



Puppy Probiotics

Team Illinois
2014





CHOCOLATE: TOO GOOD TO BE BAD?



Theobromine



- Caffeine derivative
- Large component in chocolate
- Stimulant
- Therapeutic applications



Theobromine Poisoning

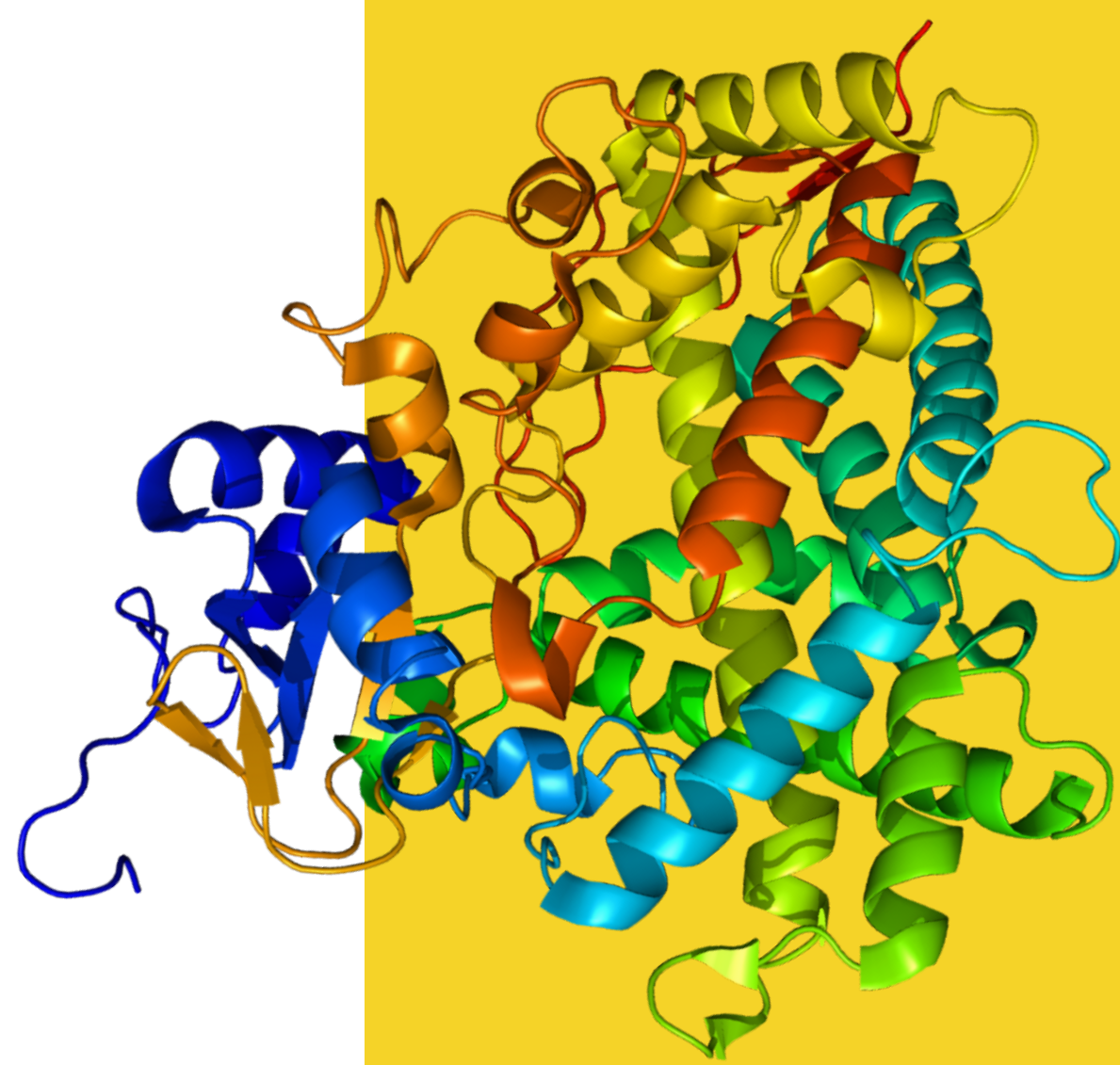


- Theobromine is toxic to small animals
- Lack of degradation enzymes
- Side effects include:
 - ▶ Vomiting
 - ▶ Tachycardia
 - ▶ Seizures
 - ▶ Cardiac arrest
 - ▶ Death



CYP1A2

- Cytochrome P450
- Involved in metabolism of xenobiotic substances
- Separate substrates in small animals



OUR Proposal



- Degrade theobromine in the small intestine
- Utilize synthetic biology
- Plasmid construct
- Mode of ingestion



