pDD-AmCyan1 Reporter

Catalog No.
632194 (Not sold separately)
Sold as a part of 632191

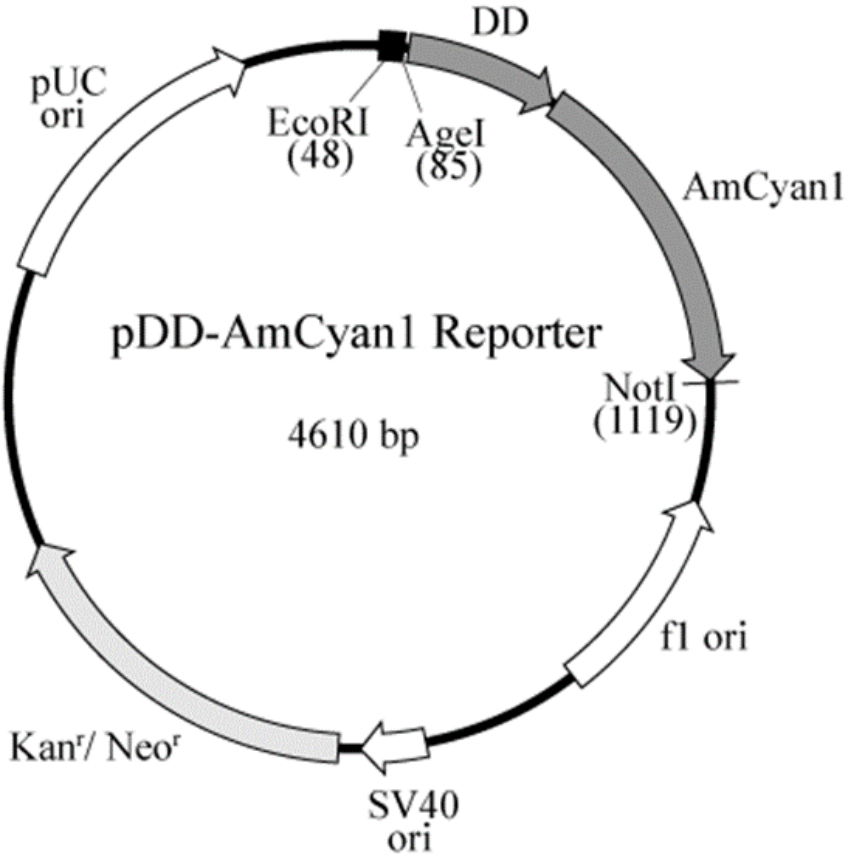

Figure 1. pDD-AmCyan1 Reporter vector map.

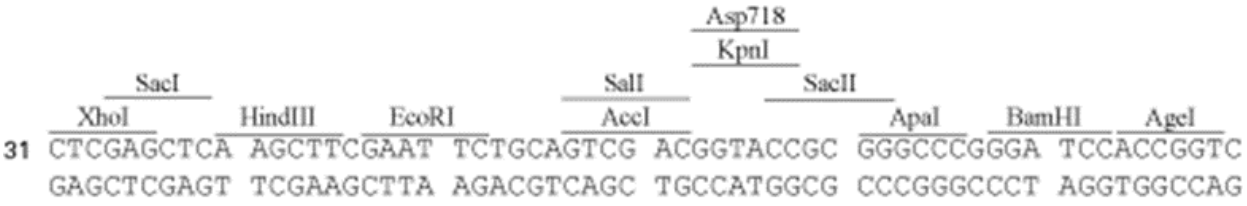

Figure 2. pDD-AmCyan1 Reporter vector multiple cloning site.

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Location of Features

- MCS (multiple cloning site): 31–89
- DD-AmCyan1
  Start codon (ATG): 97–99; Stop codon: 1114–1116
  DD (FKBP-L106P destabilization domain; 1): 97–420
  AmCyan1 (*Anemonia majano* cyan fluorescent protein): 427–1113
- f1 origin of replication (for packaging the noncoding strand of DD-AmCyan1): 1366–1821 (complementary)
- SV40 origin of replication: 2162–2300
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
  Neomycin phosphotransferase coding sequences: 2346–3140
- pUC origin of replication: 3725–4368

Description

pDD-AmCyan1 Reporter is a promoterless reporter vector that allows the functional analysis of different promoters and promoter/enhancer combinations inserted into its multiple cloning site (MCS). The vector encodes the reporter protein DD-AmCyan1, a ligand-dependent, destabilized cyan fluorescent protein that minimizes background fluorescence from leaky promoters. This reporter can be used to monitor promoter activity in live cells and *in vivo*. A promoter must be cloned into the MCS, located upstream of the DD-AmCyan1 coding sequence. Without the addition of a functional promoter, the vector will not express DD-AmCyan1.

DD-AmCyan1 Reporter

AmCyan1 (excitation and emission maxima: 458 and 489 nm, respectively) is a human codon-optimized variant of the wild-type *Anemonia majano* cyan fluorescent protein (AmCyan) that exhibits enhanced emission characteristics (Matz et al. 1999; Haas, Park, and Seed 1996). DD-AmCyan1 is a modified version of AmCyan1 that is tagged on its N-terminus with the ProteoTuner DD, which causes rapid, proteasomal degradation of DD-AmCyan1 (Banaszynski et al. 2006). However, when the membrane-permeant, stabilizing ligand Shield1 is added to the medium, it binds to the DD and prevents degradation of the DD-AmCyan1 reporter protein, thereby causing it to accumulate inside the cell.

In the absence of Shield1, the DD causes the degradation of any DD-AmCyan1 reporter protein produced prior to promoter activation, thus minimizing background fluorescence caused by leaky promoters. To analyze promoter activity, the inducer of choice is added to the medium along with Shield1, which effectively stabilizes the reporter protein, allowing it to accumulate. As a result, only the reporter molecules expressed during promoter induction will contribute to the fluorescence signal, providing a considerably higher signal-to-noise ratio than that obtained with non-destabilized or constitutively destabilized reporter systems.

The promoter's activity level can be directly correlated to the fluorescence level.

Vector Elements

The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. pDD-AmCyan1 can be transfected into mammalian cells using any standard transfection method.

Antibiotic Selection

A neomycin-resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418 (Gorman, 1985). This cassette consists of the SV40 early promoter, a Tn5 kanamycin/neomycin resistance gene, and herpes simplex virus thymidine kinase (HSV TK) polyadenylation signals. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

Additional Information

Propagation in *E. coli*

- Recommended host strains: DH5α, HB101, and other general-purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high
- Plasmid incompatibility group: pMB1/ColE1

Excitation and Emission Maxima of AmCyan1

- Excitation: 458 nm
- Emission: 489 nm

References

Banaszynski, L. A., Chen, L. chun, Maynard-Smith, L. A., Ooi, A. G. L. & Wandless, T. J. A Rapid, Reversible, and Tunable Method to Regulate Protein Function in Living Cells Using Synthetic Small Molecules. *Cell* **126,** 995–1004 (2006).

Gorman, C. (1985) In DNA Cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190.

Haas, J., Park, E. C. & Seed, B. Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr. Biol.* **6,** 315–24 (1996).

Matz, M. V. *et al.* Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat. Biotechnol.* **17,** 969–973 (1999).

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