

Strategies for the production of soluble interferon-alpha consensus and potential application in Arboviruses and SARS-CoV-2

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The image displays a nucleotide sequence of the HF-IFN construct. The sequence is presented in a grid where each row represents a nucleotide position. The sequence is color-coded to highlight specific regions: grey for the His-tag, blue for the Fh8 tag, orange for the TEV hydrolysis site, red for the cIFN sequence, and green for the STOP codon. A underlined sequence at the bottom refers to the Bam HI restriction site.

CATCATCACCAACCACCATATGCCGAGCGTTCAAGAAGTTGAAA
AACTGCTGCATGTTCTGGATCGTAATGGTATGGTAAAGTTAG
CGCAGAAGAACTGAAAGCATTGCCGATGATAAGCAAATGTCC
GCTGGATAGCAATAAAATCAAGGCCTTATCAAAGAGCACGA
TAAAAAACAAAGATGGCAAGCTGGATCTGAAAGAAACTGGTTAG
CATTCTGAGCAGCGAAACCTGTATTTCAAGGATCCTGTGAT
CTGCCGCAGACACATAGCCTGGTAATCGTCGTGCACTGATTCT
TGCTGGCACAGATGCGTCGTATTAGCCCCTTAGCTGTCTGAA
AGATCGTCATGATTGGTTTCCGCAAGAGGAAATTGATGGC
AACCAAGTTTCAAGAAAGCACAGGCAATTAGCGTTCTGCATGAA
ATGATTCAAGCAGACCTTAACCTGTTAGCACCAAGATAGCA
GCGCAGCATGGGATGAAAGCCTGCTGGAAAAATTCTATACCG
AACTGTATCAGCAGCTGAATGATCTGGAAGCATGTTATTCA
AGAGGTTGGTGTGAAGAAACACCGCTGATGAATGTTGATA
TATTCTGGCCGTGAAAAAAATACCTTCAGCGCATTACCTGTAT
CTGACCGAAAAAAAGTATAGCCCCTGTGCATGGGAAGTTGTT
CGTGCAGAAATTATGCGTAGCTTACGATTAGCACCAATCTGC
AAGAACGTCGTCGCAAGAATAATAA

Figure S1. a: Nucleotide sequence of the HF-IFN construct and site for TEV hydrolysis in the pET28a vector. Grey: His-tag; Blue: Fh8 tag; Orange: TEV hydrolysis site; Red: cIFN sequence and Green: STOP codon. The underlined sequence refers to the Bam HI restriction site.

CATCATCACCAACCACCATGATGACGCCGGCAATTCAACAAACGT
TAGCCAAAATGGGCATCAAAAGCAGCGATATTCAAGCCCGCGC
CTGTAGCTGGCATGAAGACAGTTCTGACTAACAGCAGCGTGT
TGTACATCACCGATGATGGTAACATATCATTCAAGGGGCCAAT
GTATGACGTTAGTGGCACGGCTCCGGTCAATGTCACCAATAA
GATGCTGTTAAAGCAGTTGAATGCCTGAAAAAGAGATGAT
CGTTATAAAGCGCCGCAGGAAAAACACGTCATCACCGTGT
ACTGATATTACCTGTGGTTACTGCCACAAACTGCATGAGCAA
TGGCAGACTACAACCGCCTGGGATCACCGTGCCTATCTTGC
TTTCCC CGCCAGGGCTGGACAGCGATGCAGAGAAAGAAAT
GAAAGCTATCTGGTGTGCGAAAGATAAAAACAAAGCGTTGA
TGATGTGATGGCAGGTAAGCGTCGCACCAGCCAGTTGCGA
CGTGGATATTGCCGACCATTACGCACTTGGCGTCCAGCTTGC
GTTAGCGGTACTCCGGCAGTTGTGCTGAGCAATGGCACACTT
GTTCCGGGTTACCAGCCGCCAAAGAGAGATGAAAGAATTCTC
GACGAACACCAAAAAATGACCAGCGGTAAAAGAAACCTGTAT
<u>TTTCAGGGATCC</u> TGTGATCTGCCGCAGACACATAGCCTGGTA
ATCGTCGTGCACTGATTCTGCTGGCACAGATGCGTCGTATTAG
CCC GTT TAGCTGTCTGAAAGATCGTCATGATTGGTTTTCCG
CAAGAGGAATTGATGGCAACCAGTTCAAGAAAGCACAGGCA
ATTAGCGTTCTGCATGAAATGATTCAAGCAGACCTTAACCTGT
TCAGCACCAAAAGATAGCAGCGCAGCATGGGATGAAAGCCTGC
TGGAAAAATTCTATACCGAACGTGATCAGCAGCTGAATGATCT
GGAAGCATGTGTTATTCAAGAGGTTGGTGTGAAGAAACACC
GCTGATGAATGTTGATAGTATTCTGCCGTGAAAAAAATACTTT
CAGCGCATTACCCCTGTATCTGACCGAAAAAAAGTATAGCCCCGT
GTGCATGGGAAGTTGTTCGTGAGAAATTATGCGTAGCTTA
GCATTAGCACCAATCTGCAAGAACGTCTCGTCGCAAAAGAAT
AATAA

