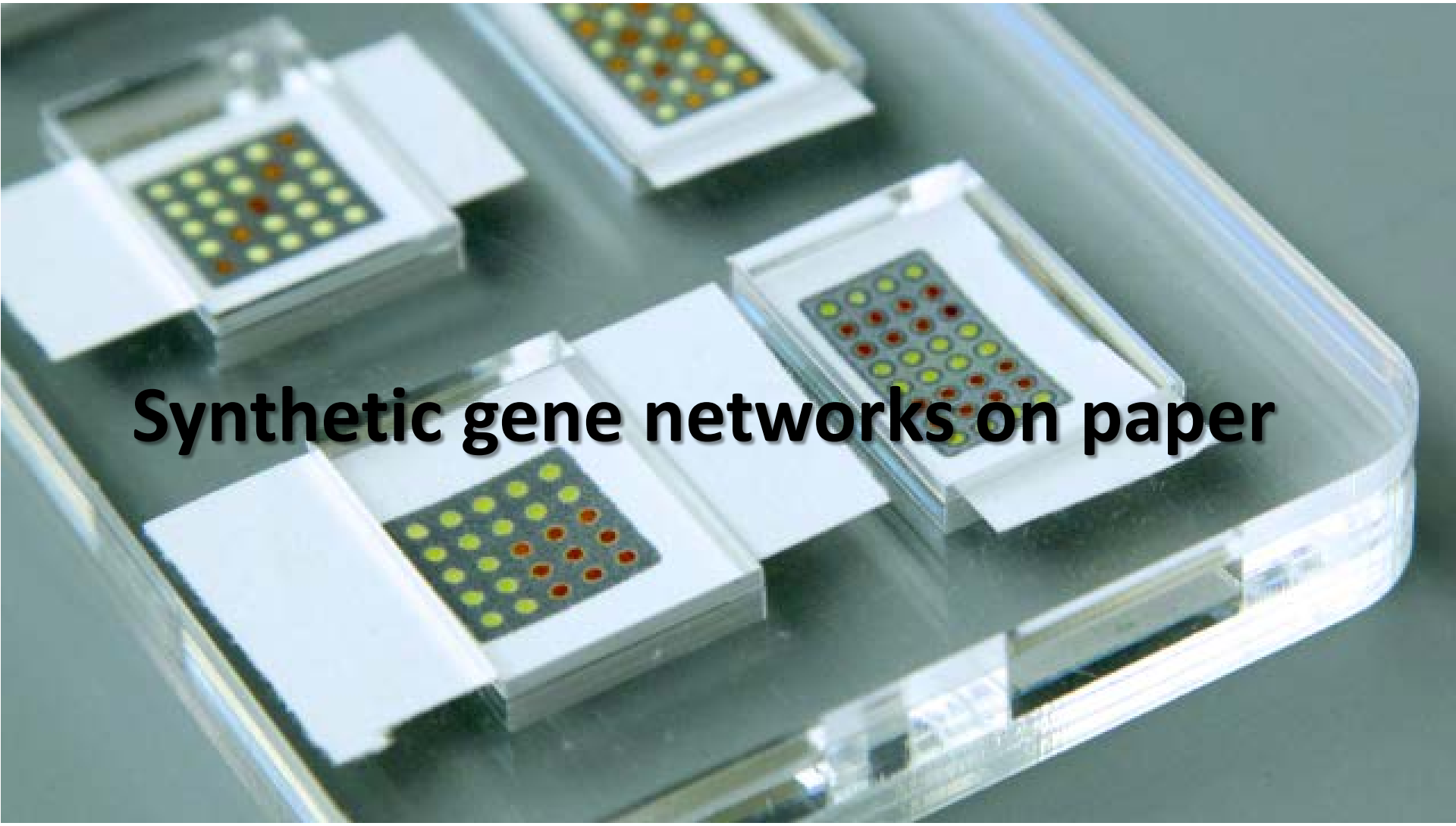


Synthetic gene networks on paper

Journal club

Caihong Zhu

25.11.2014

Synthetic biology

Definition: "designing and constructing biological devices and biological systems for useful purposes."_*Wekipedia*

Interdisciplinary science, combination of biotechnology, evolutionary biology, molecular biology, system biology and biophysics

- First used by Stéphane Leducs's publication of « Théorie physico-chimique de la vie et générations spontanées » (1910) and « La Biologie Synthétique » (1912)
- Described by Wacław Szybalski in 1974: Up to now we are working on the descriptive phase of molecular biology. ... But the real challenge will start when we enter the synthetic phase of research in our field.... in the synthetic biology, in general.

Synthetic gene networks stands in the center of synthetic biology

Milestones in synthetic gene networks

Toggle switches: **Gardner et al, Nature, 2000**

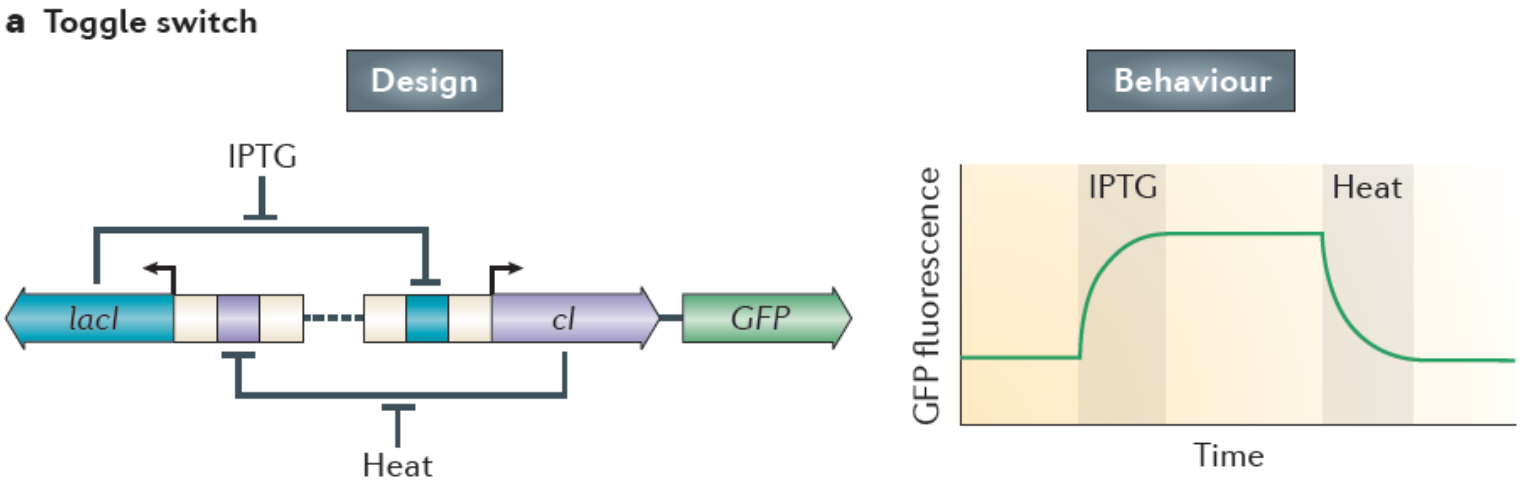


Figure from Cameron, et al, Nat Rev Microbiol, 2014

Milestones in synthetic gene networks

Repressilator: **Elowitz et al, Nature, 2000**

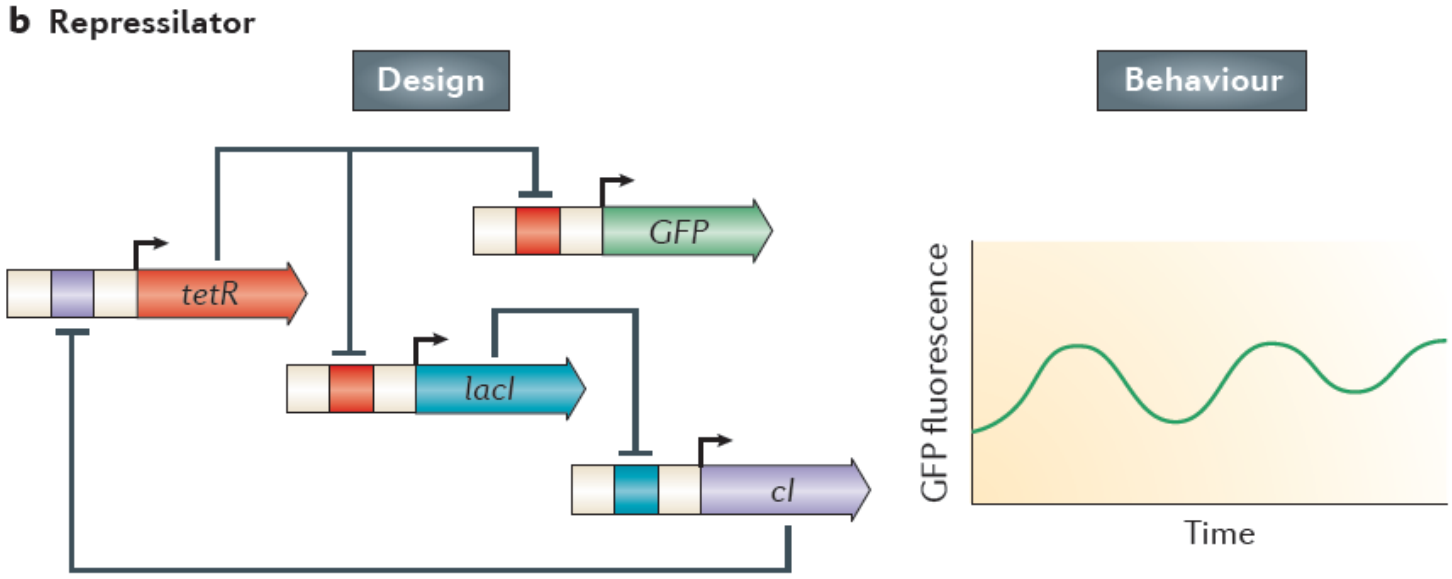
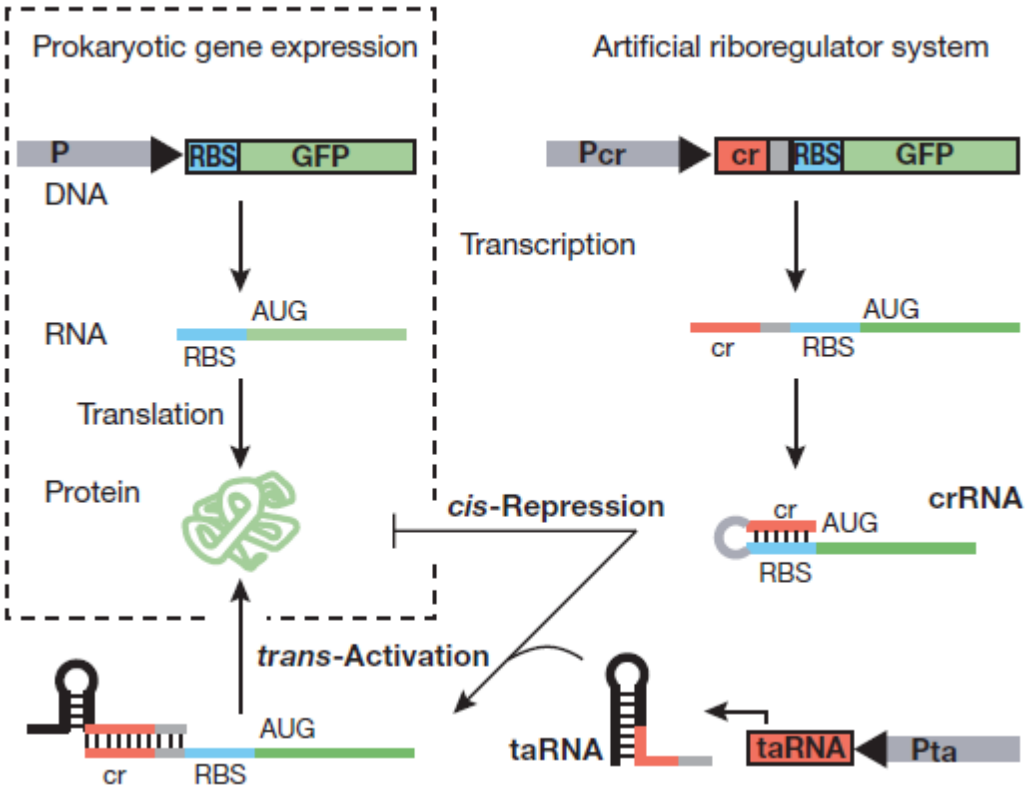


