Vector Information

In-Fusion® Ready pBacPak-NTerm 6xHN Vector Information

PT3909-5

Sold as part of Catalog No. 631410

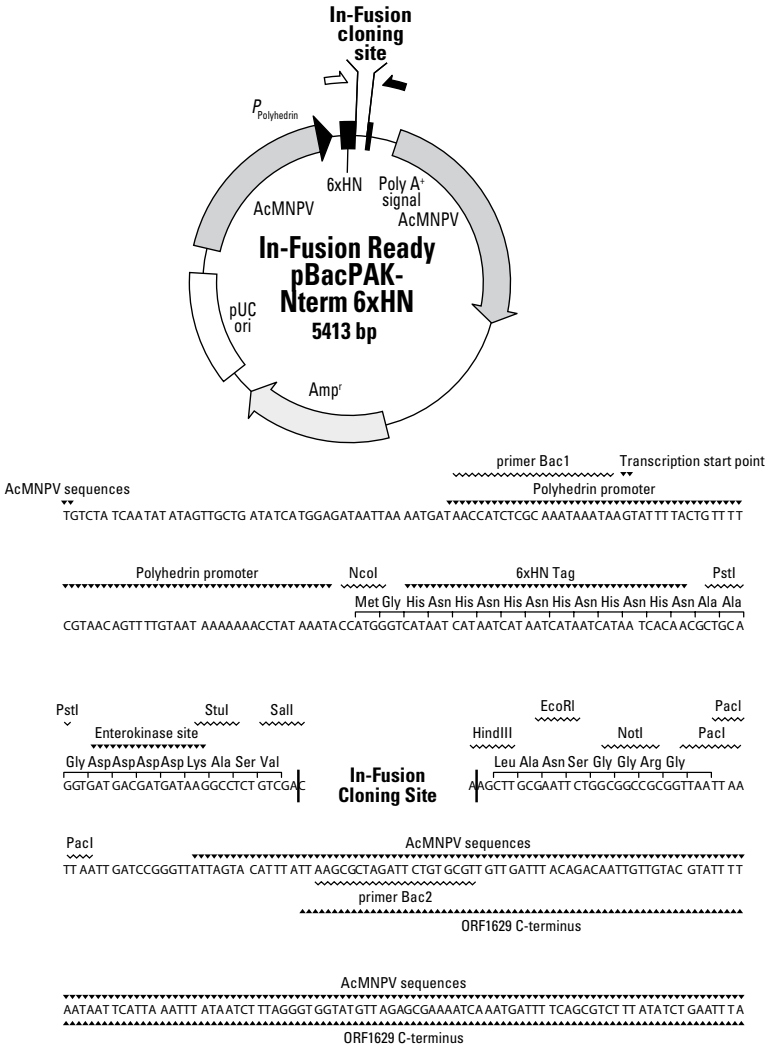

Figure 1. Restriction Map and In-Fusion Cloning Site (MCS) of In-Fusion Ready pBacPAK-Nterm 6xHN Vector.* All sites shown are unique. The Pac I sites at the end of the multiple cloning sites provide translational stop codons in all three reading frames. The M13 origin of replication in pBacPAK-Nterm 6xHN Vector can be used to package the coding strand of the target gene for sequencing and mutagenesis of the insert. In-Fusion Ready pBacPAK-Nterm 6xHN Vector also contains a pUC origin of replication and an ampicillin resistance gene for propagation in *E. coli*. The unshaded and filled arrows on the upstream and downstream side of the In-Fusion cloning site, respectively, represent the positions of the recommended forward (Bac1) and reverse (Bac2) primers.

Description

Available as part of the In-Fusion Ready BacPAK Vector Set (Cat. No. 631410), In-Fusion Ready pBacPAK-Nterm 6xHN Vector is a transfer vector designed for high-level expression of a cloned gene driven by the strong AcMNPV polyhedrin promoter. Flanking AcMNPV sequences allow recombination with viral DNA to transfer the expression cassette to the polyhedrin locus of the viral DNA. The In-Fusion Ready pBacPAK-Nterm 6xHN Vector has been prelinearized with *Sal* I and *Hin*d III, allowing easy In-Fusion cloning of PCR products in frame with the N-terminal 6xHN IMAC tag and adjacent enterokinase tag.

These PCR products can also be cloned into other prelinearized vectors, including In-Fusion Ready pBacPAK-Cterm 6xHN Vector, which places the 6xHN at the C-terminus of the protein of interest.

The In-Fusion Ready pBacPAK-Nterm 6xHN Vector contains coding sequences for an N-terminal 6xHN purification and detection tag, $(His\text{-}Asn)_6$, suitable for immobilized metal affinity chromatography (IMAC) protein purification using TALON® resin, as well as coding sequences for an enterokinase cleavage site $(Asp)_4$-Lys. Proteins containing the 6xHN tag and other

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histidine tags can be detected using Clontech's Universal His Western Blot Kit 2.0 (Cat. No. 635642).

In-Fusion Ready pBacPAK-Nterm 6xHN Vector contains a pUC origin of replication, an M13 origin for single-stranded DNA production, and an ampicillin resistance gene in *E. coli.*

Note on primer design and maintenance of reading frame: In-Fusion Ready pBacPAK-Nterm 6xHN Vector has been linearized using *Sal* I and *Hin*d III at the indicated "In-Fusion Cloning Site" and thus no longer contains the cytosine and adenosine residues shown inside the vertical lines. In order to maintain the correct reading frame and restore the *Sal* I and *Hin*d III sites, these nucleotides should be incorporated into the In-Fusion primers. If you prefer not to recreate these sites, you may replace these in the primers with other nucleotides. Details on the design of primers for In-Fusion cloning can be found in the In-Fusion Ready BacPAK Vector Set User Manual (PT3908-1).

Location of features

- AmpR: 2393–3253
- AcMNPV sequences: 50–1480
- ORF1629 C-terminus: 63–1406 (complementary)
- M13 single strand DNA origin: 1533–2006
- AcMNPV sequences: 4189–5334
- 6xHN tag: 5343–5377
- Polyhedrin mRNA polyadenylation signal: 376–381
- Transcription start point: 5286–5286
- Polyhedrin promoter: 5264–5333
- pUC plasmid replication origin: 3401–4044

Primer Locations

Bac1 Primer: 5331–5350	aaccatctcgcaaataaata
Bac2 Primer: 150–131	acgcacagaatctagcgctt

Propagation in *E. coli*

- Suitable host strains: DH5α and other general-purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Additional Information

See the In-Fusion Ready BacPAK Vector Set User Manual (PT3908-1) for In-Fusion cloning techniques. See the BacPAK Baculovirus Expression System User Manual (PT1260-1) for generation of baculoviruses and related protocols. See the BacPAK Baculovirus Rapid Titer Kit User Manual (PT3153-1) for titration of recombinant baculoviruses.

***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.