

# Walk-through GEMs PnB Designer

A web application to design Prime and Base  
Editor guide RNAs for animals and plants

# GEMs PnB Designer

User Interface:



Please select your editing strategy:

Prime editing ▼

Please select the genome of the species, you are working with:

▼

Please select the running mode:

▼

Search Reset

[Instructions](#)

The logo features a purple DNA double helix on the left, with a yellow and grey diagonal bar crossing it. To the right of the DNA is the text "geml" in a bold, lowercase, sans-serif font, followed by "PnB Designer" in a smaller, regular, lowercase, sans-serif font.

# geml PnB Designer

Select Editing Strategy

Please select your editing strategy:

Prime editing

Prime editing

Base editing

Please select the running mode:

Search Reset

[Instructions](#)

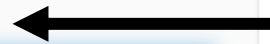


Please select your editing strategy:

Prime editing ▼

Please select the genome of the species, you are working with:

Human (hg38)  
Mouse (mm10)  
Zebrafish (GRCz11)  
Rice (MSU7)  
Thale Cress (TAIR9)  
Common Grape (IGGP12Xv2)



'Genome panel'



Please select your editing strategy:

Prime editing

Please select the genome of the species, you are working with:

Human (hg38)

Please select the running mode:

Single Sample Run

Multi Sample Run

← Select Running Mode

[Instructions](#)

Prime editing with PnB Designer

# Multi Sample Run



Please select your editing strategy:

Prime editing ▼

Please select the genome of the species, you are working with:

Human (hg38) ▼

Please select the running mode:

Multi Sample Run ▼

Choose CSV File

Browse... No file selected

Show all possible Oligos

Search Reset

[Instructions](#)

[Prime Editing template file](#)

← Browse sample file from your computer

# Templates for import file (.csv)

Prime editing:

| Variant      | Chromosome | GenomicLocation | Edit   | GeneOrientation | PBS | RTT |
|--------------|------------|-----------------|--------|-----------------|-----|-----|
| HEK3_1CTTins | 9          | 107422356       | insCTT | +               | 13  | 13  |
| HEK3_1Tdel   | 9          | 107422356       | delT   | +               | 13  | 13  |
| HEK3_1Ains   | 9          | 107422356       | insA   | +               | 13  | 13  |

Base editing:

| Variant   | Chromosome | GenomicLocation | SNP | GeneOrientation |
|-----------|------------|-----------------|-----|-----------------|
| Variant 1 | 2          | 58161573        | G>A | -               |
| Variant 2 | 2          | 58226727        | G>A | -               |
| Variant 3 | 3          | 10028690        | G>A | +               |





Please select your editing strategy:

Prime editing ▼

Please select the genome of the species, you are working with:

Human (hg38) ▼

Please select the running mode:

Multi Sample Run ▼

Choose CSV File

Browse... testfile\_HEK3\_RNF2.csv

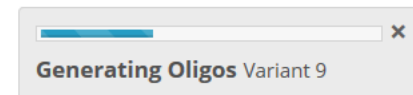
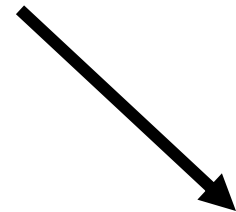
Upload complete

Show all possible Oligos

Search Reset

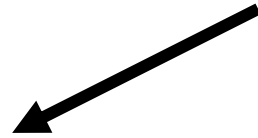
[Prime Editing template file](#) [Instructions](#)

Loading bar shows progress of computation





How many Variants can be targeted?



Please select your editing strategy:  
Prime editing

Please select the genome of the species, you are working with:  
Human (hg38)

Please select the running mode:  
Multi Sample Run

Choose CSV File  
Browse... testfile\_HEK3\_RNF2.csv  
Upload complete

Show all possible Oligos

Search Reset

[Prime Editing template file](#) [Instructions](#)