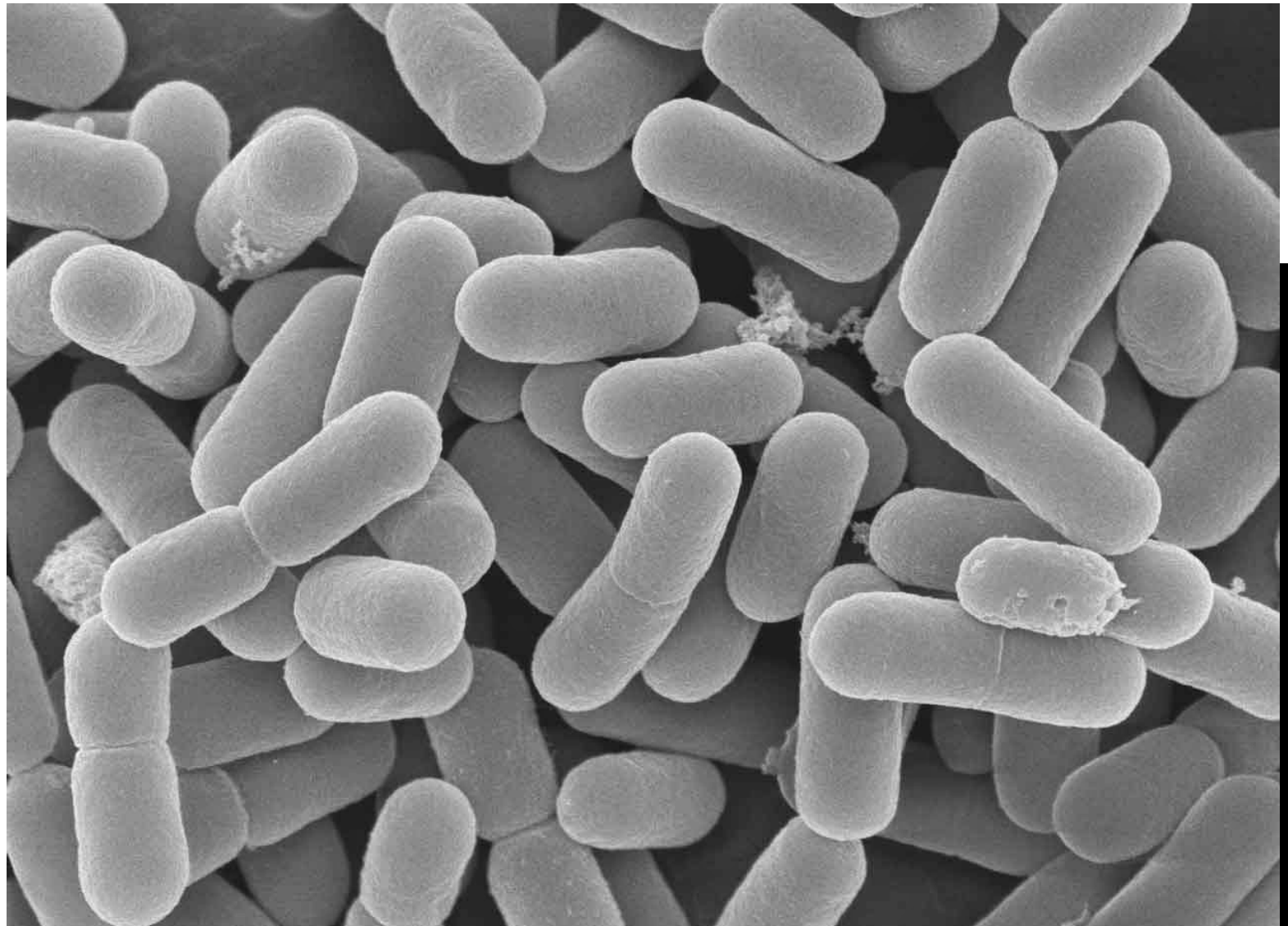


OUR SOLUTION

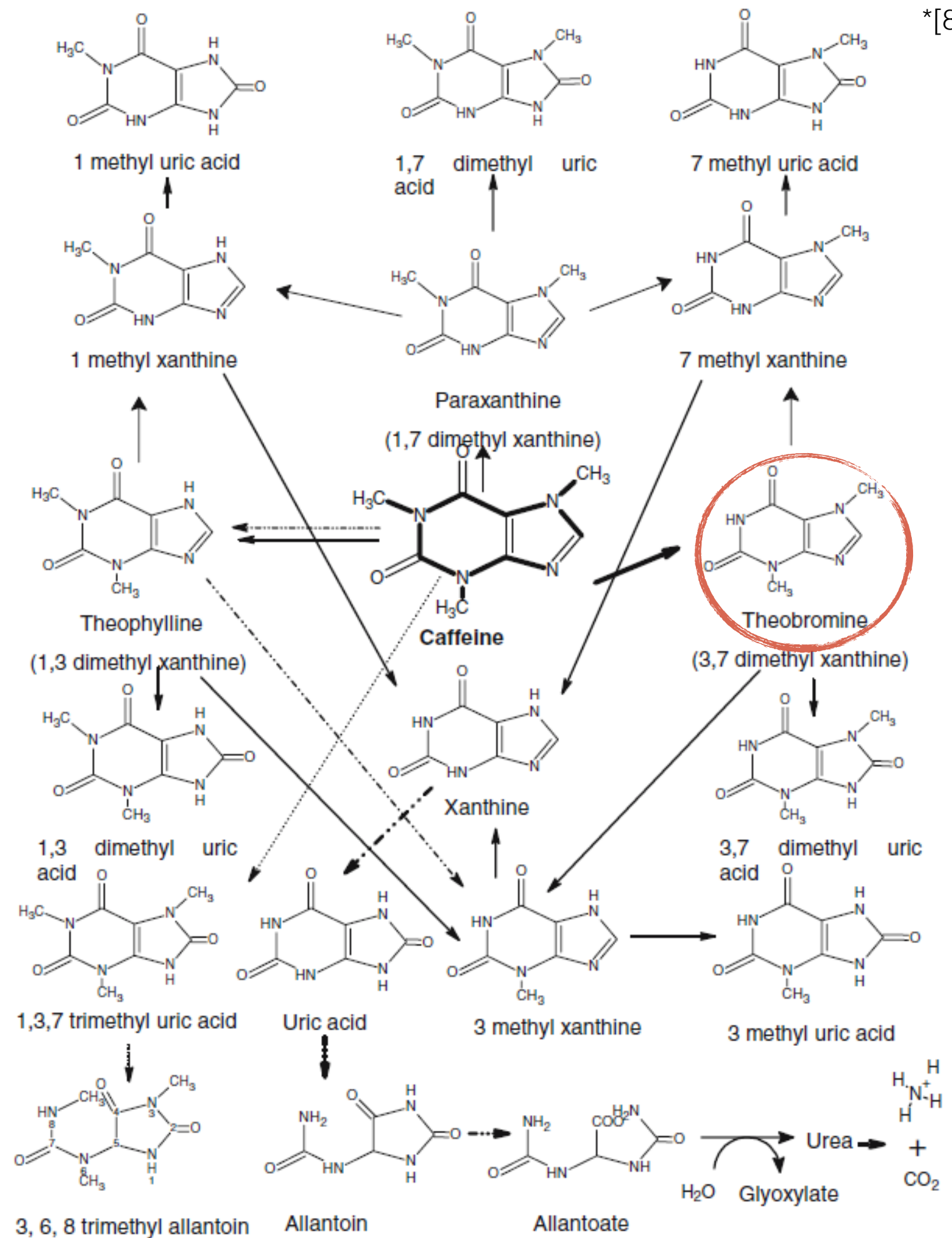
- Novel probiotic which degrades theobromine
- Use previously characterized pathways
- Implement in *Lactobacillus*
- Yogurt as a delivery method



