae.com/) joined the OpenPlant stand at Latitude Festival (Jul 2016), a mixed arts festival in the east of England which attracts 10,000 people.
- Roger Castells-Graells (PhD student, JIC), won a University of East Anglia Engagement Award in recognition of his work on the OpenPlant Fund project "Accessible 3D Models of Molecules".
- OpenPlant supported open source hardware documentation and repository: www.DocuBricks.com.
- Establishment of the Biomaker Challenge: 28 £1K micro-projects to promote interdisciplinary exchange in Cambridge and Norwich.
- Supports development of Biomakespace in old MRC Laboratory of Molecular Biology Building under leadership of Jenny Molloy, due to open summer 2017.
- Cambridge-JIC iGEM2016 team were awarded a gold medal and were the winners of the Best Plant Synthetic Biology prize at the iGEM Giant Jamboree, Boston.
- OpenPlant supports the OpenMTA initiative, which now has a website at http://www.openmta.org/. This includes a simple description of the OpenMTA.
- OpenPlant and the Earham DNA Foundry co-sponsored a BBSRC-GCRF funded workshop in South Africa and produced a report on capacity building for the future bioeconomy in Africa.
- OpenPlant has established a working group to develop new, open curriculum materials based on cell-free and other fast and frugal technologies, including:
- Two cell-free expression workshops with Vincent Noireaux (2016) and Keith Pardee (2017).

Presentations
- American Society for Plant Biology Meeting, Austin, Texas, US, June 2016.
- 17th International Conference on the Cell and Molecular Biology of Chlamydomonas Kyoto, Japan, July 2016.
- 5th Annual Sc2.0 and Synthetic Genomes Conference, Edinburgh, July 2016.
- Carbon Capture discussion meeting, Woods Hole, MA, USA, August 2016.
- GarNET meeting, Cardiff, September 2016.
- Genomics-Enabled Accelerated Crop Breeding, Cold Spring Harbor, New York, October 2016.
- CESR Bioengineering Horizon Scanning workshop, Cambridge, UK, Nov 2016.
- OpenPlant presentations at SBUK & BBSRC Workshop, Nov 2016.
- Plant Omics and Biotechnology for Human Health, Ghent, Belgium, Nov 2016.
- Plant Genetic Resources and Sustainable Development Goals, Rockefeller Foundation Bellagio Center, Italy, Nov 2016.
- Synthetic Biology for Natural Products, Cancun, Mexico, Mar 2017.
- Directing Biosynthesis V, Warwick, poster presentation, Mar 2017.
- Science AAAS Synthetic Biology Forum, Cambridge, Mar 2017.
- SBLC OpenPlant presentation, London, Mar 2017.
- SB7.0, Singapore, Jun 2017.
- ISDB2017, Singapore, Jun 2017.
- SEED, Vancouver, Canada, Jun 2017.
- Plant Transformation and Biotechnology IV, Vienna, Austria, June 2017.
- PBVAB 2017, Albufeira, Portugal, Jun 2017.
- Gordon Research Conference on Plant Metabolic Engineering, Waterville, NH, US, Jul 2017.
- CeBiTec Colloquim, Bielefeld University, Germany Sep 2017.



DNA PARTS

Synthesis of DNA parts

COMMUNITY STANDARD

With wide support from the international plant science community, we have established a common genetic syntax for exchange of DNA parts for plants, extensible to all eukaryotes (Patron et al. 2015). This common syntax for plant DNA parts is at the core of RFC 106, posted at OpenWetWare, and accepted as an official standard for DNA parts in the iGEM synthetic biology competition.

The Phytobrick standard is a consolidated and consistent standard for Type IIS restriction endonuclease based assembly of DNA parts to make synthetic genes. It is based on the widely used "Golden-Gate"-type standard, and allows highly efficient assembly of multiple standard parts into genes without the need to isolate DNA fragments. A range of existing techniques such as Gibson assembly, MoClo and Golden Braid can be used for higher order multiple-gene assemblies, however we have developed a simple and flexible protocol for assembly of plant vectors, the Loop Assembly technique.

The Phytobrick standard is general, and applicable to all plants, and other eukaryotes

Principal contacts: Nicola Patron & Jim Haseloff

OPEN SCIENCE

OpenMTA to promote free exchange of DNA parts

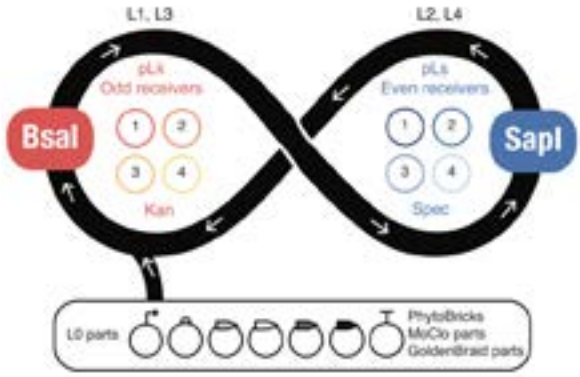
OPENPLANT & BIOBRICKS FOUNDATION

Current IP practices and restrictive licensing threaten to restrict innovation as the scale of DNA systems increases. We believe that the field needs to explore new “two-tier” intellectual property models that will protect investment in applications, while promote sharing of DNA components and freedom-to-operate for small companies in commercial applications of Synthetic Biology.

We are collaborating with the Biobricks Foundation on an Open Materials Transfer Agreement (OpenMTA). This is a simple, standardized legal tool that enables individuals and organizations to share their materials and associated data on an open basis.

The primary purpose of the OMTA is to eliminate or reduce transaction costs associated with access, use, modification, and redistribution of materials and associated data. This in turn will help minimize waste and redundancy in the scientific research process and promote access to materials and associated data for researchers in less privileged institutions and world regions. (http://www.openmta.org)

Principal contact: Linda Kahl



DNA ASSEMBLY

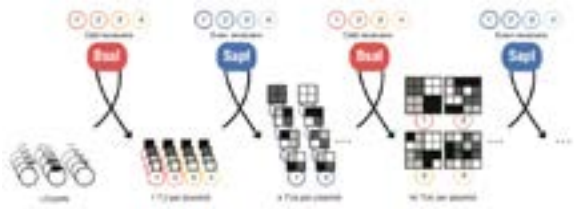
Loop Assembly for efficient & simple construction of DNA

CAMBRIDGE, PUC CHILE

As part of a collaboration between the University of Cambridge and the Universidad Católica de Chile, Pollak and Federici have devised a new method for gene assembly based on two Type IIS restriction endonuceases, BsaI and SapI. Loop Assembly allows rapid and efficient production of large DNA constructs, is compatible with widely used Level zero (L0) DNA parts such as Phytobricks, and can be easily automated.

Loop Assembly requires the alternating use of two Type IIS enzymes, Bsa1 (6-base-pair recognition sequence, 4 base overhang) and Sap1 (7 base-pair recognition sequence, 3 base overhang), and two sets of complementary plasmid vectors that allow efficient and ordered construction of 1, 4, 16, 64 gene fragments.

Principal contacts: Bernardo Pollak & Fernan Federici



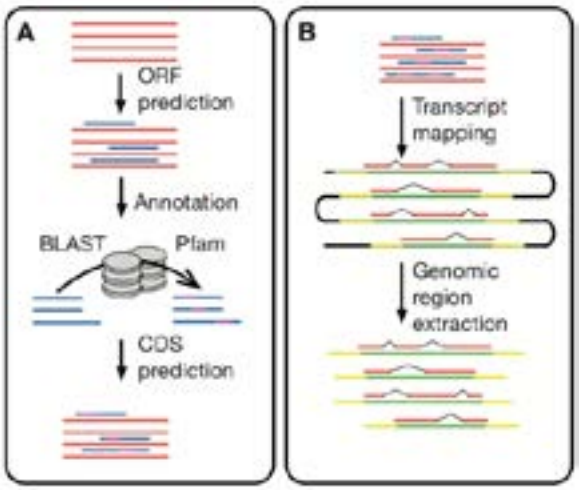
AUTOMATION

Automated Loop assembly and validation

CAMBRIDGE, PUC CHILE, EARLHAM INSTITUTE

Like other "Golden-gate"-based protocols, Loop Assembly does not require purification of individual DNA fragments, side products are recut during the ligation reaction to drive efficient formation of end-products. Loop Assembly is well suited to automation. OpenPlant researchers at the Earlham Institute and Cambridge are developing methods using acoustic-focusing non-contact liquid handling robots, which increases speed and scale of assembly, while reducing consumable costs and allowing reactions to be performed in nanolitre volumes.

Principal contacts: Bernardo Pollak & Nicola Patron



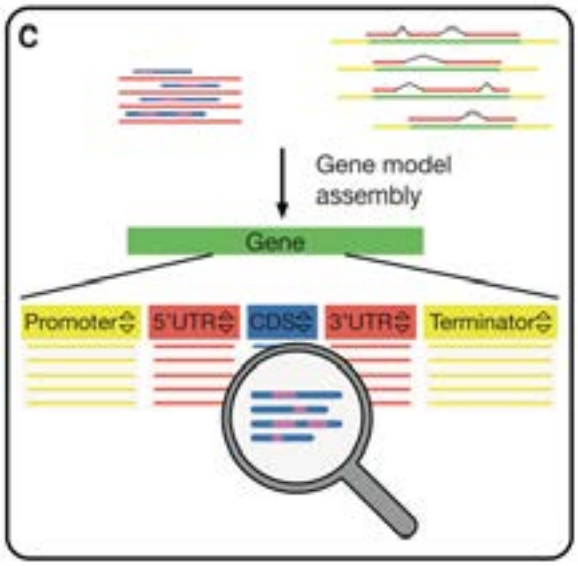
GENOMICS

MarpoDB: a gene-centric database

CAMBRIDGE

For work with the model plant *Marchantia polymorpha*, we have produced MarpoDB, which is an open source database for MarpoDB describes that presents the Marchantia genome from an engineer's perspective, rather than a geneticist's. The database handles the Marchantia genome as a collection of parts. This is highly useful for automatically mining new parts, and managing part description, and part characterisation. We think that this break from standard genome database architecture is essential for tackling the refactoring of synthetic plant genomes. MarpoDB also provides a useful container for gene expression data, and integration of cellular features via Plant Ontology terms. (http://marpodb.io)

Principal contact: Mihails Delmans



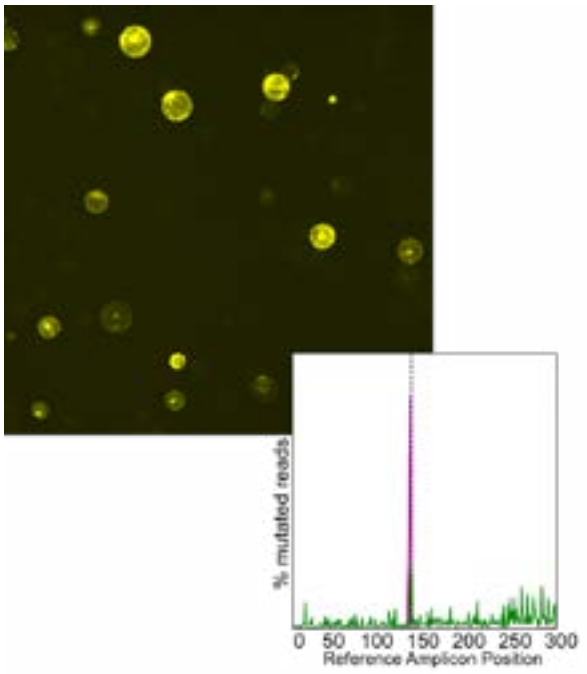
DNA PARTS

Mining the Marchantia genome for DNA parts

CAMBRIDGE

MarpoDB has been designed to facilitate the definition and extraction of synthetic DNA elements to be synthesised as standardised DNA parts. For example, we have identified core promoter candidates, and extracted these from the Marchantia genome. The extracted sequences have been domesticated, removing Bsa1 and Sap1 recognition sequences if necessary, and sent for DNA synthesis. The refactored parts will be cloned into pUAP1, a specially prepared vector designed for public distribution.

Principal contacts: Mihails Delmans & Susana Sauret-Gueto



GENOME EDITING

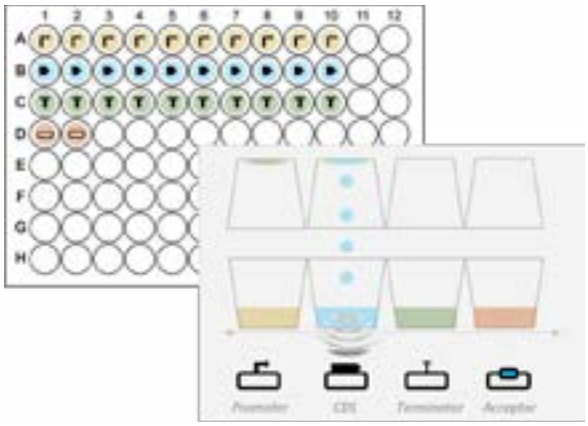
Expanded toolkit for plant genome engineering

EARLHAM

Molecular tools adapted from the Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR) loci that confer adaptive immunity in bacteria and archaea have been applied for genome engineering in eukaryotes. RNA-guided Cas (CRISPR Associated) proteins have been used to induce targeted mutagenesis at endogenous loci in numerous plant species. However, the efficiency of editing varies between species and between targets, mutations are often observed at non-target loci and use of wild-type Cas9 limits engineering to target loci containing a canonical NGG motif.

Oleg Raitskin has developed an expanded toolbox of molecular tools for RNA-guided Cas-mediated plant genome engineering to improve specificity and to increase the number of potential target sites available in plant genomes. To compare and quantify the efficiency and specificity of multiple Cas9 variants, Oleg coupled automated DNA assembly to a transient workflow using protoplasts and Illumina MiSeq targeted resequencing.

Principal contacts: Oleg Raitskin & Nicola Patron



GENE ASSEMBLY

Nanoscale automated DNA assembly and verification

EARLHAM

We have automated and miniaturized Type IIS–mediated assembly of plasmid constructs. We use a modular setup that harnesses an automated freezer working in a 96-well-plate format, and a suite of liquid handling robots to array source plates, assemble larger constructs using acoustic energy from standard DNA parts and perform the downstream microbiological workflow.

Multiple assemblies can be verified in parallel using miniaturized Nextera XT libraries on an Illumina MiSeq Platform or using a novel 'SMRTGate' protocol co-developed with the Liverpool GeneMill (D'Amore et al (2017) BioTechniques 63:1 13–20).

Principal contacts: Nicola Patron & Anthony Hall

Principal contacts: Nicola Patron and Anthony Hall

a simple model plant for bioengineering

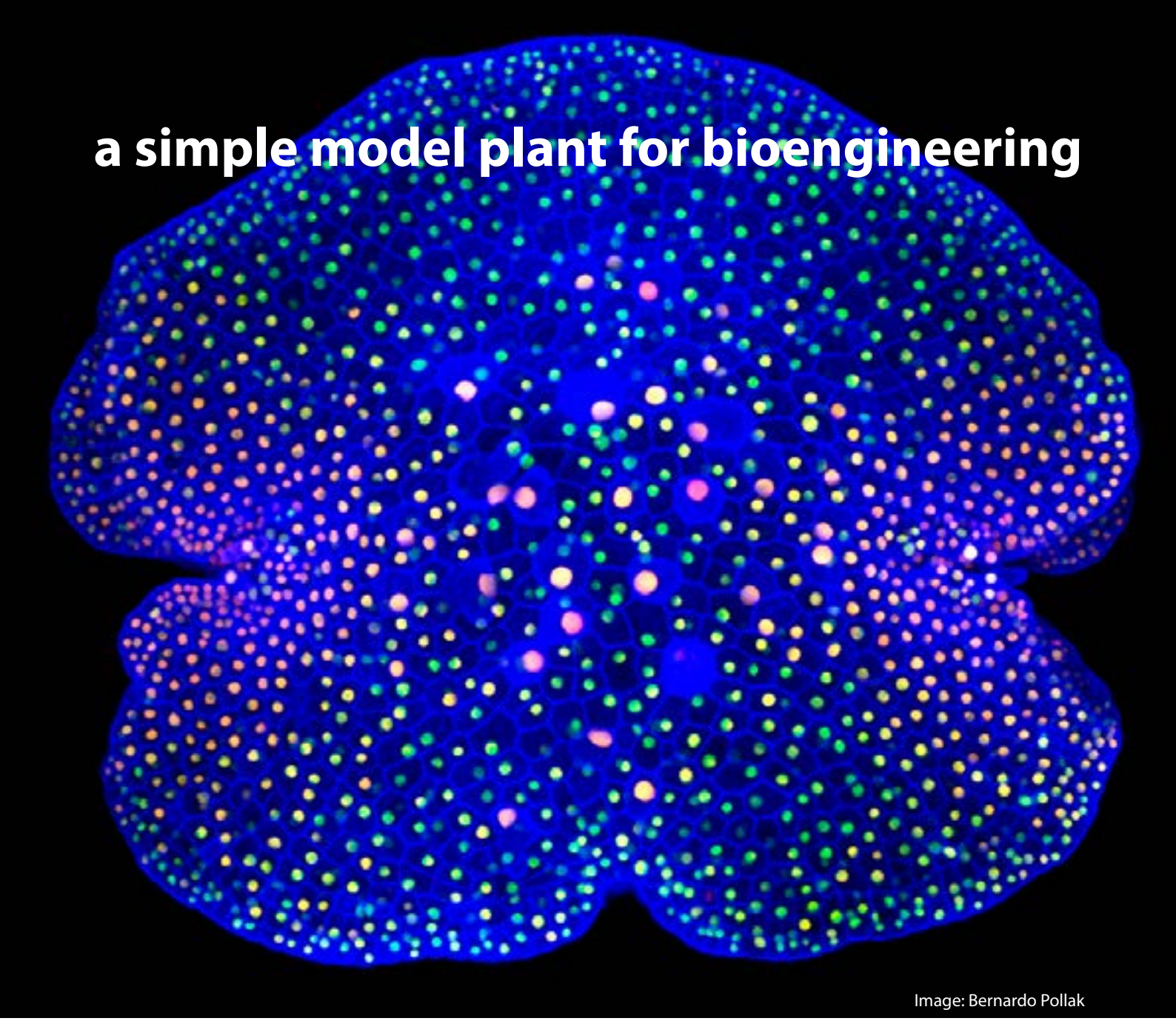


Image: Bernardo Pollak

The liverworts (or Marchantiophyta) are descendants of the earliest terrestrial plants. The group is characterised by morphological simplicity. Liverworts have been a largely neglected area of plant biology, but show great promise as model plant systems after recent developments in transformation methods, genome characterisation and biotechnology.

Marchantia polymorpha is the best characterised liverwort. It is a thalloid liverwort, forming a body of sheet-like tissues that possess distinct upper and lower surfaces. The upper surface has a modular structure, with repeated cellular units that form simple cell complexes adapted for photosynthesis and gas exchange. Like other Bryophytes, the gametophyte or haploid generation is dominant phase of the life cycle. Marchantia has a global distribution, and is often found as a weed in horticulture. The plants grow vigorously on soil or artificial media. Marchantia plants spontaneously produce clonal vegetative propagules, or gametogenesis can be induced by exposure to far red light. Male and female plants can be sexually crossed to produce spores. The plants are extraordinarily prolific. A single cross can produce millions of propagules in the form of single-cell spores. Spores can be harvested in huge numbers and stored indefinitely in a cold, desiccated state. Each spore can germinate to produce a new plant, and, unlike higher plants, can undergo the entire developmental sequence to produce an adult plant under direct microscopic observation.

Sequencing efforts have provided a draft of the ~280Mbp genome. Most of the major gene families present in more advanced plants are represented by a single or few orthologues in Marchantia, meaning that there is low genetic redundancy. The apparent simplicity of genetic networks in liverworts, combined with the growing set of techniques for genetic manipulation, culture and microscopy, are set to make this primitive plant a major new system for analysis and engineering.

OpenPlant has adopted Marchantia as a simple testbed for plant synthetic biology.

