LECTURE 9: SEMESTER PROJECT

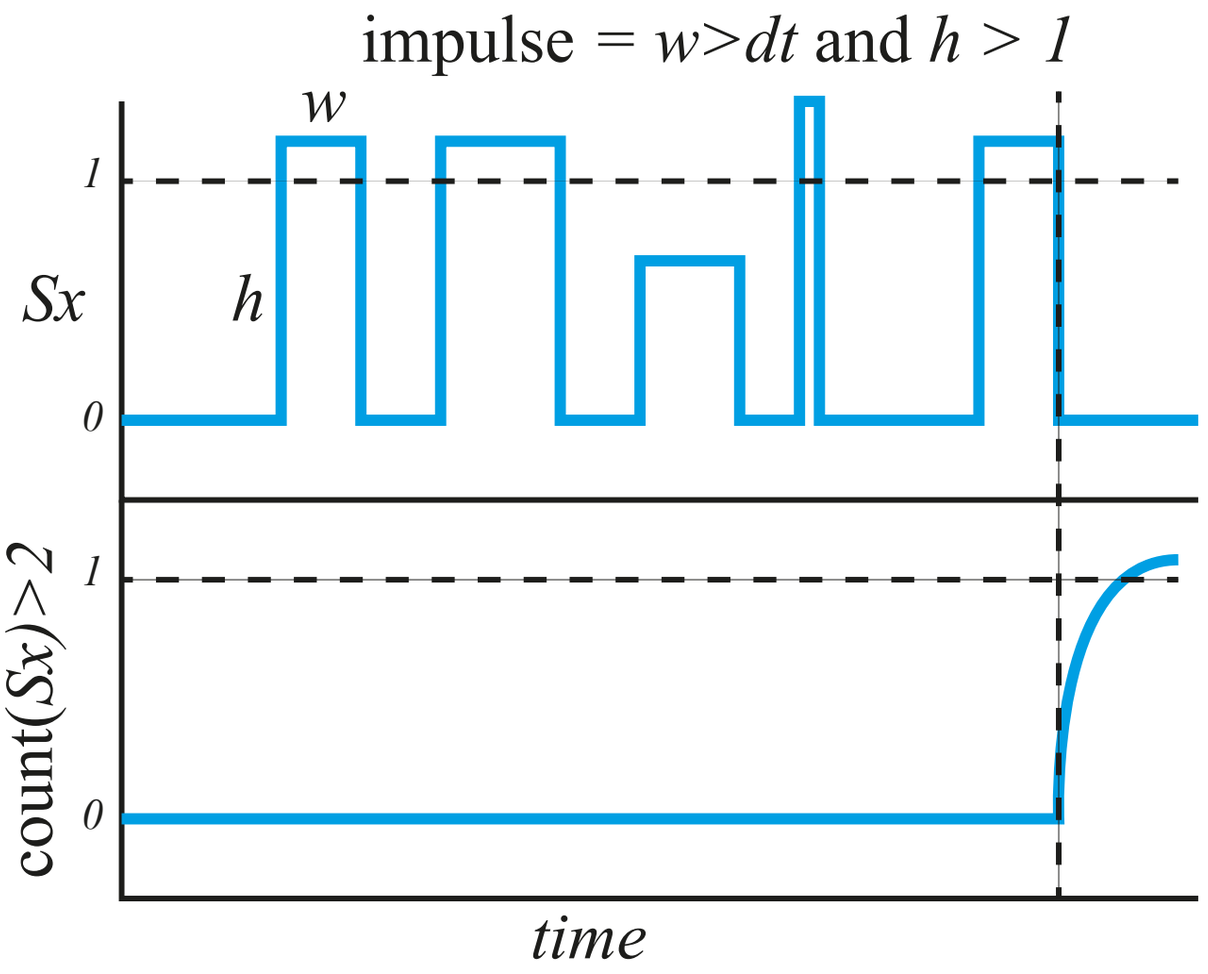
Introduction to cellular system modelling

Daniel Georgiev

Summer 2015

OUTLINE

- Literature review - Genetic circuits that count
- cis acting Riboswitches
- trans acting RNA
- Assignment - Genetic circuits that subtract
- Discussion

