hp-Vector Expression Systems for *Bacillus megaterium*

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Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Content

An efficient alternative to *E. coli*: Stable protein production with high yield – suited not only for industrial scale.

MoBiTec offers this expression system as an easy-to-handle kit with *E. coli/B. megaterium* shuttle vectors and - to be ordered separately - *B. megaterium* protoplasts ready for transformation.

1. Introduction

1.1. General features of *Bacillus megaterium*

First described over 100 years ago, *B. megaterium* has recently been gaining more and more importance in scientific as well as industrial applications. The source of its significant name "*megaterium*" is its large size of the vegetative cells (over 10 µm) and its spores. The capability of sporulation has made *B. megaterium* an important tool for examining spore-mediated disease and cell development.

B. megaterium is able to grow on a wide variety of carbon sources and thus has been found in many ecological niches such as waste from meat industry or petrochemical effluents. Also, the degradation of persistent insecticides by *B. megaterium* has been documented (Saxena *et al*., 1987; Selvanayagam and Vijaya, 1989) offering potential applications as detoxifying agent. One of the genetic regulatory elements for carbon utilization is the xylose operon. It has been described by Rygus and Hillen (1991) and is used in the expression system MoBiTec is offering in this kit.

Further, several *B. megaterium* proteins are of importance. For example, a family of P_{450} cytochrome monooxygenases is similar to eukaryotic P_{450} playing a role in many diseases. Industrial applications of enzymes excreted by *B. megaterium* are diverse, starting from amylases used in bread industry to penicillin amidase which is used for the generation of new synthetic antibiotics.

An overview about the features of this unique organism is given in review articles as "Prime time for *Bacillus megaterium*" (Vary, 1994), “A short story about a big magic bug” (Bunk *et al.*, 2010) and “*Bacillus megaterium* - from simple soil bacterium to industrial protein production host“ (Vary *et al.*, 2007).

1.2. *Bacillus megaterium* as expression host

In molecular biology, *B. megaterium* has proven to be an excellent host for the expression of non-homologous DNA. All cloning vectors of the *B. megaterium* system (all are derivatives of the original pWH1520 (Rygus and Hillen, 1991; Malten *et al.*, 2004; Barg *et al.*, 2005; Biedendieck *et al.*, 2007)) rely on the above mentioned xylose operon used as regulatory element. Remarkable improvement work was done by R. Biedendieck.

In contrast to other bacilli strains *B. megaterium* has the advantage, that no alkaline protease is present. This fact enables excellent production and secretion of foreign

proteins without degradation (Meinhardt *et al.*, 1989; Rygus and Hillen, 1991). In addition, due to its Gram-positive character there are no endotoxins found in the cell wall. Protein yields are exceptionally good, also if inexpensive substrates are used.

Recombinant plasmids are structurally and segregationally stable. For example, the *B. megaterium* glucose dehydrogenase gene (*ghd*) has been cloned back into a *B. megaterium* expression vector. The vector and the production of Ghd remained stable without selective pressure over a period of three weeks with daily subculturing (Meinhardt *et al.*, 1989).

Several proteins have successfully been overproduced in *B. megaterium* (see chapter 3). Rygus and Hillen (1991) describe cloning and expression of the genes lacZ from *E. coli*, *gdh* from *B. megaterium*, *mro* (mutarotase) from *Acinetobacter* and human *puk* (a urokinase-like plasminogen activator, rscuPA). Using the xylose operon the genes were 130- to 350-fold induced without proteolysis. Such a system offers unique possibilities for the industrial production of proteins. Further, it is of great interest to manufacturers in the biomedical field. In a diagnostic test for AIDS, the HIV coat protein is commercially produced by *B. megaterium* (Ginsburgh *et al.*, 1989).

1.3. General features of the hp-Vector expression systems

With our new hp-vectors the yields of recombinant proteins can be 10 times enhanced in comparison to the basic plasmids carrying the native promoter system. All plasmids have established multiple cloning sites (MCS) for versatile cloning.

Furthermore, we offer vectors encoding C- or N-terminal His-or Strep-tags for easy purification and a vector with 2 ribosome binding sites (2RBS) for simultaneous dual expression. The protein secretion with LipA or YocH signal peptides is increased up to ninefold. Induction of protein production of all vectors is achieved by the tightly regulated and efficiently inducible xylose operon.

