



TriLink[®]

BIOTECHNOLOGIES

part of Maravai LifeSciences

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

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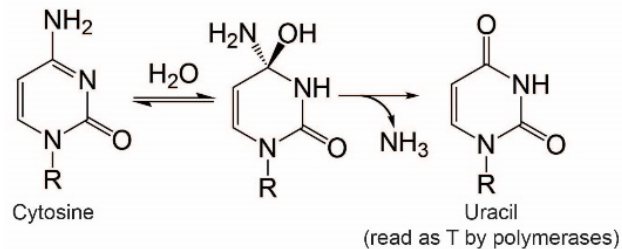
- An alternative to traditional genome editing tools
 - Traditional tools:
 - CRISPR/Cas9, zinc-fingers and TALENs
 - These modalities create double stranded breaks to stimulate homologous recombination
 - Require a DNA donor for gene correction

Base editor deaminases convert one base to another

- No double stranded cuts
- No donor DNA required
- Reduced indel formation

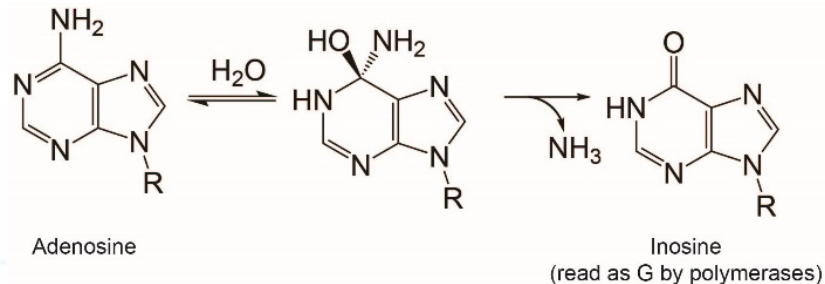
Cytidine base editors (CBEs)

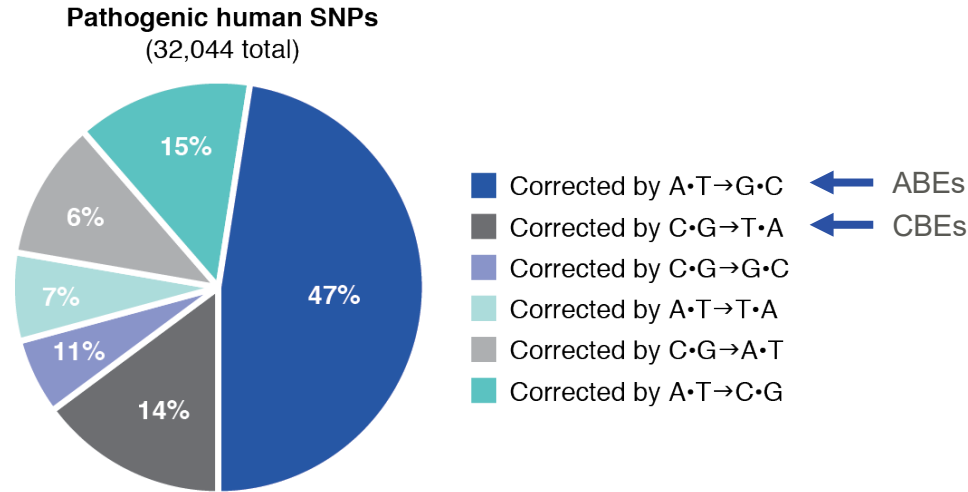
Convert C:G base pairs to T:A base pair



Adenine base editors (ABEs)

