pDRIVE-hCCKAR

A plasmid with the native human CCKAR promoter

Catalog # pdrive-hcckar

For research use only

Version # 10D26-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT115 *E. coli* bacteria transformed by pDRIVE-hCCKAR.
- GT115 genotype is: *F- mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80lacZΔM15 ΔlacX74 recA1 rspL (StrA) endA1 Δdcm uidA(ΔMluI)::pir-116 ΔsbcC-sbcD.*
- 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' 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 *BspL*U11 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

hCCKAR promoter- Complete promoter size: 724 bp

Cholecystokinin (CCK) is a gut peptide hormone known to stimulate post-prandial gallbladder contraction and pancreatic enzyme secretion. CCK also plays an important role in pancreatic carcinogenesis. The CCK-A receptor (CCKAR) is predominantly expressed in human pancreatic cancer[1]. A 0.7 kb fragment of the CCKAR promoter was reported to drive the expression of the luciferase gene selectively in pancreatic cancer cells and not in normal cells[2]. This fragment lacks a typical TATA box but contains two GC-Box motifs[3].

1. Moonka R. *et al.* 1999. Cholecystokini-A receptor messenger RNA expression in human pancreatic cancer. J Gastrointest Surg. 3(2): 134-40. **2. Li Z. *et al.* 2005.** Suppression of pancreatic tumor progression by systemic delivery of a pancreatic-cancer-specific promoter driven Bik mutant. Cancer Lett. 236(1):58-63. **3. Takata Y. *et al.* 2002.** Promoter analysis of human cholecystokinin type-A receptor gene. J Gastroenterol. 37(10):815-20.