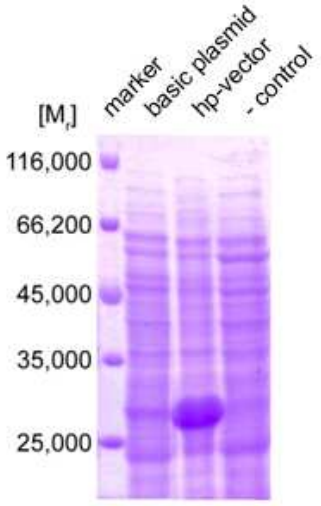

Fig. 1. Soluble protein fractions 6 h after induction of heterologous gene expression.

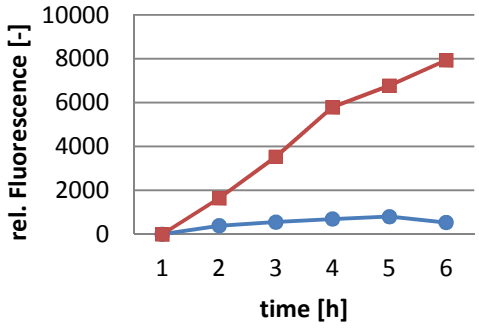

Fig. 2. Relative fluorescence mediated by the optimized hp-Vector (red; square) and the basic plasmid (blue; circle), respectively, over time [h].

2. Summary of Advantages

- *B. megaterium* not pathogenic
- No endotoxins found in the cell wall
- Tightly regulated and efficiently inducible *xylA* operon (up to 350-fold)
- Stable, high yield protein production
- No indication of proteolytic instability even up to 5 h after induction, since alkaline proteases such as e.g. in *B. subtilis* are not produced
- High performance vectors with optimized promoter sequence
- Protein yield up to 10 times better than protein production with basic plasmid
- Encoding C- or N-terminal His- or Strep-tag for versatile purification (native, 6xHis-tag, strep-tag)
- 2RBS (ribosome binding site) vector for simultaneous dual expression
- Secretion with LipA or YocH signal peptide up to ninefold increased
- Stable, high yield protein production
- Tightly regulated and efficiently inducible *xylA* operon
- Removable purification tags due to TEV and Factor Xa sites
- Compatible with all *Bacillus subtilis* vectors
- MoBiTec host strains have been found to be asporogenic on common media
- System might be suitable also for other *Bacillus* ssp.

3. Protocols

3.1. Cloning the DNA fragment of interest

The *E. coli / B. megaterium* shuttle vectors are supplied as lyophilized DNA. Follow standard protocols for propagation of the plasmid in *E. coli*, plasmid mini preparation, restriction endonuclease cleavages and ligation of the DNA fragment of interest into the vector (Sambrook and Russell, 2001). After ligation of the insert the vectors should be propagated in *E. coli* (amp^r) before transforming the *Bacillus* protoplasts (tet^r).

3.2. General remarks on the handling of *B. megaterium*

Strains will grow well on rich media such as LB medium, plates and liquid, at 30 °C and 37 °C. Make sure to aerate liquid cultures well by vigorous agitation in baffled shaking flasks.

We found MS941, WH320 and derived strains to be asporogenic on common medium - they will die on plates, kept at 4 °C, within two weeks, so prepare glycerol stocks (30 % w/v) as a backup and streak the working cultures on fresh plates every 7 - 10 days.

Positive clones carrying the plasmid of interest can be selected by adding 10 µg/ml of tetracycline to the growth medium.

To prove successful overexpression of the target gene harvest small samples of the culture just before and at intervals after induction of recombinant gene expression with xylose. To obtain crude extracts for gel analysis, the bacilli have to be lysed using lysozyme. Simple boiling of cells in sample buffer (Laemmli, 1970), which is quite convenient for *E. coli*, does not work with *B. megaterium*.

3.3. Transformation of *B. megaterium* protoplasts

For recombinant protein production *B. megaterium* protoplasts are transformed with the plasmids coding for the protein of interest. After transformation it is advisable to screen at least three different clones for protein production as the yield can vary among different clones.

