Synthetic Gene Networks That Count

Ari E. Friedland, Timothy K. Lu, Xiao Wang, David Shi, George Church, James J. Collins.

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Review + Motivation

- What do we need for a counting?

  Memory + Mechanism
- Natural memory:
  - DNA sequence.
  - Level of protein concentration. (Temporary, but could be not very short in time)
- There are a lot of stable mechanisms in a cell.
- Steps to biocomputers:
  - Whole-cell biocomputing. TRENDS in Biotechnology 19 (2001).
    - Logical operation AND and OR.
  - Programmable single-cell mammalian biocomputers. Nature 487 (2012).
    - An idea of digital computations with NOT, AND, NAND and N-IMPLY expression logic in single mammalian cells.
- Why can’t cells count? They can!

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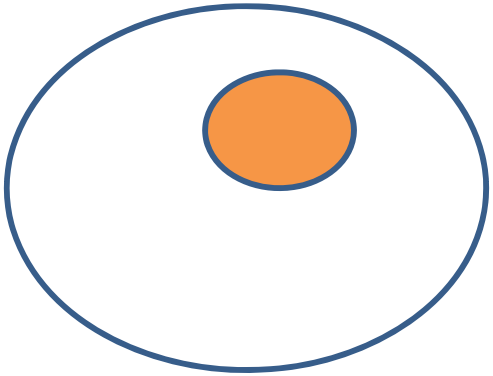
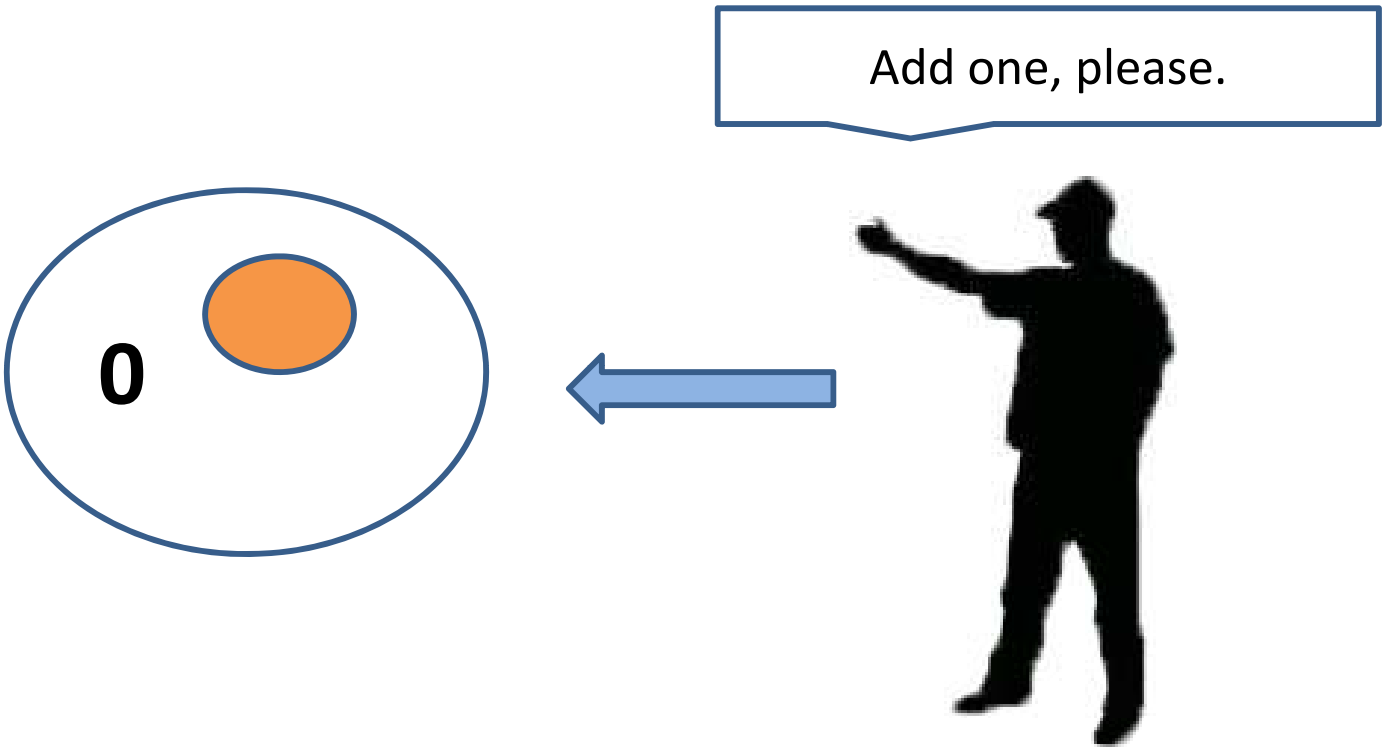
- Why can’t cells count? They can!

Counting mechanism

I’m a cell. I’ve got a memory.
Now I remember number **0**!

