

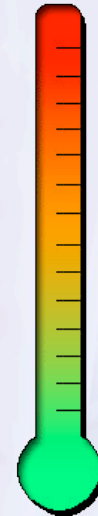
BLUE WATER



i G E M T e a m

University of Toronto & University of Waterloo

Cells-See-Us
Thermometer



Presentation Overview:

- Project Overview
- Project Design and Philosophy
- Reaction Mechanisms
- Construction
- Results

Project Overview

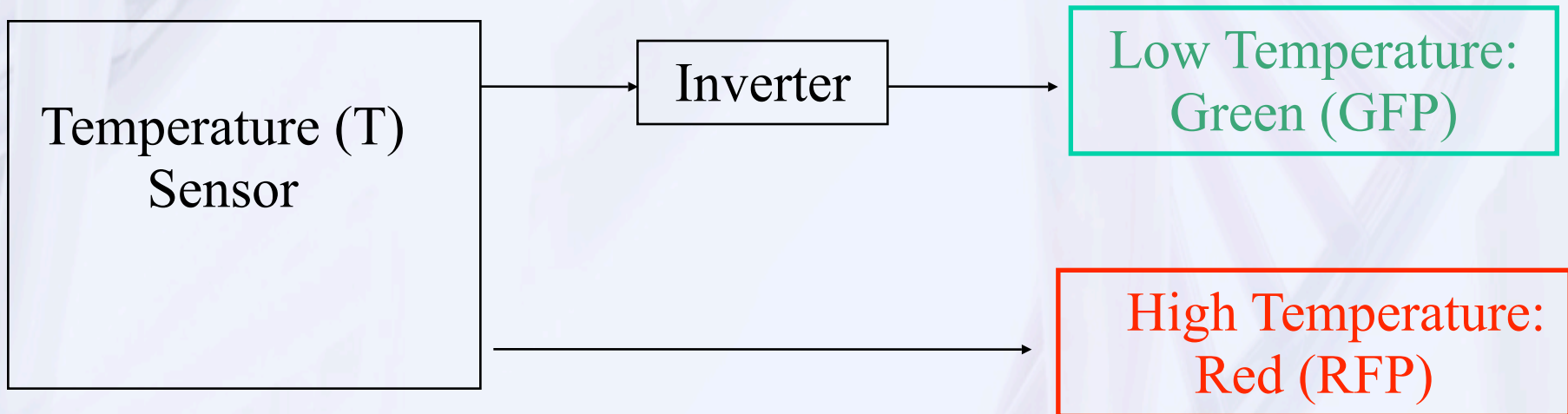
Cell-See-Us Thermometer:

- a bio-synthetic device that displays changes in temperature through colour
- **red** when **hot**, and **green** when **cold**

Applications of a bio-synthetic thermometer

- map temperature gradients with spatial resolution down to micrometers

Project Principle



GFP: Green Fluorescent Protein

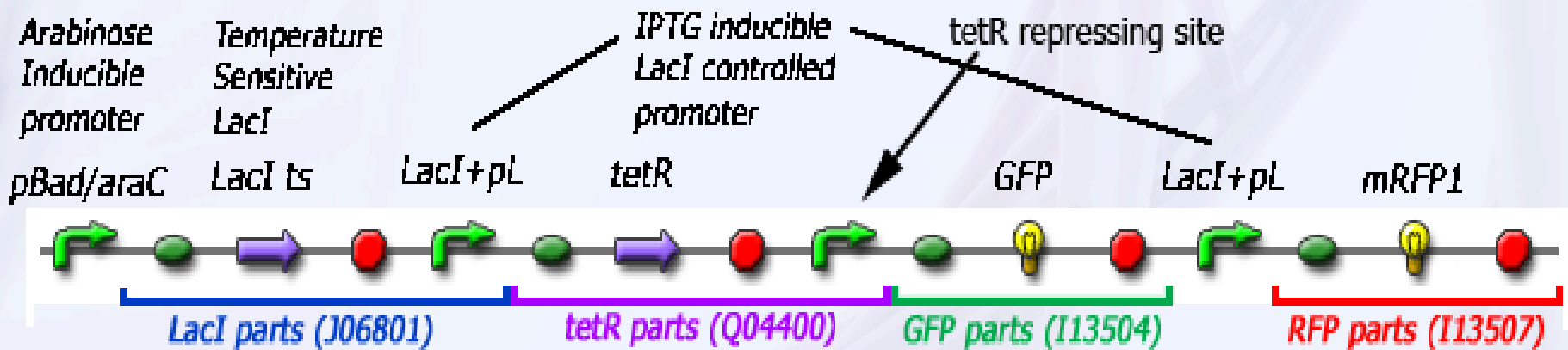
RFP: Red Fluorescent Protein

Design Philosophy

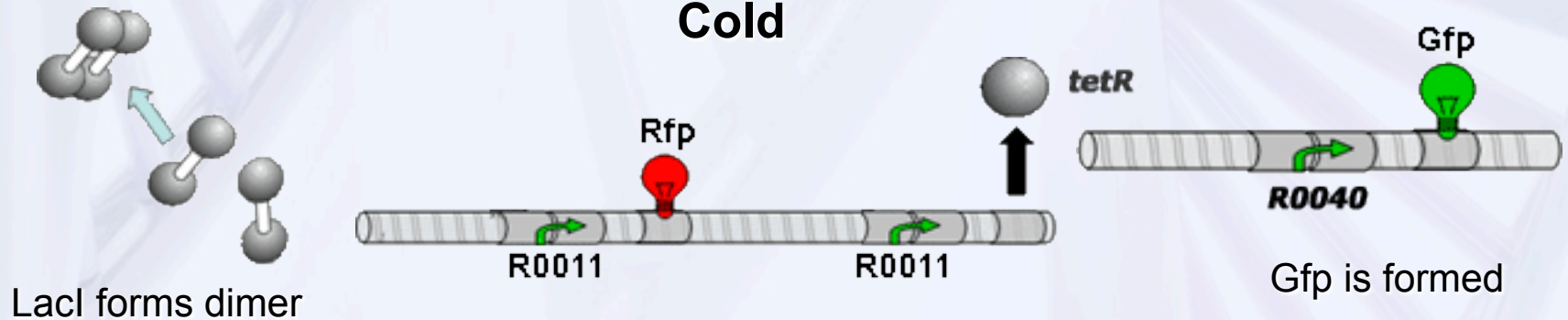
- Allow for modular testing of each vital component of the construct
- Many external control points for system modulation
- Construction flexibility

Design Philosophy

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- Many external control points for system modulation
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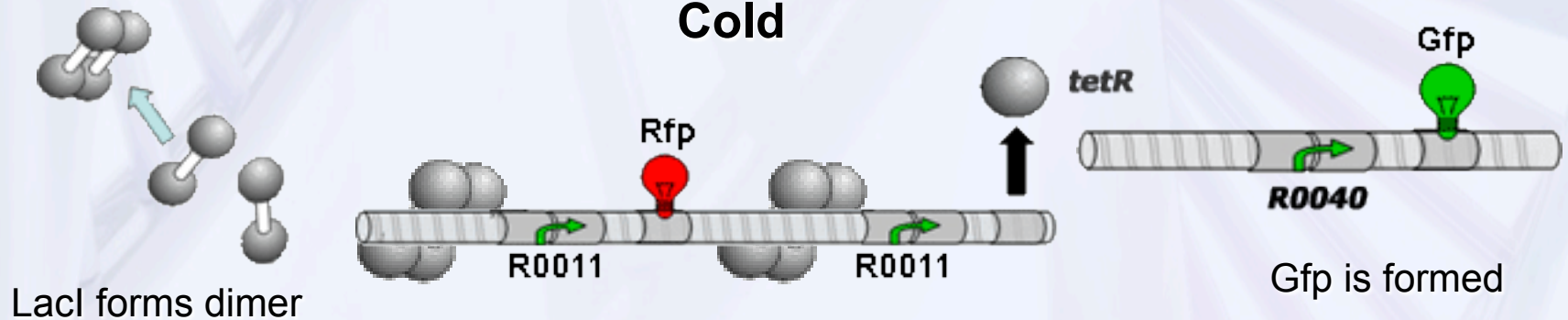


Reaction Mechanism



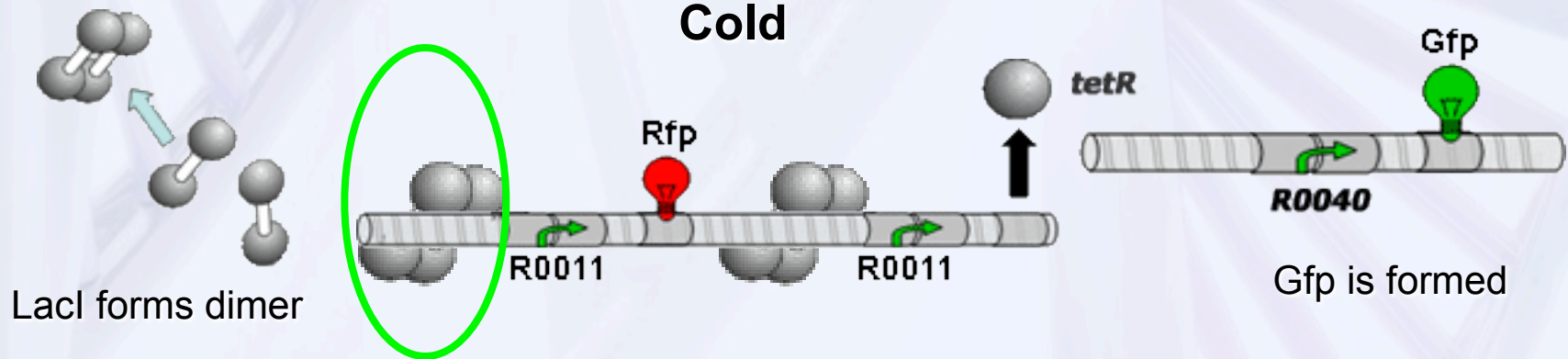
LacI binds to Rfp-repressing site (R0011)
and *tetR*-repressing site (R0011).
No Rfp or *tetR* is made