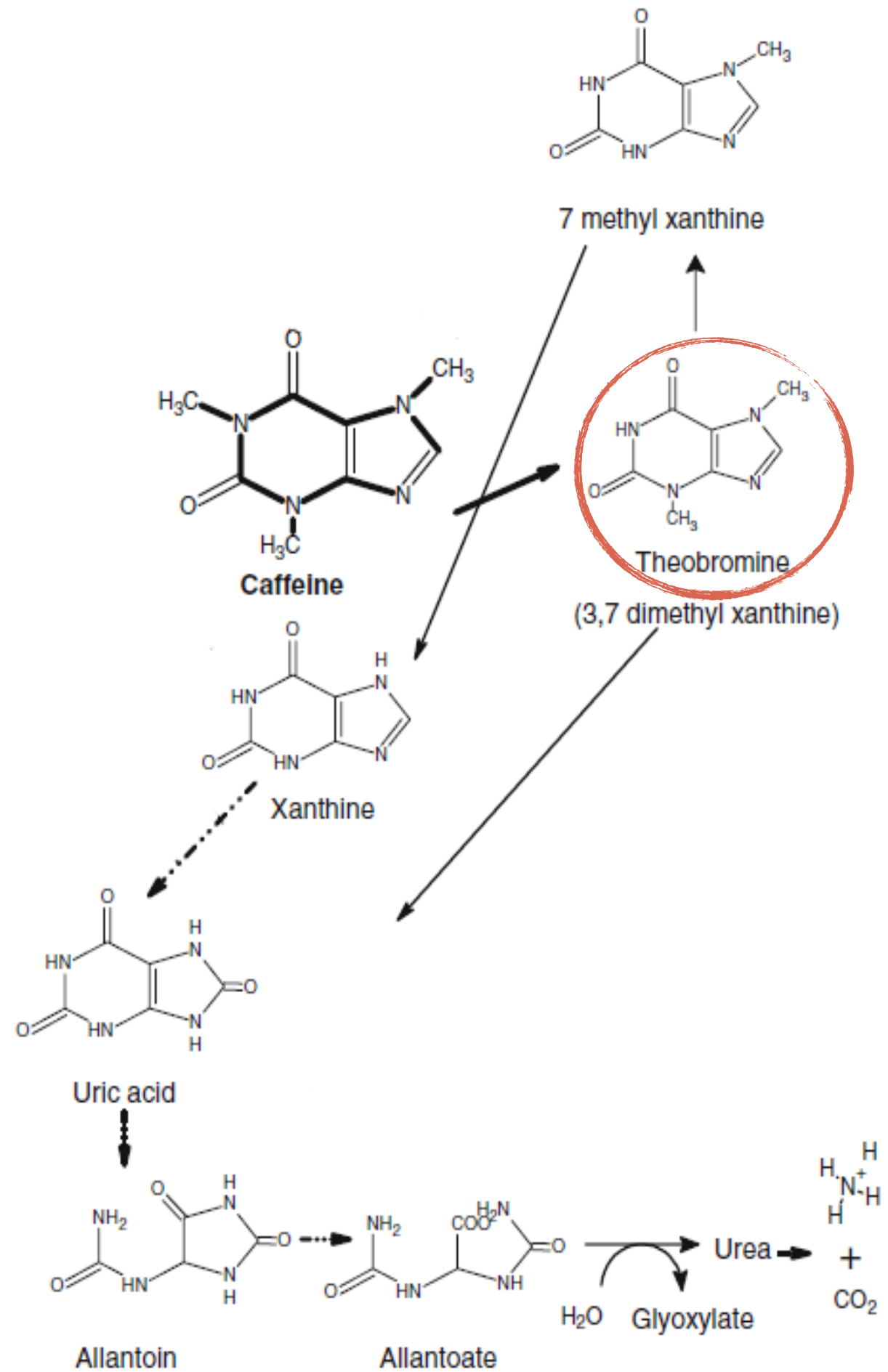
ANALYSIS

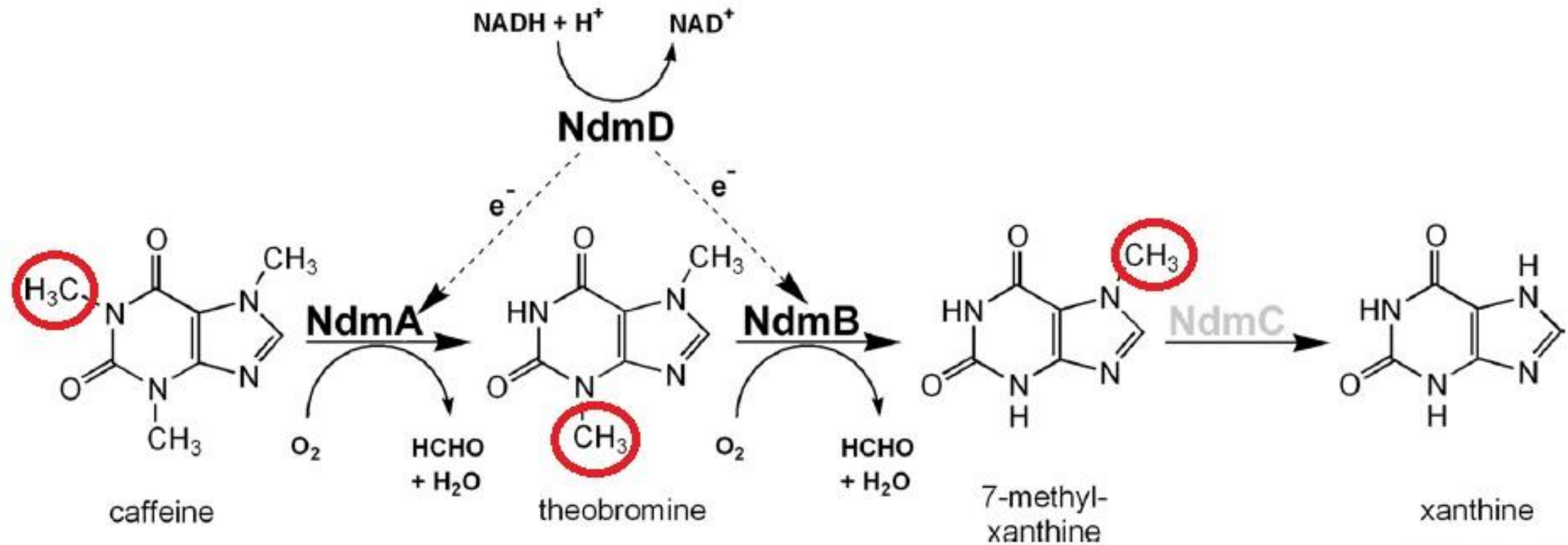


Overall Xanthine Pathway



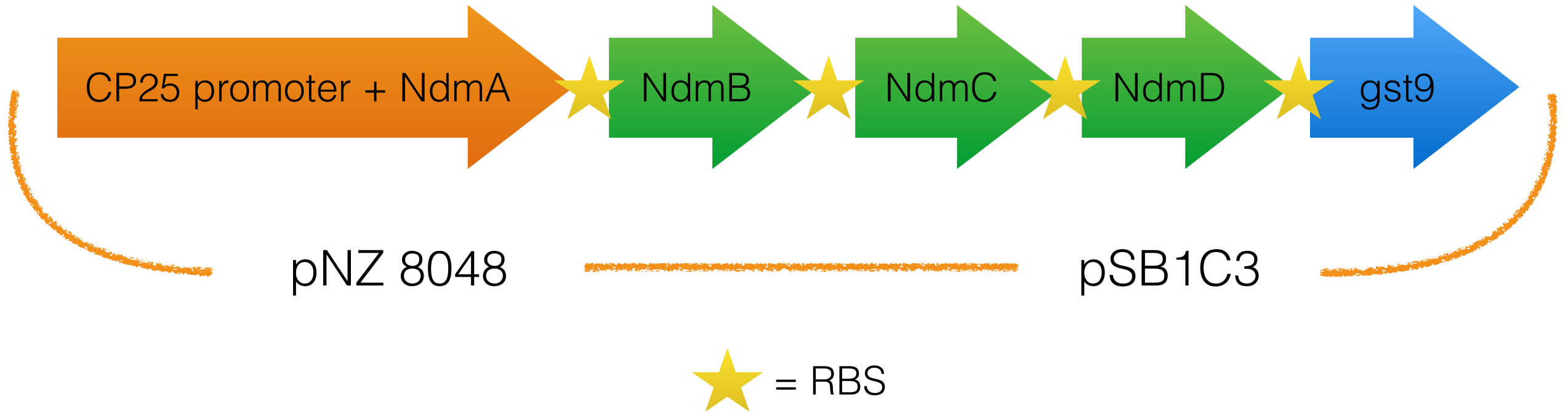
Overall Xanthine Pathway



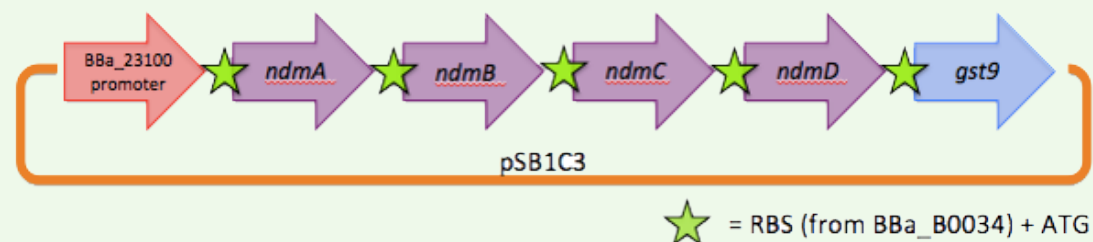


Design Pathway





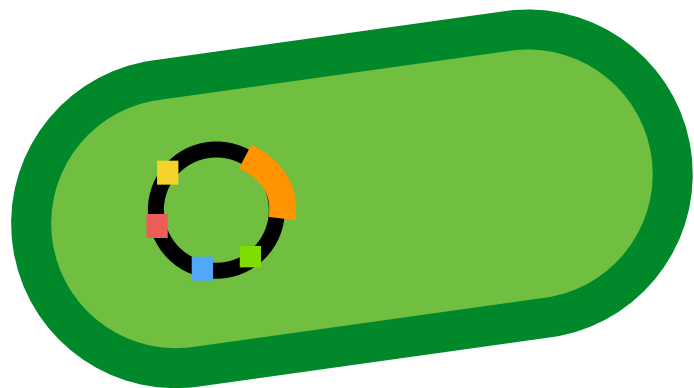
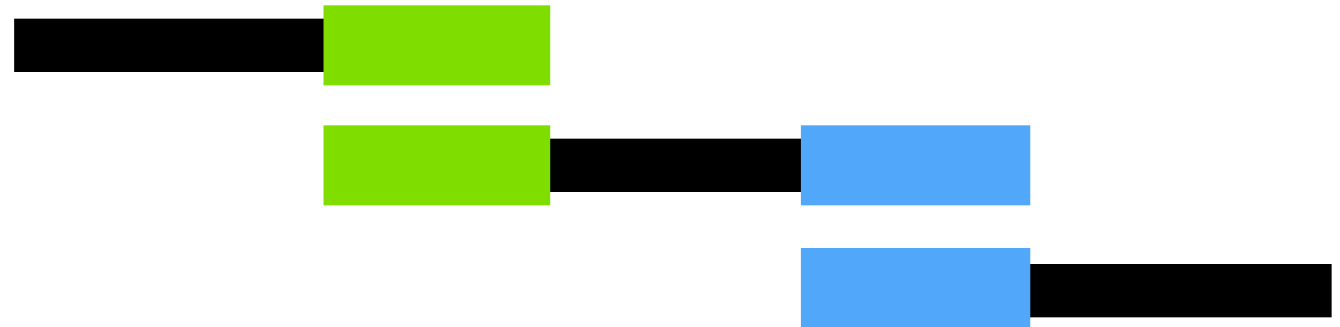
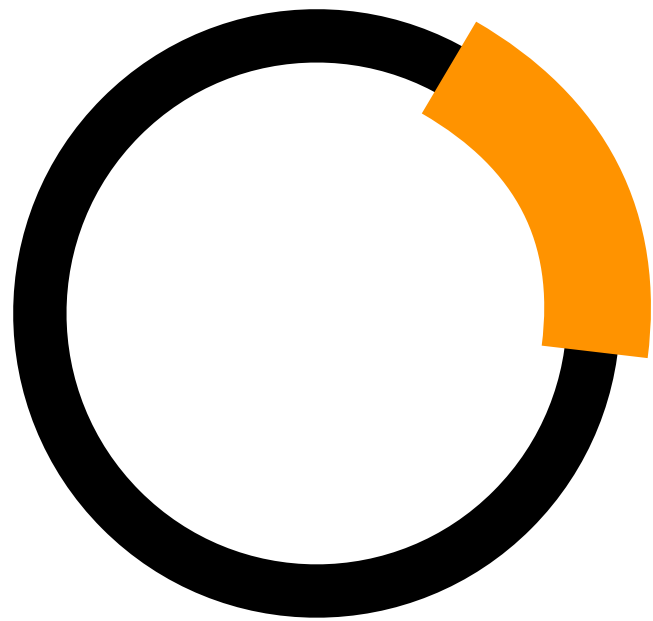
- UT Austin 2012 pDCAF
 - Contains the N-demethylation operon



