pUNO1Fc-SARS2-S2

Plasmid designed for the production of the SARS-CoV-2 Spike S2::Fc fusion protein

Catalog code: p1fc-cov2-s2

https://invivogen.com/sars2-spike-s2-tag-production-vectors

For research use only

Version 21C17-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized **pUNO1Fc-SARS2-S2** (plasmid DNA)
- 2 x 1 ml of **Blasticidin** (10 mg/ml)

Storage and Stability

- Product is shipped at room temperature.
- Store lyophilized DNA at -20°C.
- Resuspended DNA stored at -20°C is stable at least for 1 year.
- Store Blasticidin at 4°C or -20°C. The expiry date is specified on the product label.

Quality control

- After purification by ion exchange chromatography, predominant supercoiled conformation is verified by electrophoresis.
- Plasmid construct is confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.

PLASMID FEATURES

SARS-CoV-2 Spike S2::hIgG1 Fc cassette

- **SV40 enhancer** is comprised of a 72-base-pair repeat and allows the enhancement of gene expression in a wide range of hosts. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids[1].
- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter[2] and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a «house keeping» gene. It is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat[3] has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.
- **Codon-optimized Spike S2 subunit ORF**

pUNO1Fc-SARS2-S2 contains the coding sequence for the S2 subunit of the Spike (S) protein from the original SARS-CoV-2 Wuhan-Hu-1 isolate. To improve expression of the S2 subunit, the gene is codon-optimized. Furthermore, to facilitate secretion of the protein the coding sequence is preceded by the native SARS-CoV-2 signal sequence.

The S protein mediates the binding and entry of SARS-CoV-2 to host cells. In particular, the S2 subunit contains various features such as a fusion peptide and heptad repeats (HR), and is thus responsible for viral-host membrane fusion[4].

For more information visit: https://www.invivogen.com/sars2-spike

- **TEV sequence** (Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser)) is cleavable by the TEV (Tobbaco Etch Virus) protease between the Gln and Gly/Ser residues.
- **Fc** is the the Fc region of the human IgG1 cloned at the C-terminus of the gene of interest and followed by a Stop codon. This tag comprises the CH2 and CH3 domains of the IgG heavy chain and the hinge region. The hinge serves as a flexible spacer between the two parts of the Fc fusion protein allowing each part of the molecule to function independently.
- **SV40 pAn** is the Simian Virus 40 late polyadenylation (pAn) signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA[5].

Antibiotic selection cassette

- **hCMV (human cytomegalovirus) enhancer & promoter** drive the expression of the blasticidin resistance in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- ***bsr* (blasticidin resistance gene)** from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic blasticidin. The *bsr* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, blasticidin can be used to select stable mammalian cells transfectants and *E. coli* transformants.
- **Human beta-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of beta-globin pAn minimizes interference and recombination events with the SV40 pAn signal[6].

General features of pUNO1Fc-SARS2-S2

- **pMB1 ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

METHODS

- **Plasmid resuspension**

- Quickly spin the tube containing the lyophilized plasmid to pellet the DNA.
- To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water.
- Store resuspended plasmid at -20°C.

- **Plasmid amplification and cloning**

Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

- **Blasticidin usage**

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied as a 10 mg/ml colorless solution in HEPES buffer.

APPLICATIONS

- **Detection and purification of the S2 subunit**

pUNO1Fc-SARS2-S2 has been designed to generate the Spike S2 subunit in mammalian cells with a C-terminal human IgG1-Fc (Fc) tag. This facilitates the detection of the secreted protein with an anti-Fc antibody, and its purification using protein G or protein L affinity resins.

