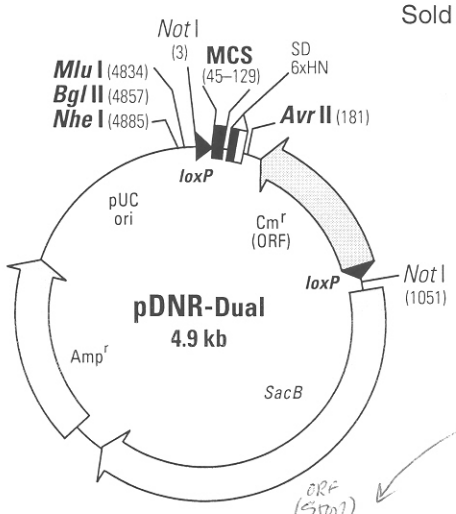
PCR → InFusion insert of ORF into vector → SalI, HindIII sites remain

pDNR-Dual Donor Vector Information PT3617-5

Sold as part of Catalog #K1677-1

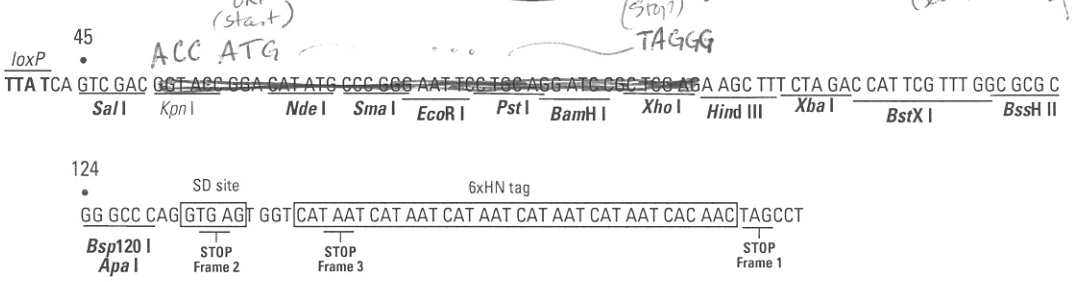
HIP Clones



SalI site remains (5' end) (KpnI gone)
all stop codons set to TAG (fusion clones have TTG leu codon instead of stop)
HindIII site remains (3' end) (XhoI gone)

TAG (stop) for "closed" format
TTG (leu) for "fusion" format

please be aware that the insert could have a SalI or HindIII site



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

(PR17853; published 17 July 2001)