Figure S1b: Nucleotide sequence of the HD-IFN construct and site for TEV hydrolysis in pET28a vector. Grey: His-tag; Blue: DsbC tag; Orange: TEV hydrolysis site; Red: cIFN sequence and Green: STOP codon. The underlined sequence refers to the Bam *HI* restriction site.

CATCATCACCAACCACCAT <u>TGTGATCTGCCGCAGACACATAGCC</u>
TGGGTAA <u>TCGTGCACTGATTCTGCTGGCACAGATGCGTCG</u>
TATTAGCCC <u>GTTCAGCTGTCTGAAAGATCGTCATGATTGGT</u>
TTTCCGCAAGAGGAATTGATGGCAACCAGTTCAAGAAAGCA
CAGGCAATTAGCGTTCTGCATGAAATGATTCAAGCAGACCTTA
ACCTGTT <u>CAGCACCAAAAGATAGCAGCGCAGCATGGGATGAAA</u>
GCCTGCTGGAAAAATTCTATACCGAACGTGATCAGCAGCTGAA
TGATCTGGAAAGCATGTGTTATTCAAGAGGTTGGTGTGAAGA
AACACCGCTGATGAATGTTGATAGTATTCTGCCGTGAAAAAA
ATACTT <u>CAGCGCATTACCCCTGTATCTGACCGAAAAAAAGTAT</u>
AGCCCGTGTGCATGGGAAGTTGTTCGTGAGAAATTATGCGT

A	G	C	T	T	T	A	G	C	A	T	A	G	C	A	A	G	A	A	C	G	T	C	G	T	C	G	C	A
A	A	G	A	A	T	A	A	T	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Figure S1c: Nucleotide sequence of the H-IFN construct in pET28a vector. Grey: His-tag; Red: IFN sequence and Green: STOP codon.

Table S1: Sequence of nucleotides used in primer design. F, "forward"; R "reverse"; AT, annealing temperature.

Gene	Oligonucleotides	AT
<i>h-ifn</i>	F: 5'CGACCATGGTCACCACTCATCATCACTGTGATCTGCCAGACAC-3' R: 5'CAGTCTGAGTCACTATTCTTGCGACGCAGACGTT-3'	52 °C
<i>hd-ifn</i>	F: 5'AGGGATCCCTGAAAATACAGGTTTCTTACCGC -3' R: 5'CGCCCATGGCCATCATCACCCACC-3'	55 °C

Table S2. a: Composition of LB-agar medium.

Nutrients	Concentration (g/L)
Tryptone	10
Yeast Extract	5
NaCl	10
Agar	20

Table S2b: Composition of the complex medium of autoinduction. *Lactose was omitted in the groups that represent negative control.