Reaction Mechanism



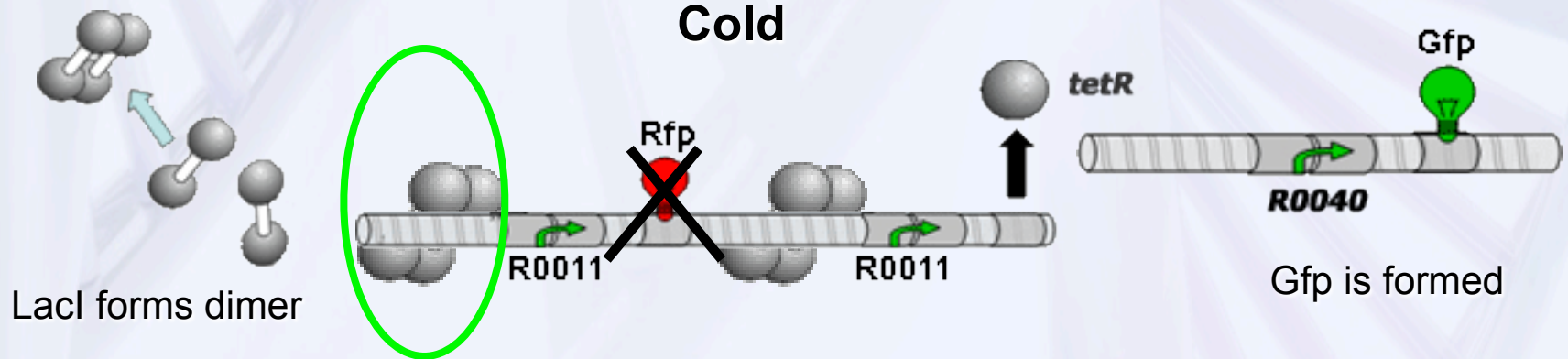
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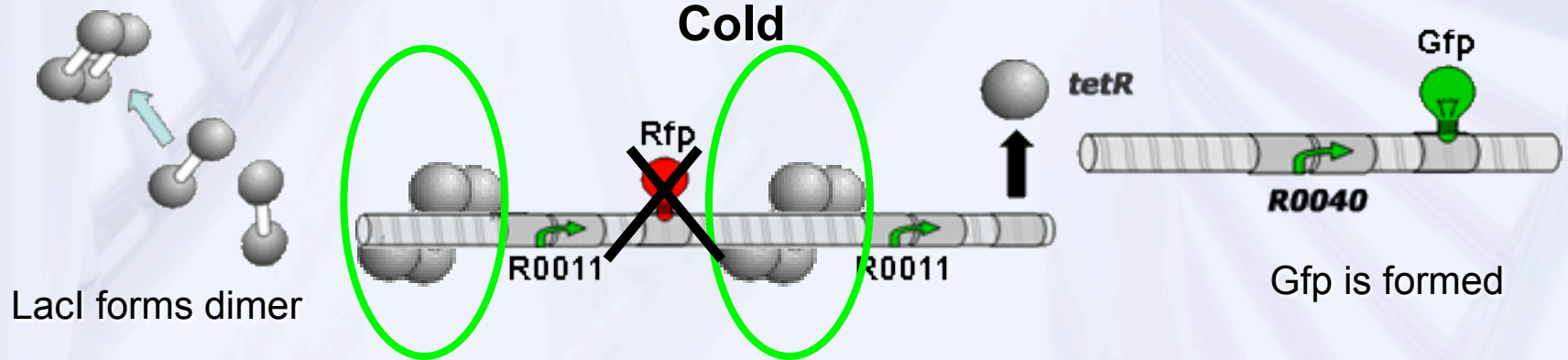
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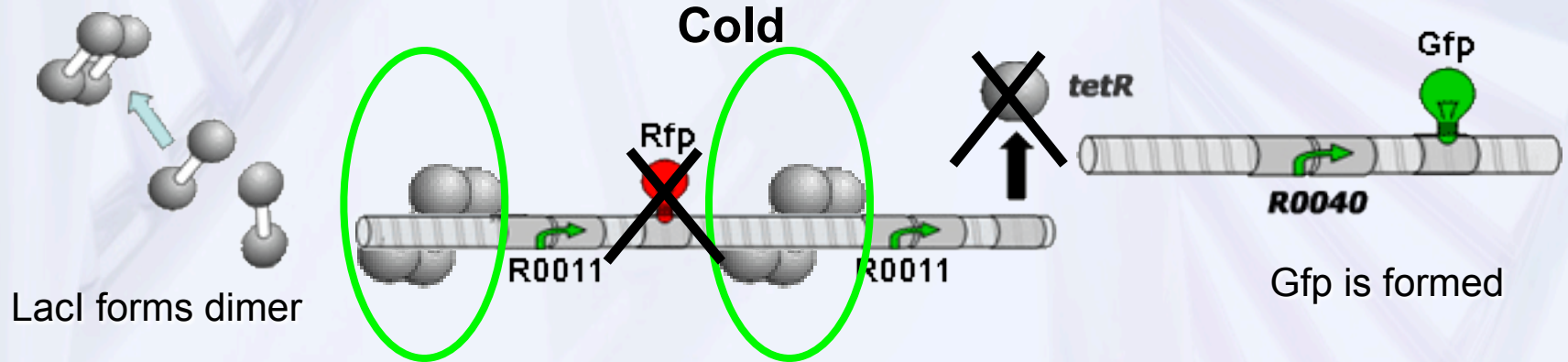
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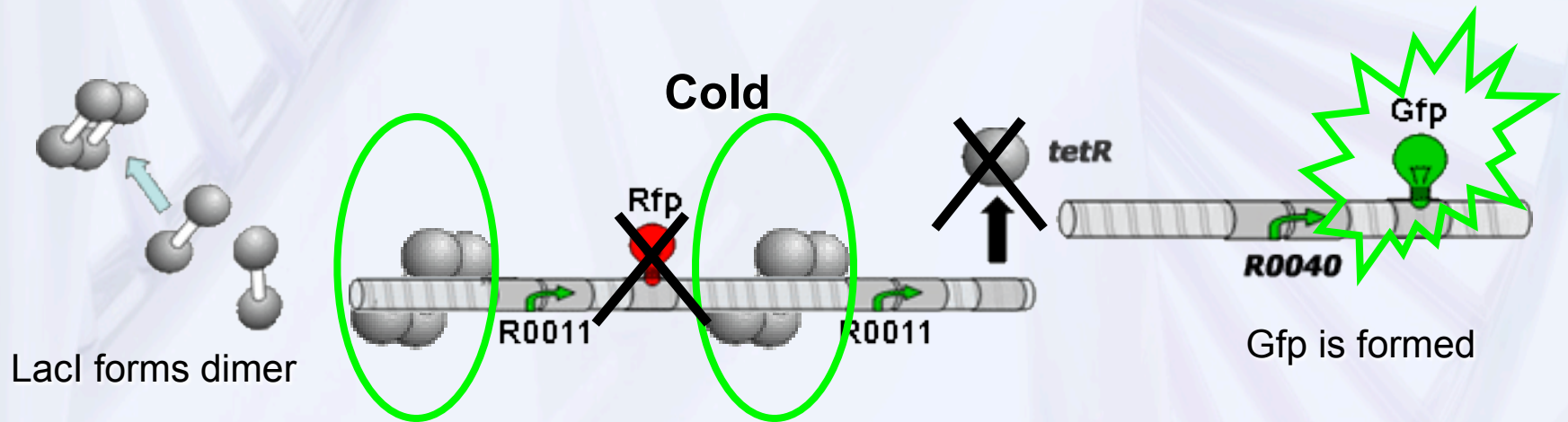
Reaction Mechanism



LacI forms dimer

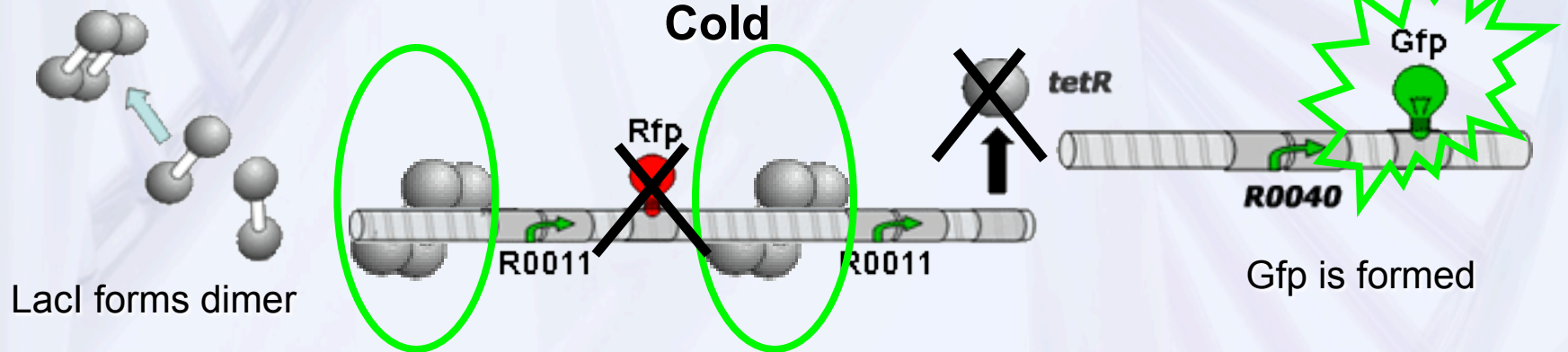
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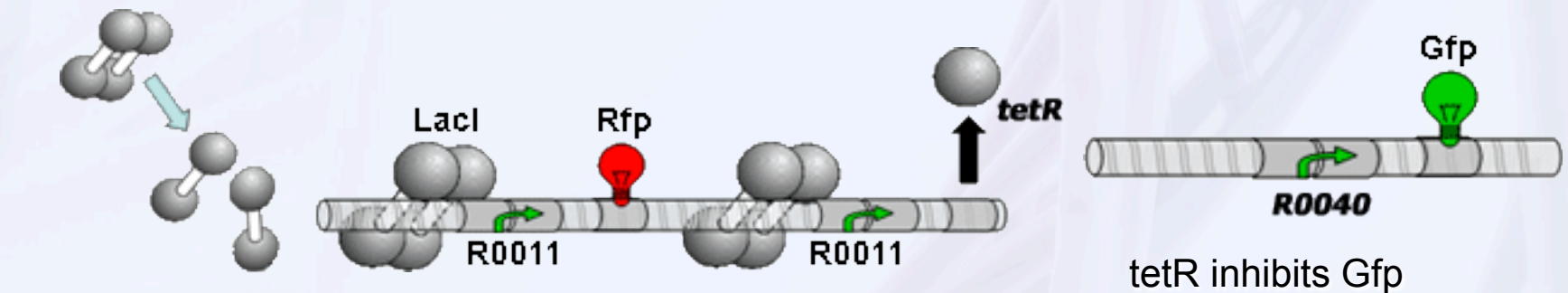
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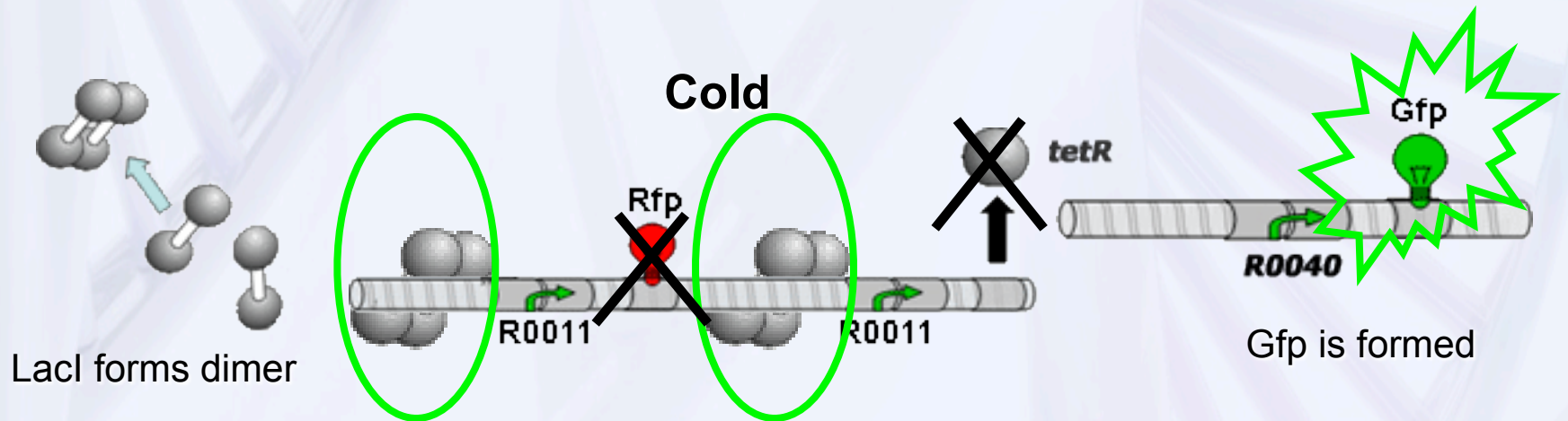
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Hot



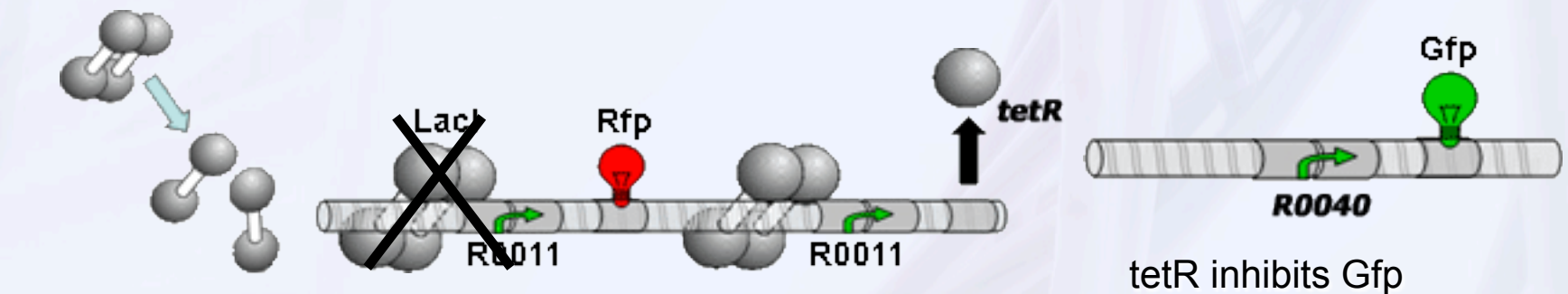
LacI monomers do not bind onto repressing sites – Rfp and tetR are formed.

