Biosafety Clearing-House (BCH)

LIVING MODIFIED ORGANISM (LMO)

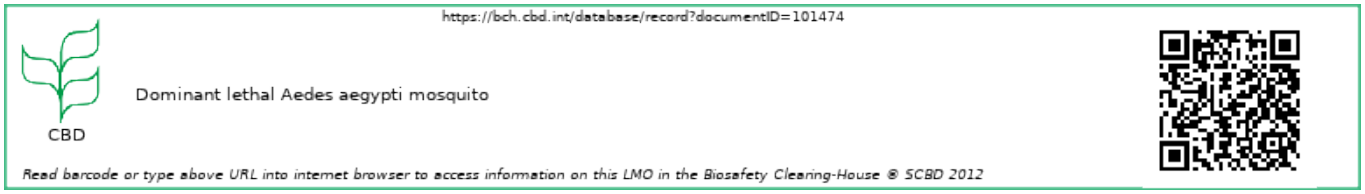
BCH-LMO-SCBD-101474-18

? Decisions on the LMO ? Risk Assessments

LAST UPDATED: 08 JAN 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

Dominant lethal Aedes aegypti mosquito EN

Transformation event

OX513A

Developer(s)

- ORGANIZATION: OXITEC LIMITED | BCH-CON-SCBD-101477-1

ORGANIZATION

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RELATED ORGANIZATION

Description

A modified strain of the *Aedes aegypti* mosquito, designated as OX513A(My1), was developed to exhibit dominant lethality in both males and females when reared in the absence of tetracycline and includes a red fluorescent protein (DsRed2) as a visible marker. In the presence of tetracycline, the synthetic tetracycline-transcriptional activator (tTAV) variant preferentially binds tetracycline instead of the tetracycline operator, thus transcription is repressed and occurs at a basal level. In the absence of tetracycline, tTAV binds the operator sequences to promote high levels of transcription. High levels of tTAV expression is toxic as it prevents the cells from producing other transcripts required for normal functioning and results in lethality. This approach is similar to sterile insect technique, which uses radiation to produce sterile males, but does not incur great fitness reductions caused by the radiation required to produce sterile males. Modified mosquitoes are reared in the presence of tetracycline, which allows for full development. Upon release, mating with the modified mosquitoes results in lethality in the next generation.	EN

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-101472-4 ORGANISM | AEDES AEGYPTI (YELLOW FEVER MOSQUITO, AEDAE) | Insects

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

Initial transformation: *Aedes aegypti* Rockefeller strain Backcrossing: *Ae. aegypti* Latin strain (from Instituto Nacional de Salud Publica, Mexico)	EN

Characteristics of the modification process

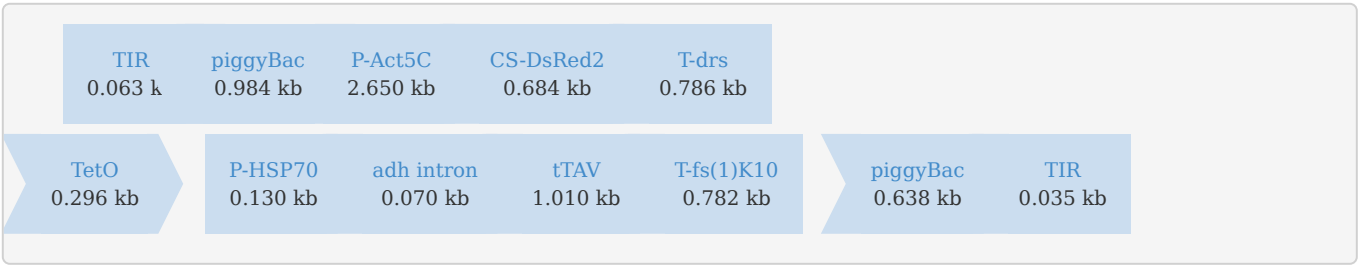
Vector

pLA513 and phsp-pBac	EN

Techniques used for the modification

Microinjection

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-101475-12 TETRACYCLINE-CONTROLLED TRANSACTIVATOR | (BACTERIA)
Protein coding sequence | Conditional lethality

BCH-GENE-SCBD-101476-5 DSRED2 FLUORESCENT PROTEIN | (CORAL ANEMONES, SEA ANEMONES)
Protein coding sequence | Selectable marker genes and reporter genes

BCH-GENE-SCBD-103761-1 ACTIN 5C GENE PROMOTOR | (COMMON FRUIT FLY)
Promoter

BCH-GENE-SCBD-103762-1 HSP70 MINIMAL PROMOTER | (COMMON FRUIT FLY)
Promoter

BCH-GENE-SCBD-103763-1 FS(1)K10 3' UTR | (COMMON FRUIT FLY)
Terminator

BCH-GENE-SCBD-103764-1 DORSOMYCIN GENE 3'UTR | (COMMON FRUIT FLY)
Terminator

BCH-GENE-SCBD-105038-3 TETRACYCLINE OPERATOR | (BACTERIA)
Binding site

BCH-GENE-SCBD-115235-1 TERMINAL INVERT REPEAT - TRICHOPLUSIA NI - CABBAGE LOOPER, CABBAGE LOOPER MOTH, CABBAGE PLUSIA, COMMON CABBAGE LOOPER, LETTUCE LOOPER

