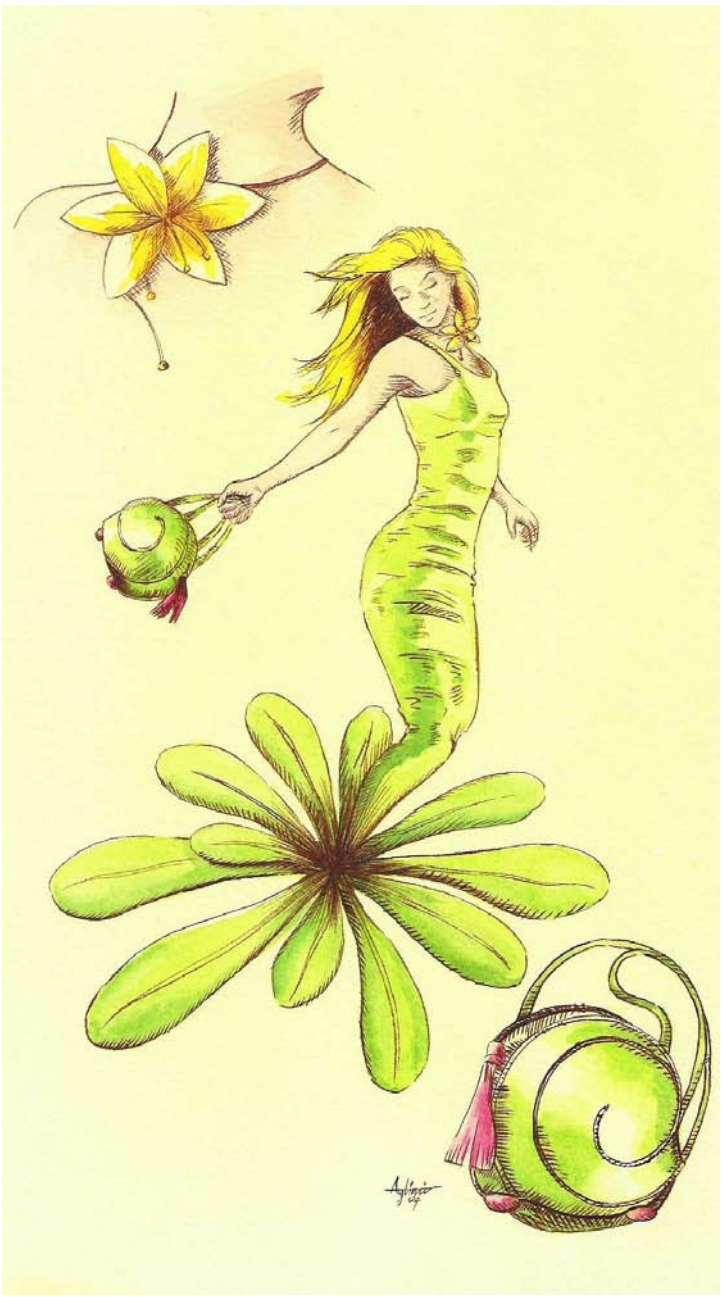
December 2004. Edition 2

GARNish

The official GARNet newsletter

Model to crop

Harvesting the benefits from Arabidopsis research



Arabidopsis Angelica

Brassica Napus

Also in this Issue - ATIDB - MIAME Compliance - AGRIKOLA - Election Results - Useful Websites

A word from our Chairman

I would first like to wish all members of the UK Arabidopsis and plant genomics communities a very happy Christmas and a prosperous and successful New Year. This is a good time of year to both reflect on the past and look forward to the future. GARNet is changing and evolving from a consortium of Arabidopsis genomics service providers with a steering committee to a democratically based coordination and community liaison organisation representing UK plant genomics in it's broadest sense.

For those of you not that aware of GARNet's history Ottoline Leyser has written a brief summary below. The annual GARNet meetings have played a central role in re-establishing a feeling of community among UK Arabidopsis groups and the continuing success of these meetings will spread to the plant genomics researchers. An account of our successful 5th meeting held at Leicester in September this year can be read on page 4.

Technology and access to technology have been a central part of the mission of GARNet and in this issue of GARNish we have articles describing Arabidopsis resources available at NASC and the JIC. Jim Beynon has provided a summary of RNAi knockout lines from AGRIKOLA. There is a detailed review of the ATIDB site which now includes useful information on locations for biBAC clones. GARNet has been very much about technology but the real point of all the technology is to find out fun stuff about biology. A good example of this is the article on page 11 by Sybille Kubis.

I would like to express my thanks to Andrew Millar and Ruth Bastow for all the hard work they have put in and warn them that it is particularly dangerous to make yourself indispensable as you may have to do it forever.

Ian Furner, University of Cambridge

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Front cover illustration by Lucio
lucioo@libero.it

Many thanks to all who contributed to this issue of GARNish

If you have any comments about GARNish or would like to contribute an article to the next issue please contact Ruth Bastow
R.M.Bastow@warwick.ac.uk

GARNet a potted history!

GARNet is the Genomic Arabidopsis Resource Network. It came officially into being in January 2000, with the establishment of the co-ordination office at York and the appointment of the first assistant co-ordinator, Karin van der Sande.

GARNet is part of a BBSRC initiative called Investigating Gene Function (IGF). The IGF initiative was aimed at smoothing the way for UK research communities into the exploitation of post-genomic technologies. Following an open meeting for Arabidopsis scientists in York in early 1999, a steering group was established to put together the Arabidopsis bid for IGF funding.

The successful bid established the main GARNet service centres at NASC for transcriptomics and bioinformatics; at IACR (originally Long Ashton, now Rothamsted) for metabolomics; and at Cambridge for proteomics. In addition, the grant funded the development of a range of functional genomics resources including large BAC libraries in binary vectors, along with a screening service; a Gene Specific Tag project; a sequenced insertion site collection for various tagging populations; and a new high copy number tagging population.

A series of annual meetings was established to promote the use of the services and resources. These proved both popular and useful for all concerned, and allowed GARNet to form highly productive links with similar projects overseas. An excellent example is in the development of the GST programme. At the first GARNet meeting, a conversation in the bar led to GARNet joining the Complete Arabidopsis Transcriptome Micro Array (CATMA) project, allowing the original modest aims to produce a few 1000 GSTs to be combined with efforts in other EU countries to make more than 20,000. This set is now available for purchase from NASC.

