

# *To Swim or Not to Swim?*

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# Problem: To Swim or Not?

Recreational swimming in bodies of water or near areas of human habitation or public pools comes with risks of infection by waterborne human pathogenic bacteria

Some major pathogens found in water:

*Escherichia* *Pseudomonas*

*Shigella*

*Salmonella*

*Vibrio*

*Leptospira*



# Assessing Water Quality

Conventional method:

Culture on selective media:

However

- 1 to 2 day for results
- Special facilities required  
(incubator, media prep, etc.)



*Additional problem: Waterborne bacteria counts can change hourly...*

# Solution: A Portable Water Quality Testing Instrument

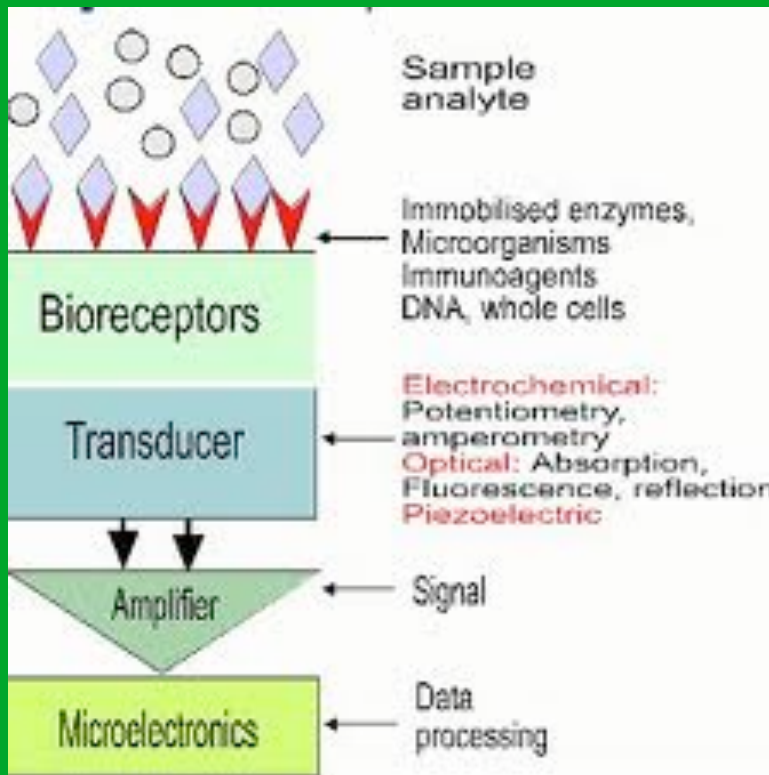
## Desired attributes

- Portable - no lab needed
- Rapid detection speed
- Selectivity for human pathogens
- Designed for an average person to use



*(like this glucometer)*

# One Solution: A Biosensor



## Advantages:

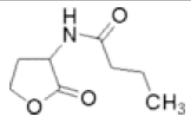
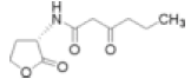
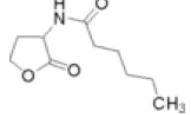
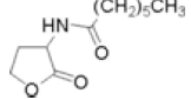
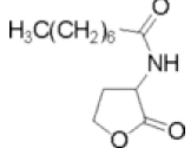
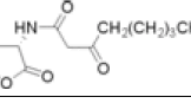
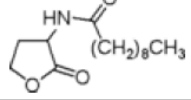
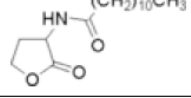
- High selectivity based on species-specific recognition
- Speed of response
- Signal amplification
- Performance – works at ambient temperature

# Bacteria can be detected through Quorum-Sensing Factors

Homoserine lactones - bacteria use to assess their numbers in the environment

- Stable chemical signatures
- Species Specific
- Concentration directly correlated with number of cells.

