PROGRAMME AND ABSTRACT BOOK

NEW BREEDING TECHNOLOGIES IN THE PLANT SCIENCES – APPLICATIONS & IMPLICATIONS IN GENOME EDITING

7 – 8 JULY 2017
UNIVERSITY OF GOTHENBURG, SWEDEN

SOWING THE SEED

SOCIETY FOR EXPERIMENTAL BIOLOGY

NEW BREEDING TECHNOLOGIES IN PLANT SCIENCES - APPLICATIONS AND IMPLICATIONS IN GENOME EDITING

ORGANISED BY:
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BADGES

Participants are required to wear name badges at all times for proof of registration, security purposes and catering identification.

Name badges will contain a barcode which will be scanned on entry each day to record attendance at meeting for SEB administrative purposes only.

CATERING

Lunch and refreshments during the satellite meeting are included in your registration fee and will be served in the breakout area.

CONFERENCE DINNER

The conference dinner on 7 July will be held at Sjömagasinet at 19:00 and the address is Adolf Edelsvärds gata 5, 414 51 Göteborg, Sweden.

CERTIFICATE OF ATTENDANCE

Delegates requiring a certificate of attendance should visit the SEB registration desk on their departure.

VENUE

Botanical Building
University of Gothenburg
Carl Skottsbergs gata 22B
413 19
Göteborg
Sweden

The scientific sessions will be taking place in the Auditorium on the ground floor.

LIABILITY

Neither the Society for Experimental Biology nor the University of Gothenburg will accept responsibility for damage or injury to persons or property during the meeting. Participants are advised to arrange their own personal health and travel insurance.

PHOTOGRAPHY

No photographs are to be taken of the speakers and their slides during the satellite meeting.

**Please note: The SEB will be taking photos during the event for promotional purposes. If you have any concerns, please visit the SEB registration desk.*

REGISTRATION

The registration desk will be open during the hours of the meeting and a SEB staff member will be on hand during the refreshment and lunch breaks should you require any assistance.

TWITTER

We're looking to increase the conversation at the meeting using Twitter so please get tweeting!
Follow the conversation **#SEBNBT17**
SEB – **@SEBiology**

PROGRAMME

FRIDAY 7 JULY

08:30 REGISTRATION

09:00
Welcome and introduction

SESSION 1 CRISPR-CAS9 SUCCESS STORIES: GENE-EDITING MADE EASY?

CHAIR: ATTILA MOLNAR

09:10
Attila Molnar
University of Edinburgh, United Kingdom
Transgene-free genome editing in plants
PS17.1

09:35
Jean-Denis Faure
AgroParisTech – INRA, France
Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid *Camelina sativa*
PS17.2

10:00
Mariette Andersson
SLU, Sweden
Efficient targeted multiallelic mutagenesis in tetraploid potato
PS17.3

10:30 REFRESHMENT BREAK

SESSION 2 POLICY AND LEGISLATIVE IMPLICATIONS FOR USE OF GENE EDITING TECHNOLOGIES

CHAIR: CRAIG CORMICK

11:00
Craig Cormick
ThinkOutsideThe, Australia
Thinking outside the box:
Public acceptance considerations
PS17.4

11:30
Piet Van der Meer
Ghent University, Belgium
Historical evolution of biosafety legislation and key definitions
PS17.5

11:50
Joachim Schiemann
Julius Kühn-Institut, Germany
Future perspectives on biotechnology legislation from an academic perspective
PS17.6

12:10
Petra Jorasch
European Seed Association, Belgium
Industrial perspective on GM legislation
PS17.7

12:30
Discussion

13:00 LUNCH

PROGRAMME

SESSION 3 INTERNATIONAL VIEWPOINTS ON THE USE OF GENE EDITING TECHNOLOGIES

CHAIR: BARRY J POGSON

14:00
Staffan Eklöf
Swedish Board of Agriculture, Sweden
Why we decided some CRISPR-Cas9 gene-edited plants are not regulated by the GMO-directive
PS17.8

14:25
Wayne Parrott
University of Georgia, United States
The prospects for gene edited plants in the USA
PS17.9