Figure from Cameron, et al, Nat Rev Microbiol, 2014

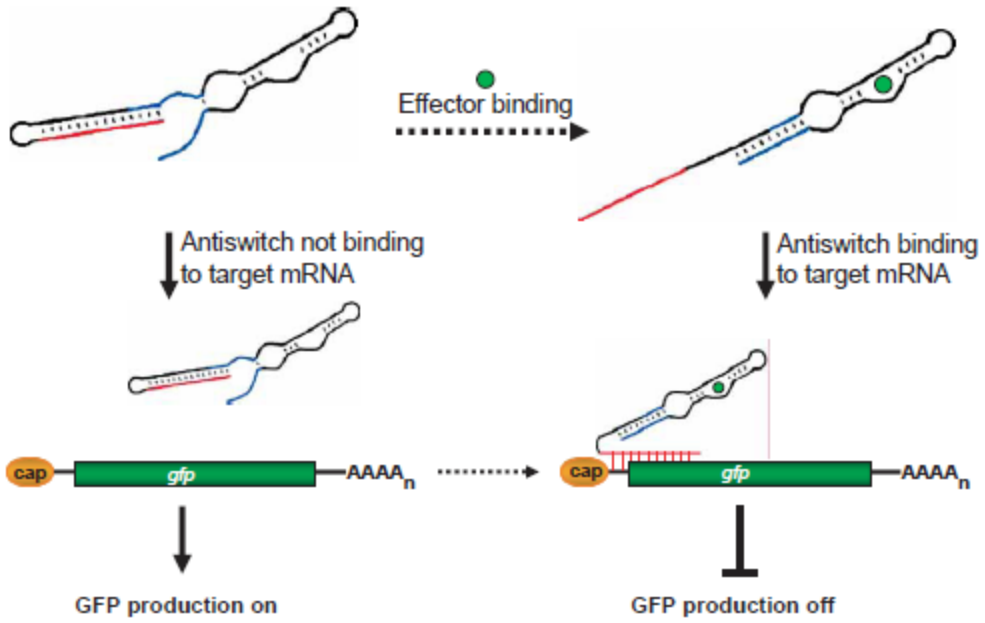
Milestones in synthetic gene networks

Riboregulator: **Isaacs et al, Nat Biotechnol, 2004**



Milestones in synthetic gene networks

Antiswitch riboregulator: **Bayer et al, Nat Biotechnol, 2005**



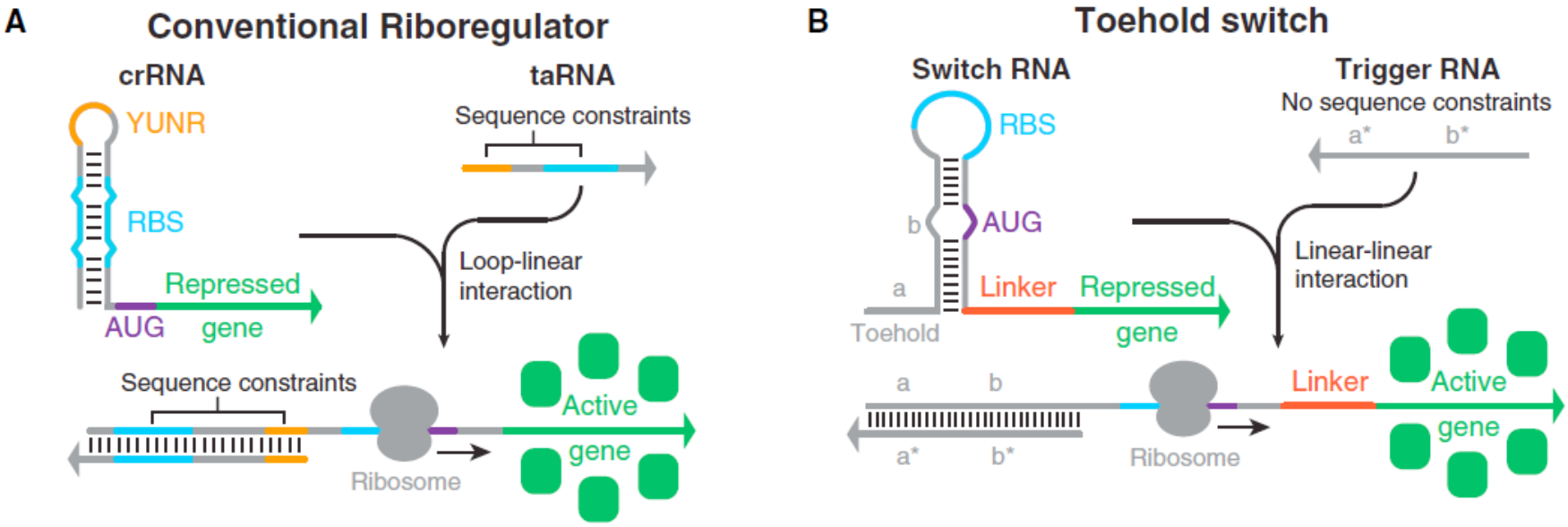
Conventional riboregulator in Synthetic gene networks

- Programmability, but limited dynamic range: 55-fold activation, 10-fold repressing (v.s protein-based transcriptional regulators, 300~400-fold modulation)
- Due to the sequence constrains, limited number of composable, high-performance parts for constructing genetic circuits
- Difficulties in integrating multiple components into a large, complex synthetic network
- New regulatory components with wide dynamice range, low system crosstalk, high flexibility are required

Toehold Switches: De-Novo-Designed Regulators of Gene Expression

Alexander A. Green,[1] Pamela A. Silver,[1,2] James J. Collins,[1,3] and Peng Yin[1,2,*]

Cell *159*, 925–939, November 6, 2014 ©2014 Elsevier Inc.



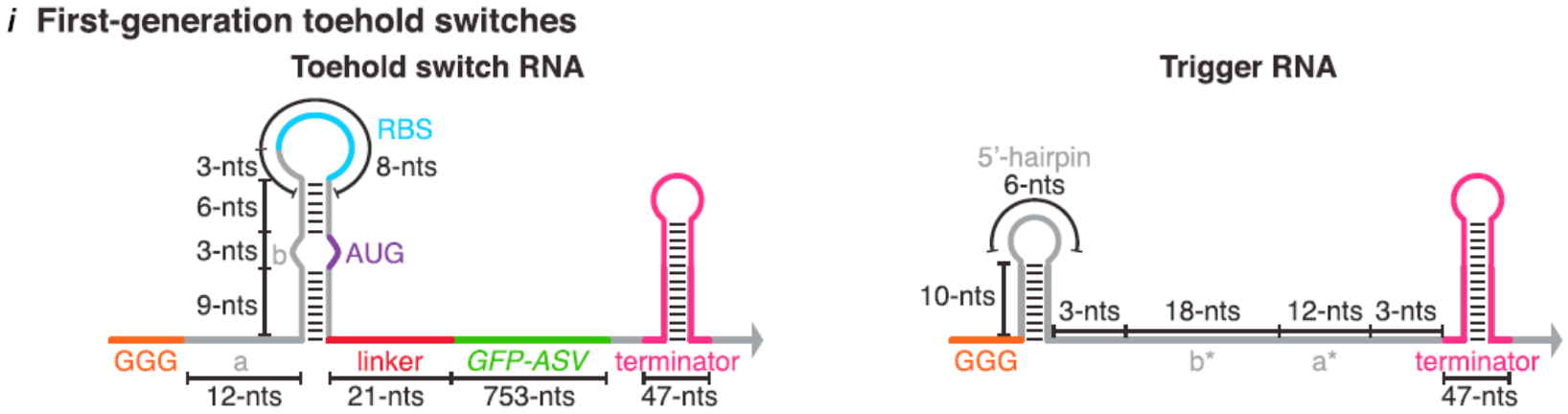
crRNA
RBS: ribosome binding site, 19 nts
YUNR: pYrimidine-Uracil-Nucleotide-puRine, 4 nts

taRNA: 26 nts complementary to YUNR, RBS of crRNA

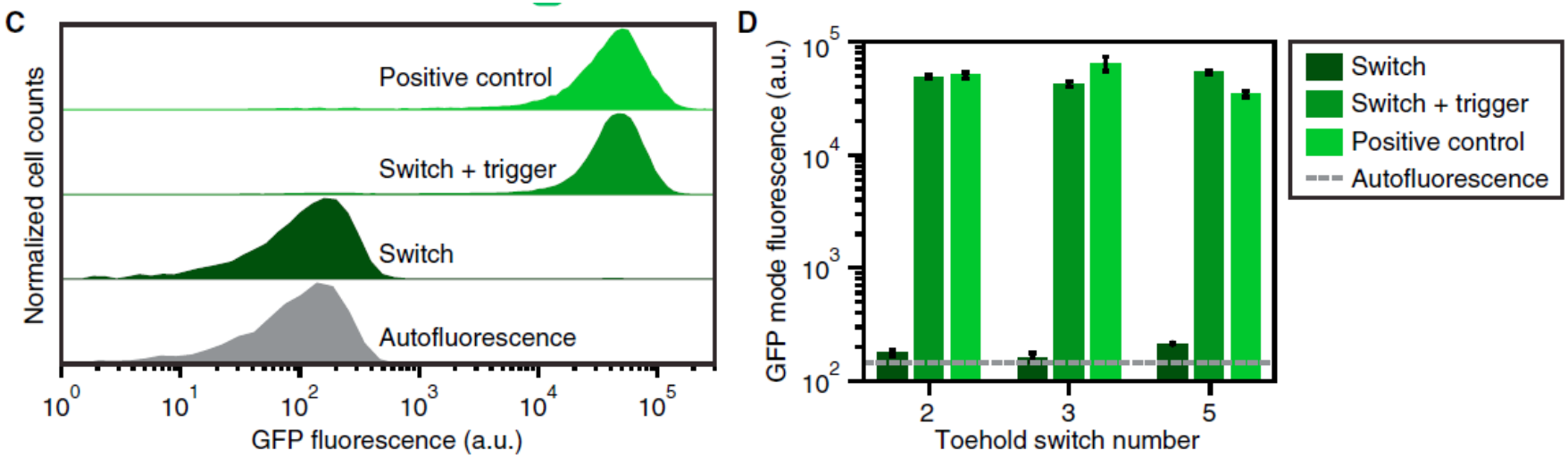
Switch RNA
21 nts linker
11 nts loop
6 nts before AUG
9 nts after AUG
12 nts toehold

Trigger RNA: 30 nts complementary to Switch RNA

Scheme of the first generation toehold switches



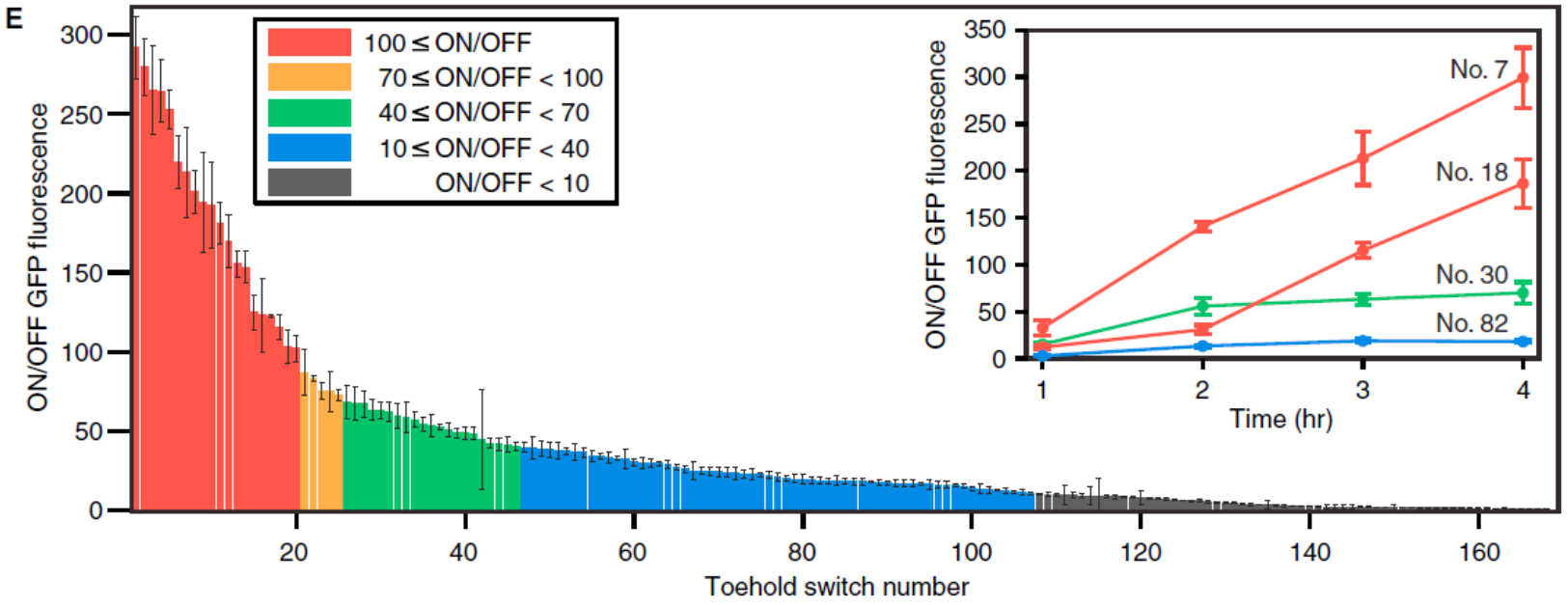
Toehold switches mediated gene expression
>100-fold modulation