Since *B. megaterium* cannot easily be transformed naturally, MoBiTec conveniently provides protoplasts of *B. megaterium* cells, which are ready for transformation (strains MS941 and WH320). MoBiTec produces these protoplasts every second month. They can be used at least 2 months after date of arrival and have to be stored at -80 °C. The protoplast suspension is supplied in 5 aliquots of 500 µl each to prevent multiple freezing and thawing of protoplasts that are not used immediately. One aliquot is provided per transformation. It is advisable to use two of the vials for the control experiments as described below.

Following you can find a standard protocol for transformation of protoplasts.

Control Experiments:

1. Negative control: protoplasts without DNA

Note: *Each lot of protoplasts undergoes this test during our quality control as well.*

This is the control demonstrating that the protoplasts have not been contaminated and vital. Split the cells after transformation and incubation. You should get an empty plate without any colonies on antibiotic plates (here: tetracycline) but a layer of cells on LB plates lacking any antibiotic.

2. Positive control: protoplasts transformed with empty plasmid (no insert; not included)

This is your control for a successful transformation and should yield lots of colonies on antibiotic plates (here: tetracycline). If this transformation works well, but you have problems with the plasmid containing your insert of interest, the problem most probably is associated with your construct.

Essential buffers are listed in chapter 4.

Transformation procedure:

1. Combine 500 µl of protoplast suspension and 3-5 µg of plasmid DNA (DNA should be purified using a commercial preparation kit. Elute the DNA from the column using water) in a 15 ml tube, one for each transformation
2. Add 1.5 ml of PEG-P (room temperature RT), mix gently and incubate for 2 minutes at RT
3. Add 5 ml SMMP, mix by rolling the tube carefully
4. Harvest cells by gentle centrifugation (in e.g. a Heraeus Biofuge/Minifuge at 3,000 rpm (1,300 x g) for 10 minutes at RT), pour off supernatant immediately after centrifugation. Supernatant does not have to be removed completely
   (*Note: do not check for a pellet - most of the time there will be none visible*)
5. Add 500 µl of SMMP to the rest of the supernatant
6. Incubate at 30 °C or 37 °C for 90 minutes with gentle shaking or rolling of tubes (max. 100 rpm) or for 45 min without followed by 45 min with shaking (300 rpm)
7. Prepare 2.5 ml aliquots of CR5-top agar in sterile tubes, one for each transformation
8. After outgrowth add all cells to 2.5 ml top agar, mix gently by rolling the tube between both hands (do not vortex!) and pour onto a prewarmed plate of LB containing the desired antibiotic
9. Incubate overnight at 30 °C or 37 °C - expect colonies of varying diameter because some will be covered with agar and others have easier access to air
   (*Note: the colonies on the top of the agar surface will be shiny*)
10. Streak several different clones on fresh plates within two days

Note*: Protein production may vary among clones due to yet unknown reasons.*

3.4 Recombinant protein production and secretion

I. Test protein production

1. Grow the recombinant *B. megaterium* cells in LB medium including antibiotic (here tetracycline) in baffled shaking flasks to an optical density at 578 nm (OD_{578nm}) of 0.3 - 0.4 at 37 °C and strong shaking (250 rpm)
2. Take a sample as control before induction
3. Induce the xylose-inducible promoter by the addition of 0.5 % (w/v) of (D)-xylose
4. Incubate at 37 °C and strong shaking (250 rpm)
5. Withdraw samples every 30 to 60 minutes until an OD_{578nm} of around 4 to 8 (depending on the growth medium and volume) is reached (now, cells have entered the stationary phase). Take samples for OD_{578nm}-measurement and protein analysis. For extracellular protein analysis take 2 ml of cell culture. For intracellular protein analysis take 3 OD equivalents
6. Centrifuge each sample to harvest cells and cell free supernatant
7. For extracellular protein analysis remove supernatant and store at 4 °C, for intracellular protein analysis completely remove supernatant and store cells at -20 °C

II. Analysis of intracellular proteins

1. Resuspend cells in 30 µl of lysis buffer
2. Incubate for 30 min at 37 °C and 1,000 rpm. Vortexing every 10 minutes increases cell lysis
3. Centrifuge for 30 min at 4 °C and 13,000 rpm to separate the insoluble fraction (pellet) from the soluble fraction (supernatant)
4. Mix 27 µl of supernatant (containing soluble proteins) with 13 µl of SDS sample buffer
5. Completely remove the supernatant. Resuspend the pelleted fraction in 30 ml of 8 % urea (w/v). Centrifuge for 30 min at 4 °C and 13,000 rpm
6. Mix 27 µl of the supernatant (containing insoluble proteins) with 13 µl of SDS sample buffer
7. Heat each sample for 5 min at 95 °C
8. Load 7.5 µl of each sample (containing cells of 0.5 OD) onto an SDS-page gel