- Adjust plasmid
 - CP25 promoter
 - Lactobacillus ori
 - Cutsites



Piecemmeal



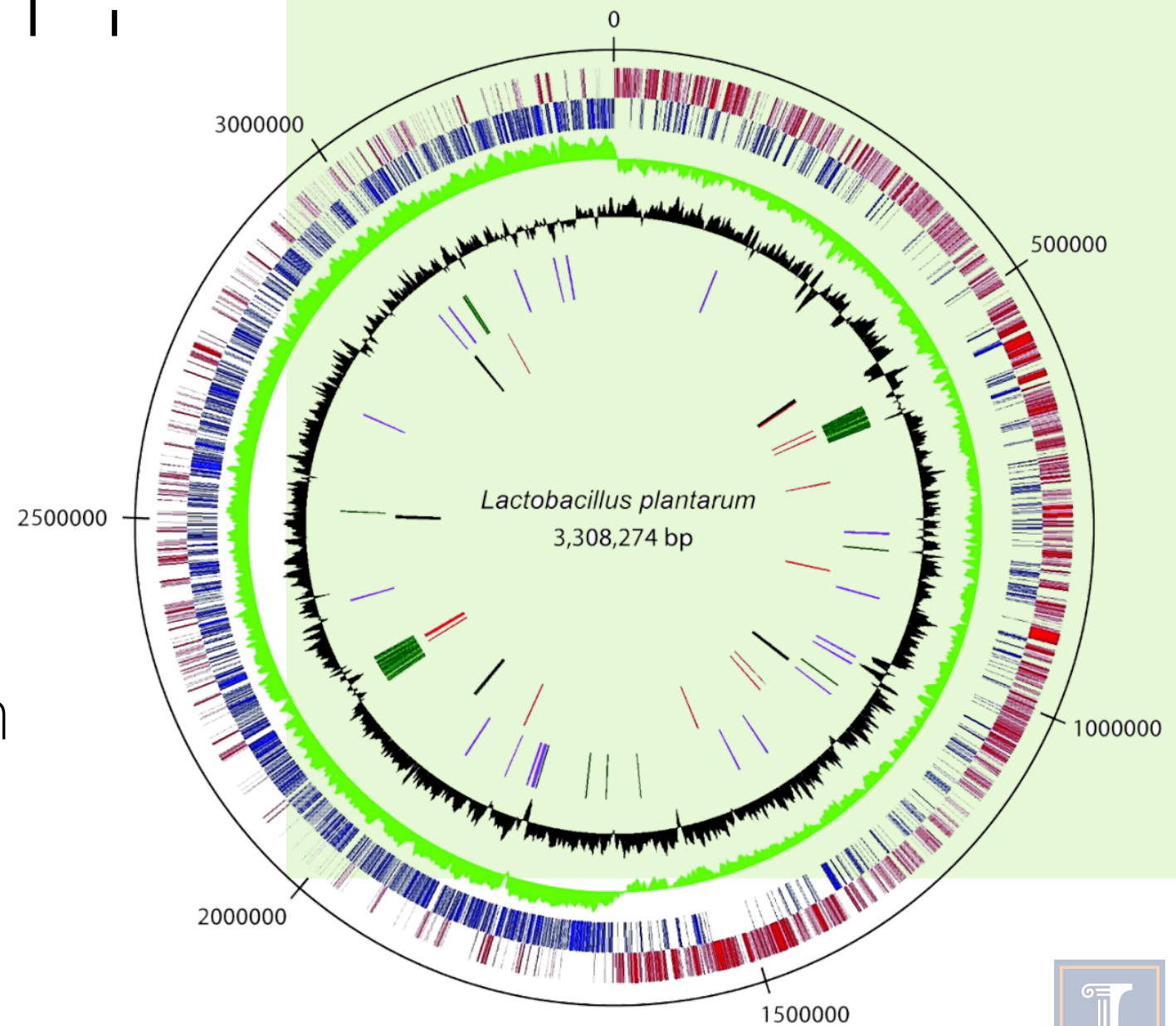
source: <http://piktochart.com>



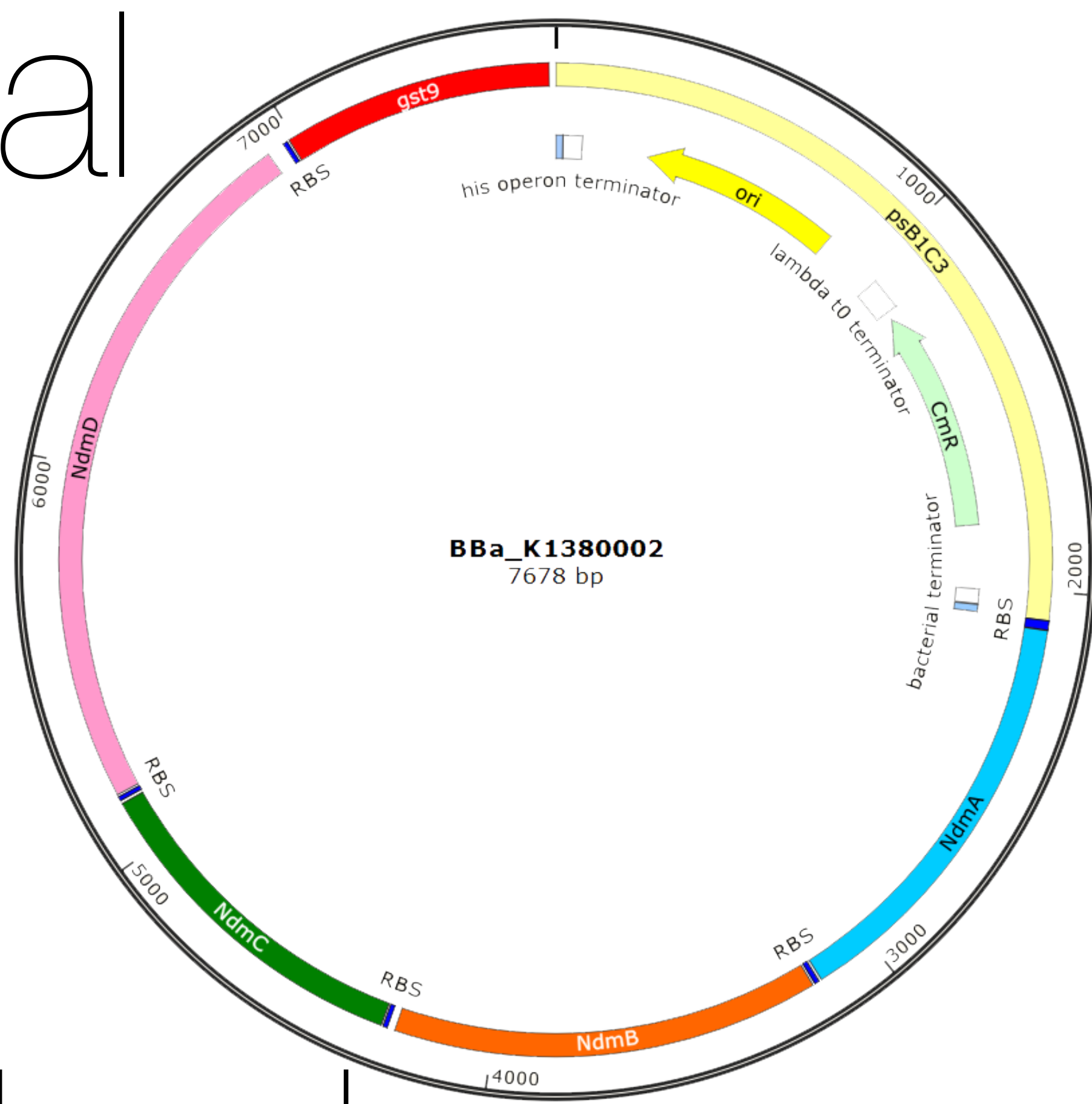
Why Plantarum?

- Large, versatile genome
- Previous probiotics
- Can survive enteric conditions
- Transformed via electroporation
- Colonization potential
- CP25 promoter

WCFS1



Original



Construct



Goal

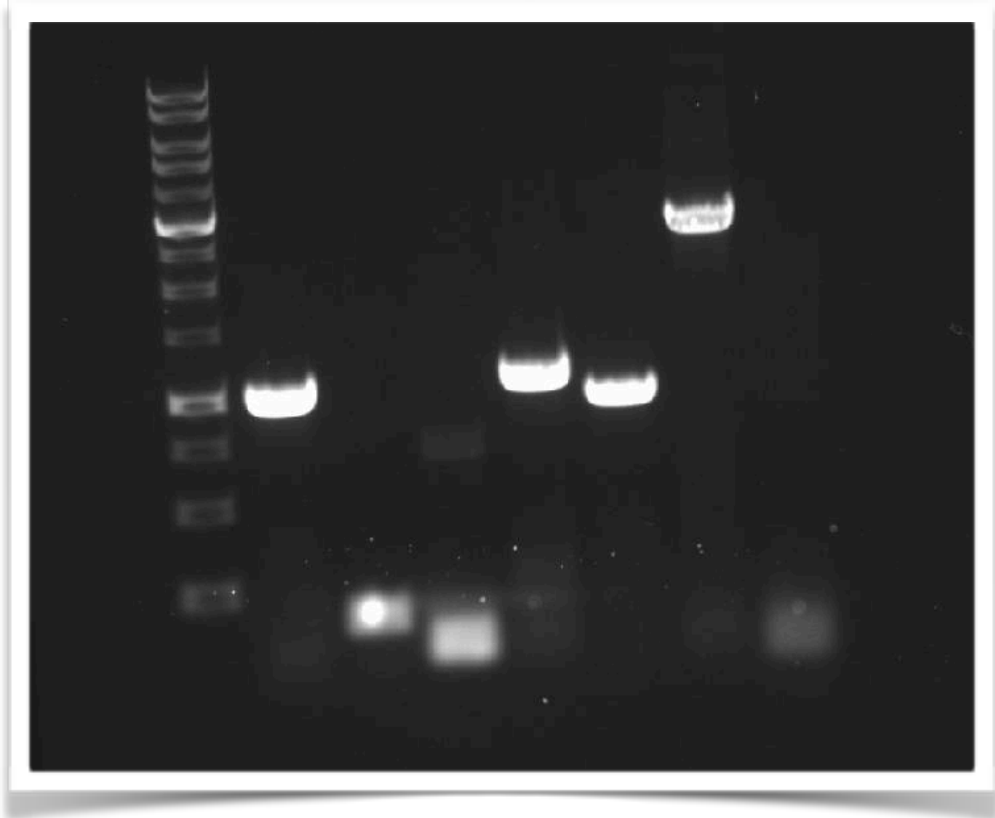


Construct

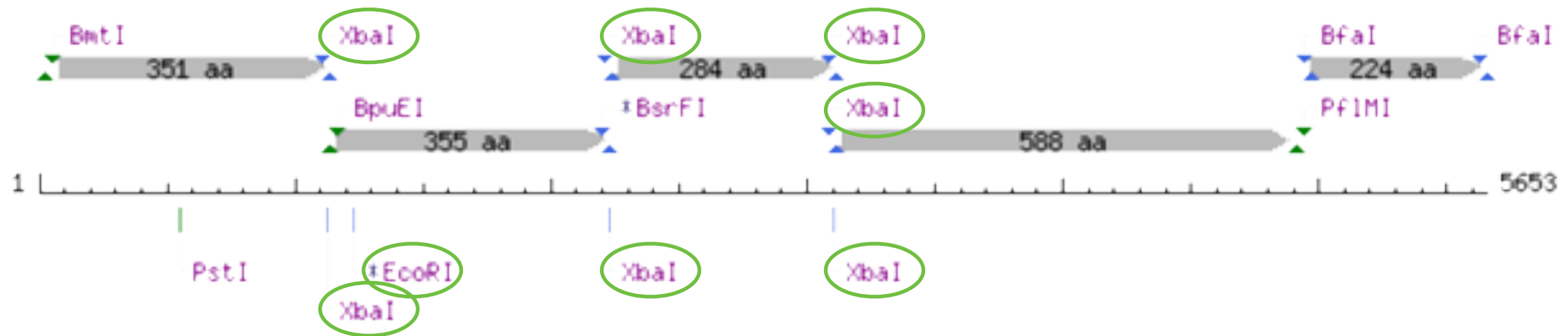


Results

(All w/ pDCAF)	None	Caffeine	Theobromine
Wildtype	+	+	+
Δ GuaB	-	+	+
Top 10	+	+	+



Results

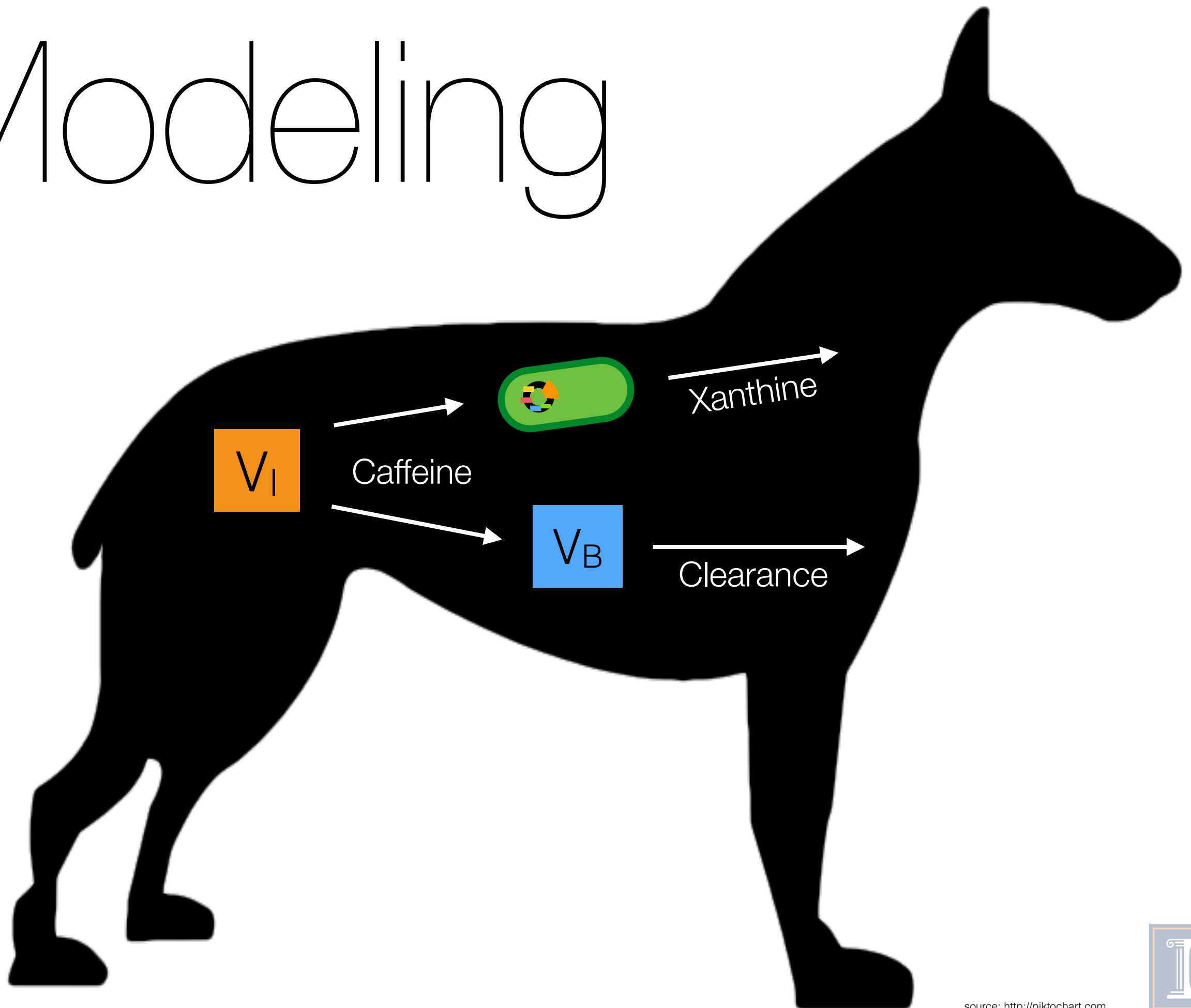


iGEM approved: EcoRI, PstI, SpeI and XbaI

- Unsuccessful Golden Gate Assembly
- CP25 included



Modeling



Basic Model – Michaelis Menten

$$\frac{dC_I}{dt} = \frac{V_{max} * C_I[t]}{k_m + C_I[t]} - (K_T * C_I[t] * \frac{A_I}{V_I})$$

