pDRIVE5SEAP-hFibronectin

A plasmid with a native tissue-specific human fibronectin promoter

Catalog # pdrive5s-hfn

For research use only

Version # 10H17-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by pDRIVE5SEAP-hFibronectin.
- GT116 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, rspL (StrA), recA1, endA1 Δdcm ΔsbcC-sbcD.*
- 4 pouches of *E. coli* Fast-Media® 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* 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

pDRIVE5-SEAP is an expression plasmid containing a native or composite promoter of interest. **pDRIVE5-SEAP** 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 are *Sda* 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 *BspL*U11 I.

- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE5-SEAP promoter drives the expression of the SEAP reporter gene which allows for testing of the promoter's activity in transient transfection experiments. Furthermore, the SEAP gene is flanked by unique restriction sites (*Nco* I and *Nhe* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Human Fibronectin promoter
Complete Promoter Size : 786bp
Plasmid backbone : pDRIVE5-SEAP
Specificity : Differentiating cells, healing tissues

Fibronectin (FN) plays an important role in organizing the extracellular matrix and facilitates cell adhesion, migration, wound healing and tumor metastasis. FN expression is generally low in undifferentiated cells and most adult tissues. However, synthesis is drastically increased after differentiation and during wound healing. Expression of FN is regulated at the transcriptional level in a cell-specific manner by a variety of transcription factors, growth factors and hormones. The FN promoter contains a TATA box, a CAAT sequence, a SP-1 binding site, a cAMP-responsive element[1] and two EGR-1 binding sites. The FN promoter can be activated *in vitro* in cell lines that normally do not express FN after induction of differentiation[2].

1. Dean DC. et al. 1987. Cloning and analysis of the promoter region of the human fibronectin gene. Proc Natl Acad Sci USA 84(7): 1876-80.
2. Suzuki M. et al. 1998. Induction of Sp1 in differentiating human embryonal carcinoma cells triggers transcription of the fibronectin gene. Mol Cell Biol. 18(5):3010-20.

PLASMID FEATURES

• **SEAP gene** encodes an engineered secreted embryonic alkaline phosphatase. The levels of SEAP in the culture medium of transfected cells expressing the reporter gene can be assayed with chromogenic or luminescent methods
• **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.
• **EM2K** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli.*
• ***Zeo*** gene confers zeocin resistance therefore allowing the selection of transformed *E. coli* carrying a pDRIVE5-SEAP 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 pDRIVE5-SEAP-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 pDRIVE5-SEAP plasmid DNA using the method of your choice.

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, 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.*

