pDRIVE-mGFAP

A plasmid with the native mouse GFAP promoter

Catalog # pdrive-mgfap

For research use only

Version # 11B07-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by pDRIVE-mGFAP
GT116 genotype is: *F- mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80lacZM15 ΔlacX74 recA1 rpsL (StrA) endA1 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 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 *Bsp*H 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 (*Nco* I and *EcoR* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Mouse glial fibrillary acidic protein promoter

Complete Promoter size: 1679 bp

Specificity: Astrocytes

The glial fibrillary acidic protein (GFAP) is an intermediate filament protein found almost exclusively in astrocytes. It is expressed throughout postnatal life and is upregulated in response to almost any damage to the central nervous system, including Parkinson's disease. The promoter of the GFAP gene was shown to direct astrocyte-specific transcription *in vitro*, *in vivo*[1], and in transgenic mice[2]. Expression of a transgene under the control of the GFAP promoter is regulated in a similar fashion as the endogenous *GFAP* gene[3].

References:

1. Vandier *et al*. 2000. Inhibition of glioma cells in vitro and in vivo using a recombinant adenoviral vector containing an astrocyte-specific promoter. Cancer Gene Ther 7:1120-6.
2. Brenner *et al*. 1994. GFAP promoter directs astrocyte-specific expression in transgenic mice. Neurosci. 14:1030-7.
3. Jakobsson J. *et al*., 2004. Lesion-dependent regulation of transgene expression in the rat brain using a human glial fibrillary acidic protein-lentiviral vector. Eur J Neurosci. 19(3):761-5.

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 **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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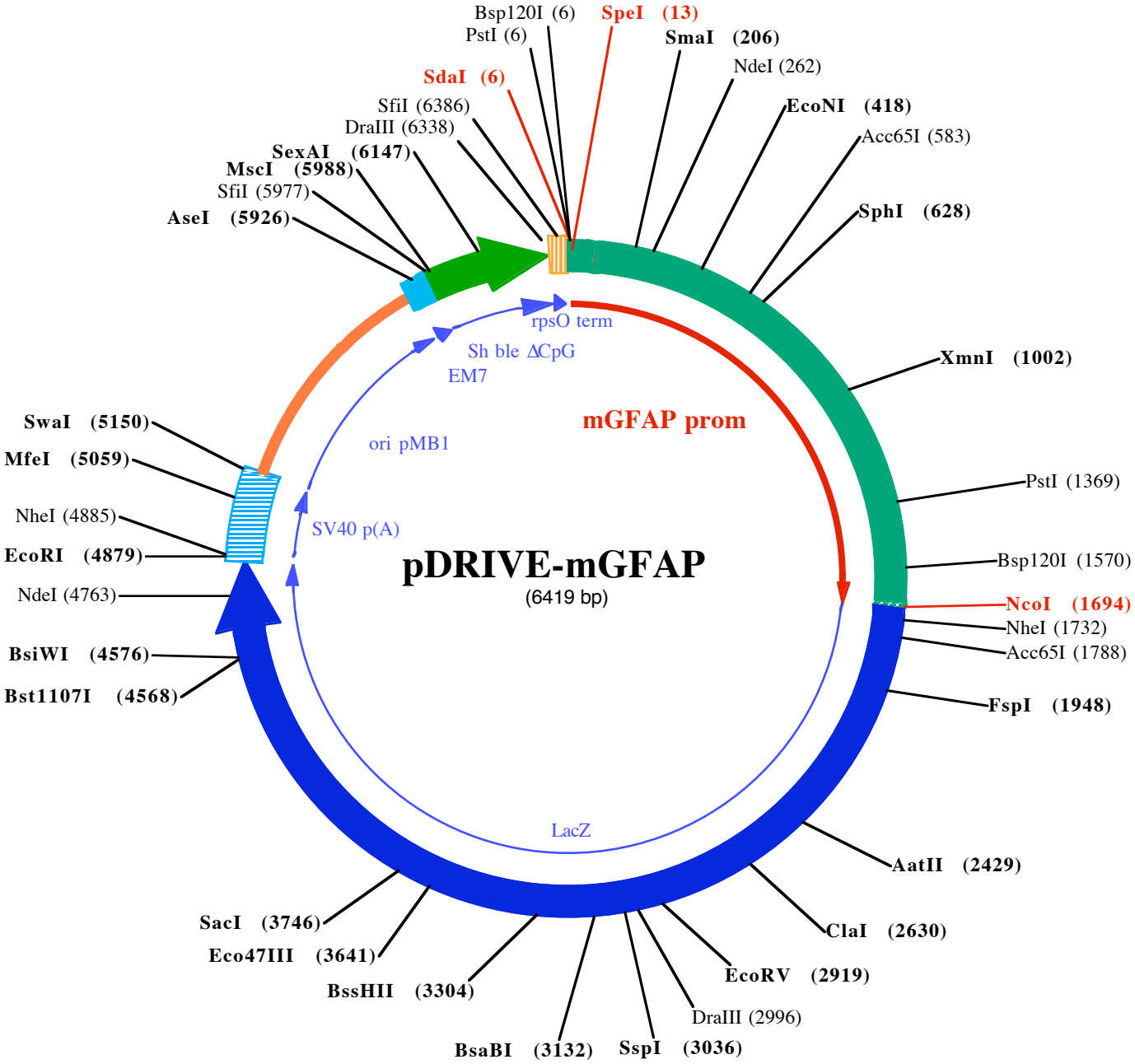
pDRIVE-mGFAP
(6419 bp)
mGFAP prom
rpsO term
Sh ble ΔCpG
EM7
ori pMB1
SV40 p(A)
LacZ
Bsp120I (6)
PstI (6)
SpeI (13)
SmaI (206)
NdeI (262)
SdaI (6)
EcoNI (418)
SfiI (6386)
Acc65I (583)
DraIII (6338)
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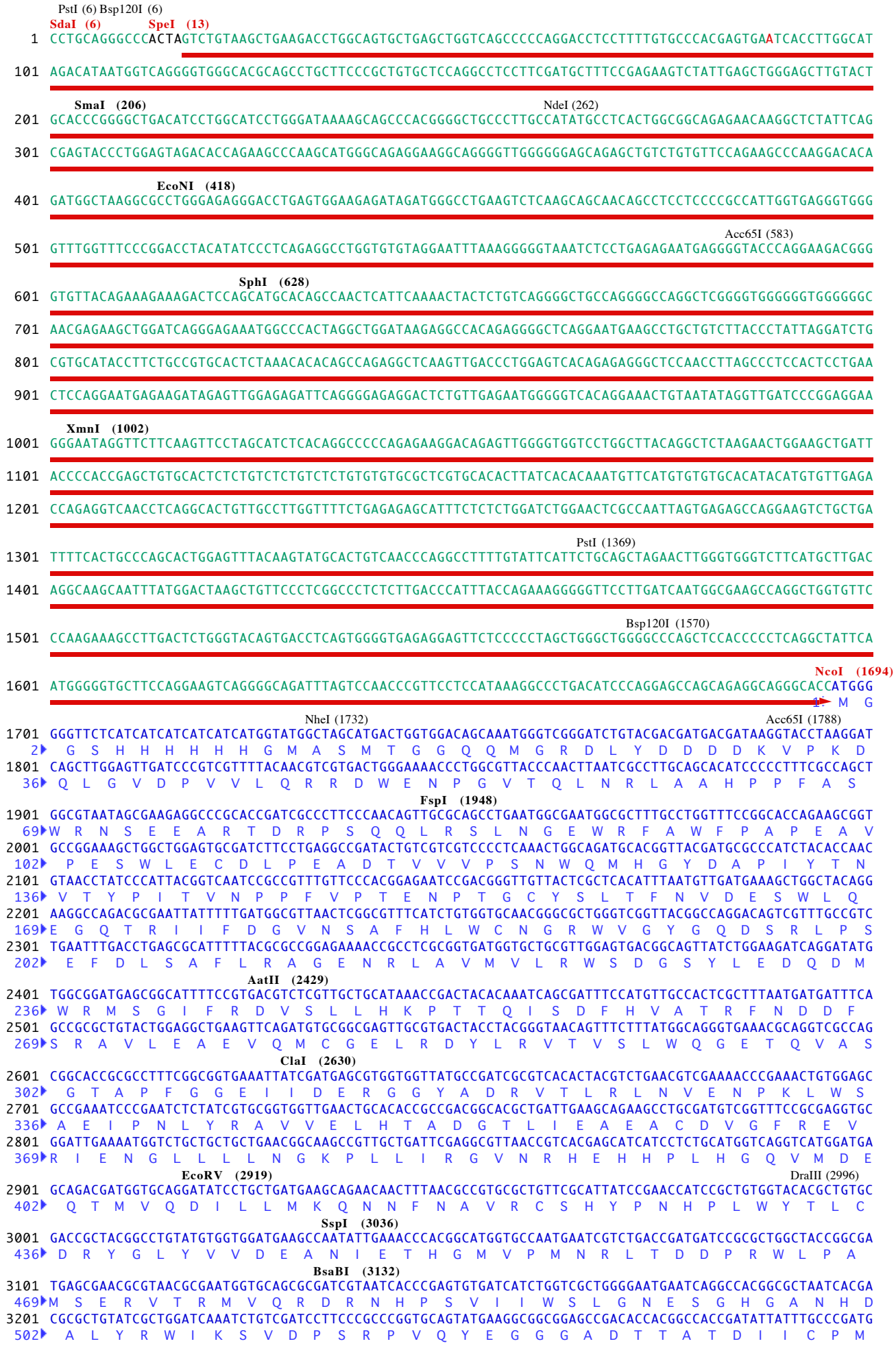
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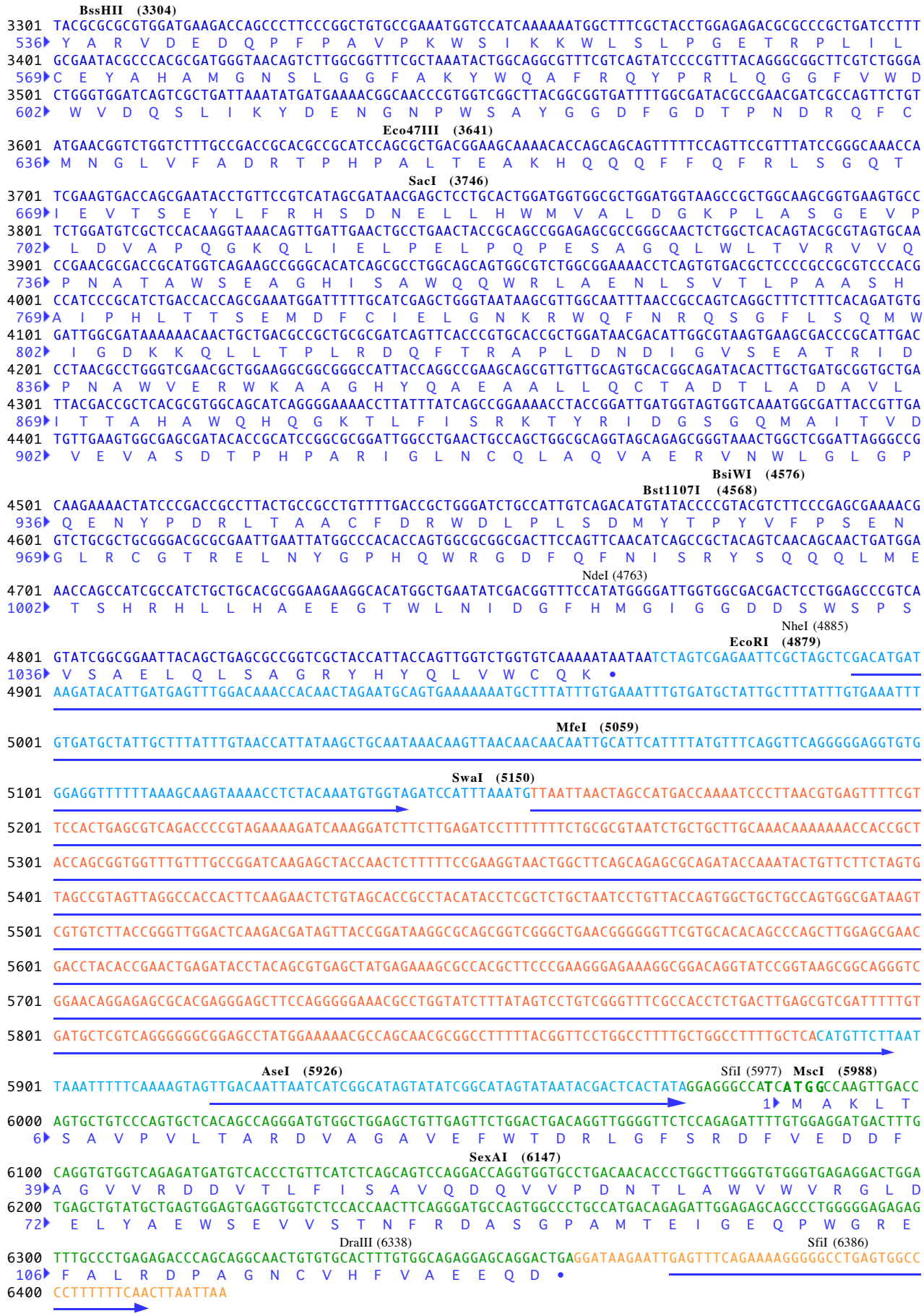
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