High-Throughput Cloning Vectors (including MBP/T4L Mutants) Multiple Cloning Sites

**Note: All high-throughput cloning vectors listed in this document are AMPICILLIN resistant
*All cloning in this vectors can be done with Froward BamHI and Reverse XhoI or STOP-XhoI

HT1: N-His-TEV-Protein-STOP Original Base Vector was pet16b
*Sequence with T7 & T7Term Primers

```
  Bgl II                    T7 Promoter              lac operator                  XbaI
AGATCTCGATCCCGCAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTCTTTAAGTT

       RBS        NcoI                 His Tag                        TEV Cleavage Site ↓        BamHI
TAAGAAGGAGATATACCATGGGCCGGGGCAGCCATCATCATCATCATCATGGCGGCGAAAACCTGTATTTTCAGGGCCCGGGATCC
                 MetGlyArgGlySerHisHisHisHisHisHisGlyGlyGluAsnLeuTyrPheGlnGlyProGlySer

   EcoRI              XhoI              *Note the XhoI site is out of frame – you need to add a STOP codon
CCGAATTCCCGGGTCGACTCGAGCGGCCGC…
ProAsnSerArgValAspSerSerGlyArg
```

HT2: Protein-His-STOP Original Base Vector was pet21b – T7 tag removed from N-terminus
*Sequence with T7 & T7Term Primers

```
  Bgl II                    T7 Promoter              lac operator                  XbaI
AGACTCCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACT

       RBS        NdeI    BamHI  EcoRI    XhoI         His Tag
TTAAGAAGGAGATATACATATG GGATCC GAATTC CTCGAG CACCACCACCACCACCACTGA
                   Met GlySer GluPhe LeuGlu HisHisHisHisHisHis *
```

HT4: N-Trx-His-TEV-Protein – TEV site replacing Enterokinase site in pet32b
*Sequence with S•Tag primer #69945-3 & T7 Term

```
T7 Promoter→                lac operator              XbaI                          RBS
TTATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAT

          Trx Tag                          MscI            His Tag
ATGAGC …315bp/105aa… CTGGCCGGTTCTGGTTCTGGCCATATGCACCATCATCATCATCATTCTTCTGGT
MetSer               LeuAlaGlySerGlySerGlyHisMetHisHisHisHisHisHisSerSerGly

    Thrombin  ↓                         S Tag                                  Bgl II
CTGGTGCCACGCGGTTCTGGTATGAAAGAAACCGCTGCTGCTAAATTCGAACGCCAGCACATGGACAGCCCAGATCTG
LeuValProArgGlySerGlyMetLysGluThrAlaAlaAlaLysPheGluArgGlnHisMetAspSerProAspLeu

    TEV Cleavage     ↓        BamHI   EcoRI   SacI      Sal I    HindIII     NotI       XhoI
GAAAACCTGTATTTTCAGGGCCCGGGATCC GAATTC GAGCTCCGTCGAC AAGCTT GCGGCCGCACTCGAG
GluAsnLeuTyrPheGlnGlyProGlySer GluPhe GluLeuArgArg GlnAla CysGlyArgThrArg…

His Tag (Not in frame)          *Note XhoI not in frame – Add STOP to reverse primer to avoid extra residues
CACCACCACCACCACCACTGA
```

Lysozyme Mutants

Plasmid Numbers		Mutant	Proven Metal Complexes		
N-Fusion	C-Fusion		Copper	Nickel	Zinc
HT5	HT7	Psuedo-WT			
HT9	HT14	D61H, K65H	✓		
HT10	HT15	R76H, R80H	✓		✓
HT11	HT16	D61H, K65H, R76H, R80H	✓	✓	
HT12	HT17	R76C, R80C			✓
HT13	HT18	R125C, E128C			

N-His-TEV-lysozyme-protein Multiple Cloning Site

```
Bgl II                    T7 Promoter              lac operator                  XbaI
AGATCTCGATCCCGCAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTCTTTAAGTT

       RBS        NcoI                 His Tag
TAAGAAGGAGATATACCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCAT
                 MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerHis

       TEV Cleavage Site ↓            Lysozyme        BamHI   NdeI    Sal I    EcoRI   XhoI    NotI
ATGGAAAACCTGTATTTTCAGGGAGGTCCGATGAAT…TATAAATCGGAATCC CATATG GTCGAC GAATTC CTCGAG GCGGCCGC
MetGluAsnLeuTyrPheGlnGlyGlyProMetAsn TyrLysSerGlySer HisMet ValAsp GluPhe LeuGlu AlaAla…
```

*XhoI is out of frame with the vectors stop codon so you need to put one in the reverse primer

N-His-TEV-Protein-Lysozyme Multiple Cloning Site

```
  Bgl II                    T7 Promoter              lac operator                  XbaI
AGATCTCGATCCCGCAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTCTTTAAGTT

       RBS        NcoI                 His Tag                      TEV Cleavage Site ↓       BamHI
TAAGAAGGAGATATACCATGGGCCGGGGCAGCCATCATCATCATCATCATGGCGGCGAAAACCTGTATTTTCAGGGCCCGGGATCC
                 MetGlyArgGlySerHisHisHisHisHisHisGlyGlyGluAsnLeuTyrPheGlnGlyProGlySer

  NdeI    Sal I    EcoRI   XhoI    Lysozyme          NotI
CATATG GTCGAC GAATTC CTCGAGTCGATGAAT … TATAAATAAGCGGCCGC
HisMet ValAsp GluPhe LeuGluSerMetAsn   TyrLys * AlaAla…
```

Maltose Binding Protein Mutants

Plasmid Numbers			Proven Metal Complexes		
N-Fusion	C-Fusion	Mutants	Copper	Nickel	Zinc
HT6	HT8	Wild-Type			
HT19	HT22	K26H, K30H			✓
HT20	HT23	A216H, K220H	✓	✓	✓
HT21	HT24	E310H, K314H			✓

N-His-TEV-MBP-Protein Multiple Cloning Site

```
Bgl II                    T7 Promoter              lac operator                    XbaI
AGATCTCGATCCCGCAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTCTTTAAGTT

      RBS        NcoI                 His Tag          TEV Cleavage Site ↓
TAAGAAGGAGATATACCATGGGCCATCATCATCATCATCATGGCGGCGAAAACCTGTATTTTCAGGGCGGCCCG
                 MetGlyHisHisHisHisHisHisGlyGlyGluAsnLeuTyrPheGlnGlyGlyPro

     MBP          BamHI   NdeI    Sal I    EcoRI   XhoI    NotI
ATGAAA…AATTCGTCGGAATCC CATATG GTCGAC GAATTC CTCGAG GCGGCCGC
MetLys AsnSerSerGlySer HisMet ValAsp GluPhe LeuGlu AlaAla…
```

N-His-Protein-MBP

```
Bgl II                    T7 Promoter              lac operator                    XbaI
AGATCTCGATCCCGCAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTCTTTAAGTT

      RBS        NcoI                 His Tag                        TEV Cleavage Site ↓       BamHI
TAAGAAGGAGATATACCATGGGCCGGGGCAGCCATCATCATCATCATCATGGCGGCGAAAACCTGTATTTTCAGGGCCCGGGATCC
                 MetGlyArgGlySerHisHisHisHisHisHisGlyGlyGluAsnLeuTyrPheGlnGlyProGlySer

  NdeI    Sal I    EcoRI   XhoI         MBP            NotI
CATATG GTCGAC GAATTC CTCGAGTCGATGAAA…AATTCGTAAGCGGCCGC
HisMet ValAsp GluPhe LeuGluSerMetLys AsnSer * AlaAla…
```