Prime Editing Off-the-Shelf (OTS) Products

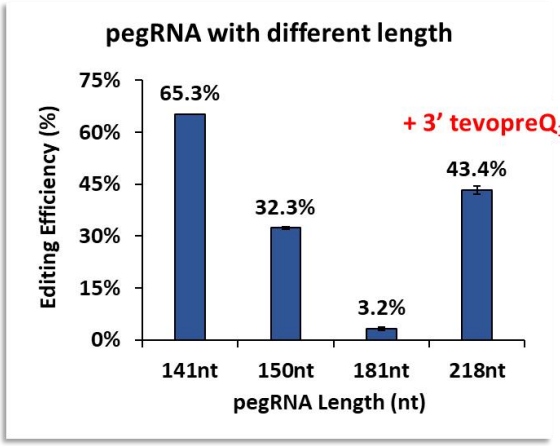
Prime editing (PE) systems minimally consist of two components: a programmable DNA nickase fused to an engineered reverse transcriptase and a pegRNA. GenScript Nucleic Acid Department provides synthetic pegRNA service; below are positive control pegRNA (KIOK16 & KIOK17) and other accessory off-the-shelf (OTS) products:

Off the Shelf (OTS) Product	Catalog No.	Purity	Quantity
PE2/PE3 mRNA	RP-A00044	/	0.2mg/ 1mg
Human HEK3 PegRNA_141nt	KIOK16	HPLC	2nmol
Human HEK3 ePegRNA (3' tevopreQ$_1$) 218nt	KIOK17	HPLC	2nmol
Human HEK3 nicking sgRNA	KIOK18	HPLC	2nmol
Human HEK3-F validation primer	KIOK19	Desalt	0.5nmol
Human HEK3-R validation primer	KIOK20	Desalt	0.5nmol

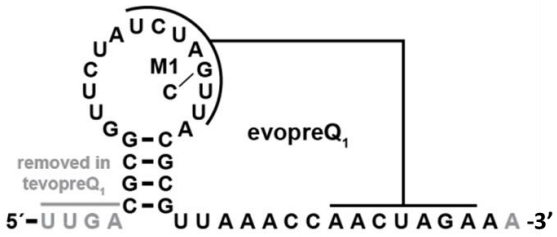
The PE2/PE3 mRNA and gRNA sequences were retrieved from Nelson, et al. Nat Biotechnology, 2022; 40: 402-410.

Gene Editing Data:

tevopreQ$_1$ motif on the 3' end increased editing efficiency



HEK293T cells (0.2 million cells) were transfected with 1ug PE2/PE3 mRNA, 30pmol nicking sgRNA and 90pmol pegRNA of different lengths (HPLC grade) targeting HEK3 gene, via electroporation method. Genome DNA were then extracted 3 days post-transfection for editing efficiency analysis (Sanger sequencing). Note that the 218nt pegRNA is an extended version of 181nt with a tevopreQ$_1$ motif (37nt) at the 3' end.



It has been reported that adding RNA structural motifs – **evopreQ$_1$** or mpknot to the 3' end of a pegRNA can protect the 3' end from exonuclease digestion (Nelson, J. W. et al., 2022). The 3' end is particularly important because it carries the RT template and PBS, degradation of it can result in truncated pegRNA-RNP which does not only fail in delivering the desired editing effect but also compete with the intact pegRNA-RNP for binding to target DNA and hence comprises the overall prime editing efficiency.

Diagram retrieved from *Nelson, J. W. et al. Engineered pegRNAs improve prime editing efficiency. Nature Biotechnology. 40, 402–410 (2022)*

Product Full Sequences:

PE2/PE3 mRNA

Kindly refer to GenScript Catalog RNA product – RP-A00044 or click here

Human HEK3 PegRNA_141nt

mG*mG*mC*rCrCrArGrArCrUrGrArGrCrArCrGrUrGrArGrUrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrArArArArArUrArArGrGrCrUrArGrUrCrGrUrUrArUrCrArArCrUrUrGrArArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCrUrUrGrGrArGrGrArArGrCrArGrGrGrCrUrUrCrCrUrUrUrCrCrUrCrUrGrCrCrGrUrGrCrUrCrArGrUrCrUrG*mU*mU*mU

Human HEK3 ePegRNA (3' tevopreQ$_1$)_218nt

mG*mG*mC*rCrCrArGrArCrUrGrArGrCrArCrGrUrGrArGrUrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrArArArArArUrArArGrGrCrUrArGrUrCrGrUrUrArUrCrArArCrUrUrGrArArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCrUrUrGrGrArGrGrArArGrCrArGrGrGrCrUrUrCrCrUrUrUrCrCrUrCrUrGrCrCrGrUrGrCrUrCrArGrUrCrUrGrUrArArUrCrCrGrUrGrCrUrUrGrUrCrUrCrUrGrCrCrArUrCrArCrUrUrArUrCrGrUrCrGrUrCrArUrCrCrUrUrGrUrArArUrCrUrArGrUrUrArCrGrCrGrUrUrArCrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCrUrUrGrGrArGrGrArArGrCrArGrGrGrCrUrUrCrCrUrUrUrCrCrUrCrUrGrCrCrGrUrGrCrUrCrArGrUrCrUrGrUrArArUrCrCrGrUrGrCrUrUrGrUrCrUrCrUrGrCrCrArUrCrArCrUrUrArUrCrGrUrCrGrUrCrArUrCrCrUrUrGrUrArArUrCrUrArGrUrUrArCrGrCrGrUrUrArCrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCrUrUrGrGrArGrGrArArGrCrArGrGrGrCrUrUrCrCrUrUrUrCrCrUrCrUrGrCrCrGrUrGrCrUrCrArGrUrCrUrG*mU*mU*mU

Human HEK3 nicking sgRNA

mG*mU*mC*rArArCrCrArGrUrArUrCrCrCrGrGrUrGrCrGrUrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrArArArArArUrArArGrGrCrUrArGrUrCrGrUrUrArUrCrArArCrUrUrGrArArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCrUrUrGrGrArGrGrArArGrCrArGrGrGrCrUrUrCrCrUrUrUrCrCrUrCrUrGrCrCrGrUrGrCrUrCrArGrUrCrUrG*mU*mU*mU

Human HEK3-F validation primer

TCTCTGACCACTGCGATATG

Human HEK3-R validation primer

TTGTAGCTACGCCTGTGATG

“m” denotes 2’O-methyl RNA, “*” denotes Phosphorothioate linkage