Nutrients	Concentration (g/L)
Glycerol	5
Glucose	0,5
Lactose*	5,0
Yeast Extract	5,0
Phytone	10
KH ₂ PO ₄	3,4
Na ₂ HPO ₄	9
NH ₄ Cl	2,7
Na ₂ SO ₄	0,7
MgSO ₄ .7H ₂ O	0,5
Ferric Citrate	100,8
CoCl ₂ .6H ₂ O	2,5
MnCl ₂ .4H ₂ O	15
CuCl ₂ .2H ₂ O	1,5
H ₃ BO ₃	3
Na ₂ MoO ₄ .2H ₂ O	2,1
Zn(CH ₃ COOH) ₂ .H ₂ O	33,8
EDTA	14,1
Thiamine	45
Kanamycin	30

Table S2c: Composition of the chemically defined medium SDAB. *Lactose was omitted in the groups that represent negative control.

Nutrients	Concentration (g/L)
Glucose	2,94
Glycerol	11,07
Lactose*	7,6
KH ₂ PO ₄	13,3
Citric Acid	1,55
(NH ₄) ₂ HPO ₄	4
MgSO ₄ .7H ₂ O	1,2
Ferric Citrate	100,8
CoCl ₂ .6H ₂ O	2,5
MnCl ₂ .4H ₂ O	15
CuCl ₂ .2H ₂ O	1,5
H ₃ BO ₃	3
Na ₂ MoO ₄ .2H ₂ O	2,1
Zn(CH ₃ COOH) ₂ .H ₂ O	33,8
EDTA	14,1
Thiamine	45
Kanamycin	30

Table S2d: Composition of chemically defined medium HDF.

Nutrients	Concentration (g/L)
Glucose	-
Glycerol	10
KH ₂ PO ₄	13,3
Citric Acid	1,7
(NH ₄) ₂ HPO ₄	4
MgSO ₄ .H ₂ O	1,2
Ferric Citrate	100,8
CoCl ₂ .6H ₂ O	2,5
MnCl ₂ .4H ₂ O	15
CuCl ₂ .2H ₂ O	1,5
H ₃ BO ₃	3
Na ₂ MoO ₄ .2H ₂ O	2,1
Zn(CH ₃ COOH) ₂ .H ₂ O	33,8
EDTA	14,1
Thiamine	45
Kanamycin	30

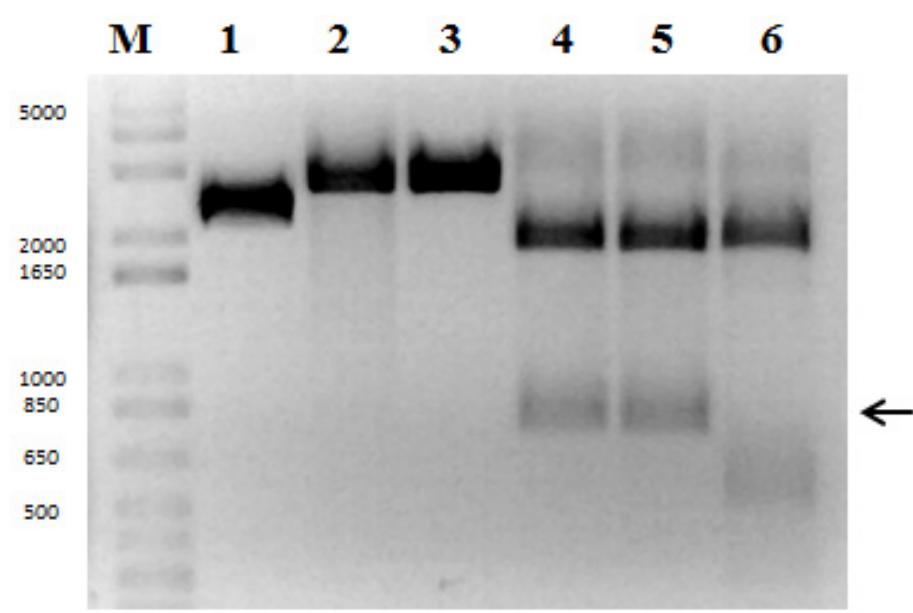


Figure S2: Restriction digest analysis for confirmation of the presence of the insert in the commercial plasmid. 1% agarose gel. (M) molecular marker 1kb DNA Ladder; (1) circular plasmid (2) linearized plasmid, digested with *Xho* I; (3) linearized, *Nco* I-digested plasmid; (4 and 5) plasmid digested with the enzymes *Xho* I and *Nco* I; (6) plasmid digested with *Xho* I, *Nco* I and *Bam* HI. The arrow indicates the released insert of 753 bp.