TECHNICAL SUPPORT
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• Subcloning the gene into another vector

For subcloning, two unique restriction sites flank the S2 subunit gene:

- *5' site: AgeI* (compatible with XmaI, BspEI, NgoMIV, and SgrAI)
- *3' site: MscI* (compatible with BaII, MIsI, MLuNI, Mox20I, and Msp20I)

REFERENCES

1. Dean DA. *et al.* 1999. Sequence requirements for plasmid nuclear import. Exp. Cell. Res. 253:713-22. **2. Kim D. *et al.*, 1990.** Use of the human elongation factor 1α promoter as a versatile and efficient expression system. Gene 91(2):217-23. **3. Takebe Y. *et al.*, 1988.** SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol. 8(1):466-72. **4. Huang, Y. *et al.* 2020.** Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 41, 1141-1149. **5. Carswell S. & Alwine J., 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58. **6. Yu J. & Russell J., 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human β-globin mRNA. Mol Cell Biol. 21(17):5879-88.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent *E. coli*	gt116-11
Protein G / Agarose	Immunoglobulin binding protein	gel-agg-2
Protein L / Agarose	Immunoglobulin binding protein	gel-protl-2

COVID-19 Product Range

HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
pUNO1-hTMPRSS2b	Expression vector	puno1-htp2b

For a complete list of InvivoGen's COVID-19 related products visit: https://www.invivogen.com/covid-19