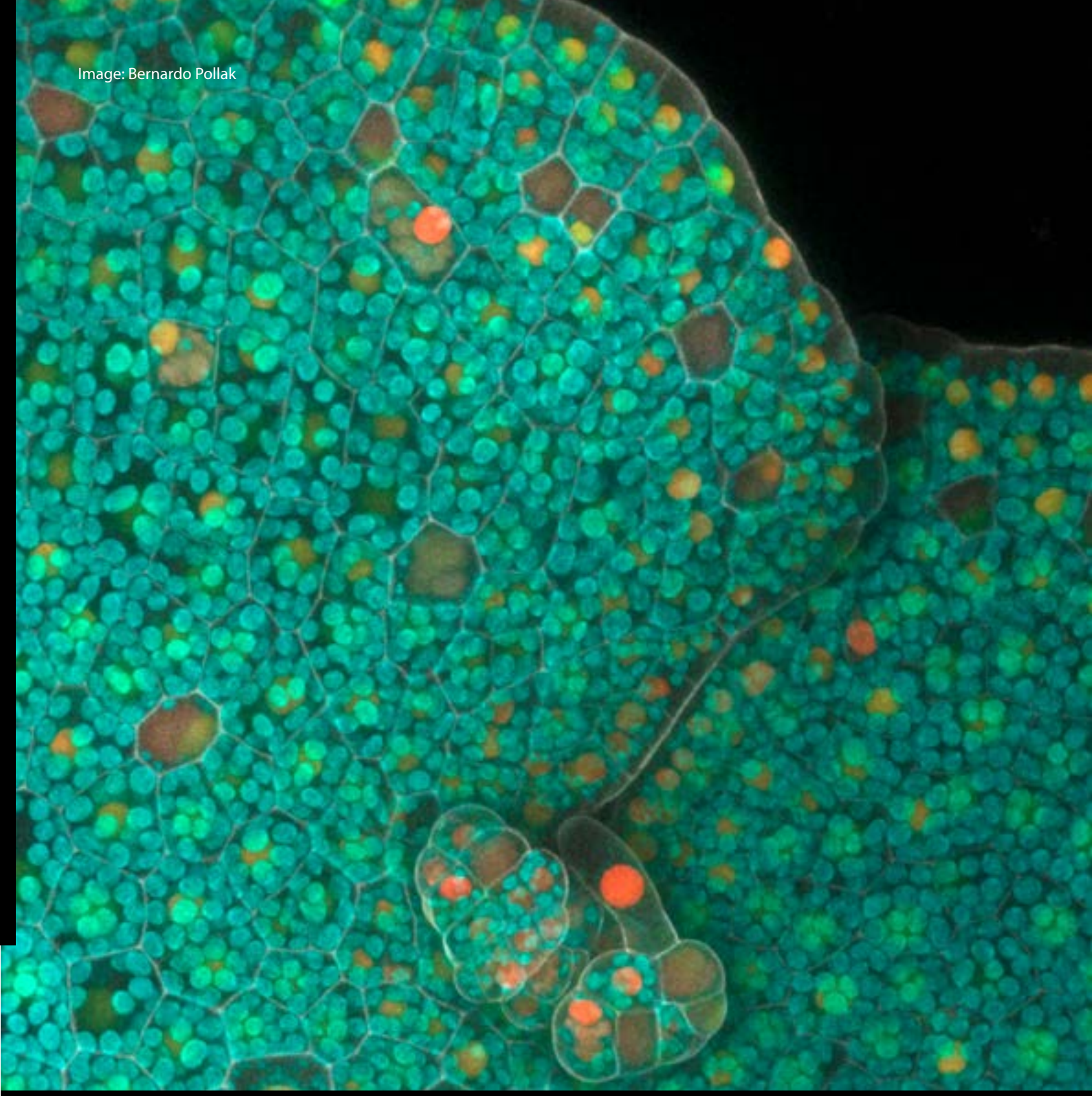


Image: Bernardo Pollak

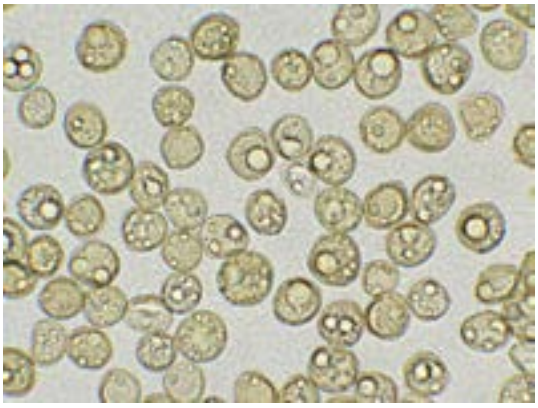
Marchantia polymorpha

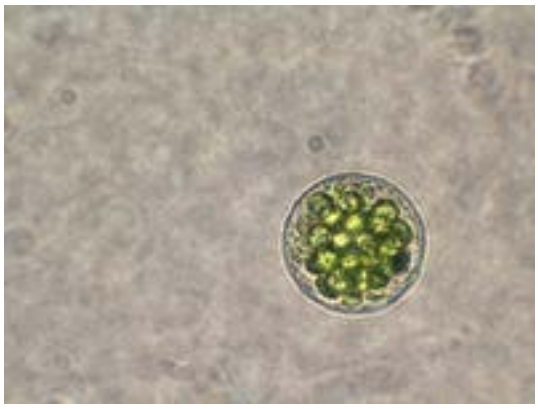
- Simple to culture and propagate
- Easy to transform and regenerate
- 280MB genome with reduced gene redundancy
- Efficient Cas9 mediated gene editing
- Haploid genetics
- Male and female gamete bearing structures induced by far-red light
- Single sexual cross results in millions of progeny spores
- Spores germnate and subsequent development is entirely exposed to observation
- Plants spontaneously produce clonal propagules (gemmae)
- Growing sporelings and gemmae can be observed directly using quantitative microscopy
- Marchantia contains the genetic machinery found in higher plants
- Best plant system for fundamental bioengineering work

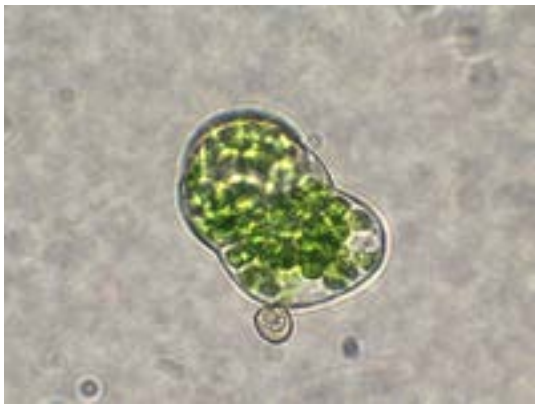
Left: Spontaneous production of clonal propagules (gemmae) during vegetative growth of *Marchantia polymorpha*

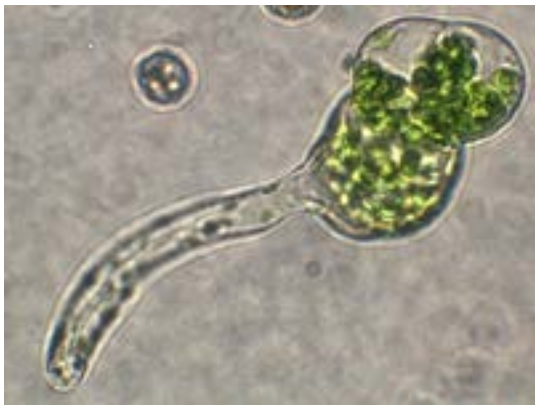
Right: Spore germination and exposed form of development in *Marchantia polymorpha*

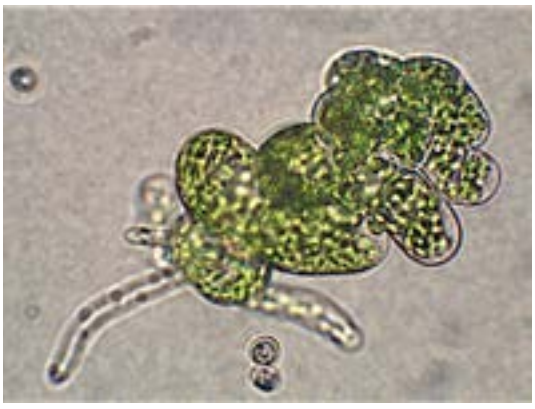
Images: Jim Haseloff

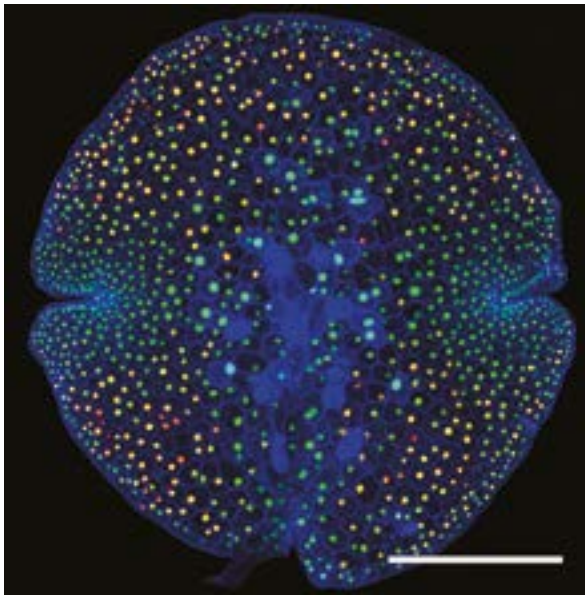












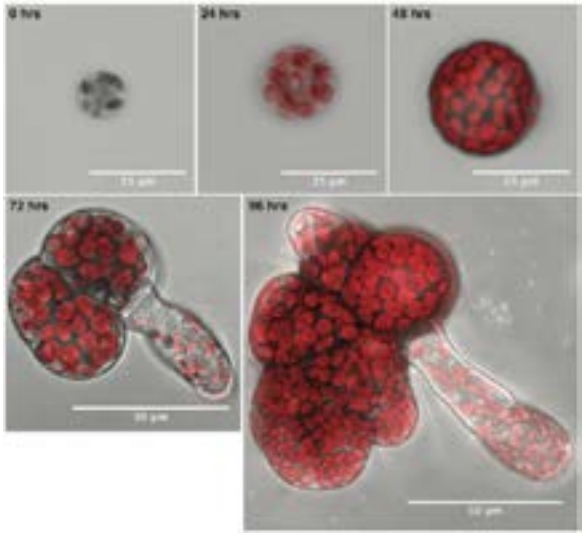
DNA PARTS

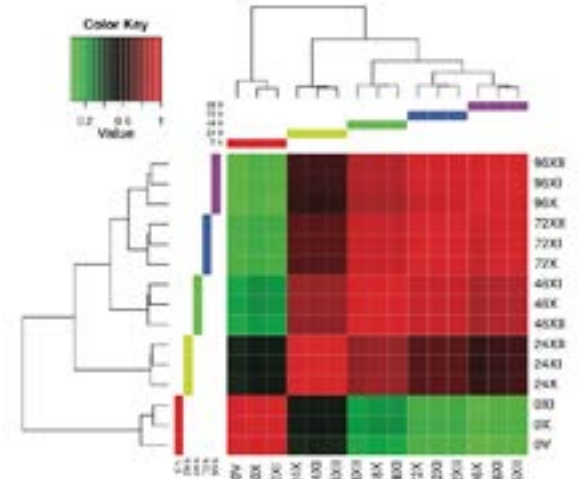
Testing synthetic promoters in Marchantia

CAMBRIDGE

Around 400 synthetic core promoters have been designed. Key genes, mainly regulatory genes and key effector genes have been identified in Marchantia. Sequences upstream of each gene, including the presumptive leader sequence, and running to the ATG start codon have been extracted. Candidate proximal promoter domains have been domesticated, cured of BsaI and SapI sites, flanked by convergent BsaI sites, and sent for DNA synthesis and cloned into a universal Level 0 plasmid vector. This pUAP1 vector was constructed by Nicola Patron, and is custom built for sharing DNA parts used in many types of Type IIS assembly procedures. Bernardo Pollak has constructed plant transformation vectors with multi-spectral fluorescent protein outputs, for ratiometric characterisation of gene expression. This allows direct observation of patterns of gene expression in transformed Marchantia tissues, as shown above for a young gemma.

Principal contacts: Susana Sauret-Gueto & Bernardo Pollak





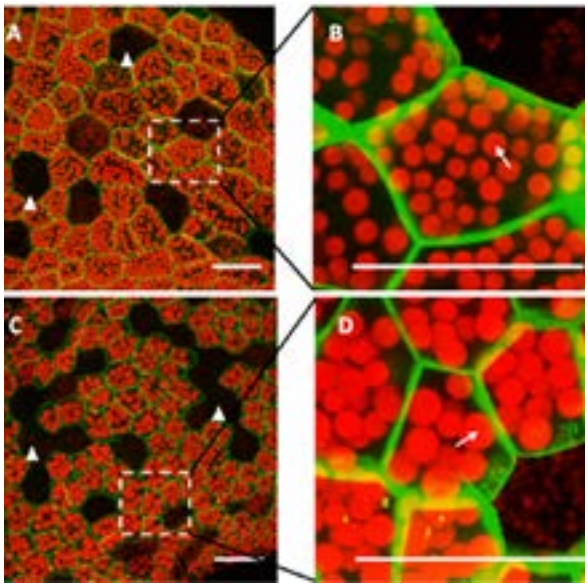
GENE EXPRESSION

Gene expression in germinating sporelings

CAMBRIDGE

Marchantia spores can be harvested and germinated in synchronised fashion. Germination is accompanied by rapid expansion, differentiation of chloroplasts, the first cell divisions, formation of a pronounced apical-basal axis and continued growth and specialisation. Samples can be collected across this period, RNAs extracted, and analysed by high-throughput RNA sequencing to obtain a map of shifting patterns of transcription during these initial phases of plant development. The transcriptome data has been used to investigate genes involved in early chloroplast differentiation and division.

Principal contact: Bernardo Pollak



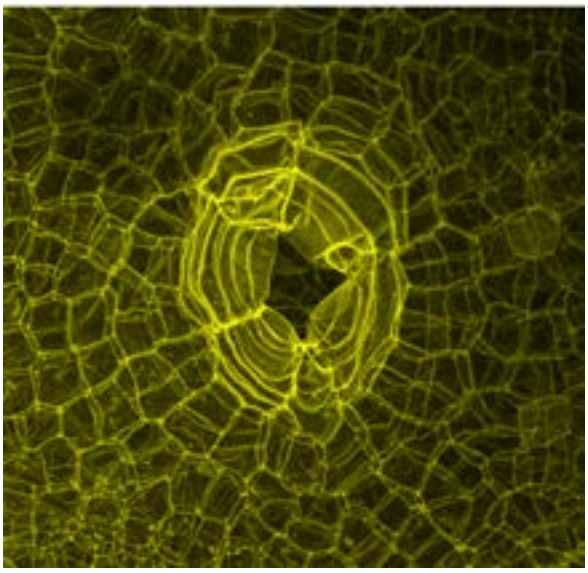
CHLOROPLASTS

Giant chloroplasts in Marchantia

CAMBRIDGE

Cas9 can be codon-modified for efficient use in Marchantia and other plants. Marchantia plants tolerate presence of the gene, and transgenic lines can be maintained, where simple delivery of a suitable guide sequence will tigger genome modification events, after Cas9-mediated cleavage of the genome. This system has been established by Bernardo Pollak. Owen Male used the sytem to target the homologues of genes known to be important for chloroplast division in higher plants. Gene knockouts produced abberant, oversized chloroplasts in the targeted lines - see the images above taken with Susana Sauret-Gueto. Eftychis Fragedakis is now targeting a wider set of genes to determine if the chloroplast numbers per cell can be further reduced, as found in Arabidopsis mutants. These manipulations may be useful for improved chloroplast transformation and homoplasty techniques.

Principal contact: Eftychis Fragedakis



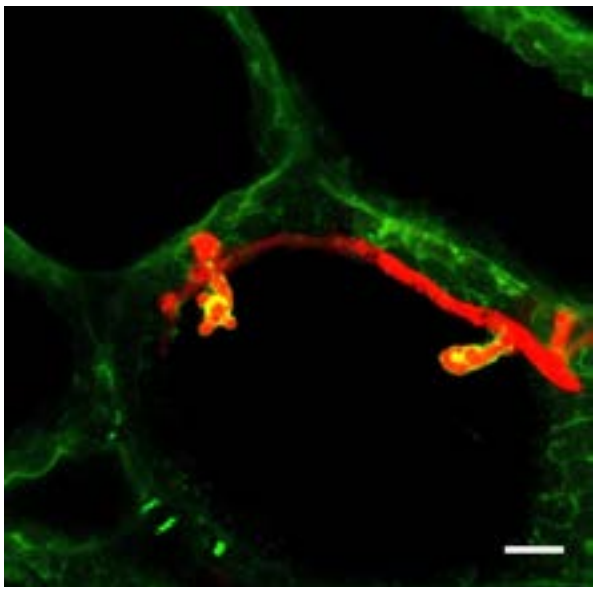
TISSUE ARCHITECTURE

Reconstruction of Marchantia air chambers

CAMBRIDGE

As Marchantia grows, cell division at growing points, or meristems, produces tissues that undergo self-organisation via additional cell divisions and differentiation events, to form air chambers. These chambers are comprised of cellular "floors", "walls", "roof" and air pore. The air chambers are packed with specialised cell filaments, that consist of highly photosynthetically active cells. The air chambers form uninterrupted arrays on the top surface of the plant, and are likely a relic of an early attempt to adapt to gas exchange and photosynthesis in a terrestrial environment. Marta Tomaselli has been applying optical clearing and image reconstruction techniques to analyse these cell complexes, including 3D printing of cellular features

Principal contact: Marta Tomaselli



PLANT SYSTEMS

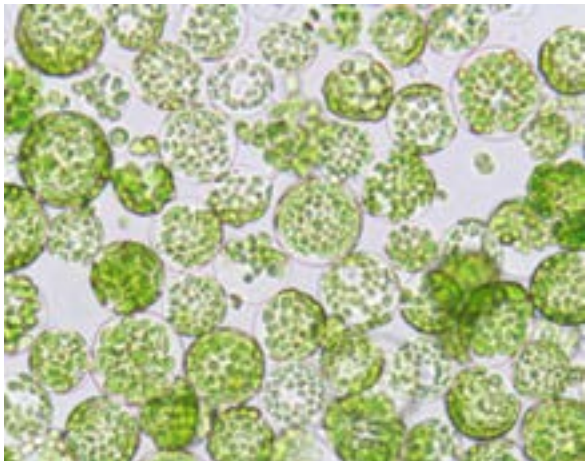
Host-pathogen interactions in lower plants

CAMBRIDGE

Numerous studies describe interactions between symbiotic microbes and early land plants like Marchantia, yet our understanding of how pathogens manipulate these plants is poorly understood. To address this, we have recently established a robust pathosystem between the filamentous oomycete pathogen Phytophthora palmivora and the model liverwort Marchantia polymorpha. We discovered that P. palmivora colonizes air chambers of the dorsal photosynthetic layer of liverworts to establish disease. Moreover, our work has revealed that P. palmivora forms intracellular structures within M. polymorpha cells. The plant recognises these structures and deploys host cellular trafficking machinery proteins. This work suggests that the formation of microbial structures in plants is evolutionary conserved and successfully exploited by pathogens. Our work lays the foundation for the identification of Marchantia pathogen-responsive promoter elements and the identification of non-vascular plant specific pathogen mechanisms.

Image: Intracellular structures formed by Phytophthora (red) inside Marchantia cells and labelled with a Marchantia protein (green/yellow) Size bar: 10 micrometer. Picture by P. Carella

Principal Contacts: Philip Carella & Sebastian Schornack



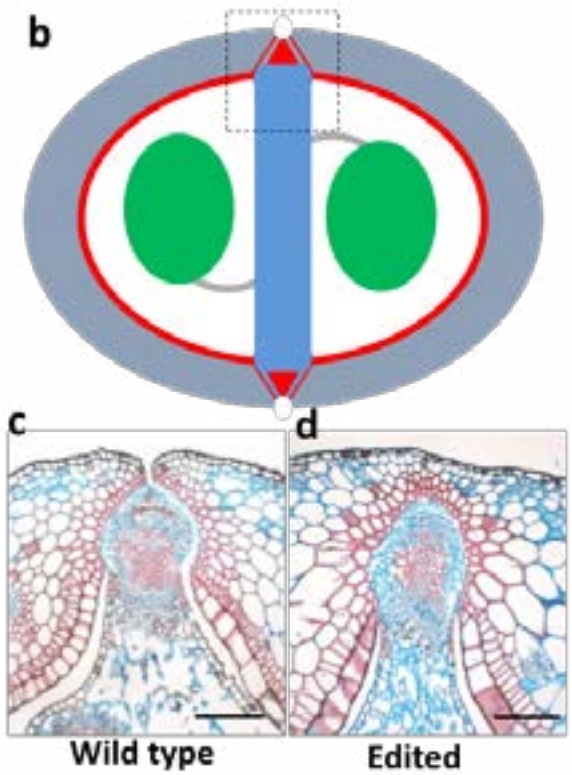
GENE EDITING

DNA-free genome engineering

JOHN INNES CENTRE & EARLHAM

Direct delivery of programmable nucleases such as Cas9 in a complex with the guide RNA, known as the ribonuclease (RNP) complex, avoids the introduction of DNA into the cell. Oleg Raitskin and Aytug Tuncel have developed protocols for the production of the ribonuclease complex and delivery to protoplasts of tobacco and potato, demonstrating DNA-free targeted mutagenesis. Regeneration of potatoes with mutations in the target genes is underway.