The original three year funding period for GARNet was extended in 2003 to allow the services to move to full cost recovery and to establish a stable structure for GARNet. Veronica Ongaro took over as assistant co-ordinator and organised the 4th GARNet meeting joint with the second Plant Genomics European meeting in York. This was to have been the last GARNet meeting, but by popular acclaim, combined with the continued need for UK co-ordination, additional funds were sought to keep this part of GARNet going. Ruth Bastow took over as assistant co-ordinator and Andrew Millar took over from me as co-ordinator. Key goals now are to keep the services up to date and to facilitate interactions between Arabidopsis and crop scientists, building on GARNet's long-standing collaboration with the Brassica community. This new co-ordination team is supported, as ever, by the GARNet steering committee, which has moved to a more sustainable and democratic format by the introduction of an annual election.

I am very happy to have been involved in something that has clearly been so useful to the UK Arabidopsis community. Long may it continue to be so!

Ottoline Leyser, University of York

News and Views

Expression atlas of Arabidopsis

Funded by the DFG, AtGenExpress undertook the ambitious task of generating a comprehensive transcriptome profile database of Arabidopsis in the autumn of 2003.
Coordinated and designed by the German Arabidopsis Functional Genomics Network (AFGN) the project aimed to collate approximately 500 developmental datasets into a publicly available reference resource. Such a large scale project has required international collaboration and contributing bodies include RIKEN, NSF, BBSRC (via GARNET and NASC) and the Max Planck Society.

The majority of samples have now been processed and it is envisaged that all data will eventually be available via TAIR and the Gene Expression Omnibus (GEO). However for those of wishing to access the data immediately a subset is available for virtual analysis via Genevestigator (https://www.genevestigator.ethz.ch/) or you can view NASC's contribution at http://affymetrix.arabidopsis.info/narrays/experimentbrowse.pl.

Election results

It must be the season for elections, as along side the long awaited ballot for the GARNet advisory committee the United States of America were also selecting their president.

After requests for potential nominees from the community the following 8 front runners emerged:-
Steven Conlan, University of Wales, Swnasea
Julie Gray, University of Sheffield
Claire Grierson, University of Bristol
Jonathan Jones, Sainsbury Laboratory Norwich,
Rod Scott, University of Bath
Gordon Simpson, Dundee University and SCRI
Matthew Terry, University of Southampton
Astrid Wingler, University College London

Throughout Novemebr you made your vote count by selecting your three favoured candidates. It was a close run ballot right up until the deadline. However, at the close of polling Claire Greirson, Jonathan Jones and Julie Gray were elected as new members of the committee.

I would like to thank all of you that took the time to cast your vote and welcome the new members to the committee. Commiserations to those that didn't make it - there is always next year!

ERA-PG moves forward

written by Sophie Laurie, BBSRC

The ERA Net in Plant Genomics is gaining momentum now, with the recent launch of the official website (http://www.erapg.org). This will be a repository for all of the information collected by the ERA PG team and will have an interactive intranet for team members. The website aims to provide an up-to-date source of information to researchers with an interest in plant genomics, together with notification of forthcoming meetings and opportunities to feed into strategic and administrative preparations for the first joint funding call. A questionnaire on the current status of plant genomic funding in the UK has recently been circulated and this will form a useful and updateable reference document, in combination with similar documents from around the EU. A similar questionnaire has been completed for administrative processes, giving an insight into the hurdles to be encountered in designing a transparent application and evaluation process for multilateral programmes, with the minimum of bureaucracy.
Currently BBSRC has earmarked funds for this multilateral call, and depending on successful progress of the preparatory stages, announcement is expected towards the end of 2006. For further information contact Sophie Laurie at the BBSRC (sophie.laurie@bbsrc.ac.uk).

And the sun shone on...

written by Keith Lindsey, University of Durham

It was glorious warm sunshine, a last desperate fling of summer, that greeted the 5th Annual GARNet Meeting. Hosted this year in the leafy suburbs that are the student residences of Leicester University, the fine venue was the perfect setting for a series of excellent scientific presentations. As is usual for these meetings, the talks were structured around particular themes, but all related to the application of functional genomics approaches to address biological questions. Many of the speakers presented examples of how the GARNet-supported central facilities in transcriptomics, proteomics and metabolomics had allowed their research to move forward rapidly. The exploitation of Brassica genomic resources was also well represented.

The Opening Plenary Session set the tone of the meeting with three important topics - RNA biology, and especially small RNAs (Pam Green), Brassica genomics (Ian Bancroft) and the identification of plant genes required for agrobacterium-mediated transformation (Angela Oldacres). There were two 'Development' sessions, covering flowering and pollen development, the shoot apical meristem, cell division control, organelle dynamics, developmental interactions with the environment, epigenetics and high throughput transcription factor analysis. The session 'Molecules Large and Small' encompassed metabolomics and protein biochemistry. 'Genomics Technologies' focused principally on microarray studies but with a fascinating insight into Brassica genetics and genomics research in China from Prof. Jinling Meng. The session on 'Nutrient Regulation' included for me a highlight of the meeting, Rodrigo Guitierrez's amazing computational visualization of interactions between nutritional supply and gene expression. In addition, there was a transcriptomics workshop for those struggling to make sense of their large data sets, and a discussion of science policy, from European activities in plant science (or lack of) to GM crops. Of course, there was also the ever popular poster session with wine reception, followed by the traditional networking in the bar. Some even found time for long, pre-dinner hikes in the Leicestershire countryside.

The meeting was very well attended with almost 200 participants, many from overseas. There was a buzz about the place, confirming in my mind the importance of promoting meetings of this size. The point of GARNet as originally envisaged was to encourage a sense of community, or perhaps I should say reinstigate it after the PMB initiatives of the 1990s. I think the popular view is that this meeting worked well, and I'm looking forward to the next one.

Make a space in your diary now for the 6th Annual GARNet Meeting 5-6 September 2005 at the John Innes Centre Norwich.

Arabidopsis Resources

AGRIKOLA: Arabidopsis Genomic RNAi Knockout Line Analysis

written by Jim Beynon, Warwick HRI

The AGRIKOLA project (co-ordinated by Ian Small, INRA, Evry and funded by the EU Framework V programme, with further support from BBSRC to Warwick HRI) aims to produce an RNAi (RNA interference) resource for the Arabidopsis community. RNAi allows a researcher to switch off a particular gene in Arabidopsis by the expression of a double stranded interfering RNA. This resource will complement the gene knockout resources as RNAi is not limited to a single Arabidopsis accession, by regulating timing of gene suppression lethality issues and problems with gene compensation can be circumvented and, with the appropriate constructs, gene families can be suppressed, overcoming redundancy.

