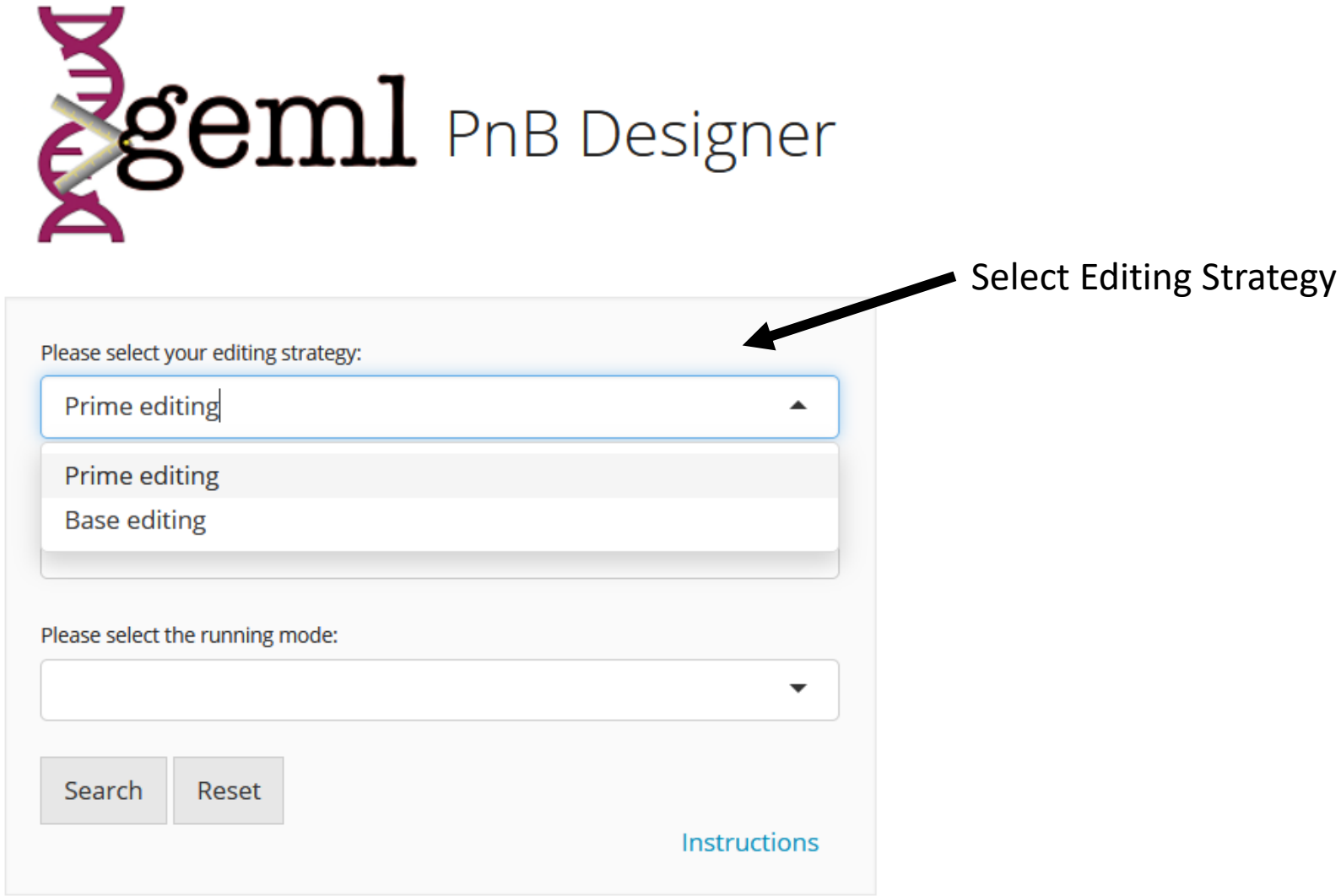
# Walk-through
# GEMLs PnB Designer

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

# GEMLs PnB Designer

User Interface:


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

geml PnB Designer

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

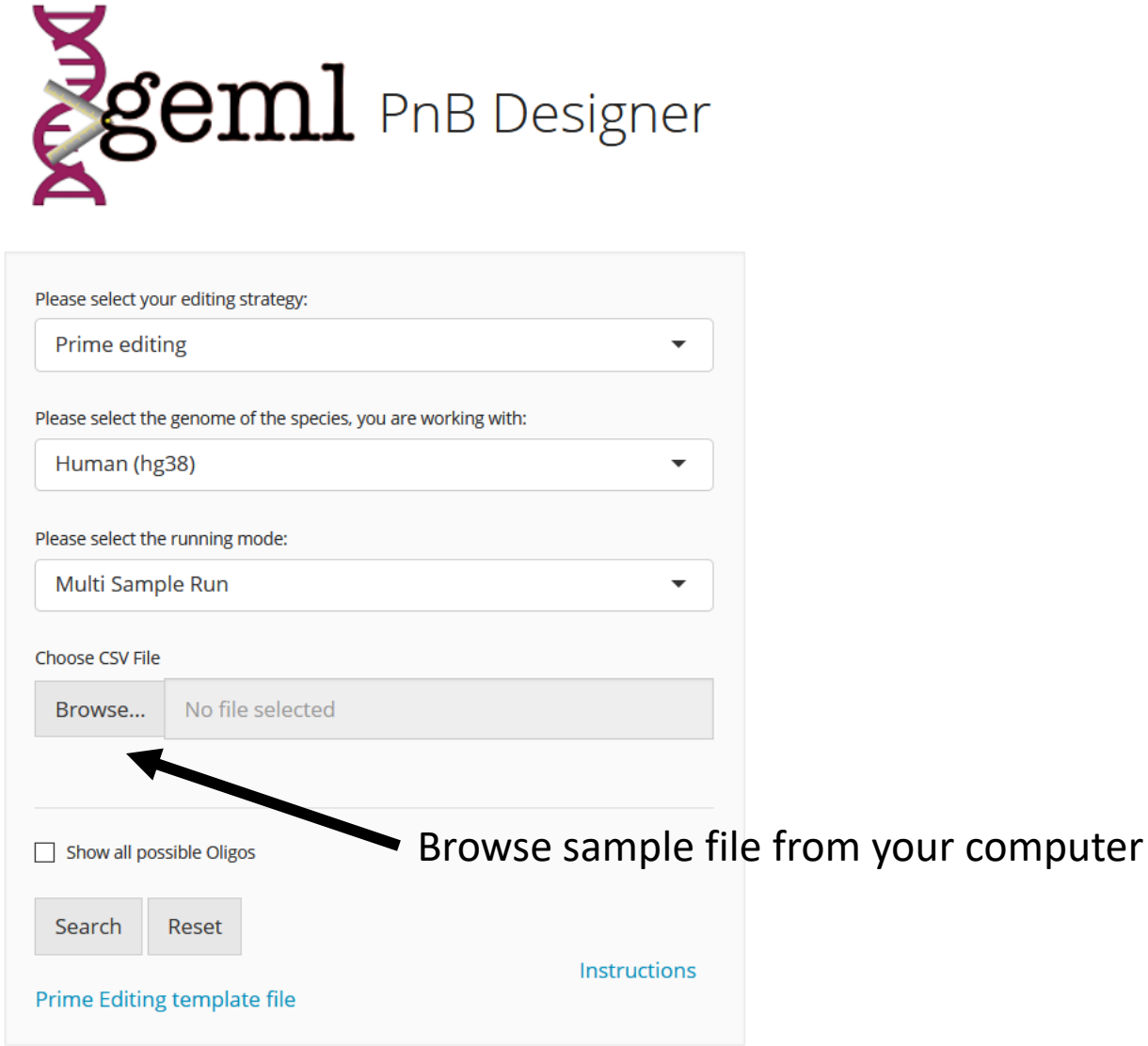
Multi Sample Run

Instructions

Select Running Mode

# Prime editing with PnB Designer

# Multi Sample Run



# 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 | + |

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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

