

***H11-hKRT14-iCre-*ployA** Cas9-KI Strategy**

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Reviewer:

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Design Date:

2019-8-18

Project Overview

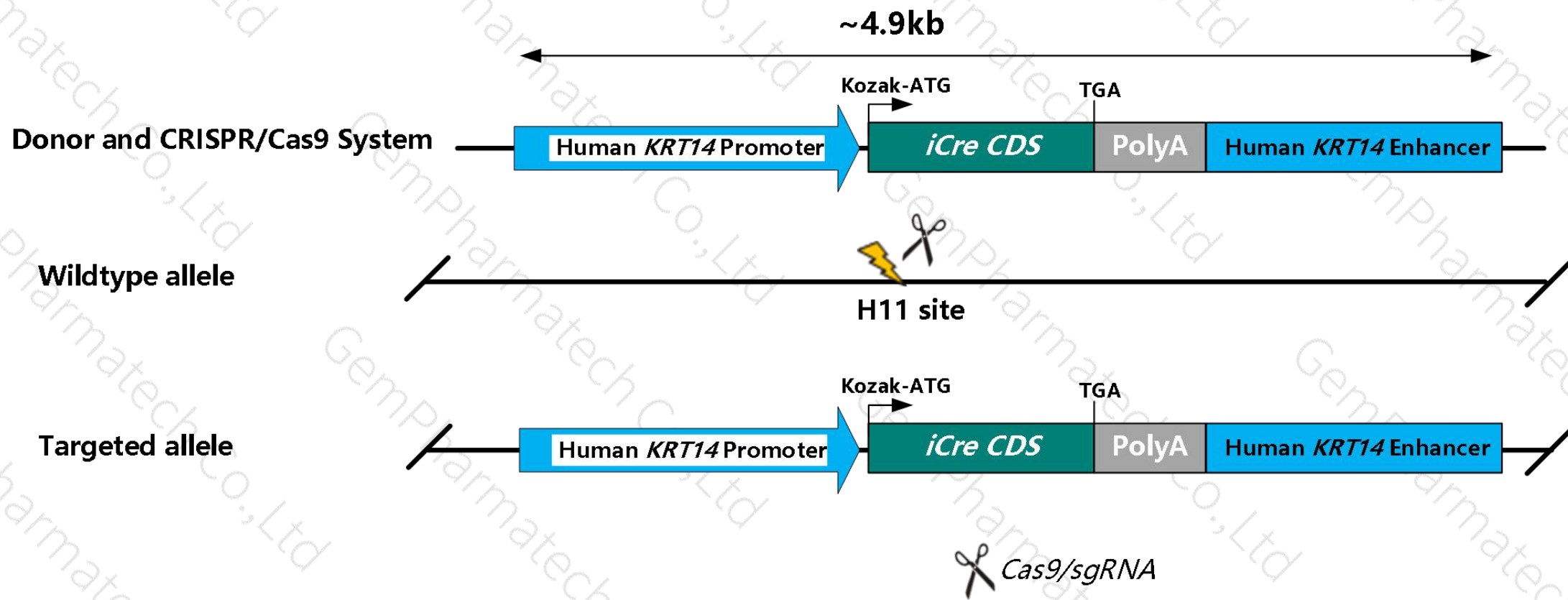
Project Name	<i>H11-hKRT14-iCre-ployA</i>
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Project type	Cas9-KI (H11)
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Strain background	C57BL/6J
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Knockin strategy

The *hKRT14-iCre-loyA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



- H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.
- In this study, the *hKRT14-iCre-ptyoA* gene fragment was inserted into H11 site of mice by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and sgRNA were constructed in vitro, Cas9, donor and sgRNA were microinjected into the fertilized eggs of C57BL/6J mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6J mice by PCR, sequencing and southern blot, then the stable inheritance of F1 positive mice model was obtained.