*What more could one ask for?*

Full Name	Molecule abbreviation	Species	Images (from Sigma Aldrich)
Butanoyl-homoserine lactone	C <sub>4</sub> HSL	<i>P. aeruginosa</i>	
<a href="#">3-oxohexanoyl-homoserine lactone</a>	<a href="#">3OC<sub>6</sub>HSL</a>	<i>V. fischeri</i>	
Hexanoyl-homoserine lactone	C <sub>6</sub> HSL	<i>C. violaceum</i>	
Heptanoyl-homoserine lactone	C <sub>7</sub> HSL	<i>E. psidii</i> R. IBSBF 435T	
Octanoyl-homoserine lactone	C <sub>8</sub> HSL	<i>B. cepacia</i> , <i>V. fischeri</i>	
3-oxooctanoyl-homoserine lactone	3OC <sub>8</sub> HSL	<i>A. tumefaciens</i>	
Decanoyl-homoserine lactone	C <sub>10</sub> HSL	<i>B. pseudomallei</i>	
Dodecanoyl-homoserine lactone	C <sub>12</sub> HSL	Synthetic	

# Biosensors? The iGEM Advantage!

## Registry of Standard Biological Parts

  
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### Part:BBa\_T9002



DNA Available  
★ 1 Registry Star

Designed by Anna Labno Group: Guests, Haseloff Lab (2005-06-21)

[Get This Part](#)

### GFP Producer Controlled by 3OC6HSL Receiver Device

The luxR based receiver, F2620 (formerly I13270), controls the production of GFP. The GFP protein generator is the same as that found in I7101.

Sequence and Features

Format: [Subparts](#) | [Ruler](#) | [SS](#) | [DS](#)    Search:    Length: 1945 bp    Context: Part only    [Get selected sequence](#)

The diagram shows a linear sequence of genetic elements. From left to right: a green arrow labeled p(tetR) R0040, a green oval labeled B0034, a purple arrow labeled luxr C0062, a red circle labeled B0010, a red circle labeled B0012, a green arrow labeled lux pR R0062, a green oval labeled B0032, a purple arrow labeled GFP E0040, a red circle labeled B0010, and a red circle labeled B0012.

Assembly Compatibility: [10](#) [12](#) [21](#) [23](#) [25](#)

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Parameters

None

Twins

[BBa\\_K204034 Planning](#)  
[BBa\\_K204700 Sent](#)

Reviews

★ 1 Registry Star  
Experience: Works

5	(77)
4	(21)
3	(44)
2	(13)
1	(5)