Convert A:T base pairs to G:C

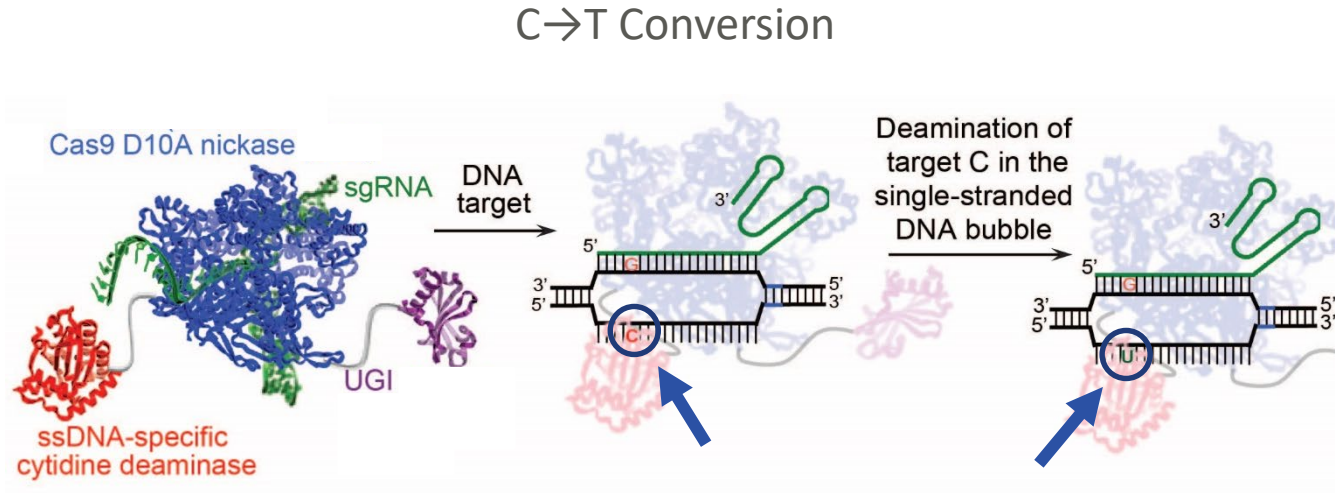




61% of pathogenic SNPs are accessible to base editing

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

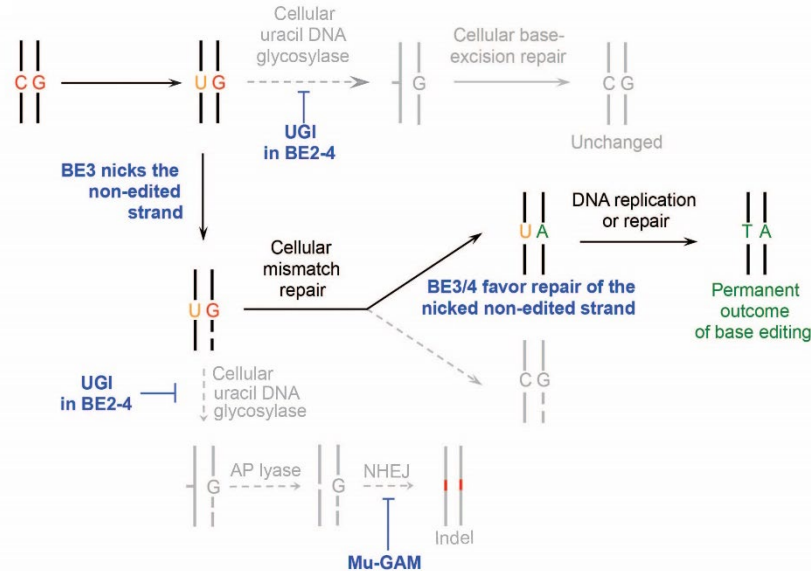
Cytosine Base Editor Mechanism (Part 1)



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

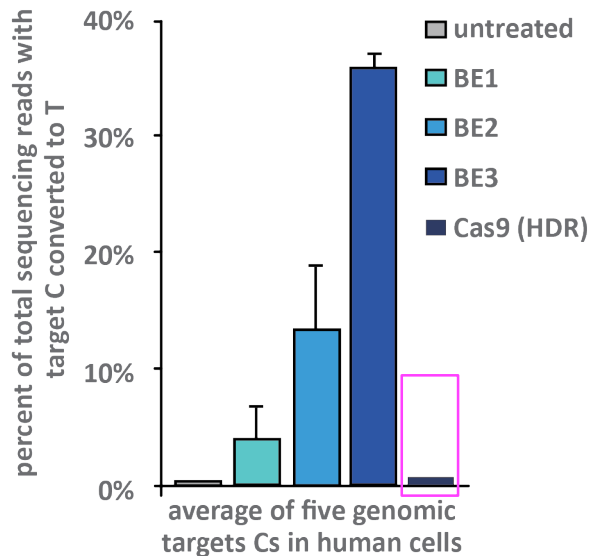
DNA Repair Makes Changes Permanent

C→T Conversion



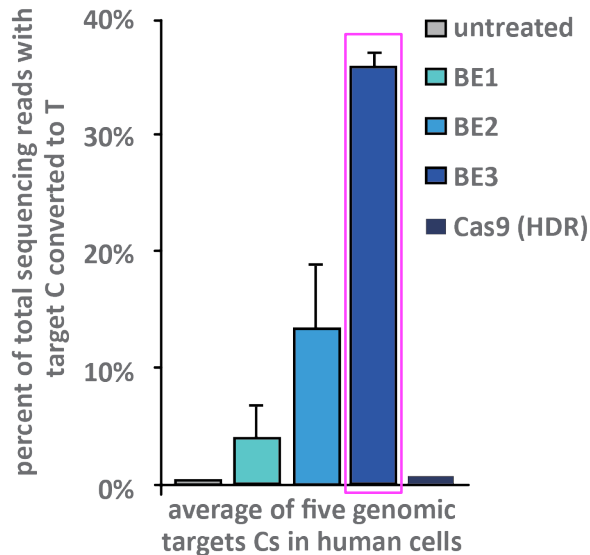
Gaudelli, Komor, Rees, Packer, Badran, Bryson, Liu Nature 551, 464 (2017)