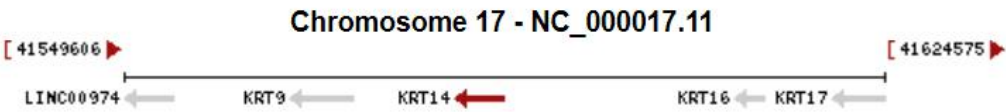
- H11 is located on Chr11. Please take the loci in consideration when breeding the Knock-in mice with other gene modified (e.g., iCre) strains, if the other gene is also on Chr11, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

KRT14 keratin 14 [*Homo sapiens* (human)]

Gene ID: 3861, updated on 4-Aug-2019

Summary

Official Symbol	KRT14 provided by HGNC
Official Full Name	keratin 14 provided by HGNC
Primary source	HGNC:HGNC:6416
See related	Ensembl:ENSG00000186847 MIM:148066
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Homo sapiens
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as	K14; NFJ; CK14; EBS3; EBS4
Summary	This gene encodes a member of the keratin family, the most diverse group of intermediate filaments. This gene product, a type I keratin, is usually found as a heterotetramer with two keratin 5 molecules, a type II keratin. Together they form the cytoskeleton of epithelial cells. Mutations in the genes for these keratins are associated with epidermolysis bullosa simplex. At least one pseudogene has been identified at 17p12-p11. [provided by RefSeq, Jul 2008]
Expression	Restricted expression toward skin (RPKM 3550.7) See more
Orthologs	mouse all

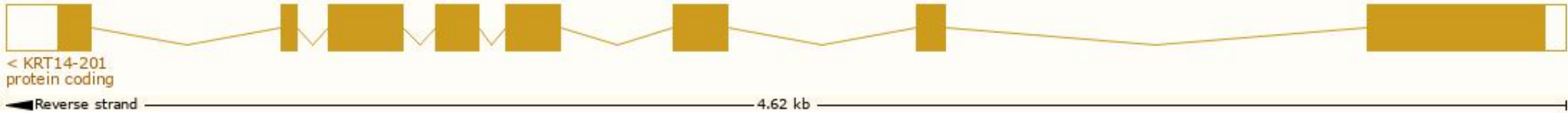


Transcript information (Ensembl)