sample

Categories

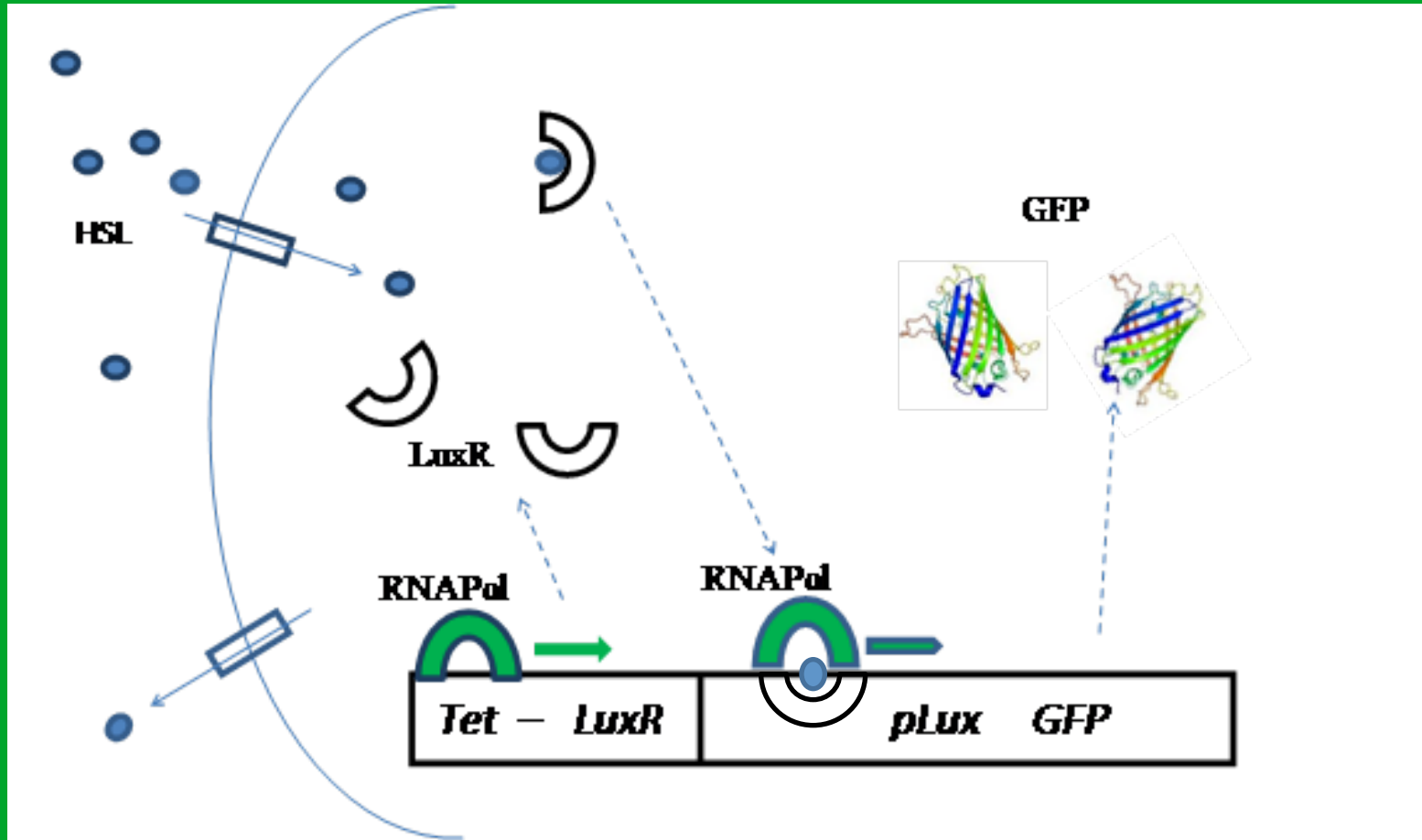
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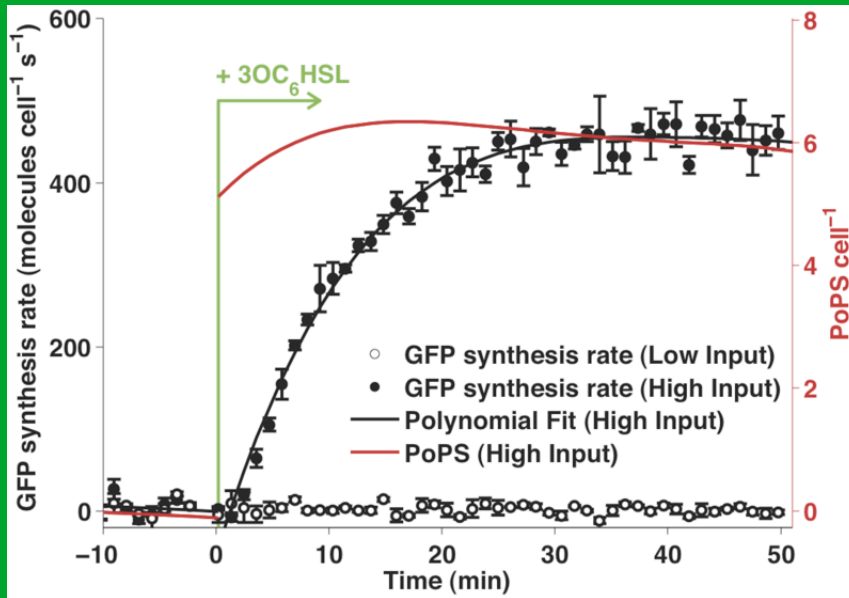
# Lux System for HSL Detection