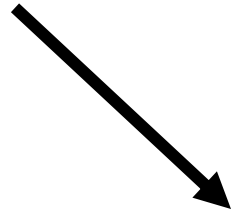
Show all possible Oligos

Search Reset

Instructions

Prime Editing template file

Loading bar shows progress of computation



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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

Instructions

Prime Editing template file

*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 | AACGAACACCTCAAAGTAATGACTAAGATG | AGG | Antisense | 8 |
| 12 | RNF2_4f_1GTAins | -1 | GTCATCTTAGTCATTACCTG | CAGGTAATGACTAAGATGAC | 1 | AACGAACACCTCATACCGTAATGACTAAGATG | AGG | Antisense | 11 |
| 15 | RNF2_4f_4Adel | -2 | gTGAGTTACAACGAACACCTC | GAGGTGTTCGTTGTAACTCAC | 2 | AGTTACAACGAACACC[T]CAGGTAATGACT | AGG | Sense | 13 |
| 14 | RNF2_4f_3-5GAGdel | -1 | gTGAGTTACAACGAACACCTC | GAGGTGTTCGTTGTAACTCAC | 1 | AGTTACAACGAACAC[CTC]AGGTAATGACT | 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 10 entries  Search:

| | Variant | Score | Protospacer.Sense. | Protospacer.Antisense. | EditPos. | Extension.coding.strand. | PAM | PAM.Strand | id |
|---|---|---|---|---|---|---|---|---|---|
| 10 | HEK3_1CTTins | -1 | GGCCCAGACTGAGCACGTGA | TCACGTGCTCAGTCTGGGCC | 1 | CAGACTGAGCACG**CTT**TGATGGC | TGG | Sense | 1 |
| 11 | HEK3_1Tdel | -29 | GGCCCAGACTGAGCACGTGA | TCACGTGCTCAGTCTGGGCC | 1 | CAGACTGAGCACG**[T]**GATGGCAGAG | TGG | Sense | 3 |
| 9 | HEK3_1Ains | -29 | GGCCCAGACTGAGCACGTGA | TCACGTGCTCAGTCTGGGCC | 1 | CAGACTGAGCACG**A**TGATGGCAG | TGG | Sense | 5 |
| 13 | RNF2_4f_1Tins | -1 | GTCATCTTAGTCATTACCTG | CAGGTAATGACTAAGATGAC | 1 | AACGAACACCTCA**A**AGTAATGACTAAGATG | AGG | Antisense | 8 |
| 12 | RNF2_4f_1GTAins | -1 | GTCATCTTAGTCATTACCTG | CAGGTAATGACTAAGATGAC | 1 | AACGAACACCTCA**TAC**CGTAATGACTAAGATG | AGG | Antisense | 11 |
| 15 | RNF2_4f_4Adel | -2 | gTGAGTTACAACGAACACCTC | GAGGTGTTCGTTGTAACTCAC | 2 | AGTTACAACGAACACC**[T]**CAGGTAATGACT | AGG | Sense | 13 |
| 14 | RNF2_4f_3-5GAGdel | -1 | gTGAGTTACAACGAACACCTC | GAGGTGTTCGTTGTAACTCAC | 1 | AGTTACAACGAACAC**[CTC]**AGGTAATGACT | AGG | Sense | 16 |
| 8 | EMX1_4f_6Tins | -6 | GAGTCCGAGCAGAAGAAGAA | TTCTTCTTCTGCTCGGACTC | 6 | TCCGAGCAGAAGAAGAAGG**T**GCTCCCATCAC | GGG | Sense | 20 |
| 5 | EMX1_4f_1TGCins | -1 | GAGTCCGAGCAGAAGAAGAA | TTCTTCTTCTGCTCGGACTC | 1 | TCCGAGCAGAAGAA**TGC**GAAGGGCTCCCATCAC | 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

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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$\sum$ = pegRNA Score

geml PnB Designer

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

Instructions

Prime Editing template file

To show all possible pegRNAs, tick this box

*22 out of 23 Variants could be targeted*

Show 10 entries Search:

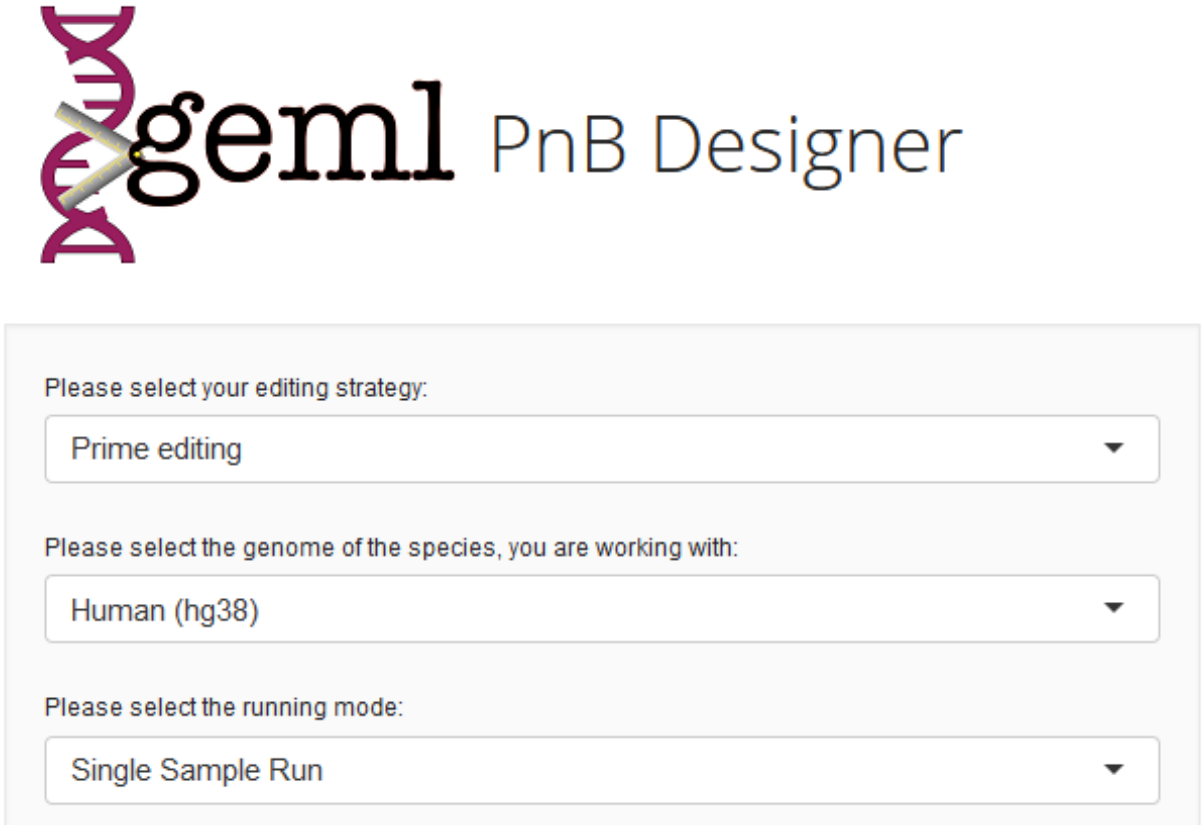
| | Variant | Protospacer.Sense. | Protospacer.Antisense. | EditPos. | Extension.Sense. | PAM | PAM.Strand | Score |
|---|---|---|---|---|---|---|---|---|
| 1 | HEK3_1CTTins | GGCCCAGACTGAGCACGTGA | TCACGTGCTCAGTCTGGGCC | 1 | CAGACTGAGCACGCTTTGATGGC | TGG | Sense | -1 |
| 2 | HEK3_1CTTins | AAAAAAAAAAAAAAAA | TTTTTTTTTTTTTTTT | 0 | | no PAM | Edit too far away, try to increase the RT length! | -999 |
| 3 | HEK3_1Tdel | GGCCCAGACTGAGCACGTGA | TCACGTGCTCAGTCTGGGCC | 1 | CAGACTGAGCACG[T]GATGGCAGAG | 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 | CAGACTGAGCACGATGATGGCAG | 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 | GAGGTGTTCGTTGTAACTCAC | 5 | AGTTACAACGAACACCTCAAGGTAATGACT | AGG | Sense | -5 |
| 8 | RNF2_4f_1Tins | GTCATCTTAGTCATTACCTG | CAGGTAATGACTAAGATGAC | 1 | AACGAACACCTCAAAGTAATGACTAAGATG | 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 | GAGGTGTTCGTTGTAACTCAC | 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