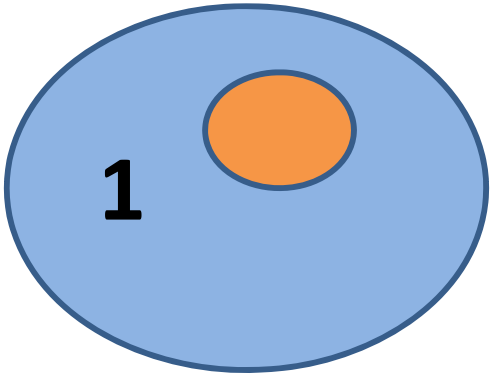


Counting mechanism

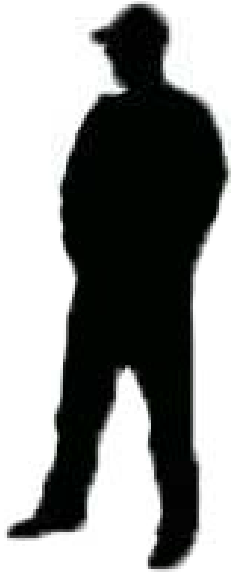


Counting mechanism

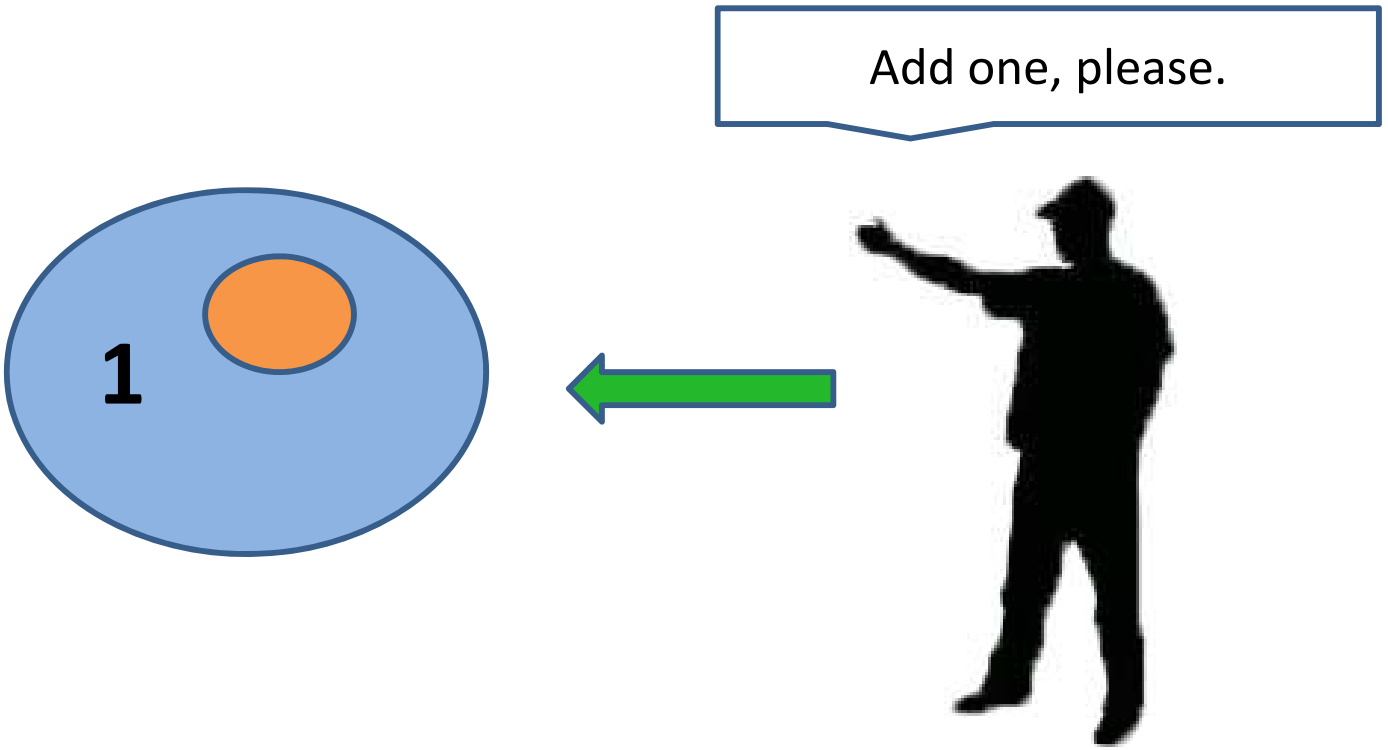
Ok.



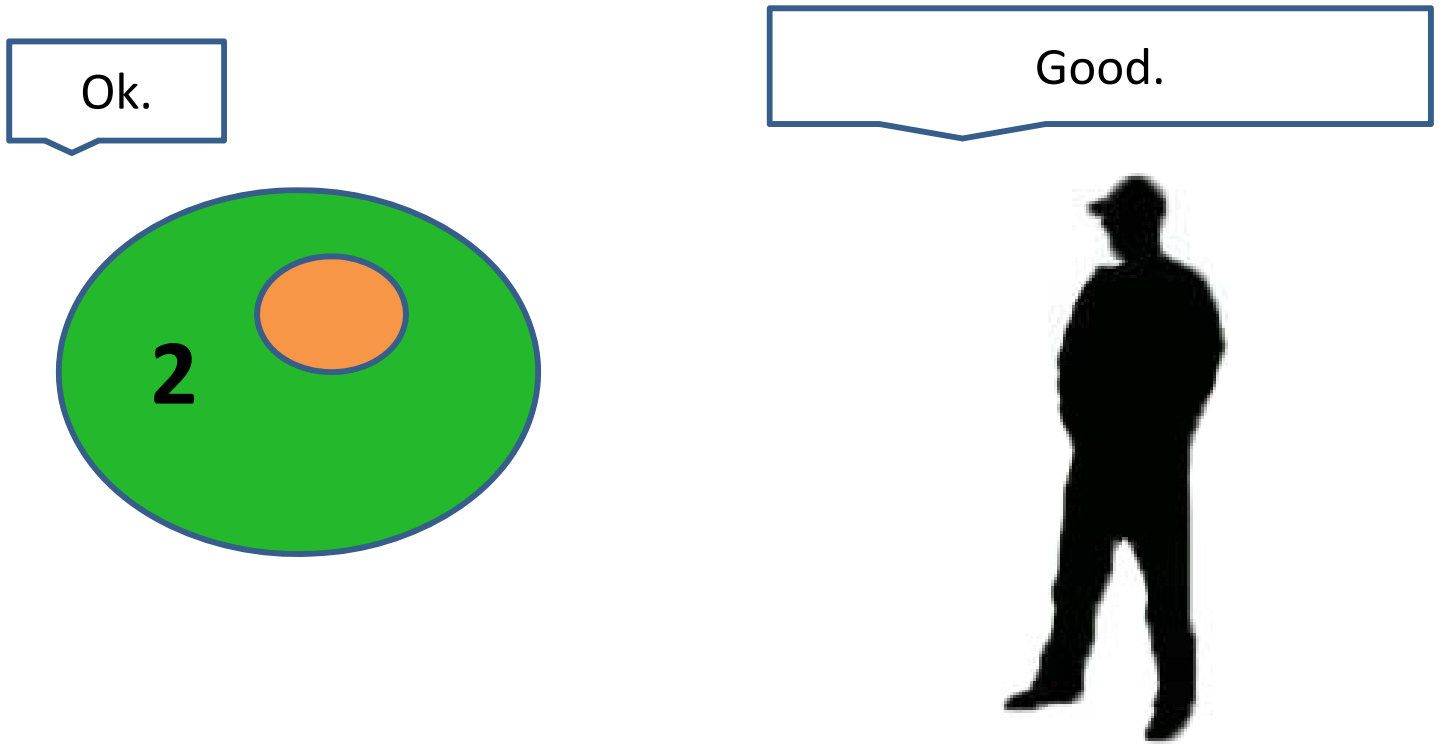
Good.