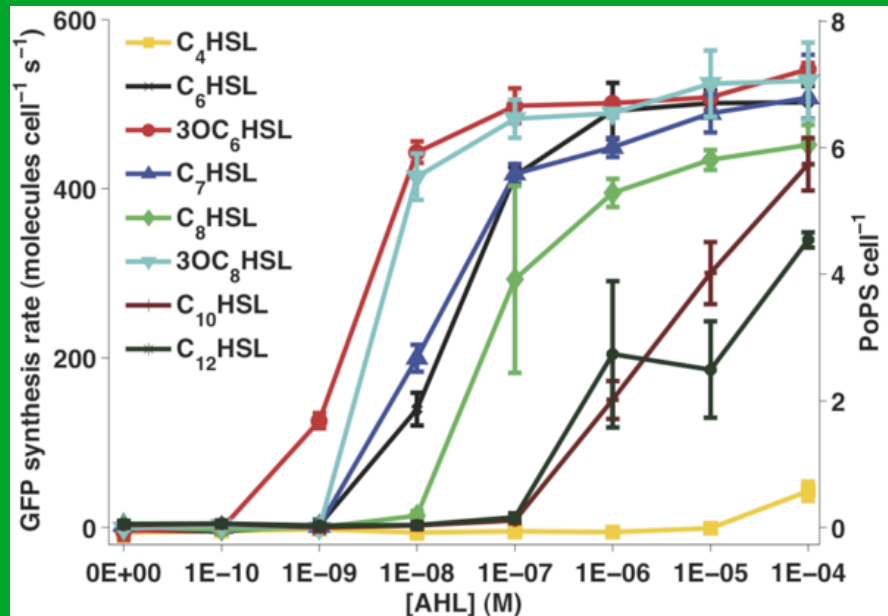
# T9002 Satisfies Detector Requirements

iGEM part designed and characterized by Ana Labno, MIT



Part responds in minutes...  
because you want to swim!

Part is highly sensitive and selective to a variety of HSL's produced by a number of potential waterborne bacteria



# Improvements on T9002-based HSL-Detection System

## Alternate chassis

- 37C growth optimum for E.coli: need to operate best at ambient temperature
- E. coli possesses the *Lac Z* gene for  $\beta$ -galactosidase

## Reporter

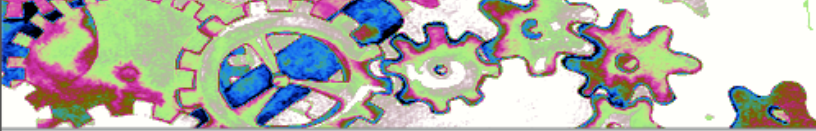
- Cost to detect absorbance as opposed to fluorescence in a handheld electronic/iGEM hybrid device

# New Chassis: Agrobacteria

## Advantages of *Agrobacterium tumefaciens*

- Lower growth optimum than *E. coli*
  - HSL synthase negative
  - HSL synthase is on the T1 plasmid
- Our host cells are T1 plasmid negative
- Not a human pathogen

# Toward a Handheld HSL detector: Addition of a New Reporter




## Registry of Standard Biological Parts

  
   
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### Part:BBa\_K498000

Designed by IvyTech\_South-Bend Team Group: iGEM10\_IvyTech-South\_Bend (2010-10-26)

Composite  
"The Blue"

DNA Planning
Experience: None

[Get This Part](#)

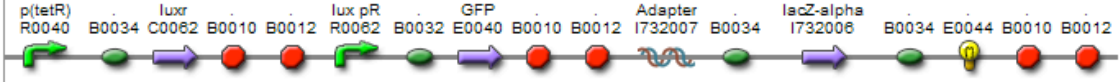
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### T9002 - LacZ

The T9002 AHL detector was ligated to a LacZ reporter gene by the conventional biobrick assembly #10.

