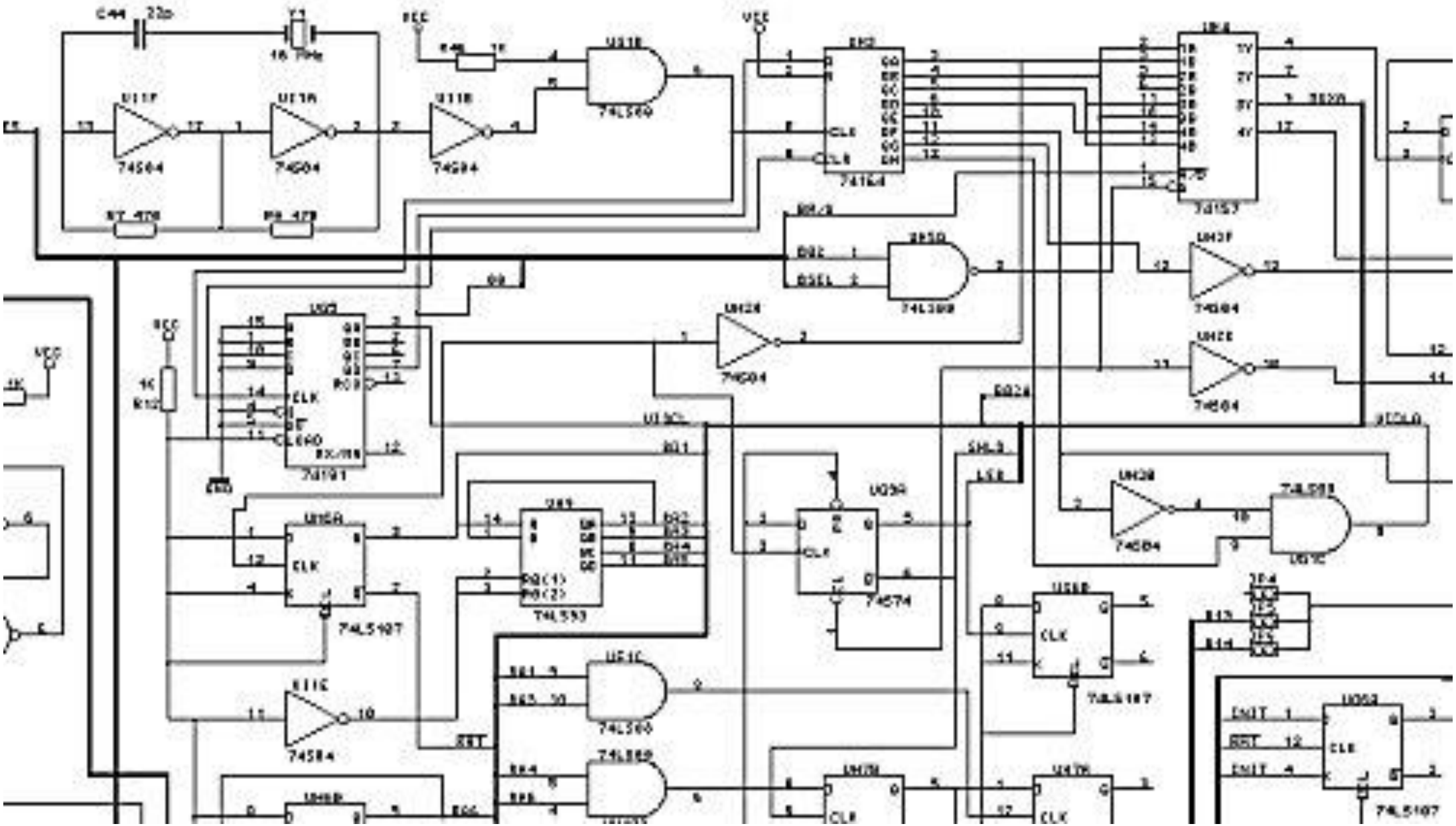
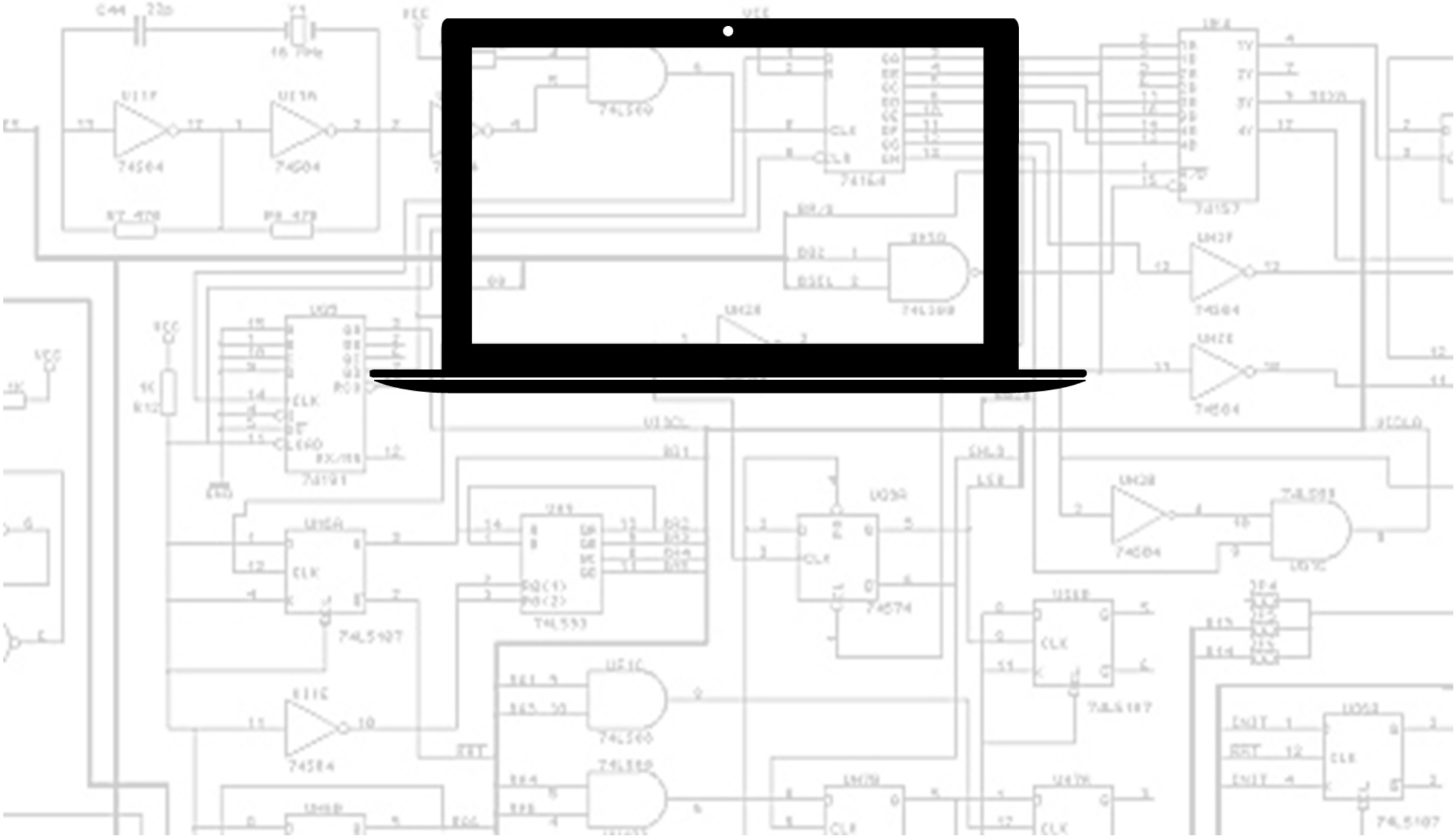
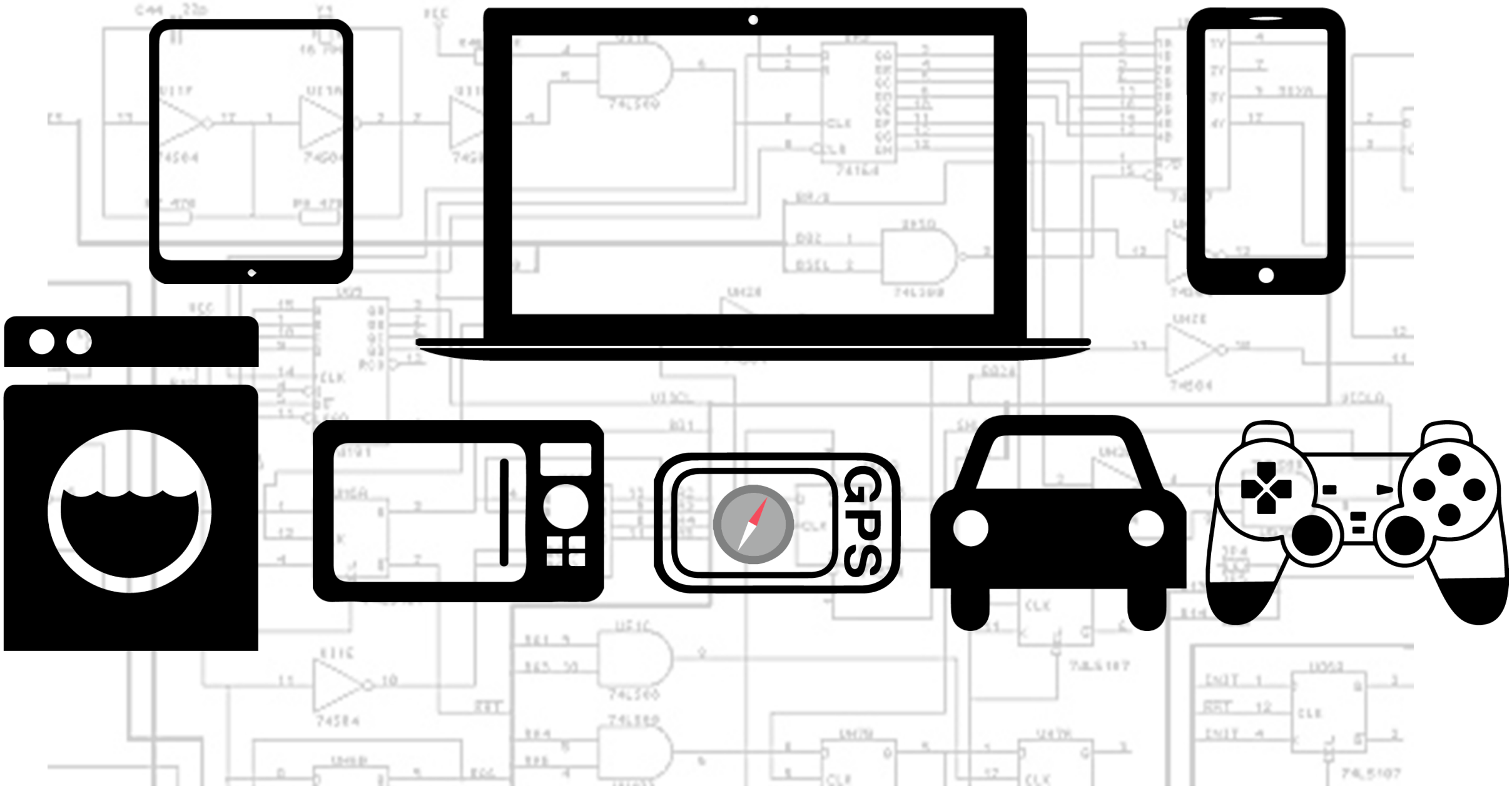
iGEM