Reaction Mechanism



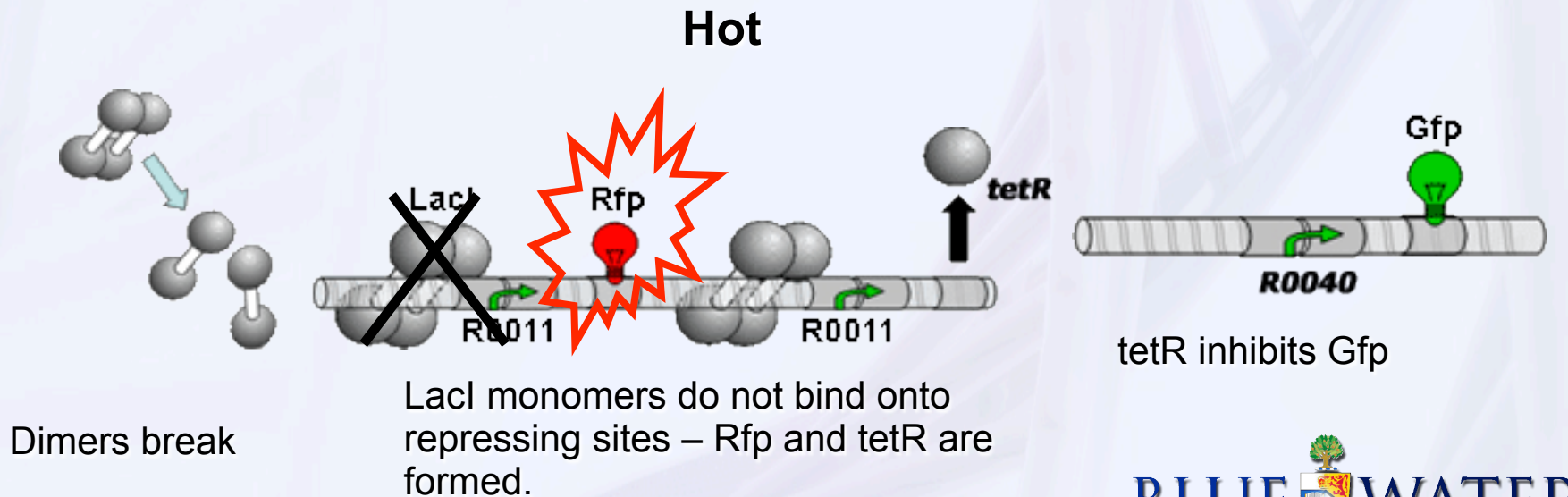
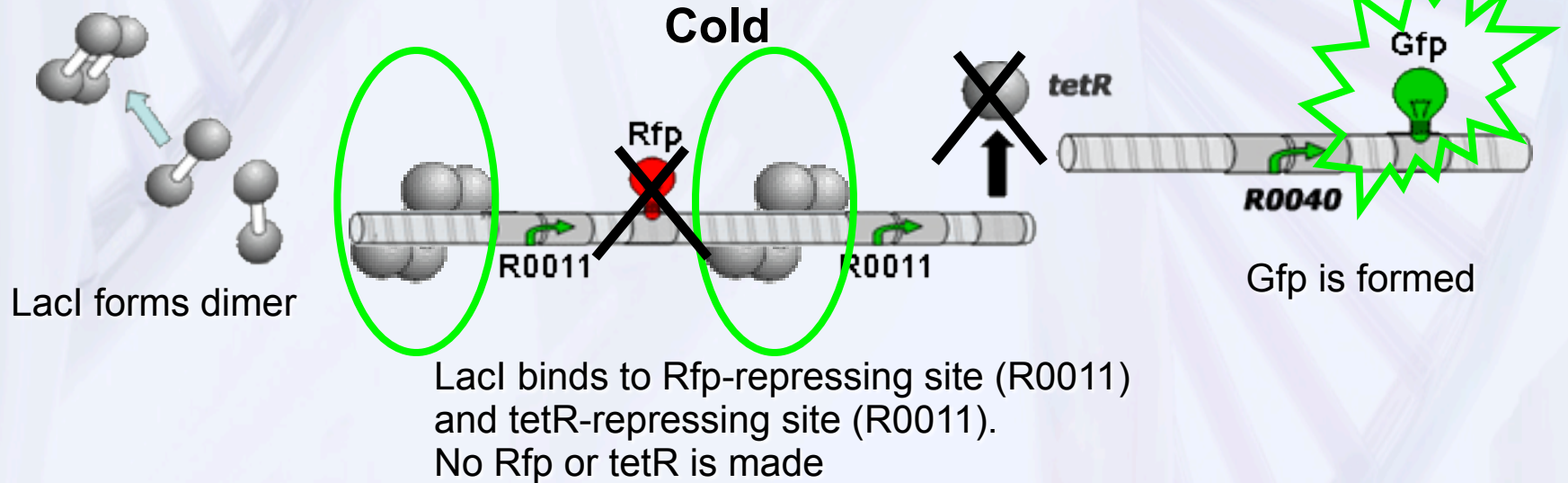
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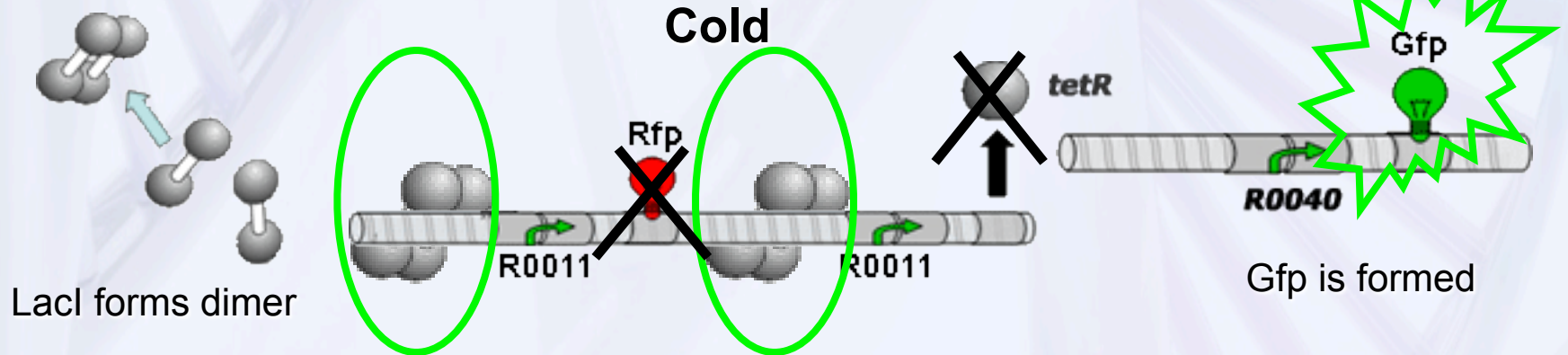


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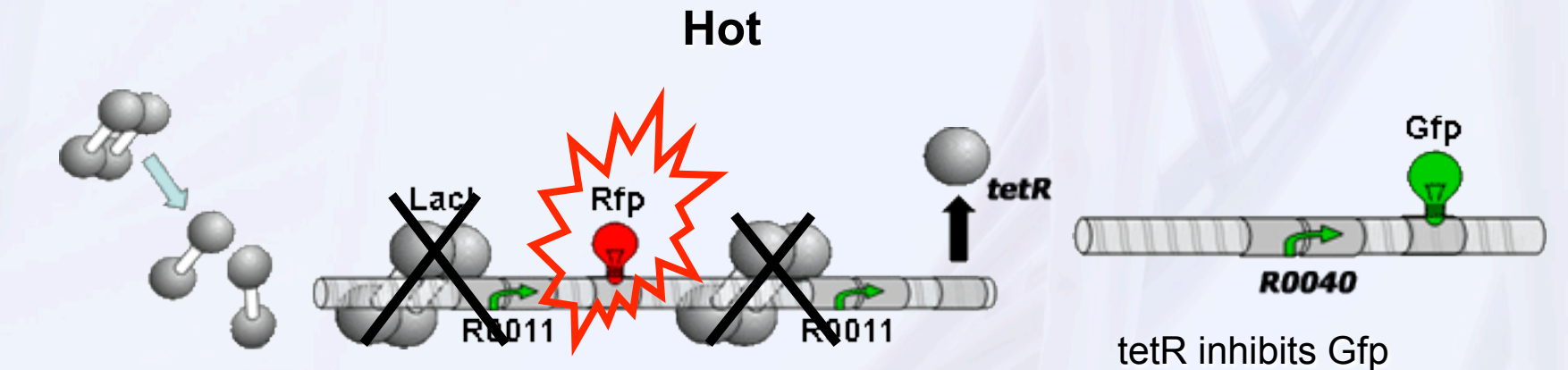
Reaction Mechanism



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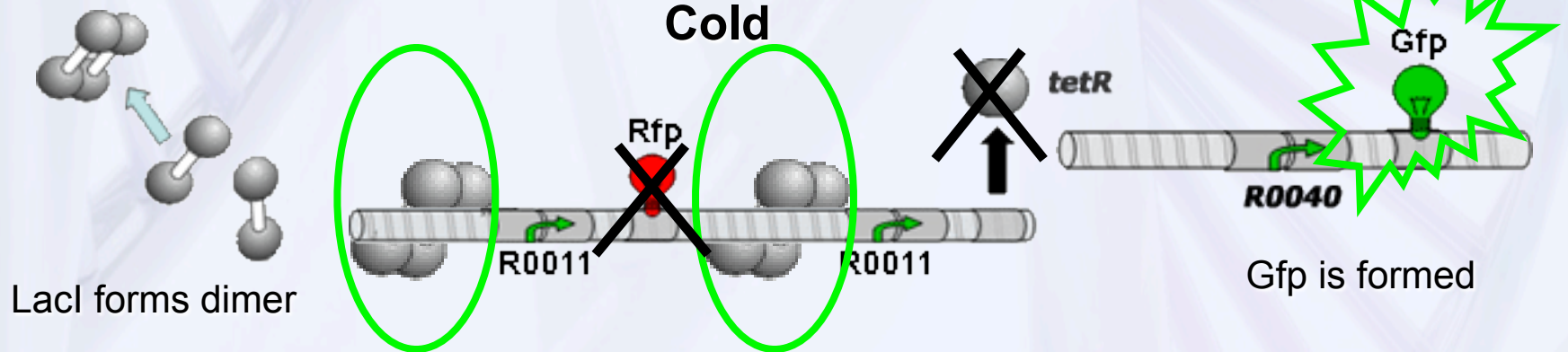
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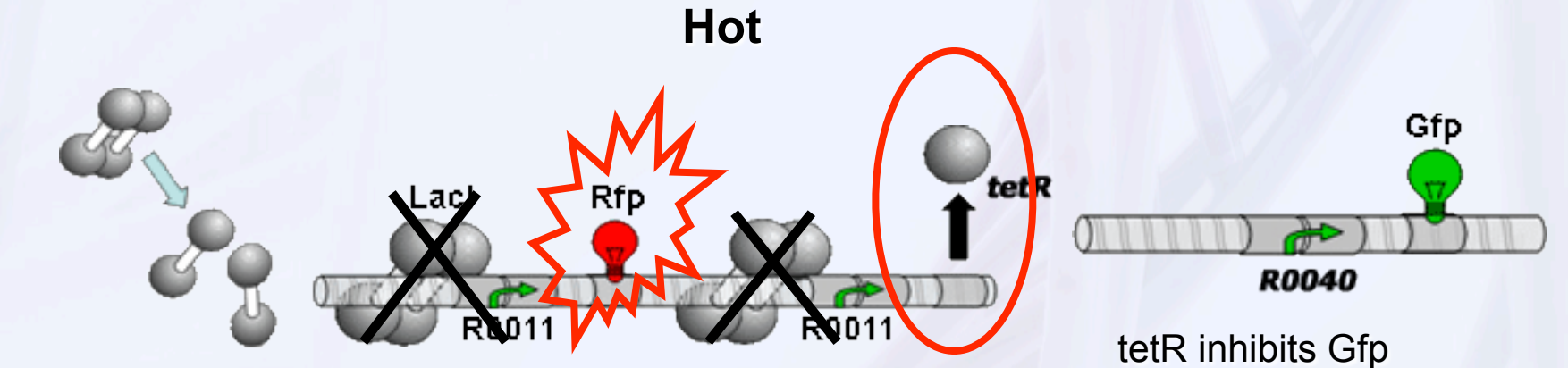
Dimers break

