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EDITORIAL

The CRISPR/Cas9 Genome Editing Revolution

Genomes encode the genetic information that controls the development and physiological functions of all living organisms on our planet, and are therefore of central interest in all aspects of biomedical research. To understand the blueprint of life, scientists have long aimed to read and manipulate the genome using a rapidly expanding toolbox. To read the genome, novel state-of-the-art sequencing technologies have made it possible to sequence any single genome rapidly and cheaply. However, methods for introducing targeted modifications of the genome have lagged behind, and though feasible, have long remained inefficient. The adaptation of the bacterial CRISPR/Cas9 system to eukaryotic cells has now changed this situation dramatically. The system, used by bacteria to cleave DNA in an RNA-dependent manner, was reported three years ago to rapidly, specifically and effectively target genomic sequences in human cells (Cong et al., 2013; Jinek et al., 2013). Since then an enormous number of publications have shown that the CRISPR/Cas9 system provides a highly effective way to edit the genomes of plants, animals and human cells, and can readily be adopted by laboratories across the world. The *Journal of Genetics and Genomics* has covered progress in this field in the past three years. In this special issue entitled Genome Editing, 12 new articles are published specifically for the benefit of readers who are interested in genome editing for agricultural and medical purposes, or simply for basic research in life sciences.

An editorial prerogative article by Dr. Daniel F. Voytas at University of Minnesota kicks off this special issue (pp. 229–232). Genome editing in plants is no longer limited to model plants such as *Arabidopsis,* but has been shown to be possible in many crop plants. While targeted mutagenesis using the CRISPR/Cas9 system is highly efficient in most plants, editing the plant genome *via* homologous recombination remains challenging. As toolkits for editing plant genomes become widely available, not only will functional genomics studies in plants no longer be difficult, but also genome-edited crops will become common at our dining tables, meeting the challenges of a growing population while reducing farmed land worldwide. Perota and colleagues from Dr. Cesare Galli's lab in the Laboratorio di Tecnologie della Riproduzione and the University of Bologna share their views on applications of genome editing in xenotransplantation, where it might, for example, reduce transplant immunogenicity (pp. 233−237).

This special issue contains reviews on plant genome editing, insect genome editing, 3D genome editing and improved procedures for CRISPR/Cas9 genome editing. "Genome Editing with CRISPR-Cas9: Can It Get Any Better?" is the question that Drs. Maximilian Haeussler and Jean-Paul Concordet at University of California and CNRS address in their review. They summarize current strategies for increasing single guide RNA (sgRNA) specificity to minimize off-target events and maximize Cas9 cleavage efficiency. They also discuss the relative merits of Cas9 and Cpf1, and make the point that the choice of cleavage enzyme depends on the specific purpose of an experiment (pp. 239−250). Zhang and colleagues from Dr. Jian-Feng Li's laboratory at the Sun Yat-sen University review recent improvements in the toolkit for CRISPR/Cas9-based genome editing in plant research; they list some key factors for improving CRISPR/Cas9 performance, and discuss strategies for reducing off-target events during plant genome editing (pp. 251−262). Huang and colleagues from Dr. Yikang S. Rong's laboratory at the Sun Yat-sen University, first describe progress in genome editing tools for model insects such as *Drosophila*, before discussing the logistics of implementing similar genome editing tools in non-model insects such as the house fly (pp. 263−272). The genomes of eukaryotic cells are organized in a 3D fashion: topologically associated domains (TADs) within discrete territories of each chromosome in the nucleus play important roles in regulating gene expression, and the CRISPR/Cas9 system has made 3D genome editing possible. Drs. Haiyan Huang and Qiang Wu at Shanghai Jiao Tong University review the application of CRISPR/Cas9 to 3D genome editing and describe how Cas9 and a pair of sgRNAs can be used to investigate the topology of chromatin TADs (pp. 273−288).