EPFL

2015





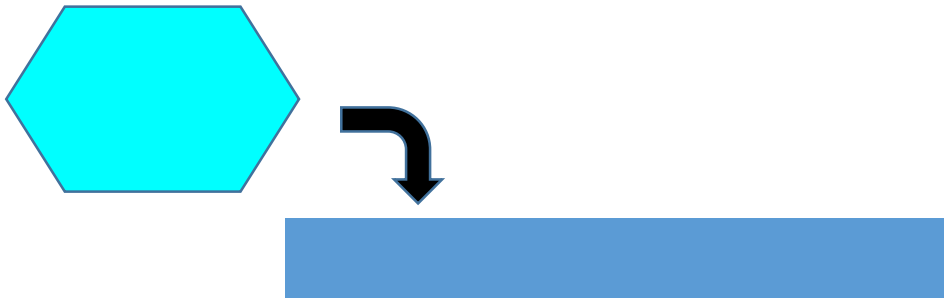

GPS

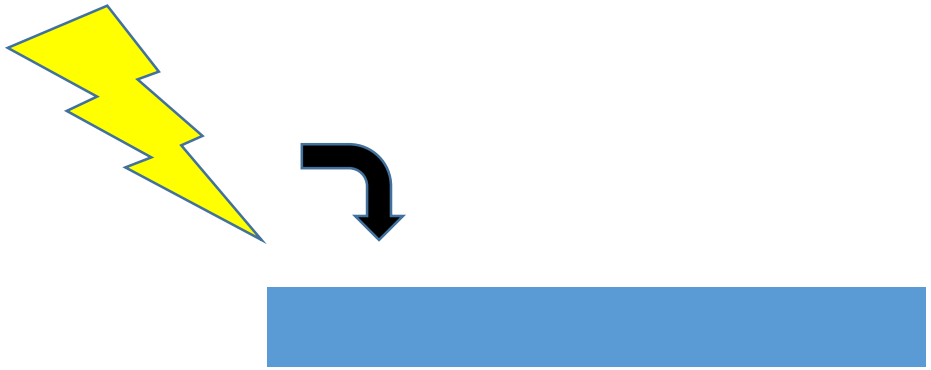
Biological logic circuits

Gene regulation networks

Biological logic circuits

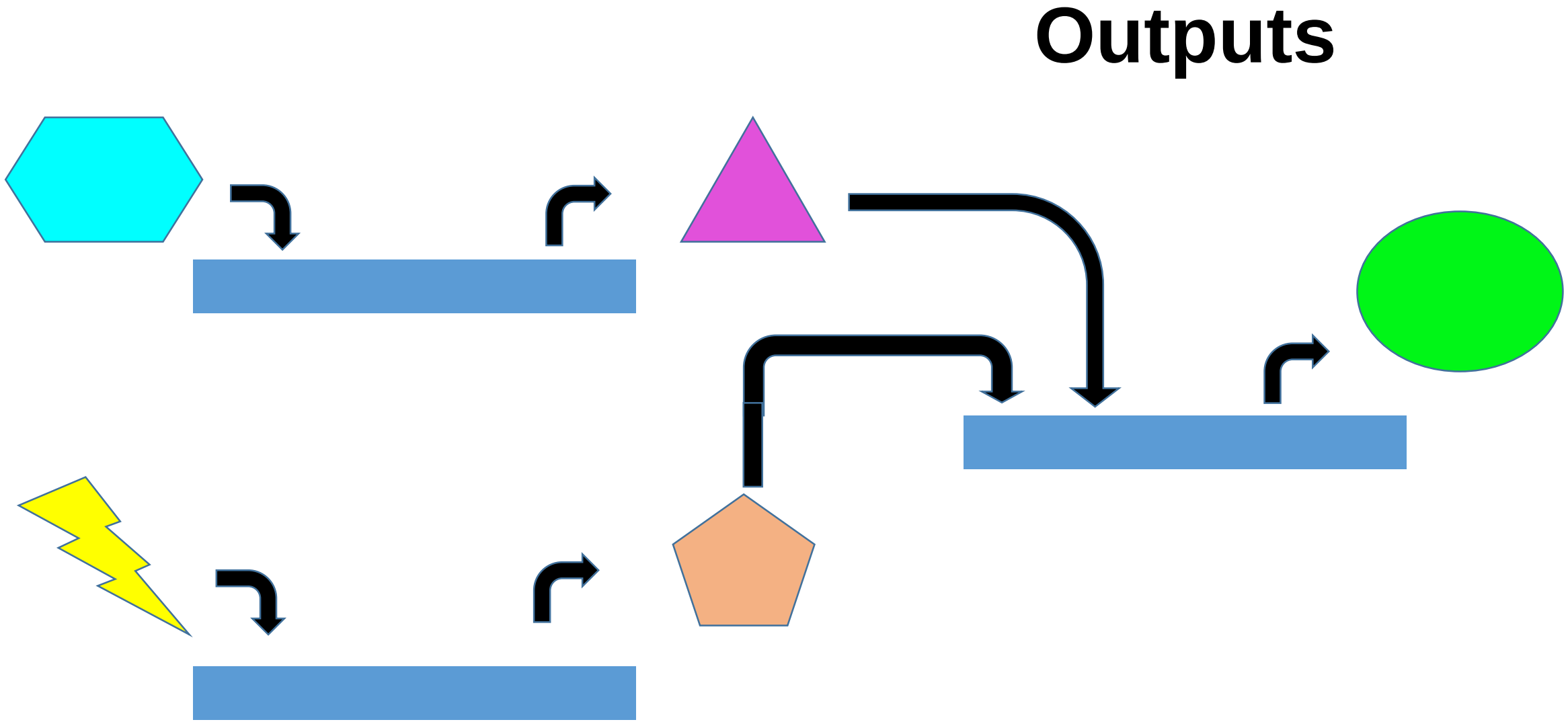
Inputs



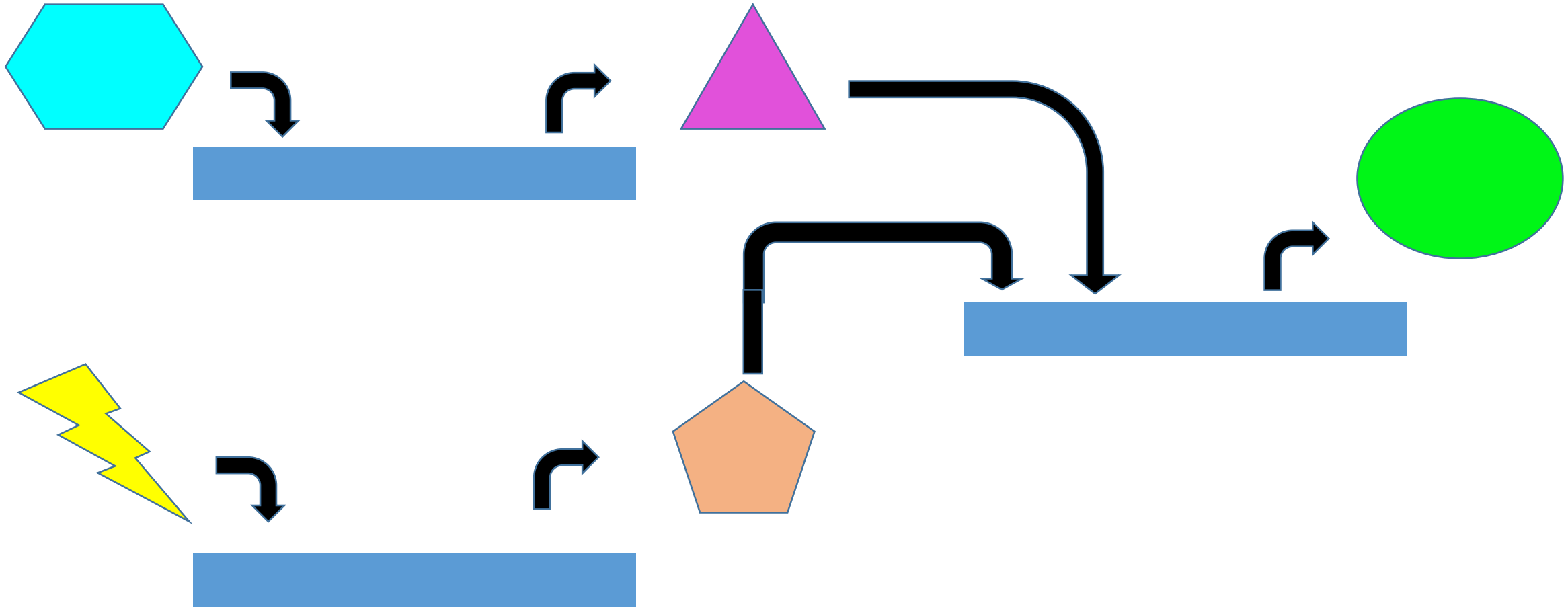


Signal processing

Biological logic circuits

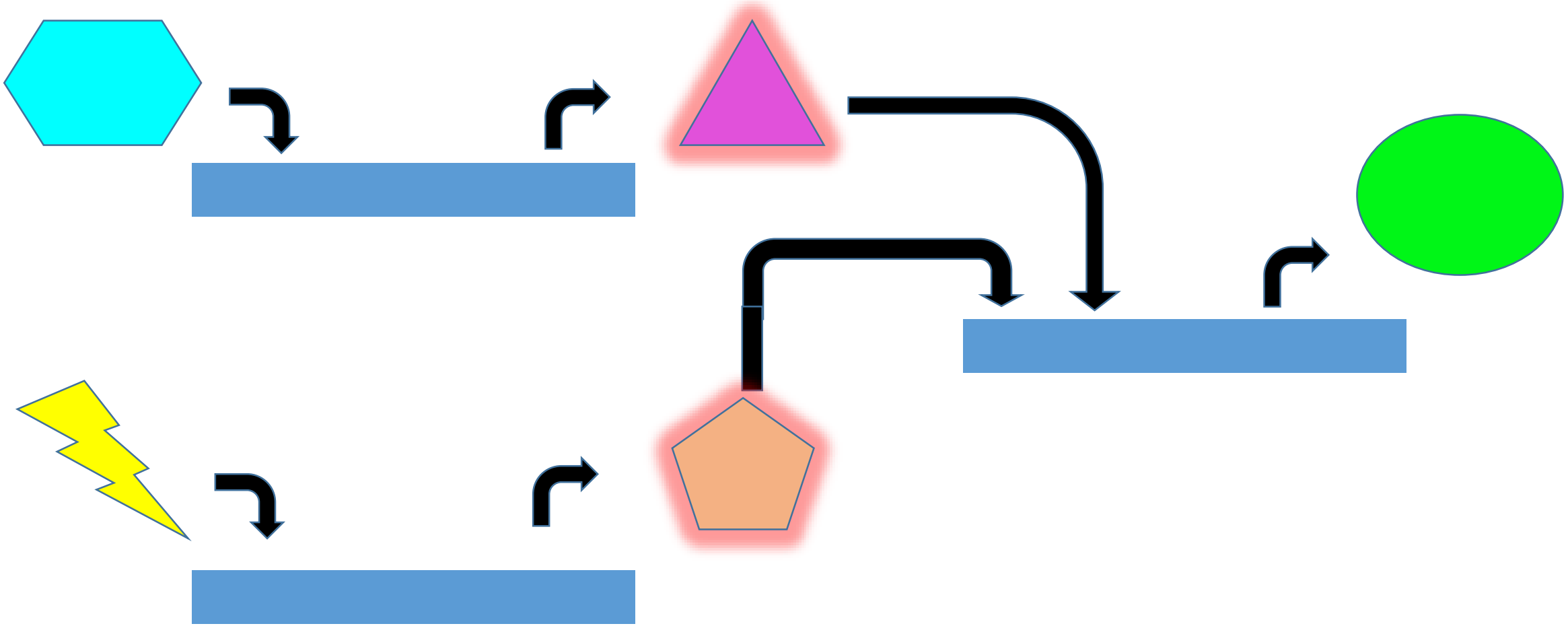


Biological logic circuits



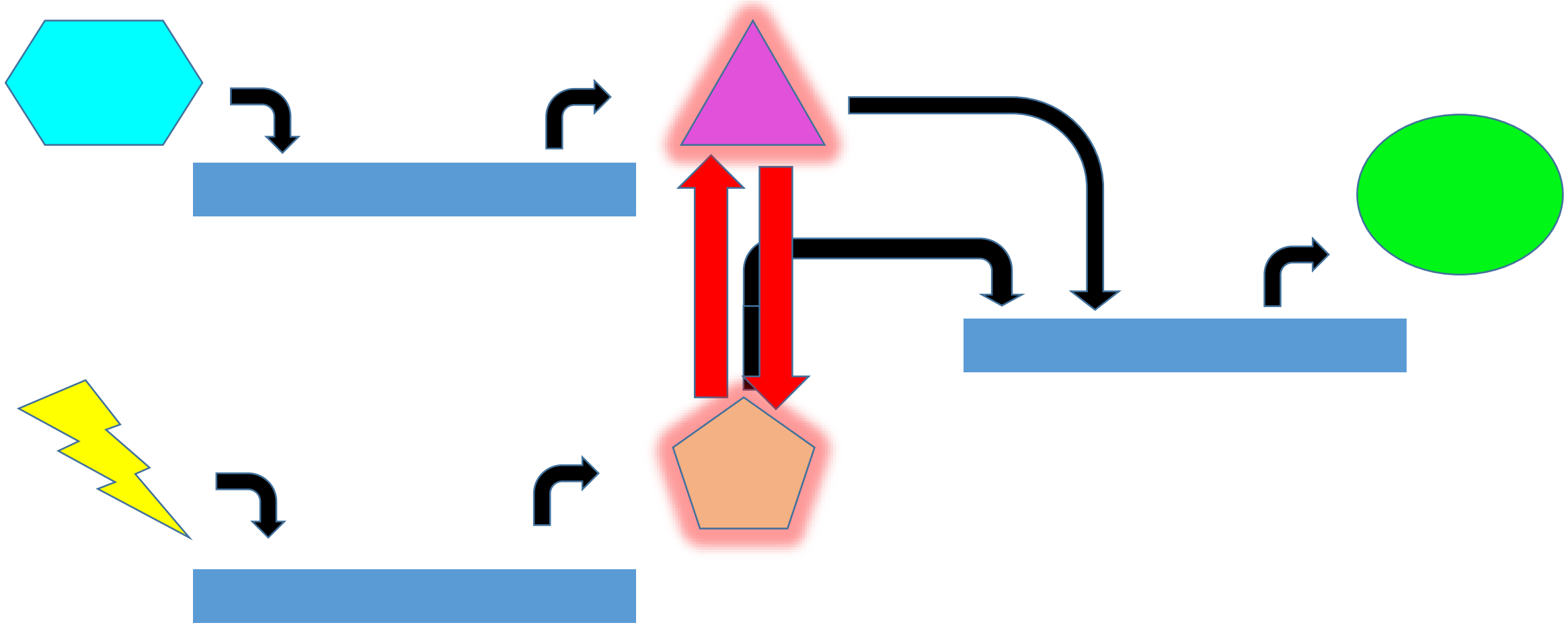
Biological logic circuits

Orthogonality



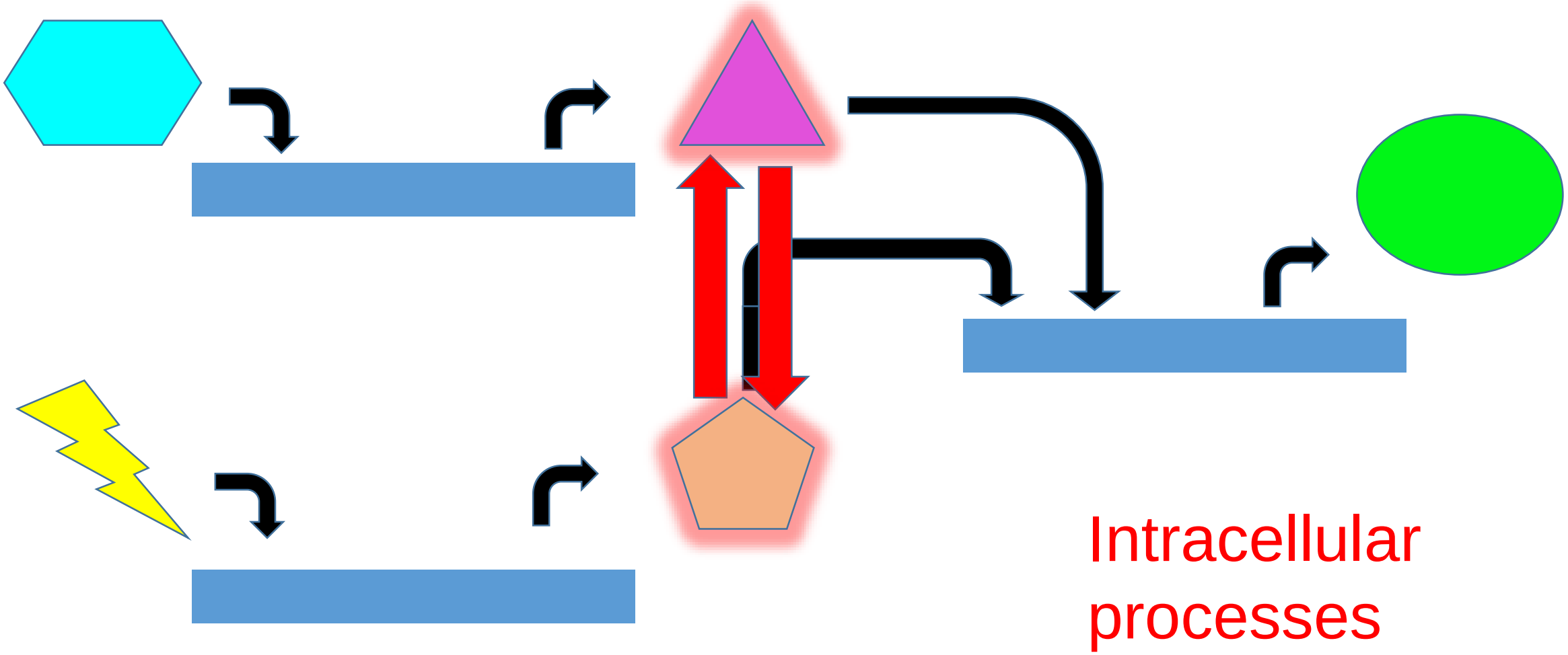
Biological logic circuits

Orthogonality

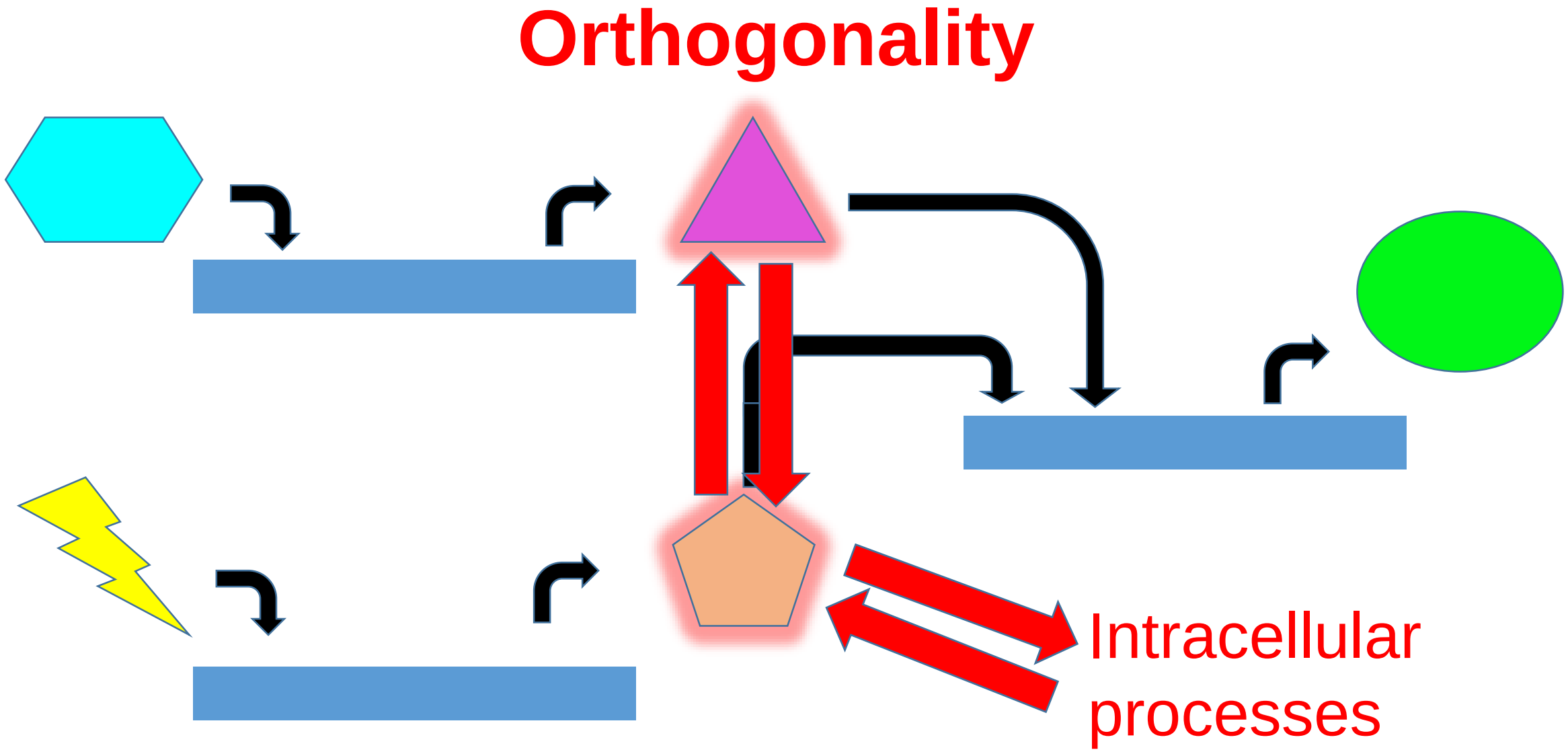


Biological logic circuits

Orthogonality

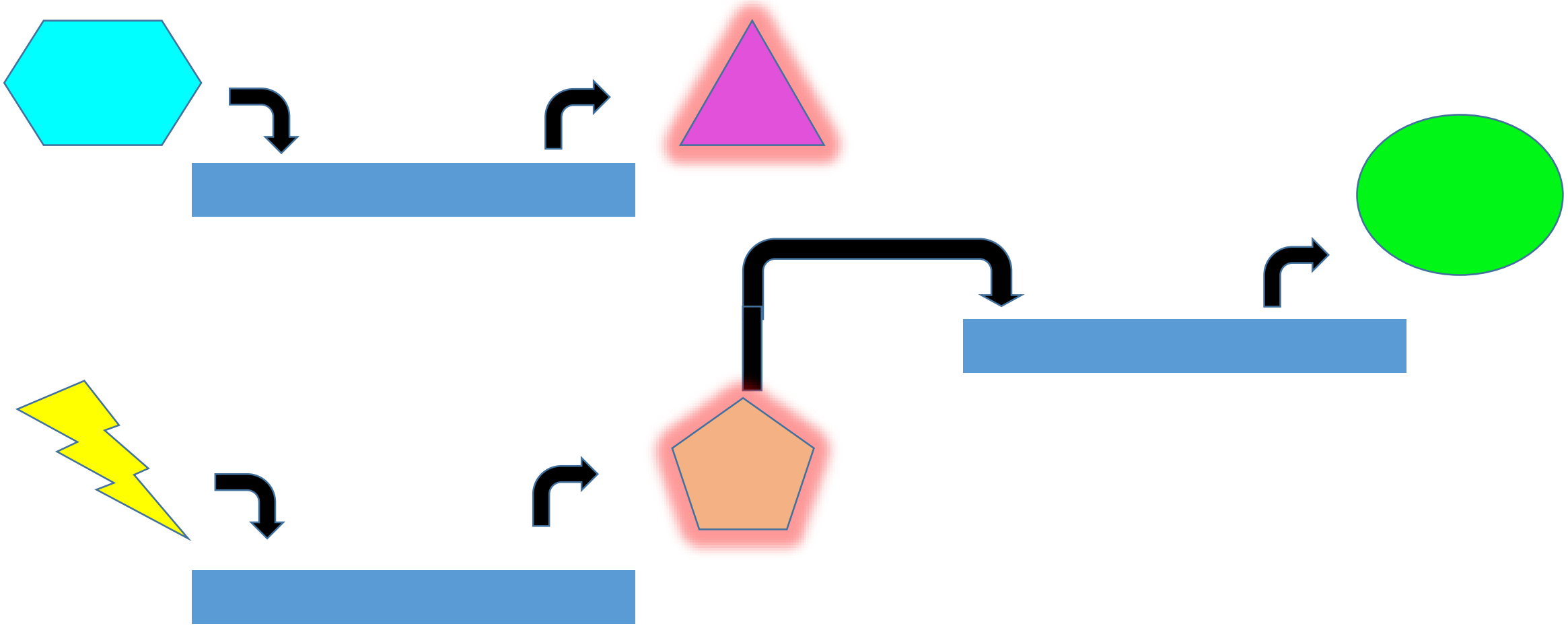


Biological logic circuits



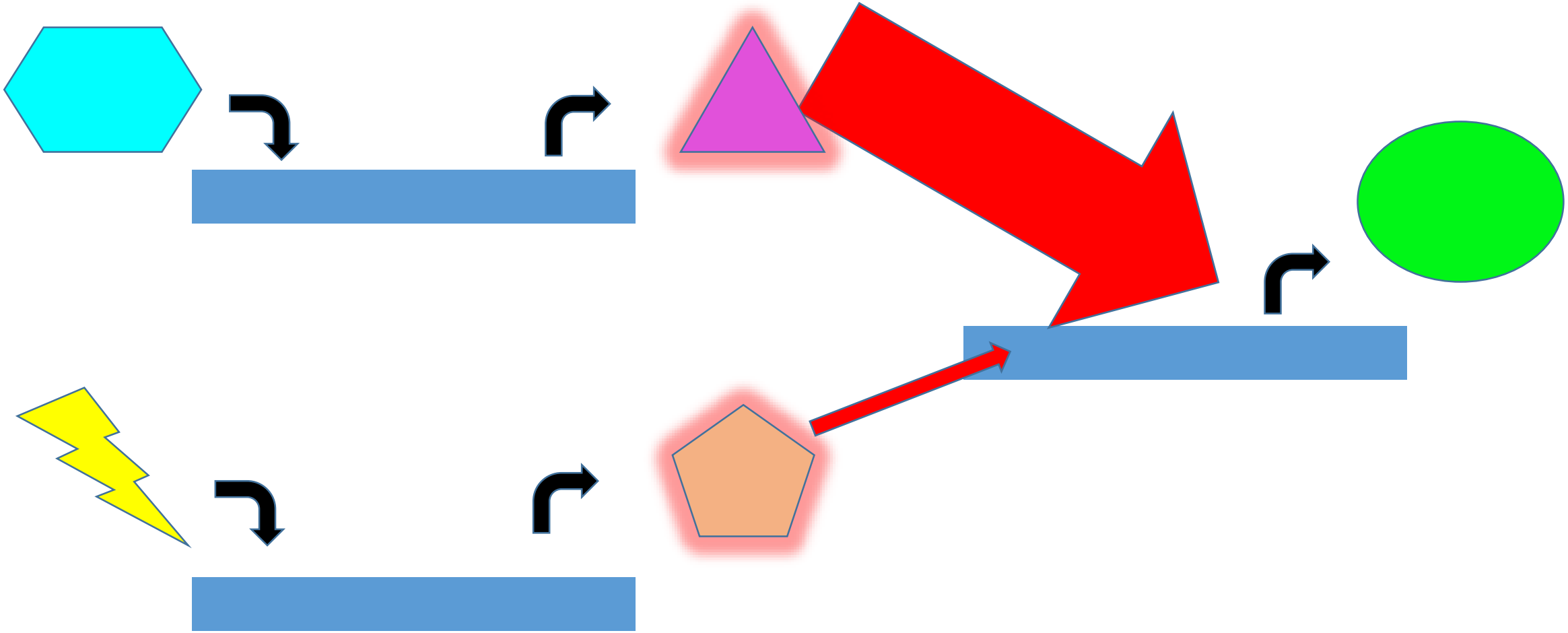
Biological logic circuits

