

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

nice!

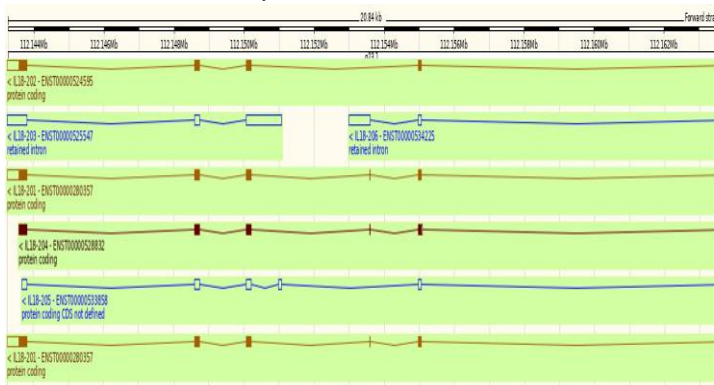
1. Summary

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

- (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,)
- (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.)
- (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
CTCCAGTGCATTTT 60

61
GCCCTCCTGGCTGCCAACTCTGGCTGCTAAAGCGGCTGCCACCTG
CTGCAGTCTACACAG 120

121
CTTCGGGAAGAGGAAAGGAACCTCAGACCTTCCAGATCGCTTCCT
CTCGCAACAAACTAT 180

181
TTGTCGCAGGAATAAAGATGGCTGCTGAACCACTAGAAGACAAT
TGCATCAACTTTGTGG 240
.....ATGGCTGCTGAACCACTAGAAGACAATTGCATCAACT
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
ATTGAATGACCAAG 360
104
CAGATTACTTTGGCAAGCTTGAATCTAAATTATCAGTCATAAGAA
ATTGAATGACCAAG 163
35 S--D--Y--F--G--K--L--E--S--K--L--S--V--I--R--N--L--N--D--Q--
54

361
TTCTTTCATTGACCAAGGAAATCGGCCTCTATTTGAAGATATGAC
TGATTCTGACTGTA 420
164
TTCTTTCATTGACCAAGGAAATCGGCCTCTATTTGAAGATATGAC
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
GTATGGCTGTAACATATCTCTGTGAAGTGTGAGAAAATTTCAACTC
TCTCCTGTGAGAACA 540
284
GTATGGCTGTAACATATCTCTGTGAAGTGTGAGAAAATTTCAACTC
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
AAATTATTTCTTTAAGGAAATGAATCCTCTGATAACATCAAGG
ATACAAAAAGTGACA 600

344
AAATTATTTCTTTAAGGAAATGAATCCTCTGATAACATCAAGG
ATACAAAAAGTGACA 403

115 K--I--I--S--F--K--E--M--N--P--D--N--I--K--D--T--K--S--D--
134

601
TCATATTCTTTCAGAGAAGTGTCCAGGACATGATAATAAGATGC
AATTGAATCTTCAT 660

404
TCATATTCTTTCAGAGAAGTGTCCAGGACATGATAATAAGATGC
AATTGAATCTTCAT 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
TTAAACTCATTTGA 720

464
CATACGAAGGATACTTTCTAGCTTGTGAAAAAGAGAGAGACCTTT
TTAAACTCATTTGA 523

155 S--Y--E--G--Y--F--L--A--C--E--K--E--R--D--L--F--K--L--I--L--
174

721
AAAAAGAGGATGAATTGGGGGATAGATCTATAATGTTCACTGT
CAAAACGAAGACTAGC 780

524
AAAAAGAGGATGAATTGGGGGATAGATCTATAATGTTCACTGTT
CAAAACGAAGACTAGC 582

175
K--K--E--D--E--L--G--D--R--S--I--M--F--T--V--Q--N--E--D--*--.193

781
TATTTAAATTTTCATGCCGGGCGCAGTGGCTCACGCCTGTAATCCC
AGCCCTTTGGGAGGC 840

841
TGAGGCGGGCAGATCACCAGAGGTCAGGTGTTCAAGACCAGCCT
GACCAACATGGTGAAA 900

901
CCTCATCTCTACTAAAAATACAAAAAATTAGCTGAGTGTAGTGAC
GCATGCCCTCAATCC 960

961
CAGCTACTCAAGAGGCTGAGGCAGGAGAATCACTTGCACCTCCGG
AGGTAGAGGTTGTGGT 1020

1021
GAGCCGAGATTGCACCATTCGCTCTAGCCTGGGCAACAACAGC
AAAACTCCATCTCAA 1080

1081 AAATAAAATAAATAAATAAAACAAATAAAAAA
1115

You have to 'reverse-complement' the reverse primer

Forward Primer: ATGGCTGCTGAACCAGTAGA Tm ~ 57
Reverse Primer: CTGTTCAAACGAAGACTAG 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)	
μL	Template DNA (typically 10 ng)	
μL	25 mM MgCl ₂	
Total reaction volume		
Pcr Step	Temperature °C	
Initial Denaturation	94 °C	
30 cycles	94 °C	1 min
Anneal Primer	57 °C	30 sec
Final Extension	72 °C	5 min

annealing temp usually lower than Tm

- 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.