Cytosine and Adenine Base Editors Expressed from Messenger RNAs Mediate Efficient Base Corrections *In Vitro* and *In Vivo*

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Abstract

New tools for genome editing raise the possibility of precisely correcting genetic defects. A variety of nucleases can stimulate homologous recombination to install desired sequences, but these approaches are inefficient and give rise to undesired indels. Here, we use transient mRNA treatment with base editors to introduce permanent single base edits with high product purity. Cytosine base editors (CBEs) use a Cas9 nickase fused to a cytosine deaminase and uracil DNA glycosylase inhibitor. C:G base pairs are converted to T:A pairs with high efficiency and minimal indels. Similarly, adenine base editors (ABEs) use an evolved deoxyadenosine deaminase fused to Cas9 nickase to convert A:T base pairs to C:G pairs. Editing efficiencies of >90% were observed without cell sorting. In contrast to viral vectors and plasmids, mRNA offers key advantages including 1) reduced risk of vector integration; 2) ability to edit hard-to-transfect, non-dividing cells; 3) ability to repeat administer *in vivo*; and 4) transient expression to maximize specificity. Here, 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. HPLC purified wild type and N1-methylpseudouridine modified editors were also tested. In cultured cells, mRNA resulted in higher editing frequencies than plasmid vectors. We also demonstrate the ability to simultaneously edit multiple sites with one base editor mRNA, and edit previously inaccessible genomic sites. Finally, we developed a mouse model by editing mouse zygotes with injected mRNA encoding BE4max variant mRNAs. This model will be used to test *in vivo* ABE corrections in future studies.

What is Base Editor?

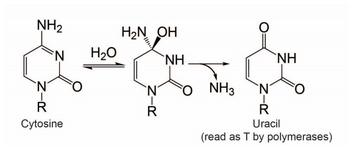
An alternative to traditional genome editing tools

- 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

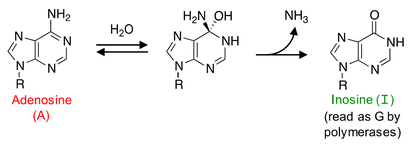
Cytidine base editors (CBEs)

» 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

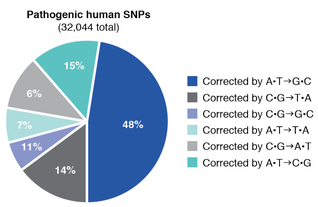


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Figure 2: Cytosine Base Editor Mechanism

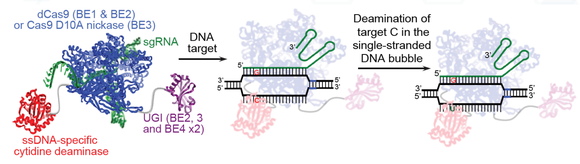

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Figure 3: Plasmids Expressing Cytosine Base Editors are More Efficient Than Cas9 + HDR in Human Cells

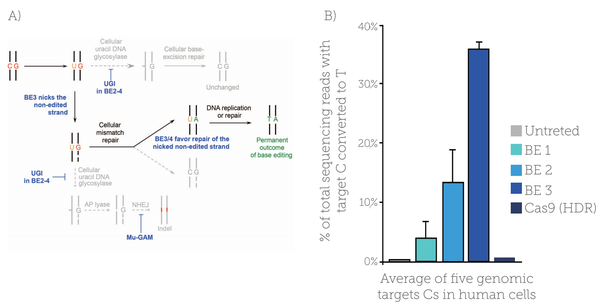

Figure adopted from Komor, Kim, Packer, Zuris, Liu Nature 533, 420 (2016)
A) Mechanism of single C to T conversion via base editing; B) Representative data from plasmid expressed proteins in human cells comparing traditional Cas9 editing to cytosine base editors

Figure 4: Adenine Base Editor Mechanism

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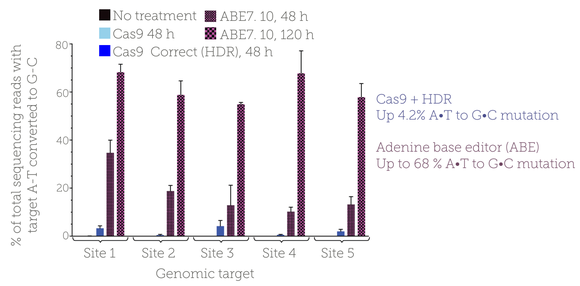
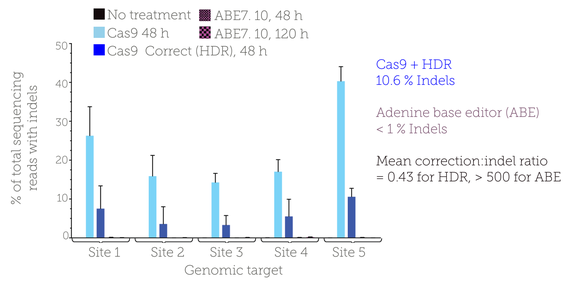
Figure 5: Adenine Base Editing by Expression from Plasmids is More Efficient than Cas9 + HDR