Homogeneity

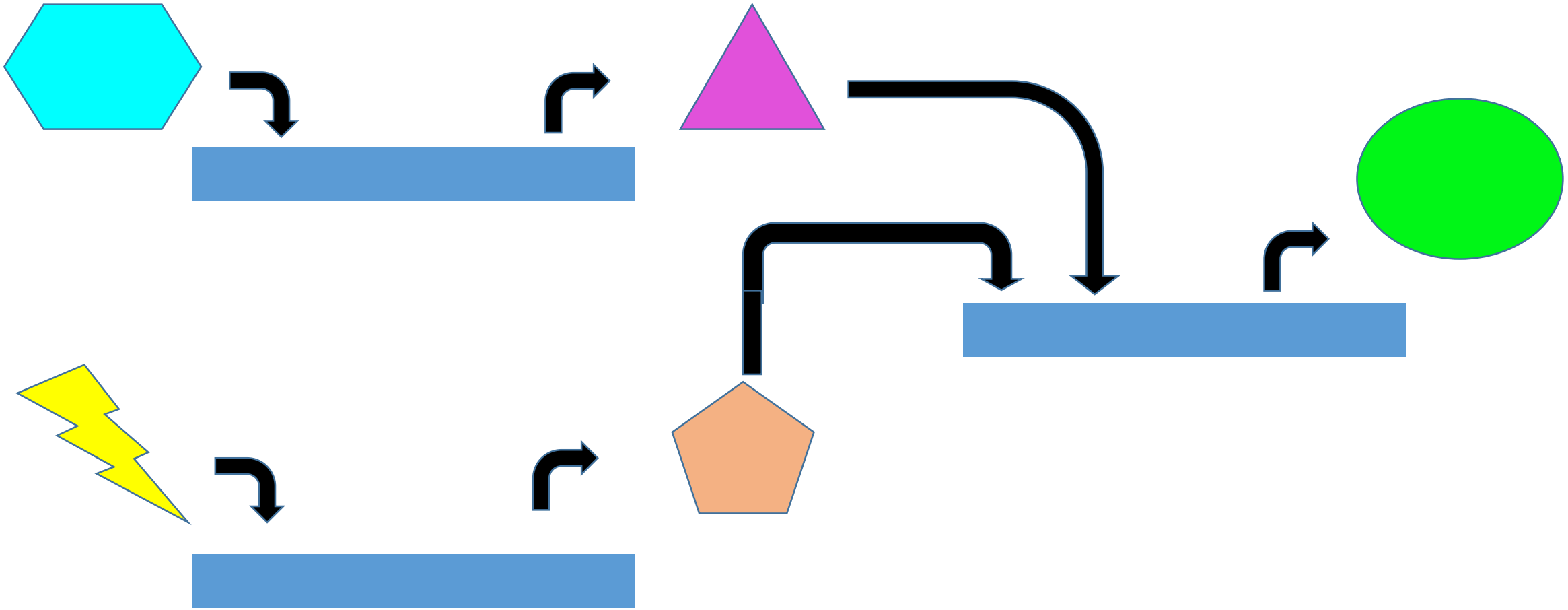


Biological logic circuits

Homogeneity

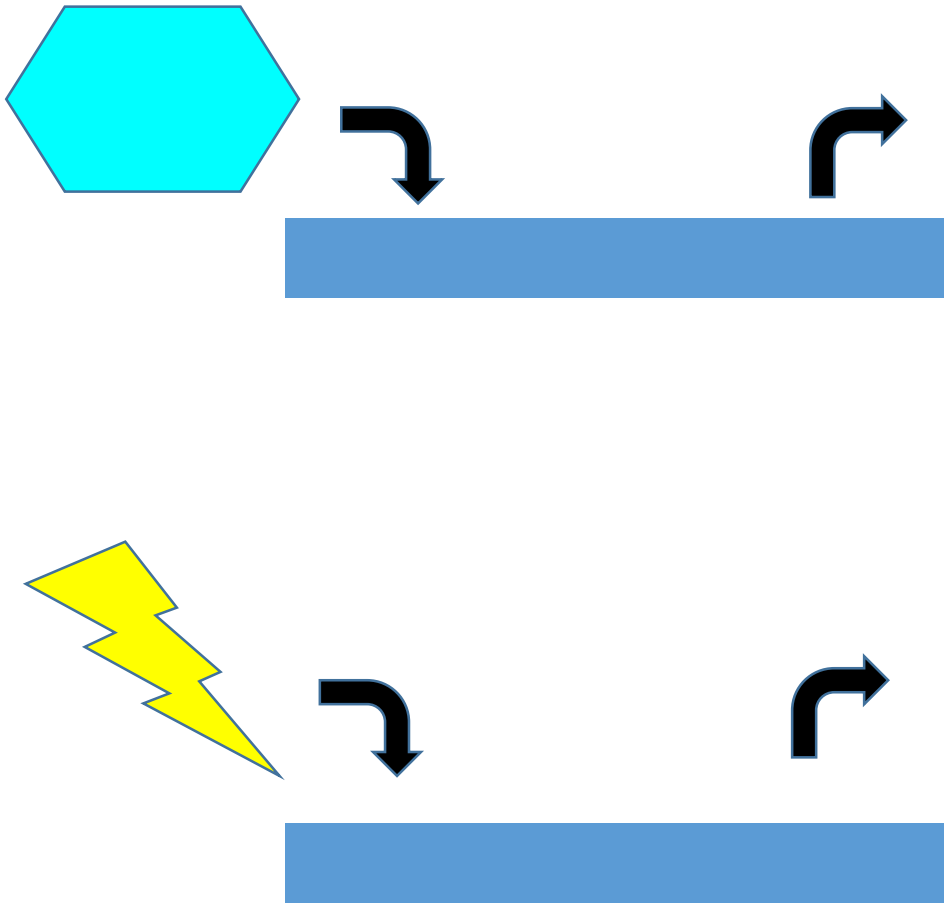


Homogeneity

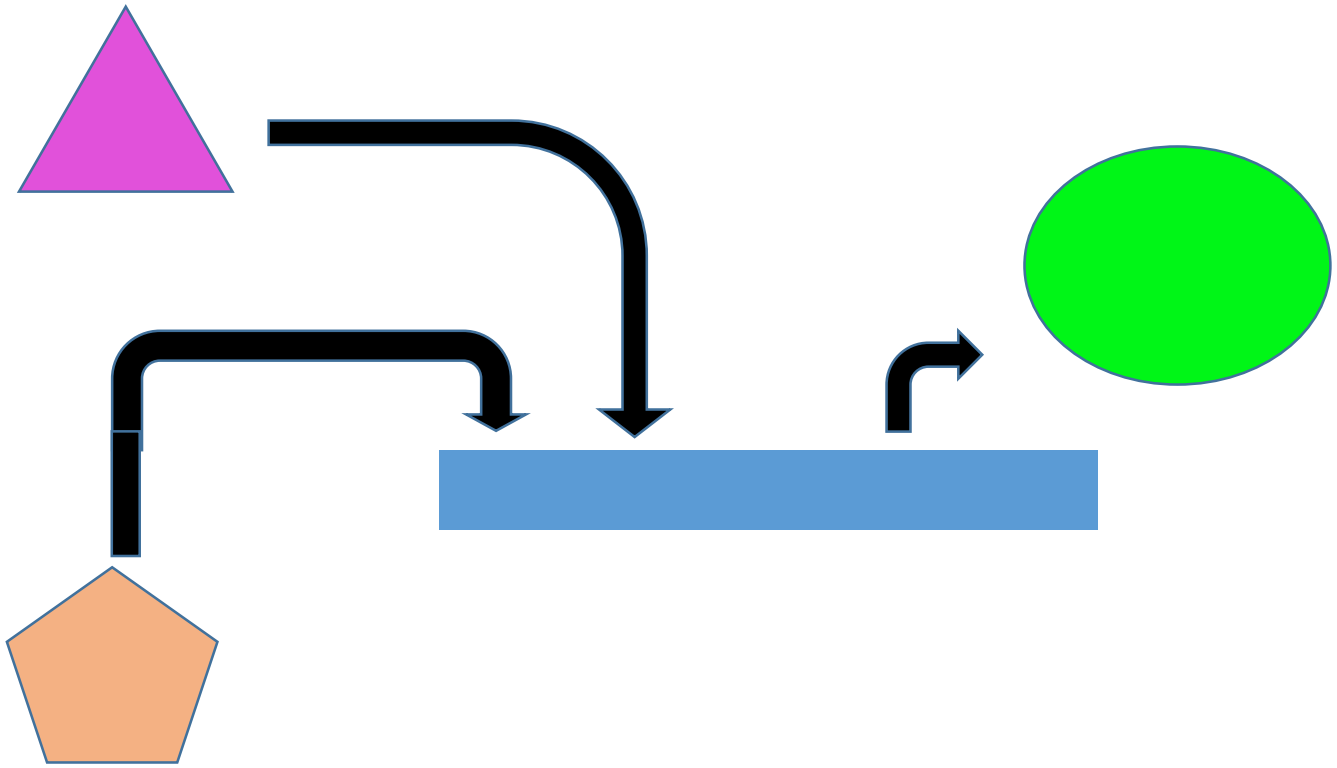


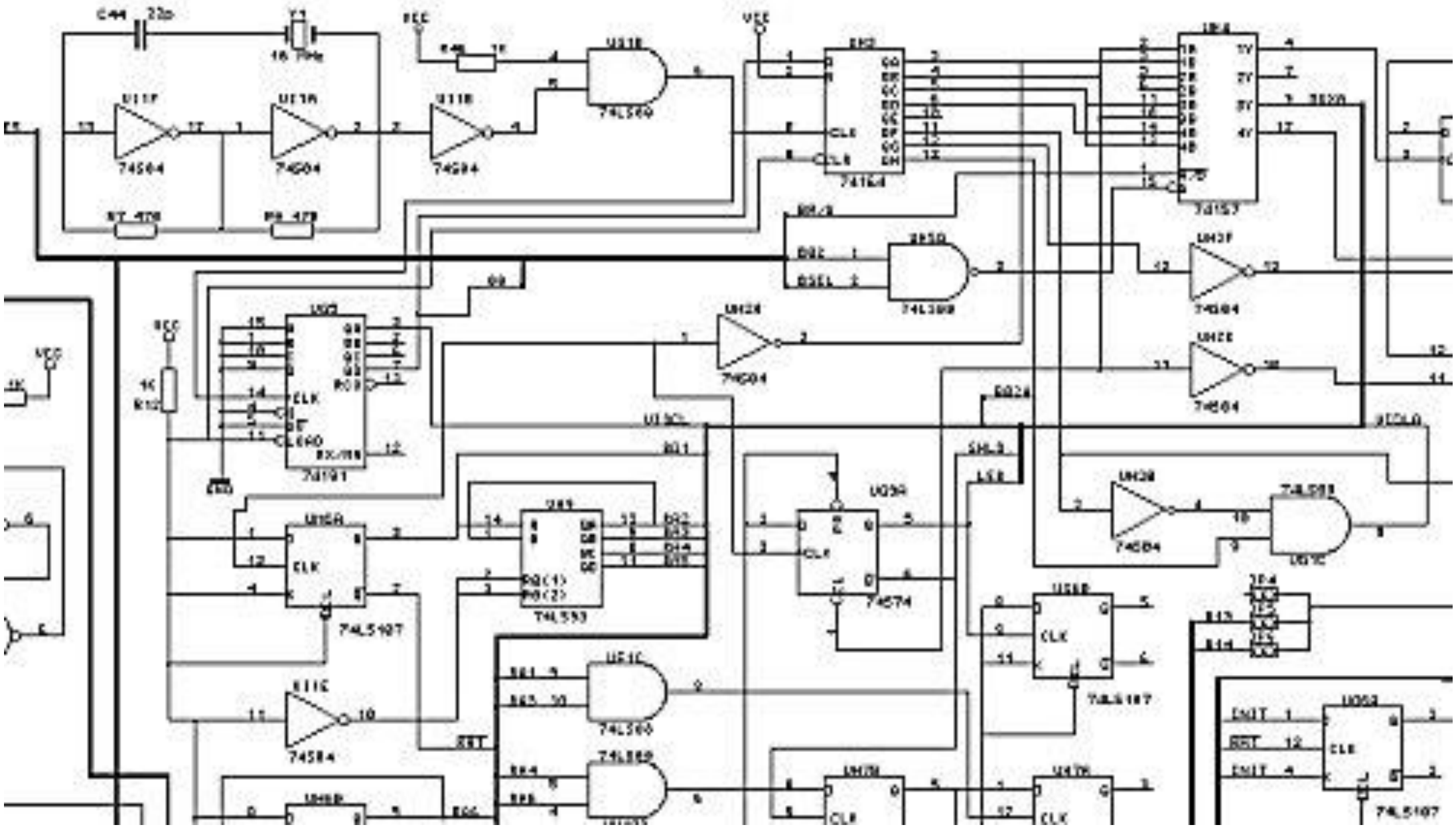
Biological logic circuits

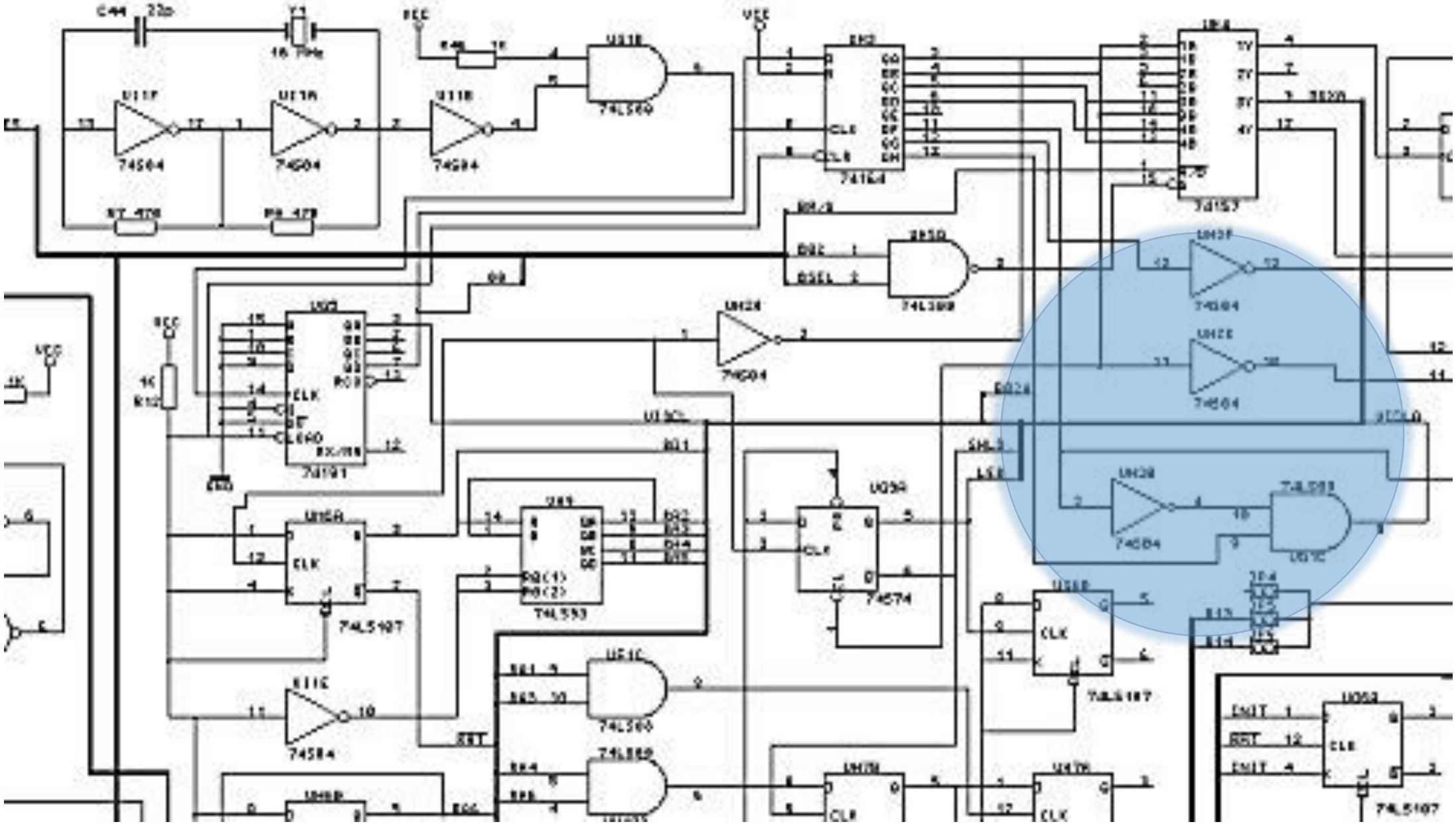
Homogeneity



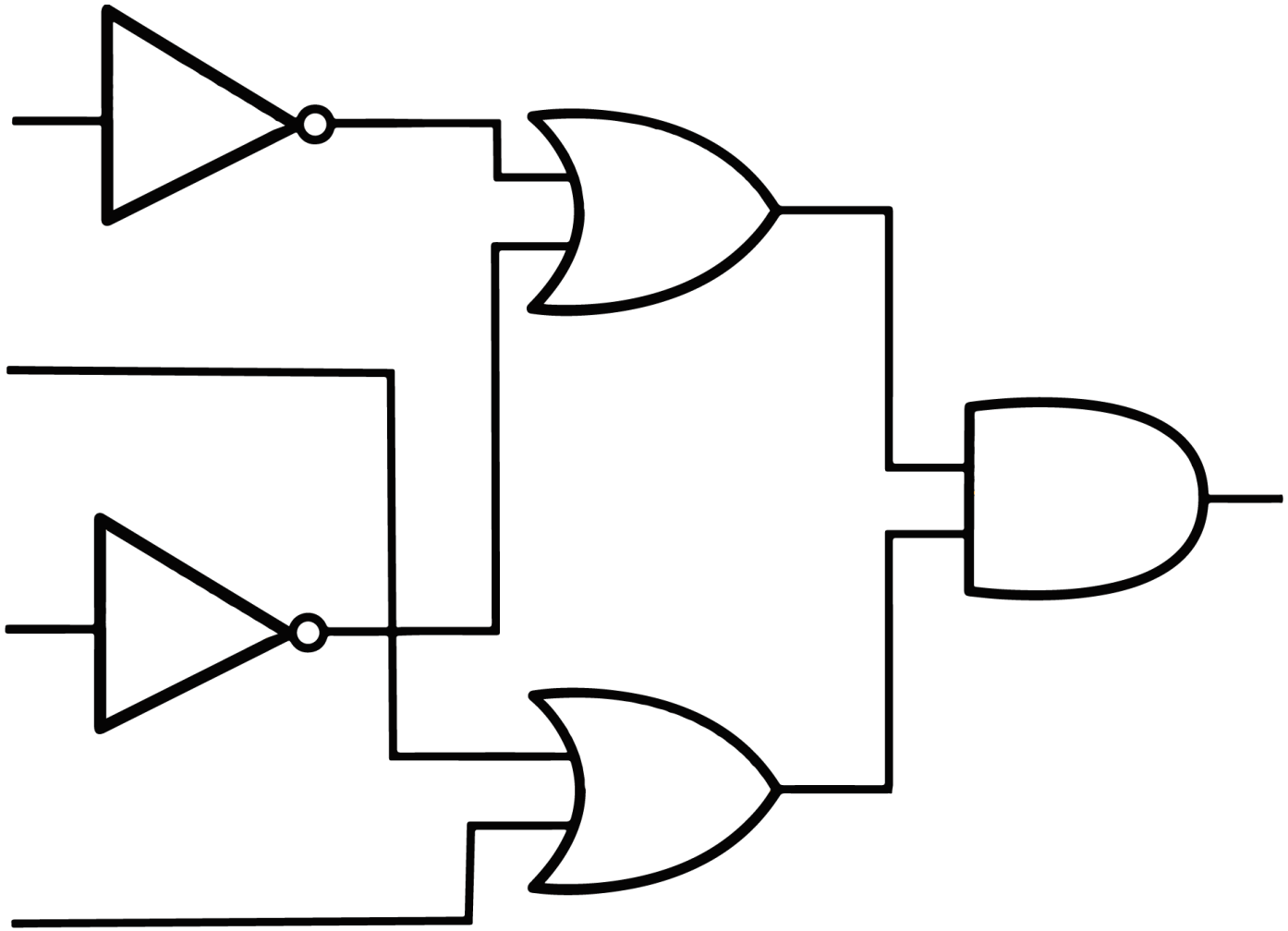
Orthogonality



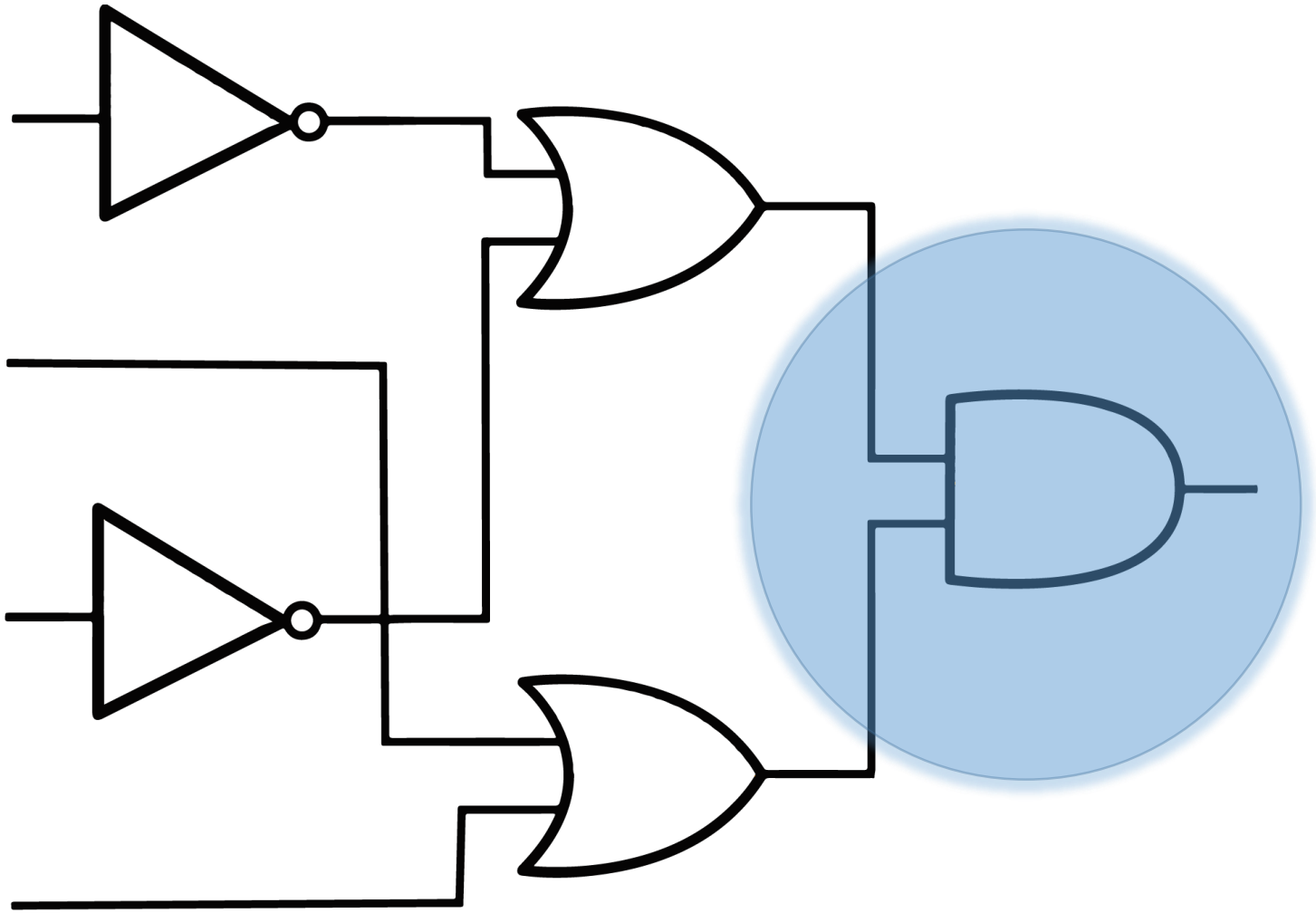




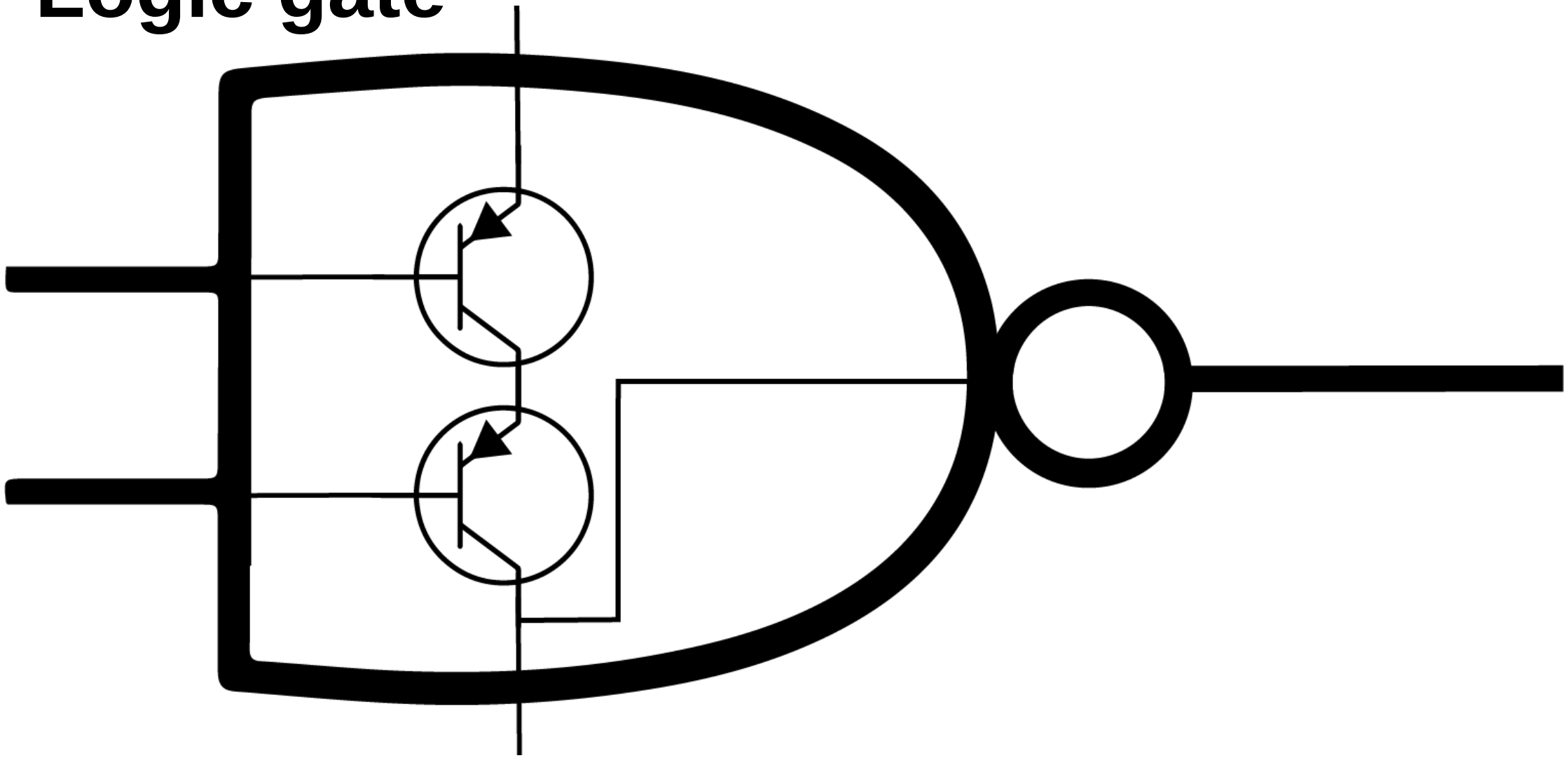
Logic circuit



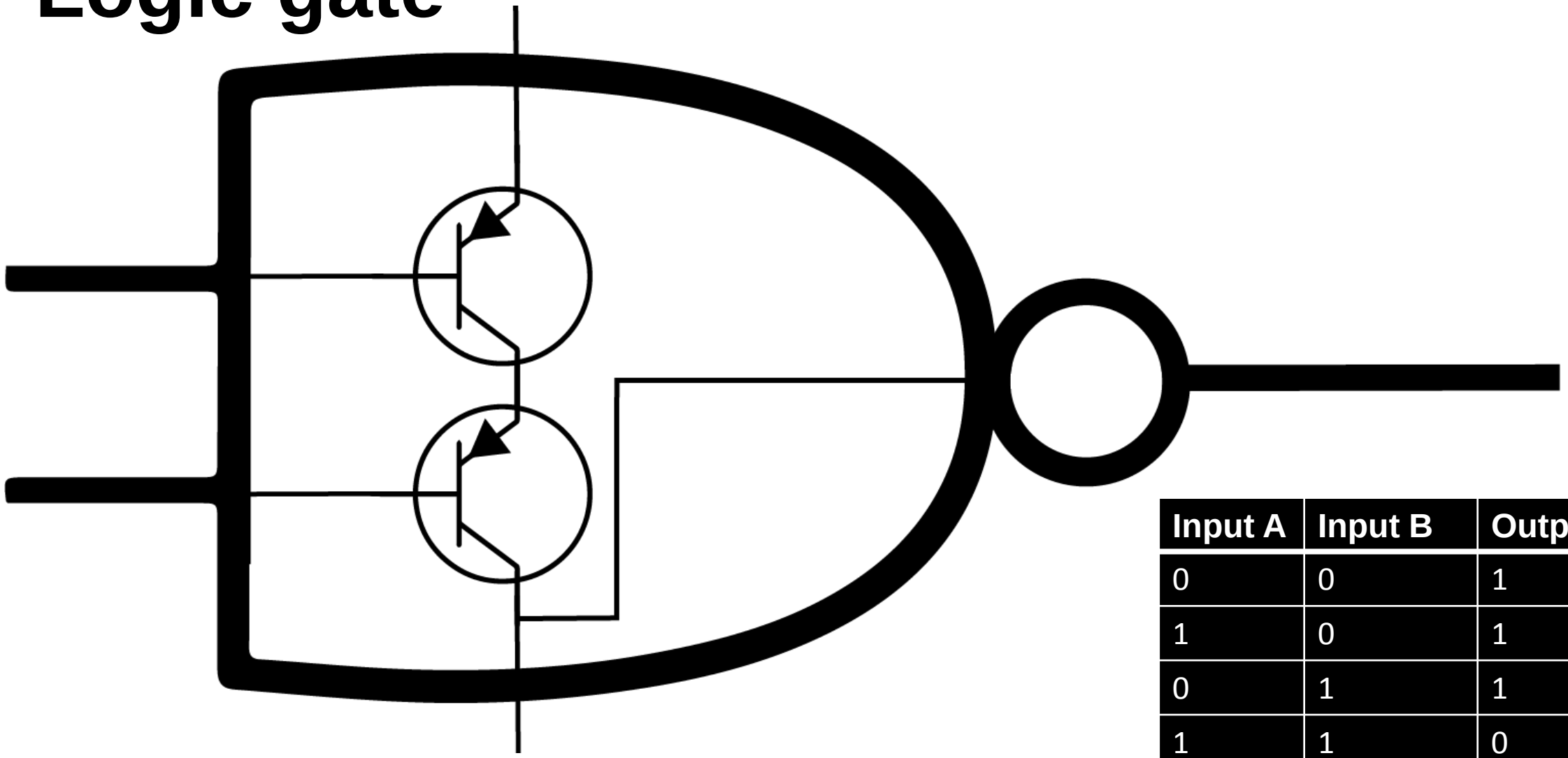
Logic circuit



Logic gate

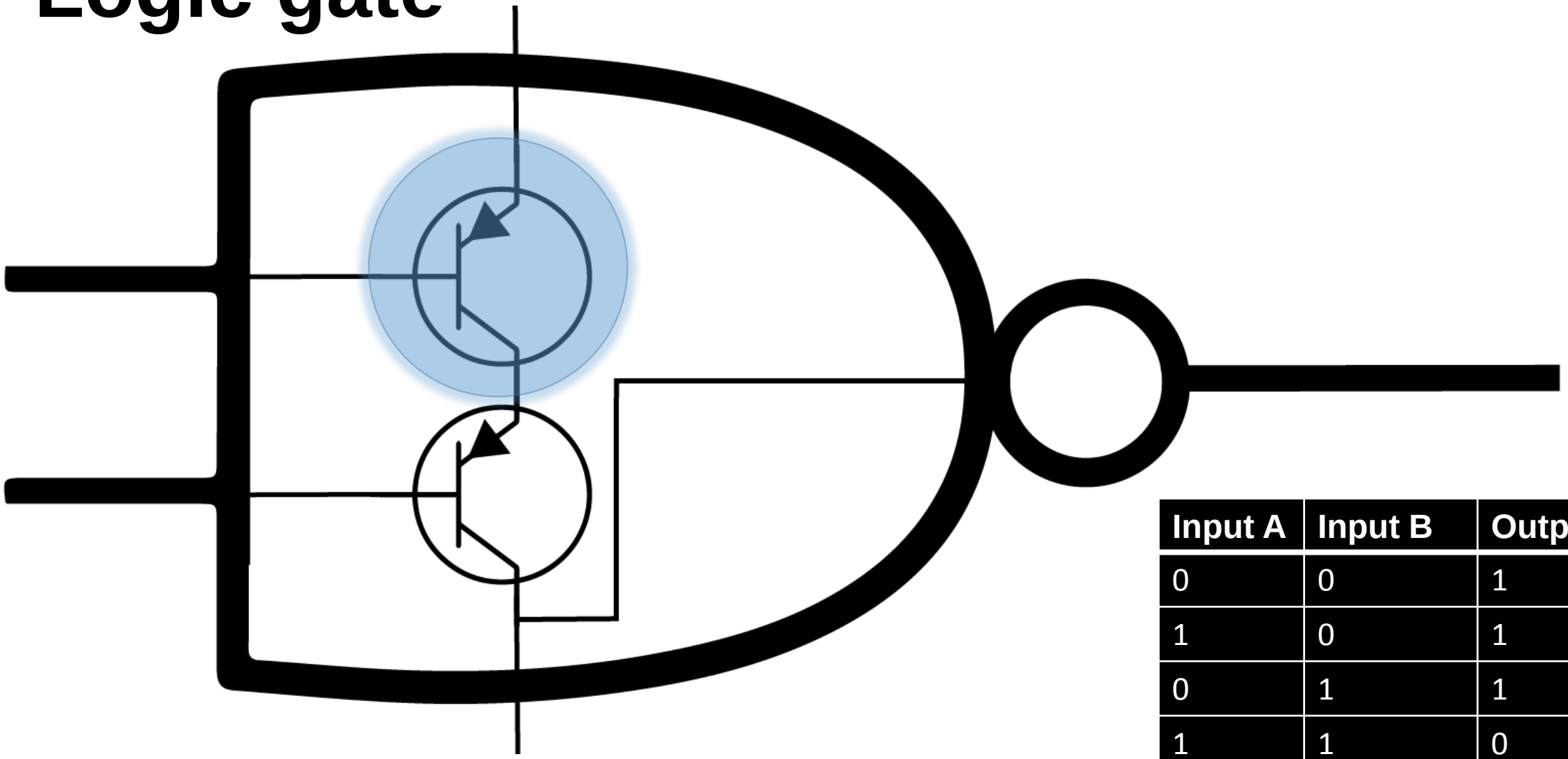


Logic gate



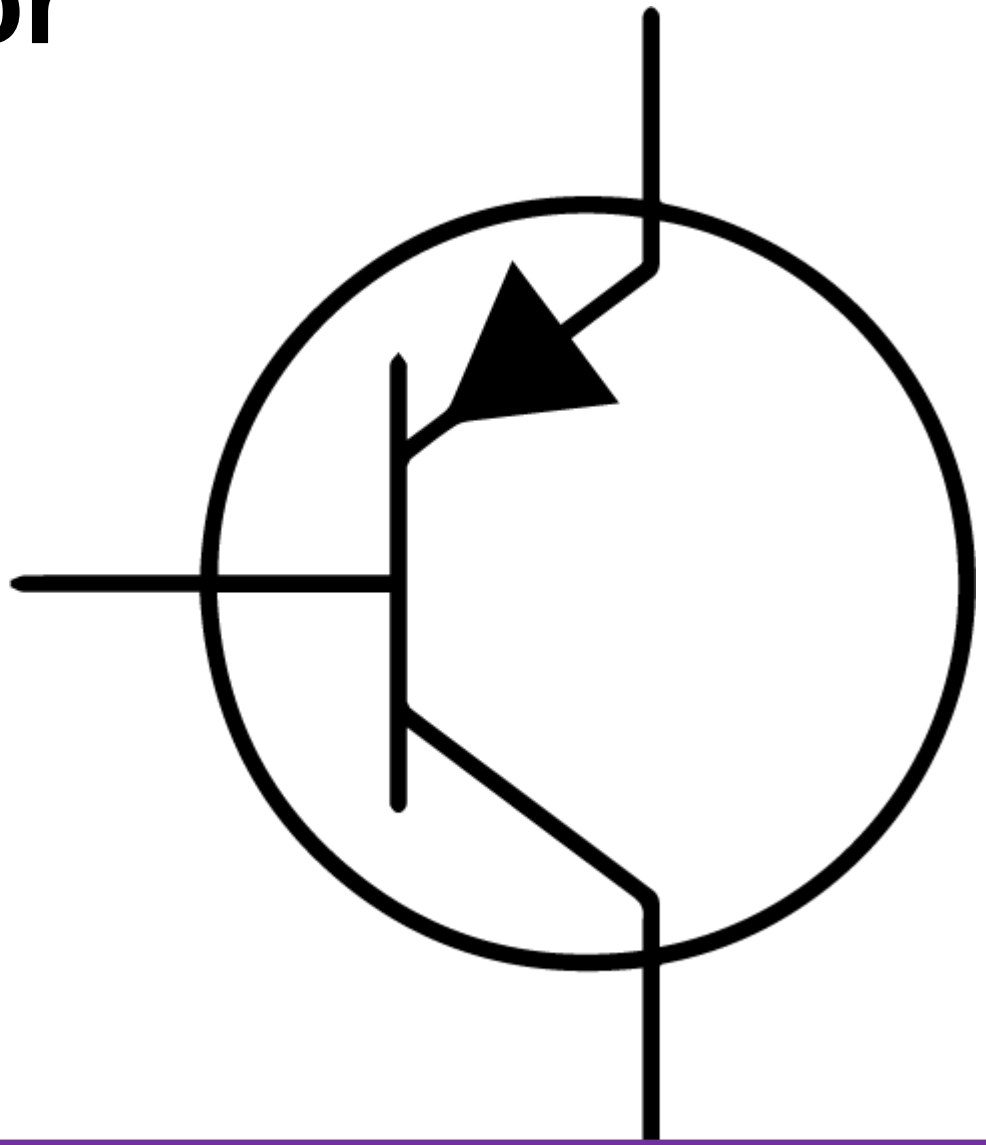
Input A	Input B	Output
0	0	1
1	0	1
0	1	1
1	1	0

Logic gate



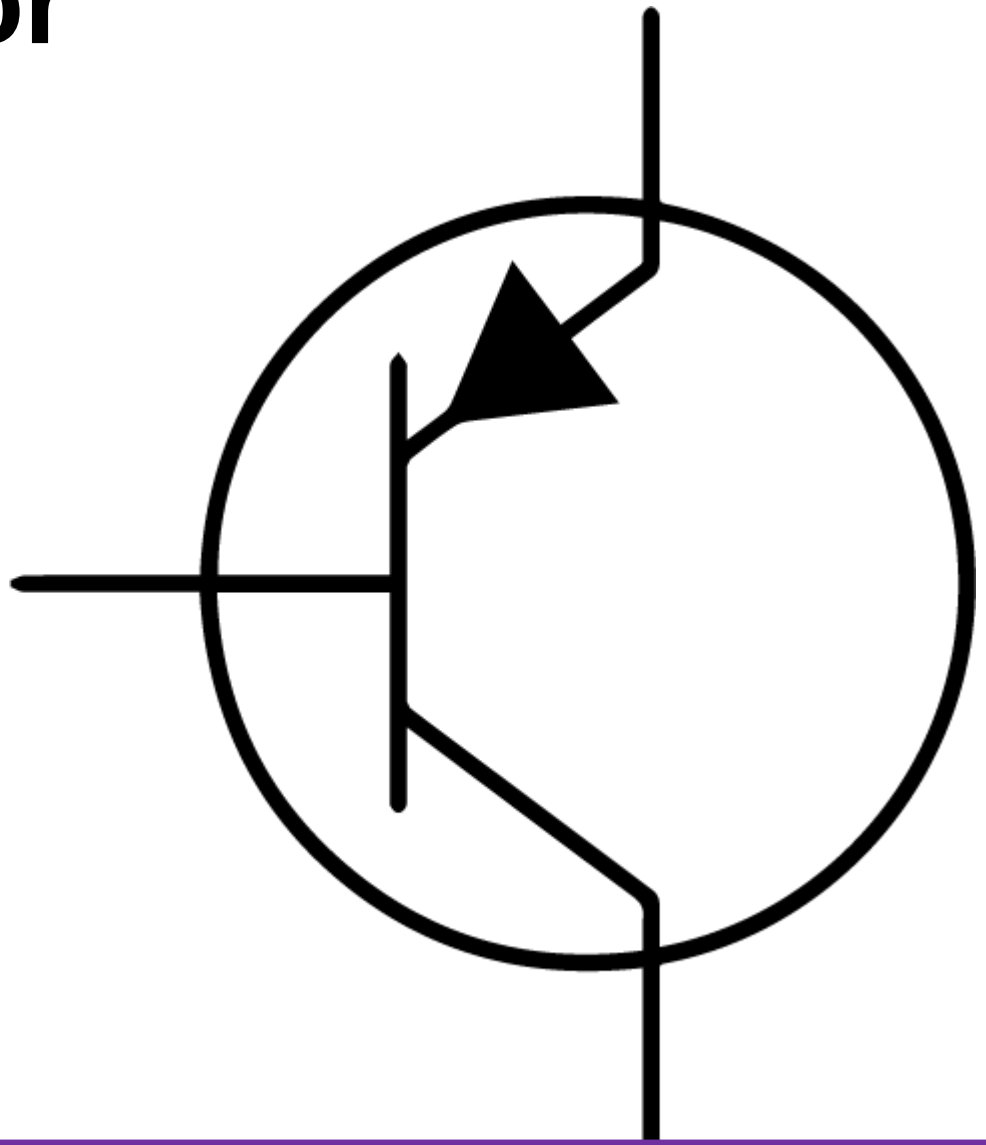
Input A	Input B	Output
0	0	1
1	0	1
0	1	1
1	1	0