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



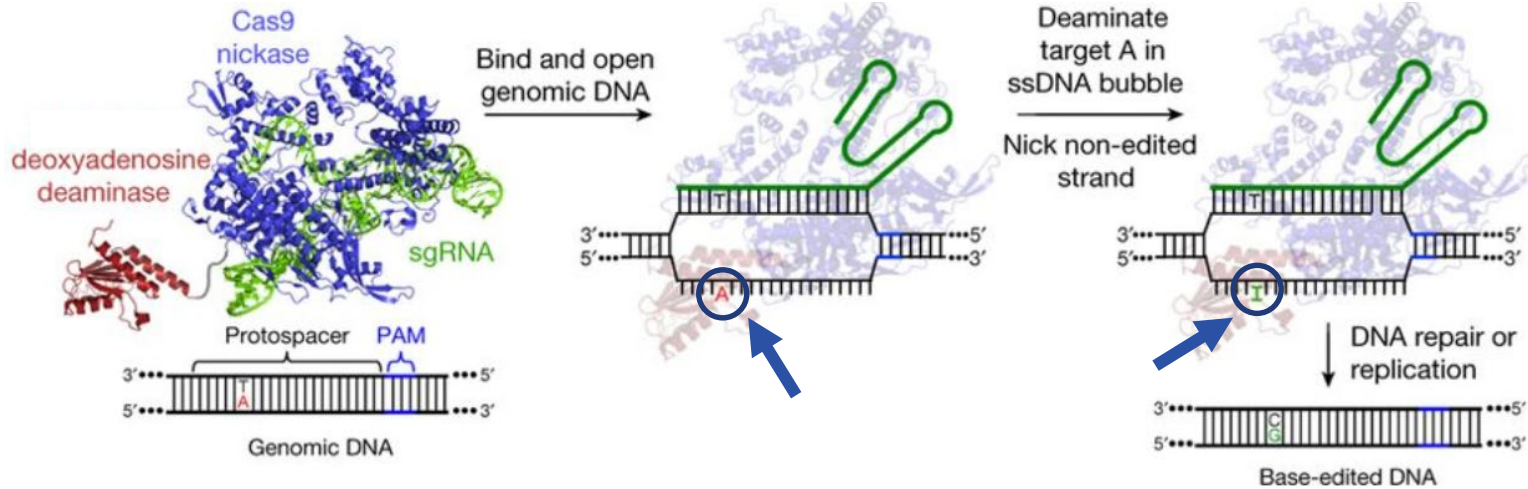
Komor, Kim, Packer, Zuris & Liu; Nature 533, 420 (2016)

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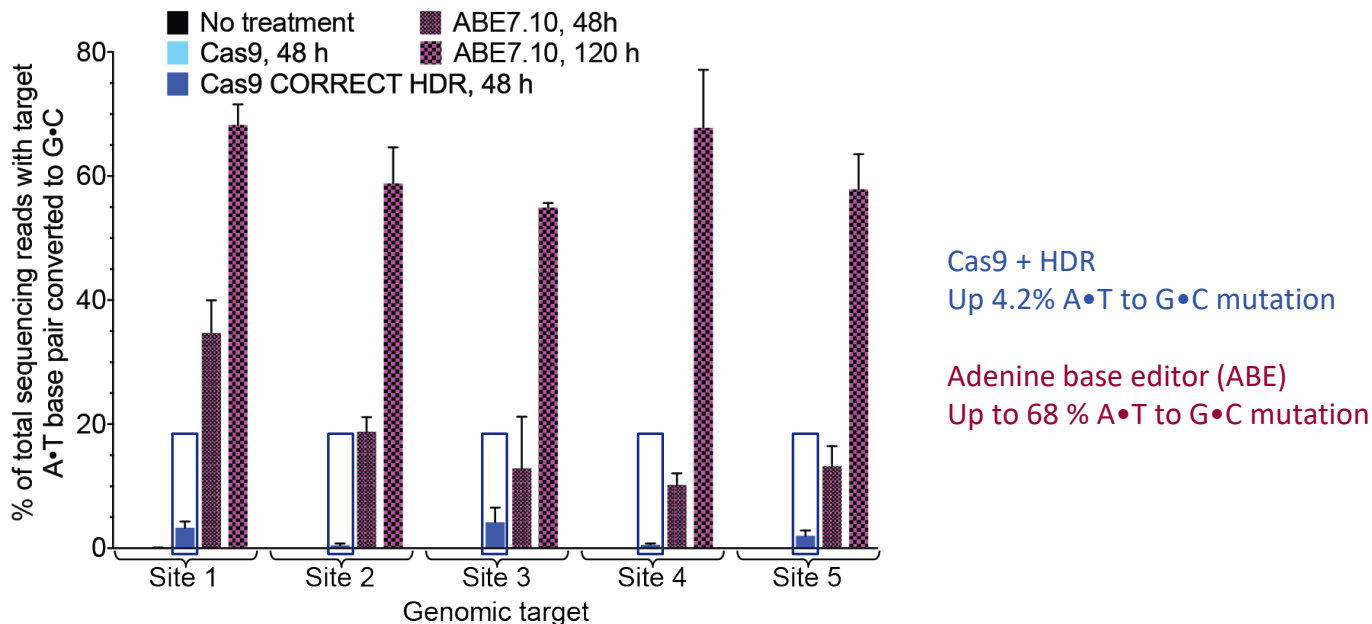
Komor, Kim, Packer, Zuris & Liu; Nature 533, 420 (2016)

