Annual Report 2016

Open Plant

BBSRC-EPSRC Synthetic Biology Research Centre

University of Cambridge, Cambridge

John Innes Centre & Earlham Institute, Norwich

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Executive Summary: In our second year of operation (Sept 2015-2016), we have made notable progress in development of: (i) **foundational technologies** for standardised DNA assembly in plants, and simple systems for plant synthetic biology, (ii) **trait development** with improved capacity for metabolic analysis and reprogramming of metabolism in plants, and (iii) activities for **outreach and responsible innovation**.

Foundational work

1. Commissioned advanced imaging/robotics equipment at the Cambridge OpenPlant lab.
2. Completion of 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.
3. High resolution map of the time course of gene expression during sporeling germination and chloroplast differentiation.
4. Construction of MarpoDB, a genecentric database for mining and describing DNA parts from Marchantia (http://marpodb.io)
5. 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 work.
6. Development of Phytobrick and UNS standards for efficient hierarchical assembly of DNA circuits.
7. Expansion of a Chlamydomonas DNA toolkit for target gene expression and assay of miRNA-dependent gene silencing.
8. Development of a suite of Cas9 variants and toolkit for targeted mutagenesis and gene deletion in multiple plant species.□□
9. Construction of series of vectors for fine tuning of protein expression using HyperTrans system
10. Methods for mining plant genomes for biosynthetic gene clusters

Trait Development

11. Design and synthesis of an artificial protein scaffold library, built to the Phytobrick standard and verified by BiFC.
12. Production of cell-specific epitope tags for identifying DNA motifs that drive gene expression in photosynthetic tissues in Arabidopsis.
13. Publication of a novel reporter for chloroplast transformation, and identification of transit peptides for chloroplast localisation of nuclear encoded products in Marchantia.
14. Identification of a large repertoire of carbohydrate active enzymes in *Euglena gracilis*.
15. Transformation of gene editing constructs into potato, to create digestion-resistant starches, and preliminary screening of transformed plantlets.
16. Gram-scale production of triterpenes for analysis and assay, using the HyperTrans system.
17. Production of the plant-derived iridoid alkaloid strictosidine in yeast.
18. Generation of a trichome-specific protein database for enzyme discovery.
19. Asteraceae P450 proteins as a toolkit for targeted modification of sesquiterpenes.
20. Development of the HyperTrans system for use in tomato.
21. Screening tomato introgression lines for regulators of monoterpene biosynthesis.
22. Yeast one-hybrid analysis for the identification of transcription factors that regulate triterpene metabolic gene clusters.
23. Characterisation of gene targets for AtMYB12 and SlMYB12 in tomato, for enhancing phenylpropanoid metabolism and high levels of resveratrol and genistin production.

24. Construction of a synthetic gene cluster for dhurrin biosynthesis in Arabidopsis roots.
25. Construction and distribution of HyperTrans DNA vectors that are compatible with the Phytobrick standard.
26. Testing of the HyperTrans system in Marchantia and BY2 cells.
27. Consultation on the design of the Norwich Research Park LeafSystems high throughput production facility, due for completion in Q2 2017.□□

Outreach and Responsible Innovation

28. Funded of 30 mini-grants that incorporate broad interdisciplinarity and collaboration between Cambridge and Norwich, including projects for SynBio training and capacity building in Africa and resources for schools and universities in South America.
29. Cambridge-JIC iGEM2015 team won a gold medal at the international Jamboree, with a hardware project entitled “OpenScope”.
30. 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.
31. Developing collaborative projects through the Virtual Institute of Responsible Innovation.
32. OpenPlant researchers contribute to a Bioengineering Horizon Scanning Exercise run by CSER. Workshop in November 2016. Outcomes will be published in a co-authored paper.
33. Responsible and Open Innovation workshop with Dr. Kathy Liddell, Law Faculty, Cambridge.
34. OpenPlant continues to work with international IP working group and collaborate with the Biobricks Foundation to implement OpenMTA and facilitate exchange of DNA parts.
35. OpenPlant participated in the inaugural meeting of BioNet group at Asilomar and supports an open technology platform for exchange and tracking of biomaterials (http://www.bionet.io).
36. OpenPlant is supporting a workshop on ‘Genetic Resources in the Age of the Nagoya Protocol and gene/genome synthesis’ in November 2016 (led by Dominic Berry, Edinburgh).
37. Organised OpenPlant All Hands meeting for scientific exchange, Newmarket.
38. Participated in Open Technology for Biology workshop, Chile.
39. OpenTechnology Week events in Cambridge, including Technology for the Bottom Billion workshop and Makethon, coordinated with the Centre for Global Equality.
40. Workshops on ethics and openness, outreach for OpenPlant scientists with the SAW Trust, and BBSRC Media Training (March 2016). Also ran joint training workshops and provided support for synthetic biology outreach activities at Edinburgh SBRC.
41. Showcased OpenPlant science in interactive exhibits and workshops at festivals and schools workshops, including Latitude Festival (Suffolk; July 2016).
42. Nineteen graduate students are participating in projects funded by the OpenPlant Fund. Three PhD students have been recruited directly to OpenPlant (Cambridge) this year.
43. Undergraduates have formed a student society for Synthetic Biology at the University of Cambridge (http://cusbs.soc.srcf.net) with OpenPlant support.
44. Students and postdocs at OpenPlant institutes are being recruited to share projects, resources and equipment, through ROC, a group of self organised, highly effective junior researchers.

Introduction

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 offers even greater potential benefits. Plants are already cultivated globally at 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. However, agricultural systems face global threats from new pathogens, climate change, soil degradation and restricted land and water use. 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. This part of the field is in its infancy and is wide open. We believe that there is a crucial need to accelerate the development of new tools and methods for plant synthetic biology, provide mechanisms for open exchange of resources, apply these standardised tools to world-leading projects in trait development, and facilitate interdisciplinary exchange, discussion, outreach and international development.

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 skills 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, Norwich and elsewhere – who have demonstrated an interest in tackling the technical aspects of surveying future technologies.

We believe that we are seeing the emergence of a new technology for the engineering of plant feedstocks that has the potential to radically alter agriculture and bioproduction. (i) Integrated and interdisciplinary approaches are required, and (ii) Synthetic Biology inspired methods are being applied rapidly to the wider plant field. OpenPlant has contributed a combination of organisation, support and direct sponsorship to a network of working groups, forums, small scale funding initiatives and research projects in order to promote these 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.

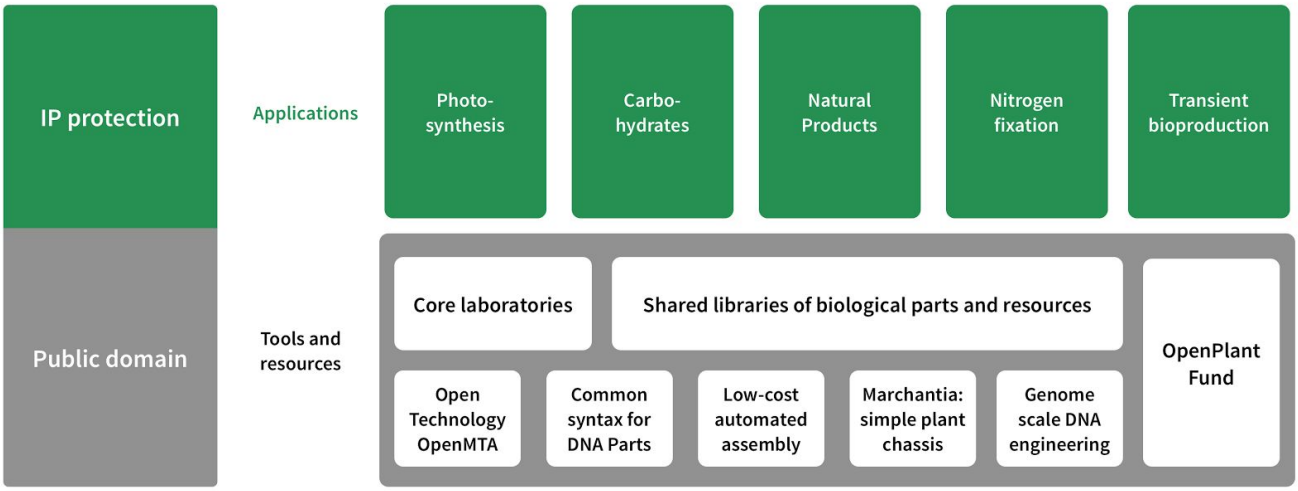

Fig. 1 OpenPlant organisation
Two tier structure of OpenPlant activities, with open technologies, simple plant systems, standardised DNA parts and assembly methods forming a basis for development of new traits.

Implementation

The OpenPlant initiative supports two tiers of activities. 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.

Further, 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.

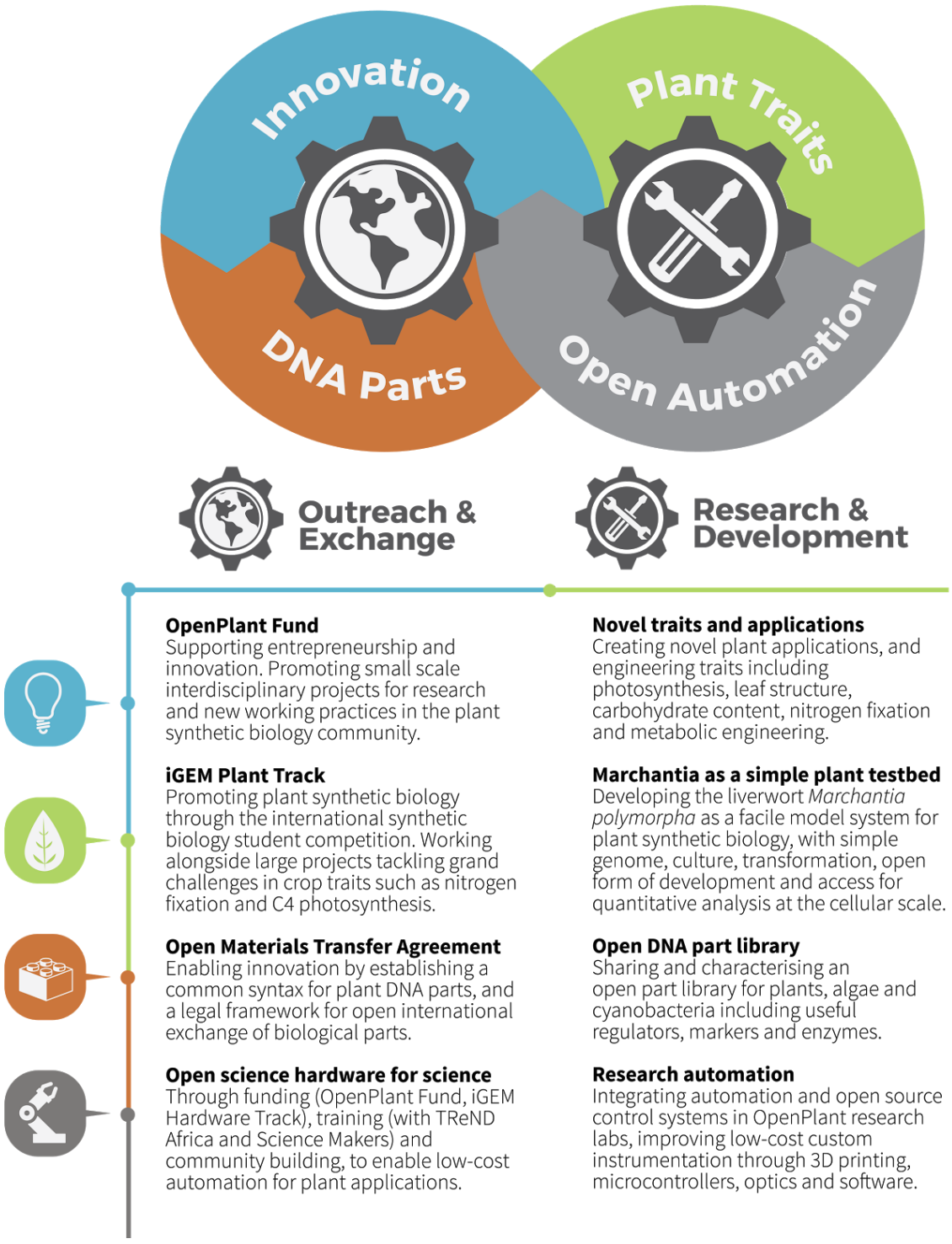

Fig. 2 Schematic view of OpenPlant activities

Improved legal mechanisms and libraries for open exchange of DNA parts (brown), along with standardised and automated assembly and biological testing (grey) underpin faster development of new plant traits (green), and facilitate wider innovation, scientific leadership and international collaboration in the field (blue).

Vision and Ambition

As part of the OpenPlant initiative, we plan to implement technologies for engineering plants:

- Open DNA registries for sharing information, and join an international web of registries with the first plant specific parts.
- A major new plant chassis for Synthetic Biology, with simple properties for high throughput screening and analysis at the micron scale.
- New DNA parts for the control and quantitative imaging of genetic circuits in plants.
- Techniques for routine genome-scale engineering in plants.
- Software tools with improved performance for automated DNA assembly, modeling of synthetic gene circuits and cellular morphogenesis.