Transistor

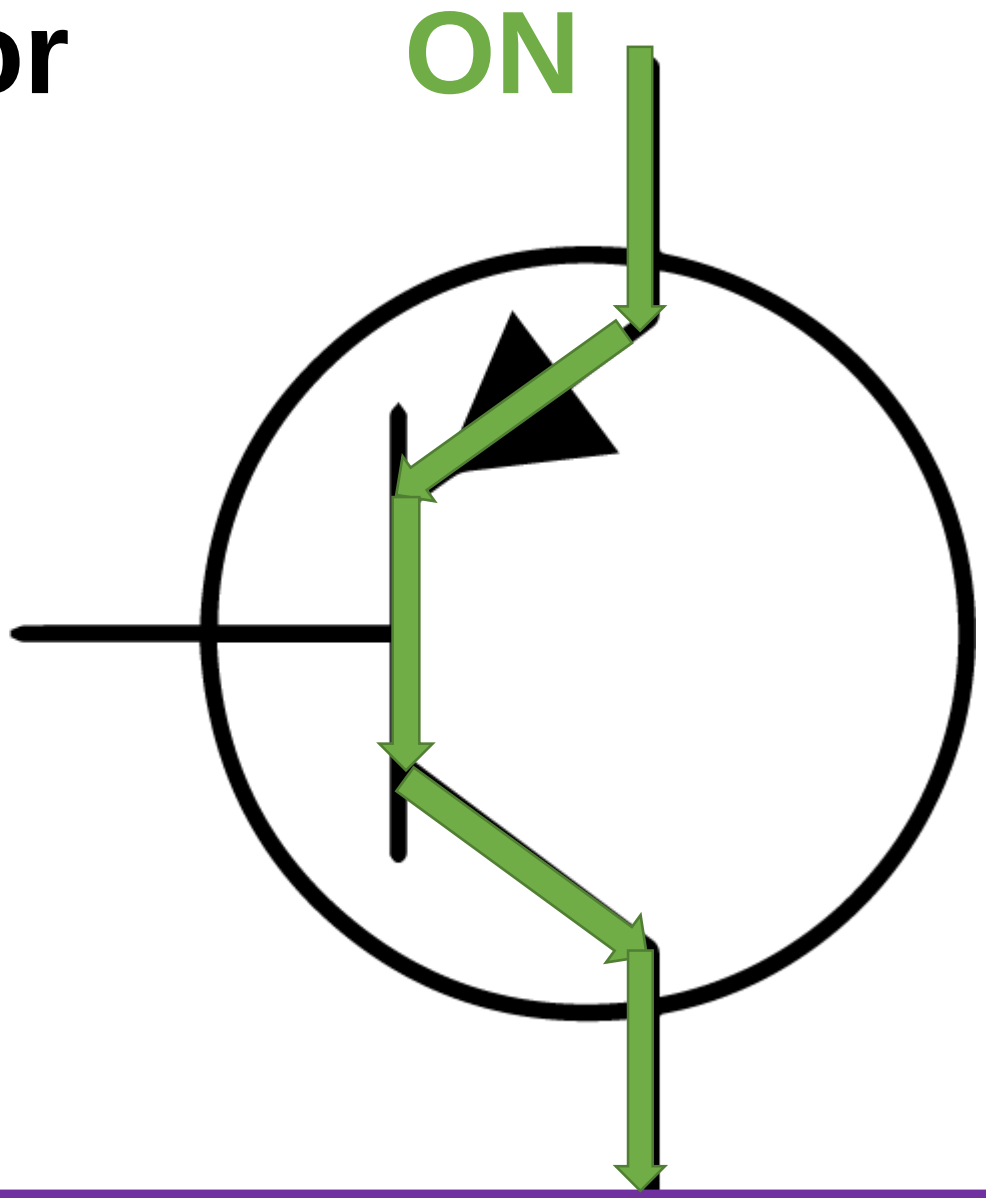


Transistor

No current

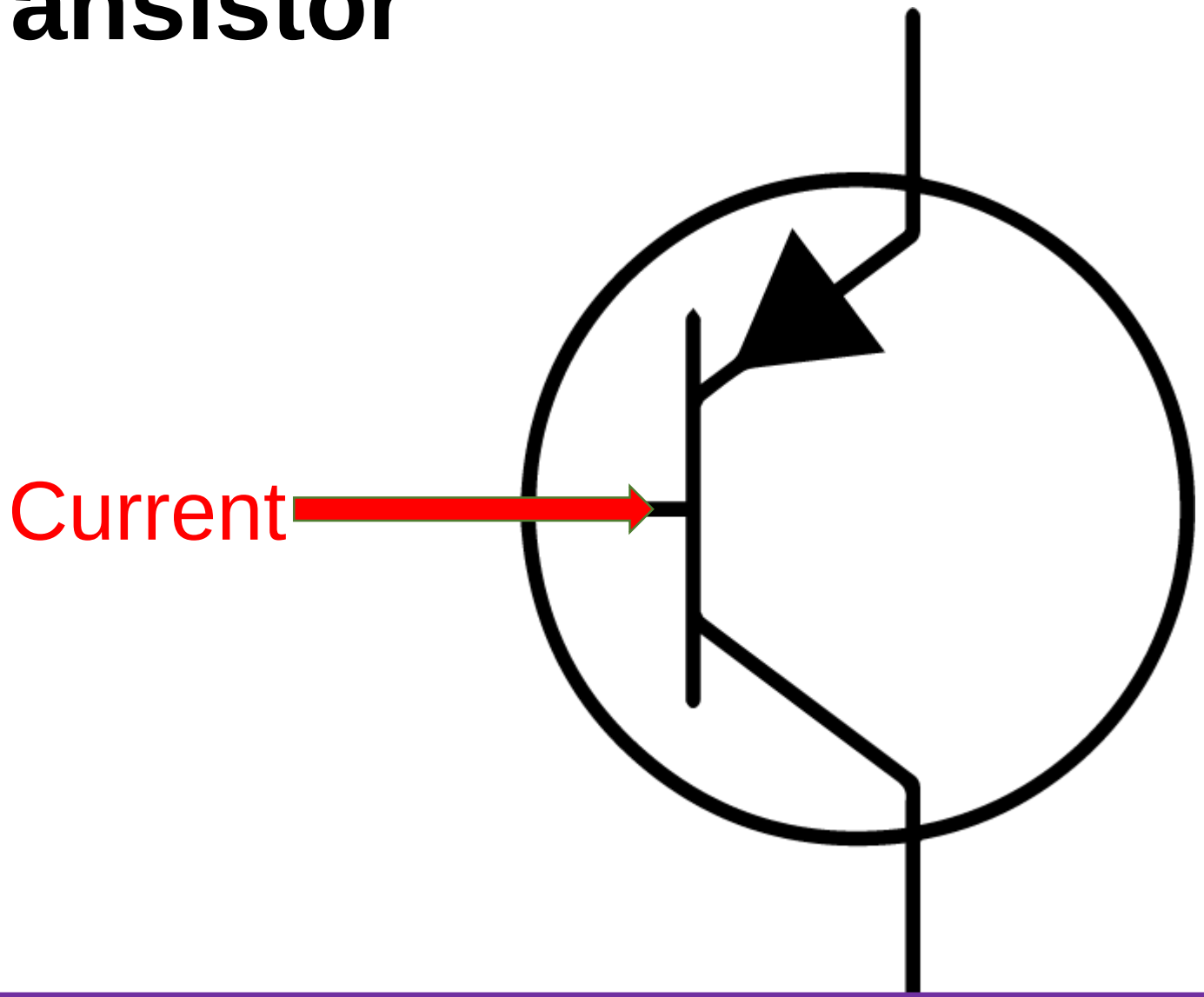


Transistor

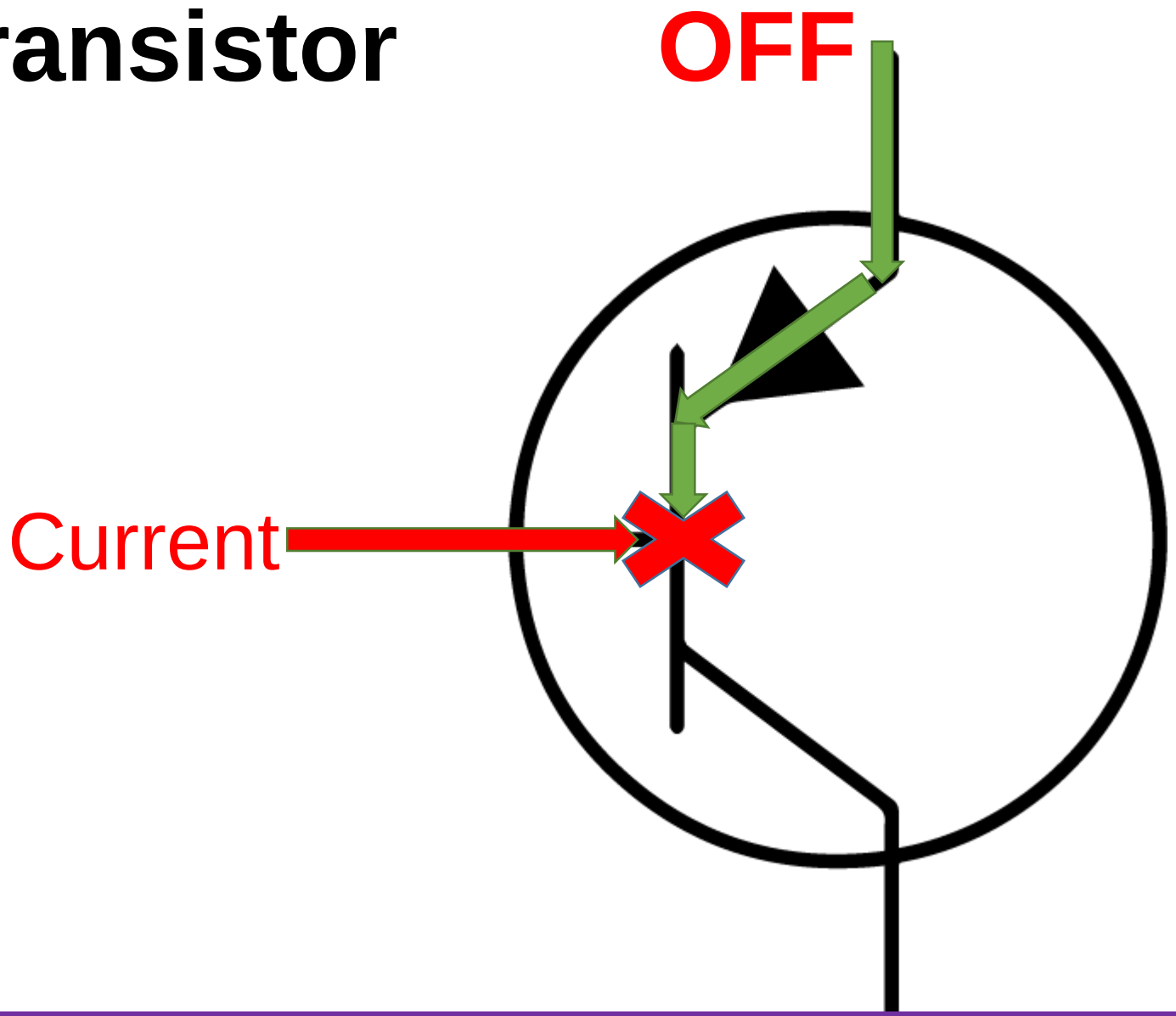


No current

Transistor



Transistor



Chassis

E. coli

S. cerevisiae

Chassis

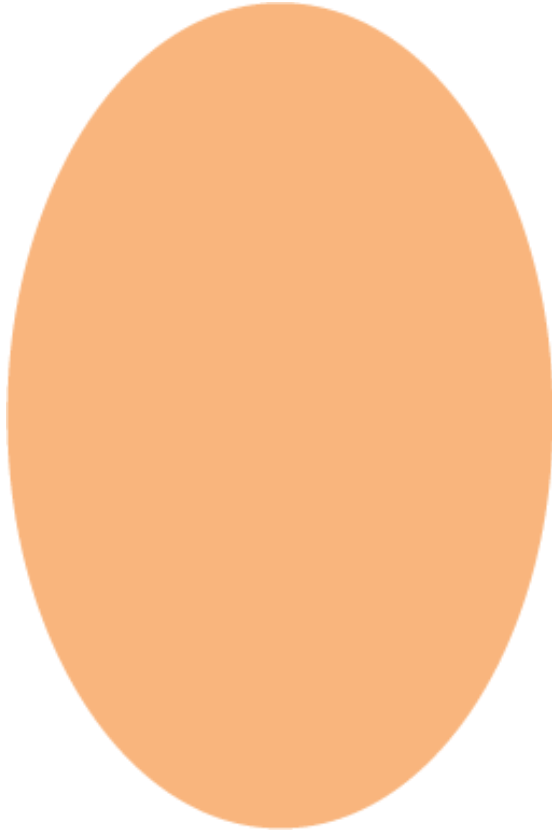
E. coli

S. cerevisiae

BioLOGIC

Logic **O**rthogonal **g**RNA **I**mplemented **C**ircuits

dCas9 binds to gRNA



GGGGTTCGCAAGTCCCACCCCAAACGATGCTGAAGGCTCAGGTTTCACAGGGACAAAAGCTTTAAACGCGAGTTCCCGCTCATAACCTGGACCGAATGCAGAATCATGCATCGTTCCACTGTGTTCGTGTCATCTA
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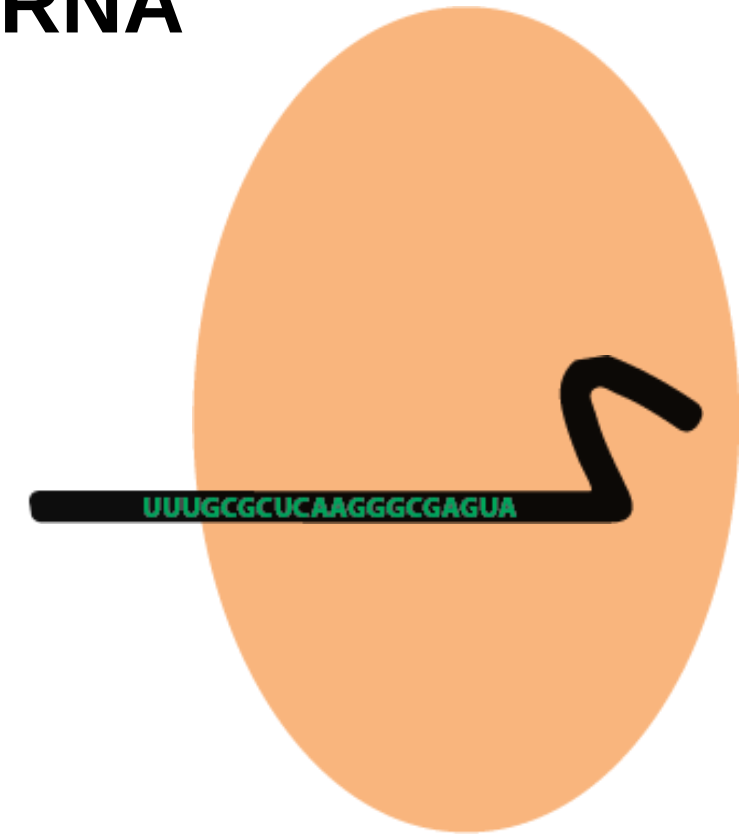
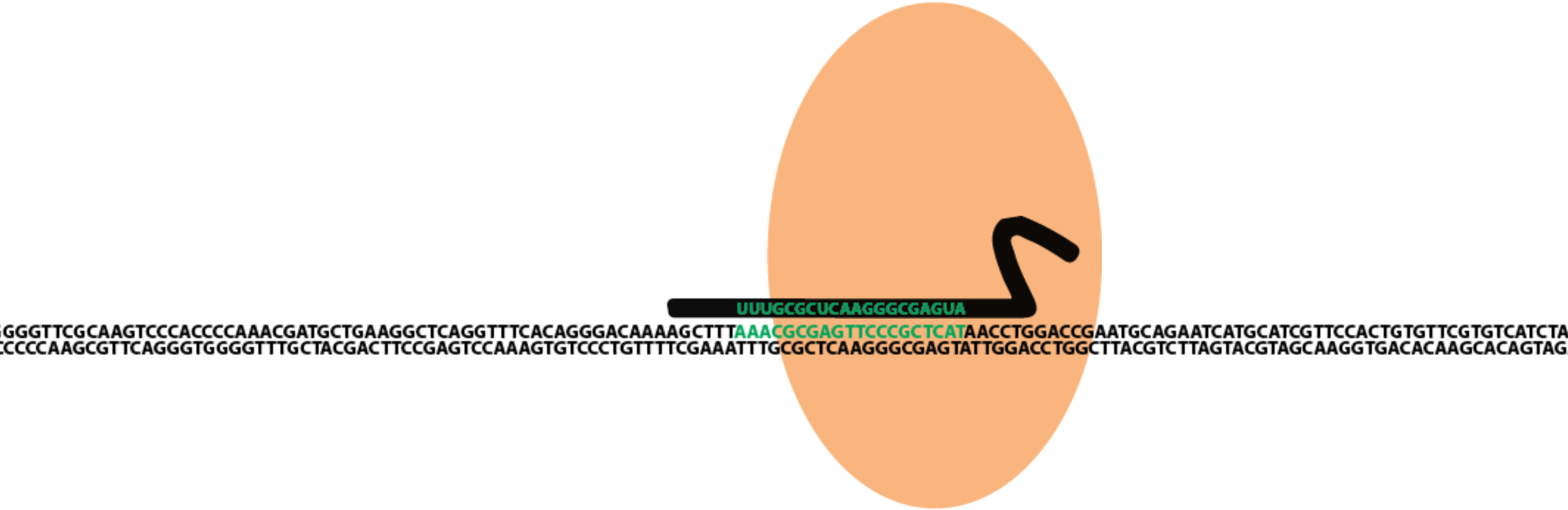
dCas9 binds to gRNA

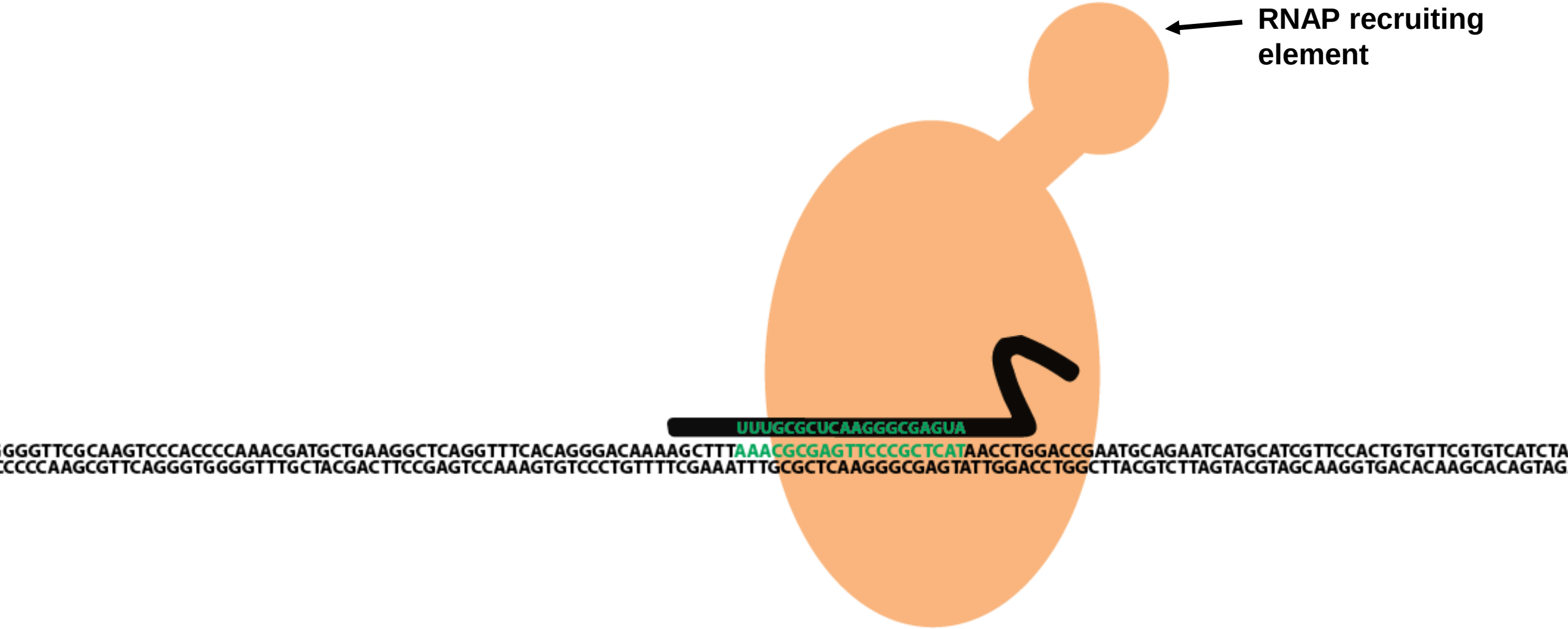


GGGTTCGCAAGTCCCACCCCAAACGATGCTGAAGGCTCAGGTTTCACAGGGACAAAAGCTTTAAACGCGAGTTCCCGCTCATAACCTGGACCGAATGCAGAATCATGCATCGTTCCACTGTGTTCGTGTCATCTA
CCCCCAAGCGTTCAGGGTGGGGTTTGCTACGACTTCCGAGTCCAAAGTGTCCCTGTTTTCGAAATTTGCGCTCAAGGGCGAGTATTGGACCTGGCTTACGTCTTAGTACGTAGCAAGGTGACACAAGCACAGTAG

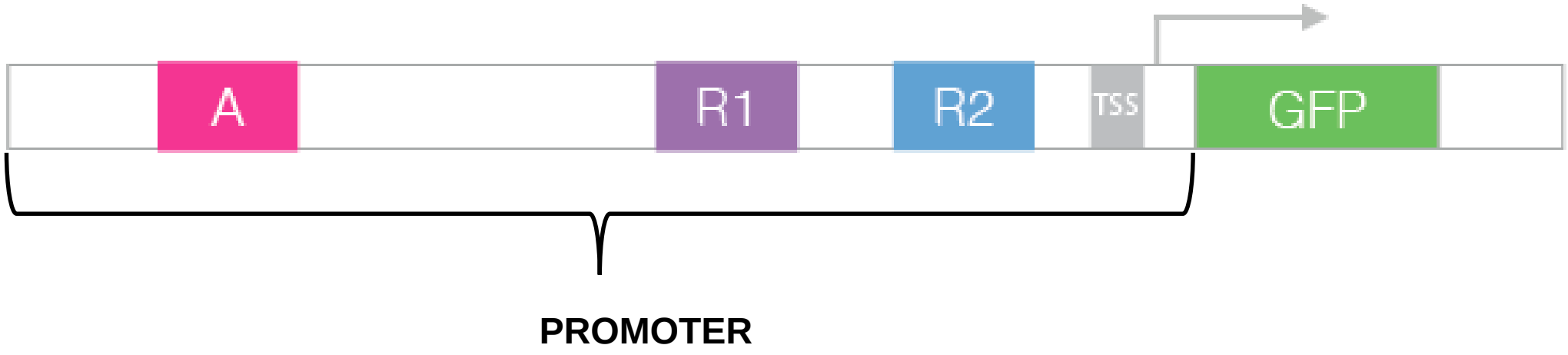
dCas9-gRNA binds to DNA



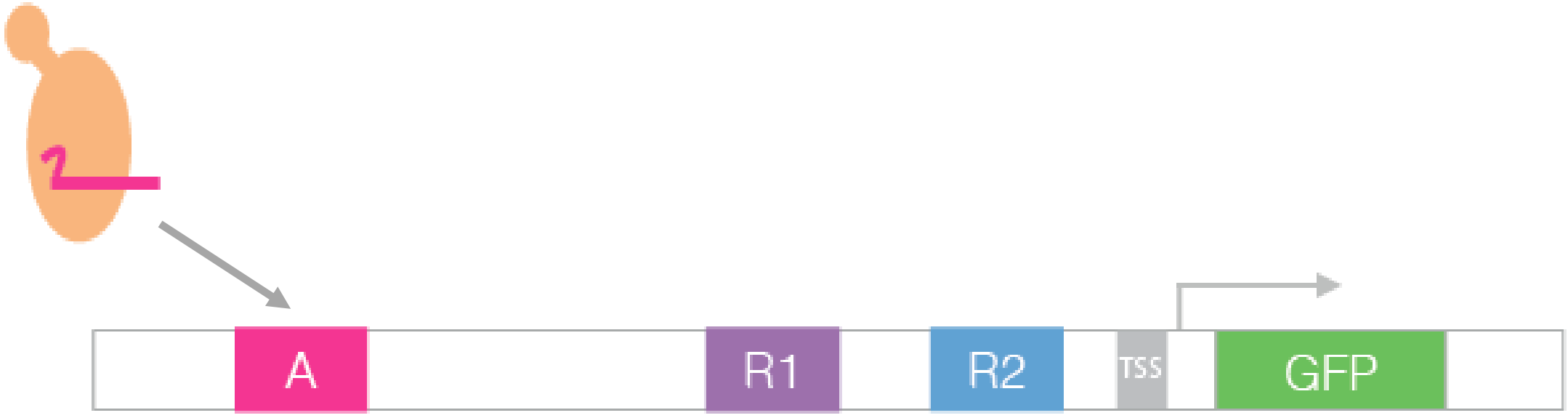
dCas9 as an artificial transcription factor



Bio transistor = promoter + gene

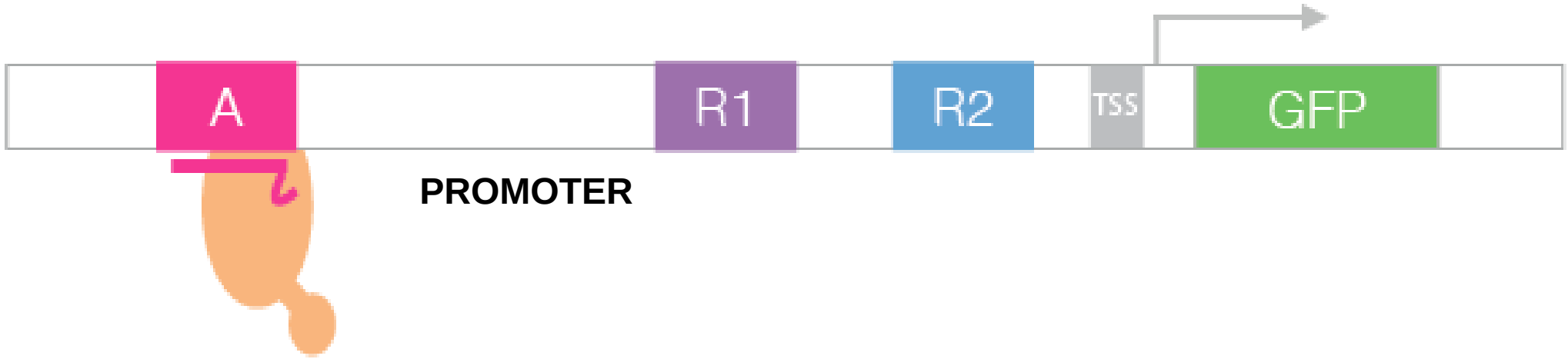


Bio transistor “ON”

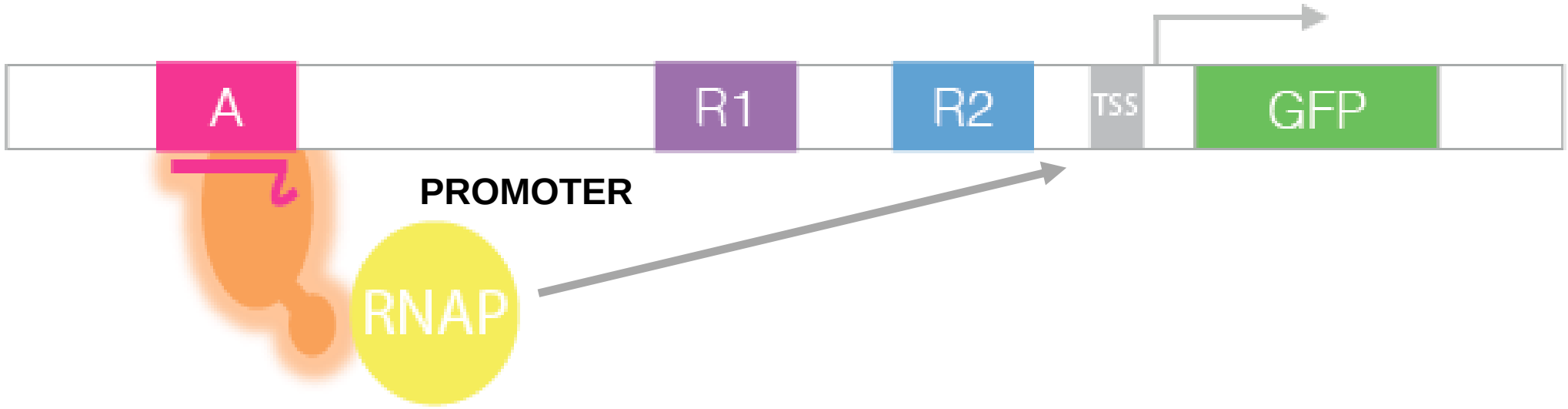


PROMOTER

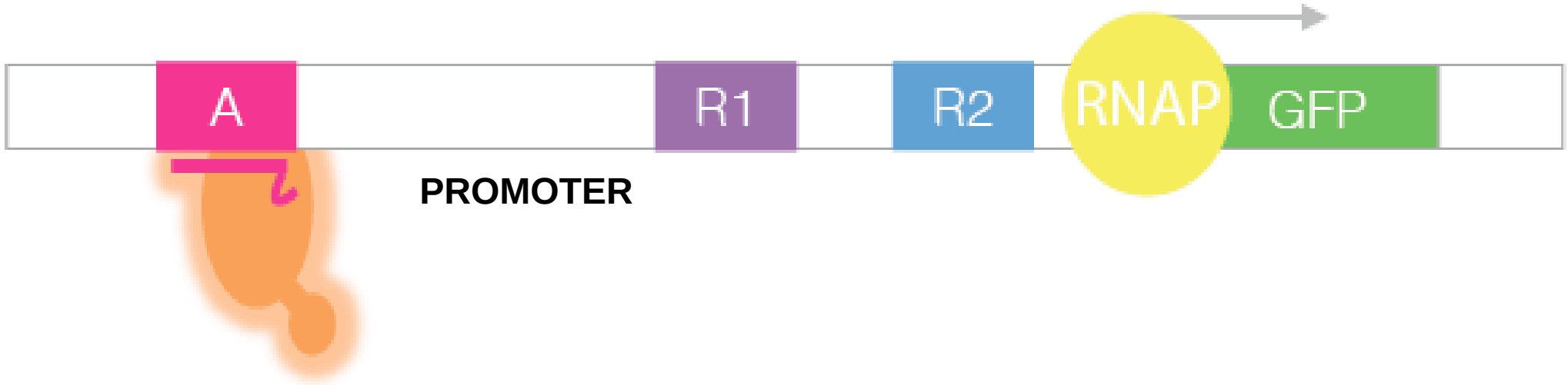
Bio transistor “ON”



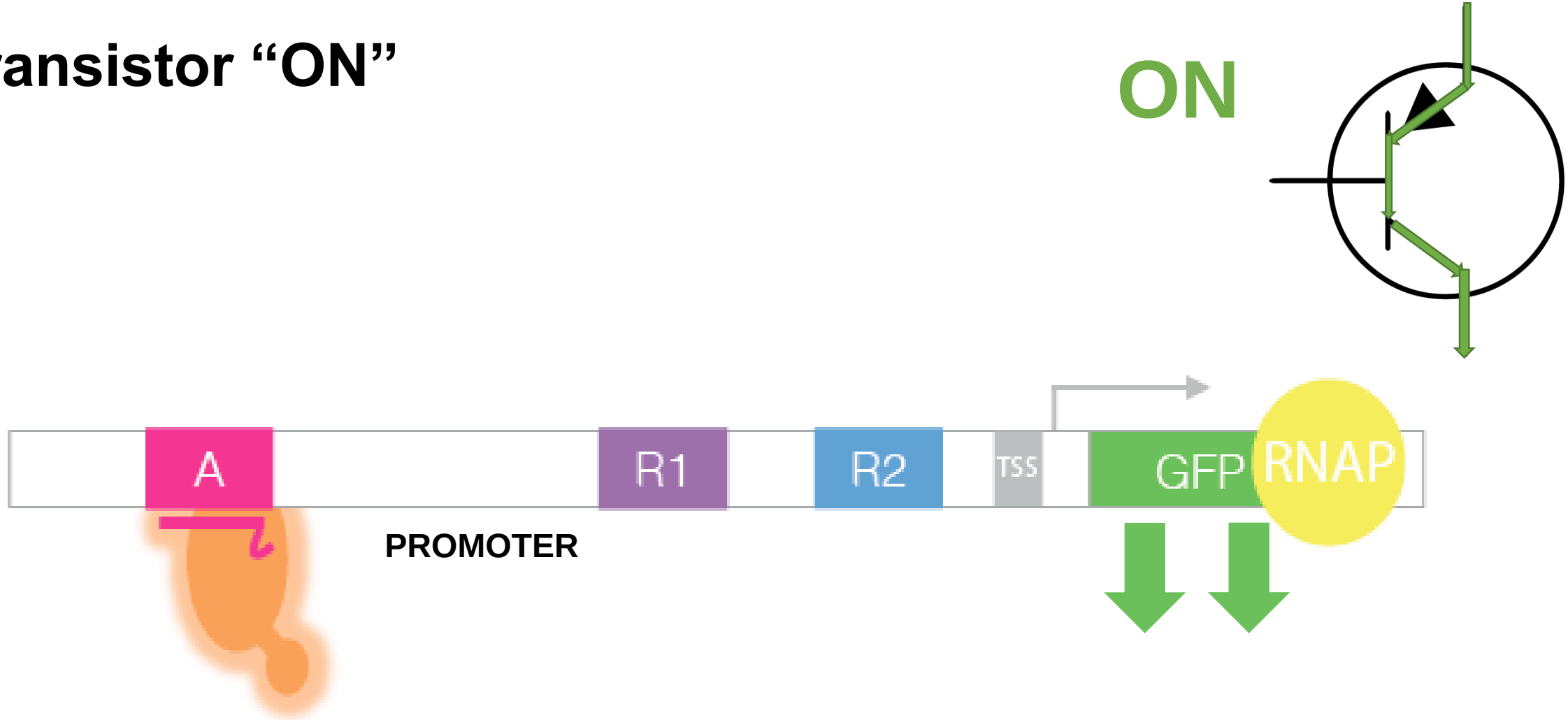
Bio transistor “ON”



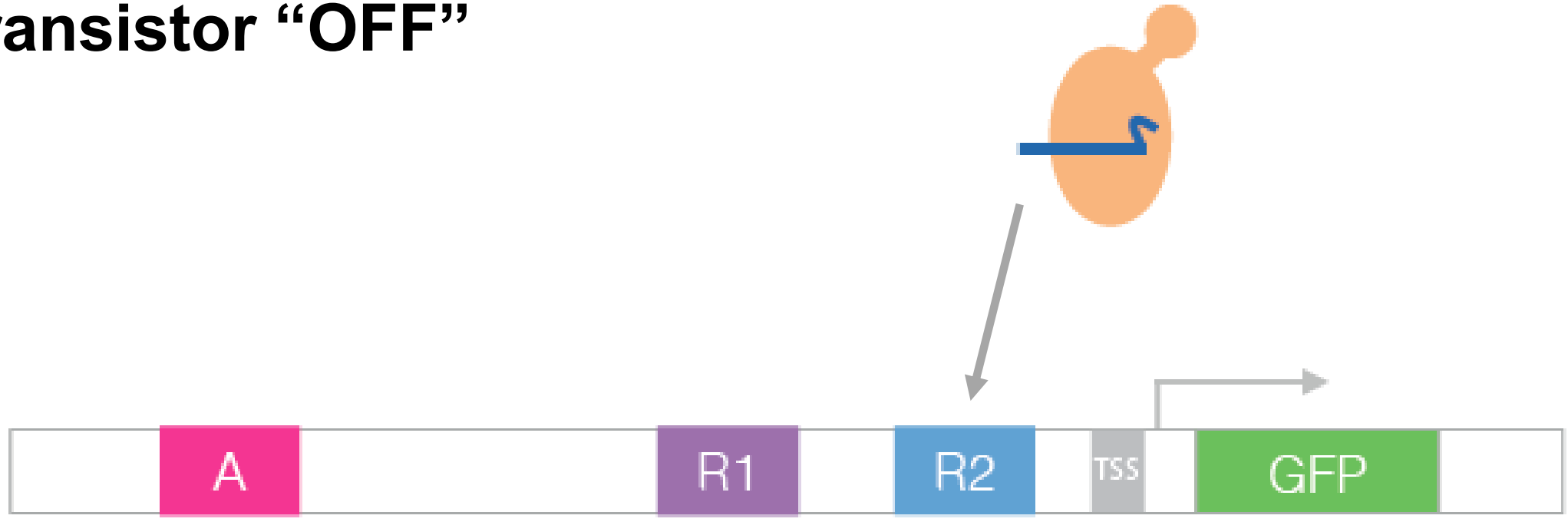
Bio transistor “ON”



