



Research Kernels

Our Latest Research Results - January 2014

Anchoring and ordering NGS contig assemblies by population sequencing (PopSeq)

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Submitted to: Nature Genetics

Next generation sequencing (NGS) is a powerful approach to understanding plant and animal genomes. Large amounts of sequence data can be easily generated at continually decreasing costs. However, to be fully leveraged for genetic studies and genomic-assisted breeding, this sequence information needs to be put in the ordered context of chromosomes representative of an organism's genome. This has typically been a long, expensive and arduous process necessitating the development of integrated physical maps along the length of individual chromosomes. In the present study, barley populations consisting of many individuals derived from two parents were used to order sequence information on the chromosomes using genetic linkage information. The resulting order of genomic sequence was as good or better than the current draft reference genome of barley, which was put together through a large international consortium over many years. The demonstrated population sequencing approach (PopSeq) is a rapid and inexpensive method to develop a working ordered assembly for complex genomes.

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Application of genotyping-by-sequencing on semiconductor sequencing platforms: A comparison of genetic and reference-based ordering in barley

Authors: M. Mascher, S. Wu, P. St. Amand, A. Rico, A. Sartori, N. Stein, J. Poland

Submitted to: PLoS ONE

Genotyping-by-sequencing is an approach that uses 'next-generation' sequencing technology for whole-genome characterization of diverse plant and animal populations for genetics and breeding studies. As new sequencing technology develops, the sequencing output is increasing while costs are decreasing. Therefore, it is important to adapt current methods to new technologies to leverage new developments and maximize output. This will increase the number of samples that can be

evaluated while decreasing the per sample cost. Particularly for plant breeding, increasing the number of samples that can be genetically profiled will enable genomic selection on larger populations and enable increased selection intensity. In the current study, we modified previous protocols that were used for sequencing on the Illumina HiSeq 2000 machine to test genotyping-by-sequencing on the Ion Torrent PGM and Ion Torrent Proton machines. We found that the new Ion Torrent platforms were suitable for GBS and gave consistent results with the established Illumina platform. We anchored the new genotyping data to the barley reference genome and found that the reference marker ordering can be used directly to establish genetic linkage maps for new populations. Overall, this study confirmed that this genotyping-by-sequencing approach can be transferred to new sequencing platforms to take advantage of technology advancements that increase sequencing output.

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Cloning and Characterization of a Critical Regulator for Pre-harvest Sprouting in Wheat

Authors: S. Liu, S.K. Sehgal, J. Li, M. Lin, H.N. Trick, J. Yu, B.S. Gill, G. Bai

Submitted to: Genetics

Pre-harvest sprouting (PHS) of wheat causes annual crop loss of \$1 billion. Consumers prefer white wheat varieties but they are more prone to PHS than red wheat. The mechanism of resistance to PHS in white wheat is not known. We found that a MOTHER OF FLOWERING TIME-like gene (*TaMFT*) regulates PHS resistance. Two nucleotide mutations in the coding region of the gene leading to mis-splicing and a truncated protein cause PHS. A DNA marker developed from the gene will facilitate effective deployment of *TaMFT* to protect grain yield and quality and extend the range of white wheat production.

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Horizontal Transfer of Methoprene by *Tribolium castaneum* and *T. confusum*

Authors: A.M. Tucker, J.F. Campbell, F.H. Arthur, K.Y. Zhu

Submitted to: Journal of Stored Products Research

In food facilities the majority of an insect population is typically within hidden locations that are difficult to reach

with conventional insecticide application methods, but as insects move in and out of these hidden areas they could pick up insecticide on their bodies and transport it back into these hidden areas. If this process results in transfer of insecticide from this individual to another and it causes negative effects on that individual then it is termed horizontal transfer. This process is important in other pest systems, but has not been previously evaluated for stored-product insects in food facilities. In a series of laboratory experiments using red flour beetle and confused flour beetle, we showed that the insect growth regulator methoprene could be transferred from a treated to an untreated individual. When red flour beetle immature stages were exposed to a treated insect normal adult emergence was reduced. The confused flour beetle is less susceptible to methoprene than the red flour beetle, so the effects of horizontal transfer were less apparent although adult emergence was still reduced. However, when immatures were able to survive exposure to methoprene treated individuals and emerge as normal adults, their reproductive ability was not affected. This is the first report indicating that methoprene can be transferred between stored-product insects and highlights a potential mechanism that might increase the effectiveness of insecticide treatments in reducing insect populations in food facilities such as mills, processing plants and warehouses. Contact Jim Campbell, telephone 785-776-2717, email James.Campbell@ars.usda.gov

High-throughput micro-plate HCl-vanillin assay for screening tannin content in sorghum grain

Authors: T.J. Herald, P. Gadgil, R. Perumal, S.R. Bean, J.D. Wilson

Submitted to: Journal of the Science of Food and Agriculture

Sorghum that contains tannin is reported to offer health promoting potential above those offered by many fruits such as blueberries. Currently, the test used to determine the amount of tannin present in sorghum is time consuming and not efficient for screening large sample sets. The objective of this research was to develop a rapid and accurate method for tannin in sorghum grain. The new test method was validated against a currently reliable method on 25 suspected tannin sorghum lines. The method was approximately eight times faster, as accurate and used fewer supplies than the current method.

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Evaluation of synergized pyrethrin aerosol for control of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae)

Authors: K. Karel, F.H. Arthur, K.Y. Zhu, J.F. Campbell, B. Subramanyam

Submitted to: Journal of Economic Entomology
Aerosol insecticides are used to control flour beetles in

milling facilities, and there are areas within flour mills where food spillage can accumulate and provide harborage sites for insect infestations. There is a need for information regarding the effect of food material on aerosol effectiveness. When the insects were directly exposed to the aerosol, mortality was high, but when they were exposed with flour, or exposed in the treated flour, mortality was greatly reduced. Larvae of both species were less susceptible to the pyrethrin aerosol in the presence of flour compared to adults and pupae, possibly because they were able to burrow into the flour and escape exposure. Results of this study show that sanitation and cleaning in conjunction with aerosol application may yield increased control of flour beetle populations in milling and food storage facilities. Contact Frank Arthur, telephone 785-776-2783, email Frank.Arthur@ars.usda.gov

SNP Meta: SNP annotation and SNP metadata collection without a reference genome

Authors: Thomas J. Y. Kono, Kiran Seth, Jesse A. Poland, and Peter L. Morrell

Submitted to: Molecular Ecology Resources

To utilize genome sequence information to make inference to biological traits it is important to have genes annotated in sequence databases. For model organisms (i.e. mouse, fly, rice) this is readily accomplished through large efforts centered on the reference genome sequence. For other species that do not have sufficient reference genome resources this approach is limited. Further, the increasing availability for low-cost DNA sequencing is making new datasets readily available. To fully leverage these sequence dataset it is important to have user-friendly tools available for annotation. Here we present a bioinformatics tool called SNP Meta that gathers information about DNA polymorphisms found in sequencing datasets and compares these sequences to reference databases for characterization and annotation. Contact Jesse Poland, telephone 785-532-2709, email [Jesse. Poland@ars.usda.gov](mailto:Poland@ars.usda.gov)

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