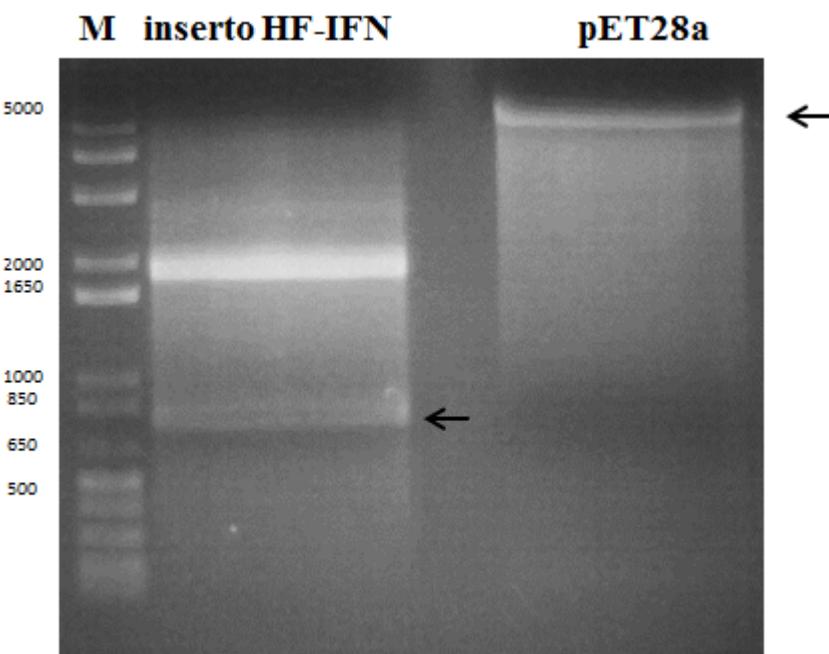


Figure S3: Preparative electrophoresis for HF-IFN insert and pET28a expression vector purification. 1% agarose gel. Plasmids were submitted to the enzymatic digestion protocol in the presence of the enzymes *Nco* I and *Xho* I. The fragments generated were used in the subsequent ligation reaction. (M) molecular marker 1kb DNA Ladder. The upper and lower arrows indicates the fragments that were purified: expression vector pET28a and HF-IFN insert, respectively.

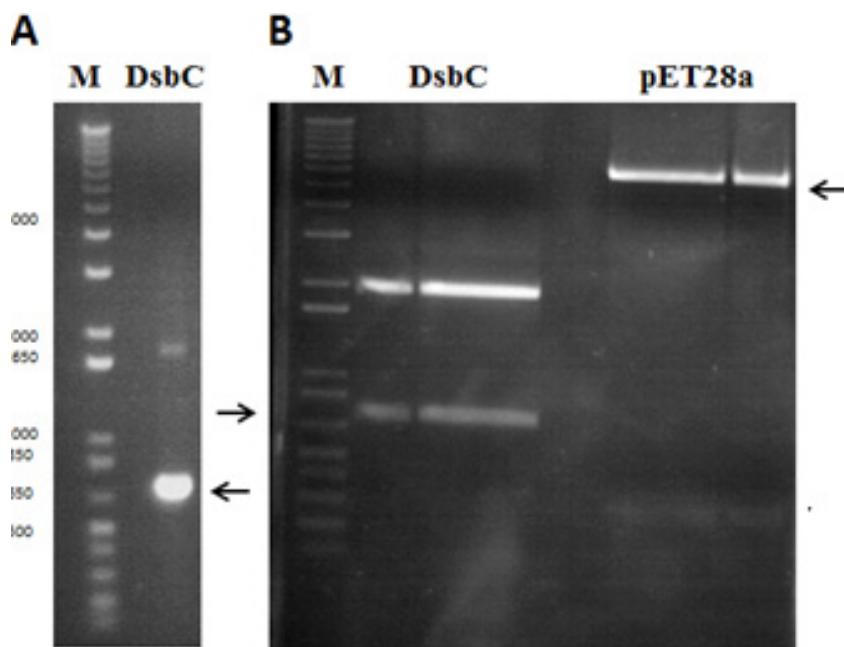


Figure S4: DsbC and pET28a cloning steps. 1% agarose gel. (A) Amplicon of the DsbC tag (648 nt). PCR was done using the commercial plasmid as DNA template; (B) preparative electrophoresis gel for purification of DsbC tag and plasmid digested with *Nco* I and *Bam* HI. The arrows indicate the fragments of interest. (M) molecular marker 1kb DNA Ladder.