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.*

TECHNICAL SUPPORT
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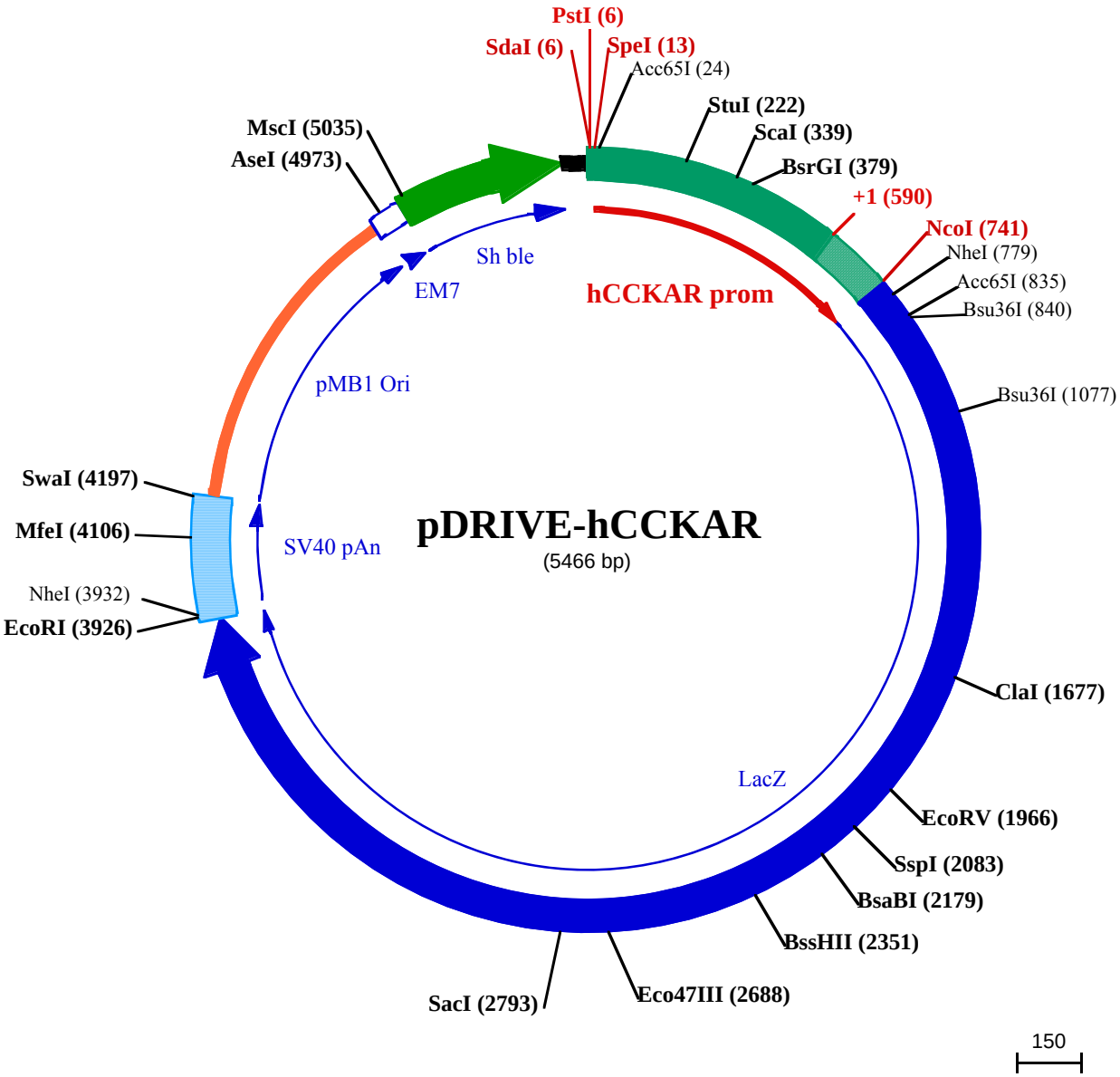
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SdaI (6)
SpeI (13)
Acc65I (24)
StuI (222)
ScaI (339)
BsrGI (379)
+1 (590)
NcoI (741)
NheI (779)
Acc65I (835)
Bsu36I (840)
Bsu36I (1077)
ClaI (1677)
EcoRV (1966)
SspI (2083)
BsaBI (2179)
BssHII (2351)
Eco47III (2688)
SacI (2793)
EcoRI (3926)
NheI (3932)
MfeI (4106)
SwaI (4197)
MscI (5035)
AseI (4973)
Sh ble
EM7
hCCKAR prom
pMB1 Ori
SV40 pAn
LacZ
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(5466 bp)
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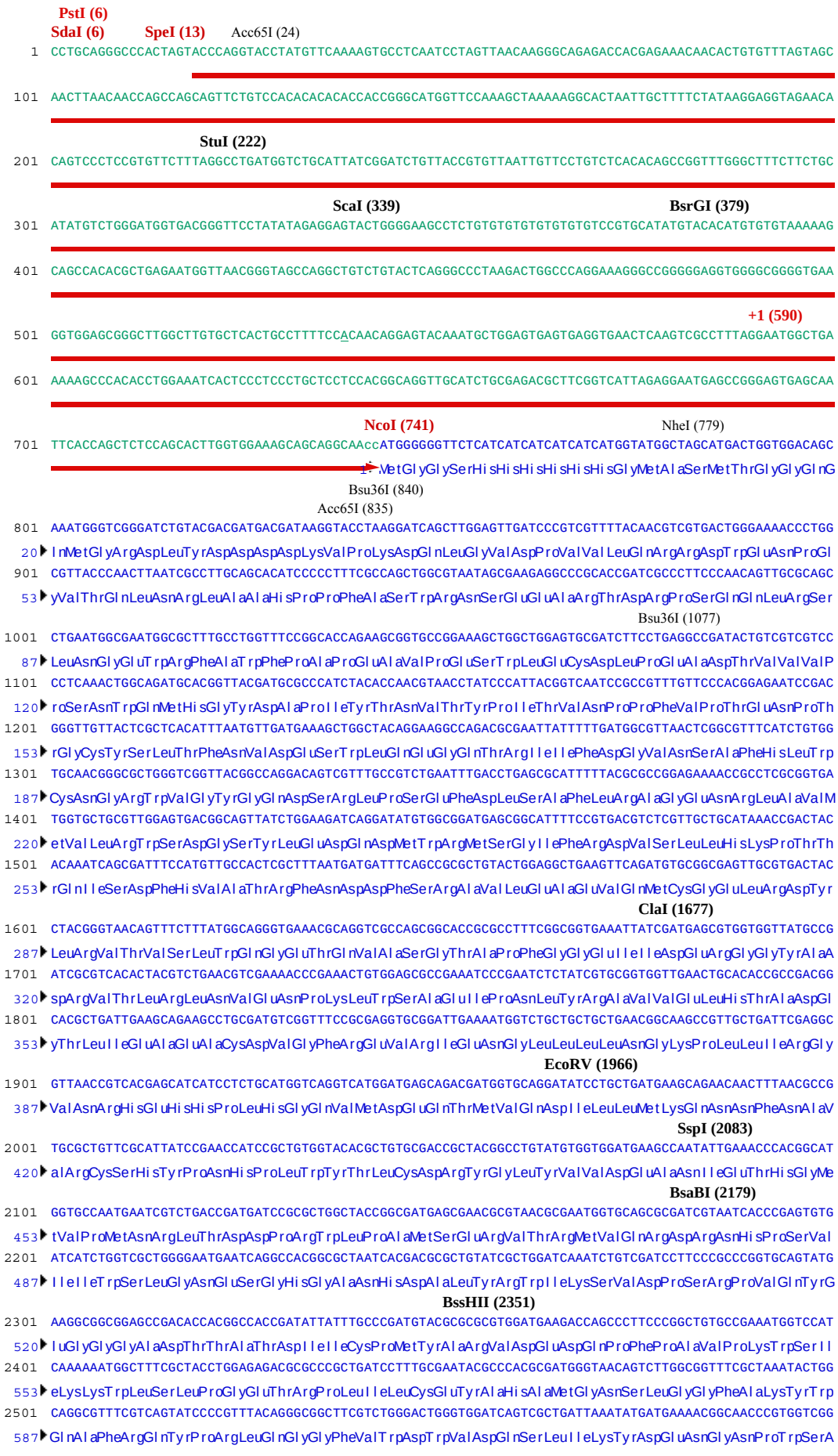
InvivoGen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StuI (222)
201 CAGTCCCTCCGTGTTCTTTAGGCCTGATGGTCTGCATTATCGGATCTGTTACCGTGTTAATTGTTCCTGTCTCACACAGCCGGTTTGGGCTTTCTTCTGC
ScaI (339)
BsrGI (379)
301 ATATGTCTGGGATGGTGACGGGTTCCTATATAGAGGAGTACTGGGGAAGCCTCTGTGTGTGTGTGTGTGTCCGTGCATATGTACACATGTGTGTAAAAAG
401 CAGCCACACGCTGAGAATGGTTAACGGGTAGCCAGGCTGTCTGTACTCAGGGCCCTAAGACTGGCCCAGGAAAGGGCCGGGGGAGGTGGGGCGGGGTGAA
+1 (590)
501 GGTGGAGCGGGCTTGGCTTGTGCTCACTGCCTTTTCCACAACAGGAGTACAAATGCTGGAGTGAGTGAGGTGAACTCAAGTCGCCTTTAGGAATGGCTGA
601 AAAAGCCCACACCTGGAAATCACTCCCTCCCTGCTCCTCCACGGCAGGTTGCATCTGCGAGACGCTTCGGTCATTAGAGGAATGAGCCGGGAGTGAGCAA
NcoI (741)
NheI (779)
701 TTCACCAGCTCTCCAGCACTTGGTGGAAAGCAGCAGGCAAccATGGGGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGC
1 MetGlyGlySerHisHisHisHisHisHisGlyMetAlaSerMetThrGlyGlyGlnG
Bsu36I (840)
Acc65I (835)
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20 InMetGlyArgAspLeuTyrAspAspAspAspLysValProLysAspGlnLeuGlyValAspProValValLeuGlnArgArgAspTrpGluAsnProGl
901 CGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGC
53 yValThrGlnLeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeuArgSer
Bsu36I (1077)
1001 CTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCC
87 LeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaAspThrValValValP
1101 CCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGAC
120 roSerAsnTrpGlnMetHisGlyTyrAspAlaProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValProThrGluAsnProTh
1201 GGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGG
153 rGlyCysTyrSerLeuThrPheAsnValAspGluSerTrpLeuGlnGluGlyGlnThrArgIleIlePheAspGlyValAsnSerAlaPheHisLeuTrp
1301 TGCAACGGGCGCTGGGTCGGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACCGCCTCGCGGTGA
187 CysAsnGlyArgTrpValGlyTyrGlyGlnAspSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValM
1401 TGGTGCTGCGTTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTAC
220 etValLeuArgTrpSerAspGlySerTyrLeuGluAspGlnAspMetTrpArgMetSerGlyIlePheArgAspValSerLeuLeuHisLysProThrTh
1501 ACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTAC
253 rGlnIleSerAspPheHisValAlaThrArgPheAsnAspAspPheSerArgAlaValLeuGluAlaGluValGlnMetCysGlyGluLeuArgAspTyr
ClaI (1677)
1601 CTACGGGTAACAGTTTCTTTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCG
287 LeuArgValThrValSerLeuTrpGlnGlyGluThrGlnValAlaSerGlyThrAlaProPheGlyGlyGluIleIleAspGluArgGlyGlyTyrAlaA
1701 ATCGCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGG
320 spArgValThrLeuArgLeuAsnValGluAsnProLysLeuTrpSerAlaGluIleProAsnLeuTyrArgAlaValValGluLeuHisThrAlaAspGl
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353 yThrLeuIleGluAlaGluAlaCysAspValGlyPheArgGluValArgIleGluAsnGlyLeuLeuLeuLeuAsnGlyLysProLeuLeuIleArgGly
EcoRV (1966)
1901 GTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAACAACTTTAACGCCG
387 ValAsnArgHisGluHisHisProLeuHisGlyGlnValMetAspGluGlnThrMetValGlnAspIleLeuLeuMetLysGlnAsnAsnPheAsnAlaV
SspI (2083)
2001 TGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGGCAT
420 alArgCysSerHisTyrProAsnHisProLeuTrpTyrThrLeuCysAspArgTyrGlyLeuTyrValValAspGluAlaAsnIleGluThrHisGlyMe
BsaBI (2179)
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487 IleIleTrpSerLeuGlyAsnGluSerGlyHisGlyAlaAsnHisAspAlaLeuTyrArgTrpIleLysSerValAspProSerArgProValGlnTyrG
BssHII (2351)
2301 AAGGCGGCGGAGCCGACACCACGGCCACCGATATTATTTGCCCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAATGGTCCAT
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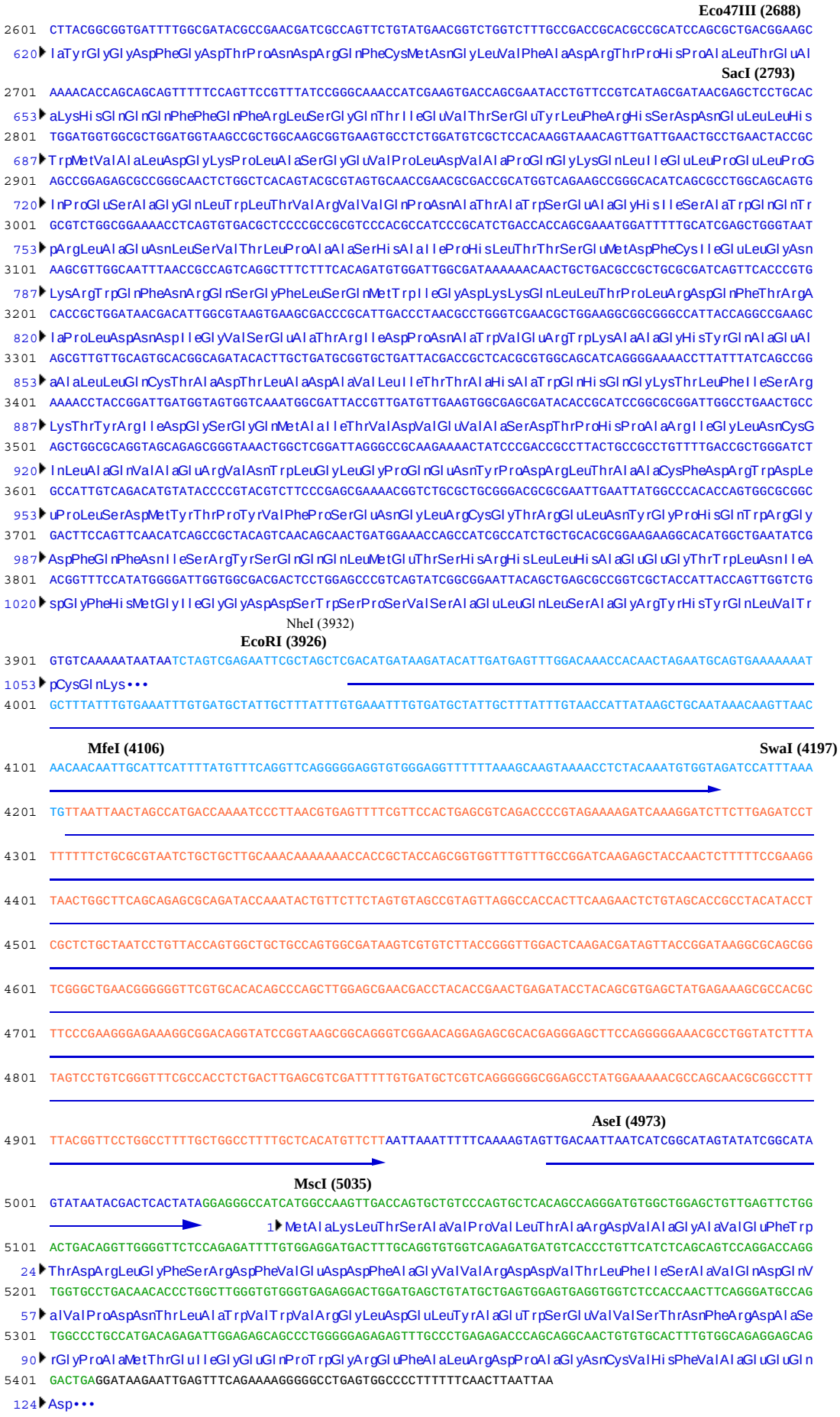
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