Adenine Base-Editing

KGD Laboratory Workshop

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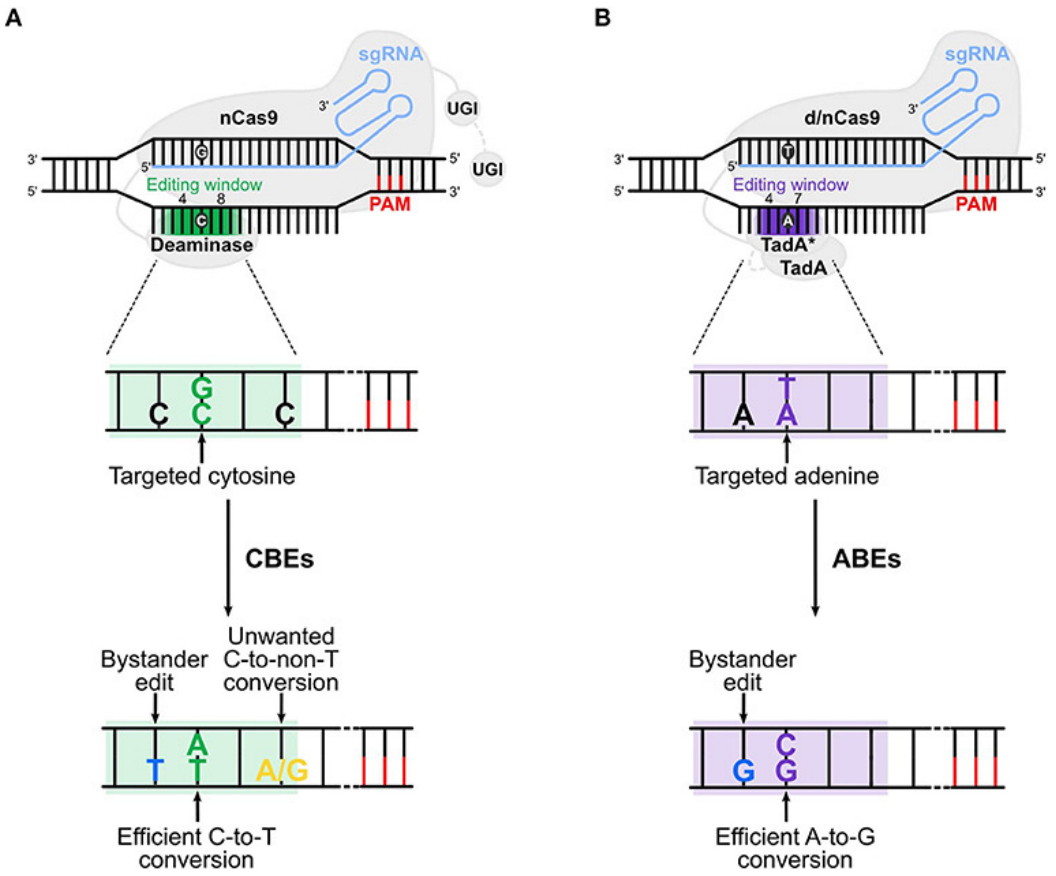
Base Editors:

Precisely targets bases in the gene.

