LIVING MODIFIED ORGANISM (LMO)

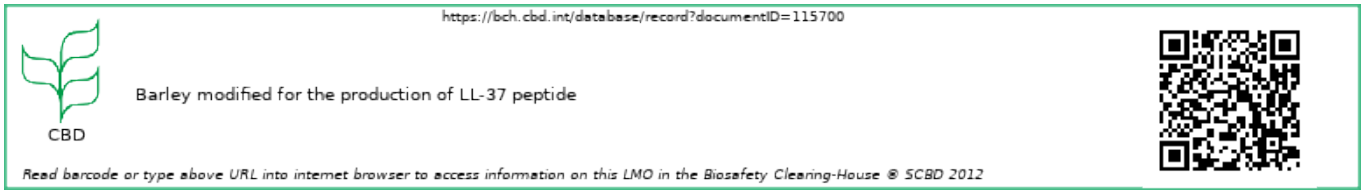
BCH-LMO-SCBD-115700-1

? Decisions on the LMO ? Risk Assessments

LAST UPDATED: 01 SEP 2020

Living Modified Organism identity

The image below identifies the LMO through its unique identifier, trade name and a link to this page of the BCH. Click on it to download a larger image on your computer. For help on how to use it go to the LMO quick-links page.



Name

Barley modified for the production of LL-37 peptide | EN

Transformation event

bHOR:MBP_LL-37

Developer(s)

- PERSON: PALACKY UNIVERSITY OLOMOUC | BCH-CON-CZ-113440-2

PERSON

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RELATED ORGANIZATION

Description

The barley was modified for the production of human LL-37 peptide, which has known broad spectrum antimicrobial activity and acts as component of the basal immune response to infection. Barley production platforms have little phenolic compound content and have low amount of proteolytic enzymatic activity. | EN

The line is one of three lines (see "Related LMOs") being tested for bioproduction and protein purification. This line contains an endosperm specific promoter and protein purification tag sequences (hexahistidine and maltose binding protein), which can be removed post-purification using an enterokinase. The hexahistidine tag allows for protein purification using immobilized metal affinity chromatography, while the maltose binding protein improves the solubility and folding of the recombinant LL-37. All lines demonstrated normal phenotypes and the LL-37 peptide was shown to be bioactive.

The modified barley additionally contains an antibiotic selection marker, *Escherichia coli* hygromycin phosphotransferase B, for hygromycin selection during transformation.

Recipient Organism or Parental Organisms

The term “Recipient organism” refers to an organism (either already modified or non-modified) that was subjected to genetic modification, whereas “Parental organisms” refers to those that were involved in cross breeding or cell fusion.

BCH-ORGA-SCBD-12110-5 ORGANISM | HORDEUM VULGARE (BARLEY, HORVU) |
Crops

Point of collection or acquisition of the recipient organism or parental organisms

Hordeum vulgare cultivar Golden promise EN

Related LMO(s)

BCH-LMO-SCBD-115698-1 | Barley modified for the production of LL-37 peptide | Palacky University Olomouc Production of medical or pharmaceutical compounds (human or animal) Resistance to antibiotics - Hygromycin Selectable marker genes and reporter genes

BCH-LMO-SCBD-115699-1 | Barley modified for the production of LL-37 peptide | Palacky University Olomouc Production of medical or pharmaceutical compounds (human or animal) Resistance to antibiotics - Hygromycin Selectable marker genes and reporter genes

Characteristics of the modification process

Vector

pBRACT209 EN

Techniques used for the modification

Agrobacterium-mediated DNA transfer

Genetic elements construct

Introduced or modified genetic element(s)

Some of these genetic elements may be present as fragments or truncated forms. Please see notes below,

where applicable.

