

pUNO-hTLR3-GFP

A plasmid expressing the human TLR3 gene fused to a GFP gene

Catalog # phtr3-gfp

For research use only

Version # 10K03-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized *E. coli* transformed with pUNO-hTLR3-GFP.
- E. coli* strain is GT116: *F mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZM15 ΔlacX74 recA1 rpsL (StrA) endA1 ΔsbC-sbcD*.
- 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar).

Storage and stability:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20°C and are stable up to 1 year.
- Store *E. coli* Fast-Media® Blas at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- hTLR3::GFP fusion gene has been fully sequenced, its fluorescence confirmed and its function tested in HEK293 cells coexpressing an NF-κB reporter plasmid (pNiFty-SEAP, cat. code: pnifty-seap).
- Plasmid construct has been confirmed by restriction analysis.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

GENERAL PRODUCT USE

pUNO-TLR-GFP plasmids express high-levels of transient or stable TLR-GFP fusion proteins in a wide range of mammalian cells. These fusion proteins can be used to study the localization of the TLRs. Transfected cells can be analyzed for GFP expression by flow cytometry.

pUNO-TLR-GFP plasmids can be used directly for *in vitro* or *in vivo* transfection experiments. They are selectable with blasticidin, an antibiotic that allows the selection of stable mammalian clones in only a few days.

TLR::GFP fusion genes are under the control of a strong and ubiquitous composite promoter, called EF1α/HTLV, comprised of the elongation factor 1 alpha (EF-1α) core promoter and the R-U5' of the human T cell leukemia virus (HTLV).

PLASMID FEATURES

• Human TLR3::GFP fusion gene (3566 bp)

TLR3 recognizes double-stranded RNA (dsRNA), a molecular pattern associated with viral infection, and poly(I:C), a synthetic analog of dsRNA¹. TLR3 signals mainly through a MyD88-independent pathway involving the TRIF/TICAM1 adapter protein that leads to the production of IFN-β².

The hTLR3::GFP fusion gene was generated by fusing at the C terminus of the human TLR3 gene to a GFP variant. A synthetic intron was added between both moieties to increase the activity of GFP. This hybrid protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).

The hTLR3::GFP fusion gene is under the control of the strong and ubiquitous hEF1/HTLV promoter. This composite promoter comprises the Elongation Factor-1α (EF-1α) core promoter³ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁴. The SV40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.

• **Blasticidin resistance (bsr) gene:** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic Blasticidin S. The *bsr* expression cassette is formed by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter, to allow blasticidin selection in both mammalian cells and *E. coli* bacteria, and the human beta globin polyadenylation signal (hβGlo pAn).

References

1. Alexopoulou L. et al. (2001). Recognition of double-stranded RNA and activation of NF-κappaB by Toll-like receptor 3. *Nature*, 413(6857):732-8
2. Yamamoto M. et al. (2002). Cutting edge: A novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-β promoter in the Toll-like receptor signaling. *J Immunol*, 169(12):6668-6672
3. Kim et al. (1990). Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene* 2: 217-223.
4. Takebe 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.* 1: 466-472.

METHODS

Growth of pUNO-transformed bacteria:

Use sterile conditions to do the following:

- 1- Resuspend the lyophilized *E. coli* by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.
- 2- Streak bacteria taken from this suspension on an blasticidin LB agar plate prepared with the *E. coli* Fast-Media® Blas agar provided (see below).
- 3- Place the plate in an incubator at 37°C overnight.
- 4- Isolate a single colony and grow the bacteria in TB supplemented with blasticidin using the Fast-Media® Blas liquid provided (see below).
- 5- Extract the pUNO plasmid DNA using the method of your choice.

