Designing genetic circuits using Cello

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What is Cello

- A program that automatically generates and designs genetic circuits depending on user's inputs and outputs
- The algorithm designs circuits using NOT and NOR logic gates
- Inputs are inducible promoters and output is the wanted product proteins
- There are currently only a few options for inputs and outputs
- The algorithm also analyzes toxicity of the product production and of the inducible and repressible signals
- The complete plasmid is included as output and can be copied as text or downloaded in sbol or ape format

Simple circuit

Verilog choose

```
1  module A(output out1,  input in1, in2, in3);
2    always@(in1,in2,in3)
3      begin
4        case({in1,in2,in3})
5          3'b000: {out1} = 1'b0;
6          3'b001: {out1} = 1'b0;
7          3'b010: {out1} = 1'b0;
8          3'b011: {out1} = 1'b0;
9          3'b100: {out1} = 1'b0;
10         3'b101: {out1} = 1'b1;
11         3'b110: {out1} = 1'b1;
12         3'b111: {out1} = 1'b1;
13       endcase
14     end
15 endmodule
```

Inputs

choose clear

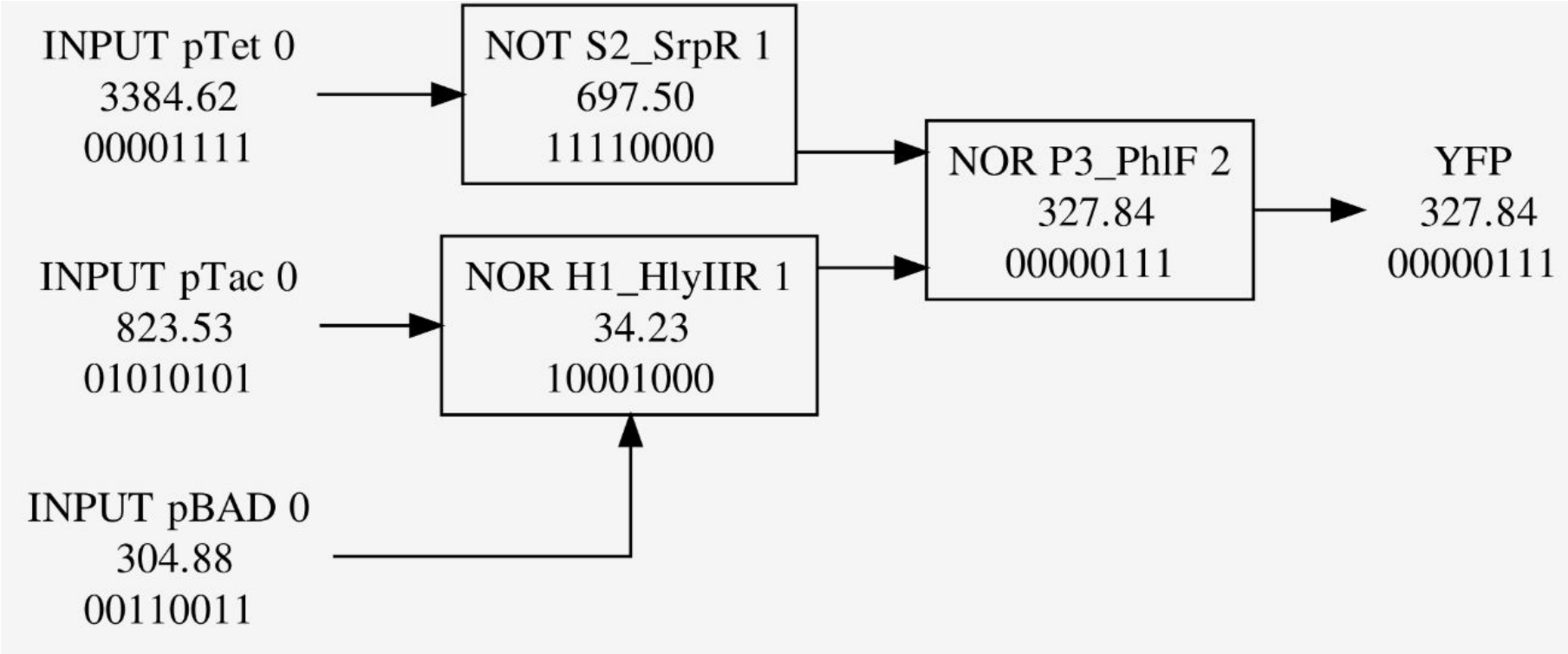
index	name	low RPU	high RPU	DNA sequence
1	pTac	0,0034	2,8	AACGATCGTTGGCTGTGTTGACAATTAAT
2	pBAD	0,0082	2,5	ACTTTTCATACTCCCGCCATTCAGAGAAC
3	pTet	0,0013	4,4	TACTCCACCGTTGGCTTTTTTCCCTATCA