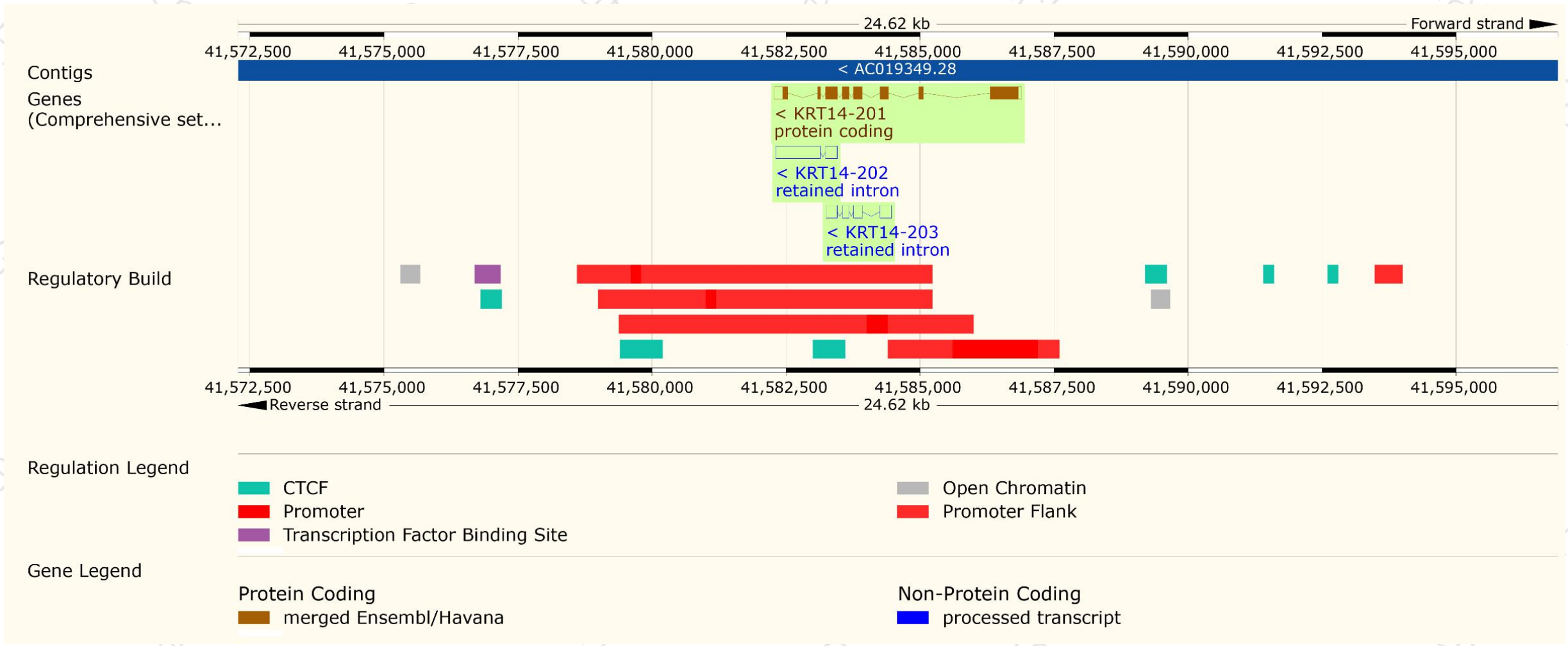
The gene has 3 transcripts, and all transcripts are shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Translation ID ▲	Biotype ▲	CCDS ▲	UniProt ▲	RefSeq Match ▲	Flags ▲
KRT14-201	ENST00000167586.7	1636	472aa	ENSP00000167586.6	Protein coding	CCDS11400	P02533	NM_000526.5	TSL:1 GENCODE basic APPRIS P1 MANE Select v0.5
KRT14-202	ENST00000441550.2	1053	No protein	-	Retained intron	-	-	-	TSL:2
KRT14-203	ENST00000476662.1	710	No protein	-	Retained intron	-	-	-	TSL:2