Sequence and Features

Format: [Subparts](#) | [Ruler](#) | [SS](#) | [DS](#)    Search:    Length: 3154 bp    Context: Part only    [Get selected sequence](#)



Assembly Compatibility: 10 12 21 23 25

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[\[edit\]](#)

Parameters


Reviews

Unrated Part	
Group Favorite	
Experience: None	
5	(77)
4	(21)
3	(44)
2	(13)
1	(5)


*sample*

Categories

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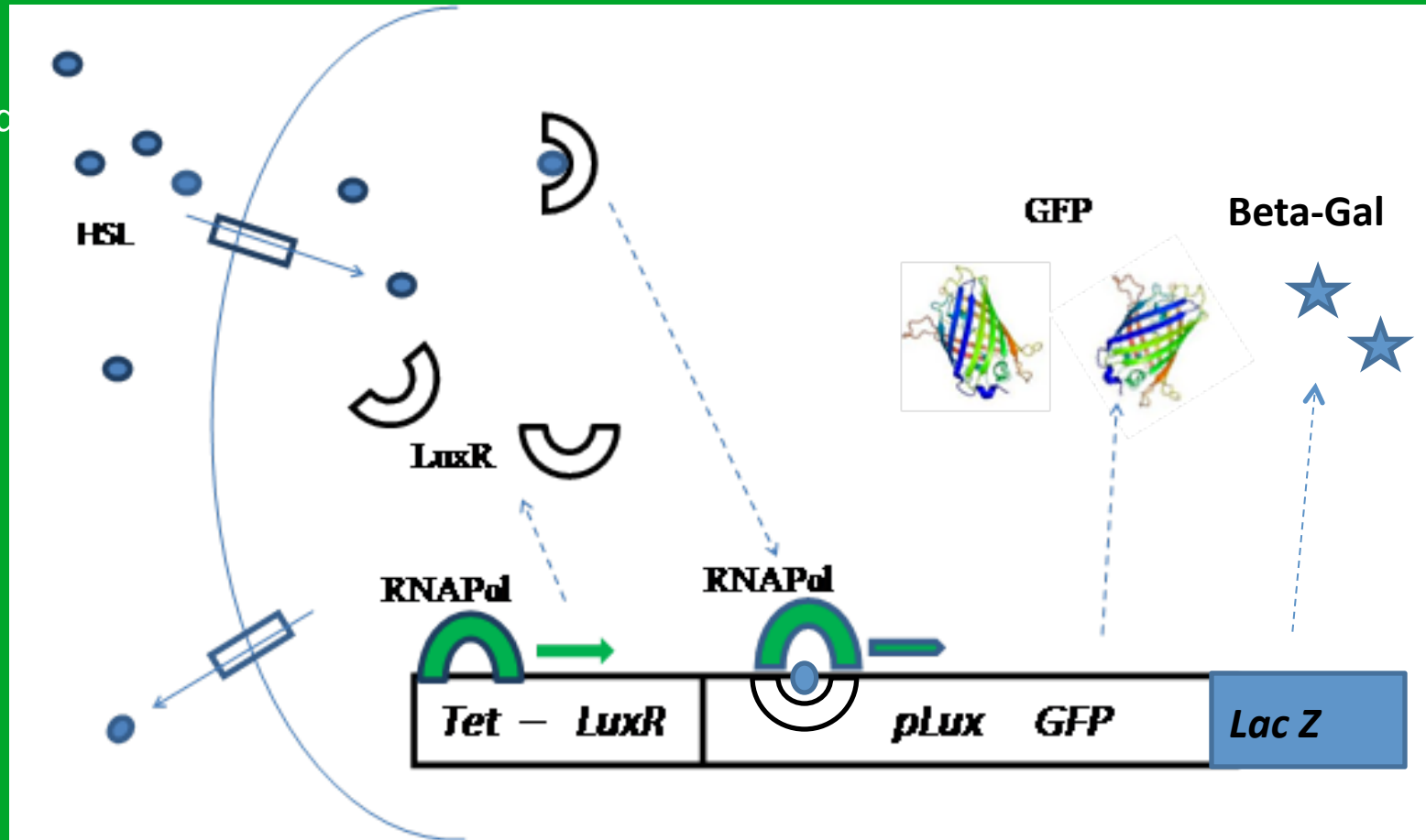


