pUNO1-Spike-dfur

Expression vector containing the Wuhan-Hu-1 D614 SARS-CoV-2 Spike (delta furin) gene

Catalog code: p1-spike-df

https://www.invivogen.com/sars2-spike-d614-expression-vectors

For research use only

Version 21C15-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized **pUNO1-Spike-dfur** (plasmid DNA)
- 2 x 1 ml of **Blasticidin** (10 mg/ml)

Storage and Stability

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

Quality control

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

PLASMID FEATURES

Wuhan-Hu-1 SARS-CoV-2 Spike (D614) cassette

- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter[1] and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes a "house-keeping"gene. It is expressed at high levels in all cell cycles and lower levels during the G0 phase. Additionally, since the promoter is not 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[2] 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.
- Codon-optimized Spike ORF

pUNO1-Spike-dfur contains the Spike (S) coding sequence from the original Wuhan-Hu-1 SARS-CoV-2 isolate. Notably, the furin cleavage site has been inactivated (dfur) by the inclusion of two mutations (R683/5A). Furthermore; to improve expression of the S protein in cell lines, the gene is codon-optimized and the last 19 amino acids, which contain an endoplasmic reticulum (ER)-retention motif (KxHxx), have been removed[3,4].

pUNO1-Spike-dfur is characterized by the following features:

- **S1 domain:** *D614*
- **S1/S2 boundary:** *R683A, R685A*

Spike (S) is a structural glycoprotein expressed on the surface of SARS-CoV-2. It mediates membrane fusion and viral entry into target cells upon binding to the host receptor ACE2 and the proteolytic activity of host proteases such as furin and TMPRSS2[5].

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

- **SV40 pAn** is the Simian Virus 40 late polyadenylation (pAn) signal and it enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[6].

Antibiotic selection cassette

- **hCMV (human cytomegalovirus) enhancer & promoter** drive the expression of the blasticidin resistance gene (*bsr*) in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the blasticidin resistance gene (*bsr*) in *E. coli*.
- ***bsr* (blasticidin resistance gene)** encodes a deaminase from *Bacillus cereus* that confers resistance to the antibiotic blasticidin. The expression of the *bsr* gene is driven by the CMV promoter/enhancer and the bacterial EM7 promoter. Therefore, Blasticidin can be used to select stable clones in mammalian cells and *E. coli* transformants.
- **Human β-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of β-globin pAn minimizes interference and possible recombination events with the SV40 pAn signal[7].

General features of pUNO1-Spike-dfur

- **pMB1 ori** is a minimal *E. coli* origin of replication.

APPLICATIONS

Stable gene expression in mammalian cells.

pUNO1 plasmids are designed for both transient and stable transfection in mammalian cell lines by selection with Blasticidin. Furthermore, they faciliate high levels of expression of the gene of interest.

Antibody screnning by flow cytometry

pUNO1-Spike-dfur has been specifically designed for mammalian cell expression of the SARS-CoV-2 S protein. Notably, due to the inactivated furin cleavage site, when this plasmid is expressed by a host cell (e.g. 293T cells) there is high surface expression of the full-length S protein[3,8]. Ideal for SARS-CoV-2 S-specific antibody screening by flow cytometry (*in-house data*).

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.

TECHNICAL SUPPORT
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REFERENCES

1. 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 **2. 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. **3. Johnson, M.C. *et al.* 2020.** Optimized Pseudotyping Conditions for the SARS-COV-2 Spike Glycoprotein. J Virol 94**. 4. Ou, X. *et al.* 2020.** Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 11, 1620. **5. Hoffmann M. *et al.*, 2020.** SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 181:1-16. **6. 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. **7. 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. **8. Walls, A.C. *et al.* 2020.** Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent *E. coli*	gt116-11
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
Anti-CoV2RBD-c1-hIgG1	Recombinant Antibody	cov2rbdc1-mab1