A→G Conversion



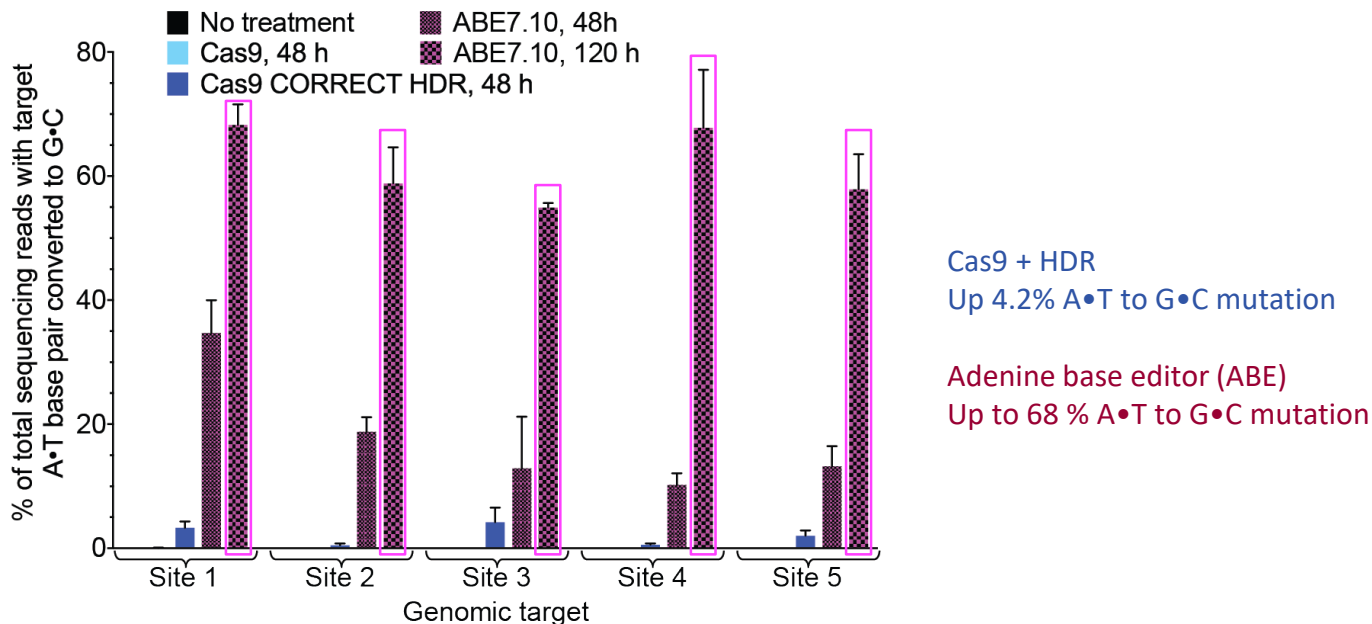
Gaudelli, Komor, Rees, Packer, Badran, Bryson, Liu Nature 551, 464 (2017)

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



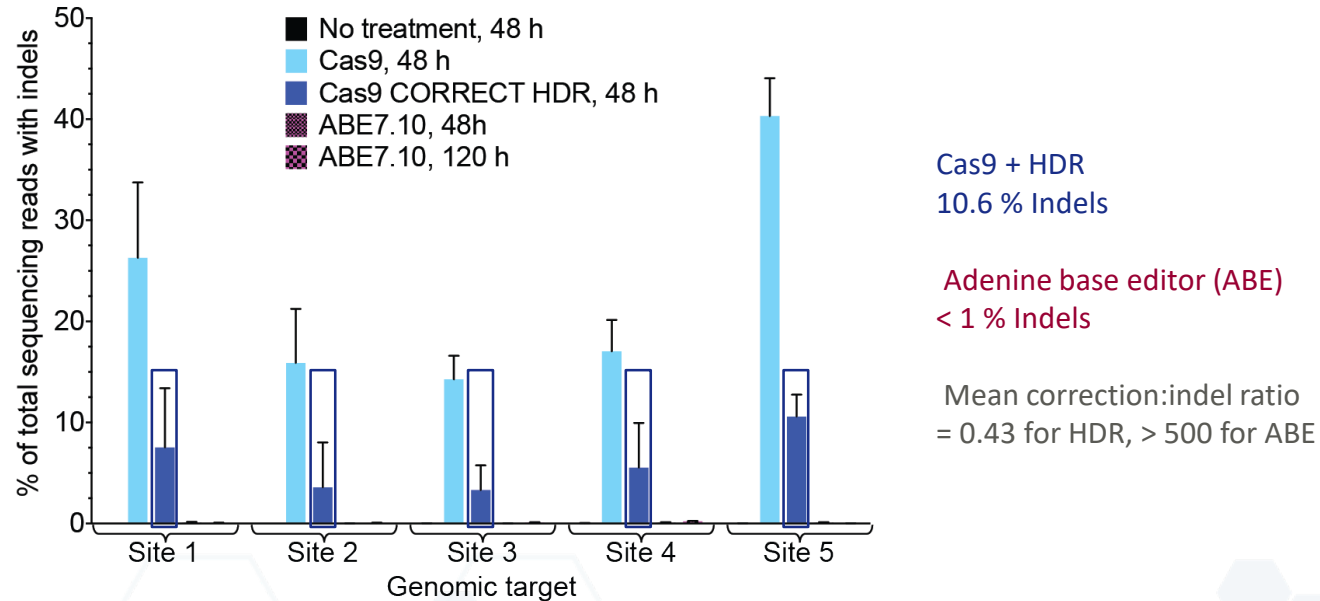
Kwart, Tessier-Lavigne et al. Nat. Protocols 12, 329 (2017) and Paquet, Tessier-Lavigne et al. Nature 533, 125 (2016)