The development of new tools and parts will directly contribute to the engineering of new traits in plants, such as:

- Altered photosynthesis and leaf structure.
- Changes in plant carbohydrate content.
- Engineered pathways for the metabolic engineering of natural products.
- New forms of symbiosis and nitrogen fixation for crop plants.
- Methods for high level production of biomolecules by transient expression.

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.

Research programme

Workpackage A: Simple Plant Chassis, Tools and Gene Delivery

Relationship to other projects/themes

The liverworts (or Marchantiophyta) are descendants of the earliest terrestrial plants. The group is characterised by morphological simplicity, and this is matched by simple underlying genome structures. Many lower plants, including liverworts, demonstrate a striking tolerance of extreme stresses, a trait that would be valuable in a production system. Liverworts have been a largely neglected area of plant biology, but show promise as new experimental systems after recent developments in transformation methods and genome characterisation. *Marchantia polymorpha* is the best characterised liverwort plant. It is a common weed, and can grow quickly and resiliently. The relative simplicity of genetic networks in Marchantia, combined with the growing set of genetic manipulation, culture and microscopy techniques, are set to make this primitive plant a major new system for analysis and engineering. We aim to establish Marchantia as a testbed for plant synthetic biology, which will provide a prototype for other OpenPlant initiatives in higher plants.

Investigators

Jim Haseloff (15 days); Giles Oldroyd (1 day); Jim Ajioka (4 days); Pietro Cicuta (6 days); Lisa Hall (2 days)

Staff Employed

Susana Sauret-Gueto (Research Manager; Haseloff lab). Started October 2015.
Linda Silvestri (Research technician, Haseloff lab). Started February 2016.
Fernan Federici (PDRA, Haseloff lab). July-Dec 2015; July-Dec 2016.
Tim Rudge (PDRA; Cicuta lab). Started August 2015.
Tom Meany (PDRA; Haseloff and Hall labs). Started October 2015.

Partners

Bernardo Pollak; Christian Boehm; Mihails Delmans

Aims

The aim of this Workpackage is to exploit the extraordinary experimental properties of Marchantia, and produce systematic collections of (i) experimental protocols and (ii) shared DNA parts. This will include a comprehensive collection of promoters, selection markers, and fluorescent and biopigment reporter genes. In addition, (iii) we will produce and distribute Marchantia lines with integrated cell fate markers in order to track physiological and morphogenetic changes. We will distribute information using laboratory-based web sites and instances of the JBEI-ICE registry, and distribute DNAs and plant material via the OpenMTA. The same methods for public distribution will be used widely in other OpenPlant workpackages.

Current milestones

A2: Establishment of microfluidic platforms for high throughput single cell imaging. □Deliverable: Production of microfluidic devices for culture, sensing and time-lapse observations (month 24, Cicuta, Hall).

A3: Establishment of Marchantia spore transformation system. □Deliverable: Validation of Marchantia spore transformation using GFP expression (month 24, Haseloff, Oldroyd, Schornack, Osbourn, Smith).

Progress to date

A1: Finished tender process and installation of: advanced imaging equipment, including (i) a Leica SP8 confocal laser scanning microscope with hybrid detectors and white light laser, (ii) upgrade to a Leica SP5 confocal laser scanning microscope for routine work, and (iii) a Keyence VHX-5000 3D surface imaging microscope and robotics equipment, including a Labcyte Echo 550 acoustic focusing liquid handler at the Cambridge OpenPlant laboratory.

A2: Dr. Tim Rudge commenced work in the Cavendish Laboratory in Pietro Cicuta's Lab on a short-term microculture project - developing approaches for quantitative measurement of gene expression in single cells. Using *E.coli* expressing GFP under the control of both constitutive and regulated promoters, he investigated correlations between the rate of growth and the rate of gene expression, and the spatial variations of both of these within the colony. It was possible to optimise confocal imaging of colonies growing from single cells up to millimetre size three dimensional colonies. Initial results indicate changes in both growth and gene expression between the core and edges of the colony, and image analysis, including optical flow algorithms was used to extract growth when cell segmentation became unfeasible. This work provides a programmable cell populations as testbeds, and allows better understanding of how physical structure in a growing clonal population can lead to morphological differentiation even in a simple prokaryote system. The biological understanding, and the experimental methods, can be ported to other cell types. This work is developing further through appointment of Dr, Lucia Parolini from November 2016, who will be looking at algal cells, and through continuing collaboration with Dr Tim Rudge, now a Lecturer at the Universidad Católica de Chile in Santiago.

Dr. Tom Meany was appointed to a shared position in Jim Haseloff and Lisa Hall's laboratories, where the OpenPlant funded position was extended by the joint award of a Wellcome Trust Interdisciplinary Fellowship. Tom has been developing Raman spectroscopy based approaches for high throughput analysis of plant oil cells in Marchantia, using a combination of microfluidic and *in planta* approaches. He has used this non-invasive microscopy approach to detect terpene-like molecules in oil bodies, and is validating these observations using micro sampling and mass spectroscopy.

A3: Ahead of schedule, Bernardo Pollak (Haseloff lab) has established a reliable agar-trap Marchantia spore transformation system, which is capable of high throughput production of transgenic plants. Further, Guru Radhakrishnan (Oldroyd lab) developed a transformation protocol for vegetative tissues, based on blender treatment and plantlet regeneration. This has primarily been applied to *Marchantia paleacea*. This is a useful technique for super-transformation experiments, and the protocols are shared.

A4: We have refactored spectral variants of fluorescent proteins for efficient expression in Marchantia, and developed a second and third generation GAL4 enhancer-trap systems. These are based on our published *in planta* cytometry method. The approach relies on the use of spectrally distinct fluorescent protein markers that allow autosegmentation of cell geometries and quantitative assignment of biological parameters on a cell-by-cell basis. The first version of the enhancer-trap vector lacked a

counter-marker for cell outlines, but was successfully tested for specific enhancer-trap patterns of gene expression. The second and third versions are being tested for improved plasma membrane-localised expression of red fluorescent proteins. We plan to scale-up the transformation screen over the next six months.

A5-6: Bernardo Pollak has generated draft genome and transcriptome sequences for the CAM-1 (male) and Cam-2 (female) isolates of *Marchantia polymorpha*. He and Mihails Delmans have annotated the genome, and constructed and published an online, gene-centric database (www.MarpoDB.io) that allows facile access to gene features from the *M. polymorpha* genome. In Norwich, the Oldroyd lab has generated genome sequence for *M. paleacea* (which, unlike *M. polymorpha*, forms symbiotic fungal associations). MarpoDB provides features that allow mining of the genome dataset for synthetic promoters, genes and terminators that can be exported directly as sequence files for synthesis of standardised Phytobrick DNA parts.

The annotated genome sequence has provided a basis for comparison with the Tak-1 genome, which is best annotated and becoming the community standard. Polymorphisms between the Tak-1 and Cam-1/2 isolates are around 1/1000 bp within genes.

In addition, the annotated genome has allowed precise analysis of transcription dynamics in germinated Marchantia spores, where changes in mRNA production have been mapped over the crucial early stages of germination, cell enlargement, chloroplast differentiation, asymmetric cell division, rhizoid and thallus formation and cell differentiation. This map, and sequence comparison between the Cam-1/2 and Tak-1 isolates is a contribution to the international four-part Marchantia genome paper that is being prepared for publication.

The availability of the MarpoDB database has allowed the extraction of core promoter sequences from genes homologous to those that play important roles in meristematic growth in higher plants. These candidate promoters have been fused to fluorescent protein coding sequences and transformed into Marchantia. Ratiometric imaging techniques have been used to quantify promoter-driven gene expression, and to validate the properties of the synthetic promoters. We are now in a position to scale up this approach to generate a comprehensive library of core promoters from all regulatory genes in Marchantia.

MarpoDB has also been useful for the identification of CRISPR sites and generation of targeted CRISPR-Cas9 gene knockouts for mutant analysis in transformed sectors and plantlets.

Liverwort diversity: Jim Haseloff attended a bryophyte field workshop in August 2016 in the Flinders Ranges, Australia. As well as being introduced to a diverse range of liverwort species (http://data.plantsci.cam.ac.uk/Haseloff/synbotany/page-2/index.html), he was able to meet a number of international bryologists, and is initiating a plant exchange between the Cambridge and Australian National Botanic Gardens, with the help of Christine Cargill, Sam Brockington and Beverely Glover. Arid zone acclimatised liverworts have a number of adaptations to temperature and desiccation that are experimentally useful, and show a wealth of morphological diversity, perhaps not seen in higher plants. We aim to build a living collection of these simple plants that will provide material for comparative studies of genome architecture and bioconstruction.

Evidence of the quality of the research

Invited presentations of the Marchantia work were made at:
International Plant Molecular Biology meeting, October 2015, Brazil;
Royal Society Discussion Meeting: Trends in Synthetic Biology and Gain of Function and implications for Regulation, November 2015, Chicheley Hall;
Cellular and Molecular Biotechnology, December 2015, IHES, Paris;
Invited seminar at MIT Synthetic Biology Center, March 2016, Boston;
Synthetic Biology Workshop, March 2016, Singapore;
Australian Synthetic Biology Congress, March 2016, Canberra;
PUC Open Technology workshop, May 2016, Santiago;
New model systems for early land plant evolution EMBO workshop, June 2016, Vienna;
British Biophysical Society Biennial Meeting, June 2016, Liverpool, and
CRA Snowbird meeting, July 2016, Utah.

Publications:

The Naming of Names: Guidelines for Gene Nomenclature in Marchantia. Bowman JL, Araki T, Arteaga-Vazquez MA, Berger F, Dolan L, Haseloff J, Ishizaki K, Kyozuka J, Lin SS, Nagasaki H, Nakagami H, Nakajima K, Nakamura Y, Ohashi-Ito K, Sawa S, Shimamura M, Solano R, Tsukaya H, Ueda T, Watanabe Y, Yamato KT, Zachgo S, Kohchi T. Plant Cell Physiol. 57:257-61, (2016).

MarpoDB: An open registry for *Marchantia polymorpha* genetic parts. Mihails Delmans*, Bernardo Pollak* and Jim Haseloff. Plant Cell Physiol. Submitted

Other evidence of impact

Distribution of Cam-1 and Cam-2 Marchantia germplasm.
Access to MarpoDB website.

Workpackage B: DNA Assembly

Relationship to other projects/themes

Standards for DNA parts and assembly underpin the development of other technologies and platforms and therefore the outcomes of workpackage B are relevant to other platforms and technologies, especially workpackage D (Genome assembly).

The genome editing tools and large-scale gene assembly technologies developed in workpackages B and D will be of direct benefit to all the trait-engineering work-packages (F, G, H, I and J). Specifically, vectors for chloroplast manipulation and methods to achieve homoplasty are being developed for use in workpackage F and molecular tools for targeted genome editing are being developed for use in workpackages G, H and I.

Investigators

Nicola Patron (5 days); Jim Haseloff (5 days); Jim Ajioka (4 days); Giles Oldroyd (0.5 days); James Locke (2 days); Christopher Howe (0.5 days)

Staff Employed

Oleg Raitskin (PDRA; Patron lab). Started January 2015.
Douglas Griffith (PDRA; Locke lab). Started July 2015.
David Willey (PDRA; Ajioka lab). Starts September 2015.
T.B.A (PDRA; Schornack lab). No candidates identified in 1st round. Re-advertise in Q4 2015

Partners

The CRISPR/Cas9 workshop held at JIC in September 2015 was co-funded by the Genomic Arabidopsis Resource Network (GARNet)

Through Workpackage I (N2 Fixation), OpenPlant interfaces with the Engineering Nitrogen Symbiosis for Africa (ENSA) project and other Gates-funded cereal engineering projects. Collaborations to accelerate the application of genome engineering technologies are being established, including through seed-funding from the Gates Cereal Engineering Consortium.

Henderson lab, University of Cambridge
Uauy lab, John Innes Centre
Wendy Harwood, John Innes Centre

Aims

Hierarchical DNA assembly methods are a necessary part of genome construction and modification. These two workpackages aim to (i) set standards for plant synthetic biology; (ii) establish registries for sharing of plant-specific DNA parts; (iii) generate collections of plant DNA parts; and (iv) create standard tools for the engineering of plant genomes.

Current milestones

B2: Installation of central database for sharing of published DNA details.
Deliverable: Installation of servers and publication of first records (month 24, Haseloff, Patron, Oldroyd).

Progress to date

B1-2: 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 work.

Last year, with wide support from the international plant science community we 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 (Rutten et al. 2015; http://hdl.handle.net/1721.1/96069), posted through the BioBricks Foundation. This was a collaborative output from the Cambridge, Valencia and Norwich Research Park iGEM teams. Following this, the iGEM foundation have agreed that parts can be submitted to the Registry of Biological Parts in RFC 106 (and are termed Phytobricks). This has set the foundation for an inaugural Plant Prize at the 2016 iGEM competition.

