Gene - IL18 (Interleukin 18) also known as - **IGIF;IL-1g; IL1F4**

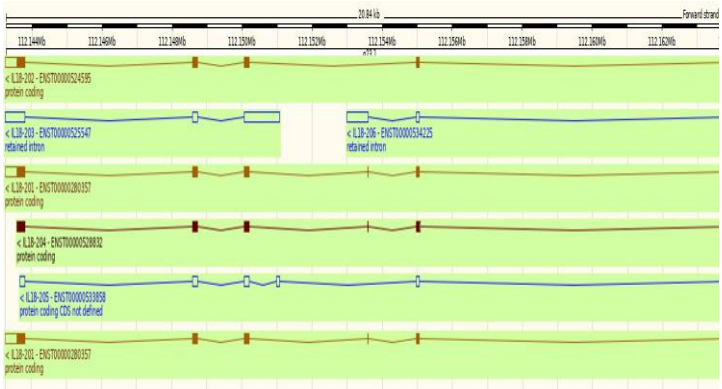
1. Summary

The protein encoded by this gene is proinflammatory cytokine of the IL-1 family that is constitutively found as a precursor within the cytoplasm of a variety of cells including macrophages and keratinocytes with 193 amino acids. Inactivity of Il18 precursor is processed to its active form by caspase-1 and is capable of stimulating interferon gamma production and regulating both TH1 and TH2 responses. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. This gene has been reviewed for its involvement in Coronavirus biology. We wish to clone in to pPiCZAC vector for expressing a recombinant hIl-18 protein using a yeast expression system.

References

1. (Kato, Z., Jee, J., Shikano, H., Mishima, M., Ohki, I., Ohnishi, H., Li, A., Hashimoto, K., Matsukuma, E., Omoya, K., Yamamoto, Y., Yoneda, T., Hara, T., Kondo, N. and Shirakawa, M. (2003) The structure and binding mode of interleukin-18. Nat Struct Biol,)
2. (Wei, H., Wang, D., Qian, Y., Liu, X., Fan, S., Yin, H. S. and Wang, X. (2014) Structural basis for the specific recognition of IL-18 by its alpha receptor. FEBS Lett, 588, 3838-43.)
3. (Le, H., Spearman, P., Waggoner, S. N. and Singh, K. (2022) Ebola virus protein VP40 stimulates IL-12- and IL-18-dependent activation of human natural killer cells. JCI Insight, 7.)

2. Gene structure, from ensemble:



3. From ensemble, 5' and 3' untranslated regions in yellow highlights

1
CCTTTGCTCCCCTGGCGACTGCCTGGACAGTCAGCAAGGAATTGT
CTCCCAGTGCATTTT 60
.....
.....

61
GCCCTCCTGGCTGCCAACTCTGGCTGCTAAAGCGGCTGCCACCTG
CTGCAGTCTACACAG 120
.....
.....

121
CTTCGGGAAGAGGAAAGGAACCTCAGACCTTCCAGATCGCTTCCT
CTCGCAACAAACTAT 180
.....
.....

181
TTGTCGCAGGAATAAAGATGGCTGCTGAACCAGTAGAAGACAAT
TGCATCAACTTTGTGG 240
.....ATGGCTGCTGAACCAGTAGAAGACAATTGCATCAACT
TTGTGG 43
.....-M--A--A--E--P--V--E--D--N--C--I--N--F--V-- 14

241
CAATGAAATTTATTGACAATACGCTTTACTTTATAGCTGAAGATG
ATGAAAACCTGGAAT 300
44
CAATGAAATTTATTGACAATACGCTTTACTTTATAGCTGAAGATG
ATGAAAACCTGGAAT 103
15 A--M--K--F--I--D--N--T--L--Y--F--I--A--E--D--D--E--N--L--E--
34

301
CAGATTACTTTGGCAAGCTTGAATCTAAATTATCAGTCATAAGAA
ATTTGAATGACCAAG 360
104
CAGATTACTTTGGCAAGCTTGAATCTAAATTATCAGTCATAAGAA
ATTTGAATGACCAAG 163
35 S--D--Y--F--G--K--L--E--S--K--L--S--V--I--R--N--L--N--D--Q--
54

361
TTCTCTTCATTGACCAAGGAAATCGGCCTCTATTTGAAGATATGAC
TGATTCTGACTGTA 420
164
TTCTCTTCATTGACCAAGGAAATCGGCCTCTATTTGAAGATATGAC
TGATTCTGACTGTA 223
55 V--L--F--I--D--Q--G--N--R--P--L--F--E--D--M--T--D--S--D--C--
74

421
GAGATAATGCACCCCGGACCATATTTATTATAAGTATGTATAAAG
ATAGCCAGCCTAGAG 480
224
GAGATAATGCACCCCGGACCATATTTATTATAAGTATGTATAAAG
ATAGCCAGCCTAGAG 283
75 R--D--N--A--P--R--T--I--F--I--I--S--M--Y--K--D--S--Q--P--R--
94