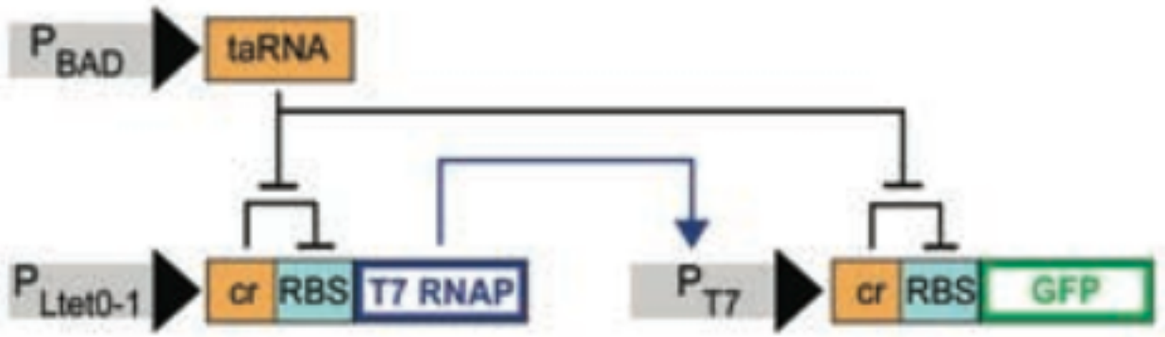


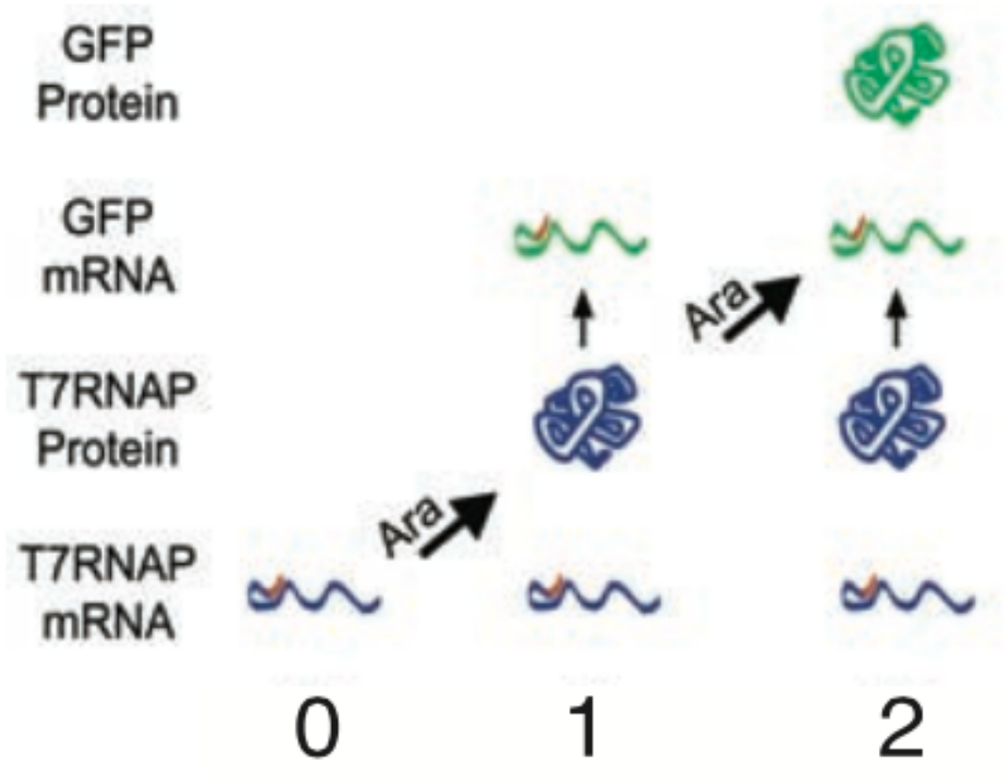
COUNTING IN GENETIC CIRCUITS

Chemical signal impulses are counted. An impulse is a signal of specific length. It is possible to have consecutive impulses or separated impulses. The output reads true when the number of impulses is greater than 2.

Science
AAAS

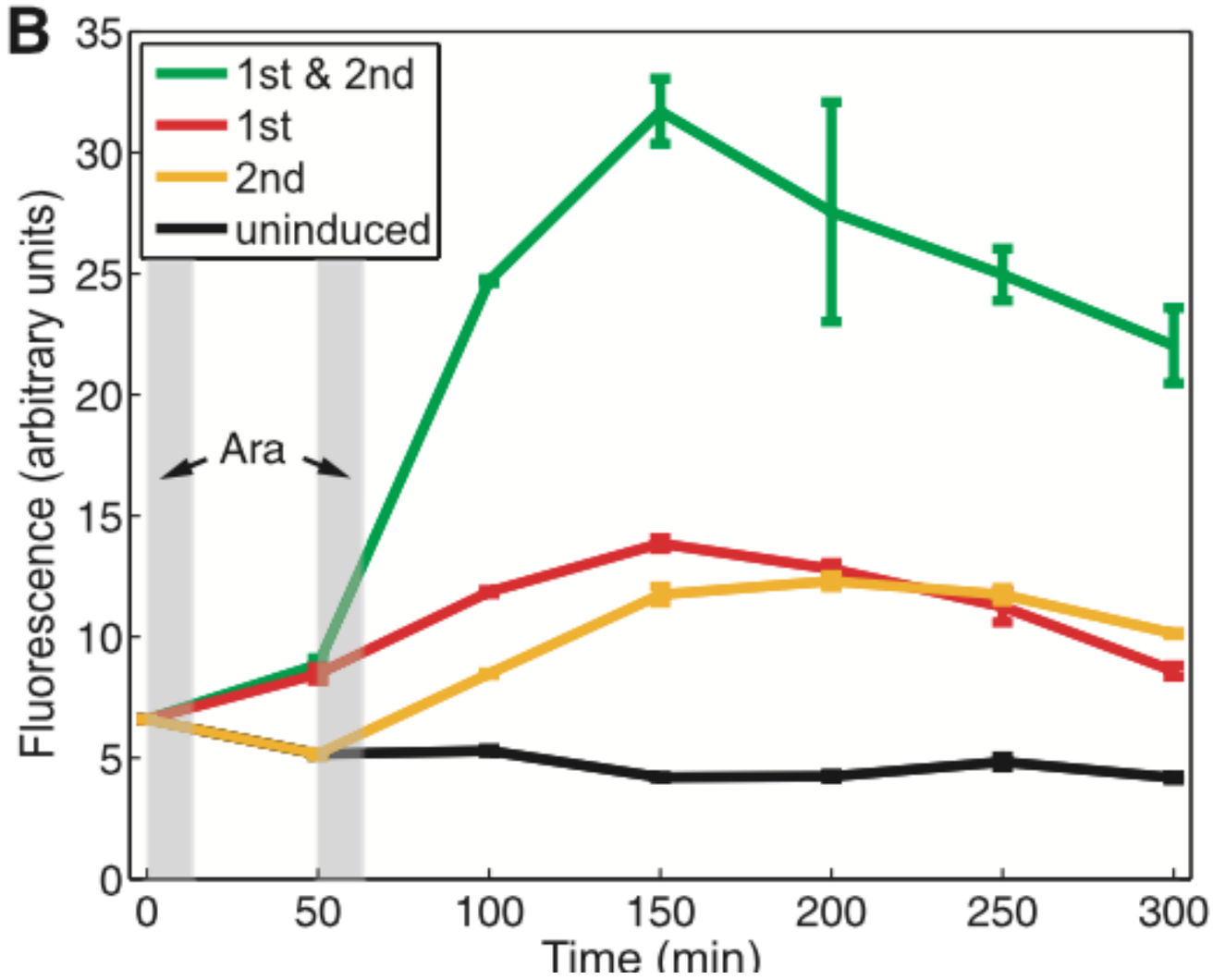
Synthetic Gene Networks That Count
Ari E. Friedland, *et al.*
Science **324**, 1199 (2009);
DOI: 10.1126/science.1172005