Selection of bacteria with *E. coli* Fast-Media Blas:

E. coli Fast-Media® Blas is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave.

- 1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask.
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
- 6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

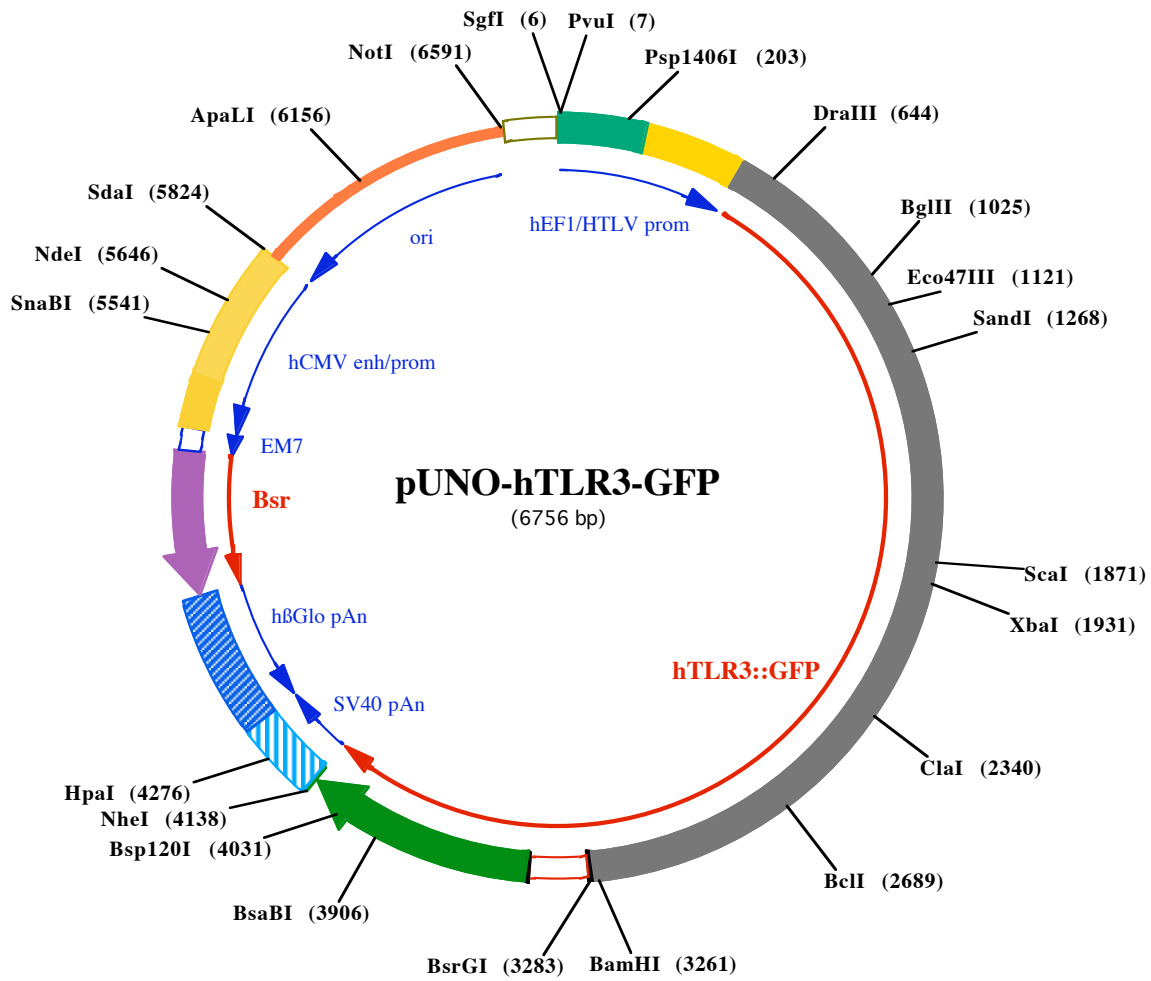
Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

TECHNICAL SUPPORT

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PvuI (7)
SgfI (6)
1 GGATCTGCATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTA
101 GAGAAGGTGGCGCGGGTAAACTGGAAAGTGATGTCGTGACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