TECHNICAL SUPPORT
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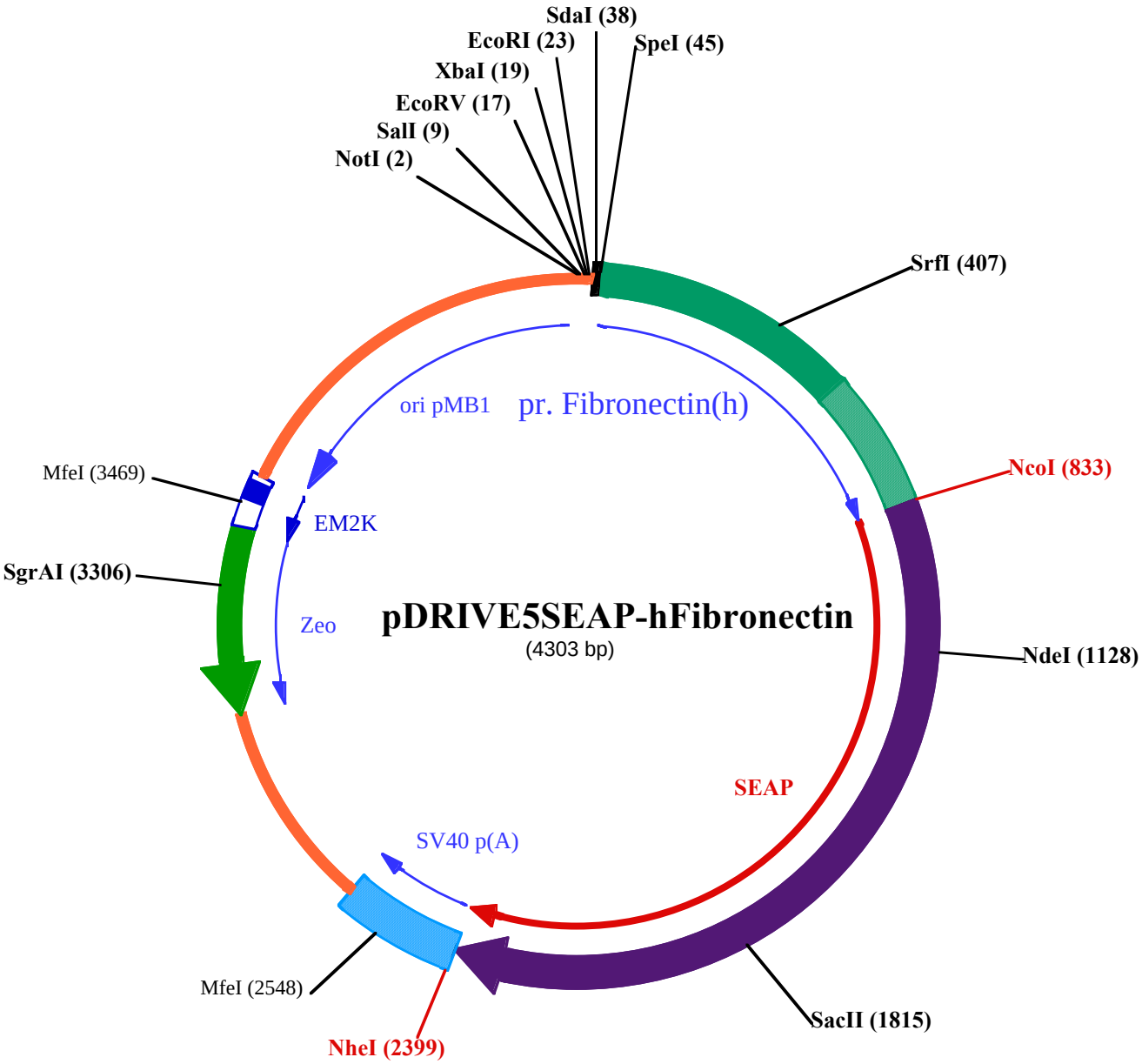
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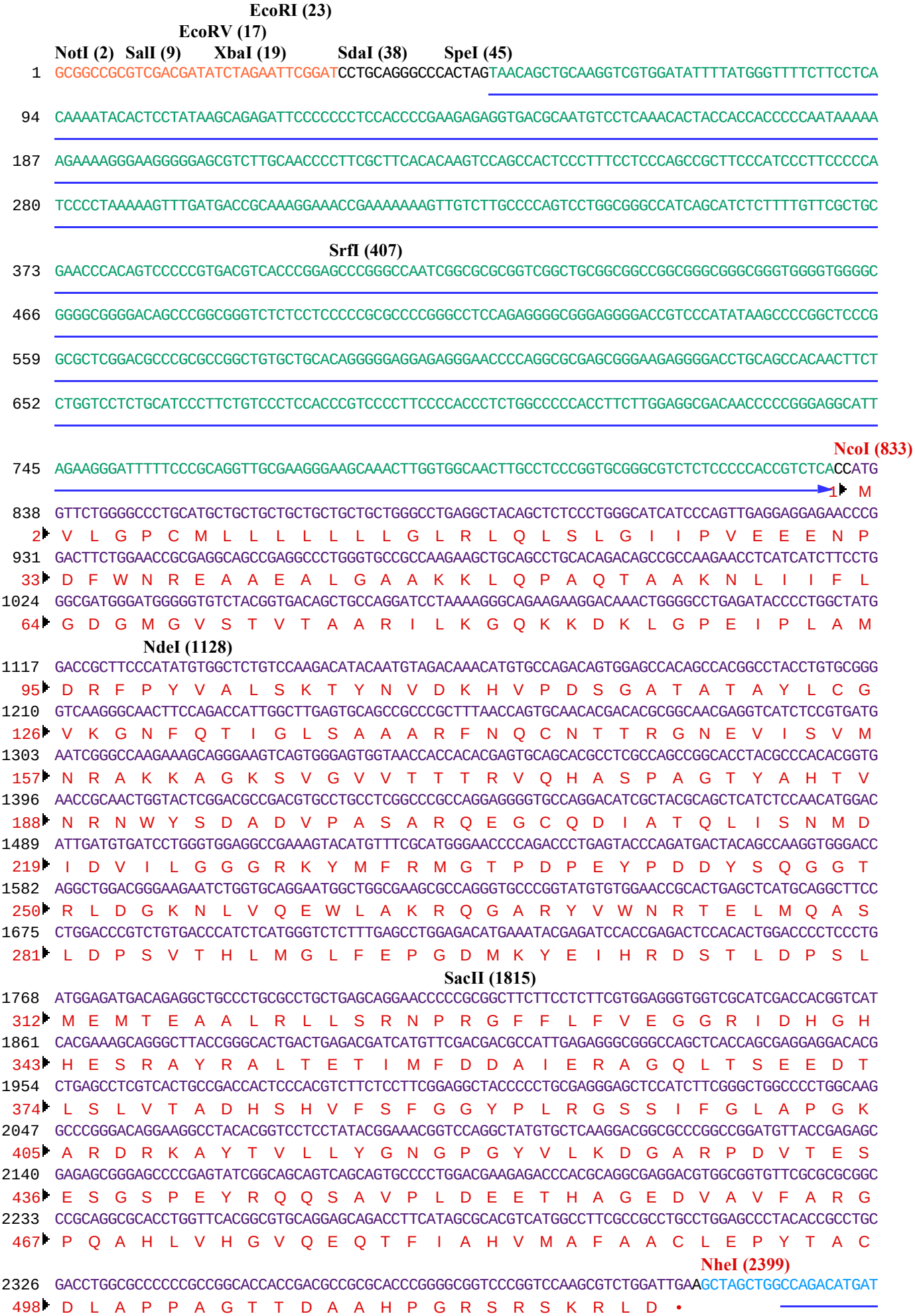
EcoRI (23)
SpeI (45)
XbaI (19)
EcoRV (17)
SalI (9)
NotI (2)
SrfI (407)
ori pMB1
pr. Fibronectin(h)
MfeI (3469)
NcoI (833)
EM2K
SgrAI (3306)
Zeo
pDRIVE5SEAP-hFibronectin
(4303 bp)
NdeI (1128)
SEAP
SV40 p(A)
MfeI (2548)
SacII (1815)
NheI (2399)

