A programmable NOR-based device for transcription profile analyses

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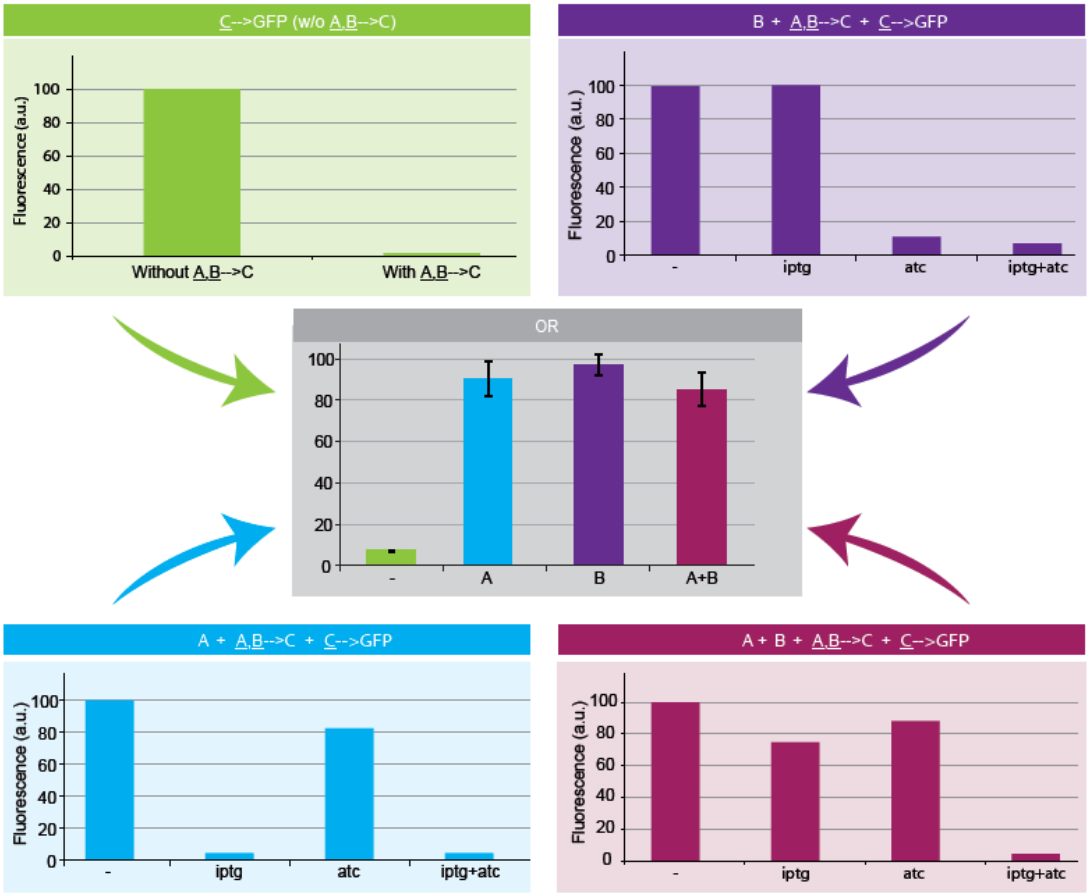
Supplementary Information

1. **Supplementary Figure S1.**
2. **Supplementary Figure S2.**
3. **Supplementary Figure S3.**
4. **Supplementary Figure S4.**
5. **Supplementary Table 1.**
6. **Supplementary Table 2.**



Supplementary Figure S1. OR-gate's control experiments.

Upper left panel, λ-Operator & λ-Repressor. Bacterial strain which normally expresses GFP under the control of a promoter harboring the λ-Operator (potentially repressible by the λ-Repressor). Left and Right bar – The depicted strain was either transformed or not with a plasmid carrying a promoter incorporating the Lac Operator and the Tet Operator controlling the expression of the λ-Repressor. The promoter is repressible by either LacI or TetR, which are not expressed by the host strain. As can be seen by the Right bar, the fluorescence was quenched only when the plasmid expressing the λ-Repressor was present, attesting that the plasmid indeed expresses a functional λ-Repressor.

Lower left panel, LacI expressing bacteria. To counteract LacI and check for potential unwanted cross-talks, four different conditions were tested – by adding to the medium nothing, IPTG, aTc or IPTG and aTc (IPTG should counteract LacI solely, while aTc should counteract TetR solely). As can be seen, only when LacI was active, *i.e.*, no IPTG was present in the medium, LacI repressed the expression of the λ- Repressor which in turn did not repress its downstream promoter which in turn expressed GFP (first and third bars).