Principal contacts: Oleg Raitskin, Aytug Tuncel, Nicola Patron & Alison M. Smith



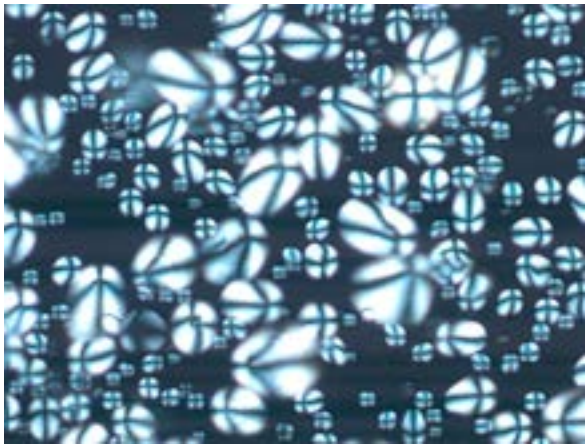
GENE EDITING

Targeted gene knock-out in crops using CRISPR/Cas9

JOHN INNES CENTRE & EARLHAM

Genome editing using CRISPR /Cas9 allows us to introduce small mutations in specific target genes to knock-out their function. The mutant plants produced are extremely valuable for determining the function of the target genes. Such gene-edited plants may also have useful characteristics that could be incorporated into improved crops. We have used this technology successfully in wheat, barley, Brassica oleracea, potato and tomato. Genome editing in crops using CRISPR / Cas9 is now being offered as a resource to the research community. This activity is funded by BBSRC through the Bioinformatics and Biological Resources (BBR) fund.

Principal contact: Wendy Harwood



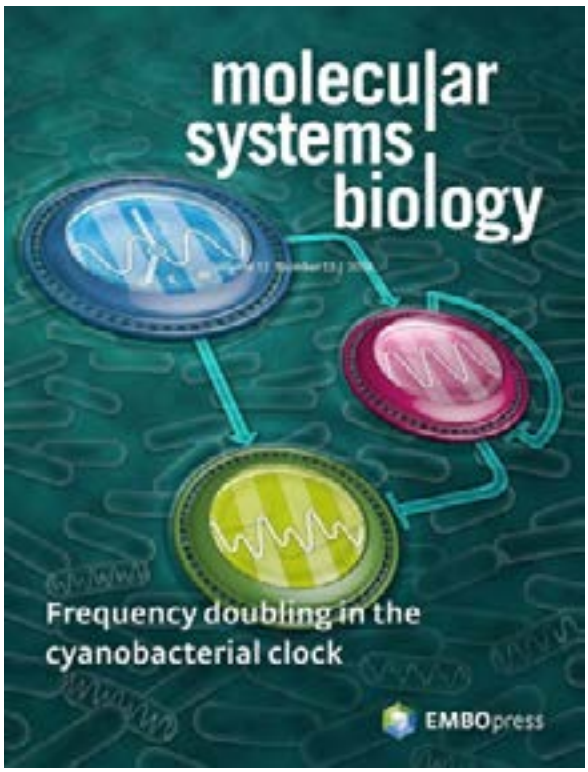
TISSUE ARCHITECTURE

Potatoes with altered starch properties

JOHN INNES CENTRE & JAMES HUTTON INSTITUTE

We are growing genome-edited potato plants expected to have altered starch polymer structures and hence improved nutritional quality. The novel starch granules in the tubers will be resistant to digestion, so when used as food the tubers will provide more dietary fibre and less elevation of blood glucose than normal potatoes. The plants were regenerated from protoplasts transformed with CRISPR-Cas9 constructs that have mutated the starch branching enzyme genes without integration of foreign DNA.

Principal contact: Alison Smith



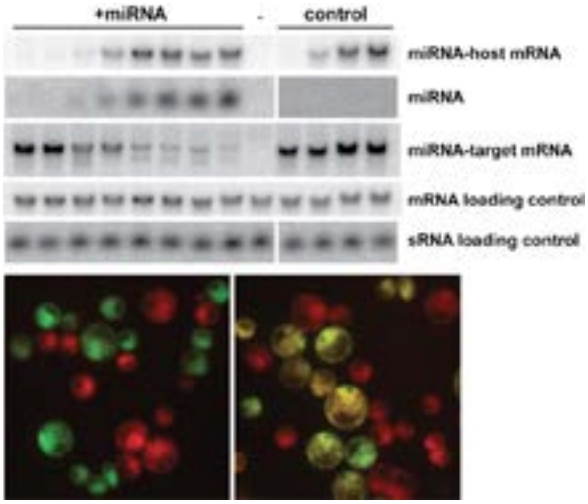
DNA CIRCUITS

Re-wiring frequency in cyanobacterial circuits

CAMBRIDGE

Rational design of oscillators is a goal of synthetic biology, but natural systems are already endowed with reliable oscillators in the form of circadian (24 hour) clocks. Understanding how to harness clocks to generate specific (non-circadian) frequencies, and how to systematically integrate clocks with other pathways will give us powerful tools, enabling the assembly of complex and dynamic synthetic circuits.
In the first stage of the project, we used single-cell time-lapse microscopy and mathematical modelling to study the coupling of the circadian clock to a circuit that controls expression of the key photosynthesis gene psbAI in the cyanobacterium S. elongatus. We observed frequency doubling in the expression of psbAI, i.e., it peaks twice a day with a period of 12 hours rather than 24 hours. We also observed two peaks in single-cell growth rates, suggesting frequency doubling can affect the global state of the cell. Using an iteration of theory and experiment, we determined the network design principles underlying the dynamics of frequency doubling (Martins et al. MSB, 2016). We are now perturbing this network to generate different frequencies, as predicted by our mathematical models.

Principal contact: James Locke



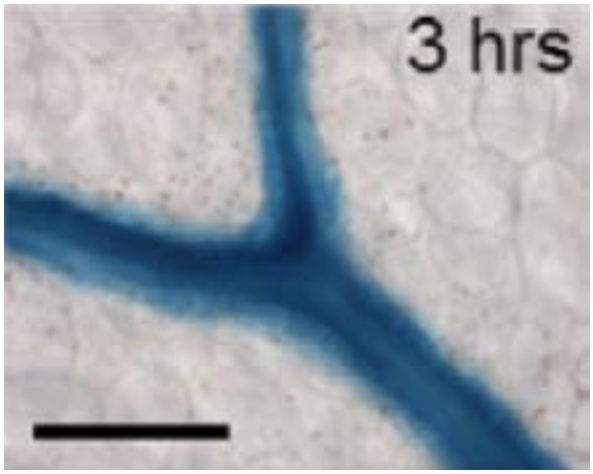
DNA CIRCUITS

Modulating gene expression in green alga by synthetic miRNAs

CAMBRIDGE

Since their discovery, miRNAs have been revealed as a promising tool for the efficient and specific modulation of gene expression, with applications ranging from human health to biotechnology. Our synthetic biology approach to characterise miRNA-mediated gene silencing in the green alga Chlamydomonas has rendered a better understanding of their action, and consequently, more control over their output. Gene silencing is followed by reporters and can be studied at single cell and population level. In addition, the standardization of DNA parts for gene expression has made possible the exchange of tools within the algal community. This work has established the basis for further engineering of gene expression in plants using miRNAs.

Principal contact: Francisco J Navarro



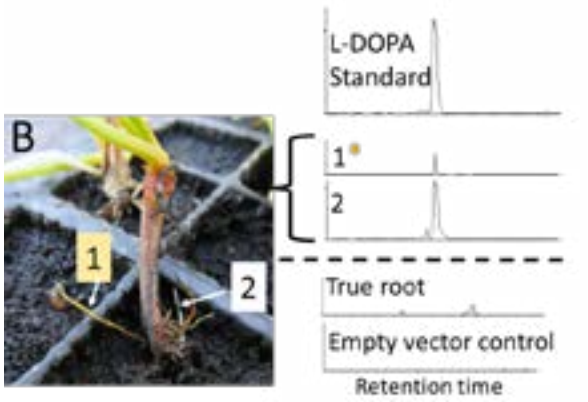
PHOTOSYNTHESIS

A synthetic module for expression in specific leaf cells

CAMBRIDGE

A unifying aspect of multicellularity is the spatial patterning of gene expression associated with different cell-types. Here we report the first DNA part that can be used to direct gene expression to specific cells of leaves. By combining transcription factor analysis with truncation and oligomerisation analysis, we identified a short tuneable fragment that can be used to control gene expression in bundle sheath cells of leaves.

Principal contact: Julian M Hibberd



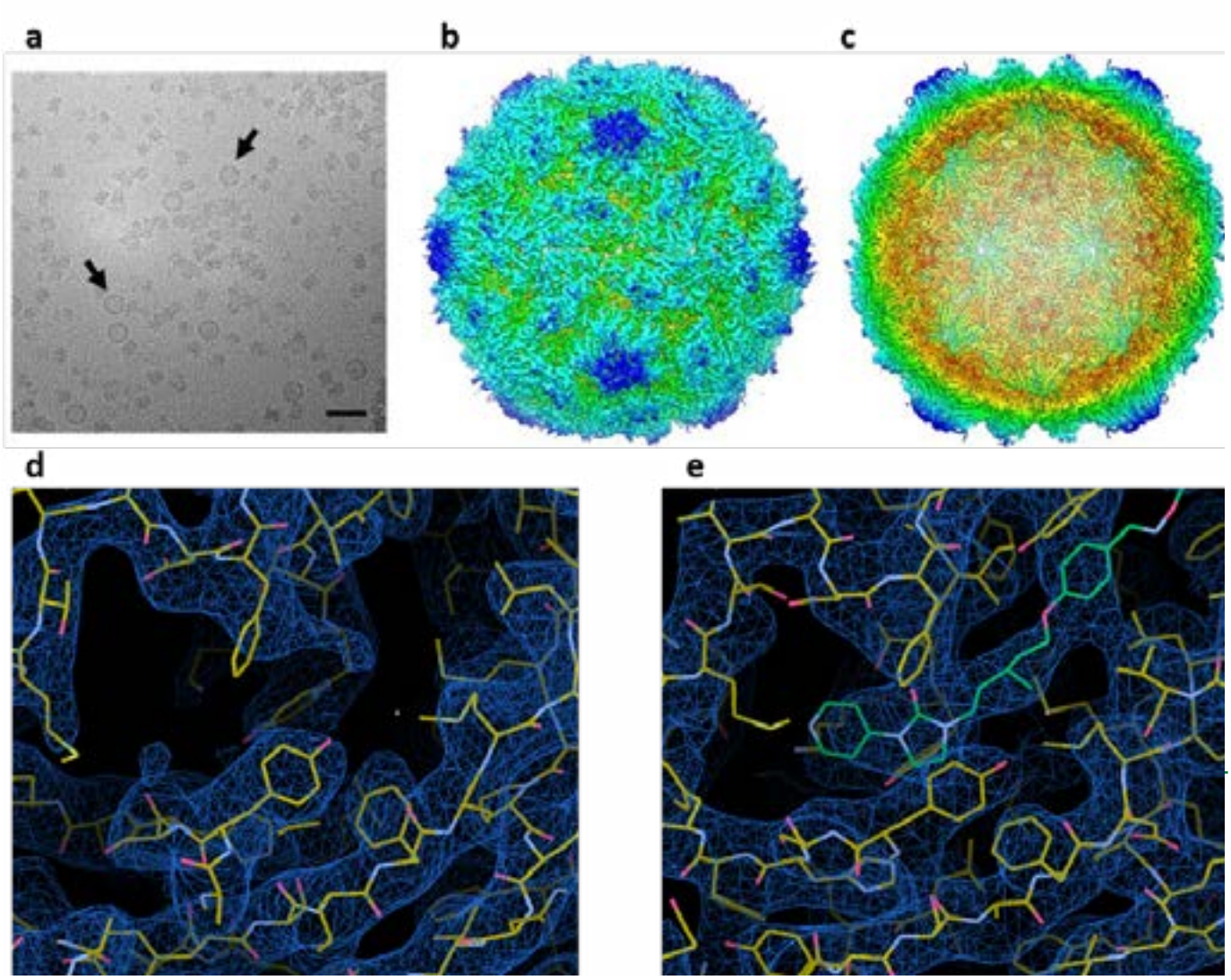
BIOPRODUCTION

Using beetroot for local production of L-DOPA

JOHN INNES CENTRE, SESVANDERHAAVE, & FONDAZIONE EDMUND MACH DI SAN MICHELE ALL'ADIGE

We have determined that beetroot can be developed as an effective production system for L-DOPA by gene editing to mutagenise the gene encoding DODA in beetroot in hairy roots. To translate these foundational experiments we have generated a mutagenized M2 population of YrYr/rr/blbl beet (yellow) by EMS mutagenesis. DODA mutants will be screened by looking for white seedlings/roots in the M2.

Principal contact: Cathie Martin



BIOPRODUCTION

Production of synthetic poliovirus particles in plants

JOHN INNES CENTRE, LEEDS, OXFORD, NIBSC

The Hypertrans® system, developed by Professor George Lomonossoff and Dr Frank Sainsbury at the John Innes Centre, has been extensively used for the rapid transient expression of proteins in plants. As part of a World Health Organisation (WHO) – funded collaboration, we have used this system to produce synthetic non-infectious poliovirus particles for use in the WHO worldwide polio eradication campaign. The synthetic particles consist only of the protein shell and lack the virus nucleic acid; however, to ensure they are stable enough to act as an effective vaccine, it was necessary to first identify mutations in the protein which stabilised the particles. The stabilised proteins were able to form particles in plants that have a structure equivalent to that of natural poliovirus. These particles were able to protect mice against poliovirus and thus have the potential to act as an effective vaccine.

Principal contact: George Lomonossoff

METABOLISM

Genome mining with plantiSMASH

JOHN INNES CENTRE-WAGENINGEN UNIVERSITY

The diversity of plant natural products is a source of great potential for industrial, agricultural and medical application. There is much to be gained from harnessing this diversity and engineering biosynthesis of high value chemicals into alternative chassis for rapid and sustainable production, discovery of novel chemicals, and synthesis of new-to-nature molecules. Advances in genome sequencing and the discovery of plant biosynthetic gene clusters have opened the doorway for systematic mining of plant genomes for new enzymes and pathways (Nützmann et al., 2016).

A collaboration between the Osbourn and Medema labs (Wageningen) has led to the development and optimisation of a customised algorithm for mining plant genomes for biosynthetic gene clusters, called plantiSMASH. plantiSMASH is a versatile online analysis platform to automate the discovery of gene clusters and biosynthesis pathways in plants (Kautsar et al. 2017). The plantiSMASH web server, precalculated results and source code are openly available at http://plantismash.secondarymetabolites.org

Principal contact: Anne Osbourn

BIOPRODUCTION

Plants as Bio-factories

JOHN INNES CENTRE

The Hypertrans® system, developed by Professor George Lomonossoff and Dr Frank Sainsbury at the John Innes Centre, has established a unique position for the UK for rapid transient expression of proteins in plants. The technology is extremely powerful and was used under licence by the Canadian company Medicago to produce 10m effective doses of H1N1 (swine flu) VLP Vaccine in just 30 days, meeting the US Defense Advanced Research Projects Agency test requirements for control of emerging diseases.

Traditional methods for vaccine production using chicken embryos take 6-9 months, and limitations in egg supplies can create bottlenecks. In contrast, plant-based production is much faster and can be rapidly up-scaled. Hypertrans® technology has also been used for expression of biosynthetic pathways, production of antibodies and antigens, and human gastric lipase enzyme for use in a model gut system, and to solve the structure of particles to near atomic resolution when no parent virus particle is available. The system has huge potential for nanotechnology and nanomedicine applications with virus-like particles delivering cargo to cells (Brillault et al., 2017) or playing a role in diagnostic and biosensor design (Lebedev et al., 2016).

When coupled with construct design tools and modular "build" techniques, the Hypertrans® system stands out as a rapid testing platform and a valuable teaching and training tool, as was demonstrated during the OpenPlant ERASynBio plant synthetic biology summer school for early career researchers in 2014. Participants were able to move through the entire design-build-test cycle, learning a multitude of technical skills, in just one week.

Principal contact: George Lomonossoff

BIOPRODUCTION

Production pipeline for small molecules

JOHN INNES CENTRE

Plants have long been recognised as a rich source of biologically active small organic molecules, and many commonly prescribed drugs are natural products or directly derived analogues. The triterpenes represent one of the most diverse families of plant natural products. However, the lack of easy access to these compounds via synthetic chemistry has hindered their exploration as potential leads for drug development. Through the utilisation of transient expression, the Osbourn lab have developed a plant based platform for the preparative production of triterpenes. The utility of this system has been demonstrated through the facile gram-scale production of the archetypal pentacyclic triterpene β-amyrin, and via the generation of novel analogues. This has afforded the opportunity to probe the structure activity relationships of biologically active β-amyrin derivatives (Reed et al. 2017). Advances in bioinformatics and the growing wealth of plant genomic data is likely to rapidly broaden the scope of potential enzymes which could be exploited through this system to access even greater chemical diversity. Such advances have the potential to reinvigorate drug discovery pipelines.

Principal contact: Anne Osbourn

BIOPRODUCTION

Scale-up for application at Leaf Systems®

NORWICH RESEARCH PARK

In January 2017, a purpose-built facility for plant production opened on the Norwich Research Park. Leaf Systems® International Ltd was designed for the scale-up of proteins, metabolites and complex natural products for research, clinical trials and bio-medical applications using the Hypertrans® expression platform. The new facility contains state of the art containment facilities to produce and engineer its feedstock plants, and incorporates industry standard downstream processing to ensure production quality and bio-security. The new facility is currently being used to scale up production of a number of test molecules.

Synthetic Biology meets the real world

OpenPlant funds interdisciplinary team-based projects that explore the intersection of electronics, 3D printing, sensor technology, low cost DIY instrumentation and biology - and policy workshops and outreach events. These projects aim to build open technologies and promote development of research skills and collaborations. They tap into existing open standards and a rich ecosystem of resources for microcontrollers, first established to simplify programming and physical computing for designers, artists and scientists. These resources provide a simple environment for biologists to learn programming and hardware skills, and develop real-world laboratory tools. Further, the OpenPlant projects provide a direct route for physical scientists and engineers to get hands-on experience with biological systems, and we are developing low-cost open reagents and protocols for easier access to cell-free DNA programmable systems.

Enabling the innovators

History
Since 2014, we have funded small interdisciplinary projects and catalysed new collaborations between several hundred students, researchers and academics across Cambridge, Norwich and beyond. A listing of recent OpenPlant projects is provided here. A more comprehensive collection of information can be found at www.biomaker.org. The projects have generated a large number of electronic prototypes, software, 3D printed devices and biological elements. We hope that these resources prove useful and can be built upon by others, especially to initiate new low-cost approaches to quantitative biology and engineering for teaching and research.

SynBio Strategic Research Initiative Fund
The University of Cambridge SynBio Fund supported eighteen innovative, open and interdisciplinary projects relevant to Synthetic Biology over 2015-16. The aim of the fund was to promote the development of Synthetic Biology as an interdisciplinary field at the University of Cambridge.
(www.synbio.cam.ac.uk)

OpenPlant Fund mini-projects
OpenPlant Fund aims to promote the development of plant Synthetic Biology as an interdisciplinary field and to facilitate exchange between The University of Cambridge, the John Innes Centre and the Earlham Institute for the development of innovative and open projects relevant to plant Synthetic Biology, and responsible innovation and outreach in this context. The projects receive £4000 over six months, with an additional £1000 for outreach or follow-on work after reporting on their progress. Funds are managed through a cost centre managed by a faculty sponsor, to help manage integration of the project with existing research loads. All outputs of the projects are open and shared.
(www.openplant.org/fund)

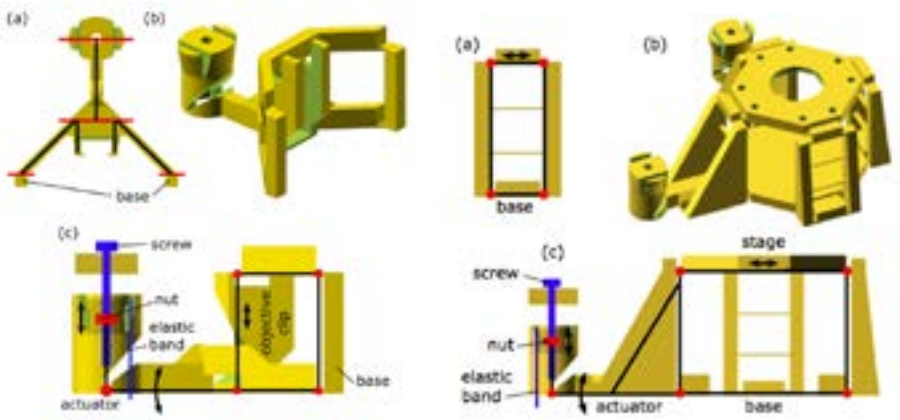
Biomaker Challenge micro-projects
Starting in Summer 2017, the Biomaker Challenge is a four-month programme challenging interdisciplinary teams to build low-cost sensors and instruments for biology. From colorimeters to microfluidics and beyond, we're looking for frugal, open source and DIY approaches to biological experiments. Participants receive a £250 Biomaker Starter Kit and a discretionary budget for additional sensors, components, consumables and 3D-printing worth up to an additional £750. Up to 50 grants will be awarded and all teams will exhibit their device at a public Open Technology event and Biomaker Fayre in October.

