



eau d'e coli  
igem 2006: mit



# meet the team



## The Undergrads:

Kate Broadbent  
Biological Engineering

Andre Green  
Biology / Physics

Stephen Payne  
Biological Engineering

Veena Venkatachalam  
Chemistry / Physics

Boyuan Zhu  
Electrical Engineering and  
Computer Science

## The Grad Students:

Barry Canton  
Austin Che  
Jason Kelly  
Reshma Shetty  
Samantha Sutton

## The Faculty:

Drew Endy  
Tom Knight

# motivations



# background

*A. thaliana*



**JMT**

jasmine

*O. basilicum*



**CCMT**

cinnamon

*A. majus*



**BAMT**

fresh / floral

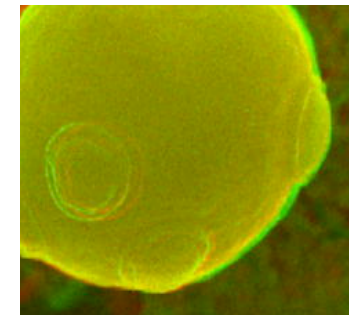
*C. breweri*



**BSMT**

wintergreen

*S. cerevisiae*



**ATFI**

banana



# background

*O. basilicum*



**CCMT**

cinnamon

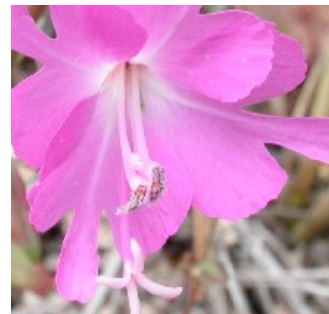
*A. majus*



**BAMT**

fresh / floral

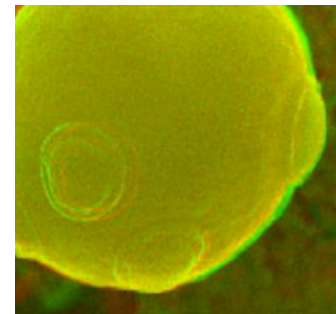
*C. breweri*



**BSMT**

wintergreen

*S. cerevisiae*



**ATFI**

banana

