# mRNA Expressing Cytosine and Adenine Base Editors Efficiently Mediate Base Corrections *In Vitro* and *In Vivo*

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## Abstract

The concept of treating genetic diseases via specific correction has been a focus in the biomedical field for decades. The ideal solution would offer a precise way of permanently fixing such mutations without introducing new errors. Early attempts at gene editing involved introduction of double stranded breaks at specific sites using zinc-finger nucleases, TALENs, and CRISPR-Cas9 nuclease to stimulate homologous recombination with an exogenous donor DNA template to correct the defect. However, these techniques also introduce indels at a high frequency. Here, we assess the potential of transient mRNA treatment to introduce permanent single base edits. Base editors offer the potential to correct single point mutations *in vivo* with an innovative modified Cas9 system. Cytosine base editors (CBEs) use a Cas9 nickase fused to a cytosine deaminase and uracil DNA glycosylase inhibitor. When directed to specific locations in the genome by a guide strand, cytosine-guanine base pairs in a small window are converted to thymine-adenine pairs with high efficiency and minimal indels. Similarly, adenine base editors (ABEs) use a laboratory-evolved deoxyadenosine deaminase fused to Cas9 nickase to convert adenine-thymine base pairs to cytosine-guanine pairs. Using base editors increases on-target editing frequency while greatly reducing off-target indel formation compared to nuclease-based methods. Compared to viral vectors and plasmids, mRNA offers key advantages including 1) reduced risk of vector integration; 2) the ability to edit hard-to-transfect, non-dividing cells, since the mRNA target is the cytoplasm not the nucleus; 3) the possibility for repeat administration *in vivo*, which is challenging for viral vectors due to capsid immune responses; and 4) transient expression, which is ideal for maximizing the specificity of genome editing applications. In this work, we compare sequence-optimized, chemically-modified CBE and ABE mRNAs in HEK293 cells. Western blot analysis showed higher expression of 5-methoxyuridine modified, sequence optimized mRNAs compared to unmodified mRNA. In cultured cells, mRNA resulted in higher editing frequencies than plasmid vectors. We demonstrate the ability to simultaneously edit multiple sites with one base editor mRNA, and edit previously inaccessible genomic sites. These results demonstrate the far-reaching potential of base editing technology. Finally, we have developed a mouse model using a BE4max variant mRNA injected into mouse zygotes which will be used to test *in vivo* ABE corrections in future studies.

## What is Base Editor?

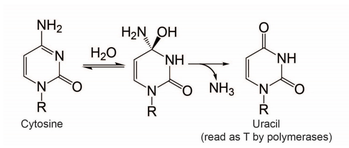
An alternative to traditional genome editing tools

- Traditional tools
  - CRISPR/Cas9, zinc-fingers and TALENs
    - » These modalities create double stranded breaks to stimulate homologous recombination
    - » They require a DNA donor for gene correction
- Base Editor
  - » Deaminases convert one base to another
  - » No double stranded cuts are made
  - » No donor DNA required
  - » Reduced Indel formation

## There Are Two Flavors of Base Editors

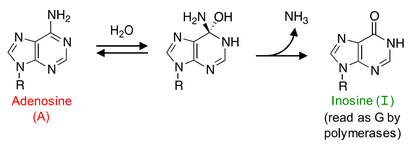
### Cytidine base editors (CBEs)

» Convert C:G base pairs to T:A base pair



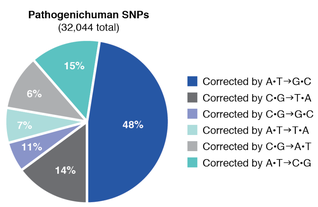

### Adenine base editors (ABEs)

» Convert A:T base pairs to G:C




Gaudelli, Komor, Rees, Packer, Badran, Bryson, Liu Nature 551, 464 (2017)

## Figure 1: Clinical Relevance of Base Editing Needs

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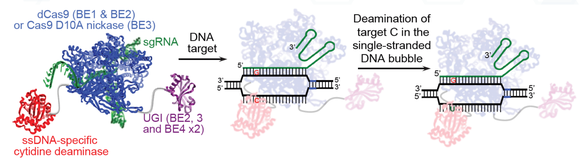
## Figure 2: Cytosine Base Editor Mechanism

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## Figure 3: Plasmids Expressing Cytosine Base Editors are More Efficient Than Cas9 + HDR in Human Cells