The Biomaker Challenge leverages additional support from the University of Cambridge Research Policy Committee through the Synthetic Biology Strategic Research Initiative and CamBridgeSens Strategic Research Network. We are actively promoting wide participation both within Cambridge and Norwich, and with external partners - including international collaborations with individuals, companies and institutions. In particular, the new Biomaker Challenge has been designed to be easily portable between institutions and open to industrial collaboration. (www.biomaker.org)

Cell-free gene expression
We are encouraging applications related to use of cell-free extracts to transcribe and translate engineered DNA. Cell-free synthetic biology is gaining popularity for prototyping genetic circuits and metabolic pathways and has many applications from production of biologics to paper-based diagnostic tests and biosensors.

Innovative projects
OpenPlant funding has proved to be a highly effective way of providing key support for independent small projects and promoting valuable new collaborations among young researchers, along with the development and documentation of open source biology, hardware and bioinstrumentation. In a short period of time, we have seen some notable outcomes. For example, our funds have provided seed money for the evolutionary development of 3D printed microscopes across several projects: "Open source 3D-printed microscope", Richard Bowman, Stefanie Reichelt, Hugh Matthews & Jeremy Baumberg, £5K; "High Performance Mechanisms for Low Cost Science", Richard Bowman, Stefanie Reichelt, Hugh Matthews, £5K; "OpenScope", Cambridge-JIC iGEM2015 team, £10K; and "OpenScope", SynBio Student Society, £5K. These early projects promoted experimentation with a novel, clever and open design. This has subsequently mutated into a family of 3D printed microscopes, optical devices and accessories - and found global use in community labs, schools, social enterprises and research labs.

1



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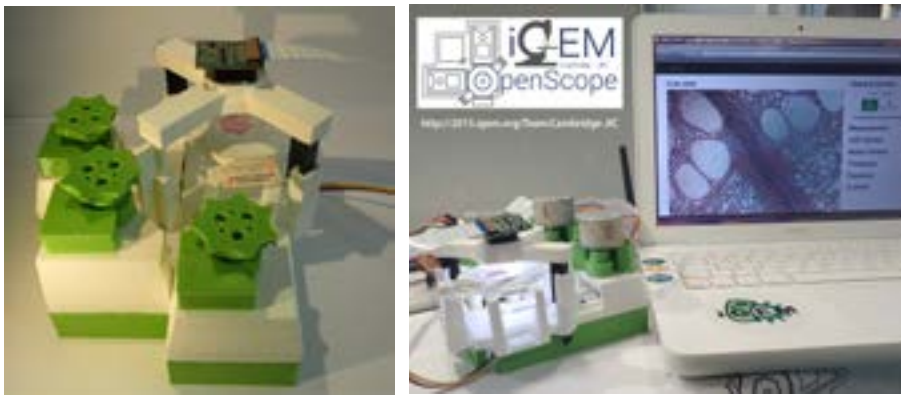

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An example: evolution and spread of open technology

OpenFlexure 3D printed microscope

1. Richard Bowman and colleagues in Cambridge develop a design for a monolithic, 3D printed microscope stage, based on a novel plastic flexure translation stage. This design and its implementation is later published as: *A one-piece 3D printed flexure translation stage for open-source microscopy* James P. Sharkey, Darryl C. W. Foo, Alexandre Kabla, Jeremy J. Baumberg, and Richard W. Bowman. **Review of Scientific Instruments** 87, 025104 (2016); http://doi.org/10.1063/1.4941068

2. The stage design is complemented by low cost Raspberry Pi board and Pi camera with inverted lens to build low cost inverted video microscopes, in the UK and beyond, eg. Public Labs in the US (https://publiclab.org/notes/mathew/04-17-2016/making-an-openflexure-microscope).

3. The Cambridge-JIC iGEM2015 team builds an upright version of the microscope in consulation with Richard. They add motorised focus and translation, and developa software package for remote image collection and data processing (http://2015.igem.org/Team:Cambridge-JIC)

4. Richard Bowman, Alex Patto and colleagues develop an award-winning social enterprise, WaterScope, around use of the low-cost microscope for rapid automated screening of mini-colonies from water-bourne bacterial contamination. The microscope technology is made freely available to all. Microscopes are now being printed in Africa (http://www.waterscope.org).

5. Richard Bowman continues to develop the microscope optics, producing versions that incorporate low-cost, high-performance objectives and cheap tube lens.

Versions of the OpenFlexure microscope are being built and modified worldwide. The stage design is modular and has been used for fibre optic alignment, and integrated into other microcope design.

The current version can be found at https://github.com/rwb27/openflexure_microscope.

OpenPlant Fund: Hardware projects

Wireless, portable, low cost, open source hardware for monitoring plant electrophysiology

Dr **Pakpoom Subsoontorn** (Plant Science, University of Cambridge), **Sakonwan Kuhaudomlarp** (JIC), **Dr Kyle Lopin** (Naresuan University, Thailand), **Dr Settha Tangkawanit** (Naresuan University, Thailand)

Plant electrical signalling regulates a wide range of physiological functions including stress responses to drought and wounding. Existing tools for monitoring such signals often require the uses of cumbersome and expensive equipment in well-controlled laboratory. We aim to create a low-cost measurement tools that can function robustly in the field, collecting electrical activity profiles from multiple plants. We have developed a tool prototype for measuring plant electrical signal coupled with radio modules for long-distance data collection. This prototype (estimated cost £40) can sense and transmit signals from Venus flytrap responding to tactile inputs (see this video for demonstration). The tool can distinguish the action potential from other disturbances.

Whiskeroscope: rodent whisker inspired sensor for use in analysis of plant tissue structure

Jan Lyczakowski (Department of Biochemistry, University of Cambridge), **Abhimanyu Singh** (Independent, previously Department of Engineering, University of Cambridge). **Christie Nel** (Independent, previously Stellenbosch University)

Understanding mechanical properties of plant biomass is crucial for multiple industries, e.g. building construction and production of lignocellulosic biofuels. Current methods to analyse mechanical properties of biomass are slow and provide little accuracy. We have developed a novel sensor to evaluate stiffness of plant stems. The device is inspired by rodent whiskers and relies on two inputs, obtained using thin steel rod, to quantify stiffness. The instrument successfully discriminated between materials with unlike mechanical properties (steel and foam) and differently aged stem samples from willow. Whiskeroscope was also applied to study Arabidopsis thaliana stems with altered composition of cell walls.

Open Labware for plant electrophysiology

Dr Carlos A. Lugo (EBI, previously The Sainsbury Laboratory), **Dr Marco Aita** (Sainsbury Laboratory, University of Cambridge), **Christian R. Boehm** (Department of Plant Sciences, University of Cambridge), **Guru Vighnesh Radhakrishnan** (John Innes Centre), **Dr Marielle Vigouroux**, (John Innes Centre)

In order to investigate electrical responses to mechanical and other external stimuli, our project consisted of replicating an open source Arduino shield which receives, amplifies and transmits "ECG"s from plant tissues into a computer or other circuits. We harnessed the electrical signals to trigger responses in a) other plants, b) other circuits. The resultant board's schematics and other experimental tools such as manipulators and signal transducers are published on a dedicated project page including files for producing boards and 3D printed parts. A number of kits are available to give away to schools and labs interested in the system.

Building a low-cost desktop plant experiment box

Dr Marco Aita (Sainsbury Laboratory, University of Cambridge), **Dr Marielle Vigouroux** (John Innes Centre), **Dr Carlos Lugo** (EBI)

Doing experiments in plant biology is a difficult task. Experimental conditions are difficult to control and often the impact of even slight variations of environmental conditions is difficult to predict. Commercial solutions to control the environment are available but quite expensive and normally are optimised for plant growth but not for running experiments. We want to build small independent "experimental boxes" which are optimised for in-vivo recording of single plant/single plate growth under different environmental conditions and subject to different stimuli. The boxes will be small in size (around 50x50x60 cm), cheap (estimate material cost <£1000 each) and flexible in features thanks to a modular design. The boxes will be under PC control and allow multiple experiment to run in parallel and in sync.

Environmental sensor networks based on plant electrical signalling

Sakonwan Kuhaudomlarp (John Innes Centre), **Dr Pakpoom Subsoontorn** (Department of Plant Sciences, University of Cambridge), **Dr Kyle Lopin** (Naresuan University, Thailand), **Dr Settha Tangkawanit** (Naresuan University, Thailand)

Tools for sensing and recording plant electrical signals could open up promising applications in agriculture and environmental engineering. Nonetheless, existing setups for monitoring plant electrophysiology often require the uses of cumbersome, expensive and specialised equipment and one would prefer to have a network of low-cost measurement tools that can function robustly in the field, capture overall electrical activities of multiple plants. Previously, our team have prototyped a plant electrical signal amplifier coupled with a radio module. Here we plan to improve upon our first prototypes, specifically, to expand detection bandwidth, to increase sampling rates, further reduce the cost and test device performance in wider range of plant species.

Plant electro-mechanics: improving low-cost plant electrophysiology for research and education

Dr Marco Aita (Sainsbury Laboratory, University of Cambridge), **Dr Marielle Vigouroux** (John Innes Centre), **Dr Carlos Lugo** (EBI), **Guru Vighnesh Radhakrishnan**, (John Innes Centre)

We prototyped a very low-cost plant electro-physiology sensor and would like to continue with further development of monitoring and data gathering capabilities of the shields, image analysis, signal long time monitoring. We will also couple the manipulators with a motor system web-application which can be used from desktop or mobile devices. All outputs will be fully open source.

Establishing 3D Printed Microfluidics for Molecular Biology Workflows

Steven Burgess (Department of Plant Sciences, University of Cambridge), **Tom Meany** (Department of Plant Sciences, University of Cambridge), **Richard Bowman** (Department of Physics, University of Cambridge), **Oleg Raitskin** (Earlham Institute), **Neil Pearson**, (Earlham Institute)

With synthesis of DNA becoming cheaper, and plasmid construction automated, the testing of biological parts is becoming a bottleneck in the design-build-test cycle. Analysis of single cells offers a procedure for rapid screening of parts and this has been facilitated by advances in microfluidics. The downside of these approaches is that they tend to rely on expensive, specialist equipment, meaning they are out of reach to most molecular biology laboratories. However, developments in 3D printing, coupled to open-source design repositories, offer the potential to address this issue. By utilising expertise in Cambridge and the NBI (Norwich Biosciences Institutes), the aim of this proposal is to integrate available open-source or low-cost commercially available components, to produce a cheap, modular microfluidic setup for single cell analysis.

Universal precise large area colony scanning stage with measurement and selection tool integration

Tobias Wenzel (Department of Physics, University of Cambridge), **Luka Mustafa** (Institute IRNAS Race), **Ji Zhou** (Earlham Institute), **Nick Pullen** (John Innes Centre), **Neil Pearson** (Earlham Institute)

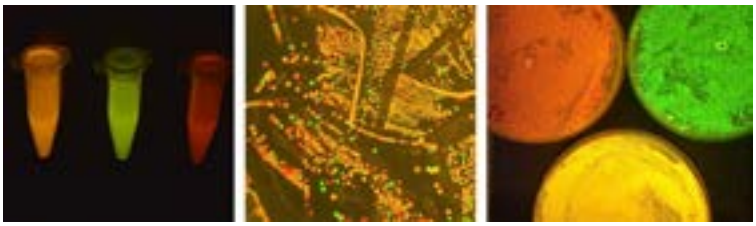
Plant or microbial cultivation and monitoring can be a time consuming and tedious process. We propose an open-source platform for automatic (flat – e.g. Marchantia) plant and colony scanning, which extends plate-reading-functionality to morphological and long-term analysis and will also be more flexible, considering growth plate size. This is an extension of the OpenScope initiative from the Cambridge 2015 iGEM team and will combine video and optical microscopy techniques with CNC technology. Outputs will include a more precise and more affordable CNC translation stag , technical interfaces that allow easy integration of open source measurement and preparation tools and new open source tool that allows to identify and pick or mark colonies and their positions. The set up will be tested on seed germination experiments and the hardware will be replicated in Norwich, Cambridge and Slovenia to lay a foundation for easy feedback and collaboration.

Development of an Open Source Autonomous Imaging Station for Distribution in High Schools, Universities, and Emerging DIY Scientific Communities

Fernán Federici (University of Cambridge/Universidad Catolica, Chile), **Neil Pearson** (Earlham Institute), **Tim Rudge** (Department of Engineering, Universidad Catolica, Chile), **Tim Marzullo** (Backyard Brains, Inc), **Juan Keymer**, (Universidad Catolica, Chile)

We propose to develop a standalone tool for imaging and analysing fluorescence in biological samples at a range of scales from individual bacteria, through colonies, plant cells and even whole organisms such as C. elegans. The system will be self contained and autonomous, including hardware and software for image capture, programmed sequences (e.g. timelapse), and quantitative analysis of samples. We also propose the development of a simple genetic toolkit for the production of fluorescent and pigmented bacteria complementing the device. The entire system, optics, frame, electronics, genetic resources and software will be open source. This robust and affordable package will enable independent, inexpensive experiments and observation for scientists in emerging scientific cultures in Latin America as well as in schools, colleges and universities.

This project also wishes to highlight the benefits of employing an open framework for academic collaborations that seek to deliver Open Access resources and information. We have formed an industry partnership with the Open Source company Backyard Brains (TM), which has significant experience in creating and distributing open educational and research technology for neuroscience in Latin America and worldwide (backyardbrains.com, backyardbrains.cl).



Light sheet microscopy of cell sheet folding in Volvox

Stephanie Hoehn (DAMPT, University of Cambridge), **Pierre Haas** (DAMPT, University of Cambridge), **Karen Lee** (JIC)

Light sheet fluorescence microscopy (LSFM) is the state-of-the-art technique to study developmental processes in vivo. LSFM causes less photo-damage than confocal microscopy enabling longer time-lapse recordings. We had previously built a LSFM setup in the Goldstein group. The purpose of this project is to improve the quality of the generated LSFM data.

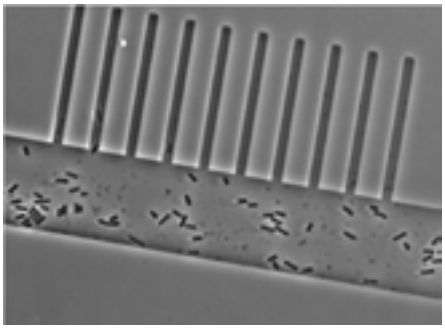
Optical sectioning is achieved by moving the sample through a light sheet and thereby creating z-stacks. In our previous setup images were recorded by a single camera. Due to light absorption and scattering the images of the sample half facing away from the camera showed a significant loss in image quality. In order to correct for this loss we have added a second camera and detection arm opposing the first one and covering the second half of the sample. This improved setup is doubling the thickness of a sample for which we can acquire useful fluorescence data. This significantly increases the variety of future applications including studies on the morphogenesis of entire embryos in the multicellular micro-alga Volvox and the development of feeding structures of the aquatic carnivorous plant Bladderwort.

Development of a Low-Cost Micro-Environment Device for Root-Nutrient Interaction

Tyler McCleery (JIC), **Ziyi Yu** (Chemistry, UCam), **Zhijun Meng** (Chemistry, UCam), **Veronica Grieneisen** (JIC)

Standard lab conditions for plant growth typically involve homogeneous nutrient conditions, but actual field conditions are rarely homogeneous. Interesting patterns in root architecture arise from heterogeneous conditions, or even dynamic conditions through time. Such patterning calls into question the underlying, likely non-linear processes among root cells that can generate diverse, plastic architecture. Indeed, understanding such phenomenon is critical for development of true synthetic plant systems. I propose development of a low-cost microfluidic device that can finely control rapid changes in the micro-

environment surrounding the root structure. A prototype of such a device could easily be tested with cut vinyl molds for PDMS rather than soft-lithography. The device would produce heterogeneous nutrient conditions along the root structure, either by laminar flow or gradient generation. The goal is to build a proof-of-concept device, and use it in conjunction with fluorescence imaging for a preliminary test of a well-documented growth response to heterogeneous nutrient conditions.



The Green Mother Machine Reloaded

Christian Schwall (Biochemistry, UCam), **Philipp Braeuninger-Weimer** (Engineering, UCam) , **Bruno Martins** (Sainsbury Laboratory, UCam), **Arijit Das** (Sainsbury Laboratory, UCam), **Chao Ye** (Sainsbury Laboratory, UCam), **Toby Livesey** (Biochemistry, UCam), **Antony Hall** (UEA)

In this project we wanted to build a microfluidic device which allows the observation of Synechococcus elongatus PCC 7942, a well-studied cyanobacterium, at the single cell level. We based our design on a well-established device called the mother machine and tailored it to the specific needs of Synechococcus elongatus. One of the biggest challenges in adapting the mother machine to Synechococcus elongatus is to keep the cells alive and to load the cells into the growth channels. Here we optimized the loading and survival of Synechococcus elongatus in the green mother machine by improving the loading protocol and the experimental setup. In addition, we tested various prototypes for the robust media switching between different media.

OpenPlant Fund: Biology projects

Developing novel selection markers for plant transformation to advance live-imaging techniques

Dr Katharina Schiessl (John Innes Centre), **Dr Fernán Federici** (University of Cambridge/Universidad Catolica, Chile), **Leonie Luginbuehl** (John Innes Centre), **Guru Rhadakrishnan** (John Innes Centre)

A total of 25 DNA parts were synthesised, including tissue specific promoters and coding sequences of fluorophores and chromophores. Level 1 and level 2 GOLDEN GATE plasmids were generated and transformed into Medicago Hairy roots. Subsequently, selection markers were tested to see if they were detectable under the stereomicroscope and images were taken using confocal microscopy. It was found that the nuclear-envelope localised fluorophore dtomato, expressed under the Lotus UBIQUITIN promoter, was detectable under the stereomicroscope and could therefore provide a novel selection marker for live imaging. Furthermore, it was found that the BEARSKIN promoter was not detectable in the lateral root cap but expressed at the base of the induced hairy root callus. No significant colour change was observed in the roots transformed with the chromoproteins.

Development of new codon optimisation tools and development of a synthetic gene expression system in the green alga Chlamydomonas reinhardtii

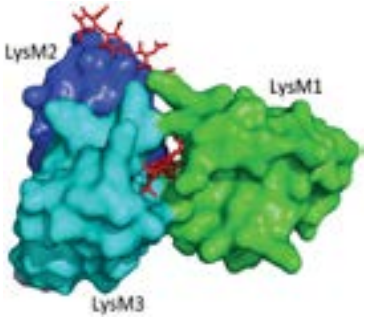
Francisco Navarro (Department of Plant Sciences, University of Cambridge), **Marielle Vigouroux** (John Innes Centre)

Most organisms share the same genetic code, based on three nucleotide codons that encode for one amino acid. However, synonymous codons (which specify a single amino acid) are not used at equal frequency by different species. We were interested in assessing the impact of codon usage in protein production in the green alga Chlamydomonas reinhardtii. We have performed sequence analysis, and developed a platform for measuring the production of a reporter protein, which can be used for testing gene variants. Our analysis, protocols, and materials will be useful for transgene design and expression in the alga.

The use of synthetic biology tools to define the roles of LysM receptor-like kinases in legumes and cereals

Feng Feng (John Innes Centre), **Ronelle Roth** (Department of Plant Sciences, University of Cambridge)

We have synthesised a number of golden gate modules including gene promoters, coding sequences and terminators and got the final constructs required for this project using Golden Gate cloning technology. Secondly, we have already expressed these constructs in Nicotiana benthamiana to check the protein expression, now we are focusing on transforming these constructs in Medicago and rice to detect defence and symbiosis phenotype.