$$\frac{dC_B}{dt} = (K_T * C_I[t] * \frac{A_I}{V_B}) - (k_{cl} * C_b[t])$$

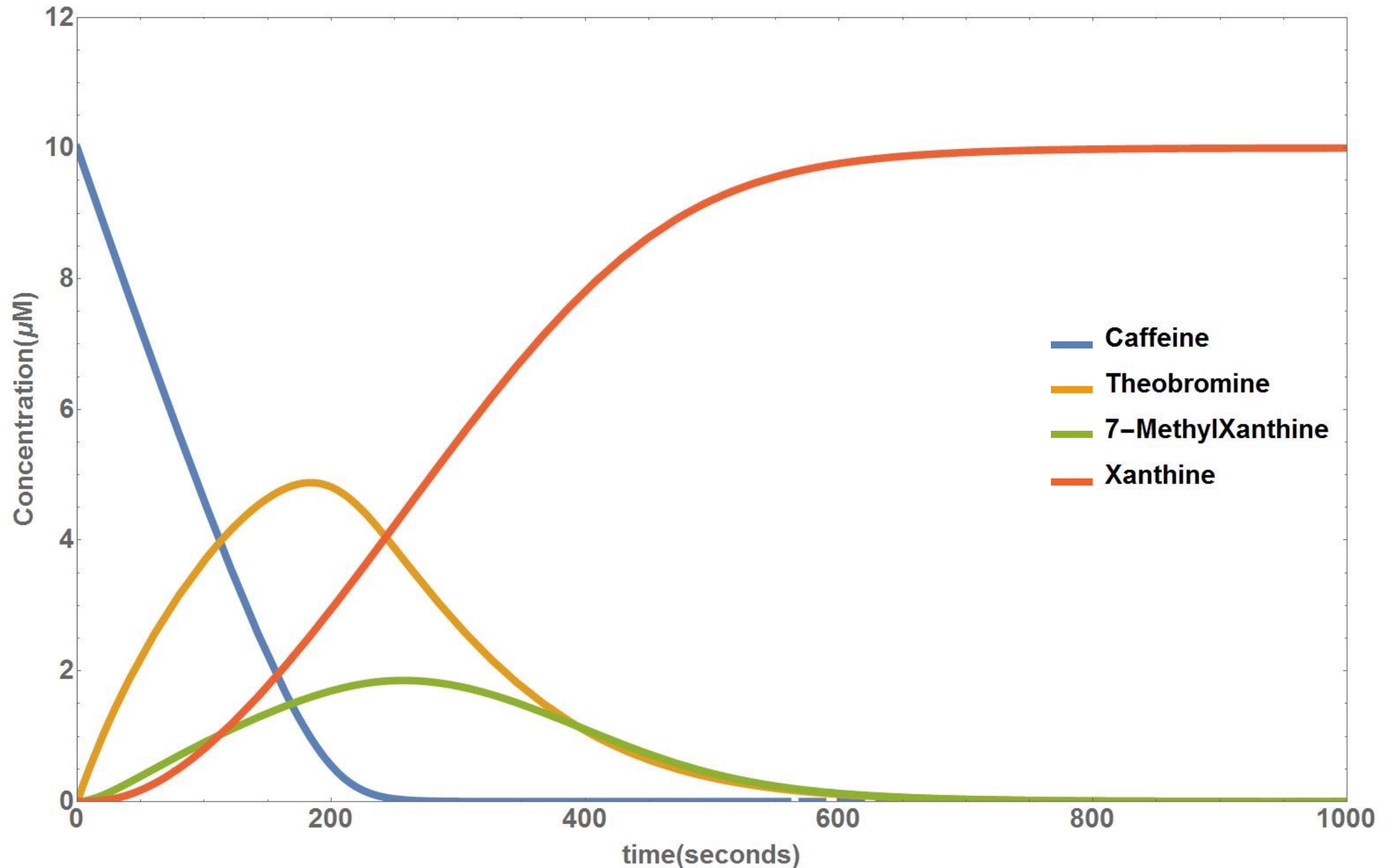
$$\frac{dX_1}{dt} = \frac{-V_{max} * X_1[t]}{k_{m_1} + X_1[t]}$$

$$\frac{dX_2}{dt} = \frac{V_{max} * X_1[t]}{k_{m_1} + X_1[t]} + \frac{V_{max} * X_2[t]}{k_{m_2} + X_2[t]}$$

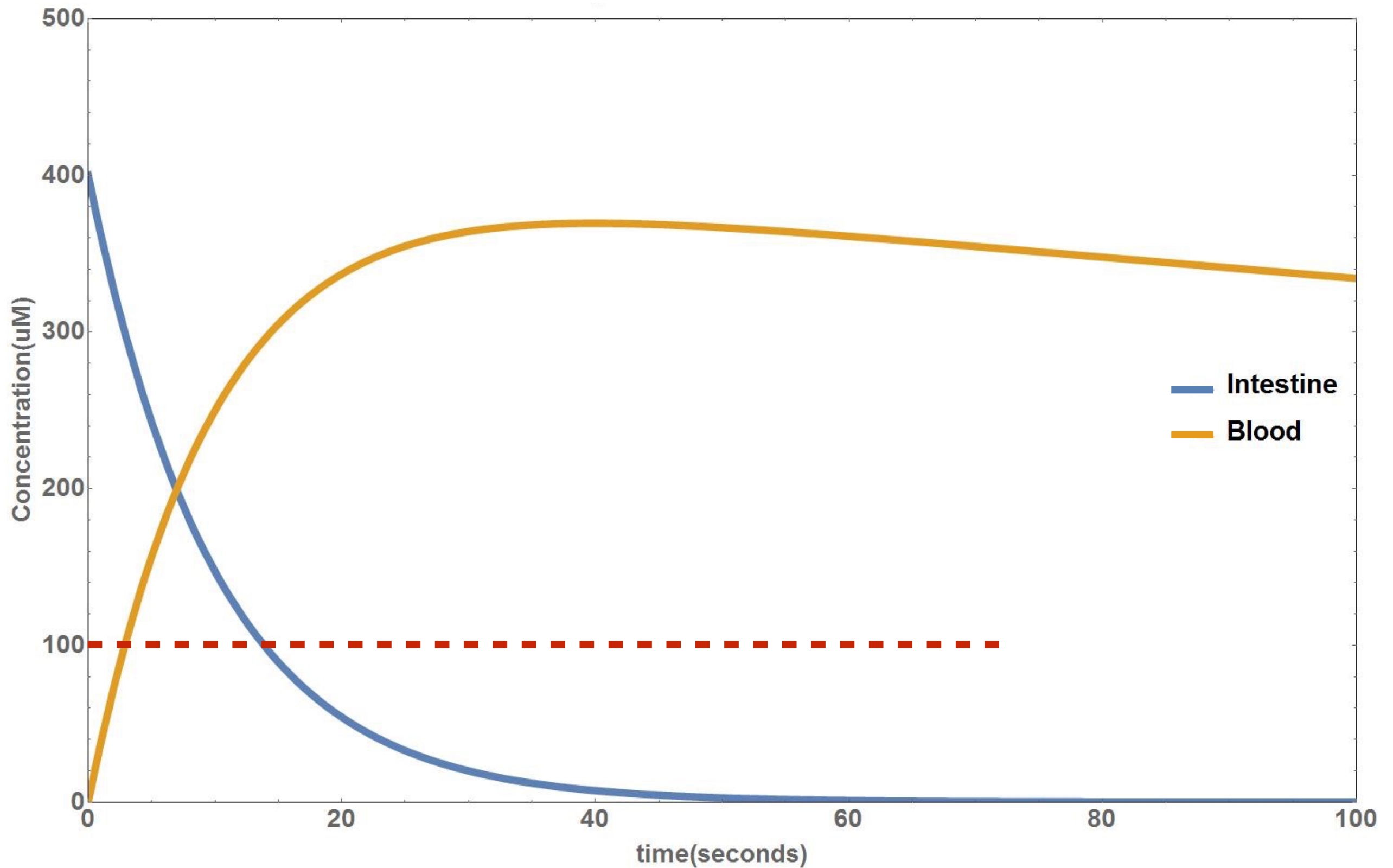
Parameter	Description
n	Number of enzyme concentration in uM
k _{cat}	Max number of substrate molecules converted into products
k _m	Rate of reaction of caffeine by bacteria
k _{cl}	Rate of clearance by blood
k _t	Rate of transfer into blood