III. Ammonium sulfate precipitation of proteins in the cell-free supernatant

1. Add 600 mg of pestled ammonium sulfate to 1.5 ml of cell free supernatant and incubate for two hours at 4 °C and shaking
2. Centrifuge at 13,000 rpm and 4 °C for 30 minutes
3. Completely remove the supernatant, centrifuge again for 1 min and make sure the protein pellet is dry
4. Add 10 µl of 8 M urea (in 50 mM Tris-HCl, pH 7.5) and 5 µl SDS sample buffer to solve the proteins again
5. Spin shortly at 13,000 rpm, heat to 99 °C for 5 minutes and load onto an SDS polyacrylamide gel for analysis
6. Determine enzymatic activities with the appropriate assays (not included in the kit)
7. Perform Western blot using appropriate antibodies (not included in the kit)

IV. Scale up protein production

1. Grow larger culture and induce as indicated above
2. Harvest cells at the time point of maximal protein overproduction, as determined by the test experiments

B. megaterium carrying a plasmid coding for GFP-Strep fusion protein was grown in semi-defined minimal medium at 37°C initially in a batch phase with 4 g/L glucose. At the end of the batch phase an exponential feeding profile was started. GFP was visualized by a lamp emitting blue light and a yellow filter using a digital camera.

4. Materials

2 × AB3 (Antibiotic Medium No. 3, DIFCO)
- 7 g AB3 (Difco) in 200 ml deion. water
- autoclave for 15 min

2 × SMM **solubilize in the given order!**
- 1.16 g maleic acid (40 mM)
- 800 mg NaOH (80 mM)
- 2.03 g $MgCl_2$ x $6H_2O$ (40 mM)
- 85.58 g sucrose (1 M)
- solubilize each component in deion. water
- mix and fill with deion. water to 250 ml
- sterilize by filtration

SMMP
- 2 × AB3 and 2 × SMM 1:1 (freshly prepared!)

PEG-P
- solubilize 20 g PEG-6000 with 1 × SMM and fill to 50 ml
- autoclave for 11 min

CR5-top-agar
prepare separately for 500 ml:

solution A
- 51.5 g sucrose
- 3.25 g MOPS
- 300 mg NaOH
- add to deionized water to 250 ml
- adjust pH to 7.3 with NaOH
- sterilize by filtration

solution B
- 2 g agar
- 100 mg casamino acids
- 5 g yeast extract
- add deionized water to 142.5 ml
- autoclave for 15 min

8 × CR5-salts
- 1.25 g K_2SO_4
- 50 g $MgCl_2 \times 6\ H_2O$
- 250 mg KH_2PO_4
- 11 g $CaCl_2$
- solubilize in 625 ml deion. water
- autoclave for 15 min

12 % proline
- 3 g L-proline
- add with deionized water to 25 ml
- sterilize by filtration

20 % glucose
- 5 g glucose
- add with deionized water to 25 ml
- sterilize by filtration or autoclave

for a 2.5 ml portion of CR5-top-agar add the following (in the given order!):
- 1.25 ml solution A
- 288 µl CR5-salts
- 125 µl 12 % proline
- 125 µl 20 % glucose

90 minutes after transformation:
- boil **solution B**
- add 713 µl to the provided CR5-top-agar
- immediately add the regenerated protoplasts and put onto prewarmed agar plates containing the corresponding antibiotic (here: tetracycline)

lysis buffer
- 100 mM Na_3PO_4
- 5 mg/ml lysozyme
- pH 6.5 (adjust with H_3PO_4
- add 1 ml of a 1 M $MgSO_4$ solution and 2 µl HS-Nuclease (5 U/µl, cat.# GE-NUC10700-01) per ml lysis buffer shortly before use

