*Tech Spec Review*

# Fighting the Red Tides

## Team Green

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# General Info

**Ulva Fasciata**

-A variety of green algae

-Naturally produces linolenic and alpha-linolenic acid. Both of which have been found to be toxic to Red Tide.

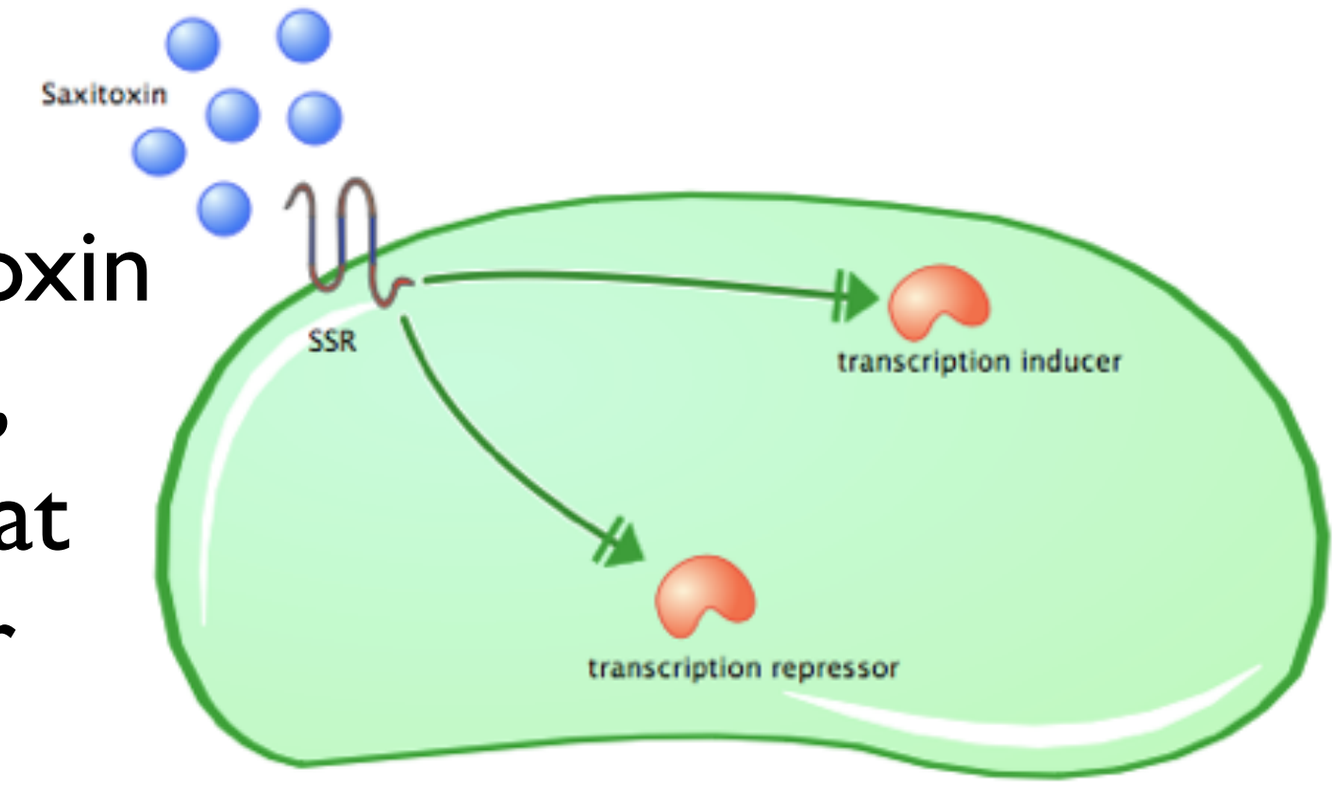
**Goal of Project:** To engineer ulva fasciata to make it an effective and feasible tool for fighting Red Tide.