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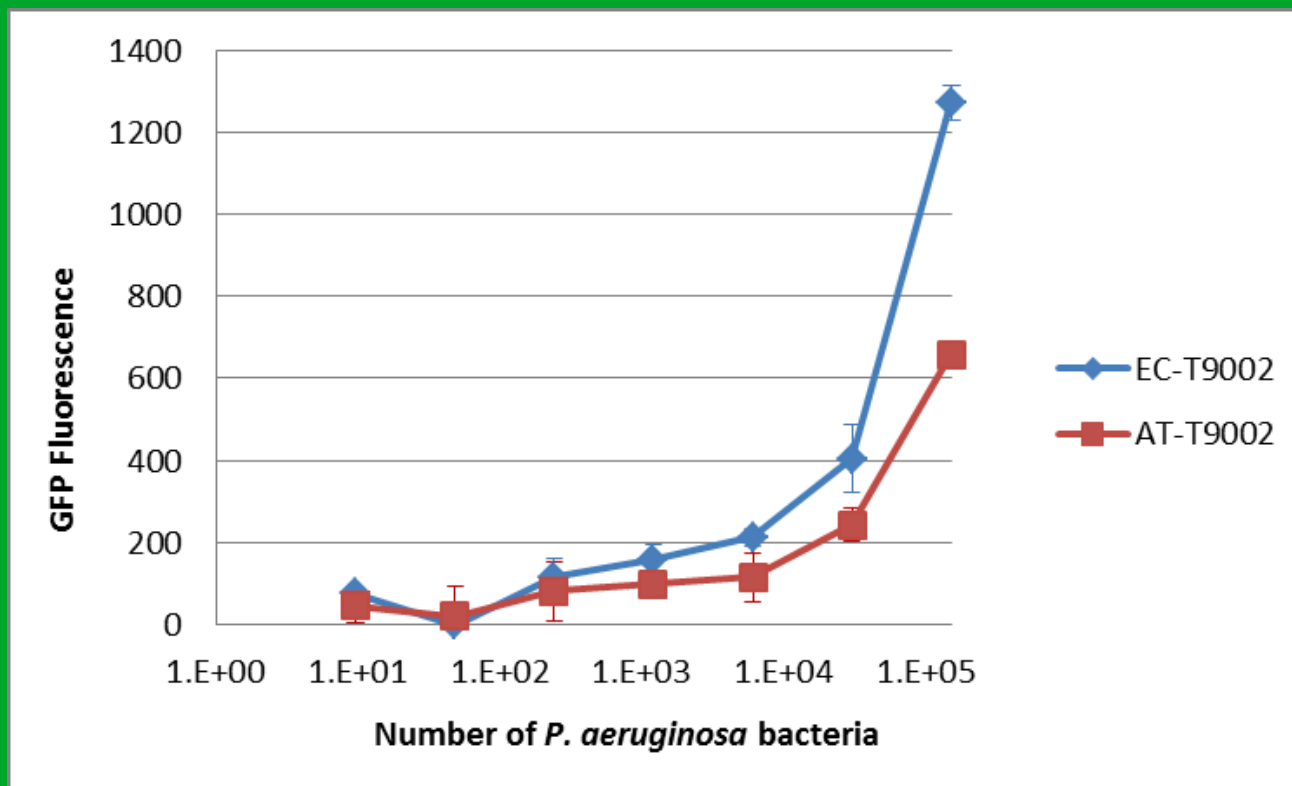


