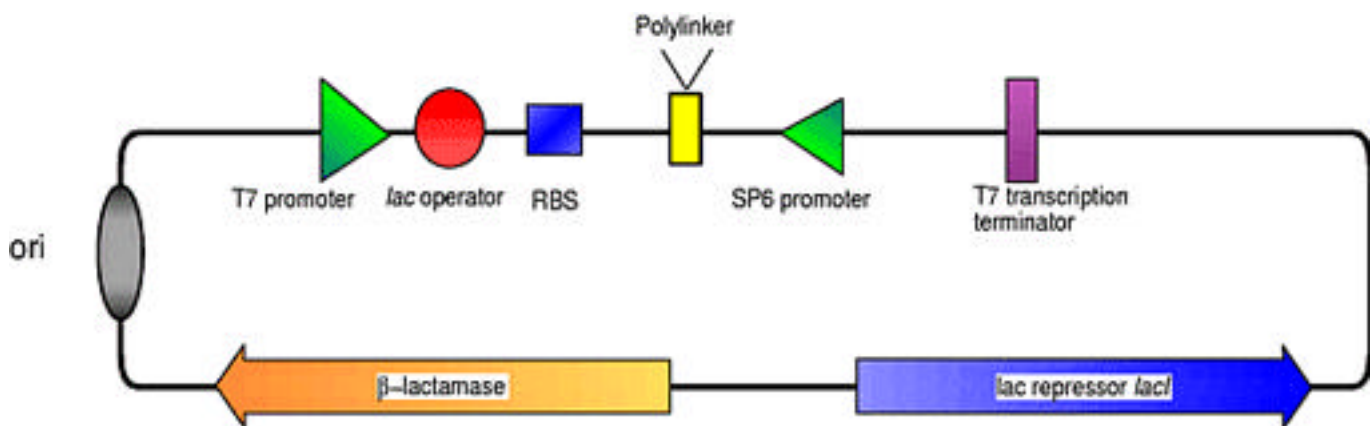


## pHAT - His-tagged expression vector



Features	position
T7 promoter	1-17
Lac operator	21-42
T7 gene 10 ribosome binding site	50-80
His-tag	98-115
Polylinker	116-175
SP6 promoter	180-198
T7 transcription terminator	277-318
LacI gene	728-1809 (reversed)
-Lactamase gene	2579-3429
Origin of replication	3504-4199

Reference:

Peränen J., Rikkonen M., Hyvönen M. & Kääriäinen L. (1996) T7 vectors with modified T7lac promoter for expression of proteins in *Escherichia coli*. *Anal. Biochem.* **236**, 371-373.

# pHAT

T7 promoter --> Lac operator XbaI  
ATTAATACGACTCACTATAGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAATTTT  
TAATTATGCTGAGTGATATCCCTTAACACTCGCCTATTGTTAAGGAGATCTTTATTAATA

rbs His-tag  
GTTTAACTTTAAGAAGGAGATATATCC**ATGA**ACACCATTTCATCACCATCACCATCACAAC  
CAAATTGAAATTCTTCCTCTATATAGGTA**CTTGTGGTAAGTAGTGGTAGTGGTAGTGGT**  
**MET**AsnThrIleHisHisHsiHisHisHisAsn

Eco47III BamHI  
SpeI NcoI NotI NsiI HindIII  
ACTAGTAGCGCTACCATGGATCCGGGCCCTCTAGATGCGGCCGCATGCATAAGCTTGAGT  
TGATCATCGCGATGGTACCTAGGCCCGGAGATCTACGCCGGCGTACGTATTCGAACTCA  
ThrSerSerAlaThrMetGluProGlyProLeuAspAlaAlaAlaCysIleSerLeuSer

<-- SP6 promoter  
ATTCTATAGTGTACCTAAATCCCAGCTTGATCCGGCTGCTAACAAAGCCCGAAAGGAAG  
TAAGATATCACAGTGGATTTAGGGTTCGAACTAGGCCGACGATTGTTTCGGGCTTTCTTC  
IleLeu\*\*\*

## Single Cutters Listed by Site Order

119	SpeI	260	EspI	1441	BclI	2010	Eco56I
127	Eco47III	337	AccIII	1455	MluI	2012	NaeI
132	NcoI	337	BspMII	1880	PflMI	2192	NdeI
136	BamHI	646	BspMI	1890	Asp718I	2447	AatII
156	Eco52I	657	PstI	1890	Acc65I	2766	XmnI
156	EagI	817	NarI	1894	KpnI	2885	ScaI
156	XmaIII	818	EheI	1898	Ecl136II	3300	Eco31I
156	NotI	820	BbeI	1898	EcoICRI	3366	Eam1105I
167	NsiI	1009	EcoRV	1900	SacI	3845	AlwNI
169	HindIII	1044	BssHII	1900	SstI	4371	SapI
260	Bpu1102I	1273	BstEII				

## Non Cutting Enzymes

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