Counting mechanism

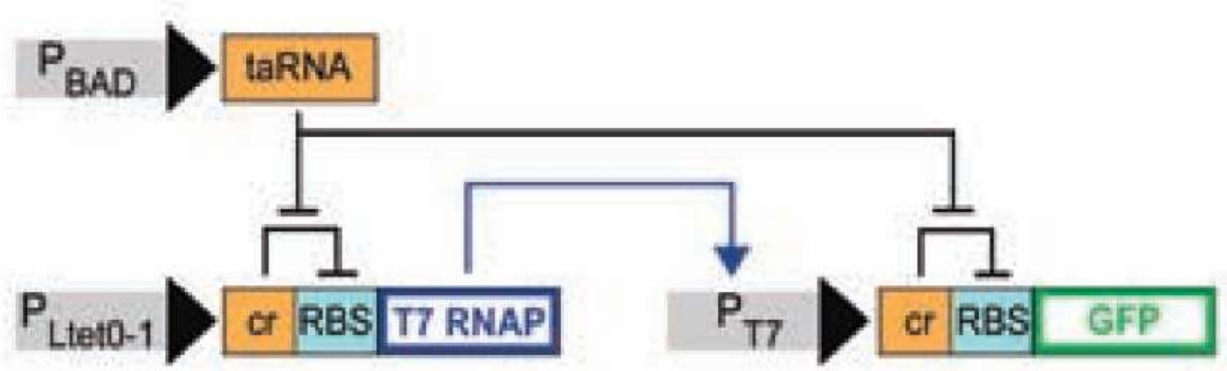


Counting mechanism



“Memory” : concentration of some protein
Regulation : extrinsical chemical stimulius

Model 1: Riboregulated transcriptional cascade(RTC), two-counter



- The cis-repressor sequence (cr) is complimentary with ribosome binding site (RBS).
- A stem-loop in a secondary structure of RNA prevents binding of ribosome (30S).
- A short noncoding taRNA binds to the cr; the stem-loop can't form; translation is allowed.
- Only T7 polymerase, which is coded by T7RNAP, can bind to promoter PT7.

- A transcription of taRNA depends on concentration pulses of arabinose.

