Highly efficient base editing in *Staphylococcus aureus* using an engineered CRISPR RNA-guided cytidine deaminase

With the support by the National Natural Science Foundation of China, the research team directed by Prof. Ji QuanJiang (季泉江) at the School of Physical Science and Technology of ShanghaiTech University, developed a highly efficient single-base editing technique in *Staphylococcus aureus*, which was published in *Chemical Science* (2018, 9: 3248—3253).

As a major human pathogen, *Staphylococcus aureus* is the leading cause of hospital—and community-acquired infections. The pathogen can cause a wide variety of infections, including minor skin infections and life-threating diseases, such as endocarditis, necrotizing pneumonia, and toxic shock syndrome. The emergence of drug-resistant *S. aureus*, such as methicillin-resistant *S. aureus* (MRSA, a Superbug), has posed a severe public crisis worldwide. Hence it is urgently needed to develop novel therapeutic means against drug-resistant *S. aureus* infections.

Before the development of the base-editing system, Ji Lab has developed a CRISPR/Cas9-mediated genome editing system pCasSA (*JACS*, 2017, 139, 3790). The method allows for rapid and efficient genetic manipulation in *S. aureus*, accelerating bacterial physiology study, such as pathogenesis and drug resistance, and boosting novel drug-target exploration and new therapeutic method development. However, the weak intrinsic homologous recombination capacity of *S. aureus* prevents the high-rate recovery of survival cells after the double-stranded break of the genome, thus hampering the applications of the pCasSA system in the strains with low transformation efficiencies, such as many MRSA strains directly isolated from patients.

To overcome the difficulty, Ji Lab designed and constructed a highly efficient and convenient base-editing system pnCasSA-BEC in *S. aureus* via engineering a fusion of a Cas9 nickase (*Streptococcus pyogenes* Cas9D10A) and a cytidine deaminase (rat APOBEC1). The pnCasSA-BEC system can achieve efficient base editing without using repair templates or sacrificing transformation CFUs. In addition, the system enables efficient gene inactivation by converting the codons of CAA, CAG, CGA, and TGG to the stop codons of TAA, TAG, TGA, and TAA in the genomes.

The development of the base editing system will dramatically accelerate bacterial physiology study and drug-target exploration. In addition, it will provide critical insights into base-editing system development in other microbes.

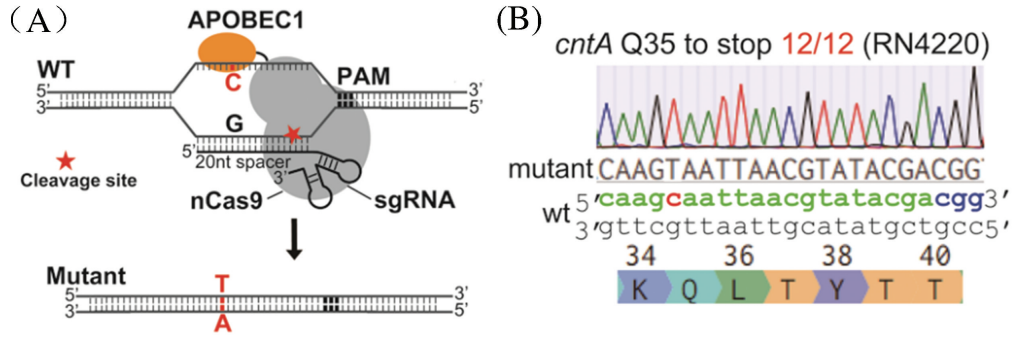

Figure (A) Scheme of the editing mechanism of the "base editor" (pnCasSA-BEC). (B) The pnCasSA-BEC system enables highly efficient base editing in the *cntA* gene in the RN4220 strain.