Bio transistor “ON”

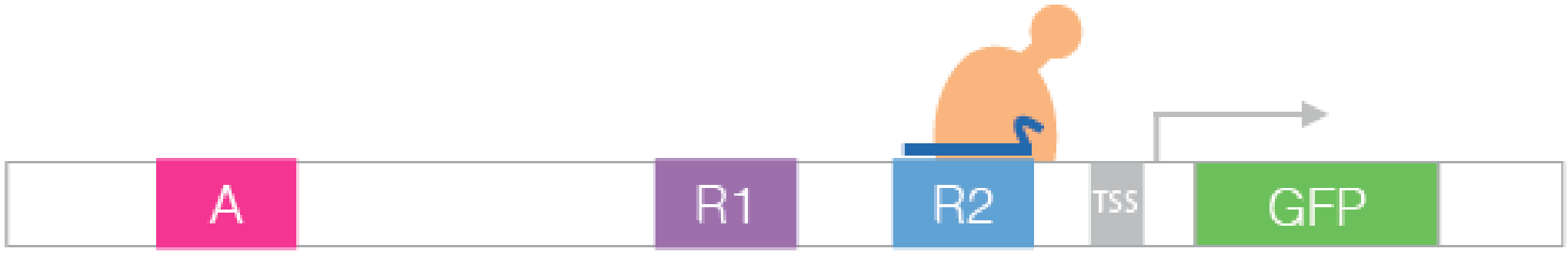


Bio transistor “OFF”



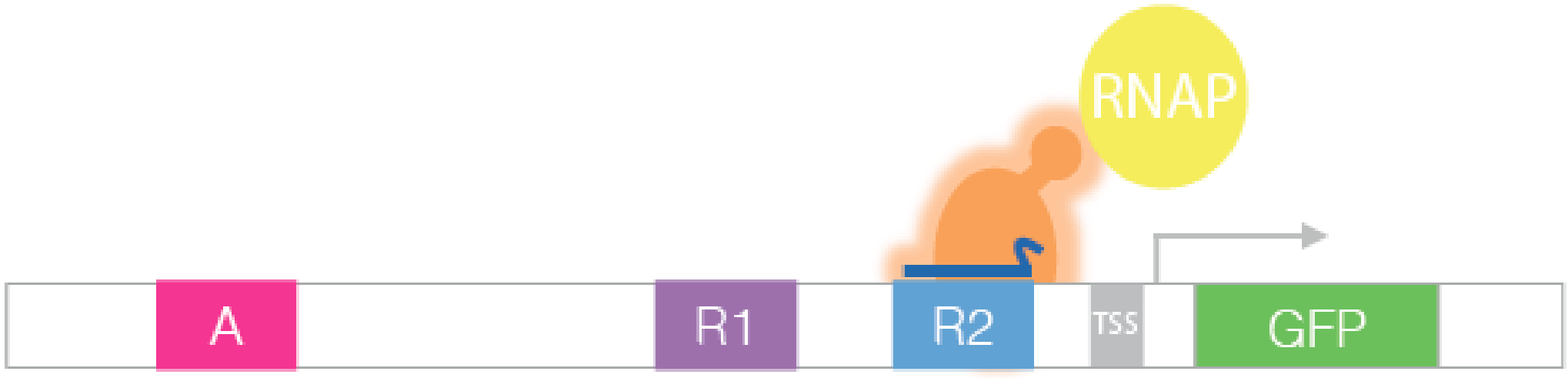
PROMOTER

Bio transistor “OFF”



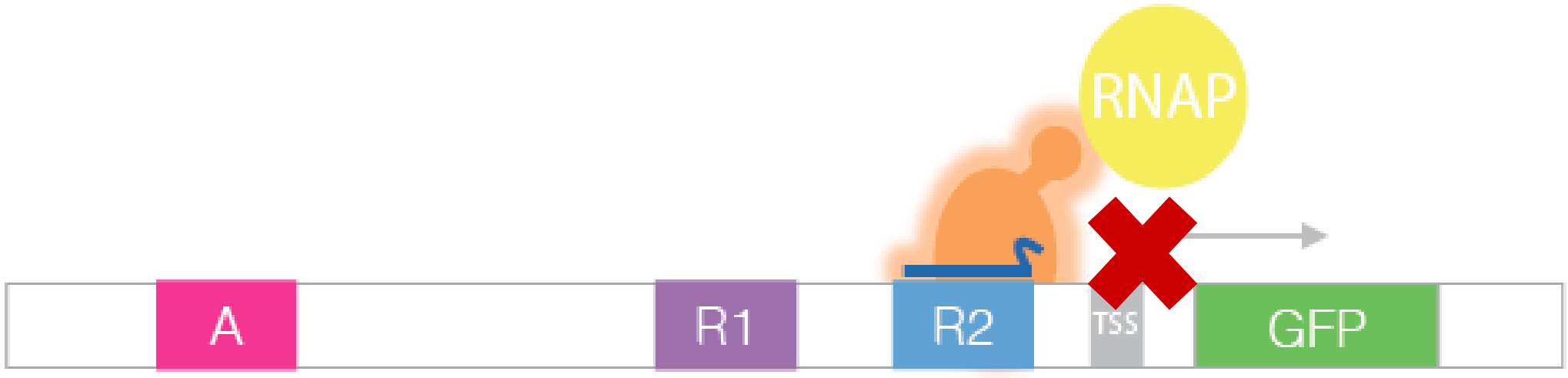
PROMOTER

Bio transistor “OFF”



PROMOTER

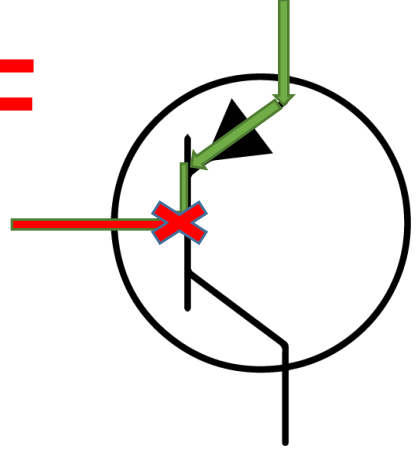
Bio transistor “OFF”

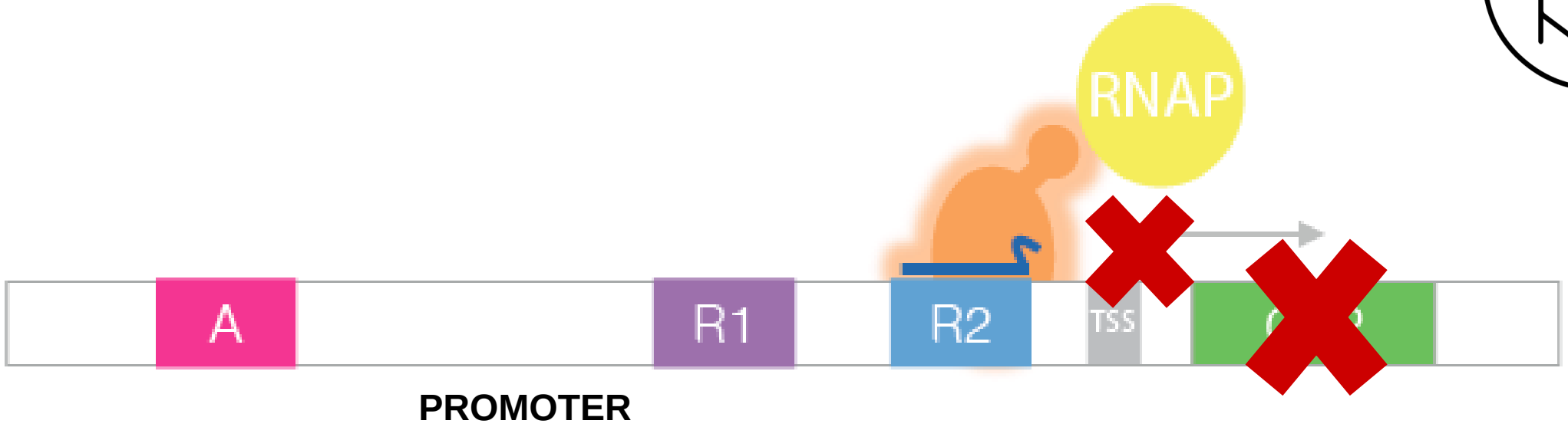


PROMOTER

Bio transistor “OFF”

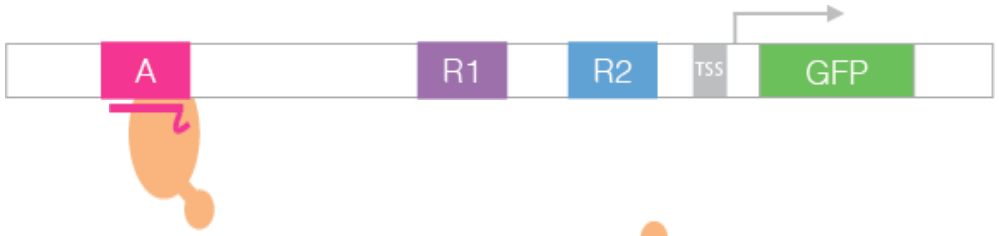
OFF



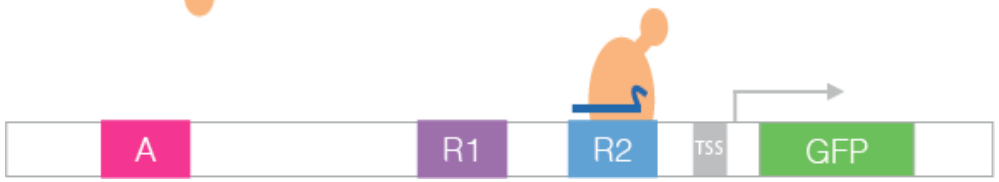


Bio transistor characterization

Turn “ON” = promoter activation



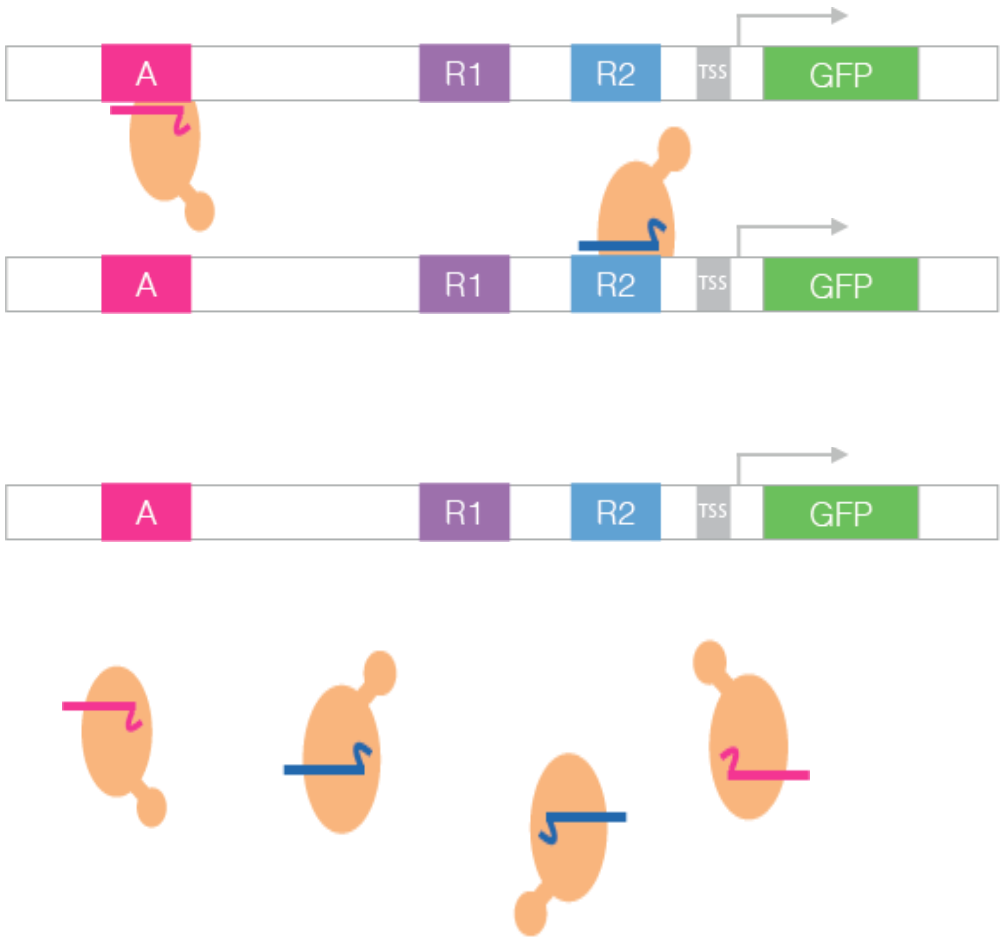
Turn “OFF” = promoter repression

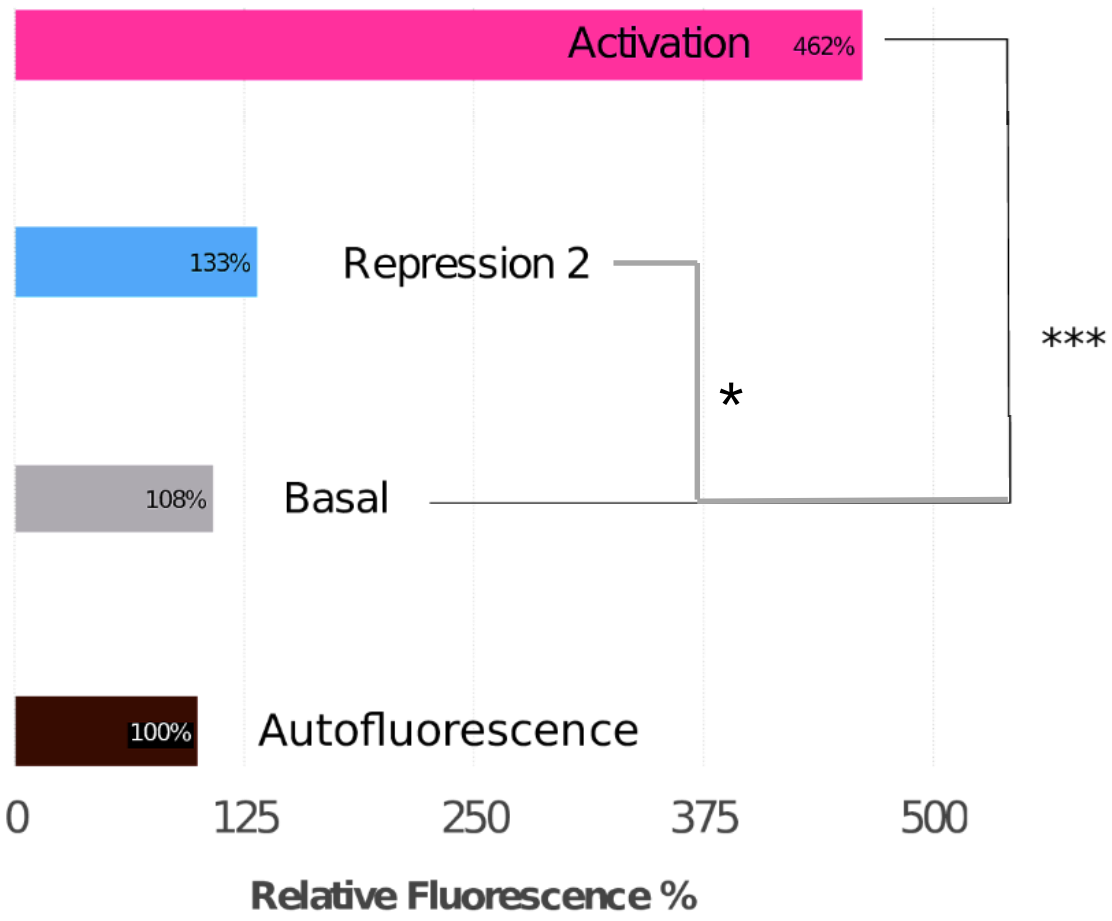


Compare to basal expression

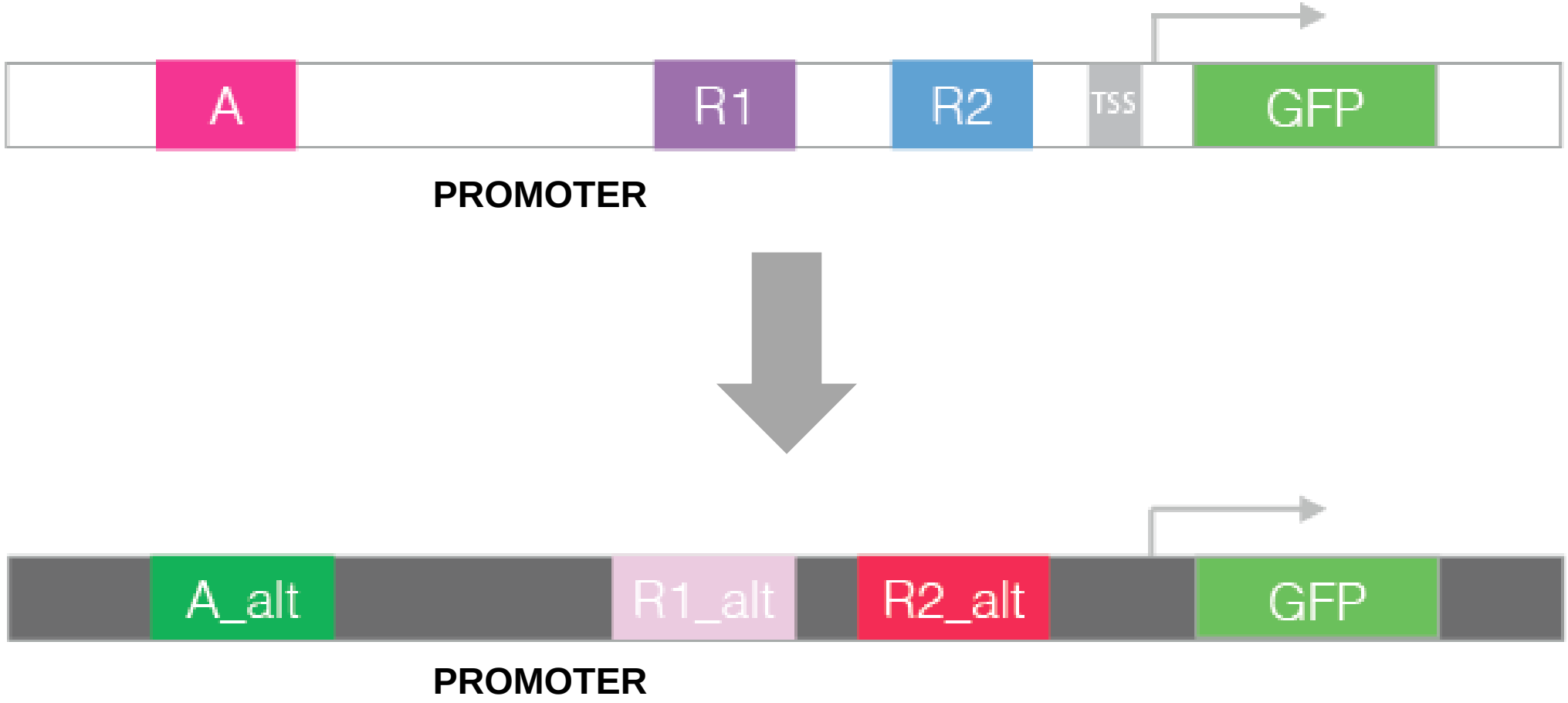
Bio transistor characterized

p values:
o >= 10% * < 10%
** < 5% *** < 1%





Multiplicability by mutation of regulation sites

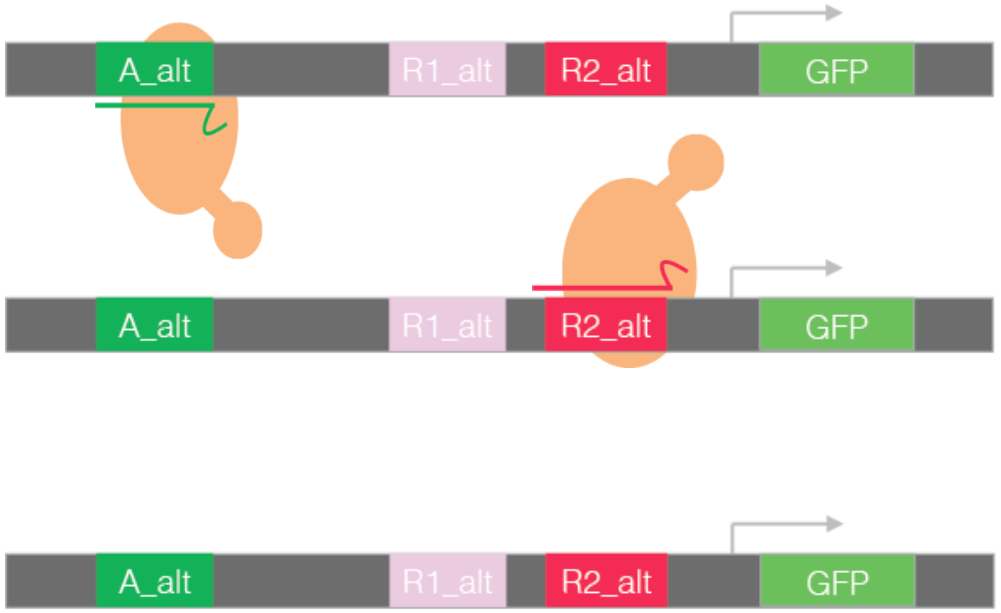


Transistor activity after mutation of regulation sites

Turn “ON” = promoter activation

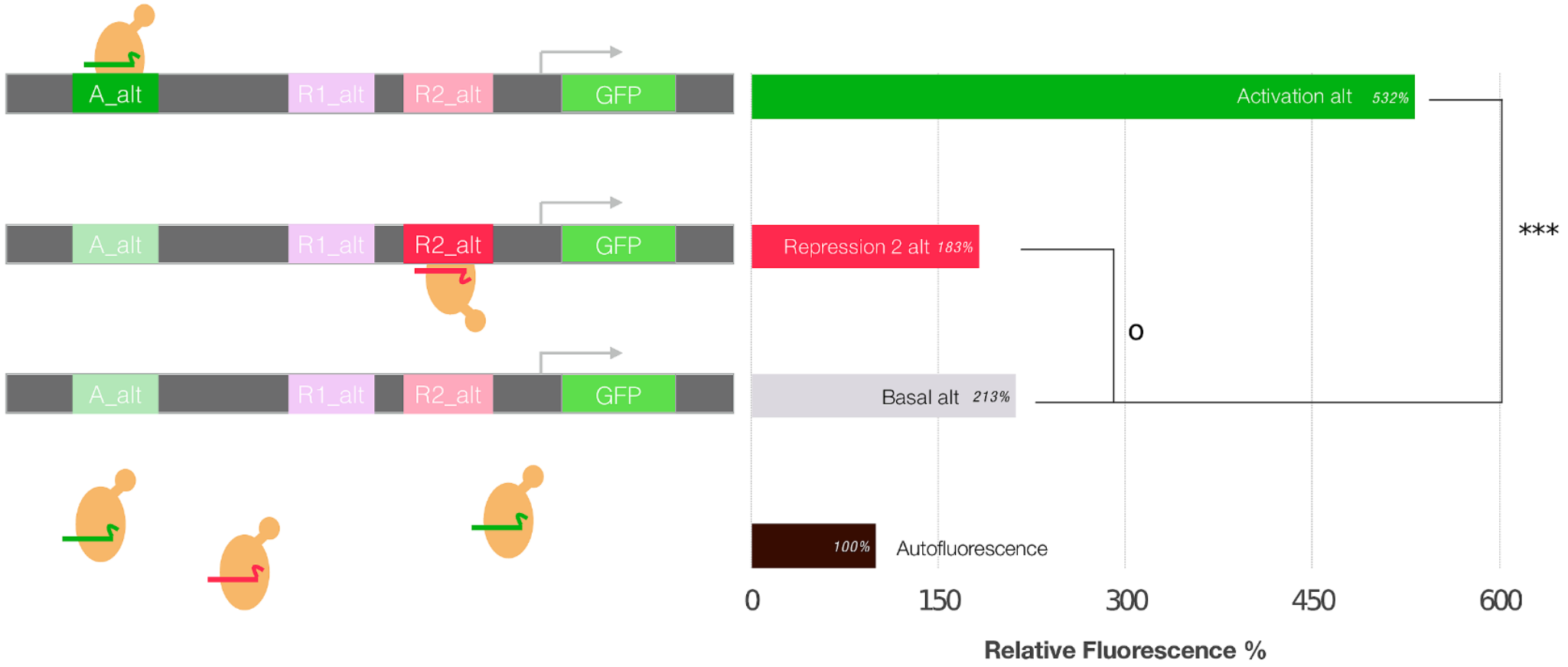
Turn “OFF” = promoter repression

Compare to basal expression

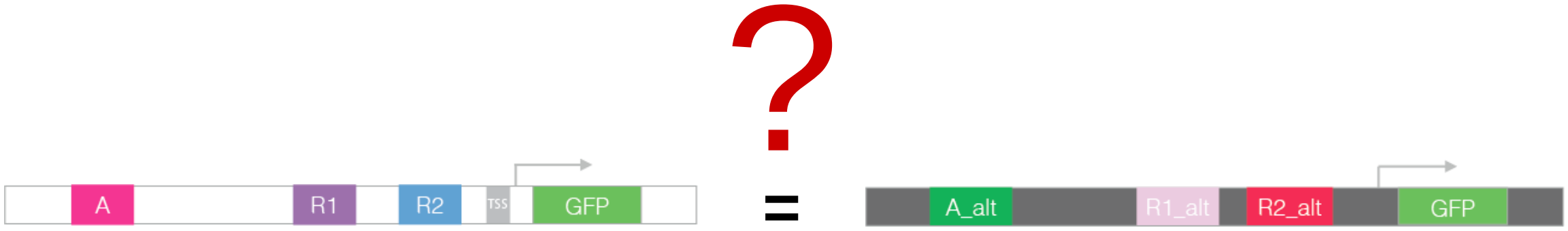


Transistor activity after mutation of regulation sites

p values:
o >= 10% *** < 10%**
**** < 5%** ***** < 1%**

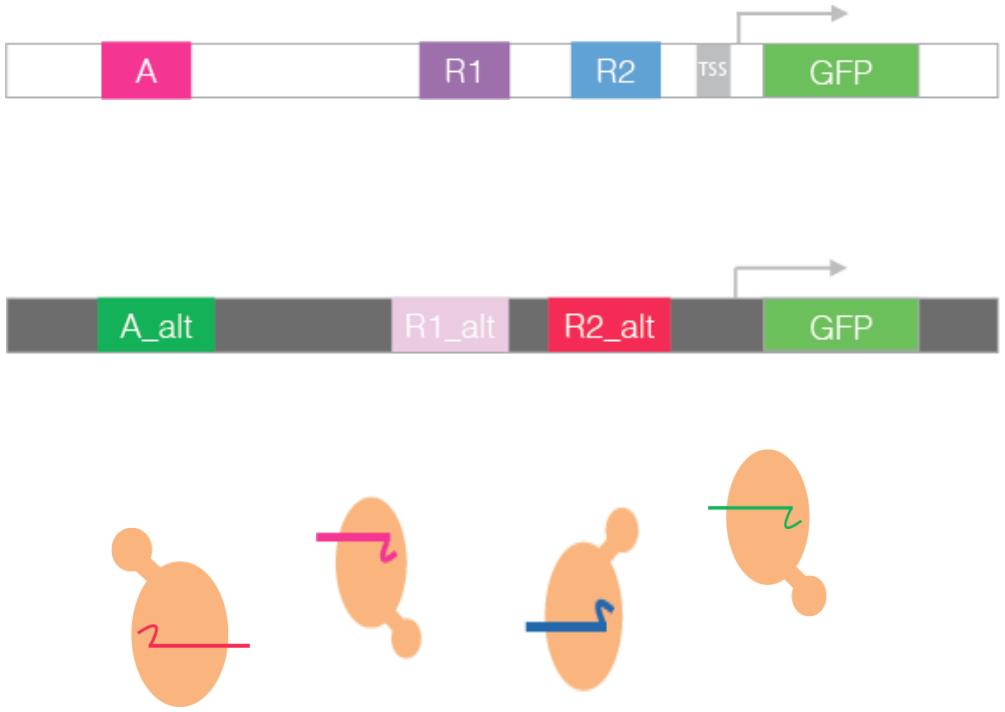


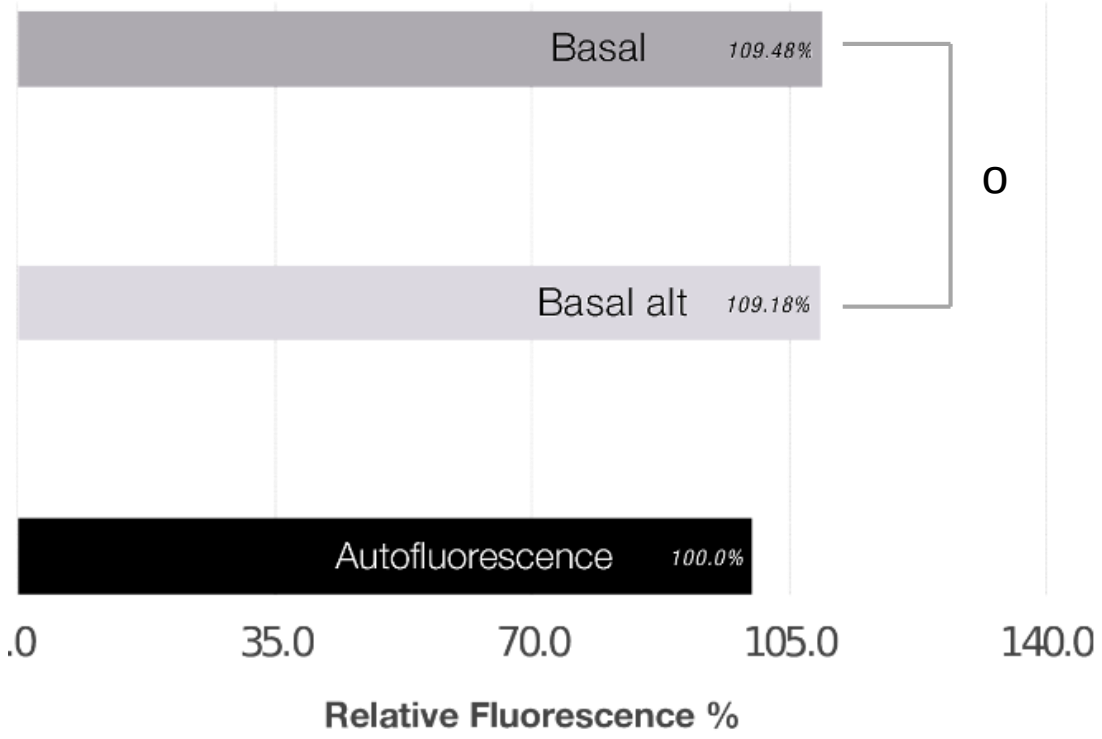
Basal level homogeneity with mutation of regulation sites