# Devices

## Saxitoxin Sensor Device [SSD]

**Input:** Saxitoxin

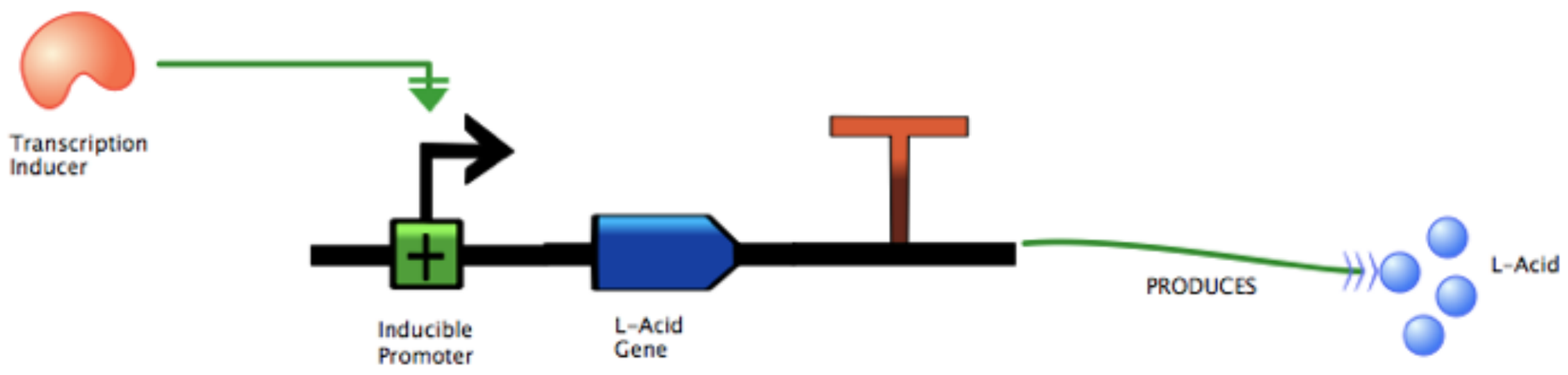
**Goal:** Binding of Saxitoxin activates signal cascade, producing an output that acts to either induce or repress transcription.



# Linolenic-Acid Up-Regulation Device [LAUD]

**Input:** Output of SSD binds to promoter, inducing transcription of the gene.

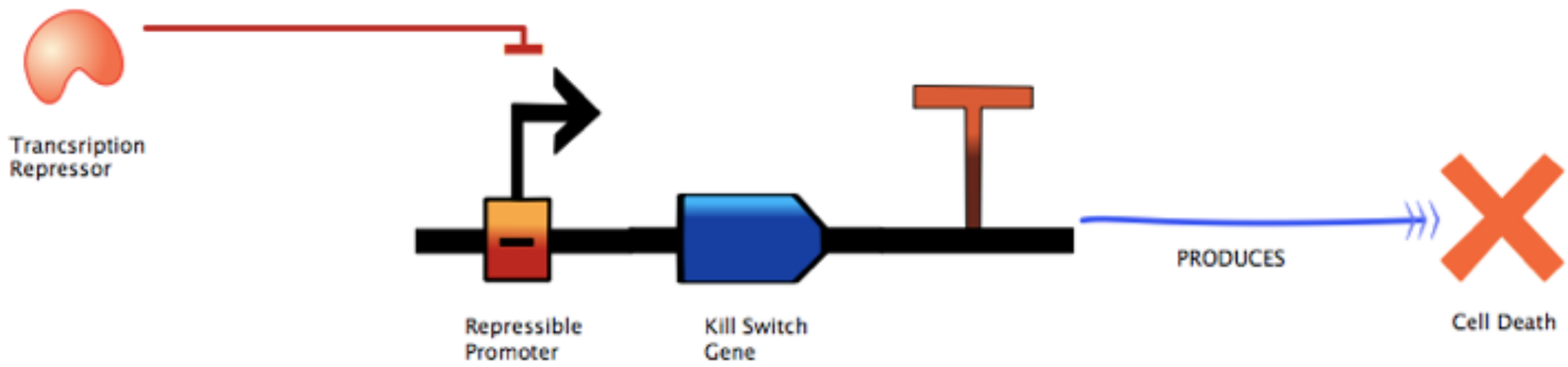
**Goal:** Increase the amount of Linolenic-Acid produced in the microalgae, killing more red tide.