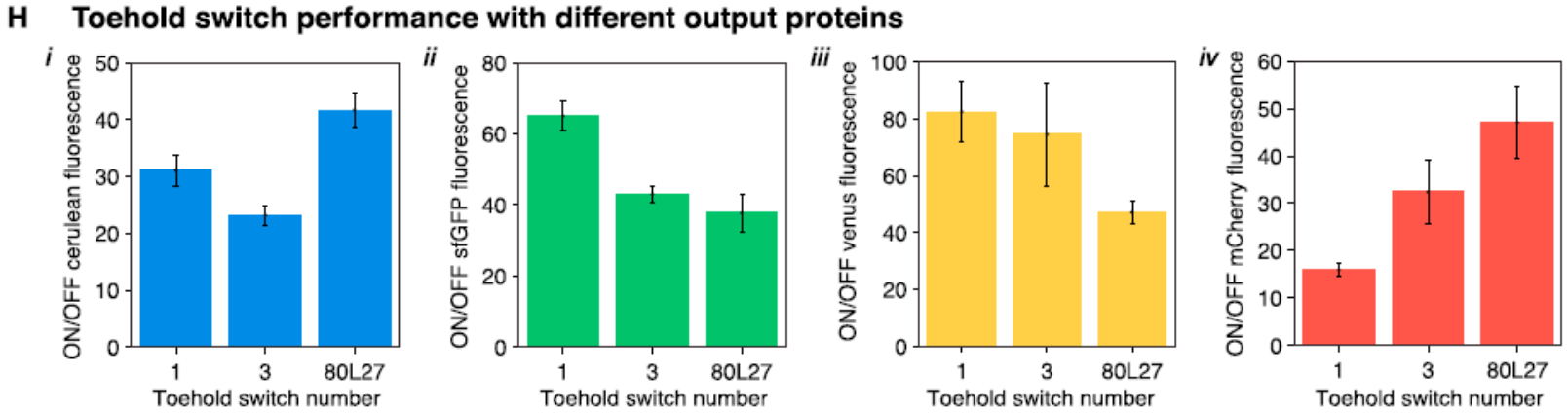


Switch RNA and Trigger RNA, induced by IPTG, T7 RNA polymerase

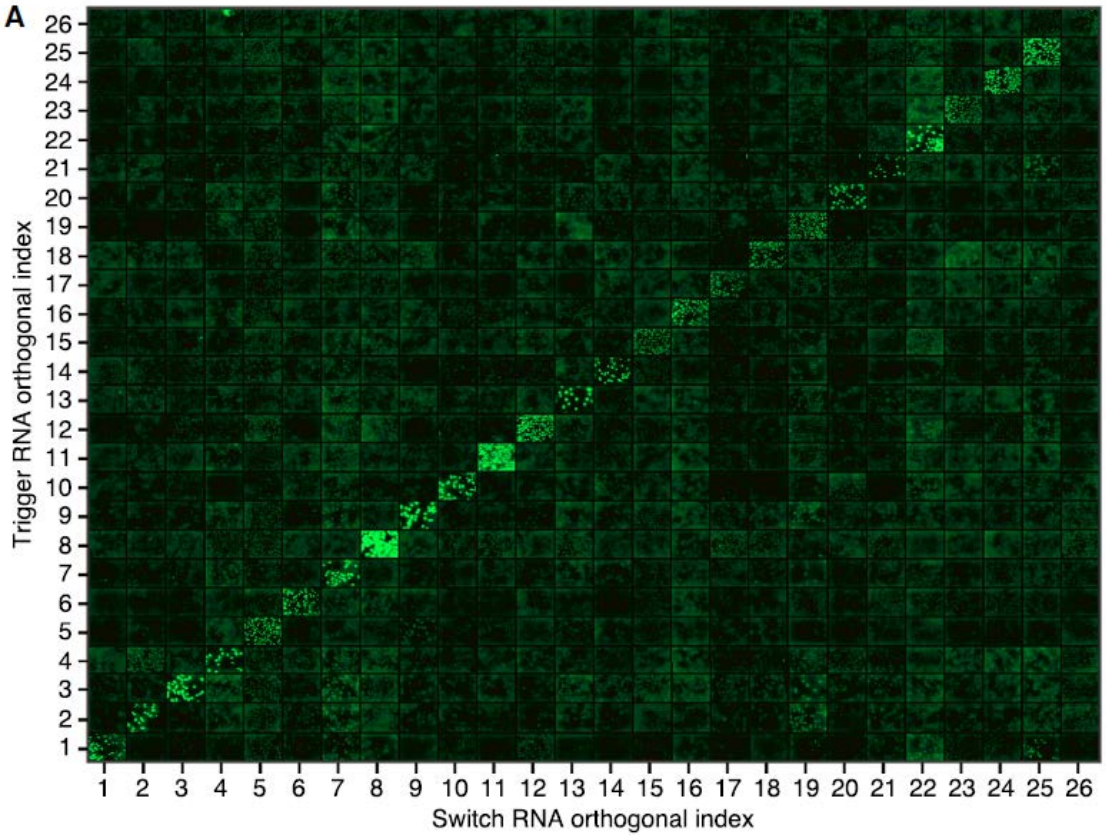
ON/OFF ratio of toehold switches mediated gene expression

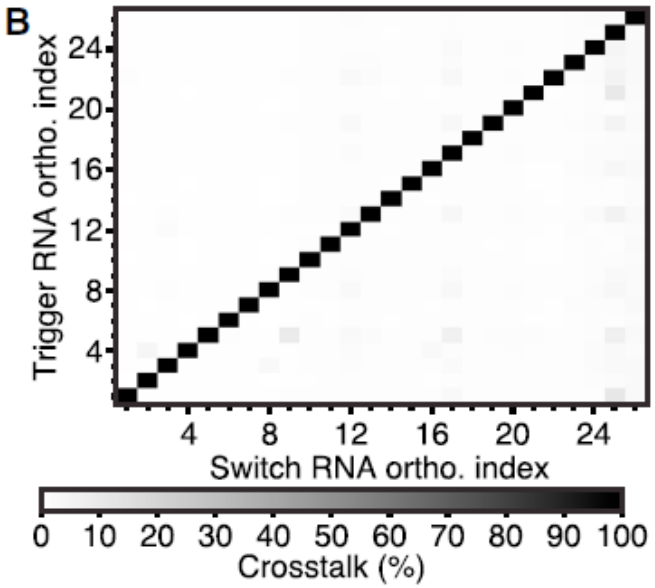
168 first generation toehold switches





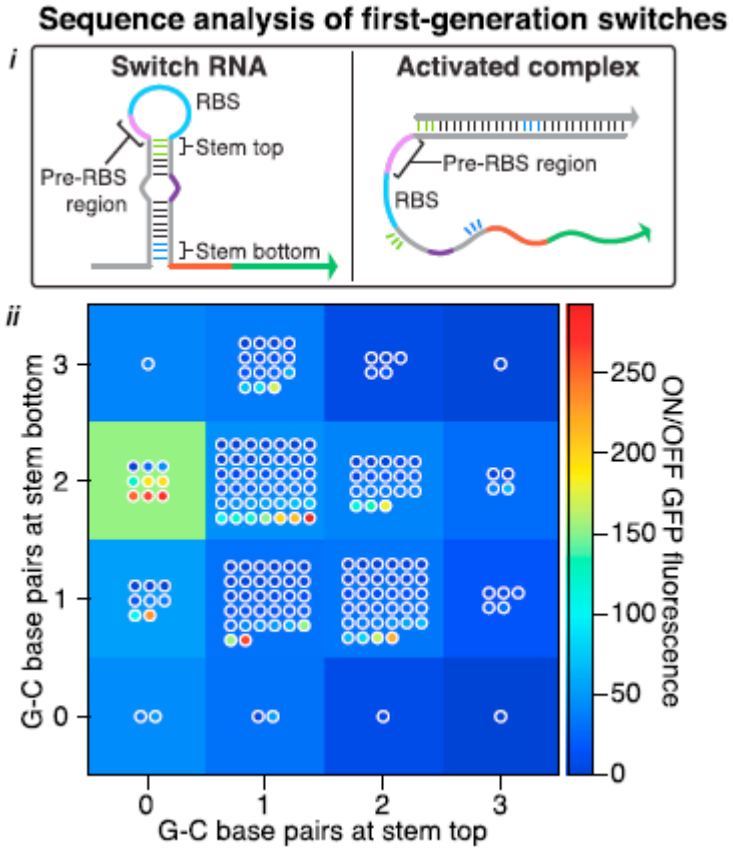
Orthogonality of toehold switch mediated gene expression

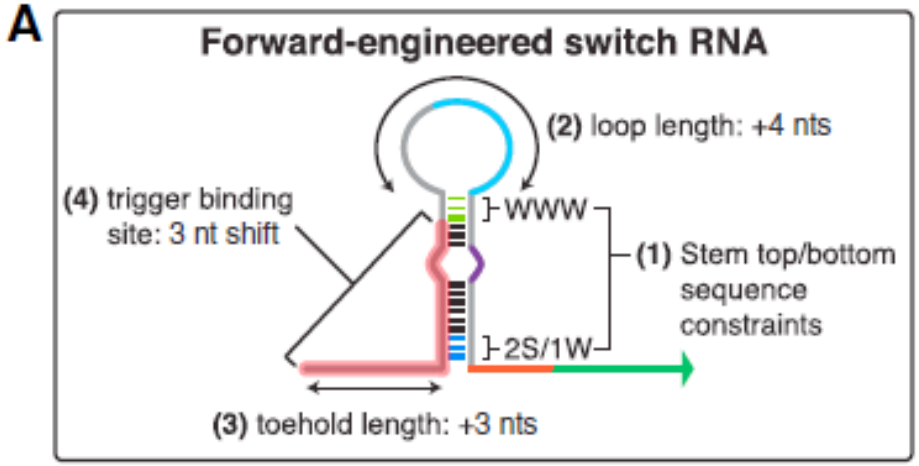




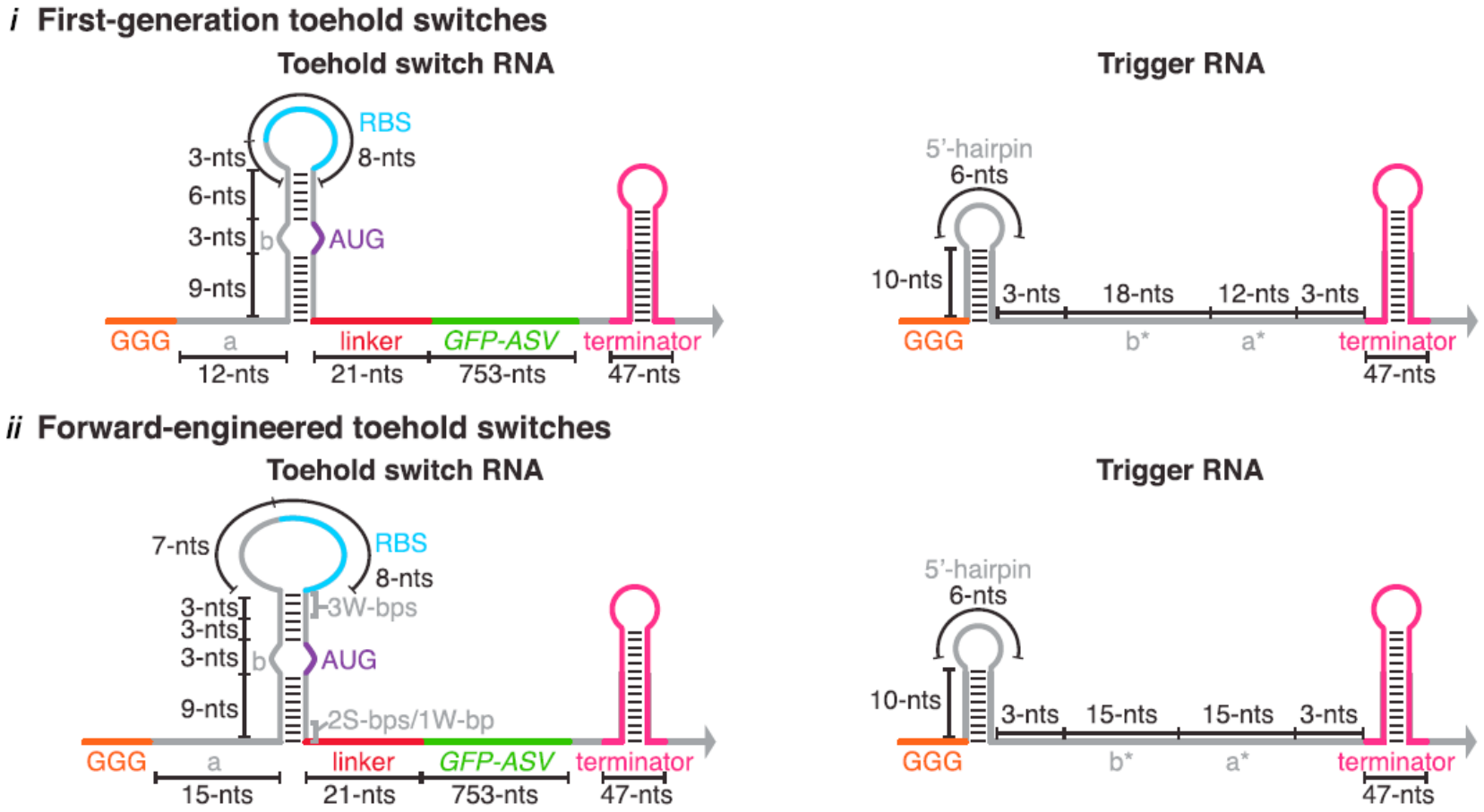
<12% crosstalk between switch RNA and noncognate triggering RNA

