pDRIVE-mCOX-2

A plasmid with the native murine COX-2 promoter

Catalog # pdrive-mcox2

For research use only

Version # 04L17-SV

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by pDRIVE-mCOX-2.
- 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® 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.
- 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' 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 *BspH* I and *BspLU*11 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 *EcoR* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Element	Name	Origin	Size bp
Promoter	COX-2	Mouse	1105
5'UTR	COX-2	Mouse	121
Enhancer	-	-	

mCOX-2 promoter

COX-2 is an inducible isoform of cyclooxygenase, a key enzyme in the conversion of arachidonic acid to prostaglandin. Expression of COX-2 is undetectable under physiological conditions but up-regulated at the transcriptional, post-transcriptional and protein levels in many malignant tumors. COX-2, which is a PKC-dependent gene, is important for the genesis of cancer since its overexpression inhibits apoptosis and increases the invasiveness of tumor cells. COX-2 promoter contains a cAMP response element and sites for AP-2 and NF-κB[1], that are both PKC-responsive cis-elements. COX-2 promoter has been used to target gene expression in pancreatic[2], gastrointestinal[3] and ovarian[4] cancers. Similar levels of expression of luciferase and HSVtk genes were obtained with COX-2 and CMV promoters.

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.

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-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.*

References:

1. Inoue H *et al.* (1995). J Biol Chem 270(42):24965-71.
2. Wesseling JG *et al.* (2001). Cancer Gene Ther 8(12):990-6.
3. Yamamoto M et al. (2001). Mol Ther. 3(3):385-94.
4. Casado E. et al. (2001). Clin Cancer Res. 7(8):2496-504.

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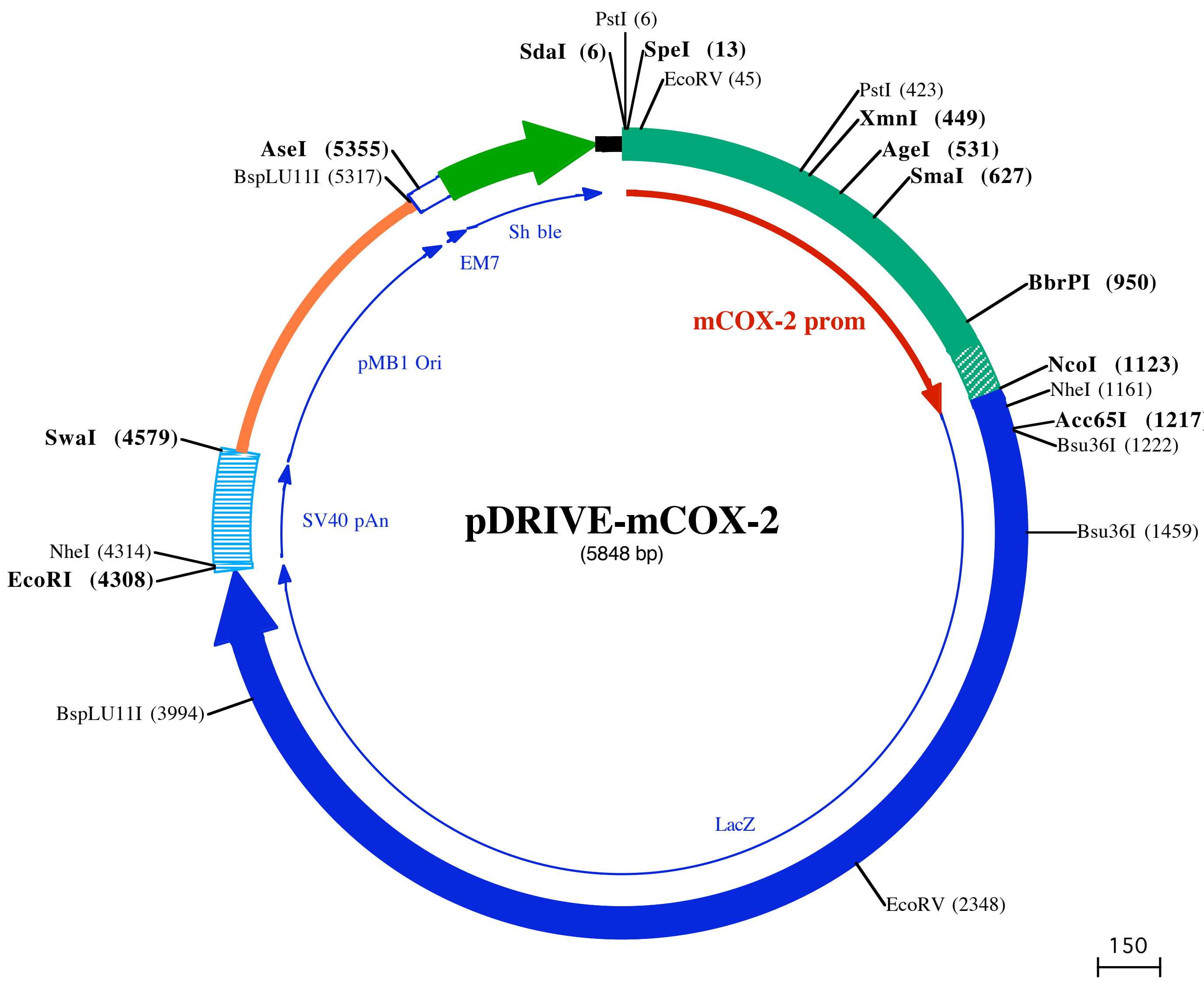
PstI (6)
SdaI (6)
SpeI (13)
EcoRV (45)
PstI (423)
XmnI (449)
AgeI (531)
SmaI (627)
BbrPI (950)
NcoI (1123)
NheI (1161)
Acc65I (1217)
Bsu36I (1222)
Bsu36I (1459)
EcoRV (2348)
BspLU11I (3994)
EcoRI (4308)
NheI (4314)
SwaI (4579)
BspLU11I (5317)
AseI (5355)
mCOX-2 prom
Sh ble
EM7
pMB1 Ori
SV40 pAn
LacZ
pDRIVE-mCOX-2
(5848 bp)
150

