Comprehensive base editing mediated by hAPOBEC3A-conjugated base editors

With the support by the National Natural Science Foundation of China, the research teams led by Prof. Yang Li (杨力) at the CAS Key Laboratory of Computational Biology, CAS-MPG Partner Institute for Computational Biology, and Prof. Chen Jia (陈佳) and Prof. Huang XingXu (黄行许), at the School of Life Science and Technology, ShanghaiTech University, developed a series of human APOBEC3A-conjugated base editors (BEs) to achieve comprehensive C-to-T base editing in all tested regions, including the regions with high methylation levels. This work was published in *Nature Biotechnology* (2018, 36: 946—949).

Base editors (BEs) are developed by combining different nucleotide deaminase family members, including cytidine deaminase family members (e.g., APOBECs) and adenosine deaminase family members (e.g., Adenosine deaminases acting on RNA, ADARs), with the CRISPR-Cas system (e.g., CRISPR/Cas9 and CRISPR/Cpf1). Various BEs have been used for targeted C-to-T/A-to-G base editing in different species. Numerous human diseases have been reported to be driven by point mutations in genomic DNA. With recently developed BEs, these disease-related point mutations can be potentially corrected, providing new therapeutic options. The newly-developed base editing technology has been highlighted by *Science* as one of its top 10 'breakthrough in 2017'.

By analyzing disease-related T-to-C mutations that can be theoretically reverted to thymines by BEs, the research team found that ~43% of them are on cytosines in the context of CpG dinucleotides. It is well known that C of CpG is usually methylated in mammalian cells, and methylation of C strongly suppresses the cytidine deamination activity of some APOBEC/AID deaminases. Consistently, previously developed BEs that are based on rat APOBEC1 (rA1) cytidine deaminase are inefficient in editing cytosines in highly-methylated regions. To develop BEs for efficient C-to-T base editing in highly methylated regions, researchers developed a series of BEs by fusing Cas9 nickase with a dozen of different APOBEC/AID deaminases, and then showed that most of them can be used for C-to-T base editing. Importantly, a novel BE based on hA3A (hA3A-BE) and its engineered versions with narrower editing windows can mediate efficient C-to-T base editing in regions with high methylation levels and other examined regions with different contexts. Overall, these newly developed hA3A-BEs in the *Nature Biotechnology* paper can comprehensively induce efficient base editing in all examined contexts, including both methylated DNA regions and GpC dinucleotides. This work has been highlighted by *Nature Methods* (2018, 15: 763).

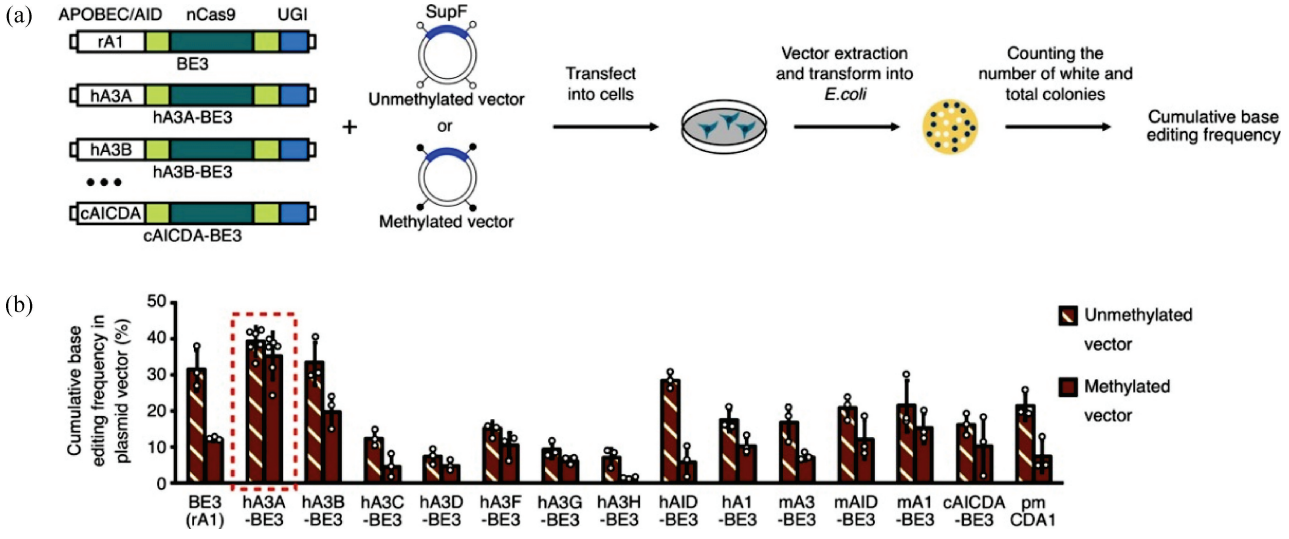

Figure Construction and screening (a) for high-efficient base editors in methylated regions (b).