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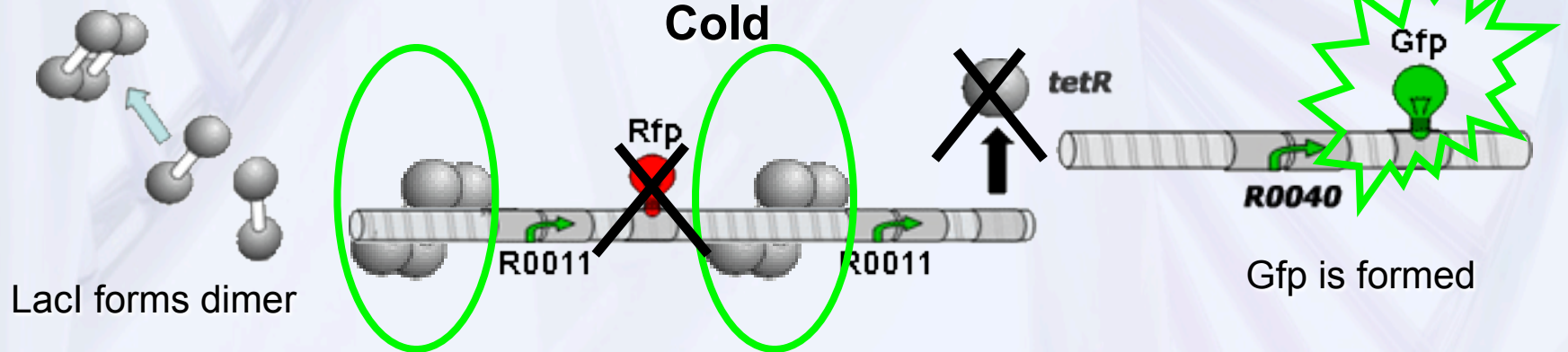
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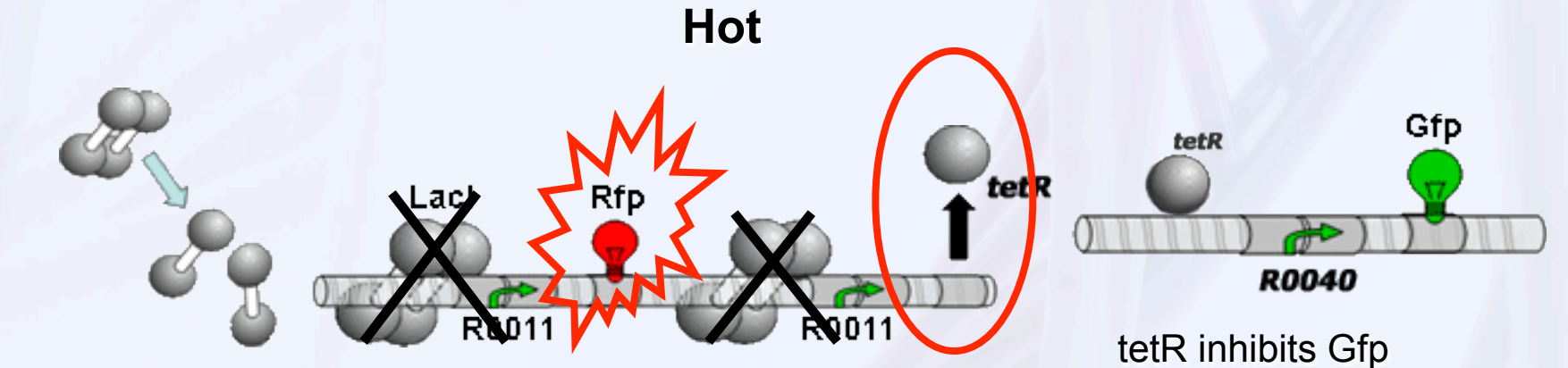
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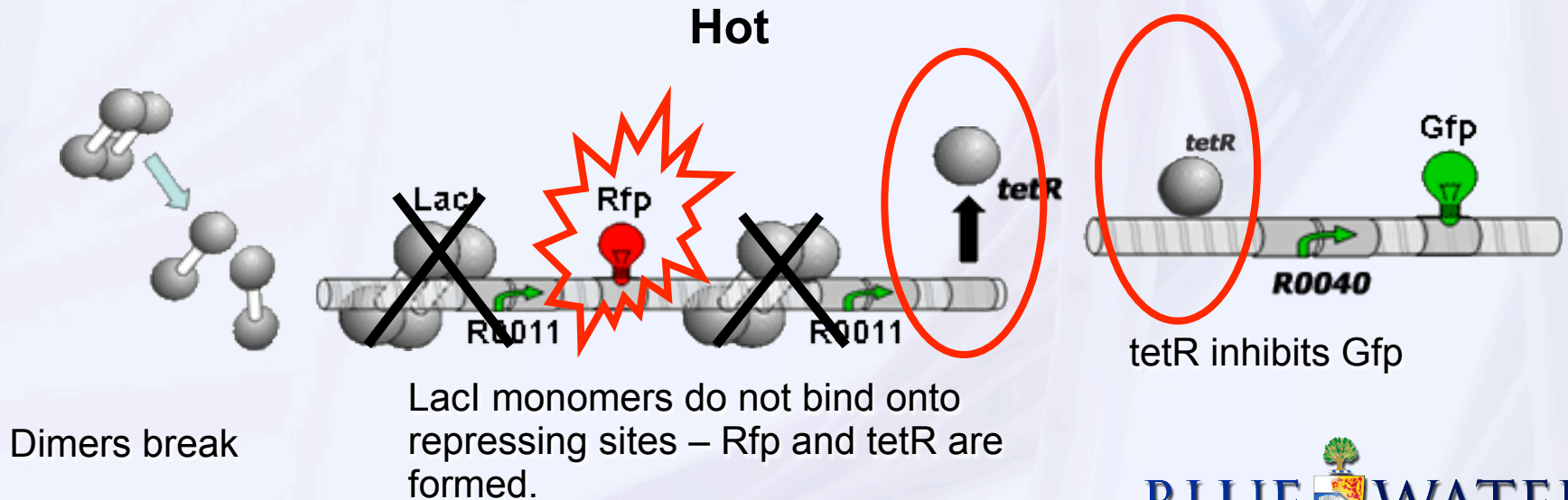
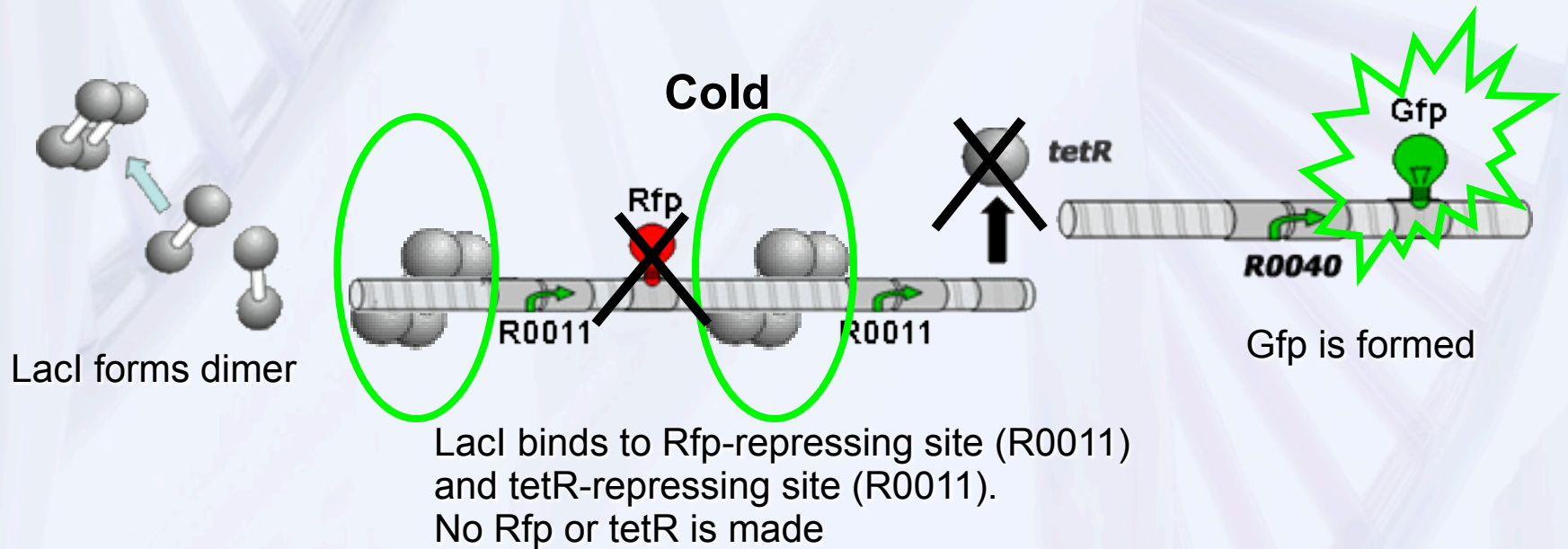
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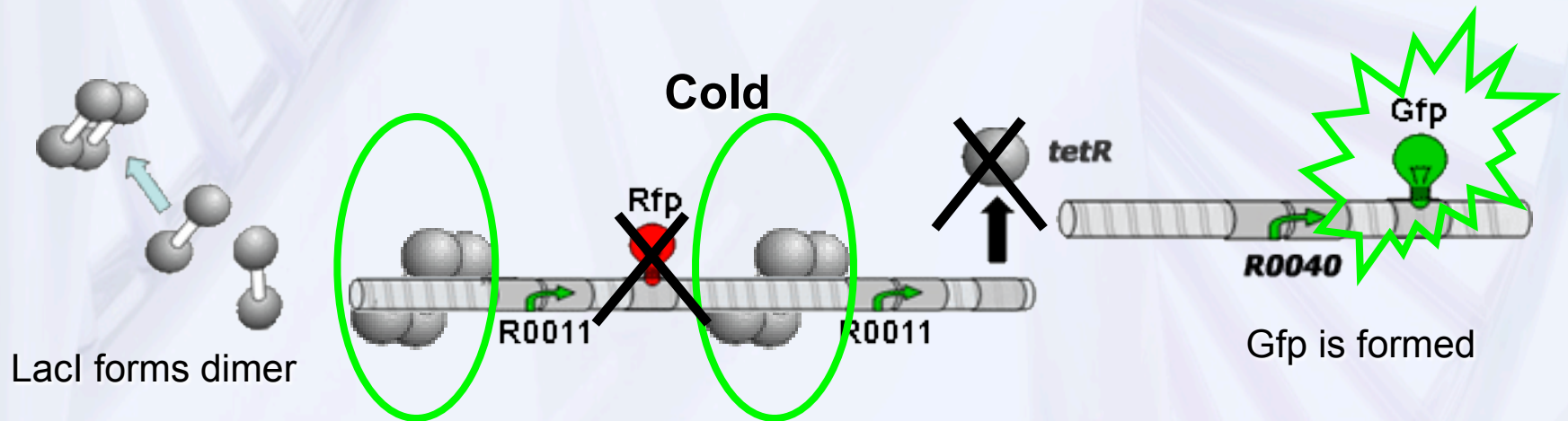
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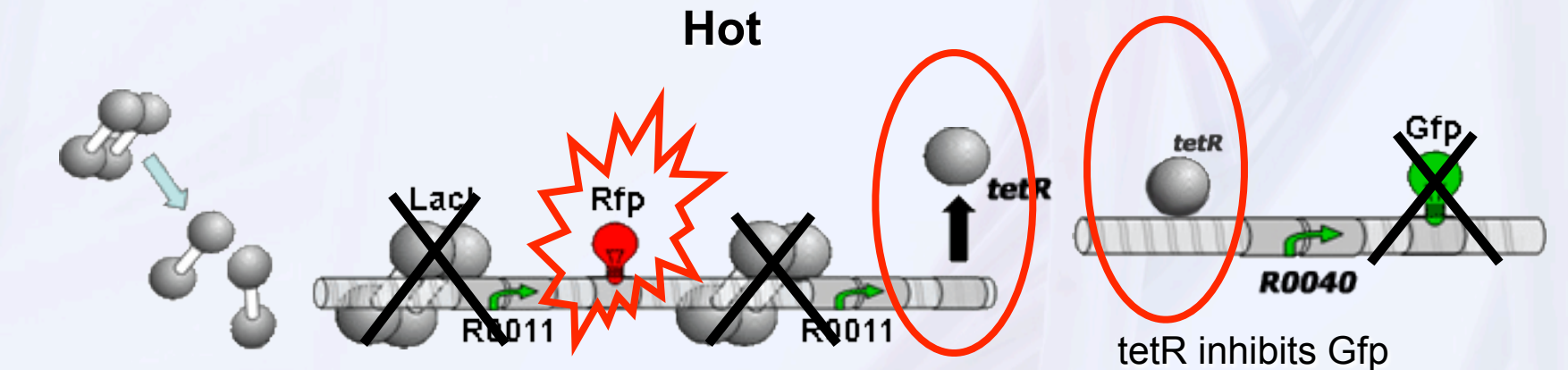
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Deterministic Model:

$$\frac{d}{dt}LacI_2 = \alpha_1 - \beta_1(LacI_2)^2 + \beta_2(\text{temperature})LacI_4 - \delta_2LacI_2 \quad (1)$$

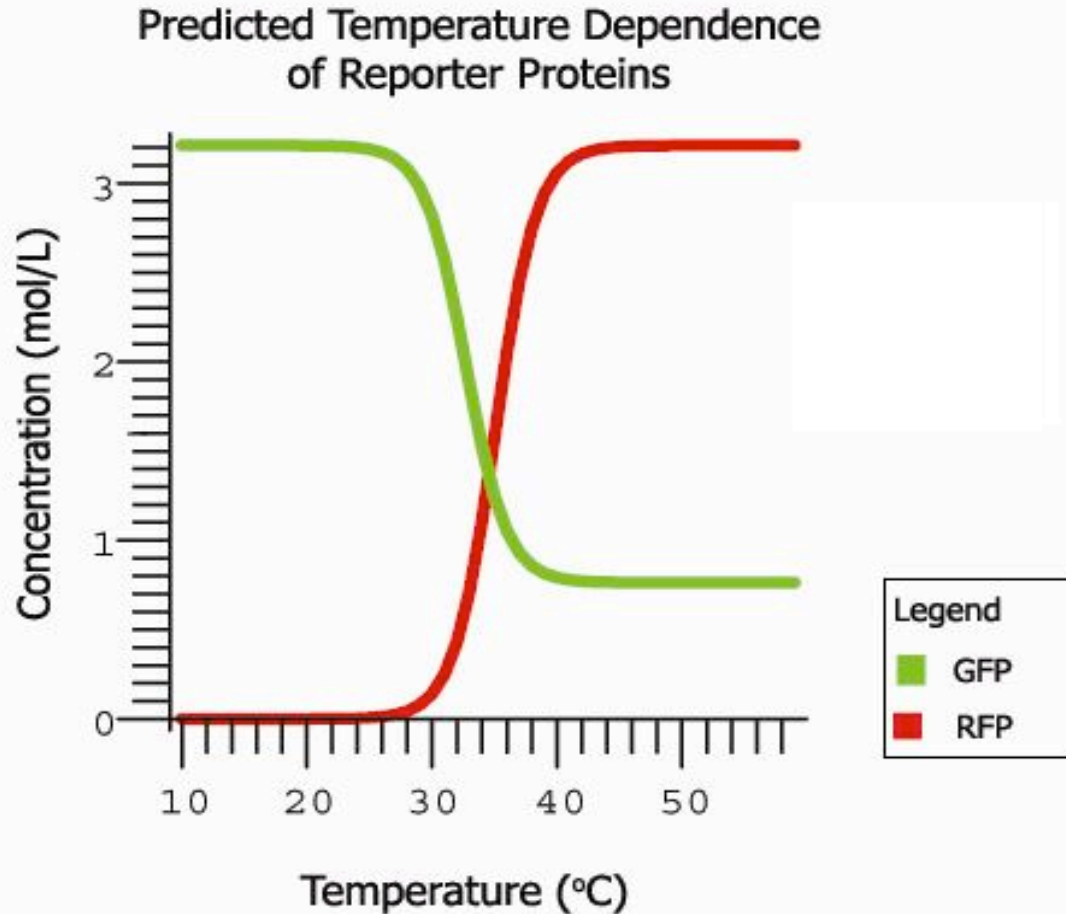
$$\frac{d}{dt}LacI_4 = \beta_1(LacI_2)^2 - \beta_2(\text{temperature})LacI_4 \quad (2)$$

$$\frac{d}{dt}tetR = \frac{\alpha_2}{k_2 + (LacI_2)^{n_2}} - \delta_t tetR \quad (3)$$