# New Reporter Addition

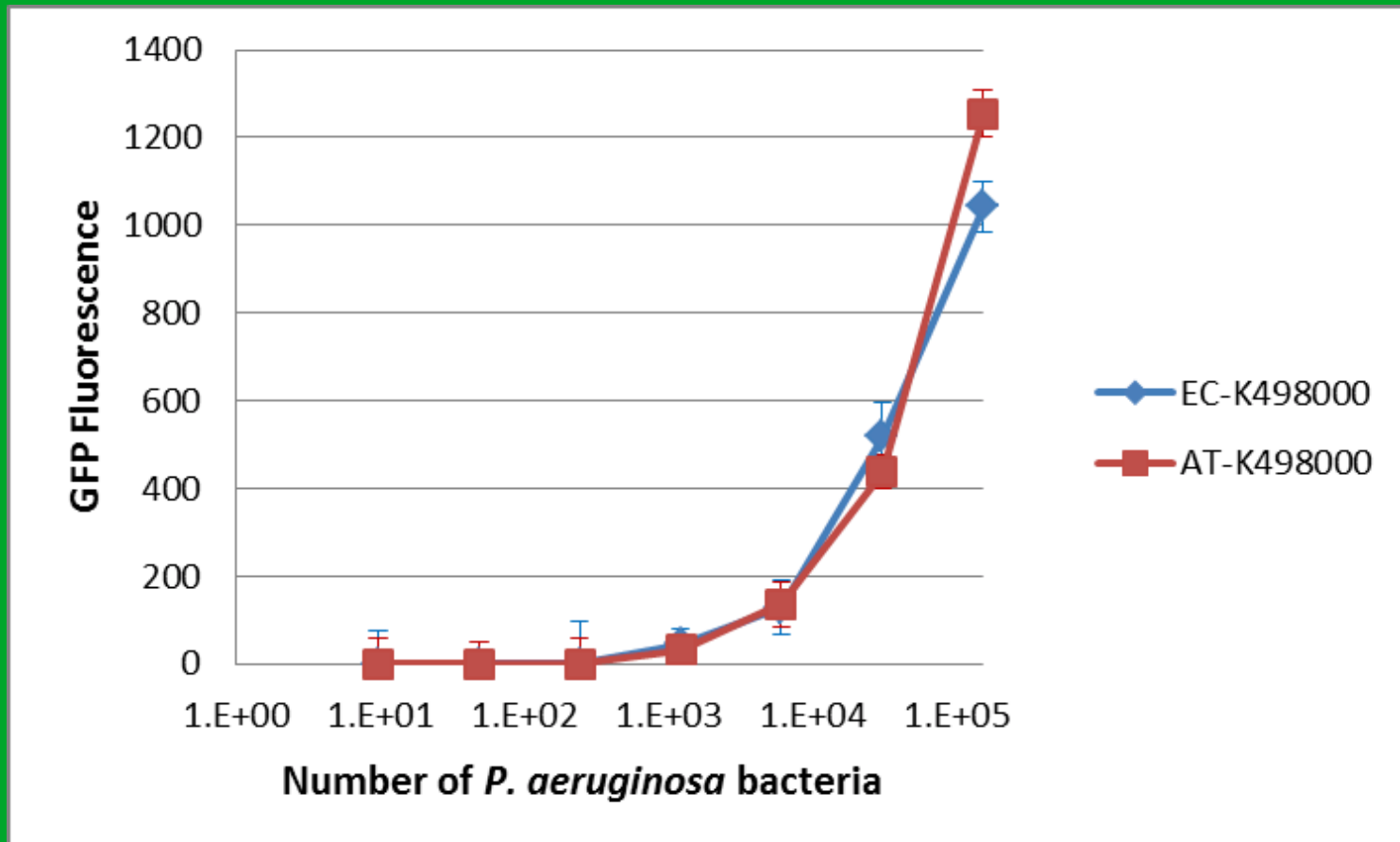
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# Comparison of the Number of Bacteria Detected by T9002 in *E. coli* and *Agrobacteria*



