UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

Illinois iGEM 2009

Bacterial Decoder

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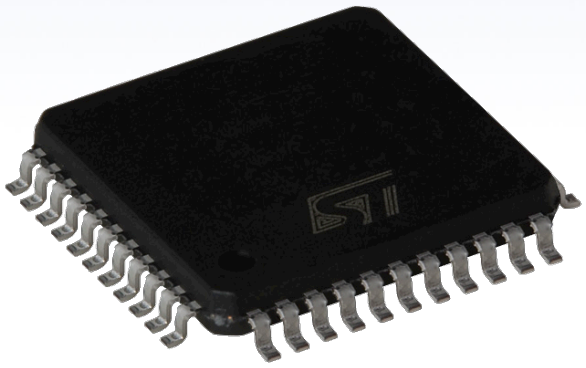
Our Goal

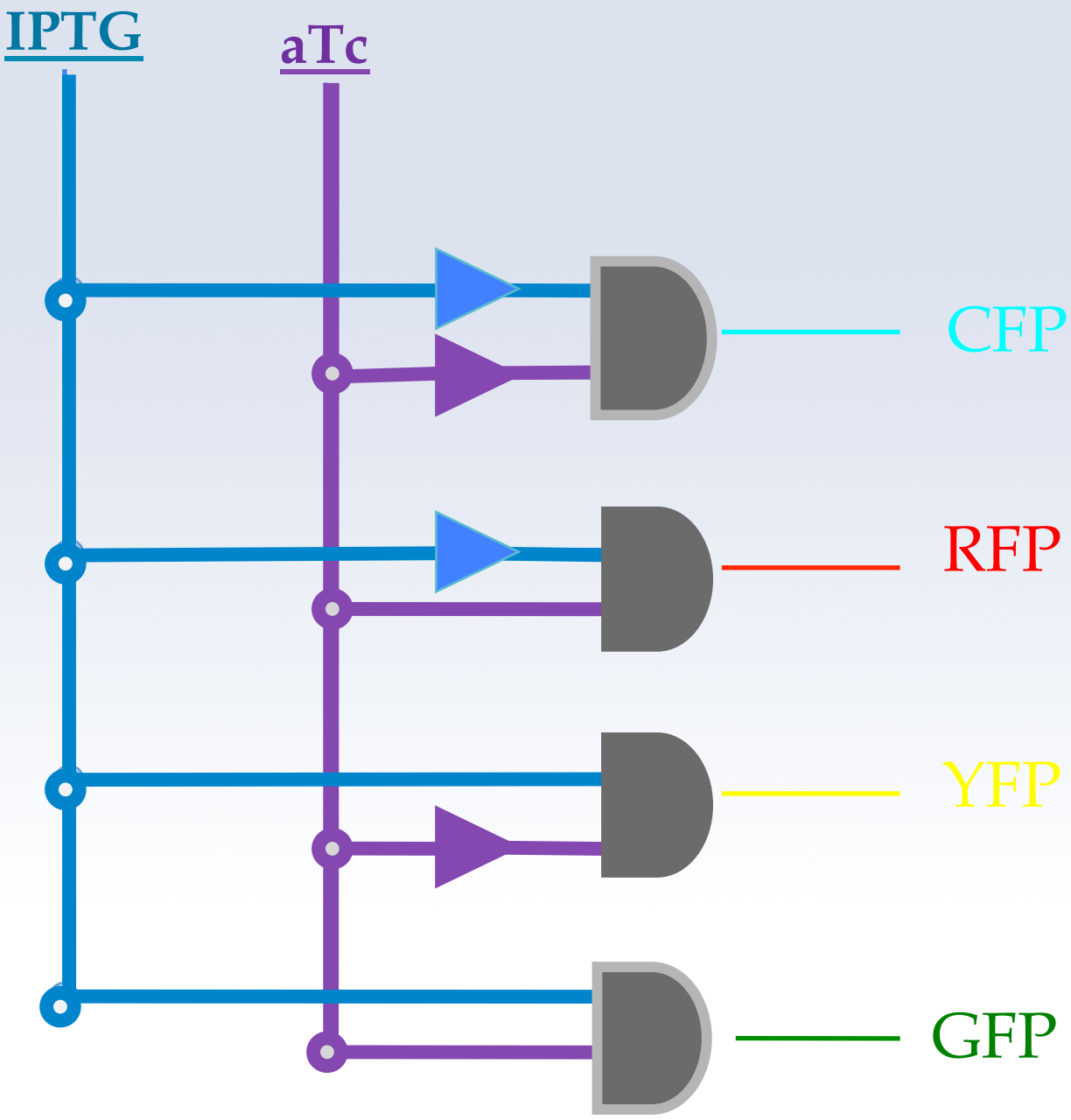
"To create a universal, **modular**, and dynamic bacterial **decoder** to be used as a tool in a variety of **cellular logic** applications, while also contributing novel **BioBricks** to the Registry of Standard Biological Parts."

