

Supporting Information

Prime Editing Guide RNA Design Automation Using PINE-CONE

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PINE CONE: Creator Of New Edits

pegRNA Design: PBS RTT Edit Cas9-HP
5' 3' 3' 5'
3' 5' Guide PAM

1: Select Organism
Manual (.txt)
Text File (.txt)
ATCGA
GCTAC
AGCAT
ATCTT

2: Select Design
Nearest Protospacers to Edit
PE2 PE3 PE3B

3: Select Input Files
Select Edit File (.csv)
Edit File: Example Input.csv
Select DNA Sequence (.txt)
DNA Sequence: DNA_seq_file.txt

4: Output File
Output Name: Example output
Example output.csv is complete
Once file(s) are selected click 'Run PINE CONE'

Run PINE CONE: click when steps 1-4 are complete

Analyze: Appears for Human (hg38) and Yeast (S288C). Maps pegRNA designs

Organism Selection: select reference genome or plasmid/text file displays selection

Select protospacer design: define near or specific* guide designs, *requires reference genome sequence

Select Prime editing strategy: displays schematic of selection

Input (.csv): File defines edit location(s) and basic pegRNA parameters (RTT, PBS length)

DNA sequence (.txt): Appears for text file and plasmid selections

Enter user defined output name

Output status: Indicates when file write is complete

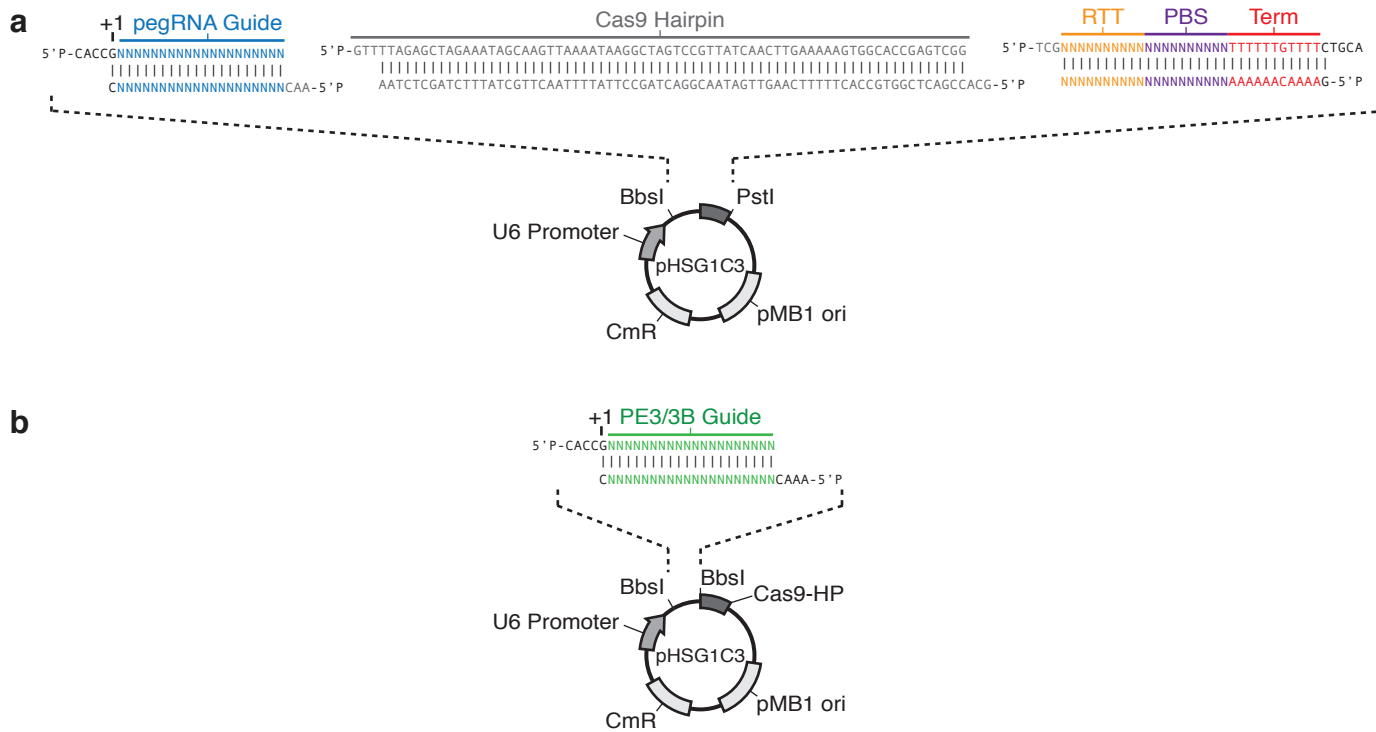
b

pegRNA Name:	Chromosome:	Position:	Edit:	RTT Length:	PBS Length:	Notes:
Names are arbitrary	Edit locus chromosome	Absolute nucleotide coordinate of edit locus	Sequence of intended edit	longer RTT's increase editing range and are necessary for insertions	Primer sequence length	Section is copied over to output for record keeping
Name	Chromosome	Position (Bp)	Edit (A,T,C,G)	RT (Bp)	PBS (Bp)	Notes:
HEK3-A	9	107422356	A	13	13	Example SNP-pegRNA
HEK3-Del	9	107422356	D5	14	13	Example Del-pegRNA
HEK3-Ins	9	107422356	iATGGAC	22	13	Example Ins-pegRNA
HEK3-A	9	107422356	A			Example Automatic RT, PBS

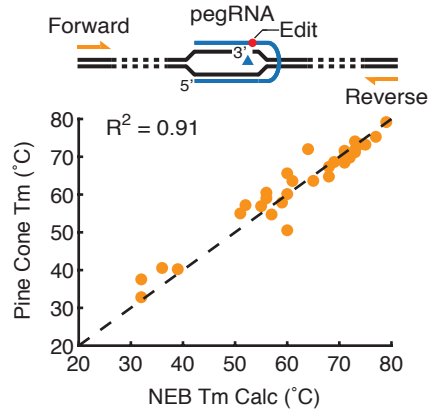
Automatic RT: Will scan for a viable RTT if left blank