# background

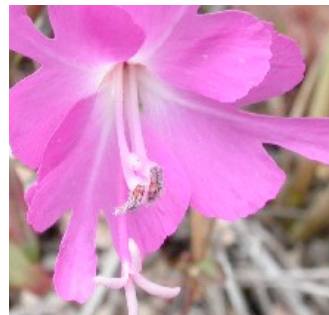
*A. majus*



**BAMT**

fresh / floral

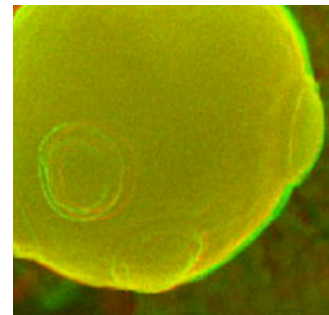
*C. breweri*



**BSMT**

wintergreen

*S. cerevisiae*

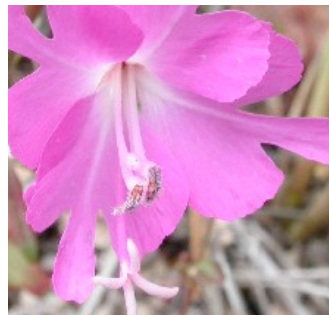


**ATFI**

banana

# background

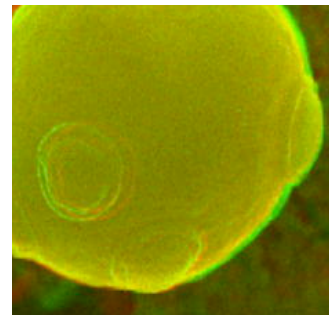
*C. breweri*



**BSMT**

wintergreen

*S. cerevisiae*

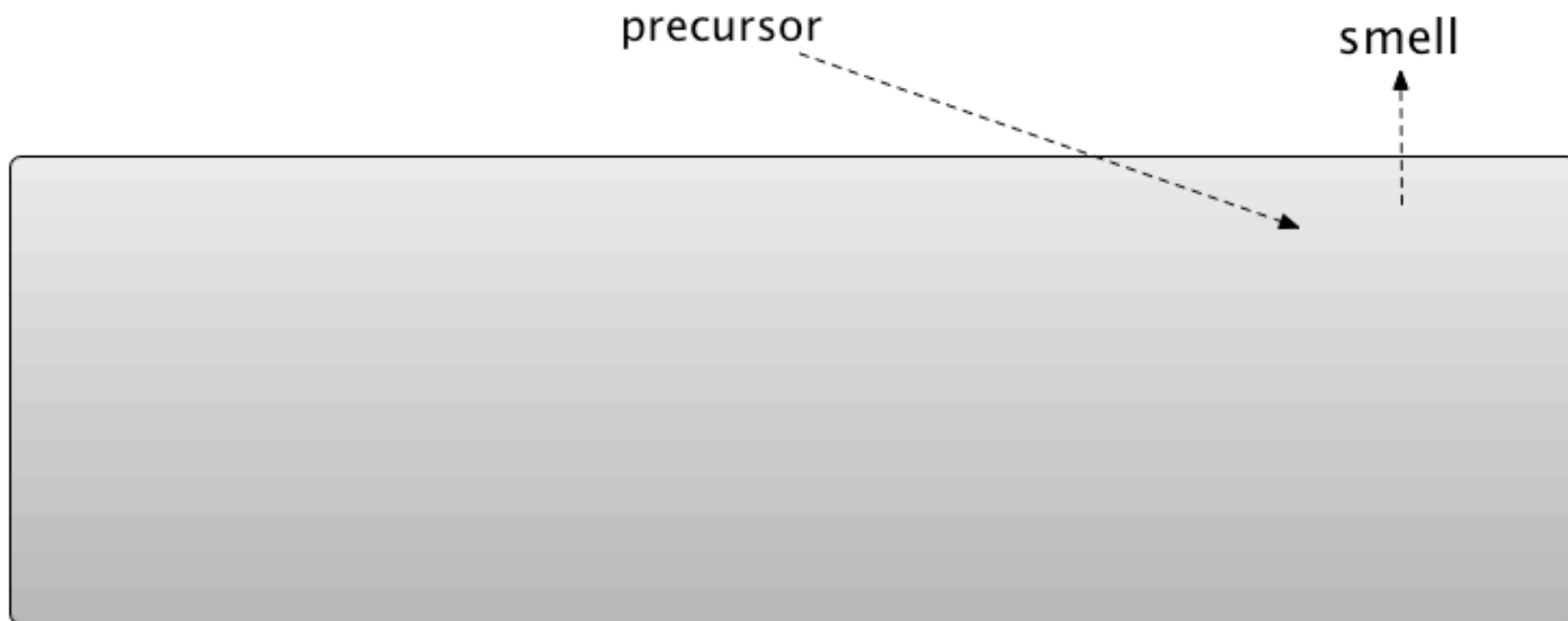


**ATFI**

banana

# timeline: initial planning

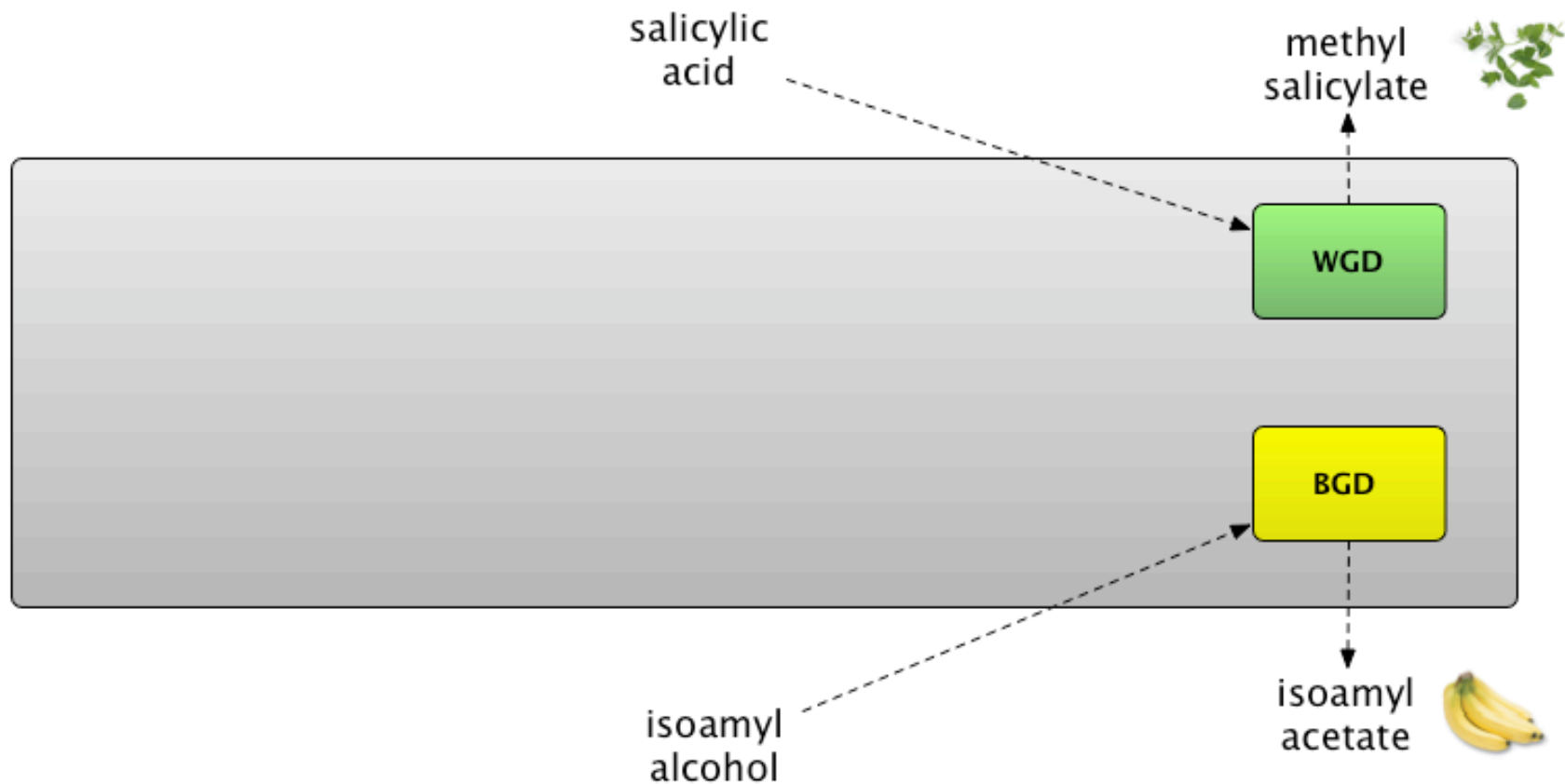
**initial planning** • scent production • self-generating scent • production regulation • eliminating indole





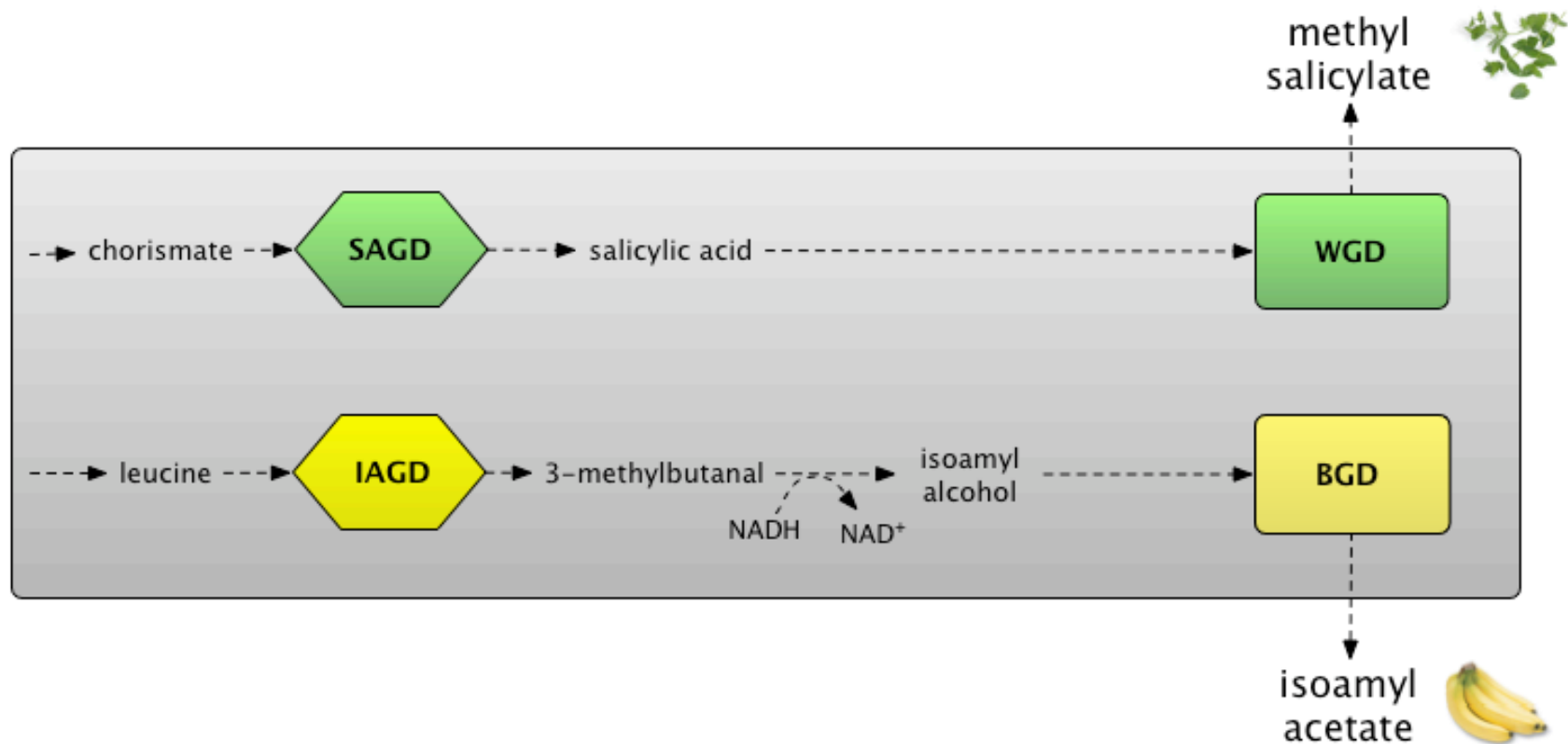
# timeline: scent production

initial planning • **scent production** • self-generating scent • production regulation • eliminating indole



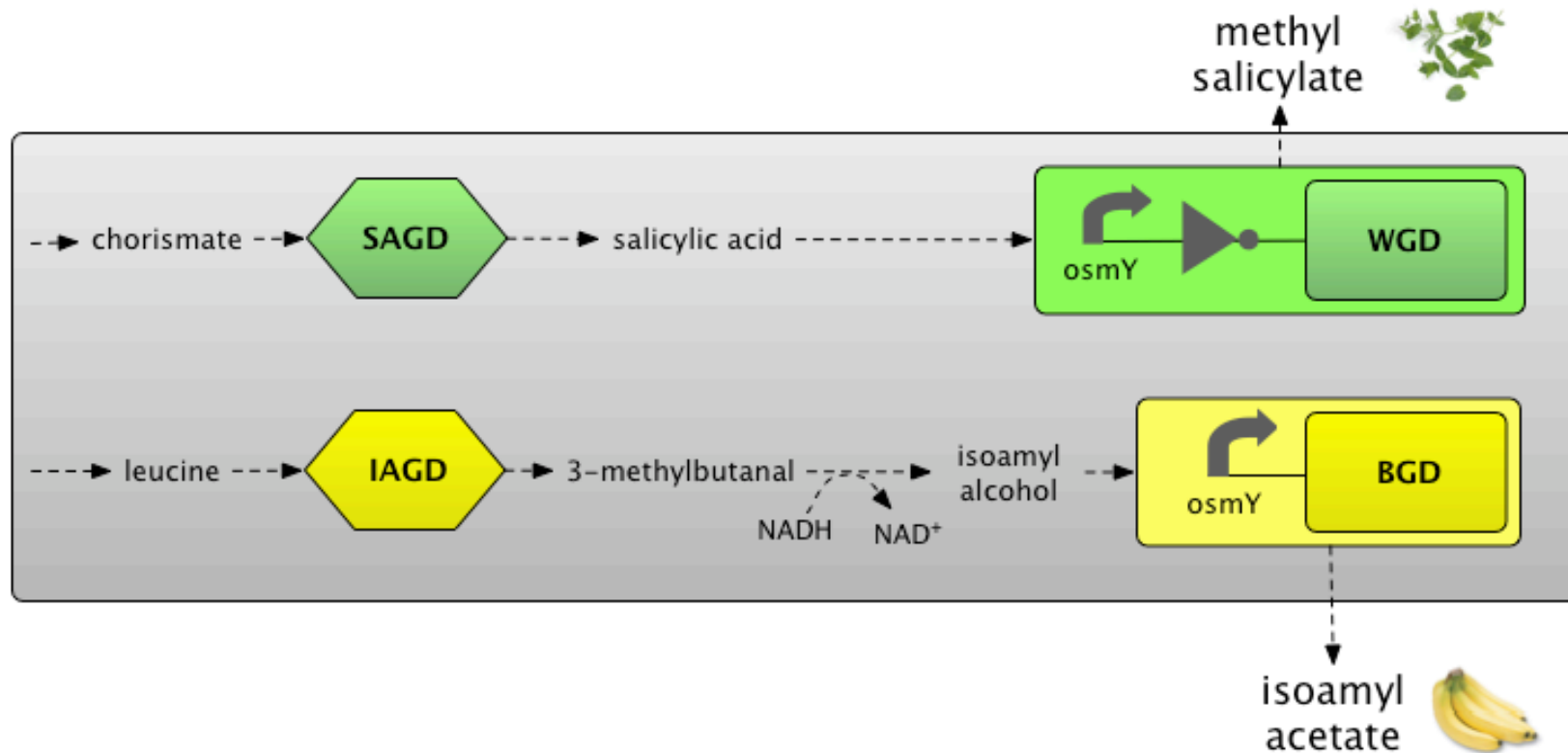
# timeline: self-generating scent

initial planning • scent production • **self-generating scent** • production regulation • eliminating indole