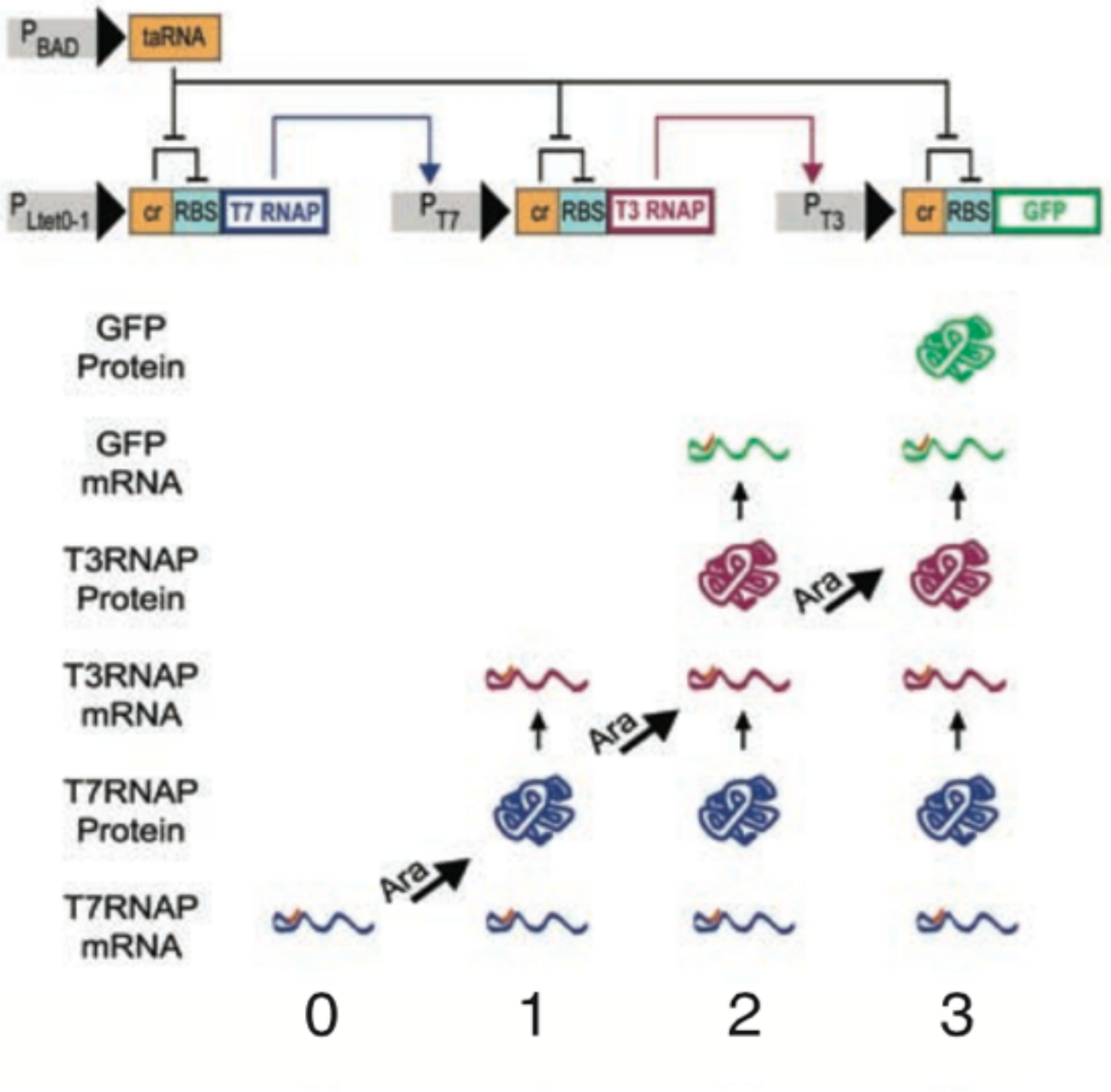
PROPOSED GENETIC CIRCUIT

Two counter proposed by Collins et al. Arabinose impulses are counted and GFP is produced following two impulse events.



