The Second-Generation, High-Fidelity Cytosine Base Editor AccuBase® Proficiently Modifies Multiple Genes in Human Primary T cell with Zero Off-Target Effects

Guanglei Li, Liling Wang, Chunju Lu, Wenwen Fan, Qi Lai, Chao Yao, Fenghua Lv, Shiguo Zhu, Xingxu Huang, Han Ying, Tianhong Xu

Download a digital copy of the poster:

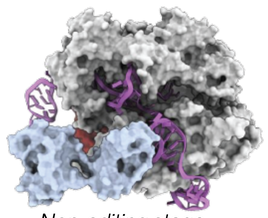
Introduction

Mitigation of any off-target events like genetic mutations, chromosomal rearrangements, and insertion-deletions (indels) has long been the primary challenge in gene editing tool development. To overcome this challenge, we utilized synthetic biology strategies to create AccuBase®, a novel cytosine base editor (CBE) featuring the highest gene editing efficiency with zero off-target effects. During the non-editing stage, AccuBase® forms a complex with the sgRNA, with deaminase buried inside the Cas domain, avoiding random tethering to the genome and generation of gRNA-independent mutation, a common issue of first-generation base editors. Once reaching the target locus, the AccuBase®-gRNA complex binds to double-strand DNA and induces conformational changes of the base editor, exposing the deaminase domain of the AccuBase® to the target loci for A-T nucleotide substitution.

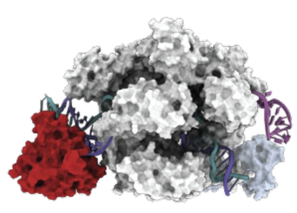
The outstanding performance of AccuBase® has been validated both in animal studies as well as cell-based assays using a variety of mammalian cell lines. In this study, we showcased the application of AccuBase® in single or multi-loci gene editing in human primary T cells and demonstrated its capacity for highly efficient gene editing without inducing off-target effects. The edited primary T cells showed enhanced ex-vivo cell expansion with no double-strand break (DSB)-induced translocations, an issue that is commonly seen in T cells edited with Cas9 nuclease. In conclusion, the AccuBase® CBE exhibits the highest on-target editing efficiency with the lowest off-target effects and is proved to be a superior solution to the unmet demands within the gene editing field, setting up a new standard for precision and effectiveness.

AccuBase®: A High-Precision Base Editor with Lowest Off-Target Effects

AccuBase® was developed by inserting deaminases into Cas9 at specific, tolerance-verified sites, a process guided by extensive transposon-based genetic screening, protein engineering, and direct evolution approach. AccuBase® forms a complex with the sgRNA (depicted as a purple double helix). In the non-editing stage, the deaminase domain (colored in red) is intricately integrated and is not in contact with any non-targeting dsDNA. Once the RNP binds to the target DNA, the conformation change is induced and converted to the editing stage, exposing the deaminase domain for editing. The “on and off switch” design can efficiently reduce off-target effects.



Non-editing stage



Editing stage

	CRISPR Nucleases	BE4Max (Broad Institute/BEAM)	AccuBase® Base Editor
DNA Double Strand Break	Yes	No	No
Target Insertion/Deletion	Yes (1bp-500bp) Low efficiency (<20%)	No	No
Single Base Editing	No	Yes	Yes
Editing Efficiency	80-100%	80-100%	80-100%
Off-Target Effects; Unwanted INDELs; Chromosomal Rearrangements	1-5% Chromosomal translocation; Large INDEL abnormalities (1 to 3 gene editing)	Off-target mutations	Close to zero
Size	4Kb	~ 5.5Kb	~5.5 Kb