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

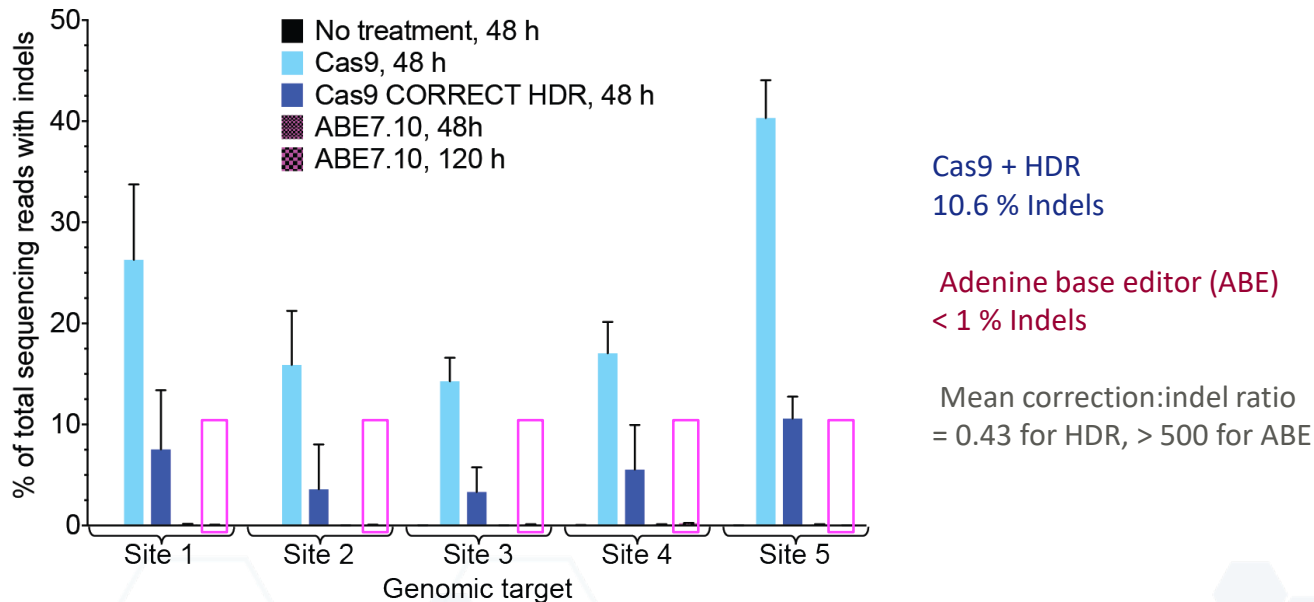


Kwart, Tessier-Lavigne et al. Nat. Protocols 12, 329 (2017) and Paquet, Tessier-Lavigne et al. Nature 533, 125 (2016)

Adenine Base Editing Produces Very Few Indels



Adenine Base Editing Produces Very Few Indels



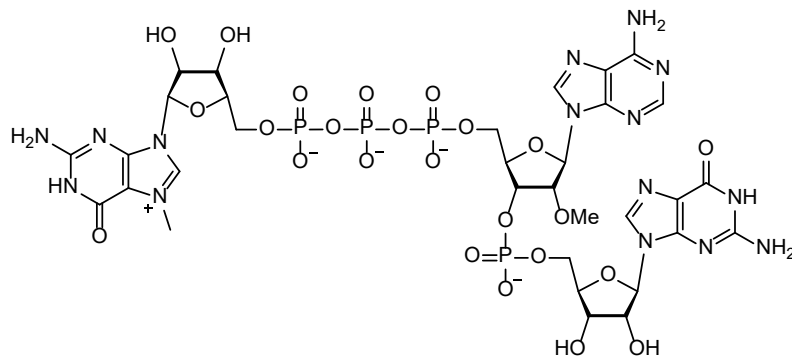
- Transient mRNA expression increases specificity of base editing
- mRNA can be introduced into the cytoplasm of difficult-to-transfect cells
- More efficient editing observed with mRNA than with plasmids