BCH-GENE-SCBD-115246-1 PIGGYBAC - TRICHOPLUSIA NI - CABBAGE LOOPER, CABBAGE LOOPER MOTH, CABBAGE PLUSIA, COMMON CABBAGE LOOPER, LETTUCE LOOPER

BCH-GENE-SCBD-115247-2 ALCOHOL DEHYDROGENASE INTRON - DROSOPHILA MELANOGASTER - COMMON FRUIT FLY

Notes regarding the genetic elements present in this LMO

The pLA513 plasmid was co-transformed with the phsp-pBac helper plasmid, which served as a source for the *piggy Bac* transposase.

Transcription of the DsRed2 protein begins at the *Drosophila melanogaster* actin 5c promoter and terminates at the *D. melanogaster* dorsomycin 3' untranslated region. The promoter drives expression of the fluorescent protein marker, which causes an accumulation of soluble protein within the cells.

Transcription of the tetracycline-transcriptional activator variant (tTAV) begins at the *D. melanogaster* heat shock protein 70 promoter and terminates at the *D. melanogaster* DNA-binding protein K10 3' untranslated region (poly-adenylation signal). The transcript initially includes a *D. melanogaster* alcohol dehydrogenase intron at the 5' end of the transcript to enhance expression of tTAV. Immediately adjacent to the tTAV cassette is a tetracycline operator, which acts as a repressible switch. In the presence of tetracycline, tTAV

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preferentially binds tetracycline rather than the operator sequences. Thus, transcription remains at a basal level and repressed. In the absence of tetracycline, tTAV binds the operator sequences and stimulates transcription. Thus, under these conditions, transcription of tTAV is expected to be strong and with production of the tTAV protein occurring at elevated levels.

Please note:
- tTAV is a synthetic construct and contains sequences from the *Escherichia coli* tetracycline repressor and the Human herpesvirus 1 viral protein 16 transactor
- DNA deletions in the *piggyBac* sequences prevent mobility of the transposon. Additionally, the construct does not introduce a transposase. Thus, re-mobilization is not expected.
- Southern blot analysis confirmed a single insertion into the mosquito genome occurred
- Inverse PCR and sequencing suggested that the insertion does not interrupt an open reading frame
- The plasmid backbone contains an ampicillin resistance gene, which was not detected in the transformants

LMO characteristics

Modified traits

Changes in physiology and/or production
Reproduction
Selectable marker genes and reporter genes

Common use(s) of the LMO

Other (Biological control)

Detection method(s)

Additional Information

Modified mosquitoes can be detected by red fluorescence under yellow light (583 nm) due to the DsRed2 protein.

The *D. melanogaster* alcohol dehydrogenase intron is spliced out of pre-mRNA, and thus also allows the distinction between cDNA (transcripts) and genomic DNA during RT-PCR analysis.

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Additional Information

Additional Information

Information on OX513A(My1)

OX513A(My1) is a bisex RIDL strain, which means that both female and male insects die unless supplied with the supplement, which in the case of OX513A(My1) is the antibiotic tetracycline.

Released bisex RIDL insects and their progeny die within a few weeks so releases must be sustained to maintain the control.

Source: Oxitec (see developer field above).

Information on the Release of Insects carrying a Dominant Lethal (RIDL) technology

Release of Insects carrying a Dominant Lethal (RIDL) is a method using recombinant DNA technology to create genetically modified insects for biological control. The dominant lethal gene kills the insects but it can be repressed by an external additive, which allows the insects to be reared in manufacturing facilities. This external additive is commonly administered orally, and so can be an additive to the insect food. The insects can also be given genetic markers, such as fluorescence, that make monitoring the progress of eradication easier.

There are potentially several types of RIDL, but the more advanced forms have a female-specific dominant lethal gene. This avoids the need for a separate sex separation step, as the repressor can be withdrawn from the final stage of rearing, leaving only males.

These males are then released in large numbers into the affected region. The released males are not sterile, but any female offspring their mates produce will have the dominant lethal gene expressed, and so will die. The number of females in the wild population will therefore decline, causing the overall population to decline.

Using RIDL means that the males will not have to be sterilized by radiation before release (as done with the "Sterile Insect Technique" (SIT) using radiation), making the males healthier when they need to compete with the wild males for mates.

Source: Wikipedia (see link below).

Other relevant website addresses and/or attached documents

? Sterile insect technique - Wikipedia *(English)*

? Late-acting dominant lethal genetic systems and mosquito control *(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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