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I. Introduction

Brevibacillus choshinensis Electro-Cells is a host cell prepared for electroporation methods. The efficiency is $10^4 \sim 10^6$ transformants/μg DNA. The method have advantages in transformation efficiency, reproducivility, and small scale of plasmid DNA. *Brevibacillus choshinensis* Electro-Cells is a competent cell for transformation of pNY326 or pNCMO2 DNA in *Brevibacillus* Expression System.

Brevibacillus choshinensis, a gram positive bacterium, has excellent ability to produce many kinds of proteins extracellularly. And the *Brevibacillus* Expression System based on this bacterium is well-suited for secretory production of heterologous proteins with high efficiency. Making use of this characteristic of the host bacterium, many successful records have been accumulated so far in this regard. This system has following merits :

- The host bacterium secretes proteins very efficiently.
- The host bacterium produces negligible amout of extracellular protease so that products remain unscathed in the culture medium.
- Proteins are produced as active forms.
- Easy to culture and sterilize the host bacterium.
- The host bacterium is amenable to genetic engineering.
- The host bacterium is guaranteed to be safe organism.

II. Contents

Brevibacillus choshinensis Electro-Cells 100 μl×10 tubes

III. Storage

− 80 ℃

Caution : *Brevibacillus Choshinensis* Electro-Cells should be stored at ＜ − 80 ℃. The transformation efficiency may decrease if the storage temperature is not controlled completely. Do not store the Electo-Cells in liquid nitrogen.

IV. Procedure

IV-1. *Brevibacillus* strains

*S*tandard genetic engineering technique is applicable.

IV-1-1. Genotypes

An essential gene for spore formation cascade is disrupted in *B. choshinensis* SP3 so that sterilization of the transformants is easily done. Furthermore, the trace activity due to intracellular protease gene (*imp*) and extracellular protease gene (*emp*) is nullified by gene disruption to secure the intactness of protein products.

IV-1-2. Control DNA

pNCMO2-BLA : Positive control plasmid for secretory production which have the gene encoding *Bacillus licheniformis* α-amylase. The size of the protein is about 55 kDa and secreted to the amount of 0.1 g/l or more.

IV-2. Transformation of *Brevibacillus*

IV-2-1. Equipments and reagents required

Brevibacillus choshinensis Electro-Cells (on ice)
Expression plasmid to express target protein
Positive control (pNCMO2-BLA)
Negative control (pNCMO2 or pNY326)
MTNm plates
Cuvette for electroporation
Culture tube
Sterilized microtube

IV-2-2. Transformation by electroporation

(1) Place the tube containing competent cells on ice
(2) Add 5 μl DNA solution (200 ～ 500 ng)* to the competent cells and mix well by flipping the tube (Never vortex!).
(3) Transfer the mixture to the electroporation cuvette. Store the cuvette on ice for 10 min.
(4) Pulse the cells under the following condition :

Electroporator : Gene Pulser II (Bio-Rad)
Charging voltage : 7.5 kV/cm
Capacitance : 25 μF
Resistance : 1000 Ω
Gap of the cuvette : 2 mm

Electroporator : Electroporator 2510 (Eppendorf)
Charging voltage : 14 kV/cm
Gap of the cuvette : 1 mm

(5) Immediately add 1 ml MT medium and then transfer the mixture into a culture tube.
(6) Incubate the tube at 30 ℃ with shaking at 120 rpm.
(7) Spread 100 μl of the mixture onto a MTNm plate. Centrifuge the remaining mixture (5000 rpm, 5 min, room temp.) to concentrate the cells. Spread the whole amount of cells onto another MTNm plate.
(8) Incubate plates for 15 ～ 18 hrs at 37 ℃. If the sizes of the colonies are small, extend the incubation further.
(9) Obtained colonies can be subjected to plasmid check or protein expression analysis.

*When constructed plasmids as the control plasmids are used, the amount of DNA should be reduced to 10 ～ 100 ng.

IV-3. Medium components

MT liquid medium

Components :

Glucose	10.0 g/L
Polypeptone	10.0 g/L
Meat extract	5.0 g/L
Yeast extract	2.0 g/L
$FeSO_4 \cdot 7H_2O$	10 mg/L
$MnSO_4 \cdot 4H_2O$	10 mg/L
$ZnSO_4 \cdot 7H_2O$	1 mg/L
$MgCl_2$	4.1 g/L

Adjust the pH to 7.0

＊Autoclave glucose separately from the rest of the ingredients. Mix them after autoclaving.

MTNm plates

Add 7.5 g of agar to 500 ml MT liquid medium and autoclave. Allow the medium to cool to about 50 ℃ before adding neomycin solution (stock sol. 10 mg/ml) to the concentration of 10 μg/ml. Mix the medium by swirling and dispense it to plates.

V. Transformation efficiency

The transformation of *B. choshinensis* cells can be done with 10 ng pNY326 plasmid by electroporation according to Procedure. Selection marker is neomycin resistance. When selected on Nm+ plate, the frequency of transformation is $> 10^5$ transformants/ug pNY326.

VI. References

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6) K. Yashiro, J. W. Lowenthal, T. E. O'Neil, S. Ebisu, H. Takagi, High-Level Production of Recombinant Chicken Interferon-g by *Brevibacillus choshinensis*. Expression and Purification, **23**, 113-120, 2001

VII. Related products

Brevibacillus Expression System (Cat.#HB100)
pNY326 DNA (Cat.#HB111)
pNCMO2 DNA (Cat.#HB112)
pNCMO2-BLA DNA (Cat.#HB113)

VIII. Notice

- This product is sold for academic research purposes only. Be sure not to use this kit for medical or diagnostic purposes. Likewise, do not use this kit as a food, cosmetics or household goods etc.
- It is forbidden to use this product for the purposes of resale and transfer, or change the formulation for the purposes of resale and transfer

Notice : Living Modified Organism

These products (catalog number HB100 and HB115) include a genetically "Living Modified Organism (LMO)" defined in "The Cartagena Protocol on Biosafety". The supplied *Brevibacillus choshinensis* Electro Cells in these kits contain a part of 2 μm plasmid DNA derived from *Saccharomyces cerevisiae*.

Please confirm the guidelines or the laws and regulations that you should obey in your country and pay attention for safe handling, storage, transport and disposal.