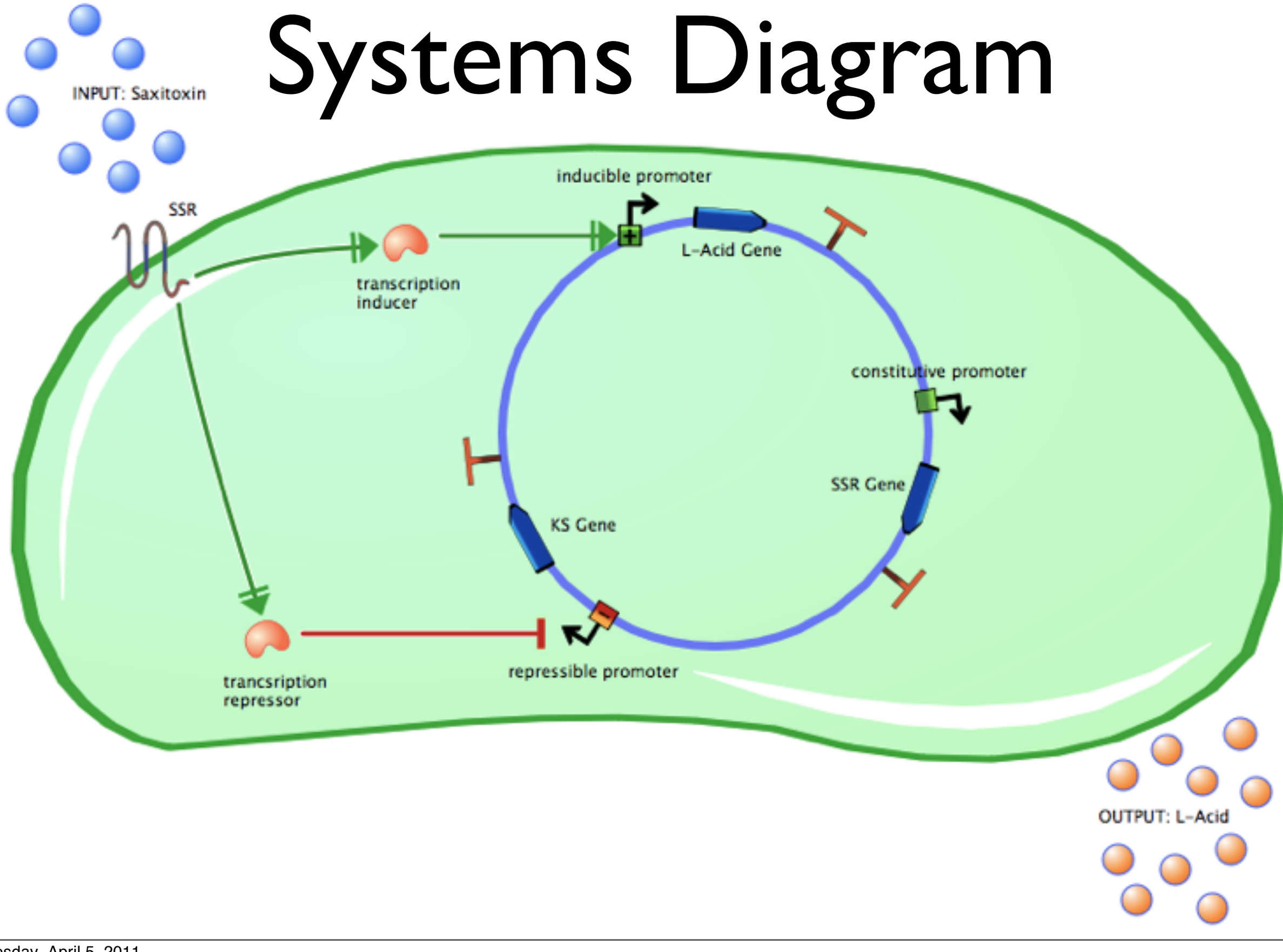
# Kill Switch Device

**Input:** Output of SSD binds to promoter, repressing transcription of gene.

**Goal:** Kill our bioengineered microalgae cells in the absence of red tide.



# Systems Diagram



# Parts

(1) Plasmid

(2) Saxitoxin-binding receptor gene

(3) Constitutive promoter for (2)

(4) Microalgae inducible promoter I for (5)

(5) Linolenic-acid Synthesis Gene

(6) Microalgae repressible promoter II for (7)