Design of the forward-engineered toehold switches

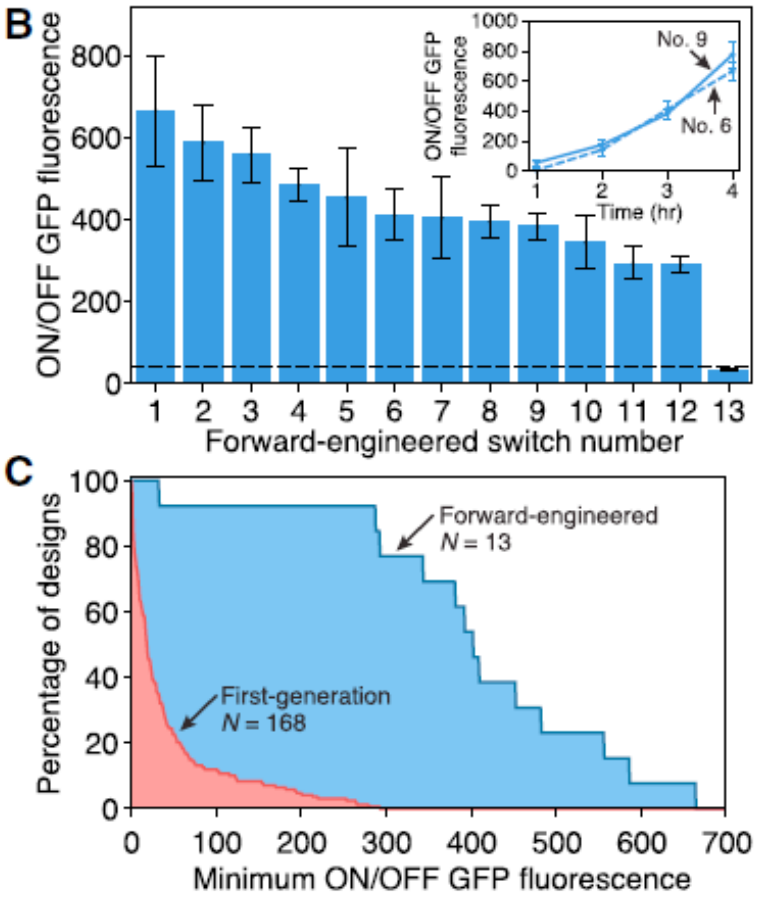


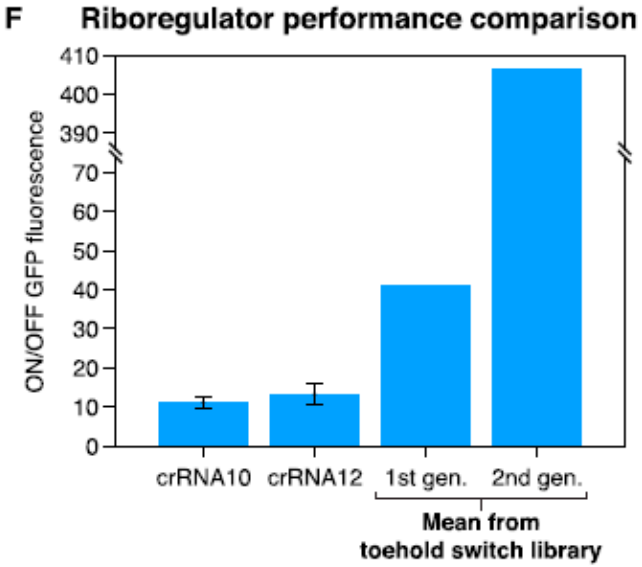


Scheme of the forward-engineered toehold switches

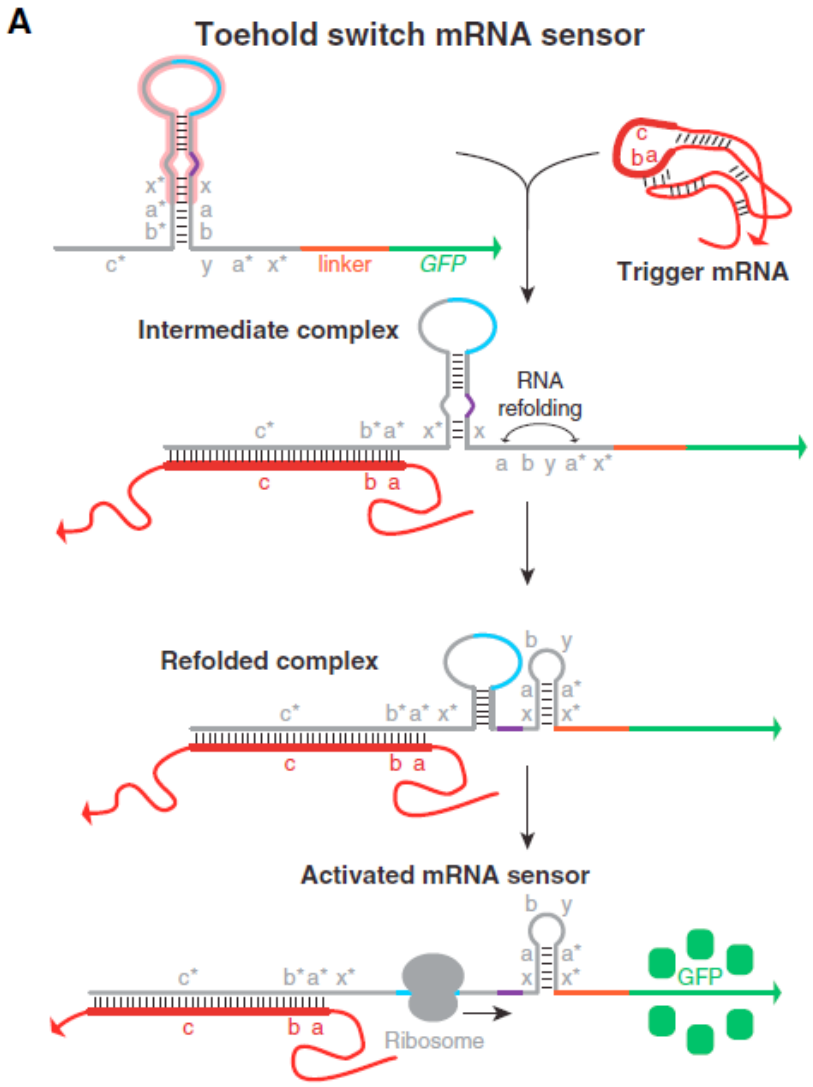


ON/OFF ratio of toehold switches mediated gene expression



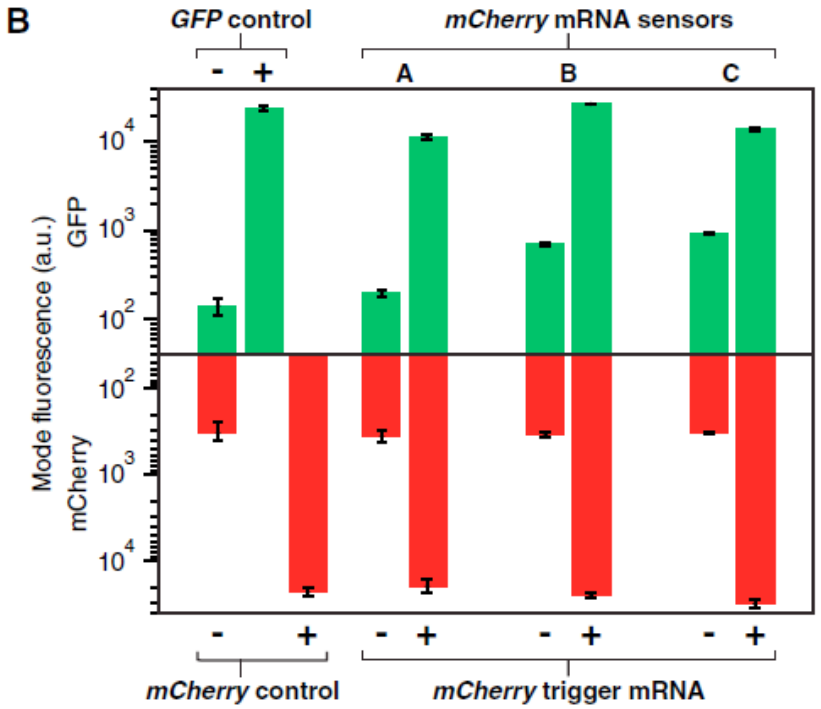


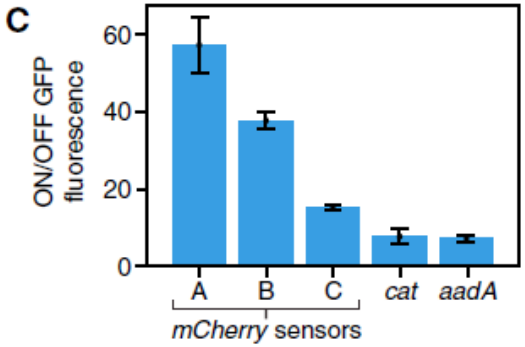
The arbitrary sequence of trigger RNAs - Toehold switches triggered by mRNA?



- Increased toehold domain from 12/15 nts to >24 nts
- Design sensor according to the previous toehold switches, common sequences
- RNA refolding mechanism to decrease the energy barrier to switch activation

Toehold switches triggered by mCherry mRNA



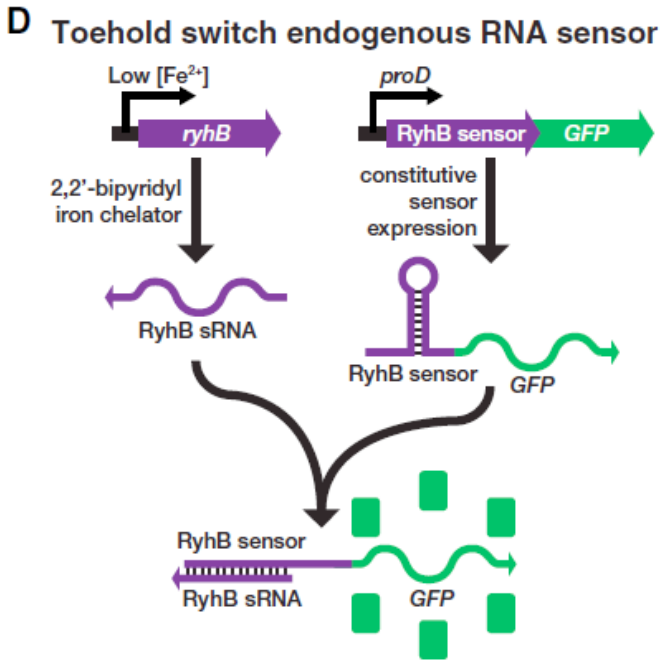


- **Sensors only express GFP upon toeswitch by mCherry mRNA**
- **Simultaneous detection of GFP and mCherry**

- ON/OFF ratio of sensros triggered by mCherry or Chloramphenicol acetyltransferase (cat) or spectinomycin resistance (aadA)

Toehold switches triggered by endogenous RNA

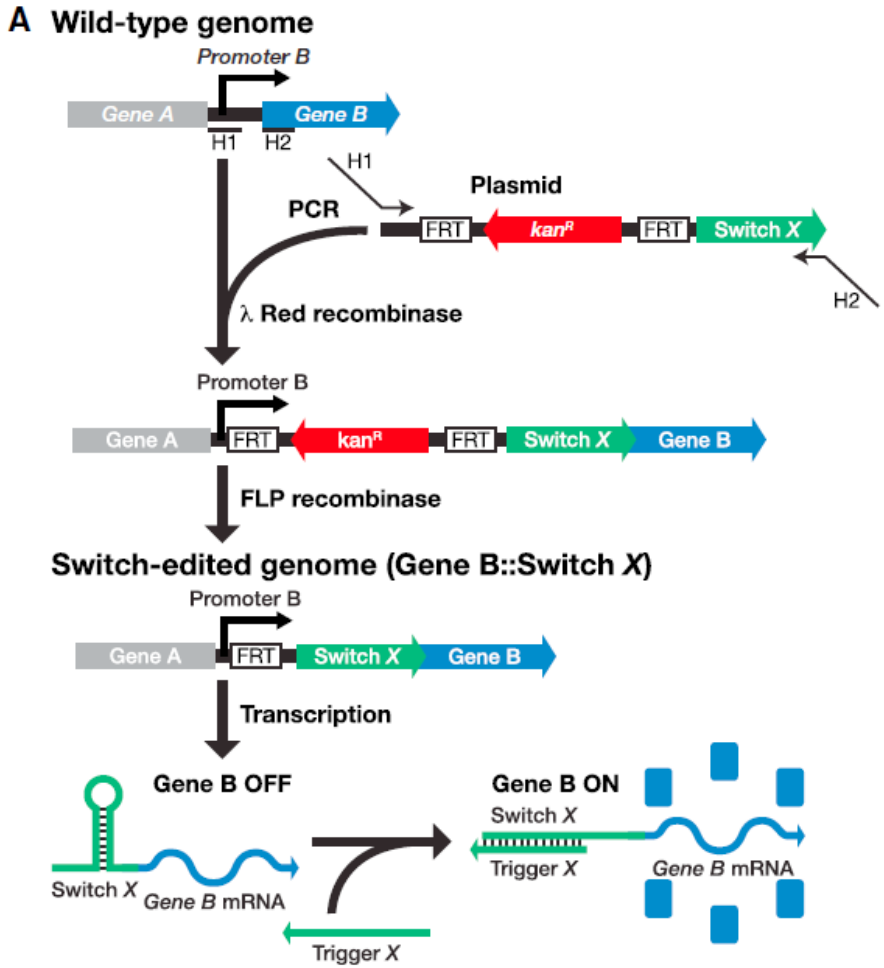
- **ryhB, 90 nts small RNA, downregulates iron-associated gene when iron level is low**
- **Increased expression of ryhB when iron level is descreased**



- ryhB sensor increased GFP expression when treated with increasing leveld of iron-chelating compound
- Control GFP construct showed decreased GFP expression

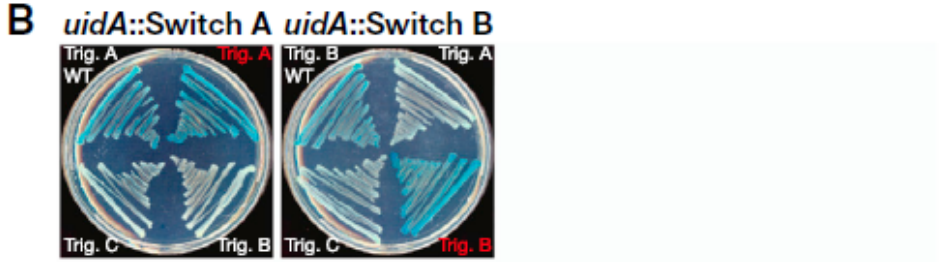
- **Toehold switch acts as endogenous RNA indicator**