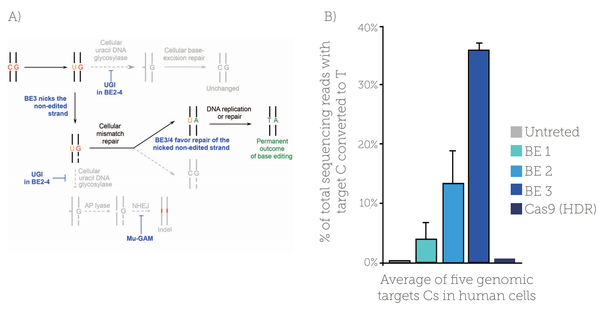


Figure adopted from Komor, Kim, Packer, Zuris, Liu Nature 533, 420 (2016)
A) Mechanism of single C to T conversion via base editing; B) Representative data from plasmid expressed proteins in human cells comparing traditional Cas9 editing to cytosine base editors

## Figure 4: Adenine Base Editor Mechanism




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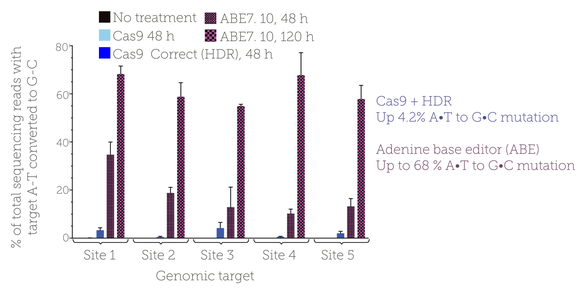
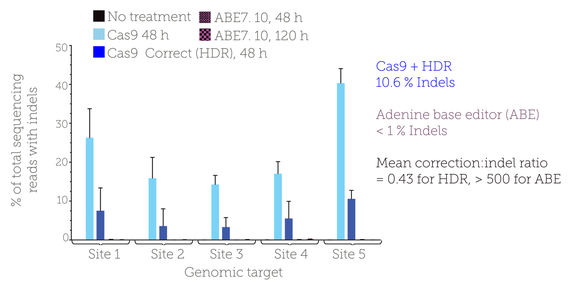
## Figure 5: Adenine Base Editing by Expression from Plasmids is More Efficient than Cas9 + HDR




Gaudelli, Komor, Rees, Packer, Badran, Bryson, Liu Nature 551, 464 (2017)
CORRECT HDR: Kwart, Tessier-Lavigne et al. Nat. Protocols 12, 329 (2017) and Paquet, Tessier-Lavigne et al. Nature 533, 125 (2016)

## Figure 6: Adenine Base Editing Produces Very few Indels



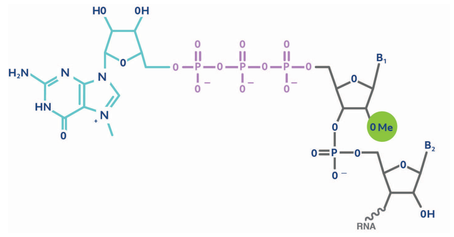

Gaudelli, Komor, Rees, Packer, Badran, Bryson, Liu Nature 551, 464 (2017)
CORRECT HDR: Kwart, Tessier-Lavigne et al. Nat. Protocols 12, 329 (2017) and Paquet, Tessier-Lavigne et al. Nature 533, 125 (2016)

## mRNA Expression of Base Editors

- Minimal risk of insertional mutagenesis
- Plasmid and viral vectors can illicit innate and adaptive immune responses
- mRNA can be introduced into the cytoplasm of difficult-to-transfect cells that do not undergo cell division
- mRNA offers transient expression of therapeutics ideal for applications such as Base Editing

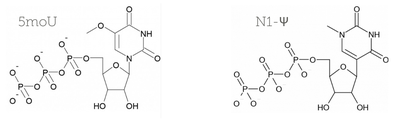
## Figure 7: Creating the Optimal Base Editor mRNAs

Cap 1 structure recognized as "self"

