

# pDRIVE-mPGK

A plasmid with the native ubiquitous murine phosphoglycerate kinase promoter

Catalog # pdrive-mpgk

For research use only

Version # 11D20-MM

## PRODUCT INFORMATION

### Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by pDRIVE-mPGK.
- GT116 genotype is: *F-*, *mcrA*,  $\Delta(mrr-hsdRMS-mcrBC)$ ,  $\Delta 80lacZ\Delta M15$ ,  $\Delta lacX74$ , *rspL* (*StrA*), *recA1*, *endA1*  $\Delta dcm$   $\Delta sbcC-sbcD$ .
- 4 pouches of *E. coli* FastMedia™ Zeo (2 TB 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* FastMedia™ Zeo at room temperature. FastMedia™ 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' site is *Sda* I which is compatible with *Nsi* I and *Pst* I. The 3' restriction site is *Bsp* HI which includes the ATG start codon, and is compatible with *Nco* 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.

## PROMOTER CHARACTERISTICS

### **Murine PGK-1 (gene : Phosphoglycerate Kinase) promoter**

Complete Promoter size: 1440bp

Specificity: Ubiquitous

Pgk-1 is an X-linked gene encoding 3-phosphoglycerate kinase, an enzyme necessary in every cell for glycolysis. The promoter region of the pgk-1 gene is rich in G and C nucleotides and contains five copies of the hexadeoxynucleotide, GGGCGG, a potential binding site for the Sp1 transcription factor, a CCAAT sequence, but no TATA box<sup>1</sup>. This promoter can efficiently drive high levels of expression of reporter genes (i.e. LacZ and GFP) and therapeutic genes, such as tumor-associated antigens<sup>2, 3</sup>. Furthermore, in contrast to the CMV promoter, the PGK promoter yields sustained expression<sup>2</sup>.

1. Adra CN. et al. 1987. Cloning and expression of the mouse pgk-1 gene and the nucleotide sequence of its promoter. *Gene* 60(1):65-74.
2. Gerolami R. et al. 2000. Gene transfer to hepatocellular carcinoma: transduction efficacy and transgene expression kinetics by using retroviral and lentiviral vectors. *Cancer Gene Ther* 7(9):1286-92.
3. Lizée G. et al., 2004. Lentivirus vector-mediated expression of tumor-associated epitopes by human antigen presenting cells. *Hum Gene Ther*. 15(4):393-404.

## PLASMID FEATURES

- **LacZ gene** encodes  $\beta$ -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* FastMedia™ 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 FastMedia™ Zeo liquid provided (see below).
- 5- Extract the pDRIVE plasmid DNA using the method of your choice.

### Selection of bacteria with *E. coli* FastMedia™ Zeo:

*E. coli* FastMedia™ Zeo is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* FastMedia™ Zeo is a TB (liquid, fas-zn-1) or LB (solid, fas-zn-s) based medium with zeocin, and contains stabilizers.

### 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 FastMedia™.**
- 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 FastMedia™ as the antibiotic will be permanently destroyed by the procedure.*

