

pNiFty2-56K-SEAP

Inducible reporter plasmid selectable with Zeocin™

Catalog code: pnf2-56ksp

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pNiFty2-56K-SEAP provided as lyophilized DNA
- 1 ml of Zeocin™ (100 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 up to 1 year.
- Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and full-length ORF sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

Interferons are key modulators of the immune response. Their pleiotropic activities are mediated by the induction of many IFN-stimulated genes (ISGs). To help study the transcriptional regulation and signal transduction of type I IFNs, InvivoGen provides several reporter systems, called pNiFty2, based on the inducible expression of the secreted embryonic alkaline phosphatase (SEAP) gene. The SEAP gene is cloned under the control of three different promoters that are activated by various transcription factors, such as IRF3, IRF5, IRF7 and NF-κB. pNiFty2-56K-SEAP features the human ISG-56K promoter.

PLASMID FEATURES

- **hISG-56K prom:** ISG-56K is the prototype ISG which expression is highly increased by Type I IFNs due to the presence of ISRE and IRF7 sites within the promoter¹. Co-expression of IGSG-56K-SEAP with constitutively activated IRF3 (saIRF3) or IRF7 (saIRF7) in HEK293 cells led to a strong increase in SEAP expression.
- **5U-140** is a synthetic 5'UTR containing an intron.
- **SEAP** is a secreted form of human embryonic alkaline phosphatase. Unlike endogenous alkaline phosphatases, SEAP is extremely heat stable and resistant to the inhibitor L-homoarginine. It catalyses the hydrolysis of pNitrophenyl phosphate (pNpp) producing a yellow end product. SEAP expression can be readily quantified by collecting samples of culture medium and measuring the hydrolysis of pNpp with a spectrophotometer at 405 nm.
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- **hEF1/HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter² and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat³. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene in vivo. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.

- **EM7** is a bacterial promoter that enables the constitutive expression of the Zeocin™ antibiotic resistance gene in *E. coli*.
- **Zeo:** Resistance to the antibiotic Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **BGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.
- **pMB1 Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

1. Der SD, et al. 1998. Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. PNAS. 95(26):15623-8. **2. Kim DW, et al., 1990.** Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. Gene 91(2): 217-23. **3. Takebe Y, et al., 1988.** SR alphapromoter: 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. Yu J & Russell JE., 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol. 21(17):5879-88.

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 H₂O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