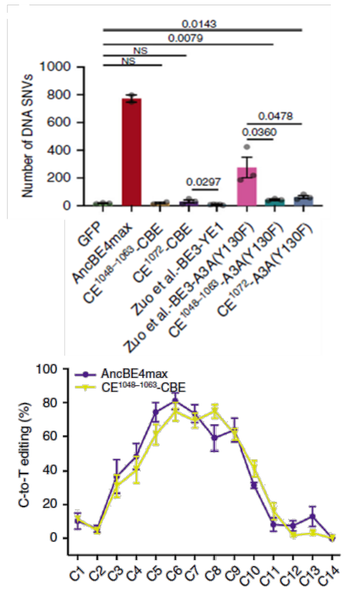
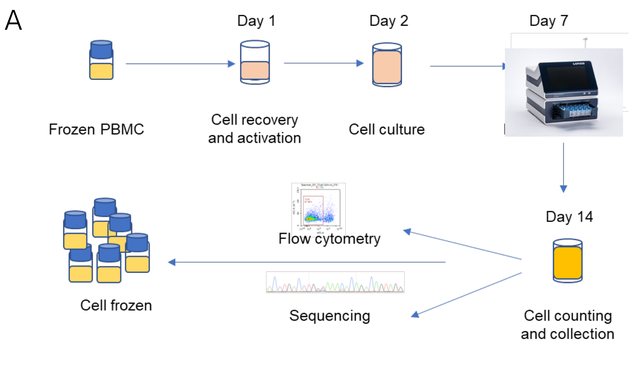
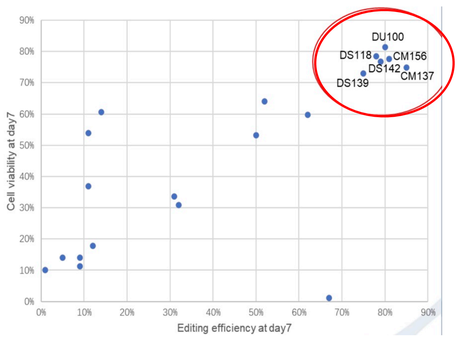
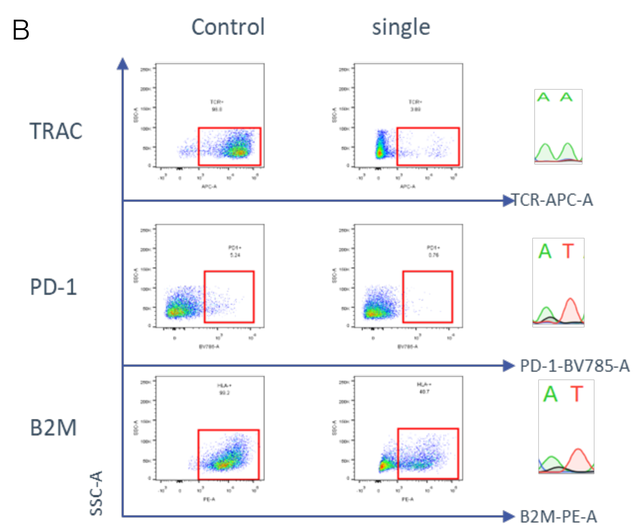


Figure 1. (A) Structure of AccuBase® CBE in non-editing (left) and editing stage (right). (B) Editing efficiency of AccuBase® in 293T cells. (C) Off-target detection for different base editors (Liu et al. Nat Commun. 2020 Nov 27;11(1):6073).

AccuBase® Base Editor Efficiently Edits Targets in Human Primary T Cells







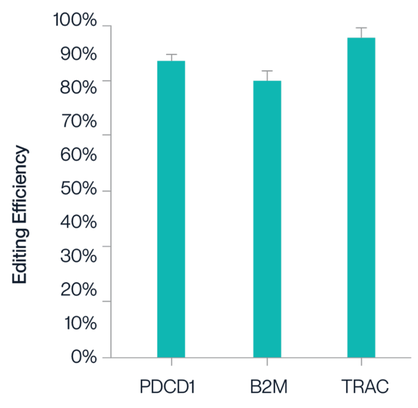


Figure 2. (A) Overview of gene editing workflow in primary T cells. The optimal gene editing condition was selected based on cell viability and editing efficiency (Circled in red). (B) TRAC, PD-1 and B2M were knocked out using AccuBase®. The efficiency was confirmed using flow cytometry (Left). Statistical analysis of the gene editing efficiency (Right), N=3