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



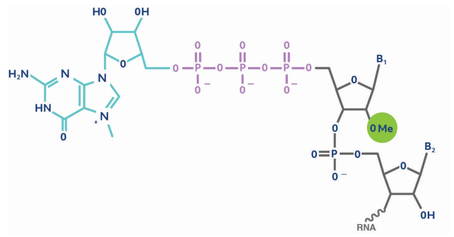
Gaudelli, Komor, Rees, Packer, Badran, Bryson, Liu Nature 551, 464 (2017)
CORRECT HDR: Kwart, Tessier-Lavigne et al. Nat. Protocols 12, 329 (2017) and Paquet, Tessier-Lavigne et al. Nature 533, 125 (2016)

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

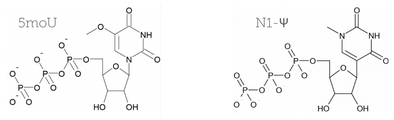


Figure 8: *CleanCap*® Co-transcriptional Capping Yields Optimal Cap 1 Structure with High Efficiency

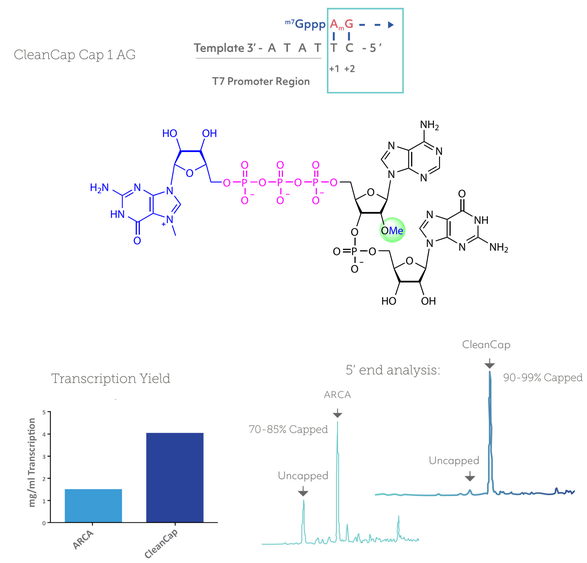
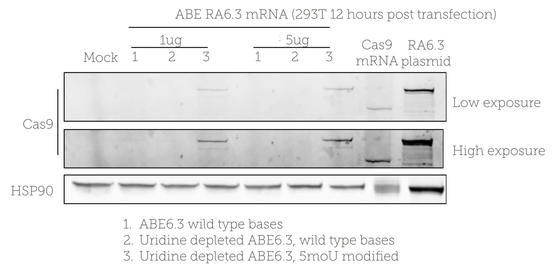


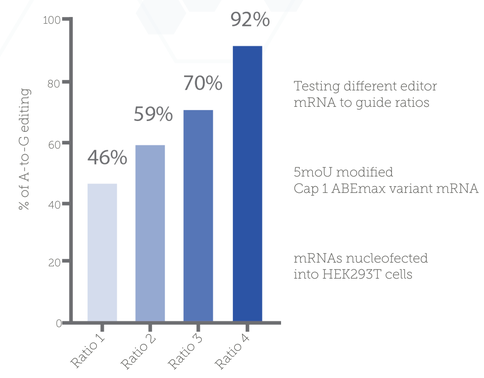
Figure 9: Uridine Depleted, 5moU Modified RNA Gives Higher Expression than Wild Type RNA in Cultured Cells



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

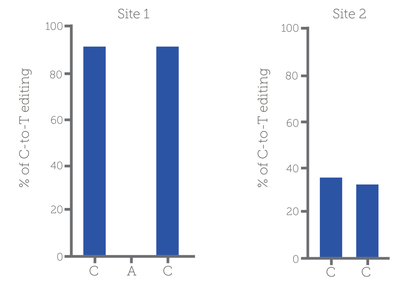
Custom mRNA optimization improves protein expression of ABE

Figure 10: Adenine Base Editing in HEK293T Cells



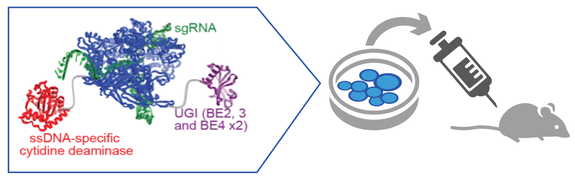
Optimizing delivery of ABE mRNA with guide strand for high editing efficiency

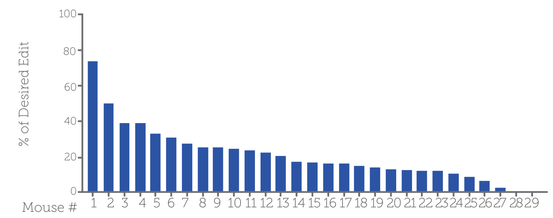
Figure 11: Multiple Sites Can Be Edited Simultaneously in Cells by Cytidine Base



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 Cap 1 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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Keystone: Emerging Cellular Therapies: Cancer and Beyond
(Q1) /Engineering the Genome
(Q2) Joint Meeting
Banff, Canada 2/20