The Bacterial Decoder

- A logic system that identifies combinations of specific inputs to express outputs
  - 2:4 3:8 4:16 $n:2^n$
- Involves AND gates, NOR gates, and inverters
  - Made from regulation on translational and transcriptional levels



IPTG	aTc	FP
0	0	C
0	1	R
1	0	Y
1	1	G



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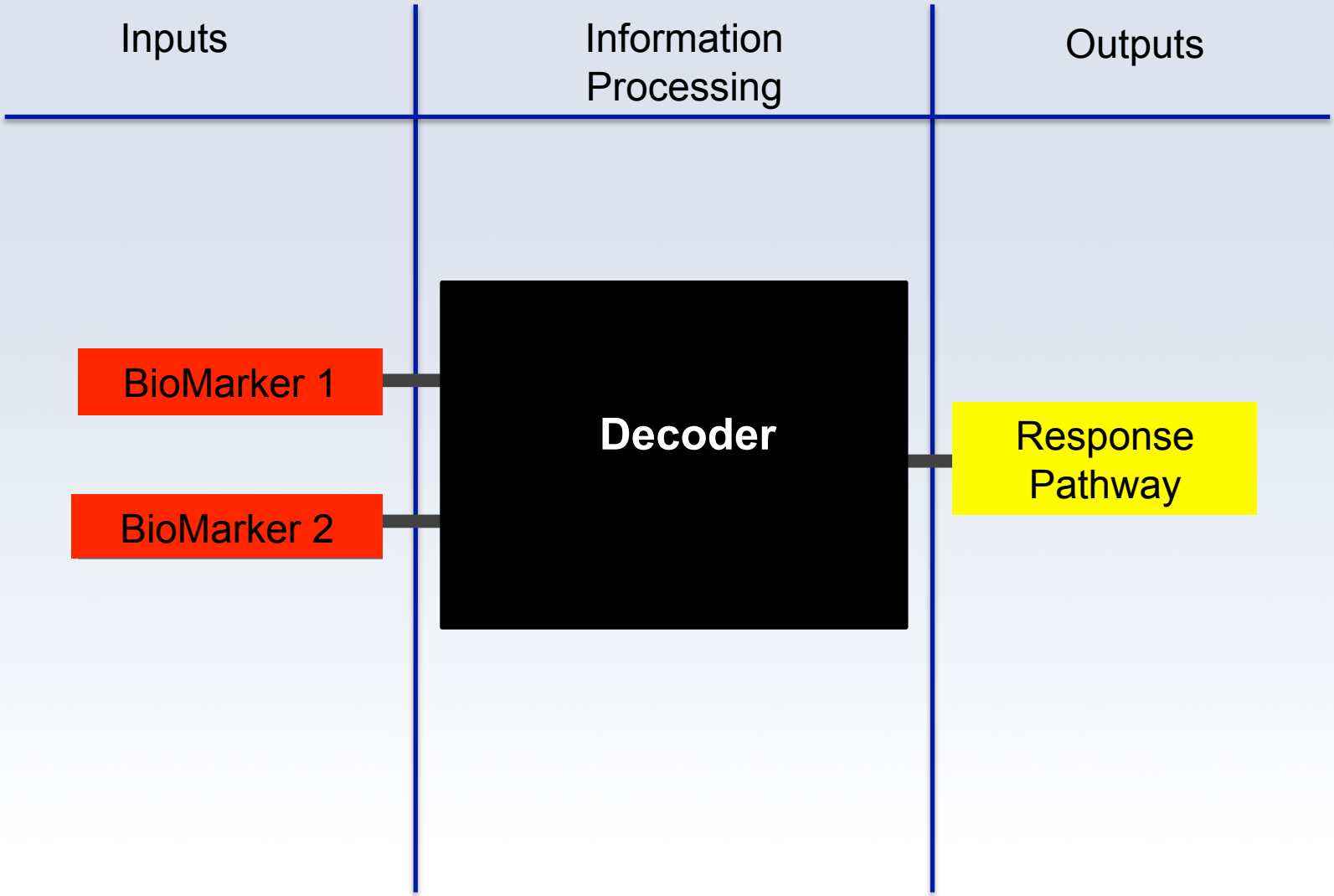
A Genetic “if” Statement

```
if (IPTG && aTc){
  return GFP;
}

else if (IPTG && !aTc) {
  return YFP;
}

else if (!IPTG && aTc) {
  return RFP;
}

else if (!IPTG && !aTc) {
  return CFP;
}
```

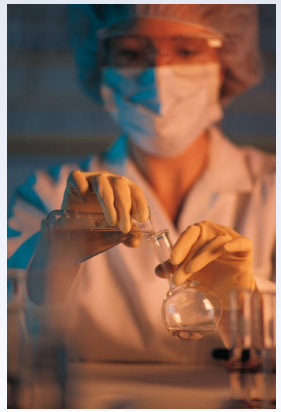

Inputs
Information Processing
Outputs
BioMarker 1
BioMarker 2
Decoder
Response Pathway

