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Supplementary Materials

www.sciencemag.org/cgi/content/full/339/13143/DC1
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RNA-Guided Human Genome Engineering via Cas9

Prashant Mali,^{1,*} Lihua Yang,^{1,3,*} Kevin M. Esvelt,² John Aach,³ Marc Guell,³ James E. D. Carlo,⁴ Julie E. Norville,³ George M. Church^{1,2,†}

Bacteria and archaea have evolved adaptive immune defenses, termed clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems, that use short RNA to direct degradation of foreign nucleic acids. Here, we engineer the type II bacterial CRISPR system to function with custom guide RNA (gRNA) in human cells. For the endogenous AAVS1 locus, we obtained targeting rates of 10 to 25% in 293T cells, 13 to 8% in K562 cells, and 2 to 4% in induced pluripotent stem cells. We show that this process relies on CRISPR components; is sequence-specific; and, upon simultaneous introduction of multiple gRNAs, can effect multiplex editing of target loci. We also compute a genome-wide resource of ~190 K unique gRNAs targeting ~40.5% of human exons. Our results establish an RNA-guided editing tool for facile, robust, and multiplexable human genome engineering.

Bacterial and archaeal clustered regularly interspaced short palindromic repeats (CRISPR) systems rely on CRISPR RNAs (crRNAs) in complex with CRISPR-associated (Cas) proteins to direct degradation of complementary sequences present within invading viral and plasmid DNA (1–3). A recent *in vitro* reconstitution of the *Streptococcus pyogenes* type II CRISPR system demonstrated that crRNA, fused to a normally trans-encoded tracrRNA, is sufficient to direct Cas9 protein to sequence-specifically cleave target DNA sequences matching the crRNA (4). The fully defined nature of this two-component system suggested that it might function in the cells of eukaryotic organisms such as yeast, plants,

and even mammals. By cleaving genomic sequences targeted by RNA sequences (4–6), such a system could greatly enhance the ease of genome engineering.

Here, we engineer the protein and RNA components of this bacterial type II CRISPR system in human cells. We began by synthesizing a human codon-optimized version of the Cas9 protein bearing a C-terminal SV40 nuclear localization signal and cloning it into a mammalian expression system (Fig. 1A and fig. S1A). To direct Cas9 to cleave sequences of interest, we expressed crRNA-tracrRNA fusion transcripts, hereafter referred to as guide RNAs (gRNAs), from the human U6 polymerase III promoter. Directly transcribing gRNAs allowed us to avoid reconstituting the RNA-processing machinery used by bacterial CRISPR systems (Fig. 1A and fig. S1B) (4, 7–9). Constrained only by U6 transcription initiating with G and the requirement for the PAM (protospacer-adjacent motif) sequence –NGG following the 20–base pair (bp) crRNA target, our highly versatile approach can, in principle, target any genomic site of the form GN₁₉GG (fig.

S1C; see supplementary text S1 for a detailed discussion).

To test the functionality of our implementation for genome engineering, we developed a green fluorescent protein (GFP) reporter assay (Fig. 1B) in human embryonic kidney HEK 293T cells similar to one previously described (10). Specifically, we established a stable cell line bearing a genomically integrated GFP coding sequence disrupted by the insertion of a stop codon and a 68-bp genomic fragment from the AAVS1 locus that renders the expressed protein fragment non-fluorescent. Homologous recombination (HR) using an appropriate repair donor can restore the normal GFP sequence, which enabled us to quantify the resulting GFP⁺ cells by flow-activated cell sorting (FACS).

To test the efficiency of our system at stimulating HR, we constructed two gRNAs, T1 and T2, that target the intervening AAVS1 fragment (Fig. 1B) and compared their activity to that of a previously described TAL effector nuclease heterodimer (TALEN) targeting the same region (11). We observed successful HR events using all three targeting reagents, with gene correction rates using the T1 and T2 gRNAs approaching 3% and 8%, respectively (Fig. 1C). This RNA-mediated editing process was notably rapid, with the first detectable GFP⁺ cells appearing ~20 hours post transfection compared with ~40 hours for the AAVS1 TALENs. We observed HR only upon simultaneous introduction of the repair donor, Cas9 protein, and gRNA, which confirmed that all components are required for genome editing (fig. S2). Although we noted no apparent toxicity associated with Cas9/gRNA expression, work with zinc finger nucleases (ZFNs) and TALENs has shown that nicking only one strand further reduces toxicity. Accordingly, we also tested a Cas9D10A mutant that is known to function as a nickase *in vitro*, which yielded similar HR but lower nonhomologous end joining (NHEJ) rates (fig. S3) (4, 5). Consistent with (4), in which a related Cas9 protein is shown to cut both strands

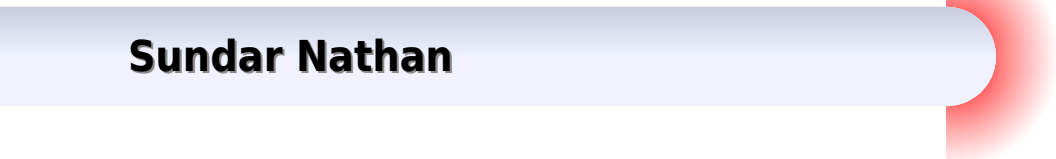
¹Department of Genetics, Harvard Medical School, Boston, MA 02115, USA. ²Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA 02138, USA. ³Biological and Biomedical Sciences Program, Harvard Medical School, Boston, MA 02115, USA. ⁴Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: gchurch@genetics.med.harvard.edu

Biology The Human Genome Guided Answers

Sundar Nathan



Biology The Human Genome Guided Answers:

Biology The Human Genome Guided Answers Book Review: Unveiling the Power of Words

In a world driven by information and connectivity, the energy of words has been evident than ever. They have the capacity to inspire, provoke, and ignite change. Such could be the essence of the book **Biology The Human Genome Guided Answers**, a literary masterpiece that delves deep into the significance of words and their impact on our lives. Written by a renowned author, this captivating work takes readers on a transformative journey, unraveling the secrets and potential behind every word. In this review, we shall explore the book's key themes, examine its writing style, and analyze its overall affect on readers.

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