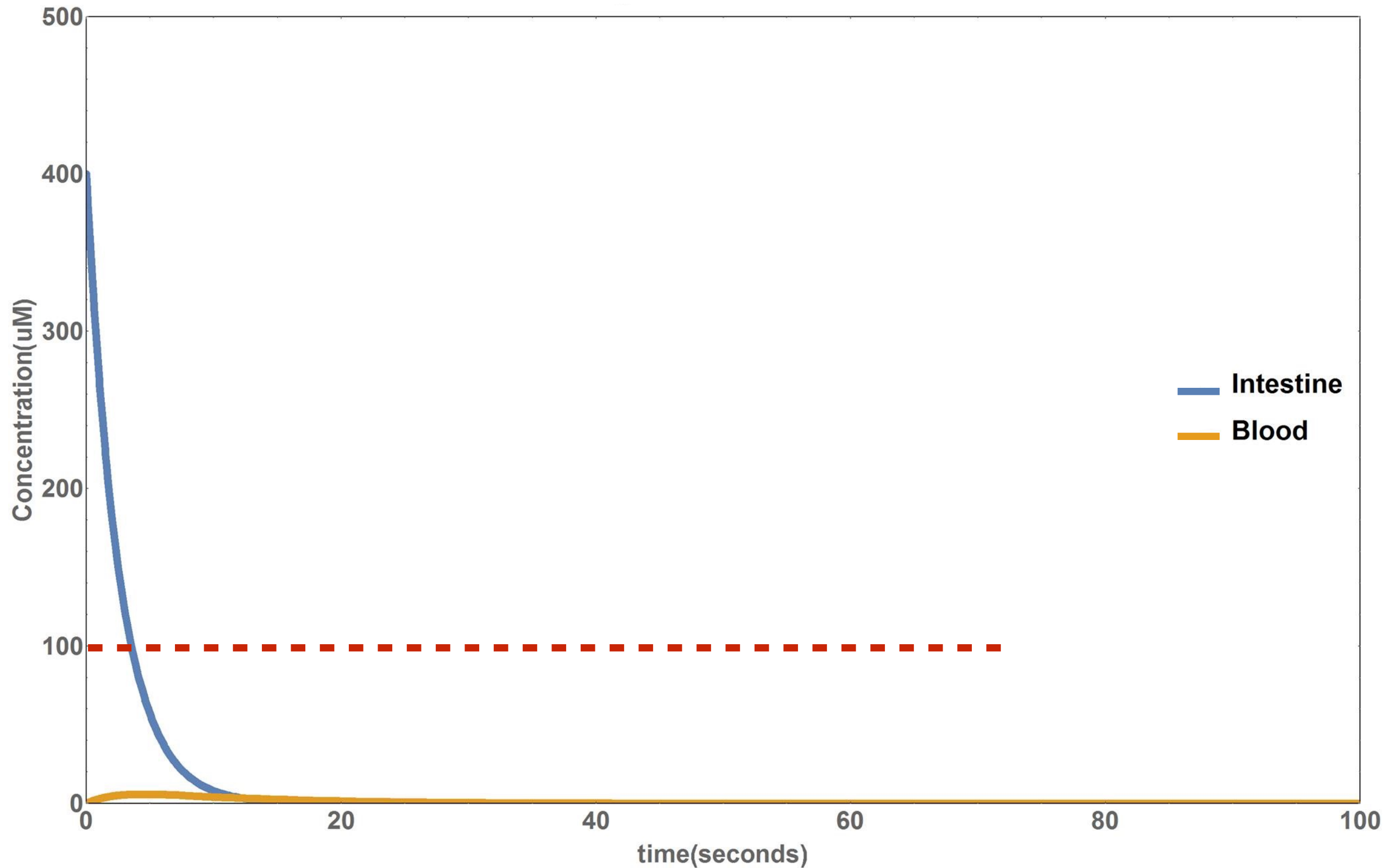
Caffeine Degradation Inside Bacteria



Caffeine Degradation Without Probiotic

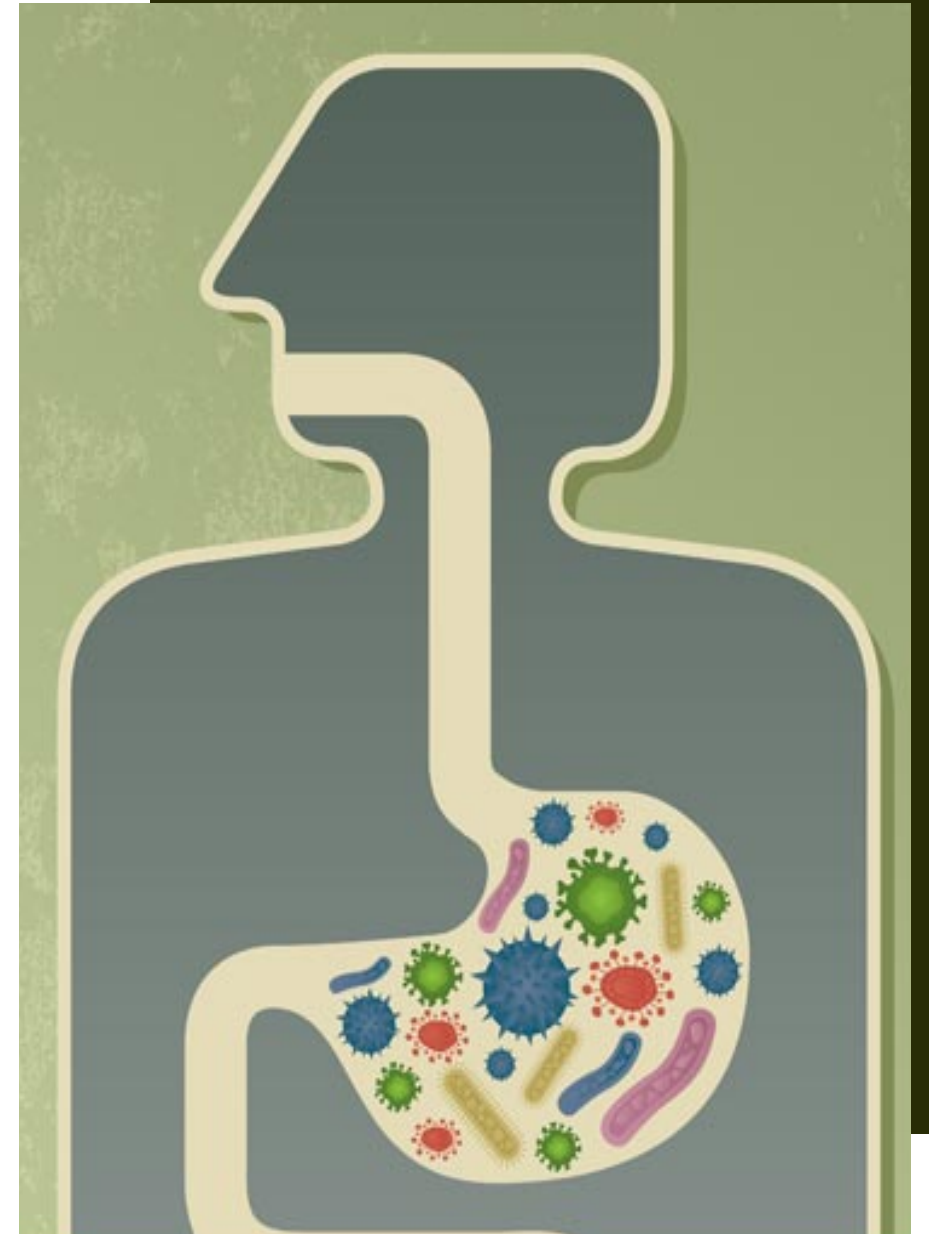


Caffeine Degradation With Probiotic



Future Directions

- Better probiotics
- Delivery system
- Reconstruct microflora
- Environmental remediation





Legality

Liability

Ethics



Cutsultant

- Expedites restriction digestion design
- Generate optimal cuts based on chosen parameters
- Parameters include the band length, available restriction enzymes and inputted sequences, and band differentiability