This advance means that the iGEM Registry provides a low cost, global method for description and distribution of Phytobrick DNAs. Agreement on a standard genetic syntax has provided a coordinated approach to Milestones A1, B1 and E1. We now have a basis for tackling our goals in automated DNA assembly and the establishment of a central database/registry for plant parts to underpin Work Packages A, B, D and E.

During this work, we have continued to synthesise and distribute Phytobrick compatible DNA parts, for example: https://www.addgene.org/Nicola_Patron/. The work described in Workpackage A has provided us with an annotated sequence database, software tools and proof of principle for our next major task - creating a large library of synthetic DNA parts for Marchantia.

The Phytobrick standard is simply a consolidated and consistent standard for Type IIS restriction endonuclease-based assembly of DNA parts to make synthetic genes. We have been deliberately agnostic about higher level gene assembly, where Gibson assembly and higher order Mo-Clo and Golden Braid techniques are generally used. Work by Fernan Federici and Bernardo Pollak has demonstrated the feasibility of using unique nucleotide sequences (UNS, first popularised in Pam Silver's laboratory) as landing pads for assembly of multigene constructions, using conserved sets of oligonucleotide adapters. This provides a flexible and low cost method of assembly in laboratories where custom oligonucleotides are expensive or difficult to obtain. We are constructing a series of plant based vectors to exploit this method of assembly.

Further, we have produced MarpoDB, which is "gene-centric" database. MarpoDB describes and 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 will be essential for tackling the refactoring of synthetic plant genomes. MarpoDB also provides a useful container for gene expression data, and we hope that this will be integrated with cellular features via Plant Ontology terms. More details about MarpoDB are provided in the Workpackage E description. Addressing Milestone B2, we have installed the database on a server at the University of Cambridge, and have published our Marchantia genome data at www.MarpoDB.io.

Evidence of the quality of the the work

Standards for plant synthetic biology: a common syntax for exchange of DNA parts. Patron NJ, Orzaez D, Marillonnet S, Warzecha H, Matthewman C, Youles M, Raitskin O, Leveau A, Farré G, Rogers C, Smith A, Hibberd J, Webb AA, Locke J, Schornack S, Ajioka J, Baulcombe DC, Zipfel C, Kamoun S, Jones JD, Kuhn H, Robatzek S, Van Esse HP, Sanders D, Oldroyd G, Martin C, Field R, O'Connor S, Fox S, Wulff B, Miller B, Breakspear A, Radhakrishnan G, Delaux PM, Loqué D, Granell A, Tissier A, Shih P, Brutnell TP, Quick WP, Rischer H, Fraser PD, Aharoni A, Raines C, South PF, Ané JM, Hamberger BR, Langdale J, Stougaard J, Bouwmeester H, Udvardi M, Murray JA, Ntoukakis V, Schäfer P, Denby K, Edwards KJ, Osbourn A, Haseloff J. New Phytologist 208:13-9, 2015

Other evidence of impact

Efforts to introduce plant chassis and standardised DNA parts at iGEM were highlighted in Synthesizing Tomorrow (Nature Plants 1, Article number: 15047 (2015).

Details of the Phytobrick standard and comprehensive instructions for how to synthesise and clone parts for distribution have been assembled on the iGEM website (http://2016.igem.org/Resources/Plant_Synthetic_Biology/PhytoBricks). A Special Award for Excellence in Plant Synthetic Biology will be awarded at the 2016 iGEM competition. Patron provided DNA resources for inclusion in the 2016 iGEM distribution kit. A plant committee has been established: Haseloff, Patron, Diego Orzaez (IBMCP, Valencia) and Dr. Heribert Warzecha (Technische Universität Darmstadt). The official introduction of the Phytobrick standard into the iGEM competition is provided a major impetus for wider recognition and adoption of the standard.

Workpackage C: New mechanisms for Regulation of Gene Expression

Relationship to other projects/themes

This project relates to the following projects:
Riboswitch in new chassis (C3) and Riboregulator circuits (C4), led by Alison Smith. Tools for transgene delivery into the alga Chlamydomonas and for the control of gene expression are common interests.
Cyanobacteria circuits (B3), led by James Locke lab. Similar methods for the quantitative measurement of circuit outputs will be implemented.
Other projects running in the RNA silencing lab. Tools, mutant strains, methods and characterization of components of the RNA silencing machinery in Chlamydomonas are being exchanged.

Investigators

David Baulcombe (5 days); Alison Smith (Cambridge; 4 days)

Staff Employed

Francisco Navarro (PDRA; Baulcombe lab). Started May 2015

Partners

An OpenPlant Fund grant has established new collaborations between OpenPlant PDRA Francisco Navarro and John Innes Centre bioinformatics specialist, Marielle Vigouroux, to develop a codon optimisation tool.

Aims

1) To develop tools of gene expression control using the endogenous RNA silencing machinery of the green alga Chlamydomonas reinhardtii.
2) To design and construct synthetic gene circuits of increasing complexity using miRNAs.
3) To evaluate the mechanism of sRNA-dependent gene silencing in Chlamydomonas by quantitative methods.

Current milestones

C1: RNA silencing modules for regulation of genes in a land plant and an alga.
Deliverable: Identification of endogenous siRNA and miRNA loci from a land plant and an alga that could serve as the backbone for construction of RNA silencing modules (month 6, Baulcombe).
Deliverable: Assembly of test modules incorporating the backbones and demonstration that they can be used to silence gene expression in a land plant and an alga (month 18, Baulcombe).
Deliverable: Analysis of variant test modules in different tissue/growth states to characterize effective silencing systems and targeting rules (month 24, Baulcombe).

Progress to date

C1.1: Complete. Endogenous miRNA precursors for synthetic gene circuit constructs were identified, characterized and modified. Please refer to 2015 report for more information.

C1.2: We have increased the number of available parts of the Chlamydomonas MoClo Kit, allowing fast and efficient construction of DNA molecules. DNA construction is based on modular assembly (Golden Gate). A similar modularity strategy has also been applied to the construction of miRNA precursors. miRNA precursors have been included in several sites of the DNA constructs, and the impact in the expression of the cassette and in miRNA maturation have also been assessed. We have

used an endogenous gene (*MAA7*) to confirm the functionality of miRNAs. Repression of *MAA7* confers resistance to the metabolic drug 5-fluoroindole offering an easy and fast reporter sytem.

Fluorescence proteins are being used as reporters of gene expression and silencing, allowing us to study the miRNA function both at population and single cell level. Nine different fluorescence proteins, which were codon optimized for expression in Chlamydomonas, have been tested to find suitable reporters. We have selected two fluorescence proteins with non-overlapping spectra that are expressed to detectable levels. Methods of fluorescence measurement have been established using fluorescence plates readers in Haseloff lab. Work in collaboration with James Locke is establishing single cell measurements.

Transgene expression is frequently suppressed in Chlamydomonas. We have tested several methods to overcome suppression in order to obtain detectable amounts of a fluorescence reporter. Selection using the antibiotic zeocin rendered transformants with high expression levels of the transgene in the wild type background. In addition, we are also using a mutant strain that confers high transgene expression without interfering with the miRNA pathway.

C1.3: We are now setting up a simple circuit in Chlamydomonas in which both miRNA and target molecule are produced from synthetic DNA constructs. These circuits carry two reporter systems: one that reports for the miRNA expression, and a second that reports for the miRNA-dependent silencing. We are currently testing the functionality of this circuit. This circuit will be used to characterize the kinetics and key parameters of the basic unit of the miRNA-dependent gene silencing, including targeting rules of miRNAs in Chlamydomonas. The use of fluorescence reporters is revealing cell-to-cell variability in the activity of the circuit described above. Understanding this variability will be useful to confer more robustness to our synthetic gene circuits.

Evidence of the quality of the research
No outputs have been published yet.

Other evidence of impact
The contribution of DNA parts from Alison Smith (Cambridge) labs is increasing the number of available parts in the Chlamydomonas MoClo Kit. This parts kit is being used as an example to test platforms for DNA parts sharing by a group of scientists in Cambridge interested in Synthetic Biology (ROC) and Open science.

Workpackage D: Genome Engineering

Relationship to other projects/themes

The genome editing tools and large-scale gene assembly technologies developed in workpackage D will be of direct benefit to all the trait-engineering work-packages (F, G, H, I and J).

Specifically, vectors for chloroplast manipulation and methods to achieve homoplasty are being developed for use in workpackage F and molecular tools for targeted genome editing are being developed for use in workpackages G, H and I.

Additionally, the standards assembly technologies from workpackage B have been applied to the technologies developed in workpackage D.

Investigators

Nicola Patron (10 days); Jim Haseloff (5 days); Jim Ajioka (4 days); Giles Oldroyd (1 day); James Locke (2 days); Christopher Howe (0.5 days); Alison Smith (2 days); Sebastian Schornack (8 days); Julian Hibberd (1 day)

Staff Employed

Oleg Raitskin (PDRA; Patron lab). Start January 2015.
Douglas Griffith (PDRA, Locke lab). Start July 2015, end November 2016
David Willey (PDRA, Ajioka lab). Start September 2015, end April 2016
Orr Yarkoni (PDRA, Ajioka lab). Start May 2016
Philip Carella (PDRA, Schornack lab). Start September 2016

Partners

The CRISPR/Cas9 workshop held at JIC in September 2015 was co-funded by the Genomic Arabidopsis Resource Network (GARNet) and sponsored by Methods in Plant Biology. A special collection of methods on Plant Genome Engineering was published in conjunction with a meeting report: http://www.biomedcentral.com/collections/plantgened

Collaborations to accelerate the application of genome engineering technologies have been established:

- Henderson lab, University of Cambridge (Schornack)
- Uauy lab, John Innes Centre (Patron)
- Harwood lab, John Innes Centre (Patron)
- Wulff lab, John Innes Centre (Patron)
- Raines lab, University of Essex (Patron)
- Banfield lab, John Innes Centre (Patron)
- McCormick lab, University of Edinburgh (Patron)

Harwood (JIC) and Patron were awarded a BBR (Targeted gene knockouts in crops using RNA-guided Cas9 nuclease BB/N019466/1). This will commence in Q4 2016.

Current milestones

None until Month 36

Progress to date

Workpackage D incorporates two main approaches: (1) the isolation and manipulation of large DNAs, and the use of CRISPR/Cas9 for targeted editing of chromosomes.

D1-3: David Willey and Orr Yarkoni have been appointed in Jim Ajioka's lab to construct yeast based systems for genome-scale DNA assembly, editing and shuttling to plant systems. The 121KB Marchantia chloroplast genome is the first target for large DNA manipulation. The size of the chloroplast genome is beyond the range of conventional plasmid cloning strategies, but is relatively small, easier to handle *in vitro* and and of great interest for metabolic engineering. In the first stage of the work, The *M. polymorpha* plastid genome annotation has been manually curated, as there have been some expected and unexpected findings, finer resolution curation is also necessary and ongoing. Several poorly annotated genes have been identified and updated. Predicted functions for a few genes: MapoCp048, MapoCp058, ORF167 (>90% homology), and a few where the nearest homolog is still >55%, (MapoCp087, MapoCp088, MapoCP005, MapoCp023).

A search for Restriction Enzyme engineering sites for a synthetic genome has been performed. Enzymes SbfI, ApaLI, AdeI, Tth111I, SexAI and CspCI have been identified as enzymes with no cut sites in areas of the genome which would be difficult to engineer, such as tRNAs and rRNAs. A MCS style approach is being used for the genome design, where these cut sites can subsequently be used to easily access the genome for further modification. DNA assembly strategy design for the synthetic plastid genome is ongoing, as manual curation of the genome has identified areas that are complex to engineer.

Last year, Christian Boehm (Haseloff lab) established plastid transformation in Marchantia. He has created the first fluorescent markers for liverwort plastid transformation. Mario Juhas from the Ajioka group has assembled elements from Christian Boehm's vector into a BAC, which could potentially be used to facilite large DNA manipulation prior to final chassis transfer into *M. polymorpha*. This will be tested in Marchantia. Further, Yarkoni has constructed 14 Fluorescent reporters have been chosen with spectra ranging from 355nm excitation to 670nm emission, essentially from UV to near infrared. The proteins AEblue, Sirius, eBFP2, eforRED, iRFP670, mCardinal, mCerulean3, mNetpurne, mOrange, mPlum, mRaspberry, mTurquoise, mVenus and tagCFP were inserted into a vector backbone developed by Christian Boehm. Reporters were optimized using a codon optimization tool developed in the Ajioka lab and adapting it for the specific requirements of the Marchantia plastid genome. Of these, 13 have now passed into final stages of assembly and will be trialed in Marchantia in the coming months.