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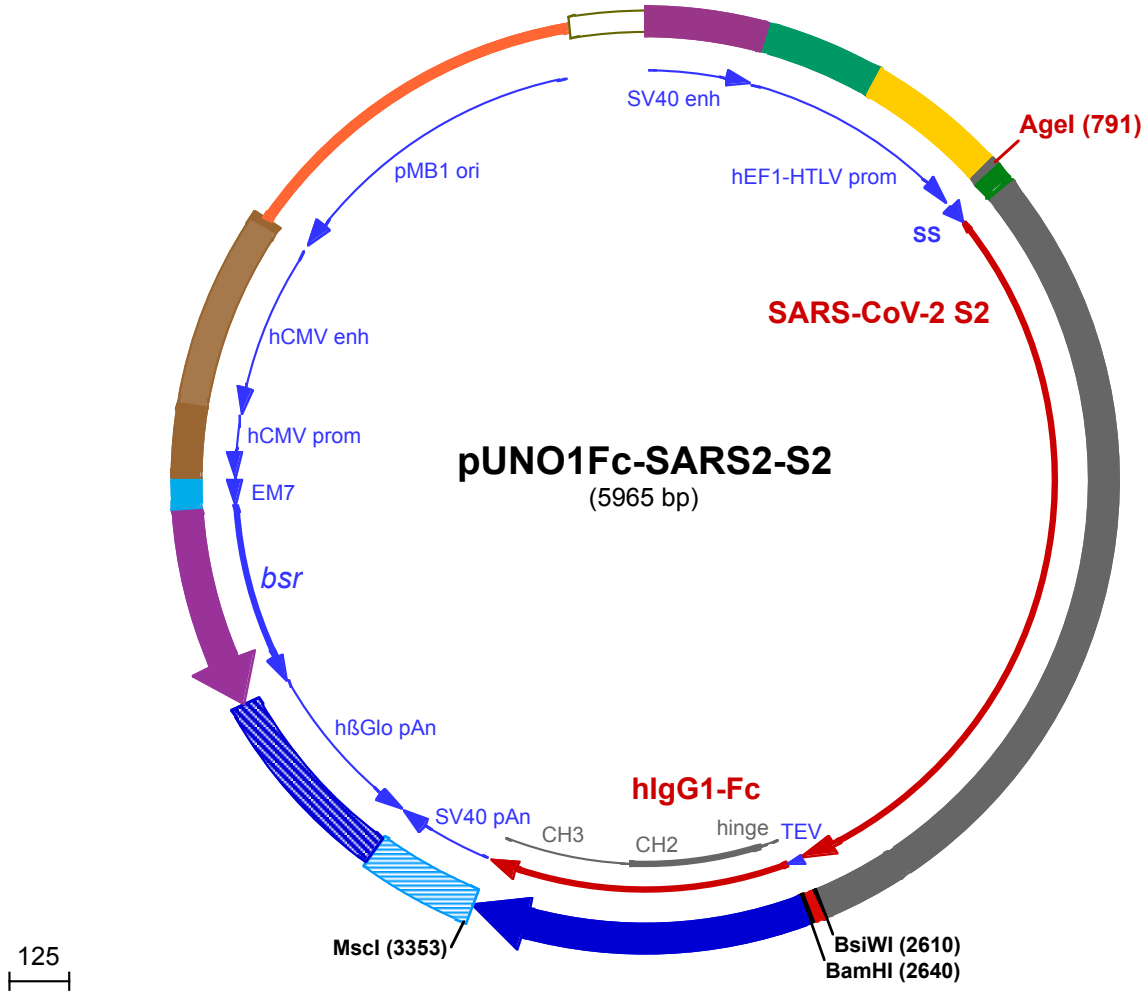
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(5965 bp)
SV40 enh
AgeI (791)
pMB1 ori
hEF1-HTLV prom
SS
SARS-CoV-2 S2
hCMV enh
hCMV prom
EM7
bsr
hßGlo pAn
hIgG1-Fc
SV40 pAn