# timeline: production regulation

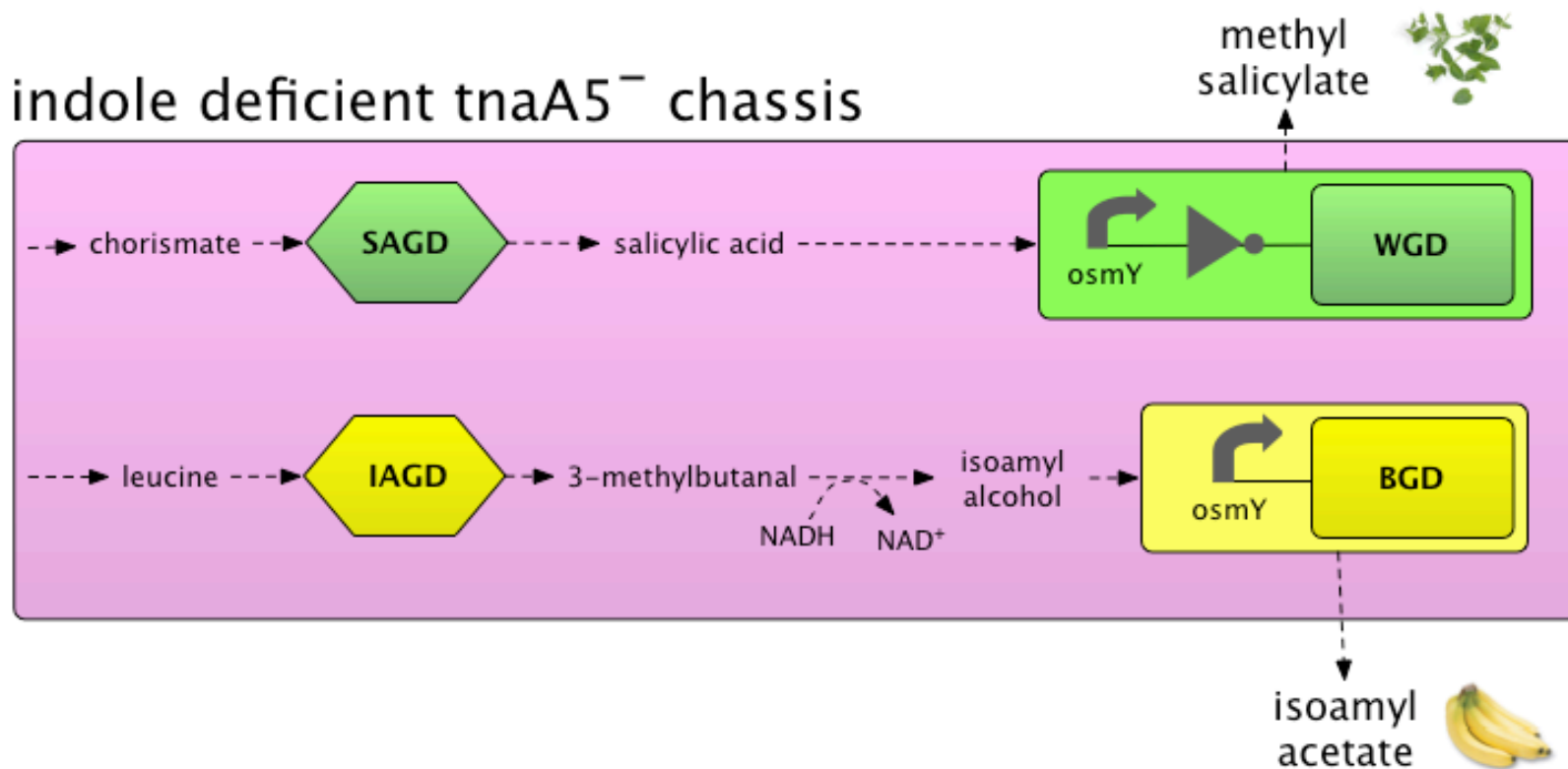
initial planning • scent production • self-generating scent • **production regulation** • eliminating indole



# timeline: indole elimination

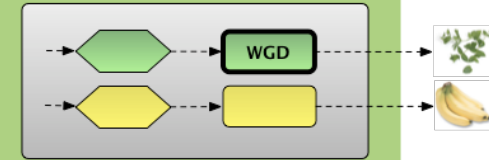
initial planning • scent production • self-generating scent • production regulation • **eliminating indole**

## indole deficient $tnaA5^-$ chassis

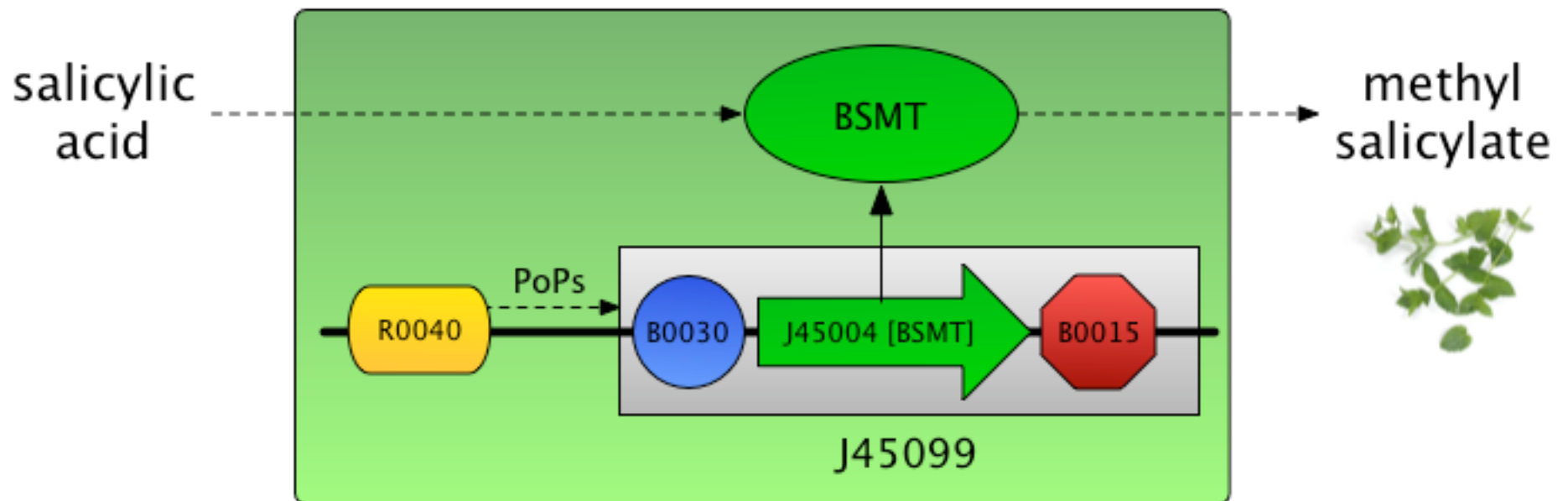




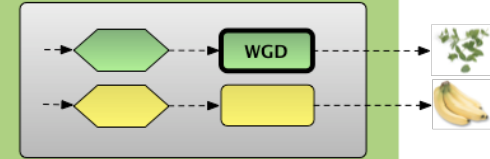
# wintergreen generating device



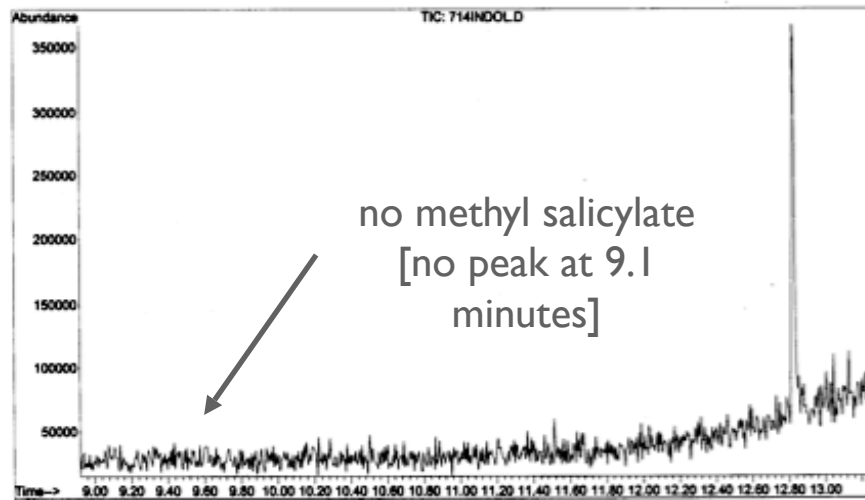
## J45100 – Wintergreen Generating Device (WGD)



