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ORTHOGONAL CRISPR-CAS GENOME EDITING AND EFFICIENT INHIBITION WITH ANTI-CRISPRS IN ZEBRAFISH EMBRYOS

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Abstract

The type II CRISPR-Cas system from *Streptococcus pyogenes* (SpCas9) is most widely used for genome editing due to its high efficiency in cells and organisms. Concentrating on a single CRISPR-Cas system can limit options for multiplexed editing. We hypothesized that CRISPR-Cas systems originating from different bacterial species could operate simultaneously and independently due to their distinct single-guide RNAs (sgRNAs) or CRISPR-RNAs (crRNAs), and specific protospacer adjacent motifs (PAMs). Additionally, we hypothesized that CRISPR-Cas activity in zebrafish could be inhibited through co-expression of anti-CRISPR (Acr) proteins. Here, we describe a simple mutagenesis screen, and confirm that CRISPR-Cas systems from SpCas9, *Streptococcus aureus* (SaCas9), and *Lachnospiraceae bacterium* (LbCas12a, previously known as LbCpf1) are highly effective in zebrafish and are orthogonal systems capable of operating simultaneously. We also demonstrated that type II Acrs are effective inhibitors of SpCas9 and SaCas9 in zebrafish. These results indicate that at least three orthogonal CRISPR-Cas systems and four anti-CRISPR proteins are functional in zebrafish embryos. These additional CRISPR-Cas systems and Acr proteins broaden the toolset for genome modification in zebrafish and enable combinatorial and intersectional strategies for spatiotemporal control of genome editing in zebrafish.

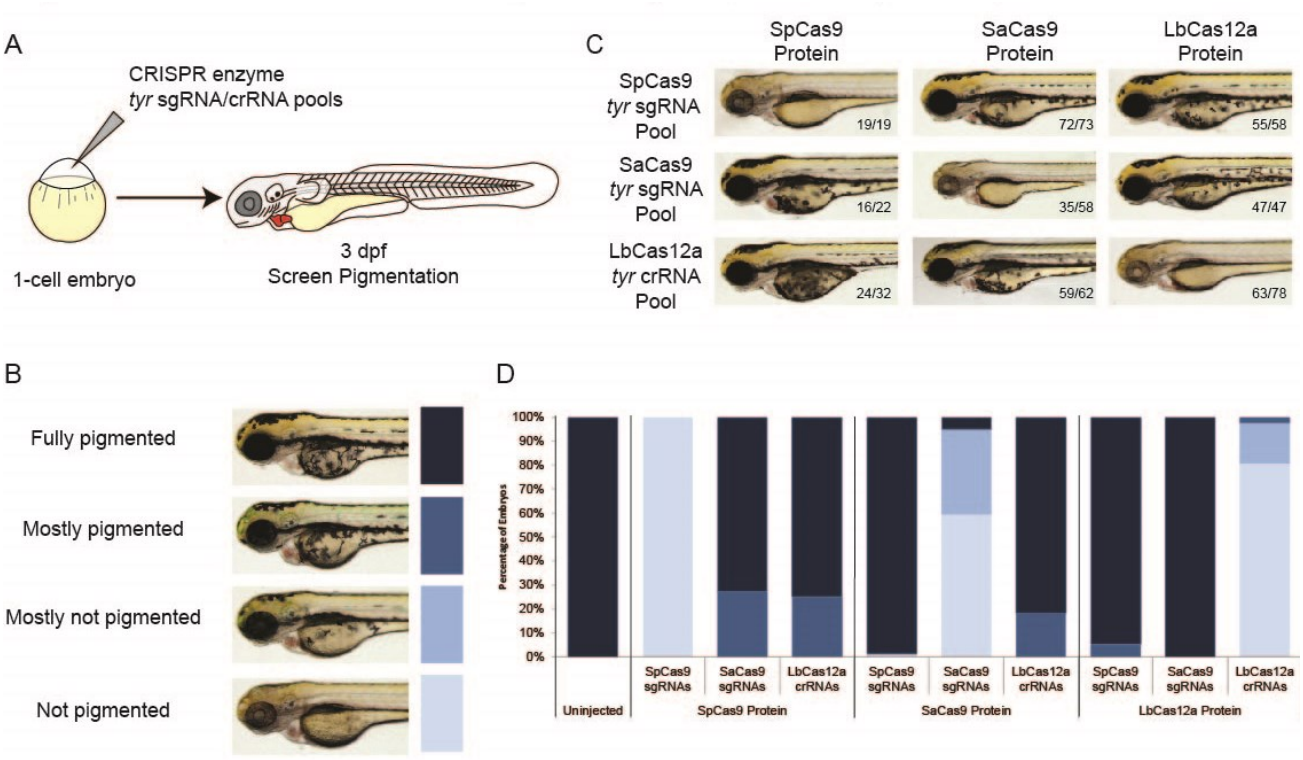

Figure 1: In vivo validation of functionality and orthogonality of SpCas9, SaCas9 and LbCas12a

A. Experimental design of the functionality and orthogonality experiment. An injection mix of CRISPR-Cas enzyme and *tyr* sgRNAs/crRNA pools is introduced into the single-cell embryo via microinjection. At 3 dpf the fish are screened for their level of pigmentation.

B. Example images of the four pigmentation categories that embryos are organized into when scored. Each category is roughly defined within a certain percentage of pigmentation: Fully pigmented=100% pigmented, Mostly pigmented=51-99% pigmented, Mostly not pigmented=6-50% pigmented, Not pigmented=0-5% pigmented. The associated colors act as the legend for panel C

C. Images of the primary phenotype of embryos by targeting the *tyr* gene with each set of sgRNAs/crRNAs. Each CRISPR-Cas system is only functional when used with its corresponding sgRNAs/crRNAs. Reference panel B as the legend.

D. Percentage of embryos from each condition exhibiting the pigmentation categories.