Use and Future Applications

Sensing for multiple water contaminants

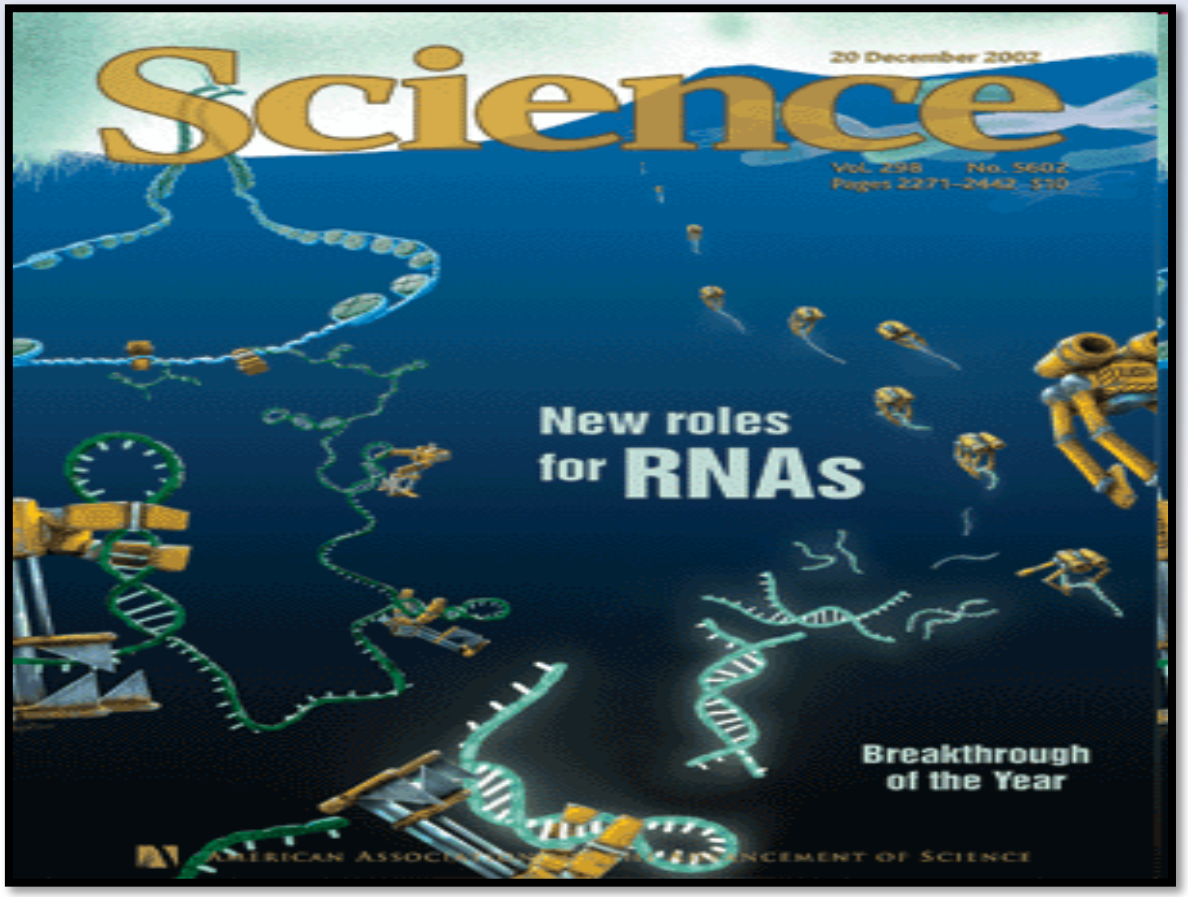
Complex logic for cellular computer

Medical diagnostics and treatment



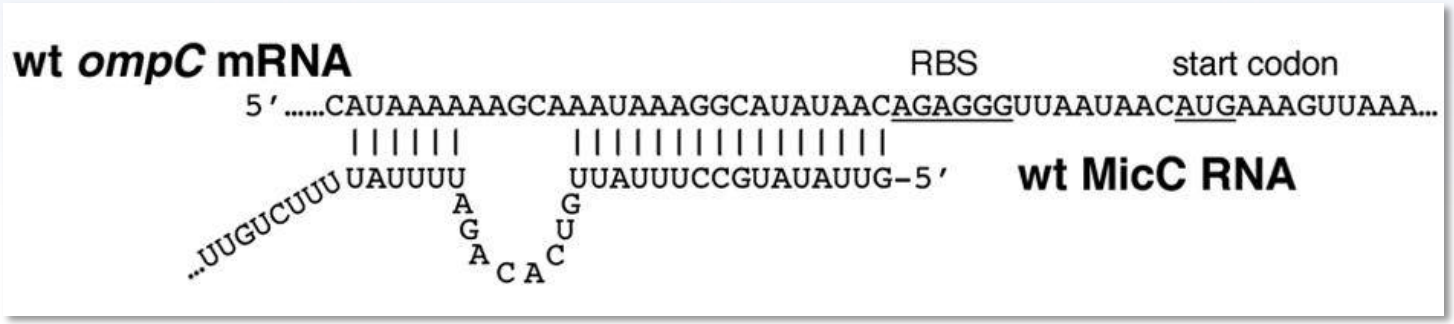
Approach

Gene regulation using transcription factors and small RNAs.