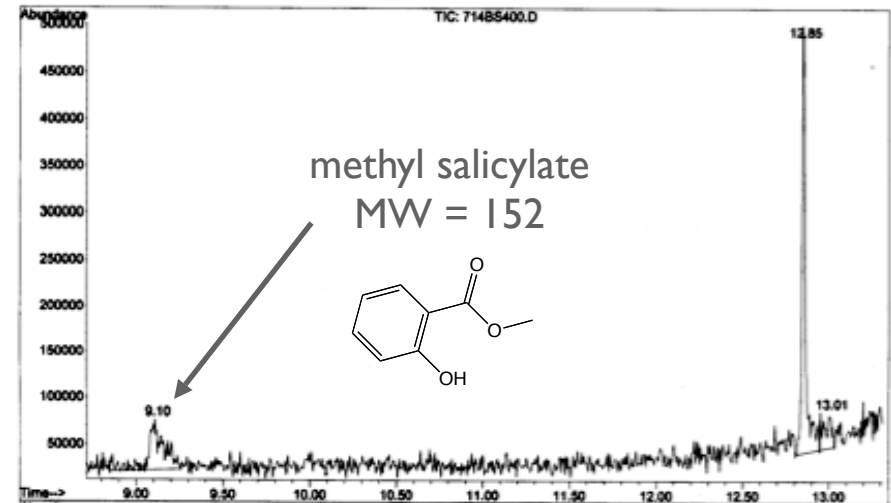
# device generates wintergreen



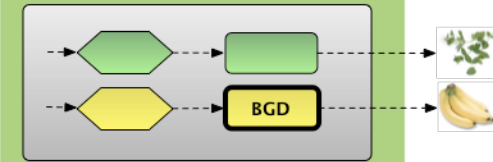
*E. coli*



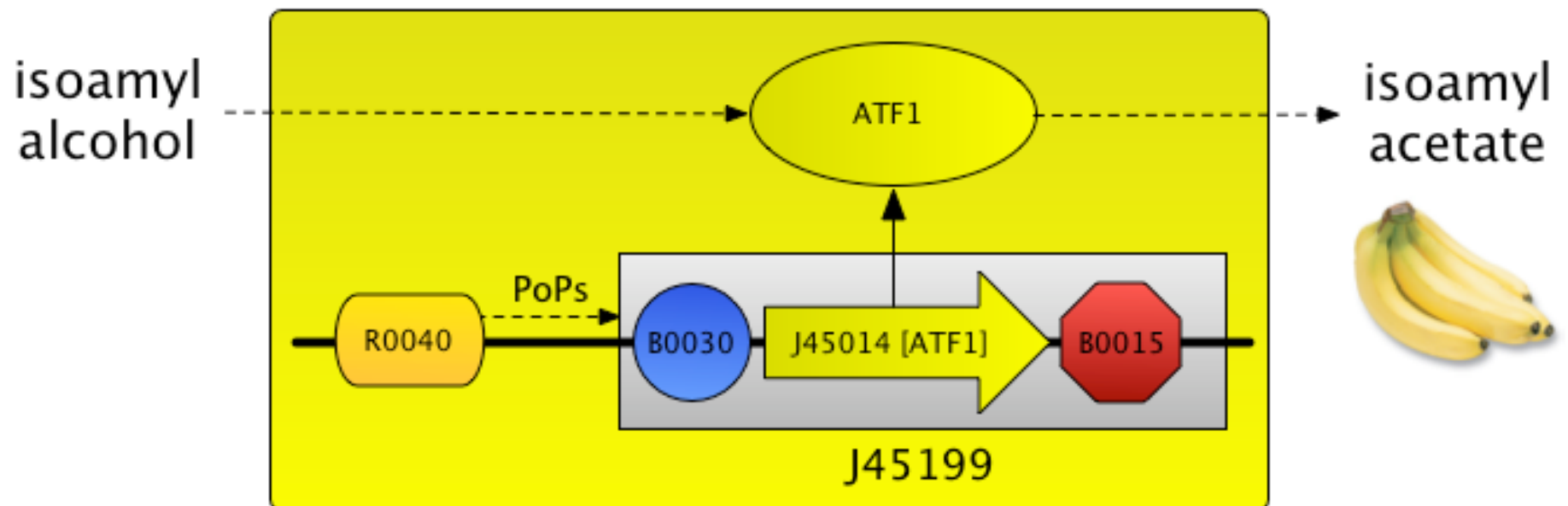
*E. coli* + precursor (SA)  
+ WGD



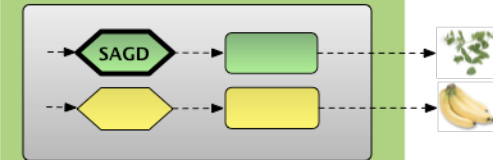
# banana generating device



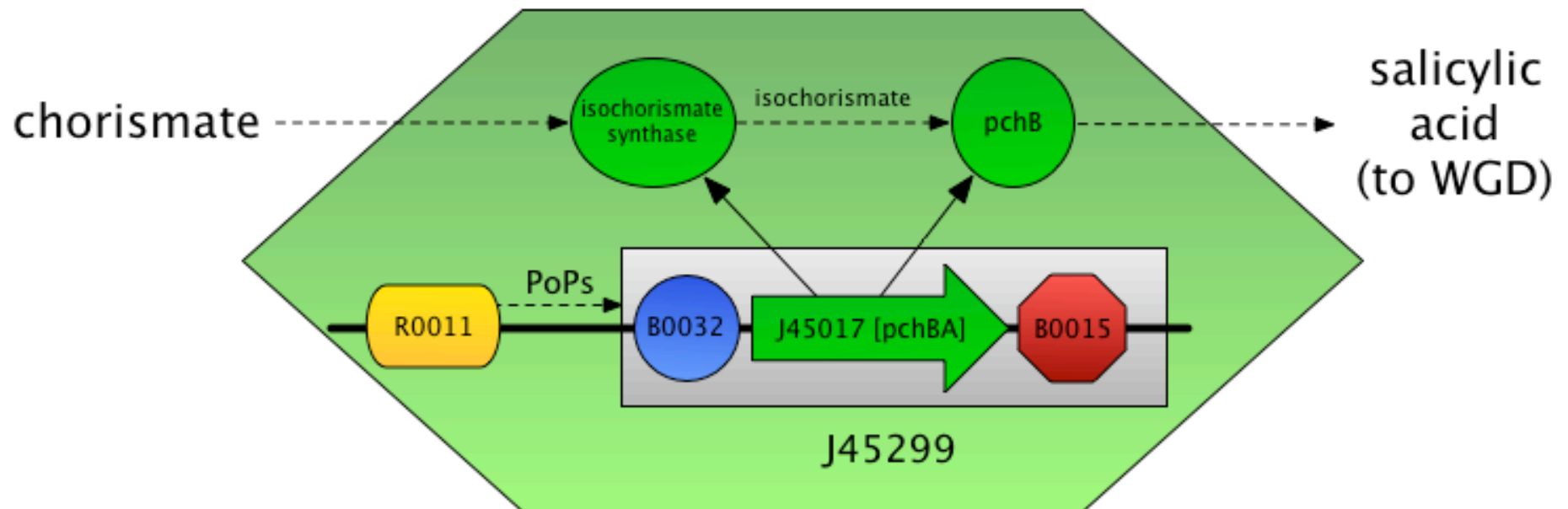
## J45200 – Banana Generating Device (BGD)



