iGarden
an open source toolkit for genetically engineering plants

Harvard iGEM 2010
iGarden
Harvard 2010
Garden Kit
how would you customize your garden?
how would you customize your garden?

environment

- less fertilizer and pesticide
- drought or flood resistance
how would you customize your garden?

environment
- less fertilizer and pesticide
- drought or flood resistance

health and safety
- allergies
- toxins

genetic safety
how would you customize your garden?

environment

- less fertilizer and pesticide
- drought or flood resistance

genetic safety

- allergies
- toxins

health and safety

living architecture

- decorative plants
- color
- flavor

fun

nutrition

- color
how would you customize your garden?

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living architecture
- genetic safety
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- decorative plants
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iGarden
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  - allergies
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iGarden

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iGarden

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iGarden

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living architecture

iGarden

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- color
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decorative plants
three chassis system

Arabidopsis thaliana
three chassis system

Arabidopsis thaliana
three chassis system

E. coli
DNA construction chassis

Arabidopsis thaliana
three chassis system

E. coli
DNA construction chassis

Agrobacterium tumefaciens
DNA transfer chassis

Arabidopsis thaliana
three chassis system

E. coli
DNA construction chassis

Agrobacterium tumefaciens
DNA transfer chassis

Arabidopsis thaliana
three chassis system

*E. coli*  
DNA construction chassis

*Agrobacterium tumefaciens*  
DNA transfer chassis

*Arabidopsis thaliana*
agrobacterium: DNA transfer chassis

Ti plasmid
agrobacterium: DNA transfer chassis

Ti plasmid

plant genome
agrobacterium: DNA transfer chassis

Ti plasmid

plant genome
BioBrick plant vectors
from *Arabidopsis* to iGarden
from *Arabidopsis* to iGarden
from *Arabidopsis* to iGarden
from *Arabidopsis* to iGarden

- grow plants
- flower dip

week 5-6
from *Arabidopsis* to iGarden

- grow plants
- flower dip
- harvest seeds

week 5-6  week 8-10
from *Arabidopsis* to iGarden

- grow plants
- flower dip
- harvest seeds
- plant on selective plates

Weeks:
- Week 5-6
- Week 8-10
- Week 11
from *Arabidopsis* to iGarden

- Grow plants: week 5-6
- Flower dip: week 8-10
- Harvest seeds: week 11
- Plant on selective plates: week 12
sprouts!
selection!

0.1% transgene integration
0.1% transgene integration
keeping plants in your garden with the genetic fence

- If the Fence Compound is present (Yes), the Death gene is OFF, resulting in Survival.
- If the Fence Compound is absent (No), the Death gene is ON, resulting in Death.
characterizing the death gene

Berkeley Barnase/Barstar Strain Grown in LB + Arabinose

Growth (OD600)

Time (min)

Arabinose concentration (Barnase inducer)
- 0%
- 1%
- 2%

strain courtesy of UC Berkeley iGEM 2007
activating the genetic fence

sensor

output

barnase gene
activating the genetic fence

sensor  input  output

fence compound

barnase gene
activating the genetic fence

sensor  input  circuit activation  output

fence compound

activated transcription factor

barnase gene
activating the genetic fence

sensor  input  circuit activation  output

fence compound

activated transcription factor

barnase protein inhibitor

barnase gene repressor

barnase gene

activating the genetic fence
personalizing our garden

helping christina
Bet v 1 is a pan-allergen present in birch tree pollen
bet v 1 is a pan-allergen present in birch tree pollen
Bet v 1 is a pan-allergen present in birch tree pollen
Bet v 1 is a pan-allergen present in birch tree pollen.
Bet v 1 is a pan-allergen present in birch tree pollen.
Bet v 1 is a pan-allergen present in birch tree pollen.
we targeted 3 pan-allergens

lipid transfer protein

bet v l

germin
targeted knockdown with RNAi

artificial microRNA

Constitutive Promoter

RS300 BioBrick

miRNA

miRNA*

mRNA transcript

splicing

completed hairpin for RNAi targeting
targeted knockdown with RNAi

**artificial microRNA**

- Constitutive Promoter
- RS300 BioBrick
- miRNA
- miRNA*

**hairpin RNA**

- Constitutive Promoter
- sense
- intron
- antisense

mRNA transcript

splicing

completed hairpin for RNAi targeting

mRNA transcript

intron splicing

Further processing by RNAi machinery
targeted knockdown with RNAi

artificial microRNA

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easy PCR construction

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- intron
- antisense
- mRNA transcript
- intron splicing
- base pairing
- Further processing by RNAi machinery

easy PCR construction

specificity

difficulty in design
targeted knockdown with RNAi

artificial microRNA

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modular
targeted knockdown with RNAi

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Constitutive Promoter

sense
intron
antisense

modular
targets many isoforms

Further processing by RNAi machinery
targeted knockdown with RNAi

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easy PCR construction
specificity
difficulty in design

hairpin RNA

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modular
targets many isoforms
ease of design
playing with pigments:
lycopene and beta carotene
lycopene metabolism

upstream metabolites → **crtl** → lycopene → **LUT2** → downstream metabolites
lycopene metabolism

upstream metabolites

| ctrl |

lycopene

downstream metabolites
brazzein protein is 2000X sweeter than sugar
miraculin is a flavor inverter
miraculin is a flavor inverter
miraculin is a flavor inverter
inducible expression of flavor proteins in *E. coli*

Brazzein + Strep-II tag

YFP tag

miraculin

brazzein

relative fluorescence

relative fluorescence

10kDa

time (2 hours)

time (2 hours)
other flavors

valencene
other flavors

valencene

methyl salycilate

MIT iGEM 2006

isoamyl acetate
iGarden version 1.0 includes 39 BioBrick parts

- 6 vector
- 5 allergy
- 9 flavor
- 3 color
- 10 genetic fence

10 constructs growing in plants!
the iGardener can find all of these parts in the iGarden kit
Would you feel comfortable changing any of the following plant characteristics through genetic engineering?

- Color
- Flavor
- Nutrition
- Allergen
- Sugar

n=60
iGarden
iGarden

personal
iGarden

personal

open source
iGarden

personal

open source

educational
iGarden

- personal
- open source
- educational
- safe
acknowledgments

kurt schellenberg
& sarah mathews
Questions?