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INVESTIGATIONS

- 1797–1803 **SWEEP: A Tool for Filtering High-Quality SNPs in Polyploid Crops**
Josh P. Clevenger and Peggy Ozias-Akins
- Identifying true SNPs in polyploid species has been difficult. For crops such as peanut (*Arachis hypogaea*) there has not been a suitable pipeline developed for efficient SNP discovery and SNPs are currently not being widely utilized in genetic studies. Clevenger and Ozias-Akins have developed a novel pipeline, SWEEP, and implemented it as a user-friendly tool. They use real data and simulated data to show that SWEEP greatly outperforms current methods for efficient filtering of high quality true SNPs in polyploid species. SWEEP can be used for other polyploid crops to identify SNPs.
- 1805–1814 **Comparative Transcriptome Analysis of the Cosmopolitan Marine Fungus *Corollospora maritima* Under Two Physiological Conditions**
Patricia Velez, Naholi D. Alejandri-Ramírez, María C. González, Karel J. Estrada, Alejandro Sanchez-Flores, and Tzvetanka D. Dinkova
- Marine beaches represent dynamic environments, where natural and anthropogenic inputs of freshwater alter salinity, which is a key variable influencing critical physiological processes. Velez *et al.* analyze the transcriptome of the strict marine fungus *Corollospora maritima* under two salinity conditions, providing the annotation of transcripts for this species and a portfolio of candidate genes for further studies. This investigation sheds light on fungal adaptation and plasticity mechanisms in marine habitats, and provides the first insights into the genomic content of this fungus.
- 1815–1826 **How Well Do Molecular and Pedigree Relatedness Correspond, in Populations with Diverse Mating Systems, and Various Types and Quantities of Molecular and Demographic Data?**
Anna M. Kopps, Jungkoo Kang, William B. Sherwin, and Per J. Palsbøll
- Kinship analyses are important pillars of ecological and conservation genetic studies with potentially far reaching implications. There is a need for power analyses that address a range of possible relationships and investigate the influence of intrinsic population characteristics. This study shows how intrinsic population characteristics, such as mating system and the number of overlapping generations, life history traits, and genetic marker characteristics can influence the power of a RCA study. Therefore species-specific power analyses are essential.
- 1827–1841 **Transcriptomic Analysis of *Musca domestica* to Reveal Key Genes of the Prophenoloxidase-Activating System**
Dianxiang Li, Yongli Liang, Xianwei Wang, Lei Wang, Mei Qi, Yang Yu, and Yuanyuan Luan
- The proPO system regulates melanization in arthropods. However, the activation mechanisms and immune functions of this system in the housefly *Musca domestica* remain unclear. In order to obtain a comprehensive perspective on the proPO systems in *M. domestica*, Li *et al.* conducted a transcriptomic analysis by sequencing mixed housefly RAN samples of normal and bacteria-challenged larvae, pupae, and adults using Illumina paired-end sequencing assays. Important genes related to the proPO system were identified from the transcriptome, including eight peptidoglycan recognition receptors, two prophenoloxidases, three prophenoloxidase activating enzymes, and eleven serine proteinase inhibitors.