# precursor biosynthesis

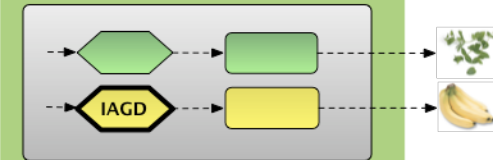


## J45300 – Salicylic Acid Generating Device (SAGD):

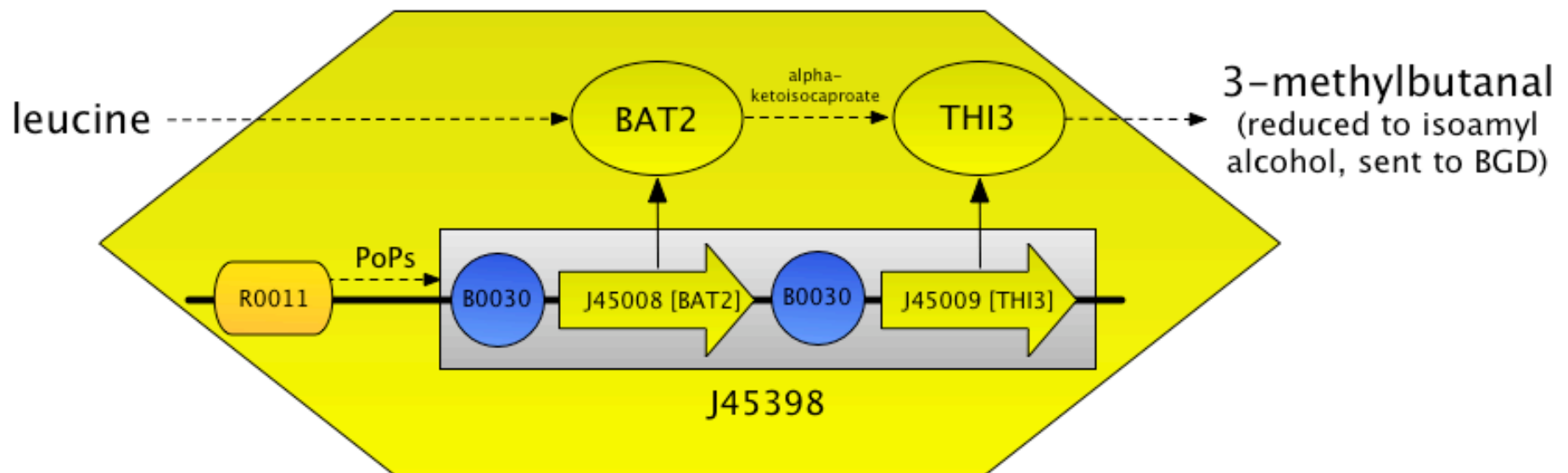




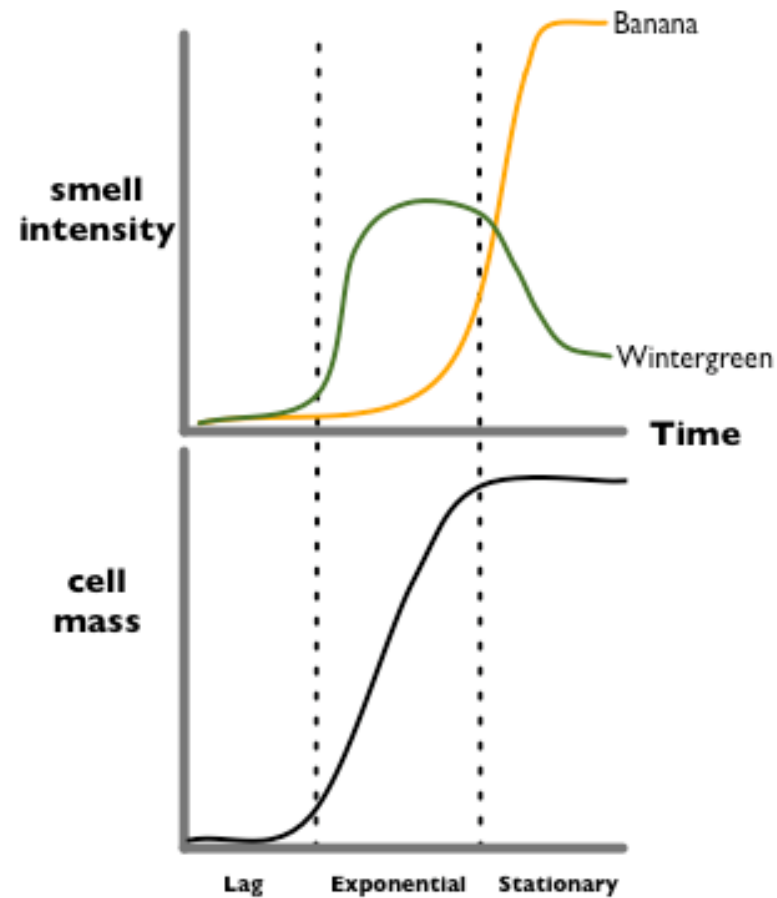
# precursor biosynthesis



## J45400 – Isoamyl Alcohol Generating Device (IAGD):

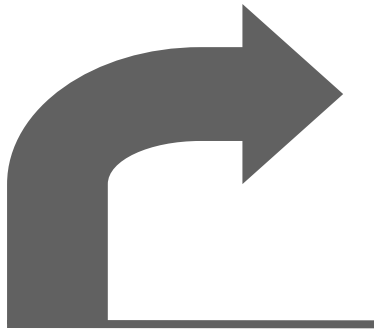


# goal: the regulated system

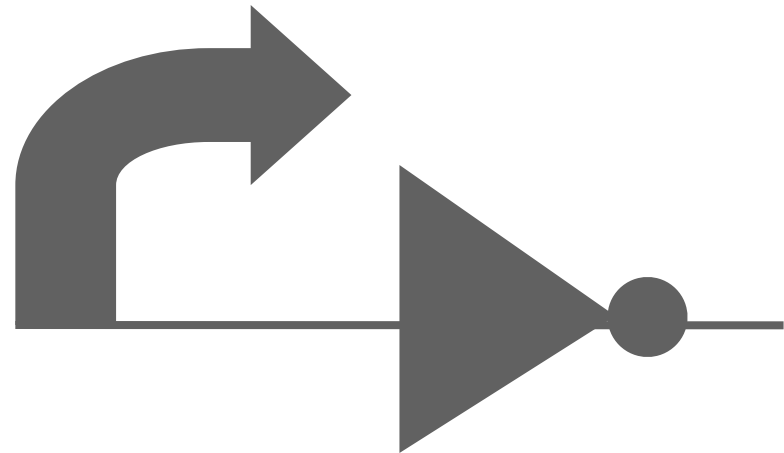


# growth dependent regulation

- ▶ *osmY*: active in stationary phase & under high osmotic pressure conditions

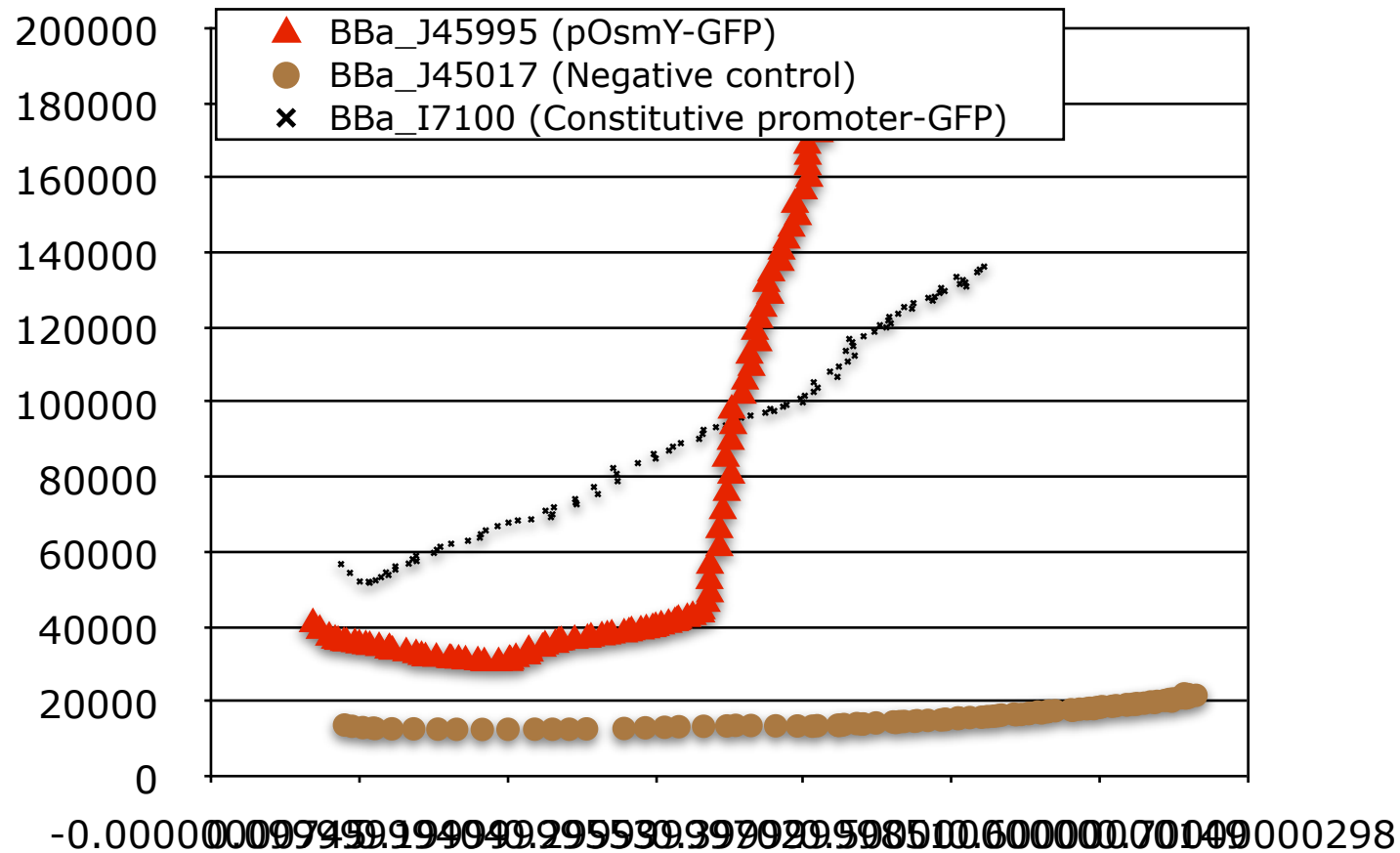


*osmY*



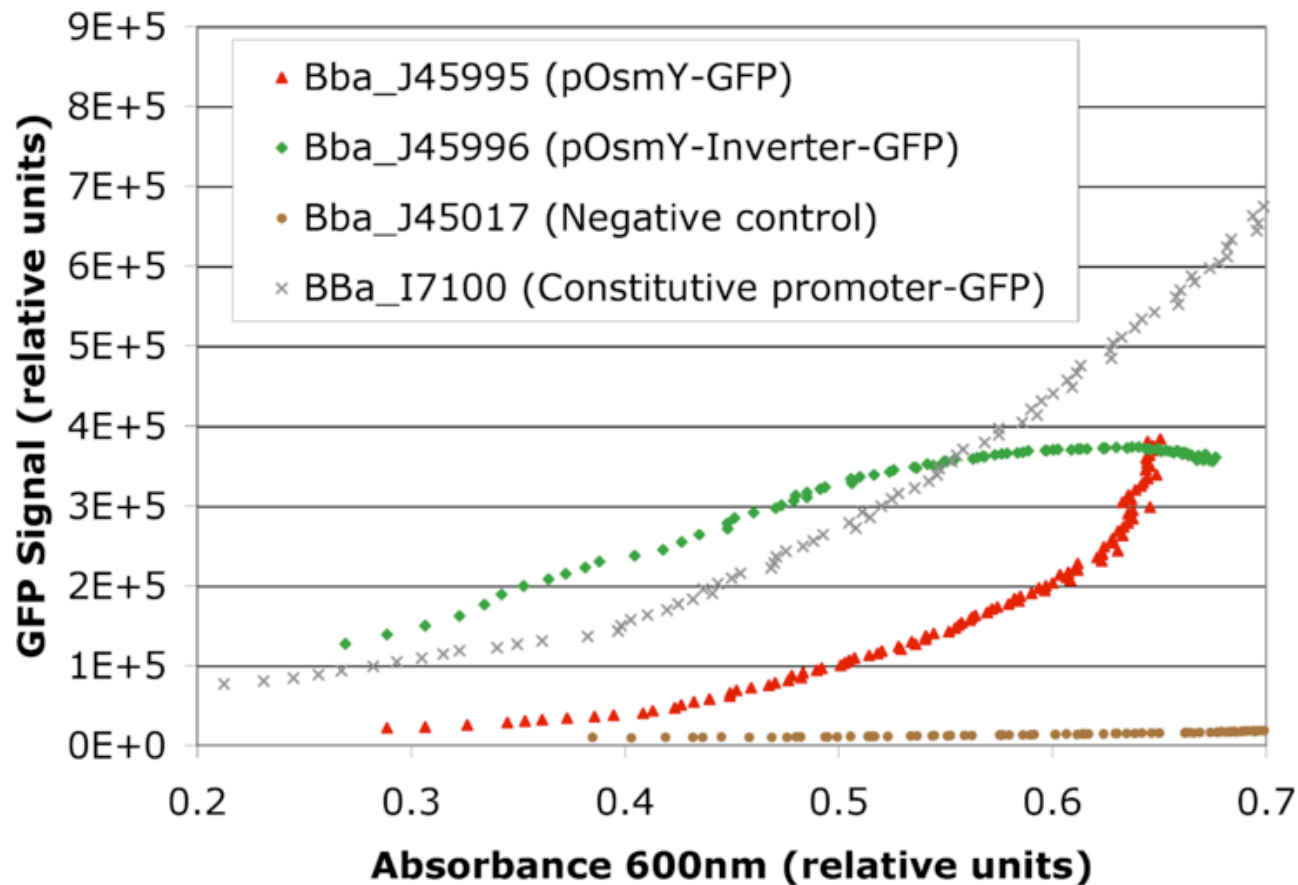
*osmY* + inverter

# regulation is growth dependent

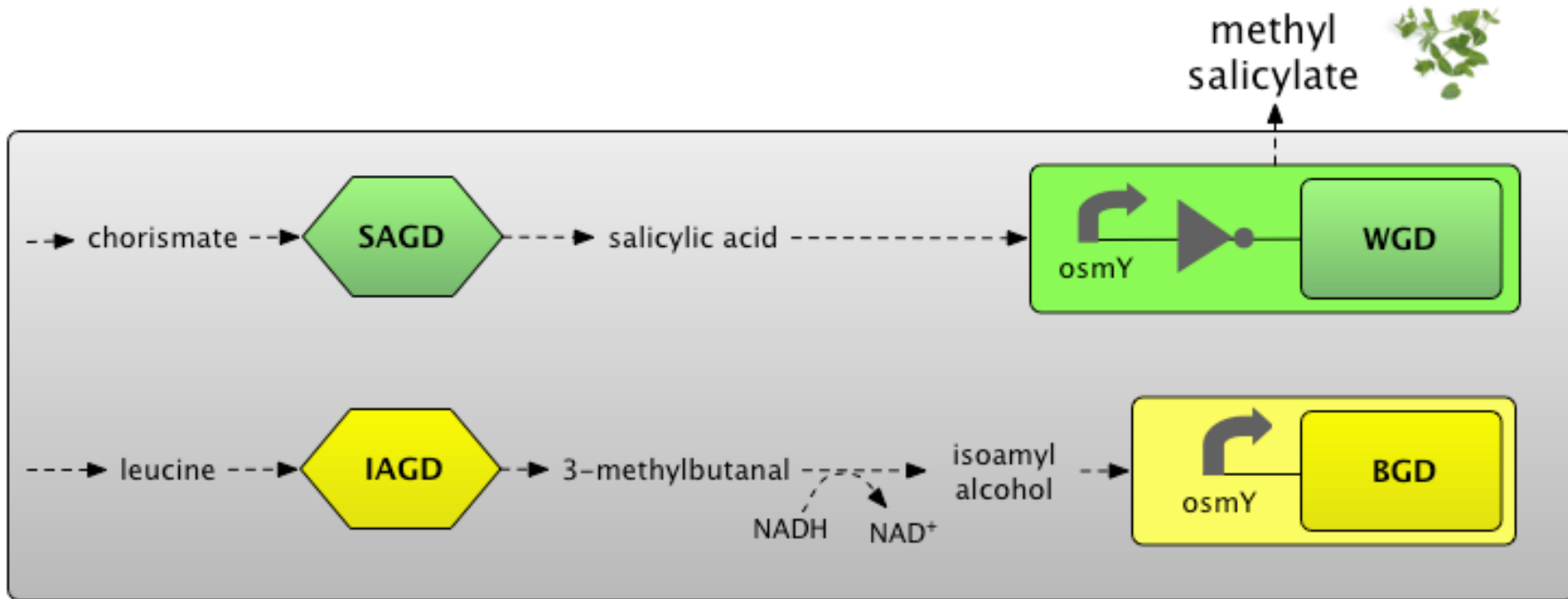




