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The purchase of the pDRIVE5Lucia-rNSE-RU5' vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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pDRIVE5Lucia-rNSE-RU5’
A plasmid with the rat NSE / RU5’ promoter
Catalog # pdrive5lc-rseru5
For research use only
Version # 14A07-MM

PRODUCT INFORMATION

Content:
- 20 µg of pDRIVE5Lucia-rNSE-RU5’ 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’ sites are EcoRI and SpeI. EcoRI is compatible with ApoI, MfeI and Tsp509I. SpeI is compatible with AavII, NheI and XbaI. The 3’ restriction site is NcoI which includes the ATG start codon, and is compatible with BspHI and BspUII1 I.
- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE promoter drives the expression of the Lucia luciferase reporter gene which allows for testing of the promoter’s activity in transient transfection experiments. Furthermore, the Lucia luciferase gene is flanked by restriction sites (NcoI and NheI) for easy replacement with a different gene of interest.

COMPOSITE PROMOTER CHARACTERISTICS

Rat NSE (Neuron-Specific Enolase) / RU5’ promoter

Complete Promoter size: 2042 bp

Specificity: Mature neurons

Neuron-specific enolase, an isoenzyme form of enolase, occurs in mature neurons and paraneurons. Transgenic mouse studies have shown that a 1.8 kb fragment of the rat NSE promoter is able to express a heterologous gene exclusively in postmitotic neurons and neuro-endocrine cells in parallel with endogenous NSE. The NSE promoter contains a TATA-like sequence, no CAAT box, and sequences for the AP-1 binding motif, AP-2 binding element, SP-1 binding sequence and cAMP response element 2. The NSE promoter was used to create transgenic mice exhibiting the neuropathological phenotypes of specific enolase gene. Brain Res Mol B 28(1):19-28. 3. Hwang DY, et al., 2004. Aberrant expressions of pathogenic phenotype in Alzheimer's diseased transgenic mice carrying NSE-controlled APPsw. Exp Neurol. 186(1):20-32.

PLASMID FEATURES

- Lucia luciferase is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase. ORF size (from the ATG to the stop codon): 634 bp
- Lucia luciferase activity can be evaluated using QUANTI-Luc™, an assay reagent containing all the components required to quantitively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases.
- 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 pDRIVE plasmid.

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 preformed in E.coli GT116 or other commonly used laboratory E.coli strains, such as DH5a.

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.