

In-Fusion® SMARTer® Directional Cloning & Screening Components

Catalog No.

639632 (Not sold separately)

Lot Number

Specified on product label.

Product Information

This package contains the components needed for cloning In-Fusion SMARTer Directional cDNA Libraries.

Package Contents

- 60 µl pSMART2IFD Linearized Vector (150 ng/µl)
- 100 µl Forward Screening Primer (10 µM)
- 100 µl Reverse Screening Primer (10 µM)

Storage Conditions

- Store all components at -20°C.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Shipping Conditions

- Dry ice (-70°C)

pSMART2IFD Linearized Vector

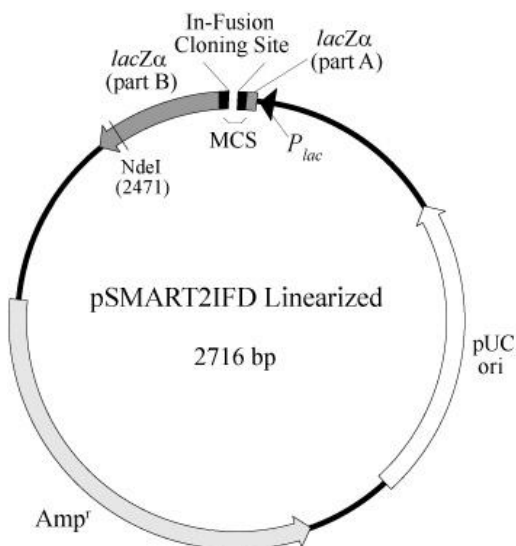


Figure 1. pSMART2IFD Linearized Vector Map

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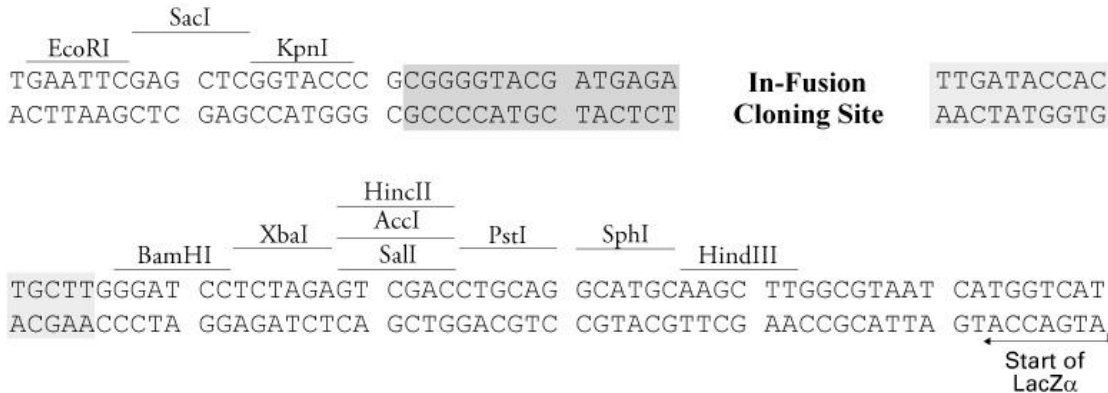


Figure 2. pSMART2IFD Linearized Vector In-Fusion Cloning Site. Each shaded region contains 15 bp of sequence that is complementary to one of the ends of the cDNA generated with the In-Fusion SMARTer PCR cDNA Synthesis Kit. The ends are different to allow for directional cDNA cloning.

Description

The pSMART2IFD Linearized Vector is a pUC19-based, high copy number, *E. coli* cloning vector. This linearized vector was generated by PCR, and contains the blunt ends shown flanking the In-Fusion Cloning Site in the sequence above. Each end of the vector shares 15 bp of complementarity (shaded above) with a different end of the cDNA generated using the In-Fusion SMARTer PCR cDNA Synthesis Kit (Cat. No. 634934). The ends are different to allow for directional cDNA cloning.

The In-Fusion Cloning Site is located within the *lacZα* gene (encoding the N-terminal fragment of β-galactosidase), the expression of which is driven by the *lac* promoter (P_{lac}). The location of the cloning site within *lacZα* allows for blue/white selection (i.e., α-complementation) after In-Fusion cloning. The vector also contains a pUC origin of replication that allows propagation in *E. coli* and temperature-dependent regulation of vector copy number, and an ampicillin resistance gene (Amp^r) for selection in *E. coli*.

Location of Features

- *lacZα* (N-terminal fragment of β-galactosidase; part A): 1–69 (complementary)
- P_{lac} (*lac* promoter): 114–143 (complementary)
- pUC origin of replication: 467–1055 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 1226–2086 (complementary)
- *lacZα* (N-terminal fragment of β-galactosidase; part B): 2432–2716 (complementary)

Additional Information

The pSMART2IFD Linearized Vector is provided as part of the In-Fusion SMARTer Directional cDNA Library Construction Kit (Cat. No. 634933), and is designed for effortless library construction with In-Fusion cloning technology.

Propagation in *E. coli*

- Recommended host strain: DH5α, XL1-Blue, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 μg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high
- Cell transformation efficiency: > 4 x 10⁹ cfu/μg

References

1. Yanisch-Perron, C., *et al.* (1985) *Gene* **33**(1):103-119.

Quality Control Data

Double-stranded SMARTer cDNA was synthesized from Control Mouse Liver Total RNA as described in the In-Fusion SMARTer Directional cDNA Library Construction Kit User Manual (PT5147-1). Three different volumes of SMARTer cDNA were each combined with 2 μ l (150 ng/ μ l) of pSMART2IFD in separate In-Fusion cloning reactions, according to the user manual. Each cloning reaction was transformed into a culture of *E. coli*. The three test reactions yielded at least 1×10^6 independent colonies, of which $\geq 75\%$ were white. Out of 15 randomly selected white colonies screened by PCR, 10 contained inserts ≥ 700 bp in length.

Plasmid Identity & Purity

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280} : 1.8–2.0

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