Cutsultant

```

bash
Arudhirs-MacBook-Pro:iGEM arudhir$ python Cutsultant9.py enzyme_list.txt PQE80L.fasta
What is the smallest band you can measure? 300
What is the large band you can measure? 4000
What is the ratio of band lengths you require? 1.2
What is the maximum number of enzymes you wish you use? 2

The ideal set of restriction enzymes to digest this plasmid to produce maximum cuts are: ('NheI', 'XbaI'), ('XbaI', 'KpnI'), and ('XbaI', 'HindIII')

The ideal set of restriction enzymes to digest this plasmid in the minimum number of enzymes are: ('XbaI',) ('BsaI', 'NheI') ('BsaI', 'PsiI')

The entire set of restriction enzymes to digest this plasmid based on the parameters given are:
[('XbaI',), ('BsaI', 'NheI'), ('BsaI', 'PsiI'), ('BsaI', 'EcoRI'), ('BsaI', 'KpnI'), ('BsaI', 'XhoI'), ('BsaI', 'HindIII'), ('BsaI', 'PstI'), ('BsaI', 'NdeI'), ('BsaI', 'SphI'), ('BsaI', 'SacI'), ('BsaI', 'NcoI'), ('NheI', 'XbaI'), ('NheI', 'NdeI'), ('XbaI', 'KpnI'), ('XbaI', 'HindIII'), ('XbaI', 'PstI'), ('XbaI', 'SphI'), ('XbaI', 'SacI'), ('XbaI', 'NcoI'), ('PsiI', 'NdeI'), ('PsiI', 'NcoI'), ('EcoRI', 'NdeI'), ('EcoRI', 'NcoI'), ('KpnI', 'NdeI'), ('XhoI', 'NdeI'), ('XhoI', 'NcoI'), ('HindIII', 'NdeI'), ('PstI', 'NdeI'), ('NdeI', 'SphI'), ('NdeI', 'SacI'), ('NdeI', 'NcoI')]

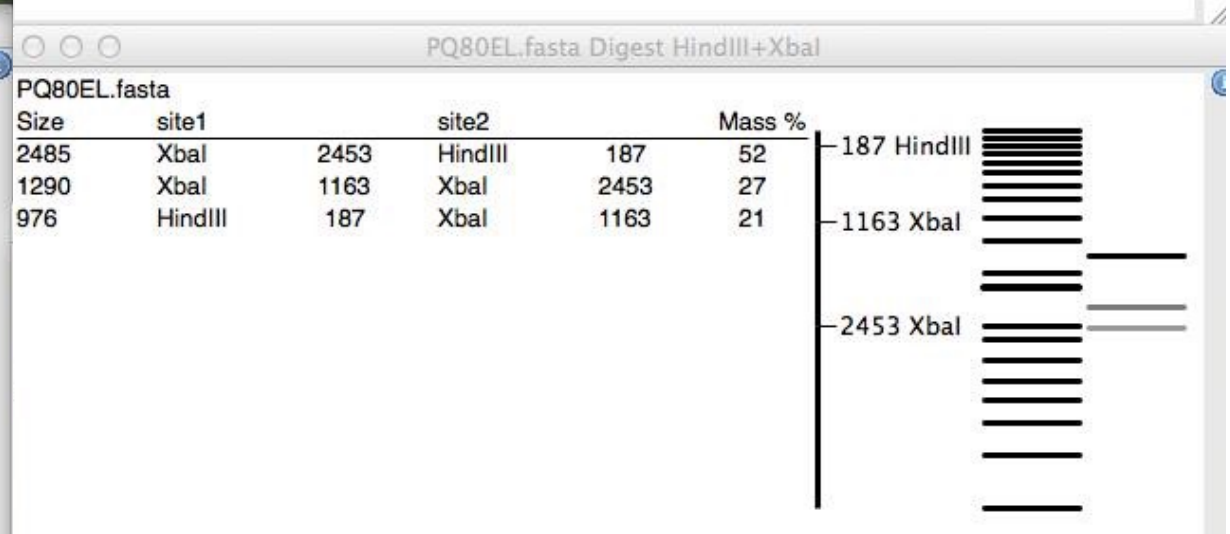
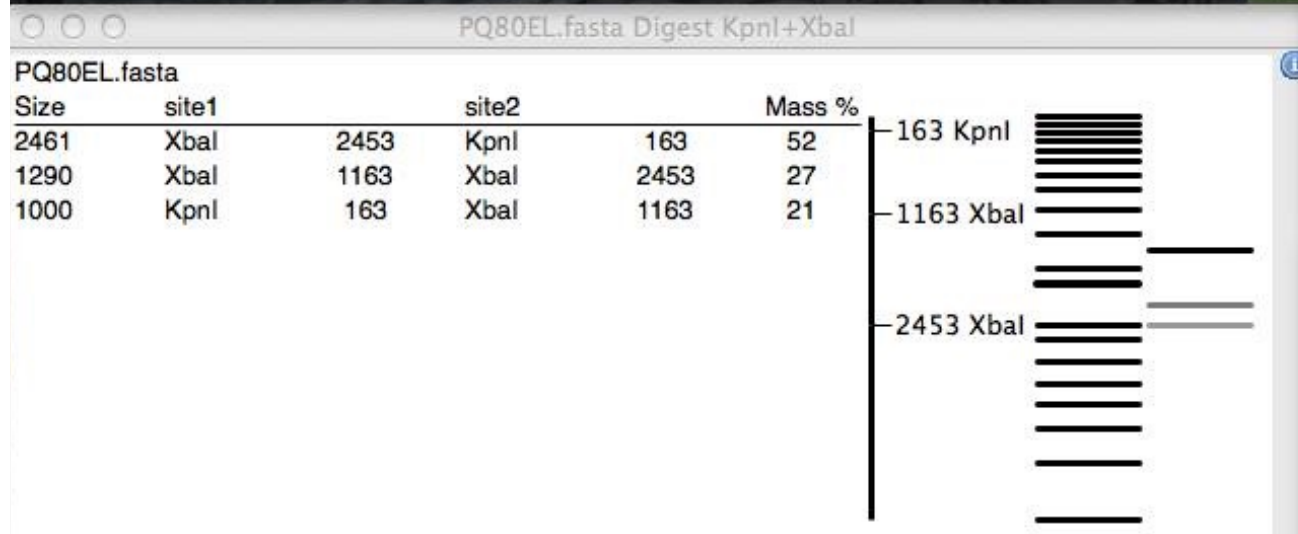
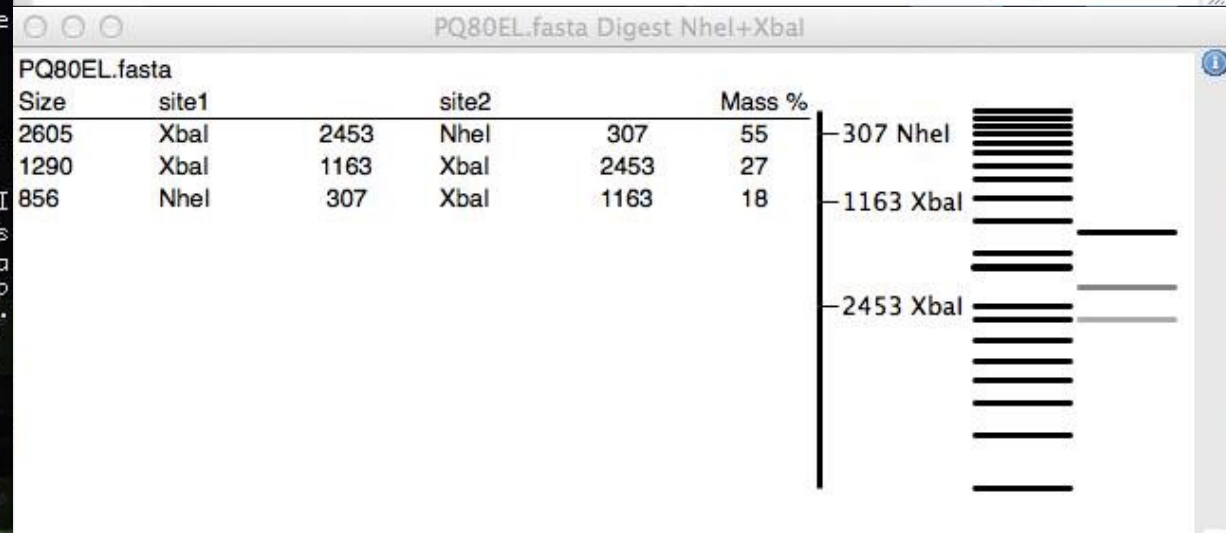
Time to execute: 0.129197120667 seconds
Arudhirs-MacBook-Pro:iGEM arudhir$
    
```

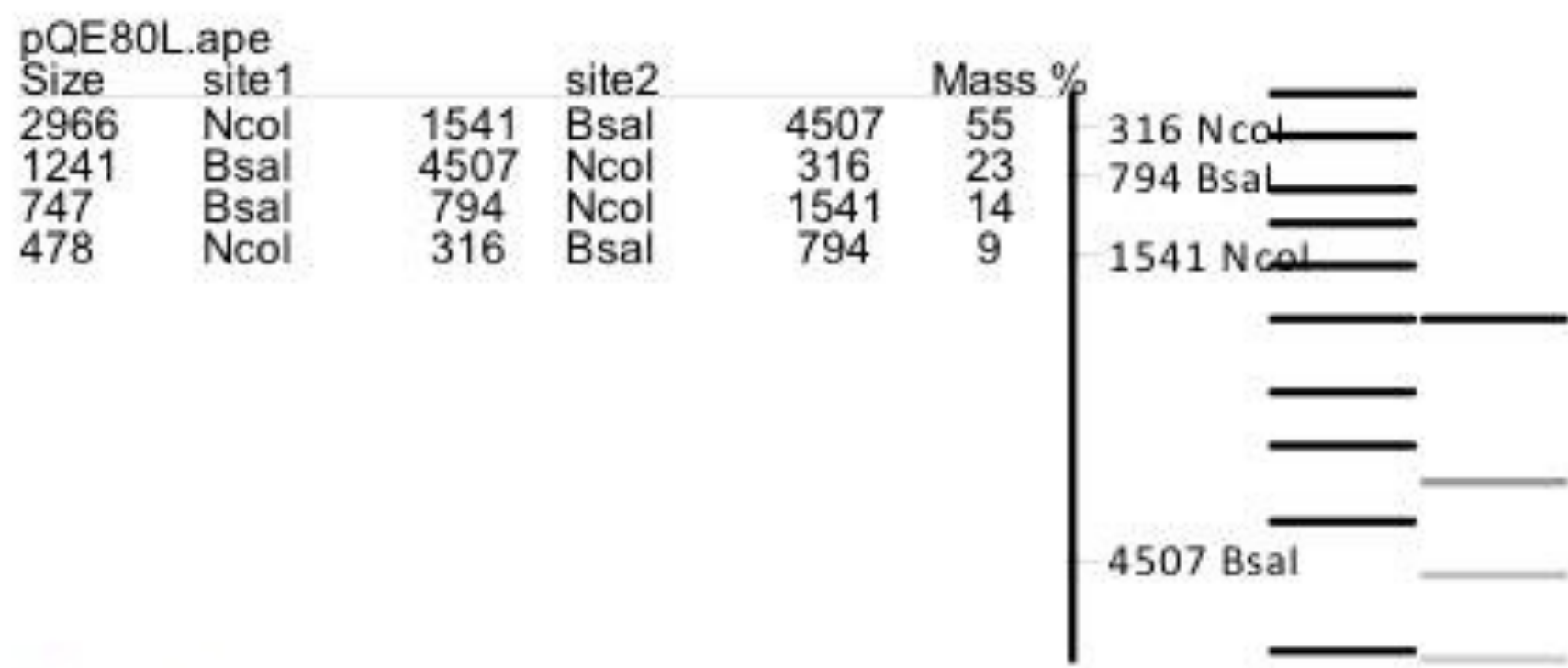
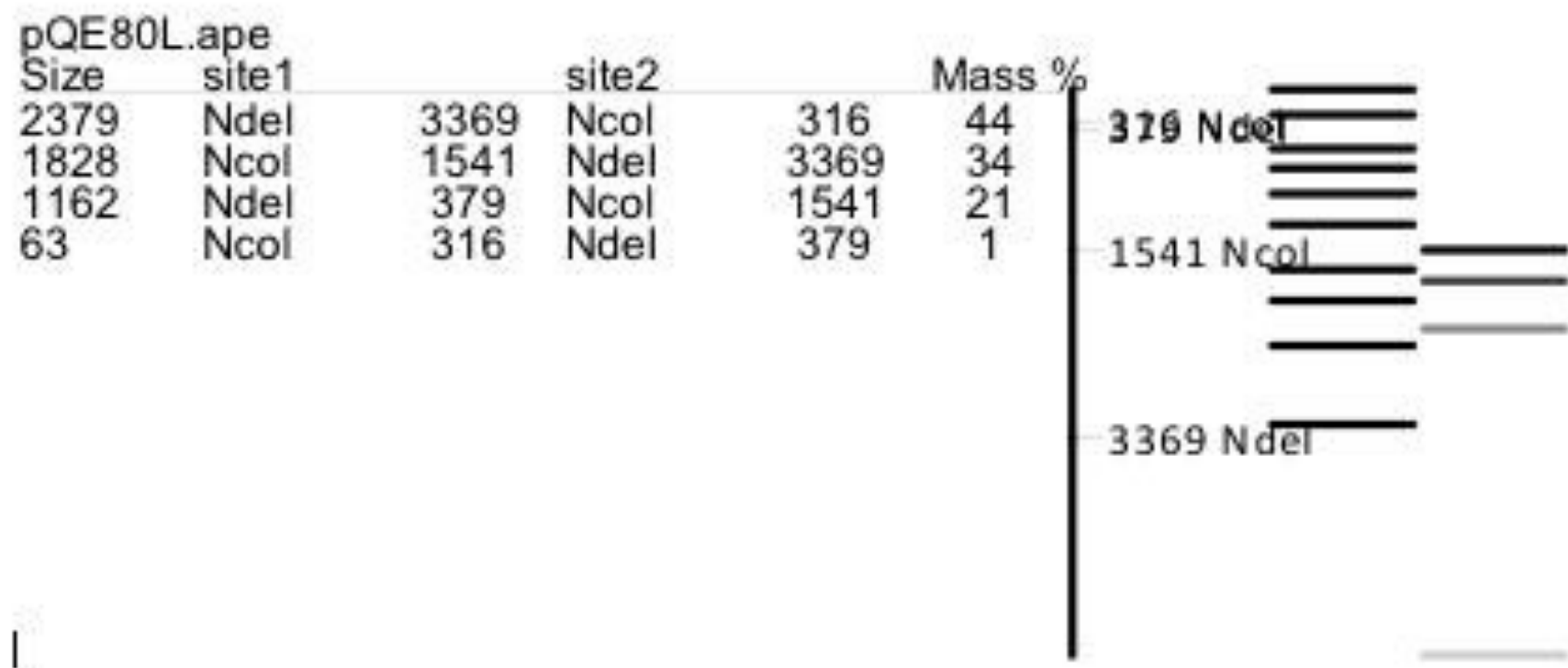
PQE80EL.fasta