D4: Oleg Raitskin (Patron lab) has developed a suite of Cas9 variants and an associated toolkit for targeted mutagenesis and gene deletion in multiple plant species. Currently these are being compared for specificity and efficiency using next-generation sequencing technologies and digital-droplet based PCR assays established last year. Assembly of plasmid vectors for targeted mutagenesis is being automated at the DNA foundry at the Earlham Institute (Patron lab). A protoplast assay for rapid assessment of constructs has been established for tobacco, Arabidopsis and barley. Recombinant Cas9 protein has been produced and purified and trials are in progress for the delivery of the protein-RNA complex to plant cells are in progress. The Patron lab has demonstrated RNA-guided Cas9-mediated targeted mutagenesis and gene deletion has been demonstrated in multiple species including Nicotiana benthamiana, Arabidopsis, tomato (collaboration with Banfield lab), potato (PDRA

Aytug Tuncel; Smith Lab (JIC); workpackage G), barley (collaboration with Harwood, Uauy & Wulff Labs, JIC; Lawrenson et al, 2015), Brassica Oleracea (collaboration with Harwood lab; Lawrenson et al, 2015). Plants (Nicotiana tabacum, Nicotiana benthamiana, arabidopsis and barley) with disrupted selection cassettes have been created to enable efficient recovery of targeted insertion events. Preliminary attempts to repair this cassette by simultaneous delivery of nucleases and repair template have begun.

Bernardo Pollack in the Haseloff lab has demonstrated RNA-guided Cas9-mediated targeted mutagenesis in Marchantia.

RNA-guided Cas9-mediated targeted mutagenesis has also been established in the Schornack lab, where an internal access website for genome editing at UCam has been set up. Sebastian Schornack is collaborating with the Henderson lab in Cambridge, advising on TALEN design.

Evidence of the quality of the research

Induction of targeted, heritable mutations in barley and Brassica oleracea using RNA-guided Cas9 nuclease. Lawrenson T, Shorinola O, Stacey N, Liu C, Østergaard L, Patron NJ, Uauy C, Harwood, W Genome Biology 16; 258, (2015).
Blueprints for Green Biotechnology: Development and Application of Standards for Plant Synthetic Biology. Patron NJ Biochem. Soc. Trans., 44, 702–708, (2016).
Synthetic Plants: in Synthetic Biology Handbook. Patron NJ Ed. Darren N. Nesbeth. CRC Press (2016).
Multi-gene Engineering with RNA-guided Cas9 Nuclease. Raitskin O, Patron NJ. Current Opinion in Biotechnology 37; 69-75, (2016).

Other evidence of impact
Invited Presentations at:
New Genomic Technologies Workshop and Genome Editing Session at the International Conference on Arabidopsis Research, Gyeongju, Korea, June 2016 (Patron)
Invited Presentations in Genome Editing Workshop BrisSynBio, Bristol, March 2016 (Patron)
JIC-TSL Submission to Nuffield Bioethics commission on Genome Editing (Patron)

Meeting review: GARNet/OpenPlant CRISPR-Cas Workshop. Parry G, Patron NJ, Bastow R, Matthewman C Methods in Plant Biology 12:16 (2016).

Workpackage E: Digital Tools

Relationship to other projects/themes
Digital Tools provides underpinning technologies for modelling, DNA assembly and distribution of part data. This supports Work Packages A, B, D and others producing vectors, tools and parts.

Investigators
James Locke (3 days); Jim Haseloff (5 days); Jim Ajioka (4 days); Nicola Patron (0.5 days); Giles Oldroyd (0.5 days)

Staff Employed
None

Partners
Mihails Delmans; Bernardo Pollak

Aims
Software tools play an increasingly important role in Synthetic Biology experiments, as we automate experiments, and the systems we construct increase in scale. In order to accurately predict the behaviour of biological systems, which are governed by multiscale parallel and feedback regulated genetic, physical and chemical interactions - we need computational models. This workpackage aims to provide software to automate DNA assembly and the quantification of gene expression in plant in addition to providing models for gene expression and cell growth.

Current milestones

E2: Software for automated quantification of gene expression *in planta*.
Deliverable: Public release* of open source software routines for automated processing of gene expression data in microbes and plants (month 24, Haseloff).

Progress to date

E1: Agreement on a standard genetic syntax for plant DNA parts (Patron et al., 2015) has provided a coordinated approach to Milestones A1, B1 and E1 and a basis for building an automated DNA assembly process and the establishment of a central database/registry for plant parts. This underpins Work Packages A, B, D and E.

The popularity of the Type IIS assembly technique across the OpenPlant community has meant that we have looked at additional database solutions. Last year we had the JBEI ICE Registry software ported to a standalone MacOS app version. We have shared the Xcode development tools with the authors, led by Nathan Hillson, JBEI. Benchling, which is a free web-based solutions that facilitates part sharing and management, has proved popular as it is robust and maintenance free, and has IIS assembly tools. In particular, the ROC group (Researchers for OpenPlant, Cambridge) have started to integrate Benchling into a shared Phytobrick-based workflow.

E2: Over the last year, Mihails Delmans (Haseloff lab) has written MarpoDB, a genecentric database for mining and describing DNA parts from Marchantia (http://marpodb.io). Part of the functionality of the database is described in Workpackage A. The database contains gene models with predicted transcripts, encoded proteins and phyllogenetic comparison data. It maintains links to the Tak-1

reference genome and nomenclature, and allows interpretation of transcriptomic data. It has formed the basis for analysis of differential gene expression in germinating Marchantia spores that will be published with the description of the Tak-1 Marchantia genome. MarpoDB uses a parts based representation for the genome, and allows export of DNA part descriptions for synthesis. Future versions of the database will incorporate more features for describing characterisation of parts and gene expression using Plant Ontology terms.

In addition, the Haseloff lab has developed 3-parameter measurement techniques for quantifying gene expression in cell suspensions in such as way that extrinsic noise is minimised and a reliable estimate of the intrinsic properties of gene promoters can be made (Rudge etal., 2016; Grant et al., 2016). This relies on software models for gene expression, cell growth, and the use of a coexpressed marker to reduce variation. A computational framework has been established to allow automated analysis of microplate reader data, and this has been made available on Github (https://github.com/HaseloffLab/Platypus).

E4: Progress continues with the improvement of the documentation and graphical user interface for CellModeller and this is documented publicly via a website and support forum, the github repository (http://haselofflab.github.io/CellModeller/). The latest features include cell-cell adhesion and cell shape, as well as algorithms for whole colony-scale segmentation from confocal microscopy datasets.

Evidence of the quality of the research

Characterization of intrinsic properties of promoters. Rudge T, Brown J, Federici F, Dalchau N, Phillips A, Ajioka J, & Haseloff J. ACS Synthetic Biology 5:89-98. (2016).
Orthogonal intercellular signaling for programmed spatial behavior. Grant PK, Dalchau N, Brown JR, Federici F, Rudge TJ, Yordanov B, Patange O, Phillips A, Haseloff J. Molecular Systems Biology 12:849-861, (2016).
MarpoDB: An open registry for *Marchantia polymorpha* genetic parts. Mihails Delmans*, Bernardo Pollak* and Jim Haseloff. Plant Cell Physiol. Submitted

Other evidence of impact
Public access to MarpoDB website.

Workpackage F: Modules for engineering photosynthesis in leaf metabolism

Relationship to other projects/themes

Workpackage F aims to employ standardised DNA parts for the assembly of a collection of tools useful for engineering photosynthesis in plants. The will be strong interactions with the standards being established in Workpackages B and D, along with application of the parts in Workpackages A, G, H and J.

Investigators

Julian Hibberd (6 days); Alex Webb (5 days); Jim Haseloff (4 days); Alison Smith (JIC; 1 day)

Staff Employed

Ivan Reyna-Llorens (PDRA; Hibberd lab). Started October 2015

Partners

Jim Ajioka; Nicola Patron; Christian Boehm

Aims

Plant leaves are biofactories that can accumulate valuable products in a number of discrete compartments both within and between cells. Furthermore, they also fine tune synthetic pathways in response to environmental signals. While significant progress has been made in defining cell specific gene expression in roots, this has not been achieved in leaves. This is a bottleneck in engineering this easily harvested organ, and there is no central repository of genetic modules to facilitate this. We aim to provide a library of elements that can be used to drive expression of both nuclear and plastid encoded genes in specific compartments of specific cells of leaves, and in addition to control that expression over the day-night cycle. These modules will be registered and made available in the OpenPlant repository.

Current Milestones

F1: Protein scaffolds for cell specific and targeted intracellular expression in leaves.
Deliverable: Artificial protein scaffolds from bacterial systems that can be assembled in planta. Public release* of DNA parts for scaffolds and cognate ligands (month 24, Hibberd).

Progress to date

The first milestone for the Hibberd laboratory is to develop artificial protein scaffolds from bacteria and assemble these in planta for metabolic engineering. We have designed, synthesized and verified parts according to the PhytoBrick standard (Patron et al., 2015) for the GBD, SH3, PDZ domains and their cognate ligands, all of which derive from metazoans. In addition, we have produced modules for the cohesin and its dockerin from bacterial systems that use the cellulosome complex. These parts were chosen based on previous work in bacterial systems where they have been used to increased flux through metabolic pathways. Each module has been placed into *Arabidopsis thaliana*, and shown to interact via BiFC coupled with Confocal Laser Scanning Microscopy. They are being prepared for public release.

The second milestone is to identify DNA motifs that generate cell specific expression in leaves. Stable transgenic lines of *Arabidopsis thaliana* have been produced, which contain epitope-tagged nuclei and ribosomes driven by cell-specific promoters. These are being characterised and selfed to identify lines that can be used for isolation of RNA that is available for translation, as well as for cell-specific

DNaseI-SEQ. By interrogating these datasets, we aim to identify short DNA sequences that can be used to drive expression of genes in specific cells of the leaf to enhance photosynthetic efficiency.

Evidence of the quality of the research

A Cyan Fluorescent Reporter Expressed from the Chloroplast Genome of *Marchantia polymorpha*. Boehm CR, Ueda M, Nishimura Y, Shikanai T, Haseloff J Plant Cell Physiol. 57(2): 291-9, (2016).

Other evidence of impact

None.

Workpackage G: Carbohydrate Engineering

Relationship to other projects/themes
This workpackage will use DNA assembly technologies and genome editing technologies developed in workpackages B and D. Nicola Patron is co-supervising the potato project described below.

Investigators
Paul Dupree (0.5 days); Rob Field (8 days); Alison Smith (JIC; 7 days); Nicola Patron (1 day)

Staff Employed
Aytug Tuncel (PDRA; Smith lab at JIC). Started January 2015.

Partners
Alison Smith obtained additional financial support from the Norwich Research Park Innovation fund. These funds have been used to establish a transformation method for potatoes in the BRACT transformation group at John Innes Centre in order to progress a CRISPR/Cas9-mediated carbohydrate engineering project.

Research into Emiliania and Prymnesium is supported by a £3.4M Innovate UK grant on 'Glycoenzymes for bio industries' - awarded jointly to University of Manchester, Newcastle University, Institute of Food Research (Norwich) and the John Innes Centre, in collaboration with industrial partners Ludger, Biocatalysts and Prozomix.

Paul Dupree is an Investigator in the Leverhulme Natural Material Innovation Centre, a £2M Leverhulme Trust project in the University of Cambridge, to improve materials from plants, such as timber, for building construction. This will provide additional support to study the properties of plants engineered in OpenPlant.

Aims
Plants provide unrivalled opportunities for provision of sugars and polysaccharides for biorefining, biofuels, animal feed, food and other industrial uses. The main goal of this workpackage is to improve the quality and increase the yield of target polymers, and to alter their structure for higher value applications. The targets will be plant cell wall polymers that important to these applications: xylan, mannan, and novel digestible glucans.

The objectives will be achieved by building a registry of polysaccharide synthesis pathway genes and transcription factors that can be co-ordinately expressed using tested promoters from this and other workpackages.

Current milestones
G4: A tool-kit of algal glucan-active enzymes.
Deliverable: Public release* of carbohydrate-active enzymes mined from red algae genomes (month 24, Field).

Progress to date
Milestones **G1-3** are scheduled to start in year 3. A PDRA will be recruited in Cambridge January 2017. A BBSRC DTP student, Jan Lyczakowski, started June 2016 and has begun preparation of aspects of **G3** xylan engineering. An OpenPlant DTP student Louis Wilson will commence an 8-week rotation in October 2016, and may continue on this project from May 2017.

G4: has been met: A complete informatics analysis has been conducted on two transcriptome data-sets generated by the Field lab for the photosynthetic protozoan Euglena gracilis, cultured under autotrophic and heterotrophic conditions (O'Neill et al., 2015). This identifies an unexpectedly large repertoire of carbohydrate-active enzymes, including many involved in storage beta-glucan metabolism and a range of what appear to be hemi-cellulose- synthesising enzymes, although Euglena is not known to produce such glycans. All data is available via the JIC web site: http://jicbio.nbi.ac.uk/euglena/

Further analyses of algae, such as Emiliania and Prymnesium, is ongoing together with an Innovate UK funded project, to assess their repertoire of polysaccharide and natural product glycosylation capabilities to feed into synthetic biology and industrial biotechnology studies.

We have employed advanced bioinformatics and structure homology prediction approaches to identify candidate algal beta-1,3-glucan phosphorylases. Heterologous expression studies are underway. An homologous bacterial phosphorylase has been crystallised and X-ray diffraction data will be acquired shortly.

