Rapid Reverse Genetic Screening Using CRISPR in Zebrafish

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FIG. 1. Pools of single guide RNAs (sgRNAs) designed to target multiple candidate genes, along with Cas9-encoding mRNA, are injected into the single-cell stage embryo. Mutagenesis (depicted as large X) occurs at each of the pool targets (depicted as boxes on chromosomes) and mutant phenotypes (depicted as a loss of synapses) can be scored in the injected (F_0) animals. Any sgRNA pool that caused a phenotype can be split (demulti-plexed) with each sgRNA being re-injected individually to determine the sgRNA/gene responsible for the phenotype. Color images available online at www.liebertpub.com/zeb

TO QUICKLY AND EFFICIENTLY assign function to genes *in vivo*, Shah *et al.*¹ developed a new CRISPR/Cas9-based reverse genetic screen in F_0 zebrafish. When injected with optimized concentrations of an engineered gene-specific single guide RNA (sgRNA) and Cas9-encoding mRNA (which together constitute the CRISPR/Cas9 system—resulting in mutations at the target site), zebrafish phenocopied known

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mutant defects across a wide range of phenotypes. To interrogate the genetic basis of electrical synapse formation, a CRISPR (clustered regularly interspaced short palindromic repeats)-based functional screen analyzed 48 genes and identified 2 new genes that were required. The screen utilized a multiplexing strategy in which pools of sgRNAs were injected and analyzed for phenotypes, and individual candidate genes were identified by demultiplexing (Fig. 1). Genes identified in the screen were confirmed in subsequent generations in stable mutant lines. The CRISPR screen, including design, synthesis, and candidate identification, took ~1 month. CRISPR-based F₀ screening *in vivo* provides an efficient, fast, and cost-effective approach to identifying new genes involved in many biological processes.

Reference

1. Shah AN, *et al.* Rapid reverse genetic screening using CRISPR in zebrafish. *Nat Methods* 2015;12:535–540.

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