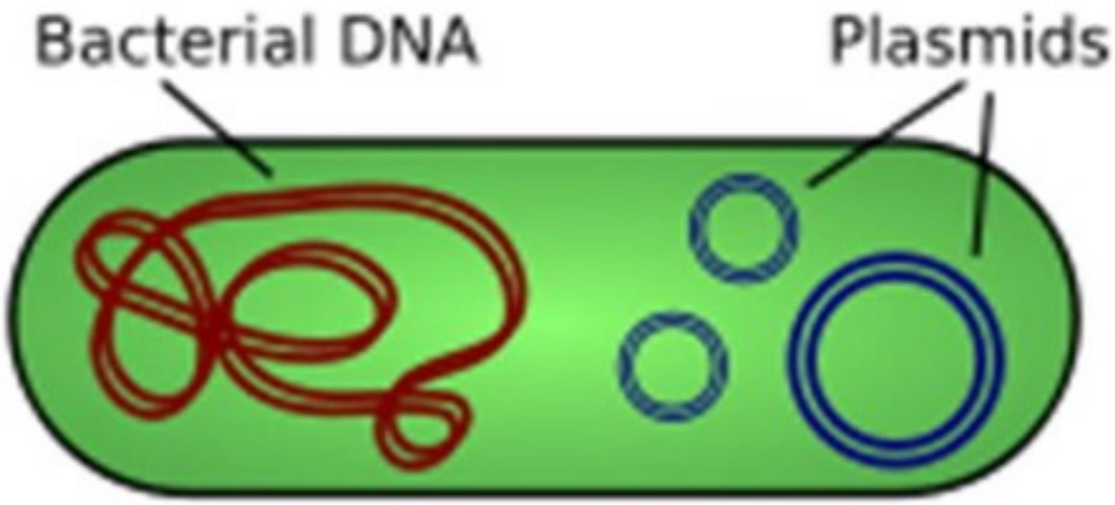
(7) Kill Switch Gene

# Devices

1. Saxitoxin-Sensor Device (SSD)
2. Linolenic-acid Up-Regulation Device
3. Kill switch

# Parts

## (1) Plasmid



# (2) Saxitoxin-binding receptor gene

- Saxitoxin-Sensor Device

- Saxiphillin: plasma protein that binds saxitoxin

- Insert DNA sequence for this extracellular binding site

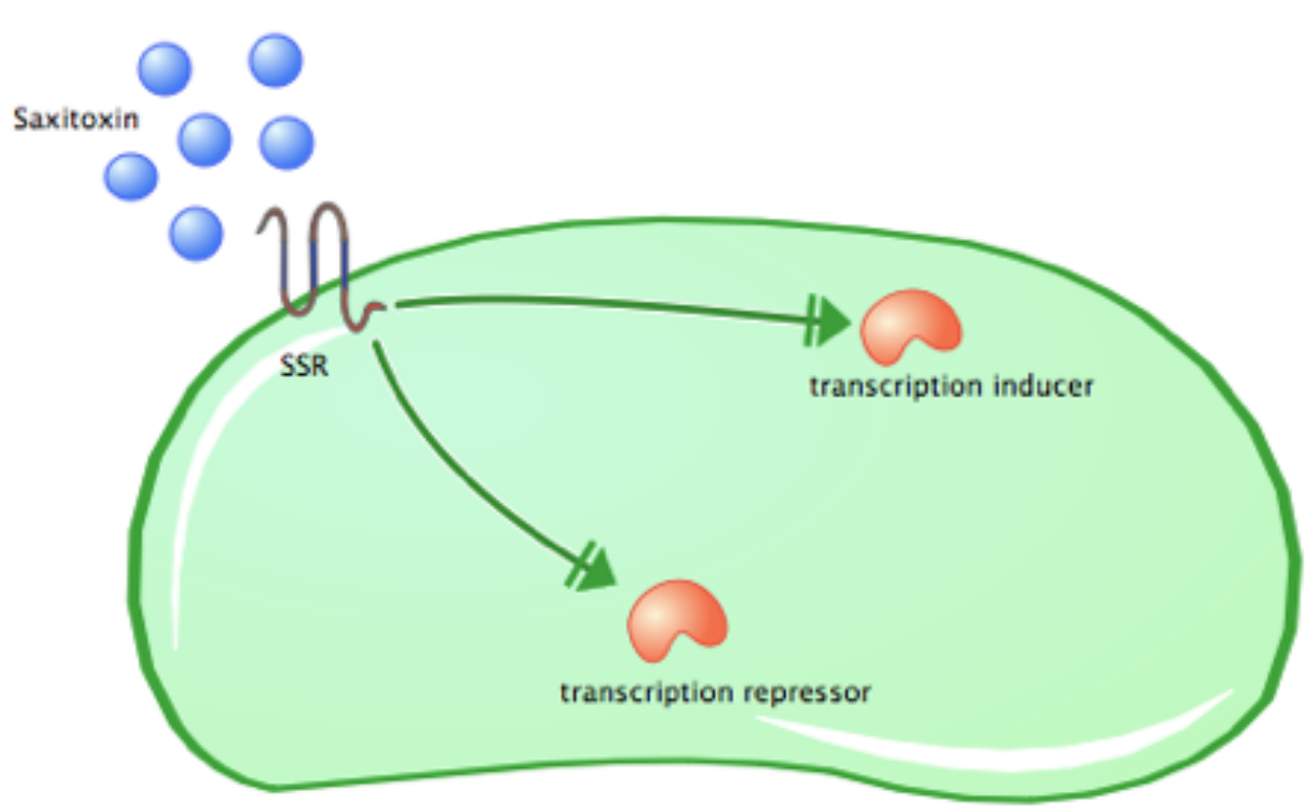
- Sequence for intracellular part of a protein that induces the promoter