Psp1406I (203)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCACGCGCCCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCTCTGAACTGCGTCCGCGCTTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCTGCTTGTCAACTCTACGTCTTTGTTTCGTTT
501 TCTGTTCTGCGCGGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGTAGGAGGCCATCATGAGACAGACTTTGCCTGTATCTACTTT

DraIII (644)
601 TGGGGGGCCTTTTGCCTTTGGGATGCTGTGTGCATCCTCCACCACCAAGTGCAGTGTAGCCATGAAGTTGCTGACTGCAGCCACCTGAAGTTGACTC
701 AGGTACCCGATGATCTACCCACAAACATAACAGTGTGAACCTTACCATAATCAACTCAGAAGATTACCAGCCGCAACTTCACAAGGTATAGCCAGCT
801 AACTAGCTTGGATGTAGGATTTAACACCATCTCAAACCTGGAGCCAGAATTGTCCAGAACTTCCCATGTTAAAAGTTTTGAACCTCCAGCACAAATGAG
901 CTATCTCAACTTTCTGATAAACCTTTGCCTTCTGCACGAATTTGACTGAACTCCATCTCATGTCCAACCTCAATCCAGAAAATTAATAATCCCTTTG

BglII (1025)
1001 TCAAGCAGAAGAATTTAATCACATTAGATCTGTCTCATAATGGCTTGTCATCTACAAAATTAGGAACTCAGGTTGAGTGGAAAATCTCCAAGAGCTTCT

Eco47III (1121)
1101 ATTATCAAACAATAAAATCAAGCGCTAAAAAGTGAAGAACTGGATATCTTTGCCAATTCATCTTTAAAAAATTAGAGTTGTCATCGAATCAAATTAATA

SandI (1268)
1201 GAGTTTTCTCCAGGGTGTTCACGCAATTGGAAGATTATTTGGCCTTTTCTGAACAATGTCCAGCTGGGTCCAGCCTTACAGAGAAGCTATGTTTGG
1301 AATTAGCAAACACAAAGCATTTCGGAATCTGTCTCTGAGTAAACAGCCAGCTGTCCACCACCAGCAATACAACCTTTCTTGGGACTAAAGTGGACAAATCTCAC
1401 TATGCTCGATCTTCTCACAACAACCTTAAATGTGGTTGGTAAACGATTCTTTGCTTGGCTTCCACAAC TAGAATATTTCTTCTAGAGTATAATAATATA
1501 CAGCATTGTTTTCTCACTTTTGCACGGGCTTTTCAATGTGAGGTACCTGAATTTGAAACGGTCTTTACTAAACAAAGTATTTCCCTTGCCTCACTCC
1601 CCAAGATTGATGATTTTTCTTTTCAAGTGGCTAAAATGTTTGGAGCACCTTAAACATGGAAGATAATGATATTCCAGGCATAAAAAGCAATATGTTACAGG
1701 ATTGATAAACCTGAAATACTTAAGTCTATCCAACCTCTTTACAAGTTTGCGAACCTTTGACAAATGAAACATTTGTATCACTTGCTCATTCTCCCTTACAC

ScaI (1871)
1801 ATACTCAACCTAACCAAGAATAAAATCTCAAAAATAGAGAGTGATGCTTTCTTGGTTGGGCCACCTAGAAGTACTTGACCTGGGCCTTAATGAAATTG

XbaI (1931)
1901 GGCAAGAACTCACAGGCCAGGAATGGAGAGGTCTAGAAAATATTTTTGAAATCTATCTTCTCACAACAAGTACCTGCAGCTGACTAGGAACTCCTTTGC
2001 CTTGGTCCCAAGCCTTCAACGACTGATGCTCCGAAGGGTGGCCCTAAAAATGTGGATAGCTCTCCTTACCATTCCAGCCTCTTCGTAACCTGACCATT
2101 CTGGATCTAAGCAACAACAACATAGCCAACATAAATGATGACATGTTGGAGGGTCTTGAGAACTAGAAAATCTCGATTTGCAGCATAACAACCTTAGCAC
2201 GGCTCTGGAACACGCAAACCCTGGTGGTCCCATTTATTTCTAAAGGGTCTGTCTCACCTCCACATCCTTAACTTGGAGTCCAACGGCTTTGACGAGAT