Small RNAs (sRNAs)

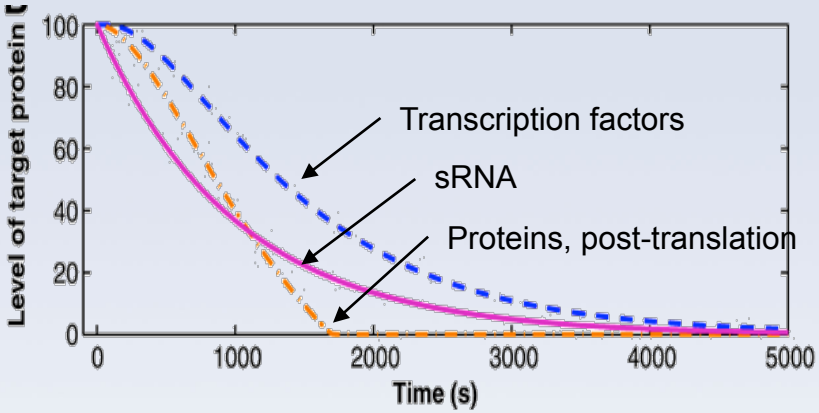
- Can repress protein synthesis
- Mechanisms of *trans*-encoded Hfq binding inhibitory sRNAs:
  - RBS occlusion
  - Degradation



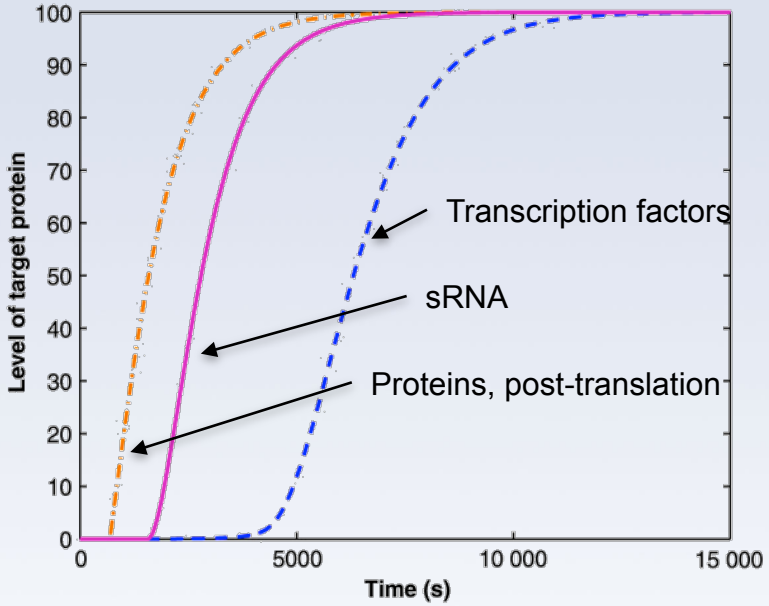
"MicC, a second small-RNA regulator of Omp protein expression in Escherichia coli." Chen S, Zhang A, Blyn LB, Storz G.IBIS Therapeutics, ISIS Pharmaceuticals, Inc., Carlsbad, California, USA

sRNA Advantages

Protein Repression vs. Time



Protein Recovery vs. Time



- Quick response times are ideal for state changes
- sRNA regulatory mechanisms are cost effective

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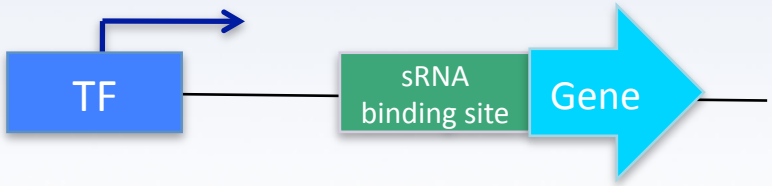
"Regulation of gene expression by small non-coding RNAs: a quantitative view", Yishai Shimoni, Gilgi Friedlander, Guy Hetzroni, Gali Niv, Shoshy Altuvia, Ofer Biham & Hanah Margalit, 2007

Implementing sRNA in a decoder

- Small RNAs are used in concert with transcription factors to regulate gene expression.
- Created a NOR gate with TF and Small RNAs.



