Brevibacillus Expression System

A Leap Forward in Protein Production

High Yields of Disulfide Bonded and Secreted Proteins
High Efficiency Secretion of Large Quantities of Proteins
Proteins are Produced in Biologically Active Forms
Good at Intracellular Production as well

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Brevibacillus Expression System

*Brevibacillus choshinensis* is a gram positive bacterium which has an exceptional ability to express heterologous proteins. The New *Brevibacillus* Expression System, from Takara Bio, uses the bacterium for high efficiency secretory production. This system produces both eukaryotic and heterologous proteins, in large quantities and in active forms. Also the *Brevibacillus* system is able to generate the protein with a minor amount of extracellular protease so the produced protein is only slightly degraded.

Successful results of proteins expressed can be seen in Table 1. This includes expression of enzymes, antigens, and cytokines. Each protein was produced at a very high level of expression and confirmed to have native biological activity. In addition, proteins from several organisms which are taxonomically far apart, such as eubacteria, archaebacteria, eukaryotes, and viruses, were successfully produced.

Normally eukaryotic proteins have intramolecular cystein residues and disulfide bonds must be formed at precise positions making it difficult to obtain efficient production of proteins using intracellular bacterial expression systems. The *Brevibacillus* system is superior when expressing assembling and expressing secreted eukaryotic proteins. In addition, *B. choshinensis* serves as an excellent host for intracellular protein production. Furthermore, it produces intracellular proteins in a soluble form in the cytoplasm without forming inclusion bodies. In several cases the *Brevibacillus* system works better than *E.coli* for expression (See page 3). Therefore, *B. choshinensis* can be used as a host in the production of heterologous proteins irrespective of origin or nature.

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Origins</th>
<th>Production (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymes</td>
<td></td>
<td></td>
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<tr>
<td>α-amylase</td>
<td><em>B. licheniformis</em></td>
<td>3.7</td>
</tr>
<tr>
<td>Sphingomyelinase</td>
<td><em>B. cereus</em></td>
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</tr>
<tr>
<td>Chitosanase</td>
<td><em>B. halodurans</em></td>
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<tr>
<td>CGTase</td>
<td><em>B. macerans</em></td>
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<tr>
<td>Chitosanase</td>
<td><em>B. circulans</em></td>
<td>1.4</td>
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<tr>
<td>Hyperthermostable protease</td>
<td><em>A. pernix</em></td>
<td>0.1</td>
</tr>
<tr>
<td>Hyperthermostable nuclease</td>
<td><em>P. horikoshii</em></td>
<td>0.7</td>
</tr>
<tr>
<td>PDI</td>
<td>human</td>
<td>1.0</td>
</tr>
<tr>
<td>Antigens</td>
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<td>Surface antigen</td>
<td><em>E. rhusiopathiae</em></td>
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<td>Surface antigen</td>
<td><em>T. pallidum</em></td>
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<tr>
<td>Cytokines</td>
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<tr>
<td>EGF</td>
<td>human</td>
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<tr>
<td>IL-2</td>
<td>human</td>
<td>0.6</td>
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<tr>
<td>NGF</td>
<td>mouse</td>
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<td>GM-CSF</td>
<td>bovine</td>
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</tr>
<tr>
<td>GH</td>
<td>flounder</td>
<td>0.2</td>
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</table>

Table 1: Example of proteins expressed using the *Brevibacillus* expression system

*Brevibacillus System Applications*

Bioclip™ is a process that uses EGF which causes sheep to shed their fleece. Developed by CSIRO livestock industries The Woolmark Company. Recombinant hEGF is industrially manufactured using the *Brevibacillus* expression system.
Construction of B. choshinensis Secretory Expression Plasmid

A comparison of intracellular protein expression systems measuring eukaryotic Dehydrogenase (DH) activity was performed using E.coli with a pET vector and the Brevibacillus expression system using the pNI vector. The pNI vector, used with the expression system, is lacking a secretory signal peptide so the expressed protein will remain in the cytoplasm. The expression in the Brevibacillus system was determined using 2 different media. The expression for E.coli tested two different induction times. The efficiency of expression was assessed through measurement of DH activity (fig. 1) as well as solubility of the expressed protein (fig. 2&3).

Fig. 1: DH activity measurement of protein expressed, using the Brevibacillus expression system (in 2 different media) or using E.coli (after 16 hour induction or no induction).

Recovery of Dehydrogenase activity is 10 to 20 times higher when expression is performed using the Brevibacillus expression system versus expression using E.coli BL21 (DE3). In conclusion, the Brevibacillus expression system produces a higher yield of active protein.

Fig. 2: Protein expression using E.coli BL21 (DE3) after 3 and 16 hours of induction in soluble fraction as well as precipitate. M: Molecular weight marker. C: pET-15b (control). 1: pET-15b-DH. sol: soluble fraction. ppt: precipitate.