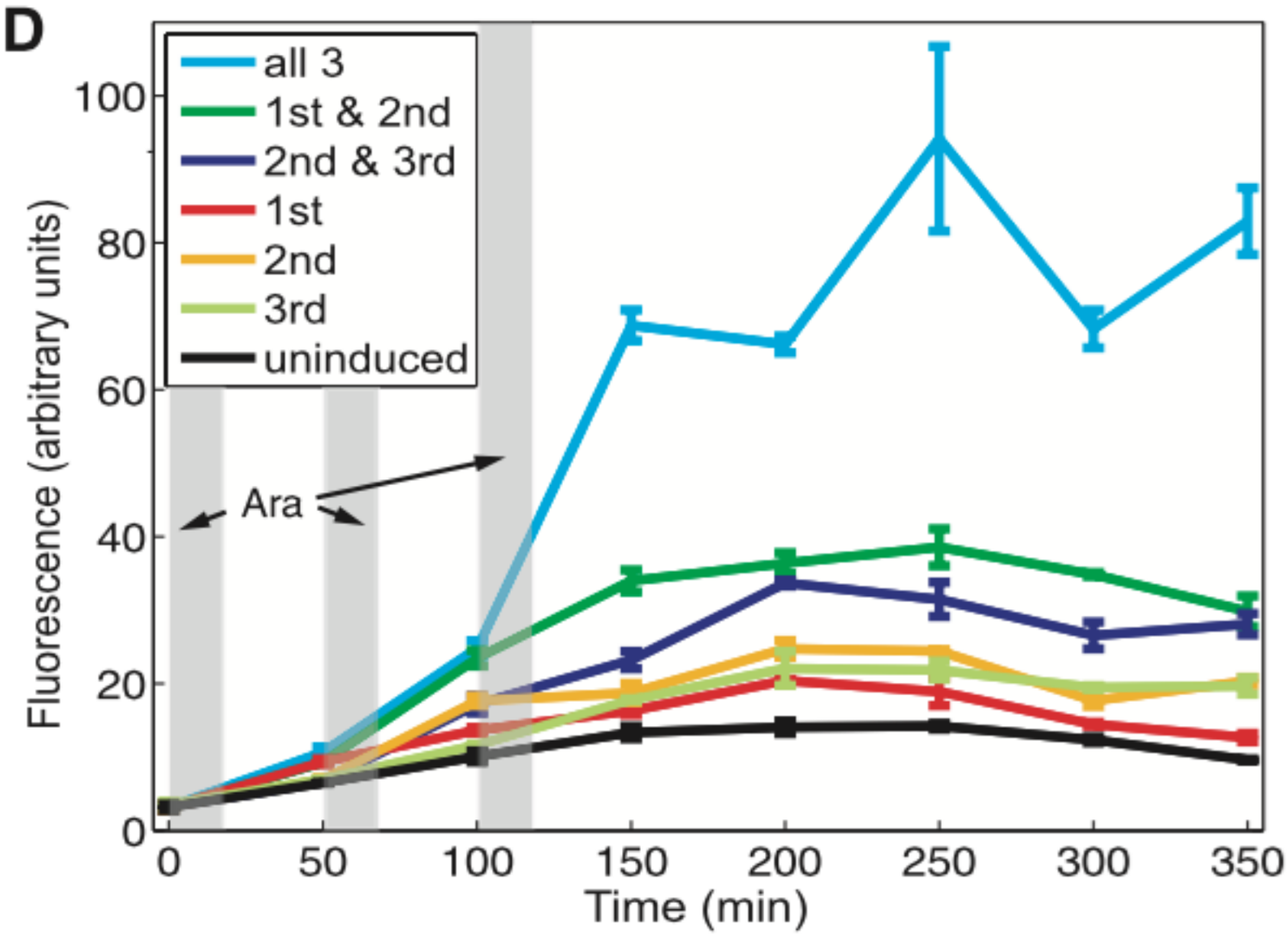
EXPERIMENTAL RESULTS FOR COUNTER CIRCUIT

Experimental results proof the genetic counter concept. The circuit is tested in various permutations to show that GFP (blue line) is expressed only following two impulses. Note the leaky expression of GFP.