Three original research papers on genome editing are among the 12 articles of this special issue. Bai and colleagues from Dr. Jinsong Li's laboratory at the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, describe a tissue-specific CRISPR/Cas9 system in mice that specifically disrupts gene function in the spermatogenic cells of mice.

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They first generated a transgenic line in which Cas9 production was restricted to spermatogenic cells before introducing a sgRNA that disrupted the *Scp3* gene in the same mice. The male founders of such mice were sterile, indicating that *Scp3* function had been abolished in the germ cells, and they showed that this male sterility was transmitted to the next generation by fertile females carrying the same edited genome (pp. 289−296). Li and colleagues from Bing Yang's laboratory at Iowa State University report their success in achieving homologous recombination in rice (*Oryza sativa*) using TALEN (transcription activator-like effector nuclease). They were able to produce herbicide resistant rice lines by homologous recombination using TALEN-based genome editing with donor DNA carrying the desired mutations. The change was stably inherited and strains with the new genome displayed stable herbicide resistance (pp. 297−305). Ear and colleagues from Dr. Shuo Lin's lab at the University of California Los Angeles established a zebrafish model of 5q-syndrome using the CRISPR/Cas9 system to modify the *RPS14* gene, and revealed an early p53-independent and late p53-dependent mechanism of erythroid failure (pp. 307−318). This model was shown to be useful for drug screening, and several drugs were identified capable of reversing the mutant fish phenotype. Whether the drugs that were effective in fish would be similarly effective in human 5q-syndrome remains to be seen.

Three methods papers offer better and easier protocols for improving the efficiency of targeted genome editing or reducing the work involved in genotyping to screen for targeted events with the CRISPR/Cas9 system. Wang and colleagues from Dr. Haoyi Wang's laboratory at the Jackson Laboratory and the Institute of Zoology, Chinese Academy of Sciences, have improved the genome editing method in mice by delivering Cas9 protein into mouse zygotes by electroporation. The improved approach can be used to introduce precise nucleotide substitutions, large deletions and small insertions into the mouse genome with high efficiency (pp. 319−327). Lemoine and colleagues from Dr. Cédric Louvet's laboratory at the Université de Nantes report a protocol with key steps required to produce a double knockout mouse with neighboring homologs *Tmem176a* and *Tmem176b* (pp. 329−340). A third procedure, described by Chenouard and colleagues from Dr. Laurent Tesson's laboratory at the Centre Hospitalier Universitaire de Nantes, is focused on the detection of targeted mutations in edited genomes. They have developed a mobility assay for heteroduplexes that involves PCR amplification of DNA of the edited genome (gDNA) followed by automated microfluidic capillary electrophoresis. This procedure efficiently discriminates between wild-type and mutant alleles at a resolution of single base pairs (pp. 341−348).

The CRISPR/Cas9 system is constantly being improved and new applications have been identified. In addition to its applications in genome editing, a unique role in live imaging of cells has emerged, bringing with it the prospect of visualizing the living genome *in situ*. Also, very recently, Komor et al. (2016) have modified the Cas9 system in such a way that bases can be changed without the need to cut the DNA; their approach significantly increases the efficiency of editing and avoids off-target events.

It is exciting to witness the rapid advances in modifying the genomes of a multitude of organisms. In this process, the CRISPR/Cas9 system has become the tool of choice for many laboratories and many purposes. Safety and ethical issues have drawn particular attention soon after the CRISPR/Cas9 system became a versatile tool for genome editing in many organisms, especially in big animals such as monkeys and pigs. On Dec. 1st of 2015, scientists including the pioneers in the CRISPR/Cas9 field, George Church, Jennifer Doudna, Emmanuelle Charpentier and Feng Zhang, gathered in Washington for the International Summit on Human Gene Editing. Experts at the summit, including Nobel laureate David Baltimore, discussed and reached common ground for ethical issues and potential social problems that may emerge due to the wide range of CRISPR/Cas9 applications. Further efforts are needed to establish specific safety and ethical guideline for all scientists working in this field.

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