Efficient knockout of TRAC and CD30 in non-dividing T cells by AccuBase®

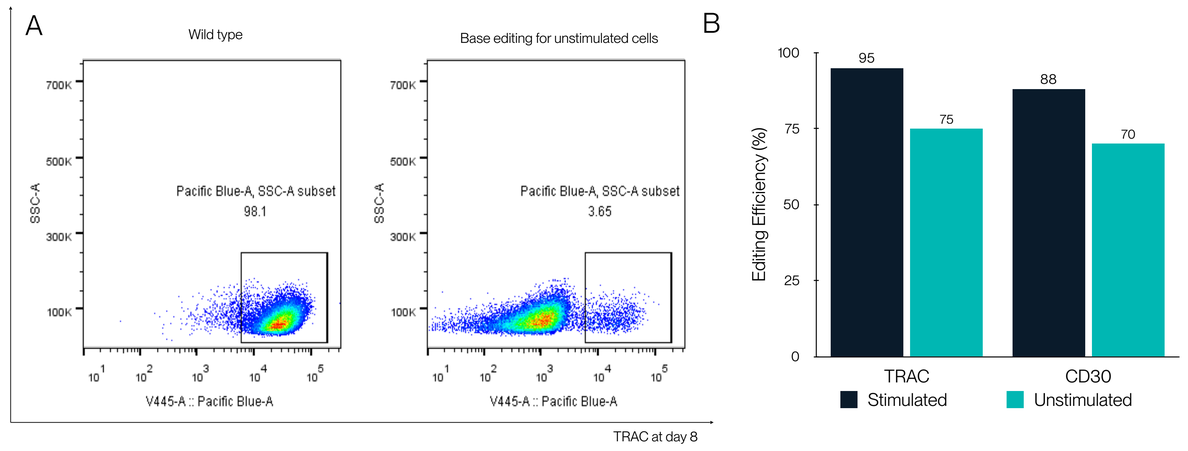


Figure 4. (A) Flow cytometry verification of TRAC and CD30 single knockout in unstimulated T cells on day 8 after nucleofection. (B) Sanger sequencing-based calculation of editing efficiency on day 4 after nucleofection.

Dual/Triple gene knockout in primary T cells using AccuBase® Base Editor

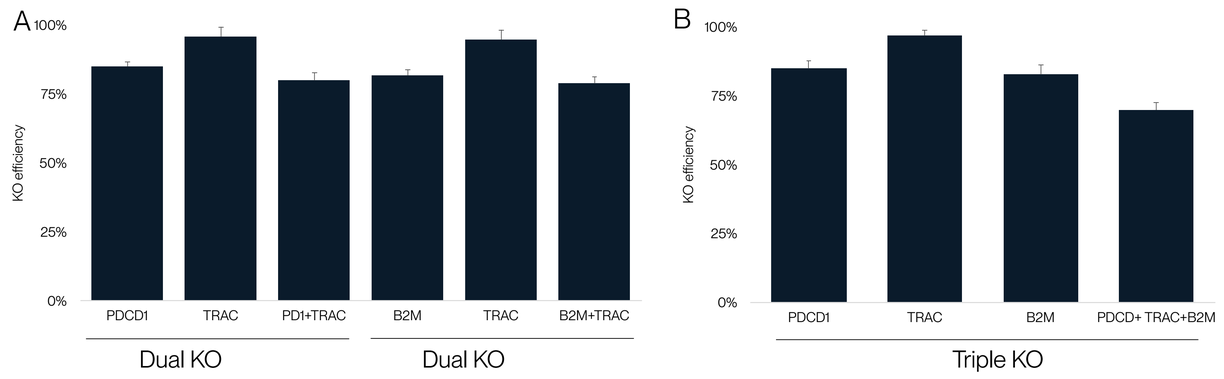


Figure 5. (A) Two genes were simultaneously edited using AccuBase®. The editing efficiency was calculated based on the flow cytometry. (B) Three genes were simultaneously edited using AccuBase®. The editing efficiency was calculated based on the flow cytometry.