5. Vector Maps

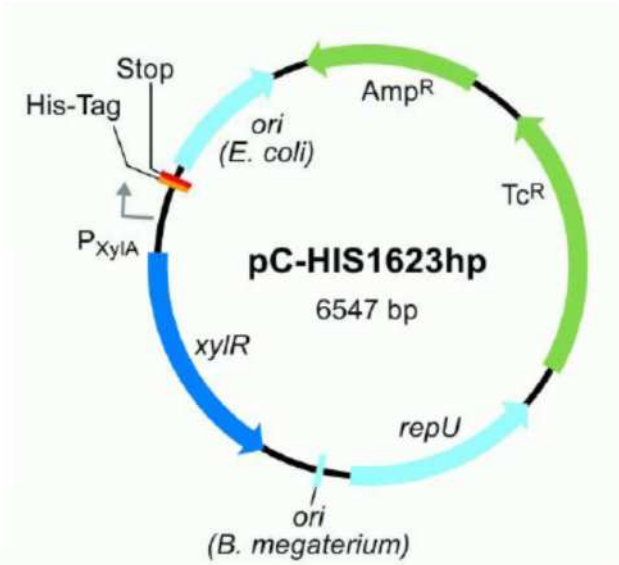

Fig. 3. Map of pC-HIS1623hp. Elements for plasmid replication in *B. megaterium* (*repU, ori* (*B. megaterium*)) and *E. coli* (ori *E. coli*); xylose-inducible promoter (P_{xylA}) and its cognate repressor (*xylR*), 6 x His-Tag, Stop-Codon, resistance genes against ampicillin (Amp^R) and tetracycline (Tc^R).

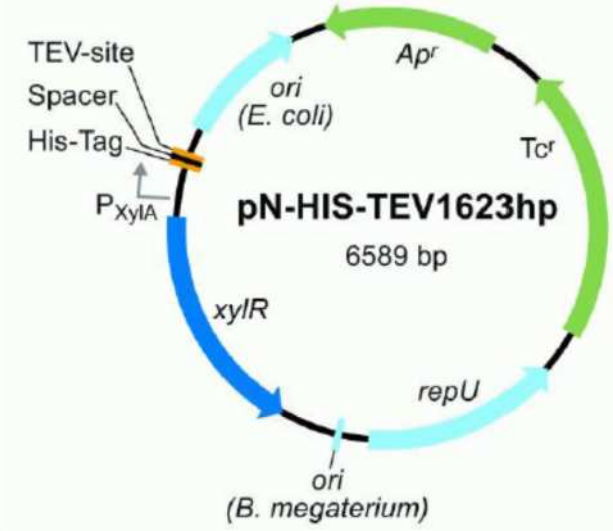

Fig. 4. Map of pN-HIS-TEV1623hp. Elements for plasmid replication in *B. megaterium* (*repU, ori* (*B. megaterium*)) and *E. coli* (ori *E. coli*); xylose-inducible promoter (P_{xylA}) and its cognate repressor (*xylR*); 6 x His-Tag, TEV-Site, resistance genes against ampicillin (Amp^R) and tetracycline (Tc^R).

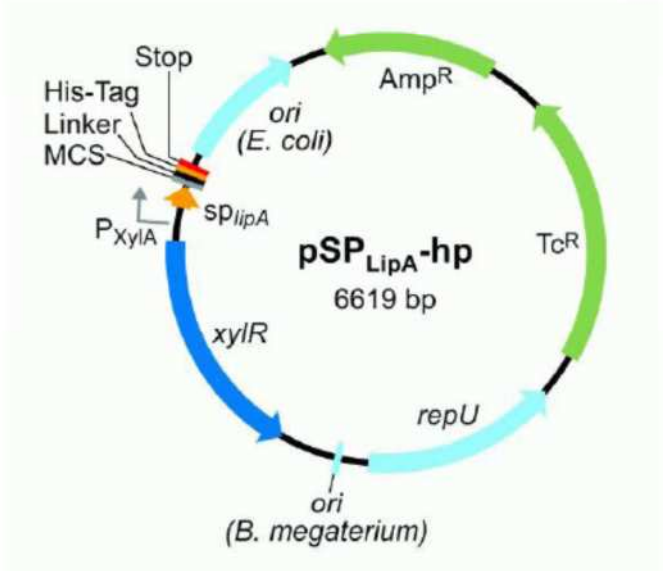

Fig. 5. Map of pSP_{LipA}-hp. Elements for plasmid replication in *B. megaterium* (*repU, ori* (*B. megaterium*)) and *E. coli* (ori *E. coli*); xylose-inducible promoter (P_{xylA}) and its cognate repressor (*xylR*); Lipase signal sequence (sp_{lipA}), multiple cloning site (MCS), linker, His-Tag, Stop-Codon; resistance genes against ampicillin (Amp^R) and tetracycline (Tc^R).