- 1843–1847 **Whole Organism Genome Editing: Targeted Large DNA Insertion via ObLiGaRe Nonhomologous End-Joining *in Vivo* Capture**
Yutaka Yamamoto, Jacob Bliss, and Susan A. Gerbi
 Targeted insertion of DNA is a goal of genome engineering, but many systems are refractory to homologous recombination. Yamamoto *et al.* use the preferred pathway of nonhomologous end-joining (used previously to create indels for gene inactivation) for precise and high efficiency integration of large DNA into the specified genomic target-site of an intact animal. The method uses site-specific cleavage, end-capture of cohesive ends, and obligate ligation-gated recombination. This approach is straightforward without additional gene manipulation; therefore it is easily applicable to a broad range of organisms.
- 1849–1855 **GFP Loss-of-Function Mutations in *Arabidopsis thaliana***
Jason L. Fu, Tatsuo Kanno, Shih-Chieh Liang, Antonius J. M. Matzke, and Marjori Matzke
 Green fluorescent protein (GFP) is used widely in biological research to monitor gene expression and protein localization in living cells. Although random and site-specific mutagenesis has been used to optimize GFP fluorescence and create novel derivatives, loss-of-function mutations that would aid in understanding GFP protein folding and chromophore formation are incompletely described. Fu *et al.* report on chemically-induced GFP loss-of-function mutations retrieved in a classical genetic screen in the plant *Arabidopsis thaliana*. Their genetic findings support previous biochemical and structural results, and identify specific amino acids of formerly unknown function that are essential for the function and stability of the GFP protein.
- 1857–1863 **Targeted Modification of Gene Function Exploiting Homology-Directed Repair of TALEN-Mediated Double-Strand Breaks in Barley**
Nagaveni Budhagatapalli, Twan Rutten, Maia Gurushidze, Jochen Kumlehn, and Goetz Hensel
 Writing and deleting in eukaryotic genomes became feasible by the discovery of programmable endonucleases like TALENs. Budhagatapalli *et al.* demonstrate the feasibility of editing the barley genome by precisely modifying a defined target DNA sequence resulting in a predicted alteration of gene function. The authors altered gene function via amino acid exchange after TALEN-mediated double strand break (DSB) induction and homology-directed repair (HDR). Predictable genetic modifications comprising only a few genomic base pairs rather than entire genes are of particular practical relevance, because they might not fall under the European regulation of genetically engineered organisms.
- 1865–1878 **Enrichment of H3K9me2 on Unsynapsed Chromatin in *Caenorhabditis elegans* Does Not Target *de Novo* Sites**
Yiqing Guo, Bing Yang, Yini Li, Xia Xu, and Eleanor M. Maine
 During meiosis in many animal species, chromosomes that do not synapse become enriched for certain histone modifications associated with heterochromatin. This process may promote formation of high quality gametes, but how? The authors mapped sites of one such modification, H3K9me2, in a *Caenorhabditis elegans* synapsis-defective mutant and investigated the effect of H3K9me2 loss on the mutant's gonad transcriptome. They also investigated H3K9me2 abundance in germline and somatic chromatin. Taken together, these results suggest the H3K9me2 plays a predominantly structural role rather than regulating transcription.
- 1879–1887 **An Updated Collection of Sequence Barcoded Temperature-Sensitive Alleles of Yeast Essential Genes**
Megan Kofoed, Karissa L. Milbury, Jennifer H. Chiang, Sunita Sinha, Shay Ben-Aroya, Guri Giaever, Corey Nislow, Philip Hieter, and Peter C. Stirling
 This article describes an updated collection of temperature-sensitive alleles for 600 essential yeast genes. This collection is a resource for functional genomic analyses of essential genes. Kofoed *et al.* show that the collection is amenable to high-throughput analysis for growth on solid or liquid media and that it is suitable for high-throughput strain construction and phenotypic analysis, using mutant allele-induced P-body formation as a readout. Together this resource adds to a growing toolbox for systematic analysis of essential genes in yeast and contributes to community efforts to create a complete map of a model eukaryotic cell.