Rana catesbeiana

# (3) Constitutive promoter (SSD)

: for Part (2) saxitoxin binding receptor gene

**Possibility:** PSAD constitutive promoter



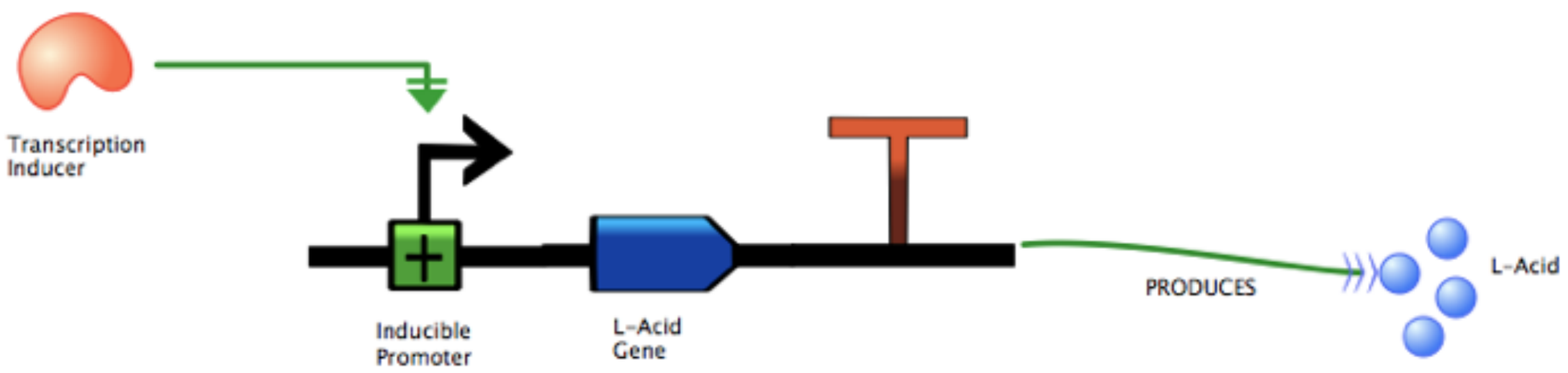
## (4) Strong inducible microalgae promoter I (LAUD)