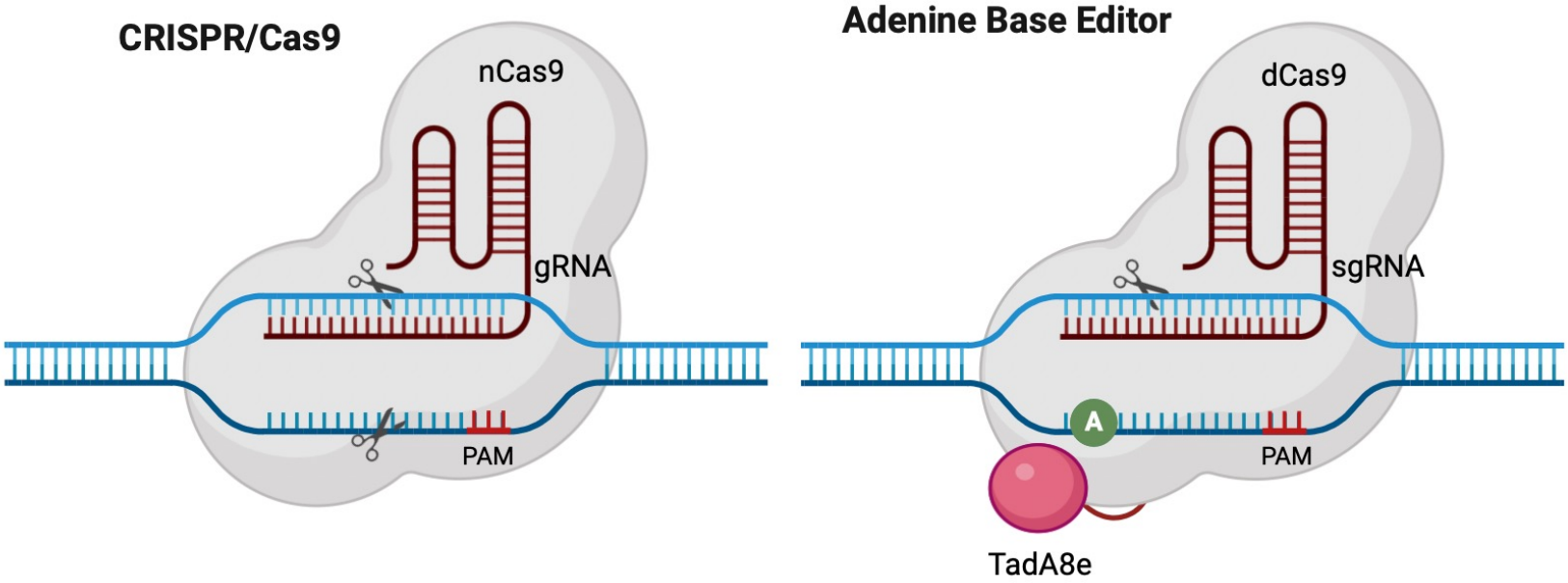
A. Cystosine Base Editors (CBEs)-
Cytosine to Thymine (C to T).

B. Adenine Base Editors (ABEs)-
Adenine to Guanine (A to G).



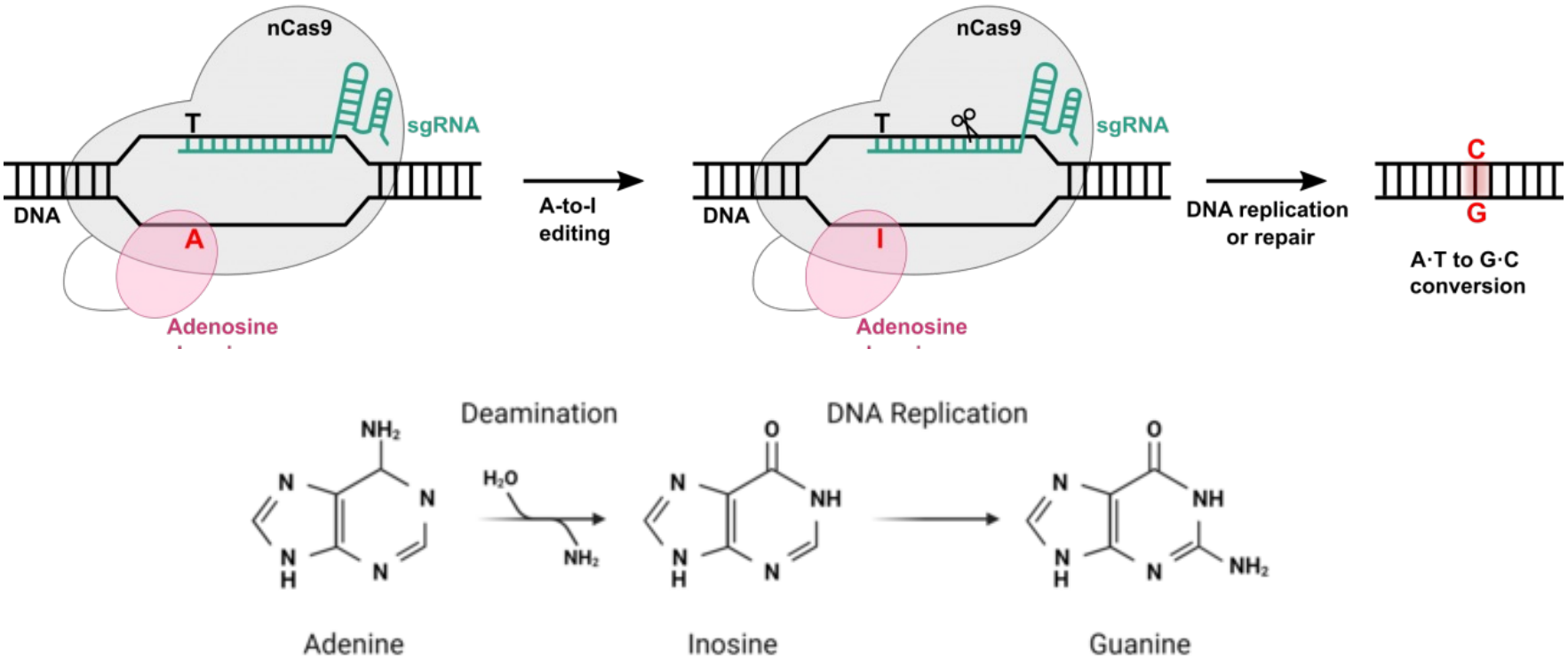
Antoniou *et al., Front. Genome Ed. 2021*