Outputs

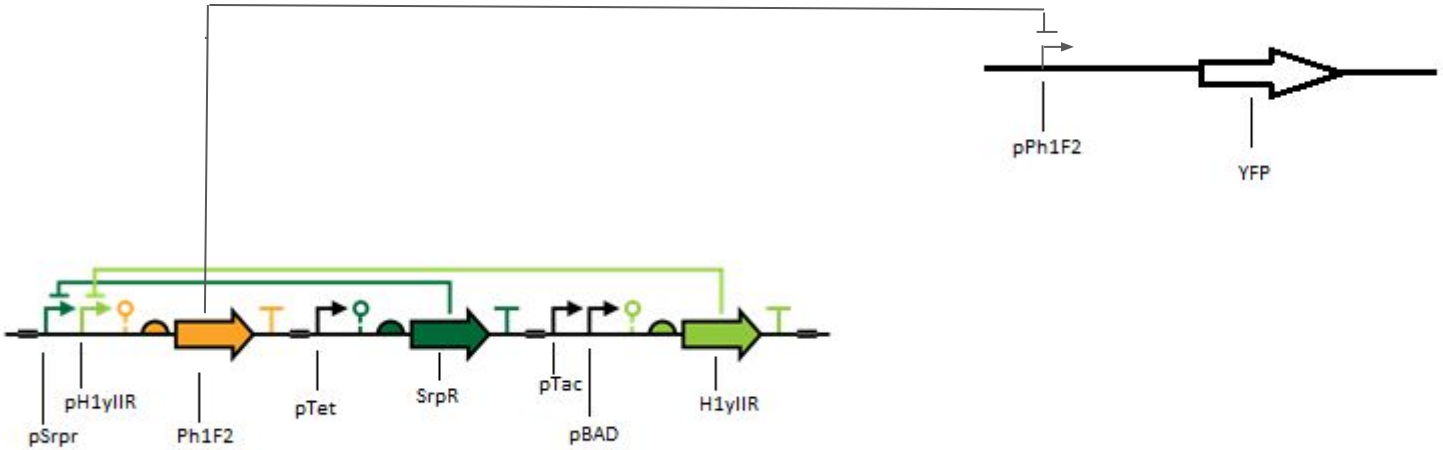
choose clear

index	name	DNA sequence
1	YFP	CTGAAGCTGTCACCGGATGTGCTTTCCGGTCTGATGAGTCCGTGAGGACG

Simple circuit



Simple circuit



How the circuit work

Three inducible promoters are used. pTet induced by TetR , pTac induced by LacI and pBAD induced by AraC.

If pTet is on, SrpR is produced. If either pTac or pBAD is on H1yIIR is produced. SrpR and H1yIIR are repressors of pSrpR and pH1yIIR. Therefore Ph1F2 is produced only if there is no SrpR or H1yIIR. Ph1F2 is a repressor of the pomoter pPh1F2 and therefore YFP is produced only if Ph1F2 is not produced.

As a result YFP is produced only if all of the promoters are on, if both pTac and pTet is on or if both pTac and pBAD is on.