14:50
Barry Pogson
Australian National University, Australia
Australian viewpoint on gene editing and future crops
PS17.10

15:15
Discussion

16:00 REFRESHMENT BREAK

16:30
PLENARY LECTURE
Stefan Jansson
Umeå University, Sweden
The first gene edited meal
PS17.11

17:00 END OF DAY 1

19:00
Conference dinner
Star quality CRISPR dinner
Location: Sjömagasinet

SATURDAY 8 JULY

08:30 REGISTRATION

SESSION 4 PRACTICAL CONSIDERATIONS FOR CRISPR-CAS9

CHAIR: GERAINT PARRY

09:00
Laurence Tomlinson
The Sainsbury Laboratory, United Kingdom
Genome editing in plant...less scary than it looks!
PS17.12

10:00
Christopher McClellan
University of Dundee, United Kingdom
Cautionary tales from the greenhouse: Confirmation of stably edited plants
PS17.13

10:20
Open discussion of practical considerations – Dealing with chimeric and off-target effects

11:00 REFRESHMENT BREAK

PROGRAMME

SESSION 5 CRISPR-CAS9 TOOLS AND RESOURCES

CHAIR: WENDY HARWOOD

11:30
Wendy Harwood
John Innes Centre, United Kingdom
A resource for targeted gene knock-out in crops
PS17.14

11:50
Markus Schmid
Umeå University, Sweden
Tools for study of flowering time using gene-editing
PS17.15

12:10
Johannes Stuttmann
Martin-Luther-Universität Halle-Wittenberg, Germany
Convenient tools for Cas9-based applications and generation of chromosomal deletions
PS17.16

12:30 LUNCH AND FINAL DISCUSSIONS

14:00 CLOSE OF MEETING

NEW BREEDING TECHNOLOGIES IN PLANT SCIENCES - APPLICATIONS AND IMPLICATIONS IN GENOME EDITING

PS17.1 TRANSGENE-FREE GENOME EDITING IN PLANTS

FRIDAY 7 JULY 2017 09:10

ATTILA MOLNAR (UNIVERSITY OF EDINBURGH, UNITED KINGDOM), DOUGLAS PYOTT (UNIVERSITY OF EDINBURGH, UNITED KINGDOM)

@ ATTILA.MOLNAR@ED.AC.UK

Plant viruses pose a ubiquitous threat to crop production by hampering the growth and fertility of infected plants and decreasing the marketability of harvested crops. In our recent research (Pyott et al., 2016) we used the CRISPR/Cas9 genome editing technology to delete a plant gene called eIF(iso)4E, which is used by certain viruses as a host factor to complete their lifecycle. We showed that deletion of eIF(iso)4E results in complete resistance to Turnip Mosaic Virus (TuMV), a major pathogen in field grown vegetable crops. In addition, we demonstrated that loss of eIF(iso)4E had no negative effect on plant growth. Importantly, this engineered resistance is heritable and does not require the presence of a transgene. We believe that similar, genome editing-based approaches will be essential for generating virus resistant crops in the near future. Other technologies to generate transgene-free designer plants will also be discussed.

PS17.2 SELECTIVE GENE DOSAGE BY CRISPR-CAS9 GENOME EDITING IN HEXAPLOID *CAMELINA SATIVA*

FRIDAY 7 JULY 2017 09:35

JEAN-DENIS FAURE (AGROPARISTECH - INRA, FRANCE), CÉLINE MORINEAU (INRA, FRANCE), YANNICK BELLEC (INRA, FRANCE), FRÉDÉRIQUE TELLIER (INRA, FRANCE), LIONEL GISSOT (INRA, FRANCE), ZSOLT KELEMEN (INRA, FRANCE), FABIEN NOGUÉ (INRA, FRANCE)