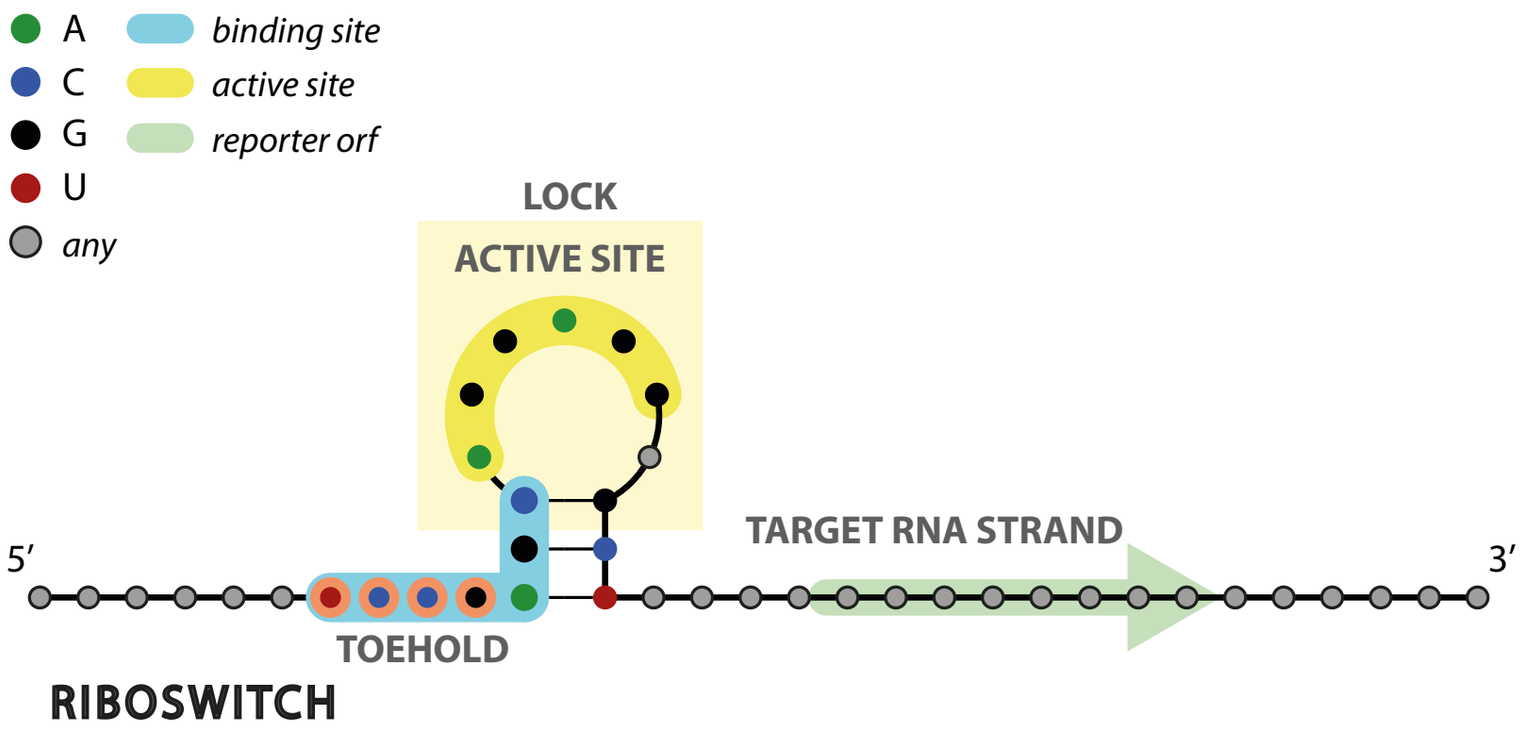
PROPOSED GENETIC CIRCUIT

Three counter proposed by Collins et al. Arabinose impulses are counted and GFP is produced following three impulse events.



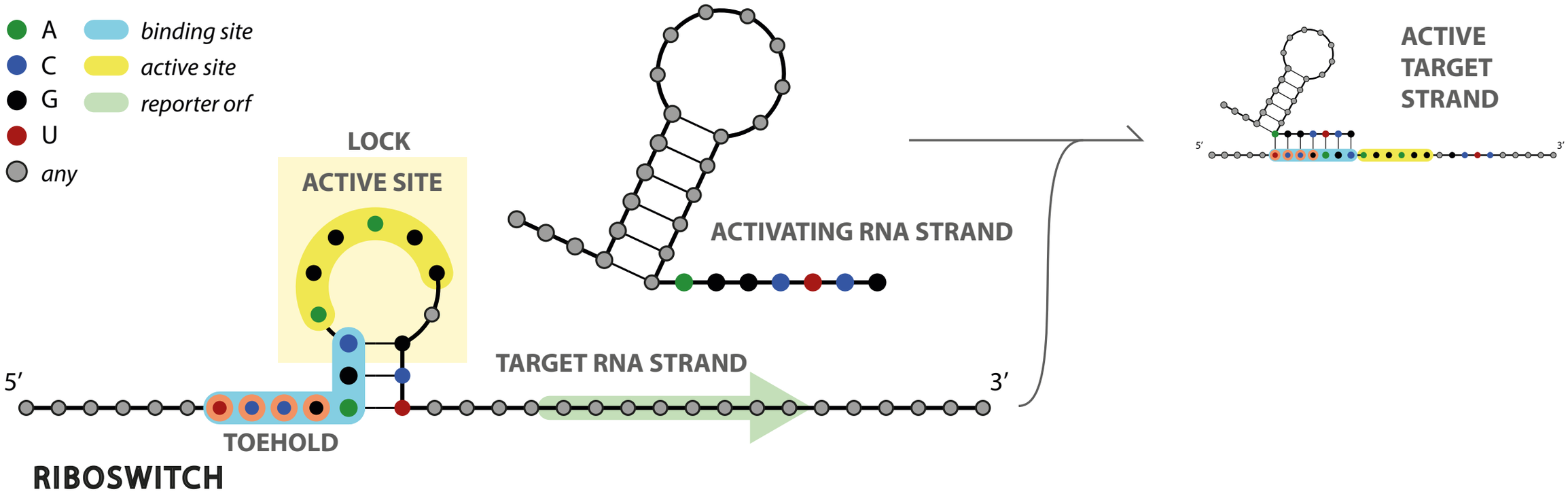
EXPERIMENTAL RESULTS FOR COUNTER CIRCUIT

Experimental results proof the genetic counter concept. The circuit is tested in various permutations to show that GFP (blue line) is expressed only following three impulses. Note the leaky expression of GFP.



