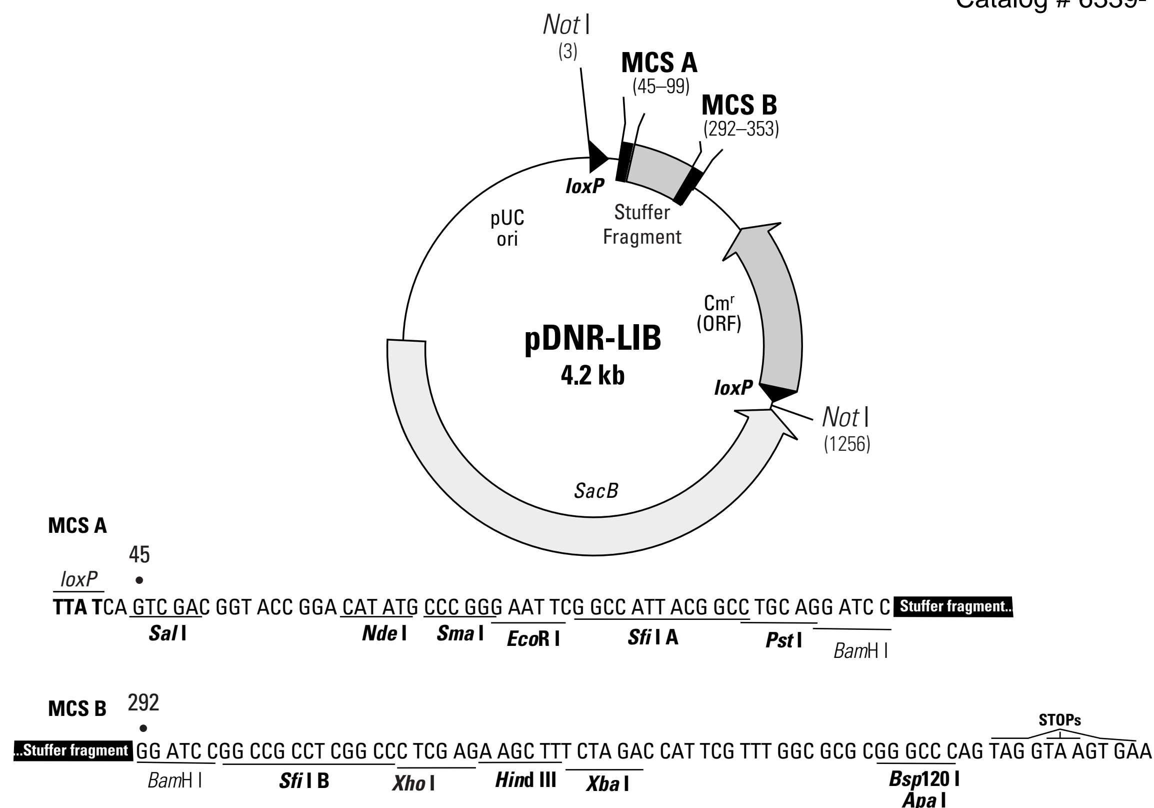
Catalog # 6339-1



Restriction Map and MCS of pDNR-LIB Vector. Unique restriction sites are shown in bold. MCS A 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 MCS A in bold. **Note:** The stuffer fragment is replaced by a cDNA insert when the library is constructed.

Description

pDNR-LIB Donor Vector is used to construct libraries compatible with CLONTECH's Creator™ System, including the Creator SMART™ cDNA Libraries. pDNR-LIB is designed for the construction of cDNA libraries with inserts that can be transferred into any Creator System acceptor vector. Cre recombinase, a 38-kDa recombinase from bacteriophage P1, mediates recombination between DNA sequences at specific locations in the donor and acceptor vectors called *loxP* sites (1,2). The pDNR-LIB vector contains two *loxP* sites, which flank the 5' end of MCS A and the 5' end of the open reading frame encoding the chloramphenicol resistance gene (Cm^r). pDNR-LIB also contains the sucrase gene from *B. subtillis* (*SacB*), which provides negative selection against both incorrect recombinants and the parental donor vector following recombination with an acceptor vector.

Use

Once you have synthesized your cDNA library and digested it with the appropriate enzyme, subclone the target library into pDNR-LIB using standard protocols and transform into *E. coli*. If desired, you may use the Creator SMART cDNA Library Construction Kit (#K1053-1) to generate libraries with maximal 5'-end sequence. The library can then be screened with conventional colony hybridization or by other screening approaches. Positive clones from the library may then be isolated and their inserts transferred to any acceptor vector in a short 15 minute reaction. When the donor vector containing your gene of interest is combined with an 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. As a result, a recombinant plasmid is created that expresses the insert from the specific expression elements for which the acceptor vector was designed. The acceptor vector also contains a bacterial promoter, located downstream of the *loxP* site, which drives expression of the

chloramphenicol marker after recombination. This provides positive selection for the recombinant plasmid. For a complete list of acceptor vectors, visit our web site at **vectors.clontech.com**. Please refer to the Creator DNA Cloning Kits User Manual (PT3460-1) for the Cre-recombinase mediated cloning procedure.

Location of Features

- *loxP* recombination sites: 9–42; 1218–1251
- MCS A: 45–99
- Stuffer fragment: 100–291
- MCS B: 292–351
- Chloramphenicol (Cm^r) open reading frame (ORF): 1189–530
- *SacB* negative selection marker: 3168–1266
- T7 RNA polymerase promoter: 4091–4109
- pUC origin of replication: 3337–3980

Primer Locations

- M13-reverse sequencing primer site: 382–362
- M13-forward sequencing primer site: 4065–4081

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101 and other general purpose strains.
- Selectable marker: plasmid confers resistance to chloramphenicol (30 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 sequence databases, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.

Notice to Purchaser

SMART™ technology is covered by U.S. Patents #5,962,271 & 5,962,272.
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