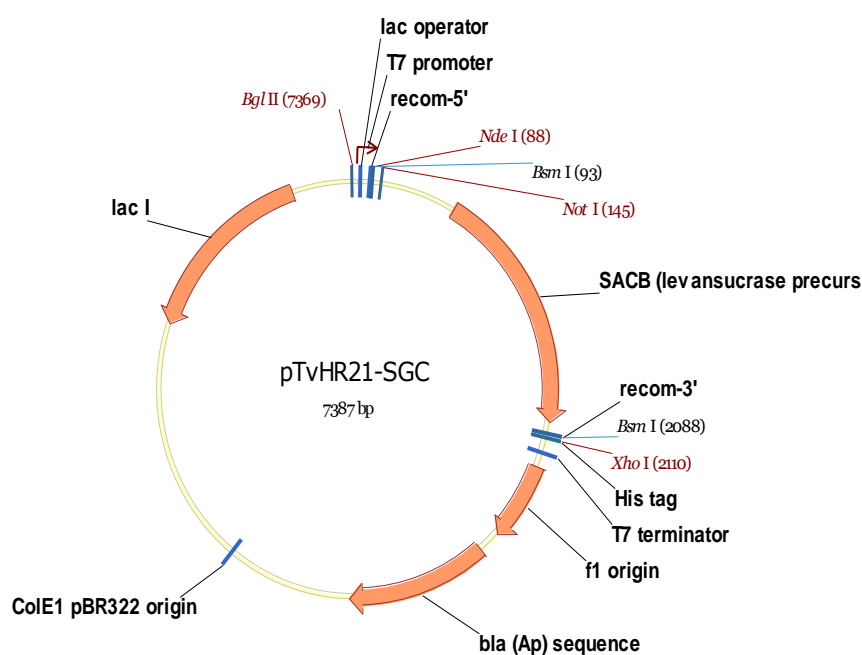


Vector information sheet

Dated: 8th May 2013

Vector Name	pTvHR21-SGC
Source	Jonathan Elkins, Oxford SGC
Sequence accession/link	(SGC)
Description	pET21a-derived expression vector with C-terminal His ₆ tag, removable with TEV protease. A “stuffer” fragment that includes the SacB gene, allows negative selection on 5% sucrose for indigested vector.
Antibiotic resistance	ampicillin
Promoter	T7 - lacO
Cloning	Cloning of PCR fragments is done by recombination using InFusion reagents (Clontech). The vector is cleaved with BsmI, and the PCR fragment includes overhangs which can recombine with identical sequences in the vector. The resulting clones have no N-terminal additions (except for initiator met).
Initiation codon	Supplied in PCR primer
C-terminal fusion – seq.	ENLYFQ*SLEHHHHHH (* - TEV cleavage site)
C-terminal fusion – MW	1946.9 (1152.2 removed by TEV cleavage)
Termination codons	In vector – following tag.
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	T7F: TAATACGACTCACTATAGGG
3' sequencing primer	T7R: GCTAGTTATTGCTCAGCGG



Polylinker region:

Recomb-5'
61 GTTTAACTT TAAGA**AGGAG ATATACATAT** GGCATTCCTG AAAGATCCAT
AACTTCGTAT

NdeI BsmI

.....**SacB**
fragment.....>

TEV cleavage site ↓
N A E N L Y F Q S L E H H H H H H *
2081 GAATGCT**GAG AACCTGTACT** TCCAATCCCT CGAGCACCAC CACCACCACC
ACTGAGATCC
BsmI Recomb-3' XhoI

Primers for recombinase cloning:

Upstream addition: AGGAGATATACATATG (add ATG only if not in sequence)

Downstream addition: GAAGTACAGGTTCTC (don't add a stop codon if you want the tag)

pTvHR21-SGC sequence:

taatacgactcactataggggaattgtgagcggataacaattcccctctagaaataatTTTgtttaact
ttaagaaggagatatacatatggcattcctgaaagatccataaacttcgtatagcatacattatacgaag
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