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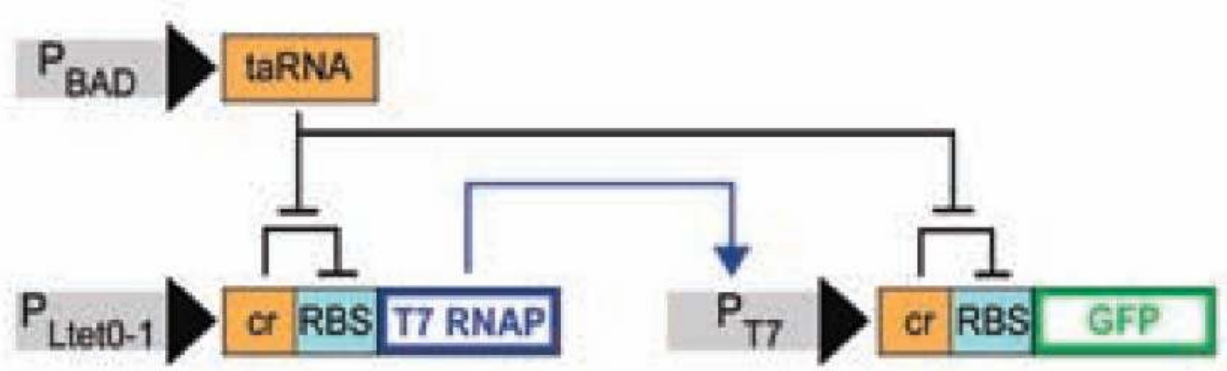
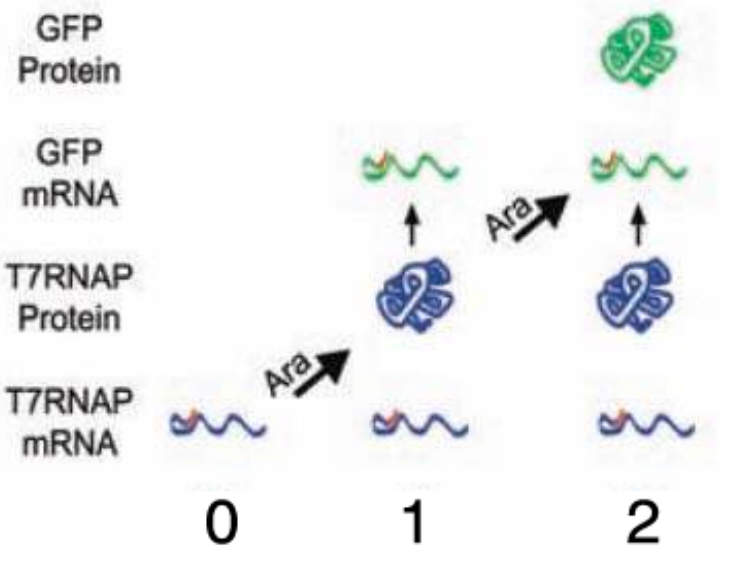
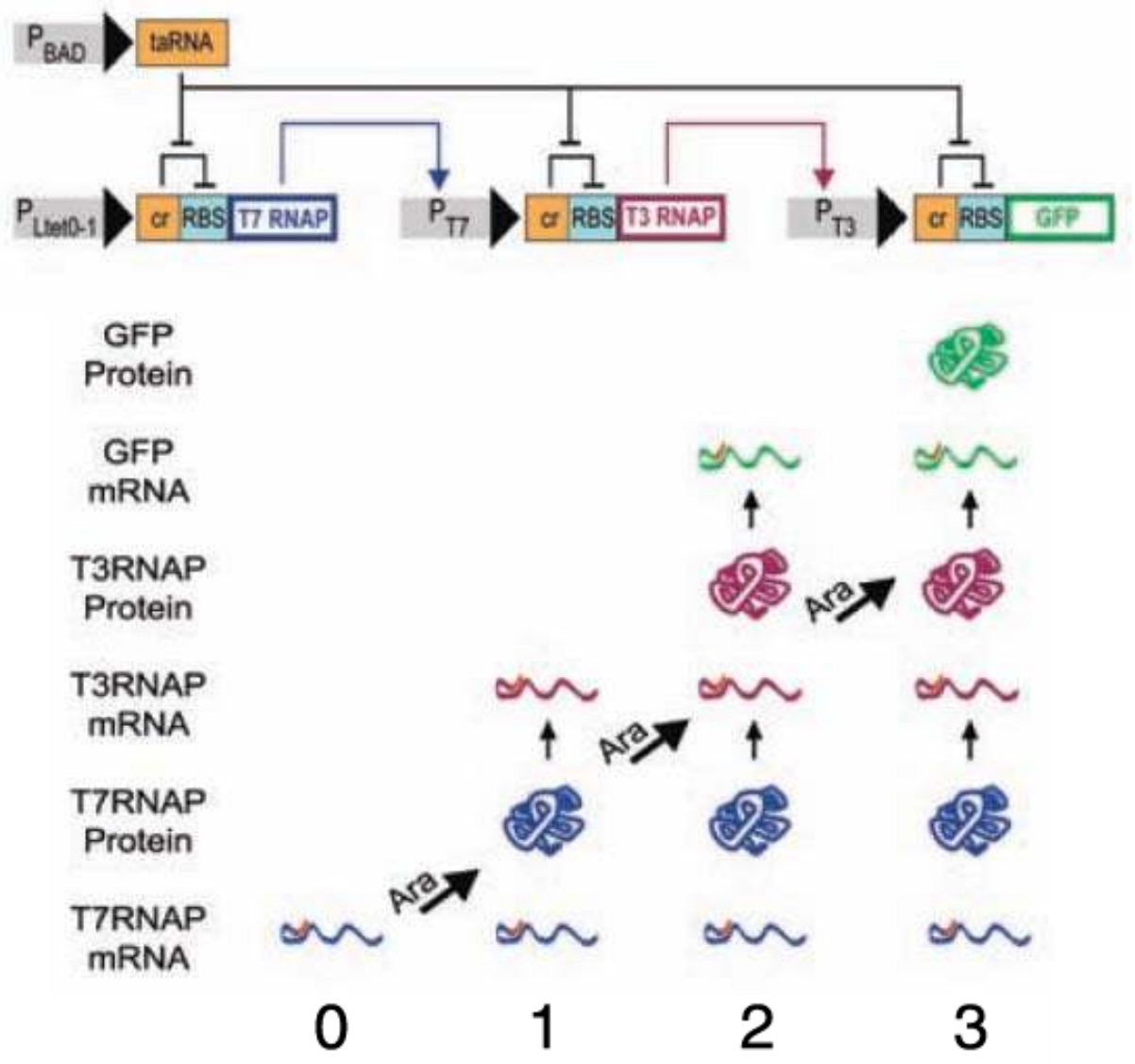