: for the Linolenic-acid synthesis gene

**Possibilities:** nitrate reductase gene promoter, HSP70A promoter, cabII-1 promoter

## (5) Linolenic-acid Synthesis Gene

: from microalgae, Ulva Fasciata

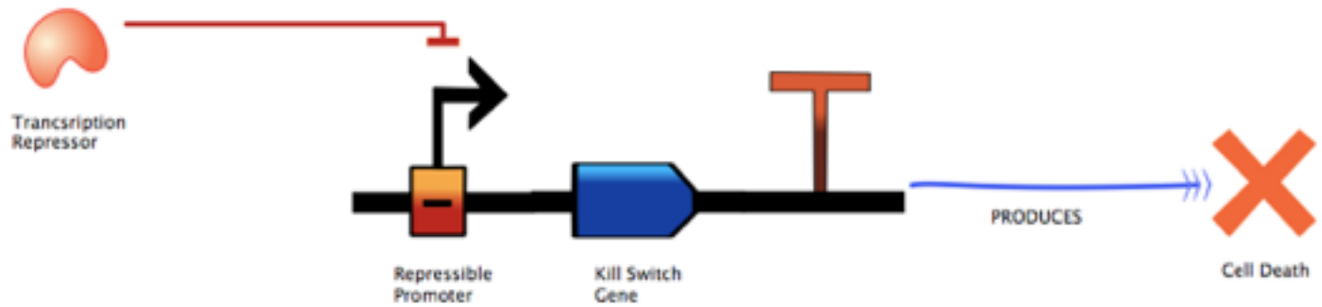


# (6) Strong microalgae repressible promoter II (KSD)

# (7) Kill Switch Gene

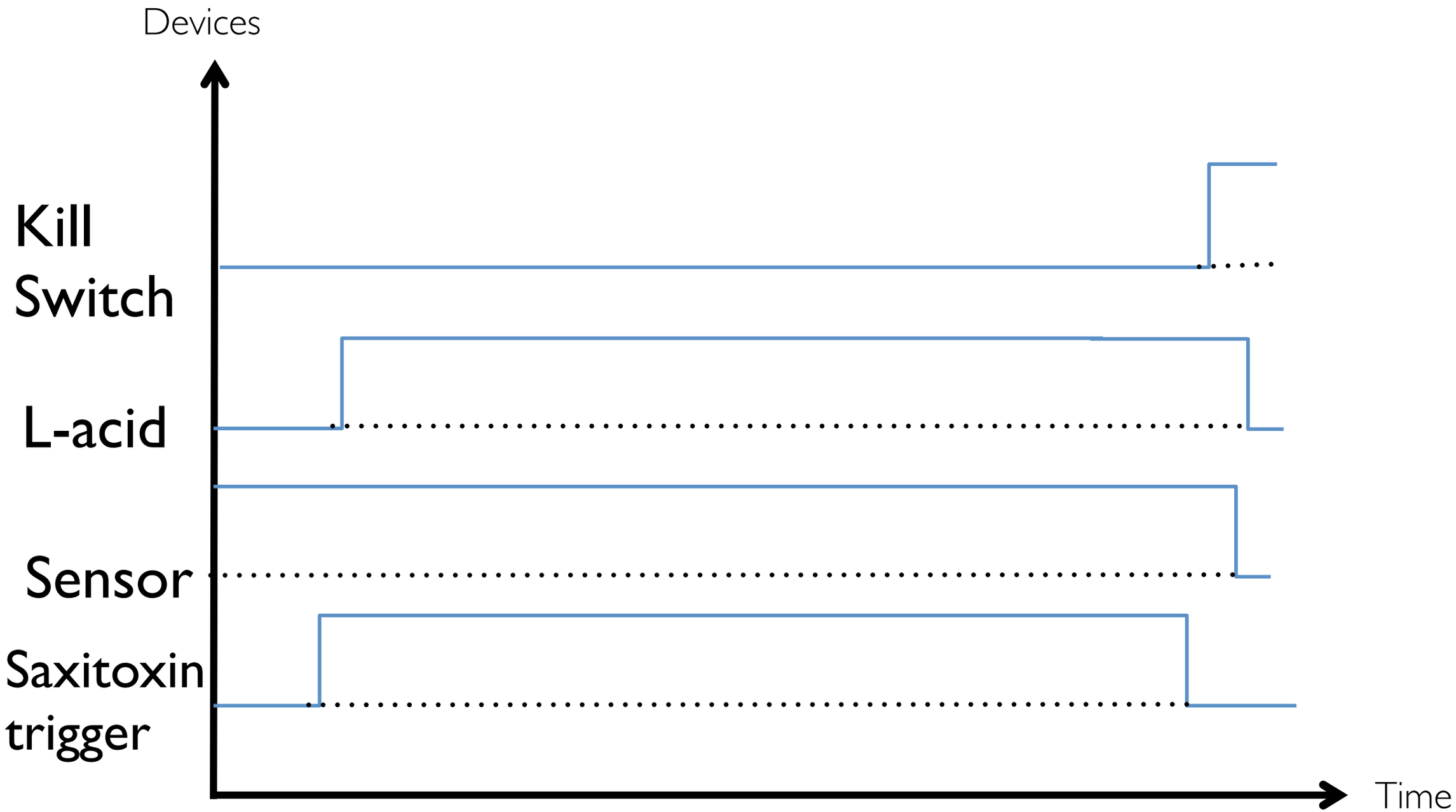
- In presence of saxitoxin
  : crRNA & taRNA OFF