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Selectively engineering gene dosage, particularly in polyploid genomes, provides an efficient tool for plant breeding. The hexaploid oilseed crop Camelina sativa,which has three closely-related expressed sub-genomes, is an ideal species for investigation of the possibility of creating a large collection of combinatorial mutants. Selective, targeted mutagenesis of the three delta-12-desaturase (FAD2) genes was achieved by CRISPR-Cas9 gene editing, leading to reduced levels of polyunsaturated fatty acids and increased accumulation of oleic acid in the oil. Analysis of mutations over four generations demonstrated the presence of a large variety of heritable mutations in the three isologous CsFAD2genes. The different combinations of single, double and triple mutants in the T3 generation were isolated, and the complete loss-of-function mutants revealed the importance of delta-12-desaturation for Camelina development. Combinatorial association of different alleles for the three FAD2 loci provided a large diversity of Camelina lines with various lipid profiles, ranging from 10 to 62% oleic acid accumulation in the oil. The different allelic combinations allowed also an unbiased analysis of gene dosage and function in this hexaploid species, but also provided a unique source of genetic variability for plant breeding.

PS17.3 EFFICIENT TARGETED MULTIALLELIC MUTAGENESIS IN TETRAPLOID POTATO

FRIDAY 7 JULY 2017 **10:00**

MARIETTE ANDERSSON (DEPARTMENT OF PLANT BREEDING SLU, SWEDEN), HELLE TURESSON (DEPARTMENT OF PLANT BREEDING SLU, SWEDEN), ANN-SOFIE FÄLT (DEPARTMENT OF PLANT BREEDING SLU, SWEDEN), NIKLAS OLSSON (DEPARTMENT OF PLANT BREEDING SLU, SWEDEN), PIA OLSSON (DEPARTMENT OF PLANT BREEDING SLU, SWEDEN), MATHIAS SAMUELSSON (LYCKEBY STARCH AB, SWEDEN), PER HOFVANDER (DEPARTMENT OF PLANT BREEDING SLU, SWEDEN)

@ MARIETTE.ANDERSSON@SLU.SE

Potato is ranked as one of the most important food crops in the world but is also one of the major crops grown for starch production. Starch produced from potatoes has many uses, both in food and technical applications, and is often chemically or physically modified to reach certain specifications. To increase the portfolio of "green-labelled" starch products we would like to replace the down-stream modified starches by starch modified in planta.

Potato is a tetraploid crop withtetrasomic inheritance and high heterozygosity, making traditional cross-breeding a long term process. Therefore, breeding technologies where only one or a few traits can be introduced into an elite background is of major interest. We have implemented CRISPR-Cas9 as a targeted mutatgenesis method in potato, using DNA transfection and transient expression in protoplasts. The method was applied to develop an amylopectin starch potato by knocking-out a granule bound starch synthase (GBSS). The amylopectin starch can be used, without further down-stream modification, in for example food and paper applications. With the novel breeding method, mutations in at least one allele was found in up to 12% of regenerated shoots and in 2% of the shoots, all four alleles were found mutated. A draw-back found with the method was a >50% frequency of vector inserts of random size.

Today, transgene-free "CRISPR-amylopectin potatoes" are grown in field trial, already three years after project start. It's no doubt that CRISPR-Cas9 and similar technologies will have a huge potential for potato breeding in the future.

PS17.4 THINKING OUTSIDE THE BOX: PUBLIC ACCEPTANCE CONSIDERATIONS

FRIDAY 7 JULY 2017 **11:00**

CRAIG CORMICK (THINKOUTSIDETHE, AUSTRALIA)

@ CRAIG.CORMICK@THINKOUTSIDETHE.COM.AU

Community attitudes to new technologies in the food chain are often driven by personal values and world-views, complicated by modern communication channels that have allowed for contested perceptions of scientific facts, alternative truths, and reinforcement of ideas - no matter how fringe. Understanding people's values however, can provide insights into not only how they underpin attitudes, but how best to reach different people. This presentation will look at research into the different values segments of the population that exist, what defines them, and importantly, show what can be done to frame technology development messages to better align with people's values to better engage with them.

PS17.5 HISTORICAL EVOLUTION OF BIOSAFETY LEGISLATION AND KEY DEFINITIONS

FRIDAY 7 JULY 2017 11:30

PIET VAN DER MEER (GHENT UNIVERSITY, FREE UNIVERSITY OF BRUSSELS, BELGIUM)

@ PIETVANDERMEER@GMAIL.COM

The first recombinant DNA applications in the early 1970s started a worldwide debate about potential benefits and risks that continues up to today. The approach most governments take with modern biotechnology is best summarised as 'maximising the benefits and minimising risks', i.e. the lead principle of the Agenda 21 and has been reaffirmed many times since.