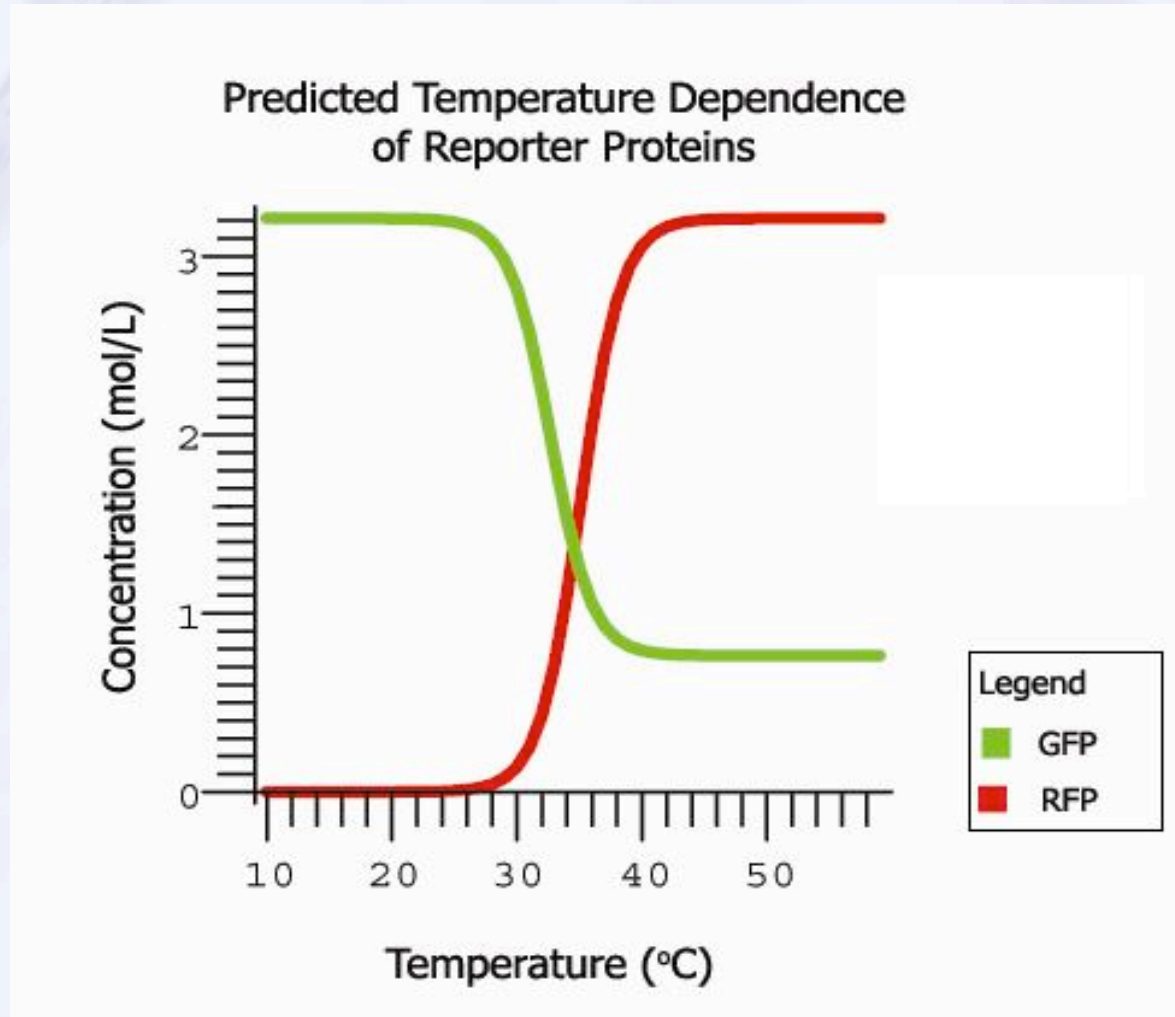
$$\frac{d}{dt}mRFP = \frac{\alpha_2}{k_2 + (LacI_2)^{n_2}} - \delta_y mRFP \quad (4)$$

$$\frac{d}{dt}GFP = \frac{\alpha_2}{k_3 + (LacI_3)^{n_3}} - \delta_g GFP \quad (5)$$

Project Model

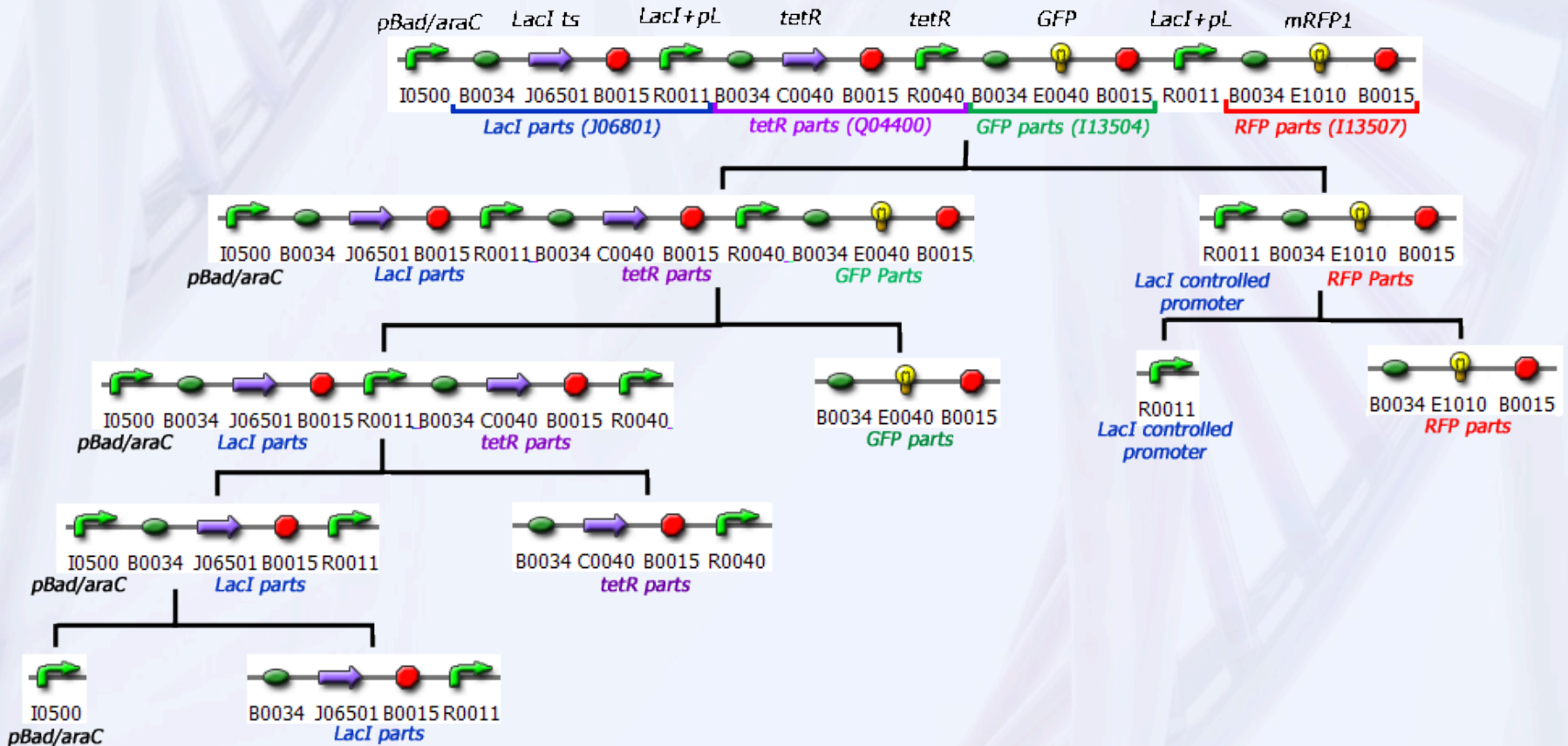


Project Model



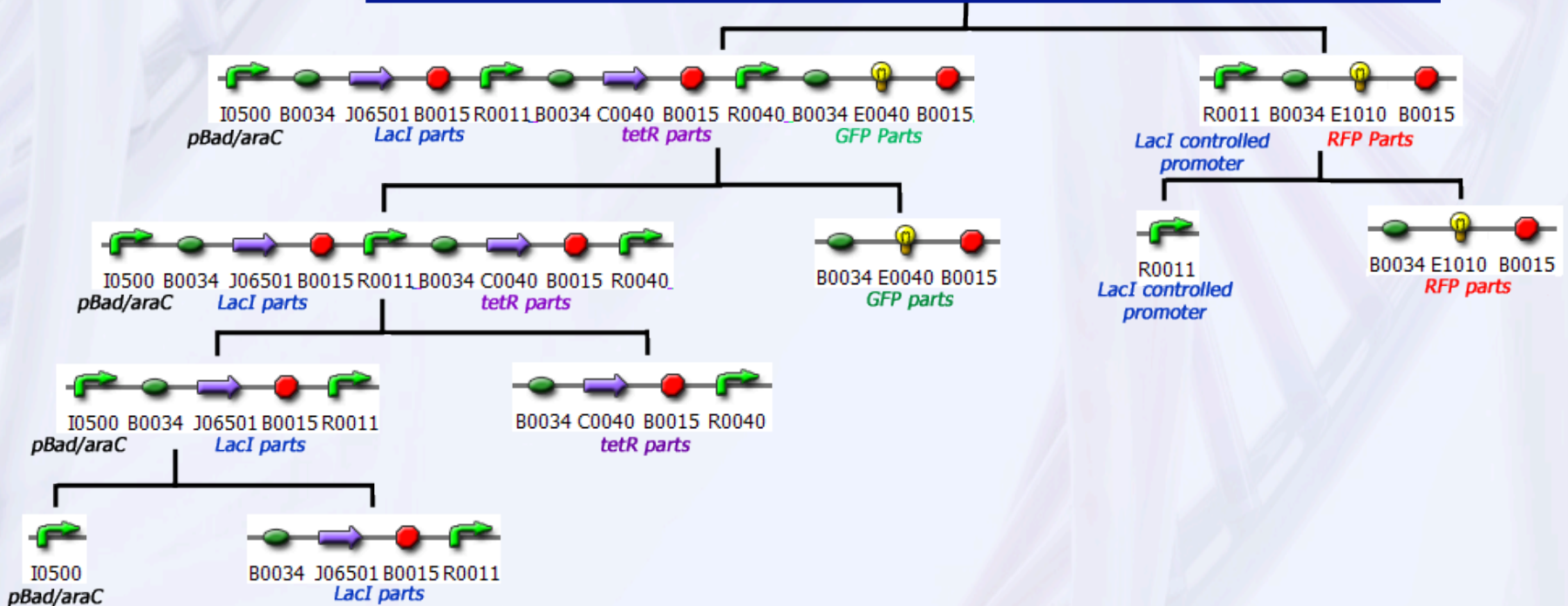
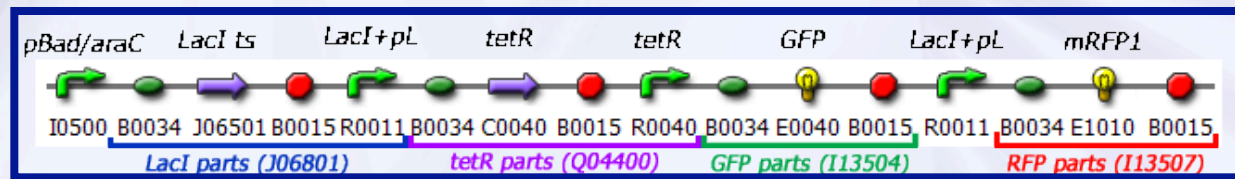
- Concentration difference in reporter proteins between 27°C and 37°C