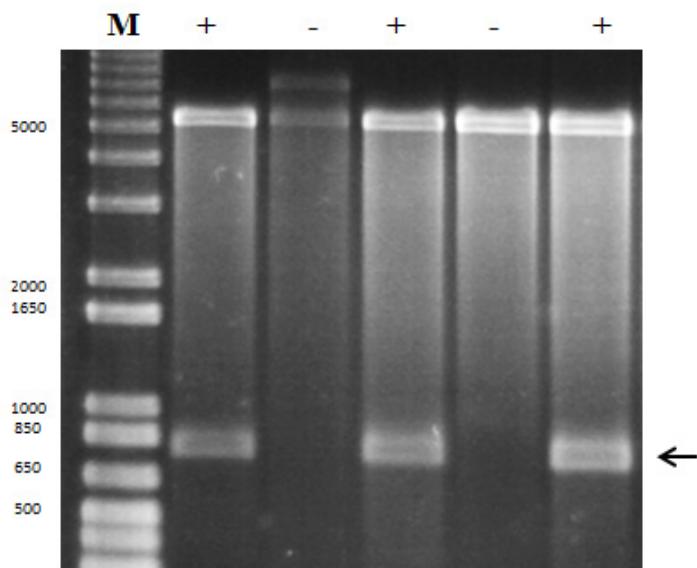


Figure S5: Selection of positive colonies for the HF-IFN insert. 1% agarose gel. Plasmids were submitted to the enzymatic digestion protocol in the presence of the enzymes *Nco* I and *Xho* I. The arrow indicates the insert released in the expected size, 753 nt. (M) molecular marker 1kb DNA Ladder. The + and - symbols respectively indicate bacterial colonies positive or negative for the HF-IFN insert.

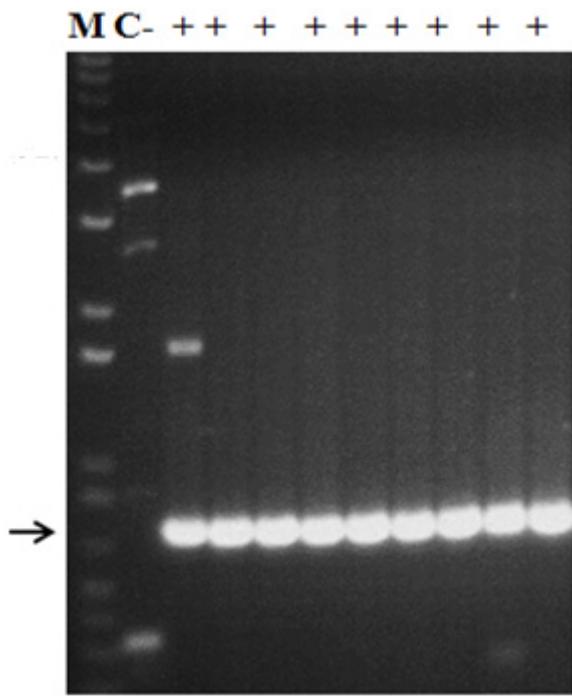


Figure S6: Positive *E. coli* colonies for plasmid pET28a-HD-IFN. 1% agarose gel. Bacterial colonies were submitted to PCR for detection of DsbC. The arrow indicates the fragment of interest. (M) molecular marker 1kb DNA Ladder. The + and - symbols indicate, respectively, bacterial colonies positive or negative for DsbC. C- indicates negative control.