CIS ACTING RIBOSWITCH

Riboswitch is an RNA device that regulates translation. If the device is on the same strand as the protein coding sequence, it is referred to as cis.



TRANS ACTING sRNA

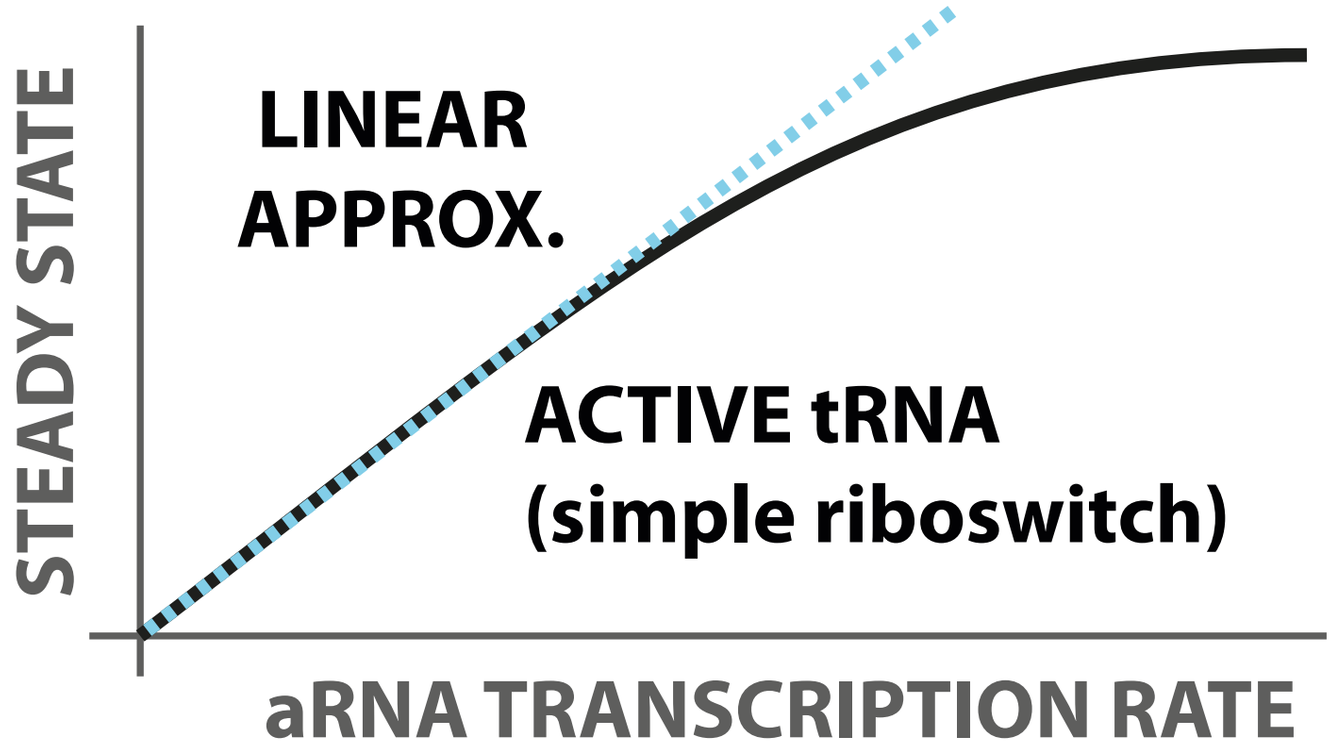
RNA from different genes can hybridise to effect their mutual secondary structures. This mechanism can be used to reveal or conceal active sites (e.g., translation initiation loci)

$$\mathrm{taRNA} + \mathrm{csRNA} \overset{k}{\rightarrow} \mathrm{aRNA}$$

trans active RNA | inactive RNA | active RNA

RIBOSWITCH CHEMICAL REACTION

A ribocounter and taRNA system react through a simple hybridisation reaction. This is of course an approximation since the actual process proceeds through base-by-base strand migration.