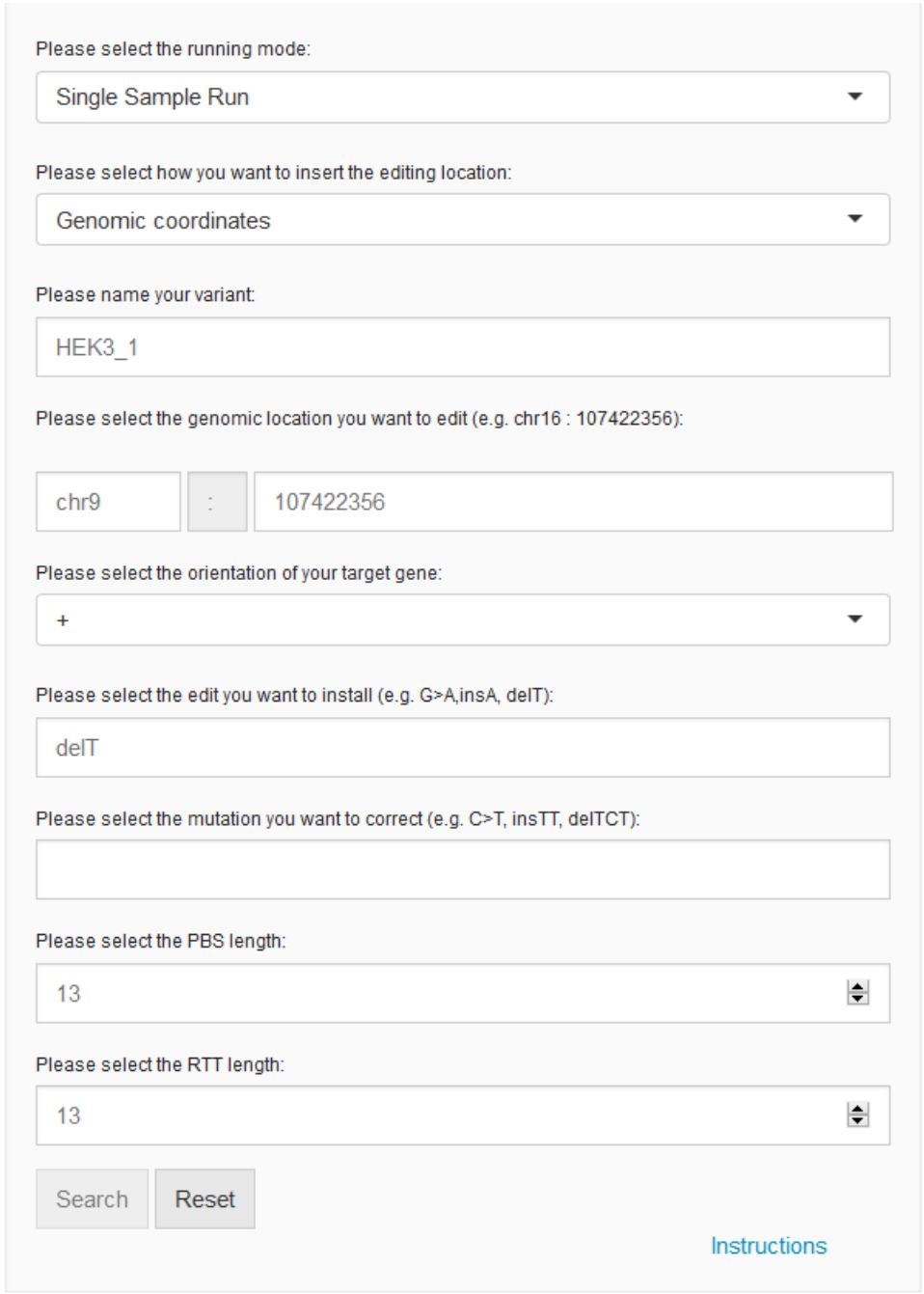


# Genomic coordinates

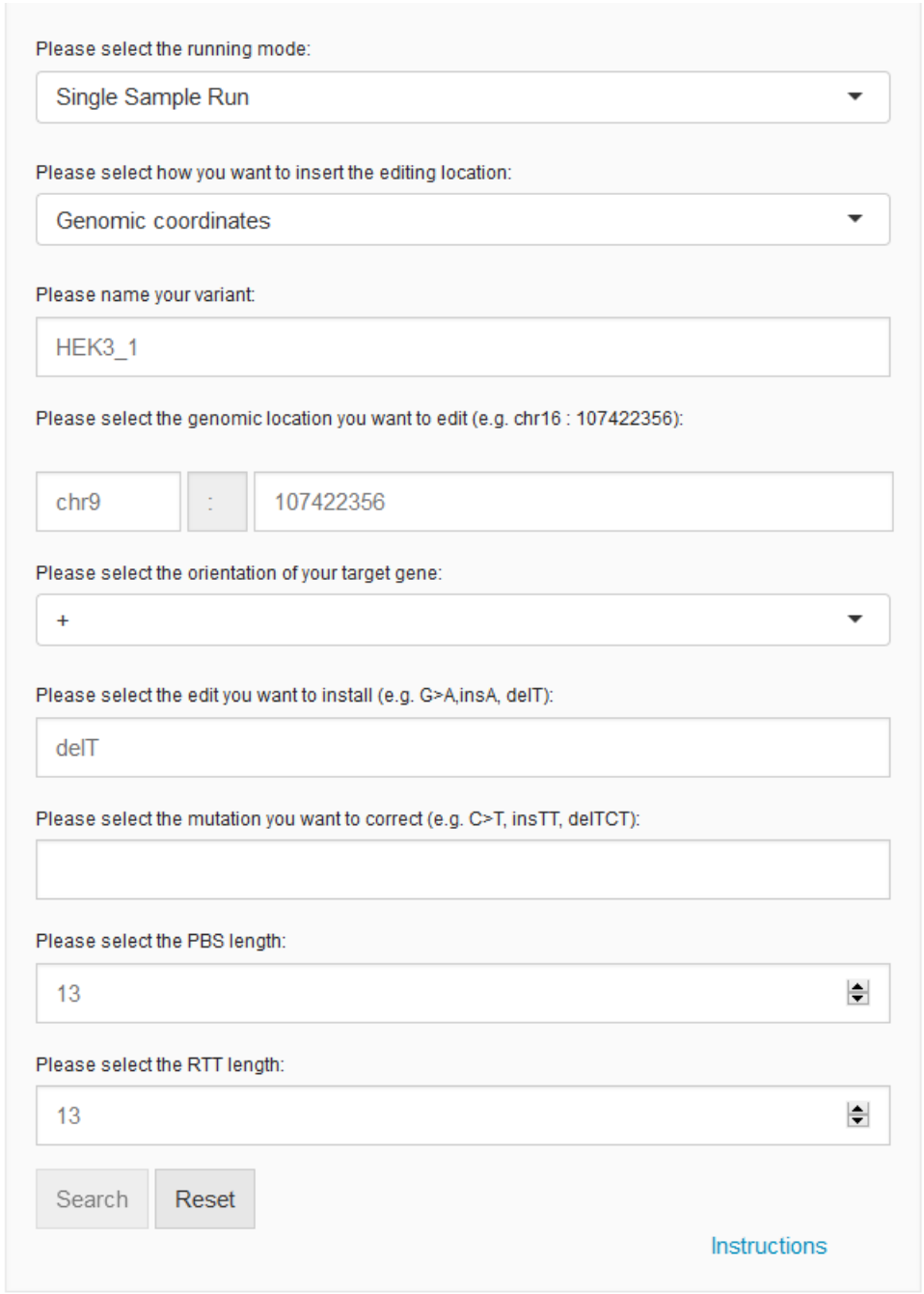
Give your sample a name