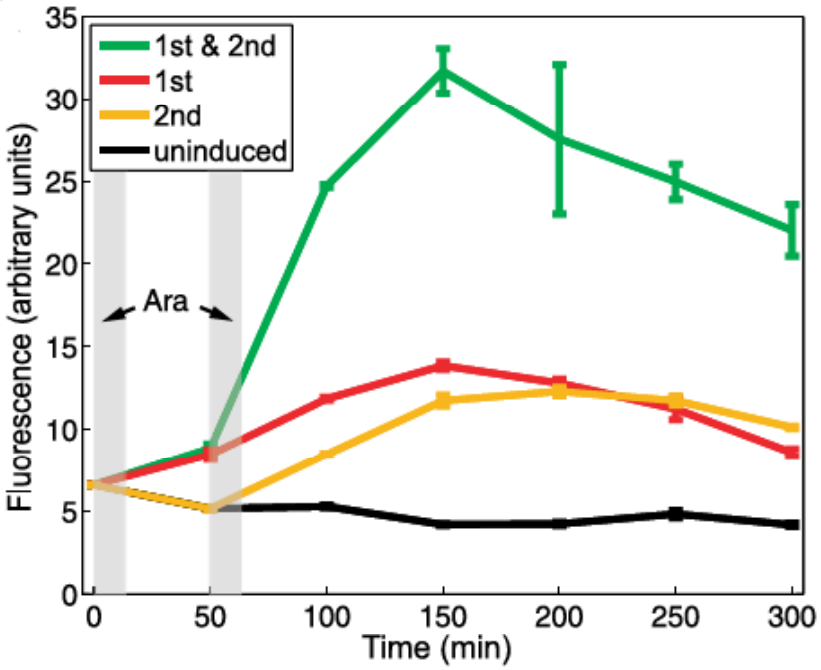


Model 1: Riboregulated transcriptional cascade(RTC), three-counter

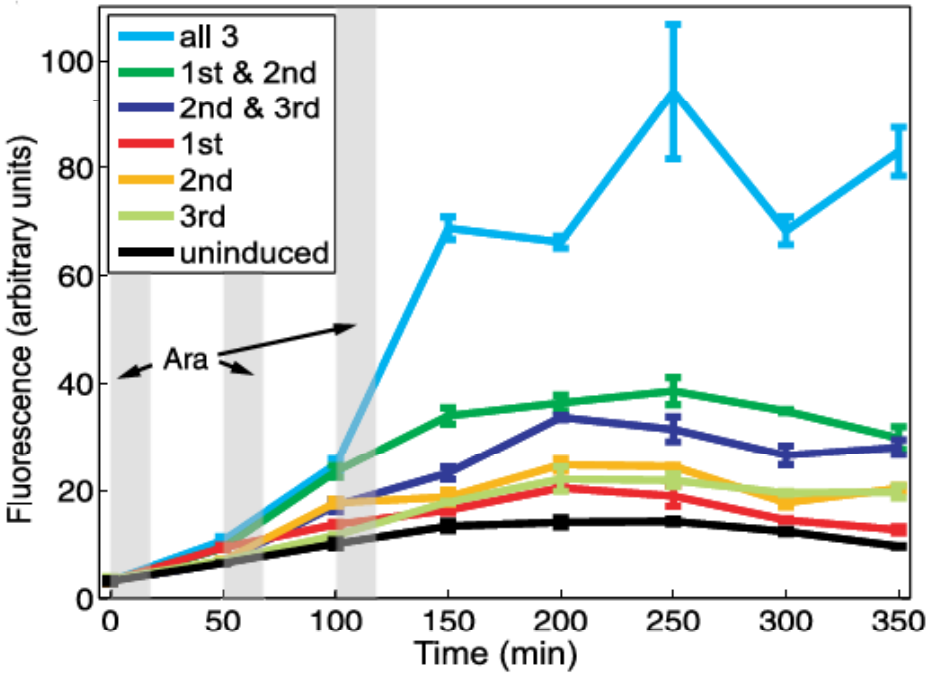


Experimental results of RTC counters

Two-counter



Three-counter

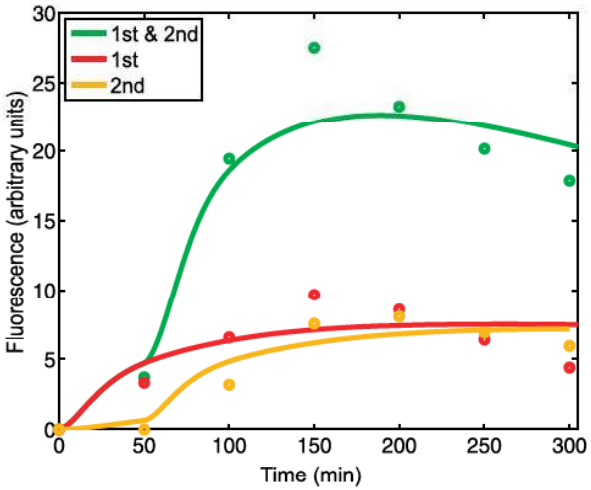


Shaded areas represent arabinose pulse

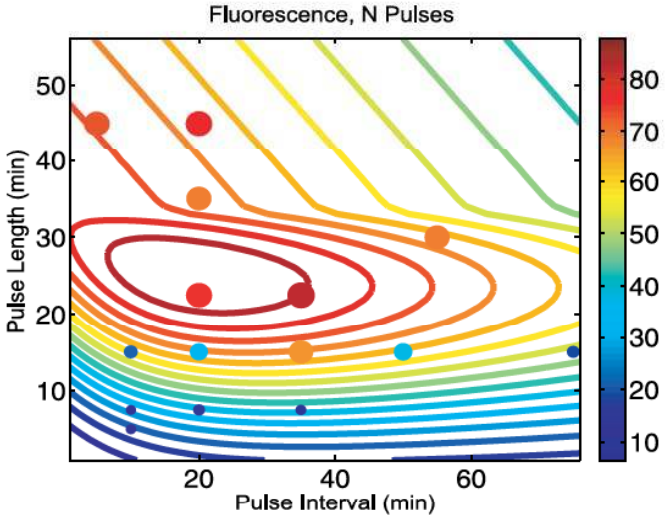
- Experimental results demonstrate that fluorescence increases **only** when all two (or three) arabinose pulses are delivered.
- Based on this results, Ari E. Friedland at el. constructed and analyzed a **mathematical model**.