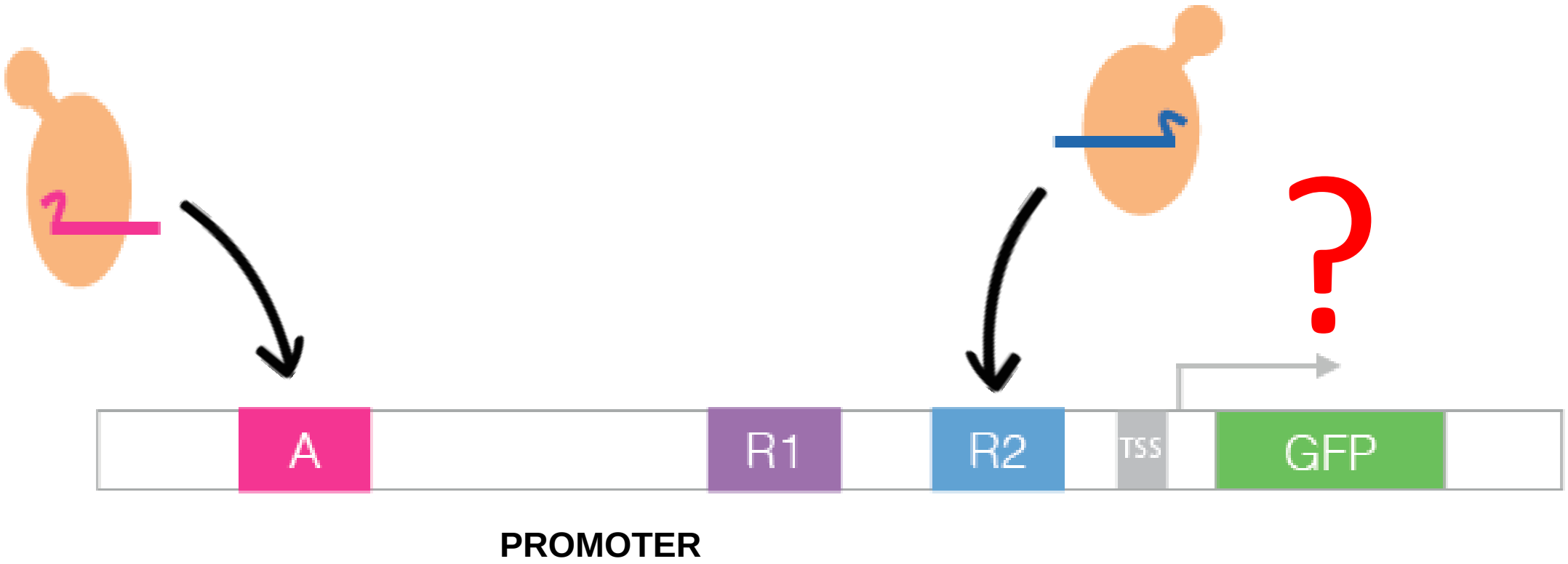
Basal level homogeneity with mutation of regulation sites

p values:
o >= 10% *** < 10%**
**** < 5%** ***** < 1%**

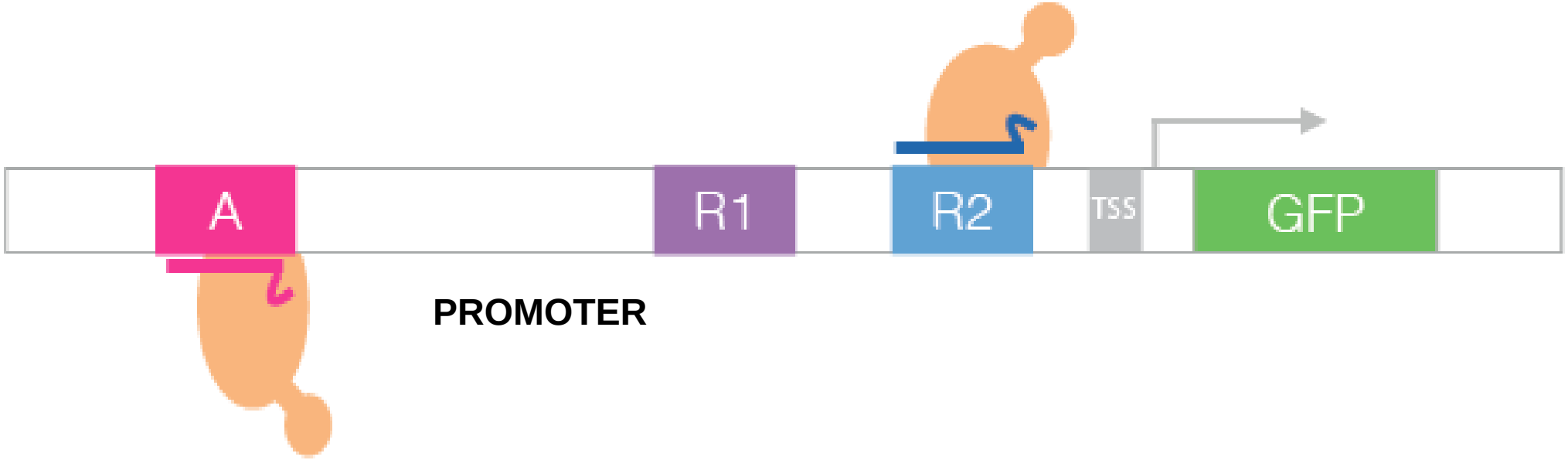




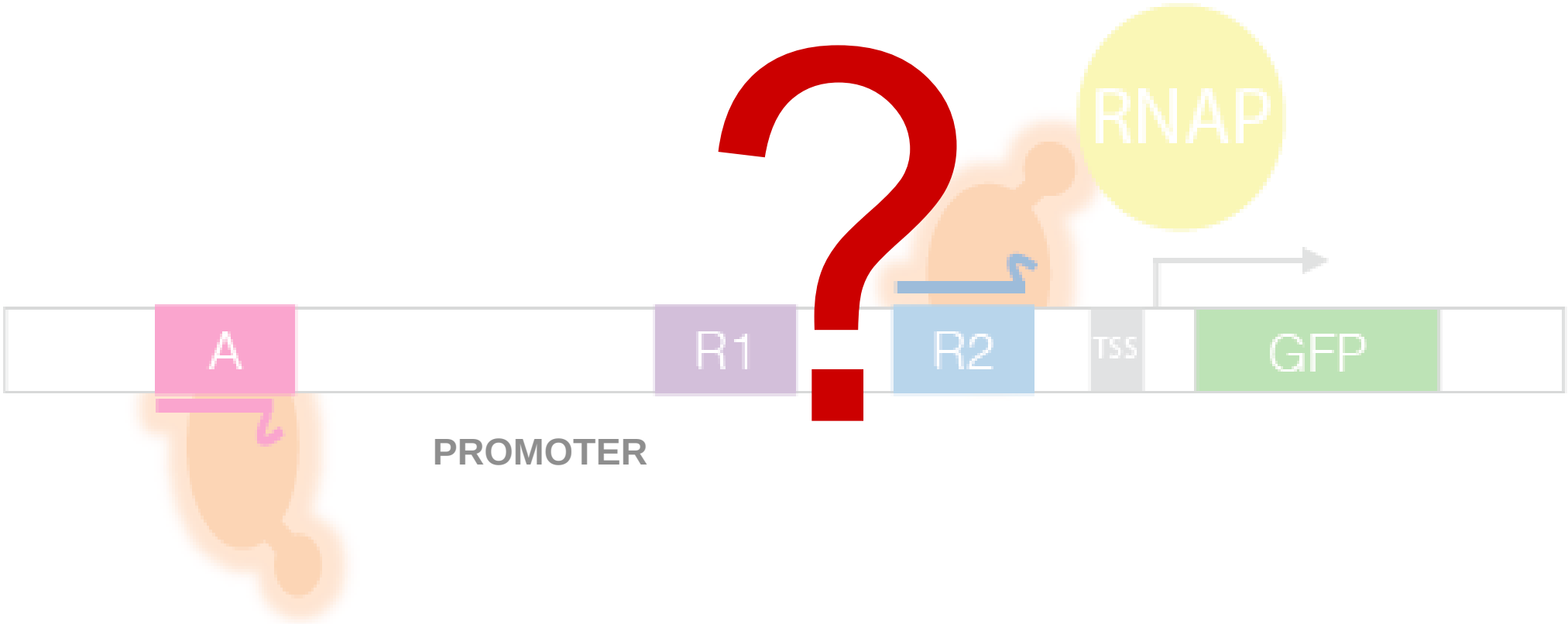
Simultaneous activation and inhibition: “ON” or “OFF”?



In S. cerevisiae inhibition prevails over activation

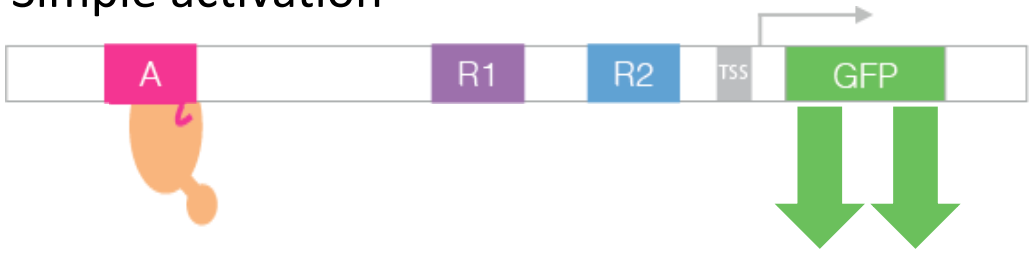


Simultaneous activation and inhibition: bio transistor “ON” or “OFF” **for E. coli?**

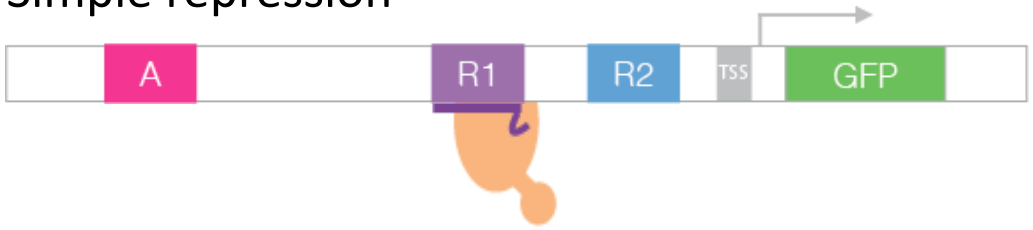


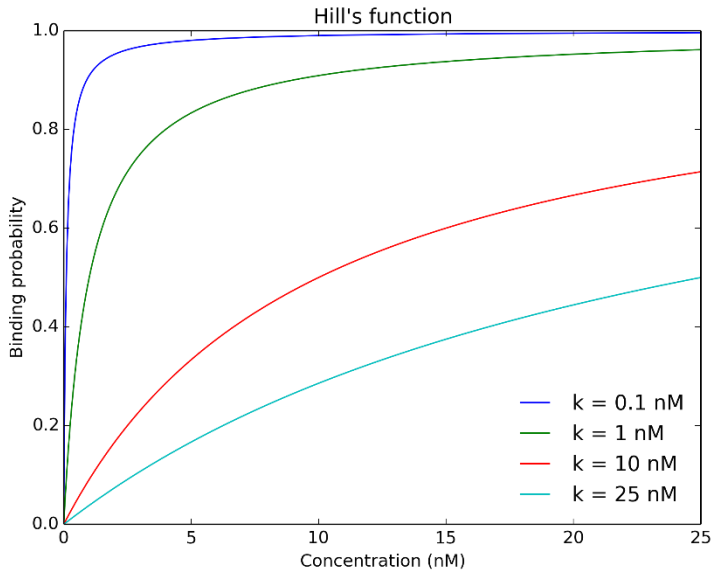
Lattice model for single binding of dCas9 :

Simple activation



Simple repression





Partition function for a lattice model with dCas9 interacting and two binding sites

$$Z = 1 + K_a[A] + K_a[R] + K_a^2[A][R]e^{-\beta j}$$

- Z = all the states of the system pondered by their Boltzmann weight
- Allows the computing of binding probabilities

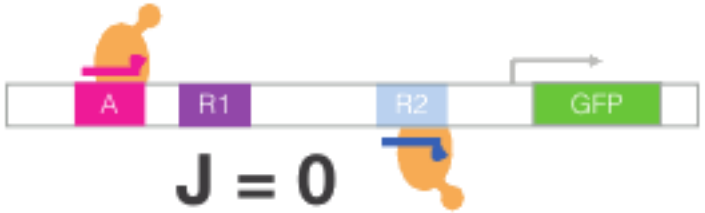
Partition function for a lattice model with dCas9 interacting and two binding sites

$$Z = 1 + K_a[A] + K_a[R] + K_a^2[A][R]e^{-\beta j}$$

- J determines the coupling energy of two dCas9s binding simultaneously

Partition function for a lattice model with dCas9 interacting and two binding sites

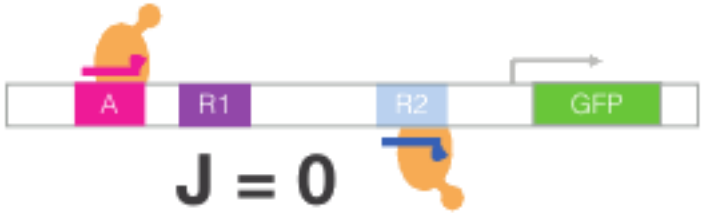
$$Z = 1 + K_a[A] + K_a[R] + K_a^2[A][R]e^{-\beta j}$$

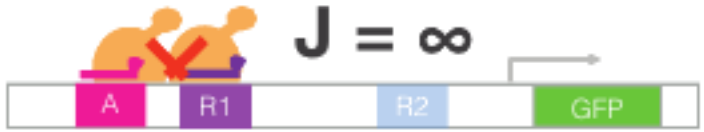


≡ Product of Hill functions for simple activation / inhibition

Partition function for a lattice model with dCas9 interacting and two binding sites

$$Z = 1 + K_a[A] + K_a[R] + K_a^2[A][R]e^{-\beta j}$$

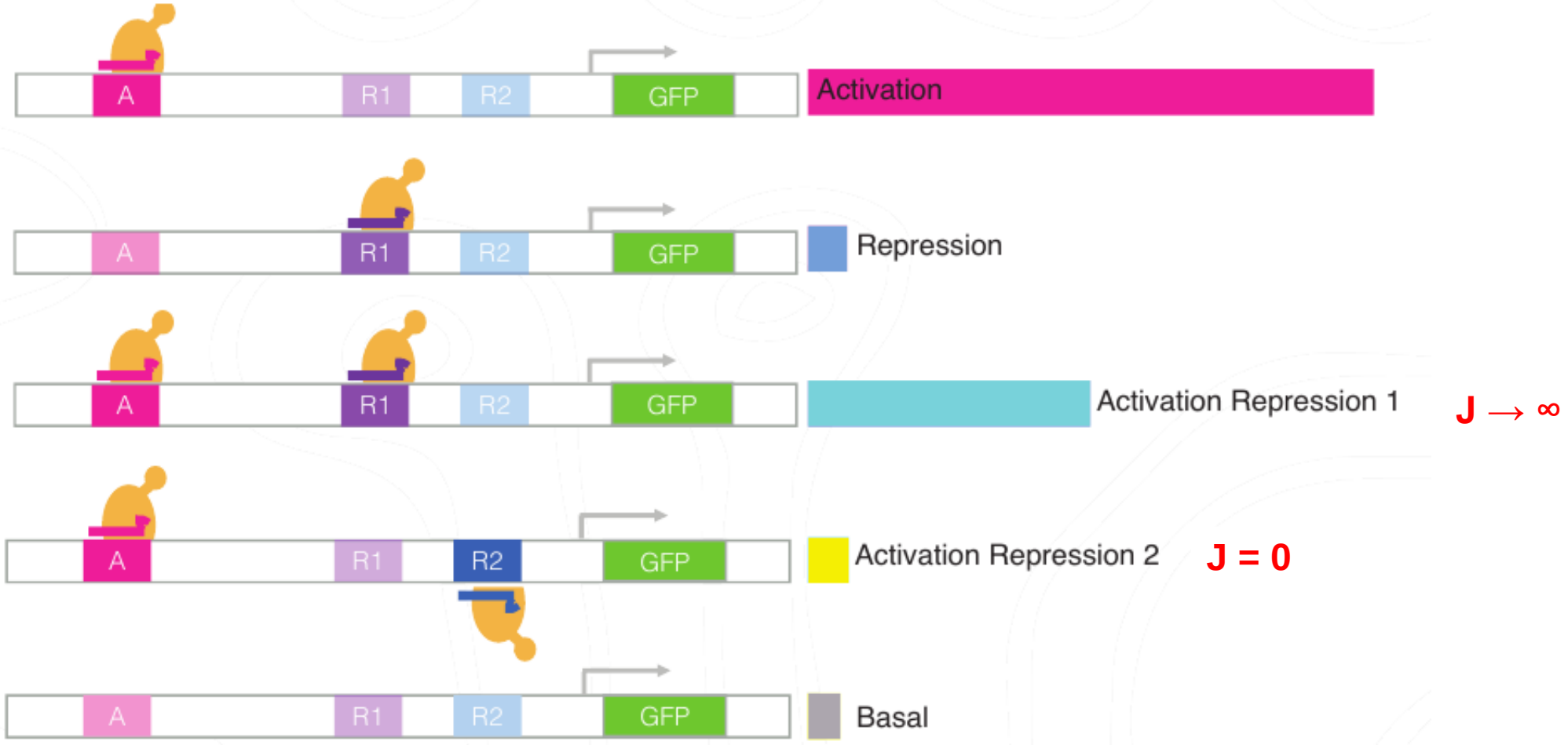




≡ Product of Hill functions for simple activation / inhibition

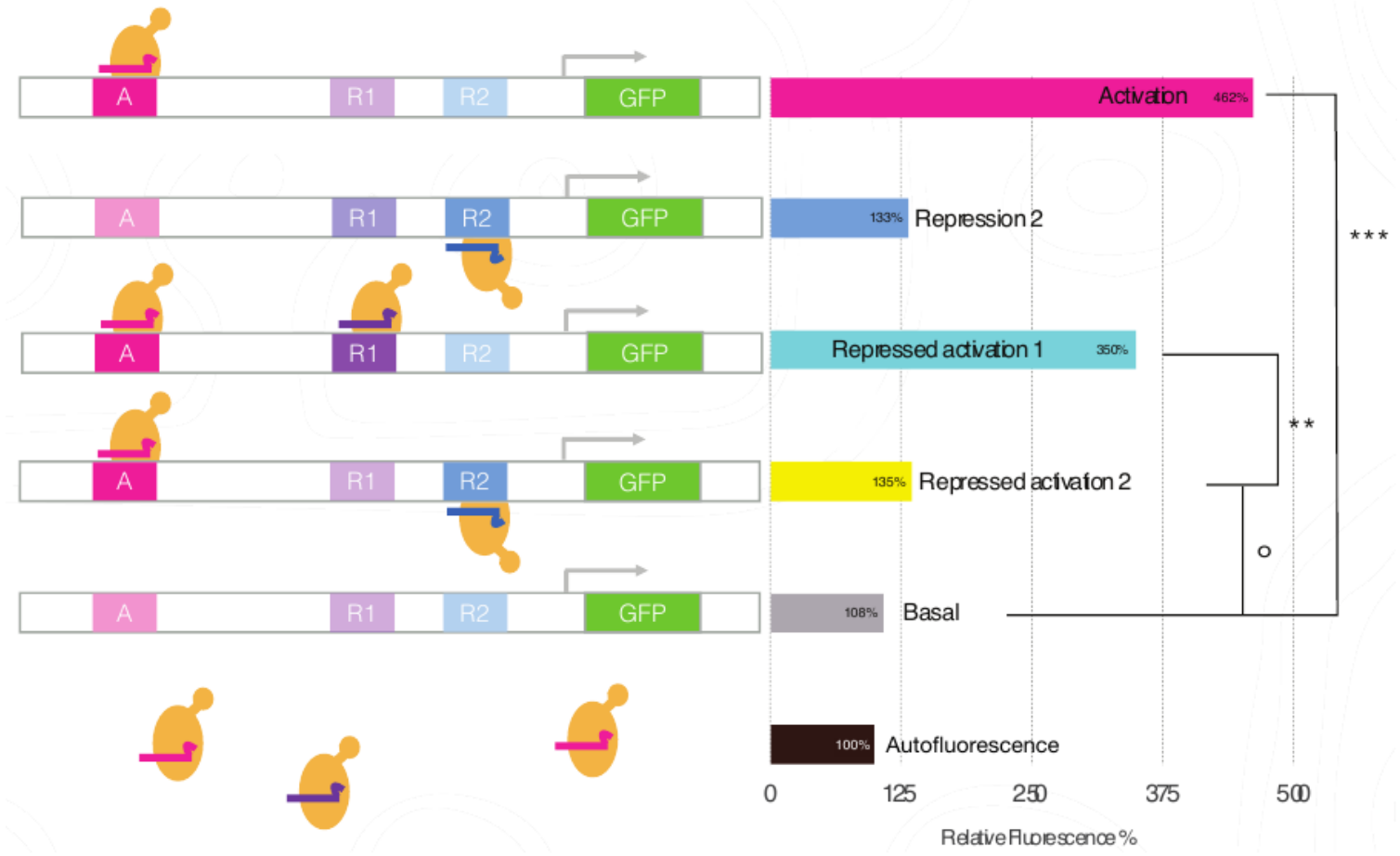
≡ Competition between 2 ligands

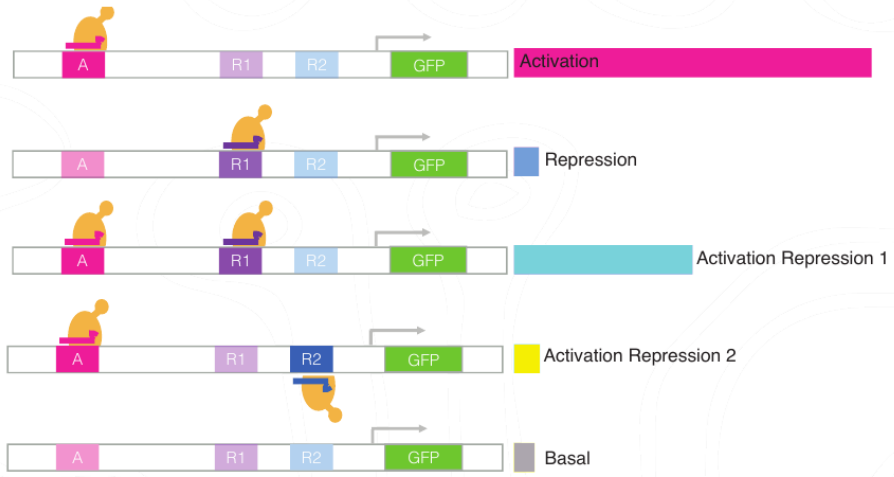
Modeling predicts basal expression levels for J = 0



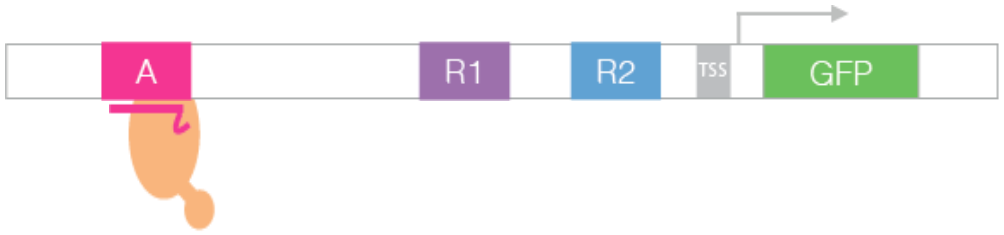
GFP expression in a.u.

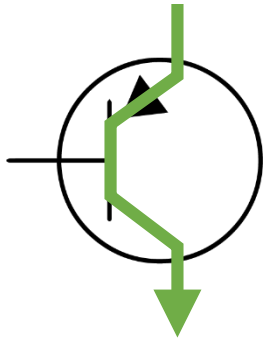
Wet lab results for simultaneous activation and repression





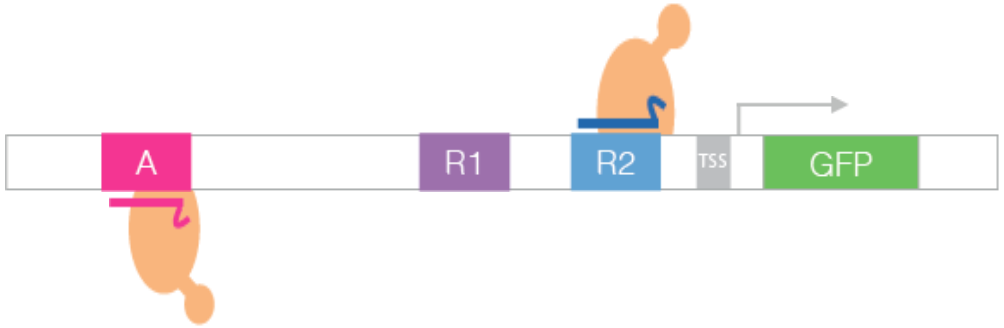
Wet lab results for simultaneous activation and repression