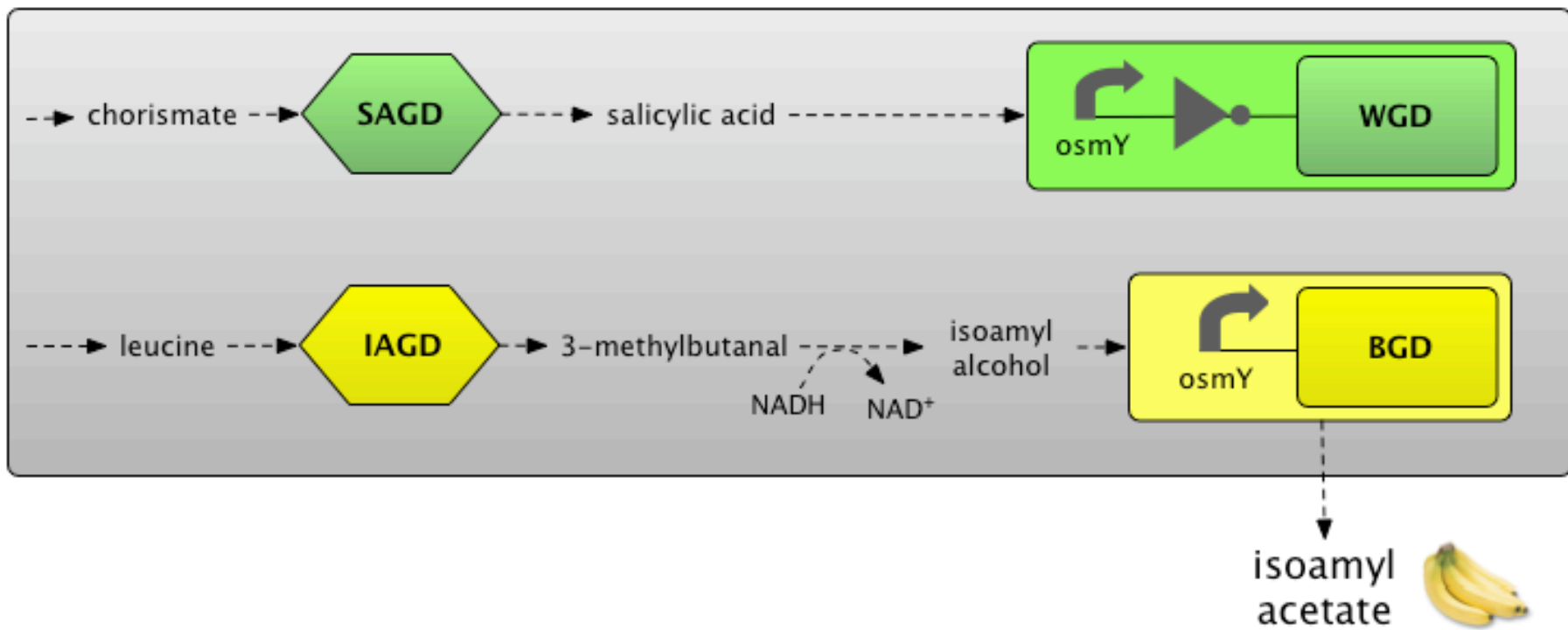
regulation is growth dependent



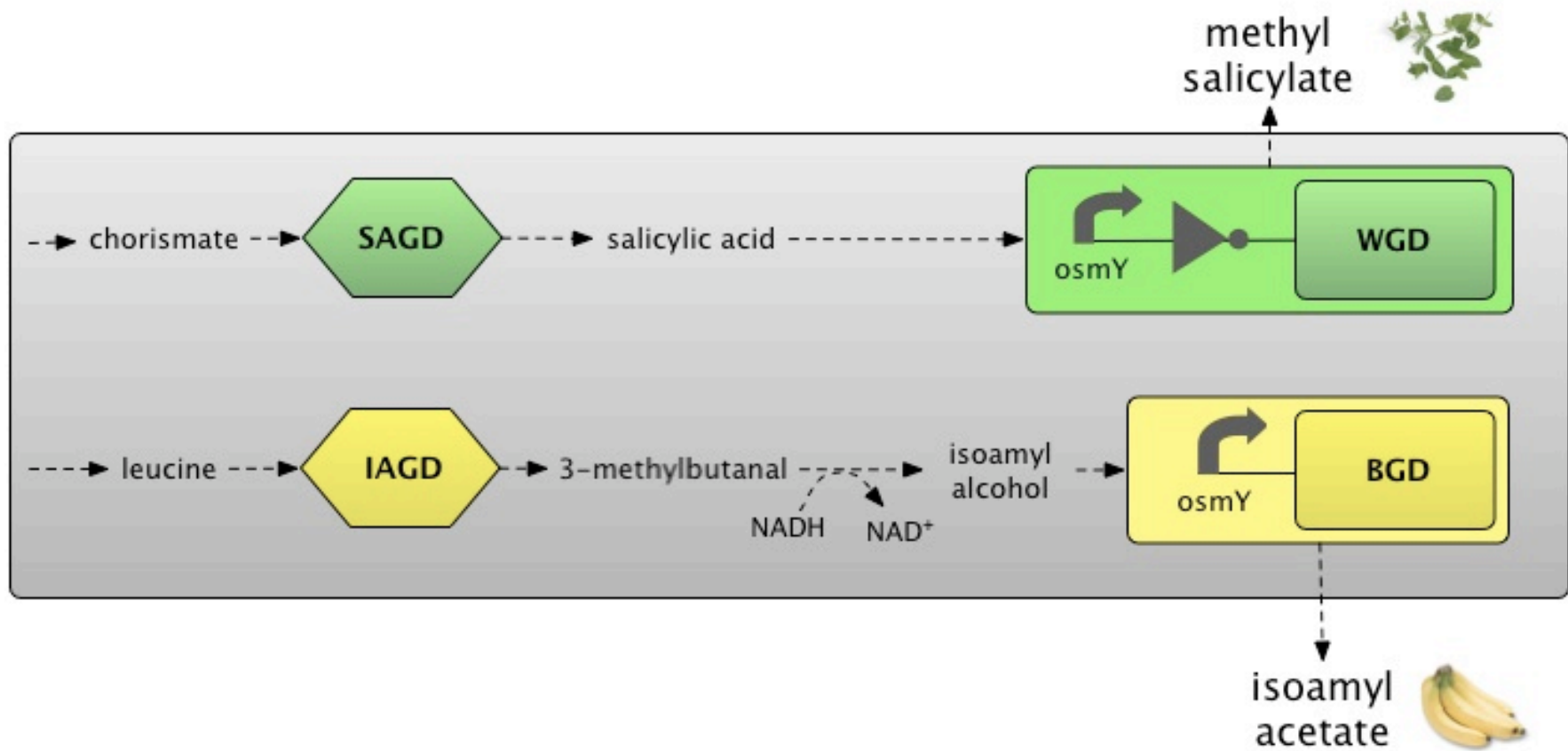
# regulated system: exponential



# regulated system: stationary

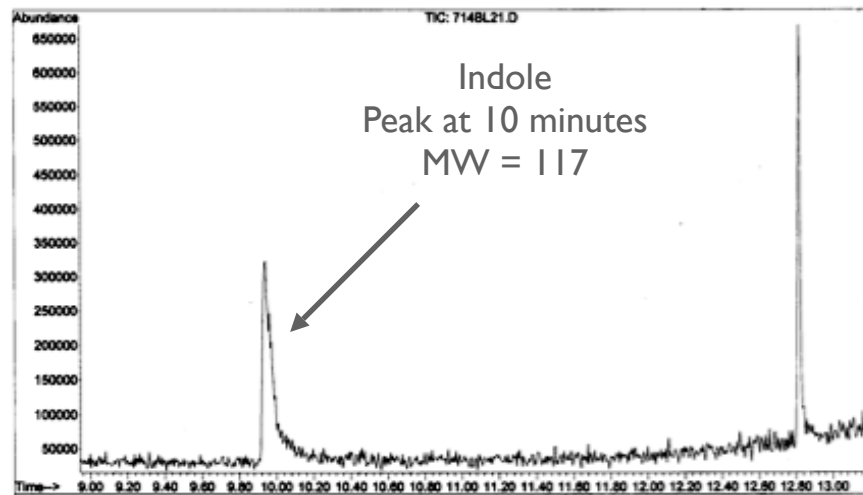


# regulated system

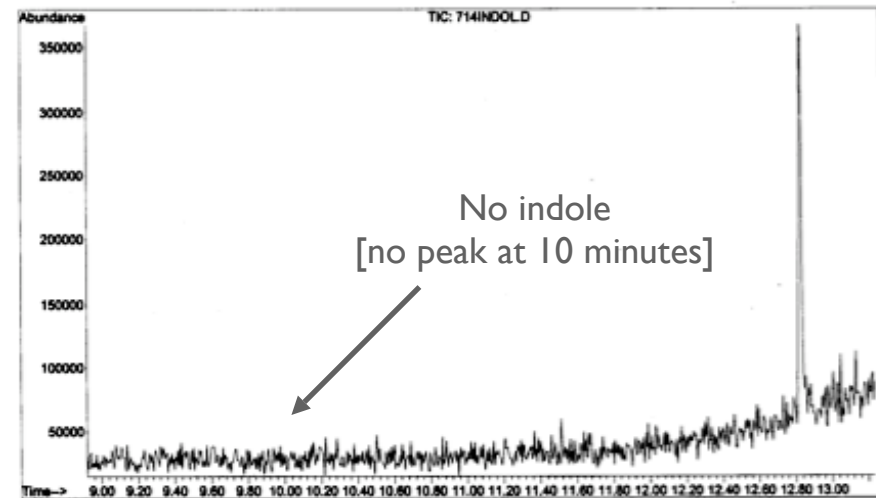


chassis (*tnaA5*<sup>-</sup>) does not produce indole

wild type *E. coli*

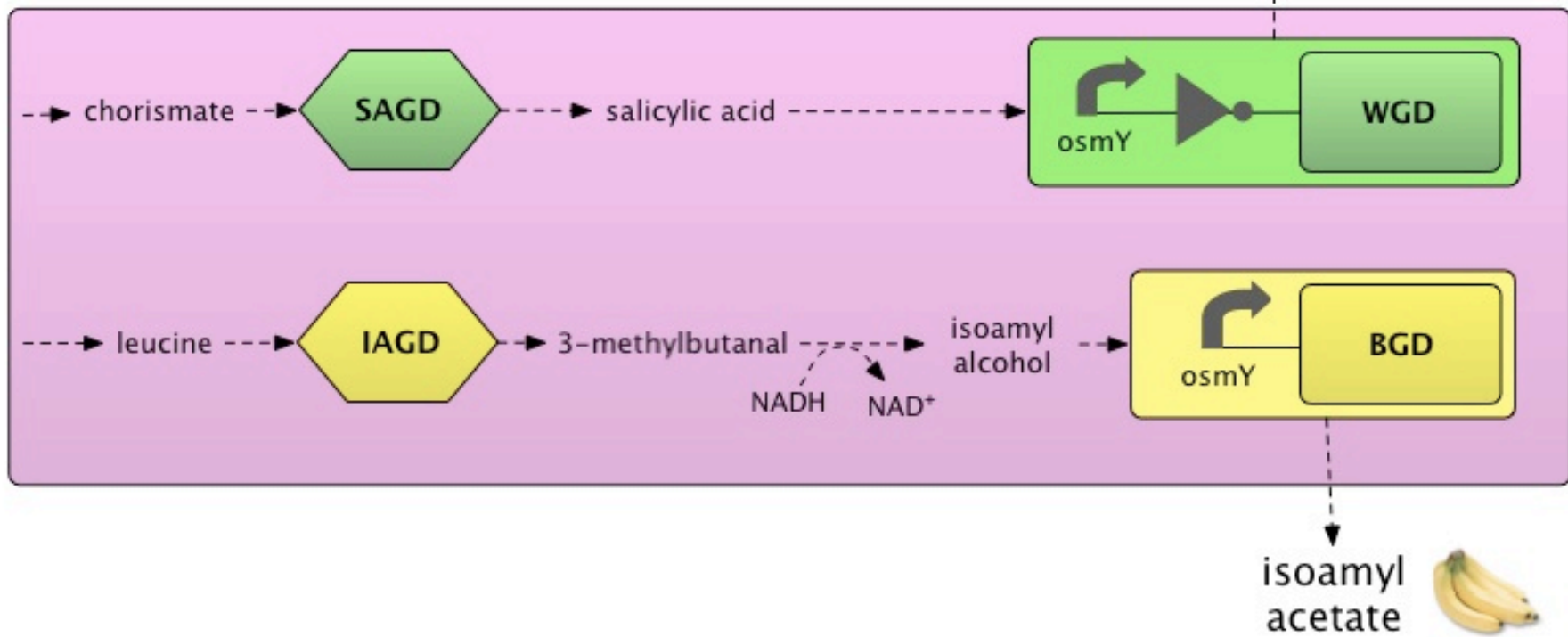


chassis: *tnaA5*<sup>-</sup> *E. coli*

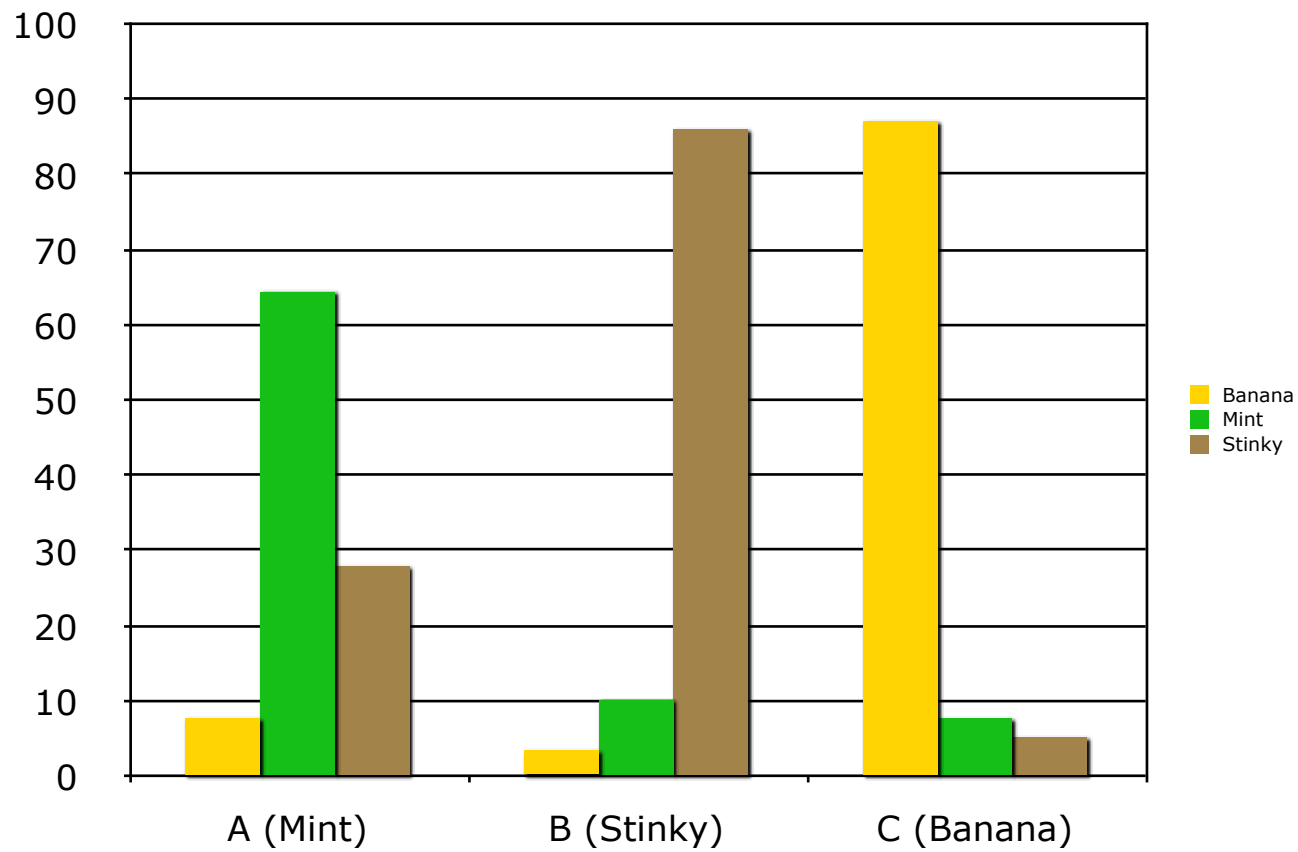


# final system

## indole deficient tnaA5<sup>-</sup> chassis



# smell test results



# future applications

- ▶ Improve the workplace environment for microbiologists working with *E. coli*
- ▶ Port our system to bacterial species involved in bioremediation.