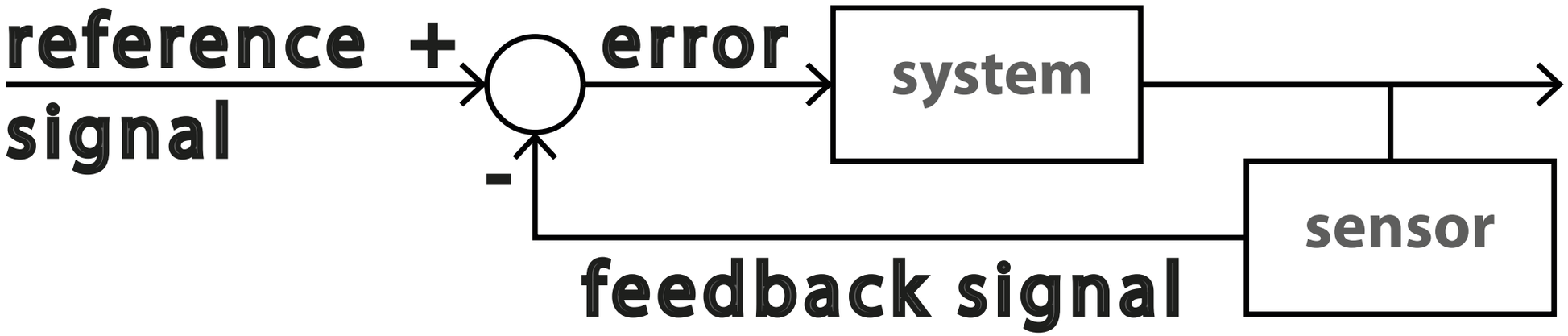


RIBOSWITCH RESPONSE

A ribocounter and taRNA system behaves like a linear gain up to a certain threshold.

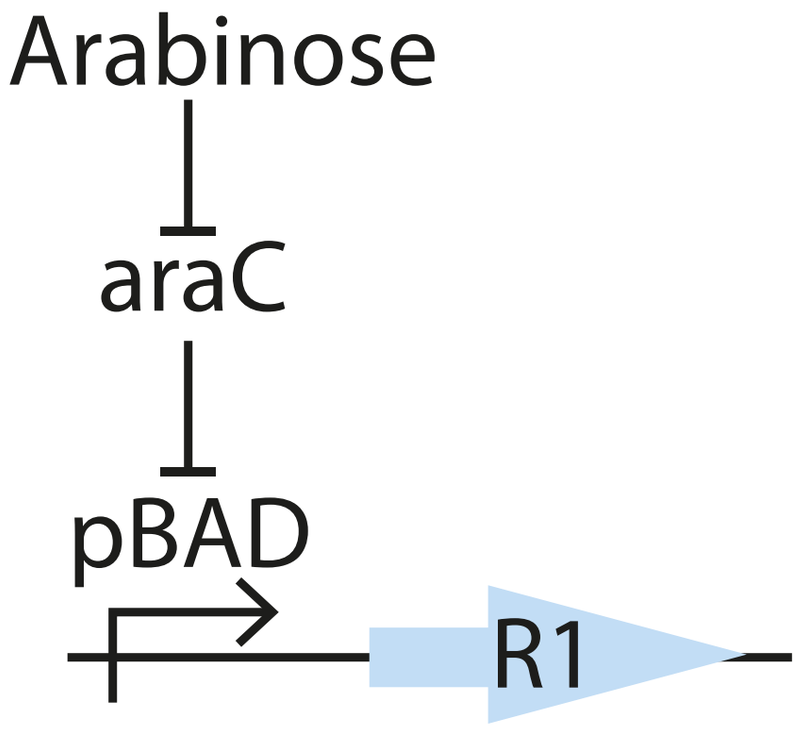
PROJECT MOTIVATION - SUBTRACTION

Subtraction is a critical function necessary to compare two signals and evaluate their difference. In negative feedback systems, subtraction is used to compute the regulation error and accordingly adjust the control input.