Prediction of the mathematical model

Two-counter

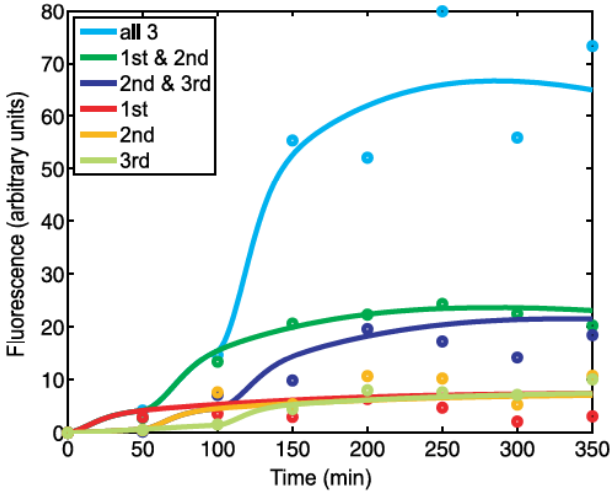


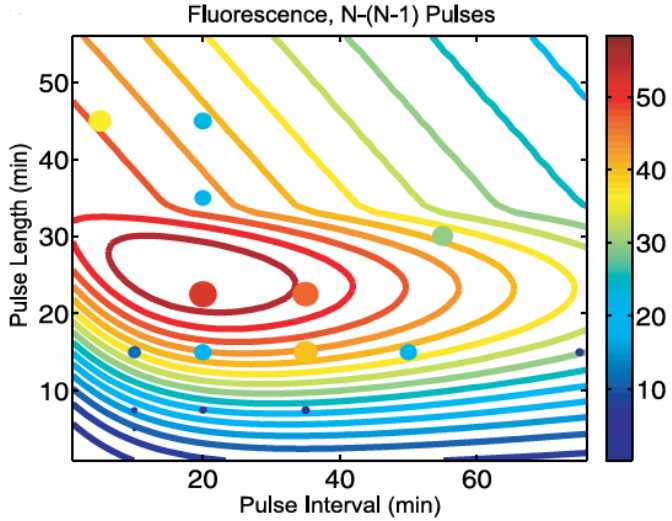
Maximum of the predicted concentration.



Three-counter: searching of optimal combination (interval-length).
Points: the difference between model and experiment.

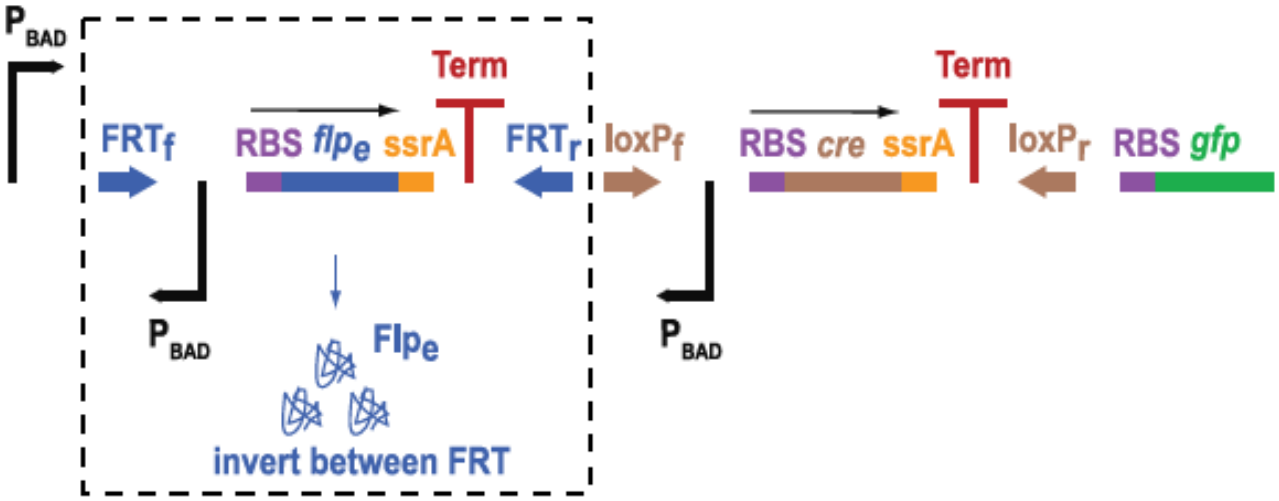
Three-counter





The absolute difference in fluorescence after three pulses and two

Model 2: Single Invertase Memory Module(SIMM), three-counter



DNA – vector

- After the first arabinose pulse only Flpe protein can express.
- Flpe is a site-specific recombinase. (Site FRT)