Construction Phase



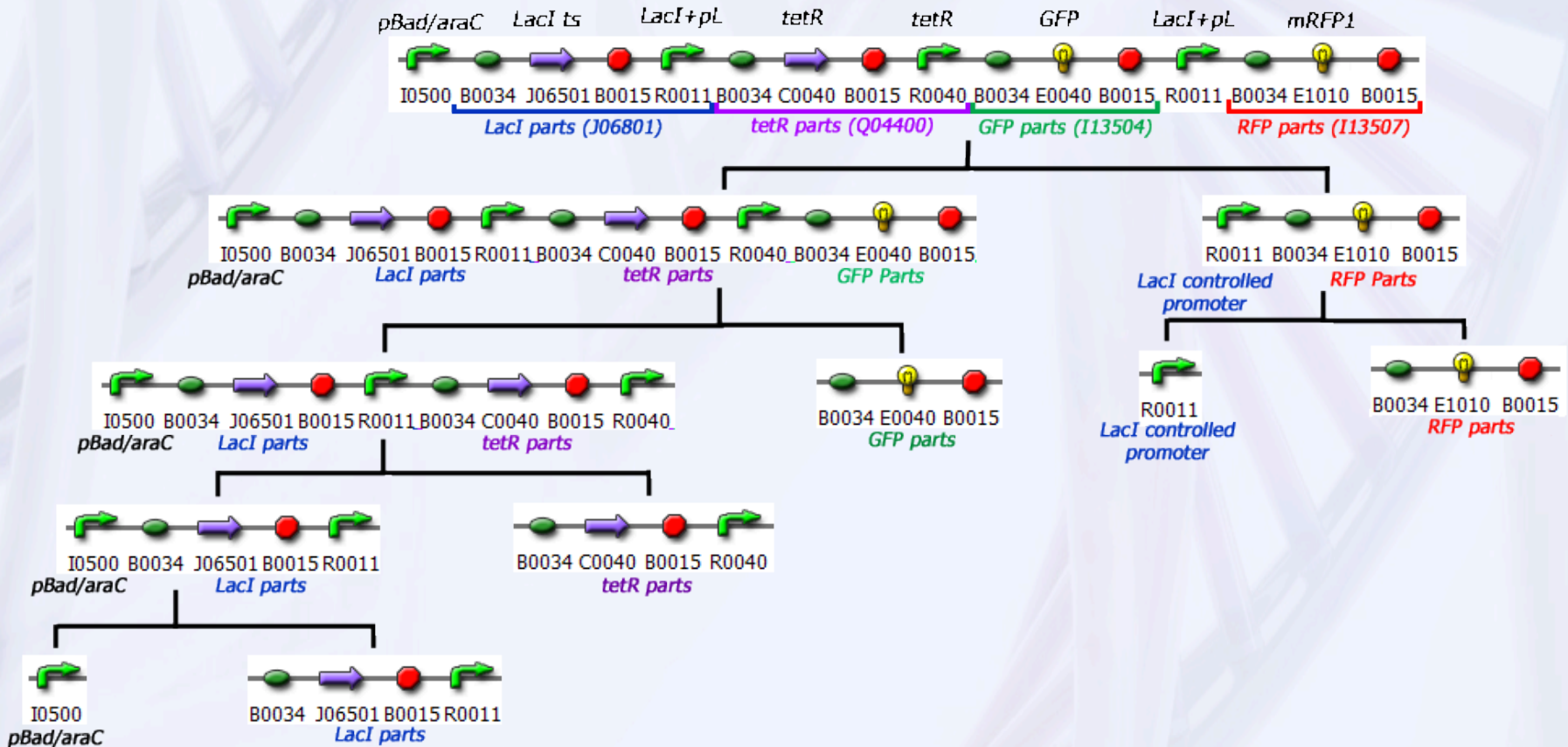
- Constructed 15 new parts (10 not shown)

Construction Phase



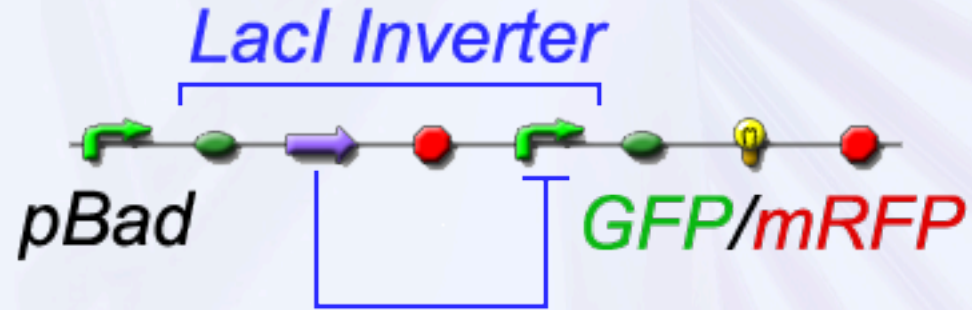
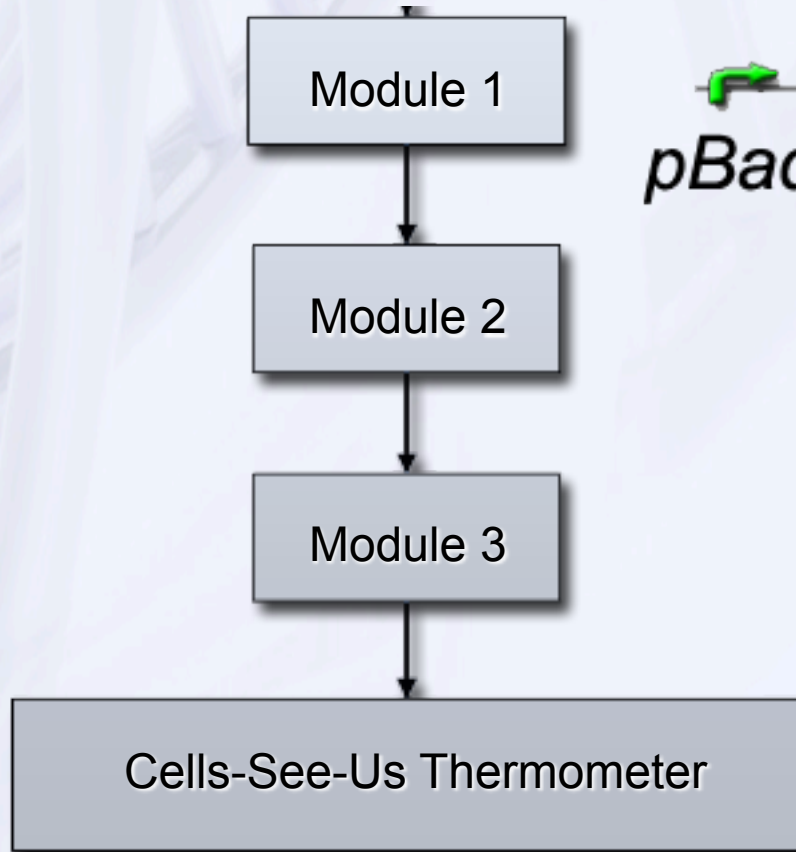
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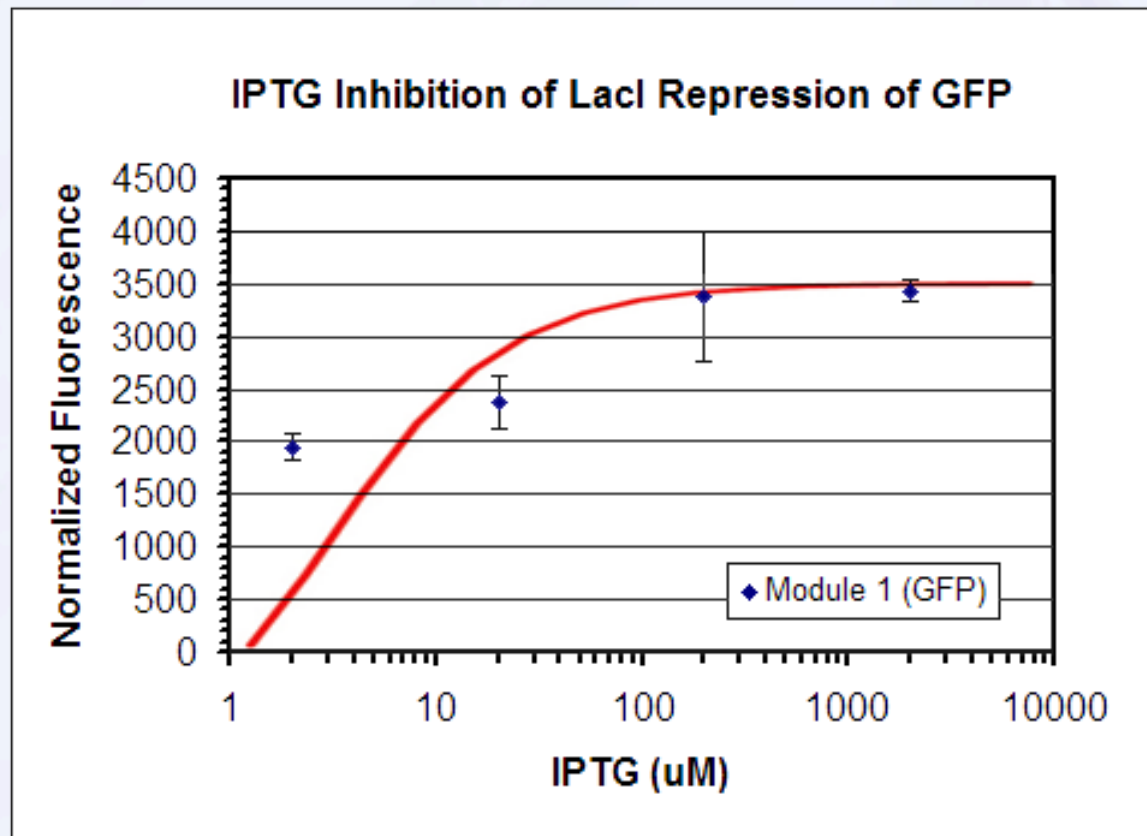
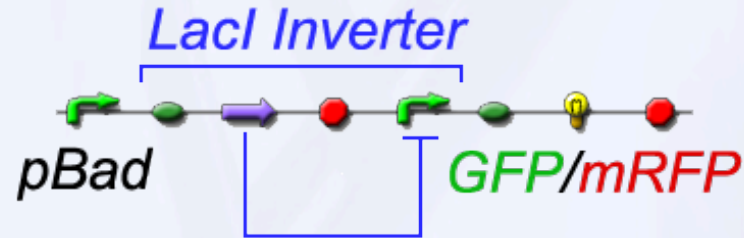


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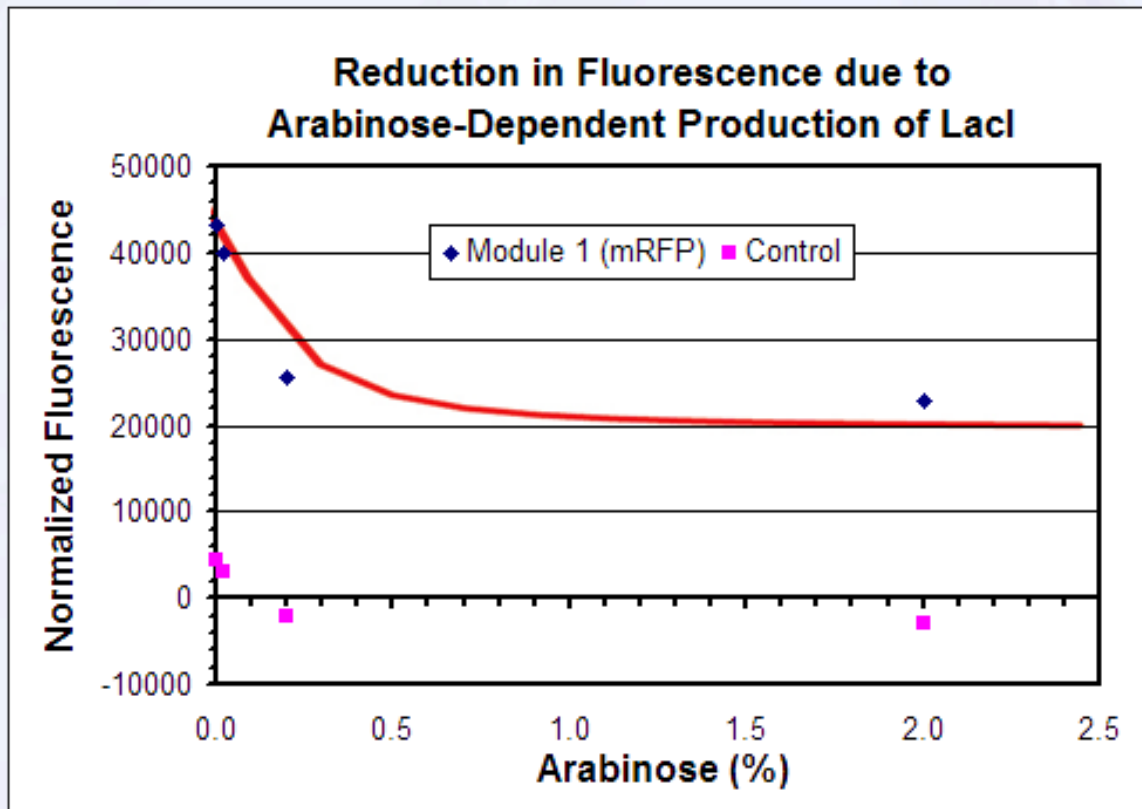
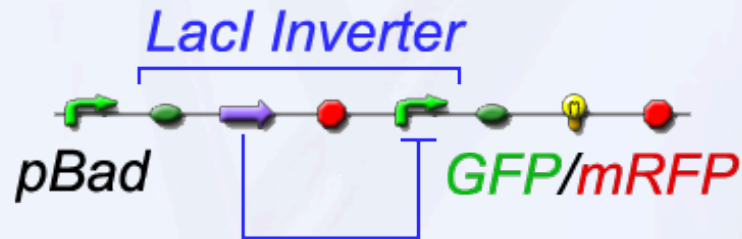
Testing Phase