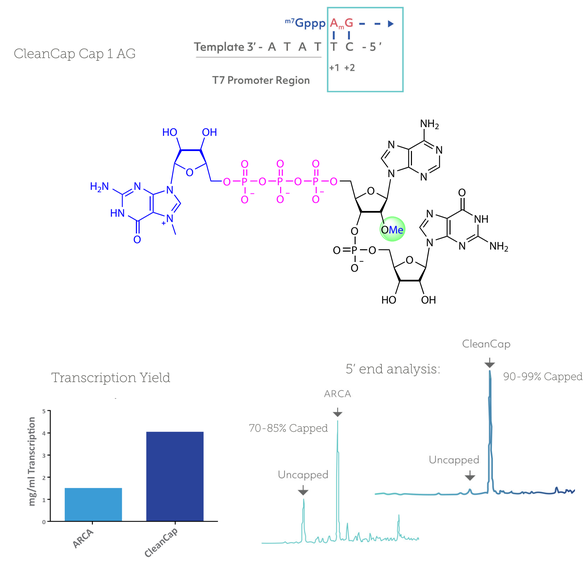



Base modification/optimization to reduce innate immune stimulation

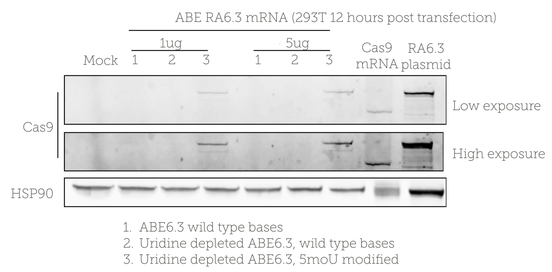
- » Uridine depletion
- » 5-methoxyuridine (5moU) modification
- » N1-methylpseudouridine (N1-Ψ) modification




## Figure 8: *CleanCap*® Co-transcriptional Capping Yields Optimal Cap 1 Structure with High Efficiency



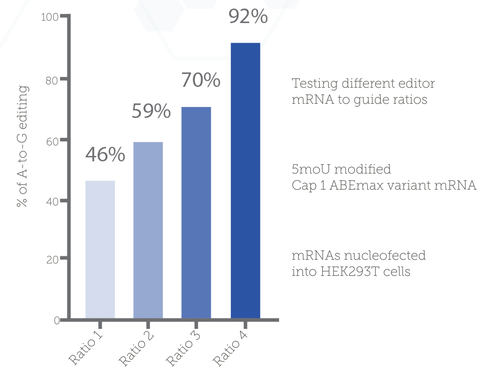

## Figure 9: Uridine Depleted, 5moU Modified RNA Gives Higher Expression than Wild Type RNA in Cultured Cells




1. ABE6.3 wild type bases
2. Uridine depleted ABE6.3, wild type bases
3. Uridine depleted ABE6.3, 5moU modified

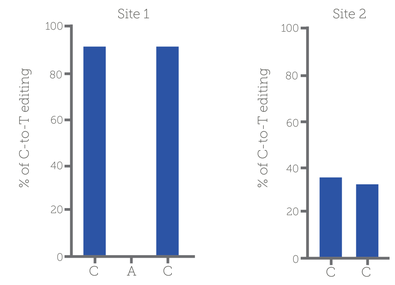
Custom mRNA optimization improves protein expression of ABE

## Figure 10: Adenine Base Editing in HEK293T Cells



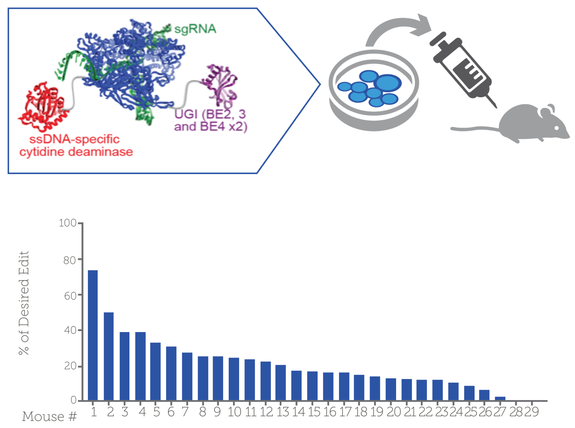

Optimizing delivery of ABE mRNA with guide strand for high editing efficiency

## Figure 11: Multiple Sites Can Be Edited Simultaneously in Cells by Cytidine Base




5moU modified Cap1 BE4max variant mRNA with 2 guides nucleofected into HEK293T cells
Site 1 and Site 2 are on different chromosomes

## Figure 12: Creating a Mouse Model for *In Vivo* Base Editing




5moU modified Cap1 BE4max variant mRNA was injected into mouse zygote to create a model mouse with a single base change. A mosaic pattern is observed in F1 animals that will be purified by subsequent breeding.

## Conclusions

- » CleanCap® co-transcriptional capping produces Cap 1 structure that mimics natural "self" RNAs
- » Uridine depleted modified mRNA yields maximal Base Editor expression
- » mRNAs expressing adenine base editors can efficiently mediate A ⟶ G changes in cultured cells
- » mRNAs expressing cytosine base editors can mediate simultaneous C ⟶ T changes at different chromosomal locations in cultured cells
- » Injection of mRNAs expressing cytosine BE4max variant into mouse zygotes followed by implantation into pseudo-pregnant females results in the birth of pups that are mosaic for the C ⟶ T change desired. Conversion can be efficient in mice.

## Contact

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