CleanCap[®] Co-transcriptional Capping Yields Optimal Cap 1 Structure with High Efficiency

CleanCap Cap 1 AG

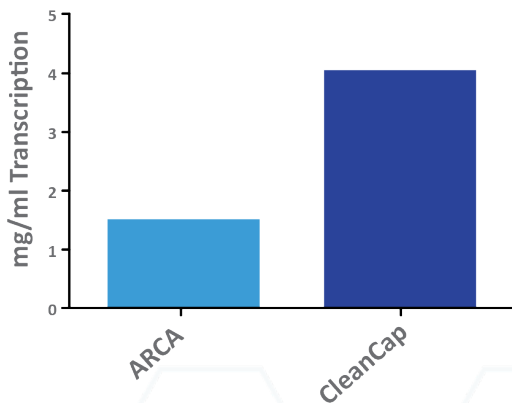


Cap 1 recognized as “self”

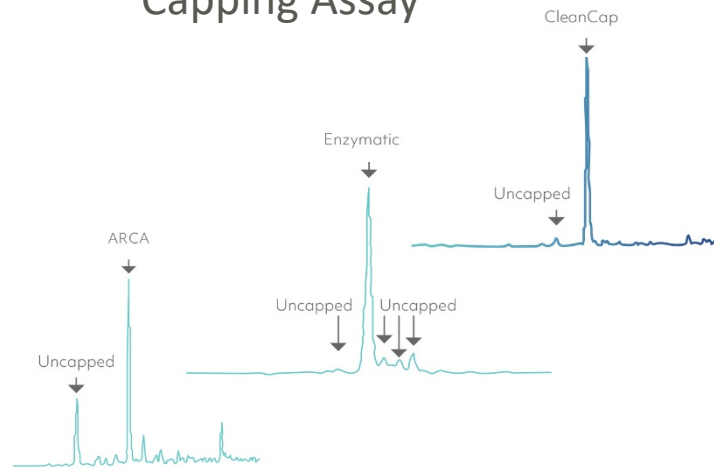


CleanCap[®] Co-transcriptional Capping Yields Optimal Cap 1 Structure with High Efficiency

Transcription Yield

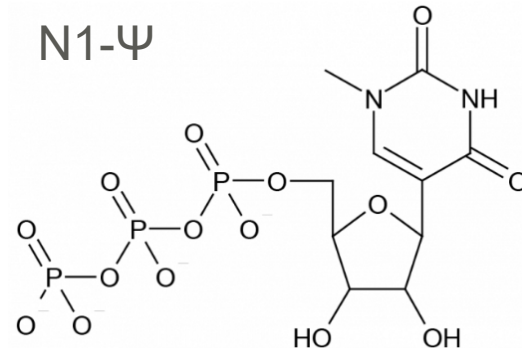
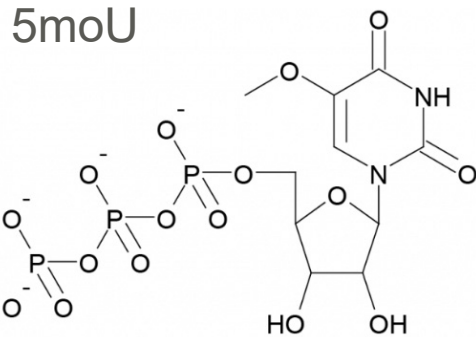