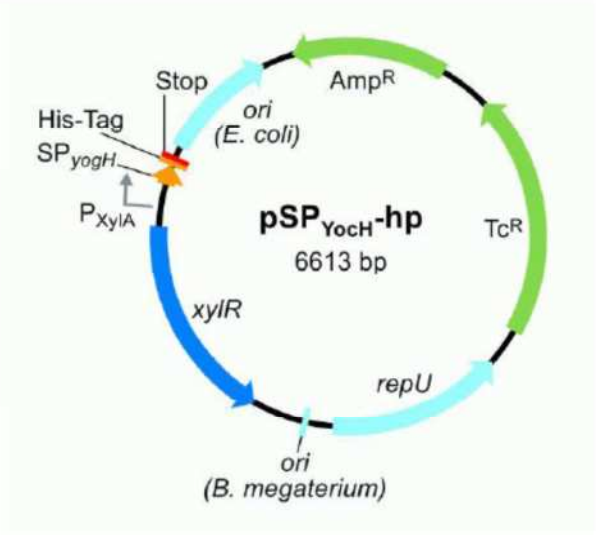

Fig. 6. Map of pSP_{YocH}-hp: Elements for plasmid replication in *B. megaterium* (*repU, ori* (*B. megaterium*)) and *E. coli* (ori *E. coli*); xylose-inducible promoter (P_{xylA}) and its cognate repressor (*xylR*); YocH signal peptide (sp_{YocH}), His-Tag, Stop-Codon, resistance genes against ampicillin (Amp^R) and tetracycline (Tc^R).

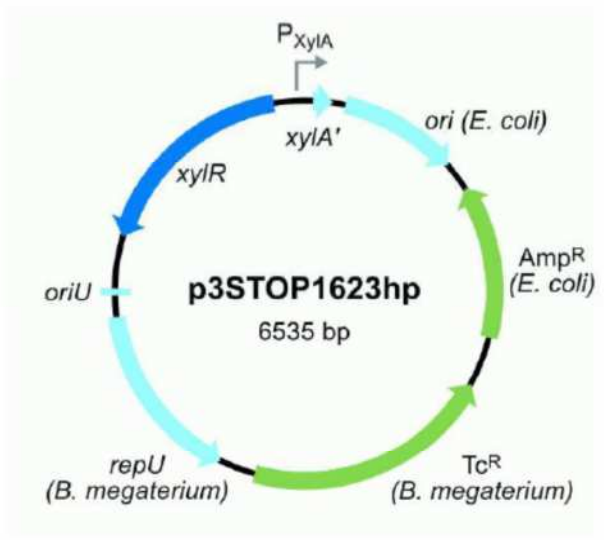

Fig. 7. Map of p3STOP1623hp: Elements for plasmid replication in *B. megaterium* (*repU, ori* (*B. megaterium*)) and *E. coli* (ori *E. coli*); xylose-inducible promoter (P_{xylA}) and its cognate repressor (*xylR*); xylose isomerase, gene incomplete (*xylA'*), resistance genes against ampicillin (Amp^R) and tetracycline (Tc^R).

For complete sequences please check www.mobitec.com

6. References

Antelmann, H., Tjalsma, H., Voigt, B., Ohlmeier, S., Bron, S., van Dijl, J. M. & Hecker, M. (2001). A proteomic view on genome-based signal peptide predictions. Genome Res. *11*, 1484-1502.

Biedendieck, R., Beine, R., Gamer, M., Jordan, E., Buchholz, K., Seibel, J., Dijkhuizen, L., Malten, M. & Jahn, D. (2007). Export, purification, and activities of affinity tagged *Lactobacillus reuteri* levansucrase produced by *Bacillus megaterium*. Appl. Microbiol. Biotechnol *74*, 1062-1073.

Biedendieck, R., Borgmeier, C., Bunk, B., Stammen, S., Scherling, C., Meinhardt, F., Wittmann, C. & Jahn, D. (2011) Systems biology of recombinant protein production using *Bacillus megaterium*. Methods Enzymol. *500,* 165-195.