- ▶ Implement our system in bacteria responsible for bad human odor in the mouth, armpits and feet.
- ▶ Implement our system in yeast used in bread and beer production



# conclusion

- ▶ Designed, built, and tested a synthetic biological system over the course of summer
- ▶ Engineered synthetic scent systems in *E. coli*
- ▶ Regulated via transcription control devices

# acknowledgments

- ▶ **Natalia Dudareva**, Purdue University: gifts of expression vectors encoding BAMT, SAMT and BSMT enzymes.
- ▶ **Eran Pichersky**, University of Michigan: suggestion of eliminating indole from *Escherichia coli* to mitigate the natural "bad" smell.
- ▶ **Mary Berlyn**, CGSC, The Coli Genetic Stock Center: *Escherichia coli* strain YYC912.
- ▶ **Brian Cook**, MIT: valuable discussions.
- ▶ **Cornelia Reimmann**, University of Lausanne: gift of an expression vector carrying the pchBA coding region.
- ▶ **Dieter Haas**, University of Lausanne: gift of the *Pseudomonas fluorescens* strain CHA0.
- ▶ **Peter Bakker**, Utrecht University, The Netherlands: gift of an expression vector carrying the pmsCEAB coding region, as well as, gift of the *Pseudomonas fluorescens* strain WCS374.
- ▶ **Herbert P. Schweizer**, Colorado State University: gift of the pUCP22 *Escherichia coli* to *Pseudomonas* shuttle vector.
- ▶ **Pamela Silver**, Harvard Medical School: gift of yeast BioBricks vector.

questions and answers

1-

2-

3-

4-

5-

6-

7-

# growth curves

