pDRIVE-hAFP
A plasmid with the human Alpha-FetoProtein promoter
Catalog # pdrive-hafp
For research use only
Version # 14K28-MM

PRODUCT INFORMATION

Content:
- 20 µg of pDRIVE-hAFP provided as lyophilized DNA
- 4 pouches of E. coli Fast-Media® Zeo (2 TB and 2 Agar)

Shipping and storage:
- Products are shipped at room temperature.
- Lyophilized DNA is stable for at least 12 months when stored at -20°C.
- Resuspended DNA is stable for 6 months when stored at -20°C. Avoid repeated freeze-thaw cycles.
- 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.

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 Pst I. Pst I is compatible with Sda I. The 3’ restriction site is Nco I which includes the ATG start codon, and is compatible with BspH I and BspLUI 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

<table>
<thead>
<tr>
<th>Element</th>
<th>Name</th>
<th>Origin</th>
<th>Size bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’ UTR</td>
<td>AFP</td>
<td>Human</td>
<td>244</td>
</tr>
<tr>
<td>3’ UTR</td>
<td>AFP</td>
<td>Human</td>
<td>31</td>
</tr>
</tbody>
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Alpha-fetoprotein promoter
The alpha-fetoprotein (AFP) gene is normally expressed in fetal but not adult livers. However, about 70% of hepatocellular carcinoma (HCC) are known to overexpress AFP, this up-regulation being at the transcriptional level. The AFP promoter has been extensively studied and shown to confer selective expression of a transgene in vitro and in vivo. The expression level of the transgene is proportional to the level of AFP expression in the transfected cells. Several studies have reported the use of AFP promoter to express a cytokine or suicide gene into HCC cells after delivery with viral or plasmid vectors.

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 pA: The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- Ori pMB1 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

Plasmid resuspension:
Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1µg/µl, resuspend the DNA in 20µl of sterile H2O. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning:
Plasmid amplification and cloning can be performed in E. coli GT116 or other commonly used laboratory E. coli strains, such as DH5α.

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).
4- Do not heat a closed container. Do not autoclave Fast-Media®.
5- Swirl gently to mix the preparation. Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.
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:
pDRIVE-hAFP (5004 bp)

- PacI (3465)
- SwaI (3736)
- EcoRI (3465)
- PstI (7)
- PacI (5002)
- NcoI (280)

Genetic components:
- SV40 pAn
- EM7
- Sh ble
- hAFP prom
- pMB1 ori
- LacZ