Bacillus Subtilis
Expression Vectors

Product Information and Instructions
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These vector systems have been developed in the laboratory of Wolfgang Schumann at the Institute of Genetics, University of Bayreuth, Germany.

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B. subtilis Expression Vectors

Plasmid-based expression vectors for highly efficient intra- and extracellular production of recombinant proteins in Bacillus subtilis

1. Introduction

Gram-positive bacteria are well known for their contributions to agricultural, medical and food biotechnology and for the production of recombinant proteins. Among them, Bacillus subtilis has been developed as an attractive host because of several reasons: (i) it is non-pathogenic and is considered as a GRAS organism (generally regarded as safe); (ii) it has no significant bias in codon usage; (iii) it is capable of secreting functional extracellular proteins directly into the culture medium (at present, about 60% of the commercially available enzymes are produced by Bacillus species); (iv) a large body of information concerning transcription, translation, protein folding and secretion mechanisms, genetic manipulation and large-scale fermentation has been acquired.

But there are also two obstacles reducing the use of B. subtilis: (i) production of a number of extracellular proteases which recognize and degrade heterologous proteins, and (ii) stable vector plasmids. The first obstacle has been largely solved by the construction of protease-deficient strains. And the second has been completely overcome by introducing plasmids using the theta-mode of replication such as those derived from the natural plasmids pAMβ1 and pBS72 (Jannière et al., 1990; Titok et al., 2003).

Quite recently, the construction and use of four different expression vectors based on the E. coli - B. subtilis shuttle vector pMTLBS72 exhibiting full structural stability was published (Nguyen et al., 2005).

The two new vectors pHT01 and pHT43 allow high-level expression of recombinant proteins within the cytoplasm, where pHT43 directs the recombinant proteins into the medium. Both vectors are based on the strong σ^A-dependent promoter preceding the groE operon of B. subtilis which has been converted into an efficiently controllable (IPTG-inducible) promoter by addition of the lac operator. Derivatives of pHT01 are available either with a 8xHis tag (pHT08), a Strep tag (pHT9) or a c-Myc tag (pHT10).
2. The pHT Vectors

All vectors use the strong promoter preceding the groESL operon of Bacillus subtilis fused to the lac operator allowing their induction by addition of IPTG. While the background level of expression of these expression cassettes is very low in the absence of the inducer, an induction factor of about 1,300 was measured using the bgaB reporter gene (Phan et al., 2005). The amount of recombinant protein produced after addition of IPTG may represent 10 and 13%, respectively, of the total cellular protein (demonstrated when fusing the htpG and pbpE genes to the groE promoter; Phan et al., 2005). High level secretion of amyQ α-amylase and cellulase A and B of Clostridium thermocellum was demonstrated. An efficient Shine-Dalgarno (SD) sequence as well as a multiple cloning site (BamHI, XbaI, AatII, SmaI) were also inserted. To obtain secretion of recombinant proteins, the coding region for the signal peptide of the amyQ gene encoding an α-amylase was fused to the SD sequence of pHT01, thereby constructing pHT43.

2.1. Vector Map pHT01

Pgrac: Pgrac promoter (consisting of the groE promoter; the lacO operator and the gsiB SD sequence)
ColE1 ori: ColE1 origin
AmpR: ampicillin resistance
lacI: lacI gene (lac repressor)
CmR: chloramphenicol resistance

Complete DNA sequence is available on request.
2.2. Vector Map pHT43

Pgrac: Pgrac promoter (consisting of the groE promoter, the lacO operator and the gspBSD sequence)
ColE1 ori: ColE1 origin
AmpR: ampicillin resistance
lacI: lacI gene (lac repressor)
CmR: chloramphenicol resistance
SamyQ: amyQ signal sequence

Complete DNA sequence is available on request.
2.3. Location of the tags in the pH01 derivatives

Location of the 8xHis tag in pH08:

\[
\text{8xHis tag} \quad \text{BamH} \mid \text{XbaI} \mid \text{SmaI}
\]
\[
P_{\text{grac}}-\text{lacO-RBS-} \quad \text{GGATCC TCTAGAgtcgacgtC}
\]

Location of the Strep tag in pH09:

\[
\text{Strep tag} \quad \text{BamH} \mid \text{XbaI} \mid \text{SmaI}
\]
\[
P_{\text{grac}}-\text{RBS-} \quad \text{GGATCC TCTAGAgtcgacgtC}
\]

Location of the c-Myc tag in pH10:

\[
\text{BamH} \mid \text{XbaI} \quad \text{c-Myc tag}
\]
\[
P_{\text{grac}}-\text{GGATCC TCTAGAgtcgacgtC}
\]

3. Protocols

Detailed protocols for *E. coli* and *Bacillus* molecular genetic handling (growth, transformation etc.) can be found in the relevant laboratory manuals such as Sambrook and Russell (2001). For transformation of *B. subtilis* we recommend the protocol of Anagnostopoulos and Spizizen (1961), slightly modified:

1. 20 ml LS medium (Spizizen’s medium* supplemented with 0.5 % glucose, 5 μg/ml DL-tryptophane, 5 μg/ml uracil, 0.01% casein hydrolysate, 0.1% yeast extract [Difco], 1 mM MgSO\(_4\), 2.5 mM MgCl\(_2\), 0.5 mM CaCl\(_2\)) are inoculated with 1 ml of an 5 ml overnight culture grown in HS medium (Spizizen’s medium* supplemented with 0.5 % glucose, 50 μg/ml DL-tryptophane, 50 μg/ml uracil, 0.02% casein hydrolysate, 0.1% yeast extract [Difco], 8 μg/ml arginine, 0.4 μg/ml histidine, 1 mM MgSO\(_4\)) at 37°C, shaking slowly at 30°C for 3 to 4 hours.

2. Incubate 1 ml of this HS culture (late log/early stationary phase; OD\(_{578}\)) with 10 μl of 0.1 M EGTA at room temperature for 5 minutes and add 1 to 2 μg plasmid DNA.

3. After shaking at 37°C for 2 hours for development of antibiotic resistance, the cells are plated on selective plates.

*Spizizen’s medium: 2 g (NH\(_4\))\(_2\)SO\(_4\), 14 g K\(_2\)HPO\(_4\), 6 g KH\(_2\)PO\(_4\), 1 g sodium citrate; add 100 ml distilled water, autoclave, then add 0.1 ml 1 M MgSO\(_4\).*

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4. References


5. Order Information, Shipping & Storage

<table>
<thead>
<tr>
<th>order #</th>
<th>description</th>
<th>amount</th>
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<tbody>
<tr>
<td>PBS001</td>
<td>pHT01 vector, lyophilized plasmid DNA</td>
<td>10 µg</td>
</tr>
<tr>
<td>PBS002</td>
<td>pHT43 vector, lyophilized plasmid DNA</td>
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<td>PBS003</td>
<td>pHT08 vector, lyophilized plasmid DNA</td>
<td>10 µg</td>
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<tr>
<td>PBS004</td>
<td>pHT09 vector, lyophilized plasmid DNA</td>
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<tr>
<td>PBS005</td>
<td>pHT10 vector, lyophilized plasmid DNA</td>
<td>10 µg</td>
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</table>

Shipped at room temperature (RT). Lyophilized plasmid DNA can be stored at 4°C. Once the DNA has been dissolved in sterile water or buffer we recommend storage at -20°C.