G5-6: PDRA Aytug Tuncel (Smith lab) is applying and testing the genome editing tools and technologies developed in the Patron lab (Workpackage D) to generate novel, commercially or nutritionally valuable glucans in model plant and crop species. The primary objective is the creation of potatoes that contain digestion-resistant starches with two major nutritional benefits: reduced calorie intake from consumption of chips, crisps and other potato-based snack 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. Constructs encoding RNA-guided Cas9 to target starch branching enzymes in the potato genome have been assembled and delivered to potatoes by the BRACT group.

Since our last annual report in 2015 we screened several potato plantlets and identified mutants which have successful editings in different isoforms of the starch branching enzymes. These mutant plants, which still retain the wild type form of the genes of interest, are currently being grown to maturity in greenhouse to be re-examined for increased gene editing. In addition, we constructed new vectors which are expected to improve editing efficiency and will soon be used for transformation to create second generation of mutants. As an alternative approach, we are also implementing and optimizing a protocol to isolate protoplasts from potato which can be subsequently transformed with the new constructs and regenerated into plants with a high chance obtaining starch branching enzyme null mutants.

Evidence of the quality of the research

O'Neill et al., 2015. **The transcriptome of Euglena gracilis reveals unexpected metabolic capabilities for carbohydrate and natural product biochemistry**. Mol. BioSyst. DOI: 10.1039/C5MB00319A.

Other evidence of impact

The above-mentioned analysis of the Euglena transcriptome has been released in the CAZy database (http://www.cazy.org/), with primary data in the process of being deposited through EBI.

Workpackage H: Tools for Engineering Plant Natural Products

Relationship to other projects/themes

The HyperTrans plant expression system (Workpackage J) is being heavily used by both the Martin and Osbourn labs. This platform supports the testing and investigation of metabolic pathways and the creation of new compounds. In turn, these projects inform and enable further optimisation of this powerful tool.

Investigators

Cathie Martin (8 days); Anne Osbourn (6 days); Paul O'Maille (0 days) – Left project after moving to a new position; Sarah O'Connor (4 days)

Staff Employed

Yang Zhang (PDRA; Martin lab at JIC). Started January 2015 – Ended January 2016
Don Nguyen (PDRA; O'Maille lab at JIC). Started February 2015 – Ended February 2016
Hans-Wilhelm Nützmann (PDRA; Osbourn lab at JIC). Started September 2014
Michael Stephenson (PDRA; Osbourn lab at JIC). Started February 2015
Benjamin Lichman (PDRA; O'Connor lab at JIC). Started February 2016

Partners

Alain Goossens, VIB, Ghent
Dr. Dae-Kyun Ro, Associate Professor, Department of Biological Sciences, University of Calgary, Canada
Norfolk Plant Sciences has a patent granted in the USA on use of AtMYB12 to modulate metabolism.
An OpenPlant Fund grant has established new collaborations between the Osbourn and Haseloff labs for producing triterpenes in Marchantia.
Dr. Hans Nützmann and Prof. Anne Osbourn have established a new collaborative project with industry partners Croda, UK (Proof of Concept Award 2016, High Value Chemicals from Plants)

Aims

Plants produce a rich and diverse array of natural products. These compounds have important ecological functions, providing protection against pests, diseases, ultraviolet-B damage and other environmental stresses. They are also exploited as pharmaceutical drugs, agrochemicals, within the food and drink industry, and for a wide variety of other industrial biotechnology applications. Although plants are potentially a tremendous source of diverse and valuable natural products, identifying the pathways for the synthesis of these compounds is more complicated than in microbes because the genomes are larger and more complex.
However, advances in sequencing technology coupled with the recent discovery that the genes for natural products pathways are in many cases organised in operon-like clusters within plant genomes; now makes it possible to access the genes and enzymes of specialised metabolism in plants far more readily. We aim to harness and exploit metabolic diversity using synthetic biology approaches.

Current milestones

None until month 36.

Progress to date

H1: The Osbourn lab has developed strategies for discovery of new plant natural product pathways and chemistries based on genome mining for biosynthetic gene clusters (Nützmann et al. 2016; Medema & Osbourn 2016). Use of the HyperTrans transient plant expression platform is enabling rapid functional characterisation of new candidate genes. The Osbourn lab has been focussing on developing the potential of this expression system for triterpene metabolic engineering and has recently been able to generate milligram-gram quantities of purified triterpene analogs, levels that are ample for both structural determination by NMR and for screening for bioactivity. This genome mining approach is also enabling the discovery of enzymes that make entirely new classes of plant natural products, including the first plant sesterterpene synthases.

The O'Connor lab at JIC has recently produced the plant-derived iridoid alkaloid strictosidine in yeast (Brown et al. 2015). PDRA Benjamin Lichman is currently discovering additional enzymes in this pathway to generate more "building blocks" for this work. He has generated a proteome database for trichomes of iridoid producing plants and is now searching this database for new candidate pathway enzymes.

H2: In this project, the O'Maille lab investigated the use of characterized cytochrome P450s in sesquiterpenoid metabolism to produce customized small-molecule products. Using a plug-and-play approach based on the understanding of the involved enzymes, means the natural metabolic diversity can be used to producing desired and/or novel products.

The work focused on a set of cytochrome P450s that oxidize sesquiterpenes in the Asteraceae family. Homology models of the P450s were generated and analysed. Libraries of mutants have been designed based on the enzymes' differences, constructed and evaluated. The product profiles of the mutants have been analysed.

H3: The Martin lab previously reported the development of new Golden Braid compatible vectors for transient induction of gene expression in tomato fruit, the creation of a new set of introgression lines (S. lycopersicum x S. lycopersicoides) and generation of RNA-seq data.

These new tools and resources were used to test candidate transcription factors controlling lycopene, alpha tocopherol and ascorbate production in tomato. So far, the Martin lab have robust evidence supporting two positive regulators of lycopene biosynthesis and a repressor of alpha tocopherol biosynthesis from tomato. They have deleted the gene encoding one of the activators of lycopene biosynthesis using CRISPR/Cas9 and are confirming its role in fruit by phenotypic characterisation of the fruit.

Yang Zhang used these tools to test the activity of a new pathway for flavone synthesis in Scutellaria baicalensis. This resulted in co-authorship on a paper published in Science Advances in April 2016.

Jie Li from Cathie Martin's group was awarded an OpenPlant project with Greg Reeves from Julian Hibberd's group in Cambridge to test the production of capsaicin in tomato. Jie has introduced VpVAN, pAMT, KASI, KASIIIb, BCAT, BCKDH, ACS1 and CS genes into N.benthamiana using the Hypertrans system to determine whether the proteins are active and also whether, in combination, they will make capsaicin.

PDRA Hans Nützmann (Osbourn lab) showed that the histone 2 variant H2A.Z is required for expression of metabolic gene clusters in A. thaliana (Nützmann and Osbourn, 2015). Wider investigations into chromatin regulation led to the identification of a chromatin mark that delineates silenced metabolic gene clusters on plant chromosomes. Knowledge about this mark was successfully applied to identify metabolic gene clusters in different plant species (Yu et al, 2016).

In collaboration with the Alain Goossens laboratory PDRA Hans-Wilhelm Nützmann was able to show that a candidate transcription factor identified by yeast-one-hybrid assays activates promoters of metabolic gene clusters in transactivation assays. The observed activity was dependent on co-incubation with a second transcription factor. The dual regulatory activity may represent a novel mechanism in the control of clustered metabolic pathway genes.

H4: During his time on the OpenPlant project, PDRA Yang Zhang (Martin lab) focussed on characterising the targets of AtMYB12 and SlMYB12 in tomato, as explained in the 2015 Annual Report.

H5: The Osbourn lab has previously shown that the promoters for a specialized metabolite gene cluster from oat (the avenacin cluster) retain their characteristic expression patterns (in the epidermal cells of root meristems) when introduced into diverse plant species as promoter-reporter constructs (Kemen et al. (2014) PNAS, 111:8679). Building on this, we have generated a synthetic gene cluster in which promoters from the avenacin pathway have been used to successfully drive the expression of a three-gene pathway for a plant defence compound (dhurrin) from sorghum in Arabidopsis thaliana roots. This proof of concept experiment will be the first step towards engineering synthetic clusters for the synthesis of other types of compound and for expression of other multi-gene traits (e.g. nitrogen fixation genes).

Evidence of the quality of the research

Publications

Brown et al., 2015. **De novo production of the plant-derived alkaloid strictosidine in yeast**. Proc Natl Acad Sci U S A. doi: 10.1073/pnas.1423555112.
Martin, C., Zhang, Y., et al., 2015. **Different ROS-scavenging properties of flavonoids determine their abilities to extend shelf life of tomato**, Free Radical Bio Med. 86, S11-S11.
Medema, M. H. & Osbourn, A. (2016) **Computational genomic identification and functional reconstitution of plant natural product biosynthetic pathways**, Nat Prod Rep. 33, 951-62
Nützmann H.W. et al. 2016. **Plant metabolic clusters – from genetics to genomics**. New Phytologist 211:771-89
Nützmann H. W., Osbourn A., 2015. **Regulation of metabolic gene clusters in Arabidopsis thaliana**. New Phytologist. doi: 10.1111/nph.13189.
Tohge et al., 2015. **Ectopic expression of snapdragon transcription factors facilitates the identification of genes encoding enzymes of anthocyanin decoration in tomato**. Plant J. doi: 10.1111/tpj.12920.
Yu N., Nützmann H.W. et al. 2016 **Delineation of metabolic gene clusters in plant genomes by chromatin signatures**. Nucleic Acids Res. 44:2255-65.
Zhang, Y., Butelli, E. & Martin, C., 2014. **Engineering anthocyanin biosynthesis in plants**, Current opinion in plant biology. 19, 81-90.

Zhang Y., et al. 2015. **Multi-level engineering facilitates the production of phenylpropanoid compounds in tomato**. Nat Commun. doi: 10.1038/ncomms9635.
Zhang, Y..et al, 2015. **Different Reactive Oxygen Species Scavenging Properties of Flavonoids Determine Their Abilities to Extend the Shelf Life of Tomato**, Plant Physiology. 169, 1568-1583.
Zhao Q., Zhang Y., Wang G., Hill L., Weng J. K., Chen X. Y., Xue H., Martin C* (2016). **A specialized flavone biosynthetic pathway has evolved in the medicinal plant, Scutellaria baicalensis**. Science Advances 2 p4 Publisher's version: 10.1126/sciadv.1501780

Conferences
PDRA Hans-Wilhelm Nützmann has given invited talks at the International Conference on Arabidopsis Research 2016, Gyeongyu, South Korea, and the Next Generation Sequencing in Plants Conference 2016, Halle (Saale), Germany

Other evidence of impact

Industry partnership and Commercialisation
Patent granted in the USA on use of AtMYB12 to modulate metabolism.
PDRA Hans Nützmann's discoveries on chromatin regulation of metabolic gene clusters led to the establishment of a new collaborative project with industry partners Croda, UK (Proof of Concept Award 2016, High Value Chemicals from Plants)
PDRA Michael Stephenson has provided valuable consultancy and knowledge exchange to BBRSC funded JIC spin off company 'Leaf Systems Ltd'.
Public engagement and outreach
Sarah O'Connor gave a talk about her work at the first Pint of Science Festival in Norwich, to a mixed audience of ~45 people.
PDRA Michael Stephenson has given a talk about his work during a Schools visit to the John Innes Centre, and has supervised a work experience placement for a local six-form student.
PDRA Michael Stephenson took part in the OpenPlant Exhibit at Latitude Festival in July 2016, talking to families about engineering plants over the three day festival.

Workpackage I: N2 Fixation

Relationship to other projects/themes

Workpackage I feeds into Workpackage A, assisting in the establishment of Marchantia as a simple plant chassis for synthetic biology through the development and testing of methods and tools, and Workpackage B by producing parts that can be included in the parts collection. Workpackage I tests and uses genome editing tools produced in Workpackage D.

Investigators

Giles Oldroyd (5 days); Jim Haseloff (3 days); Sebastian Schornack (1 day); Nicola Patron (0.5 days)

Staff Employed

Pierre-Marc Delaux (PDRA; Oldroyd lab), Started September 2014 - Ended August 2015 (to take up an independent young investigator position at CNRS in Toulouse). PDRA position in the Oldroyd lab is currently vacant.

Partners

Gates-funded ENSA project (Engineering Nitrogen Symbiosis for Africa)
Three OpenPlant Fund grants have been funded for new collaborations between the Oldroyd group and groups in Cambridge, to explore the evolution of symbiosis signalling using Marchantia paleacea as a model, to develop modules for studying LysM receptor-like kinases and to develop novel cell reporters for high resolution imaging.

Aims

We have initiated an engineering strategy to transfer the recognition of rhizobial bacteria from legumes to cereals, as the first step towards engineering N-fixing cereal crops. This is a strategically important challenge and this Gates and BBSRC-funded programme represents one of the most ambitious engineering strategies in plant signalling. Marchantia provides a fantastic platform for testing synthetic biology approaches in engineering symbiosis signalling that is directly linked to a strategic programme in cereals.

Current milestones

I2: Assembly of genetic components required for engineering Nod factor signalling. □Deliverable: A toolkit of transcription factors and signalling components for engineering synthetic responses to Nod factors (month 24, Patron, Oldroyd).