Decoder Schematic

IPTG	aTc
0	0
0	1
1	0
1	1

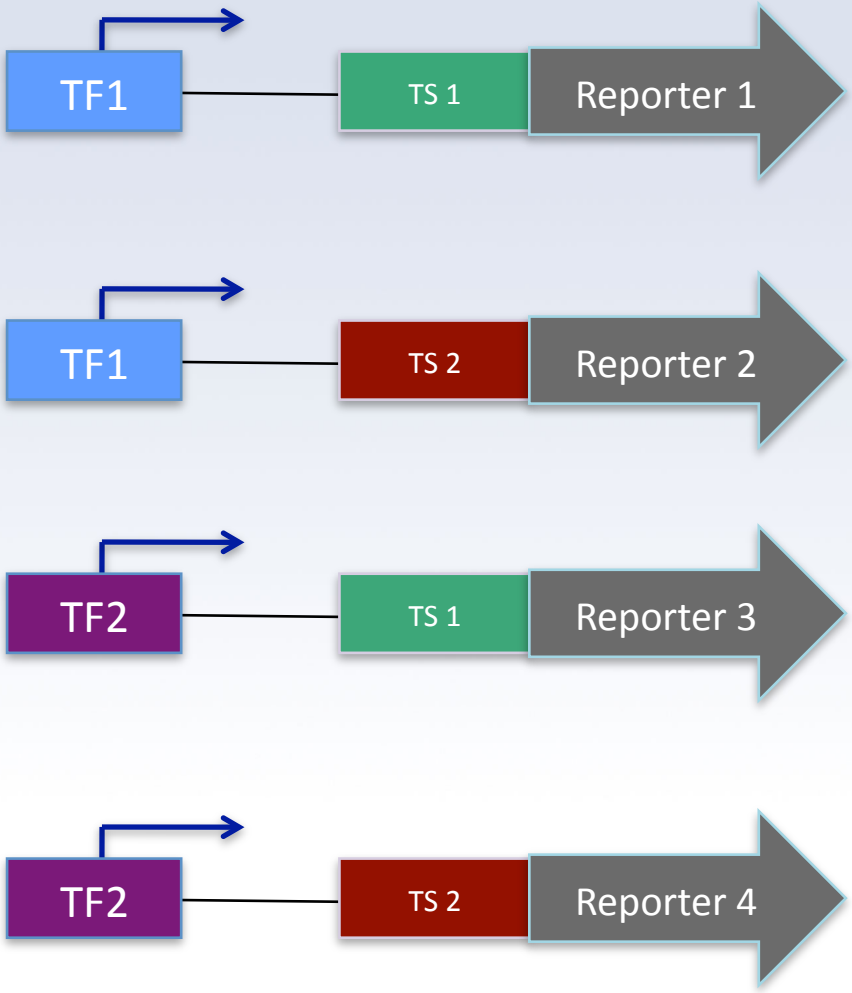
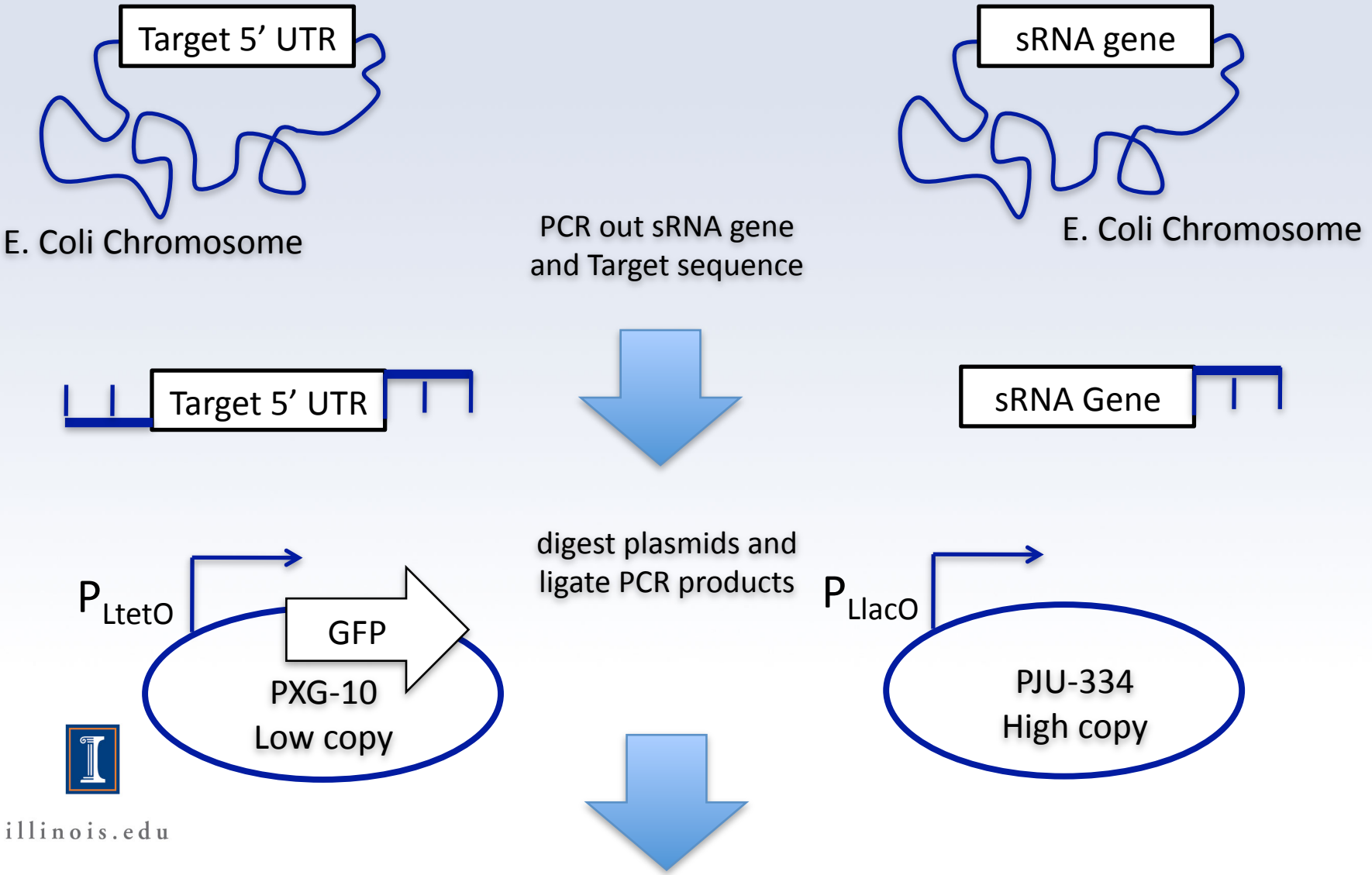


