

# Vector information sheet

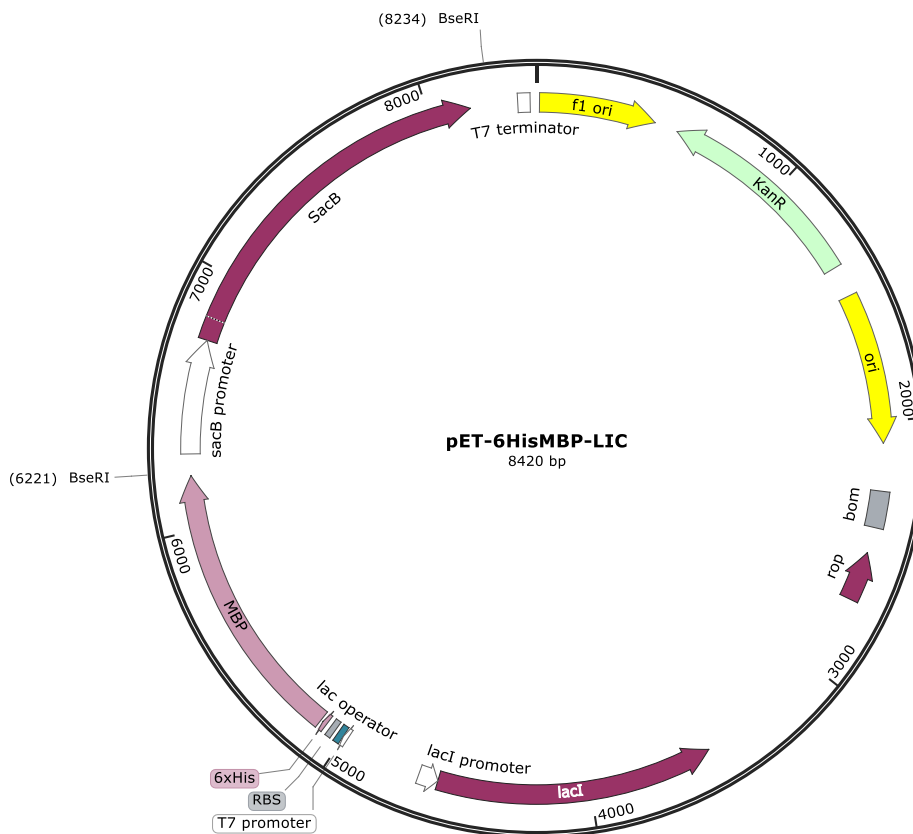
Date: Aug 2020

Vector Name	pET-6HisMBP-LIC Vector
Source	Designed by Levon Halabelian and constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto
Description	pET-6HisMBP-LIC is derived from pET28a (Novagen) and p6HISMAL-MHL (Peter Loppnau, SGC Toronto). It is used for T7 promoter driven expression of recombinant proteins with the addition of N-terminal 6 x His and MBP fusion tags
Antibiotic resistance	Kanamycin, 50ug/ml
Promoter	T7 - lacO
Cloning Methods	Insertion of DNA sequence into the cloning/expression region is preformed using Infusion enzyme mediated seamless cloning (Clontech cat # 639605). Vector is linearized by BseRI. Insertion of target sequence involves replacement of a SacB stuffer gene, which provides for negative selection of the original plasmid on 5% sucrose.
N – terminal fusion 6x His-MBP Tags	MKSSHHHHHGGSSMKIEEGKLVWINGDKGYNGLAIEVGGKFEKDTGKIKVTVEHPD KLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAV RYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYF TWPLIAADGGYAFKYENGYDIKDVGVNDNAGAKAGLTLVLDLIKHKHMNADTDYSI AEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGIN AASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELVKDPRIAATMENA QKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNA
5' primer additions	5' cagactaatgctgca---3'
3' primer additions	5' caagcttcgcatca --- 3'
5' sequencing primer- MBP-nF	5' tggaaaacgccagaaag---3'
3' sequencing primer- T7-R	5' atgctagtattgctcagcgg---3'

### Features of pET-6HisMBP-LIC:

- f1 origin: 12-467
- Kan R coding sequence: 560-1375
- lacI promoter and coding sequence: 3515-4675
- T7 promoter: 4984-5002
- lac operator: 5003-5027
- N-terminal 6 x His tag: 5083-5100
- N-terminal MBP-tag: 5110-6210
- BseR1: 6221 & 8234
- SacB promoter and coding sequence: 6295-8162
- T7 terminator: 8348-8395

### Map of pET-6HisMBP-LIC



**pET-6HisMBP-LIC cloning/expression region:**

```

                  T7 FWD                      lac operator
                  .....▶ ~~~~~
4968 ctgatcccg cgaaattaat acgactcact ataggggaat tgtgagcgga
    gagctagggc gctttaatta tgctgagtga tatcccctta acactcgct

~~~~~
5018 taacaattcc cctctagaaa taattttggt taactttaag aaggagatat
    attgttaagg ggagatcttt attaaaaaca attgaaattc ttcctctata
  
```

```

N-Tag:      6xHis      MBP
M K S   S H H   H H H   H G S S   M K
         ───────────────────────────────────────────────────▶
  
```

```

5068 accatgaaat cttctcacca tcaccatcac catggttctt ctatgaaat
    tggtagcttta gaagagtggg agtggttagtg gtaccaagaa gatactttta
  
```

(---1102 base pairs continued MBP coding sequences---)

```

          BseRI
6211 aatgctgca/attatgagtt ctcctc  ----- SacB(1.87 kb) -----
     ttacgacgt/taataactcaa gaggag
  
```

```

BseRI           2 x stop codons
8219 gaggagatca tgcaca/tgat gacgaagctt gcggccgcac tcgagcacca
     ctctcttagt acgtgt/acta ctgcttcgaa cgccggcgtg agctcgtgga
  
```

**Full sequence: 8420 bp**

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