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

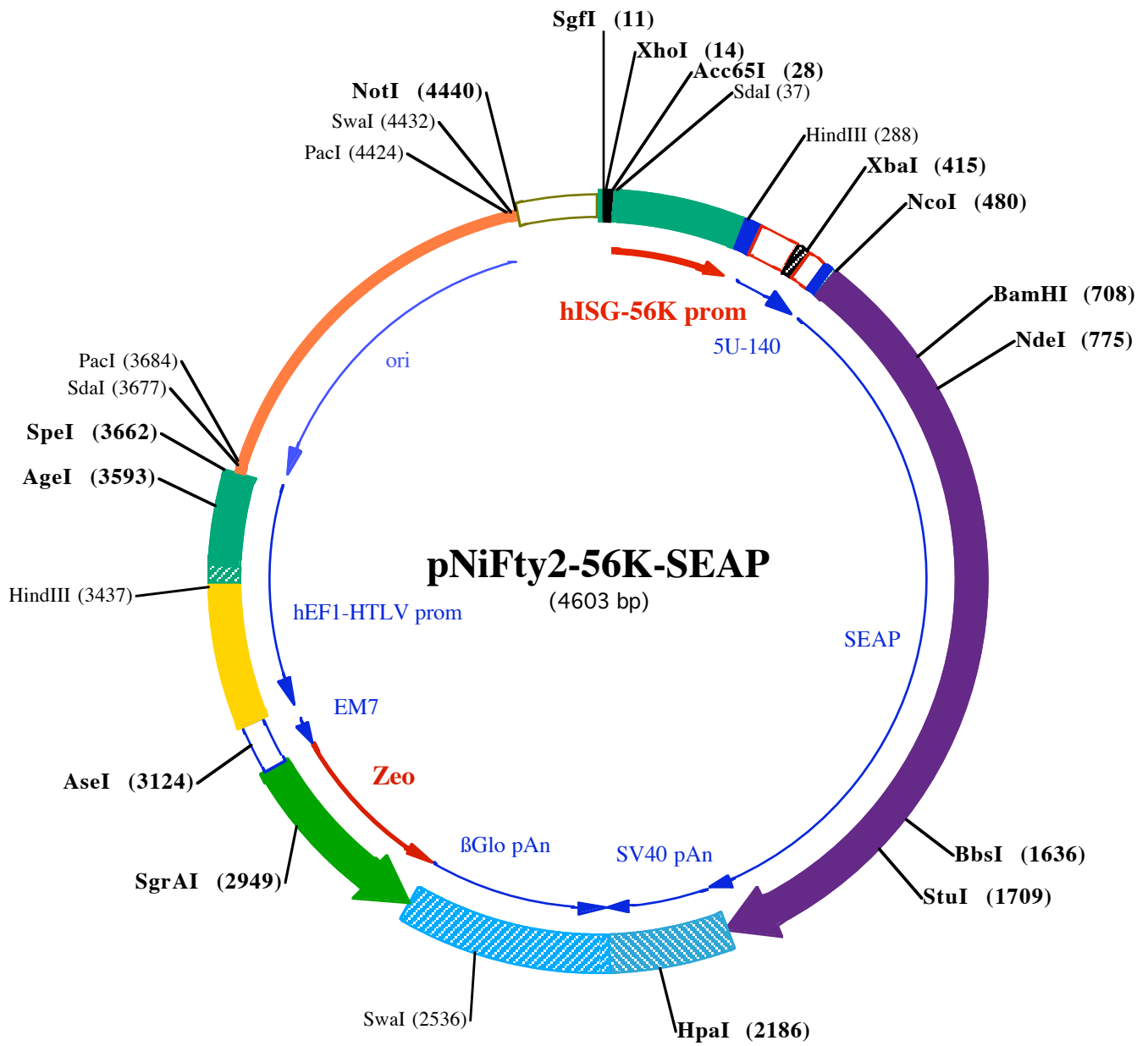
Zeocin™ usage

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong : +852 3-622-34-80
E-mail: info@invivogen.com





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SgfI (11) XhoI (14) SdaI (37) Acc65I (28)

1 GGATCTGCGATCGCTCGAGTGAATTTCTGGTACCTGCAGgACTAGAAACATCTATGTTGCGAGGTCTGCAGTTTATCTGTTTTAAATAGAAACAAAGTTT

101 CATTCCCCACCCCCCGTCAGCAGGAATTCGCTAGCTTTAGTTTACATTTCCCTTTTCGGTTTCCCTAGGTTTCAACTTGAAGGACACACCACA

201 GCTTACACCATTGGCTGCTTTAGCTCCCTTATATAACACTGTCTTGGGGTTTAAACGTAACGAAAATCCACAAGAGAATAGCAAGCTTTCGCTT HindIII (288)

301 CTCCTCTGTGAGTTTGtaagtcactgactgtctatgcctgggaanggtgggcaggagatggggcagtgaggaaaagtggcactatgaaccTGCa

XbaI (415) NcoI (480)

401 GCCTAGGAATGCATCTAGAcattgtactaaccttcttctcttctcctcctgacagGTTGGTGTACAGTAGCTTCCACATGGTTCTGGGGCCCTGCA 1MetValLeuGlyProCysM

501 TGCTGCTGCTGCTGCTGCTGCTGGGCTGAGGCTACAGCTCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCCGAGCAGC

74AlaAlaArgIleLeuLysGlyGlnLysLysAspLysLeuGlyProGluIleProLeuAlaMetAspArgPheProTyrValAlaLeuSerLysThrTyrA

79etLeuLeuLeuLeuLeuLeuLeuGlyLeuArgLeuGlnLeuSerLeuGlyIleIleProValAlaGluGluAsnProAspPheTyrAsnArgGluAlaAla

601 CGAGGCCCTGGTGGCCCAAGAAGCTGCAGCTGCACAGACAGCCCAAGAACCTCATCTTCTGGCGATGGGATGGGGGTGTCTACGGTGACA

40BAlaAlaLeuGlyAlaLysLysLeuGlnProAlaGlnThrAlaAlaLysAsnLeuIleIlePheLeuGlyAspGlyMetGlyValSerThrValThr

BamHI (708) NdeI (775)

701 GCTGCCAGGATCTAAAAGGGCAGAAGAAGGACAAACTGGGGCTGAGATACCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACA

74AlaAlaArgIleLeuLysGlyGlnLysLysAspLysLeuGlyProGluIleProLeuAlaMetAspArgPheProTyrValAlaLeuSerLysThrTyrA

801 ATGTAGACAACATGTGCCAGACAGTGGAGCCACAGCCACGGCTACCTGTGGGGTCAAGGCAACTTCCAGACCATTTGGCTTGTAGTGCAGCCGCCCG

107snValAspLysHisValProAspSerGlyAlaThrAlaThrAlaTyrLeuCysGlyValLysGlyAsnPheGlnThrIleGlyLeuSerAlaAlaAlaAr

901 CTTTAAACCAGTGCACACAGCAGCGGCAACGAGGTCTCCGTGATGAATCGGGCAAGAAGCAGGGAAGTCACTGGGAGTGGTAACCCACACACGA

140gPheAsnGlnCysAsnThrThrArgGlyAsnGluValIleSerValMetAsnArgAlaLysLysAlaGlyLysSerValGlyValValThrThrThrArg

1001 GTGCAGCAGCCTCGCCAGCCGGCACCTACGCCACACGGTGAACCCGCACTGGTACTCGGACGCCGACGTGCTGCTCGCCCGCCAGGAGGGGTGCC

174ValGlnHisAlaSerProAlaGlyThrTyrAlaHisThrValAsnArgAsnTyrTyrSerAspAlaAspValProAlaSerAlaLeuGlyCysG

1101 AGGACATCGCTACGCAGCTCATCTCAACATGGACATTGATGTGATCTGGTGGAGGCCGAAAGTACATGTTTCCGATGGGAACCCAGACCTGAGTA

207rPheAsnIleAlaThrGlnLeuIleSerAsnMetAspIleAspValIleLeuGlyGlyArgLysTyrMetPheArgMetGlyThrProAspProGluTyr

1201 CCCAGATGACTACAGCCAAGGTGGACACAGGCTGGACGGGAAGAATGTGGTGCAGAAATGGCTGGCAAGCCAGGCTGCGGATGTGTGGAAACCC

240rProAspAspTyrSerGlnGlyThrArgLeuAspGlyLysAsnLeuValGlnGluTyrLeuAlaLysArgGlnGlyAlaArgTyrValTyrAsnArg

1301 ACTGAGCTCATGACGCTTCCCTGGACCCGTCTGTGACCCATCTCATGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCAGACTCCACAC

274ThrGluLeuMetGlnAlaSerLeuAspProSerValThrHisLeuMetGlyLeuPheGluProGlyAspMetLysTyrGluIleHisArgAspSerThrL

1401 TGGACCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCTGCTGAGCAGGAACCCCGCGGCTTCTTCTTCTCGTGGAGGGTGGTTCGATCGACCA

307euAspProSerLeuMetGluMetThrGluAlaAlaLeuArgLeuLeuSerArgAsnProArgGlyPhePheLeuPheValGluGlyArgIleAspHi

1501 CGGTCATCACGAAAGCAGGGCTTACCGGCCTGACTGAGACGATGTGTTGACGACGCCATTGAGAGGGCGGCCAGCTCACCAGCGAGGAGGACCG

340rGlyHisHisGluSerArgAlaTyrArgAlaLeuThrGluTyrIleMetPheAspAspAlaIleGluArgAlaGlyLysLeuThrSerGluGluAspThr

BbsI (1636)

1601 CTGAGCCTCGTCACTGCCGACCACTCCACGTCTTCTCCTCGAGGCTACCCCTGCGAGGGAGCTCCATCTTGGGCTGGCCCTGGCAAGGCCGGG

374LeuSerLeuValThrAlaAspHisSerHisValPheSerPheThrGlyGlyTyrProLeuArgGlySerSerIlePheGlyLeuAlaProGlyLysAlaArgA

StuI (1709)

1701 ACAGGAAGGCTACACGGTCTCTATACGGAACCGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCGGATGTTACCGAGAGCGAGAGCGGGAGCCC

407spArgLysAlaTyrThrValLeuLeuTyrGlyAsnGlyProGlyTyrValLeuLysAspGlyAlaArgProAspValThrGluSerGlySerGlySerPr

1801 CGAGTATCGGCAGCAGTCAAGCAGTCCCTGGACGAAGAGACCCAGCAGGCGAGGAGCTGGCGGTGTTGCGCGCGGCCCGCAGGCGCACCTGGTTCC

440oGluTyrArgGlnGlnSerAlaValProLeuAspGluGluThrHisAlaGlyGlyAspValAlaValPheAlaArgGlyProGlnAlaHisLeuValHis

1901 GCGGTGCAGGAGCAGACCTTATAGCGCACGTCTGCGCTTCCGCGCTGCTGGAGCCCTACACCGCTGCGACCTGGCGCCCGCCCGCCGACCCCG

474GlyValGlnGluGlnThrPheIleAlaHisValMetAlaPheAlaAlaCysLeuGluProTyrThrAlaCysAspLeuAlaProProAlaGlyThrThrA