Toehold switches regulate the translation of endogenous RNA



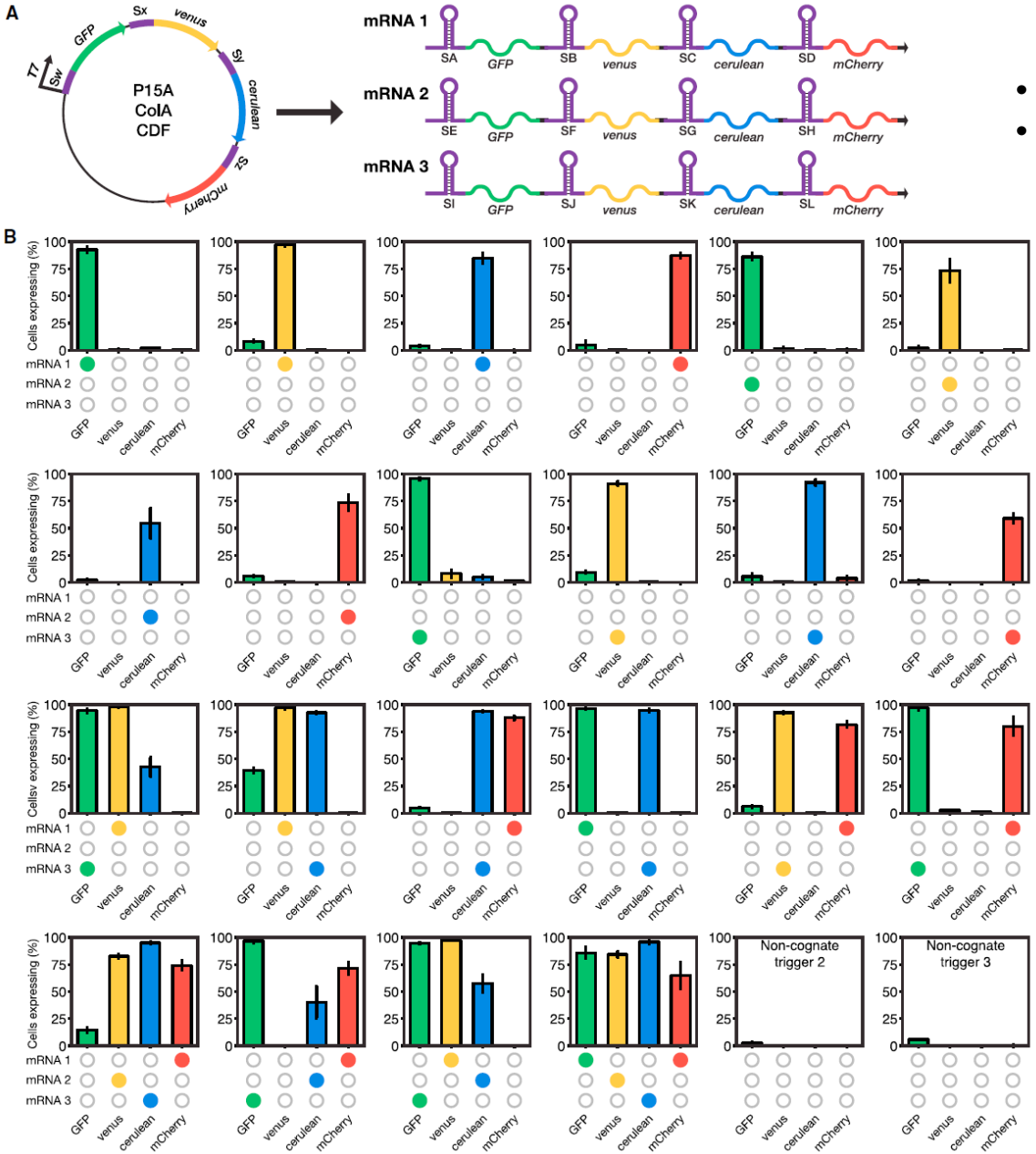
- **Knock-in a switch into genome**
- **Add trigger to stimulate the expression of endogenous genes**

Toehold switches regulate the translation of endogenous RNA



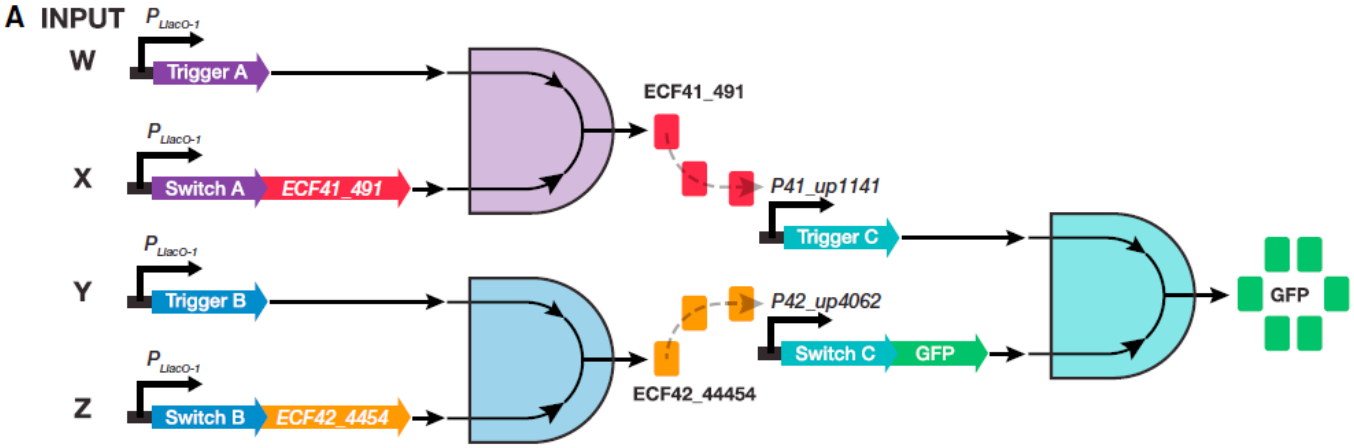
- **uidA, β-glucoronidase, stained by X-Gluc**

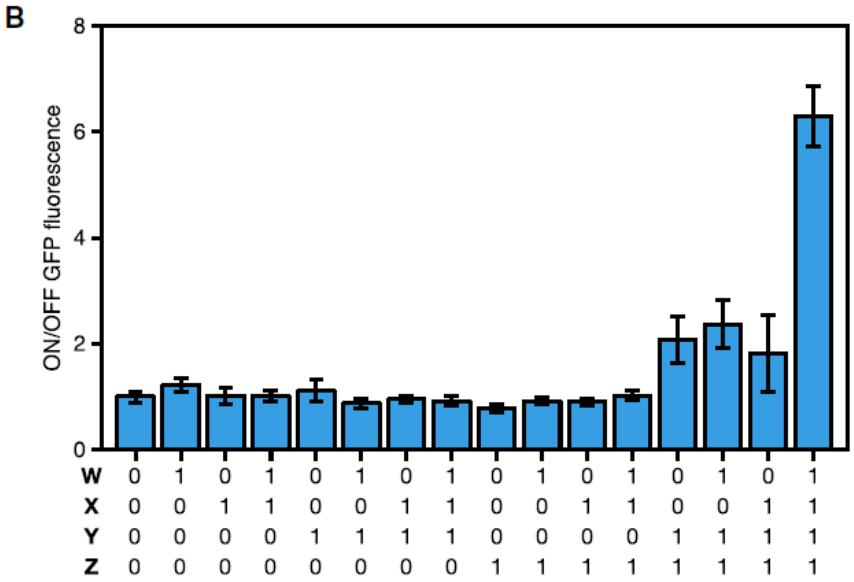
Multiplexing regulation by toehold switches



- **12 toehold switches**
- **4 fluorescent proteins**

Implementation of a 4-Input AND Circuit





ECF: transcription factors

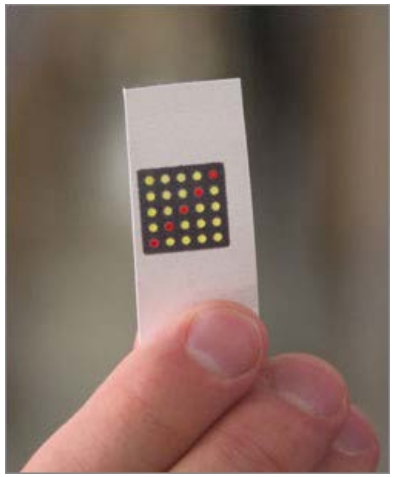
Toehold switches in synthetic gene networks

- Versatile and powerful platform for posttranscriptional regulation, high performance, high dynamic range. High orthogonality
- Can be used to monitor the endogenous gene expression and to regulate endogenous gene expression
- Multiplexing
- All were done in cultured E.Coli

Paper-Based Synthetic Gene Networks

Keith Pardee,[1,2] Alexander A. Green,[1,2] Tom Ferrante,[1] D. Ewen Cameron,[2,3] Ajay DaleyKeyser,[1] Peng Yin,[1] and James J. Collins[1,2,3,*]

Cell *159*, 940–954, November 6, 2014

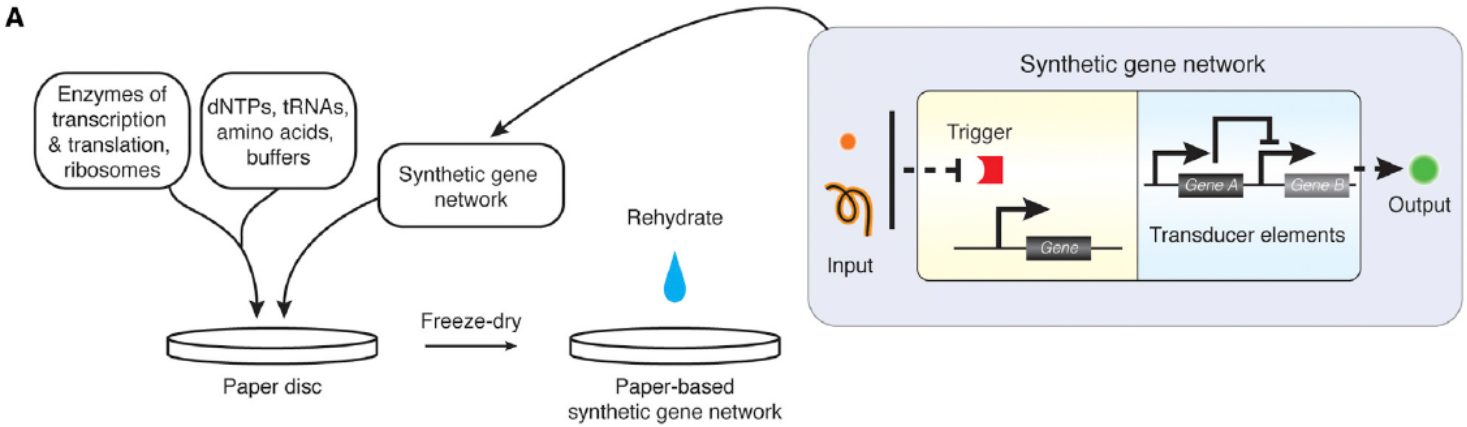


- Cell-based synthetic gene networks outside of laboraroy are restricted due to the biosafety and practicality of the cellular host

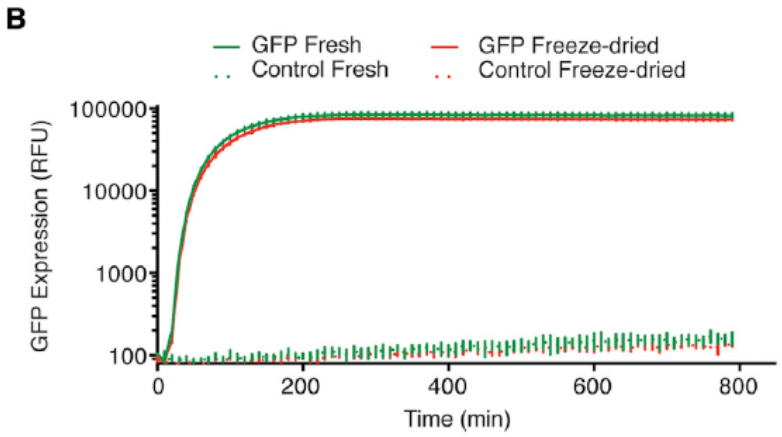
- Synthetic gene networks have been studied in solution-phase reactions using freshly frozen cell-free systems, often liposomes. But solution-phase reastions are still not suitable and practical for handling outside of the lab

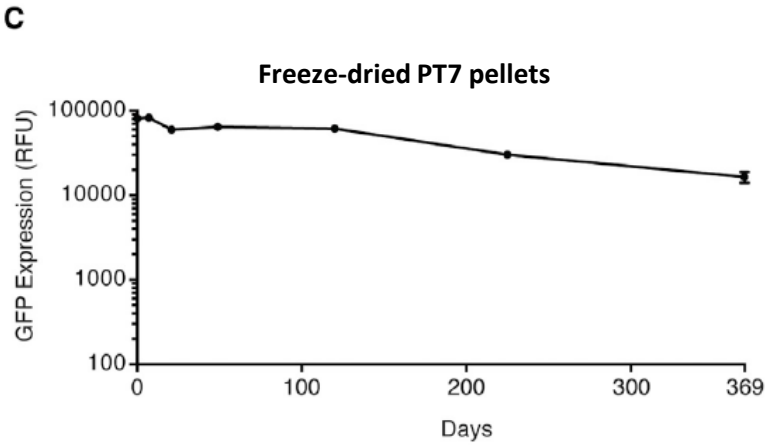
- Paper-based measurement or diagnostics mostly rely on chemical reaction

Overview of synthetic gene networks on paper



GFP expression in solution phase from fresh and freeze-dried PT7 cell-free reactions





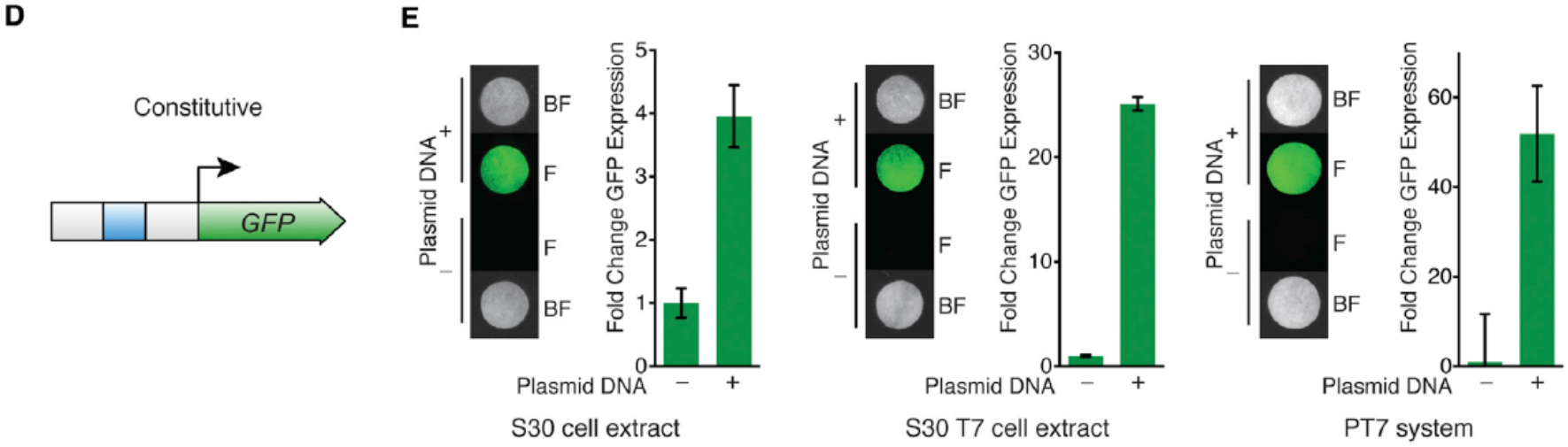
PT7 system: Ribosome + 35 purified bacterial proteins

