pDRIVE-mROSA

A plasmid with the native mouse ROSA promoter

Catalog # pdrive-mrosa

For research use only

Version # 05H18-MT

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-mROSA GT100 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, 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. Furthermore, the *LacZ* gene is flanked by unique restriction sites (*Bsp*H I and *EcoR* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Element	Name	Origin	Size bp
Promoter	ROSA	Mouse	1926
5'UTR	ROSA	Mouse	457
Enhancer	-	-	-

The ROSA26 promoter, initially identified by random retroviral gene trapping in mouse embryonic stem cells[1], directs expression of reporter[2] and recombinase genes[3] 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 mouse 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.**

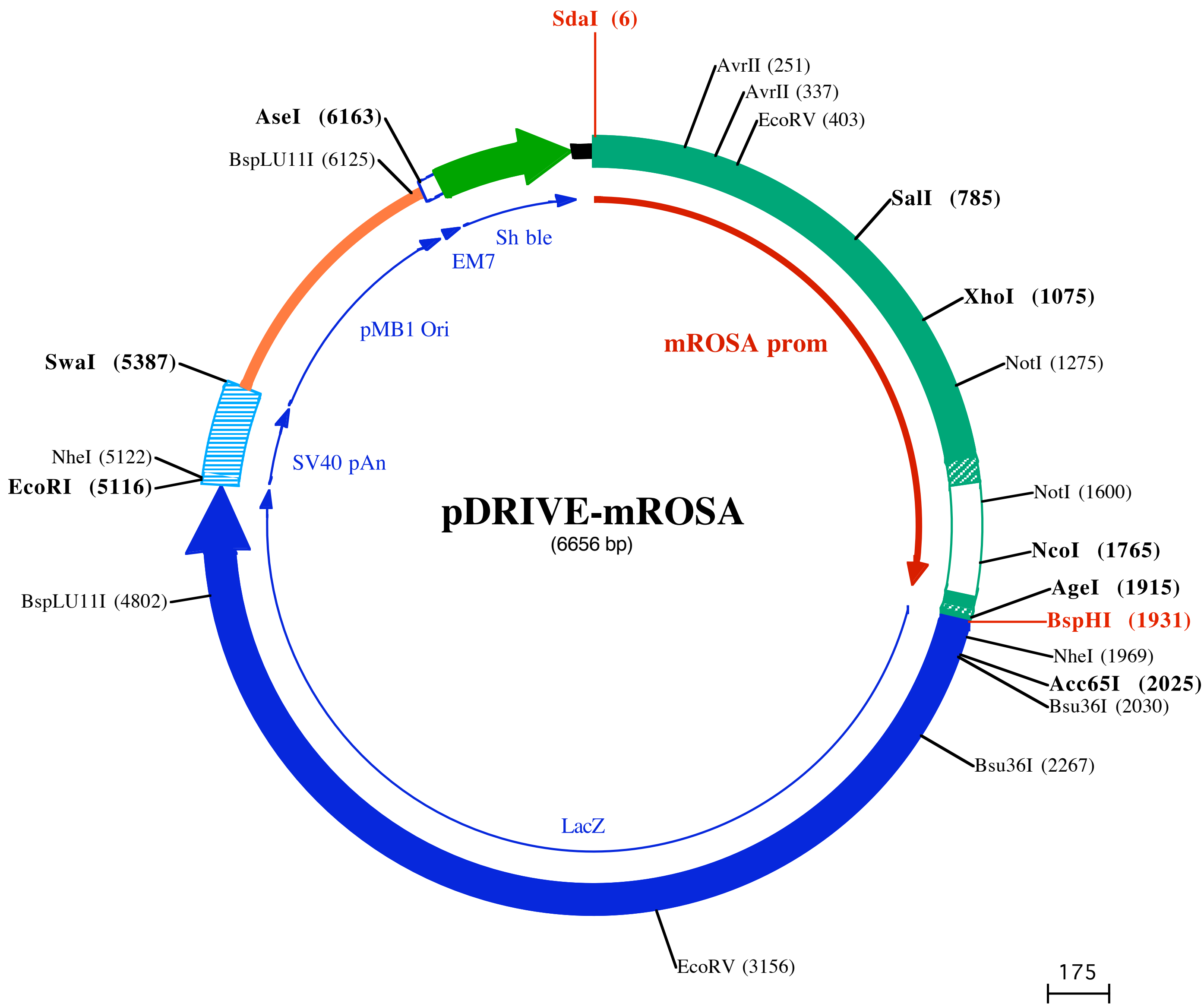
5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.