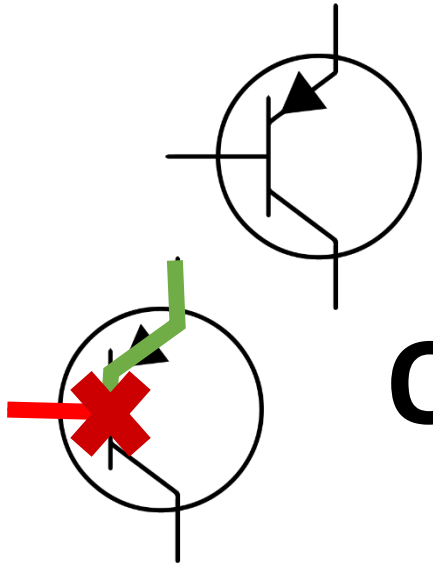




ON

VS





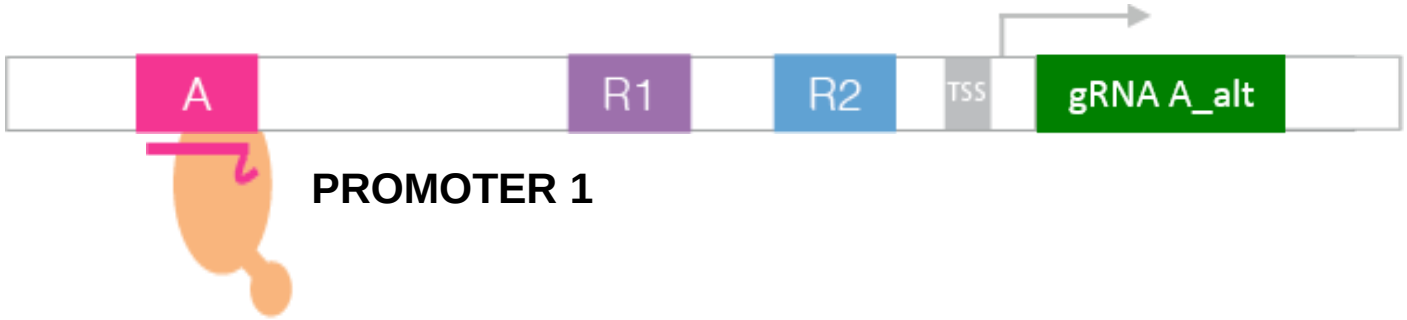
OFF

Chaining 2 bio transistors

PROMOTER 1

PROMOTER 2

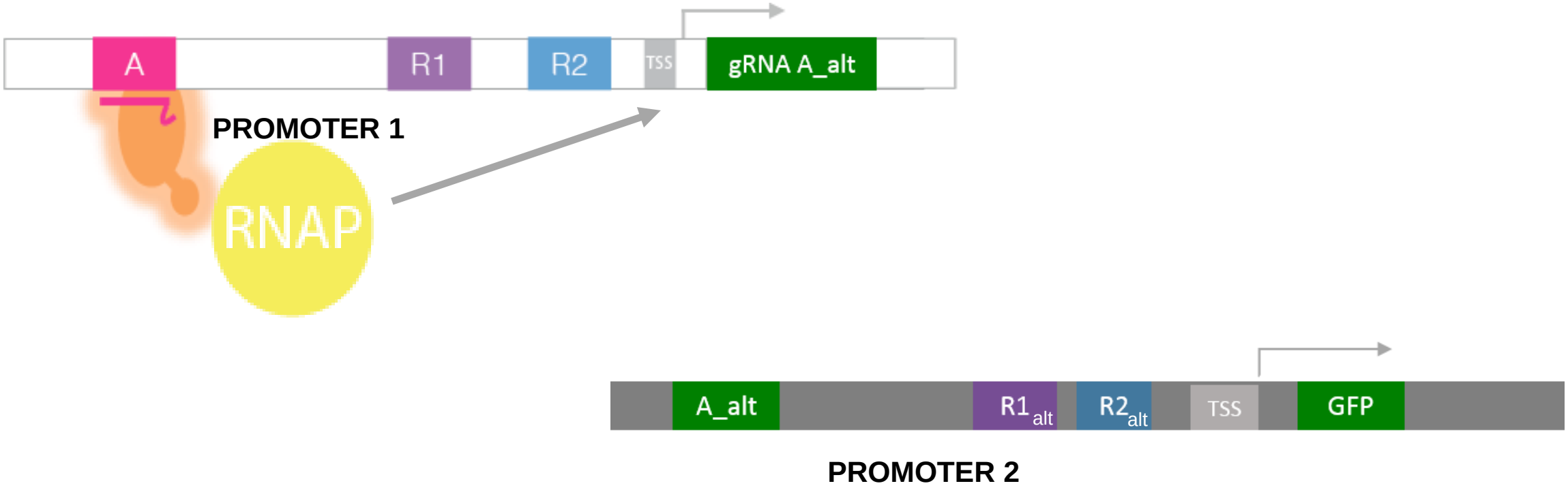
Chaining 2 bio transistors



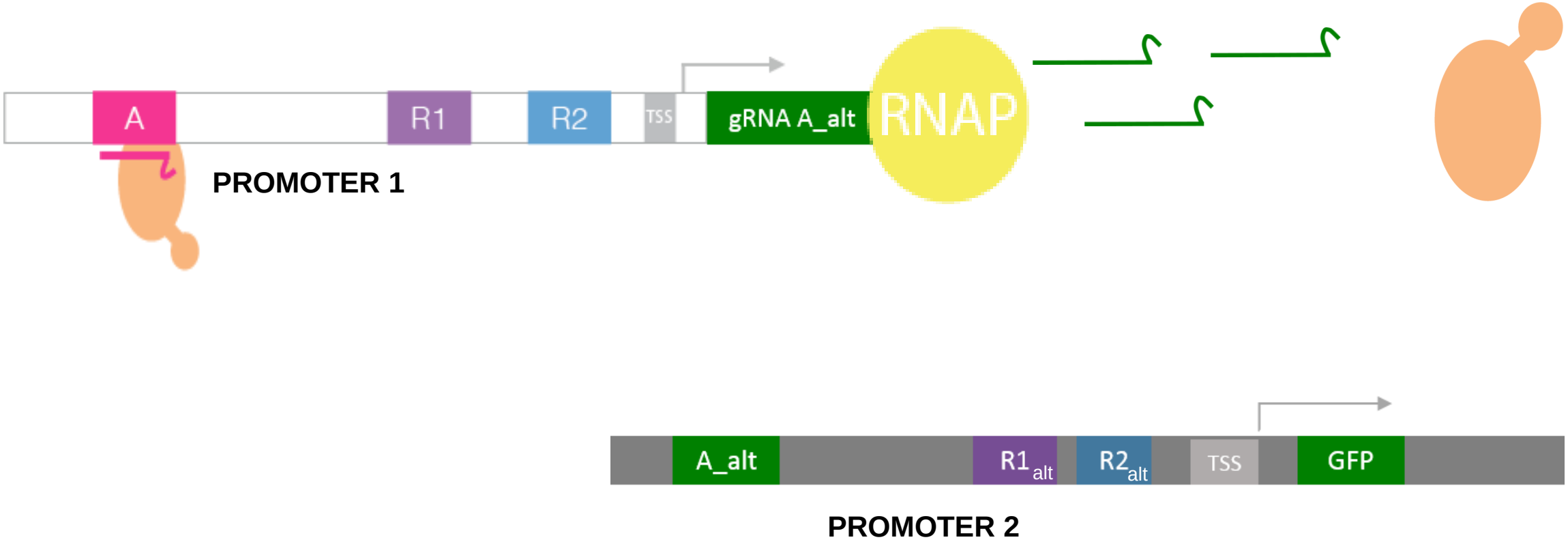
PROMOTER 1

PROMOTER 2

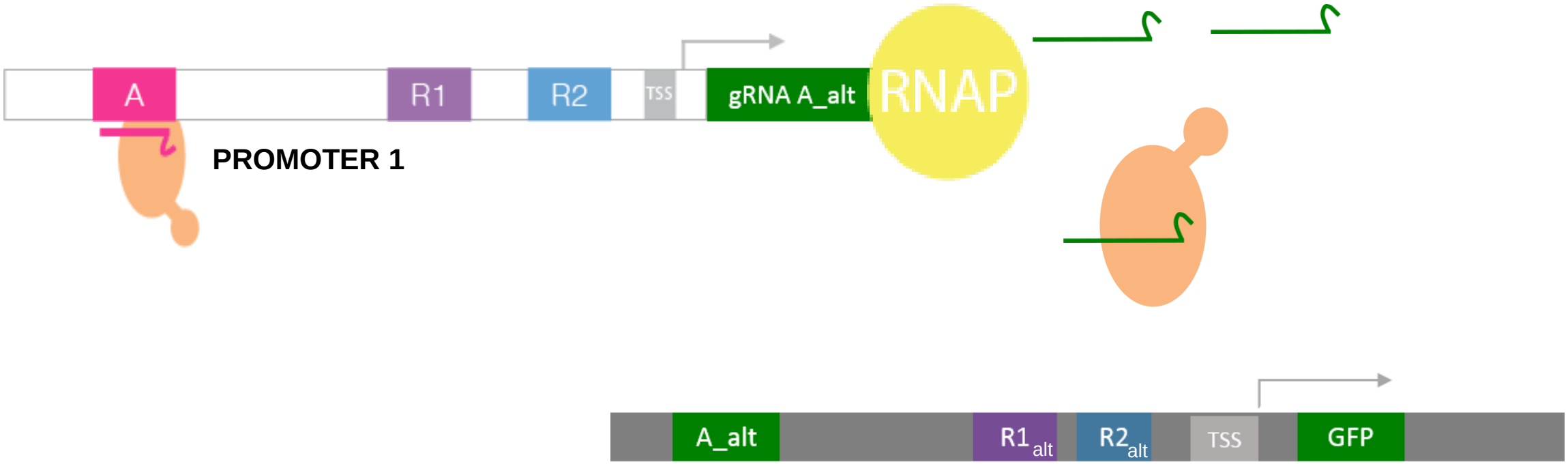
Chaining 2 bio transistors



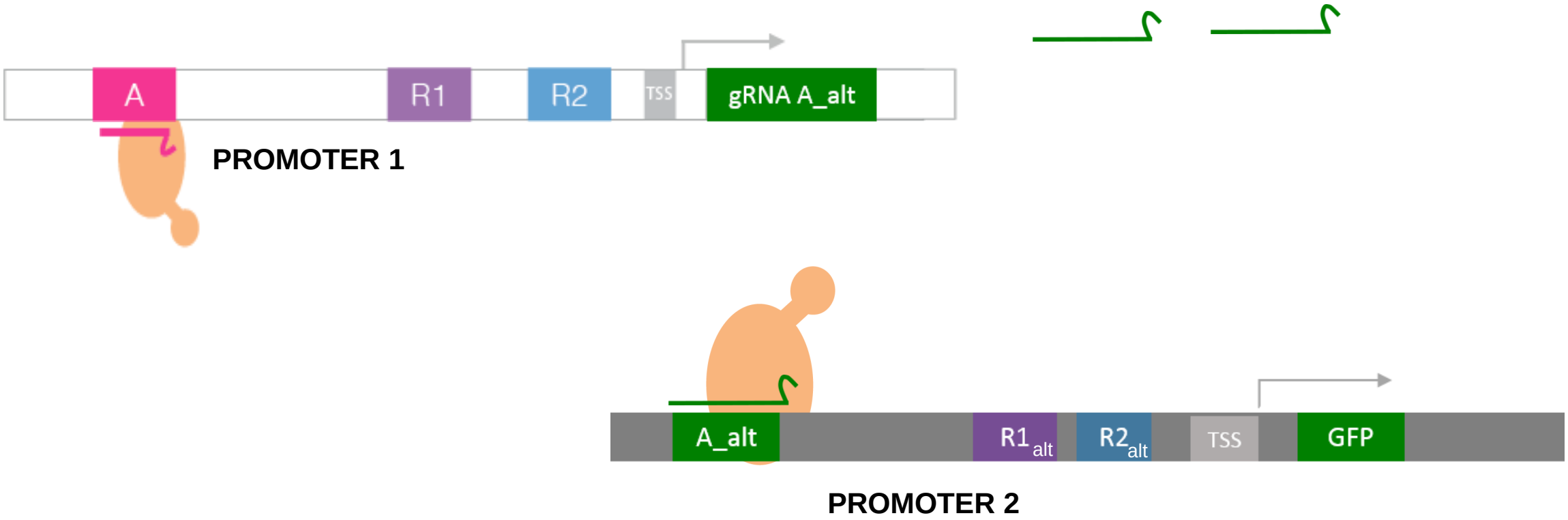
Chaining 2 bio transistors



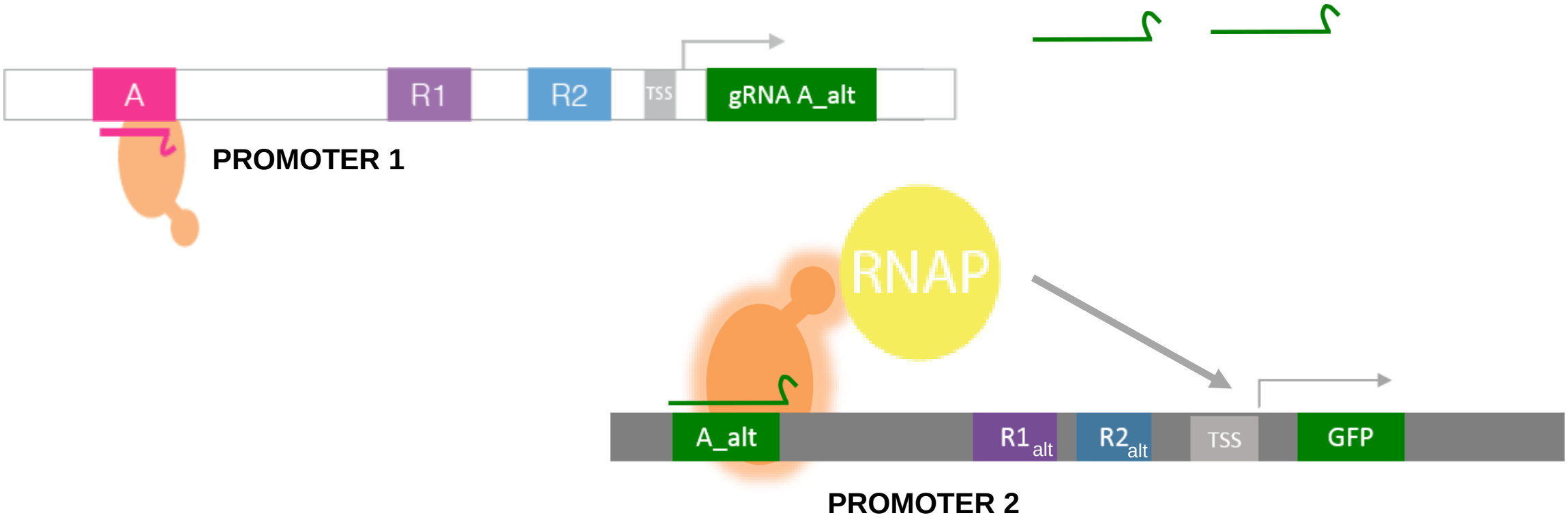
Chaining 2 bio transistors



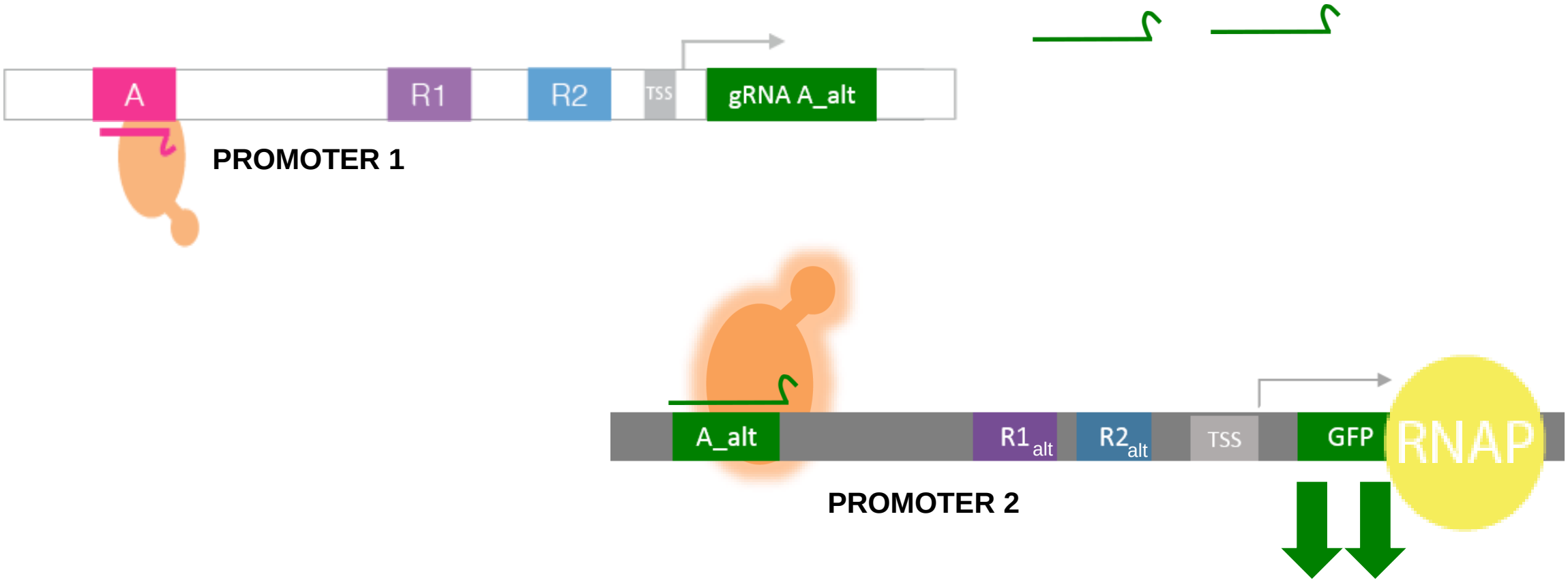
Chaining 2 bio transistors



Chaining 2 bio transistors



Chaining 2 bio transistors

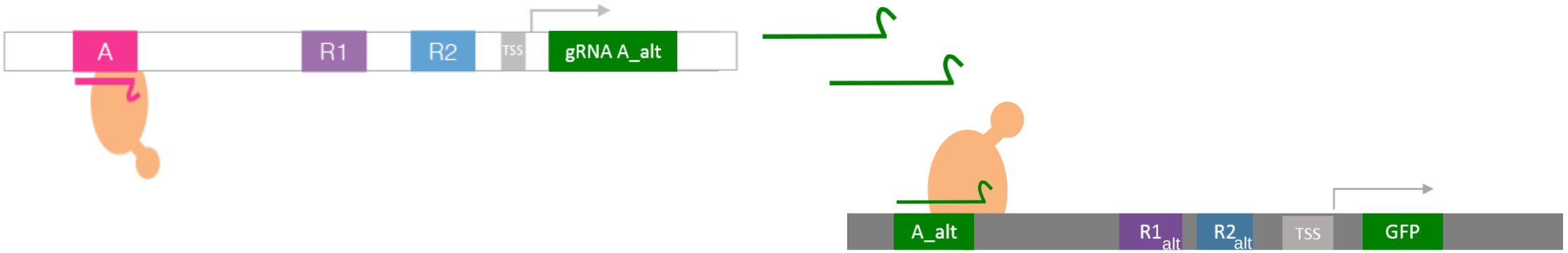


Chaining 2 bio transistors “ON”

Turn “ON” transistor 2 via transistor 1

=

Promoter 1 activation → promoter 2 activation

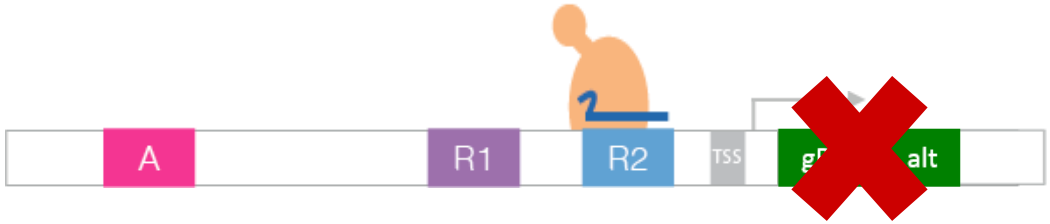


Chaining 2 bio transistors “OFF”

Turn “OFF” transistor 2 via transistor 1

=

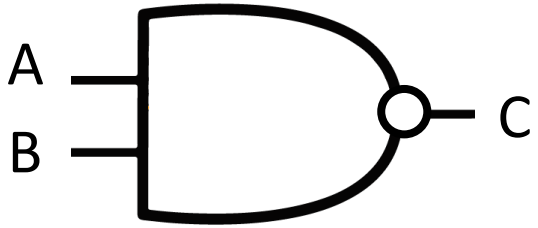
Promoter 1 repression → promoter 2 basal level



Chaining 2 bio transistors

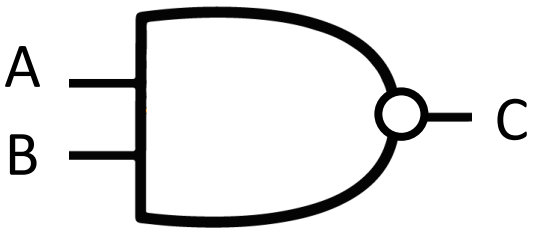
p values:
o >= 10% * < 10%
** < 5% *** < 1%

Making a NAND gate with dCas9 transistors



A	B	C
0	0	1
0	1	1
1	0	1
1	1	0

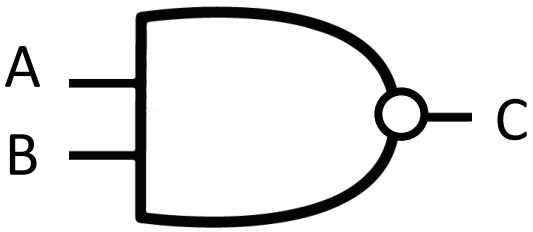
Making a NAND gate with dCas9 transistors



A	B	C
0	0	1
0	1	1
1	0	1
1	1	0

A	B	C
0	0	?
0	1	?
1	0	?
1	1	?

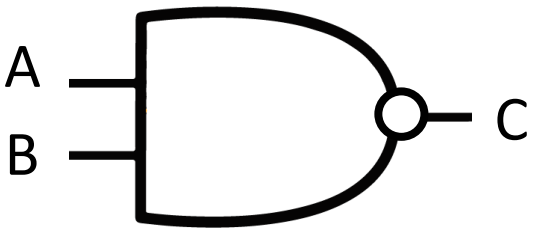
Making a NAND gate with dCas9 transistors



A	B	C
0	0	1
0	1	1
1	0	1
1	1	0

A	B	C0	C1
0	0	?	?
0	1	?	?
1	0	?	?
1	1	?	?

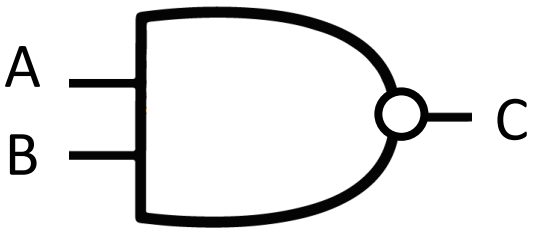
Making a NAND gate with dCas9 transistors

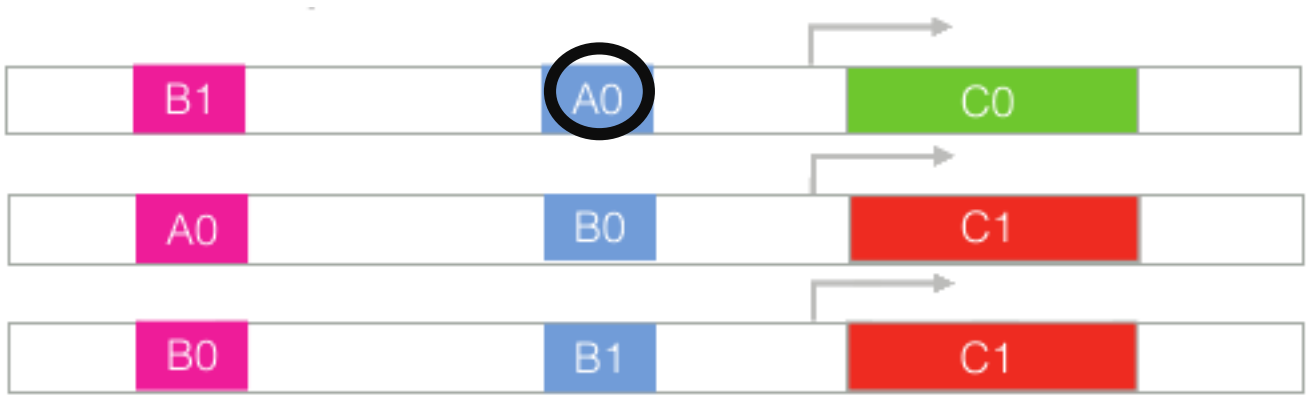


A	B	C
0	0	1
0	1	1
1	0	1
1	1	0

A	B	C0	C1
0	0	?	?
0	1	?	?
1	0	?	?
1	1	?	?

Making a NAND gate with dCas9 transistors

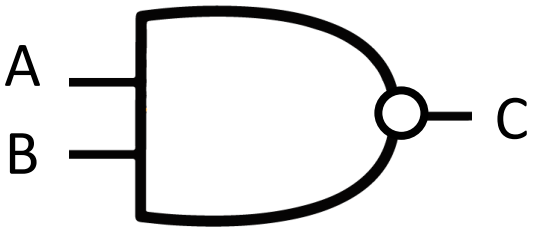


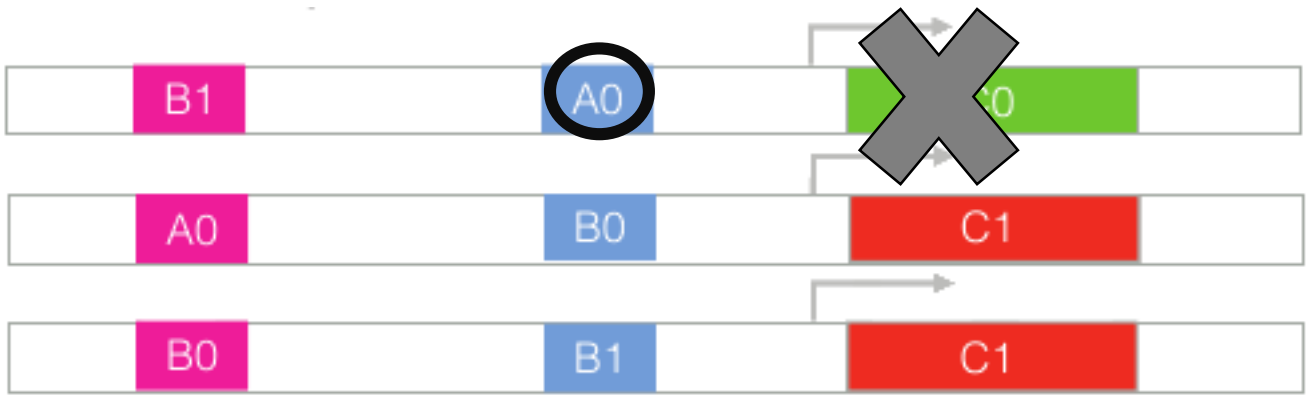


A	B	C
0	0	1
0	1	1
1	0	1
1	1	0

A	B	C0	C1
0	0	?	?
0	1	?	?
1	0	?	?
1	1	?	?

Making a NAND gate with dCas9 transistors

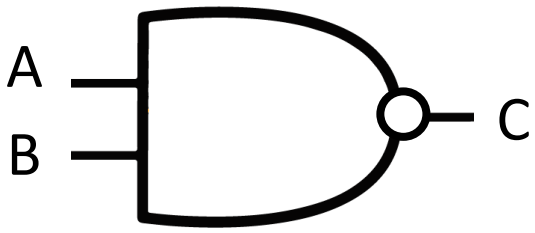




A	B	C
0	0	1
0	1	1
1	0	1
1	1	0

A	B	C0	C1
0	0	R	?
0	1	?	?
1	0	?	?
1	1	?	?

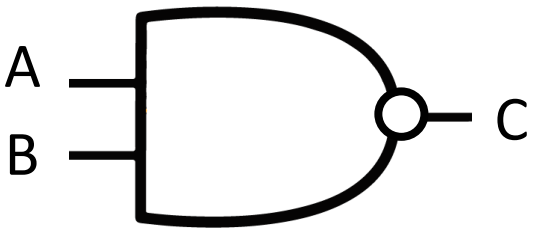
Making a NAND gate with dCas9 transistors



A	B	C
0	0	1
0	1	1
1	0	1
1	1	0

A	B	C0	C1
0	0	R	R
0	1	?	?
1	0	?	?
1	1	?	?

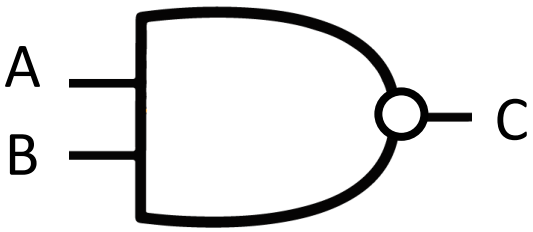
Making a NAND gate with dCas9 transistors



A	B	C
0	0	1
0	1	1
1	0	1
1	1	0

A	B	C0	C1
0	0	R	R + A ≈ A
0	1	?	?
1	0	?	?
1	1	?	?