2001 ACGCCGCGACCCGGGGGCTCCCGTCCAAGCGTCTGATTTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGCAAACCACTA

507spAlaAlaHisProGlyArgSerArgSerLysArgLeuAsp•••

HpaI (2186)

2101 GAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAAACAACAATTG

2201 CATTCAATTTATGTTTCAGGTTCAAGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTTCAAATACAGCATAG

2301 CAAAACCTTAACTCCAAATCAAGCCTCACTTGAATCCTTTCTGAGGATGAATAAGGCATAGGCATCAGGGCTGTTGCCAATGTGCATTAGCTGTT

2401 TGCAGCCTCACCTCTTTCATGGAGTTTAAAGATATAGTGATTTTCCAAGGTTTGAAGTACGCTCTTCAATTTCTTTATGTTTTAAATGCATGACTCC

Swal (2536)

2501 ACATTCCTTTTGTAGTAAATATTCAGAATAATTTAAATACATCATTGCAATGAAAAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCT

2601 TCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGAACCTTAAATAGAAATTTGGACAGCAAGAAAGCAGCTTCTAGCTTATCTCAGTCC

127•••Gly•••AspG

2701 TGCTCCTCTGCCCAAAGTGACGACAGTTGCCGGCGGGTCCGCGAGGGCAACTCCCGCCCGCAGGCTGCTCGCCGATCTGGTCATGGCCGGCCGG

122IleGluGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluArgGlyTyrProGlnGluGlyIleGluThrMetAlaProGlySer

2801 AGGCGTCCCGGAAGTTCTGTGGACGACCTCCGACCACTCGCGGTACAGCTGCTCAGGCGCGCACCCACCCAGCCAGGGGTGTTGTCGGCCACCC

89rAlaAspArgPheAsnThrSerValValGluSerTyrGluAlaTyrLeuGluAspLeuGlyArgValTyrValTyrAlaLeuThrAsnAspProValVal

SgrAI (2949)

2901 CTGGTCTGGACCGCTGATGAACAGGGTCACTGCTCCCGGACCAACCGCGGAAGTCTCTCCACGAAGTCCCGGGAGAACCAGCGGCTCGGTC

56GlnAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThrT

3001 CAGAATCTGACCGCTCCGCGACCTCGCGCGGTGAGCAGCCGGAACCGGCACTGGCTCAACTGGCCATGATGGCCCTCTATGATGAGTCTATTATA

22rPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMet

AseI (3124)

3101 ATGCCGATATACTATGCCGATGATTAATTGTCACTACTGTTGTAGGCGCGGTACAGCTTGGATCTGTAACGGCGCAGAACAGAAACGAAACAAAG

3201 ACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGTCTAGTCTAGGTAGGCTCCAAGGGAGCGCCGACAAAGCCCGGCTCTGACCTGA

3301 GCTTTAACTTACCTAGACGGCGGACGAGTTCAGGAGGCCACAGGGGGAGCGGCAGAACCGGACTCAACCGCGTGGATGGCGGCTCAGGTAGG

HindIII (3437)

3401 GCGGGCGGCGGTGAAGGAGAGATGCGAGCCCTCGAAGCTTCACTGTGTTCTGGCGGAAACCCGTTGCAAAAAGAACGTTACGGGCACTACTGCA

3501 CTTATATACGGTTCTCCCCACCCTCGGGAAAAAGGCGGAGCCAGTACACGACATCACTTTCCAGTTTACCCCGGCCACCTTCTCTAGGCACCGGTTCT **AgeI (3593)**

3601 AATTGCCGACCCCTCCCCCAACTTCTCGGGGACTGTGGGCGATGTGCGCTCTGCCCACTGACTAGTGGGCCCTGCAGGTTAATTAAGAACATGTGAGCA **SpeI (3662)** SdaI (3677) PacI (3684)

3701 AAAGGCCAGAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAA

3801 GTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCCTGCCGCTTACCGG

3901 ATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGTTTTCTCATAGCTACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGC

4001 TGTGTGCACGAACCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAG

4101 CAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATT

4201 TGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGTTTTTTTGT

4301 TGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGAAAACCTACGTTAAG

4401 GGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGTTTTTTGTGTAATCG **PacI (4424)** **Swal (4432)** **NotI (4440)**

4501 TAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATC

4601 GAA