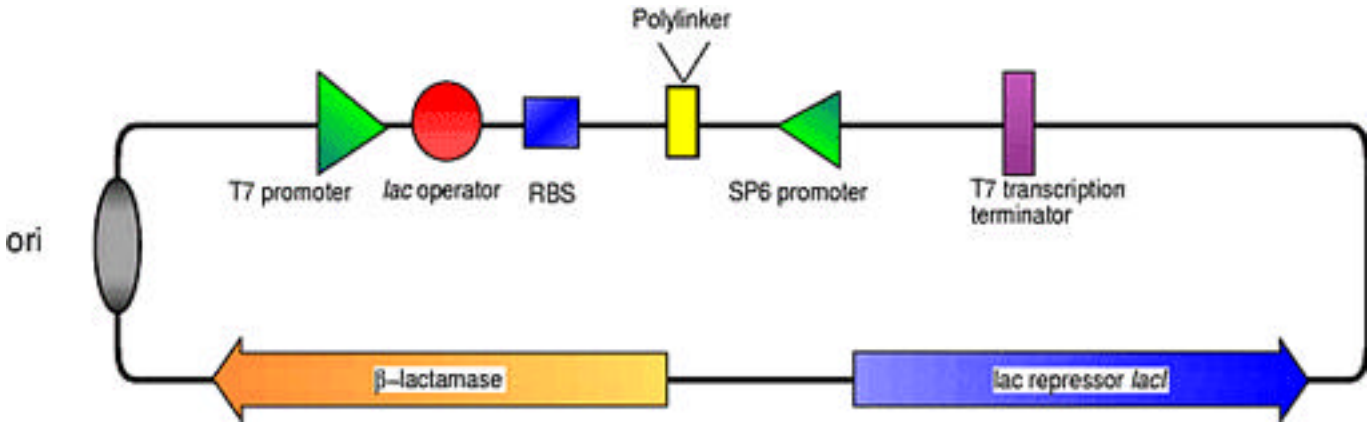
pHAT2 - His-tagged expression vector



Features	position
T7 promoter	1-17
Lac operator	21-42
T7 gene 10 ribosome binding site	50-80
His-tag	92-109
Polylinker	111-179
SP6 promoter	184-202
T7 transcription terminator	281-322
LacI gene	732-1813 (reversed)
β-Lactamase gene	2583-3433
Origin of replication	3507-4203

Reference:

Peränen J., Rikkonen M.,Hyvönen M. & Kääriäinen L. (1996) T7 vectors with modified T7*lac* promoter for expression of proteins in *Escherichia coli* . *Anal. Biochem.* **236**, 371-373.

pHAT2

T7 promoter --> **Lac operator** XbaI

```
ATTAATACGACTCACTATAGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAATTTT
TAATTATGCTGAGTGATATCCCTTAACACTCGCCTATTGTTAAGGAGATCTTTATTAAAA
```

rbs **His-tag** NcoI

```
GTTTAACTTTAAGAAGGAGATATATCC**ATG**AGTCATCACCATCACCATCACTCCATGGAT
CAAATTGAAATTCTTCCTCTATATAGGTACTCAGTAGTGGTAGTGGTAGTGAGGTACCTA
                          **MET**SerHisHisHsiHisHisHisSerMetAsp
```

EcoRI SalI XhoI BamHI NotI NsiI HindIII

```
ATCGAATTCGTCGACCTCGAGGGATCCGGGCCCTCTAGATGCGGCCGCATGCATAAGCTT
TAGCTTAAGCAGCTGGAGCTCCCTAGGCCCGGGAGATCTACGCCGGCGTACGTATTCGAA
IleGluPheValAspLeuGluGlySerGlyProSerArgCysGlyArgMetHisLysLeu
```

<-- SP6 promoter

```
GAGTATTCTATAGTGTCACCTAAATCCCAGCTTGATCCGGCTGCTAACAAAGCCCGAAAG
CTCATAAGATATCACAGTGGATTTAGGGTCGAACTAGGCCGACGATTGTTTCGGGCTTTC
GluTyrSerIleValSerProLysSerGlnLeuAspProAlaAlaAsnLysAlaArgLys
```

Single Cutters Listed by Site Order

111	NcoI	173	HindIII	1277	BstEII	2014	Eco56I
122	EcoRI	264	EspI	1445	BclI	2016	NaeI
128	SalI	264	Bpu1102I	1459	MluI	2196	NdeI
134	XhoI	341	AccIII	1884	PflMI	2451	AatII
136	SciI	341	BspMII	1894	Asp718I	2770	XmnI
140	BamHI	650	BspMI	1894	Acc65I	2889	ScaI
160	NotI	661	PstI	1898	KpnI	3304	Eco31I
160	EagI	821	NarI	1902	Ecl136II	3370	Eam1105I
160	Eco52I	822	EheI	1902	EcoICRI	3849	AlwNI
160	XmaIII	824	BbeI	1904	SacI	4375	SapI
171	NsiI	1048	BssHII	1904	SstI		

Non Cutting Enzymes

AflII	AgeI	AscI	AsuII	AvrII	BalI
BglII	BsmI	Bsp1407I	Bsu36I	ClaI	Csp45I
CspI	CvnI	DraIII	Eco47III	Eco72I	EcoNI
FseI	HpaI	I-PpoI	MfeI	Mlu113I	MscI
MstII	NheI	NruI	PacI	PinAI	PmaCI
PmeI	RleAI	SacII	SauI	SfiI	SgrAI
SmaI	SnaBI	SpeI	SplI	SpoI	SrfI
SstII	StuI	SunI	SwaI	Tth111I	XmaI