A CRISPR Approach to Genetic Screening

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Identifying genes involved in biological processes is essential for understanding cellular and organismal function. While the genomes of many organisms have been sequenced, the function of a large fraction of genes even in genomes as well-studied as human and mouse are unknown. Perhaps the most widely used method to discover gene function is the forward genetic screen, in which chemical mutagens are used to cause random mutations throughout the genome, followed by tedious work to identify mutant animals and the mutated gene. This process can take several years, even using modern high-throughput sequencing technology to facilitate mutation identification. To develop a more efficient way to screen for gene function, research technician Arish Shah and colleagues in the lab of Dr. Cecilia Moens (Basic Sciences Division) turned to the CRISPR (clustered regularly interspaced short palindromic repeats) genome editing system. CRISPR involves a nuclease called Cas9 that is targeted to specific DNA sequences by engineered single guide RNAs (sgRNAs). The authors took advantage of this for genetic screening by expressing Cas9 and sgRNAs that cause small insertions or deletions (indels) in zebrafish. "We show the utility of the system by examining 48 genes for their requirement in electrical synapse formation and find two new genes that are involved in the process. Our screen took ~1 month to complete, showing that this can greatly speed up discovery," said Dr. Adam Miller, a postdoctoral fellow involved in the study.

The authors first assessed the potential of CRISPR to cause indels in single zebrafish genes by targeting a gene involved in retinal pigmentation and two genes involved in neuronal migration. This was done by injecting sgRNAs and mRNA encoding Cas9 into one-cell zebrafish embryos. Each gene disruption produced the expected phenotype, despite that fact that embryos were mosaic for a number of mutations due to a delay in Cas9 protein expression following injection.

Having confirmed that CRISPR could be used to screen for known phenotypes in injected embryos, the authors next wanted to ask whether they could screen for new genes involved in a process of interest. The authors chose to screen for electrical synapse formation because little is known about the genes required for their formation. The authors used the Mauthner circuit (M) in the zebrafish spinal cord due to the accessibility of its electrical synapses. CRISPR targeting of the *gjd1a* gene, previously found to be required for M electrical synapse formation, with two separate sgRNAs resulting in a >95% decrease in synapse number in 95% and 60% of injected embryos. This

difference in efficiency was subsequently found to be a consequence of the sequence composition of the sgRNA target.

To screen a large number of genes simultaneously, the authors tested the efficiency of pooling sgRNAs for a single injection with Cas9. The most efficient *gjd1a* sgRNA was pooled with sgRNAs targeting five genes not involved in electrical synapse formation and injected. All pool-injected embryos displayed loss of synapses, suggesting that pooling of sgRNAs is effective. Following up on this, they designed a set of 48 sgRNAs targeting genes potentially involved in synapse formation. These were injected in pools of six and eight, with each sgRNA present in two pools. This led to six pools and a total of nine genes likely to be involved in synaptogenesis. Injection of individual sgRNAs from the pools recapitulated the pooled phenotype in a subset of injections, and allowed the authors to identify two genes not previously implicated in electrical synapse formation. The genes were subsequently confirmed in stable mutant lines. From sgRNA synthesis to phenotypic screening of embryos injected with individual sgRNAs, the screen took 3 weeks. Furthermore, sequencing of the target sites for each sgRNA showed that most targets were successfully disrupted.

"Looking forward this approach can certainly be expanded to many different phenotypes," said Dr. Miller. "Ongoing work in lab has found that the technique can be used to examine genes involved in neuronal migration, cell polarity, and cancer metastasis. Moreover, our screen of 50 genes was a pilot, and we hope to expand the screen to hundreds, perhaps even up to 1,000 genes in future work. CRISPR reverse genetic screening greatly increases the speed of doing genetics in a vertebrate system."

Shah AN, Davey CF, Whitebirch AC, Miller AC, Moens CB. 2015. Rapid reverse genetic screening using CRISPR in zebrafish. *Nat Methods* 12(6):535-540.

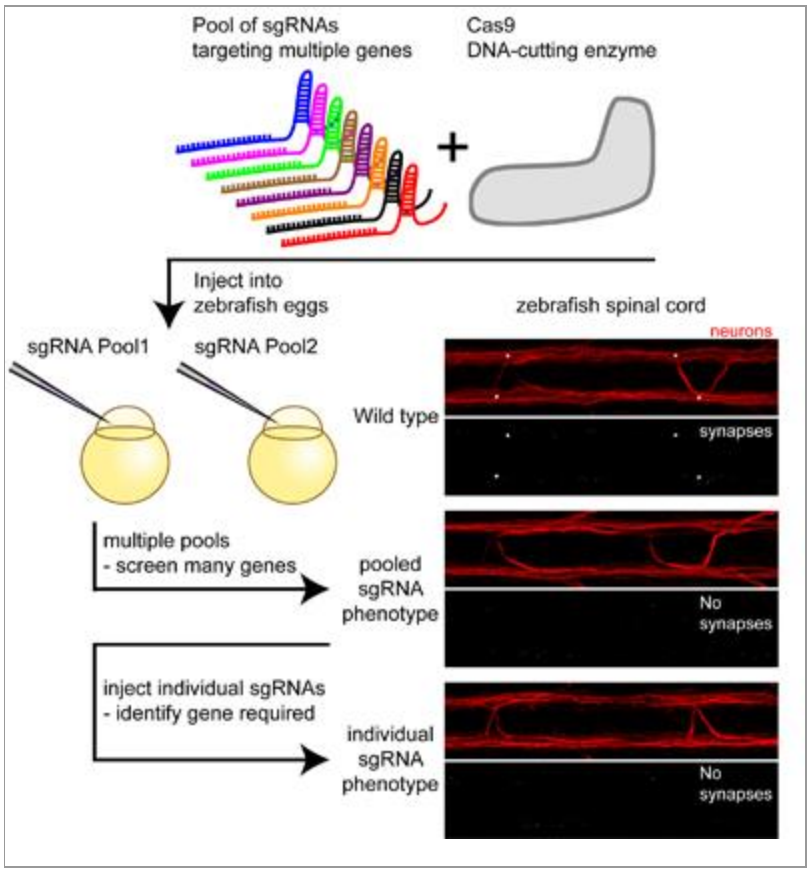

Image provided by Dr. Adam Miller

Schematic of the screening strategy. A pool of sgRNAs and mRNA encoding Cas9 is injected into zebrafish eggs. The phenotype resulting from the sgRNA pool is then analyzed. Following this, individual sgRNAs from the pool and Cas9 are injected to identify the gene(s) responsible for the pool's phenotype.