Capping Assay

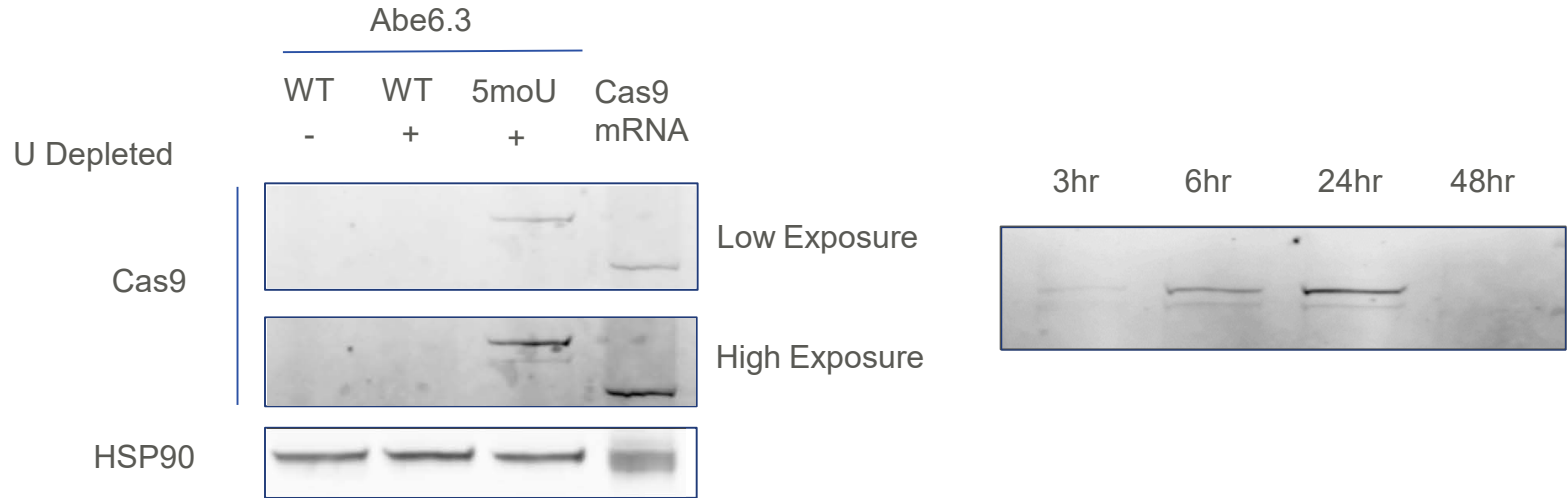


Base Modification/Sequence Optimization to Reduce Innate Immune Stimulation

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



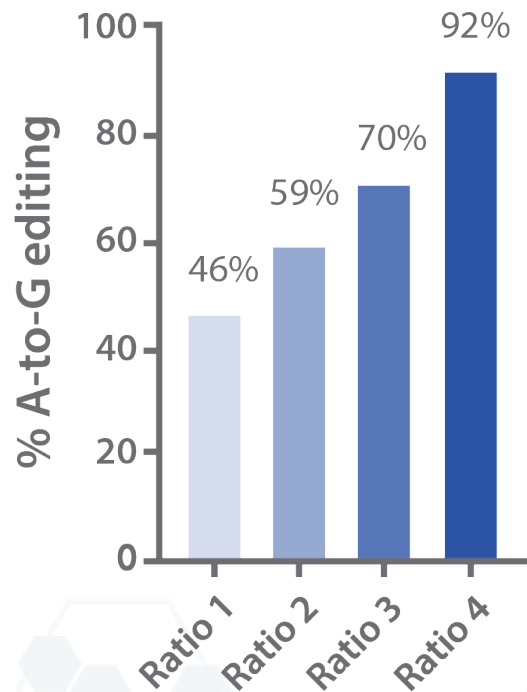
Uridine Depleted, 5moU Modified RNA Gives Higher Expression than Wild Type RNA in Cultured Cells