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TEV
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BsiWI (2610)
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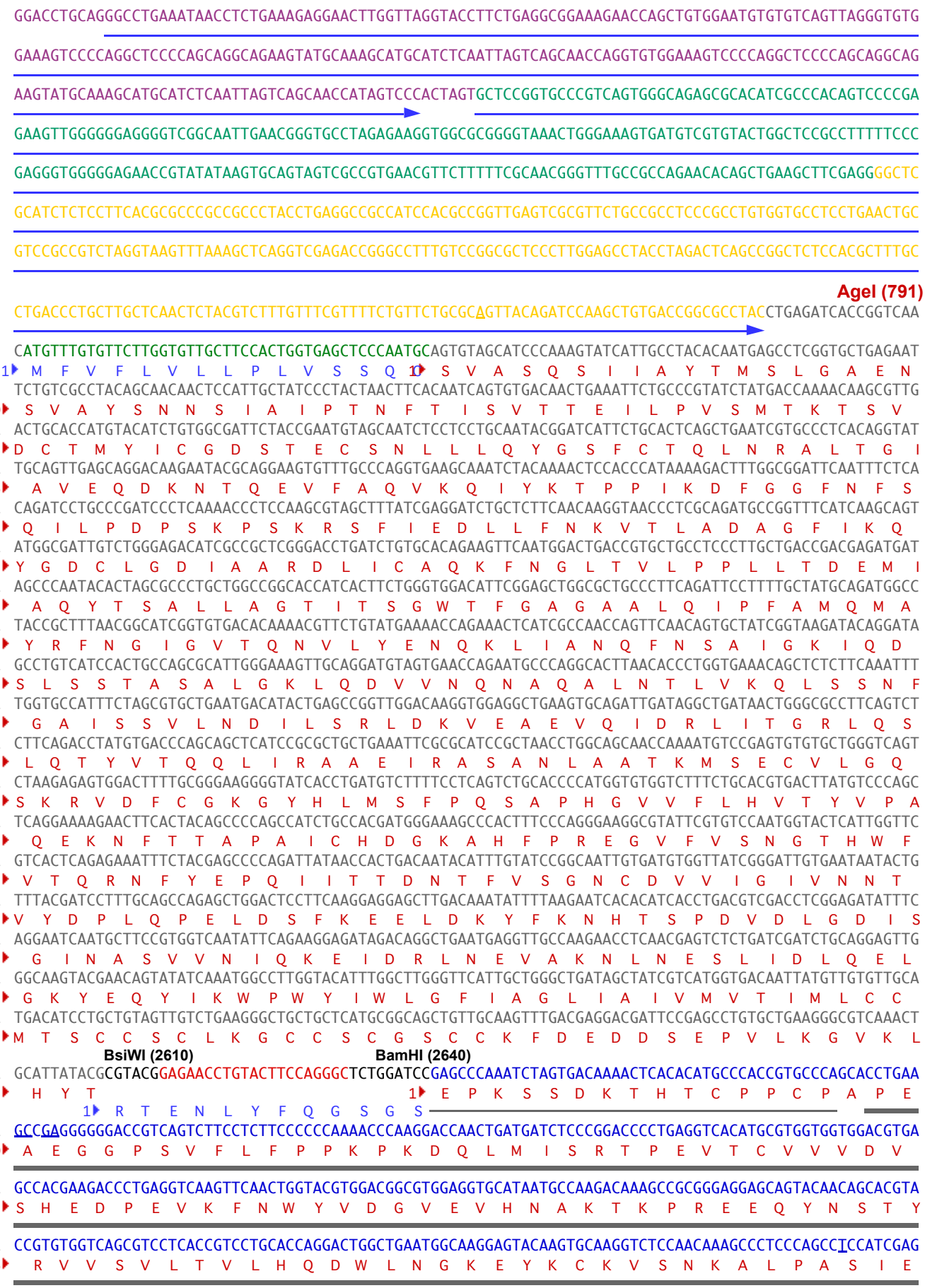
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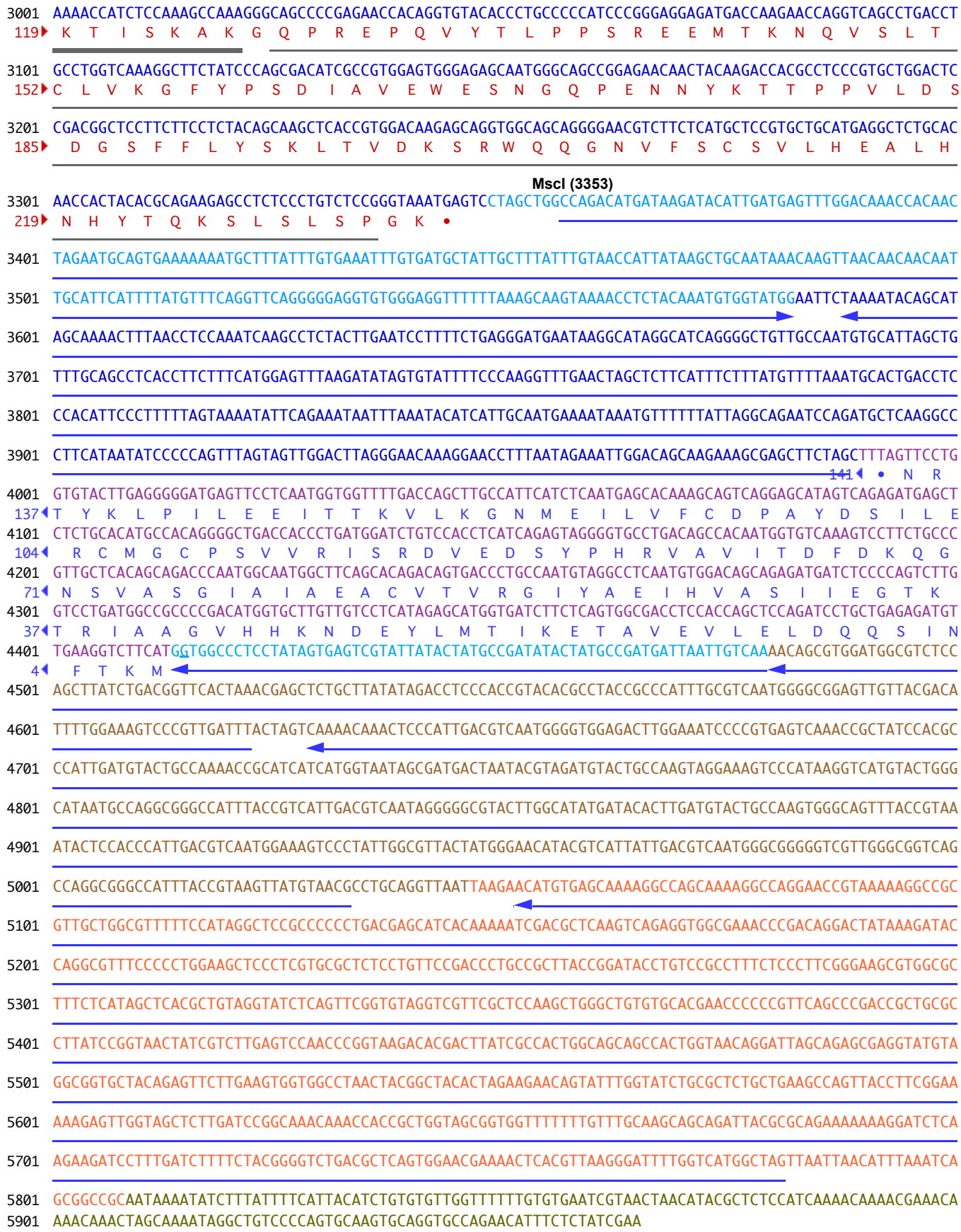
MscI (3353)