Diagram of Method



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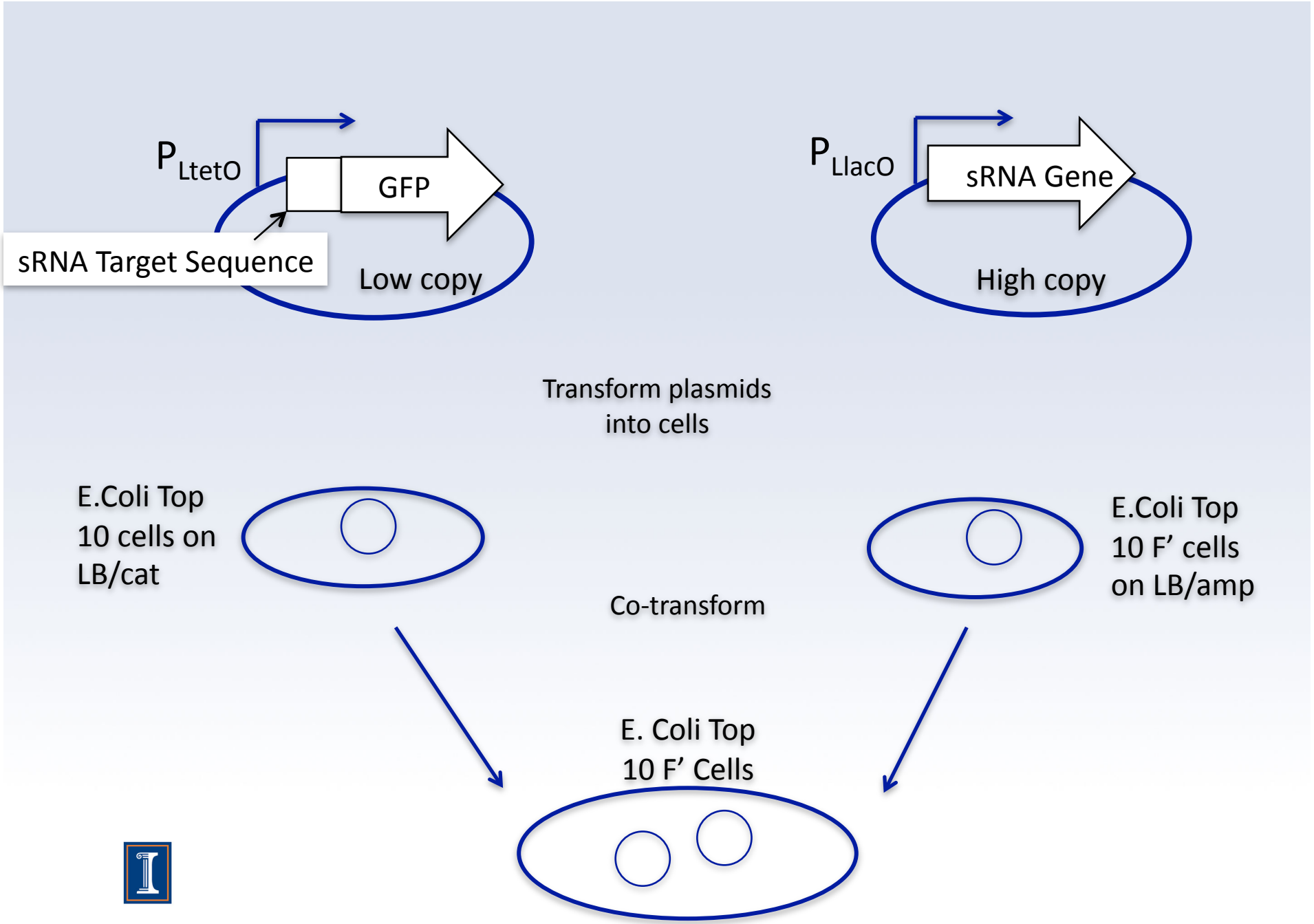