## TECHNICAL SUPPORT

Toll free (US): 888-457-5873

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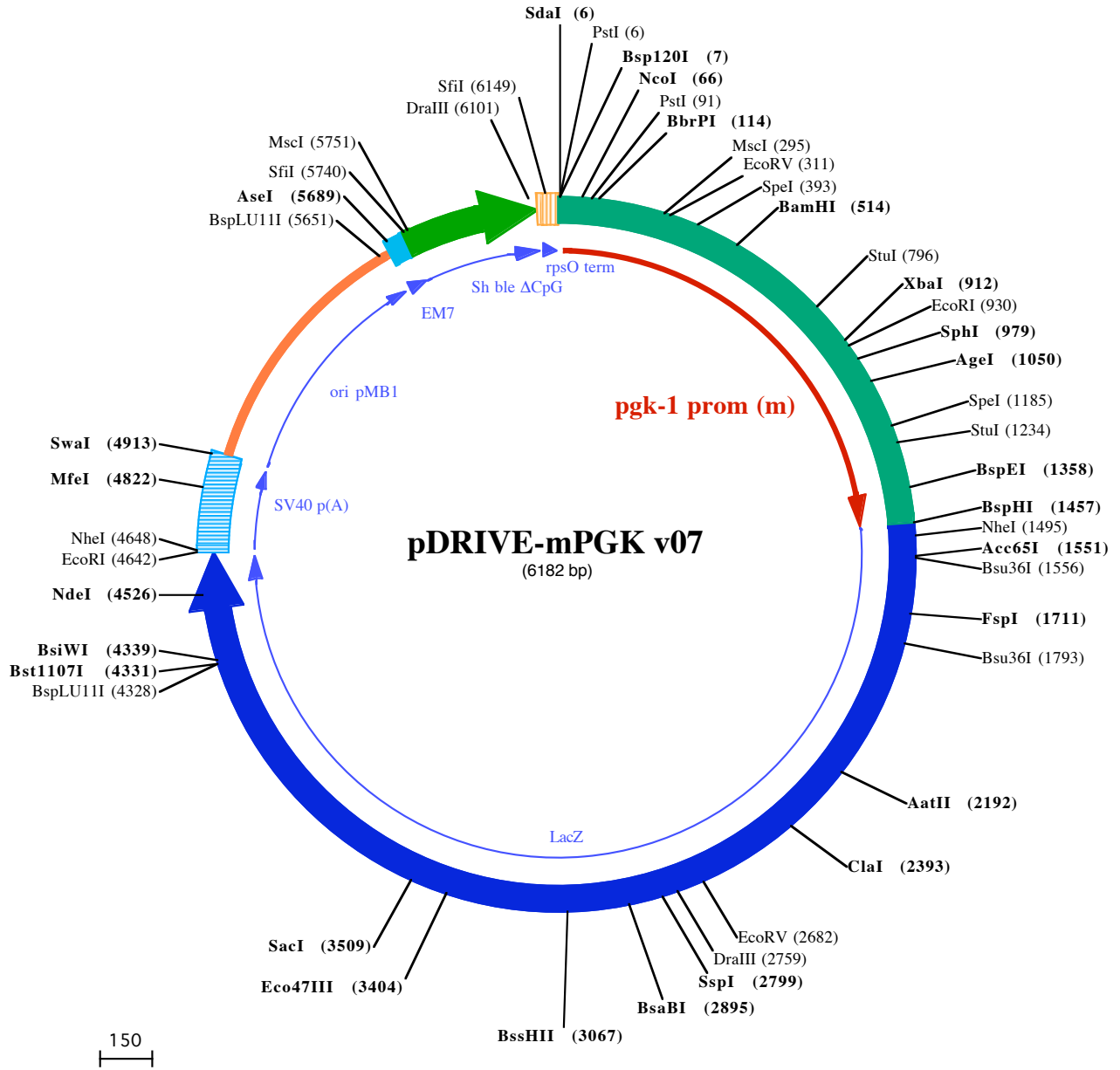
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PstI (6) Bsp120I (7)  
SdaI (6) NcoI (66) PstI (91)  
1 CCTGCAGGGCCACTAGGTCGCTTGCATGTATCCGATTTCCAGACTGCACACTCTGCTATTGGATAACCATGGGGCTCTCAGCATTCTGCAGTCCTTTGT

BbrPI (114)  
101 CTCTTCCATGTCACGTGGCTCCTGTTACCCAGTCTTTTTCCGCTCTGTTCTCTTAAACTGTTTCCCTTCTGGACCTGCTCTCTCTCCATGTA

MscI (295)  
201 TATGCTTATATAAAAAGTCATAGGATAGAAAACACATGGTATTGCTTTCTGAGTACTTCACATAATATAGTAACCTCC AGAGGTGCTTTGGCC AG

EcoRV (311) SpeI (393)  
300 TCACTCTGGATATCTGCCAGTGAGAGAGGTGAAAAG AAAAACAGGAGAGTGAACAAGGCTTCCATTCTCATGTGCCCTTCAATTTCTAACTAGT  
399 TGTCTGTCTCAGACCGTCAGGCAAGCACTTTACCACTGAGCACCGTCTTAGCCAAATGAGTGTCTAGTAGAGATTTAAAGTTTTGTTTTTTTAA

BamHI (514)  
499 ACAGTGTGGA GATTGGATC CACGGCT CTGGCCCGATTTTACCACTGAGCTACACTCCAAAGCAGTCGAAATCACAGTGGCCAGGATTGAAA  
595 TGATCACTTAGATGCTTTCAGTCTTGATAAGACACTAAATCTTTGCTATCAGTACTTTCATCTTTAATAACAGAACGTACTTAGGAATTTATGAGCA

StuI (796)  
695 TTGTTAGTTAGCATGACACATGCTATATGTATTGCTCATTATGAATAATGTAACCACAGCAATTACATTGTACTTTTTATTATAAAAGGGGGAGGGGAA  
795 GGCCTGCTCTTTTTAACTTCTGAGAGGTTTCGATTACTAAGTAAGACCTTATGTAGACTTCCATTTGGGAGCTGAGAAAAGCAGAGGATTCAAAAGGG

