Mosquito Intelligent Terminator

An environment-friendly pesticide for killing wrigglers

National Chiao Tung University Taiwan
MOTIVATION:
Mosquitoes pose a public health threat in certain areas of the world.

A mosquito carries disease-causing viruses and parasites.

Yellow fever, Malaria, Dengue fever
IDEA: Killing Wrigglers to Prevent Spreading of Diseases

Life cycle of a Mosquito

- Mosquito
- Eggs
- Pupa
- Wriggler
IDEA:
Construct a Terminator

An *E. coli* which equipped with CRY weapon to kill wigglers

Mosquito Intelligent Terminator
Cry proteins are toxic to very specific species of insects yet harmless to humans and the natural hunters of many crop pests.

Cry11Aa was cloned from *Bacillus thuringiensis* and is highly toxic to certain larvae, such as *Aedes*, *Culex* and *Anopheles* larvae.
Spraying of Terminators into foul water
After wrigglers eat Terminators......
Uh……
......DIE !!
The E.coli Terminator kill the wigglers successfully. But………
IDEA:
Terminator Population Control
GOAL: A Mosquito Intelligent Terminator

Mosquito Intelligent Terminator

Population Control system

Low-temperature release system

CRY weapon system
CIRCUIT DESIGN: CRY weapon system

A
Constitutive promoter
37°C induced RBS
RBS tetR STOP STOP

tetR

B
CRY weapon system

Ptet RBS cry RBS GFP STOP STOP

C
Ptet RBS LuxI RBS LuxR STOP STOP

LuxI LuxR SAM AHL

D
Plux RBS ccdB RBS mRFP STOP STOP
CIRCUIT DESIGN:
Low-temperature Release System

Low-temperature release system

A
Constitutive promoter
37°C RBS tetR STOP STOP
37°C RBS tetR

B
Ptet RBS cry RBS GFP STOP STOP

C
Ptet RBS LuxI RBS LuxR STOP STOP
SAM AHL

D
Plux RBS ccdB RBS mRFP STOP STOP
CIRCUIT DESIGN: Population Control

Population Control

A
Constitutive promoter
37°C induced RBS
RBS
tetR
STOP
STOP
tetR

B
Ptet
RBS
cry
RBS
GFP
STOP
STOP

C
Ptet
RBS
LuxI
RBS
LuxR
STOP
STOP

SAM
AHL

D
Plux
RBS
ccdB
RBS
mRFP
STOP
STOP
Part I: CRY Weapon System

CRY weapon system
cry11Aa (1932bp) was cloned from *Bacillus thuringiensis* and ligated into TA vector.
CRY Weapon System: Biobrick of cry11Aa

- PCR construction of Biobrick parts (add E,X,S,P sites)

Biobrick of cry11Aa Gene (BBa_K332011)
Part II: Low-temperature Release System

Low-temperature release system
Low-temperature Release System: How Does It Work?
Low-temperature Release System: How Does It Work?

Constitutive promoter

37°C induced RBS

tetR

STOP STOP

Ptet

RBS cry RBS mGFP STOP STOP

37°C
Low-temperature Release System: How Does It Work?

A. Constitutive promoter

B. TetR

CRY

GFP

37°C
Green fluorescence intensity was measured by flow cytometer.
Low-temperature Release System: Dynamic model

\[
\frac{d[\text{TetR}]}{dt} = \alpha_{\text{Temp}} - \gamma_{\text{TetR}}[\text{TetR}] - d(t)[\text{GFP}]
\]

\[
\frac{d[\text{GFP}]}{dt} = \frac{\alpha_{\text{B}}}{1 + [\text{TetR}]^n} - \gamma_{\text{GFP}}[\text{GFP}] - d(t)[\text{GFP}]
\]

- **\( \alpha \)**: production rate of the corresponding protein
- **\( \gamma \)**: decay rate of the corresponding protein
- **\( d(t) \)**: dilution rate during cell division

A

Constitutive promoter

37°C induced RBS

**tetR**

STOP  STOP

B

Ptet

RBS  **cry**  RBS  mGFP  STOP  STOP

\( \alpha \): production rate of the corresponding protein
\( \gamma \): decay rate of the corresponding protein
\( d(t) \): dilution rate during cell division
Parameters of our model were determined by the nonlinear regression based on the minimum sum of square residuals.
The relative translational activity of temperature-sensitive RBS BBa_K115002 at 25°C, 30°C, 37 °C and 40°C were estimated.
Population Control
Population control:
Three Statues

1. Reproduction status at 37°C in laboratory
2. Self-maintain status at room temperature
3. Self-destruction status by AHL administration
Population control: I: Reproduction Status at 37°C in Laboratory

Constitutive promoter

A

37°C induced RBS

tetR

STOP

STOP

Bacteria Culture (T ≥37°C)

C

RBS

LuxI

RBS

LuxR

STOP

STOP

D

RBS

ccdB

RBS

mRFP

STOP

STOP
Population control:
II: Self-maintain Status at Room Temperature

Constitutive promoter

A

Spray to environment (T<37°C)

RBS
LuxI
RBS
LuxR
STOP
STOP

37°C

B

Ptet

C

tetR

RBS
Luxl
RBS
LuxR
STOP
STOP

D

Plux

RBS
ccdB
RBS
mRFP
STOP
STOP
Population control: II: Self-maintain Status at Room Temperature

Constitutive promoter

A

Spray to environment (T<37°C)

37°C

B

Ptet

RBS LuxI RBS LuxR STOP STOP

LuxI LuxR

SAM AHL

C

D

Plux

RBS ccdB RBS mRFP STOP STOP
Population control:
II: Self-maintain Status at Room Temperature

Constitutive promoter

Spray to environment (T<37°C)

A

Constitutive promoter

37°C

B

tetR

STOP

STOP

C

Ptet

RBS

LuxI

RBS

LuxR

STOP

STOP

D

Plux

RBS

ccdB

RBS

mRFP

STOP

STOP

AHL

LuxR

ccdB

RFP

37°C

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Population control: II: Self-maintain Status at Room Temperature
Population control: Dynamic Model

\[
\frac{d[AHL]}{dt} = k_2 [LuxI][bac] - \gamma_{AHL}[AHL]
\]

\[
\frac{d[bac]}{dt} = \frac{\alpha_{bac}}{1 + [ccdB]^{n1}} - \gamma_{bac}[bac]
\]
Population control: Dynamic Model

Bacterial population vs. Time graph.
Population control:
III: Self-destruction status by AHL administration
New protocol: for generation of RBS library with different translational activity
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New protocol: for generation of RBS library with different translational activity
Advantages

1. **Economical** The Terminator adapts to the environment easier than *Bacillus*, thus avoids repetitive spraying and reduces spraying cost.

2. **Safe** Cry proteins are toxic to very specific species of insects yet harmless to humans and the natural hunters of many crop pests.

Customizable with more than one hundred crystal proteins targeting various insect species, the intelligent terminator system can be replaced with different cry genes for killing other insect species.
Contributions

Create a new Cry biobrick: BBa_K332011

Create and quantify a low temperature release device: BBa_K332032

Quantify the relative translation activity of temp-sensitive RBS: BBa_K115002

Design a new protocol for generation of RBS library with different translational activity: BBF RFC=79
Our contributions will make mosquitoes cry!!
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ACS Lab
Automatic Control Signal Processing and Systems Biology Lab
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Thank You for Your Attention
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