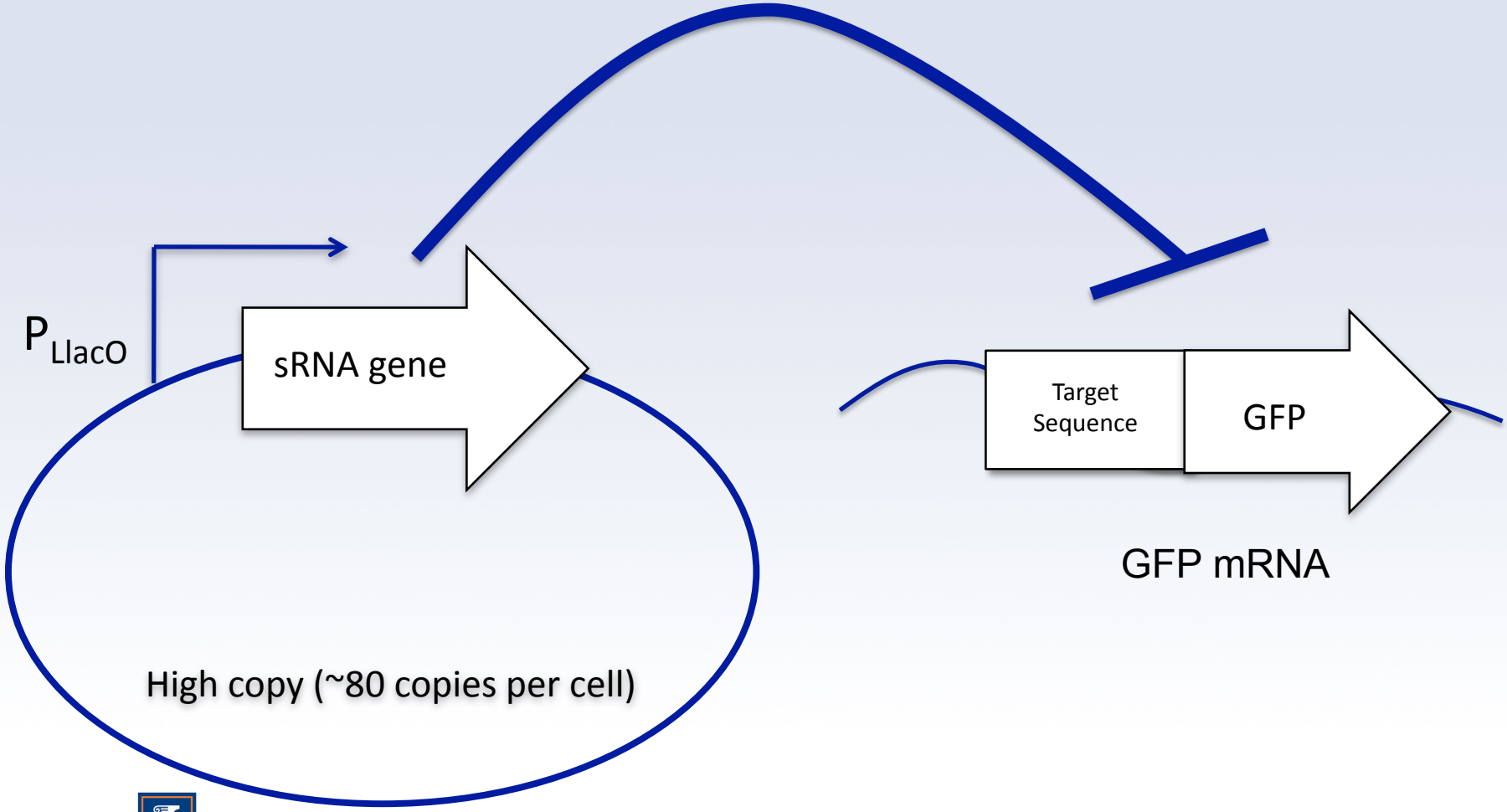
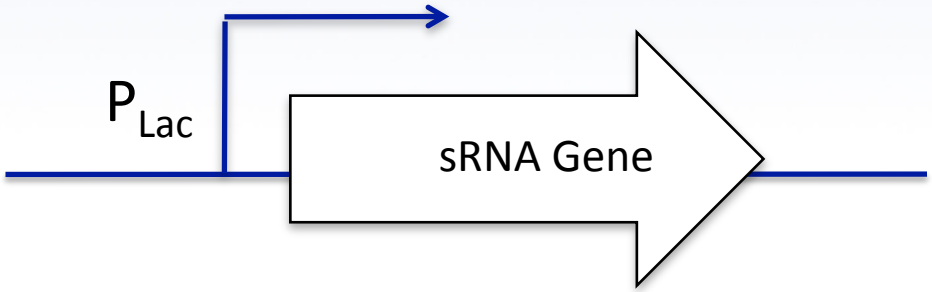