Freeze-dry – rehydrate with H_2O – 37°C incubation

Freeze-dry – room temperature – rehydrate with H_2O – 37°C incubation

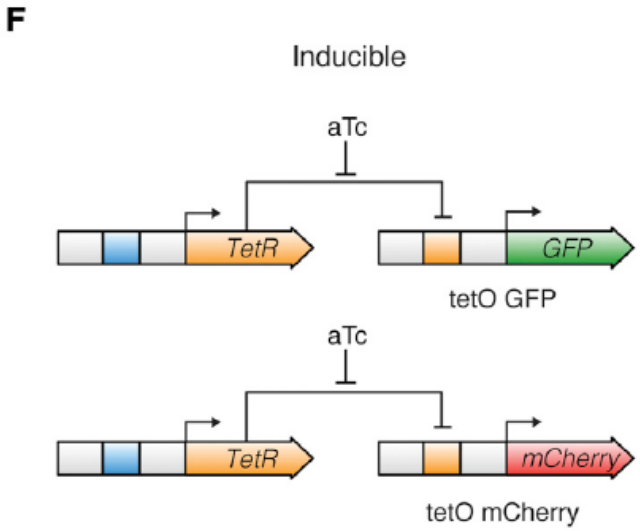
GFP expression on cellulose matrix paper

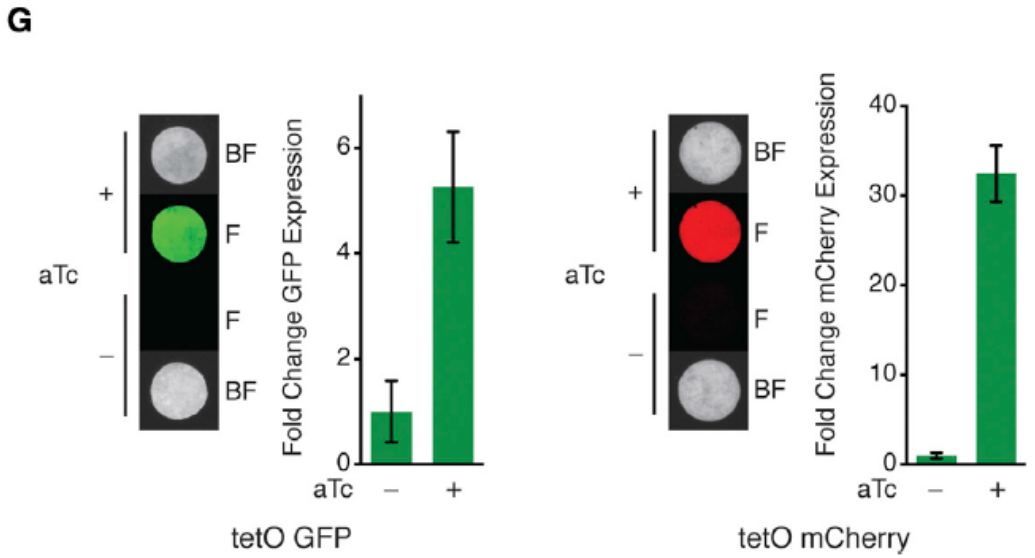
2mm paper discs, 1.8ul reaction
Freeze-dry
Rehydrate with 1.8ul H_2O
Incubate at 37 °C



Synthetic gene networks on cellulose matrix paper

2mm paper discs, 1.8ul reaction
Freeze-dry
Rehydrate with 1.8ul Tetrocycline analog: anhydrotetracycline (aTc)
Incubate at 37 °C, 2 hours

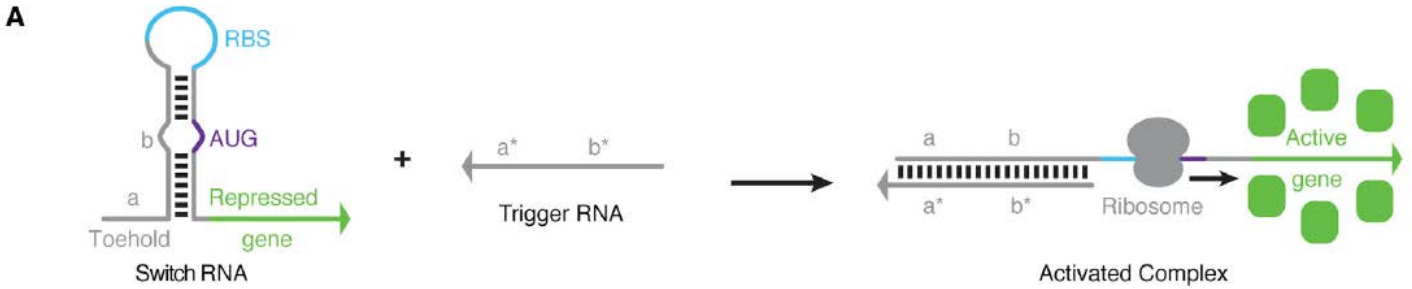


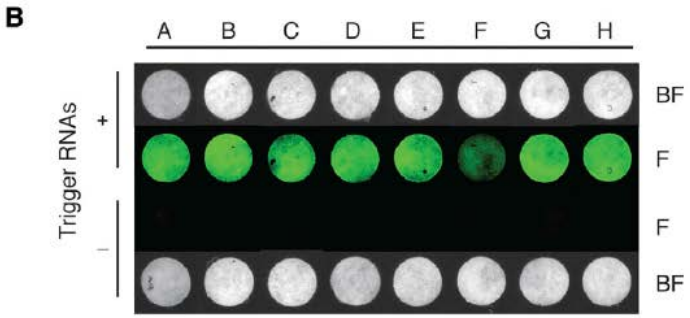


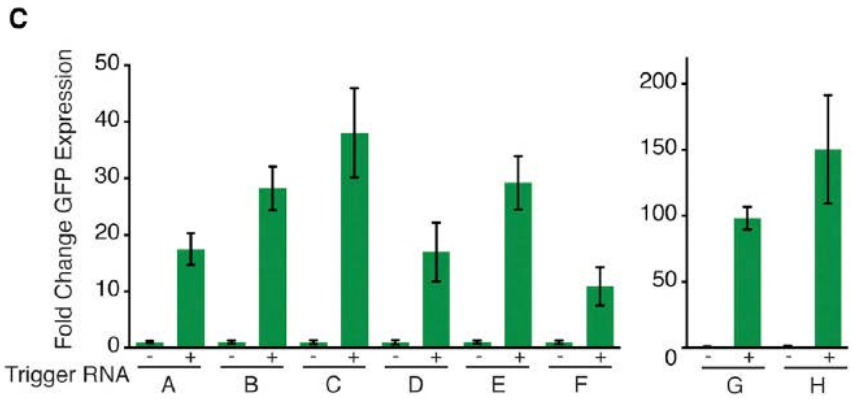
S30 E.coli cell extract

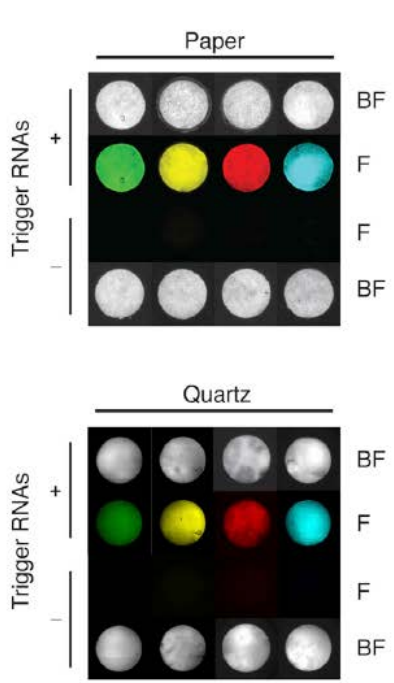
Toehold switches on cellulose matrix paper

2mm paper discs, 1.8ul reaction
Freeze-dry
Rehydrate with 1.8ul trigger RNAs
Incubate at 37 °C, 2 hours

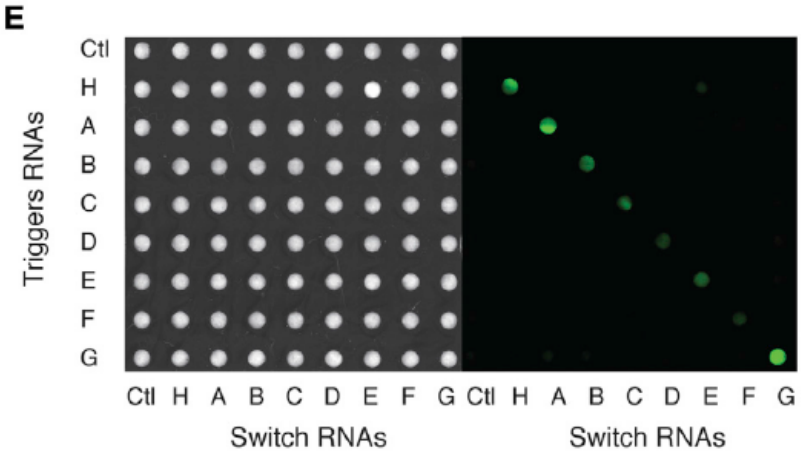


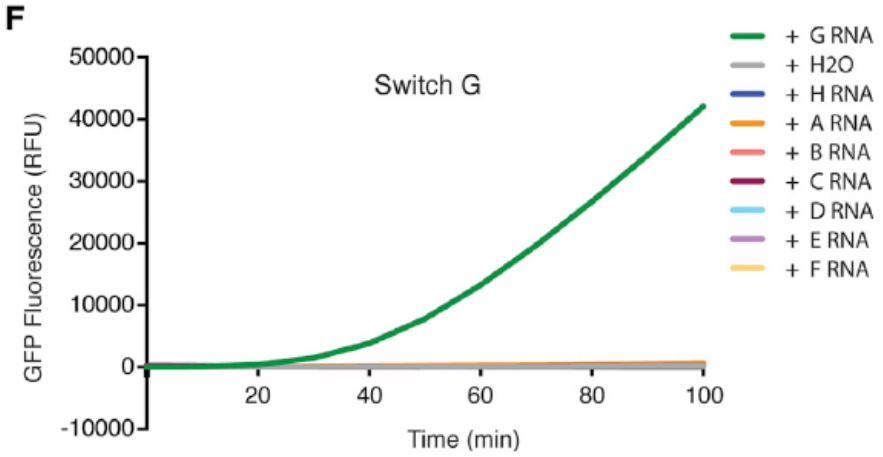






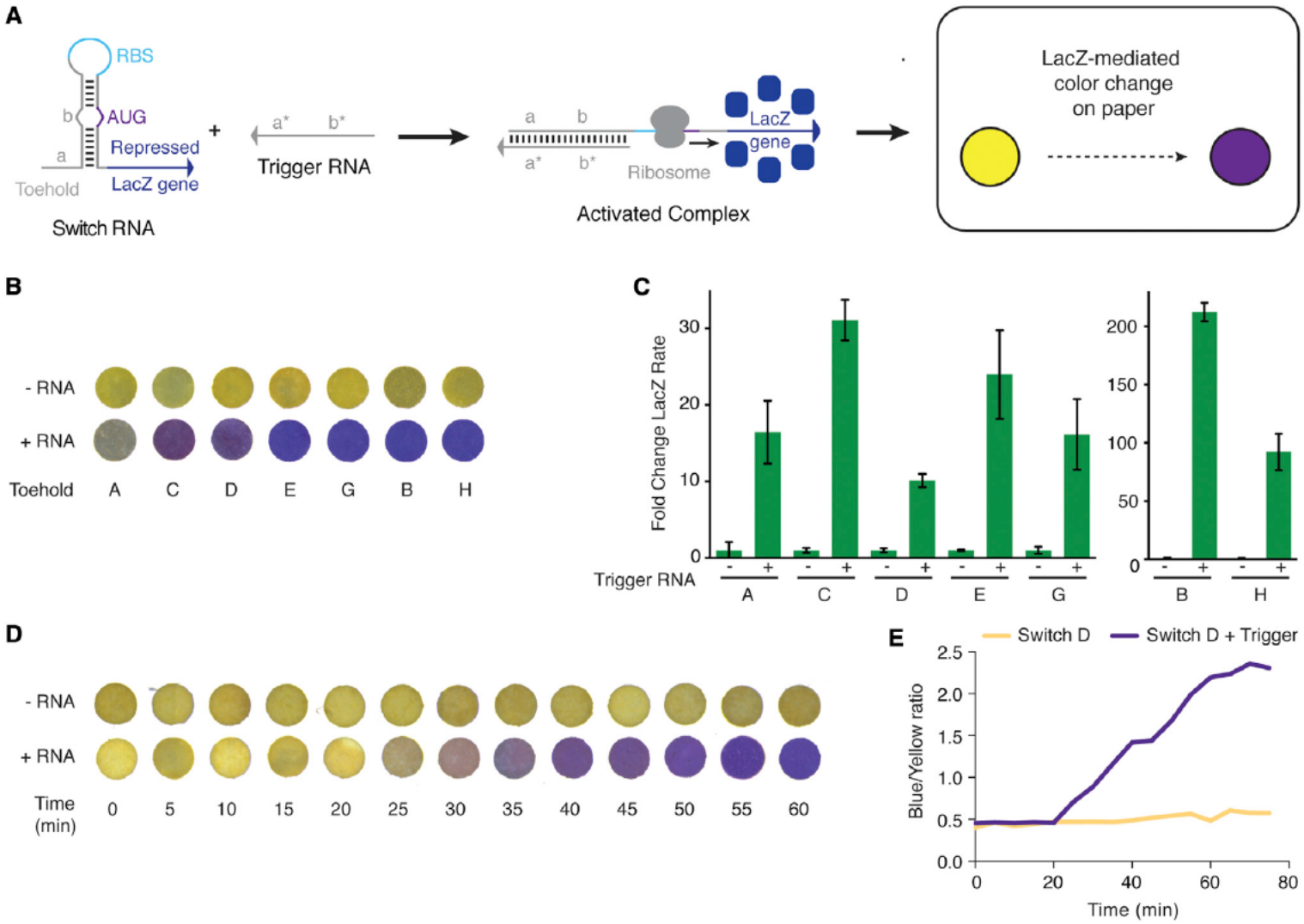
Orthogonality of toehold switches on cellulose matrix paper