XbaI (912) EcoRI (930) SphI (979)  
895 GATGACATTTGCAAAGGCTAGAAAAGGCGCTGGGAATTTACCGGGTAGGGGAGGCGCTTTTCCAAAGCAGTCTGGAGCATGCGCTTTAGCAGCCCC

AgeI (1050)  
995 GCTGGGCACTTGGCGCTACACAAGTGGCTCTGGCTCGCACACATTCCACATCCACGGTAGGCGCAACCGGCTCCGTTCTTTGGTGGCCCTTCGC

SpeI (1185)  
1094 GCCACCTTCTACTCTCCCTAGTCAGGAAGTTC CCCCCGCCCG CAGCTCGCTCGTGCAGGACGTGACAAATGGAAGTAGCAGCTCTCACTAGTC

StuI (1234)  
1192 TCGTGCAGATGGACAGCACCCTGAGCAATGGAAGCGGGTAGGCCCTTTGGGGCAGCGCCAATAGCAGCTTTGCTCCTTCGTTTTGGGCTCAGAGGCT

BspEI (1358)  
1292 GGGAAAGGGTGGGTCCGGGGCGGGCTCAGGGCGGGCTCAGGGCGGGCGGGCGGGCGCCGAAGTCTCCG GAGGCCCGCATTCTGCACGCTTCAAAA

BspHI (1457)  
1391 GCGCACGTCTGTGCGCTGTTCTCTCTCTCTCATCTCCGGCCCTTTCGACCTCACGGTGTGGCCATCATGAGCGGTTCTCATCATCATCATCATG  
1 M S G S H H H H H H

NheI (1495) Bsu36I (1556) Acc65I (1551)  
1490 GTATGGCTAGCATGACTGGTGGACAGCAAATGGTCCGGATCTGTACGACGATGACGATAAGGTACCTAAGGATCAGCTTGGAGTGTATCCCGTCTGTTTT  
111 G M A S M T G G Q Q M G R D L Y D D D D K V P K D Q L G V D P V V L  
1590 ACAACGCTGCTGACTGGGAAAACCTGGCGTTACCAACTTAATCGCCTTGCAGCAGATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACC  
44 Q R R D W E N P G V T Q L N R L A A H P P F A S W R N S E E A R T

FspI (1711)  
1690 GATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCAATGGCGTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGAAAAGCTGGCTGGAGTGGCATC  
78 D R P S Q Q L R S L N G E W R F A W F P A P E A V P E S W L E C D

Bsu36I (1793)  
1790 TTCCTGAGGCCGATACTGCTGCTGCTCCCTCAAACCTGGCAGATGCACGGTTACGATCGCCCATCTACACCAACGTAACCTATCCCATACGGTCAATCC  
111 L P E A D T V V P S N W Q M H G Y D A P I Y T N V T Y P I T V N P  
1890 GCGCTTTGTTCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACCGCAATATTTTTGAT  
144 P F V P T E N P T G C Y S L T F N V D E S W L Q E G Q T R I I F D  
1990 GCGTAACTCGGCTTTCATCTGTGGTGAACGGCGTGGTTCGGTACGGCCAGGACAGTCTGTTTGGCTGTAATTTGACCTGAGCGCATTTTTAC  
178 G V N A S C F H L W C N R V W V G Y G Q D S R L P S E F D L S A F L

AatIII (2192)  
2090 GCGCCGGAGAAAACCGCTCGCGGTGATGGTGTGCTGGTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTCCGTGA  
211 R A G E N R L A V M V L R W S D G S Y L E D Q D M W R M S G I F R D  
2190 CGTCTGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTAAATGATGATTTAGCCGCGCTGACTGGAGGCTGAAGTT  
244 V S L L H K P T T Q I S D F H V A T R F N D D F S R A V L E A E V  
2290 CAGATGTGCGGCGAGTTGCTGACTACTACGGTAACAGTTTCTTTATGGCAGGGTGAAACCGAGGTCGCCAGCGGCACCGCCCTTTCGGCGGTGAAA  
278 Q M C G E L R D Y L R V T V S L W G G E T Q V A S G T A P F G G E

ClaI (2393)  
2390 TTATCGATGAGCGTGGTGGTTATGCCGATCGCGTACACTACGCTGTAACGCTGAAAAACCGGAACTGTGGAGCGCGAAATCCCGAATCTCTATCGTG  
311 I D E R G G Y A D R V T L R L N V E N P K L W S A E I P N L Y R A  
2490 GGTGGTTGAACTGCACACCGCCGACGGCAGCTGATTGAAGCAGAAGCTGCGATGTCGGTTTCCGGAGGTCGGATTGAAAATGGTCTGCTGCTGCTG  
344 V V E L H T A D G T L I E A E A C D V G F R E V R I E N G L L L L

