Vector information sheet.

<table>
<thead>
<tr>
<th>Vector Name</th>
<th>pTvHR21-SGC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Jonathan Elkins, Oxford SGC</td>
</tr>
<tr>
<td>Sequence accession/link</td>
<td>(SGC)</td>
</tr>
</tbody>
</table>

**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*$^*$SEHHHHHHH

(* - 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: GCTAGTTATTGCTAGCGG

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![Diagram of pTvHR21-SGC vector](attachment:image.png)

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Opher Gileadi, Structural Genomics Consortium
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Polylinker region

Recomb-5’

61 GTTTAACTT TAAGAGGAG 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 H H *

2081 GAATGC

BsmI  Recomb-3’

GCTGAG AACCTGTACT TC CAAATCCCT CGAGCACCAC CACCACACC ACTGAGATCC

XhoI

Primers for recombinase cloning:

Upstream addition: AGGAGATACATAT (add ATg only if not in sequence)

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