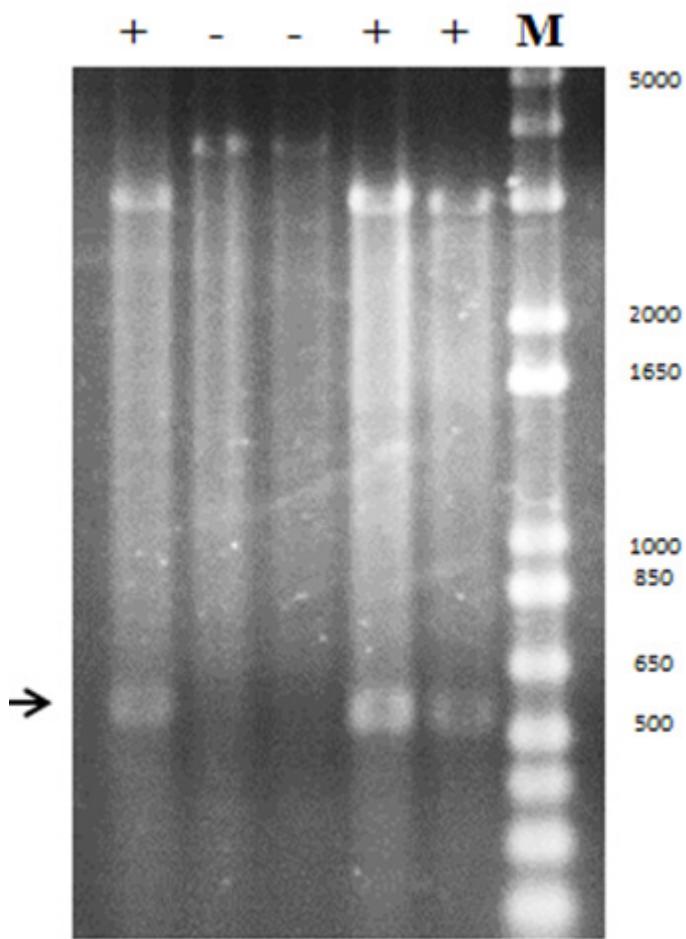


Figure S7: Electrophoresis gel for PCR-amplified H-IFN insert detection and selection of bacterial colonies. 1% agarose gel. Plasmids from bacterial colonies were submitted to the enzymatic digestion protocol in the presence of the enzymes *Nco* I and *Xho* I. The arrow indicates the presence insert in the expected size, 504 nt. (M) molecular marker 1kb DNA Ladder. The + and - symbols respectively indicate bacterial colonies positive or negative for the H-IFN insert, which does not have the Fh8 tag fused to the target protein.

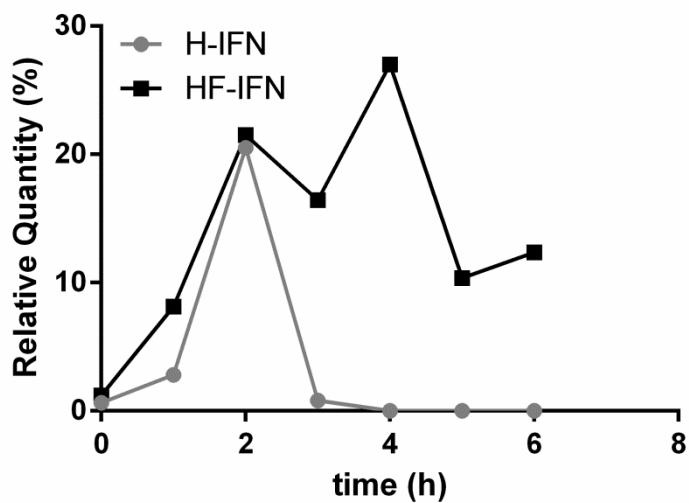


Figure S8: Quantification analysis of H- and HF-IFN in the soluble fraction. The relative amount of the target protein was determined by densitometry (Equation 1).