Complex circuit

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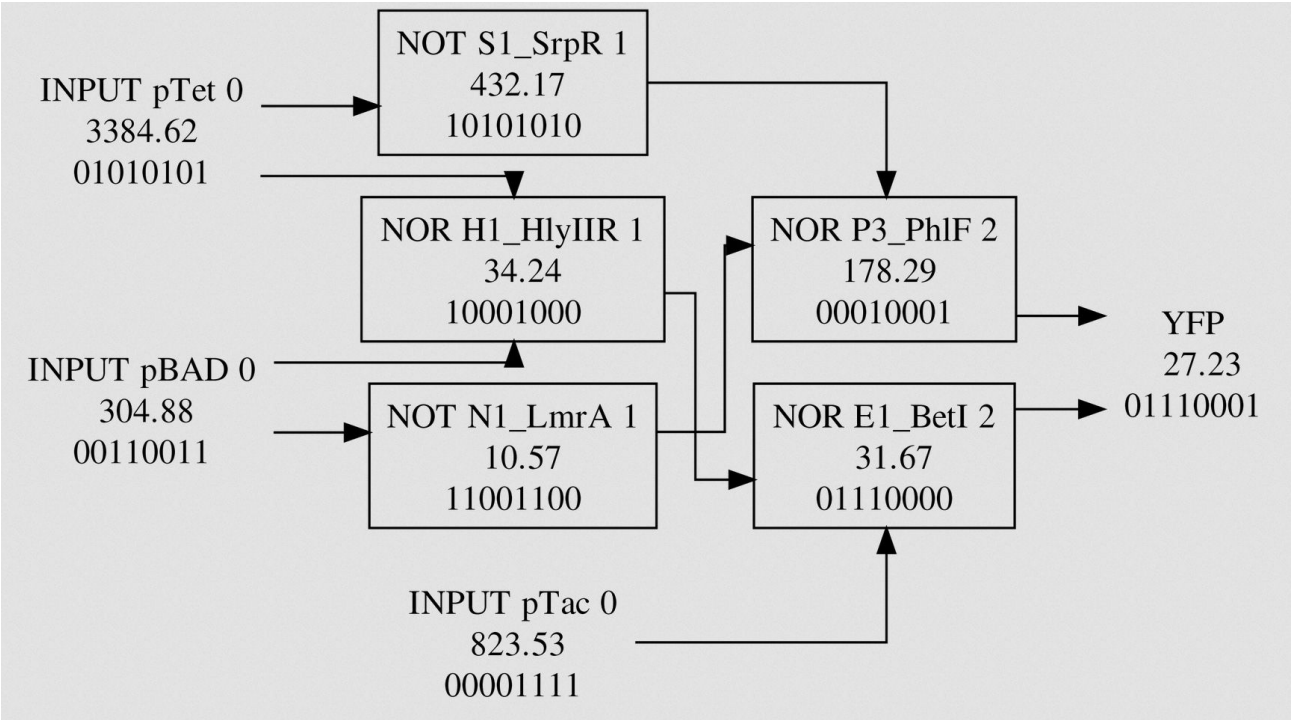
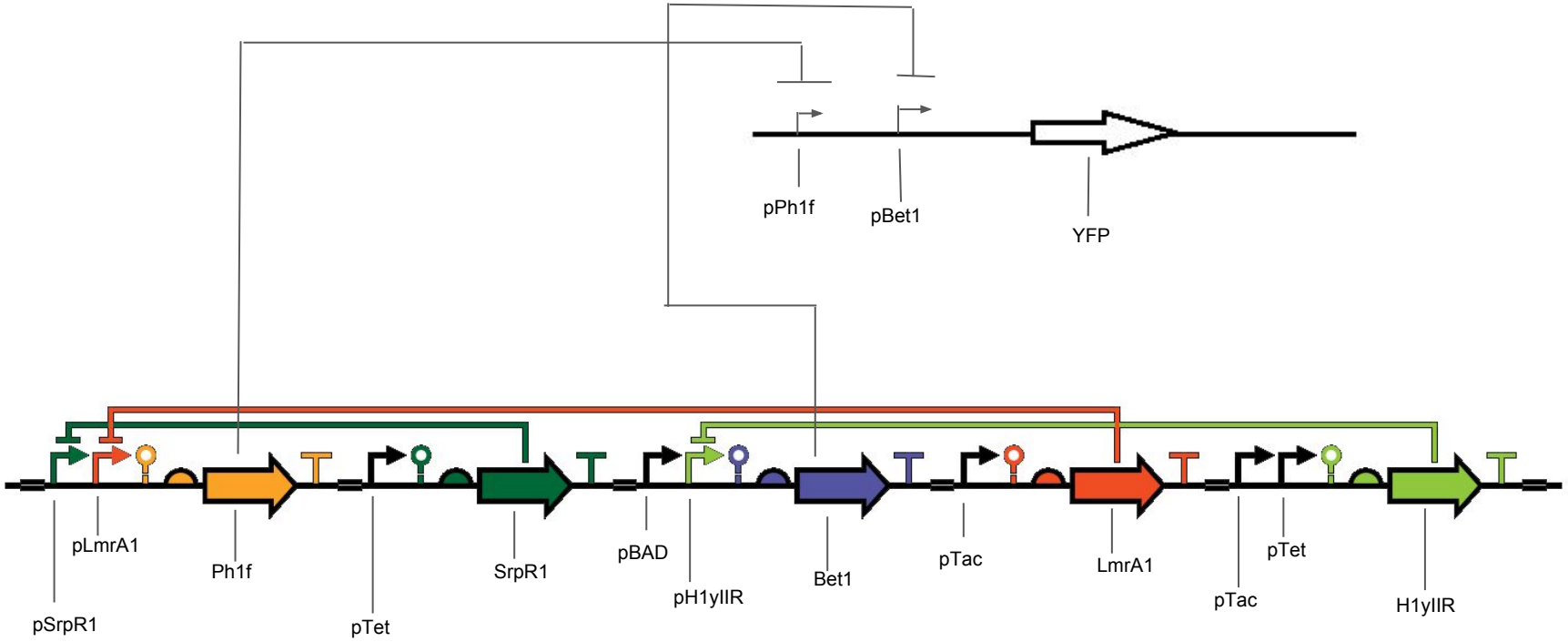


Complex circuit



Implementing the design in cells

Cello gives the sequence information needed for plasmid construction.

Two plasmids has to be done. One with the circuit and another with the output.

The sequences that cello is giving are inserted to *E. coli* plasmids with a selection marker. Then the plasmids are inserted to *E.coli.*

Sources

Nielsen AA, Der BS, Shin J, Vaidyanathan P, Paralanov V, Strychalski EA, Ross D, Densmore D, Voigt CA. Genetic circuit design automation. Science. 2016 Apr 1;352(6281):aac7341. doi: 10.1126/science.aac7341.