P LtetO
GFP
sRNA Target Sequence
Low copy
P LlacO
sRNA Gene
High copy
Transform plasmids into cells
E.Coli Top 10 cells on LB/cat
E.Coli Top 10 F' cells on LB/amp
Co-transform
E. Coli Top 10 F' Cells

Genetic Circuit



sRNA preparation

- sRNAs should have a blunt 5' end
  - Incompatible with the standard BB format
- Under standard lac promoter
  - BBa_R0010
- Typically expressed alone under a promoter



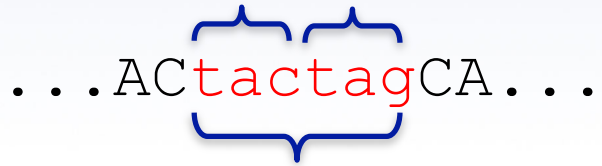
Target sequence preparation

- RBS containing target sequences are fused upstream of the GFP reporter
- Standard BioBrick “prefixes and suffixes” would not allow for an in frame fusion.

Standard BioBrick assembly

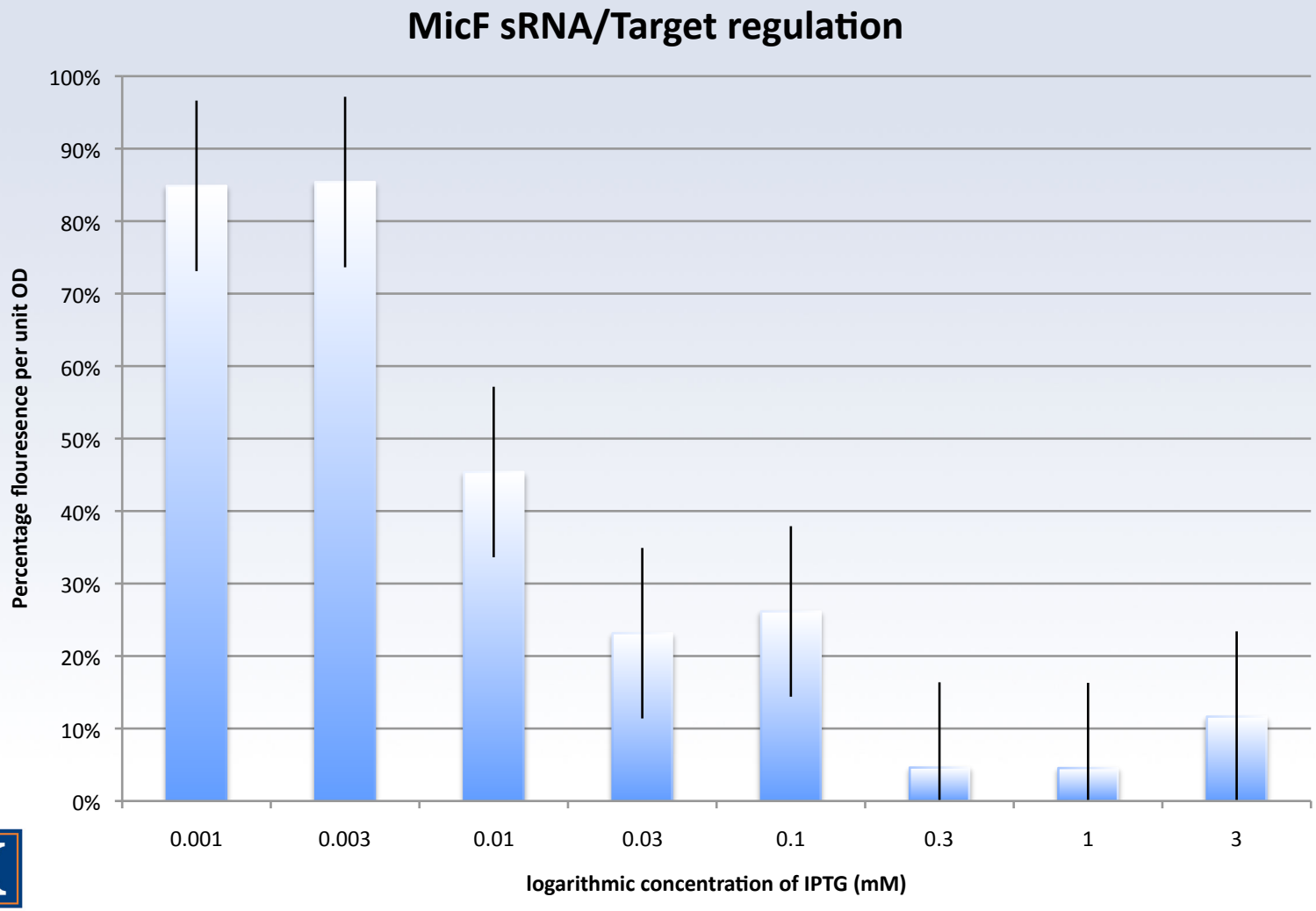
8bp → Frame shift

Freiburg Assembly Standard



6bp → No frame shift

MicF regulation



Submitted Parts

sRNA gene BioBricks	5' Target Sequence BioBricks
MicC	OmpC
MicF	OmpF
MicA	OmpA
SgrS	Ptsg
GcvB	Oppa & Dppa
DicF	Ftsz
	galK

Status and Conclusion

- Biology is hard.
- We created several of the constructs for the decoder, but didn't have time to get it in working order
- We are submitting 14 novel BioBricks - the first sRNAs!

Advisors

Dr. Ido Golding

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Dr. Chris Rao

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Acknowledgments

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ENGINEERING AT ILLINOIS

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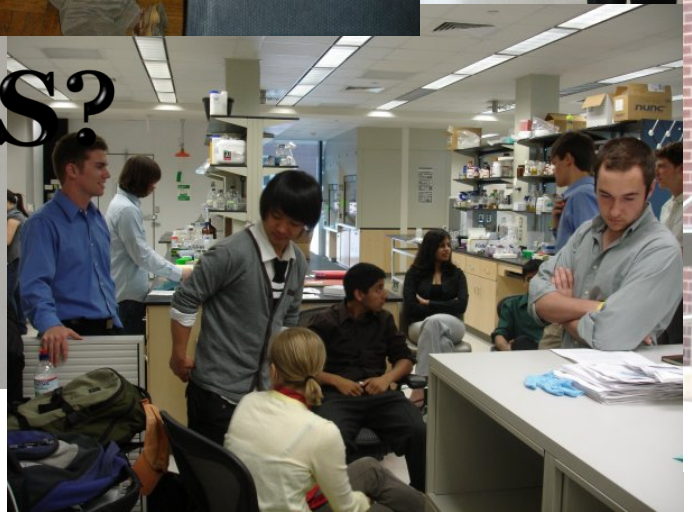


QUESTIONS?