CRISPR/Cas9 vs. Adenine Base Editor



Non-homologous end joining (NHEJ)
Homology directed repair (HDR)

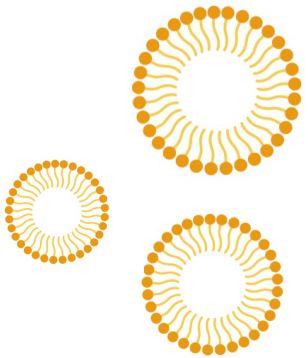
Adenine Base Editors (ABE) converts Adenine to Inosine.



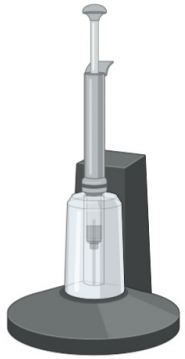
S. Park, 2020
M. Llado, *2023*

Requirements for ABE:

1. Enzyme: ABE8e
2. Single guide RNA
3. Mode of delivery:
    a. Electroporation
    b. Lipofection
4. Cells to be edited:
    a. iPSC-RDEB
    b. iPSC-RDEB-FB.



Lipofectamine Messenger Max®



Neon Transfection System®

How to determine the sgRNA for ABE?

Step 1:

Use ~25 bases of your gene of interest.

Target base is flanked.

Step 2:

Use **CRISPR RGen Tools** to determine possible sgRNA.

Step 3:

Choose which suggested sgRNA targets your base of interest.

Example: sgRNA for RDEB iPSC and FB

DNA Sequence: **COL7A1 c.1573C>T, p.Arg525Ter**
AGCTGCCCGGGCAGCGGGTG**T**GAGTGTCCTGGAG

CRISPR RGen Tools Site: CRISPR RGEN Tools (rgenome.net)

Conditions: ABE, 13 to 17 editing window

ABE Protocol for iPSC:

(Imogen Brooks; PI: Dr. Joanna Jackow, King's College London)

Step 1:

Prepare:

A. Lipofectamine+ media
B. ABE8e+sgRNA+ media
C. Media (NC)

(10 min)

Step 2:

Combine:

A+B

A+C

(5 min)

Step 3:

Add:

AB and AC to cells

(15 min)

How to determine the % editing efficiency?

Step 1:

Send samples for sequencing.

Step 2:

Use **EditR** to estimate the gene editing efficiency.

Step 3:

Compare the negative control with the edited cells.

moriaritylab.shinyapps.io/editr_v10/

New Chrome available

EditAR

Load Example Data

Please upload your Sanger sequencing file here

Upload .ab1 File

Browse... FB-EB3.NC_COL7A1.ab1

Upload complete

Please enter in your ~20bp guide sequence in 5'-3' orientation

Enter gRNA sequence

GACACTCACACCCGCTGCCCGGG

Guide sequence is reverse complement

Manually enter the 5' start and 3' end of the good sequencing below to trim the sanger sequening read. To determine which values to use, click the 'Data QC' tab and cursor over the 'Data QA: Signal and noise plot'.