EcoRV (2682)  
2590 AACGGCAAGCCGTTGCTGATTGAGGCGTTAACCGTCACGAGCATCATCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGC  
378 N G K P L L I R G V N R H E H H P L H G Q V M D E Q T M V Q D I L

DraIII (2759)  
2690 TGATGAAGCAGAACAACCTTAAACGCCGTGCGCTGTTCCGATTATCCGAACATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGATGTGGTGA  
411 L M K Q N N F N A V R C S H Y P N H P L W Y T L C D R Y G L Y V V D

SspI (2799)  
2790 TGAAGCCAATATTGAAACCCACGGCATGGTCCAAATGAATCGTCTGACCGATGATCCGCGTGGTACCGGCGATGAGCGAACCGGTAACCGGAATGGTG  
444 E A N I E T H G M V P M N R L T D D P R W L P A M S E R V T R M V

BsaBI (2895)  
2890 CAGCGCATCGTAATACCCGAGTGTATCATCTGGTCTGGGGAATGAATCAGGCCACGGCGTAATCACGACGCGCTGTATCGCTGGATCAAATCTG  
478 Q R D R N H P S V I I W S L G N E S G H G A N H D A L Y R W I K S

BssHIII (3067)  
2990 TCGATCCTTCCCGCCGGTGCAGTATGAAGCGCGGAGCCGACACCACGGCCACCGATATTTTCCCGATGTACGCGCGTGGATGAAGACCAGCC  
511 V D P S R P V Q Y E G G G A D T T A T D I I C P M Y A R V D E D Q P  
3090 CTTCCGGCTGTGCCAAATGGTCCATAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCGCTGATCCTTTGCGAATACGCCACCGATGGGTAAAC  
544 F P A V P K W S I K K W L S L P G E T R P L I L C E Y A H A M G N  
3190 AGTCTGGCGGTTTCGCTAAATACTGGCAGGCGTTTTCGTCAGTATCCCGGTTTACAGGGCGGCTTCTGCTGGGACTGGGTGGATCAGTCCGCTGATTAAT  
578 S L G G F A K Y W Q A F R Q Y P R L Q G G F V W D W V D Q S L I K  
3290 ATGATGAAAACGGCAACCGTGGTGGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCCAGTCTGTATGAACGGTCTGCTTTTGGCAGCCG  
611 Y D E N G N P W S A Y G G D F G D T P N D R O F C M N G L V F A D R

**Eco47III (3404)**  
3390 CACGCCGATCCAGCGCTGACGGAAGCAAACACCCAGCAGCAGTCTTTCCAGTTCCTGTTATCCGGGCAAACCATCGAAGTGACCAGCGAATACCTGTT  
644▶ T P H P A L T E A K H Q Q Q F F Q F R L S G Q T I E V T S E Y L F

**SacI (3509)**  
3490 CGTCATAGCGATAACGAGCTCCTGCACCTGGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGTCGCTCCACAAGGTAAC  
678▶ R H S D N E L L H W M V A L D G K P L A S G E V P L D V A P Q G K  
3590 AGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGCCGGCAACTCTGGCTCACAGTACGCGTAGTGAACCGAACCGCACCATGTCAGAAGC  
711▶ Q L I E L P E L P Q P E S A G Q L W L T V R V V Q P N A T A W S E A  
3690 CGGGACATCAGCGCCTGGCAGCAGTGGCGTCTGGCGAAAACCTCAGTGTGACGCTCCCGCGCGTCCACGCCATCCCGCATCTGACCACCGCGAA  
744▶ G H I S A W Q Q W R L A E N L S V T L P A A S H A I P H L T T S E  
3790 ATGGATTTTGCATCGAGCTGGTAATAAGCGTTGGCAATTAACCGCCAGTCAGGCTTTCTTTTACAGATGGATTGGCGATAAAAAACAACCTGCTGA  
778▶ M D F C I E L G N K R W Q F N R Q S G F L S Q M W I G D K K Q L L  
3890 CGCCGCTGCGCATCAGTTCACCCGTCACCGCTGGATAACGACATTGGCGTAAGCGAACCGCATTGACCTAACCGCTGGGTGAAACGCTGGAA  
811▶ T P L R D Q F T R A P L D N D I G V S E A T R I D P N A W V E R W K  
3990 GCGCGCGGGCATTACCAGCCGAAGCAGCGTGTTCAGTGCACCGCAGATACACTTGTGTGATGCGGTGCTGATTACGACCGCTCAGCGTGGCAGCAT  
844▶ A A G H Y Q A E A A L L Q C T A D T L A D A V L I T T A H A W Q H  
4090 CAGGGAAAACTTATTATCAGCCGAAAACCTACCGATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCAGCGATACCCG  
878▶ Q G K T L F I S R K T Y R I D G S G Q M A I T V D V E V A S D T P  
4190 ATCCGCGCGGATTGGCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAACATCCCGACCGCTTAC  
911▶ H P A R I G L N C Q L A Q V A E R V N W L G L G P Q E N Y P D R L T

