CRISPR/Cas9 used to mimic mitotic recombination to help map out genes

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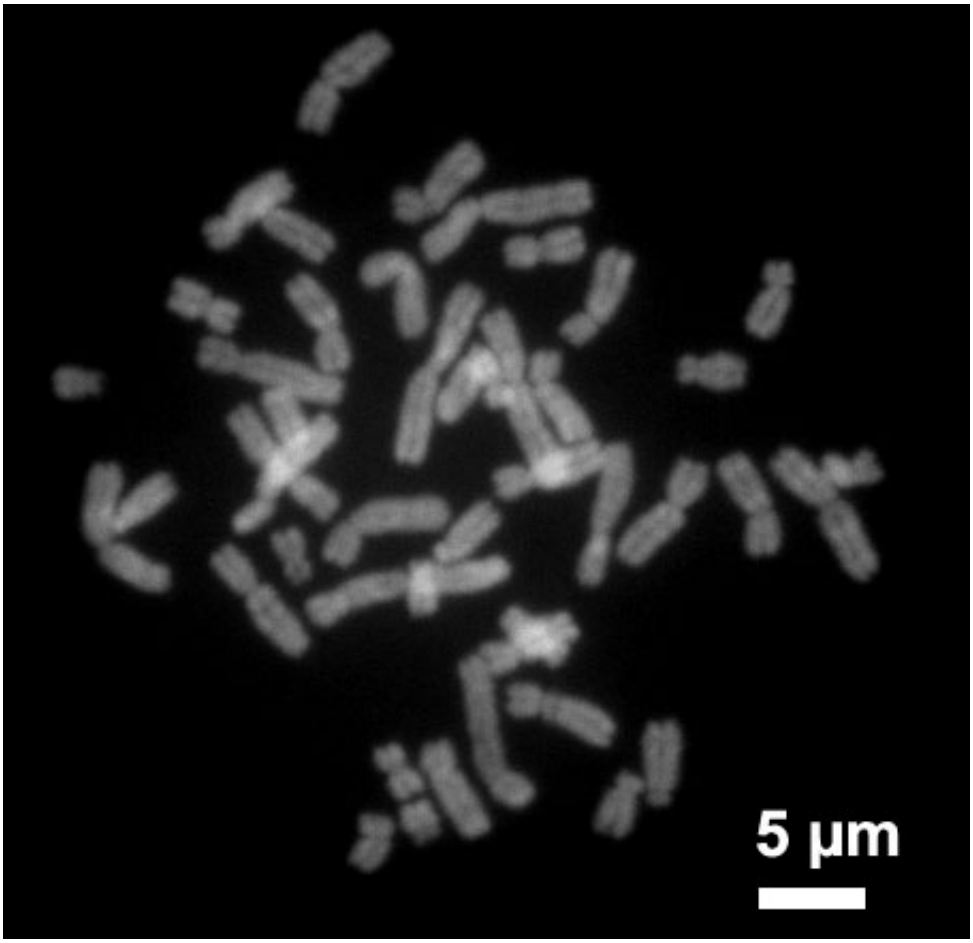

Human chromosomes during metaphase. Credit: Steffen Dietzel/Wikipedia

(Phys.org)—A small team of researchers with the University of California has developed a way to use CRISPR/Cas9 to mimic mitotic

recombination in yeast cells leading to a new way to map out genes and by doing so, determine their functions. In their paper published in the journal *Science*, Meru Sadhu, Joshua Bloom, Laura Day and Leonid Kruglyak describe how they developed the technique, how they tested it and how the technique may be used going forward.

In the past, trying to figure out what individual genes do has been a long hard process—researchers have had to look for meiotic recombination that occurred naturally shuffling genetic material. But this approach has been slow and generally required a very large number of progeny and still did not always provide the results that were sought. In this new effort, the researchers have developed a technique that mimics a natural process that occurs only rarely in cell division, but which allows for a more detailed understanding of what has occurred.

The team started with the knowledge that double-strand breaks that sometimes occur as a chromosome is about to split has on occasion allowed researchers to zero in on phenotype information due to the way the cells go about repairing the damage—called homologous recombination, the other chromosome is used to replace the lost sequence sometimes leading to a re-grown chromosome with only one set of genes, rather than the normal two, which of course caused the gene to lose its role and in so doing reveal its function. Because CRISPR/Cas9 works by snipping sequences, the team set out to discover if it might be used to cause the same type of splits on demand, allowing the researchers to control the action whenever they chose. Each time it was done, the researchers could examine the repaired sequence and if there were missing functions, they could match it with the missing genes, thereby identifying the gene responsible for a given trait.

The team worked with yeast sensitive to manganese, picking out non-fluorescent cells and in so doing were able to show that the technique worked as envisioned, possibly paving the way for a new approach to

mapping genotypes to specific loci.

More information: M. J. Sadhu et al. CRISPR-directed mitotic recombination enables genetic mapping without crosses, *Science* (2016). DOI: 10.1126/science.aaf5124

Abstract

Linkage and association studies have mapped thousands of genomic regions that contribute to phenotypic variation, but narrowing these regions to the underlying causal genes and variants has proven much more challenging. Resolution of genetic mapping is limited by the recombination rate. We developed a method that uses CRISPR to build mapping panels with targeted recombination events. We tested the method by generating a panel with recombination events spaced along a yeast chromosome arm, mapping trait variation, and then targeting a high density of recombination events to the region of interest. Using this approach, we fine-mapped manganese sensitivity to a single polymorphism in the transporter Pmr1. Targeting recombination events to regions of interest allows us to rapidly and systematically identify causal variants underlying trait differences.