





Biosafety Clearing-House (BCH)

LIVING MODIFIED ORGANISM (LMO)

BCH-LMO-SCBD-115698-1

? 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.

https://bch.cbd.int/database/record?documentID=115698



Barley modified for the production of LL-37 peptide



Read barcode or type above URL into internet browser to access information on this LMO in the Biosafety Clearing-House @ 5CBD 2012

Name

Barley modified for the production of LL-37 peptide

ΕN

Transformation event

bHOR:LL-37

Developer(s)

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

PERSON

Palacky University Olomouc

Institute of Molecular and Translational Medicine

Olomouc

77900, Czech Republic
Phone: +420 585 632 111
Email: reception@imtm.upol.cz

Website: https://veda.upol.cz/en/workplaces/science-centres/the-institute-of-molecular-and-

translational-medicine/

RELATED ORGANIZATION

Description

The barley (*Hordeum vulgare*) 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.

ΕN

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 no protein purification tag sequences. 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

ΕN

Related LMO(s)

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

BCH-LMO-SCBD-115700-1 | Barley modified for the production of LL-37 peptide | Palacky University Olomouc Production of medical or pharmaceutical compounds (human or animal) Protein purification Resistance to antibiotics - Hygromycin Selectable marker genes and reporter genes Show detection method(s)

Characteristics of the modification process

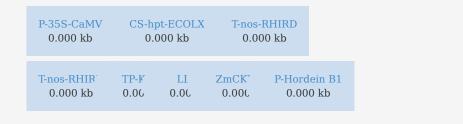
Vector

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-100269-8 NOPALINE SYNTHASE GENE TERMINATOR

Terminator

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

CORN, MAIZE

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

Promoter

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'): *Zea mays* cytokinin dehydrogenase 1 (ZmCKX1sp) signal peptide, 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 (ER). Prior to export from the ER, the KDEL sequence is cleaved from the peptide. ZmCKX1sp facilitates the transit of LL-37 through the ER and excretion into the apoplast.

EN

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.
- 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)

Additional Information

The LL-37 peptide was only detected in the grains of the modified barley and not the roots or leaves (see "Molecular Farming in Barley: Development of a Novel Production Platform to Produce Human Antimicrobial Peptide LL-37").

ΕN

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

- ? Molecular Farming in Barley Development of a NovelProduction Platform to Produce Human AntimicrobialPeptide LL-37.pdf (English)
- $\ref{eq:continuous}$ John Innes Centre Crop transformation (BRACT vectors) (<code>English</code>)
- ? pBract209 sequence map.doc (<code>English</code>)
- ? EU Joint Research Centre Deliberate Release into the Environment and Placing on the EU Markets of GMO GMO Register (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.

Secretariat of the Convention on Biological Diversity

413 rue Saint-Jacques, suite 800 Montreal, Québec, H2Y 1N9

Canada

Fax: +1 514 288-6588 Email: secretariat@cbd.int