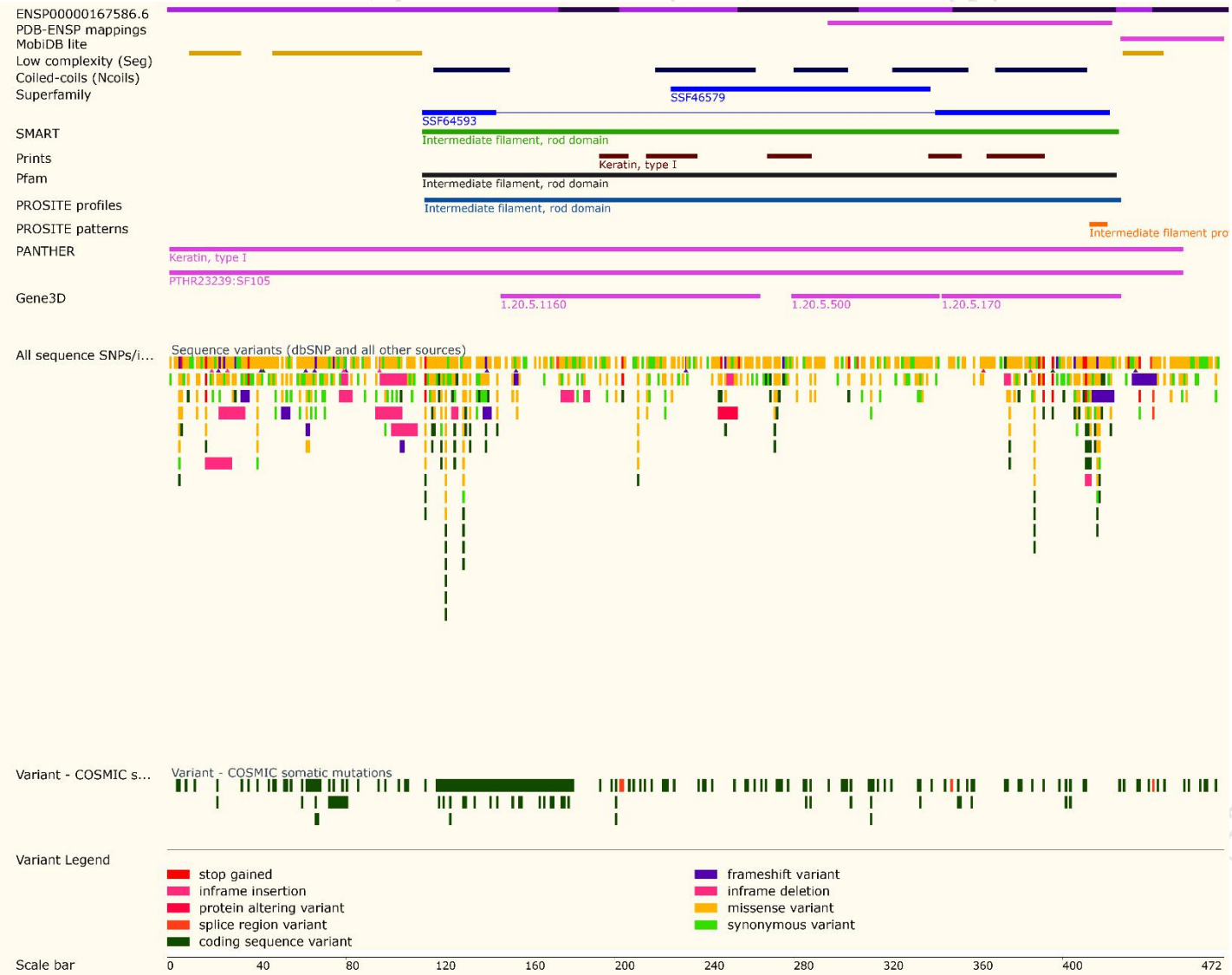
The strategy is based on the design of *hKRT14-201* transcript, The transcription is shown below



Genomic location distribution



Protein domain



iCre Sequence^[1] (1056bp)

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGG
ATGCCACCTCTGATGAAGTCAGGAAGAACCTGATGGACATGTTCAGGGACAGGCAGGCCTTCTCTGAACACAC
CTGGAAGATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAACAACAGGAAATGGTTCCCTG
CTGAACCTGAGGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACA
GCACCTGGGCCAGCTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCC
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GCACTGACTTTGACCAAGTCAGATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAGGAACCTGGCCTTC
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TCTGGCCACTCTGCCAGAGTGGGTGCTGCCAGGGACATGGCCAGGGCTGGTGTGTCCATCCCTGAAATCATGCA
GGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCAGAAACCTGGACTCTGAGACTGGGGCCATGG
TGAGGCTGCTCGAGGATGGGGACTGA

Promoter Sequence of Human *KRT14* (2500bp) ^[2]



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GTCAGGACTGACAACCCTTTAACCCCTGATGCCCCAGAAAATGCCCTCCCTTAAAATGTCTGAGTACCATGCTTCTTTGGCATCAGGGAGGGGAGGGGAGTGTGGTGTCTCTAAG
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GGCAAGTTGATGACCAGCTCCCAGCCTCAGCTGCCATTTCTGTAAAAGGGGCATAATGATAGTATCTACCTCA
TGGGCTTGTTGGGAG

- [1] Madison BB, Dunbar L, Qiao XT, Braunstein K, Braunstein E, Gumucio DL. *cis* Elements of the Villin Gene Control Expression in Restricted Domains of the Vertical (Crypt) and Horizontal (Duodenum, Cecum) Axes of the Intestine. J Biol Chem. 2002 Sep 6;277(36):33275-83. Epub 2002 Jun 13.
- [2] Robert Vassar, et.al.(1989). Tissue-specific and differentiation-specific expression of a human K14 keratin gene in transgenic mice. PNAS. 86:1563-1567.

If you have any questions, you are welcome to inquire.
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