Biedendieck, R., Bunk, B., Fürch, T., Franco-Lara, E., Jahn, M. & Jahn, D. (2010) Systems biology of recombinant protein production in *Bacillus megaterium* in "Advances in Biochemical Engineering / Biotechnology" (Scheper, T., Ed.); Biosystems Engineering (Wittmann, C., Ed.) Springer, Berlin, Heidelberg *120,* 133-161

Biedendieck, R., Yang, Y., Deckwer, W., Malten, M. & Jahn, D. (2007). Plasmid system for the intracellular production and purification of affinity-tagged proteins in *Bacillus megaterium*. Biotechnol. Bioeng. *96*, 525-537.

Borgmeier, C., Biedendieck, R., Hoffmann, K., Jahn, D. & Meinhardt, F. (2011) Transcriptome profiling of *degU* expression reveals unexpected regulatory patterns in *Bacillus megaterium* and discloses new targets for optimizing expression. Appl. Microbiol. Biotechnol. *92*, 583-596.

Bunk, B., Biedendieck, R., Jahn, D. & Vary, P.S. (2010) *Bacillus megaterium* and other Bacilli: Industrial Applications. In Flickinger M. C. (Ed.) Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology. Volume 1 pp 429-443. John Wiley & Sons. Inc., Hoboken, NJ.

Bunk, B., Schulz, A., Stammen, S., Münch, R., Warren, M. J., Rohde, M., Jahn, D. & Biedendieck, R. (2010). A short story about a big magic bug. Bioeng. Bugs *1:2*, 1-7.

Eppinger, M., Bunk, B., Johns, M.A., Edirisinghe, J.N., Kutumbaka, K.K., Koenig, S.S.K., Creasy, H.H., Rosovitz, M.J., Riley, D.R., Daugherty, S., Martin, M., Elbourne, L.D.H., Paulsen, I., Biedendieck, R., Braun, C., Grayburn, S., Dhingra, S., Lukyanchuk, V., Ball, B., ul-Qamar, R., Seibel, J., Bremer, E., Jahn, D., Ravel, J. & Vary, P.S. (2011) Genome sequences of the biotechnologically important *B. megaterium* strains QM B1551 and DSM319. J. Bacteriol. *193*, 4199-4213.

Hueck, C. J., Kraus, A., Schmiedel, D & Hillen, W. (1995). Cloning, expression and functional analyses of the catabolite control protein CcpA from *Bacillus megaterium*. Mol. Microbiol. *16*, 855-864.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature *227*, 680-685.

Lee, J., Sohn, H., Shin, K., Jo, M., Kim, J., Lee, S., Shin, J., Kum, E. & Kwon, G. (2006). Isolation of a soil bacterium capable of biodegradation and detoxification of endosulfan and endosulfan sulfate. J. Agric. Food Chem. *54*, 8824-8828.

Malten, M., Biedendieck, R., Gamer, M., Drews, A., Stammen, S., Buchholz, K., Dijkhuizen, L. & Jahn, D. (2006). A *Bacillus megaterium* plasmid system for the production, export, and one-step purification of affinity-tagged heterologous levansucrase from growth medium. Appl. Environ. Microbiol. *72*, 1677-1679.

Meinhardt, F., Stahl, U. & Ebeling, W. (1989). Highly efficient expression of homologous and heterologous genes in *Bacillus megaterium*. Appl. Microbiol. Biotechnol *30*, 343-350.

Puyet, A., Sandoval, H., López, P., Aguilar, A., Martin, J. & Espinosa, M. (1987). A simple medium for rapid regeneration of *Bacillus subtilis* protoplasts transformed with plasmid DNA. FEMS Microbiol. Lett. *40*, 1-5.

Rygus, T. & Hillen, W. (1991). Inducible high-level expression of heterologous genes in *Bacillus megaterium* using the regulatory elements of the xylose-utilization operon. Appl. Microbiol. Biotechnol. *35*, 594-599.

Rygus, T., Scheler, A., Allmansberger, R. & Hillen, W. (1991). Molecular cloning, structure, promoters and regulatory elements for transcription of the *Bacillus megaterium* encoded regulon for xylose utilization. Arch. Microbiol. *155*, 535-542.