Warwick HRI, as part of GARNet, joined the CATMA (Complete Arabidopsis Thaliana Micro-Array) consortium. CATMA combined resources from the national programmes of several EU countries and resulted in the production of some 24,000 genome sequence tags (GSTs) from the Arabidopsis genome. Each GST was designed to be 150-500 bp in length and no more than 70% identical to any other Arabidopsis sequence. These are being used to produce microarrays around the EU and are available from NASC (http://nasc.nott.ac.uk).

The CATMA GSTs are an excellent starting point from which to create an RNAi resource. The AGRIKOLA consortium aims to clone all of the CATMA GSTs into GATEWAY pDONR entry vectors and make them freely available to the research community via NASC. This phase of the project is essentially complete and the partners hope to ship the entry clones to NASC by the end of 2004. The next step is to clone the GSTs into the hpRNA (hairpin RNA) plant transformation vector pAGRIKOLA to induce gene knockouts in Arabidopsis plants. Two types of hpRNA vector will be used; one will express the hpRNA from the 35S promoter for constitutive gene suppression and the other will control hpRNA expression from an inducible promoter. The 35S constructs are nearly complete and will be submitted to NASC shortly after the entry clones. The inducible versions of the hpRNA vectors will be constructed early in 2005 and will be submitted to NASC on completion. There are four labs involved in generation of the clones: Ian Small (INRA, Evry), Pierre Hilson (VBI, Gent), Javier Paz-Aras (Centro Nacional de Biotecnología, Madrid) and Jim Beynon (Warwick HRI).

A further component of the project is the use of up to 5000 hpRNA clones to produce transgenic plants using Arabidopsis accession Columbia. The first 4,000 will be using the 35S constructs and the remainder using the inducible construct. From each construct up to 10 independent transformants are being selected and grown for seed production. The seeds are being stored directly into NASC tubes for immediate distribution to NASC. Production of transformants is now well underway in each lab and shipments of seed to NASC will begin shortly. There are three labs carrying out this procedure (Murray Grant, Imperial College; Thomas Altmann, Max-Planck-Institut für molekulare Pflanzenphysiologie, Golm; Jim Beynon, Warwick HRI).

Further details on the project can be obtained from the AGRIKOLA website (http://www.agrikola.org).

Useful Websites

GARNet http://garnet/arabidopsis.org.uk
New look GARNet website provides a comprehensive portal to Arabidopsis genomic resources in the UK and further a field. In addition an online version of GARNIsh and a diary of scientific meetings are also available via this site

MapMan http://gabi.rzpd.de/projects/MapMan/
MapMan is a user-driven tool that displays large datasets such as expression data from Arabidopsis Affymetrix arrays onto diagrams of metabolic pathways or other processes. For an example of it use see Thimm *et al.* Plant J. 2004 Mar;37(6):914-39.

Comprehensive Systems-Biology Database http://csbdb.mpimp-golm.mpg.de
The Comprehensive Systems-Biology Database (CSB.DB), provides a useful portal into systems biology by allowing statistical analysis of gene expression data. CSB.DB maintains databases of various model organisms, namely *Escherichia coli*, *Saccharomyces cerevisiae* and *Arabidopsis thaliana* (this include NASC affymetrix data). Comprehensive analyses of these data sets can be achieved via various tools provided by CSB.DB allowing parallel evaluation of a compendium of data.

MPSS http://mpss.udel.edu/at/
A useful resource for analysis of Arabidopsis MPSS (massively parallel signature sequencing) expression data. The website contains a database of 17 libraries of MPSS data from Columbia-0 at various developmental stages. The database can be searched via gene name, location or MPSS signature allowing users look at the expression profile of their favourite gene(s)

Genevestigator https://www.genevestigator.ethz.ch/
A user friendly site containing a wide range of tools for analysing and querying a large database of microarray data from sources including NASC, AtGenExpress, FCGZ and the Gruissiem lab.

QTL Resources

Natural http://www.natural-eu.org
EU funded project to exploit the natural variation of Arabidopsis to identify genes and gene variants of relevance for complex plant adaptive traits. Resources available via this site include RILs and marker polymorphisms.

Natural Variation http://naturalvariation.org
US based consortium looking into quantitative traits in Arabidopsis. Resources available via this site include a range of RILs (using Columbia as the reference) along with genomic and computational tools for QTL analysis.