Quick analytical system for plastid genome modifications

Mario Juhas (Department of Pathology, University of Cambridge)

We set out to provide the synthetic biology community with a quick Pulsed-Field Gel Electrophoresis (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 and the protocol has been published in: Juhas, M. and Ajioka, J.W., 2016. Integrative bacterial artificial chromosomes for DNA integration into the Bacillus subtilis chromosome. Journal of Microbiological Methods, 125, pp.1-7.

Channeling targeted DNA double strand breaks into alternative repair pathways

Dr Ian Henderson, **Dr Natasha Yelina**, **Patrick Diaz** (Department, of Plant Sciences, University of Cambridge), **Dr Sebastian Schornack** (The Sainsbury Laboratory, University of Cambridge), Meiogenix (Paris)

We have expressed TAL DNA binding domains fused to the FokI nuclease under meiotic promoters (e.g. DMC1, SPO11) in Arabidopsis. The aim of this work is to target DNA double strand breaks to specific sites in the genome, in order to bias initiation of meiotic recombination. Our preliminary data show that while these nucleases are expressed in meiotic-stage floral buds they do not support wild type levels of crossover recombination when the endogenous nuclease (SPO11-1) is mutated. Additionally these transgenic lines show occurrence of developmental phenotypes, leading us to the hypothesis that the resulting DSBs enter a mutagenic pathway. To investigate this in this project we are performing whole genome DNA sequencing and mutation discovery. This has been performed using support from the OpenPlant project and bioinformatic mutation discovery is ongoing. In parallel we have crossed these nuclease lines to mutants in canonical and alternative end joining pathways to test the hypothesis that we can shunt DSBs into crossover recombination via removing competing repair pathways. These lines will be grown and DNA sequencing repeated, in addition to phenotypic analysis in the next part of this project.

Engineering Marchantia polymorpha chloroplasts for the production of high-value specialised terpenes

Aymeric Leveau (John Innes Centre), **Tessa Moses** (John Innes Centre), **Christian R. Boehm** (Department of Plant Sciences, University of Cambridge)

Originally, three independent operon-like synthetic constructs should be built to achieve de novo synthesis of mono-, sesqui- and triterpenes in M. polymorpha chloroplasts. GoldenGate modules of coding sequences to be expressed in M. polymorpha were synthesized. However, two major issues were encountered during the project, including problems with transforming M. polymorpha chloroplasts with large constructs, and an assembly defect of the 2A peptide system used for generating the clusters. To circumvent these obstacles, constructs allowing nuclear transformation of M. polymorpha and subsequent chloroplast targeting of the proteins were designed and a new 2A peptide system has been created and is currently being evaluated.

Hot Tomato: Complementation of the Capsaicin Biosynthetic Pathway to Engineer Spicy Tomatoes

Greg Reeves (Department of Plant Sciences, University of Cambridge), **Chris Boursnell** (Department of Plant Sciences, University of Cambridge), **Jie Li** (John Innes Centre)

This proposal seeks to utilise synthetic biology approaches to overexpress capsaicin pathway enzymes missing from tomatoes but found in chilli peppers, yielding spicy tomatoes. Transient expression in tomato fruit and leaves would be used for fast screening and validation of the key genes mentioned above. The project would utilise the current models for the capsaicin pathway as a blueprint and would provide a clearer picture of capsaicinoid evolution in Solanaceae. This would demonstrate that the path to capsaicin production is relatively straightforward and that other members of Solanaceae may be evolving capsaicin production. This proposed experiment offers a tool to building synthetic pathways in plants through complementation of existing components and furthers understanding the evolution of secondary metabolites in plants.

Implementation of a synthetic transcriptional AND gate in the chloroplast of Chlamydomonas reinhardtii

Christian Boehm (Department of Plant Sciences, University of Cambridge),**Payam Mehrshahi** (Department of Plant Sciences, University of Cambridge), **Hannah Laeverenz-Schlogelhofer** (Department of Physics, University of Cambridge)

Chloroplasts are among the most attractive substrates for biological engineering and one of the major limitations to realisation of its potential has been a lack of suitable systems for controlling the expression of transgenes from the chloroplast genome. Over the past decade, several conditional expression systems have been developed responding to a single input only. In order to enable more sophisticated control over chloroplast gene expression based on multiple conditions, we propose to develop a synthetic transcriptional AND gate in the chloroplast of Chlamydomonas reinhardtii. The nuclear component of the proposed circuit is composed of two chloroplast-targeted halves of split T7 RNA polymerase, which are conditionally expressed under control of two different input promoters. Co-induction of the two polymerase halves will lead to expression of a fluorescent transgene.

Advancing the ability to image single RNA molecules at the cellular level

Susan Duncan (John Innes Centre), **Susana Sauret-Gueto** (Department of Plant Sciences, University of Cambridge), **Christian Boehm** (Department of Plant Sciences, University of Cambridge)

Plant biology currently lags behind other fields in the study of cell-to-cell variation and subcellular localisation of mRNA. Susan Duncan (John Innes Centre) helped to establish the first Single molecule Fluorescent In situ hybridisation (smFISH) method for plants where each RNA molecule can be visualised as a single fluorescent dot in Arabidopsis thaliana root meristem tissue (Duncan et al., Plant methods, 2016 in press). This technique revealed subcellular localisation of coding and non-coding RNA and provided data to enable the estimation of the frequency of transcriptional firing events. The high level of back ground autofluorescence emitted by many green plant tissues currently limits smFISH analysis to a single tissue type. With the support of OpenPlant we propose to promote and optimise this existing technique. In addition, we aim to adapt the methodology for use in other Arabidopsis tissues and to enable RNA imaging in the liverwort Marchantia polymorpha.

Establishing a Procedure for Rapid Identification of Genetic Parts for Use in Algal Biotechnology

Kher Xing Chan (Cindy) (Department of Plant Sciences, University of Cambridge), **Steven Burgess** (Department of Plant Sciences, University of Cambridge), **Marielle Vigouroux** (John Innes Centre)

We propose to run a pilot experiment to investigate the feasibility of using DNase-SEQ to identify of regulatory elements in Chlamydomonas reinhardtii; with the view to producing a genetic toolkit for this alga. DNase-SEQ is a powerful approach to identify transcription factor (TF) binding sites (He et al. 2014) which can then be utilised as genetic parts. To date there have been no reports of DNase-SEQ being applied to C. reinhardtii so the first stage of the project will be to establish the procedure. As a test case we will focus on identifying regulatory elements that control the induction of the algal carbon concentrating mechanism (CCM). We propose to develop an open access, online tool to facilitate the bioinformatics pipeline for DNase-SEQ.

A synthetic biology approach to investigating arbuscular mycorrhizal symbiosis in Marchantia paleacea

William Summers (Department of Plant Sciences, University of Cambridge), **Uta Paszkowski** (Department of Plant Sciences, University of Cambridge), **Giles Oldroyd** (John Innes Centre), **Andrew Breakspear** (John Innes Centre), **Guru Radhakrishnan** (John Innes Centre)

D14-LIKE (D14L) encodes an alpha/beta hydrolase receptor that has been well characterised for its role in the perception of the smoke constituent karrikin; whilst in recent years it has been heavily studied for functions in development and light responses. Recently however it has also been identified as being vital for the establishment of arbuscular mycorrhizal (AM) symbiosis in rice (Oryza sativa). Mutation of this gene results in a complete breakdown in communication between the plant and fungus (Gutjahr et al 2015). The evolutionary origin of the AM symbiosis coincides with the occurrence of the early land plants with affinity to liverworts approximately 450 million years ago. The liverwort lineage includes members of the Marchantiacea of which some species, such as Marchantia paleacea, engage in AM symbioses; whilst others, including Marchantia polymorpha, do not.

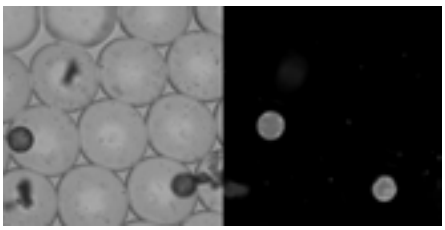
Here, we propose to determine the relevance of the ancient D14L for AM-symbiosis. The approach is two-fold and involves (1) genetic complementation of the rice d14l mutant with synthesized homologs of M. paleacea and M. polymorpha. (2)the CRISPR-Cas9-based editing of the M. paleacea locus to assess the functional requirement of MpD14L for AM symbiosis. The project utilises gene synthesis, Golden Gate cloning, the CRISPR/Cas9 system and established protocols for liverworts available in the Oldroyd laboratory.

Plant-ProChip 2.0: High throughput transformation of plant protoplast

Ivan Reyna-Llorens (Plant Sciences, UCam), **Steven Burgess** (Plant Sciences, UCam), **Ziyi Yu** (Chemistry, UCam), **Gregory Reeves** (Plant Sciences, UCam), **Christian R. Boehm** (Plant Sciences, UCam)

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.



Translating Nitrogen Use Efficiency from models to crops

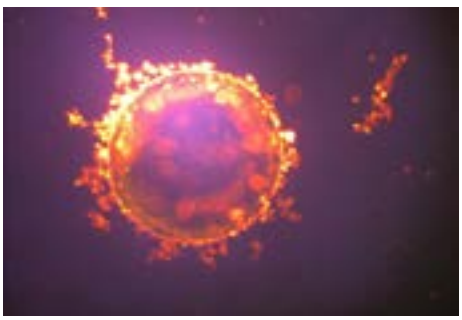
Mariana Fazenda (Plant Sciences, UCam), **Matthew Milner** (NIAB), **Mario Caccamo** (NIAB), **Dan Swan** (Earlham Institute)

Optimizing biological nitrogen (N) use is pivotal to maximizing crop yields and ameliorating the adverse environmental impacts of excess agricultural N application. Most cereal crops only take up about 50% of the applied N (Robertson, 1997). The other 50% of applied nitrogen is lost either to soil microbes, leeched from the soil during rains or chemically lost to the environment. New opportunities exist to provide gains in efficiency via the translation of basic research into application in crop species. The proposal sought to help better indentify orthologous genes as well as indentify how much variation exists in nitrogen use efficiency targets in wheat. We proposed to collect RNA Seq data on the eight parents of a widely distributed and publically available mapping population developed at NIAB (MAGIC elite) to add experimental evidence to help build connections between current knowledge in model species and wheat. The RNA samples are currently awaiting sequencing and the collaborators have visited and given talks at their respective sites.

DNA-mediated fusion of spheroplasts with synthetic liposomes

Lorenzo Di Michele, (Physics, UCam), **Martin Howard** (JIC), **Pietro Cicuta** (Physics, UCam)

We have very good control over phospholipid liposome (vesicle) formation, transport, and fusion; we also know how to lyse the external cell membrane of gram negative bacteria, yeast and single cell algae, all of which then form a 'spheroplast' state, from which the whole cell can be recovered under appropriate culture. Removing the external cell membrane/wall is indeed a standard step in various protocols for uptake of material into the cells. The key idea of our proposal is to demonstrate a 'hybrid' system, engineering controlled adhesion and fusion of artificial liposomes to spheroplast cells. This could represent a new high throughput and selective tool for delivering cargo into cells, not limited to genetic material and very flexible in terms of size and chemical nature of the cargo.



OpenPlant Fund: Software projects

Documentation Tool for Open Plant Technologies

Tobias Wenzel (Department of Physics, University of Cambridge), **Johan Henriksson** (EMBL-EBI), **Carlos Lugo** (EMBL-EBI), **Luka Mustafa** (Shuttleworth Foundation Fellow, IRNAS)

We have successfully built an open source hardware documentation software and an online repository called DocuBricks (DocuBricks.com). We arrived at a software tool that is (according to feedback of users) easy to use and helpful in a wide range of hardware projects and saves documentations in a modular and accessible XML format. The database is citable and the first biology related documentations have been uploaded – many more are to follow from Open Plant Fund projects and the Open Science Hardware Movement. We will continue to develop DocuBricks to serve as a high quality repository for Open Science Hardware.

Open Pi-Image: A low cost-open source plant growth imaging and analysis platform

Dr Dan MacLean (The Sainsbury Laboratory), **Prof Alex Webb** (Department of Plant Sciences, University of Cambridge)

We have designed and constructed a near infrared image capture system based on a Raspberry Pi computer, PiNoir camera and custom 3D printed parts. This runs an extensible and modular open source software suite we developed called Open Pi Image that controls automated image capture and spawns image analysis. The Pi software can be accessed on any external system (e.g. a laptop) via a web server running on the Pi and the system can be embedded in inaccessible places. Open Pi Image is designed to incorporate new user provided scripts for analysis and can be easily extended and customised.

Facilitating synthetic biology literature mining and searching for the plant community

Dr Robert Davey (Earlham Institute), **Dr Ksenia Krasileva** (Earlham Institute/TSL), **Dr Nicola Patron** (Earlham Institute), **Richard Smith-Unna** (CU), **Dr Peter Murray-Rust** (CU)

A two-day workshop in March 2016 centred on novel methods for discovering information about plants from the existing literature ("Content Mining"). Most people were running within an hour and a typical example was "find all you can about diseases of oats" using EuropePubMedCentral (with over 1 million Open Access papers). This retrieves about 500 papers, which were further filtered for chemicals, diseases, species, etc. and displayed within a minute or two, significantly increasing the speed of knowledge-driven scientific discovery. Participants contributed code to the project and helped construct scrapers and dictionaries to extract more information from papers related to plant synthetic biology. The group will run a second workshop and are seeking external grant funding for further collaboration.

BIOMAKER.ORG

ELECTRONICS : 3D PRINT : SENSORS : INSTRUMENTATION : BIOLOGY

New funding model for interdisciplinary exchange, project-based learning and DIY bioinstrumentation

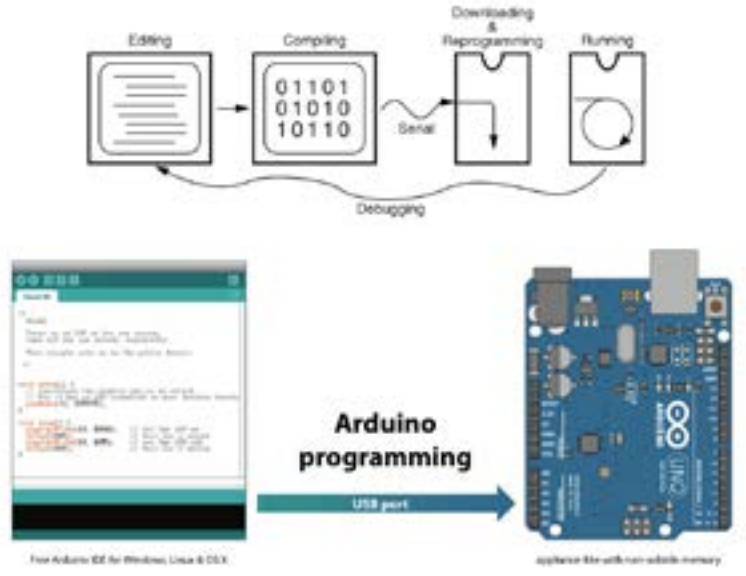
The Biomaker Challenge is an interdisciplinary team-based opportunity to explore the intersection of electronics, 3D printing, sensor technology, low cost DIY instrumentation and biology. The Biomaker Challenge aims to promote collaboration between disciplines, tapping into commodity electronics and open technologies for instrumentation to build research skills and collaborations.

We have chosen Arduino-based hardware (www.arduino.cc) as our starting point. The Arduino community has established open standards and rich ecosystem of resources for simple microcontrollers, first established to simplify programming and physical computing for designers and artists. Arduino circuit boards can be plugged into the USB port of any laptop, and a simple cross-platform programming environment used to program the board. A program is simply loaded to non-volatile memory on the Arduino board, which will execute this program loop whenever the board is powered on - behaving as a dedicated appliance or instrument. Arduino boards include many input/output ports, and are intended to interface with sensors and actuators.

The Arduino system provides a simple environment for learning programming and hardware skills, and developing real-world laboratory tools for biologists. Further, the Biomarker Challenge provides a direct route for other scientists and engineers to get hands-on experience with biological systems.

The effort is sponsored by BBSRC/EPSRC through OpenPlant Synthetic Biology Research Centre (www.openplant.org) and the University of Cambridge Research Policy Committee through the Synthetic Biology Strategic Research Initiative (www.synbio.cam.ac.uk) and CamBridgeSens, the Sensors Strategic Research Network (www.sensors.cam.ac.uk).

The Biomaker Challenge Starter Kits contain teaching materials to allow anyone with no previous experience to learn programming and interfacing to the Arduino microcontroller board



Biomaker Starter Kit

Each team in the Biomaker Challenge receives a Starter Kit that will allow even inexperienced individuals to develop skills, and provide a platform for exploring more challenging applications. The kit includes:

ARDX Prototyping Kit. The ARDX Starter Kit for Arduino is a great learning resource with components to build 13 different projects. The kit provides a manual with instructions arranged as a series of lessons. These provide a simple way of learning how to wire electronic circuits and programming the Arduino microcontroller. For example, the kit comes complete with a set of paper circuit templates that you lay over the breadboard and push the components through - to remove the worry of wiring the project incorrectly. No experience necessary.

Grove Modular Sensor/Actuator Kit. Grove is a modular electronics platform for Arduino-based quick prototyping that does not involve soldering. Simply plug the Grove modules into the Grove shield and leverage the example code provided for each Grove module. Grove is a modular, ready-to-use tool set. Much like Lego, it takes a building block approach to assembling electronics. The Grove Starter Kit contains 10 of the most popular Grove modules and an Arduino shield with Grove connectors.

Sidekick Basic Component Starter Kit. This contains basic components to build 7 different projects, and include an additional small circuit breadboard and more hook-up wire. The kit is provided by SeeedStudios (http://wiki.seeed.cc/Sidekick_Basic_Kit_for_Arduino_V2/).

Giant Prototyping Board for Arduino. The Gtronics Proto Shield Plus allows you to plug in Arduino boards, and to integrate these with custom shields and components on a large plug board - minimising tangled hook-up wires. On-board push buttons and a LCD are provided to facilitate debugging of program flow and to interrogate hardware during testing.

Programmable Touchscreen. The Biomaker Starter Kit will contain a 4D Systems 3.2" gen4 touch-responsive programmable display from 4D Systems (with memory card, Arduino interface and programmer), with information about programming environments. An Arduino library for direct serial communication with the display is available - along with more sophisticated Workshop4 development tools, including ViSi-Genie, a graphical programming tool that allows simple access to a wide range of display widgets like gauges, switches, sliders, readouts, etc., for creating customised interfaces for Arduino-based instruments. The programmable displays can be easily adapted for Raspberry Pi board computers. These programmable touchscreens allow the simple prototyping of sophisticated user interfaces, to match the flexible and programmable control of hardware by microcontroller-based instruments.

The teams are also provided with additional support of up to £750 over the summer, for additional components and materials, including access to a 3D printing service with both fused deposition modelling (FDM) and stereolithography (SLA) printing services, and teams will be expected to share their projects on Github. The Biomaker Challenge culminates in a public Open Technology exhibition. All teams will be expected to demonstrate their creations at this public event. Prizes will be awarded for especially creative and/or enabling projects.

For more information, go to
https://www.biomaker.org

Practices for responsible innovation

BIOMAKESPACE PLANNING

INTERDISCIPLINARY WORKSHOPS

The global cultivation of crops and pastures are driven by global population pressure, and are responsible for unsustainable impacts on natural environments. An overarching aim of the OpenPlant project is to provide a map of feasible technical approaches for improving bioproduction and agriculture – considering possible economic models, opportunities and social implications. This includes consideration of the adoption of different forms of IP ownership, open source technologies, new business models in biotechnology, scientific codes of practice, responsibility for design and implementation, bioengineering accreditation, third world exchange, design for sustainability, decentralisation, UK policy development, evaluation of environmental impact (at the point of conception and design, rather than implementation), guidelines for best practice in new biological systems and real-world agronomy.

Responsible Research and Innovation (RRI) activities are integrated into the OpenPlant SBRC through a number of cross-cutting activities. Central to this are efforts to create mechanisms for the exchange of resources and information by developing enabling tools for sharing such as standards and IP solutions, DNA part collections, shared protocols, and an open community for plant synthetic biology; along with OpenPlant Fund workshops to strengthen synthetic biology capacity in Latin America and Africa.