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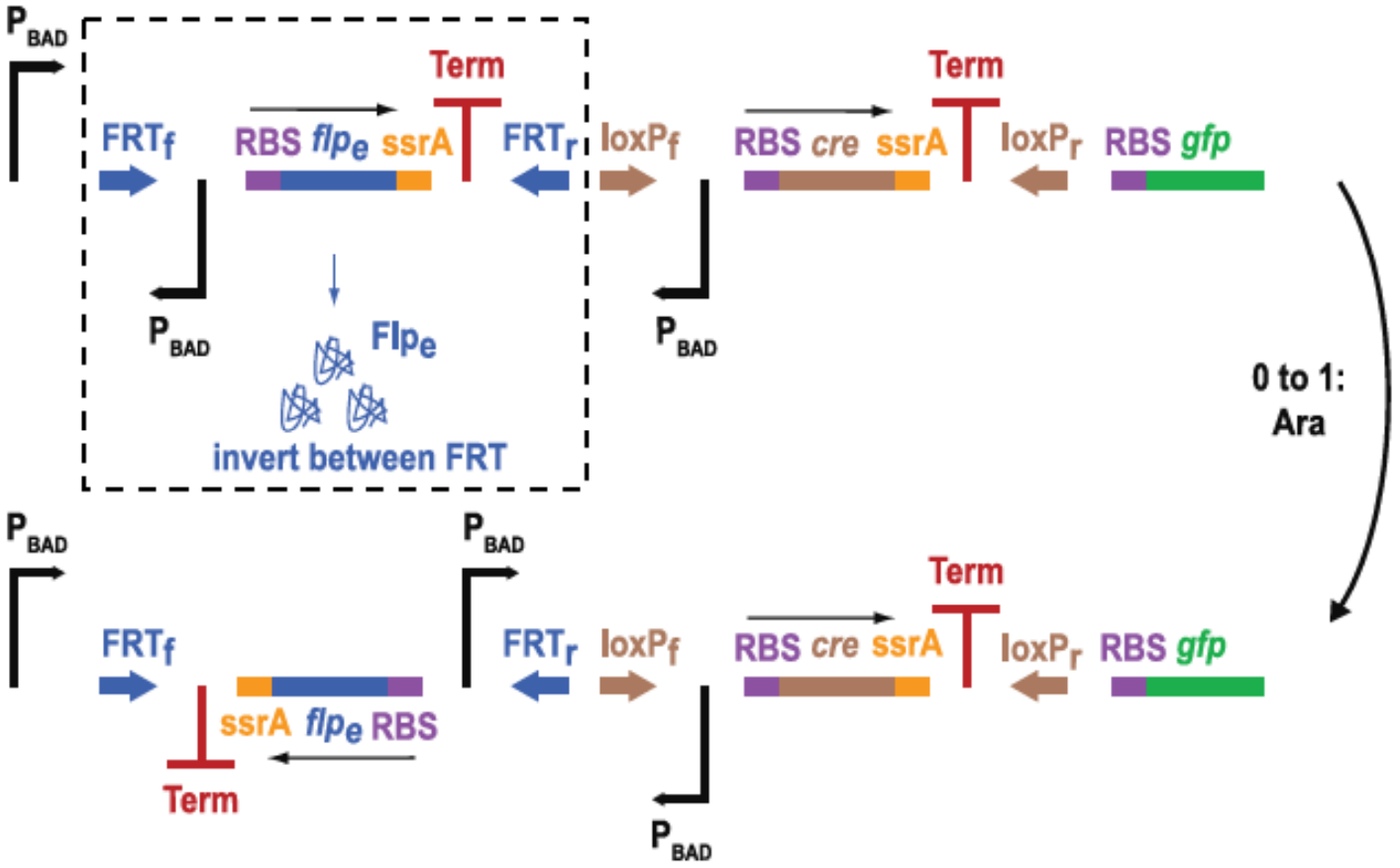

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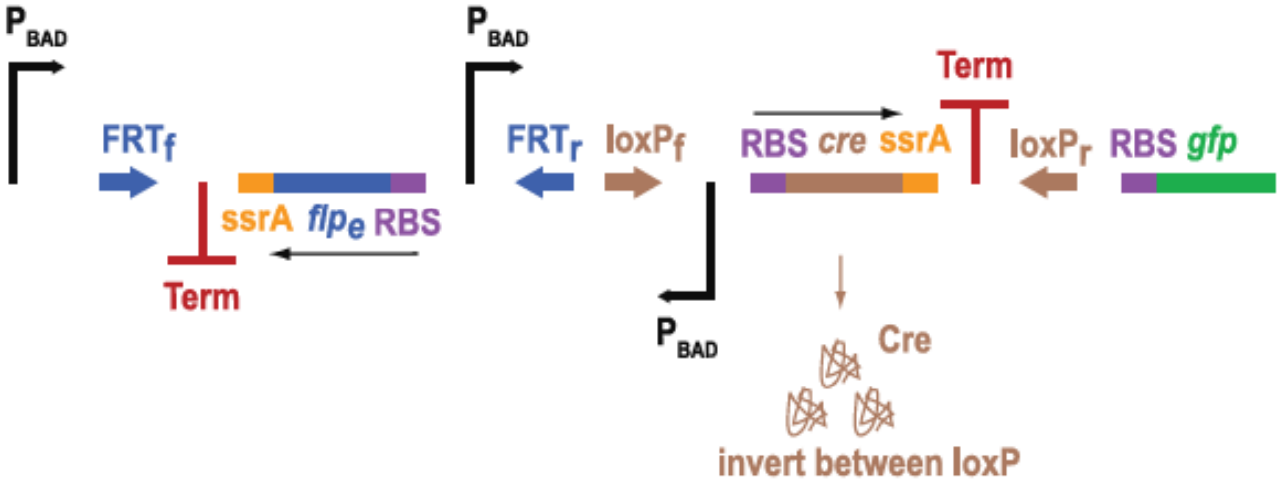

- After the second arabinose pulse only Cre protein can express.
- Cre is a site-specific recombinase. (Site lox)