Select chromosome and genomic location

Select the target strand



# Genomic coordinates

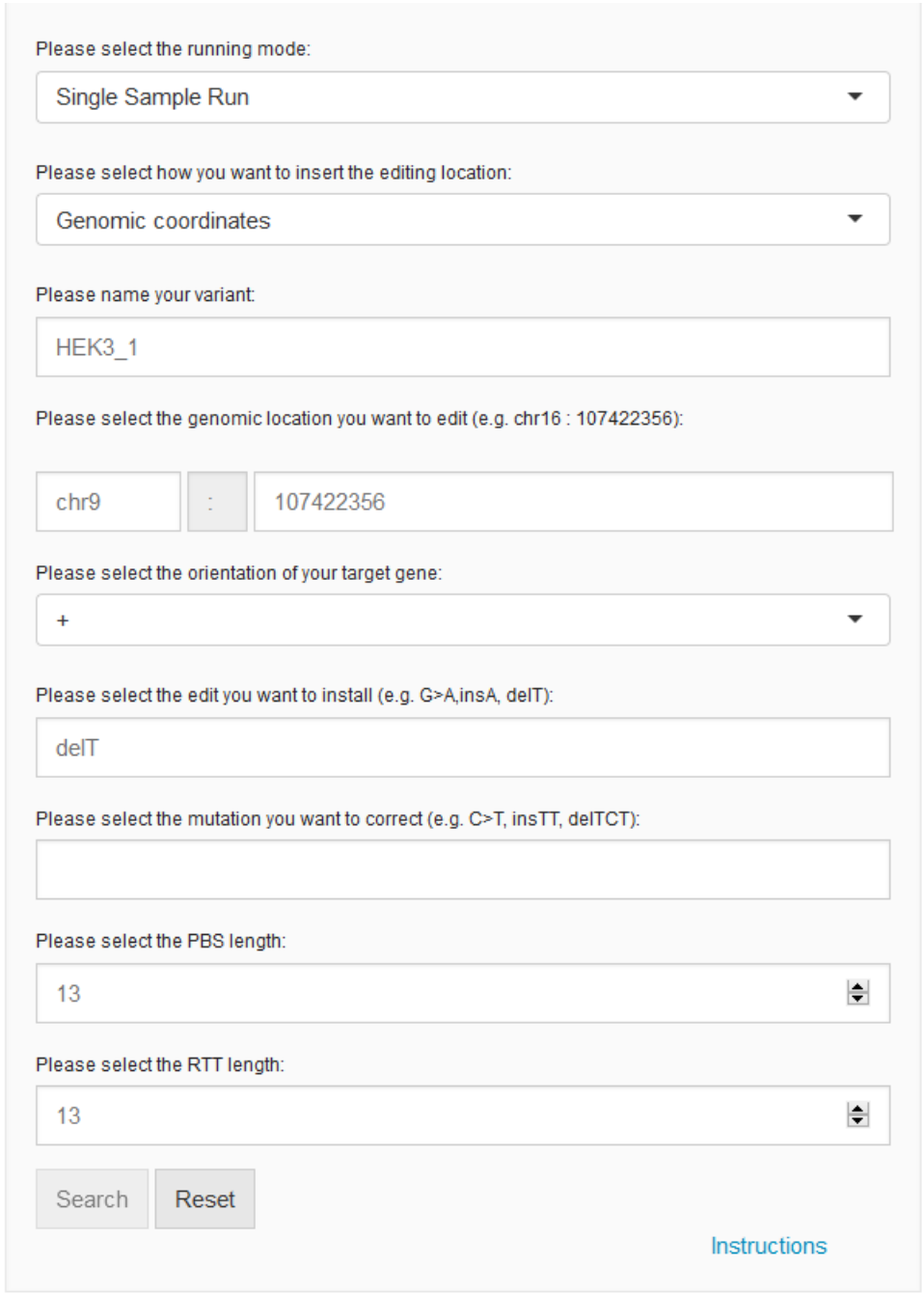



Insert specific edit here