Arabidopsis Resources

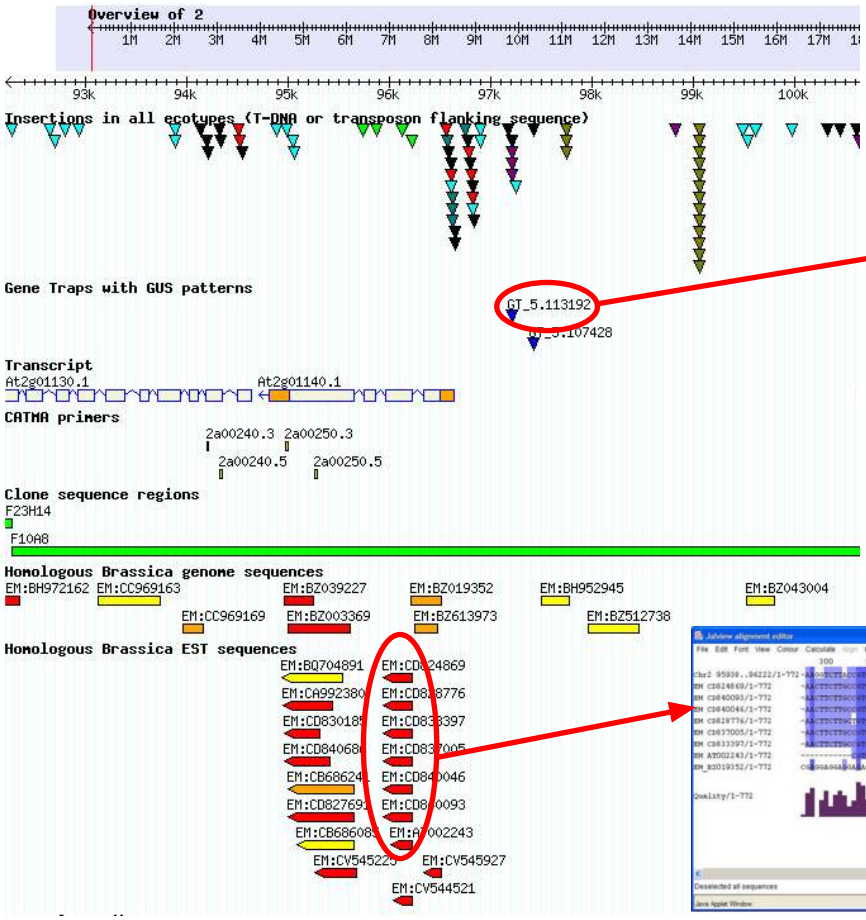
Arabidopsis thaliana Insertion Database

written by Martin Trick, John Innes Centre

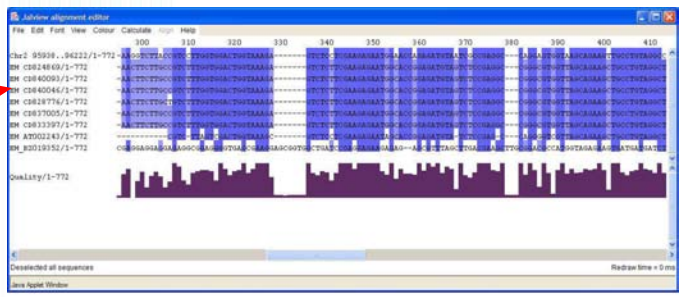
John Innes Centre

Arabidopsis thaliana Insertion Database

ATIDB, at http://atidb.org, is an Arabidopsis database and genome browser being developed at the John Innes Centre. Using the GMOD group's open source Generic Genome Browser, it represents a complementary resource to the AtEnsembl project described in the last issue of GARNish. In addition to the eponymous insertion data, the results of other GARNet-funded activities are deposited here – all in one, easy to use and customisable interface offering a high level of data integration. At its core is the TIGR v5 genome sequence and associated gene model annotation. Several extra layers of annotation have been added, starting with the positions of knockouts generated by a number of insertional mutagenesis programmes. A refined algorithm for the calculation of the insertion point has been developed in order to increase accuracy where poor sequence reads, failed vector clipping or spurious iPCR products may all have introduced ambiguity. All such placements are awarded a confidence score, and the user can quickly re-validate them with an integrated, real-time BLAST facility. Where the transposon system has been used as a Gene Trap, the expression data is fully integrated and searchable and all insertion line objects have links to appropriate stock centres.



new line name	GT.113192
picture file	 350x280
pictureNotes	
root cap	0
root ram	0
root epidermis	0
root cortex	0
root vascular	0
hypocotyl epidermis	0
hypocotyl cortex	0
hypocotyl vascular	0
cotyledon vascular	1
cotyledon rest	0
seedling leaf vascular	0
seedling leaf hydathode	0
seedling leaf rest	0
wounding	0
stipules	0
shoot apex	1
find similar Seedling Patterns (% similarity)	[100|95|90|85|80]



Also available as feature tracks are locations of the CATMA primer-pairs and Affymetrix ATH-1 GeneChip probes. Recent enhancements to ATIDB have included the addition of Gene Ontology terms to the underlying gene annotation and currently under development is integration with the InterPro database. Of interest to many in the GARNet community, the JAtY BAC library has been mapped *in silico* to the genome sequence and is now represented in ATIDB as a virtual clone path. Researchers can thus easily look for coverage of their favourite region in this transformation-competent clone library (for details, see http://www.getcid.co.uk). For those working at the model–crop interface, nearly 650,000 Brassica genomic and EST sequences have been similarly mapped. Clicking on a Brassica feature launches a real-time multiple alignment, allowing the power of comparative genomics to inform on the quality of the current annotation. A unique feature of the GBrowse interface is the ability for users to transiently upload their own data and thus overlay an in-house, proprietary view of the genome onto the public dataset. Finally, as well as the web interface suitable for casual browsing and searching, the underlying database is also accessible to programmers through a rich and versatile interface that allows sophisticated data-mining.

Arabidopsis Resources

JIC Genome Lab

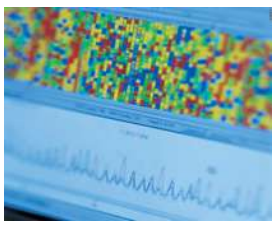
written by Jonathan Clarke, JGL

Genomics technologies have revolutionized molecular genetics. The Genome Laboratory aims to support these technologies with services, technical support and by acting as a research partner in genomics grant applications. Based in the Genome Centre at the John Innes Centre, the Genome Laboratory is tightly integrated into on-going genomics research projects in plants and microbes.

The Genome Laboratory is actively involved in the reduction to practice of emerging genomics technologies and in the development of new applications run on existing platforms. An emphasis on generic technologies allows the Genome Laboratory to support genomics across a wide range of plants and microbes.

The Genome Laboratory supports five platform technologies: DNA sequencing, DNA Libraries, Genotyping, Microarrays and Mutation Detection. In addition to these core platforms the Genome Laboratory supports the GARNet GeTCID clone distribution service and the Arabidopsis Transposon Insertion Service. The Genome Laboratory is a not-for-profit service provider and with a commitment to providing Cost Effective Genomics Solutions.

The DNA Sequencing Services is focused on supporting both small scale individual sequencing projects of one or more samples to large scale projects of greater than 10,000 samples. An on-line submission, tracking and data retrieval system allows the user to follow a sample from submission to completion. On-going projects include large scale Brassica BAC end sequencing and Streptomycese Cosmid end sequencing.

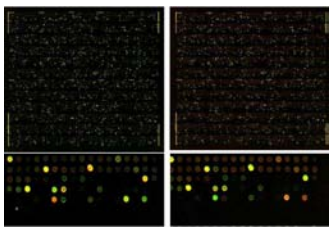


The DNA Library Service undertakes large and small insert library construction eg. BAC, Cosmid, EST and subclone libraries. The service supports plating and colony picking, library replication, sample pooling, rearraying (cherry-picking) and gridding on to filters. Projects running in the Laboratory include the construction of two potato BAC libraries and five Streptomycese cosmid libraries.

Library screening and the management of stock libraries is undertaken by the GeTCID Service. The stock Arabidopsis and Brassica libraries generated by GARNet have been extended to include libraries from additional Arabidopsis ecotypes and the IGF Wheat and Brachypodium BAC libraries. Clone distribution and in-house screening services are available on all stock libraries. Screening services have been extended to support libraries generated by the Genome Laboratory or independently. Currently screens are in progress on a *P. vulgaris* library for NIAB.

The Genotyping Service offers low and high resolution mapping in a wide range of Arabidopsis ecotypes based on stock INDEL markers. The service also supports mapping and genotyping in wheat, based on stock SSRs. The service also supports an on-line data submission, tracking and data retrieval system for ready reactions, running AFLP, SSR and INDEL reactions, prepared by the researcher, on ABI capillary detection system. The service has recently generated maps for six Arabidopsis RIL populations which will be available from NASC for Natural Variation projects.