No Off-Target Effects or Chromosomal Translocation in Multiplex Edited T Cells

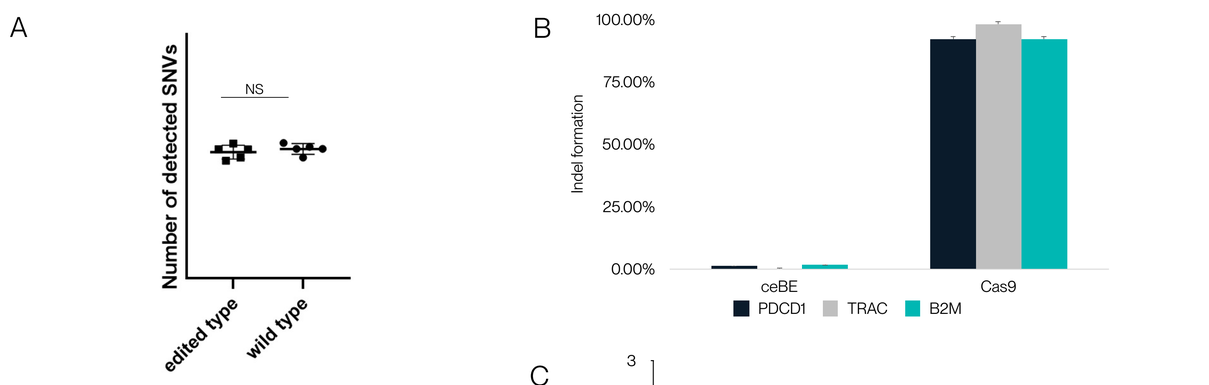


Figure 6. (A) Detection of SNVs were detected using whole genome sequencing. No significant difference was found between the edited group and wild type group. (B) No significant indels were found for the AccuBase® edited groups. (C) Cas9-edited cells could contain translocation, and it was not found in AccuBase®-edited groups.

Quantification of Residual AccuBase® Using AccuBase® ELISA Kit

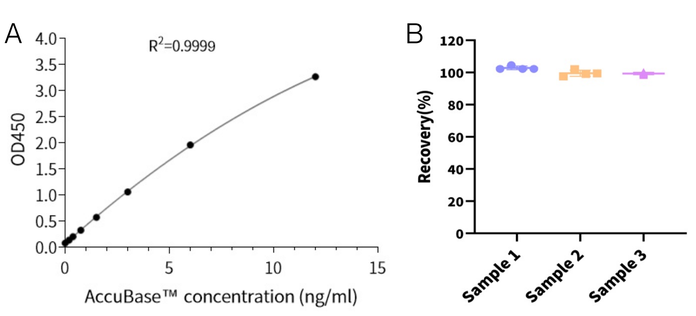


Figure 7. (A) Example 8-point standard curve for AccuBase® ELISA. The quantitative range of this ELISA kit is 187.5 pg/mL - 12 ng/mL. (B) The recovery rate of the kit was analyzed by testing three AccuBase® samples with known concentrations in dilution buffer using the same AccuBase® ELISA kit. Results show that in dilution buffer, the recovery rate of various AccuBase® sample concentrations is between 80% and 120%, indicating that this reagent kit has high accuracy.

Advantages of AccuBase® Base Editor

- Minimized off-target effects to background level.
- Validated performance in primary cells and in vivo.
- Multiple gene editing with high efficiency and safety.

Product Information

AccuBase® Base Editor, GMP-Grade
Cat. No. GMP-KD-0001

AccuBase® Base Editor, Research-Grade
Cat. No. KD-0001

AccuBase® Base Editor ELISA Kit
Cat. No. ACB-EE00B

Conclusion

In this study, we used AccuBase® Base Editor to perform multiplex gene modifications in primary human T cells. The knockout efficiencies for individual genes were as follows: 89% for PDCD1, 81% for B2M, and 96% for TRAC. When applied to create a triple knockout, the editing efficiency reached 60%, and this was confirmed through flow cytometry analysis.

Remarkably, the edited T cells exhibited normal expansion characteristics and did not display the double-strand break-induced translocations typically observed in T cells edited with Cas9 nuclease. Equally significant, our analysis revealed the absence of any off-target effects in the AccuBase®-edited T cells.

support@kactusbio.us | kactusbio.com