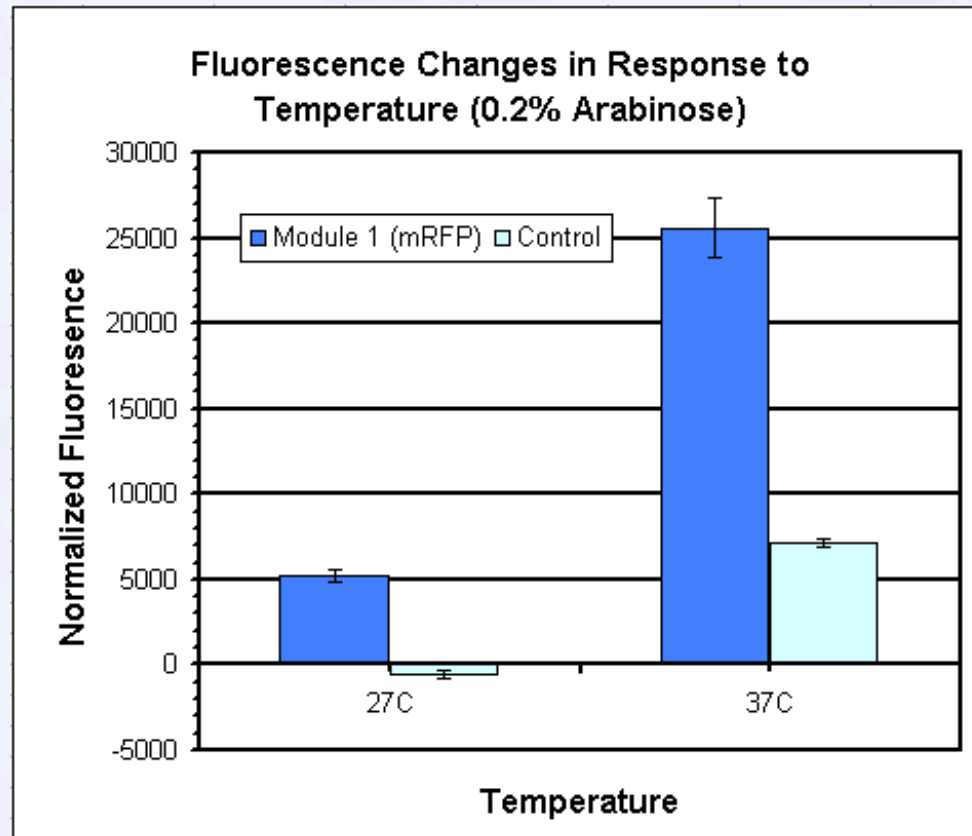
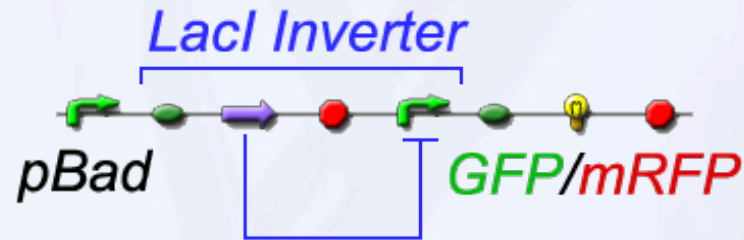
Module 1 – Reporter Functionality



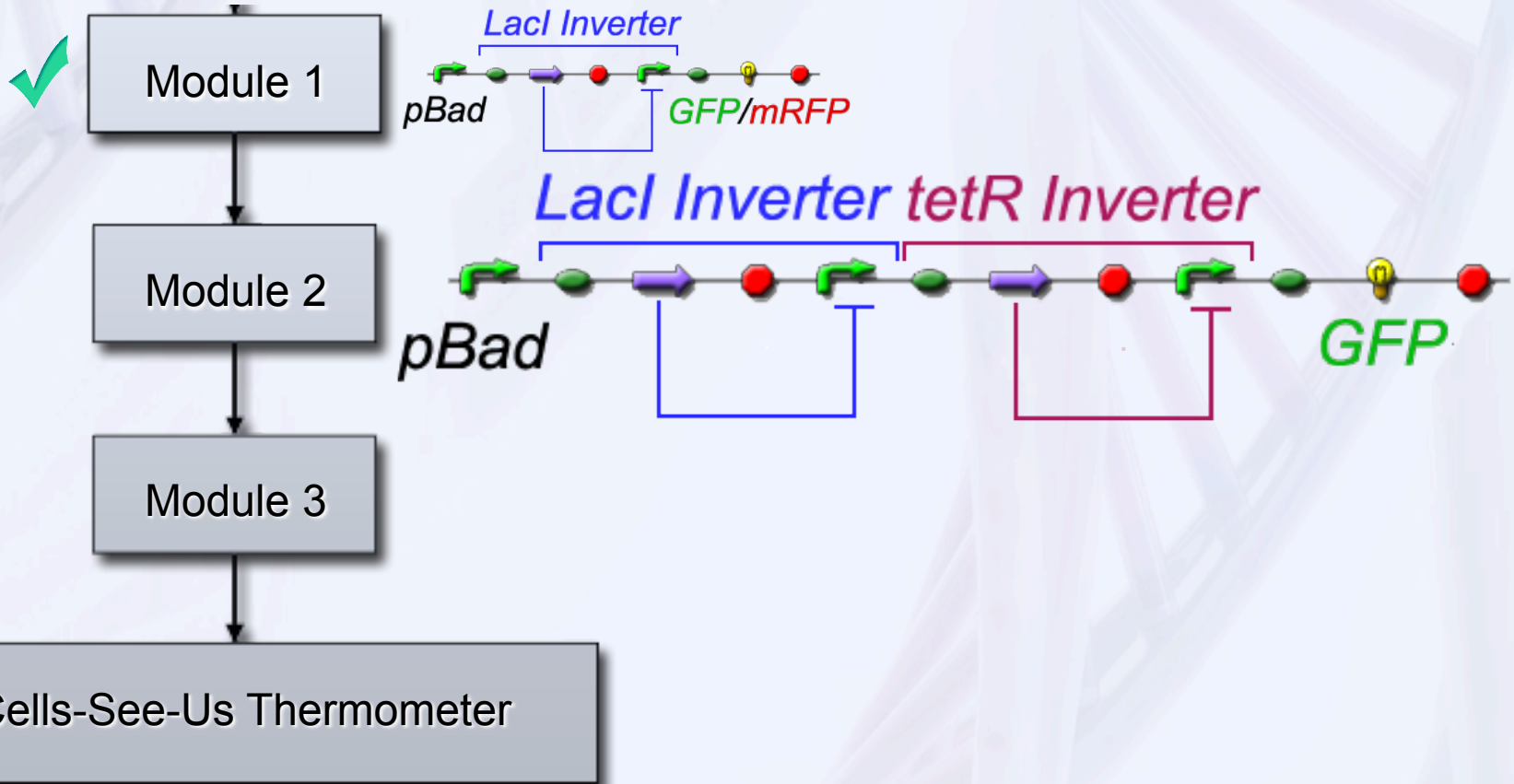
Module 1 – Production of LacI



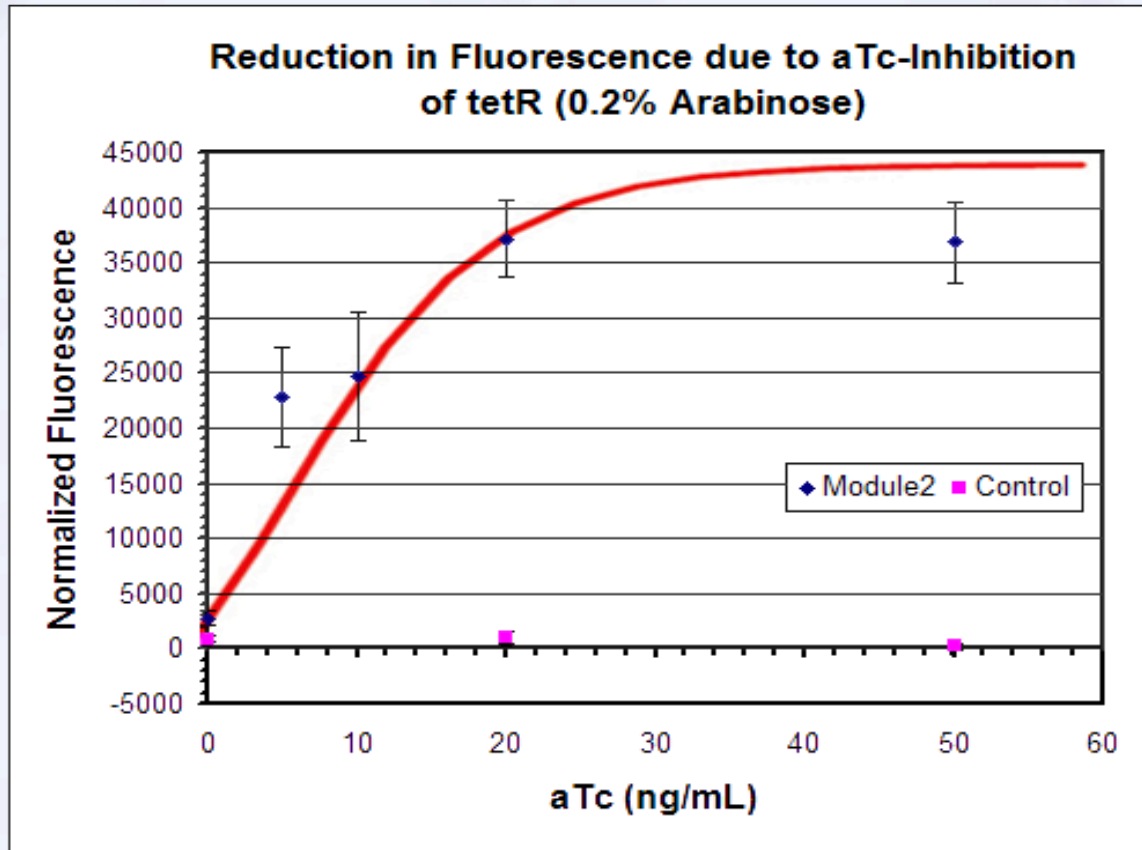
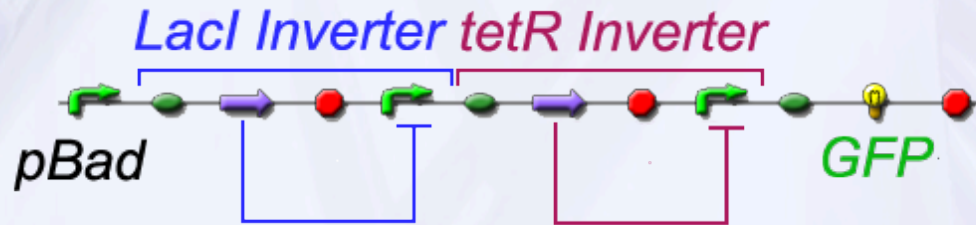
Module 1 – Temp. Dependence



