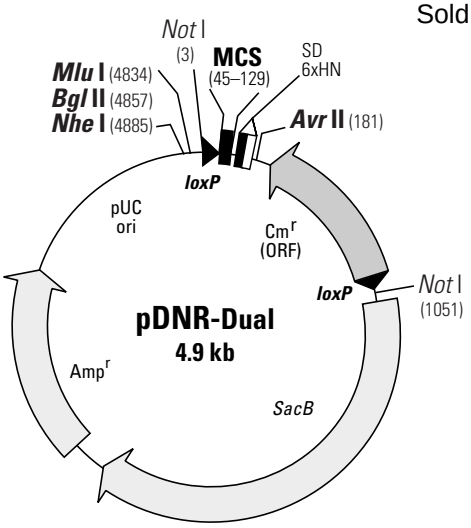
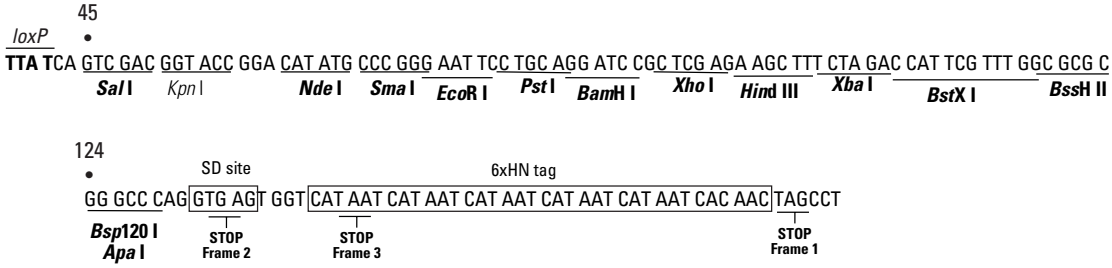
Sold as part of Catalog #K1677-1





Restriction Map and MCS of pDNR-Dual Vector. The MCS is shown in frame with the *loxP* site. The last four nucleotide bases of the *loxP* site can be seen at the left hand side of the map in bold.

Description

pDNR-Dual Donor Vector, like all donor vectors, is designed to transfer a target gene into any acceptor expression vector in the presence of Cre recombinase. In addition, pDNR-Dual makes it possible to attach 3' tags to the transferred gene via intron splicing, when combined with a specialized acceptor vector. Cre, a 38-kDa recombinase protein from bacteriophage P1, mediates recombination between DNA sequences at specific locations called *loxP* sites (1, 2). The pDNR-Dual Donor Vector contains two *loxP* sites, which flank the 5' end of the MCS and the 5' end of the open reading frame encoding the chloramphenicol resistance gene (Cm^r). This vector also contains the ampicillin resistance gene, which is the marker for propagation and selection of the donor vector in *E. coli*, and a splice donor (SD) site. In addition, pDNR-Dual contains the sucrase gene from *B. subtillis* (*SacB*), which provides negative selection against incorrect recombinants and the parental donor vector following recombination. pDNR-Dual also includes a 6xHN affinity tag directly downstream of the SD site. 6xHN-tagged fusions permit purification using CLONTECH's TALON® Metal Affinity Resin (#8901) and detection using CLONTECH's 6xHN Polyclonal Antibody (#8940-1). 6xHN tagged proteins have equal or higher affinity for IMAC resins than 6xHis tagged proteins, resulting in higher purification yields.

When the donor vector containing your gene of interest is combined with any acceptor vector and Cre recombinase, Cre molecules attach to *loxP* sites located on both the donor and acceptor vectors. Cre then mediates the transfer of the DNA fragment located between the two *loxP* sites in the donor vector, to the acceptor vector. When combined with a specialized acceptor vector containing a splice acceptor (SA) site, the Creator pDNR-Dual Cloning Kit can be used to rapidly generate recombinant expression plasmids for the addition of 3' tags to a gene of interest. The SD site is transferred from pDNR-Dual along with the gene of interest. The SD site mediates the fusion of the gene to the tag in the acceptor vector through intron splicing, which occurs when the construct is expressed in eukaryotic cells. As a result, a transcript is created that expresses the tag as a fusion to the 3' end

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of the gene of interest. All acceptor vectors also contains a bacterial promoter, located downstream of the *loxP* site, which drives expression of the chloramphenicol marker after recombination. For a complete list of acceptor vectors, visit our web site at **vectors.clontech.com**.

Use

Subclone your gene of interest into the pDNR-Dual Donor Vector using standard methods. When using the Creator pDNR-Dual Cloning Kit for the addition of 3' tags to a gene of interest, the sequence cloned into pDNR-Dual must be in frame with the SD site, and lack stop codons and a 3' UTR for correct expression of the protein-tag fusion. Then refer to the Creator DNA Cloning Kits User Manual (PT3460-1) for the Cre-recombinase mediated cloning procedure. Chloramphenicol and sucrose selection lets you harvest recombinant colonies that contain your desired recombinant construct. After selection, recombinant plasmids can be further propagated in chloramphenicol or the antibiotic that is appropriate for the resistance marker of the acceptor vector.

The 6xHN tag in pDNR-Dual can also be used to tag genes directly from the donor vector, through *in vitro* transcription/translation, using the T7 promoter upstream of the MCS. In addition, the tag will be expressed, with the gene of interest, after transfer to a standard (non-SA site) acceptor vector. For correct expression, the 3' end of the gene must be cloned in frame with the 6xHN tag with no intervening stop codons. If you do not wish to express your gene with the 6xHN tag and you are transferring your insert to a standard acceptor vector, ensure that the 3' end of your gene of interest is **not** in frame with the 6xHN tag. Note that when the gene is transferred to an acceptor vector containing an SA site, the 6xHN tag will be automatically spliced out of the final transcript.

Location of Features

- *loxP* recombination sites: 9–42; 1015–1048
- MCS: 45–129
- Splice donor (SD) site: 132–136
- 6xHN epitope tag: 141–176
- Chloramphenicol (Cm^r) open reading frame (ORF): 986–327
- *SacB* negative selection marker: 1057–2972
- Ampicillin-resistance gene:
  Start codon: 3103–3105; stop codon: 3961–3963
- pUC origin of replication: 4111–4574

Primer Locations

- M13-forward sequencing primer site: 4839–4855
- T7 RNA polymerase primer/promoter: 4865–4883

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101 and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Sauer, B. (1994) *Curr. Opin. Biotechnol.* **5**:521–527.
2. Abremski, K. & Hoess, R. (1984) *J. Biol. Chem.* **259**:1509–1514.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.