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      PstI (6)
      SdaI (6)        SpeI (13)                         EcoRV (45)
   1  CCTGCAGGGCCCACTAGTTGGCCAACACAAACACAGTAGGAAGATATCCAACACTATCAAAAACATCACCTCTCTAGGCAAATAATTTTTTATCAAACAC
 101  TGTTTCTGAATGAAGTGAATTTTTTTTTAAGGTTAGGGAGAATAAGGCTAGTTATGGAAAAATAACTATTACCTTTTGATCCGTTGCCATAACATACTTT
 201  CTTGTAAACATGGACAGAAAAAGTTTTTAAAAGGTACATTTGAAAACAAATTCTTTCTAGTGATACATCCCGTGAAAAGAGTTGCTGATCAAAATGATAA
 301  AAGCTATGTAACAGCAGGGGGAAAATACCTTAAAGCAATGCTGTGGACACTTAGCATTCCGATGAAGTGGAGCTCAGCATCTGTCCCTGGGAAAGGCGAG
                        PstI (423)                  XmnI (449)
 401  TGCCAGGGGCTCCTGTGACTGCAGAACCCGGAGGGTAGTTCCATGAAAGACTTCAACCTAATTCCACCAGTACAGATGTGGACCCTGACAGAGGACACTC
                                   AgeI (531)
 501  ATTTCATTAAAAATAGAAGAAATTAAATTAACCGGTAGCTGTGTGCGTGCTCTGAGCAGCGAGCACGTCAGACTGCGCCCCAGTGGGGAGAGGTGAGGGG
                              SmaI (627)
 601  ATTCCCTTAGTTAGGACCTTAGATCCCGGGAGGGGAAGCTGTGACACTCTTGAGCTTTTAGGCCCCCACTGGATGCGCGACTGGGAGGAAACCGGAGACC
 701  CCAAAGAGCGCCAGACTAGGCGCAGACTCAGCGAACCACAGGGCGCCTGGAGGGATGGAGAGGGCGGTGCAGCTCTCTTGGCACCACCTGGGGCAGCCAA
 801  GGGCAGCTTCCCGGCTTCCTTCGTCTCTCATTTGCGTGGGTAAAAGCCTGCCGCTGCGGTTCTTGCGCAACTCACTGAAGCAGAGAGGGGGAAAAGTTGG
                                                       BbrPI (950)
 901  TGGGGGTTGGGGAAAGCCTAAGCGGAAAGACAGAGTCACCACTACGTCACGTGGAGTCCGCTTTACAGACTTAAAAGCAAGGTTCTCCCCATTAGCAGCC
1001  AGTTGTCAAACTGCGAGCTAAGAGCTTCAGGAGTCAGTCAGGACTCTGCTCACGAAGGAACTCAGCACTGCATCCTGCCAGCTCCACCGCCACCACTACT
                                NcoI (1123)                          NheI (1161)
1101  GCCACCTCCGCTGCCACCTCTGCCATGGGGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGGATCTGT
                             1▸MetGlyGlySerHisHisHisHisHisHisGlyMetAlaSerMetThrGlyGlyGlnGlnMetGlyArgAspLeuT
                              Bsu36I (1222)
                          Acc65I (1217)
1201  ACGACGATGACGATAAGGTACCTAAGGATCAGCTTGGAGTTGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCG
  26▸ yrAspAspAspAspLysValProLysAspGlnLeuGlyValAspProValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsnAr
1301  CCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGC
  59▸ gLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeuArgSerLeuAsnGlyGluTrpArg
                                                                        Bsu36I (1459)
1401  TTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCAGATGC
  93▸ PheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaAspThrValValValProSerAsnTrpGlnMetH
1501  ACGGTTACGATGCGCCCATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCAC
 126▸ isGlyTyrAspAlaProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValProThrGluAsnProThrGlyCysTyrSerLeuTh
1601  ATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTC
 159▸ rPheAsnValAspGluSerTrpLeuGlnGluGlyGlnThrArgIleIlePheAspGlyValAsnSerAlaPheHisLeuTrpCysAsnGlyArgTrpVal
1701  GGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCGTTGGAGTG
 193▸ GlyTyrGlyGlnAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuArgTrpSerA
1801  ACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCCA
 226▸ spGlySerTyrLeuGluAspGlnAspMetTrpArgMetSerGlyIlePheArgAspValSerLeuLeuHisLysProThrThrGlnIleSerAspPheHi
1901  TGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCT
 259▸ sValAlaThrArgPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyrLeuArgValThrValSer
2001  TTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGTC
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2101  TGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGA
 326▸ euAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspGlyThrLeuIleGluAlaGl
2201  AGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGAGCAT
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                                                    EcoRV (2348)
2301  CATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAACAACTTTAACGCCGTGCGCTGTTCGCATTATC
 393▸ HisProLeuHisGlyGlnValMetAspGluGlnThrMetValGlnAspIleLeuLeuMetLysGlnAsnAsnPheAsnAlaValArgCysSerHisTyrP
2401  CGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGGCATGGTGCCAATGAATCGTCT
 426▸ roAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAsnIleGluThrHisGlyMetValProMetAsnArgLe
2501  GACCGATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCGAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGCTGGGG
 459▸ uThrAspAspProArgTrpLeuProAlaMetSerGluArgValThrArgMetValGlnArgAspArgAsnHisProSerValIleIleTrpSerLeuGly
2601  AATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAGCCGACA
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 559▸ uProGlyGluThrArgProLeuIleLeuCysGluTyrAlaHisAlaMetGlyAsnSerLeuGlyGlyPheAlaLysTyrTrpGlnAlaPheArgGlnTyr
2901  CCCCGTTTACAGGGCGGCTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTTTG
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3001  GCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGCTGACGGAAGCAAAACACCAGCAGCAGTT
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 726 lnLeuTrpLeuThrValArgValValGlnProAsnAlaThrAlaTrpSerGluAlaGlyHisIleSerAlaTrpGlnGlnTrpArgLeuAlaGluAsnLe
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3501 CGCCAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACTGCTGACGCCGCTGCGCGATCAGTTCACCCGTGCACCGCTGGATAACGACA
 793 ArgGlnSerGlyPheLeuSerGlnMetTrpIleGlyAspLysLysGlnLeuLeuThrProLeuArgAspGlnPheThrArgAlaProLeuAspAsnAspI
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3901 AGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAACTATCCCGACCGCCTTACTGCCGCCTGTTTTGACCGCTGGGATCTGCCATTGTCAGACATGTA
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                  NheI (4314)
           EcoRI (4308)
4301 TAGTCGAGAATTCGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTT

4401 GTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCAT

                                                                                     SwaI (4579)
4501 TTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGATCCATTTAAATGTTAATTAACTAGCCAT

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4701 TGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAG

4801 CGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTT

4901 ACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGT

5001 TCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGG

5101 CGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCG

5201 CCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTT

                     BspLU11I (5317)               AseI (5355)
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5401 TAGGAGGGCCATCATGGCCAAGTTGACCAGTGCTGTCCCAGTGCTCACAGCCAGGGATGTGGCTGGAGCTGTTGAGTTCTGGACTGACAGGTTGGGGTTC
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5701 GATTGGAGAGCAGCCCTGGGGGAGAGAGTTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACTTTGTGGCAGAGGAGCAGGACTGAGGATAAGAATTG
  96 uIleGlyGluGlnProTrpGlyArgGluPheAlaLeuArgAspProAlaGlyAsnCysValHisPheValAlaGluGluGlnAsp•••
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