The Genome Laboratory is an Affymetrix Approved European Service Centre. In addition to Affymetrix GeneChips the service provides access Agilent Printed Microarrays and Operon Printed Microarrays. A new on-line sample submission system allows the status of samples passing through the processing pipeline to be tracked. The processing service includes cDNA and cRNA synthesis and QC. GCOS data files and .txt files can be downloaded or sent for analysis. The Genome Laboratory supports the Genespring analysis software, including quarterly training courses. In collaboration with the Department of Computational Biology the Genome Laboratory supports experimental design and bespoke data analysis.



The Mutation Detection Service exploits a range of genomics technologies for the isolation and characterization of mutants. Services include positional cloning in Arabidopsis, transcript based cloning of deletion and insertion mutants and reverse genetic screens of deletion (fast neutron) and EMS populations by deletion PCR and TILLING (based on the users populations). The GARNet Arabidopsis Thaliana Insertion Service (ATIS) supports the use of transposon lines for functional genomics. In addition the service undertakes transposon and T-DNA insertion site verification. On-going projects include TILLING in Medicago, the positional cloning of three EMS mutants in and the transcript based cloning of a recalcitrant T-DNA insertion mutant in Arabidopsis.

The Genome Laboratory runs Plant DNA, Plasmid, Cosmid and BAC isolations and high throughput PCR services. All of the services, technical support and consultation are also available through the Research Hotel facility which can host up to twenty-five scientists undertaking large scale genomics projects or developing new genomics applications and technologies.

For further information on the services, support and the Research Hotel available from the Genome Laboratory contact Jonathan Clarke at enquiries@jicgenomeLaboratory.co.uk or visit the website http://www.jicgenomeLaboratory.co.uk

John Innes Centre Genome LABORATORY

Arabidopsis Resources

Arabidopsis.info – Just look on the web

NASC

The European Arabidopsis Stock Centre

NASC provides seed and information resources to the International Arabidopsis Genome Programme and the wider research community. (Old NASC home page)

About NASC | Address & Staff | Ask a Question | Background lines | Feedback | Growing Arabidopsis | Links | PLANET | Plant Science Division | UKPGRG | University of Nottingham | What is Arabidopsis? |

Stock Catalogue

- Search Catalogue
- Browse Catalogue
- How to Order
- Ordering FAQ
- Stock Overview
- Price Information
- Check Order Progress
- Seed Donation Form
- MTA FAQ

Transcriptomics

- Affymetrix Chip Service
- Apply for the Service
- AffyWatch (CDs)
- Genechip Spot Histories
- CATMA project
- Presentations
- GARNet

Genomics

- Arabidopsis Ensembl
- InsertWatch
- UKCropNet
- AGR (we recommend Ensembl)
- RI Map Data

Proteomics

- Proteomics database

Stock Catalogue

NASC now maintains over 300,000 accessions of *Arabidopsis thaliana* representing over half a million genotypes.

To **search** the catalogue click the button below.

Search Catalogue

To **browse** for a particular category of line click the button below. Useful for populations such as **SALK, SAIL, and JIC lines**.

Browse Catalogue

News

Latest Additions to the Stock Catalogue:

Cell autonomy (CAUT) lines - donated by Dr. Ian Furner - July 23rd 2004.
SAIL Lines from Syngenta - June 16th 2004.

Latest News:

Proteomics database
MIAME-Plant released Sept 19th 2004.
Arabidopsis Ensembl v 4.1 released Sept 16th 2004.
MASC report 2004
Alternative spot history for NASCarrays data at the AMPL.
Credit Card orders now accepted.
CATMA microarray slides now available.
Arabidopsis visual similarity prototype (IMEDIA Project INRIA).
What was new

Transcriptomics

The NASC Affymetrix service processes Affymetrix Gene Chips on behalf of customers through the UK's GARNET programme.

Affymetrix Service

All data produced by the service is available through the NASCArrays database and as a CD subscription service called AffyWatch. To access the data, use data mining tools, and apply for the service click on the button above.

Genomics

NASC have developed a new genomic resource based on the Ensembl genome browser which contains both TIGR and MIPS annotations.

Arabidopsis Ensembl

A.thaliana **TIGR assembly** - parallel display of TIGR and MIPS annotations, and all other features such as inserts stocked at NASC.

A.thaliana **MIPS assembly** - MIPS assembly and annotation with no other features.

The University of Nottingham

QLRI-CT-2001-00006 PLANET A NETWORK OF EUROPEAN PLANT DATABASES

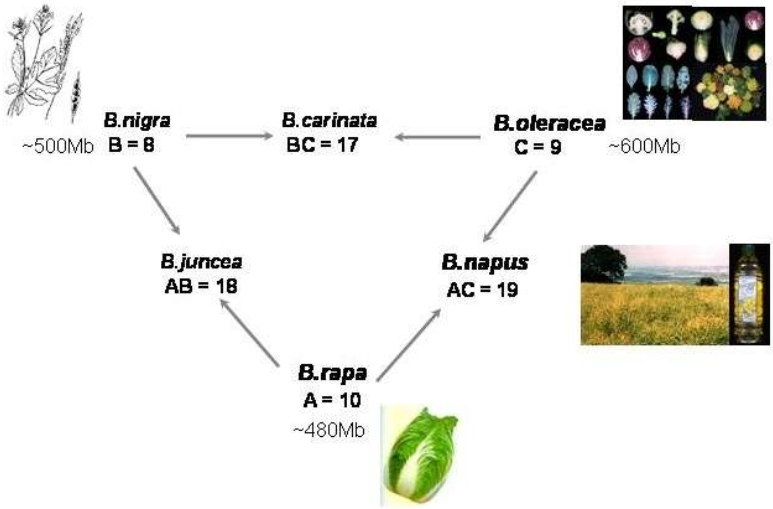
Brassica Resources

Brassica - an Opportunity to Harvest the Benefits from Arabidopsis Research

written by Graham King, University of Warwick

The recent 'Review of BBSRC-funded research relevant to Crop Science' [1] recommended that BBSRC should "seek to re-balance its plant science research portfolio to place greater emphasis on crop science, and to promote the transfer of knowledge from plant science to crop science". It also recommended that the Council "should seek to increase the proportion of the basic plant science budget that addresses the priority of knowledge transfer from plant to crop science, whilst maintaining the current level of support for basic plant science."
Over the next few years, the challenge for the plant science community is to deliver the benefits of model plant genomics to consumers, farmers, processors, retailers and other stakeholders, in order to build their confidence in the value of plant science. The UK is in an enviable position to capitalise on the investment and information from Arabidopsis. The UK *Brassica* Research Community (UK-BRC) has brought together a wide range of researchers in the public and private sector to encourage this knowlege transfer. In addition, the Defra Oilseed Rape Genetic Improvement Network (OREGIN) provides a focus for direct interaction between a wider range of stakeholders and the research community.