22 out of 23 Variants could be targeted

Show 10 entries

Search:

|    | Variant           | Score | Protospacer.Sense.    | Protospacer.Antisense. | EditPos. | Extension.coding.strand.          | PAM | PAM.Strand | id |
|----|-------------------|-------|-----------------------|------------------------|----------|-----------------------------------|-----|------------|----|
| 10 | HEK3_1CTTins      | -1    | GGCCCAGACTGAGCACGTGA  | TCACGTGCTCAGTCTGGGCC   | 1        | CAGACTGAGCACGCTTTGATGGC           | TGG | Sense      | 1  |
| 11 | HEK3_1Tdel        | -29   | GGCCCAGACTGAGCACGTGA  | TCACGTGCTCAGTCTGGGCC   | 1        | CAGACTGAGCACG[T]GATGGCAGAG        | TGG | Sense      | 3  |
| 9  | HEK3_1Ains        | -29   | GGCCCAGACTGAGCACGTGA  | TCACGTGCTCAGTCTGGGCC   | 1        | CAGACTGAGCACGATGATGGCAG           | TGG | Sense      | 5  |
| 13 | RNF2_4f_1Tins     | -1    | GTCATCTTAGTCATTACCTG  | CAGGTAATGACTAAGATGAC   | 1        | AACGAACACCTCAAGTAATGACTAAGATG     | AGG | Antisense  | 8  |
| 12 | RNF2_4f_1GTains   | -1    | GTCATCTTAGTCATTACCTG  | CAGGTAATGACTAAGATGAC   | 1        | AACGAACACCTCATACCGTAATGACTAAGATG  | AGG | Antisense  | 11 |
| 15 | RNF2_4f_4Adel     | -2    | gTGAGTTACAACGAACACCTC | GAGGTGTCGTTGTAATCAC    | 2        | AGTTACAACGAACACC[T]CAGGTAATGACT   | AGG | Sense      | 13 |
| 14 | RNF2_4f_3-5GAGdel | -1    | gTGAGTTACAACGAACACCTC | GAGGTGTCGTTGTAATCAC    | 1        | AGTTACAACGAACACC[CTC]JAGGTAATGACT | AGG | Sense      | 16 |
| 8  | EMX1_4f_6Tins     | -6    | GAGTCCGAGCAGAAGAAGAA  | TTCTTCTTCTGCTCGGACTC   | 6        | TCCGAGCAGAAGAAGAAGGTGCTCCCATCAC   | GGG | Sense      | 20 |
| 5  | EMX1_4f_1TGCins   | -1    | GAGTCCGAGCAGAAGAAGAA  | TTCTTCTTCTGCTCGGACTC   | 1        | TCCGAGCAGAAGAATGCGAAGGGCTCCCATCAC | GGG | Sense      | 25 |
| 7  | EMX1_4f_5Gdel     | -5    | GAGTCCGAGCAGAAGAAGAA  | TTCTTCTTCTGCTCGGACTC   | 5        | TCCGAGCAGAAGAAGAAG[G]GCTCCCATCAC  | GGG | Sense      | 30 |

Showing 1 to 10 of 23 entries

Previous 1 2 3 Next

Download Results

Download ready to clone oligos with cloning sites



# Output Table (One entry with the highest score for each Variant):

Show  entries

Search:

|    | Variant           | Score | Protospacer.Sense.    | Protospacer.Antisense. | EditPos. | Extension.coding.strand.                   | PAM | PAM.Strand | id |
|----|-------------------|-------|-----------------------|------------------------|----------|--|-----|------------|----|
| 10 | HEK3_1CTTins      | -1    | GGCCCAGACTGAGCACGTGA  | TCACGTGCTCAGTCTGGGCC   | 1        | CAGACTGAGCACG <b>CTT</b> TGATGGC           | TGG | Sense      | 1  |
| 11 | HEK3_1Tdel        | -29   | GGCCCAGACTGAGCACGTGA  | TCACGTGCTCAGTCTGGGCC   | 1        | CAGACTGAGCACG <b>[T]</b> GATGGCAGAG        | TGG | Sense      | 3  |
| 9  | HEK3_1Ains        | -29   | GGCCCAGACTGAGCACGTGA  | TCACGTGCTCAGTCTGGGCC   | 1        | CAGACTGAGCACG <b>A</b> TGATGGCAG           | TGG | Sense      | 5  |
| 13 | RNF2_4f_1Tins     | -1    | GTCATCTTAGTCATTACCTG  | CAGGTAATGACTAAGATGAC   | 1        | AACGAACACCTC <b>AA</b> AGTAATGACTAAGATG    | AGG | Antisense  | 8  |
| 12 | RNF2_4f_1GTains   | -1    | GTCATCTTAGTCATTACCTG  | CAGGTAATGACTAAGATGAC   | 1        | AACGAACACCTC <b>TAC</b> CGTAATGACTAAGATG   | AGG | Antisense  | 11 |
| 15 | RNF2_4f_4Adel     | -2    | gTGAGTTACAACGAACACCTC | GAGGTGTTGTTGTAACCTCAC  | 2        | AGTTACAACGAACACC <b>[T]</b> CAGGTAATGACT   | AGG | Sense      | 13 |
| 14 | RNF2_4f_3-5GAGdel | -1    | gTGAGTTACAACGAACACCTC | GAGGTGTTGTTGTAACCTCAC  | 1        | AGTTACAACGAACAC <b>[CTC]</b> AGGTAATGACT   | AGG | Sense      | 16 |
| 8  | EMX1_4f_6Tins     | -6    | GAGTCCGAGCAGAAGAAGAA  | TTCTTCTTCTGCTCGGACTC   | 6        | TCCGAGCAGAAGAAG <b>AGG</b> TGCTCCCATCAC    | GGG | Sense      | 20 |
| 5  | EMX1_4f_1TGCins   | -1    | GAGTCCGAGCAGAAGAAGAA  | TTCTTCTTCTGCTCGGACTC   | 1        | TCCGAGCAGAAG <b>AATG</b> CGAAGGGCTCCCATCAC | GGG | Sense      | 25 |
| 7  | EMX1_4f_5Gdel     | -5    | GAGTCCGAGCAGAAGAAGAA  | TTCTTCTTCTGCTCGGACTC   | 5        | TCCGAGCAGAAGAAG <b>[G]</b> GCTCCCATCAC     | GGG | Sense      | 30 |

Showing 1 to 10 of 23 entries

Previous  2 3 Next

Variant name

pegRNA Score  
(See next page)

Edit position  
(See Anzalone et. al., 2019)

Extension on the coding strand  
with highlighted edit

# The pegRNA Score

The higher the better!

Computed based on recommendations from David Liu's Lab:

(-28) 5' C in 3' extension

(-50) Poly-T tracts (TTTTT or more) in the 3' extension

(-6) 4 or less bp homology in the RT after the edit

(-1) per 1 bp increase of Edit Position

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$\Sigma$  = pegRNA Score

Please select your editing strategy:

Prime editing

Please select the genome of the species, you are working with:

Human (hg38)

Please select the running mode:

Multi Sample Run

Choose CSV File

Browse... testfile\_HEK3\_RNF2.csv

Upload complete

Show all possible Oligos

Search Reset

[Prime Editing template file](#) [Instructions](#)

To show all possible pegRNAs, tick this box

22 out of 23 Variants could be targeted

Show 10 entries

Search:

|    | Variant         | Protospacer.Sense.    | Protospacer.Antisense. | EditPos. | Extension.Sense.                 | PAM    | PAM.Strand  | Score |
|----|-----------------|-----------------------|------------------------|----------|----------------------------------|--------|---|-------|
| 1  | HEK3_1CTTins    | GGCCCAGACTGAGCACGTGA  | TCACGTGCTCAGTCTGGGCC   | 1        | CAGACTGAGCACGCTTGTATGGC          | TGG    | Sense   | -1    |
| 2  | HEK3_1CTTins    | AAAAAAAAAAAAAAAA      | TTTTTTTTTTTTTTTT       | 0        |                                  | no PAM | Edit too far away, try to increase the RT length! | -999  |
| 3  | HEK3_1Tdel      | GGCCCAGACTGAGCACGTGA  | TCACGTGCTCAGTCTGGGCC   | 1        | CAGACTGAGCACGTTGTATGGCAGAG       | TGG    | Sense   | -29   |
| 4  | HEK3_1Tdel      | AAAAAAAAAAAAAAAA      | TTTTTTTTTTTTTTTT       | 0        |                                  | no PAM | Edit too far away, try to increase the RT length! | -999  |
| 5  | HEK3_1Ains      | GGCCCAGACTGAGCACGTGA  | TCACGTGCTCAGTCTGGGCC   | 1        | CAGACTGAGCACGATGTATGGCAG         | TGG    | Sense   | -29   |
| 6  | HEK3_1Ains      | AAAAAAAAAAAAAAAA      | TTTTTTTTTTTTTTTT       | 0        |                                  | no PAM | Edit too far away, try to increase the RT length! | -999  |
| 7  | RNF2_4f_1Tins   | gTGAGTTACAACGAACACCTC | GAGGTGTCGTTGTAACCTCAC  | 5        | AGTTACAACGAACACCTCAGGTAATGACT    | AGG    | Sense   | -5    |
| 8  | RNF2_4f_1Tins   | GTCATCTTAGTCATTACCTG  | CAGGTAATGACTAAGATGAC   | 1        | AACGAACACCTCAAGTAATGACTAAGATG    | AGG    | Antisense   | -1    |
| 9  | RNF2_4f_1Tins   | AAAAAAAAAAAAAAAA      | TTTTTTTTTTTTTTTT       | 0        |                                  | no PAM | Edit too far away, try to increase the RT length! | -999  |
| 10 | RNF2_4f_1GTAins | gTGAGTTACAACGAACACCTC | GAGGTGTCGTTGTAACCTCAC  | 5        | AGTTACAACGAACACCTCATACGGTAATGACT | AGG    | Sense   | -5    |