LacZ expression on cellulose matrix paper

_visible by naked eyes

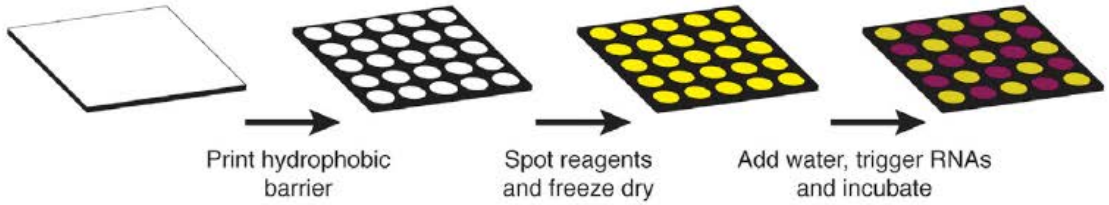


LacZ expression on cellulose matrix paper

_low cost and ease to manufacture

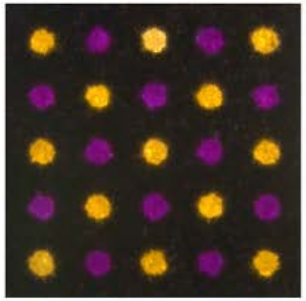
Standard computer printer+ chromatography paper + wax-based ink
Electronic optic reader

F



G

toehold switch E



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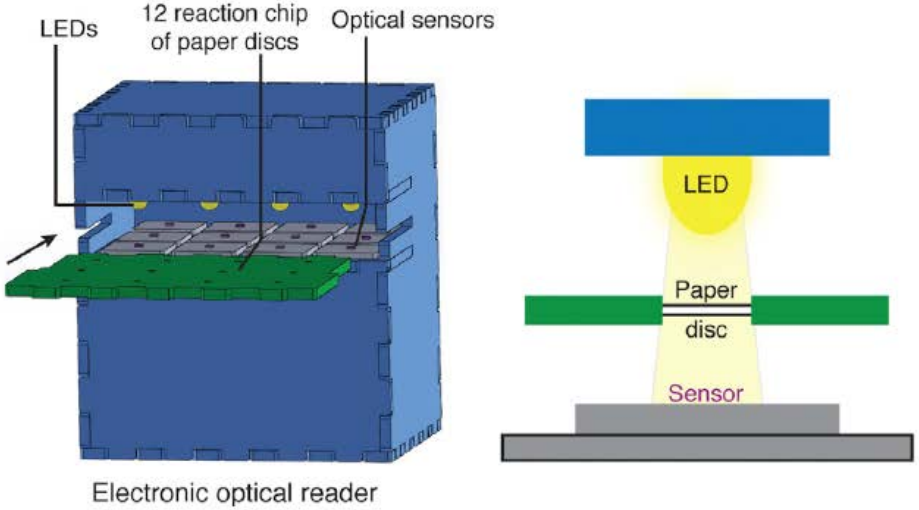
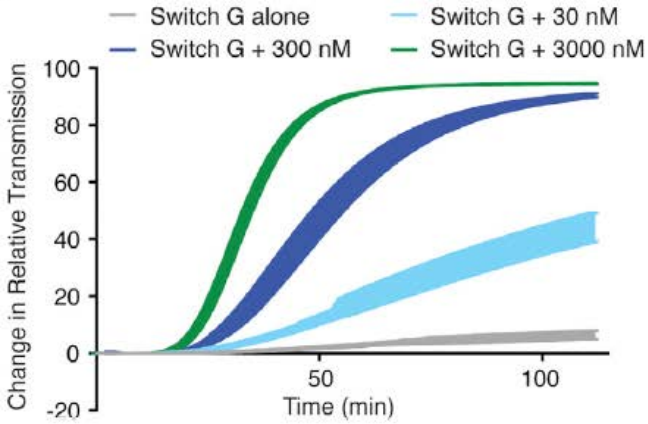
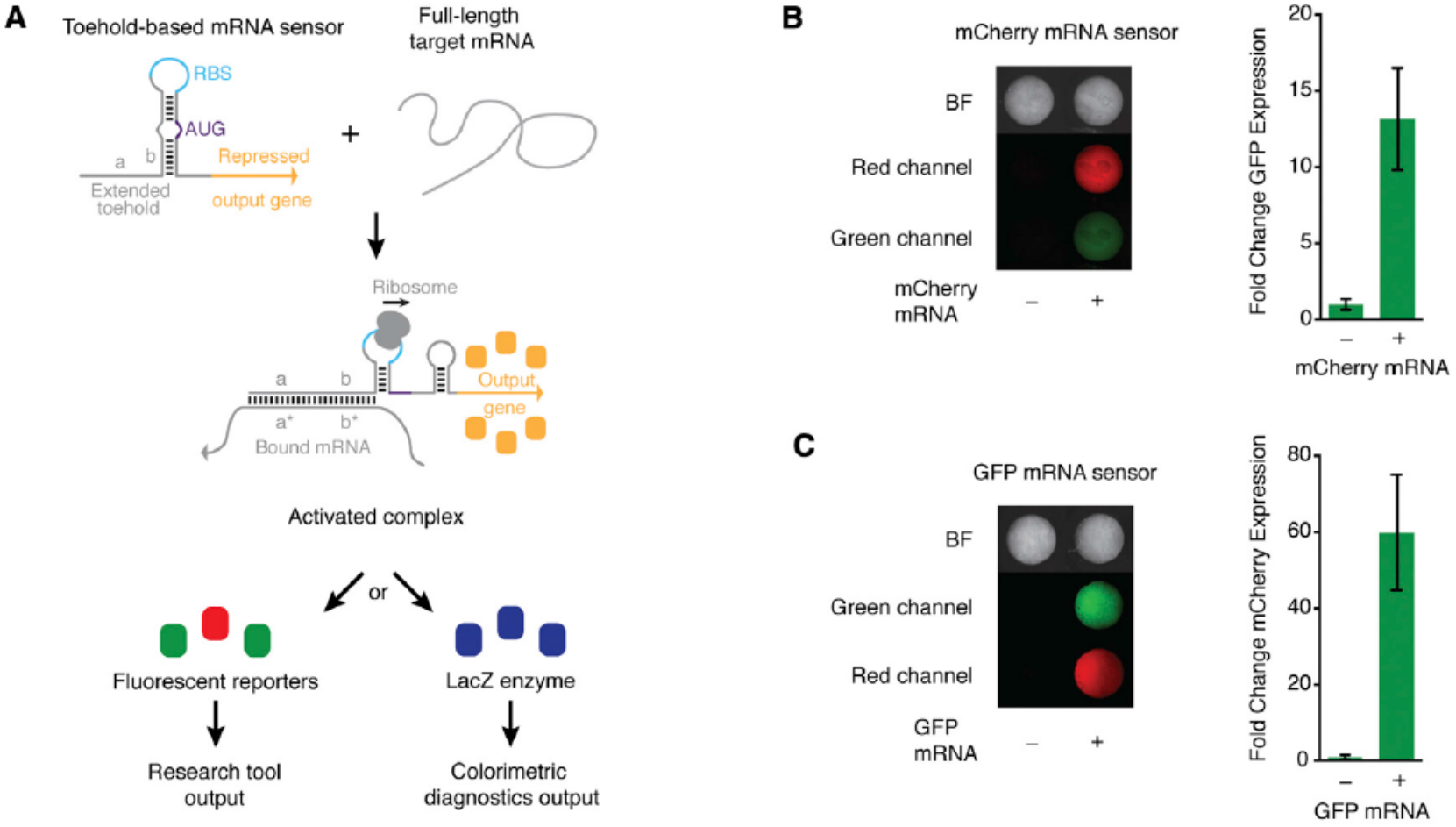


I

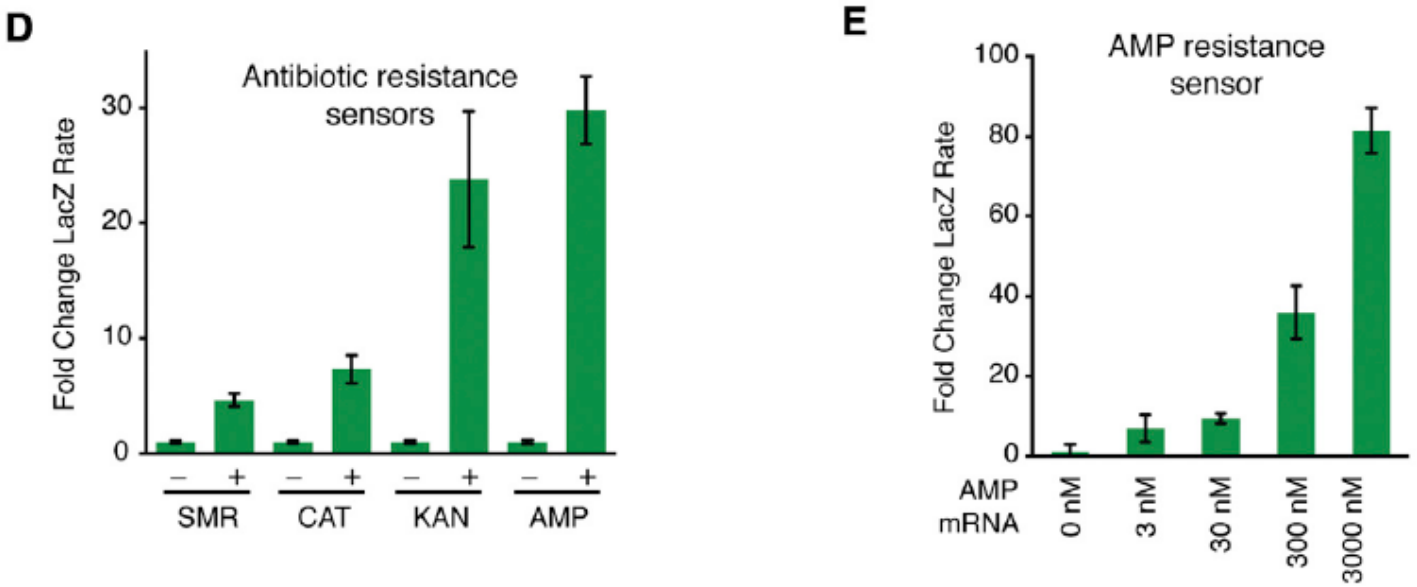


Diagnostics by toehold switches on paper



Diagnostics by toehold switches on paper

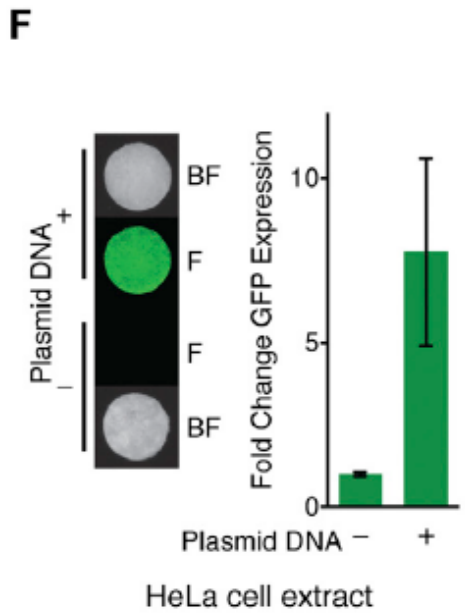
_antibiotic resistance genes



Purified mRNAs

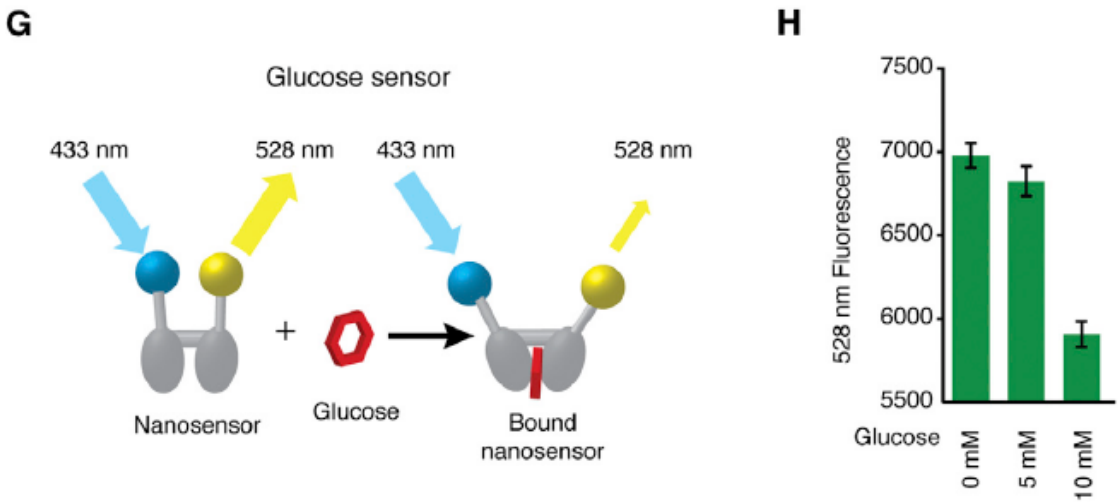
Diagnostics by toehold switches on paper