- In absence of saxitoxin
  : crRNA & taRNA ON

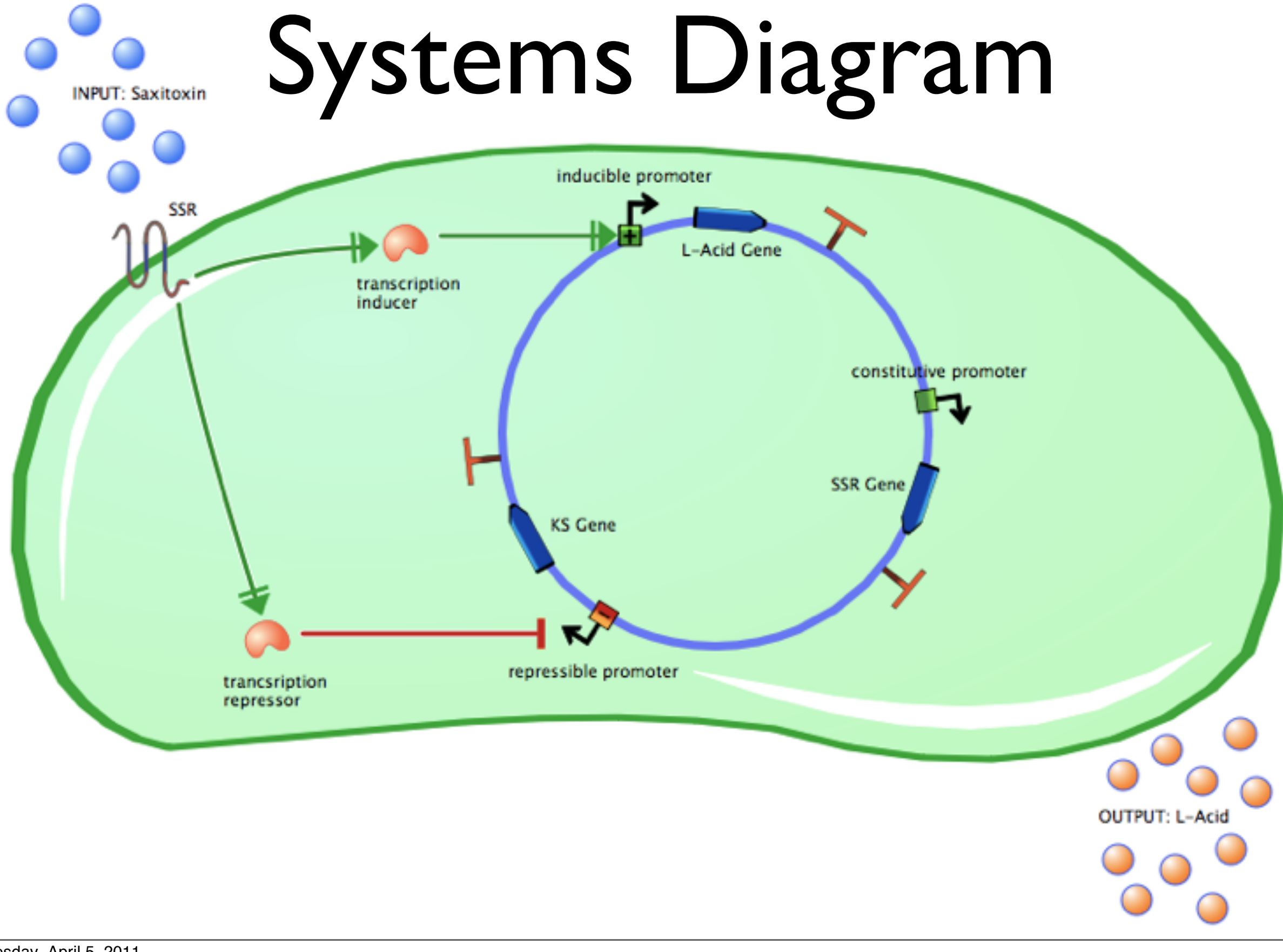



When crRNA and taRNA are both expressed,
a protein is produced that kills the cell

# Timing Diagram



# Systems Diagram



# Testing/Debugging

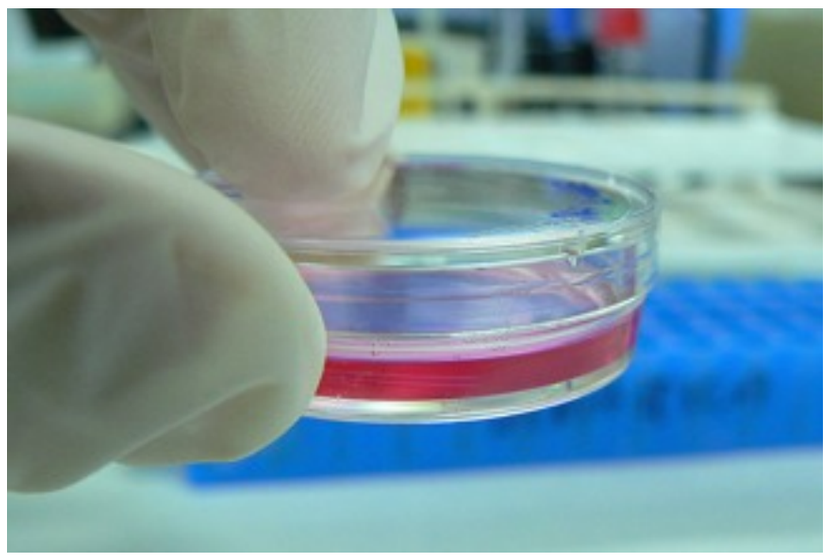
**First Test:** How do we know if the algae we use contain the vector plasmids?

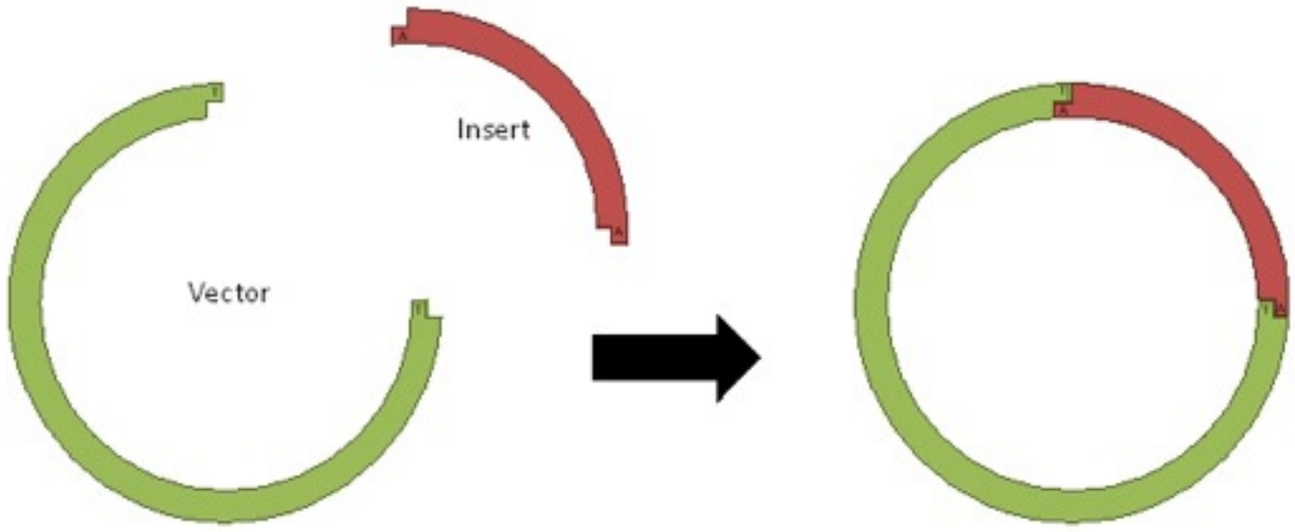
**Answer:** Selectable marker gene, OPD, which encodes for a crucial enzyme in the pyrimidine biosynthesis pathway