Sequence: 4751, Insert@: 3024<2>

Circular: Dam/Dcm

Sequence view showing positions 1, 88, 175, 262 with corresponding DNA sequence.





The Great Race!

```
iGEM — bash — 91x28
bash
Time to execute: 0.129197120667 seconds
Arudhirs-MacBook-Pro:iGEM arudhir$ clear

Arudhirs-MacBook-Pro:iGEM arudhir$ python Cutsultant9.py enzyme_list.txt PQE80L.fasta PQE80L-GFP.fasta
What is the smallest band you can measure? 300
What is the large band you can measure? 4000
What is the ratio of band lengths you require? 1.2
What is the maximum number of enzymes you wish you use? 2

November 2014
The ideal set of restriction enzymes to digest this plasmid to produce maximum cuts are: ('BsaI', 'NcoI'), ('NheI', 'XbaI'), and ('XbaI', 'HindIII')

The ideal set of restriction enzymes to digest this plasmid in the minimum number of enzymes are: ('BsaI', 'KpnI'), ('BsaI', 'PstI'), ('BsaI', 'SphI')

The entire set of restriction enzymes to digest this plasmid based on the parameters given are:
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Time to execute: 0.132457971573 seconds
```



Cutsultant

1.43 Seconds

Todd

4.5 minutes

Thank you!

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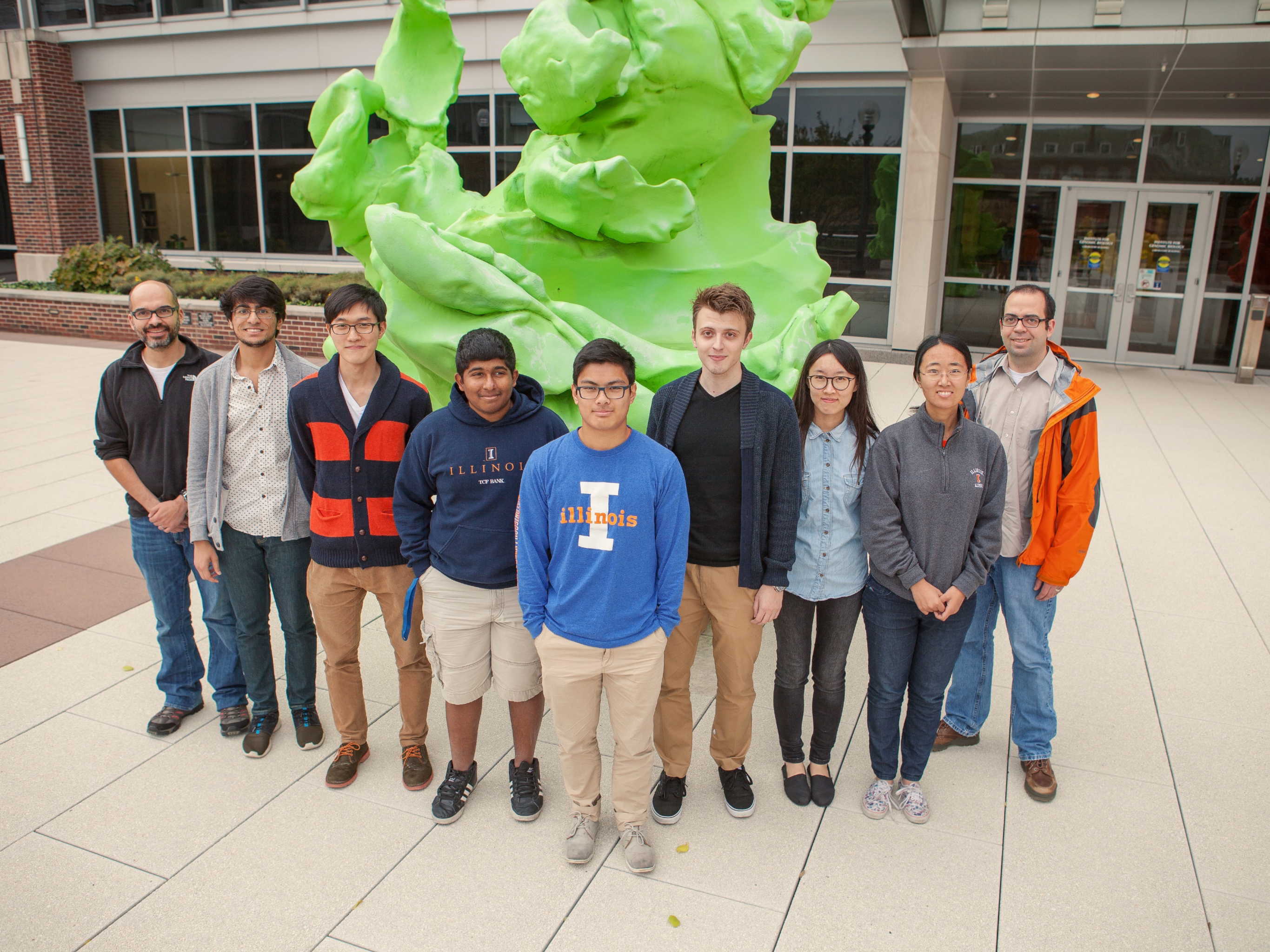


Citations



- [1] **Chen, Q., Wang, C.-H., Deng, S.-K., Wu, Y.-D., Li, Y., Yao, L., ... Li, S.-P.** (2014). Novel three-component Rieske non-heme iron oxygenase system catalyzing the N-dealkylation of chloroacetanilide herbicides in sphingomonads DC-6 and DC-2. *Applied and Environmental Microbiology*, 80(16), 5078–85. doi:10.1128/AEM.00659-14
- [2] **Dash, S. S., & Gummadi, S. N.** (2006). Catabolic pathways and biotechnological applications of microbial caffeine degradation. *Biotechnology Letters*, 28(24), 1993–2002. doi:10.1007/s10529-006-9196-2
- [3] **Kouamé, B., Marcel, G., André, K. B., & Séraphin, K.** (2011). Potential Food Waste and By-products of Coffee in Animal Feed, 7(4), 74–80.
- [4] **Madyastha, K. M., Sridhar, G. R., Vadiraja, B. B., & Madhavi, Y. S.** (1999). Purification and partial characterization of caffeine oxidase--A novel enzyme from a mixed culture consortium. *Biochemical and Biophysical Research Communications*, 263(2), 460–4. doi:10.1006/bbrc.1999.1401
- [5] **Pandey, A., Soccol, C. R., Nigam, P., Brand, D., Mohan, R., & Roussos, S.** (2000). Biotechnological potential of coffee pulp and coffee husk for bioprocesses. *Biochemical Engineering Journal*, 6(2), 153–162. doi:10.1016/S1369-703X(00)00084-X
- [6] **Quandt, E. M., Hammerling, M. J., Summers, R. M., Otoupal, P. B., Slater, B., Alnahhas, R. N., ... Barrick, J. E.** (2013). Decaffeination and measurement of caffeine content by addicted *Escherichia coli* with a refactored N-demethylation operon from *Pseudomonas putida* CBB5. *ACS Synthetic Biology*, 2(6), 301–7. doi:10.1021/sb4000146
- [7] **Yu, C. L., Kale, Y., Gopishetty, S., Louie, T. M., & Subramanian, M.** (2008). A novel caffeine dehydrogenase in *Pseudomonas* sp. strain CBB1 oxidizes caffeine to trimethyluric acid. *Journal of Bacteriology*, 190(2), 772–6. doi:10.1128/JB.01390-07
- [8] **Yu, C. L., Louie, T. M., Summers, R., Kale, Y., Gopishetty, S., & Subramanian, M.** (2009a). Two distinct pathways for metabolism of theophylline and caffeine are coexpressed in *Pseudomonas putida* CBB5. *Journal of Bacteriology*, 191(14), 4624–32. doi:10.1128/JB.00409-09





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