Tools of the trade

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Prime editing sensors enable multiplexed genome editing

To understand the consequences of genetic variants, researchers need the ability to precisely generate these mutations in model systems. Prime editing satisfies these requirements, enabling the generation of almost any small variant in its natural genomic context. However, prime editing is limited by large variation in editing efficiency among different prime editing guide RNAs (pegRNAs), which provide the instructions for engineering mutations of interest. To overcome this challenge, we developed an approach that we call the prime editing sensor[1], wherein a synthetic copy of the endogenous target site — the 'sensor' — enables the simultaneous evaluation of pegRNA editing activity and abundance in a pooled format (Fig. 1).

Prime editing is a 'search-and-replace' genome-editing tool[2]. The system consists of a prime editor protein — a nicking Cas9 fused to reverse transcriptase — that takes direction from the information encoded in a pegRNA. Within each pegRNA is a protospacer (the 'search' sequence) that directs the prime editor to a site of interest, as well as a 3′ extension (the 'replace' sequence) that provides the template for reverse transcriptase to synthesize a DNA strand containing the desired mutation.

A key obstacle in prime editing is deciding among the hundreds of possible pegRNA designs. For any desired mutation, many design combinations are possible through variation in the protospacer and the two sub-components of the 3′ extension: the so-called primer binding site, and the reverse transcriptase template. Most pegRNAs will not edit efficiently, and searching the vast pegRNA design space to identify efficient pegRNAs is challenging. Machine learning methods that predict pegRNA efficiency continue to progress[3,4], but even these algorithms are not guaranteed to yield a high-efficiency pegRNA.

Our prime editing sensor approach solves this problem with a construct design that places a copy of the endogenous target site next to the pegRNA. The 'sensor' recapitulates the sequence of the native genomic target, and thus the prime editor edits both our surrogate sensor site and the endogenous target site (Fig. 1). Next-generation sequencing can sequence the prime editing sensor cassette to identify the pegRNA and quantify the editing outcomes at the sensor, which can be used as a proxy for editing at the endogenous locus. In multiple contexts, we have shown that editing at the sensor is highly correlated with endogenous editing in the same cell population. Additionally, with a multiplexed format, we can design multiple pegRNAs for each variant of interest to comprehensively scan the pegRNA design space and identify pegRNAs that edit with high efficiency at the sensor.

We applied the prime editing sensor approach to investigate over 1,000 clinically observed variants of *TP53* — the most frequently mutated gene in cancer. To this end, we designed up to 30 pegRNA–sensors for each variant, with a range of design parameters, using a Python package that I created and named PEGG. We screened this library of nearly 30,000 pegRNA–sensors in A549 cells (a lung adenocarcinoma line) that stably express the prime editor protein and identified *TP53* variants that differentially inactivate the p53 pathway.

A key advantage of the prime editing sensor approach is that it enables filtering out low-efficiency pegRNAs on the basis of sensor editing. This step removed substantial noise in our dataset and helped to identify previously underappreciated variants in the oligomerization domain of p53 that increase cell fitness and inactivate the p53 pathway. Furthermore, we found that variants in the oligomerization domain did not produce the same effect when exogenously overexpressed from a transgene, highlighting the importance of assessing genetic variants in their native context using genome editing.

We envision that the prime editing sensor approach will enable our group and others to uncover the genetic determinants, both coding and non-coding, of diseases such as cancer at unprecedented scale and resolution.

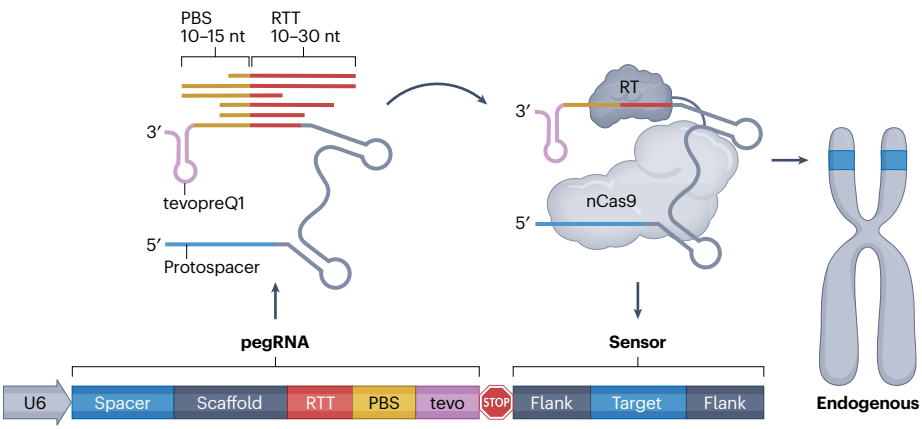

Fig. 1 | Prime editing sensors enable an integrated readout of editing activity in a pooled format. nCas9, nicking Cas9; PBS, primer binding site; pegRNA, prime editing guide RNA; RT, reverse transcriptase; RTT, reverse transcriptase template; tevo, tevopreQ1; tevopreQ1, engineered pegRNA motif (RNA pseudoknot) that improves editing. Reproduced from ref. 1.

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Competing interests

The author declares no competing interests.