Fig. 3: Protein expression using B. choshinensis in 2 different media (TMNm & 2SLNm) in soluble fraction as well as precipitate. M: Molecular weight marker. C: pNI (control). 1-2: pNI-DH. sol: soluble fraction. ppt: precipitate.

TaKaRa Bio recommends

High fidelity polymerase for PCR amplification of the target gene:
PrimeSTAR HS DNA polymerase (R010A; R044A or R040A)

For efficient synthesis of long cDNA fragments:
PrimeScript RTase (2680A or 6110A)

For protein purification:
Talon Resin (635501 or 635600) or His60 (635659)

For insert cDNA cloning:
DNA Ligation Kit <Mighty Mix> (6023) or DNA Ligation Kit Ver.2.1 (6022) and Takara’s restriction enzymes
Choosing a Brevibacillus Vector?

<table>
<thead>
<tr>
<th>Vector Name</th>
<th>Vector Type</th>
<th>Expression Vector</th>
<th>Lac Operator</th>
<th>His-Tag</th>
<th>Sec Signal Peptide</th>
<th>Construct in</th>
<th>Protease cleavage site</th>
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<tbody>
<tr>
<td>pNC-HisE (5,263 bp)</td>
<td>Shuttle Vector</td>
<td>Secretary</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>E.coli</td>
<td>Enteropeptidase</td>
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<td>Yes</td>
<td>Yes</td>
<td>E.coli</td>
<td>Thrombin</td>
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<td>pNC-HisF (5,260 bp)</td>
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<td>Yes</td>
<td>Yes</td>
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<td>pNI-His DNA (5,079 bp)</td>
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<td>Yes</td>
<td>NO</td>
<td>E.coli</td>
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<tr>
<td>pNY326 (3.4 Kb)</td>
<td>Expression</td>
<td>Secretary</td>
<td>NO</td>
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<td>pNCMO2 (5.24b)</td>
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<td>pNCMO2-BLA</td>
<td>Positive Control</td>
<td>Secretary</td>
<td>Has the gene encoding Bacillus licheniformis amylase (55 kDa)</td>
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Vectors for the Brevibacillus System

Vectors sequences can be found here: http://www.takara-bio.com/products/download_e.htm

Brevibacillus Expression System Kit

<table>
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<tr>
<td>Brevibacillus Expression system</td>
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<td>kit</td>
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Kit Components

<table>
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<tr>
<th>Expression Vectors</th>
<th>Control Vector</th>
<th>Competent cells (for electroporation)</th>
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<tr>
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<td>pNCMO2 DNA</td>
<td>Brevibacillus choshinensis Electro-cells</td>
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Ordering Information

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<th>Quantity</th>
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<tr>
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<td>HB100</td>
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<td>Brevibacillus choshinensis Electro-cells</td>
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<td>pNC-HisE DNA</td>
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<td>pNC-HisF DNA</td>
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<td>pNC-HisT DNA</td>
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<td>pNI-His DNA</td>
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<tr>
<td>pNY326 DNA</td>
<td>HB111</td>
<td>10 µg</td>
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License Notices

[514] His-Tag Sequence This product is covered by the claims of U.S. Patent No. 5,284,933, 5,310,663 and their foreign counterpart patent claims. Protein Purification Technology of His-Tag used in some of plasmid vectors and Brevibacillus expression vectors is licensed from Hoffmann-La Roche, Inc., Nutley, NJ and/or Hoffmann-La Roche Ltd., Basel, Switzerland and is provided only for the use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Qiagen Strasse 1, D-40724 Hilden, Germany.

[539] Brevibacillus Expression System 1. B. choshinensis SPP. pNY326, pNCMO2, pNCMO2-BLA, pNC-HisE, pNC-HisF, pNC-HisT, pNI-His, pNi and pNi-His were developed and manufactured by Higoata Shoyu Co., Ltd. and sold by TAKARA BIO INC. 2. The use of this product is allowed for academic research only. You have to conclude a license agreement if you use this product or the products produced by using this product for commercial purpose. Contact us if you would like to ask any question about license etc. If any questions are received, either Higoata Shoyu Co., Ltd. or an agent for it will answer. TaKaRa Bio Inc. Tel +81-77-543-7247... 3. All rights of intellectual property related to this product are owned by Higoata Shoyu Co., Ltd. This product is covered by the claims of U.S. Patent No. 5,284,933, 5,310,663 and their foreign counterpart patent claims. Protein Purification Technology of His-Tag used in some of pCold vectors and Brevibacillus expression vectors is licensed from Hoffmann-La Roche, Inc., Nutley, NJ and/or Hoffmann-La Roche Ltd., Basel, Switzerland and is provided only for the use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Qiagen Strasse 1, D-40724 Hilden, Germany.

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