Brassica species play an important role in UK and global agriculture and horticulture, as well as being the closest crop relatives to Arabidopsis. The species are characterised by a wide range of adaptations that have been domesticated domesticated into crops including oilseed rape (Canola) and swede (*B. napus*); cabbage, cauliflower, broccoli, Brussels sprout (*B. oleracea*); turnip, chinese cabbage and pak choi (*B. rapa*). They contribute both to the economy and health (e.g. *via* anti-oxidants, vitamins, anti-carcinogenic compounds, etc.) of the nation. Crop improvement has been identified as a key route to ensuring the population benefits from these foods and plant products. *Brassica* is typical of many crop species in having a larger and more complex genome than the model. The genomic relationships are well characterised, as shown in the 'triangle of U' (left), and these have been exploited to understand the basis of chromosome evolution since divergence from a common progenitor shared with Arabidopsis. A wide range of genetic and genomic resources are available from *Brassica*, as well as the easy access to information derived from Arabidopsis (see article on use of Affymetrix chip page 10). Reference linkage maps, a wide range of QTL relevant to basic processes and crop phenotypes and sequence-based information are all available. In addition, there is increasing emphasis on characterising and utilising the diverse allelic variation present in genetic resource collections.



The Challenges
Considerable progress has been made in the genetic analysis of a wide range of agronomic and related plant traits in *Brassica*. Many of these have a complex quantitative inheritance which is exacerbated by interactions between genes, plant development and the environment. Despite the advantages of using information from Arabidopsis, the current challenge remains the need to identify key genes and understand their regulation in crop plants. There is a pressing requirement to resolve recently identified functional loci (major genes and QTLs) in terms of locus-specific copies of candidate or novel genes located in the context of physical map contigs and the emerging complete genome sequence.

***Brassica* genomics - where are we now ?**
For many crop species, the advances made in genomic analysis are limited by the 'resolution gap' between a large number of genetic trait loci and gene and regulatory sequences. The development of BAC physical contigs in the BBSRC IGF programme represented a crucial first stage through anchoring the *Brassica* genome to >1200 genes throughout the Arabidopsis genome. This project delivered a framework of BAC contigs for the A and C diploid genomes.
The latest release of EMBL/Genbank includes 404Mb of *Brassica oleracea* DNA sequence, with additional large datasets of *B. napus* and other ESTs. This is set to increase rapidly as the *Brassica rapa* Sequencing Project gains momentum, under the auspices of the Multinational *Brassica* Genome Project. This includes investment from BBSRC in the first stages of BAC-end and comparative sequencing, as well as major initial contributions from S. Korea and Australia. In addition, a public-domain *B. oleracea* EST programme established under the BBSRC-supported UK-Canada interaction between Warwick-HRI and AAFC Saskatoon will be releasing data in the new year.

A White Paper for *Brassica* is currently being prepared in order to provide a focus for the international community on agreed challenges and objectives, as well as to encourage additional investment from countries that are major producers of brassica crops (this will be available at http://www.brassia.info).

[1] http://www.bbsrc.ac.uk/news/reports/crop_sci_review12_05_04.html

Brassica Resources

www.brassica.info

www.brassica.info was set up in 2002 to collate and exchange information relating to *Brassica* genomics and genetics. The site has been adopted by the Multinational *Brassica* Genome Project to provide a comprehensive portal to relevant resources and background information. An international email list is open to all interested researchers and stakeholder (with 300+ subscribers worldwide). In addition, there is a *Brassica* Genome Gateway (http://brassica.bbsrc.ac.uk/), maintained by JIC.
http://www.brassica.info

UK-BRC

The UK-Brassica Research Community (UK-BRC) brings together a wide range of public and private sector researchers in the UK. Annual meetings are held (with >80 attending in 2004), as well as participation in joint GARNet meetings. A UK-BRC email list currently has >100 subscribers, who also receive postings from www.brassica.info
http://www.brassica.info/ukbrc/index.htm

OREGIN

The Defra Oilseed Rape Genetic Improvement Network (OREGIN) was established to act as a focus for collation and dissemination of information relevant to *Brassica* oilseed crop improvement, with clear involvement of a range of public and private sector stakeholders. Resources being developed and placed in the public domain include reference *B. napus* diversity sets and mapping population (from IMSORB) and pathogen isolate collections.
http://www.oregin.info

Brassica IGF Project
BAC physical map contigs, anchored to Arabidopsis

The BBSRC IGF *Brassica* project was successful in developing BAC-based physical contigs of the A and C genomes (*B. rapa, B. oleracea* that together comprise the oilseed rape *B. napus*). BAC clones were fingerprinted and contigs anchored to the Arabidopsis genome sequence through hybridisation to 1300 gene specific probes. All data, clones and filters are available in the public domain.
http://brassica.bbsrc.ac.uk/IGF/

tigr db
brassica oleracea

An approx. 0.5x genome coverage of *B. oleracea* was sequenced by TIGR as part of the NSF annotation programme for Arabidopsis. The data are available and searchable in EMBL/GenBank, TIGR and BrassicaDB. The original clones (2-12kb long) are available from WarwickHRI Genomics Resources Centre.
http://www.brassica.info/tigr_clones/tigr_bog_clones.htm,
http://www.tigr.org/tdb/e2k1/bog1/

B. oleracea EST sequences and clones

A public domain EST programme is underway in a collaboration between UK (Warwick HRI) and Canadian (AAFC, Saskatoon) labs. This will add to existing data from *B. napus,* developed in France. The aim is to generate data for up to 30k clones, with the first tranche of sequences available in Jan 2005. Libraries are available for additional sequencing. Contact: guy.barker@warwick.ac.uk
http://grc.warwick.ac.uk

***Brassica* Microsatellite Information Exchange**

An increasing number of SSR (microsatellite) genetic markers are becoming available in the public domain. Charlotte Allender at Warwick HRI has collated primer and associated information, including summaries of map locations and polymorphism data.
http://www.brassica.info/ssr/SSRinfo.htm

Reference Mapping Populations

Reference mapping populations (doubled haploid or RI) are the bedrock of *Brassica* genetics and genomics. An increasing number are now available in the public domain, and a number of labs are ensuring that the linkage maps are integrated with common public domain SSR and other sequence-tagged markers. Many are available via Warwick HRI Genomics Resource Centre.
http://grc.warwick.ac.uk
http://www.brassica.info/resources/cwg_notes_resources.htm