InvivoGen


EcoRI (23)
EcoRV (17)
NotI (2)
SalI (9)
XbaI (19)
SdaI (38)
SpeI (45)
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94 CAAAATACACTCCTATAAGCAGAGATTCCCCCCCTCCACCCCGAAGAGAGGTGACGCAATGTCCTCAAACACTACCACCACCCCCAATAAAAA
187 AGAAAAGGGAAGGGGGAGCGTCTTGCAACCCCTTCGCTTCACACAAGTCCAGCCACTCCCTTTCCTCCCAGCCGCTTCCCATCCCTTCCCCCA
280 TCCCCTAAAAAGTTTGATGACCGCAAAGGAAACCGAAAAAAAGTTGTCTTGCCCCAGTCCTGGCGGGCCATCAGCATCTCTTTTGTTCGCTGC
SrfI (407)
373 GAACCCACAGTCCCCCGTGACGTCACCCGGAGCCCGGGCCAATCGGCGCGCGGTCGGCTGCGGCGGCCGGCGGGCGGGCGGGTGGGGTGGGGC
466 GGGGCGGGGACAGCCCGGCGGGTCTCTCCTCCCCCGCGCCCCGGGCCTCCAGAGGGGCGGGAGGGGACCGTCCCATATAAGCCCCGGCTCCCG
559 GCGCTCGGACGCCCGCGCCGGCTGTGCTGCACAGGGGGAGGAGAGGGAACCCCAGGCGCGAGCGGGAAGAGGGGACCTGCAGCCACAACTTCT
652 CTGGTCCTCTGCATCCCTTCTGTCCCTCCACCCGTCCCCTTCCCCACCCTCTGGCCCCCACCTTCTTGGAGGCGACAACCCCCGGGAGGCATT
NcoI (833)
745 AGAAGGGATTTTTCCCGCAGGTTGCGAAGGGAAGCAAACTTGGTGGCAACTTGCCTCCCGGTGCGGGCGTCTCTCCCCCACCGTCTCACCATG
1 M
838 GTTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGCTGGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCCAGTTGAGGAGGAGAACCCG
2 V L G P C M L L L L L L L G L R L Q L S L G I I P V E E E N P
931 GACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTGCCGCCAAGAAGCTGCAGCCTGCACAGACAGCCGCCAAGAACCTCATCATCTTCCTG
33 D F W N R E A A E A L G A A K K L Q P A Q T A A K N L I I F L
1024 GGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTGGGGCCTGAGATACCCCTGGCTATG
64 G D G M G V S T V T A A R I L K G Q K K D K L G P E I P L A M
NdeI (1128)
1117 GACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGG
95 D R F P Y V A L S K T Y N V D K H V P D S G A T A T A Y L C G
1210 GTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCGCTTTAACCAGTGCAACACGACACGCGGCAACGAGGTCATCTCCGTGATG
126 V K G N F Q T I G L S A A A R F N Q C N T T R G N E V I S V M
1303 AATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTGCAGCACGCCTCGCCAGCCGGCACCTACGCCCACACGGTG
157 N R A K K A G K S V G V V T T T R V Q H A S P A G T Y A H T V
1396 AACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTGCCAGGACATCGCTACGCAGCTCATCTCCAACATGGAC
188 N R N W Y S D A D V P A S A R Q E G C Q D I A T Q L I S N M D
1489 ATTGATGTGATCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCAGACCCTGAGTACCCAGATGACTACAGCCAAGGTGGGACC
219 I D V I L G G G R K Y M F R M G T P D P E Y P D D Y S Q G G T
1582 AGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTGGAACCGCACTGAGCTCATGCAGGCTTCC
250 R L D G K N L V Q E W L A K R Q G A R Y V W N R T E L M Q A S