# Testing/Debugging

**Method:**

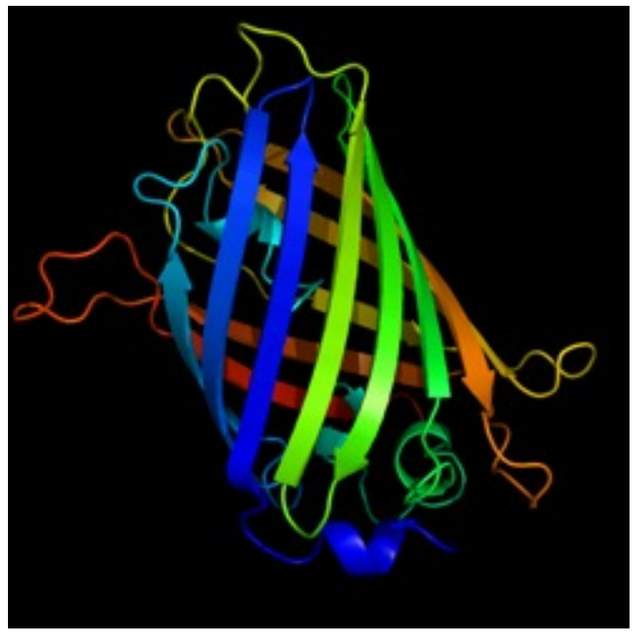
1. Select for OPD-minus microalgae mutants
2. Isolate wild-type OPD gene and insert into plasmids
3. Introduce plasmids into mutant cells
4. Select for the algae that have taken up the plasmid by growing in medium without uracil





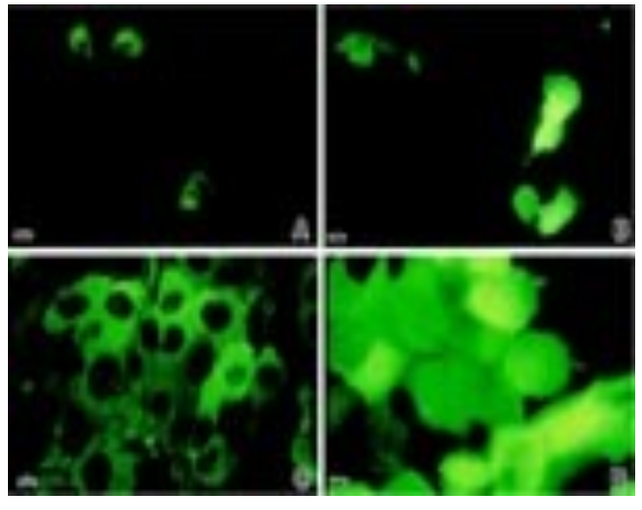
# Testing/Debugging

**Second Test:** What if the gene for linolenic acid is not being expressed?



**Answer:** Use GFP gene to determine if linolenic acid gene is being expressed

Splice gene into the plasmid in the region that encodes the gene for linolenic acid: now when linolenic acid gene is expressed, GFP is expressed as well



# Concerns

- Breakdown of the molecules after killing red tides

- Some of the parts are unknown

- Applying recombinant DNA technology to a new organism

# Impact

***Minimal success would still bring big impact.***

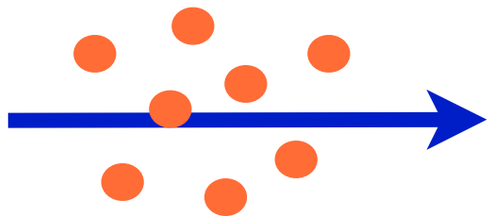
- Current technologies are inferior (dumping toxic chemicals into the ocean)

- Damages from red tides are huge.

# Summary

**Goal:** Removing Red Tides

Microalgae
(Ulva Facsiata)



L-acid
(toxic to red tides)

Red tides
DIE

**Approach:**

Up-regulation (making more powerful)
Kill Switch to control GM population

# TEAM GREEN!

# References


http://www.google.com/url?q=http%3A%2F%2Fwww.jstage.jst.go.jp%2Farticle%2Fbbb%2F71%2F1%2F265%2F_pdf&sa=D&sntz=1&usg=AFQjCNG3yKmPJJCx-9Rnh_2lmj7KwIFhGQ

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