Governments maximise benefits through tools as research strategies. Many millions of Euros have been and are invested in public biotechnology research aimed at strengthening agricultural production, health care and environmental protection. Governments minimise risks through biosafety regulations, for which the basis was laid in the 1975 Asilomar conference, i.e.: recombinant DNA techniques use existing natural processes to transfer genes between unrelated organisms, and the use of these techniques themselves do not confer risks. However, these techniques can result in novel genetic combinations beyond mating and natural recombination, and it can only be assessed on a case by case basis whether those novel genetic combinations pose risks. Consequently, organisms with such novel genetic combinations are subject to prior risk assessments. Many biosafety regulations are currently being reviewed for effectiveness and efficiency. These reviews also touch on the question whether the definitions are still adequate vis-a-vis new developments such as "new breeding techniques".

The paper will discuss that some organisms developed through genome editing will fall under the existing definitions of GMO/LMO, while other organisms do not, as they do not contain novel genetic combinations beyond mating and natural recombination.

PS17.6 FUTURE PERSPECTIVES ON BIOTECHNOLOGY LEGISLATION FROM AN ACADEMIC PERSPECTIVE

FRIDAY 7 JULY 2017 11:50

JOACHIM SCHIEMANN (JULIUS KÜHN-INSTITUT, GERMANY), THORBEN SPRINK (JULIUS KÜHN-INSTITUT, GERMANY), FRANK HARTUNG (JULIUS KÜHN-INSTITUT, GERMANY)

@ JOACHIM.SCHIEMANN@JULIUS-KUEHN.DE

Genome editing is a transformative technology with general applicability providing a very wide range of potential uses to tackle societal challenges. Worldwide, several genome-edited plants and products thereof are already approved as non-regulated articles and are reaching the market. In January 2017 the U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA) have published four documents related to the pre-market regulatory oversight of a variety of biology-based agricultural tools, including genetically engineered plants and plants and animals derived from certain newer precision breeding techniques, such as genome editing. EASAC, the European Academies Science Advisory Council, has published a Report on Genome Editing: scientific opportunities, public interests and policy options in the EU. In this report, EASAC takes a broad perspective on the research advances, applications, policy implications and priorities for EU strategy for promoting innovation and managing regulation. EPSO, the European Plant Science Organization, has highlighted that in the implementation of the EU biotechnology regulatory framework there is a disproportionate focus on the genetic improvement technique used. This has led to the misinterpretation that GMOs are merely defined by the use of certain techniques. This is incorrect. Whether or not the resulting organism is a GMO depends on the fact if a novel combination of genetic material has been produced beyond the natural barriers of mating and recombination. This is not the case for several new plant traits obtained by genome editing. Based on the documents mentioned above future perspectives on biotechnology legislation will be discussed.

PS17.7 INDUSTRIAL PERSPECTIVE ON GM LEGISLATION

FRIDAY 7 JULY 2017 **12:10**

PETRA JORASCH
(EUROPEAN SEED ASSOCIATION, BELGIUM)

@ PETRAJORASCH@EUROSEEDS.EU

Plant breeders have always strived to create new variations of plant characteristics to provide solutions for disease and pest resistance, to achieve higher yields, to increase tolerance to environmental stress, and to breed new plant varieties that meet consumer expectations. The rediscovery of Mendel's laws of heredity, in the early 1900s, turned the first plant breeding efforts from an art into science, and specialised farmer-breeders emerged, building a business concept on their efforts. From that point in time, scientific breakthroughs in agricultural and biological sciences have accelerated.

Governmental policy must be firmly based on sound scientific principles to avoid the risk of impeding innovation in plant breeding. The seed industry takes the position that plant varieties developed through the latest breeding methods should not be subject to different, or additional, regulatory oversight if they are similar, or indistinguishable from, varieties that have been, or could have been, produced through traditional breeding methods or might also have been obtained from natural processes without human intervention.