BCH-GENE-SCBD-115696-1 LL-37 PEPTIDE - HOMO SAPIENS - HUMAN
Production of medical or pharmaceutical compounds (human or animal)

BCH-GENE-SCBD-14991-8 HYGROMYCIN B PHOSPHOTRANSFERASE GENE | (BACTERIA)
Protein coding sequence | Resistance to antibiotics (Hygromycin),Selectable marker genes and reporter genes

BCH-GENE-SCBD-103023-2 KDEL ER RETENTION SIGNAL
Transit signal

BCH-GENE-SCBD-115697-1 CYTOKININ DEHYDROGENASE 1 SIGNAL PEPTIDE - ZEA MAYS - MAIZE, CORN, MAIZE

BCH-GENE-SCBD-100269-8 NOPALINE SYNTHASE GENE TERMINATOR
Terminator

BCH-GENE-SCBD-100287-7 CAMV 35S PROMOTER
Promoter

BCH-GENE-SCBD-115695-2 MALTOSE BINDING PROTEIN AFFINITY TAG - ESCHERICHIA COLI - ECOLX
Protein purification

BCH-GENE-SCBD-103022-3 HEXAHISTIDINE TAG
Protein coding sequence | Protein purification

BCH-GENE-SCBD-101595-2 HORDEIN B1 PROMOTER | (BARLEY)
Promoter

Notes regarding the genetic elements present in this LMO

Gene cassettes The DNA insertion contain the following two gene cassettes: 1) *Escherichia coli* hygromycin B phosphotransferase (*hph*); and 2) *Homo sapiens* LL-37 peptide. Gene expression Transcription of *hph* is under control of the *Cauliflower mosaic virus* 35S promoter and the *Agrobacterium tumefaciens* nopaline synthase (*nos*) terminator. Due to the nature of the viral promoter, transcription is expected to occur at high levels. Transcription of the human LL-37 peptide occurs from the *Hordeum vulgare* hordein B1 (bHOR) promoter and terminates at the *nos* terminator. The transcript contains the following (from 5' to 3'): a *Zea mays* cytokinin dehydrogenase 1 (ZmCKX1sp) signal peptide, a synthetic hexahistidine affinity tag, an *E. coli* maltose binding protein affinity tag, the LL-37 peptide and a synthetic KDEL ER retention signal. The bHOR promoter restricts expression to the barley endosperm. The synthetic eukaryotic KDEL sequence facilitates the transport of LL-37 peptide to the endoplasmic reticulum. Prior to export from the ER, the translated peptide is cleaved from the peptide. ZmCKX1sp facilitates the transit of LL-37 through the ER and excretion from the cell. The final protein will retain the hexahistidine and maltose binding protein tags for purification. Note - *Agrobacterium tumefaciens* strain AG1 was used in the transformation of barley immature zygotic embryos. - The bHOR promoter sequence has the following accession number GenBank: X87232.1.	EN

- The final LL-37 peptide is not expected to retain the signal peptides.

LMO characteristics

Modified traits

Resistance to antibiotics
Hygromycin
Production of medical or pharmaceutical compounds (human or animal)
Selectable marker genes and reporter genes

Common use(s) of the LMO

Pharmaceutical
Research

Detection method(s)

External link(s)

? A generic protocol for the expression and purification of recombinant proteins in Escherichia coli using a combinatorial His6-maltose binding protein fusion tag.pdf *(English)*

Additional Information

Due to the inclusion of the hexahistidine affinity tag, the LL-37 protein can be isolated using immobilized metal affinity chromatography. If using amylose affinity chromatography, the maltose binding protein domain may not bind efficiently and may not produce proteins with sufficient purity. Therefore, the maltose binding protein tag is not expected to contribute to protein purification (see "External links" above).	EN

Additional Information

Additional Information

Cathelicidin antimicrobial peptide is the only cathelicidin protein found in humans and is location on chromosome 3p21. The sequence contains 4 exons and is translated to hCAP18, a pre-pro-protein, containing signal peptide, a conserved pro-sequence (cathelin-like domain) and a C-terminal antimicrobial peptide, LL-37. The active LL-37 peptide is produced from proteolytic cleavage from hCAP18 and its primary structure is based on 37 amino acid residues (~ 18kDa), which form an amiphiphatic alpha-helix (secondary) structure.

Other relevant website addresses and/or attached documents

? Joint Research Centre - Deliberate Release into the Environment and Placing on the EU Markets of GMO - GMO Register *(English)*

? John Innes Centre - Crop Transformation (BRACT vectors) *(English)*

? pBract209 sequence map.doc *(English)*

? Molecular Farming in Barley Development of a NovelProduction Platform to Produce Human AntimicrobialPeptide LL-37.pdf *(English)*

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Further Information

Questions about the Cartagena Protocol on Biosafety or the operation of the Biosafety Clearing-House may be directed to the Secretariat of the Convention on Biological Diversity.

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