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

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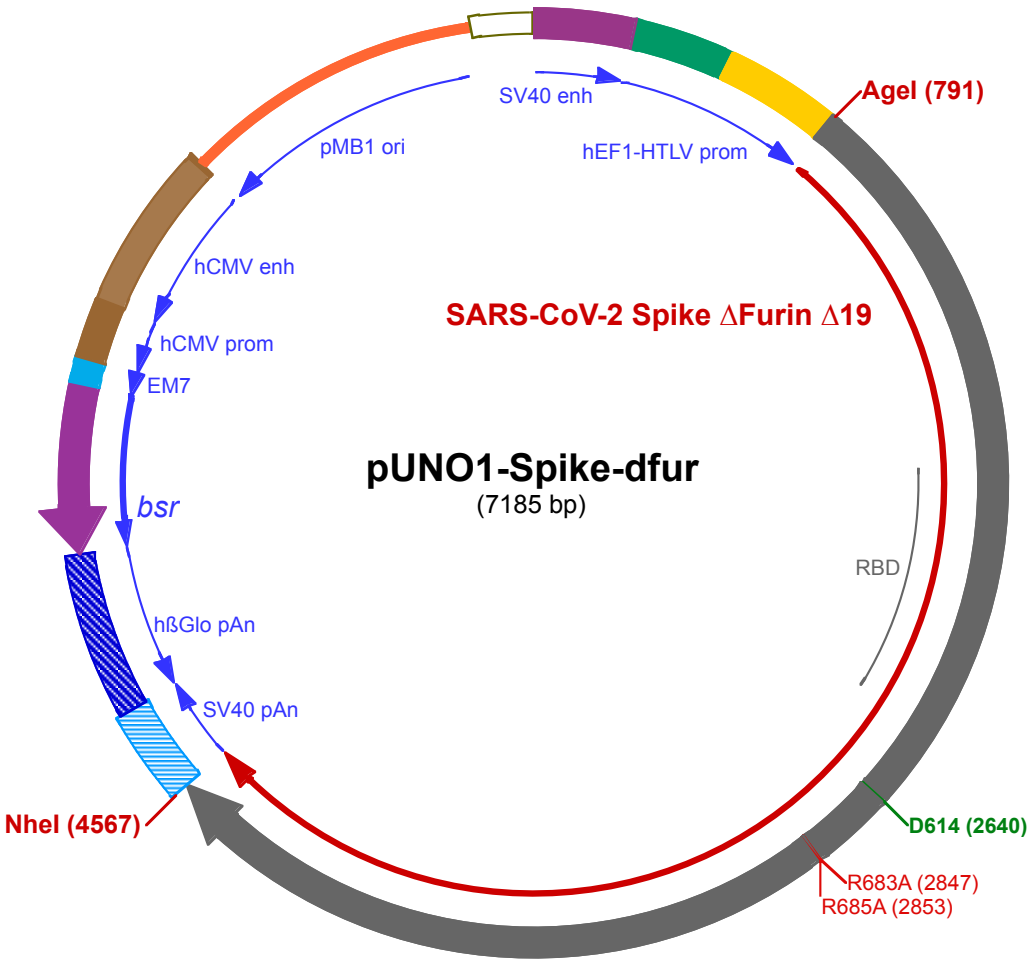
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pMB1 ori
hEF1-HTLV prom
hCMV enh
SARS-CoV-2 Spike ΔFurin Δ19
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pUNO1-Spike-dfur
(7185 bp)