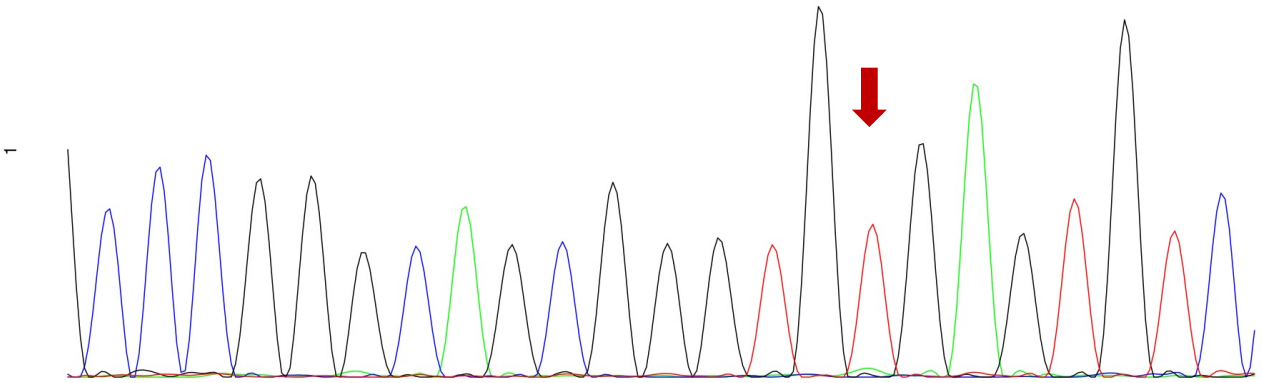
For example, if clean sequencing starts at the 56th bp of the chromatogram and ends at 405th bp, enter those respective values.

5' start

3' end

Instructions | Data QC | Predicted Editing | Download Report

gRNA Protospacer



	C	C	C	G	G	G	C	A	G	C	G	G	G	T	G	T	G	A	G	T	G	T	C
T	1	1	1	0	1	0	2	1	1	2	1	2	2	93	1	92	1	2	2	95	1	92	3
G	3	3	2	98	98	93	2	2	94	4	97	96	97	4	98	2	95	1	93	3	98	4	1
C	94	95	95	2	1	2	94	1	2	93	1	0	0	1	1	0	1	0	0	0	1	3	95
A	1	0	2	0	1	4	3	96	3	1	1	1	1	2	1	5	3	97	4	2	0	1	1

moriaritylab.shinyapps.io/editr_v10/

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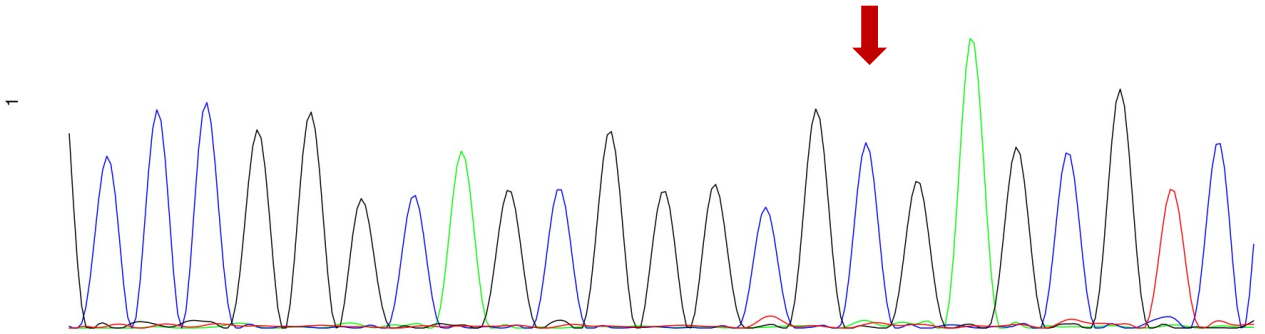
gRNA Protospacer

ABE has achieved 92% on-target editing efficiency.



Bystander edits

	C	C	C	G	G	G	C	A	G	C	G	G	G	T	G	T	G	A	G	T	G	T	C
T	2	2	2	1	1	0	2	1	2	2	1	2	0	9	1	3	2	1	2	5	2	88	4
G	3	3	3	95	98	94	1	2	94	5	97	96	97	3	97	1	91	1	93	2	96	2	1
C	95	95	95	2	1	2	94	1	2	92	1	1	2	89	1	92	2	1	2	92	2	7	95
A	0	1	0	2	0	4	3	95	2	1	1	1	1	0	1	4	4	97	3	1	1	2	1

Summary

Adenine base editing (ABE) is a powerful gene editing tool that can be used to correct point mutations in the genes.

Careful designing of single guide RNAs (sgRNA) is key to achieve a high on-target editing efficiency.

Bystander edits and off-target effects are unavoidable, thus their effects should be determined.