Showing 1 to 10 of 86 entries

Previous 1 2 3 4 5 ... 9 Next

Download Results

86 instead of 23 entries (also pegRNAs with lower pegRNAs Score are shown)

# Single Sample Run



Please select your editing strategy:

Prime editing

Please select the genome of the species, you are working with:

Human (hg38)

Please select the running mode:

Single Sample Run

# Genomic coordinates

Give your  
sample a  
name



Select chromosome  
and genomic location



Select the target  
strand



Please select the running mode:

Please select how you want to insert the editing location:

Please name your variant:

Please select the genomic location you want to edit (e.g. chr16 : 107422356):  
 :

Please select the orientation of your target gene:

Please select the edit you want to install (e.g. G>A, insA, delT):

Please select the mutation you want to correct (e.g. C>T, insTT, delTCT):

Please select the PBS length:

Please select the RTT length:

[Instructions](#)

# Genomic coordinates

Insert specific  
edit here

OR

Insert Mutation here  
for correction

Please select the running mode:

Single Sample Run

Please select how you want to insert the editing location:

Genomic coordinates

Please name your variant:

HEK3\_1

Please select the genomic location you want to edit (e.g. chr16 : 107422356):

chr9 : 107422356

Please select the orientation of your target gene:

+

Please select the edit you want to install (e.g. G>A, insA, delT):

delT

Please select the mutation you want to correct (e.g. C>T, insTT, delTCT):

Please select the PBS length:

13

Please select the RTT length:

13

Search Reset

[Instructions](#)



# Genomic coordinates

Please select the running mode:

Single Sample Run

Please select how you want to insert the editing location:

Genomic coordinates

Please name your variant:

HEK3\_1

Please select the genomic location you want to edit (e.g. chr16 : 107422356):

chr9 : 107422356

Please select the orientation of your target gene:

+

Please select the edit you want to install (e.g. G>A, insA, delT):

delT

Please select the mutation you want to correct (e.g. C>T, insTT, delTCT):

Please select the PBS length:

13

Please select the RTT length:

13

Search Reset

[Instructions](#)

Define prime binding site (PBS) length



Define reverse transcriptase template length here (recommended 10-16 nt)  
Anzalone et al. 2019



# Sequence Input

Please select your editing strategy:

Prime editing ▼

Please select the genome of the species, you are working with:

Human (hg38) ▼

Please select the running mode:

Single Sample Run ▼

Please select how you want to insert the editing location:

Sequence input ▼

Please name your variant:

HEK3\_1

Upstream Sequence > 75 nt:      Edit:      Downstream Sequence > 75 nt:

:CAGCCCTGGCCTGGGTCAA      delTCC      TTGGGGCCCAGACTGAGCAC

Please select the PBS length:

13 ▼

Please select the RTT length:

13 ▼

Search    Reset

[Instructions](#)

## Positions of tested Prime editing sites

- HEK3 +1 : chr9 107422356 (+)
- RNF2 +1 : chr1 185087640 (+)
- DNMT1 +1 : chr19 10133644 (-)
  - In Anzalone et al. edit is on (+) Strand
    - -> Here use reverse complement Sequence and change direction for deletions
- EMX1 +1: chr2 72933870 (+)

# Base editing with PnB Designer

Coming soon...