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
Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
E-mail: info@invivogen.com
Website: www.invivogen.com

InvivoGen
3950 Sorrento Valley Blvd. Suite A
San Diego, CA 92121 - USA


SdaI (6)
AvrII (251)
AvrII (337)
EcoRV (403)
SalI (785)
XhoI (1075)
NotI (1275)
NotI (1600)
NcoI (1765)
AgeI (1915)
BspHI (1931)
NheI (1969)
Acc65I (2025)
Bsu36I (2030)
Bsu36I (2267)
EcoRV (3156)
BspLU11I (4802)
EcoRI (5116)
NheI (5122)
SwaI (5387)
BspLU11I (6125)
AseI (6163)
mROSA prom
Sh ble
EM7
pMB1 Ori
SV40 pAn
LacZ
pDRIVE-mROSA
(6656 bp)
175

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SdaI (6)
   1 CCTGCAGGTGAAGACGTTACACAAGTAACATGAGAAAGCAGAAAATGCAGGTCATCCACGCACCCCTGACCCAGGCCAGCAGGGCGGGCTGCAGCATCAG
 101 TACACAGGAGAAAGATCCTTATTCCTAAGAATGAGAAAGGCAAAGGCGCCCGATAGAATAAATTAGCATAGAAGGGGCTTTCCCAGGAGTTAAAACTTTC
                                                          AvrII (251)
 201 CTTCTGAGCGATTACCTACTAAAACCAGGGCTTTTGCCCACTACCATTTACCTAGGATCTTGGCTTGCACGGATTCATAGGGGCATATCCCTCCCCCTCT
                                            AvrII (337)
 301 TCTTTAGAGTCGTTCTTAAAAGATCGCTCTCCACGCCCTAGGCAGGGAAAACGACAAAATCTGGCTCAATTCCAGGCTAGAACCCTACAAATTCAACAGG
EcoRV (403)
 401 GATATCGCAAGGATACTGGGGCATACGCCACAGGGAGTCCAAGAATGTGAGGTGGGGGTGGCGAAGGTAATGTCTTTGGTGTGGGAAAAGCAGCAGCCAT
 501 CTGAGATAGGAACTGGAAAACCAGAGGAGAGGCGTTCAGGAAGATTATGGAGGGGAGGACTGGGCCCCCACGAGCGACCAGAGTTGTCACAAGGCCGCAA
 601 GAACAGGGGAGGTGGGGGGCTCAGGGACAGAAAAAAAAGTATGTGTATTTTGAGAGCAGGGTTGGGAGGCCTCTCCTGAAAAGGGTATAAACGTGGAGTA
                                                                                          SalI (785)
 701 GGCAATACCCAGGCAAAAAGGGGAGACCAGAGTAGGGGGAGGGGAAGAGTCCTGACCCAGGGAAGACATTAAAAAGGTAGTGGGGTCGACTAGATGAAGG
 801 AGAGCCTTTCTCTCTGGGCAAGAGCGGTGCAATGGTGTGTAAAGGTAGCTGAGAAGACGAAAAGGGCAAGCATCTTCCTGCTACCAGGCTGGGGAGGCCC
 901 AGGCCCACGACCCCGAGGAGAGGGAACGCAGGGAGACTGAGGTGACCCTTCTTTCCCCCGGGGCCCGGTCGTGTGGTTCGGTGTCTCTTTTCTGTTGGAC
                                                                                 XhoI (1075)
1001 CCTTACCTTGACCCAGGCGCTGCCGGGGCCTGGGCCCGGGCTGCGGCGCACGGCACTCCCGGGAGGCAGCGAGACTCGAGTTAGGCCCAACGCGGCGCCA
1101 CGGCGTTTCCTGGCCGGGAATGGCCCGTACCCGTGAGGTGGGGGTGGGGGGCAGAAAAGGCGGAGCGAGCCCGAGGCGGGGAGGGGGAGGGCCAGGGGCG
                                                                                NotI (1275)
1201 GAGGGGGCCGGCACTACTGTGTTGGCGGACTGGCGGGACTAGGGCTGCGTGAGTCTCTGAGCGCAGGCGGGCGGCGGCCGCCCCTCCCCCGGCGGCGGCA
1301 GCGGCGGCAGCGGCGGCAGCTCACTCAGCCCGCTGCCCGAGCGGAAACGCCACTGACCGCACGGGGATTCCCAGTGCCGGCGCCAGGGGCACGCGGGACA
1401 CGCCCCCTCCCGCCGCGCCATTGGCCTCTCCGCCCACCGCCCCACACTTATTGGCCGGTGCGCCGCCAATCAGCGGAGGCTGCCGGGGCCGCCTAAAGAA
                                                                                                   NotI (1600)
1501 GAGGCTGTGCTTTGGGGCTCCGGCTCCTCAGAGAGCCTCGGCTAGgtaggggatcgggactctggcgggagggcggcttggtgcgtttgcggggatgggc
1601 ggccgcggcaggccctccgagcgtggtggagccgttctgtgagacagccgggtacgagtcgtgacgctggaaggggcaagcgggtggtgggcaggaatgc
                                                                  NcoI (1765)
1701 ggtccgccctgcagcaaccggagggggagggagaagggagcggaaaagtctccaccggacgcggccatggctcgggggggggggggcagcggaggagcgc
1801 ttccggccgacgtctcgtcgctgattggcttcttttcctcccgccgtgtgtgaaaacacaattgtactaaccttcttctctttcctctcctgacagGTGT
             AgeI (1915)         BspHI (1931)                              NheI (1969)
1901 GAAACAGGAAGAGAACCGGTAGGAGGGCCATCATGAGCGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAAATGGGTCG
                                1 ▶ MetSerGlySerHisHisHisHisHisHisGlyMetAlaSerMetThrGlyGlyGlnGlnMetGlyAr
                            Bsu36I (2030)
                        Acc65I (2025)
2001 GGATCTGTACGACGATGACGATAAGGTACCTAAGGATCAGCTTGGAGTTGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAA
  23 ▶ gAspLeuTyrAspAspAspAspLysValProLysAspGlnLeuGlyValAspProValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGln
2101 CTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCG
  57 ▶ LeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeuArgSerLeuAsnGlyG
                                                                          Bsu36I (2267)
2201 AATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTG
  90 ▶ luTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaAspThrValValValProSerAsnTr
2301 GCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTAC
 123 ▶ pGlnMetHisGlyTyrAspAlaProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValProThrGluAsnProThrGlyCysTyr
2401 TCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGC
 157 ▶ SerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnThrArgIleIlePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyA
2501 GCTGGGTCGGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCG
 190 ▶ rgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuAr
2601 TTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGC
 223 ▶ gTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMetSerGlyIlePheArgAspValSerLeuLeuHisLysProThrThrGlnIleSer
2701 GATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAA
 257 ▶ AspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyrLeuArgValT
2801 CAGTTTCTTTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCAC
 290 ▶ hrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAlaProPheGlyGlyGluIleIleAspGluArgGlyGlyTyrAlaAspArgValTh
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 323 ▶ rLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspGlyThrLeuIle
3001 GAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTC
 357 ▶ GluAlaGluAlaCysAspValGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuLeuAsnGlyLysProLeuLeuIleArgGlyValAsnArgH
                                                                 EcoRV (3156)
3101 ACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAACAACTTTAACGCCGTGCGCTGTTC
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3201 GCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGGCATGGTGCCAATG
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 457▸ AsnArgLeuThrAspAspProArgTrpLeuProAlaMetSerGluArgValThrArgMetValGlnArgAspArgAsnHisProSerValIleIleTrpS
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 590▸ rgGlnTyrProArgLeuGlnGlyGlyPheValTrpAspTrpValAspGlnSerLeuIleLysTyrAspGluAsnGlyAsnProTrpSerAlaTyrGlyGl
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 623▸ yAspPheGlyAspThrProAsnAspArgGlnPheCysMetAsnGlyLeuValPheAlaAspArgThrProHisProAlaLeuThrGluAlaLysHisGln
3901 CAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGG
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4801 GACATGTATACCCCGTACGTCTTCCCGAGCGAAAACGGTCTGCGCTGCGGGACGCGCGAATTGAATTATGGCCCACACCAGTGGCGCGGCGACTTCCAGT
 957▸ AspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyProHisGlnTrpArgGlyAspPheGlnP
4901 TCAACATCAGCCGCTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTTCCA
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                                NheI (5122)
                   EcoRI (5116)
5101 TAATAATCTAGTCGAGAATTCGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTG
1057▸ •••
5201 TGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATT

                                                                                               SwaI (5387)
5301 GCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGATCCATTTAAATGTTAATTAA
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                                 BspLU11I (6125)                    AseI (6163)
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  27▸ euGlyPheSerArgAspPheValGluAspAspPheAlaGlyValValArgAspAspValThrLeuPheIleSerAlaValGlnAspGlnValValProAs
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  60▸ pAsnThrLeuAlaTrpValTrpValArgGlyLeuAspGluLeuTyrAlaGluTrpSerGluValValSerThrAsnPheArgAspAlaSerGlyProAla
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6601 AAGAATTGAGTTTCAGAAAAGGGGGCCTGAGTGGCCCCTTTTTTCAACTTAATTAA
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