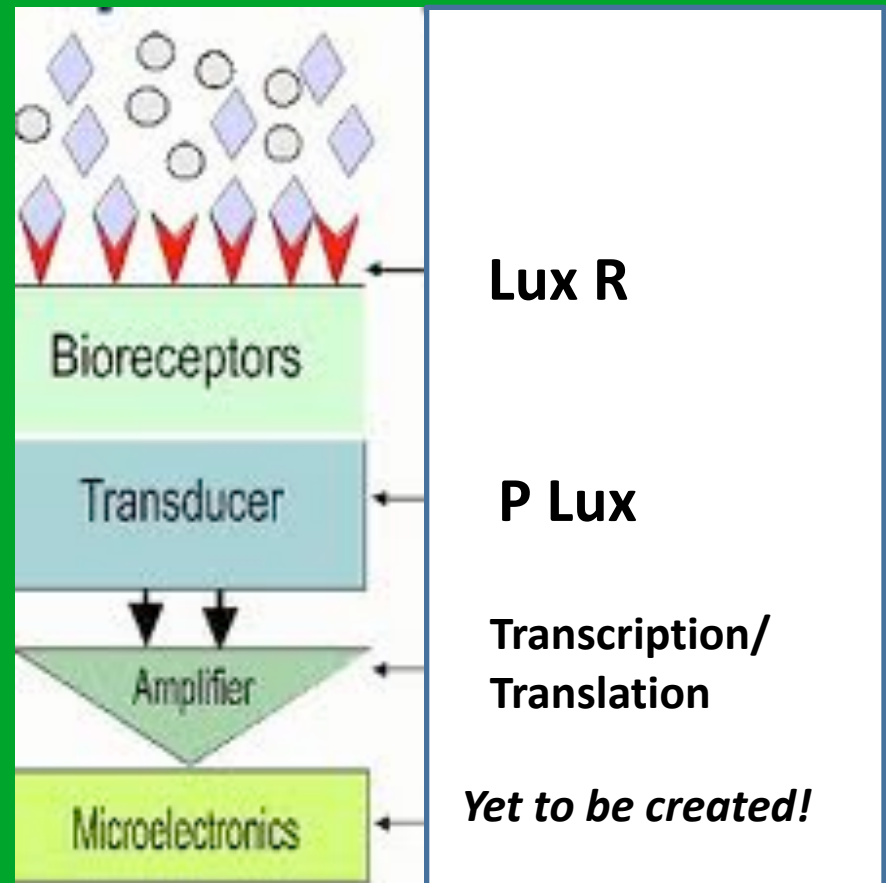
# Comparison of the Number of Bacteria Detected by T9002 in K498000 in *E. coli* and *Agrobacteria*



# Conclusions

The iGEM HSL detector system T9002 works as well in *A. tumefaciens* as in *E. coli* and thus could serve as the basis of our biosensor.

*Lac Z* (B-galactosidase ) activity in K498000 has yet to be evaluated...





# Credits: Thank You!

iGEM Community  
(Randy, Meagan, Tom, et al.)  
MIT  
Anna Labno for T9002  
Zhan Jian for LacZ

Ivy Tech Community College  
Perkins Foundation  
Lilly Endowment

Amr Mentash  
George Twaddle

Picture credits

Bathers: HomeAway.com and Eurotimes.net; Beach: pcbdaily; bacteria: ifixh20.com; agar plate: stupy09/PicassaWeb;  
lab: netl.doe.gov.; glucometer: firefighter-emt.com