ClaI (2340)
2301 CCCAGTTGAGGTCTTCAAGGATTTATTTGAACTAAAGATCATCGATTTAGGATTGAATAATTTAAACACACTTCCAGCATCTGTCTTTAATAATCAGGTG
2401 TCTCTAAAGTCATTGAACCTT CAGAAGAATCTCATAACATCCGTTGAGAAGAAGGTTTTCCGGCCAGCTTT CAGGAACCTGACTGAGTTAGATATGCGCT
2501 TTAATCCCTTTGATTGCACGTGTGAAAGTATTGCCTGGTTTGTAAATTGGATTAACGAGACCCATACCAACATCCCTGAGCTGTCAAGCCACTACCTTTG

BclI (2689)
2601 CAACACTCCACCTCACTATCATGGGTTCCAGTGAGACTTTTTGATACATCATCTTGCAAAGACAGTGCCCCCTTTGAACTCTTTTTCATGATCAATACC
2701 AGTATCCTGTTGATTTTTATCTTTATTGACTTCTCATCCACTTTGAGGGCTGGAGGATATCTTTTTATTGGAATGTTTCAGTACATCGAGTTCTTGGTT
2801 TCAAAGAAATAGACAGACAGACAGAACAGTTTGAATATGCAGCATATAAATTCATGCCTATAAAGATAAGGATTGGGTCTGGGAACATTTCTTTCAAT
2901 GGAAAAGGAAGACCAATCTCTCAAATTTGTCTGGAAGAAAGGACTTTGAGGCGGGTGTTTTTGAACTAGAAAGCAATTGTTAACAGCATCAAAGAAGC
3001 AGAAAAATATTTTTGTTATAACACACCATCTATTAAGACCCATTATGCAAAGATTCAAGGTACATCATGCAAGTTCAACAAGCTATTGAACAAAATC

3101 TGGATTCCATTATATTGGTTTTCTTGAGGAGATTCCAGATTATAAACTGAACCATGCACTCTGTTTGCAGAGGAATGTTTAAATCTCACTGCATCTT

3201 GAACTGGCCAGTTTCAGAAAGAACGGATAGGTGCCTTTTCGTCATAAATTGCAAGTAGCACTTGGATCCAAAACTCTGTGCATCTGTACAAGGgtaagtca

BamHI (3261) **BsrGI (3283)**

3301 ctgactgtctatgctctggaaaggggtggcaggagatggggcagtgaggaaaagtggcactatgaaccACTAGTTTGACAATTAATCATAAGCATAGT

3401 ATAATACTCACTACTATAGcaattgtactaaccttcttctcttctctctgacag**GAGGAG**CCATCATGGCCGCTATGGAGATCGAGTGCCGCATCA

3500 CCGGCACCCTGAACGGCTGGAGTTCGAGCTGGTGGCGGGGAGAGGGCACCCCGAGCAGGGCCGCATGACCAACAAGATGAAGAGCACCAAAGGCGC

3600 CCTGACCTTCAGCCCTACCTGCTGAGCCACGTGATGGCTACGGCTTACCCTTCGGCACCTACCCAGCGGCTACGAGAACCCCTTCTGCACGCC

3700 ATCAACAACGGCGGTACACCAACACCCGCATCGAGAAGTACGAGGACGGCGGCTGCTGCACGTGAGCTTACGCTACCGCTACGAGGCCGGCCGCTGA

3800 TCGCGCAGTTCAAGGTGATGGGCACCGGCTTCCCGAGGACAGCGTGATTTACCAGACAAGATCATCCGAGCAACGCCACCGTGGAGCACCTGCACCC

BsaBI (3906)