_Mammalian cells based system



Diagnostics by FRET on paper

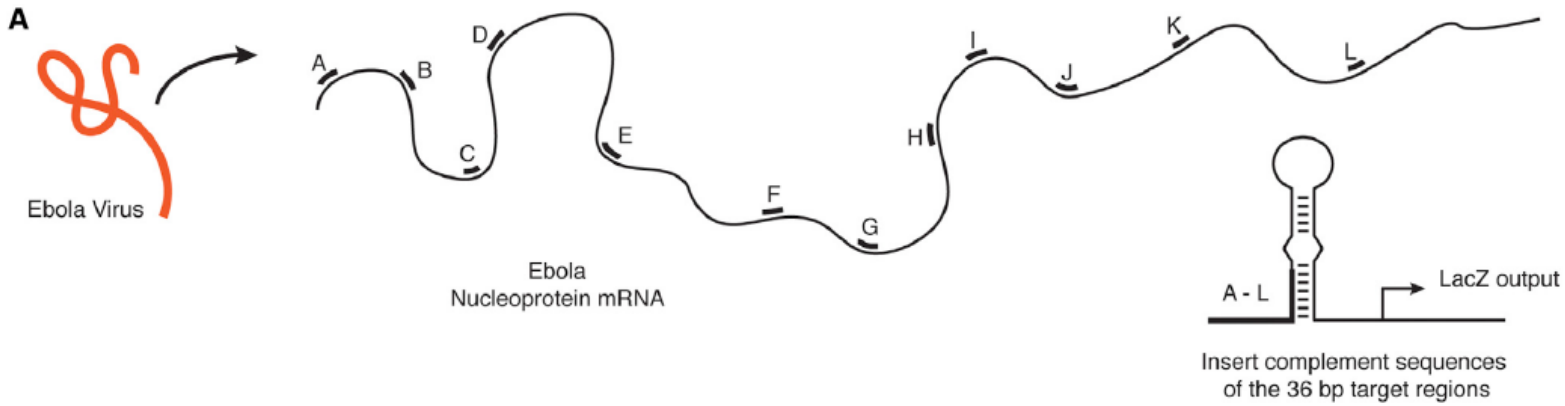
_Glucose measurement

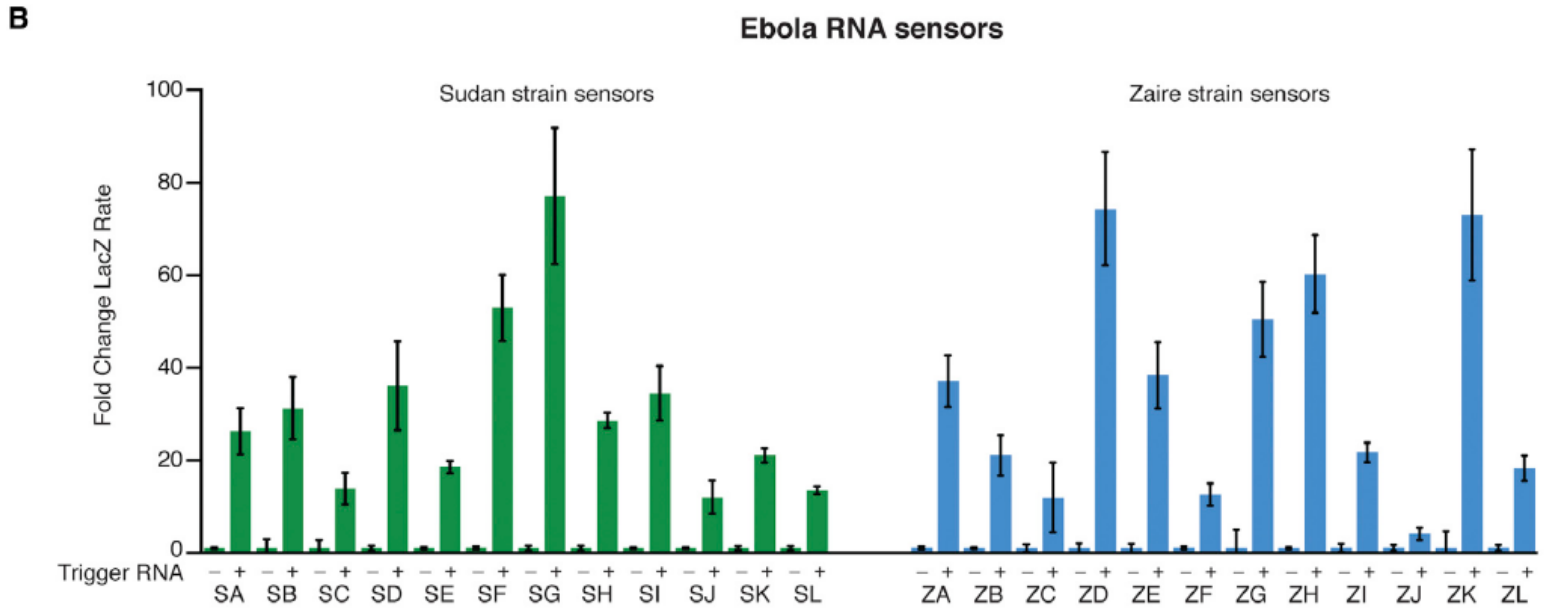


- FLIPglu-30μΔ13V: express CFP, Venus, and periplasmic glucose binding
- Reaction in freeze-dried Hela cell extract on paper

Rapid sensor prototyping

_strain typing for Ebola

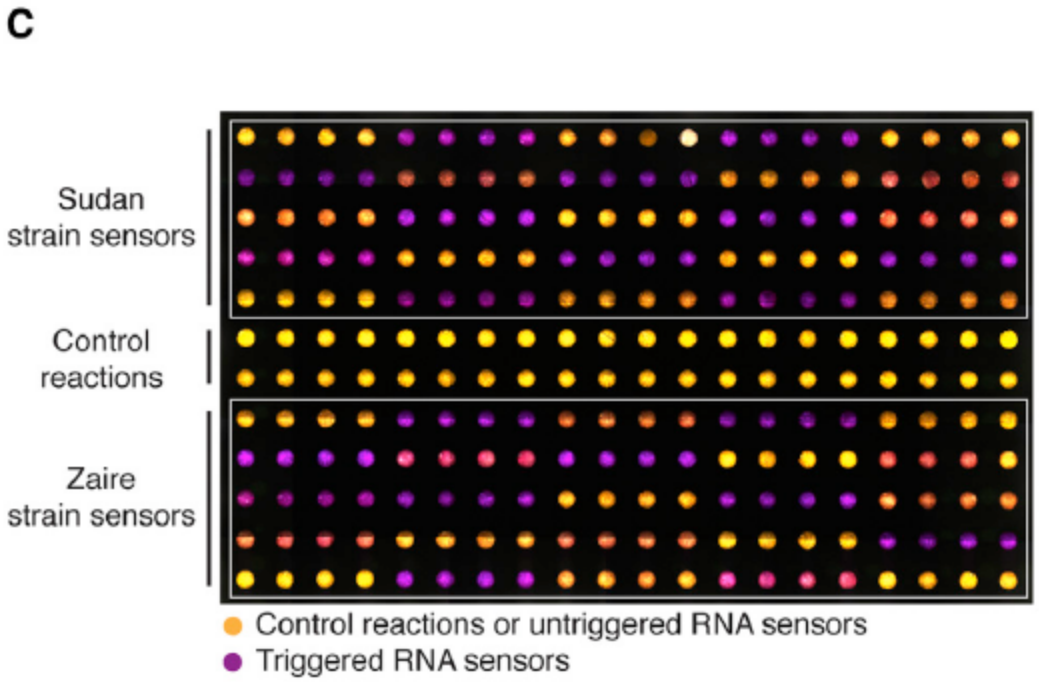


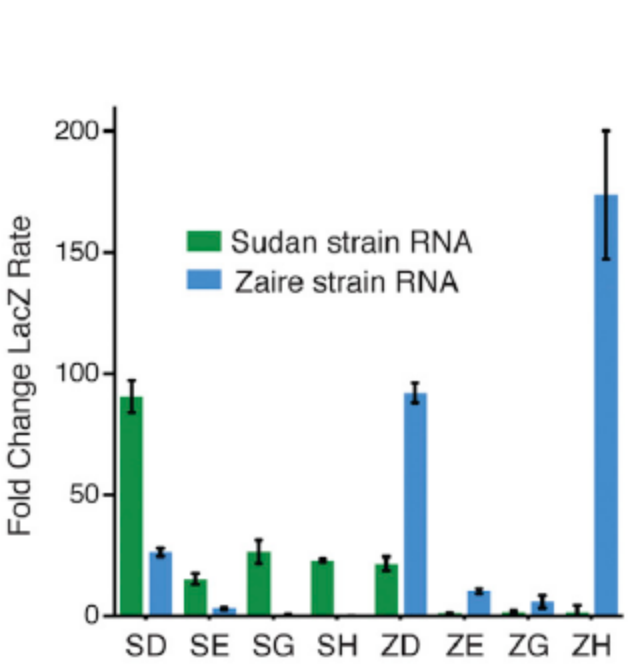


24 sensors

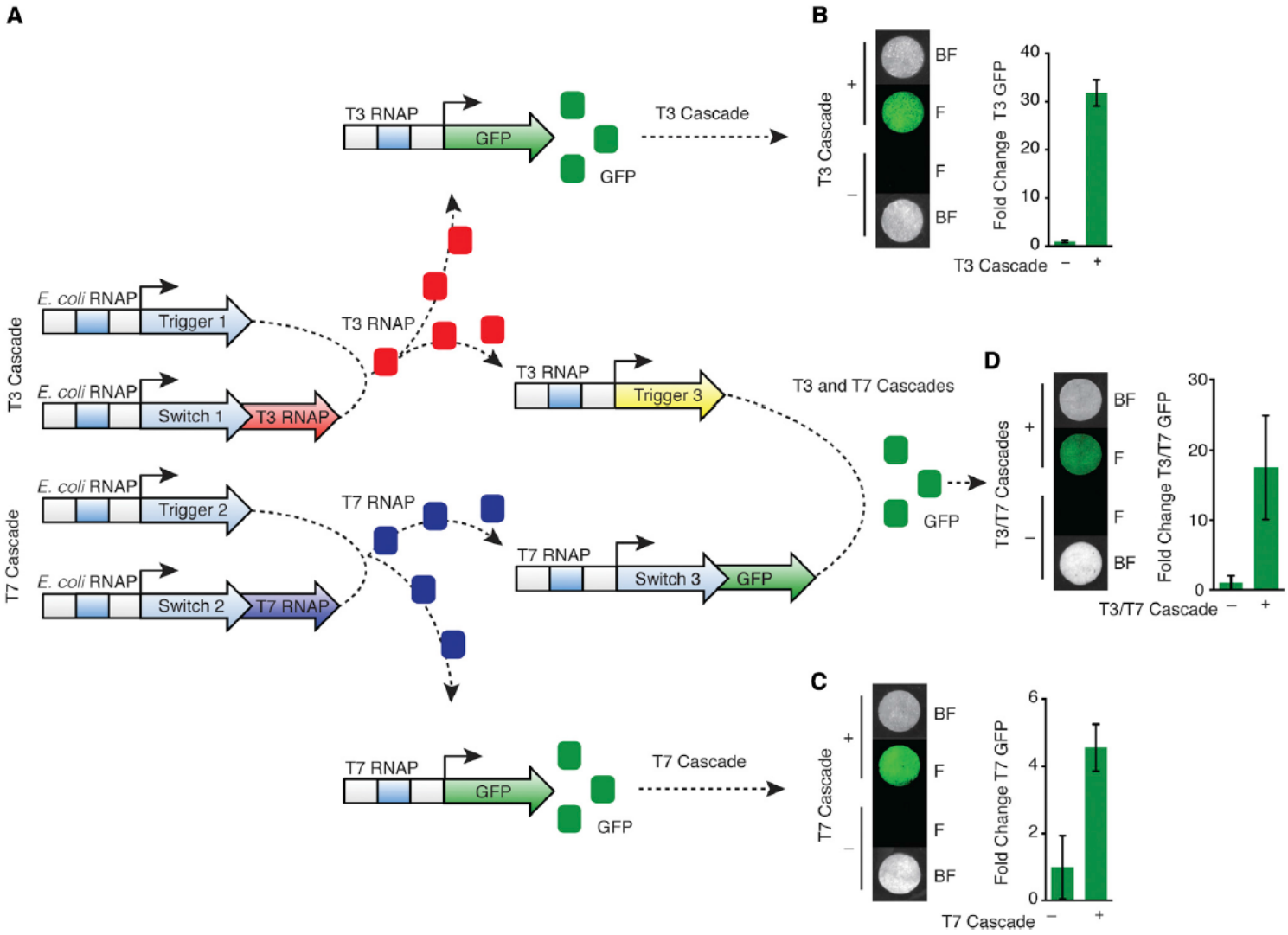
Rapid sensor prototyping

_strain typing for Ebola (<12 h)





Complex synthetic gene networks on paper



Summary

Synthetic gene networks on paper or other porous materials

Integrated toehold switches or FRET into paper

Sensitivity: nanomolar, picomolar

Synthetic gene networks on paper has potential to translate existing constructs designed for basic research and biotechnology into portable and readily accessible molecule tools

Synthetic biology has been predicted to heal us, feed us and fuel us.

-David Willetts

Thank you!