Automatic PBS: Will estimate PBS by GC content if blank

Supplemental Figure 1: PINE-CONE User Interface (UI) and Input (.CSV) format. (a) PINE-CONE's UI includes of 4 primary steps. (1) Selection of organism determines if sequence is retrieved from a reference genome or is accessed locally via "Manual (.txt)" or "Plasmid (.txt)" selections. (2) Selection of protospacer preference and editing strategy determines the design format of pegRNAs and 'accessory' sgRNAs. (3) Input file selection or select locally an accompanying DNA sequence text file (.txt). (4) User defined arbitrary name of output file. This file is written in the same path as PINE-CONE. Following input of information, click "Run PINE-CONE". This will initiate API requests and design of pegRNAs. A green status is displayed once the output file is done writing. If pegRNAs are designed for Human (hg38) or Yeast (S288C) an 'analyze' will appear mapping the location of pegRNAs to their respective reference genomes. **(b)** Input file format (.CSV) includes an arbitrary pegRNA name, chromosome, position, edit sequence, RTT length and PBS length preference and user notes. The figure includes an example single base edit, deletion and insertion. If RT and/or PBS are left blank, PINE-CONE will estimate an optimal design.



Supplemental Figure 2: PINE-CONE oligonucleotide output for cloning of pegRNA and PE3/PE3B sgRNAs. (a) Cloning strategy for pegRNAs using pHSG1C3. pHSG1C3 (Addgene # 164423) enables cloning of pegRNAs via digestion with BbsI and PstI. 5' phosphorylated (5'P) Oligonucleotide duplexes encoding a guide (blue, +1 is the transcriptional start of the U6 promoter), Cas9 hairpin (gray) and RTT/PBS and U6 terminator (Orange, Purple Blue respectively) are ligated into BbsI and PstI digested pHSG1C3. (b) PE3 or PE3b guides (green) are cloned into BbsI digested pHSG1C3. Oligonucleotide formatting can also be found in Supplemental table 2.