Sambrook, J. & Russell, D.W. (2001). Molecular cloning: a laboratory manual. (New York: Cold Spring Harbor Laboratory Press).

Saxena, A., Zhang, R. W. & Bollag, J. M. (1987). Microorganisms capable of metabolizing the herbicide metolachlor. Appl. Environ. Microbiol. *53*, 390-396.

Stammen, S., Müller, B.K., Korneli, C., Biedendieck, R., Gamer, M., Franco-Lara, E. & Jahn, D. (2010) High yield intra- and extracellular protein production using *Bacillus megaterium*. Appl. Environ. Microbiol. *76*, 4037-4046.

Vary, P. (1992). Development of genetic engineering in *Bacillus megaterium*. Biotechnology *22*, 251-310.

Vary, P. S. (1994). Prime time for *Bacillus megaterium*. Microbiology (Reading, Engl.) *140 (Pt 5)*, 1001-1013.

Vary, P. S., Biedendieck, R., Fuerch, T., Meinhardt, F., Rohde, M., Deckwer, W.-D. & Jahn, D. (2007) *Bacillus megaterium* – from simple soil bacterium to industrial protein production host. Appl. Microbiol. Biotechnol. *76*, 957-967.

Wittchen, K. & Meinhardt, F. (1995). Inactivation of the major extracellular protease from *Bacillus megaterium* DSM319 by gene replacement. Appl. Microbiol. Biotechnol. *42*, 871-877.

7. Order Information, Shipping and Storage

Order #	Description	Amount
BMEG30	*Bacillus megaterium* vector p3STOP1623hp, lyophilized	10 µg
BMEG31	*Bacillus megaterium* vector pC-HIS1623hp, lyophilized	10 µg
BMEG32	*Bacillus megaterium* vector pN-HIS-TEV1623hp, lyophilized	10 µg
BMEG33	*Bacillus megaterium* vector pSP_{LipA}-hp, lyophilized	10 µg
BMEG34	*Bacillus megaterium* vector pSP_{YocH}-hp, lyophilized	10 µg
BMEG35	*Bacillus megaterium* vector p3STOP1623-2RBShp, lyophilized	10 µg
BMEG36	*Bacillus megaterium* vector pC-STREP1623hp, lyophilized	10 µg
BMEG37	*Bacillus megaterium* vector pN-STREP-Xa1623hp, lyophilized	10 µg
BMEG38	*Bacillus megaterium* vector pN-STREP-TEV1623hp, lyophilized	10 µg

Shipped at RT, store lyophilized vectors at 4 °C, reconstituted vectors at -20 °C!
Vectors are *E. coli / B. megaterium* shuttle vectors.

Related Products

Order #	Description	Amount
BMEG02	*Bacillus megaterium* protoplast, strain WH320	5 x 500 µl
BMEG50	*Bacillus megaterium* protoplast, strain MS941	5 x 500 µl
PR-ETA10010-01	MobiTEV Protease, recombinant, His-Tag	1000 U
PR-ETA10010-05	MobiTEV Protease, recombinant, His-Tag	10 x 1000 U
PR-ETA10050-01	TEV Protease(TurboTEV), recombinant, GST- & His-Tag	10 µg (100 U)
PR-ETA10050-02	TEV Protease(TurboTEV), recombinant, GST- & His-Tag	50 µg (500 U)
PR-ETA10050-03	TEV Protease(TurboTEV), recombinant, GST- & His-Tag	100 µg (1000 U)
PR-ETA10050-04	TEV Protease(TurboTEV), recombinant, GST- & His-Tag	1 mg (10.000 U)
GE-NUC10700-01	HS-Nuclease, recombinant Endonuclease (encoded by the same gene as Benzonase®)	50000 U

Protoplasts are shipped on dry ice, store protoplasts at -80 °C!

8. Contact and Support

MoBiTec GmbH ◆ Lotzestrasse 22a ◆ D-37083 Goettingen ◆ Germany

Customer Service - General inquiries & orders
phone: +49 (0)551 707 22 0
fax: +49 (0)551 707 22 22
e-mail: order@mobitec.com

Technical Service - Product information
phone: +49 (0)551 707 22 70
fax: +49 (0)551 707 22 77
e-mail: info@mobitec.com

MoBiTec in your area: Find your local distributor at www.mobitec.com

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