The OpenPlant Forum is an important vehicle for bringing together a multidisciplinary community to discuss important questions in Responsible Research and Innovation. Smaller meetings such as the OpenPlant All-Hands meeting, ROC meetings, and interdisciplinary workshops provide opportunities to explore issues related to responsible innovation. To support these activities and enable our PDRAs to contribute more extensively, we deliver workshops on RRI, ethics and argumentation, and openness attended by OpenPlant-funded PDRAs and many associates. OpenPlant participates in quarterly meetings of the Virtual Institute of Research and Innovation (VIRI) in Cambridge. These meetings bring together members of the science departments with members of the Centre for the Study of Existential Risk (CSER) and the Centre for Science and Policy (CSaP) to discuss matters related to RRI and to discuss opportunities for collaboration. Resulting from these collaborations, OpenPlant researchers from all three institutes have become involved in a Bioengineering Horizon Scanning Exercise organised by CSER.

PUBLIC EXHIBITIONS

PUBLIC ENGAGEMENT

Public Perceptions of Synthetic Biology

JOHN INNES CENTRE, UNIVERSITY OF EAST ANGLIA

OpenPlant joined forces with synthetic biologists and social scientists from the University of East Anglia to organise a scoping workshop on public engagement in synthetic biology, looking at: (i) the diversity of synthetic biology engagement processes; (ii) public views and concerns identified in these processes; (iii) the opportunities moving forward. The workshop, held in July 2017, was attended by synthetic biologists and social scientists from the UK Synthetic Biology Research Centres and the Eastern Academic Research Consortium (Eastern ARC) as well as a representative from the NGO, Society Inside. The workshop highlighted the vast range of synthetic biology engagement activities, and thus the models available for all to use. Additionally, the workshop collected suggestions on how the community can build upon the work that has already taken place.

Principal contact: Colette Matthewman

PUBLIC ENGAGEMENT

DNA Dave, the robot!

JOHN INNES CENTRE, SAW TRUST

In December 2016, a group of enthusiastic scientists met with artist Molly Barrett to begin work on a robot that would explain the processes of transcription and translation to a lay audience. The world was introduced to DNA Dave, the robot, at the 2017 Cambridge Science Festival. Regardless of age, the public were really excited to discover what the robot could do, and the process of transcription and translation of DNA to proteins was well explained by operating Dave's buttons, cogs and switches. One parent commented "I love how accurately you simplify the process for the young ones". Most adults admitted that they, as well as their children, learnt something new. The creation of the robot was funded through an Outreach Grant from the Biochemical Society.

Principal contact: Jenni Rant

SAW TRUST WORKSHOP

Global Garden Workshop

NORWICH

A Global Garden workshop was run as a collaboration between OpenPlant researchers at the John Innes Centre, the Science Art Writing (SAW) Trust, Social Scientist Dr Nick Lee (Social Scientist, Warwick Integrative Synthetic Biology Centre) and the Writers Centre Norwich. The workshop was advertised to the public, and explored biodiversity, traditional and modern uses of plants, access and benefit sharing and feelings on natural vs synthetic products. Participants were immersed in the theme through practical science, art, poetry and a set of case studies that raised a variety of questions leading to discussions of issues around the use of plants as sources of drugs and other high value products. This co-learning experience highlighted to researchers the values, concerns and optimisms of publics in relation to the use of plants as a source of natural products.

Principal contact: Jenni Rant

MAKING

Norwich Science Makers Network

NORWICH

OpenPlant has supported the establishment of a Norwich Science Makers meetup group to bring together a cross disciplinary network of people to learn from each other, share ideas and skills and shape interdisciplinary and collaborative project plans. The network will provide an umbrella under which a variety of activities can be captured, and can feed into programmes such as the OpenPlant Fund and Biomaker Challenge. The first meetup will be in September.

https://www.meetup.com/Norwich-Science-Makers/

Principal contact: Colette Matthewman

WEB COMMUNICATION

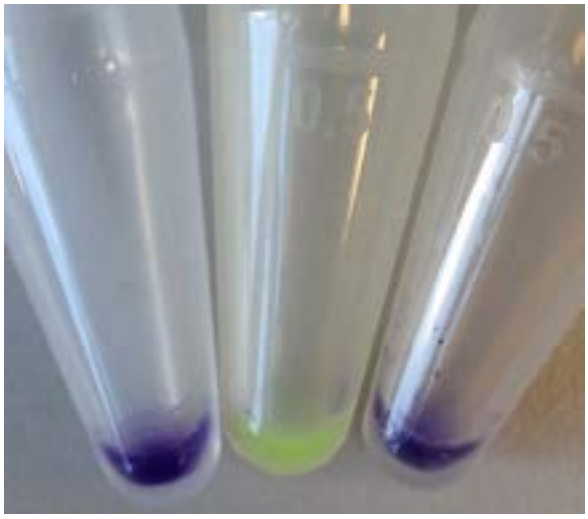
Workspace for exchange

CAMBRIDGE

As part of the work in Cambridge establishing the Biomaker Challenge, we have been experimenting with new ways of sharing inforrmation between individuals who might be working in different disciplines and at different locations. Facebook has recently released a workplace-specific version of their social media and exchange plaform, called Workplace. The Workplace version of the Facebook web service allows the building of groups and networks online, with features that are relevant for use in a work environment, but without many of the distractions found on Facebook. For example, Workplace is limited to connections across the same email domain, apart from specific shared company groups. Facebook and Workplace are not connected.

In addition, we have adopted a number of other off-the-shelf solutions for networking and exchanging information. Benchling has become our preferred means of compiling and exchanging DNA sequence related information. We have adopted Github as a means of documenting hardware and software projects. Meetup is our preferred platform for events management and notification. Squarespace is proving to be an excellent platform for easy building and maintenance of websites.

Principal contact: Jim Haseloff



TRAINING

Cell-free biology workshops

CAMBRIDGE-NORWICH

Recent technical advances in the preparation of microbial cell-free extracts have given rise to a new class of highly efficient systems for gene expression that are cheap to deploy and have huge potential benefit for the provision of a wide variety of diagnostics, sensors, vaccines and research materials. Further, the extracts can be stored desiccated, stable for over a year, and reactivated at the point of use by hydration. The cell free extracts can be programmed by the addition of DNA to allow rapid and simple prototyping of gene circuits for diagnostics or bioproduction.

In vitro biology provides a number of key advantages for the design, assembly and testing of DNA encoded circuits for diagnostics and environmental sensing. Cell-free extracts avoid the complications, delays and regulatory uncertainty associated with uncontained of GMOs, while providing opportunities for high level, low cost training and capacity building.

The emerging technology enables engineering of DNA circuits without the need for genetic modification and in a low cost manner that makes it accessible for researchers in low resource settings. OpenPlant is sponsoring efforts to develop new educational and training materials for use in the UK and developing countries.

Principal contact: Jim Haseloff

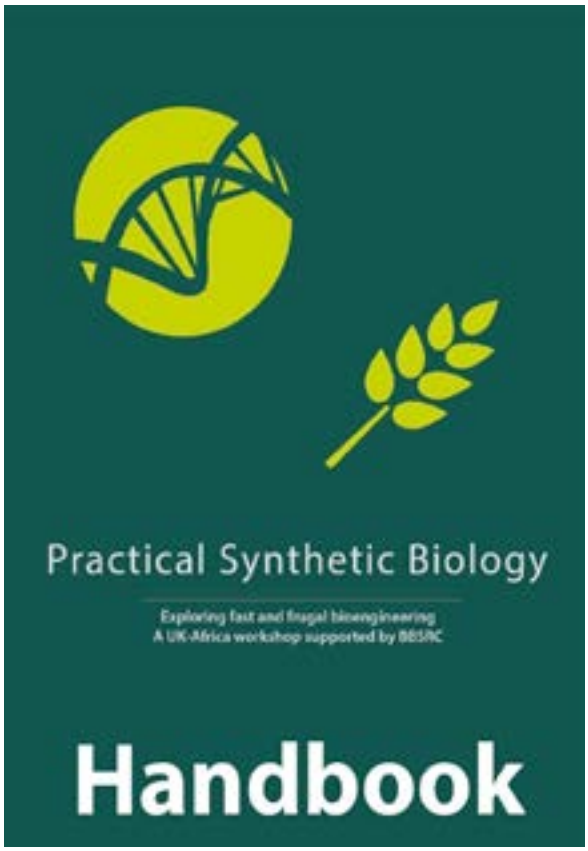
MAKING

Building the Biomakespace in Cambridge

CAMBRIDGE

A Biomakespace in Cambridge is being built by a group of researchers, scientists, engineers, technologists and curious minds - to create an innovation space for biology and biological engineering. This effort is being driven by OpenPlant personnel and supported by OpenPlant initiatives. The Biomakespace is situated within the historic old MRC Laboratory of Molecular Biology building.
It intends to make bioengineering and manufacturing technologies accessible to a wide spectrum of innovators and enthusiasts to develop projects and ideas in an informal setting, with space for experimental biology and fabrication tools focused on scientific applications. The space aims to build a cross-disciplinary and cross-sector community for synthetic biology in the city, with a focus on open technology and innovation. It will also provide activities such as training and skills sharing sessions, networking events and foster links with innovation and seed funding schemes and local bioincubator spaces and accelerator programmes. (https://biomake.space)

Principal contact: Jenny Molloy



GLOBAL CHALLENGE

Bakubung Report

AFRICA

Synthetic biology and open-source applied biology tools that are pragmatic, safe and cost-effective have the potential to stimulate bioeconomic growth and address African challenges in healthcare, agriculture, education and the environment. OpenPlant is recruiting leading international experts to explore the latest developments in synthetic biology, bioengineering and DIY biology, their potential as training tools for students and future innovators, and practical opportunities for deployment in Africa.
This effort has been started with a symposium and workshop that was held in Pretoria and Bakubung, South Africa in February 2017, supported by the BBSRC and Global Challenges Research Fund (GCRF), a £1.5bn UK fund dedicated to the support of challenge-driven research in developing countries. The workshop resulted in the publication of a report that canvassed the difficulties and opportunities for promoting innovation in the emerging bioeconomy in Africa. In response, OpenPlant is coordinating an international programme for development of open materials for biological education in low resource environments. (https://www.openplant.org/global-challenges/)

OpenPlant Fund: Responsible Innovation projects

The Big Algal Open Experiment

Dr Paolo Bombelli (Biochemistry, University of Cambridge), **Dr Brenda Parker** (Biochemical Engineering, UCL), **Dr James Lawrence** (Biochemical Engineering, UCL), **Marc Jones** (PhD student in Computational and Systems Biology, John Innes Centre)

Algae are amazing: they recycle over half of the carbon dioxide we exhale, and form the basis of many food chains, yet we still understand very little about how they grow. In future, we may wish to cultivate algae for food, fuel, or to clean up wastewater so we need to understand more about their biology! With this in mind, we have set up the Big Open Algae Experiment to help us enhance our knowledge by performing the biggest parallel algae experiment in history. We are inviting universities and citizen scientists to participate in an open-source data collection experiment on outdoor microalgal growth. Up and down the UK, we'll be running experiments using a bioreactor we have designed and asking people to submit their recordings of how well the algae are growing. Following and recording the algal growth will be easy and fun. This thank to a smart-phone app: the Alg-app . The Alg-app will enable everyone having access to a smartphone to get involved. During the OpenPlant Fund project, bioreactors, the website and app were constructed (http://bigalgae.com/about). The project has since been on the road at Latitude Festival 2016 and exhibited at London Zoo, further experiments with schools and universities are planned for the future and the concept is being developed into an 'Algaegotchi' pet with the Iaac Advanced Architecture Group in Barcelona.

Responsible Innovation and Open innovation with Large BioResources: Goals, Challenges and Proposals

Dr Kathy Liddell (Centre for Law, Medicine and Life Sciences, Faculty of Law, University of Cambridge), **Dr John Liddicoat** (Centre for Law, Medicine and Life Sciences, Faculty of Law, University of Cambridge), **Dr Rob Doubleday** (Centre for Science and Policy), **Dr Nicola Patron** (Earlham Institute).

On 28 January 2016, the Centre for Law, Medicine and Life Sciences together with the Centre for Science and Policy, and OpenPlant hosted a workshop on responsible and open innovation with large bio-resources. The central question the workshop tackled was: whether, and to what extent, policies of openness are appropriate for successful innovation with bio-resources in synthetic biology and genomics. Closely related to this question was: how does one implement openness effectively in bio-resources intellectual property policies? Discussions were stimulating and highlighted the different approaches taken by the two fields. The outcomes have since been published as an Open Access report on SSRN (https://ssrn.com/abstract=2888871).

Strengthening synthetic biology capacity in Kenya through bioinformatics training

Richard Smith-Unna (CU), **Dr Vicky Schneider** (Earlham Institute), **Dr Jelena Aleksic** (TReND), **Richard Pilling** (Intel)

From 30th November to 5th December 2015, 37 students from nine African countries attended our course, held at ICIPE in Nairobi, Kenya. The course involved six days of theory and practical work, starting from the principles of Unix and programming, through to advanced scientific programming and visualisation. Towards the end of the week students worked on specific analysis methods in various areas of genetics and genomics, with a special focus session on synthetic biology. An ongoing student-led study group, coordinated online, will help the students keep the momentum from the course going and the course also repeated with a new cohort in 2016. The course materials are available at https://github.com/jelena121/NGS_analysis_icipe.

Setting up an open synthetic biology lab in Abuja, Nigeria

Richard Smith-Unna (CU), **Dr Chinyere Okoro** (Sanger Institute), **Dr Ibukun Akinrinade** (University of Bingham, Nigeria), **Dr Jelena Aleksic** (TReND), **Dr Vicky Schneider** (Earlham Institute)

The team were able to develop a synthetic biology lab in Bingham University, Abuja, Nigeria by collecting over 550 kg of equipment donations from Institutes in Switzerland and the UK and shipping to Nigeria in May 2016. This included molecular biology equipment such as a PCR machine, centrifuges and consumables. Preparations for the workshop is now in top gear as logistics are been arranged and course materials are being prepared. A course was run in January 2017 providing a robust introduction to molecular biology and gene editing techniques (e.g. cloning, CRISPR, DNA, RNA and protein methods). The course also included a Science Policy Lecture supported by the European Molecular Biology Organization.

Co-lab OpenPlant - interdisciplinary workshops of science art and design

Dr Paolo Bombelli (Department of Biochemistry, University of Cambridge), **Dr Paloma Portela Torres** (UCL), **Lena Asai** (Goldsmiths, London), **Juan Manuel García Arcos** (CRI, Paris), **Ke Fang** (CRI, Paris)

Co-lab OpenPlant ran a series of three workshops and a hackathon event during 2016, with the objectives of creating new ideas around plant synthetic biology applications and fostering further collaboration by establishing links between designers, artists and scientists. Some projects have continued and one, VRICKS, achieved it's own OpenPlant Fund to develop accessible 3D models of molecules for schools.

Synthetic Biology for Schools: A multidisciplinary approach

Dr Colette Matthewman (John Innes Centre), **Dr Jenni Rant** (The SAW Trust), **Dr Tim Rudge** (Universidad Catolica, Chile), **Tim Marzullo** (Backyard Brains, Inc), **Juan Keymer** (Universidad Catolica, Chile), **Nadia Radzman** (John Innes Centre), **Samantha Fox** (John Innes Centre), **Lawrence Pearce** (John Innes Centre), **Dr Nicola Patron** (Earlham Institute), **Dr Fernán Federici** (University of Cambridge/ Universidad Catolica, Chile), **Lalitha Sundaram** (Department of Pathology, University of Cambridge), **Dr Steven Burgess** (Department of Plant Sciences, University of Cambridge), **Dr Ben Miller** (School of Biological Sciences, University of East Anglia)

The synthetic biology community in Norwich and Cambridge are working on several ideas for developing educational materials, tools and practicals to bring multidisciplinary science and synthetic biology into schools. To increase their overall impact, we propose to create a complete package of activities, supporting information and hardware that can be successfully used in schools to introduce synthetic biology with a focus on plant chassis, and to provide learning opportunities across a wide range of disciplines. Our intention within the scope of this project is to target the resources for local schools, but subsequently we can look for national and international opportunities for dissemination.

Workshop on Genetic resources in the age of the Nagoya Protocol and gene/genome synthesis

Prof Jim Haseloff (University of Cambridge), **Dr Dominic Berry** (University of Edinburgh), **Dr Deborah Scott** (University of Edinburgh)

The ongoing improvement of gene and whole genome sequencing and synthesis technologies presents possibilities of new practices, and demands discussion and debate in light of the long history of global bioresource management. This workshop in November 2016 acted as a venue for collecting information on current developments, sharing views, highlighting potential areas of concern, and establishing grounds upon which to build better understanding of the interactions between and implications of the Nagoya Protocol and gene synthesis for collection, circulation, and use of genetic resources. A report is in preparation.

Developing Cell-Free Genetic Circuits and their Electronic Counterparts as Educational Tools for SynBio Students

Cambridge University Synthetic Biology Society

There is a notable absence of Synthetic Biology (SynBio) concepts and methods within the STEM courses at Cambridge, particularly in the first two years of study. In addition, the course structures preclude any interdisciplinary student research. The need for a society bridging departmental divides and giving students a platform on which to develop experience in SynBio outside the conventional course structure was clear. In 2015, CUSBS was established with the aim of increasing understanding and involvement in SynBio within the student body, and to allow students from different backgrounds to share ideas and skills. This project will allow us to work on understanding gene regulation by engineering oscillating genetic networks in cell-free TX-TL systems. Generally, the project aims to develop along two parallel "biological" and "physical" branches designed to be mutually informative and complementary. Theoretical design of genetic networks will run alongside the practical realisation of their electronic counterparts, allowing students to gain experience in circuit design, and informing their view on biological systems. Do cells compute information in terms of AND, OR, IF gates? If yes, how do they implement these genetically and molecularly? How are bistable switches, hysteretic systems, all-or-none responses, proportional control mechanisms etc. implemented in living cells? Can we reconstruct simple and complex networks motifs outside of a cell?

Accessible 3D Models of Molecules

Roger Castells Graells (JIC), **Vanessa Bueno** (Earlham Institute, **Elisabeth Gill** (Engineering, UCam), **Charlie Owen** (JIC)

This project aims to create kits of 3D models of molecules for schools and outreach activities. The models will be used to facilitate the understanding of viral structures, polymers and synthetic biology projects. The kits will include complete structures and also pieces to be assembled as 3D puzzles and will be a tool for teachers and researchers to teach about their subject in an interactive manner.

Developing teaching resources for rapid, open and combinatorial genetic circuit fabrication in cell-free systems

Fernan Federici (Plant Sciences, UCam and PUC, Chile), **Nicola Patron** (Earlham Institute), **Bernardo Pollak** (Plant Sciences, UCam)

We propose to develop an efficient system for the fabrication of elementary genetic function and the assembly of higher order circuits to be tested in cell-free systems. We will adopt and promote the OpenPlant syntax to facilitate community-based expansion of these resources (ie distributed development) and provide instructions for simple mathematical model building to fit the data obtained with these resources. We will develop open source hardware to track the dynamics of genetic reactions using fluorescent proteins that are excited by the same emission source (low cost 470 nm LEDs and Raspberry Pi Camera). Finally, we will develop a NCBE-style manual and slides accompanying the resources to facilitate their use in classrooms.

OpenPlant Global Challenges

Synthetic Biology in Africa

The OpenPlant obtained funding for a 'Practical Synthetic Biology' workshop in Africa - to exploit recent technical advances in biology that have given rise to cell-free and transient expression systems that are cheap to deploy and have large potential benefit for diagnostics, sensors, vaccines and research materials. The workshop was help in collaboration with the University of Pretoria, which has initiated the construction of the Future Africa campus, intended to provide a hub for Africa's leading scientists and scholars.

The workshop found:

1. The field of Synthetic Biology is introducing low-cost, breakthrough technologies for a wide range of practical challenges including diagnostics, environmental conservation, microbial bioproduction, crop improvement and human health. These are of critical importance to the future well-being and economic development of sustainable societies across Africa.
2. Synthetic biology offers new tools and approaches:
    - Standardised, modular DNA parts and rapid assembly of genetic circuits for reprogramming biological systems.
    - Cell free expression systems that do not require containment, and can be freeze-dried and stored at ambient temperatures to eliminate the need for refrigeration.
    - Transient gene expression in contained hosts, and transgene-free genome editing to avoid the costs, resources and regulatory hurdles associated with the deployment of genetically modified organisms.
    - Legal frameworks, repositories and open technologies for the open exchange of genetic materials.
3. These new technologies are relatively low-cost, but their adoption in Africa is limited by deficits in technical training, poor access to new research materials, inadequate laboratory facilities, and lack of strategic partnerships with other African and international research institutions.
4. The UK and Africa share a common goal with the need to develop improved synthetic biology training in schools, universities, community labs and industry.
5. International efforts to develop open standards and protocols for DNA parts and tools will provide a major impetus for technology transfer to Africa.
6. We recommend that (i) biotechnology is fertile area for UK-Africa exchange, and that (ii) capacity-building based on open technologies and exchange be a major component of any funding initiative.
7. Synthetic biology can provide better solutions for: (i) rapid-response production of vaccines and biologics, (ii) point-of-use diagnostics and field biosensors, (iii) agricultural crop improvement using non-transgenic (genome editing) tools, and (iv) harnessing local biodiversity to build a sustainable bioeconomy.
8. In each of these applications, the development of practical solutions and social impact requires:
    - Standardised curricula for training and biotechnology education in resource-poor communities and institutes.
    - Building local expertise through exchange and shared knowledge.
    - Establishing in-country facilities for generation and exchange of open-source tools and materials.