- 1889–1897 **Comparative Genomics Reveals Chd1 as a Determinant of Nucleosome Spacing *in Vivo***
Amanda L. Hughes and Oliver J. Rando
 Eukaryotic genomes are packaged into chromatin, which consists of repeating nucleosomes separated by accessible linker DNA. Although many features of the nucleosome landscape are quite conserved, quantitative aspects of nucleosome packaging differ between species, as for example the average length of linker DNA can differ significantly between closely-related species. Hughes and Rando take advantage of the difference in linker length between two Hemiascomycete species – *Saccharomyces cerevisiae* and *Kluyveromyces lactis* – to investigate the “molecular ruler” responsible for this difference in chromatin state. The results demonstrate a role for sequence evolution of a chromatin remodeler in establishing quantitative aspects of the chromatin landscape in a species-specific manner.
- 1899–1908 **Metabolic Impacts of Using Nitrogen and Copper-Regulated Promoters to Regulate Gene Expression in *Neurospora crassa***
Shouqiang Ouyang, Consuelo N. Beecher, Kang Wang, Cynthia K. Larive, and Katherine A. Borkovich
 Few metabolite-regulated promoters have been described for *Neurospora crassa* and there is a paucity of studies that explore the metabolic consequences of using these promoters to control gene expression in fungi. In this study, *nit-6* is developed as a nitrogen-regulated promoter in *N. crassa*. Using proton NMR, the metabolome of *N. crassa* is investigated under inducing and repressing conditions for *nit-6* and the copper-regulated promoter *tcu-1*. The results show that conditions used for *tcu-1* do not significantly alter the primary metabolome and that the minor metabolic adjustments for *nit-6* can be explained by different nitrogen sources used for induction and repression.
- 1909–1918 **GABP α Binding to Overlapping ETS and CRE DNA Motifs Is Enhanced by CREB1: Custom DNA Microarrays**
Ximiao He, Khund Sayeed Syed, Desiree Tillo, Ishminder Mann, Matthew T. Weirauch, and Charles Vinson
 He *et al.* explore the cooperative interactions of two transcription factors (TFs), GABP α and CREB1, upon binding DNA. To this end, they designed a Protein Binding Microarray (PBM) containing >170,000 DNA sequences representing variations of the ETS \leftrightarrow CRE motif recognized by these TFs. The authors show that CREB1 enhances GABP α binding to the canonical ETS \leftrightarrow CRE motif two-fold, and up to 23-fold for specific SNPs located at opposite sides of the ETS motif, suggestive of two distinct allosteric mechanisms of cooperative binding. Custom-designed PBMs will aid not only in understanding mechanisms underlying TF interactions, but also in functional interpretation of genetic variants impacting human diseases.
- 1919–1924 **Reagent and Data Resources for Investigation of RNA Binding Protein Functions in *Drosophila melanogaster* Cultured Cells**
Stephanie E. Mohr, Yanhui Hu, Kirstin Rudd, Michael Buckner, Quentin Gilly, Blake Foster, Katarzyna Sierzputowska, Aram Comjean, Bing Ye, and Norbert Perrimon
 RNA binding proteins (RBPs) are involved in many functions. To facilitate functional characterization of RBPs, Mohr *et al.* generated an RNA interference (RNAi) library for *Drosophila* cell-based screens comprised of reagents targeting known or putative RBPs. They then screened the library using a total ATP assay and high-throughput imaging in *Drosophila* S2R+ cultured cells. Altogether, the authors provide resources in the form of an initial curated list of *Drosophila* RBPs; a high-quality RNAi screening library; and total ATP and image data. The data, including more than 200,000 images, are easily accessible online.
- 1925–1935 **Rapid Identification of Chemoresistance Mechanisms Using Yeast DNA Mismatch Repair Mutants**
Irene Ojini and Alison Gammie
 Chemoresistance is a significant medical problem and is the primary reason for chemotherapeutic failure and patient mortality. Ojini and Gammie exploit the mutator phenotype of mismatch repair defective yeast to develop a rapid method for identifying anti-cancer drug resistance mechanisms. The utility of this method is further enhanced by identifying compounds capable of preventing the emergence of drug resistant variants. The success of this strategy suggests a potentially general and powerful method for the identification of genetic pathways involved in the development of drug resistance and the rational design of effective drug combinations.

MUTANT SCREEN REPORT

1937–1944 **Genetic Regulation of Dna2 Localization During the DNA Damage Response** *Askar Yimit, Michael Riffle, and Grant W. Brown*

Dna2 is a multifunctional protein that is recruited to nuclear foci during both DNA replication and DNA repair. Yimit *et al.* screened the nonessential gene deletion collection in unperturbed conditions and in the presence of phleomycin-induced DNA double-strand breaks, identifying 37 genes that alter Dna2 localization to nuclear foci. The authors complemented the analysis by determining the subset of proteins that colocalize with Dna2 in nuclear foci in response to double-strand DNA breaks. Together, the data comprise a useful resource for understanding the regulation of Dna2 intracellular localization.