Progress to date

I1: After screening five *Marchantia spp.* and close relatives for their ability to form arbuscular mycorrhizal symbiosis, we choose to focus our efforts on a *Marchantia paleacea* (*M. paleacea*) isolate. A rapid transformation protocol was previously developed and we have now successfully moved this transformation protocol onto a 96 well format, creating high throughput *M. paleacea* transformation.

I2. Because we needed to develop a new liverwort platform that was able to associate with AM fungi, we could not rely on the existing liverwort genome sequences. We have been sequencing the genome of *M. paleacea* using paired end libraries and illumina sequencing. This has provided a genome of sufficient quality for our studies in this project. For the 250Mb *M. paleacea* genome we have 27,530 scaffolds and an N50 of 87kb. Clearly with so many scaffolds the genome is still quite fragmented and we are currently exploring using PacBio sequencing as a means to integrate

scaffolds. However, our analysis of the existing genome reveals many complete gene models with good levels of promoter sequences attached. Thus for many genes the existing genome provides sufficient resolution.

I3: We have also been developing CAS9 knockouts in *M. paleacea.* For this we have designed a number of different constructs to test the efficiency of CAS9 endonucleases in *M. paleacea.* We are using the *NOP1* gene as our target as this has been shown by researchers in Cambridge to be required for air chambers on the thalli of *M. polymorpha.* Mutation of this gene creates an easily scorable and non-lethal phenotype. We have generated a number of constructs that test different promoters to drive Cas9; codon optimisation of Cas9; different U6 promoters and different sgRNA backbones. All constructs have been transformed into *M. paleacea* and efficiency of CAS9-directed knockouts will be assessed. Using this information we will develop symbiotically relevant CAS9-directed mutants.

Evidence of the quality of the research

Delaux, PM, Radhakrishnan, G, Jayaraman, D, Cheema, J, Malbreil, M, Volkening, J, Sekimoto, H, Nishiyama, T, Melkonian, M, Pokorny, L, Rothfels, C, Sederoff, H, Stevenson, D, Surek, B, Zhang, Y, Sussman, M, Dunand, C, Morris, R, Roux, C, Wong, G, Oldroyd, G, Ane, JM (2015). Algal ancestor of land plants was preadapted for symbiosis. *Proc. Natl. Acad. Sci USA* 112: 13390-13395

Delaux, Radhakrishnan, and Oldroyd. 2015. Tracing the evolutionary path to nitrogen-fixing crops. *Curr. Op. Plant Biol.* DOI:10.1016/j.pbi.2015.06.003

Other evidence of impact

None

Workpackage J: Virus-based systems for bioproduction

Relationship to other projects/themes

Workpackage J provides a plant expression technology for use by others within the OpenPlant consortium and throughout the world. The technology is already well integrated and being used in Workpackage H. Work to extend the range of hosts for protein expression is carried out through interaction with Workpackage A.

Investigators

George Lomonossoff (8 days); Sarah O'Connor (1 day); Anne Osbourn (0.5 days)

Staff Employed

Eva Thuenemann (PDRA; Lomonossoff lab). Started November 2014

Partners

LeafSystems® International Limited

Aims

The CPMV-HT technology, and its associated pEAQ vectors (Sainsbury & Lomonossoff, 2008; Sainsbury et al., 2009), developed by George Lomonossoff (JIC) has established a unique position for the UK for rapid transient expression of proteins in plants through Agrobacterium-mediated infiltration of Nicotiana benthamiana leaves. CPMV-HT is a highly flexible system and will be developed for a range of applications in the field of plant synthetic biology.

Current milestones

J1: A series of expression vectors with defined translational characteristics.
Deliverable: Distribution of new viral expression cassettes with fine-tuned levels of translation efficiency (month 24, Lomonossoff, Osbourn).

Progress to date

J1: A series of vectors has been made to enable fine-tuning of protein expression levels by making changes in the 5'- and 3'-UTRs and these modified vectors have been made available to the OpenPlant consortium. Alternate versions of the CPMV-HT sequences have been made compatible with the common plant syntax (Patron et al., 2015) by the Patron and Osbourn groups.

J2: The CPMV-HT system has recently been tested in Marchantia (in collaboration with Jim Haseloff's group, Workpackage A) and has been used successfully in the BY2 cell pack system (developed by Fraunhofer Institute, Aachen, Germany) by the Lomonossoff group. The BY2 system in particular has potential for high-throughput screening of CPMV-HT expression constructs. A training workshop will be run at JIC in July 2016 for anyone interested in the system.

Staff involved in this workpackage are advising on the design of a translational facility (LeafSystems® International Limited) for scaling up production using the HyperTrans system, which is due to be for completed in Q2 2017. Information about the new facility has been communicated at various meetings and conferences.

Evidence of the quality of the research

Publications

Lebedev N., Griva I., Dressick W. J., Phelps J., Johnson J. E., Meshcheriakova Y., Lomonossoff G. P., Soto C. M. (2016) **A virus-based nanoplasmonic structure as a surface-enhanced Raman biosensor**. *Biosensors and Bioelectronics* **77**, 306-314.

Saxena P., Lomonossoff G. P. (2016) **Production of virus-like particles in plants in "Viral Nanotechnology"** CRC Press, Taylor & Francis Group, Boca Raton FL, USA, 251-262.

Saxena P., Thuenemann E. C., Sainsbury F., Lomonossoff G. P.(2016) **Virus-Derived Vectors for the Expression of Multiple Proteins in Recombinant Proteins from Plants** in "Methods and Protocols" Springer New York Heidelberg Dordrecht London **1385** 39-54.

Marsian J., Lomonossoff G. P. (2016) **Molecular pharming-VLPs made in plants**. *Current Opinion in Biotechnology* **37** 201-206.

Peyret H., Lomonossoff G. P. (2015) **When plant virology met Agrobacterium: the rise of the deconstructed clones**. *Plant Biotechnology Journal* **13**, 1121-1135.

Conferences

In the past year numerous talks at various meetings have been given by members of the Lomonossoff group.

Other evidence of impact

Industry partnership and Commercialisation

George Lomonossoff and Eva Thuenemann are involved in scientific committees advising plans for development of the translational facility LeafSystems® International Limited.

The HyperTrans technology is being used under licence by the Canadian company Medicago for production of specific vaccines. Medicago have built scale-up facilities in the US and Canada and have demonstrated proof of concept for commercial production. The smaller LeafSystems® facility will be available in Norwich in 2017. This will enable JIC to step up our capacity to make known and novel molecules for translational research.

Since the start of OpenPlant (Jul 2014), over 50 pEAQ vector kits containing the HyperTrans technology have been sent out to laboratories worldwide under MTAs.

Public engagement, outreach and education

A case-study about the use of plants as biofactories was produced and published by Pearsons as an A-level teachers' resource (Published March 2016: ISBN 9781447977414, ISBN 9781447977452).

TV interviews were given by George Lomonossoff about the CPMV-HT technology and the new Leaf Systems® facility.

A detailed protocol for the use of the CPMV-HT system for expression of fluorescent Bluetongue Virus particles was produced for publication in Methods in Molecular Biology book on Virus-Derived Nanoparticles for Advanced Technologies (publication expected in 2017)

<u>Workpackage K: OpenPlant Fund for interdisciplinary exchange</u>

Relationship to other projects/themes

The OpenPlant Fund provides support for projects relevant to all work packages and fosters interdisciplinary exchange within and between the teams working on the different packages at each OpenPlant institution. The open hardware development and training component also especially supports OpenPlant pathways to international exchange.

Investigators
Jim Haseloff (4 days); Anne Osbourn (1 day)

Staff Employed
Colette Matthewman (Project Manager). Started October 2014.
Jenny Molloy (Project Coordinator). Started February 2015.

Partners
Ionscope Ltd (CEO has contributed in-kind technical assistance, participated in judging panel and provided training for investment pitches to OpenPlant Fund applicants)
Cambridge Consultants (Representative has contributed in-kind technical assistance to teams and participated in judging panel)
Microsoft Research (Representative has contributed in-kind technical assistance to teams and participated in judging panel)

Aims
The OpenPlant Fund was established to support seed projects on a competitive basis through the annual distribution of up to twenty £5000 grants following a lightweight application process and public pitching event. The aim of the fund is 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 Sainsbury Laboratory for the development of open technologies and responsible innovation in the context of Synthetic Biology.

Also within in this work package we aim to promote open source hardware for science through supporting technical development and also the necessary training required to deliver and implement such hardware in synthetic biology laboratories.

Current milestones
K1: Annual funding round to support small-scale innovative research projects.
Deliverable: Distribution of awards and public documentation of project results (annually, months 12-60, Haseloff, Osbourn).
K2: Annual support for open source hardware development and training.
Deliverable: Co-sponsorship of student training, and development and documentation of open source hardware and bioinstrumentation (annually, months 12-60, Haseloff, Osbourn).

Progress to date
The milestones and deliverables for this work package are annual and are on schedule.

K1: Sixteen proposals were funded in the 2015 OpenPlant Fund, and fourteen fourteen were funded in the 2016 OpenPlant Fund (see Appendix 2 for a full list). The topics ranged from DNA part development to open lab hardware, schools outreach, international capacity building, IP policy, software and more. All teams are multidisciplinary and span Cambridge and Norwich. The teams were supported prior to making their applications by mixer events with lightning talks, pitch training and

networking opportunities combined with substantial effort on the part of the management team in making connections and linking collaborators. Several new collaborations arose directly as a result.

The management team made a decision to run future rounds as themed opportunities in order to more effectively seed interactions around the key tools, technologies and outreach initiatives required for plant synthetic biology. Ideas for focus areas were collected at the All-Hands Meeting in May 2016 and include:

- Imaging
- Microfluidics
- Functional materials
- Education and public engagement

These will be translated to one-day mixer events featuring time for presentations from a diverse range of potential collaborators, followed by brainstorming time with the intention that teams coalesce around ideas and develop them into proposals for submission several weeks afterwards.

K2: iGEM teams in both Cambridge and Norwich were facilitated by OpenPlant-supported labs and OpenPlant provided £10k in sponsorship to the Cambridge-JIC team to enter the biohardware track in 2015 and undertake a project competing for the new Plant Special Prize in 2016.

Evidence of the quality of the research

K1: The 2016 OpenPlant Fund projects are underway and not due to report until later this year, a list of titles can be found along with 2015 project summaries in Appendix 2. Further details are available at http://www.openplant.org.

The 2015 OpenPlant Fund projects are now in their second phase of funding to enable them to extend the project, disseminate the results and conduct follow-on and outreach experiments, including journal publications. The early-stage research highlights from the first batch of projects have been:

Development of hardware/software tools and prototypes

The funded hardware and software projects aimed to prototype practical solutions for researchers, thus most are still at the prototype stage and under active testing for research purposes. Most projects aim to do further quality testing or extend the capabilities of their tool in the second phase of their projects and we anticipate publications on either the tools themselves or research performed using the tools in the near future. A selection of tools now available for the research community as a result of the OpenPlant Fund includes:

1. Two plant electrophysiology devices for monitoring plant signalling both remotely for field work through incorporation of radio modules and at low-cost in the lab or in educational settings.
2. A near infrared image capture system based on a Raspberry Pi computer, PiNoir camera and custom 3D printed parts, running Open Pi Image, an extensible and modular open source software suite developed during the project that controls automated image capture and spawns image analysis. This is already being deployed in the Webb Lab for study of circadian rhythms in plants.
3. A novel sensor to evaluate stiffness of plant stems which successfully differentiated differently aged stem samples from willow and has been used in research on *Arabidopsis thaliana* stems with altered composition of cell walls.

4. An open source hardware documentation software and an online repository called DocuBricks (DocuBricks.com). Feedback from users demonstrates that the tool is easy to use and helpful in a wide range of hardware projects and saves documentations in a modular and accessible XML format.

DNA parts and biological tools

Several projects linked biology to design and assessment of DNA parts or developed and characterised new parts to expand the toolkit of plant synthetic biology. Results of note include:

1. 25 novel selection markers for plant transformation were synthesized, including tissue specific promoters and coding sequences of fluorophores and chromophores designed to . advance live-imaging techniques.The most promising result used the Lotus UBIQUITIN promoter, which rendered marker genes detectable under the stereomicroscope and could therefore provide a novel selection marker for live imaging.
2. A platform for measuring the production of a reporter protein, which can be used for testing gene variants to assess impact of codon usage in protein production in the green alga *Chlamydomonas reinhardtii*. The project performed sequence analysis, and developed which analysis, protocols, and materials will be useful for transgene design and expression
3. Golden gate modules including gene promoters, coding sequences and terminators to define the roles of LysM receptor-like kinases in legumes and cereals were designed and constructed. The constructs have been expressed in *Nicotiana benthamiana* and the team is now focusing on transforming the constructs into *Medicago* and rice to detect defense and symbiosis phenotypes.
4. A quick Pulsed-Field Gel Electrophoresis (PFGE)-based analytical system for plastid genome modifications was developed and published, along with publicly available educational resource to help people use the technique (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)

K2:

(i) Cambridge-JIC iGEM 2016 team

The Cambridge-JIC iGEM 2016 project is currently underway. The team are designing a regulatable molecular vector for *Chlamydomonas reinhardtii* using CRISPR/Cas9 system and will take this to the iGEM Jamboree in October 2016.