Regulatory policy will determine utilisation of methods across companies and across crops. An overly high regulatory burden will limit utilisation to the largest companies and cash crops as well as to a limited number of traits. Farmers' access to a wide choice of better varieties-and, consequently, the availability of improved and sufficient products for consumers-would be in danger.

PS17.8 WHY WE DECIDED SOME CRISPR-CAS9 GENE-EDITED PLANTS ARE NOT REGULATED BY THE GMO-DIRECTIVE

FRIDAY 7 JULY 2017 **14:00**

STAFFAN EKLÖF
(SWEDISH BOARD OF AGRICULTURE, SWEDEN)

@ STAFFAN.EKLOF@JORDBRUKSVERKET.SE

Being a regulator means working at the interface between regulations and their intentions on one side and reality on the other. Regulators should ideally carry the intentions of the political realm to everyday life, in an effective, predictable and non-discriminatory way. I will shortly present the Swedish system as a background.

After receiving questions on whether some specific CRISPR-Cas9 gene-edited plants are regulated or not, my authority experienced an uncertain legislative situation. Based on our service obligation towards the citizens we finally chose to make interpretations and stressed two things. That the interpretation concerns only these very types of modifications and that it can change in future with the establishment of EU-common guide lines.

Articles and annexes in Directive 2001/18/EC decide what constitutes a GMO as well as which GMOs are regulated and which are exempted. A step-wise process was followed. First we assessed whether the categories of modification fulfil the requirements for being a GMO. We concluded that they all did so. After that we assessed whether they are defined as mutations and thus exempted. Some did count as produced via mutagenesis, some not. Thereafter we assessed whether the techniques involved the use of recombinant nucleic acid molecules. That would revoke the exemption for those plants produced by mutagenesis. Finally, all the plants had in previous generations contained T-DNA, why it had to be assessed whether this fact mattered.

Our interpretation was that some of the modifications are not regulated and some are. Other interpretations can be made.

PS17.9 THE PROSPECTS FOR GENE EDITED PLANTS IN THE USA

FRIDAY 7 JULY 2017 **14:25**

WAYNE PARROTT
(UNIVERSITY OF GEORGIA, UNITED STATES)

@ WPARROTT@UGA.EDU

Genome editing refers to series of technologies that make double-stranded breaks in DNA at a pre-determined location, followed by DNA repair. The results of genome editing can range from replicating the results of conventional mutagenesis, to replacing alleles, as happens in conventional plant breeding, to the insertion of transgenes at a pre-determined location in a chromosome. The prospects for acceptability vary by application. Theoretically, any one or more of three agencies could regulate edited plants: USDA, EPA, and FDA. The USDA did not regulate the first genome-edited plants and fungi to come out of a laboratory, as the USDA lacks legal authority to do so—USDA can only regulate plants that contain DNA derived from a pest or pathogen. The USDA is in the process of updating its regulations, and has proposed explicit exceptions for genome-edited plants that mimic conventional mutagenesis. The FDA is currently seeking comments on genome-edited plants, so its final position is not predictable. EPA regulates based on intent—if the intent is to control a pest or disease, EPA can regulate it, but thus far, EPA has not stated a position on gene-editing. The greatest factor that will determine the prospects for gene-edited plants will be the grocery manufacturers. They have voiced the need for genome editing to be regulated, or they fear consumers will not trust the technology. Furthermore, the latest trend in the food industry is transparency, and it is not at all clear that consumers will distinguish between genome-editing and genetic modification.

PS17.10 AUSTRALIAN VIEWPOINT ON GENE EDITING AND FUTURE CROPS

FRIDAY 7 JULY 2017 **14:50**

BARRY J POGSON (AUSTRALIAN NATIONAL UNIVERSITY, AUSTRALIA)

@ BARRY.POGSON@ANU.EDU.AU

The impact of this fast moving technology on science, policy and industry in the Australian context will be discussed. The talk will consider what is happening in the policy and regulatory space with respect to GMOs and NBTs. What is the potential for the science and its impact on agriculture in Australia? What is the appropriate regulatory environment for NBTs and what processes are underway with respect to the regulatory framework?