1675 CTGGACCCGTCTGTGACCCATCTCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTG
281 L D P S V T H L M G L F E P G D M K Y E I H R D S T L D P S L
SacII (1815)
1768 ATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCCGCGGCTTCTTCCTCTTCGTGGAGGGTGGTCGCATCGACCACGGTCAT
312 M E M T E A A L R L L S R N P R G F F L F V E G G R I D H G H
1861 CACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACG
343 H E S R A Y R A L T E T I M F D D A I E R A G Q L T S E E D T
1954 CTGAGCCTCGTCACTGCCGACCACTCCCACGTCTTCTCCTTCGGAGGCTACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAG
374 L S L V T A D H S H V F S F G G Y P L R G S S I F G L A P G K
2047 GCCCGGGACAGGAAGGCCTACACGGTCCTCCTATACGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGC
405 A R D R K A Y T V L L Y G N G P G Y V L K D G A R P D V T E S
2140 GAGAGCGGGAGCCCCGAGTATCGGCAGCAGTCAGCAGTGCCCCTGGACGAAGAGACCCACGCAGGCGAGGACGTGGCGGTGTTCGCGCGCGGC
436 E S G S P E Y R Q Q S A V P L D E E T H A G E D V A V F A R G
2233 CCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCATAGCGCACGTCATGGCCTTCGCCGCCTGCCTGGAGCCCTACACCGCCTGC
467 P Q A H L V H G V Q E Q T F I A H V M A F A A C L E P Y T A C
NheI (2399)
2326 GACCTGGCGCCCCCCGCCGGCACCACCGACGCCGCGCACCCGGGGCGGTCCCGGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGAT
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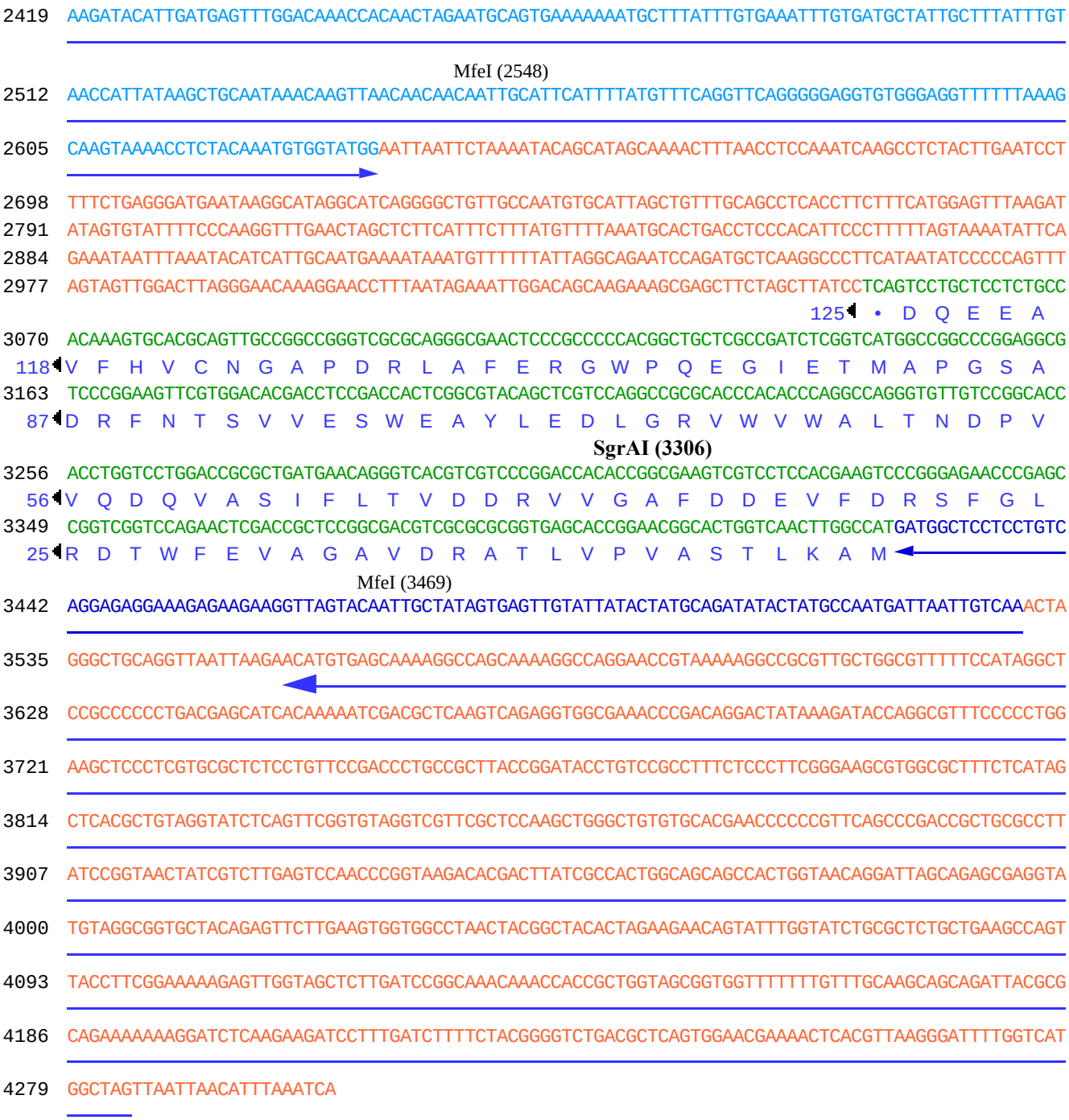
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MfeI (2548)
2512 AACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAG
2605 CAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCT
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2884 GAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTT
2977 AGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCC
125 • D Q E E A
3070 ACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCG
118 V F H V C N G A P D R L A F E R G W P Q E G I E T M A P G S A
3163 TCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACC
87 D R F N T S V V E S W E A Y L E D L G R V W V W A L T N D P V
SgrAI (3306)
3256 ACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGC
56 V Q D Q V A S I F L T V D D R V V G A F D D E V F D R S F G L
3349 CGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCCTGTC
25 R D T W F E V A G A V D R A T L V P V A S T L K A M
MfeI (3469)
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3628 CCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGG
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