**Bst1107I (4331)**  
**BspLU11I (4328) BsiWI (4339)**  
4290 TGCCGCTGTTTTGACCCTGGGATCTGCCATTGTGACACATGTATACCCGCTAGCTCTCCGAGCGAAAACGGTCTGCGCTGCGGGACGCGGAATTG  
944▶ A A C F D R W D L P L S D M Y T P Y V F P S E N G L R C G T R E L  
4390 AATTATGGCCACACCGAGTGGCGGCGACTTCCAGTTCACATCAGCCGCTACAGTCAACAGCAACTGATGAAACCGCATCCCATCTGCTGCACG  
978▶ N Y G P H Q W R G D G F N I S R Y S Q Q Q L M E T S H R H L L H

**NdeI (4526)**  
4490 CGGAAGAAGGCACATGGCTGAATATCGACGGTTCCATATGGGATTGGTGGCAGCAGCTCCTGGAGCCGTCAGTATCGCGGAATTACAGCTGAGCGC  
1011▶ A E E G T W L N I D G F H M G I G G D D S W S P S V S A E L Q L S A

**NheI (4648)**  
**EcoRI (4642)**  
4590 CGGTCGCTACCATTACCAGTGGTCTGGTGTCAAAAAATAATCTAGTCGAGAACTCGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAA  
1044▶ G R Y H Y Q L V W C Q K •  
4690 CCACAAC TAGAATG CAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACC

**MfeI (4822)**  
4790 ATTATAAGCTGCAATAAACAAGTTAAACAACAATTCATTTCATTTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACC

**SwaI (4913)**  
4890 TCTACAAATGTGGTAGATCCATTTAAATGTTAATTAAGTCCATGACCAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAA  
4990 AAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGAACAACAAAAAACCCGCTACCAGCGGTGGTTTTGTTGCCGGATC  
5090 AAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTACGACAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACCTCAA  
5190 GAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCAGGTTGGACTCAAGA  
5290 CGATAGTTACCGGATAAAGCGCAGCGGTGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACTAC  
5390 AGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCT  
5490 TCCAGGGGAAACCGCTGGTATCTTTATAGTCTGCTGGGTTTCCGCACCTCTGACTTGGCGTCTGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTA

**BspLU11I (5651)** **AseI (5689)**  
5590 TGGAAAAACGCCAGCAACCGCCCTTTTACGGTTCCTGGCCTTTTGTGCTGCTTTTGTGCTCAGTGTCTTAATTAATTTTTCAAAGTAGTTGACAAT

**SfiI (5740) MscI (5751)**  
5690 TAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAAGGAGGGCCATCATGGCCAAGTTGACCAGTGTGTCCAGTGTCTCACAGCCAGG  
5790 GATGTGGCTGGAGCTGTTGAGTCTGACTGACAGGTTGGGTTCTCCAGAGATTTTGTGGAGGATGACTTTGCAGGTGTGGTCAGAGATGATGTACCC  
15▶ D V A G A V E F W T D R L G F S R D F V E D D F A G V V R D D V T  
5890 TGTTTCATCTCAGCAGTCCAGGACCGGTGGTGCCTGACAACACCTGGCTTGGGTGTGGGTGAGAGGACTGGATGAGCTGTATGCTGAGTGGAGTGGGT  
48▶ L F I S A V Q D Q V V P D N T L A W V W V R G L D E L Y A E W S E V  
5990 GGTCTCCACCAACTTCCAGGATGCCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGAGAGAGTTTGCCTGAGAGACCCGACGAGGCAAC  
81▶ V S T N F R D A S G P A M T E I G E Q P W G R E F A L R D P A G N

**DraIII (6101)** **SfiI (6149)**  
6090 TGTGTGCACTTTGTGGCAGAGGAGCAGGACTGAGGATAAGAATTGAGTTTCAGAAAAGGGGGCCTGAGTGGCCCCTTTTTCAACTTAATTA  
115▶ C V H F V A E E Q D •