Supplemental Figure 3: PINE-CONE design of PCR primers flanking the edit site. PINE-CONE designed primers tm correlate with a commercially available New England Bio Labs (NEB) Tm calculator (<https://tmcalculator.neb.com/>).

pegRNA-HEK3, Chr9, Position: 107422356, Edit: T-to-A



pegRNA-RNF2, Chr1, Position: 185087640, Edit: G-to-T



pegRNA-FANCF, Chr11, Position: 22625787, Edit: C-to-A



pegRNA-VEGFA, Chr6, Position: 43769426, Edit: T-to-A



pegRNA-EMX1, Chr2, Position: 72933874, Edit: G-to-T



pegRNA-CTNNB1(D6), Chr3, Position: 4122608, Edit: D6



pegRNA-CTNNB1(D3), Chr3, Position: 4122608, Edit: D3



pegRNA-ES1, Chr22, Position: 37623379, Edit: TC-to-GA



pegRNA-ES2 Chr2, Position: 32039449, Edit: G-to-C



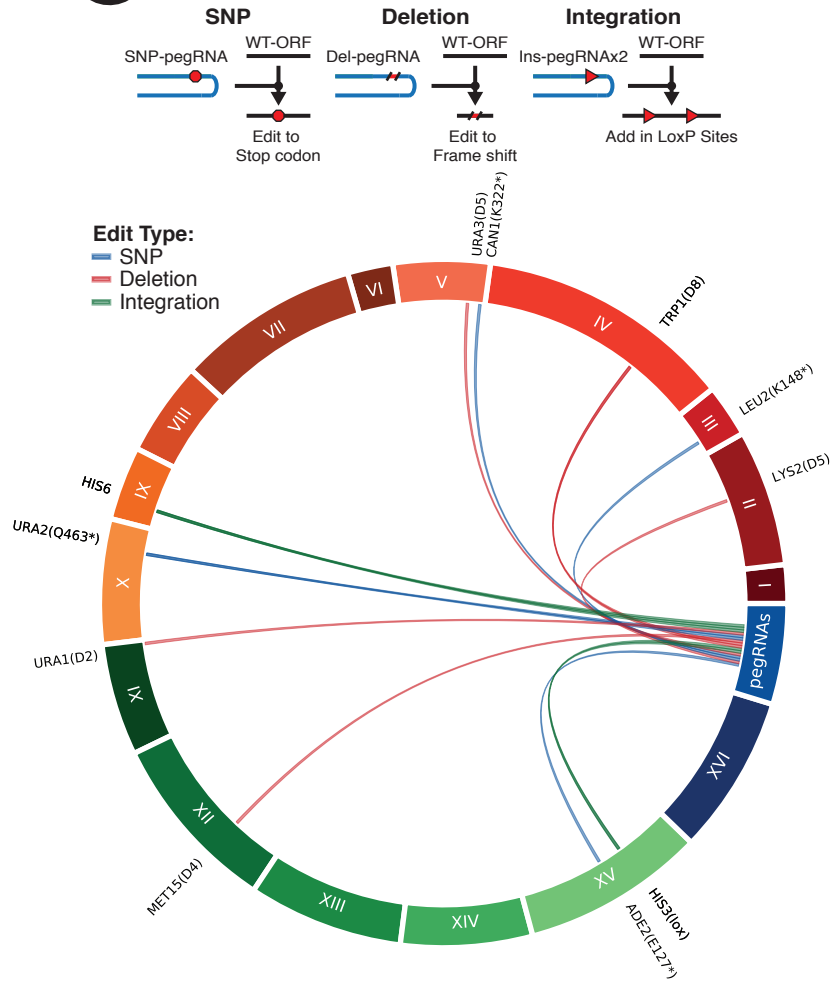
pegRNA-ES3, Chr19, Position: 44865305, Edit: G-to-C