3900 TATGGGCGATAACGATCTGGATGGCAGCTTACCCGCACCTTACGCTGCGGACGGCGCTACTACAGCTCCGTGGTGGACAGCCACATGCACTTCAAG

Bsp120I (4031)

4000 AGCGCCATCCACCCAGCATCTGCAGAACGGGGGCCCATGTTCCGCTTCCGCCGTGGAGGAGGATCACAGCAACACCGAGCTGGGCATCGTGGAGT

NheI (4138)

4100 ACCAGCACGCTTCAAGACCCCGGATGCAGATGCCTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACACTAGAATGC

HpaI (4276)

4200 AGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAAACAATTCATTCA

4300 TTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAAC

4400 TTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTGTTGCCAATGTGCATTAGCTGTTGCAGC

4500 CTCACCTTCTTCATGGAGTTTAAAGATATAGTGTATTTCCCAAGTTTGAAGTACTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTC

4600 CCTTTTTAGTAAATATTAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTTCATAA

4700 TATCCCCAGTTTAGTAGTTGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCTGGTGTACTT

4800 GAGGGGATGAGTTCTCAATGGTGGTTTTGACCAGCTTGCCATTATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGATGAGCTCTCTGCAC

135 LeuP roI l eLeuGl uGl u l l eThr Thr LysVal l eLeuLysGl yAsnMetGl u l l eLeuVal l PheCysAspP roAl aTyrAspSer l l eLeuGl uArgCysM

4900 ATGCCACAGGGGCTGACCACCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAAATGGTGTCAAAGTCTTCTGCCCTTGCTCA

101 e tGl yCysP roSer Val l eAl Arg l l eSer ArgAspVal l Gl uAspSer TyrP roHi sArgVal l Al aVal l l eThr AspPheAspLysGl nGl yAsnSer Va

5000 CAGCAGACCAATGGCAATGGCTTCAGCACAGACAGTACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCAAGTCTTGGTCTGTAT

68 l Al aSer Gl y l l eAl a l l eAl aGl uAl aCysVal l Thr Val l ArgGl y l l eTyrAl aGl u l l eHi sVal l Al aSer l l e l l eGl uGl yThr LysThr Arg l l e

5100 GGCGCCCGACATGGTGTCTTGTCTCATAGAGCATGGTGTCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATGTTGAAGGTC

35 Al aAl aGl yVal l Hi sHi sLysAsnAspGl uTyrLeuMetThr l l eLysGl uThr Al aVal l Gl uVal l LeuGl uLeuAspGl nGl nSer l l eAsnPheThr L

5200 TTCATGATGGCCCTCTATAGTGAAGTCTATTACTATGCGGATATACTATGCGGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGCTTATC

1 ysMet

5300 TGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTGCGTCAATGGGGCGGAGTTGTTACGACATTTGGAA

5400 AGTCCCGTTGATTTACTAGTCAAAAACAACTCCCAATTGACGTCAATGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGAT

SnaBI (5541)

5500 GTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTGATGACTGGGCATAATGC

NdeI (5646)

5600 CAGGGGGCCATTTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGATGACTGCCAAGTGGGCAGTTTACCGTAAATACTCCA

5700 CCCATTGACGTCAATGAAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGGGGGGGTCTTGGCGGTCAGCCAGGCGG

SdaI (5824)

5800 GCCATTTACCGTAAGTTATGTAACGCCCTGCAGGTTAATTAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGG

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6000 TCCCCTGGAAGCTCCCTCGTGCCTCTCTGTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCAT

ApaLI (6156)

6100 AGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCGACCGCTGCGCCTTATCCG

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6400 GGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATC

NotI (6591)

6500 CTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACACAGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGC

6600 AATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAC

6700 TAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTATCGAA