

pDRIVE-hROSA

A plasmid with the native human ROSA promoter

Catalog # pdrive-hrosa

For research use only

Version # 05H18-MT

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-hROSA GT100 genotype is: *F*-, *mcrA*, Δ (*mrr-hsdRMS-mcrBC*), Φ 80*lacZ* Δ M15, Δ *lacX74*, *recA1*, *endA1*.
- 4 pouches of *E. coli* Fast-Media® Zeo

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® is 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.
- Promoter activity has been confirmed by transient transfection of 293 cells as well as other selected cell lines.

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' site is *Sda* I. *Sda* I is compatible with *Nsi* I and *Pst* I. The 3' restriction site is *Bsp*H I which includes the ATG start codon, and is compatible with *Nco* I, *Afl* III and *Sty* 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.

PROMOTER CHARACTERISTICS

Element	Name	Origin	Size bp
Promoter	ROSA	Human	2815
5'UTR	ROSA	Human	1323
Enhancer	-	-	-

The ROSA26 promoter, initially identified by random retroviral gene trapping in mouse embryonic stem cells¹, directs expression of reporter² and recombinase genes³ in all cells throughout embryonic development and in adult tissues. This TATA-less promoter is very effective *in vitro* in a very broad range of mammalian cell lines. The strength of the ROSA26 promoter is ascribed to the 10 potential Sp1 sites found within the CpG island extending from the proximal promoter to the the first half of intron 1, the highest number of Sp1 sites ever recorded in any natural promoter. The human ROSA promoter provided by InvivoGen contains at its 3' end a synthetic intronic sequence featuring a consensus splice acceptor site.

1. Zambrowicz BP., Imamoto A. *et al.* 1997. Proc Natl Acad Sci USA. 94:3789-94
2. Kisseberth WC., Brettingen NT., Lohse JK., Sandgren EP. 1999. Dev Biol. 214:128-38.
3. Farley FW, Soriano P, Steffen LS, Dymecki SM. 2000. Genesis. 28:106-10

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 **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. *E. coli* Fast-Media® Zeo can be ordered separately (reference # fas-zn-l, 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.

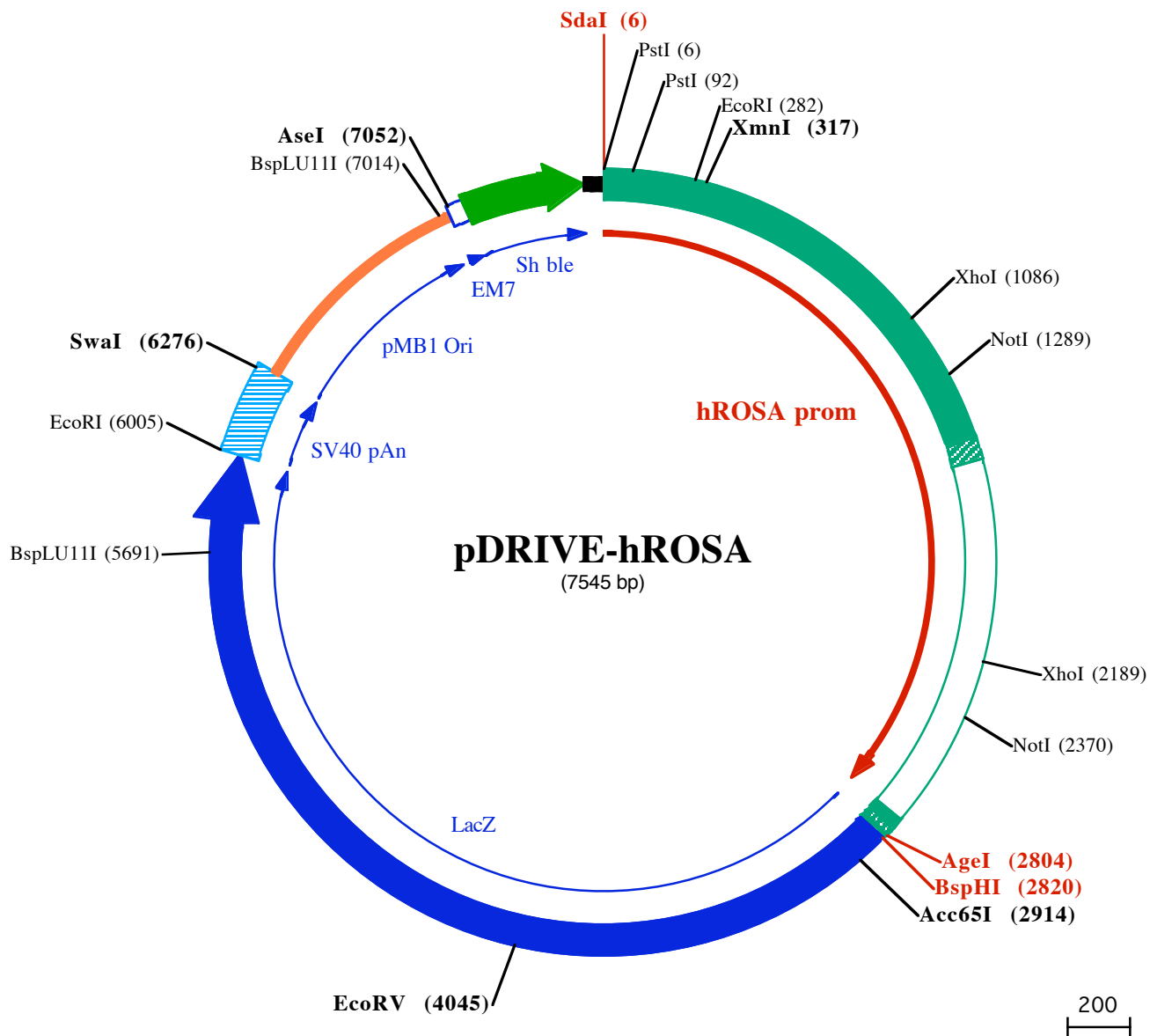
Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

TECHNICAL SUPPORT

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3401 TACGGCCAGGACAGTCGTTTCCGCTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACCGCTCGCGGTGATGGTCTGCGTTGGAGTGACG
194 TyrGI yGI nAspSer ArgLeuP roSer GI uPheAspLeuSer Al aPheLeuArgAl aGI yGI uAsnArgLeuAl aVal MetVal LeuArgTrpSer AspG
3501 GCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGCTCTGTTGTCATAAACCGACTACACAATCAGCGATTTCCATGT
227 I ySer TyrLeuGI uAspGI nAspMet TrpArgMet Ser GI yI ePheArgAspVal Ser LeuLeuHi sLysP roThr Thr GI nI eSer AspPheHi sVa
3601 TCCACTCGCTTAAATGATGATTTACGCCGCTGTACTGGAGGCTGAAGTTTCAGATGTGCGCGGAGTTGCGTGACTACCTACGGGTAAACGTTTCTTTA
260 I Al aThr ArgPheAsnAspAspPheSer ArgAl aVal LeuGI uAl aGI uVal GI nMetCysGI yGI uLeuArgAspTyrLeuArgVal Thr Val Ser Leu
3701 TGGCAGGGTAAACGACAGTCCGCGAGCGGCACCGCCTTTCCGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTACACTACGCTCTGA
294 TrpGI nGI yGI uThr GI nVal Al aSer GI yThr Al aProPheGI yGI yGI uI eI eAspGI uArgGI yGI yTyrAl aAspArgVal Thr LeuArgLeuA
3801 ACGTCGAAAACCCGAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCCTGGTGGTGAAGTGCACACCGCCGACGGCAGCTGATTGAAGCAGAAGC
327 snVal GI uAsnProLysLeuTrpSer Al aGI uI eP roAsnLeuTyrArgAl aVal Val GI uLeuHi sThr Al aAspGI yThr LeuI eGI uAl aGI uAl
3901 CTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTGAGGGCTTAACCGTCACGAGCATCAT
360 aCysAspVal GI yPheArgGI uVal A rgl I eGI uAsnGI yLeuLeuLeuLeuAsnGI yLysP roLeuLeuI eArgGI yVal AsnArgHi sGI uHi sHi s

EcoRV (4045)

4001 CCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCTGCTGATGAAGCAGAACAACCTTAAACGCCGTGCGCTGTTCCGATTATCCGA
394 ProLeuHi sGI yGI nVal MetAspGI uGI nThr MetVal GI nAspI l eLeuLeuMetLysGI nAsnAsnPheAsnAl aVal A r gCysSer Hi sTyrProA
4101 ACCATCCGCTGTGGTACAGCTGTGCGACCGCTACGGCTGTATGTGGTGGATGAAGCAATATTGAAACCCACGGCATGGTGCCAAATGAATCGTCTGAC
427 snHi sP uThr ArpTrpTyrLeuI l eLeuCysAspGI uTyrAl aHi sAl aMetVal yAsnSer LeuGI yGI yPheAl aLysTyrGI uGI nGI nPheTh
4201 CGATGATCCGCGCTGGTACCGCGATGAGCGAACCGTAACCGAATGGTGCAGCGCATCGTAATACCCGAGTGTGATCATCTGGTCCGCTGGGGAAT
460 rAspAspP roArgTrpLeuP roAl aMe tSer GI uArgVal Thr ArgMetVal GI nArgAspArgAsnHi sP roSer Val I l eI l eTrpSer LeuGI yAsn
4301 GAATCAGGCCACGGCCTAATCACGACGCGCTGTATCGTGGATCAATCTGCTGATCCTTCCCGCCCGTGCAGTATGAAGCGCGCGGAGCCGACACCA
494 GI uSer GI yHi sGI yAl aSer GI yAl aSer Hi sAspAl aLeuTyrArgTrpI l eLysVal Ser Val AsnArgP roVal GI nTyrGI uGI nGI nPheTh
4401 CGGCCACCGATATTATTTGCCGATGTACGCGCGCTGGATGAAGACCAGCCCTTCCCGCTGTGCCGAAATGGTCCATCAAAAAATGGCTTTCGCTACC
527 hrAl aThrAspI l eI l eCysProMetTyrAl aArgVal AspGI uAspGI nP roPheProAl aVal P roLysTrpSer I l eLysLysTrpLeuSer LeuP r
4501 TGGAGAGACGCGCCGCTGATCCTTTGCGAATACGCCACCGCATGGTAACAGTCTTGGCGGTTTCGCTAAATACTGGCAGCGGTTTCGTCAGTATCCC
560 oGI uSer GI yHi sGI yAl aSer GI yAl aP roLeuAspVal Al aP roGI nGI yLysGI nLeuI l eGI uLeuP roGI uLeuP roGI uSer Al aGI yGI nL
4601 CGTTTACAGGGCGGCTTCGCTGGGACTGGTGGATCAGTCGCTGATTAATATGATGAAAACGGCAACCCGTTGGTGGCTTACGGCGGTGATTTTGGCG
594 ArgLeuGI nGI yGI yPheVal TrpAspTrpVal AspGI nSer LeuI l eLysTyrAspGI uAsnGI yAsnP roTrpSer Al aTyrGI yGI yAspPheGI yA
4701 ATACCGCAACGATCGCCAGTCTGTATGAACGGTCTGGTCTTTGCCAGCCGACGCCGATCCAGCGCTGACGGAAGCAAAACACCAGCAGCAGTTTTT
627 spThr P roAsnAspArgGI nPheCysMetAsnGI yLeuVal P heAl aAspArgThr P roHi sP roAl aLeuThr GI uAl aLysHi sGI nGI nPheP h
4801 CCAGTCCGTTTATCCGGCAAACCATCGAAGTGACCAGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGT
660 eGI nPheArgLeuSer GI yGI nThr I l eGI uVal Thr Ser GI uTyrLeuPheArgHi sSer AspAsnGI uLeuLeuHi sTrpMetVal Al aLeuAspGI y
4901 AAGCCGCTGGCAAGCGGTGAAGTGCCTTGGATGTGCTCCACAAGTAAACAGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGCCGGGCAAC
694 LysP roLeuAl aSer GI yAl aP roLeuAspVal Al aP roGI nGI yLysGI nLeuI l eGI uLeuP roGI uLeuP roGI uSer Al aGI yGI nL
5001 TCTGGCTCACAGTACCGTAGTGCAACCGAACCGACCGCATGGTCAGAAAGCCGGGCACATCAGCGCTGGCAGCAGTGGCTGTGGCGAAAACCTCAG
727 euTrpLeuThr Val A r gVal Val GI nP roAsnAl aThr Al aTrpSer GI uAl aGI yHi sI l eSer Al aTrpGI nGI nTrpArgLeuAl aGI uAsnLeuSe
5101 TGTGACGCTCCCCCGCGTCCCACGCCATCCCGCATCTGACCACCGCAAAATGGATTTTGCATCGAGCTGGTAATAAGCGTTGGCAATTTAACCGC
760 rVal Thr LeuP roAl aAl aSer Hi sAl aI l eP roHi sLeuThr Thr GI uMetAspPheCysI l eGI uLeuGI yAsnLysArgTrpGI nPheAsnArg
5201 CAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACCTGCTGACGCGCTGCGGATCAGTTACCCGTCACCCTGGATAACGACATTG
794 GI nSer GI yPheLeuSer GI nMe tTrpI l eGI yAspLysLysGI nLeuLeuThr P roLeuArgAspGI nPheThr ArgAl aP roLeuAspAsnAspI l eG
5301 GCGTAAGTGAAGCGACCCGATGACCTAACCCCTGGTTCGAAAGCTGGAAGCGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGAGTGCACGGC
827 I yVal Ser GI uAl aThr ArgI l eAspP roAsnAl aTrpVal GI uArgTrpLysAl aAl aGI yHi sTyrGI nAl aGI uAl aLeuLeuGI nCysThr Al
5401 AGATACACTTGTGATGCGGTGCTGATTACGACCGCTCACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAACCTACCGGATTGATGGT
860 aAspThr LeuAl aAspAl aVal LeuI l eThr Thr Al aHi sAl aTrpGI nHi sGI nGI yLysThr LeuPheI l eSer ArgLysThr TyrArgI l eAspGI y
5501 AGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCAGCGATACCCGATCCGCGCGGATTGGCTGAACTGCCAGCTGGCGAGGTAGCAGAGC
894 Ser GI yGI nMe tAl aI l eThr Val AspVal GI uVal Al aSer AspThr P roHi sP roAl aArgI l eGI yLeuAsnCysGI nLeuAl aGI nVal Al aGI uA

BspLU11I (5691)

5601 GGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAATATCCCGACCGCCTTACTGCCCGCTGTTTTGACCGCTGGGATCTGCCATTGTGACAGCATGTATAC
927 r gVal AsnTrpLeuGI yLeuGI yProGI nGI uAsnTyrP roAspArgLeuThr Al aAl aCysPheAspArgTrpAspLeuP roLeuSer AspMe tTyrTh
5701 CCCGTACGCTTCCCAGAGCAAAACGGTCTGCGCTGCGGGACGCGCAATTAATGAAATGAGCCACACCAAGTGGCGCGGCGACTTCCAGTTCAACATCAGC
960 r P roTyrVal PheP roSer GI uAsnGI yLeuArgCysGI yThr ArgGI uLeuAsnTyrGI yProHi sGI nTrpArgGI yAspPheGI nPheAsnI l eSer
5801 CGCTACAGTCAACAGCAACTGATGAAACACGACCTCGCCATCTGCTGCACGCGGAAGAGGCACATGGCTGAATATCGACGGTTCCATATGGGGATTG
994 ArgTyrSer GI nGI nLeuMe tGI uThr Ser Hi sArgHi sArgHi sAl aGI uGI uGI yThr TrpLeuAsnI l eAspGI yPheHi sMe tGI yI l eG
5901 GTGGCGACGACTCCTGGAGCCGTCAGTATCGCGGAAATACAGCTGAGCGCGGCTGCTACCATACCAAGTTGGTCTGGGTGCAAAAATAATAATCTAG
1027 I yGI yAspAspSer TrpSer ProSer Val Ser Al aGI uLeuGI nLeuSer Al aGI yArgTyrHi sTyrGI nLeuVal TrpCysGI nLys•••

EcoRI (6005)

6001 TCGAGAATTCGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTG
6101 ATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACACAACAATTGCATTCATTTT

SwaI (6276)

6201 ATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGATCCATTTAAATGTTAATTAACAGCCATGAC
6301 CAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGGATCCTTTTTTCTGCGCGTAATCTGC
6401 TGCTTGCAAAACAAAAAACACCGCTACCAGCGGTGTTTGTGGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTACGACAGAGCGC
6501 AGATACCAAATACTGTTCTTCTAGTGTAGCCGATGTAGGCCACCACTTCAAGAACTCTGTAGCACCAGCTACATACCTCGCTGCTAATCCTGTTACC
6601 AGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCGGTTGGACTCAAGACGATAGTTACCGGATAAAGCGCAGCGGTGGGCTGAACGGGGGTTCCG
6701 TGACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGG
6801 ACAGGTATCCGGTAAGCGGACGGTCCGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAAACCGCTGGTATCTTTATAGTCTGTGCGGTTTCGCCA
6901 CCTCTGACTTGAGCGTGCATTTTTGTGATGCTGTCAGGGGGCGGAGCCTATGGAAAACGCCAGCAACCGCGCTTTTTACGGTTCTGGCCTTTTGC

BspLUII (7014)

AseI (7052)

7001 TGGCCTTTTGCTCACATGTTCTTAATTAATTTTCAAAGTAGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAG
7101 GAGGGCCATCATGGCCAAGTTGACCAAGTGTGACCAAGTGTGCTCCAGTGTCTCACAGCCAGGGATGTGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTGGGGTTCTCC
1▶MetAl aLysLeuThr SerAl aValProValLeuThrAl aArgAspValAl aGlyAl aValGluPheTrpThrAspArgLeuGlyPheSer
7201 AGAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGTCACCTGTTCATCTCAGCAGTCCAGGACCAGGTGGTGCCTGACAAACCCCTGG
31▶ArgAspPheValGluAspAspPheAl aGlyValValArgAspAspValThrLeuPheIleSerAl aValGluAspGlnValValProAspAsnThrLeuA
7301 CTTGGTGTGGGTGAGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGGTGGTCTCCACCAACTTCAGGGATGCCAGTGGCCCTGCCATGACAGAGAT
64▶IaTrpValTrpValArgGlyLeuAspGluLeuTyrAl aGluTrpSerGluValValSerThrAsnPheArgAspAl aSerGlyProAl aMetThrGluIle
7401 TGGAGAGCAGCCCTGGGGGAGAGATTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACTTTGTGGCAGAGGAGCAGGACTGAGGATAAGAATTGAGT
97▶eGlyGluGluGlnProTrpGlyArgGluPheAl aLeuArgAspProAl aGlyAsnCysValHisPheValAl aGluGluGluGlnAsp●●●
7501 TTCAGAAAAGGGGCTGAGTGGCCCTTTTTCACCTAATTA