Brassica Diversity Fixed Foundation

To provide reference material to assess genetic diversity in the *Brassica* genepools for genome (marker allele), trait variation and association studies, Diversity Fixed Foundation Sets are being established for *B. oleracea*, C genome spp. (at Warwick HRI) and *B. napus* (in OREGIN) with a further set in planning stage for *B.rapa*.
http://www.brassica.info/diversity/diversity_sets.htm

Arabidopsis.info

Brassica 'tracks' are being added to the Arabidopsis Ensembl viewer at NASC. The first of these allows location of *B. napus* ESTs in relation to Arabidopsis gene models. During 2005 *B. oleracea* EST, IGF *Brassica* probes and other tracks are due to be added.
http://atensembl.arabidopsis.info/Arabidopsis_thaliana_TIGR/

IMSORB

Integrated Marker System for Oilseed Rape Breeding (IMSORB) is an EU programme co-ordinated by JIC with Chinese, German and industrial partners. This is developing SNP markers, high resolution map and QTL analysis.
http://brassica.bbsrc.ac.uk/IMSORB/

News from NASC

A chip for all species

John Hammond, Martin Broadley, Philip J White and NASC
written by Sean May

Affymetrix GeneChip® arrays are clearly the gold standard in transcriptome analysis and have already generated vast quantities of useful data for the Arabidopsis community. However, they are only currently available to the general public for a few plant species beyond Arabidopsis (Barley, Soy, Grape, Wheat*, Tomato*). Because of this short-term deficiency, we have developed a method to study the transcriptome of a plant species, *Brassica oleracea L.*, for which no GeneChip® array is currently available. This was done both as a proof of concept exercise and as a pragmatic tool for gene analysis within a species with particular scientific interest.

We first tested the hypothesis that *B. oleracea* genomic DNA should hybridise to probes on the *Arabidopsis thaliana (L.)* Heynh ATH1-121501 GeneChip® array in silico by determining the sequence homology between publicly-available *B. oleracea* sequence data and *A. thaliana* GeneChip® PM probes. This allowed us to estimate the likelihood/risk of performing an actual genechip hybridisation given the costliness of the process. Our results were positive, reflected the estimated state of sequencing for the Brassica genome, and indicated that almost all of the known genes in Brassica would specifically cross-hybridisation with the available Affymetrix probe set.
Subsequently, we generated biotin-labelled *B. oleracea* genomic DNA and performed a modified hybridisation to the ATH1-121501 GeneChip® array. Perfect match (PM) *A. thaliana* oligo probes which hybridised to the *B. oleracea* genomic DNA above an empirically-determined threshold value were selected as a 'mask' to isolate probes for subsequent *B. oleracea* transcriptome analyses.

Using this method, we identified 192,645 of the 250,206 available PM probes as suitable for *B. oleracea* transcriptome analyses. These probes represented 22,707 of the available 22,746 probe sets (~genes), each probe set represented by a minimum of two PM probes per probe set for quality control.
We tested the method in a biological context by studying the transcriptional response of *B. oleracea* to a mineral nutrient (phosphorus; P) stress. The virtual (masked / DNA-selected) Brassica chip was far more discriminatory than a simple RNA hybridisation to an un-masked chip. In an un-masked chip, the standard algorithms used for analysis of RNA hybridisations frequently eliminate positive signal components by treating them as if they were noise.
Multiple genes were regulated under P starvation, including the 'usual suspects' - homologues of known P-responsive genes in *A. thaliana*, notably P translocator and P metabolism genes. We therefore conclude that this method will allow the transcriptome of a wide range of plant species to be studied even in the absence of custom arrays or primary sequence data. For some species this may be the only way ahead, whereas for others it may simply be a useful advance preview of full genome chips to come. The full study is being submitted for publication in a peer-reviewed journal (enquiries to sean@arabidopsis.info).
* Release date - December 2004.

The need to conform

written by Beatrice Schildknecht & Simon Jupp

Standard and Ontologies
Plant databases are expanding rapidly in number, size and complexity, and are reshaping the way we think about data storage and management. These information-rich databases face the challenge of accurately and consistently documenting features such as genomic information, phenotypes, anatomical parts, and microarray experimental information. Comparisons between database objects have remained difficult, as precise or structured data annotation is incomplete, and plant-specific standards have never been defined.

It has become increasingly important and desirable to have tools in place for cross-database querying and improved connections and comparisons (interoperabitlity) between these plant databases. The terminology used to describe comparable objects in different databases can be quite variable, and limits the ability to accurately and successfully query the information. One solution is to utilise various ontologies and standards, giving the research community a controlled and structured vocabulary to describe and represent their data.

What is an 'Ontology' and where would I find or use one ?
An Ontology is a classification methodology used to represent an area of knowledge where the essential facts are combined with structuring rules that describe the relationship between the facts. There are several standards and ontologies available, such as Plant Ontology (http://www.plantontology.org), that provides taxon-specific, and general terms for describing plant anatomy, structure and growth and developmental stages. For microarray experiments, MIAME (http://www.mged.org/Workgroups/MIAME/miame.html) is an ontology that describes the Minimum Information About a Microarray Experiment that is needed to "enable the interpretation of the results of the experiment".

MIAME/Plant
With the recent massive accumulation of plant gene expression data, it has become increasingly urgent to develop and implement a standard way of defining microarray experimental parameters. A multinational group of scientists, including bioinformaticists from the Nottingham Arabidopsis Stock Centre, has set out to extend the MIAME standard and to establish a list of controlled vocabularies specifically for plant microarray experiments (Whitepaper: http://arabidopsis.info/info/miame.html). The objective of MIAME/plant is therefore to provide such a conceptual structure in the context of plant genomics. The MIAME/plant specific additions to MIAME are included essentially in the "Experiment Package", "Protocol" and "BioMaterials" objects of the MGED core ontology.

At NASC
NASC has recently adopted MIAME/Plant and Plant Ontology in its Transcriptomics and Germplasm databases to structure and annotate its experimental and phenotypic descriptions. These will immediately benefit the plant research community by allowing users to query the database using defined terminologies, and standardise the way microarray experiments and Germplasm stocks are annotated and manipulated.

NASC is also currently implementing a plant ontology browser and search tool, accessible through the web that will allow ontological-based text searching of NASC stocks. This tool will shortly appear in the 'what's new' section of http://arabidopsis.info and on the database itself. The ontological descriptions of Stocks will also be available through web services from the PLANet project (http://www.eu-plant-genome.net), allowing easier cross database queries with other plant databases that implement the ontologies.

GARNet Service Success

Which Tocs make which proteins Tic?

written by Sybille Kubis University of Leicester

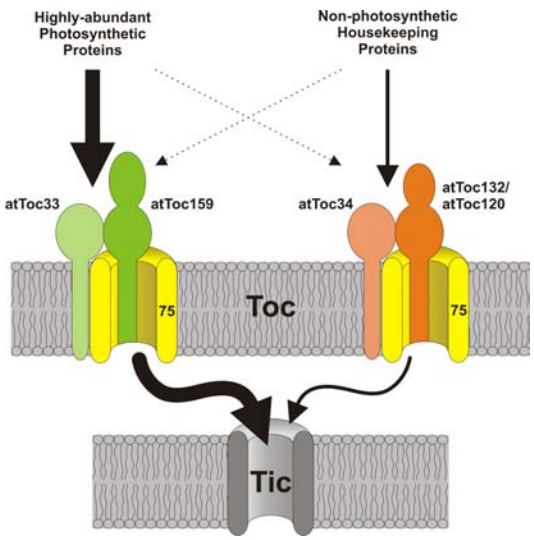
Our research group at Leicester, headed by Dr. Paul Jarvis, are looking at chloroplast protein import in Arabidopsis, focusing on the function, interaction and substrate-specificity of the different components involved in import. With assistance from several of the GARNet services we have been able to gain a better understanding of these processes as illustrated below.

Chloroplast protein import is facilitated by multimeric translocation complexes in the outer envelope (the Toc complex) and the inner envelope (the Tic complex).Toc159 and Toc34 are related GTPases involved in preprotein recognition. Together with Toc75 (the channel forming component) they form the core Toc complex. Recent advances have revealed that in Arabidopsis there are several homologous genes for many of the components: for Toc34 there are two homologues, atToc33 and atToc34, and for Toc159 there are four homologues, atToc159, atToc132, atToc120 and atToc90.

Utilising knockout mutants for each of the different homologues, we undertook comprehensive and comparative analysis of the two Toc34 and four Toc159 homologues, as well as of other components of the Toc/Tic machinery [1,2,3,4]. Many of the mutant alleles were supplied by NASC, and the proteomics service offered by GARNet was used to characterize the *ppi1* and *toc132* mutants [1,2], advancing our understanding of the substrate-specificity of chloroplast protein import significantly. A summary of our findings from these studies is given below.

The first indication for substrate-specificity came from an atToc159 knockout mutant, called *ppi2* (Bauer *et al.* 2000, Nature 403, 203-207), which has a severe albino phenotype and blocked chloroplast development. Since *ppi2* exhibits a strong reduction of photosynthetic proteins and a specific down-regulation of photosynthetic genes, it was proposed that atToc159 is a receptor for highly abundant photosynthetic proteins.
Our detailed studies of the *ppi1* mutant (a knockout of the main Arabidopsis Toc34 isoform, atToc33) showed that it is specifically defective in the expression, import and accumulation of photosynthetic proteins [1]. Using proteomics to characterize *ppi1* chloroplasts, all proteins found to be depleted in *ppi1* were components of the photosynthetic apparatus [1]. Furthermore, import rates for two photosynthetic precursors were greatly reduced in *ppi1*, whereas a non-photosynthetic precusor was imported normally. All these data suggested that atToc33 and atToc159 are more important for the import of photosynthetic precursors, leading us to questions if the other isoforms (atToc34, atToc132 and atToc120) are more important/specific for the import of constitutive precursors?

Our detailed study of the Toc159 homologues revealed that the *toc132 toc120* double homozygotes exhibit a severe phenotype similar to *ppi2*, indicating that an important role is shared by atToc132 and atToc120 [2]. In agreement with the proposed substrate-specificity, root plastids were most strongly affected in the double mutant, whereas chloroplast were much more developed (with distinct thylakoids and grana) than those in *ppi2*. Furthermore, overexpression of *atTOC159* failed to rescue the double mutant, whereas overexpression of either *atTOC132* or *atTOC120*, could. Biochemical data from Ivanova *et al.* (2004, Mol. Biol. Cell 15, 3379-3392) provides further support for the hypothesis of substrate-specificity;Toc complexes containing atToc132 and atToc120 were found to contain mainly atToc34, and atToc132 was shown to bind preferentially to transit peptides of non-photosynthetic precursors. In agreement with these data, *ppi3* (a knockout of the other Toc34 isoform, atToc34) has no visible phenotype in green, aerial tissues, but root growth is retarded indicating a relatively higher importance of this isoform in non-photosynthetic tissues [3].



Taken together, the data strongly support a model whereby the different Toc34 and Toc159 isoforms exhibit specialized function in the import of subsets of precursors (albeit with some overlap in specificity), as summarized in the figure shown here (reprinted from [5], with permission from Elsevier). The existence of distinct receptor systems would ensure that the import of less abundant, but equally important non-photosynthetic, constitutive precursors is not out-competed by the bulk flow of highly abundant, photosynthetic ones.

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Society for Experimental Biology

Plant Frontier Meeting

University of Sheffield, UK 21st - 23rd March 2005

Plant Systems Biology: Linking Theory and Experiment

In association with GARNet and the IPCR

Speakers to include: David Rand, Jan Traas, Luis Serrano, Oliver Thimm

Plant Meristems

Scientific organiser

Prof Keith Lindsey (keith.lindsey@durham.ac.uk)

Speakers to include: Ben Scheres, Caroline Dean, Cris Kuhlemeier, George Coupland, Keith Lindsey, Maria Costa, Miltos Tsiantis, Nick Battey, Ottoline Leyser, Rudiger Simon, Tom Beeckman, Yka Helariutta

The Visible Plant Cell: Biosensors and Bioreporters

Scientific organiser

Dr Stephen Rolfe (s.rolfe@sheffield.ac.uk)

Speakers to include: Alison Roberts, Andrew Fleming, Fred Sack, Gergo Angenent, Jim Haseloff, John Runion, Liam Dolan, Loren Looger, Malcolm Bennett, Patrick Hussey, Steve Rolfe, Ulrich Schurr

Phenotypic Plasticity and the Changing Environment

Scientific organiser

Dr Owen Atkin (OKA1@york.ac.uk)

Speakers to include: Angela Hodge, Anne Borland, Gail Taylor, Glyn Bengough, Hans Lambers, Hendrik Poorter, Ichiro Terashima, Kouki Hikosaka, Murray Badger, Norio Murata, Owen Atkin, Paul Quick, Rowan Sage, Steve Long

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