PS17.11 THE FIRST GENE EDITED MEAL

FRIDAY 7 JULY 2017 **16:30**

STEFAN JANSSON (UMEÅ UNIVERSITY, SWEDEN)

@ STEFAN.JANSSON@UMU.SE

In November 2015, The Swedish Board of Agriculture announced their opinion that Arabidopsis plants that have been modified using CRISPR-Cas9 where DNA where only a piece of a gene has been deleted but no novel DNA added, does not fall within the scope of the GMO legislation. This historical decision opened up the use of genome edited deletion mutants in agriculture. In the summer of 2016, such genome-edited Brassica plants were grown in a garden in Bjurfors, Sweden, under rather primitive conditions and on August 16, for (probably) the first time ever, field-grown CRISPR-mutants were harvested and cooked.

The meal got significant attention and has so far been reported in ca 300 media in ca 40 countries. In this talk, the reasoning behind the various kinds of responses and follow-ups to the meal will be described. Special focus will be put on the consequences of the decision and the meal, in particular intriguing issues around the fact that there will now be plants grown in Sweden (and other parts of the world) which are not covered by the GMO legislation, while the legal status of the very same plants in other EU countries is still unclear. This legal limbo challenges core values of EU like free movement of goods, and pinpoints the inability of EU to adjust its legislation around GMOs to developments in science and technology. The talk will also give an introduction to the conference dinner.

PS17.12 GENOME EDITING IN PLANT... LESS SCARY THAN IT LOOKS!

SATURDAY 8 JULY 2017 **09:00**

LAURENCE B TOMLINSON (THE SAINSBURY LABORATORY, UNITED KINGDOM), BAPTISTE CASTEL (THE SAINSBURY LABORATORY, UNITED KINGDOM), JONATHAN DG JONES (THE SAINSBURY LABORATORY, UNITED KINGDOM)

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The Cas9 protein (CRISPR-associated protein 9), derived from type II CRISPR (clustered regularly interspaced short palindromic repeats) bacterial immune systems, has emerged as a powerful and simple tool for engineering the genome in diverse organisms. Due to ease of use, CRISPR-Cas9 has been widely adopted for genome editing in plants. CRISPR-Cas9 edited plants include Arabidopsis, barley, *Brassica oleracea*, cotton, dandelion, flax, lettuce, liverwort, corn, petunia, populus, rice, sorghum, soybean, sweet orange, tomato, wheat, and tobacco. There are however several elements to consider when designing any CRISPR Cas9 construct. One of the crucial step is the design of the gRNA; this process is now made easy with the use of web-based tools. Another element to consider that might have an impact on the editing efficiency is the structure of the trans-activating crRNA (tracrRNA). Lastly the choice of the promoter of Cas9, Cas9 allele and its terminator had also been shown to be critical. Finally, I will review the different ways to select transformants. The aim of this talk is to give the latest insight into the practical considerations when designing a CRISPR Cas9 construct.

PS17.13 CAUTIONARY TALES FROM THE GREENHOUSE: CONFIRMATION OF STABLY EDITED PLANTS

SATURDAY 8 JULY 2017 **10:00**

CHRISTOPHER MCCLELLAN (UNIVERSITY OF DUNDEE, UNITED KINGDOM), ABDELLAH BARAKATE (UNIVERSITY OF DUNDEE, JAMES HUTTON INSTITUTE, UNITED KINGDOM), JENNIFER STEPHENS (JAMES HUTTON INSTITUTE, UNITED KINGDOM), CLAIRE HALPIN (UNIVERSITY OF DUNDEE, UNITED KINGDOM)

@ CHRISTOPHER.MCCLELLAN@HUTTON.AC.UK

Plant biomass is an emerging source of feedstocks for producing 'green' industrial products like biofuel. Altering genes involved in the synthesis of lignin, a cell wall component, could improve conversion of cellulose into glucose (saccharification) and improve the usefulness of crop residues as industrial feedstocks. Most crop plants are not amenable to large-scale genetic screens due to growth time, plant size and genome complexity. We have utilised CRISPR/Cas9 technology to specifically target multiple genes involved in lignin biosynthesis to alter cell wall composition in barley and wheat. While reducing the number of plants involved in obtaining a desired mutation, CRISPR/Cas9 can still involve screening hundreds of individuals, and confirming mutations in multiple genes can involve screening hundreds more. Therefore, an outline of the screening and confirmation process for finding mutations generated through CRISPR/Cas9 will be presented, from mutation detection in the initial population through to generating a stable, homozygous mutant plant. I will discuss the workflow used to find and confirm mutations in five lignin biosynthesis genes in barley and one in wheat, with a discussion on the strategies and trade-offs involved in obtaining mutations in CRISPR/Cas9 transformants.

PS17.14 A RESOURCE FOR TARGETED GENE KNOCK-OUT IN CROPS

SATURDAY 8 JULY 2017 **11:30**

WENDY A HARWOOD (JOHN INNES CENTRE, UNITED KINGDOM), TOM LAWRENSON (JOHN INNES CENTRE, UNITED KINGDOM), PENNY HUNDLEBY (JOHN INNES CENTRE, UNITED KINGDOM), ALISON HINCHLIFFE (JOHN INNES CENTRE, UNITED KINGDOM), SADIYE HAYTA (JOHN INNES CENTRE, UNITED KINGDOM), MONIKA CHHETRY (JOHN INNES CENTRE, UNITED KINGDOM), MARK SMEDLEY (JOHN INNES CENTRE, UNITED KINGDOM)

@ WENDY.HARWOOD@JIC.AC.UK

The BRACT crop transformation facility at the John Innes Centre has a long history of providing crop transformation resources to the research community. We have now demonstrated efficient targeted gene knock-outs in a range of crops including barley, wheat, Brassica oleracea, potato and tomato using RNA-guided Cas9. This capability can be provided to the research community on a cost recovery basis and free of charge to some UK research groups thanks to funding from BBSRC. We are able to provide design, construct assembly, transformation and initial screening for mutations if required. Training can also be provided and we are developing specific training courses to cover the entire process from design through to screening for mutations.

In this presentation we will describe the resources available in more detail, illustrate the processes involved and present efficiency data in a range of crops.

PS17.15 TOOLS FOR STUDY OF FLOWERING TIME USING GENE-EDITING

SATURDAY 8 JULY 2017 **11:50**

MARKUS SCHMID (UMEÅ UNIVERSITY, SWEDEN)

@ MARKUS.SCHMID@UMU.SE

In many plants the correct timing of the transition from vegetative growth to flowering is critical to ensure reproductive success. Because of its importance, flowering time is regulated by an intricate genetic network that integrates endogenous and environmental signals. CRISPR/Cas9 technology provides the means to dissect this genetic network and study the function of individual genes with unprecedented precision. For this purpose, we have developed a CRISPR/Cas9 toolkit based on the GreenGate vector series, which employs the Golden Gate cloning principle to assemble multiple pre-cloned building blocks into functional units suitable for plant transformation. Examples of how this toolkit was used to interrogate the function of specific flowering time genes or to construct artificial transcriptional regulators using a catalytically inactive version of Cas9 (dCas9) in *Arabidopsis thaliana* will be presented.

PS17.16 CONVENIENT TOOLS FOR CAS9-BASED APPLICATIONS AND GENERATION OF CHROMOSOMAL DELETIONS

SATURDAY 8 JULY 2017 **12:10**

JOHANNES STUTTMANN (MARTIN-LUTHER-UNIVERSITÄT HALLE-WITTENBERG, GERMANY), JANA ORDON (MARTIN-LUTHER-UNIVERSITÄT HALLE-WITTENBERG, GERMANY), JOHANNES GANTNER (MARTIN-LUTHER-UNIVERSITÄT HALLE-WITTENBERG, GERMANY)

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The functionality of Cas9-based, RNA-guided nucleases has by now been shown in many plant systems. We have developed numerous tools for routine application of the technology: Agrobacterium-compatible plant transformation vectors encoding for Cas9 (or variants) and up to eight different guide RNAs are assembled in two steps (four days), without any PCR, in a highly efficient manner. Making use of the simple multiplexing, the frequency and feasibility of generating chromosomal deletions by paired nucleases was tested. Data on assembly and testing of nuclease constructs, as well as mutation and deletion frequencies in different plant species, will be discussed.

AUTHOR INDEX

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