pegRNA-ES4, Chr22, Position: 46308575, Edit: C-to-G



Supplemental Figure 4: pegRNAs designs by PINE-CONE match previously validated pegRNAs. A series of loci previously targeted and validated by *Anzalone et al.*, *Schene et al.* and *Kim et al.* (References 18,19,20) were provided as input to PINE-CONE. Alignments of in silico designed pegRNAs and designs with PINE-CONE designs (top) and published bottom. +1 is the 'G' transcriptional start from the U6 promoter added by PINE-CONE.



Supplemental Figure 5: PINE-CONE design of pegRNAs targeting various marker loci in yeast (S288C). (a) Strategies for targeting open reading frames (ORFs) on the yeast genome. Knock-out of various ORFs via point mutation introduction of stop codons, introduction of short deletions resulting in frame shift mutations and Insertion of LoxP sites flanking the ORF. (b) PINE-CONE Mapping of pegRNA targeting various marker loci with point mutations (blue chords), targeted deletion (Red chords), or integration of LoxP sites (green chords).

Supplemental Table 1: PINE-CONE organism selection

Organism:	Species:	Ref. Genome:	Host Link:
Human	<i>H.sapiens</i>	hg38	https://genome.ucsc.edu/index.html
Yeast	<i>S.cerevisiae</i>	SacCer3,(S288C)	https://genome.ucsc.edu/index.html
Mouse	<i>M.musculus</i>	mm10	https://genome.ucsc.edu/index.html
Zebrafish	<i>D.erio</i>	danRer11	https://genome.ucsc.edu/index.html
Roundworm	<i>C.elegans</i>	ce11	https://genome.ucsc.edu/index.html
Fruitfly	<i>D.melanogaster</i>	dm6	https://genome.ucsc.edu/index.html

PINE-CONE is capable of retrieving reference genome sequences for multiple species

Supplemental Table 2: PINE-CONE Cloning Oligo Format

Oligo:	Sequence: (5'→3')	Notes:
pegRNA Guide Top	CACCGNNNNNNNNNNNNNNNNNNNN	'N' Sequence varies between pegRNAs
pegRNA Guide Bottom	AACNNNNNNNNNNNNNNNNNNNNNC	'N' Sequence varies between pegRNAs
pegRNA Cas9 Hairpin Top	GTTTTAGAGCTAGAAATAGCAAGTTAAA ATAAGGCTAGTCCGTTATCAACTTGAAA AAGTGGCACCGAGTCGG	Constant for all pegRNA outputs
pegRNA Cas9 Hairpin Bottom	GCACCGACTCGGTGCCACTTTTTCAAGT TGATAACGGACTAGCCTTATTTAACTT GCTATTTCTAGCTCTAA	Constant for all pegRNA outputs
pegRNA RTT-PBS Top	TCGNNNNNNNNNNNNNNNNNNNTTTTT TGTTTTCTGCA	Sequence and length of 'N' varies for different pegRNAs
pegRNA RTT-PBS Bottom	GAAAACAAAAANNNNNNNNNNNNNNN NNNN	Sequence and length of 'N' varies for different pegRNAs
PE3/3B sgRNA guide Top	CACCGNNNNNNNNNNNNNNNNNNNN	'N' Sequence varies between PE3 and PE3B sgRNAs
PE3/3B sgRNA guide Bottom	AACNNNNNNNNNNNNNNNNNNNNNC	N' Sequence varies between PE3 and PE3B sgRNAs

General format of oligonucleotides for cloning pegRNAs and PE3 or PE3B sgRNA guides into pHSG1C3. N = A, T, C or G. Cas9 hairpin oligos are constant across pegRNA designs and thus are not included directly in the PINE-CONE output to avoid redundancy.