Making a NAND gate with dCas9 transistors

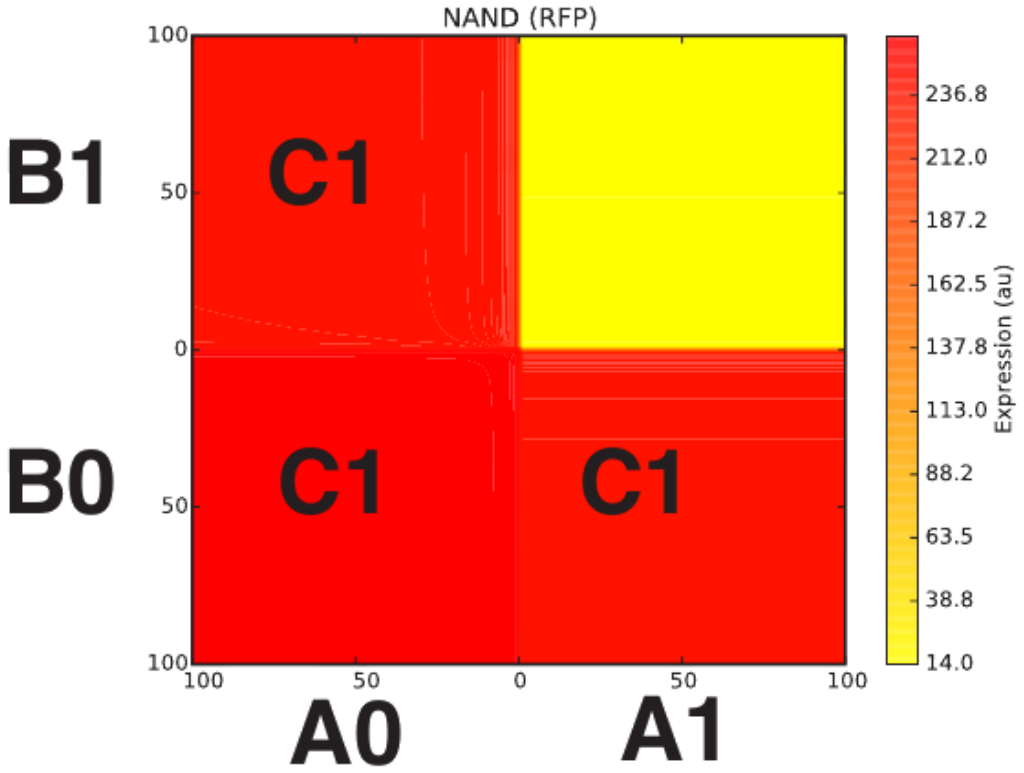


A	B	C
0	0	1
0	1	1
1	0	1
1	1	0

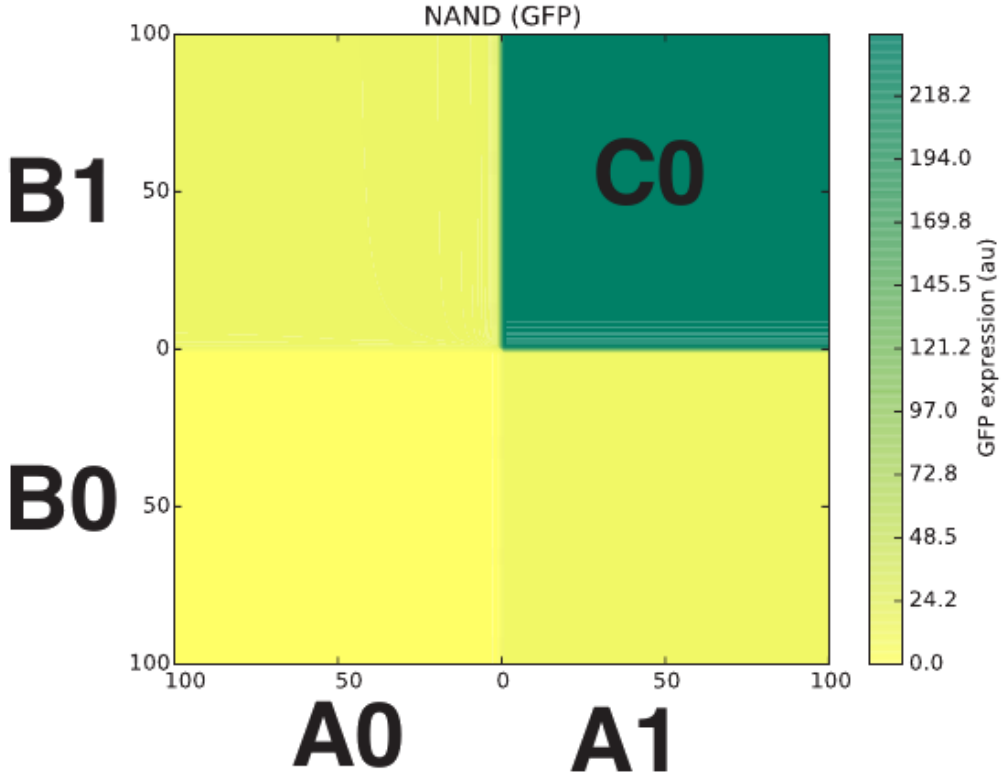
A	B	C0	C1
0	0	R	R + A ≈ A
0	1	R	A + R ≈ A
1	0	B ≈ R	R + A ≈ A
1	1	A	B + R

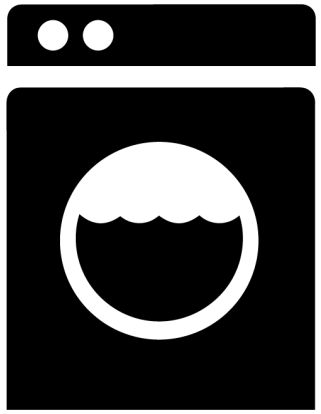
Modeling the NAND gate

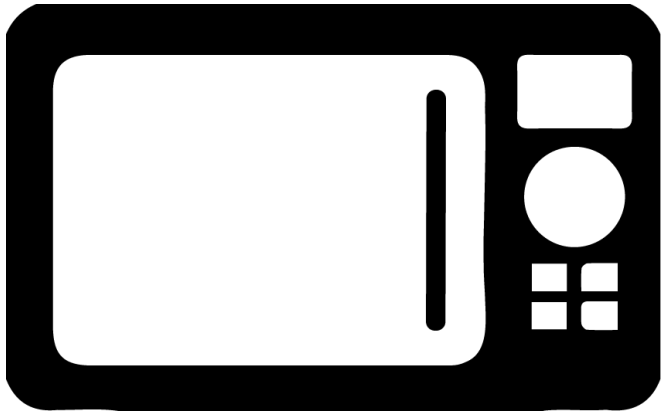
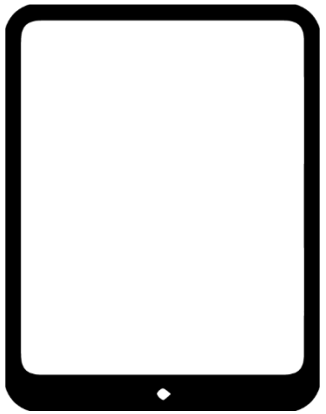
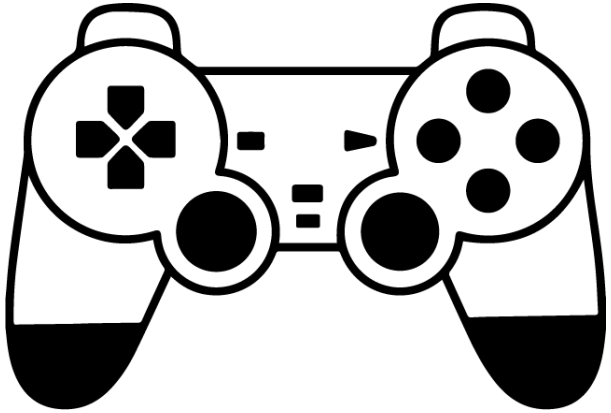
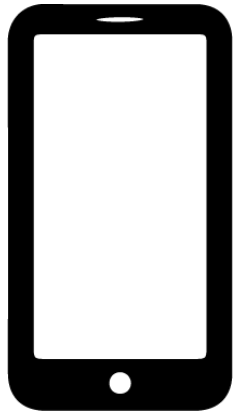
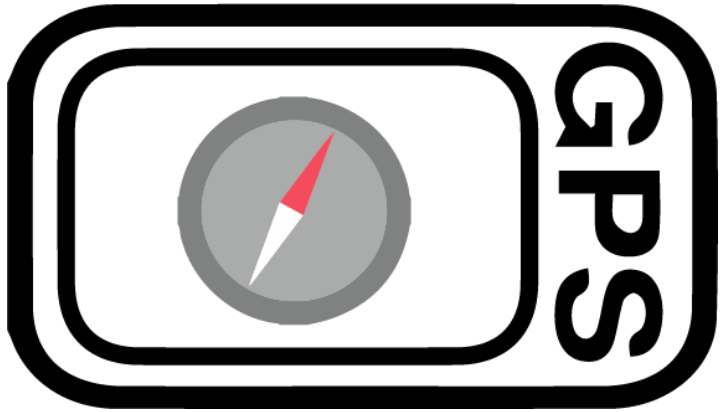
RFP levels



GFP levels



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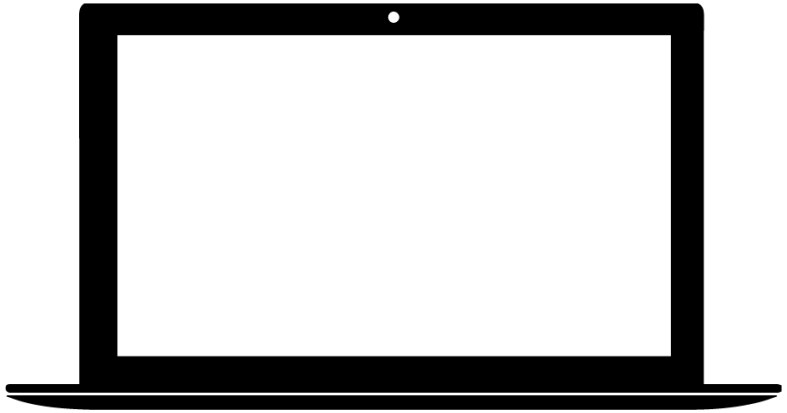
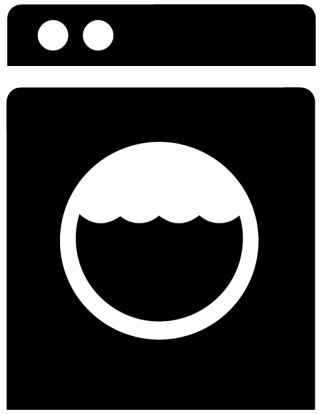
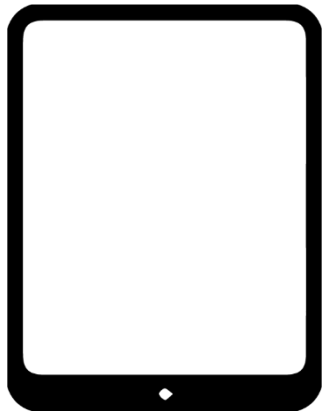
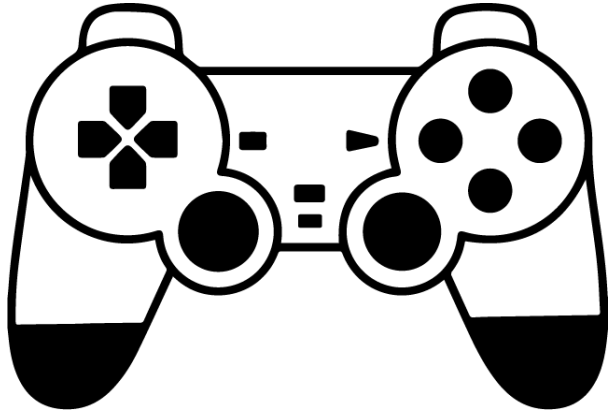
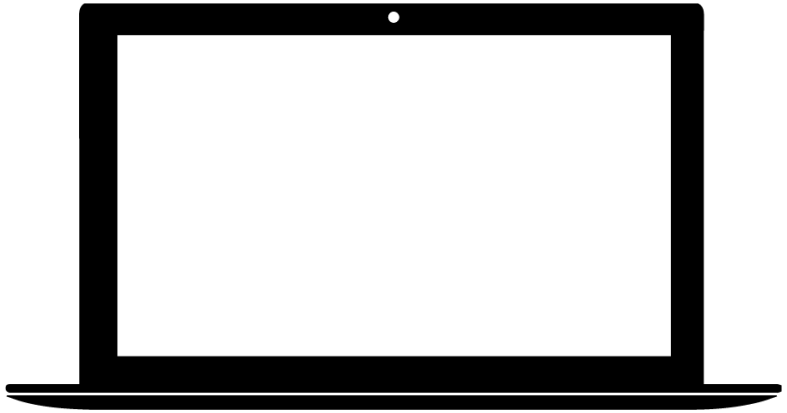










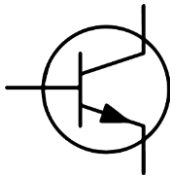
Human Practices

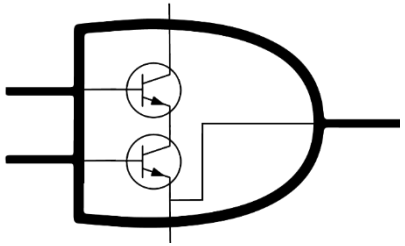
Survey in Switzerland

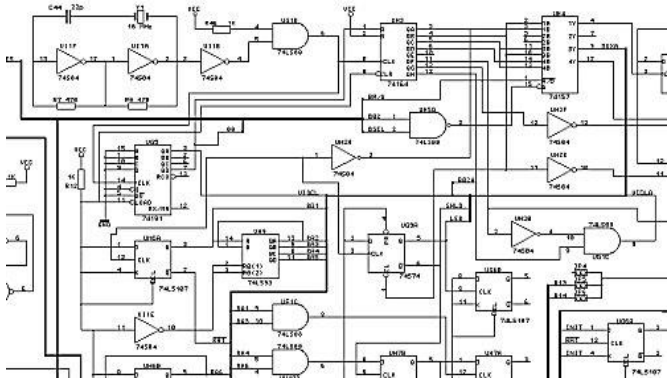
Meet the experts

High school day

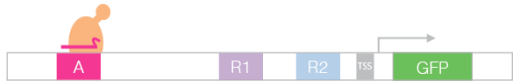
ELECTRONICS

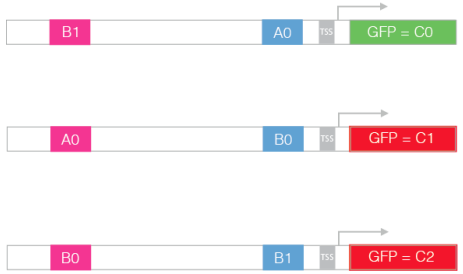


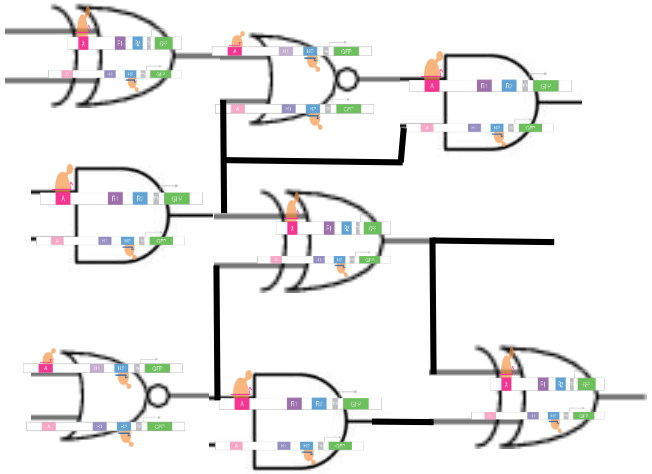




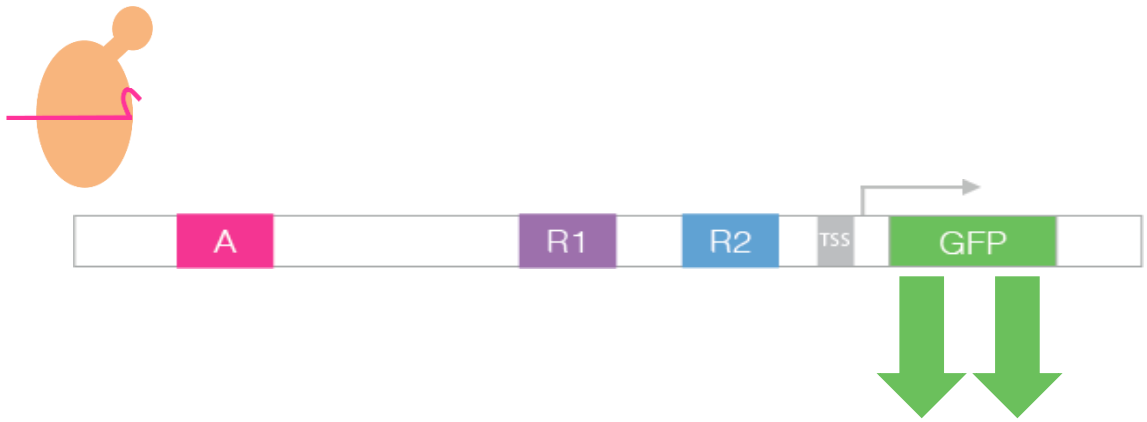
BIOLOGY







The Bio Transistor



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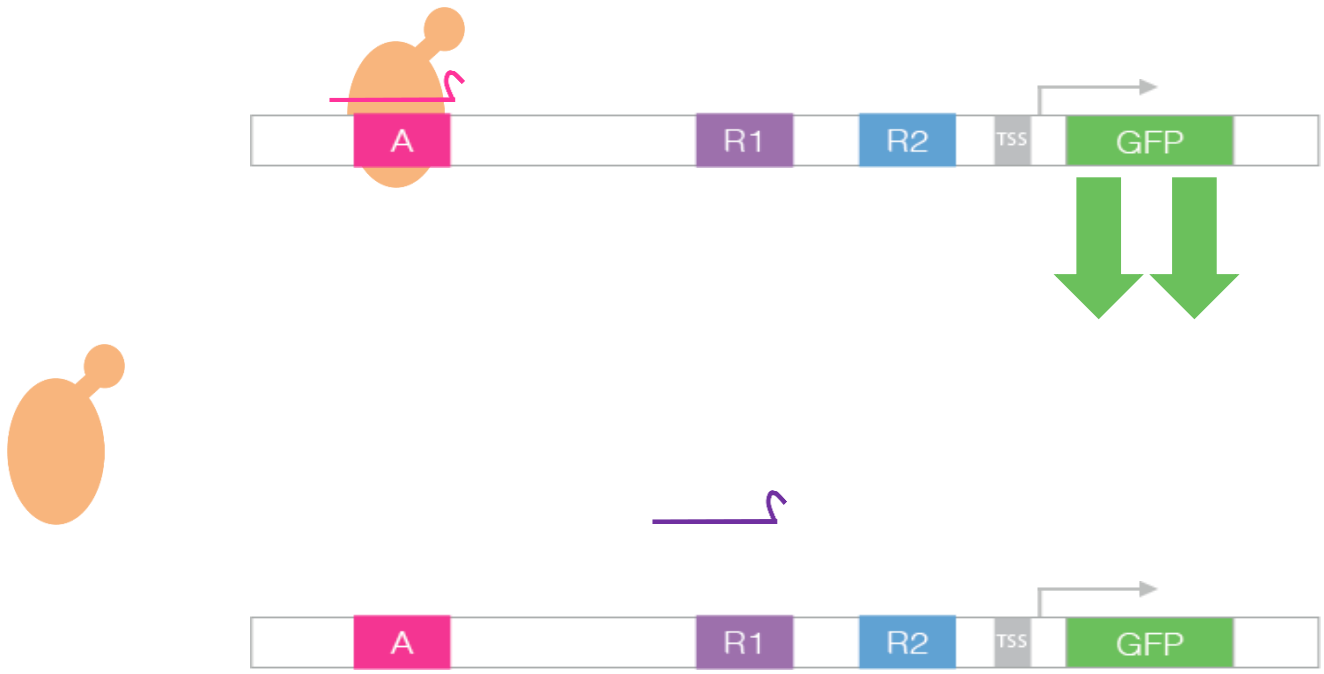

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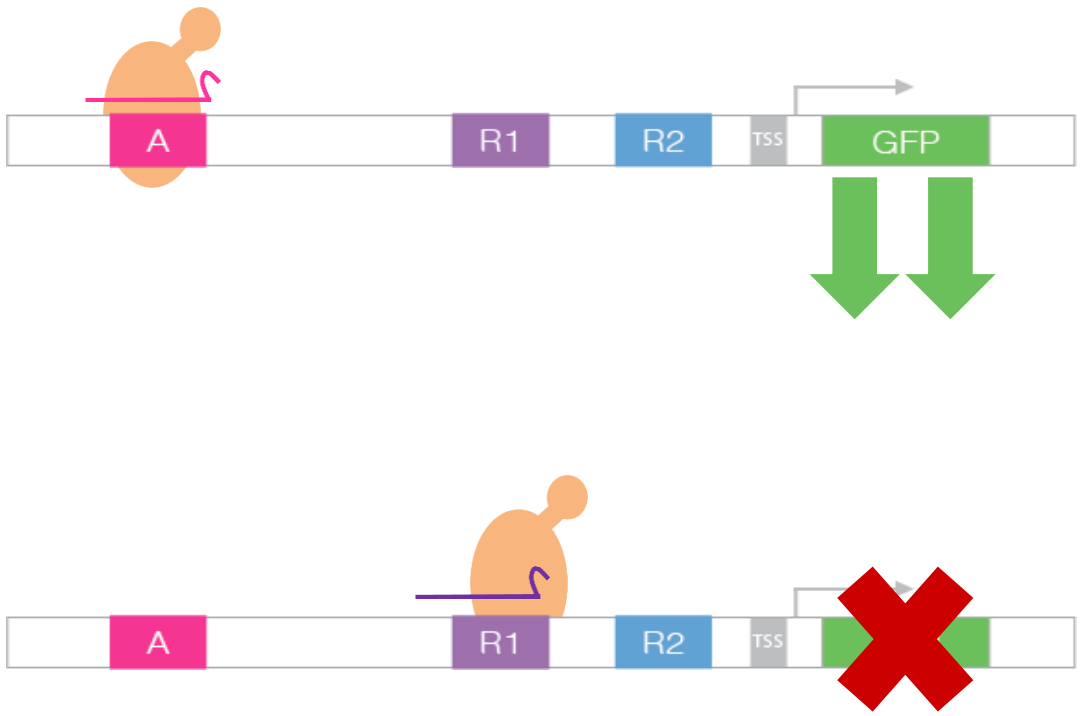

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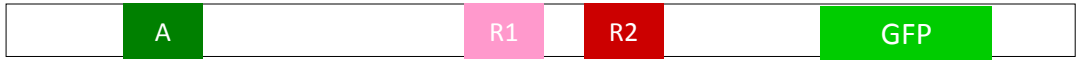
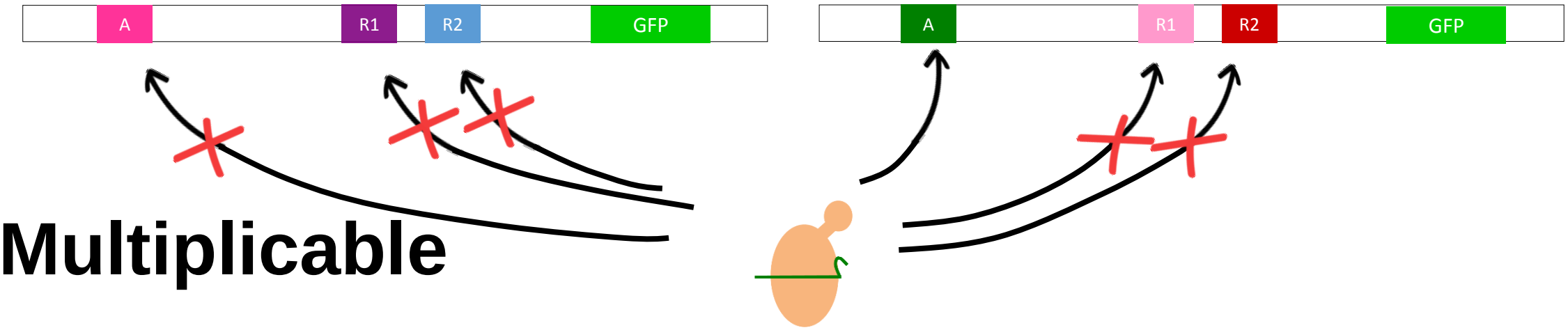


Multiplicable

Homogeneous

The Bio Transistor

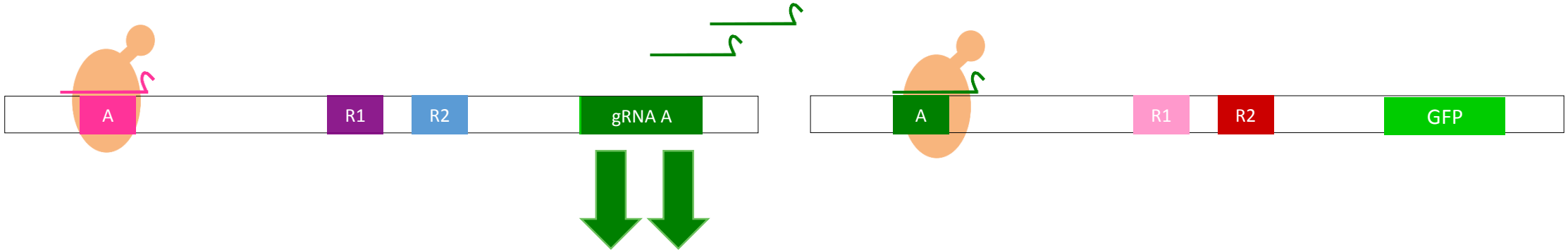


Multiplicable

Homogeneous

Orthogonal

The Bio Transistor



Multiplicable

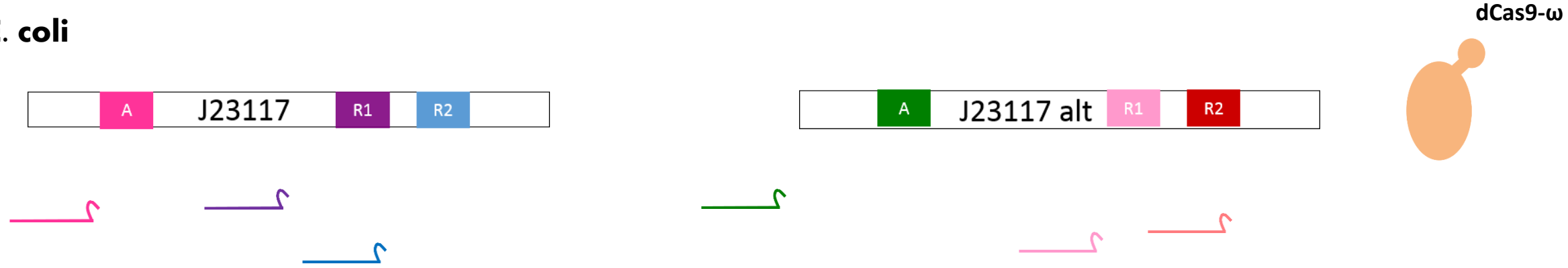
Homogeneous

Orthogonal

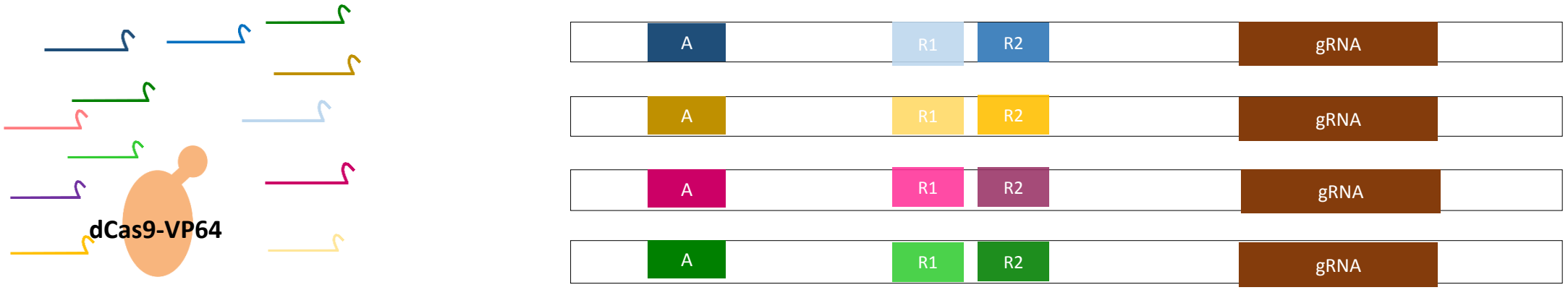
Chainable

Parts

E. coli

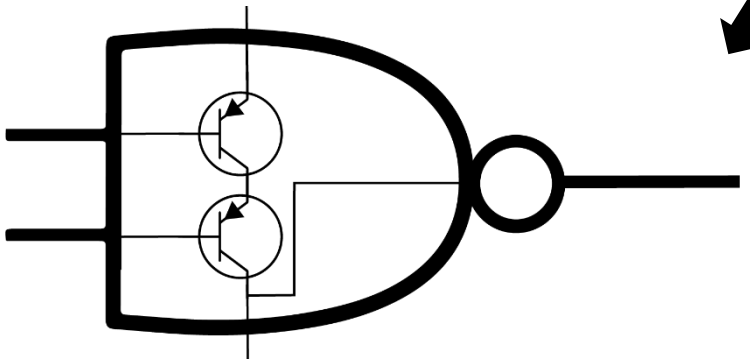


S. cerevisiae



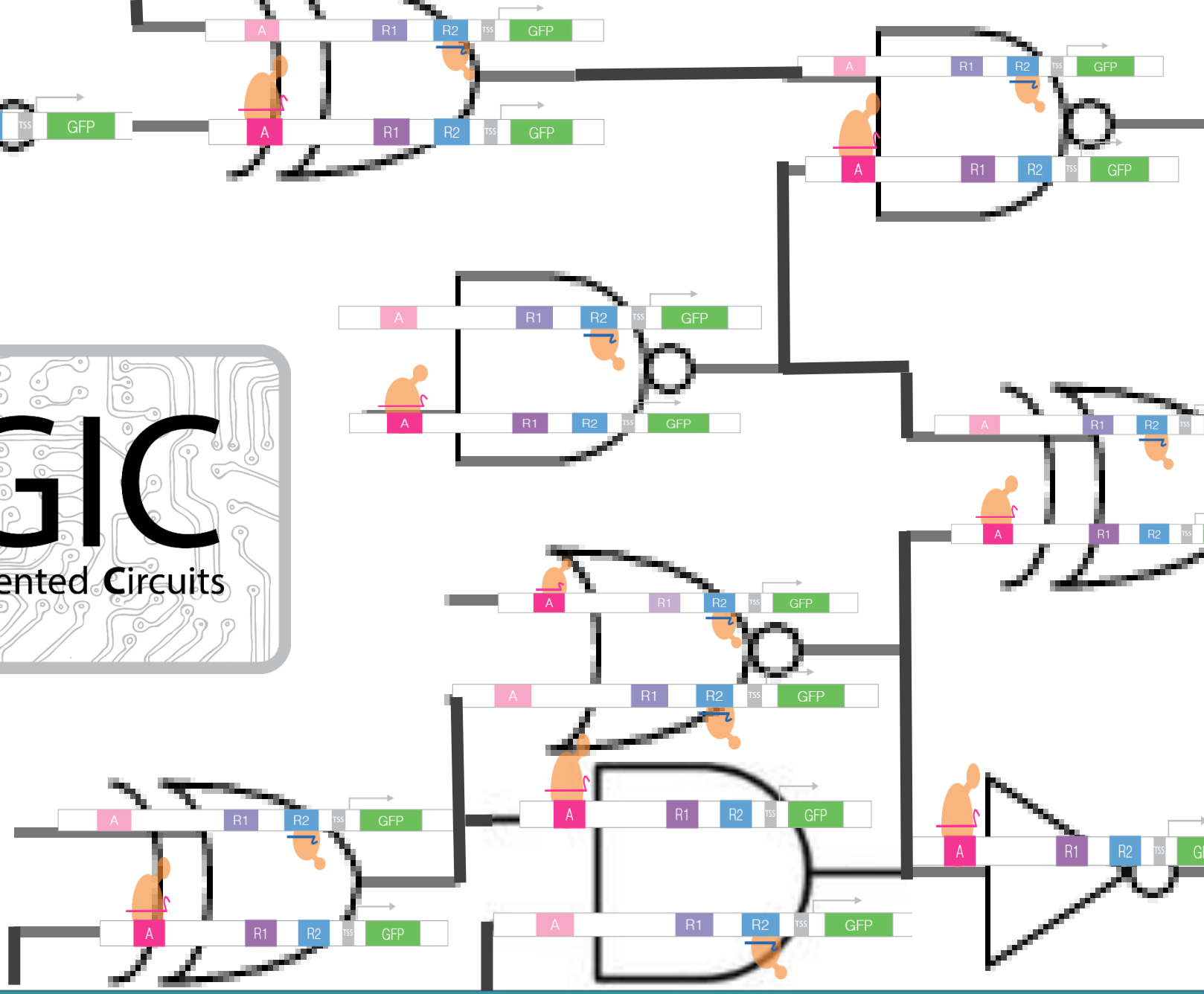
Next steps

Wet Lab ⇌ Modeling



Bio LOGIC

Logic **O**rthogonal **g**RNA **I**mplemented **C**ircuits



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