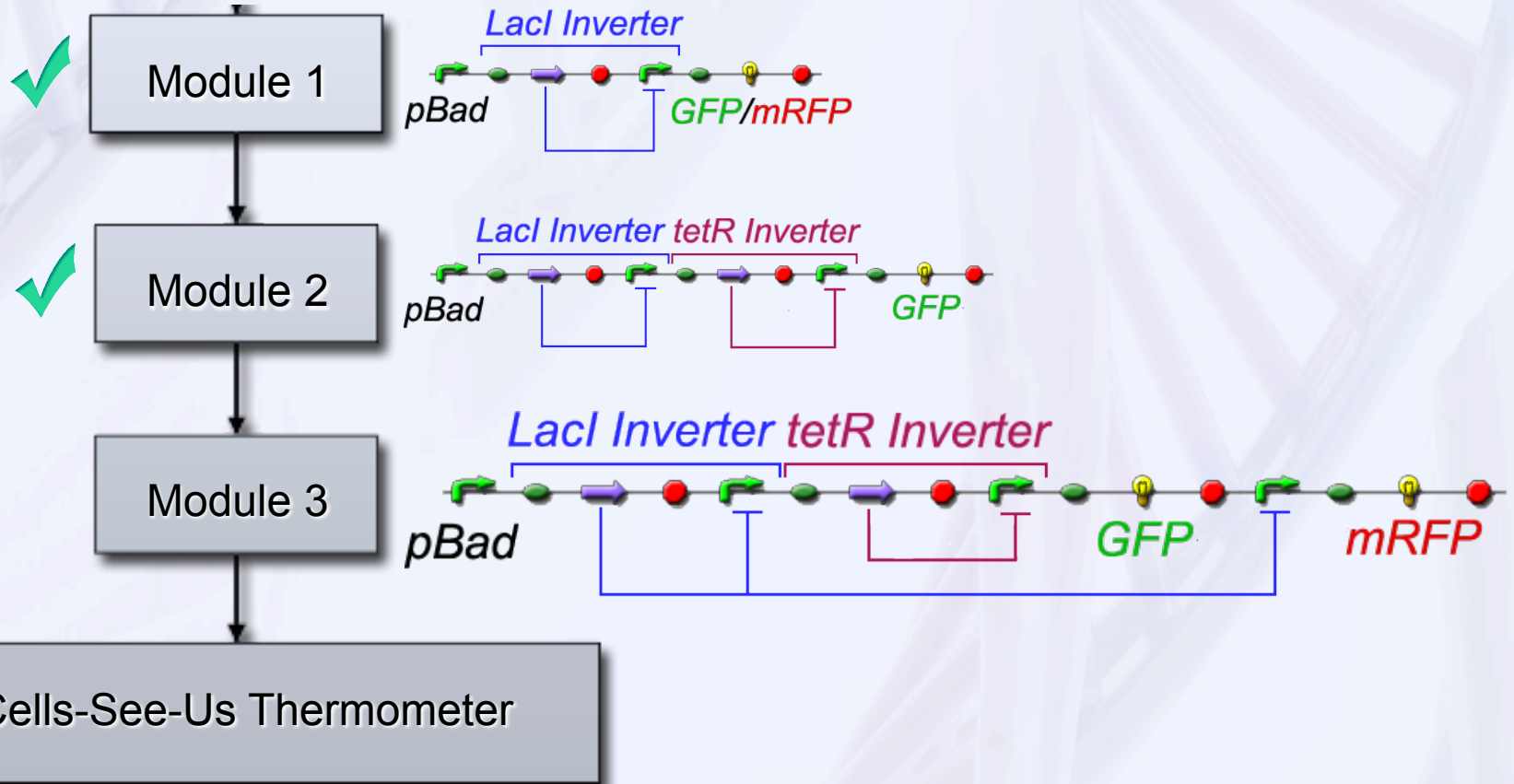
Testing Phase



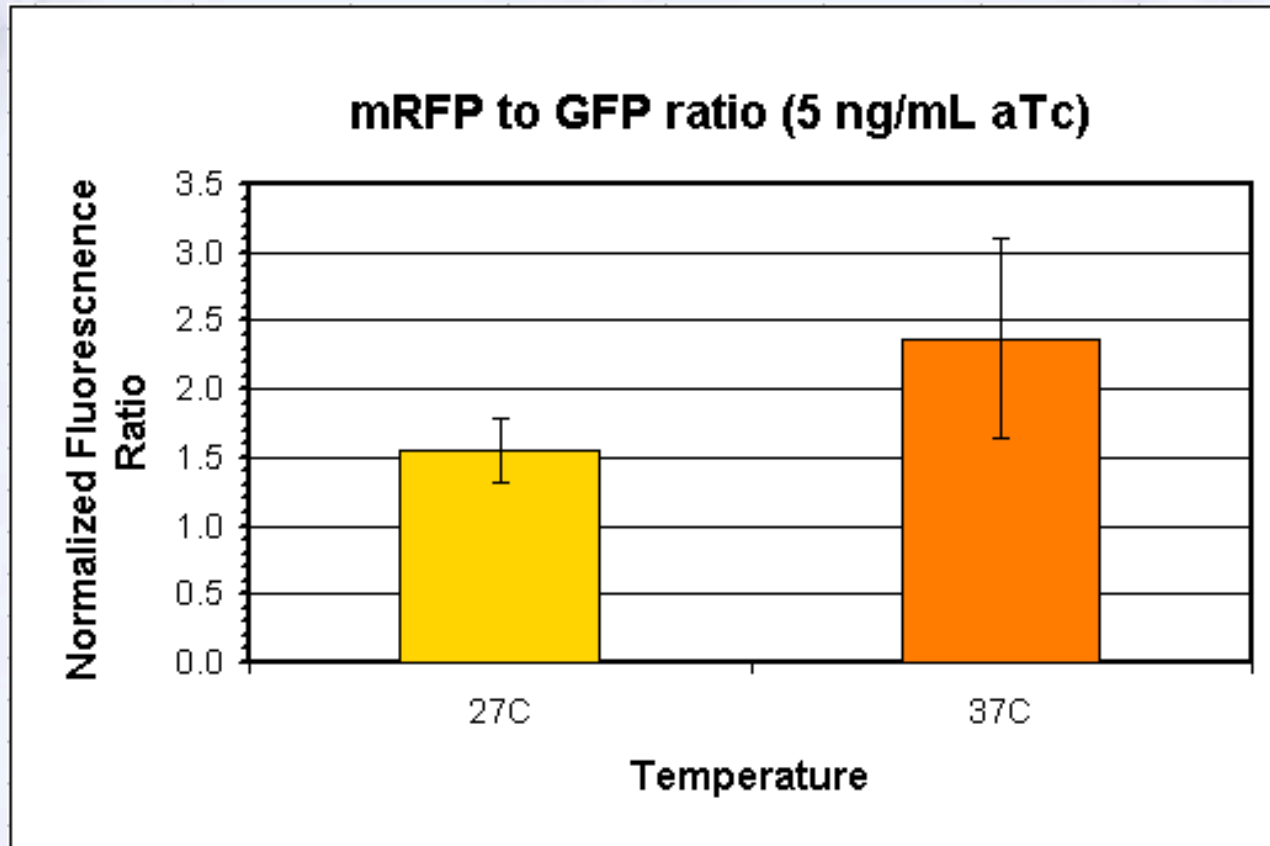
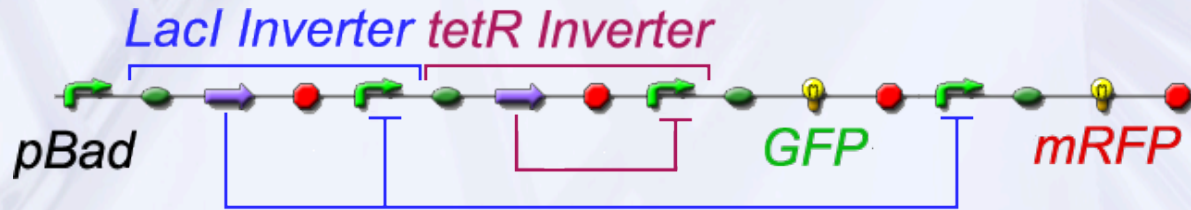
Module 2 – tetR Functionality



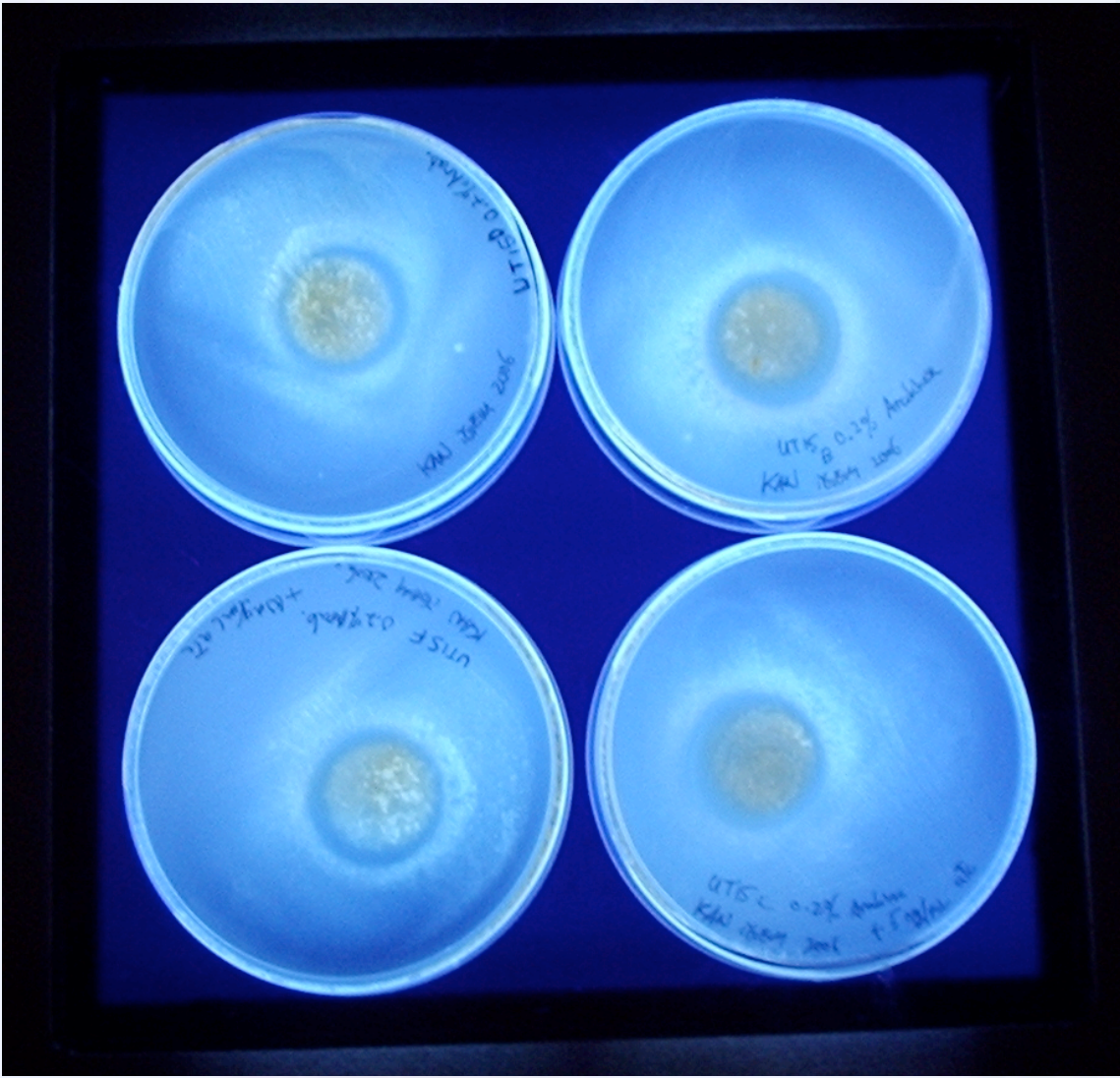
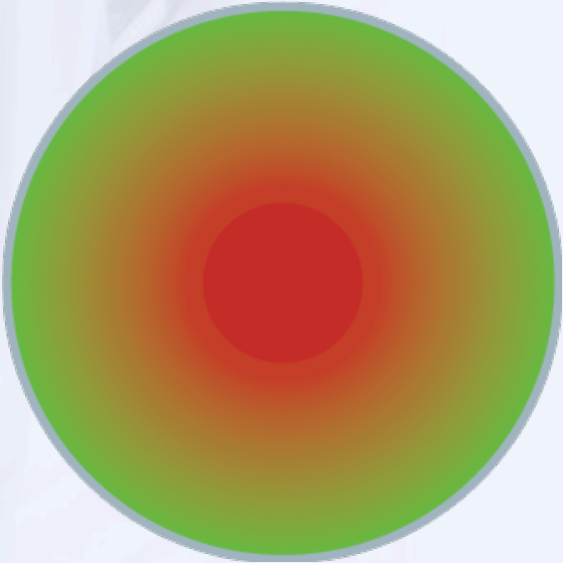
Testing Phase



Module 3 – Temperature Test



Heat Gradient Plate Test



Before...



And After...



Conclusions

- Completed construction of designed genetic device
- Basic functionality of the Cell-See-Us Thermometer was achieved:
 - o Both red and green fluorescence
 - o Temperature-dependent fluorescence levels

Acknowledgements

Sponsors:



university of toronto
engineering society



University of Waterloo
Mathematics Endowment Fund



Waterloo Engineering
Endowment Fund

Special thanks to:

- University of Toronto Davies' Lab for their generous donations of lab space, equipment, and supplies
- Brian Ingalls for his generous support in modeling

Thank you iGEM organizers
and MIT for hosting!



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- Charles Yoon (Toronto)
- Nancy Xu (Waterloo)

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- Natalie Yeung (Toronto)
- Konstantin Savitsky (York)
- Anne Tran (Waterloo)
- Conrad Lochovsky (Toronto)
- Jovan Lukovich (Toronto)
- Nick Ngai (Toronto)
- Tara khiabani (Toronto)
- HoKwon Kim (Toronto)
- ChengChuan Qu (Toronto)
- Adnan Najmi (Toronto)
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- George Ye (Toronto)
- Ram Puri (Toronto)
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- Arash Mirrahimi (Toronto)
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- Michael Leung (Toronto)
- Christina Lucey (Waterloo)
- Linda Chen (Waterloo)
- Rohan Gidvani (Waterloo)
- Jeffrey Wong (Waterloo)

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- Prof. Stephen Davies (Toronto)
- Prof. Brian Ingalls (Waterloo)
- Seema Nagaraj (Toronto)
- Lance DaSilva (Waterloo)
- Patrick Tsui (Waterloo)