The single strand dna nick in the non-target

The nextstage of improvement of base editors was achieved by converting dCas9to anickase through replacement of either amino acid aspartate (D)by alanine (A) at position 10 (D10A; also described as nCas9), orreplacement of amino acid histidine (H) by alanine atposition 840 (H840A). The modified forms of Cas in the form of nCas9 and H840A both producenicks inopposite strands, and have been suitably utilized insingle base gene editing18. For instanceD10A mutant of Cas9 retains a domain that generates a single strand DNA nick inthe non-target strand instead of creating double strand breaks at the desiredsite; this would simulate ‘ mismatch repair’, so that a unmodified opposite DNA strand would mimic a DNA strandundergoing synthesis, where the strand containing the edited base is used as atemplate (C®U), taking U as T (Figure 6). Therefore, BE3 had thefollowing three components in addition to sgRNA, which will guide the editor tothe target site: (i) an AID/APOBEC1deaminase, that was fused to a (ii)nickase (nCas9) that was deficient for nuclease activity nCas9(D10A), and (iii) a UGI that was linkedto nCas9through a 4 amino acids linker. The importance ofUGI in base editing was demonstrated by showing that the UGI-deleted BE3 (BE3-? UGI) wasless competent in base editingcompared to originalBE3, and produced not only lower frequencyof desired C®T editing, butalso produced a higher frequency of unwanted indels.

A number of improved BE3variants were also developed (Table 2), which resulted in much more efficientconversion of the G: U intermediate to desired A: U and A: T products10, 15. Another problem associated with BE1and BE2 wasthe occurrence of more than one Cs within the base-

editing window, so that the cytosine deaminase will convert even a non-targeted C into U. Thisproblem was overcome by the development of a number of BE3 variants with SpCas9(NGG), where even the non-NGG PAM sequence could be used for base editing (Table2; also see later). It was also shownthat addition of another copy of UGI to BE3 further reduced the frequency ofindels, so that BEs with more than one UGI were developed and were described as4th generation base editors, the BE4, which werefound to be more efficient19.

BE4 or SaBE4 were further improved by adding
Gam to thecassette, so thatthe use of BE4-Gam resulted in a further 1. 5 to 2. 0 folddecrease in the indel frequency (Table 1).