We have organised follow-up workshops and meetings, focusing on knowledge transfer for cell-free biology.

2014-2019

OpenPlant Fund

The OpenPlant Fund supports workshops in Africa and Cambridge to support teaching efforts, capacity building and development of open resources for technology transfer.

2016

Cell-free workshops

OpenPlant identifies the potential of cutting edge developments in the field to contribute to education and innovation in low resource environments., using non-GMO reagents that do not require a cold-chain. The same technology can be applied to paper-based assays and field tests. Workshops in cell-free biology are held in Cambridge

2017

Bakubung workshop

OpenPlant and the Earlham Foundry coordinate a workshop in South Africa to explore the limits and opportunities for new technologies to affect development of a modern bioeconomy in Africa. We conclude that OpenPlant can contribute positively through promoting open practices and exchange, and facilitating access to new technologies that offer radical improvements for learning and research in the new biology.

2017-2019

Biomaker Challenge

OpenPlant initiates a new model for project-based learning, the Biomaker Challenge. This offers relatively low-cost, broad participation, entry-level access to new fields to promote interdisciplinarity and a common set of tools and skills for training. Further, the model is easily transportable between institutions and countries. The Biomaker Challenge produces new prototypes for bioinstrumentation, and we wish to add cell-free systems to facilitate addition of DNA-based programming to the Challenge.

2018

Future Africa coalition

OpenPlant is planning future workshops and meetings to recruit a coalition of international experts in cell-free biology and curriculum development, and to produce open source frameworks and materials for teaching in low resource environments. These include working practices for *in vitro* DNA assembly, distribution of non-GM freeze-dried reagents and low-cost instrumentation for quantitative analysis.

Leadership group

Prof. Anne Osbourn, Norwich Director
Anne investigates plant natural products - function, synthesis and mechanisms underpinning metabolic diversification. An important advance from the Osbourn laboratory has been the discovery of gene clusters for specialized metabolic pathways in plants, a finding that has opened up new opportunities for elucidation of new pathways and chemistries through genome mining and for the development of synthetic/refactored clusters for improved/high-value plant traits. She has also developed and co-ordinates the Science, Art and Writing (SAW) initiative, a cross-curricular science education programme for enabling engagement of scientists with society.

Prof. Jim Haseloff, Cambridge Director
Jim and his lab engineered the first synthetic RNA enzymes with targeted substrate specificity, developed fluorescent proteins for plants, new misexpression systems in plants, new 3D microscopy and visualisation methods and computer models for plant morphogenesis. He has pioneered the application of Synthetic Biology approaches in plants, including new quantitative imaging techniques, genetic circuits for cell-cell communication, and adoption of lower plant systems for bioengineering.

Prof. Sir David Baulcombe, Principal Investigator
David's group was the first to identify small interfering (si)RNAs as the specificity determinant of RNA silencing and through their genetic analyses have identified many components of RNA silencing pathways. Relevant to this application the group has unravelled many aspects of the role of RNA silencing in virus defense and other aspects of genetic and epigenetic regulation. His work has been recognised through several awards including the 2008 Lasker Award for Basic Medical Science, the 2010 Wolf Prize for Agriculture and the 2012 Balzan Prize for Epigenetics.

Prof. Dale Sanders, Principal Investigator
Dale's research investigates how plant cells respond to changes in their environment and how they store the nutrients they acquire. He is a leading authority on the mechanisms for the transport of chemical elements across cell membranes in plants. These mechanisms have key roles in the control of crucial crop traits such as nutritional value of foods, seed germination, response to drought and how plants cope with toxic compounds in the soil.

Coordination group

Dr. Colette Matthewman, Manager
Colette is the Norwich-based Project Manager for OpenPlant. With a research background in the plant sciences, she has a broad overview of OpenPlant research activities, and coordinates events, training, and outreach to build new synergies and increase the impact of the centre. She is a member of an OpenPlant working group exploring new IP solutions for biotechnology and is leading a project to develop resources for school pupils to learn about synthetic biology.

Dr. Jenny Molloy, Coordinator
Jenny is the Cambridge-based Coordinator for OpenPlant and the University of Cambridge Synthetic Biology Strategic Initiative. Jenny is a molecular biologist by training and researched genetic control of mosquito populations while becoming increasingly interested in the role and impacts of open source in science. She enjoys being an enabler of open approaches and her role involves coordination of events and activities including the IP working group and OpenPlant Fund, through which the centre is developing new legal tools for sharing and a wide variety of innovative open technologies.

Dr. Susana Sauret-Gueto, Cambridge Research Manager
Susana is an experienced molecular biologist and microscopist. She has established new facilities for robotic liquid-handling and advanced microscopy in the Cambridge OpenPlant laboratory, and is coordinating the sharing of standardised Marchantia resources. These include libraries of DNA parts and transformed plant lines. With a scientific background in plant growth and development, she supports researchers and strengthens the integration of research projects. Susana is the main organiser of the ROC Group (Researchers with OpenPlant Cambridge).

Research leaders

Dr. Fernan Federici, OpenPlant International Fellow
Fernan is an assistant professor at PUC (Santiago, Chile) and manages a research group that explores spatial patterning, open science and educational efforts across Latin America. Fernan has a long association with Cambridge as a Gates Scholar and research fellow. He is continues to play a pioneering role, contributing open technologies for bioengineering, science and education across Latin America, and exploring international collaborations with OpenPlant colleagues.

Dr. Nicola Patron, Earlham Institute
Nicola is a Group Leader in Synthetic Biology at the Earlham Institute. Her work aims to develop technologies to engineer photosynthetic organisms for the biosynthesis of materials and therapeutics and to improve plants for increased production and nutritive value. Her broader interests are in understanding the function of DNA sequences and the mechanisms and consequences of gene transfer. As a SynBio LEAP fellow Nicola was recognized as an emerging leader in synthetic biology with a desire to ensure that synthetic biology has positive social impact; she is interested in the complex questions of ownership and intellectual property that surround genetic sequences and biomolecules.

Dr. Jim Ajioka, University of Cambridge
Jim's lab works on large scale DNA assembly of synthetic circuits in Gram positive bacteria and protozoan biology. He leads a Wellcome Trust programme to build and employ novel biosensors, using Synthetic Biology techniques. Jim's lab is also funded by the EPSRC for foundational work such as generalised codon optimisation, robust switches and counters and big DNA manipulation. The lab's work on big DNA extends to the collaboration with the Haseloff lab on plant plastids.

Prof. Sarah O'Connor, John Innes Centre
Sarah uses transcriptomic and genomic data to elucidate the alkaloid pathways of Madagascar Periwinkle, a medicinal plant that produces compounds that are used to treat a variety of cancers and other diseases. Plants synthesize thousands of complicated molecules that they use to protect themselves from predators, attract pollinators and communicate with other plants. Thousands of years ago, humans realized that many of these plant-derived molecules also have a powerful impact on human health and well-being. Advances in genomic and transcriptomic sequencing have rapidly advanced understanding of the complex metabolic pathways that produce these high-value chemicals.

Prof. Rob Field, John Innes Centre
Rob has 30 years' experience in glycobiology and associated (bio)chemistry. His interests lie in understanding and exploiting carbohydrate recognition, in the design of enzyme inhibitors as probes plant and microbial metabolism, and for the development of lectin-binding anti-adhesive agents to impact on cell adhesion by microbial pathogens (trypanosomes, Campylobacter, flu virus). These activities are underpinned by synergistic synthetic chemistry and synthetic biology efforts aimed at providing new routes to scalable bespoke carbohydrate production.

Prof. Paul Dupree, University of Cambridge
Paul studies plant cell wall polysaccharide synthesis, structure and function. These carbohydrates have important functions in the human diet, agriculture, bioenergy, paper and packaging and for building construction using timber. He has developed a range of innovative techniques for quantitative analysis of polysaccharides, such as PACE for studies of polysaccharide structures and enzyme activities, and DASH capillary electrophoresis of oligosaccharides using DNA sequencers. Having discovered a number of the enzymes that synthesise cell walls, he is now engineering plants to produce novel polysaccharide structures. This approach will generate plants with modified cell walls for improved material properties, and will enable producuction of high value plant products.

Prof. Giles Oldroyd, John Innes Centre
Giles is a leading investigator in plant-symbiotic interactions, with a particular focus on the signalling processes that allow the establishment of nitrogen-fixing and arbuscular mycorrhizal associations. His work has provided the genetic underpinnings to understand the symbiosis signalling pathway that allows rhizobial recognition in legumes and mycorrhizal associations in most plants. He leads an international programme funded by the Bill and Melinda Gates Foundation and the BBSRC that is attempting to engineer cereal recognition of rhizobial bacteria as the first step towards engineering nitrogen-fixing cereals.

Prof. Christopher Howe, University of Cambridge
Chris has long experience with the biochemistry and molecular biology of photosynthetic bacteria and chloroplasts, with a particular emphasis on electron transfer reactions. His lab has pioneered the development of 'biophotovoltaic' technology – the direct production of electricity from photosynthetic microorganisms – which underpins his contribution to OpenPlant. He has also made influential contributions to our understanding of the evolution of chloroplast genomes in organisms ranging from plants to protists. He is a scientific advisor to two local companies working in microbial biotechnology.

Prof. Cathie Martin, John Innes Centre
Cathie uses genetics, biochemistry and molecular biology to investigate the basis of cellular specialisation in plants. This includes many aspects of metabolic specialisation, particularly phenylpropanoid metabolism and its regulation. She has used this to effectively engineer the production of polyphenol bioactives in crops, demonstrating healthpromoting properties in preclinical studies. Her expertise on transcriptional regulators of metabolic pathways has been applied in a wide range of plant species, establishing effective plant production systems of natural products including natural colours and bioactives from Chinese medicinal plants.

Prof. Alison Smith, John Innes Centre
Alison (JIC) studies starch and sucrose metabolism. Her recent work is on starch degradation in Arabidopsis leaves at night, the control of flux through this pathway and its relationship to carbon availability and growth. Her lab also studies pathways of starch metabolism in crops, including potato and wheat. A major current interest is the relationship between starch synthesis and grain yield in wheat.

Prof. Alison G Smith, University of Cambridge
Alison (CAM) focuses on metabolism of plants and algae, particularly biosynthetic pathways for high value products such as vitamins, pigments and lipids. She has been developing tools for improved genetic manipulation of microalgae, in particular by generating regulatory genetic circuits using vitamin responsive promoters and riboswitches. By taking a synthetic biology approach to generate standard parts and workflows for optimal transgene expression, the aim is to establish microalgae as suitable platforms for industrial biotechnology production. In addition, she has established the Algal Innovation Centre in Cambridge that allows scale up of algal cultivation.

Prof. Alex Webb, University of Cambridge
Alex's lab is investigating how plants measure time by studying the circadian clock. They identify how the circadian clock provides benefits to plants to maximize their growth and productivity. As part of these studies they discovered that the regulation of photosynthesis, carbon metabolism and growth are regulated by the circadian clock. They use molecular genetic, transcriptomic, imaging and physiological techniques to understand circadian mechanisms. They also develop new engineering approaches for systems biology in collaboration with Engineers. They are collaborating with Bayer to convert our biological discoveries in to real world solutions for crop improvement.

Prof. Julian Hibberd, University of Cambridge
Julian's research aims to understand how C4 photosynthesis operates and to provide insight into the molecular mechanisms driving its evolution. The group uses a mixture of wet-lab, computational and synthetic approaches to answer these questions. His work includes the demonstration that C3 plants possess characteristics of C4 photosynthesis, the identification of cis-elements that underpin the expression of multiple C4 genes in evolutionarily independent C4 lineages, and technologies to allow specific cell types to be marked and isolated in leaves of C3 species.

Dr. Sebastian Schornack, Sainsbury Laboratory Cambridge University
Sebastian studies processes underlying the interaction of microbes with plants, especially plant processes targeted by microbial effector proteins. He is credited with the discovery of DNA base-specific TAL effector repeat-binding in promoter elements of target host genes. This discovery led to generation of customised TAL based transcription modulators and nucleases with unrivalled DNA binding specificity, that are now being widely exploited in animals and plants.

Prof. George Lomonossoff, John Innes Centre
George is a project leader in the Department of Biological Chemistry JIC, with more than 30 years post-doctoral experience working with plant viruses and plant virus-derived expression systems, including the CPMV HyperTrans™ system for which he was named BBSRC Innovator of the Year 2012. This expression system is used in over 200 academic institutions, and employed for commercial production of human vaccines. He has considerable experience of large collaborative projects and international collaborations in the field of biotechnology. He also has extensive experience in handling intellectual property issues, is a named inventor on several patents and acts as a consultant for several companies.

Dr. James Locke, Sainsbury Laboratory Cambridge University
James is an expert in mathematical modelling and single cell analysis of genetic networks. He developed the first model of the plant circadian clock, and experimental data and modelling to correctly predict a new feedback loop. He co-developed a high-throughput time-lapse single cell analysis and tracking system for bacteria, and used the system to discover a new mode of prokaryotic gene regulation; stochastic frequency modulated pulsing. He is studying stochasticity and signal integration at the single cell level in B. subtilis, plants and Cyanobacteria

Prof. Pietro Cicuta, University of Cambridge
Pietro's group uses optical tweezers, microrheology, advanced confocal microscopy and image analysis methods to address dynamics both in colloidal and cellular systems. The lab's research includes self-assembly of phospholipids, including physical properties of lipid bilayers, hydrodynamic synchronisation of motile cilia, including model colloidal systems and living ciliated cells, particularly human airways; and physical mechanisms of regulating gene expression in bacteria.

Prof. Lisa Hall, University of Cambridge
The main theme of research in Lisa's Analytical Biotechnology Group is in heterogeneous analytical systems, with a primary but not exclusive focus on molecular sensors, the latter including both chemical and biological systems. The activities are concerned with interfacing these systems and/or principles of mechanism and action, with transduction technologies to achieve diagnostic devices and monitoring capability. This research is directed towards environmental, medical and industrial application, with the group pro-active in responding to and advising industry of existing capability and future direction.

Scientific Advisory Board

Dr. Tom Knight, Ginkgo Bioworks, Chair of Scientific Advisory Board
Tom is widely regarded as father of the Synthetic Biology field from his work at MIT, which followed seminal work in early networking technology and artificial intelligence at MIT Computer Science and AI Laboratory, a part of the MIT School of Engineering. He has an extraordinary record of serial innovation in different fields, and is orginator of the first widely used standard for DNA parts, and was a driving force behind the start of iGEM and the Registry of Standard parts, an open competition and social network for assembly and sharing of DNA parts. He now runs a prominent synthetic biology start-up in Boston, Ginkgo Bioworks - engineering high value biochemical pathways in microbes. Tom is widely respected as one of the brightest and most original thinkers in the field. He is extremely well connected, and is also very

Dr. Tim Fell, Synthace, Co-Chair of Scientific Advisory Board
Tim is an experienced technology venture entrepreneur with an R&D background in both the physical and life sciences. Before Synthace, he was Chief Operating Officer of CellCentric, a leading epigenetics drug discovery company, Chief Technology Officer of Arrow Therapeutics and co-founder and General Manager of DNA microarray tools company, Oxford Gene Technology (Operations). Prior to this, Tim spent 13 years performing highly interdisciplinary research at the University of Oxford holding post-doctoral positions in three different departments (Biochemistry, Engineering and Materials). He has a D.Phil in Semiconductor Materials and an MBA from London Business School. Tim is the Chairman of the UK BioIndustry Association's Synthetic Biology Advisory Committee and also a member of the Synthetic Biology Leadership Council.

Prof. Christina Smolke, Stanford University
Christina is one of the few "dyed-in-the-wool" synthetic biologists exploring plant systems, outside OpenPlant. She has a very high profile in the field, with a fast-track career at Berkeley-Caltech-Stanford - working on the engineering of RNA-based control mechanisms and natural product biosynthesis. She's been president of the Society for Biological Engineering, and has string of awards to her name: NIH Director's Pioneer Award, National Institutes of Health (2012), World Technology Award in Biotechnology (Individual), World Technology Network (2009), Alfred P. Sloan Foundation Fellow, Alfred P. Sloan Foundation (2008), National Science Foundation CAREER Award, National Science Foundation (2006),

Prof. Drew Endy, Stanford University
Drew is a well-known evangelist for synthetic biology. As well as his scientific work at MIT and Stanford, Drew has provided early leadership and support for many open biotechnology programs. He was the co-founder of the iGEM competition, OpenWetWare.org, the Biofab and Bionet, efforts to share high-quality standard biological parts. Drew is founder and President of the BioBricks Foundation, which supports open technical and legal standards. He has worked with Congress, the White House, DARPA, OECD, the National Academy, etc. on policy matters. Drew is a "big ideas" person, with an exceptional track record in promoting successful international open science initiatives in synthetic biology.

Dr. David Rejeski, Woodrow Wilson Institute
David directs the Science and Technology Innovation Program (STIP) at the Woodrow Wilson Center in Washington DC, including synthetic biology (www.synbioproject.org). STIP focuses on emerging technologies and the critical choices innovation presents to public policy. He has graduate degrees in public administration and environmental design from Harvard University and Yale University and a degree in industrial design from the Rhode Island School of Design. He founded and co-directed a non-profit involved in renewable energy technologies, was head of the Future Studies Unit at the US Environmental Protection Agency, and worked at the White House Council on Environmental Quality (CEQ) and the Office of Science and Technology (OSTP) on a variety of technology, R&D, and policy initiatives.

Prof. François Kepes, Genepole, CNRS, France
François is a noted leader in the Synthetic Biology field in Europe. He studies and engineers genome architecture. For this purpose he uses various approaches including bioinformatics and molecular, systems and synthetic biology. François Képès is a Research Director at CNRS. He is the Founding director of the Epigenomics Project (Genopole), an Institute of Complex Studies that is dedicated to the emerging disciplines of Systems and Synthetic Biology. He is a Team Leader at the institute of Systems and Synthetic Biology (Genopole, CNRS, UEVE). He is a permanent Invited Professor at Imperial College London. He is a member of the National Academy of Technologies of France.

Prof. Susan Rosser, University of Edinburgh
Susan Rosser is a Professor and PI of the SynthSys-Mammalian: Edinburgh Mammalian Synthetic Biology Research Centre. Susan studied microbiology and genetics at the University of Dundee before a PhD working on the mechanisms of multiple antibiotic resistance. Susan then moved to the Institute of Biotechnology at the University of Cambridge to work on biotransformations of cocaine and high explosives. She then became a lecturer in biotechnology at the University of Glasgow before being promoted to Professor in 2012, followed by a Chair in Synthetic Biology at the University of Edinburgh.

Dr. Scott Steedman, British Standards Institute
Director at the British Standards Institution (BSI), where he is responsible for the work of the UK National Standards Body, representing the UK internationally and for advising industry and government on the role of standardization in the economy. Prior to joining BSI in January 2012, Scott spent around twenty years working on major infrastructure and building projects in the UK and around the world for consulting and contracting companies, including GIBB, Whitbybird, High-Point Rendel and Foster Wheeler Energy. Formerly a lecturer at Cambridge University, he has specialised in natural disasters, forensic engineering, risk and innovation strategy. Scott chaired the European Council for Construction, Research and Innovation for over a decade, and is former Vice President of the Royal Academy of Engineering.

Research Council Programme Managers

Dr. Ceri Adams-Tong, BBSRC
Strategy and Policy Manager at the Biotechnology and Biological Sciences Research Council (BBSRC), covering Synthetic Biology, Systems Biology, Genomics and Mathematical Biology - until May 2017.

Dr. Rowan McKibben, BBSRC
Head of Science Strategy: Exploiting New Ways of Working at the Biotechnology and Biological Sciences Research Council (BBSRC)

Dr. Alex Broomsgrove, EPSRC
Portfolio Manager for Synthetic Biology and Process systems at the Engineering and Physical Sciences Research Council (EPSRC).