481
GTATGGCTGTAACTATCTCTGTGAAGTGTGAGAAAATTTCAACTC
TCTCCTGTGAGAACA 540
284
GTATGGCTGTAACTATCTCTGTGAAGTGTGAGAAAATTTCAACTC
TCTCCTGTGAGAACA 343
95 G--M--A--V--T--I--S--V--K--C--E--K--I--S--T--L--S--C--E--N--
114

541
AAATTATTTCCTTTAAGGAAATGAATCCTCCTGATAACATCAAGG
ATACAAAAAGTGACA 600
344
AAATTATTTCCTTTAAGGAAATGAATCCTCCTGATAACATCAAGG
ATACAAAAAGTGACA 403
115 K--I--I--S--F--K--E--M--N--P--P--D--N--I--K--D--T--K--S--D--
134

601
TCATATTCTTTCAGAGAAGTGTCCCAGGACATGATAATAAGATGC
AATTTGAATCTTCAT 660
404
TCATATTCTTTCAGAGAAGTGTCCCAGGACATGATAATAAGATGC
AATTTGAATCTTCAT 463
135 I--I--F--F--Q--R--S--V--P--G--H--D--N--K--M--Q--F--E--S--S--
154

661
CATACGAAGGATACTTTCTAGCTTGTGAAAAAGAGAGAGACCTTT
TTAAACTCATTTTGA 720
464
CATACGAAGGATACTTTCTAGCTTGTGAAAAAGAGAGAGACCTTT
TTAAACTCATTTTGA 523
155 S--Y--E--G--Y--F--L--A--C--E--K--E--R--D--L--F--K--L--I--L--
174

721
AAAAAGAGGATGAATTGGGGGATAGATCTATAATGTTCACTGTT
CAAAACGAAGACTAGC 780
524
AAAAAGAGGATGAATTGGGGGATAGATCTATAATGTTCACTGTT
CAAAACGAAGACTAG. 582
175
K--K--E--D--E--L--G--D--R--S--I--M--F--T--V--Q--N--E--D--*-.193

781
TATTAAAATTTCATGCCGGGCGCAGTGGCTCACGCCTGTAATCCC
AGCCCTTTGGGAGGC 840
.....
.....

841
TGAGGCGGGCAGATCACCAGAGGTCAGGTGTTCAAGACCAGCCT
GACCAACATGGTGAAA 900
.....
.....

901
CCTCATCTCTACTAAAAATACAAAAAATTAGCTGAGTGTAGTGAC
GCATGCCCTCAATCC 960
.....
.....

961
CAGCTACTCAAGAGGCTGAGGCAGGAGAATCACTTGCACTCCGG
AGGTAGAGGTTGTGGT 1020
.....

.....

1021
GAGCCGAGATTGCACCATTGCGCTCTAGCCTGGGCAACAACAGC
AAAACTCCATCTCAAA 1080
.....
.....

1081 AAATAAAATAAATAAATAAACAAATAAAAAATTCA
1115
.....
.....

Forward Primer: ATGGCTGCTGAACCAGTAGA Tm ~ 61
Reverse Primer: CTGTTCAAAACGAAGACTAG Tm ~57

4. PCR Protocol

The PCR provides a means of amplifying DNA sequences and can be used to generate microgram quantities of DNA and can amplify the DNA from a single cell into amounts sufficient for cloning or sequencing.

Amount	Component	Final Concentration
µL	Water	31.5 µL
µL	10x PCR Buffer (P2317)	1x
1 µL	Deoxynucleotide Mix	200 µM
µL	Forward primer	0.5 µM
µL	Reverse primer	0.5 µM
0.5 µL	*Taq* DNA Polymerase (D1806)	0.05 units/µL
µL	Template DNA (typically 10 ng)	200 pg/µL
µL	25 mM $MgCl_2$	0.5 mM
	Total reaction volume	**50 µL**
Pcr Step	**Temperature °C**	**Duration**
Initial Denaturation	94 °C	4 min
30 cycles	94°C	1 min
Anneal Primer	57°C	30 sec
Final Extention	2°C	5 min

5. The final step is to check the DNA sequence which involves synthesizing DNA sub-fragments of all possible lengths and separating them on a Agarose Gel Electrophoresis and subsequent Ethidium Bromide Staining and now we can read the sequence directly

- Agarose (precast gels, powder, etc.)
- Buffer such as MOPS-EDTA-sodium acetate, tris-acetate-EDTA (TAE) or tris-borate-EDTA (TBE)
- Gel loading solution and sample loading buffer for RNA
- Electrophoresis stain or dye such as ethidium bromide.

We can now separate those fragments into four groups depending on the last base by using Sanger Sequencing Method, this method allow us to generate the fragments of the segment of DNA to be sequenced by using four different dideoxy analogs one for each four bases, allows the generation of four sets fragments in four separate reactions and now the reaction can be read off, starting at the bottom of gel and reading upwards.