OR

Insert Mutation here for correction

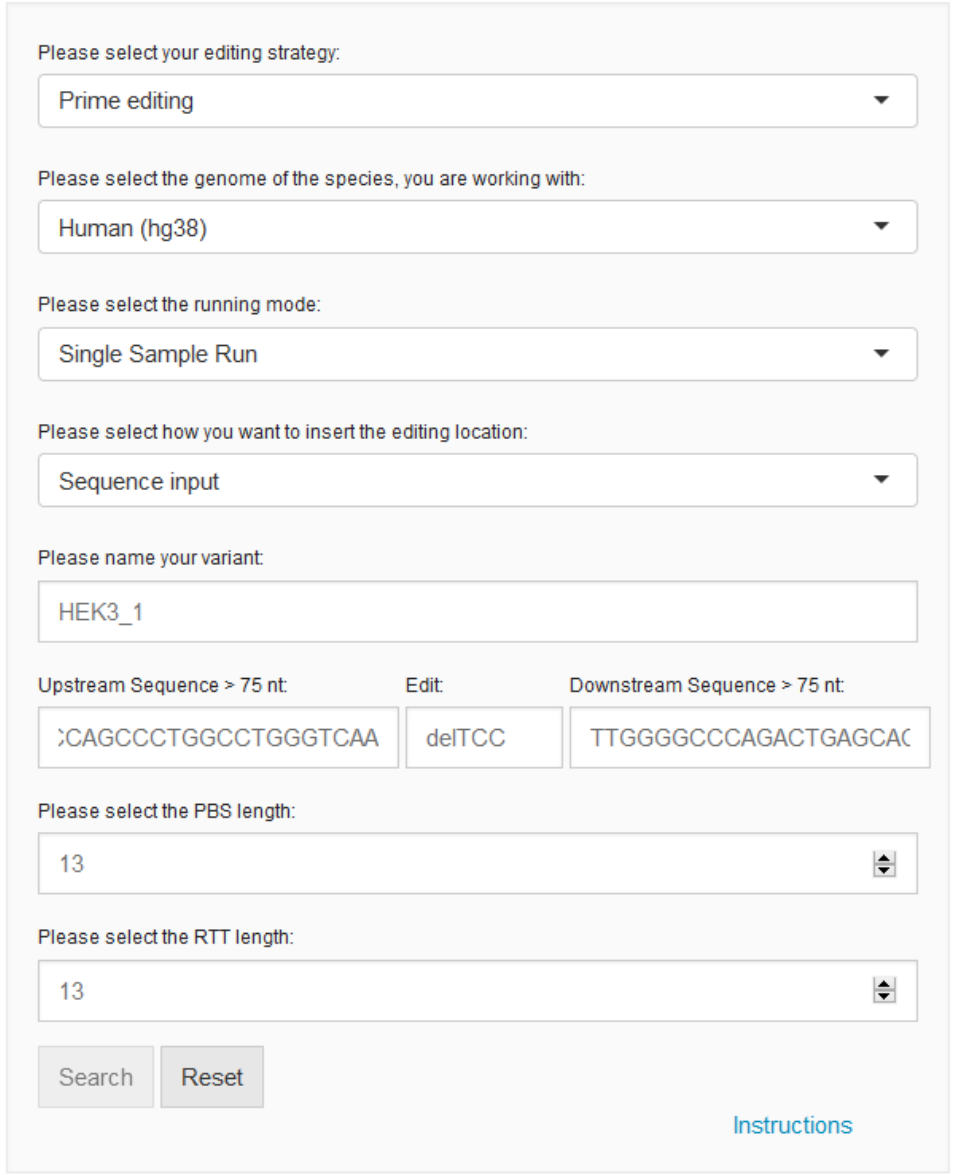
# Genomic coordinates




Define prime binding site (PBS) length

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

# Sequence Input



# 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…