

# pDRIVE-Tyr-mTyr

A plasmid with a composite promoter based on the murine Tyrosinase promoter and the Tyrosinase enhancer

Catalog # pdrive-tyrmtyr

**For research use only**

Version # 12J08-MM

## PRODUCT INFORMATION

### Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by pDRIVE-Tyr-mTyr
- GT116 genotype is: *F' mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZM15 ΔlacX74 recA1 rpsL (StrA) endA1 ΔsbcC-sbcD*
- 4 pouches of *E. coli* Fast-Media® Zeo (2TB and 2 Agar)

### Shipping and storage:

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

### Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

## GENERAL PRODUCT USE

pDRIVE is an expression plasmid containing a native or composite promoter of interest. pDRIVE may be used to:

- **Subclone a promoter of interest into another vector.** Unique restriction sites are present at each end of the promoter allowing convenient excision. The 5' sites include *Sda* I, *Pst* I, and *Spe* I. *Sda* I is compatible with *Nsi* I and *Pst* I. *Spe* I is compatible with *Avr* II, *Nhe* I and *Xba* I. The 3' restriction site is *Nco* I which includes the ATG start codon, and is compatible with *Bsp*H I and *Bsp*LU11 I.
- **Compare the activity of different promoters** in transient transfection experiments. Each pDRIVE promoter drives the expression of the *LacZ* reporter gene which allows for testing of the promoter's activity in transient transfection experiments. Furthermore, the *LacZ* gene is flanked by unique restriction sites (*Nco* I and *Eco*R I) for easy replacement with a different gene of interest.

## COMPOSITE PROMOTER CHARACTERISTICS

Element	Name	Origin	Size bp
Promoter	Tyr	Murine	258
5'UTR	Tyr	Murine	78
Enhancer	Tyr	Murine	201

### Composite Tyrosinase promoter

Tyrosinase, the rate-limiting enzyme in melanin synthesis, is expressed specifically in pigment-producing cells. Expression of tyrosinase is regulated by cyclic AMP (cAMP). The M-box 70-bp upstream from the TATA-box and the E-box located downstream the TATA-box, near to the initiator site, are involved in the regulation of the tyrosinase promoter activity by cAMP. Microphthalmia, a basic helix loop helix transcription factor, binds to these regulatory elements and modulates the transcriptional activity of the tyrosinase promoter. cAMP elevating agents, such as forskolin and a-MSH, lead to a strong stimulation of transcriptional activity of the tyrosinase promoter by increasing binding of microphthalmia to the M-box and to the E-box<sup>1</sup>. Melanoma-specific expression of a transgene can be achieved by using the tyrosinase promoter<sup>2</sup>, this targeted expression being further amplified by the melanocyte-specific enhancer.

1- Bertolotto C. *et al.* 1996. *J Cell Biol.* 134(3): 747-55.

2- Vile R. *et al.* 1994. *Gene Ther.* 1(5): 307-16

## PLASMID FEATURES

- **LacZ gene** encodes β-galactosidase an enzyme that catalyzes the hydrolysis of X-Gal, producing a blue precipitate that can be easily visualized under a microscope.
  - **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
  - **pMB1 Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.
  - **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
  - **Sh ble** gene confers zeocin resistance therefore allowing the selection of transformed *E. coli* carrying a pDRIVE plasmid.
- Note:** Stable transfection of clones cannot be performed due to the absence of an eukaryotic promoter upstream of the *Sh ble* gene.

## METHODS

### Growth of pDRIVE-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 a zeocin LB agar plate prepared with the *E. coli* Fast-Media® Zeo 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 zeocin using the Fast-Media® Zeo liquid provided (see below).
- 5- Extract the pDRIVE plasmid DNA using the method of your choice.

**Note:** For long-term storage of the pDRIVE-transformed bacteria, prepare a 20% glycerol stock of the bacteria grown in the overnight liquid culture and freeze at -80°C.

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

*E. coli* Fast-Media® Zeo is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* Fast-Media® Zeo is a TB (liquid) or LB (solid) based medium with zeocin, and contains stabilizers.

*E. coli* Fast-Media® Zeo can be ordered separately (catalog code # fas-zn-1, fas-zn-s).

### Method:

- 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

Toll free (US): 888-457-5873

Outside US: (+1) 858-457-5873

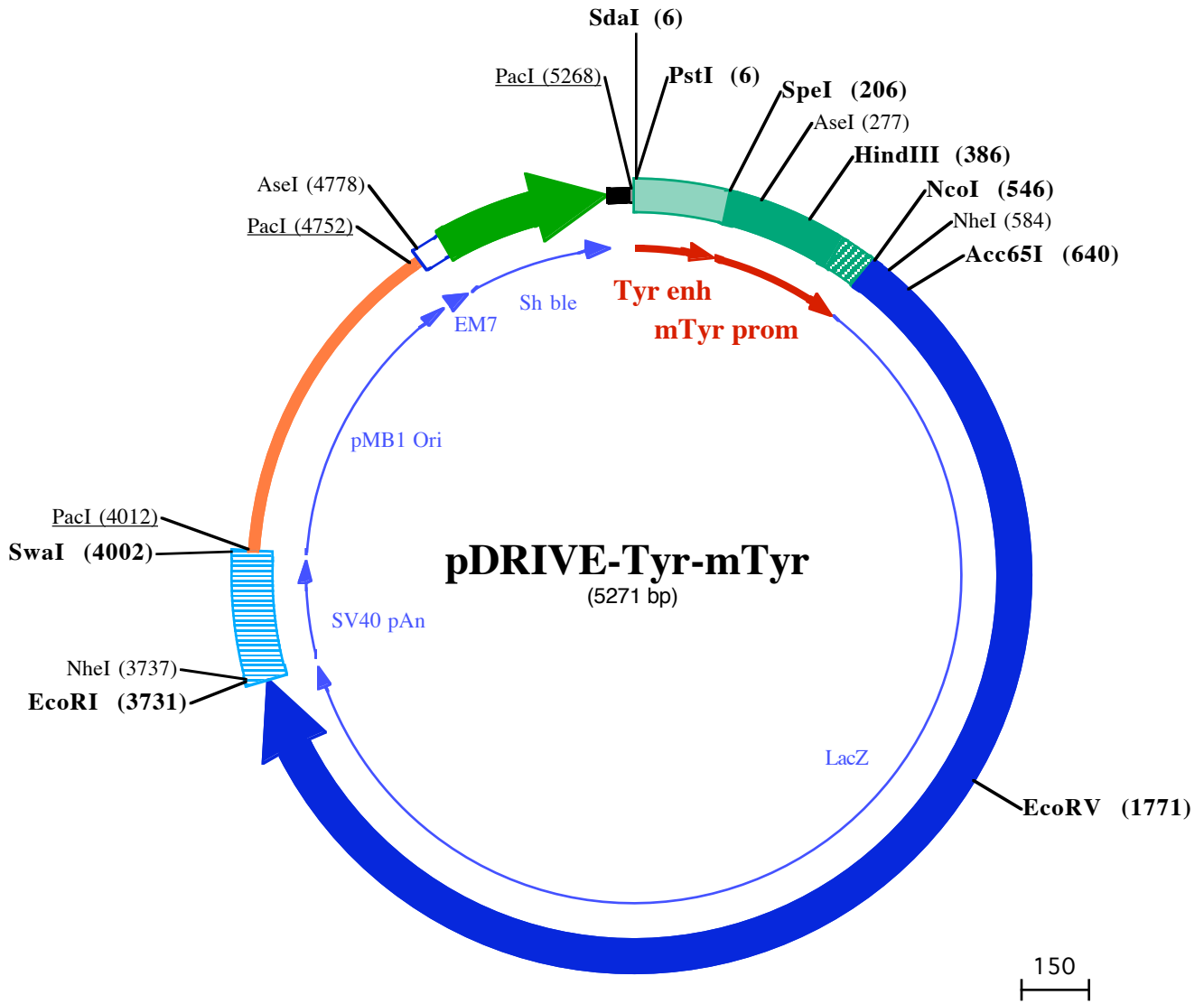
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**PstI (6)**  
**SdaI (6)**  
1 CCTGCAGGTCATAGTTCCTGCCAGCTGACTTTGTCAAGACAGTGATGTCTGTGTTCCAGCAGTTGTTCTGAGTATCCTTTTATTATCCACTGTCTTTT  
101 TTCTTAAATTCACCCCAACATTGTAATAGTCTTTCTTAAACTCTGTTCAAAGAACCAGCTTGAGTGTGTACAGTCTTCTGCTGGGGTCTGGC

**SpeI (206)** **AseI (277)**  
201 AAACCACTAGTGACCTTTATTCCATAAGAGATGATGTATTCTTGATACTTCTCATTTGCAAAATCCAATTATTATTAATTTTCATATCAATTAGAATAA

**HindIII (386)**  
301 TATATCTTCTTCAATTTAGTTACCTCACTATGGGCTATGTACAAACTCCAAGAAAAAGTTAGTCATGTGCTTTGCAGAAGATAAAAAGCTTAGTGTAATAA  
401 CAGGCTGAGAGTATTTGATGTAAGAAGGGGAGTGGTTATATAGTCTTAGCCAAAACATGTGATAGTCACTCCAGGGTGGCTGAAAAAGAAAGTCTGTGA

**NcoI (546)** **NheI (584)**  
501 CACTCATTAACCTATTGGTGCAGAAATTTGAATGATCTAAAGGAGACCATGGGGGCTTCCATCATCATCATCATGGTATGGCTAGCATGACTGGTGG  
▶▶MetGlySerHisHisHisHisHisHisSlyMetAlaSerMetThrGly

**Acc65I (640)**  
601 ACAGCAAATGGGTCGGGATCTGTACGACGATGACGATAAGGTACCTAAGGATCAGCTTGGAGTTGATCCCCTGTTTTACAACGTCGTGACTGGGAAAA  
18▶yGlnGlnMetGlyArgAspLeuTyrAspAspAspAspLysValProLysAspGlnLeuGlyValAspProValValLeuGlnArgArgAspTrpGluAsn  
701 CCTGGCGTTACCCAACTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGC  
52▶ProGlyValThrGlnLeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnLeuAla  
801 GCAGCCTGAATGGCGAATGGCGCTTTCCTGGTTCGCGCACCAGAAGCGGTGCCGGAAGCTGGCTGGAGTGCATCTTCTGAGGCCGACTGTGCGT  
85▶rGlySerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaAspThrValVa  
901 CGTCCCTCAAACCTGGCAGATGACGGTTACGATGCGCCATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTCCACGGAGAAT  
118▶IValProSerAsnTrpGlnMetHisGlyTyrAspAlaProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValProThrGluAsn  
1001 CCGACGGGTTGTTACTCGCTCACATTAATGTTGATGAAAGCTGGCTACAGGAAGCCAGACGCGAATATTTTTGATGGCGTTAACTCGGCGTTTCATC  
152▶ProThrGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyAlaValProGluSerValAspLeuAlaPheHisSLe  
1101 TGTGGTGCAACGGCGCTGGGTGCGTTACGGCCAGGACAGTCTGTTGCGCTGTAATTTGACCTGAGCGCATTTTTACGCGCCGAGAAAAACCGCTCGC  
185▶euTrpCysAsnGlyArgTrpValGlyTyrGlyGluAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAla  
1201 GGTGATGGTGTGCGTTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGCTCTGTTGCTGCATAAACCG  
218▶aValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspGluAspMetTrpArgMetSerGlyIlePheArgAspValSerLeuLeuHisLysPro  
1301 ACTACAAAATCAGCGATTTCCATGTTGCCACTCGCTTAATGATGATTTACGCGCGCTGACTGGAGGCTGAAAGTTTCAGATGTGCGCGGATTTGCGGTG  
252▶ThrThrGlnIleSerAspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGlyGluLeuArgA  
1401 ACTACCTACGGGTAACAGTTCTTTATGGCAGGGTGAACGCGAGGTGCGCAGCGCCGCGCTTTCGCGGTTGAAATATTCGATGAGCGTGGTGGTTA  
285▶spTyrLeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyTrpAlaProPheGlyGlyGluIleIleAspGluArgGlyGlyTy  
1501 TGCCGATCGCGTACACTCGTCTGAACGTCGAAAACCGGAACTGTGGAGCGCGAAATCCCGAATCTCTACTGTCGCGTGGTTGAACCTGCACACCGCC  
318▶rAlaAspArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHisThrAla  
1601 GACGGCAGCTGATTTGAAGCAGAAGCCTGCGATGTGCGTTCCGCGAGGTGCGGATTTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTC  
352▶AspGlyThrLeuIleGluAlaGluAlaCysAspValGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuAsnGlyLysProLeuLeuIleA

**EcoRV (1771)**  
1701 GAGGCGTTAACCGTCACGAGCATCATCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCTGCTGATGAAGCAGAACAACCTTAA  
385▶rGlyValAsnArgHisGlyHisHisProLeuHisGlyGluValMetAspGluGlnThrMetValGlnAspIleLeuLeuMetLysGluAsnAsnPheAs  
1801 CGCCGTGGCTGTTCCGATTATCCGAACCATCCGCTGGTACACGCTGTGCGACCGCTACGGCCTGTATGGTGGATGAAGCCAATATTGAAACCCAC  
418▶nAlaValArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAsnIleGluThrHis  
1901 GGCATGGTGCAATGAATCGTCTGACCGATGATCCGCGTGGCTACCGCGATGAGCGAACCGGTAACCGCAATGGTGCAGCGCATCGTAATCACCCGA  
452▶GlyMetValProMetAsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluArgValThrArgMetValGlnArgAspArgAsnHisProS  
2001 GTGTGATCATCTGGTTCGCTGGGGAATGAATCAGGCCACGGCCTAATCACGACGCGCTGATCGCTGGATCAAATCTGTCGATCCTTCCGCGCGGTGCA  
485▶erValIleIleTyrSerLeuGlyAsnGlySerGlyHisGlyAlaAsnHisAlaAsnAlaLeuTyrArgTrpIleLysSerValAspProAlaProValGly  
2101 GTATGAAGGCGGCGGAGCCGACACCAGCCACCGATATTATTTGCCGATGTACGCGCGCTGGATGAAGACCAGCCCTTCCGCGCTGTGCCGAAATGG  
518▶nTyrGluGlyGlyAlaAspThrThrAlaThrAspIleIleCysProMetTyrAlaArgValAspGluAspGluProPheProAlaValProLysTrp  
2201 TCCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCGCTGATCCTTTCGCAATACGCCACGCGATGGTAACAGTCTTGGCGGTTTCGCTAAAT  
552▶SerIleLysLysTrpLeuSerLeuProGlyGluThrArgProLeuIleLeuCysGluTyrAlaHisSAlaMetGlyAsnSerLeuGlyGlyPheAlaLysT  
2301 ACTGGCAGGCGTTTCGTCAGTATCCCGTTCACAGGCGCGCTTCTGCTGGGATGGTGGATCAGTCGCTGATTAATATGATGAAAACGGCAACCCGTG  
585▶yTrpGlnAlaPheArgGlnTyrProArgLeuGlnGlyGlyPheValTrpAspTrpValAspGlnSerLeuIleLysTyrAspGluAsnGlyAsnProTr  
2401 GTCGGCTTACGGCGGTGATTTGGCGATACGCCGAACGATCGCCAGTCTGTATGAACGCTTGGTCTTTCGCCAGCCGACGCCGATCCAGCGCTGACG  
618▶pSerAlaTyrGlyGlyAspPheGlyAspThrProAsnAspArgGlnPheCysMetAsnGlyLeuValPheAlaAspArgTrpHisSProAlaLeuThr  
2501 GAAGCAAAACACAGCAGCAGTTTTCCAGTTCGTTTTATCCGGCAAACTCCGAAAGTGAACGACCGCAATACCTGTCGATGACGCGGTAACGAGCTCC  
652▶GluAlaLysHisGlnGlnGlnPhePheGlnPheArgLeuSerGlyGlnThrIleGluValThrSerGluTyrLeuPheArgHisSerAspAsnGluLeuL  
2601 TGCACTGGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTGGATGTGCTCCACAAGGTAAACAGTTGATTGAACCTGCCTGAAC  
685▶euHisSTrpMetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspValAlaProGluGlyLysGlnLeuIleGluLeuProGluLe  
2701 ACCGCAAGCGGAGCGCGCGCAACTTGCCTCACAGTACGCGTAGTGCAACGAAACGCGACCGCATGGTGAAGCCGCGGCAACCTTATGATGAACTGCGGATGCGCAG  
718▶uProGlnProGluSerAlaGlyGlnLeuTrpLeuThrValArgValValGlnProAsnAlaThrAlaTrpSerGluAlaGlyHisSleSerAlaTrpGln  
2801 CAGTGGCGTCTGGCGAAAACCTCAGTGTGACGCTCCCGCGCGCTCCACGCCATCCCGCATCTGACCACAGCGAAATGGATTTTGCATCGAGCTGG  
752▶GlnTrpArgLeuAlaGluAsnLeuSerValThrLeuProAlaAlaSerHisSAlaIleProHisSLeuThrThrSerGluMetAspPheCysIleGluLeuG  
2901 GTATAAGCGTTGGCAATTAACCGCAGTACGGCTTTCTTACAGATGTGGATGGCGGATGAAAGCAAAACAACTGCTGACGCGCGGCAACCTTATGATCA  
785▶lyAsnLysArgTrpGlnPheAsnArgGlnSerGlyPheLeuSerGlnMetTrpIleGlyAspLysLysGlnLeuLeuThrProLeuArgAspGlnPheTh  
3001 CCGTGACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGATTTGACCTAACGCTGGTTCGAACGCTGGAAGCGCGCGGCCATTACAGGCC  
818▶rArgAlaProLeuSerAsnAspIleGlyValSerGluAlaThrArgIleAspProAsnAlaTrpValGluArgTrpLysAlaAlaGlyHisSTrpGlnAla  
3101 GAAGCAGCGTTGTTGTCAGTGCACGCGAGATACACTTGTGTAGCGGTGCTGATTACGACCGCTACCGCTGAGCAGTACAGGCAAAACCTTATTTATCA  
852▶GluAlaAlaLeuLeuGlnCysThrAlaAspThrLeuAlaAspAlaValLeuIleThrThrAlaHisSAlaTrpGlnHisGlnGlyLysThrLeuPheIleS  
3201 GCCGAAAACCTACCGATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCGGCGCGGATTGGCCTGAA  
885▶erArgLysThrTyrArgIleAspGlySerGlyGlnMetAlaIleThrValAspValGluValAlaSerAspThrProHisSProAlaArgIleGlyLeuAs  
3301 CTGCCAGTCGGCAGAGTACGAGCGGGTAACTGGCTGGATTAGGGCCGCAAGAAAACCTTCCGACCGCTTACTCCGCGCTGTTTTGACCGCTGG  
918▶nCysGlnLeuAlaGlnValAlaGluArgValAsnTrpLeuGlyLeuGlyProGlnGluAsnTyrProAspArgLeuThrAlaAlaCysPheAspArgTrp  
3401 GATCTGCCATTGTACAGACATGTATACCCGTACGCTTCCGAGCGAAAACGGTCTGCGCTGCGGACCGCGAATGAATATGCCCCACACAGTGGC  
952▶AspLeuProLeuSerAspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyProHisSlnTrpA

3501 GCGGCGACTTCCAGTTCAACATCAGCCGCTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCACATGGCTGAA  
985▶ r g G l y A s p P h e G l n P h e A s n I l e S e r A r g T y r S e r G l n G l n L e u M e t G l u T h r S e r H i s A r g H i s L e u L e u H i s A l a G l u G l u G l y T h r T r p L e u A s  
3601 TATCGACGGTTCCATATGGGGATTGGTGCGGACGACTCCTGGAGCCCGTCAGTATCGGCGGAATACAGCTGAGCGCCGGTCGCTACCATTACCAGTTG  
1018▶ n l l e A s p G l y P h e H i s M e t G l y l l e G l y G l y A s p A s p S e r T r p S e r P r o S e r V a l S e r A l a G l u L e u G l n L e u S e r A l a G l y A r g T y r H i s T y r G l n L e u

NheI (3737)

**EcoRI (3731)**

3701 GTCTGGTGTCAAAAATAATAATCTAGTCGAGAATTCGCTAGCTCGACATGATAAGATACATTGATGAGTTGGACAAACCACAAC TAGAATGCAGTGAAA  
1052▶ V a l T r p C y s G l n L y s •••  
3801 AAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAG

**SwaI (4002)**

3901 TTAACAACAACAATTGCATTCTTTTATGTTTCAGGTT CAGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTAGATCCAT

PacI (4012)

4001 TTA A A T G T T A A T T A A C T A G C C A T G A C C A A A A T C C C T T A A C T G A G T T T T C G T T C C A C T G A G C G T C A G A C C C G T A G A A A A G A T C A A A G G A T C T T C T T G A G

4101 A T C C T T T T T T T C T G C G C G T A A T C T G C T G T T G C A A A C A A A A A A C C A C C G C T A C C A G C G G T G G T T T G T T T G C C G G A T C A A G A G C T A C C A A C T C T T T T C C

4201 G A A G G T A A C T G G C T T C A G C A G A G C G C A G A T A C C A A A T A C T G T T C T T A G T G T A G C C G T A G T T A G G C C A C C A C T T C A A G A A C T C T G T A G C A C C G C C T A C A

4301 T A C C T C G C T C T G T A A T C C T G T T A C C A G T G G C T G C T G C C A G T G G C G A T A A G T C G T G T C T T A C C G G T T G G A C T C A A G A C G A T A G T T A C C G A T A A G G C C

4401 A G C G G T C G G G C T G A A C G G G G G T T C G T G C A C A G C C C A G C T T G G A G C G A A C G A C C T A C C G A A C T G A G A T A C C T A C A G C G T G A G C T A T G A G A A A G C G C

4501 C A C G C T T C C C G A A G G G A A A G G C G G A C A G G T A T C C G G T A A G C G G C A G G G T C G G A A C A G G A G A G C G C A C G A G G G A G C T T C A G G G G A A A C G C C T G G T A T

4601 C T T T A T A G T C C T G T C G G G T T T C G C C A C C T C T G A C T T G A G C G T C G A T T T T T G T G A T G C T C G T C A G G G G G C G G A G C C T A T G G A A A A C G C C A G C A A C G C G G

PacI (4752)

AseI (4778)

4701 C C T T T T T A C G G T T C C T G C C T T T T G C T G C C T T T T G C T C A C A T G T T C T T A A T T A A A T T T T C A A A G T A G T T G A C A A T T A A T C A T C G G C A T A G T A T A T C G

4801 G C A T A G T A T A A T A C G A C T C A C T A T A G G A G G C C A T C A T G G C C A A G T T G A C C A G T G C T G T C C C A G T G C T C A C A G C C A G G G A T G T G G C T G G A G C T G T T G A G T

4901 T C T G G A C T G A C A G G T T G G G G T T C T C C A G A G A T T T T G T G G A G G A T G A C T T T G C A G G T G T G G T C A G A G A T G A T G T C A C C C T G T T C A T C T C A G C A G T C C A G G A

5001 C C A G G T G G T G C C T G A C A A C C C T G G C T T G G G T G T G G G T G A G A G A C T G G A T G A G C T G T A T G C T G A G T G G A G T G A G G T G G T C C C A C C A A C T T C A G G G A T

5101 G C C A G T G G C C C T G C C A T G A C A G A G A T T G G A G A G A G C C C T G G G G G A G A G A G T T T G C C C T G A G A G A C C C A G C A G G C A A C T G T G T G C A C T T T G T G G C A G A G G

PacI (5268)

5201 A G C A G G A C T G A G G A T A A G A A T T G A G T T T C A G A A A A G G G G C C T G A G T G G C C C T T T T T T C A A C T T A A T T A A