(ii) Cambridge-JIC iGEM 2015 team

The 2015 team project was entitled ‘OpenScope’ - an open source microscope.

The team undertook a two week crash course in Plant Sciences featuring lectures and activities with faculty from Cambridge and Norwich, including OpenPlant PIs Dr. Jim Ajioka (Pathology & Arsenic Biosensor, University of Cambridge), Dr. Jim Haseloff (Plant Sciences & OpenPlant, University of Cambridge), Dr. George Lomonossoff (JIC) and Dr Nicola Patron (TSL Norwich). Inspired by a 3D printed microscope designed by Dr Richard Bowman (Department of Physics, University of Cambridge), they chose to build an ultra low-cost 3D-printed motorised web-streaming autofocus microscope for synthetic biologists,. This allows researchers to tailor the microscope to their needs - allowing imaging on any lab-bench, in the incubator, fume-hood or the field using remote access and battery power, or use many OpenScopes for parallel rapid preliminary screening. The microscope

offers fluorescence imaging for schools and laboratories with small budgets, based on low-cost (£200 in its most expensive set-up) and easily sourced components.

Functionalities developed include Brightfield (max. 0.4 µm optical resolution, 1 µm movement resolution in xy-plane), Fluorescence (qualitative, GFP imaging) and Darkfield. The stage of the microscope can be translated using 3 stepper motors, which in turn can be controlled via the Arduino using WebShell. OpenScope is internet accessible from anywhere in the world via WebShell and MicroMaps, which also allows unambiguous and accurate distance measurements anywhere within its field of view, easy high quality image and time-lapse capture at any time. An ImageJ plugin was developed to combine the power of ImageJ's annotation tools with the simplicity of OpenScope.

OpenScope was awarded a gold medal at the iGEM Giant Jamboree in Boston, and was nominated by the judging team for four awards:

- Best Hardware Project
- Best Software Tool, Undergrad
- Best Applied Design, Undergrad
- Best Poster, Undergrad

A working set-up of OpenScope and the software was demonstrated to huge interest from other teams and supervisors. A number of team supervisors mentioned that they were considering using OpenScope as an alternative to commercial microscopes in their teaching laboratories, both to reduce costs and as setting up the microscope would be an educational experience in itself.

Workpackage L: OpenPlant Forum: responsible innovation

Relationship to other projects/themes

This workpackage spans all other workpackages in OpenPlant. The annual Forum meeting encourages attendance from all OpenPlant participants and OpenPlant Fund recipients, and all workpackages should be represented. SAW workshops are coordinated by Dr Jenni Rant and opportunities exist for all workpackages and OpenPlant Fund projects to interact.

Investigators

David Baulcombe (1 day); Dale Sanders (0.5 days); Jim Haseloff (4 days); Anne Osbourn (2 days)

Staff Employed

Colette Matthewman (Project Manager, Norwich)
Jenny Molloy (Project Coordinator, Cambridge)

Partners

Jenni Rant - The SAW Trust
Linda Kahl – BioBricks Foundation

Aims

This workpackage involves activities of the annual OpenPlant Forum, the annual working group and workshops and other public engagement activities with the SAW Trust. The OpenPlant Forum will provide a platform for exploring the potential applications of reprogrammed biological systems, and a framework for exploring the wider implications of the potentially disruptive new technologies. Each year, in association with the Forum, a working group will be established for in depth investigation of a topic relevant to the Forum theme.

The SAW Trust provides training in project design to scientists working in collaboration with professional artists and writers who come together as teams to deliver projects themed on the scientists' research topics.

Current milestones

L1.1: Annual symposia on a series of themes related to plant synthetic biology
Deliverable: Devise and convene annual meetings (annually months 12-60, Haseloff, Osbourn).
L1.2: Recruitment of annual working groups
Deliverable: Appoint working groups around the symposia themes, with membership rotating to suit (annually, months 12-60, Haseloff, Osbourn, Baulcombe, Sanders).
L1.3: Annual published report
Deliverable: Document the themed output of the working group and symposium speakers (annually, months 12-60, Haseloff, Osbourn).
L2: SAW workshops
Deliverable: Co-sponsor SAW Trust workshops (annually, months 12-60, Osbourn).

Progress to date

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 (the common syntax; Patron et al., 2015) and IP solutions (OpenPlant IP Working Group; Open MTA),

resources such as DNA parts collections (see workpackage reports) and shared protocols (OpenPlant protocols shared on http://protocols.io/), and building an open community for plant synthetic biology (e.g. through OpenPlant Forum; OpenPlant Fund workshops to strenghten synthetic biology capacity in 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 workshop (e.g. Co-lab OpenPlant workshops) provide further opportunities for discussions on issues related to RRI. To support these activities and enable our PDRAs to contribute more extensively, we delivered a workshop on RRI, ethics and argumentation, and openness attended by all OpenPlant-funded PDRAs and some associates.

Jenny Molloy coordinates quarterly meetings of the Virtual Institute of Research and Innovation (VIRI) in Cambridge, which Colette Matthewman attends when able. 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.

The OpenPlant Fund grant proposals have proved a great resource for the development of more targeted RRI activities, enabling the following workshops:

- Responsible Innovation and Open Innovation with Large BioResources
- Genetic resources in the age of the Nagoya Protocol and gene/genome synthesis
- Co-lab OpenPlant - interdisciplinary workshops of science, art and design

The milestones for Workpackage L are annual and have been completed for the second year, with the organisation of the second OpenPlant Forum (L1.1), the continuation of the IP working group (L1.2), generation of a report from the working group (L1.3; Appendix 3), and the completion of several activities with the SAW Trust (L2).

L1: One of the key deliverables for addressing responsible innovation is the arrangement of an annual themed Forum to discuss wider questions in the field of plant synthetic biology. The 2016 OpenPlant Forum focussed on the theme of reprogramming agriculture.

There are huge opportunities for delivering social, environmental and economic benefits through efforts to reprogramme plants and agriculture, but there are both technical and social bottlenecks that affect progress in this field. The 2016 OpenPlant Forum brought together over one hundred people from various disciplines to hear some of the recent advances in crop and feedstock engineering, discover tools to support innovation in this field, and to discuss and reflect on ethical, legal, social, and economic considerations. Sessions included reprogramming feedstocks, innovation in crops, ELSA, creating tools and developing methods for plant synthetic biology, and enabling innovation through openness and exchange.

The Forum was coupled to an industry showcase and networking event to develop new interactions between academic researchers and companies working in the synthetic biology space.

L1.2 & **L1.3**: Twenty four experts were recruited to the first working group on the topic of IP solutions for OpenPlant and the wider SynBio community. The group was convened at the OpenPlant Forum in July 2015 and continued exchanging ideas and information at monthly teleconferences until December 2016. The purpose of these meetings was to refine the design goals of the Open Materials Transfer Agreement (OpenMTA) - a collaboration between the BioBricks Foundation and OpenPlant to enable open exchange of plasmids and other biological materials. A report has been completed and will be distributed as part of a 'soft launch' of the OpenMTA in the final quarter of 2016 (Appendix 3). Smaller groups were convened throughout 2016 to discuss specific challenges in successfully introducing a mechanism for open transfer within institutions, which requires both strategic partnerships and strategic timing. Progress towards a launch is well underway but a meeting of the larger working group has not been considered timely until the point at which they could add most value to ensuring the OpenMTA is implemented and meets its aims. Co-organisers are in active discussion about the next steps in this process. The topic of the OpenPlant working group will be reviewed for 2017 in light of the good progress made with the OpenMTA to determine if focus on another topic would be fruitful.

L2: Building on activities from the first year of OpenPlant, in the second year we have strengthened the collaboration between OpenPlant and The SAW Trust and increased the scale and impact of outreach and public engagement activities. OpenPlant exhibits were run at the Youth STEMM Awards mid-year conference (Jan 2016), the Cambridge Science Festival (Mar 2016; with OpenPlant Fund Whiskeroscope project), and Latitude Festival (July 2016). For these exhibits, we have designed and developed a modular set of activities and displays that we can mix and match for different events. This enables us to easily adapt to different size events, and shift the focus of the exhibit for different events. These activities will be exhibited at the Norwich Science Festival in October.

A key achievement was securing a place to exhibit in the Kids Area at Latitude Festival in July 2016. Latitude is a mixed arts festival that attracts over 10,000 visitors a year, who enjoy the rich mix of thought-provoking performances and interactive workshops. Combined efforts of OpenPlant, the SAW Trust, and OpenPlant Fund grant award winners, The Big Algal Open Experiment, led to the creation of stand entitled "The Power of Plants". This was an exhibit that led visitors on a journey looking at traditional uses of plants, how plant selective breeding has produced the food crops that we recognise today, tracking the evolution of our relationship with plants through science to introduce the synthetic biology approach, and some of the modern uses of plants and algae that bioengineering enables. The exhibit attracted much attention and was received with great positivity. The first day of the festival, Schools Day, saw the exhibit fully booked with 1 hour workshops for school and public groups repeated throughout the day. We ran an open, drop-in exhibit for the second and third days of the festival.

OpenPlant Fund grant award winners, Carlos Lugo and Marielle Vigouroux, worked with The SAW Trust to develop and deliver a 1-day workshop for Year 6 pupils at Stapleford Community Primary School (South Cambridgeshire). More details can be found on the OpenPlant blog: http://openplant.org/blog/2016/02/openplant-science-art-and-writing-workshop-a-success/

In March 2016, Jenni Rant ran a workshop to introduce OpenPlant PDRAs and associates to The SAW Trust methods of exploring science through art and poetry, and to give them a chance to experience these themselves. Jenni has also run a train the trainer workshop with the UK Centre for Mammalian Synthetic Biology, Edinburgh.

Evidence of the quality of the research

Jenny Molloy and Linda Kahl. OpenPlant Intellectual Property Working Group Meeting Report. 2016. Appendix 3

Other evidence of impact

Blog posts summarising OpenPlant Forum 2016 were published on the OpenPlant website and in the PLOS Synbio Community blog:
https://openplant.org/blog/2016/09/openplant-forum-2016-reprogramming-agriculture-with-synbio/
http://blogs.plos.org/synbio/2016/08/02/seven-developments-in-synbio-science-patents-and-ethics-openplant-forum-2016/
Blog posts summarising OpenPlant activities at Latitude Festival:
https://openplant.org/blog/2016/09/the-power-of-plants-openplant-visits-latitude-festival/
https://www.ucl.ac.uk/biochemeng/news/biochemeng-news-publication/brenda-parker-latitude-2016
OpenPlant web pages showcasing OpenPlant activities out in society:
https://openplant.org/openplant-in-society/

Workpackage M: Governance and Management

Relationship to other projects/themes
This work package is related to all themes in the centre as it involves coordination between all projects and groups and also outreach activities.

Investigators
David Baulcombe (3 days); Dale Sanders (3 days); Jim Haseloff (34 days); Anne Osbourn (5 days)

Staff Employed
Colette Matthewman (Project Manager, Norwich)
Jenny Molloy (Project Coordinator, Cambridge)

Partners
N/A

Aims
This workpackage will be responsible for the overall management and coordination of the project. This will involve key participants and coordinators from within the projects and external advisors. The Coordination and Management groups will play the major role in monitoring progress, and contingency planning. This workpackage will be responsible for running project management meetings, and ensuring coordination of activities between the Cambridge and Norwich sites. It will also be responsible for coordinating the OpenPlant Forum and associated pump-priming and outreach activities.

Progress to date
M1-2: Coordination of activities between the Cambridge and Norwich sites has continued to work effectively with regular communications between the coordination group. Quarterly management group meetings are held, with a progress update report produced for the meeting. The minutes from the management meetings are shared with the Science Advisory board via Basecamp.

M3: The second Advisory Board Meeting took place on 27 July 2016. There was unanimous agreement that Tim Fell will be Co-Chair of the SAB alongside Tom Knight. The following comments and action points were raised:

- Response of the SAB to the progress of OpenPlant was positive.
- Recommendations were made to help refine the OpenPlant tagline, single slide and statement of intent.
- Bring together a group to look into crowd-sourcing feedback to consolidate information on the practicality of synthesising - what can be synthesised by different companies.
- Norwich, Cambridge and Edinburgh have purchased Labcyte Echo instruments. Share common resources and protocols for automation.
- Pursue links or representation on RRI Platform Network and SBLC Governance subgroup
- Continuing from ongoing efforts, one action is to draft a format or template for sharing parts data and continue to pursue platform solutions.
- Investigate coordinating with other SBRCs to run an OpenPlant Fund like initiative, including a networking session and SBUK 2016

- Prepare for GCRF within OpenPlant in order to respond to calls at short notice

M4: OpenPlant held a 1-day All-Hands meeting in May 2016, which was well attended by both PIs and PDRAs from the OpenPlant research centre. The main focus of the meeting was on tools and technologies being developed / used in OpenPlant labs and mechanisms for sharing information around these. The meeting provided a chance for PDRAs to update the network on their progress and for discussions around cross-cutting topics. The following five topics were points of discussion:

1. Tools and technologies (biological, hardware, software, automation etc.)
2. Open Innovation and exchange of resources
3. Opportunities for research and application
4. Training and community building
5. Commercialisation, engagement and impact

Several action points came out of the meeting, including new scientific collaborations, suggested topics for themed OpenPlant Fund calls, the establishment of a Slack account as an online communication forum for OpenPlant, and a PDRA led forum for discussion around methods and tools for sharing information.

A meetup group called “Researchers related to OpenPlant in Cambridge” (ROC) has been established in Cambridge. This group are holding monthly meetups discussing topics including sharing of information and resources. Information from these meetings is fed into a Slack discussion thread to share with researchers that are not able to attend the meetings. A PDRA-led meeting took place at the John Innes Centre prior to the OpenPlant Forum to bring together Norwich and Cambridge researchers and expand the ongoing discussions. The outcomes of this meeting were fed into a panel discussion at the Forum on enabling innovation through openness and exchange. Actions that have already been taken as a result of these discussions are the establishment of an OpenPlant project to share protocols on protocols.io: https://www.protocols.io/groups/openplant-project, and the establishment of an OpenPlant Benchling account for testing out DNA parts information sharing prior to public release.

M5: The OpenPlant Fund has now awarded 30 projects and management has been handled through the management team and judging committees for each round, including external participants. Substantial management effort has been put in throughout 2015-16 to form teams and collaborations for these open calls. The OpenPlant management team made a decision to run future rounds as themed opportunities in order to more effectively seed interactions around the key tools, technologies and outreach initiatives required for plant synthetic biology. Ideas for focus areas were collected at the All-Hands Meeting in May 2016. For more details on the projects see Workpackage K and Appendix 2.

M6: The OpenPlant Forum took place at the John Innes Conference Centre, 25-27 July with just over 100 attendees. There was a good representation from the consortium and national and international external participants from other projects, institutions, organisations and companies, including several post-docs from the Warwick SBRC.

The focus was on reprogramming agriculture, and invited speakers included Allan Green (CSIRO), Johnathan Napier (Rothamsted), Matthew White (AB Sugar), Spencer Adler (Bioeconomy Capital), and Tom Knight (Ginkgo Bioworks). Discussion panels were highly multidisciplinary, and included Monique Simmonds (Kew Gardens), Wieke Betten (Ethicist, VU Amsterdam), Dominic Berry (Science

Historian, University of Edinburgh), Tobias Wenzel (Department of Physics, University of Cambridge), and Peter Murray-Rust (ContentMine). There was also an opportunity for the OpenPlant PDRAs to present their research, either orally or as a poster. This gave the SAB a chance to see the latest research progress.

The next OpenPlant Forum will be held in Cambridge from 24-26 July 2017.

M7: Public engagement and outreach through SAW Trust activities are being coordinated by Jenni Rant and Colette Matthewman. Building on last year's success, further activities were organised in this second year, these included a large increase in involvement from OpenPlant PDRAs and OpenPlant Fund grant recipients. A successful application to Latitude Festival earned OpenPlant a 3-day exhibit at this mixed-arts festival. Further details on 2016 activities are provided in the Workpackage L progress report.

M8: The Cambridge-JIC iGEM team were supported to enter the hardware track and were awarded a gold medal at the iGEM jamboree for their OpenScope, a 3D-printed microscope using a Raspberry Pi Camera. This year the team are focusing on chloroplast transformation in *Chlamydomonas* and competing for a new Plant prize that was proposed by a group including OpenPlant PIs.

Evidence of the quality of the research
A summary report of the 30 OpenPlant Fund projects was generated and distributed at the Forum.

Full reports from each of the 2015 OpenPlant Fund projects have been submitted, and webpages are being developed to publish these on the OpenPlant website. These will be public by the end of September.

Other evidence of impact
Several blog posts have been published based on the OpenPlant Forum and outreach activities. More information can be found in the Workpackage L report.

Leadership and Management

The management structure is unchanged from the original grant proposal, and is as outlined in the 2015 annual report.

The Scientific Advisory Board is chaired by Tom Knight. The second SAB annual meeting was held in July 2016, and Tim Fell was nominated as Co-Chair of the SAB alongside Tom Knight.

Training and Career Development

OpenPlant have recruited three high-calibre candidates for the OpenPlant PhD studentships. They will start in October 2016 with two rotation projects in OpenPlant labs, before choosing final projects to focus on for the remainder of their PhD. They will be joining a community of highly motivated and proactive PhD students and PDRAs already involved in OpenPlant and will benefit from the opportunities at the University of Cambridge, as well as those made available through OpenPlant.

Training and career development was highlighted in the BBSRC response to the 2015 annual report as a point to focus on for this second year of OpenPlant. OpenPlant PDRAs have access to a range of excellent training and career development opportunities at their host institutes and have funding available to attend external workshops critical to their projects and personal development. To broaden the opportunities available, Colette and Jenny have been working with organisers of training and career activities in their respective institutes to ensure that events relevant to OpenPlant researchers are accessible to participants from both sites. This has been very successful, with numerous courses being opened up to wider participation from OpenPlant researchers, including the following:

- Perfecting Your Proposal: It Must Be PitchCraft, John Innes Centre, Feb 2016
- “Everyone needs a bigger network” networking skills workshop, John Innes Centre, June 2016
- Career Direction in Agri-Tech and FarmRound networking event, University of Cambridge, May/July 2016
- JIC-Taiwan Agricultural Biotechnology Research Centre (ABRC), Student and PostDoc Workshop on “Identifying and Engineering Beneficial Bioproducts in Plant Systems”, John Innes Centre, July 2016
- Various programming and technical workshops offered at the Earlham Institute

A Slack account established for online communication between the wider OpenPlant community has proven to be a good tool for sharing information about training opportunities across the sites.

To complement existing training and career development opportunities at the OpenPlant institutes, OpenPlant have funded and organised several workshops.

- Two-day CRISPR-Cas workshop held at John Innes Centre (September, 2015). Meeting report published (Parry et al., 2016; http://www.ncbi.nlm.nih.gov/pubmed/26823675)
- Workshop on Responsible Research and Innovation: Ethics and openness in Synthetic Biology (March 2016; Norwich). Attended by all OpenPlant PDRAs.
- BBSRC Media Training workshop run for OpenPlant (March, 2016; Norwich)
- Workshop on outreach with the SAW Trust (March, 2016; Norwich)

- LithographX courses, one day was for biologists and computer scietists wanting to use the tool, and a second was for those wanting to develop tools and automate protocols based on LithographX (August 2016; Cambridge)
- Cambridge Synthetic Biology Meetup activities: Monthly Café Synthetique meetings and monthly Science Makers events

The OpenPlant Fund continues to play a major role in enabling researchers, with the support of their group leaders, to independently apply and obtain funding for collaborative interdisciplinary projects of interest that they might not otherwise be able to pursue. Applicants gain experience in starting up and shaping collaborations, proposal writing and pitching through the application process. Many projects also create further training opportunities, for example a two-day workshop on 'Facilitating synthetic biology literature mining and searching for the plant community' (March 2016, Earlham Institute), and the Co-lab project is running several interdisciplinary workshops, providing a co-learning experience in both bioengineering and design principles (July 2016 in Cambridge, September 2016 Norwich).

One-on-one and group discussions with PIs and PDRAs have helped to identify areas where additional training is desired. One area of common interest that was identified is for training in commercialisation and entrepreneurship in synthetic biology. Colette is in communication with contacts in both Norwich and Cambridge to identify and shape relevant training opportunities in this area.

Training of undergraduate students through the iGEM competition continues to play a key role in OpenPlant's aim of supporting development of open tools and as a pathway to international exchange. The Cambridge-JIC iGEM teams from 2015 and 2016 are supported by OpenPlant-supported labs in both Cambridge and Norwich. The Cambridge-JIC iGEM 2015 team project was entitled 'OpenScope' - an open source microscope. The team chose to build an ultra low-cost 3D-printed motorised web-streaming autofocus microscope for synthetic biologists, allowing researchers to tailor the microscope to their needs and operate it using remote access. OpenScope was awarded a gold medal at the iGEM Giant Jamboree in Boston, and was nominated for four awards by the judging team.

The NRP-UEA iGEM2015 team, House of Carbs, was supported by Nicola Patron through her role as an advisor. The team achieved a Gold Medal and were nominated for two awards by the judging team, including the Best Education and Public Engagement, Undergrad. The team hosted the JIC-Cambridge team on a visit to the John Innes Centre to discuss their project with Norwich researchers.

The Cambridge-JIC iGEM 2016 project is currently underway. The team are designing a regulatable molecular vector for Chlamydomonas reinhardtii using CRISPR/Cas9 system and will take this to the iGEM Jamboree in October 2016.

OpenPlant Programme Manager Colette Matthewman has led on putting together a summary of all training and international activities across the SBRCs and the IKC for the Science and Technology subgroup of the SBLC.

Added Value

OpenPlant initiative provided support for Open Technology Week in June 2016 in Cambridge. The week included activities for developing fundamental tools for synthetic biology and beyond, including a Maker Faire, Open Technology and Technology for the Bottom Billion workshops, and an Open Technology for Development Makeathon. These activities focused on access, openness and enabling technologies and attracted a strongly interdisciplinary group of participants. The 2016 OpenPlant Forum was held in Norwich and showcased the work of OpenPlant to a broader audience and sought to engage with the synthetic biology community and external experts in a way that will add value to the OpenPlant programme. The forum promoted the theme of Reprogramming Agriculture, and discussions were seeded that will help shape the future of OpenPlant in the UK and international plant synthetic biology community.

OpenPlant is working closely with The Science Art Writing Trust (SAW) to take a different approach towards stimulating discussion and co-learning on themes relevant to the use of synthetic biology to address grand challenges with different groups of participants. Although the approach has been used mainly in schools so far, we have demonstrated that SAW is a highly effective vehicle for engaging people of all ages in discussions about science. It uses science as a meeting place for exploration, and draws on visually exciting scientific images and cross-disciplinary approaches involving art and poetry to demystify synthetic biology and capture the imagination of groups from primary school to adulthood. This approach gives people the space to explore the concepts, potentials and ethics of synthetic biology through various media that are accessible to people who are less engaged with science. SAW activities run within the last year, including with the University of Edinburgh SBRC, are outlined in Workpackage L description. Plans are being developed to expand activities in the coming year, working closely with social scientists to define key areas for discussion and exploration.

Anne Osbourn has led on the establishment of a scientific image library for the Norwich Research Park (http://images.norwichresearchpark.ac.uk/). This image library is being expanded to include images relevant to synthetic biology for all OpenPlant participants. The images are open access and free to use. They will provide a valuable platform for further SAW activities on synthetic biology themes.

Impact

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 offers the even greater potential benefits of complex metabolism, huge scale and low costs. The OpenPlant initiative aims to (1) promote innovation by accelerating the development and exchange of underpinning tools and techniques in plant synthetic biology, and to (2) facilitate outreach, policy discussion and international development.

The first two years have seen substantial progress in promoting a two-tier approach to managing intellectual property, as we explore ways of implementing less restrictive models for distributing low-level tools and components for plant biotechnology. Innovation in a young field like Synthetic Biology requires freedom to operate. We believe that our steps to facilitate free exchange of DNA parts and tools will substantially speed the take-up of new technologies in plant synthetic biology, and foster innovation and entrepreneurship in the UK and beyond.

The introduction of standards for the assembly of characterised DNA sequences and the establishment of a Registry of Standard Parts were landmarks in microbial engineering. Improvements in the ability to reprogram plants will impact a wide range of industries including textiles, fuels, sugars, fine chemicals, drugs and food. The publication of a common genetic syntax that enables the exchange of standard DNA parts for plants and other eukaryotes was a major outcome of the first year of OpenPlant (Patron *et a*l., 2015). The standard has been ratified by an international consortium of scientists, and in this second year, we have seen it accepted as the new Phytobrick standard for the sharing of plant DNA parts via the Registry of Biological Parts and the establishment of an inaugural Plant Award at in the 2016 iGEM competition. We see the standard being applied to the production of parts for genome editing, the engineering of novel traits and to enable coordinated development of supporting hardware and software for bioengineering.

The social acceptance of genetic modification in field-grown crop plants remains a significant barrier to the adoption of plant synthetic biology in the UK. OpenPlant research, public engagement and outreach efforts promote (i) models for decentralised ownership and control of key technologies, (ii) use of *cis*-genics and precision gene editing technologies, (iii) development of new crop traits with improved properties, sustainable production, resource management and environmental impact, and (iv) aid international development and technical exchange for agriculture and sustainable land use.

Future Plans

- Large scale synthesis and distribution of Marchantia DNA parts
- Delivery of lab automation pathways
- Marchantia enhancer trap screen and formal description of cellular anatomy
- Continued development of trait-based technologies
- International implementation of the OpenMTA agreement
- Build a network of UK-African scientists for Synthetic Biology
- Look for opportunities to promote open technologies by technical exchange and resource sharing in Latin America and Africa