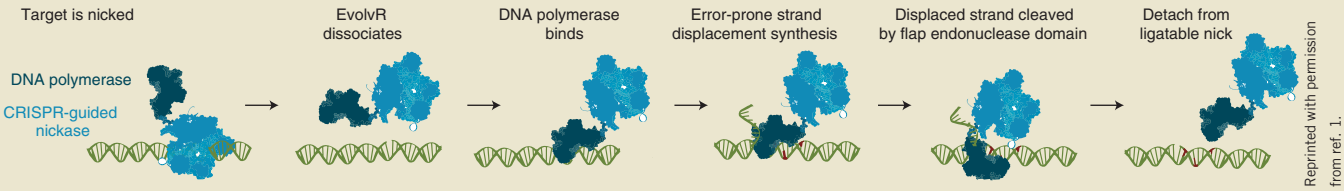
EvolvR-ing to targeted mutagenesis



Directed evolution of novel molecular and cellular functions often makes use of tools for genetic diversification, such as error-prone polymerases and saturation mutagenesis. Writing in *Nature*, Halperin *et al.*[1] describe a new mutagenesis tool, called EvolvR, that introduces semi-random mutations in a small region downstream of any genomic or plasmid site that can be targeted by CRISPR-Cas9. A fusion of a nicking variant of Cas9 (nCas9) and an error-prone DNA polymerase I (PolI3M), EvolvR effectively recruits the mutagenesis activity of the polymerase to the Cas9 target site.

In the first step, nCas9 creates a single-strand break at a site determined by its guide RNA and disassociates from the DNA. PolI3M then binds at the nick and extends the 3′ DNA end, while its native endonuclease activity degrades the displaced strand. To make this system work, the authors needed to add mutations both in nCas9, to reduce its affinity for DNA, and in PolI3M, to increase its error rate. Fusion of the two enzymes was also necessary for substantially enhanced mutagenesis.

Using an optimized form of EvolvR that includes a bacteriophage thioredoxin-binding domain, Halperin *et al.*[1] achieve a mutation rate of 10^{-5} to 10^{-6} mutations per nucleotide per generation in a 56-bp window, with higher rates closer to the nick. Low activity at an off-target control gene is also observed.

EvolvR offers a new combination of features compared with previous directed evolution systems. Targeted mutagenesis was recently demonstrated using a fusion of catalytically dead Cas9 and activation-induced deaminase (AID)[2,3]. But AID preferentially deaminates cytosines to uracil, while EvolvR can introduce all four bases. However, EvolvR still exhibits some substitution bias, with adenines and thymines composing >80% of introduced mutations. In this respect, it is less versatile than multiplex activation genome engineering (MAGE)[4], which can add any base throughout the genome using user-designed oligonucleotides.

EvolvR also facilitates continuous diversification across multiple bacterial generations. Achieving this with MAGE requires repeated addition of DNA constructs. "My first thought upon reading this paper was, finally, somebody has come up with a way to do Lamarckian-like evolution! By focusing mutation continuously in a given region of a genome, particular phenotypes can be accessed without the burden of fitness decreases elsewhere," says Andrew Ellington at the University of Texas at Austin.

Halperin *et al.*[1] apply EvolvR to select mutations that confer antibiotic resistance in plasmid and genomic genes. A wide array of other applications can be envisaged, from evolving new protein activities to studying cellular diversification in response to exogenous stimuli. It will also be necessary to determine whether EvolvR can function effectively in eukaryotic cells, although this seems promising given earlier results with dCas9-AID fusion proteins. The EvolvR construct itself is likely to be improved by modifications that increase the mutation rate and the length of the targetable window. As Ellington notes: "The current version is already useful, and there are no inherent limitations to improving it."

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1. Halperin, S.O. *et al. Nature* **560**, 248–252 (2018).
2. Ma, Y. *et al. Nat. Methods* **13**, 1029–1035 (2016).
3. Hess, G.T. *et al. Nat. Methods* **13**, 1036–1042 (2016).
4. Wang, H.H. *et al. Nature* **460**, 894–898 (2009).

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