CRISPR/Cas genome engineering system generates valuable conditional mouse models

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Whitehead Institute researchers have used the gene regulation system CRISPR/Cas to engineer mouse genomes containing reporter and conditional alleles in one step. Animals containing such sophisticated engineered alleles can now be made in a matter of weeks rather than years and could be used to model diseases and study gene function.

"We've used CRISPR/Cas to mutate genes before, but the nature of the targeted mutations has been unpredictable," says Whitehead Founding Member Rudolf Jaenisch. "Now we can make specific deletions defined by two cuts. We can use this to make conditional mice in one step, and we can easily and very efficiently insert pieces of DNA up to three thousand base pairs It used to be much more work to make such mice."

The CRISPR/Cas (for "clustered regularly interspaced short palindromic repeat/CRISPR-associated) system is based on an immune defense against viral invaders in bacteria and archaea. Scientists recently have adapted that defense to alter the genomes of mouse and human cells quickly and efficiently. Until now, however, researchers had yet to use CRISPR/Cas to create one of the most useful tools for genetic research: the conditional mutant mouse.

A conditional mutant mouse's genome contains a gene or collection of genes that can be turned on or off using a particular signal. By turning the genes on or off, scientists can tease apart the role of certain genes in biological functions and diseases.

Previously, scientists created such model organisms using a complex and time-consuming process that requires using embryonic stem cells (ESCs). Unfortunately, scientists have only been able to efficiently manipulate the ESCs of mice and rats, a restriction that has hobbled this type of research.

Using CRISPR/Cas, Jaenisch and his lab have created mice with conditional alleles, as well as mice that carry multiple tagged genes that report whether these genes are being expressed. Their work is described in the September 12 issue of the journal *Cell*.

The researchers' experiments also allay concerns regarding CRISPR/Cas's off-target activity.

"Recent studies in human cancer cell lines raised some concerns on the specificity of CRISPR/Cas," says Chikdu Shivalila, a co-author of the Cell paper and a graduate student in the Jaenisch lab, "Our study shows that the non-specific DNA cleavages could happen, but they are rare and predictable."

The Jaenisch lab's latest work opens up a number of avenues for future research.

"The methods we described in this work will greatly accelerate the speed of generating gene modified animals," says Hui Yang, a postdoctoral researcher in the Jaenisch lab and co-author. "I'd like to use CRISPR/Cas to establish sophisticate disease models using this method."

Because CRISPR/Cas does not rely on ESCs, it can be used to genetically modify any animal, including livestock.

"We haven't tried it yet, but I'd like to adapt the CRISPR/Cas system for genome engineering in large animals, such as primate for disease

modeling, or cattle for agricultural purposes," says Haoyi Wang, a co-author and a postdoctoral researcher in the Jaenisch lab. "If so, this method could be very important economically, too."

More information: "One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas mediated genome engineering" *Cell*, September 12, 2013.

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