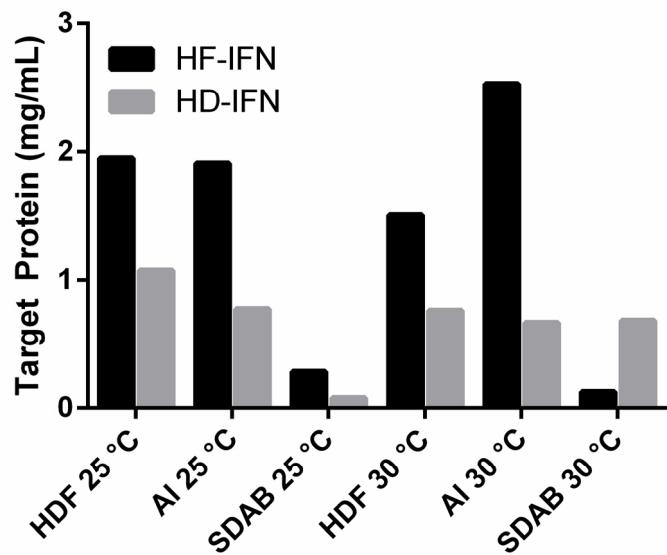


Figure S9. Comparison of HF-IFN and HD-IFN maximum production in different culture media and temperatures. The values reported refer to the maximum concentration of HF-IFN and HD-IFN reached in each culture media at 25 °C or 30 °C.

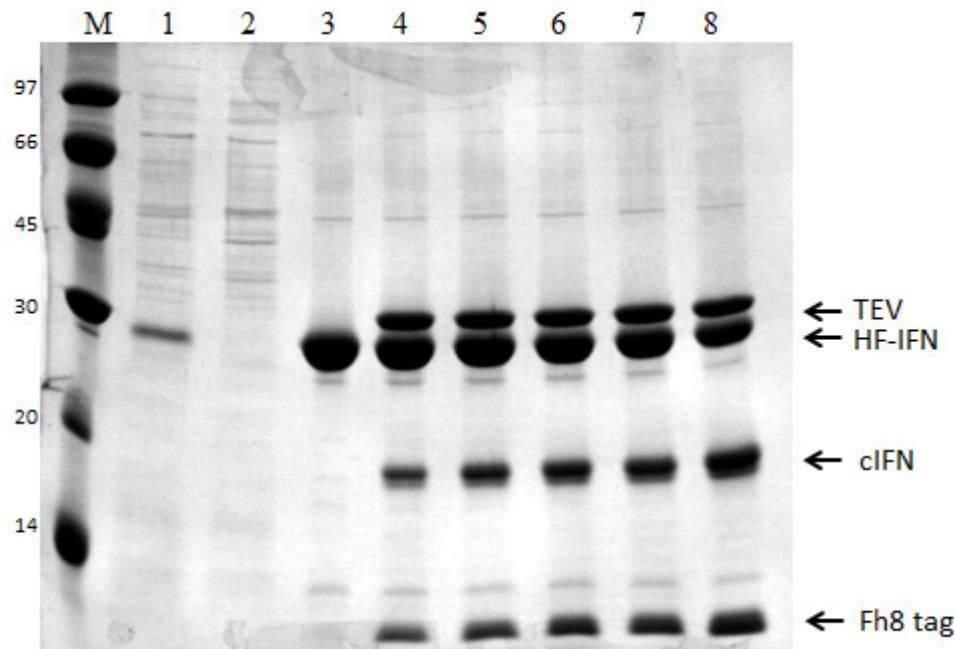


Figure S10. Enzymatic kinetics of HF-IFN hydrolysis in the presence of TEV. HF-IFN and TEV (5:1 proportion) were incubated in 20 mM Tris-HCl pH 8, containing 150 mM NaCl, 20% glycerol and 2 mM β -mercaptoethanol. (M) molecular weight standard (kDa); (1) non-hydrolyzed HF-

IFN; (2) non-induced BL21 (DE3) extract, negative control; (3) HF-IFN in the absence of TEV; (4) HF-IFN incubation with TEV for 1 hour; (5) HF-IFN incubation with TEV for 2 hour; (6) HF-IFN incubation with TEV for 3 hour; (7) HF-IFN incubation with TEV for 4 hour and (8) HF-IFN overnight enzymatic incubation with TEV. TEV has an approximate weight of 33 kDa; HF-IFN 28.8 kDa; cIFN 19.4 kDa and Fh8 tag 9.4 kDa. SDS-PAGE electrophoresis gel 15%.