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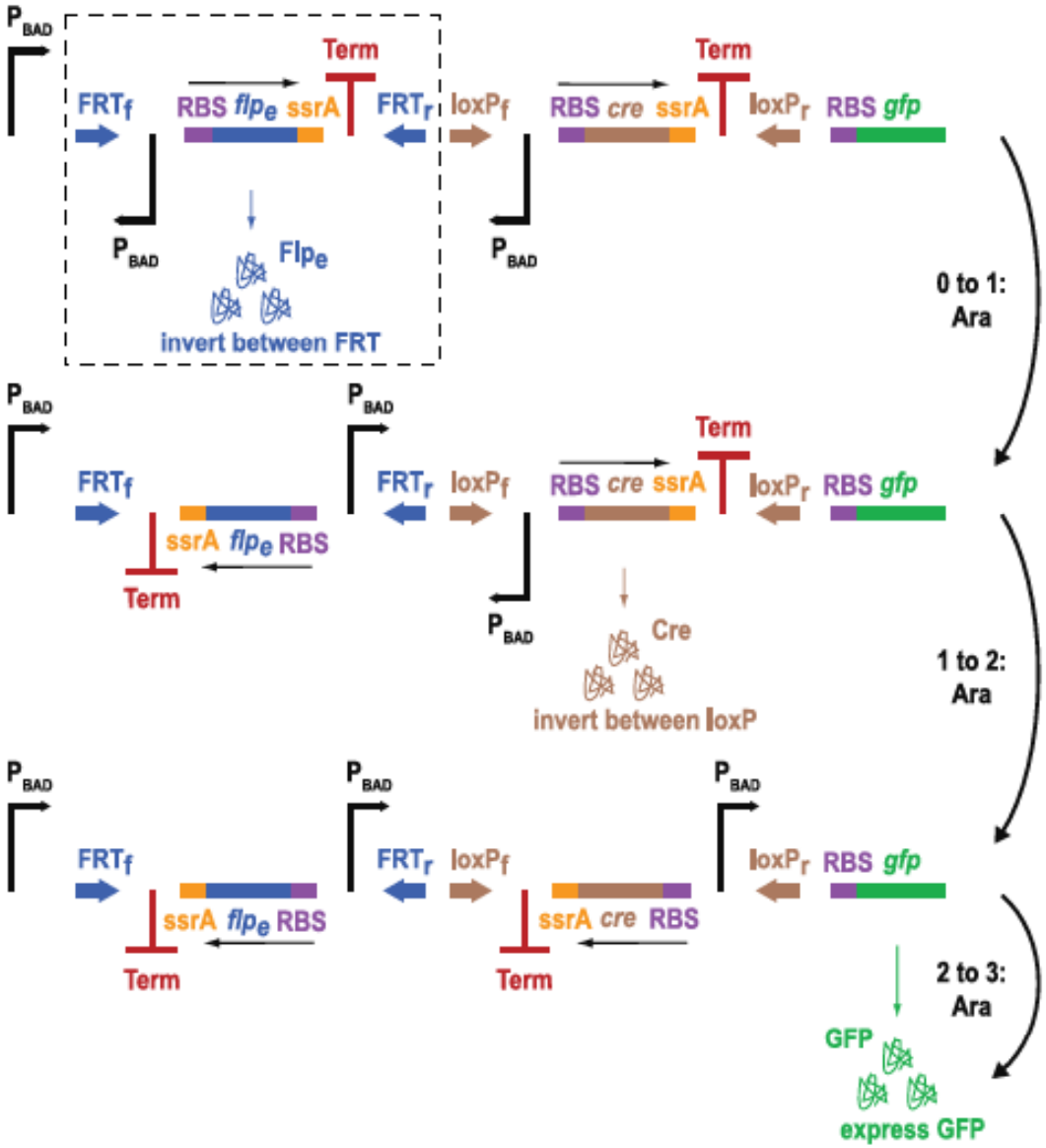
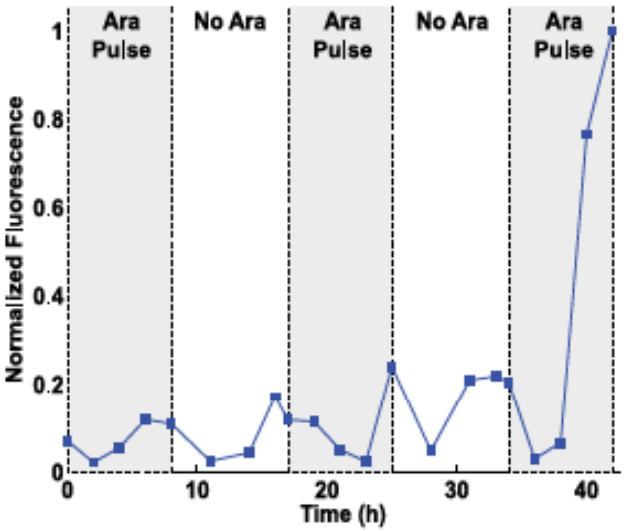


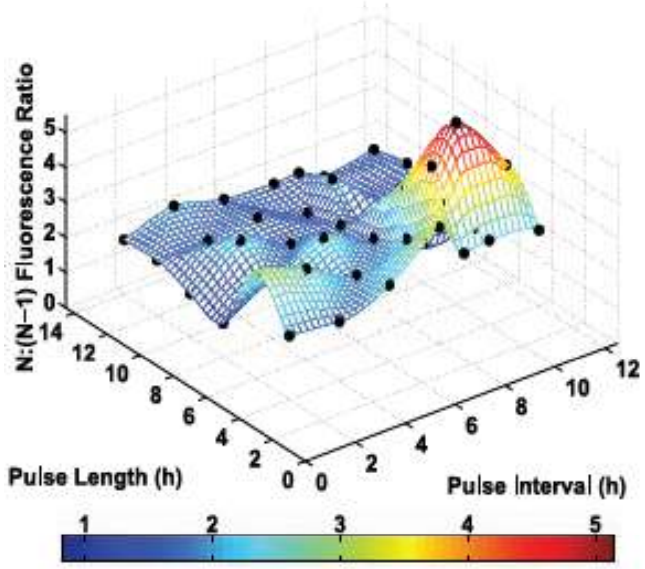
Counting cascade.
Result: GFP expresses after 3 Ara pulses.

The same cascade was built for two-counter

Experimental results and Modelling of SIMM counters



Fluorescence in SIMM three-counter aftres 3 pulses.



GFP fluorescence ratios between the single-inducer DIC three-counter exposed to three pulses of arabinose (N) versus two pulses of arabinose (N – 1) with varying arabinose pulse lengths and intervals; experimental results are represented by black dots.

Conclusion + Future

- Imitation of computer ticks.
- Temporary effect.
- Unfortunately, cell mechanisms have got mistakes and this counters too.
- There is a dubious future for biocompurets which are based on this counters.

Thank for your attention!