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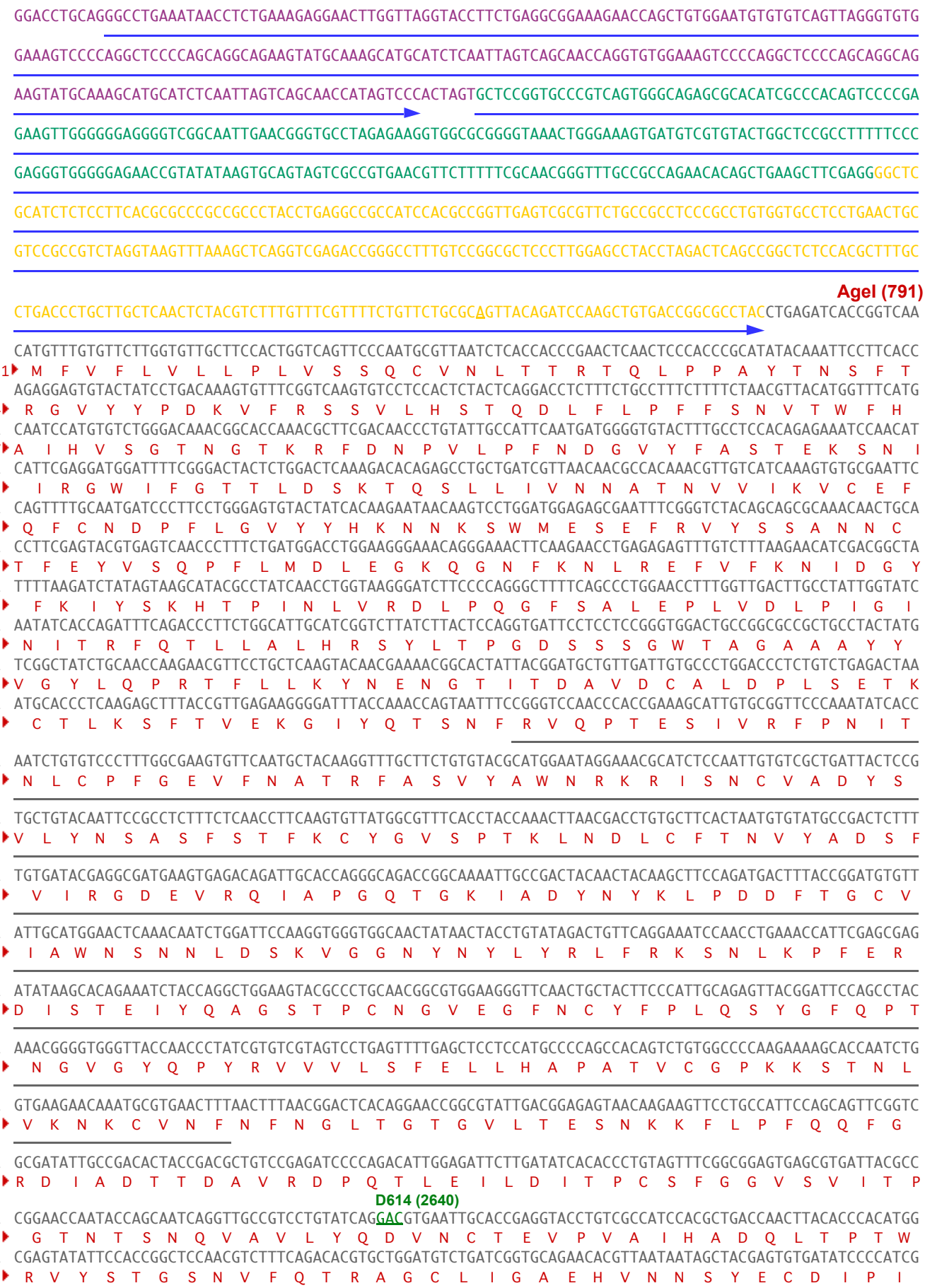
D614 (2640)
R683A (2847)
R685A (2853)
150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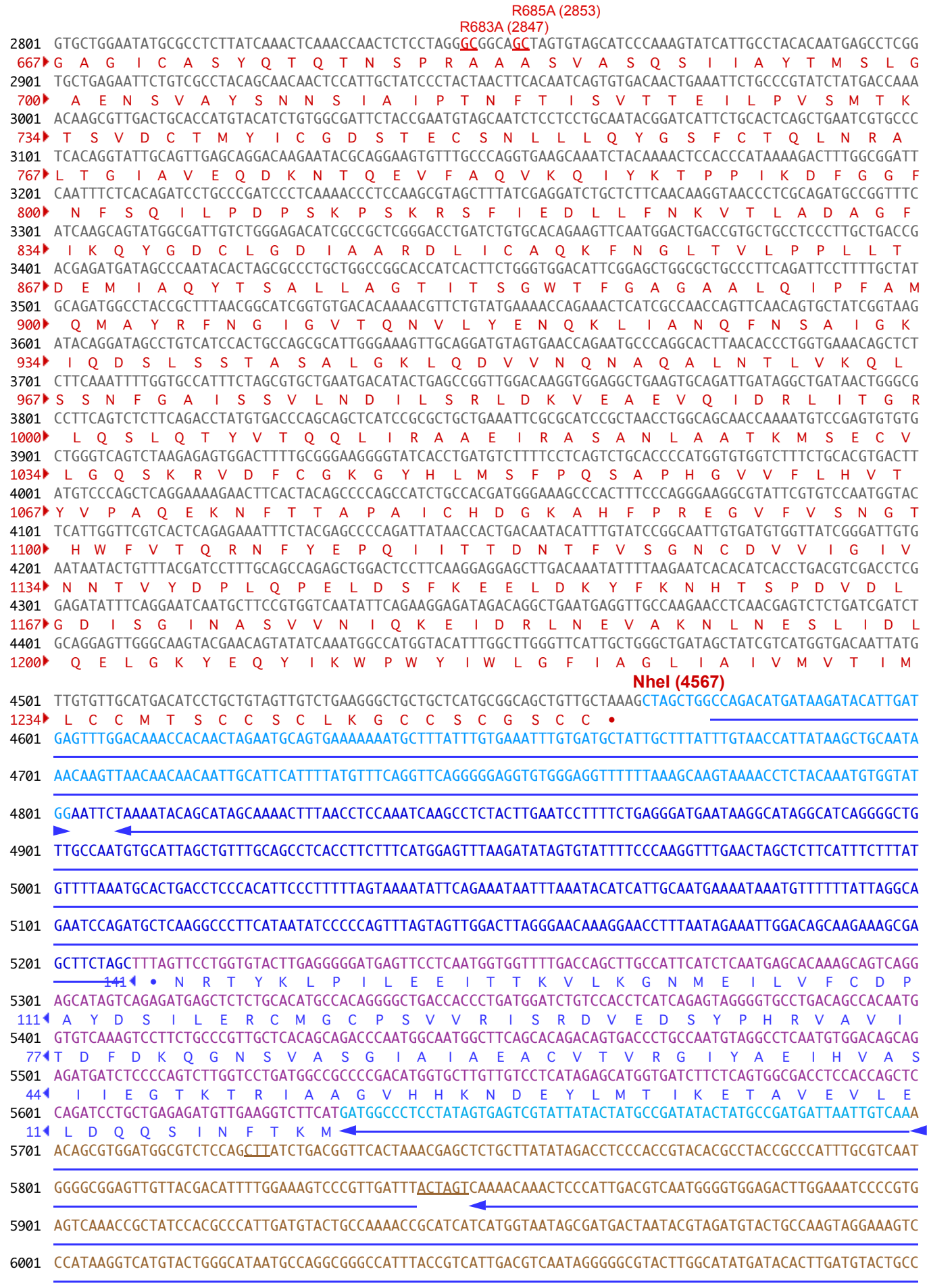
D614 (2640)


R685A (2853)
R683A (2847)
NheI (4567)

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