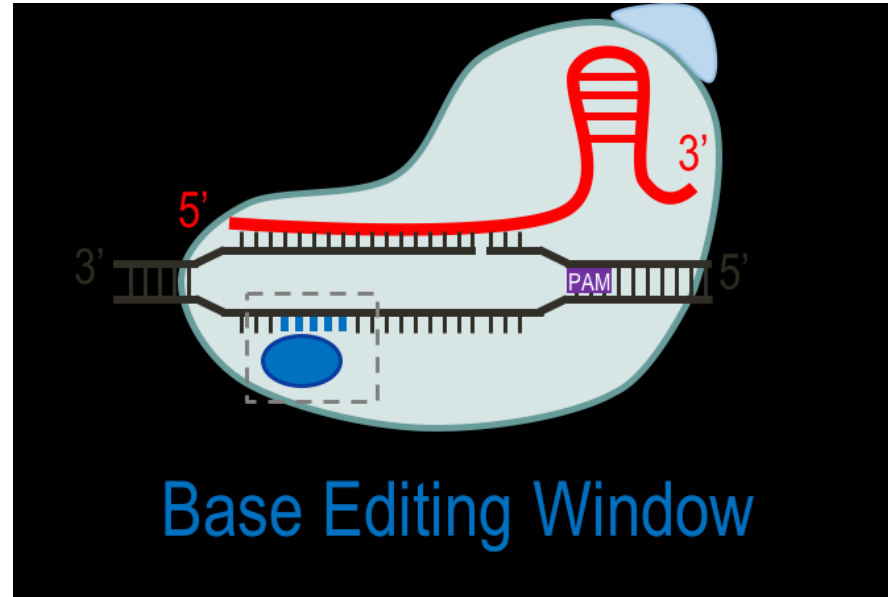
293T Cells at 12 hours

Adenine Base Editing in HEK293T Cells

Optimizing Guide: mRNA Ratio

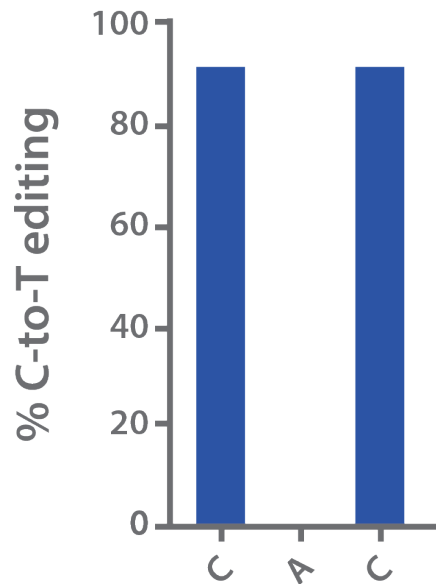


Base Editors have an Editing Window

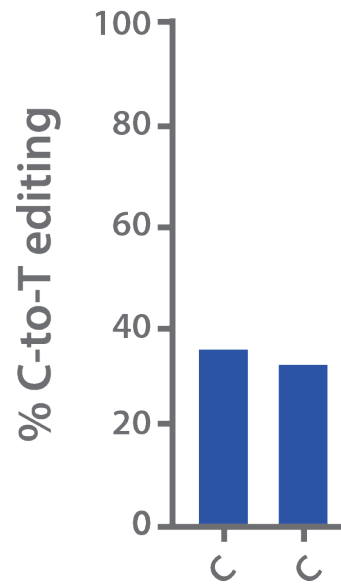


Multiple Sites Can Be Edited Simultaneously in Cells by Cytidine Base Editor

Chromosomal
Location 1

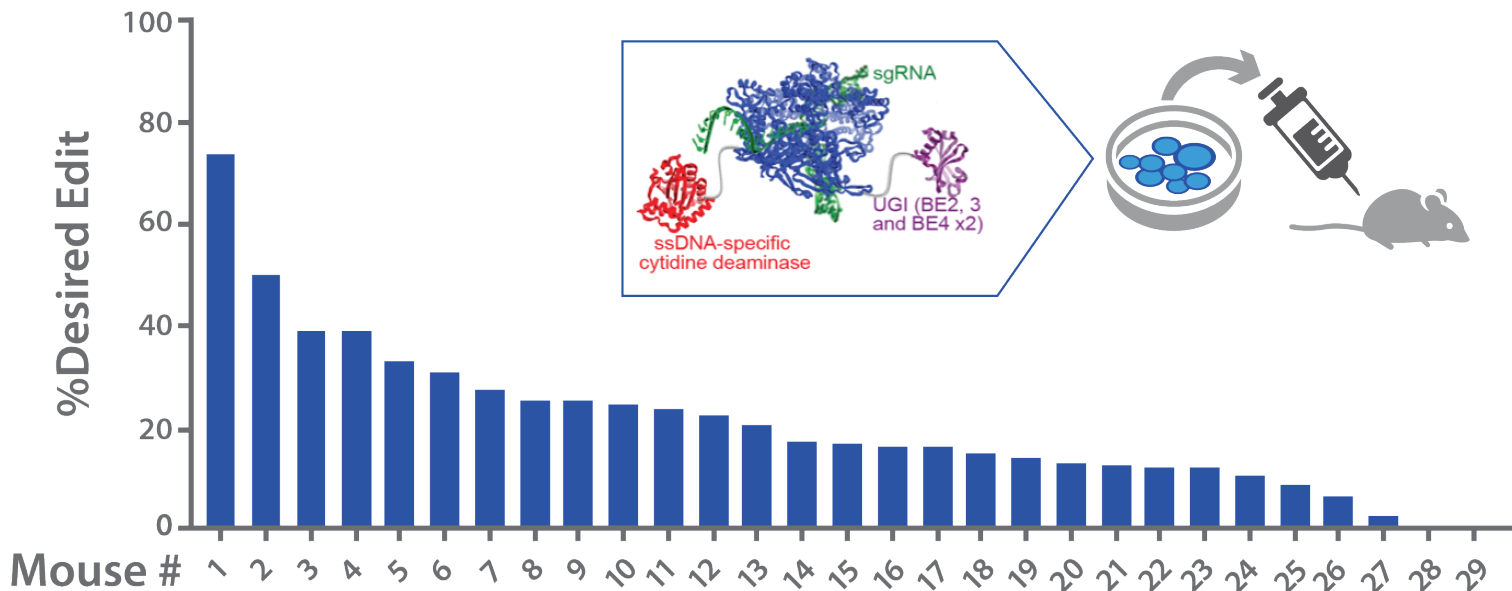


Chromosomal
Location 2



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

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.

- CleanCap® co-transcriptional capping produces Cap 1 structure that mimics natural “self” RNAs
- Uridine depleted modified mRNA yields maximal base editor expression
- Expression of base editors from mRNA is more efficient than from plasmid
- 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.

Grand Opening of a New Expanded TriLink Facility



Grand Opening November 20, 2019, San Diego

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Wen Xue

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Talk posted at www.trilink/BaseEditor