Research Associates

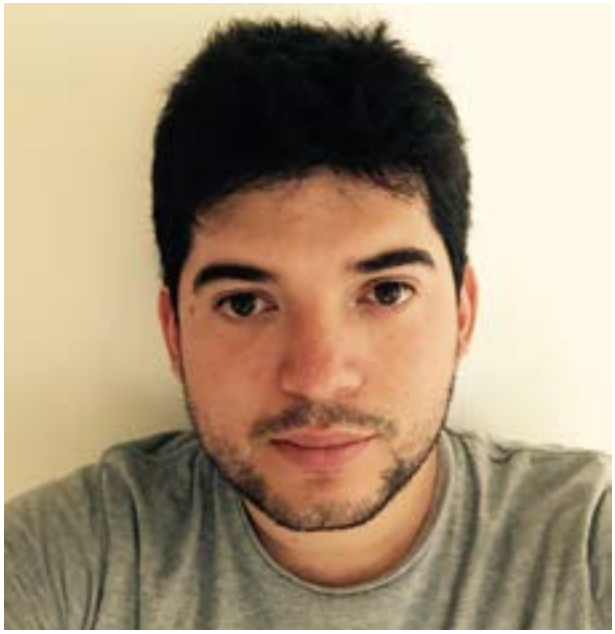
Dr Philip Carella
Postdoctoral Fellow, Schornack Lab, Sainsbury Laboratory, University of Cambridge

I recently completed my PhD in Dr. Robin Cameron's lab (McMaster University, Canada), where I studied phloem-mediated long-distance immune signalling induced by a bacterial pathogen in Arabidopsis thaliana. Feeling a need to branch out a little, I joined Dr. Sebastian Schornack's group (Sainsbury Laboratory, University of Cambridge, UK) to study interactions between filamentous microbes and non-vascular early land plants. Our goal is to identify core developmental processes required for the colonization of early land plant tissues by filamentous microbes and to understand how these processes evolved into the defense and symbiotic programs employed by higher plants. Our work will generate transcriptomics data, fluorescent marker lines and microbe inducible promoters for cell biology, and other molecular-genetic tools that will enable the OpenPlant community to explore early land plant biology.

Dr Eftychis Frangedakis
Postdoctoral Fellow, Haseloff Lab, University of Cambridge

Eftychis did his PhD at Oxford University focusing on the evolution of developmental mechanisms in land plants. During his doctoral research he developed a strong interest and fascination for bryophytes. He then moved to the University of Tokyo to work with the least studied group of bryophytes, hornworts. After a short detour in Hong Kong he is now back to the UK working on the development of new synthetic biology tools in Marchantia. In particular, he is developing tools for engineering the chloroplast genome, where work with the liverwort allows the benefits of single-cell handling through spores, facile transformation and regeneration, and access to a full set of genetic and optical tools for manipulation and quantitative screening of the organism.

Dr Henry Temple
Postdoctoral Fellow, Dupree Lab, University of Cambridge



Plant cell walls represent the most abundant renewable source on the planet, but only a small fraction of this biomass is used by humans. With ongoing interest in use of cell wall polysaccharides, we are just starting to understand their biosynthesis in plant cells. Synthesis of polysaccharides occur mainly through the activity of glycosyltransferase (GTs) enzymes which transfer an activated sugar in the form of a nucleotide-sugar onto a specific growing polysaccharide acceptor. I have great interest in the different processes that govern cell wall biosynthesis. In my Master's and PhD thesis I worked on characterisation of Golgi localised nucleotide sugar transporters (NSTs) responsible for the incorporation of substrates used by GTs enzymes. Now I'm working in Professor Dupree's laboratory as a Postdoctoral Research Associate on a very exciting project, where our goal is to manipulate polysaccharides synthesis by developing genetic tools expressing different GT activities (and other required activities) under tissue specific promoters to evaluate whether it's possible to engineer polysaccharide synthesis, proportions/structures and assess the consequences of these changes in the extracellular matrix.

Marta Tomaselli
PhD student, Haseloff Lab, Schornack lab, University of Cambridge

I did my bachelor and master in Biotechnology in Pisa, where I discovered how fascinating plants can be. In the past, I have worked with CRISPR/Cas9 systems in two different plant models: *Arabidopsis thaliana* and *Marchantia polymorpha*. These were my first experiences related to synthetic biology and they really got me involved. I started as an OpenPlant PhD student at the University of Cambridge in 2016. During a rotation in the Haseloff Lab, I developed optical clearing techniques for microscopy of Marchantia gemmae. These tools allow 3D reconstruction of the plant tissue. In my second rotation in the Schornack lab, I focused focused on plant-pathogen interactions: looking for pathogen-responsive promoters in Marchantia. These sequences can be exploited to generate new reporter lines.
In the future, I wish to continue working with Marchantia and exploit this plant as a model to implement new synthetic circuits. I think that the OpenPlant Community is a great resource for a PhD student, since a lot of different topics are covered by senior researchers, who can help answer questions and provide suggestions about your own project.

Dr Bruno Martins
Postdoctoral Fellow, Locke Lab, University of Cambridge

I am a post-doctoral researcher in James Locke's group at the Sainsbury laboratory. I am interested in how cells discriminate between different environmental states, integrate dynamic outputs from different gene circuits, and make decisions. In my current research, I use a combination of theory and time-lapse microscopy experiments to understand the dynamical coupling of the cyanobacterial circadian clock to other networks, in both endogenous and synthetic systems.
Before coming to Cambridge, I did a PhD in Peter Swain's lab at the University of Edinburgh. In my PhD I used mathematical modelling to gain insight into two simple, yet ubiquitous, sensing and transductions mechanisms: allosteric sensing and phosphorylation-dephosphorylation cycles. I studied the input-output dynamics of these mechanisms in terms of the fundamental constraints inherent in their design.

Dr Francisco Navarro
Postdoctoral Fellow, Baulcombe Lab, University of Cambridge

Fran's work focuses on the function of small RNA (sRNA) molecules and their use as regulatory elements in synthetic gene circuits. sRNA molecules most likely evolved as a defense mechanism against viruses and retro-transposons, and were co-opted for fine-tuning of gene expression. Their small size and predictable targeting rules make them perfect tools for regulating gene expression in synthetic gene circuits. This project is carried out in the green alga *Chlamydomonas reinhardtii*, which is amenable to genetic manipulation and possesses a sRNA pathway that resembles that of higher plants. Chlamydomonas provides a testbed for plant RNA-based genetic devices.
Fran completed his PhD in the laboratory of Prof. Jose Manuel Siverio (University of La Laguna, Spain), studying nitrate assimilation in *Hansenula polymorpha*, a methylotrophic yeast with important biotechnological applications. This was followed by a postdoc in the laboratory of Sir Paul Nurse, at The Rockefeller University, USA, and the London Research Institute, on cell size control and regulation of gene expression by RNA-binding proteins in *Schizosaccharomyces pombe*.

Linda Silvestri
Research Technician, Haselhoff Lab, University of Cambridge

As the Research Technician for the Haseloff group, I work closely with Susana Sauret-Gueto, Research Lab Manager, to ensure the smooth running of the lab. I am responsible for *Marchantia polymorpha* tissue culture and am working on the standardisation of existing protocols for the propagation, transformation and short and long term storage solutions, including cryopreservation.
This work will enable and facilitate the high-throughput screenings of Marchantia lines, such as the Enhancer Trap lines; a project on which several lab members collaborate. A summer student joined us for 8 weeks to work on this project and I helped with her supervision and provided laboratory training.

Dr Lukas Müller
Postdoctoral Fellow, Webb and Haseloff Labs, University of Cambridge

I'm interested in the circadian clock and its effect on physiological and agricultural performance in plants. In the OpenPlant project I am investigating the circadian clock in Marchantia polymorpha and analyze the regulation of clock behavior and outputs in this relative of early land plants. In particular, I am focusing on the primary metabolism as an excellent proxy for systemic processes and vegetative growth.

I apply fluorescent imaging tools with computational time-lapse analysis to obtain cell-specific read-outs for the whole plant in real-time. This data is intended to set the stage for both physiological engineering and systems biology approaches. Part of my project is to engineer fluorescent proteins that are standardised and improved reporters for dynamic changes in gene expression.

Dr Noam Chayut
Postdoctoral Fellow, Martin Lab, John Innes Centre

I am interested in the interface between applied plant breeding and plant metabolism. In my master's thesis we used classical breeding of passionfruit with the goal of releasing new varieties, now used by farmers. In my PhD thesis we studied carotenoid metabolism in melons and established a molecular marker now used routinely by melon breeders. More importantly, we suggested a novel non-transgenic path toward pro-vitamin A carotenoid biofortification of food crops. The objective of the current OpenPlant project is to develop pre-breeding lines of beetroot for the production of L-DOPA.
L-DOPA is used to treat Parkinson's symptoms; however, the current costs of chemical synthesis make it unavailable for deprived populations worldwide. L-DOPA, a product of tyrosine hydroxylation, is an intermediate metabolite in biosynthesis of violet and yellow betalain pigments, in Beta vulgaris (table beet). L-DOPA natural steady state levels are very low, usually undetectable. We intend to block the turnover of L-Dopa in beetroot to allow its accumulation to levels that could enable low-tech accessible production in a plant system.

Louis Wilson
PhD student, Dupree lab, University of Cambridge

I started as an OpenPlant PhD student at the University of Cambridge in September 2016. I am interested in all parts of plant biochemistry, but my projects tend to focus on the characterization and manipulation of enzymes and catalytic pathways.
In my first rotation project, I worked with Prof. Alison G Smith in Cambridge on metabolic gene clusters, developing methods for the expression of higher plant clusters in algae and yeast, and the detection of potential clusters endogenous to algae themselves. I am working with Paul Dupree to study and engineer cell wall-modifying enzymes for improved crops, food and materials. I have been using OpenPlant heterologous expression systems and a transient expression construct from the Lomonossoff lab to assess the stability of glycosyltransferases *in vitro*, with the aim of finding better enzymes for further study and exploitation. Increasing our understanding of these enzymes may ultimately permit the creation of designer fibres and saccharides, as well as being able to manipulate the properties of plant cell walls.

Dr Susana Sauret-Gueto
Research manager, Haseloff Lab, University of Cambridge

Dr. Susana Sauret-Gueto is an experienced molecular biologist and microscopist, with a scientific background in plant growth and development. In the OpenPlant Cambridge laboratory, she coordinates the establishment of semiautomated workflows to accelerate the generation and characterisation of genetically engineered Marchantia lines. Susana is establishing a new facility for robotic liquid-handling around the Echo acoustic liquid handler, and an advanced microscopy facility. The microscopy hub includes a Keyence digital microscope for real-time 3D reconstruction of Marchantia plants, as well as a series of fluorescent microscopes with different resolution capabilities, including a Leica SP8 confocal microscope. She is specially interested in the sector analysis project in order to dissect gene function and autonomy at the cell and tissue level. Susana is also the main organiser of the ROC Group (Researchers with OpenPlant Cambridge), which brings together synthetic biology researchers from across Cambridge.

Dr Orr Yarkoni
Postdoctoral Fellow, Ajioka Lab, University of Cambridge

I've been involved in Synthetic Biology for better part of the last decade. My PhD work at Newcastle University focused on facilitating bio-electronic interface via engineered pathways as part of a larger collaborative grant to create a bio-robotic hybrid device. My more recent work at the University of Cambridge was on developing a field-use whole-cell Arsenic Biosensor for deployment in South Asia.
I'm relatively new at working with plants and the opportunity to reengineer the *Marchantia polymorpha* plastid as part of the Open Plant initiative is a great point of transition into this sphere. The main focus of my contribution to Open Plant is to reconstruct the entire 121kb plastid genome in a way that makes it easier to manipulate, facilitating future work on plastid transformation in Marchantia and, in time, other plants. I am also working together with Haydn King from the Ajioka Lab on creating a codon optimised reporter toolkit for use in the Marchantia plastid, consisting of a 13 fluorescent reporters across a wide spectrum ranging from near UV to near infrared.

Dr Aytung Tuncel
Postdoctoral Fellow, Smith Lab, John Innes Centre

I am applying the genome editing tools to generate novel, commercially or nutritionally valuable glucans in model crop species. The primary objective of my OpenPlant project is to generate potatoes that contain digestion-resistant starches with two major nutritional benefits: reduced calorie intake from consumption of chips, crisps and other potato-based foods and increased supply of complex carbohydrates to the microbiota of the lower gut that reduces risk of several diseases including colorectal cancer and type II diabetes.

More specifically, the project involves knocking out the gene(s) of starch branching enzymes I and/or II using crispr-CAS9 method thereby increasing the ratio of amylose to amylopectin (linear to branched starch chains) in tubers without significantly compromising the starch yield. The engineered starch will be less accessible to starch degrading enzymes, thus more resistant to digestion.

Dr Michael Stephenson
Postdoctoral Fellow, Osbourn Lab, John Innes centre

I am a chemist, with a background in natural product total synthesis, medicinal chemistry, and pharmacy. In the Osbourn group we are interested in plant secondary metabolites, and this places us at the very interface between biology and chemistry. I bring expertise in small organic molecule extraction, purification, and structural characterisation. This strengthens the group's ability to functionally characterise biosynthetic enzymes; something which is important for many areas of research within the Osbourn lab. As a medicinal chemist I am interested in applying these techniques to engineer chemical diversity, and to explore the structure activity relationships of bioactive triterpenes. I have been involved in isolating and characterising several novel triterpenes structures arising from co-expression of 'un-natural' combinations of biosynthetic enzymes. In addition, I have solved the structure of a number of novel and usual triterpene scaffolds, produced by oxidosqualene cyclases under investigation within the group.

Dr Ivan Reyna-Llorens
Postdoctoral Fellow, Hibberd Lab, University of Cambridge

My research involves using synthetic biology and evolution for improving agricultural traits, more specifically to improve photosynthesis. C3 photosynthesis can be very inefficient as Rubisco interacts with oxygen in a wasteful process known as photorespiration. In order to increase yields, photorespiration should be reduced considerably. Fortunately, some plants have evolved such mechanism already. C4 photosynthesis results from a series of anatomical and biochemical modifications in the leaf that lead to photosynthesis being compartmentalized between mesophyll and bundle sheath cells. This division of labour generates a CO_2 enriched environment where photorespiration is effectively abolished. C4 plants therefore produce more yield and use water and nitrogen more efficiently. In order to engineer this trait, cell specific genetic circuits need to be developed. Unfortunately there is a limited number of genetic parts driving cell specificity in leaves. My main objective in OpenPlant is to generate a library of leaf specific motifs that can be used to drive the expression of both nuclear and plastid encoded genes in specific compartments and specific cells of leaves.

Dr Oleg Raitskin
Post-doc, Patron Group, Earlham Institute

My project involves optimization of CRISPR/Cas9 methodology of genome editing in plants. CRISPR/Cas9 is a method of choice to perform genome engineering. There are however significant limitations which prevent broader implementation of this technology in plants.

These limitations include variable efficiency of editing at different targets, off target activity, inefficient inheritance of the created mutations, ability to edit simultaneously several targets, limited selection of targets/PAM repertoire and the need to segregate Cas9 and sgRNA from the created mutations. Numerous configurations of CRISPR/Cas9 designed to address these limitations had been published. Our aim is to establish a uniform testbed and toolkit, where many of these configurations are tested under the same conditions and their editing efficiency and off target activity will be assessed. In order to minimize variability in transgenic expression we established an editing essay in plant protoplasts.

Dr Hanz-Wilhelm Nützmann
Postdoctoral Fellow, Osbourn Lab, John Innes Centre

Plants produce a wide variety of specialised metabolites. These molecules play key roles in the interaction of plants with their biotic and abiotic environment. In addition to their ecological functions, plant-derived specialised metabolites are major sources of pharmaceuticals and other high-value compounds. Recently, it was discovered that the genes for the biosynthesis of several major classes of these compounds are physically co-localised in so called 'gene clusters' in plant genomes. Such clustering of non-homologous genes contrasts the expected arrangement of genes in eukaryotic genomes. The co-localisation of functionally-related genes enables the formation of fundamentally different mechanisms of gene regulation in comparison to the control of dispersed genes. The purpose of this project is to improve our understanding of the transcriptional control of plant metabolic gene clusters. The focus within OpenPlant will be on chromatin related regulatory processes that govern the expression of gene clusters.

Dr Eva Thuenemann
Postdoctoral Fellow, Lomonossoff Lab, John Innes centre

Plants can be used as a production platform for high-value products such as vaccines, enzymes and metabolites, thereby providing a potentially fast and cost-effective alternative to other cell culture techniques. Developed within the Lomonossoff group, HyperTrans (HT) is a technology for rapid, high-level transient expression of proteins in plants. One key application of HT in the Lomonossoff group has been the production of virus-like particles for use as vaccines, scaffolds for nanotechnology and in fundamental research of virus assembly.
In addition to my research project, I was involved in the planning stages for the new John Innes Centre spin-out, Leaf Systems International Ltd, which opened on the Norwich Research Park in January 2017 and will enable translation of research to indsutry through scale-up of plant-based production of proteins and metabolites. I have also participated in various outreach activities, such as a TV interview for regional news, the Great British Bioscience Festival, JIC's Speed Science event as well as a work experience day for school children, amongst others.

Dr Benjamin Lichman
Postdoctoral Fellow, O'Conor Lab, John Innes Centre

Plants are incredible chemical factories, capable of producing a host of complex molecules that synthetic chemists struggle to produce. These compounds are produced by plants to interact with their environment, but they also have great significance for humans, as we use them for fragrances, agrichemicals and medicines. This knowledge can then be used to produce natural products and novel chemicals in microbial or plant based platforms. I am currently working with catnip and catmint (*Nepeta cataria* and *N. mussinii*), plants famous for their intoxicating effect on cats. The origin of this activity is the nepetalactones, a group of volatile compounds from the iridoid family of natural products. Along with their role as feline attractants, nepetalactones have also been reported to have both insect pheromone and insect repellent properties, in some cases having activities superior to DEET. The biosynthetic origin of these compounds is currently unknown. We have been using transcriptomics and proteomics to discover enzymes in the Nepeta nepetalactone biosynthesis pathway.

Bernardo Pollak
PhD student, Haseloff Lab, University of Cambridge

I am a final year PhD student at Plant Sciences in the Haseloff lab, with a BA in Biochemistry from Pontificia Universidad Católica de Chile. I am studying the molecular genetics involved in meristem establishment and maintenance in the simple plant model system *Marchantia polymorpha*. I have experience with next-generation sequencing technologies, bioinformatics, genome assembly, microscopy and genome editing. Recently, I have developed Loop assembly, a novel recursive method for rapid construction of gene circuits.
As a side-project, I have been collecting water samples around the world and have isolated several strains of bioluminescent bacteria (>15) from the Pacific, Atlantic, Mediterranean and Caribbean oceans. I have performed a preliminary characterization of these strains and found differences of brightness of about 100 fold in comparison to *Vibrio fischeri*, and constructed new bioluminescent reporter genes.

Mihails Delmans
PhD Student, Haseloff Lab, University of Cambridge

Mihails is a 3rd year PhD student, with an Engineering background as an undegraduate. His research topic is the regulation of cell proliferation in Marchantia gemmae. In collaboration with Bernardo Pollak, he has developed an open source gene-centric database platform for managing genome data and synthetic DNA parts for Marchantia. He maintains a strong interest in enginnering approaches to biological problems, and explots his considerable expertise with electronics, optics and 3D printing to build and modify instrumentation for observing Marchantia cell dynamics.

His PhD research combines the construction of new marker genes, expression in Marchantia gemma, quantitative imaging and software analysis in order to map the dynamics of growth in gemmae. He has found evidence of long distance control of cell proliferation which can be deregulated by surgical manipulations.

Dr. Jenni Rant
SAW Trust Coordinator

Whilst training as a PhD student and working as a plant pathologist at the John Innes Centre, Jenni became interested in science communication and spent time out of the laboratory volunteering for the Science Art and Writing (SAW) Trust (reg charity no.1113386). Twelve years on and she has transitioned to running SAW fulltime as a social enterprise specialising in working with researchers on the design of innovative outreach activities. SAW delivers cross-disciplinary projects, providing accessible and inclusive starting points for people with varied interests and learning styles to explore scientific concepts and cutting edge research themes. SAW works in partnership with OpenPlant to deliver a range of activities, including workshops in schools, with adult groups, exhibits at science festivals and music festivals. We have also worked with SynthSys and the UK Centre for Mammalian Synthetic Biology at the University of Edinburgh to train scientists, teachers, writers and artists in the delivery of SAW workshops. See www.sawtrust.org for more information about work with OpenPlant.

Notes:

OpenPlant Synthetic Biology Research Centre