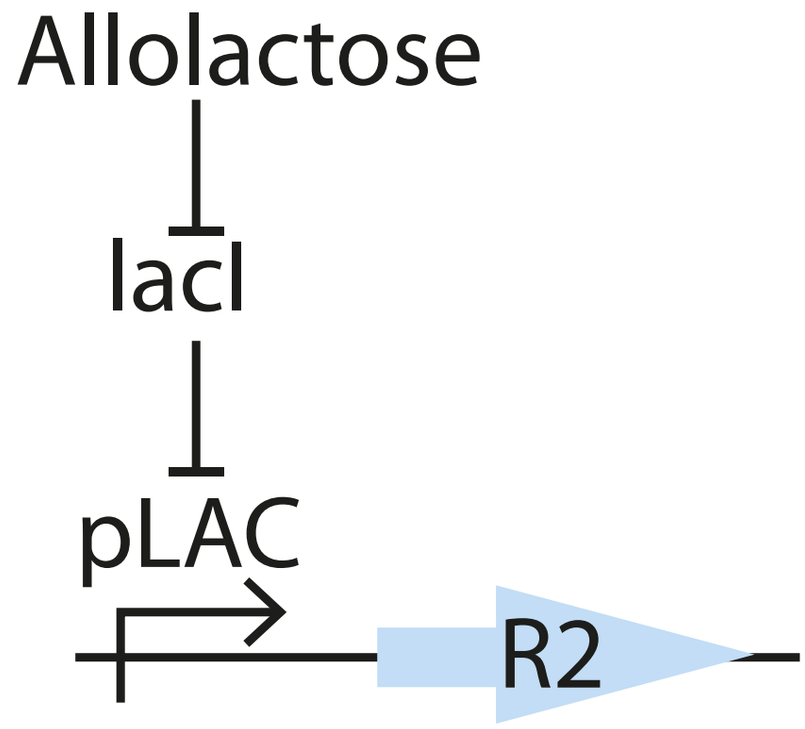


PROJECT ASSIGNMENT - WHAT IS GIVEN

INPUTS



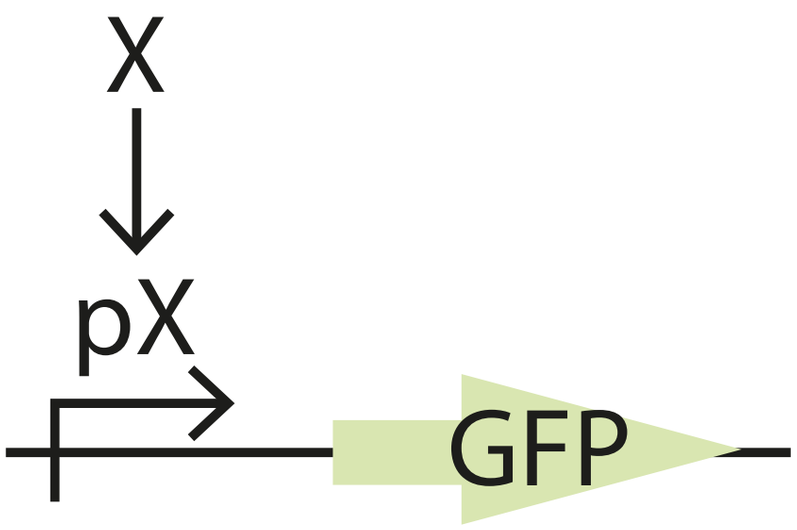
arabinose impulse sensor
taRNA1 expressed from pBAD promoter



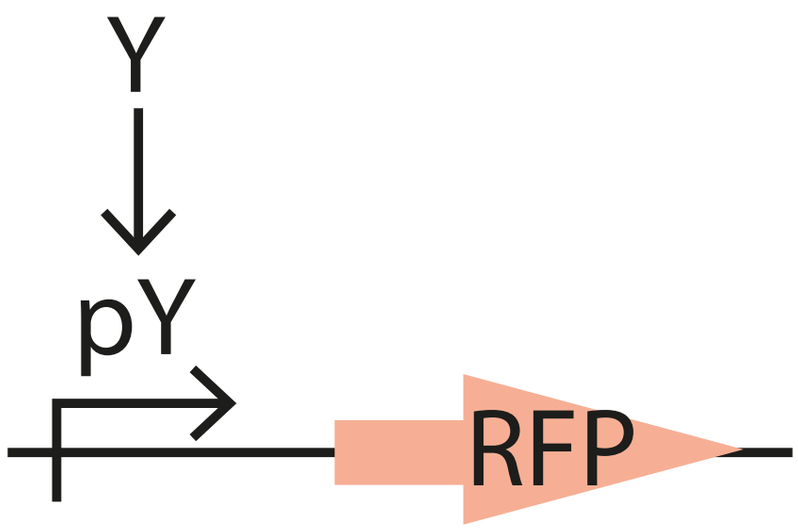
allolactose impulse sensor
taRNA2 expressed from pLAC promoter

PROJECT ASSIGNMENT - WHAT IS GIVEN

OUTPUTS



GFP reporter
Green fluorescent protein activated by a regulatory protein X



RFP reporter
Red fluorescent protein activated by a regulatory protein Y

ASSUMPTIONS

1) Arabinose and Allolactose impulses last 10-20 minutes.
2) The time between impulses is 10-30 minutes.
3) RNA half-life is 5 minutes.
4) Protein half-life (including dilution due to cell division) is 20-60 minutes.
5) Maximal transcription rate is 1 RNA every 2 seconds.
6) Maximal translation rate is 1 Protein every 60 seconds.

PROJECT ASSIGNMENT - DESCRIPTION OF TASK

Propose a realistic genetic network that will subtract the number allolactose pulses from the number of arabinose pulses and display the difference in red fluorescence (if negative) and green fluoresce (if positive).

In other words, the input/output relationship is described by the following logic table:

# of Allolactose pulses \ # of Arabinose pulses	1	2	3
1	0	1	2
2	1	0	1
3	2	1	0

In the above table, the color intensity indicates fluorescence of either GFP or RFP. The numbers in the coloured cells indicate the relative intensity. So the green number 2 implies fluorescence should be 2 times as high as the green number 1 (and same for red numbers).

PROJECT ASSIGNMENT - THINGS TO KEEP IN MIND

1) MISSING INFORMATION: As is typical in Synthetic Biology, the project assignment alone does not provide all the information you need. You will need to consult literature or the course instructor to fill in the gaps as you come across them. This is a part of the project, to see you well you identify and fill in the missing information.

2) TIME SCALE: The output should be realised on the same time scale (tens of minutes) as the input. It is very easy to make fancy genetic devices that, however, take a long time to execute. You may need to use “seep-up” devices to accomplish this goal.

3) ROBUSTNESS: Just because something works when using boolean logic schematics or deterministic models doesn’t mean it will work in reality. Your final proof of concept should prove your design in stochastic simulation.

4) SIMPLICITY: You’ve seen the size of transcription networks that are implemented in real cells. If your design involves a large number of components, it is likely not going to work. You should try to keep the number of components and the number of steps to a minimum. During your final presentation, I will ask how you considered simplicity in your design.