Upper right panel, TetR expressing bacteria. To counteract TetR and check for potential unwanted cross-talks, four different conditions were tested – by adding to the medium nothing, IPTG, aTc or IPTG and aTc (IPTG should counteract LacI solely, while aTc should counteract TetR solely). As can be seen, only when TetR was active, *i.e.*, no aTc was present in the medium, TetR repressed the expression of the λ-Repressor which in turn did not repress its downstream promoter which in turn expressed GFP (first and second bars).

Lower right panel, LacI & TetR expressing bacteria. To counteract LacI and/or TetR, four different conditions were tested – by adding to the medium nothing, IPTG, aTc or IPTG and aTc (IPTG should counteract LacI solely, while aTc should counteract TetR solely). As can be seen, only when LacI and TetR were counteracted by the addition of both IPTG and aTc, neither transcription factor repressed the expression of the λ-Repressor which in turn repressed its downstream promoter which in turn expressed GFP (fourth bar).

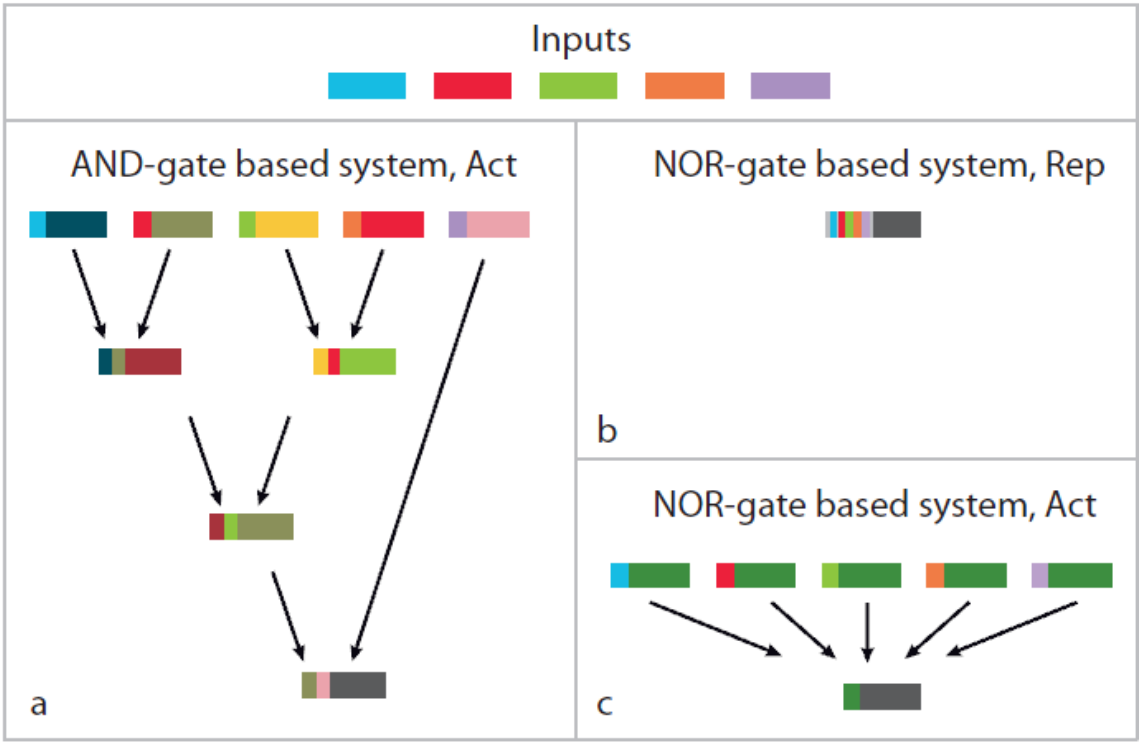
Supplementary Figure S2. AND-gate's control experiments and system's kinetics.

Upper left panel, LacI expressing bacteria. To counteract LacI and check for potential unwanted cross-talks, four different conditions were tested – by adding to the medium nothing, IPTG, aTc or IPTG and aTc (IPTG should counteract LacI solely, while aTc should counteract TetR solely). As can be seen, only when LacI was active, *i.e.*, no IPTG was present in the medium, LacI repressed the expression of the λ-Repressor which in turn did not repress its downstream promoter which in turn expressed GFP (first and third bars). The fifth condition tested the addition of the plasmid carrying a promoter incorporating the Tet Operator controlling the expression of the λ-Repressor. Addition of the plasmid should result the GFP's quenching – given that the strain does not express TetR, λ-Repressor's expression is enabled. λ-Repressor will in turn repress the expression of the GFP.

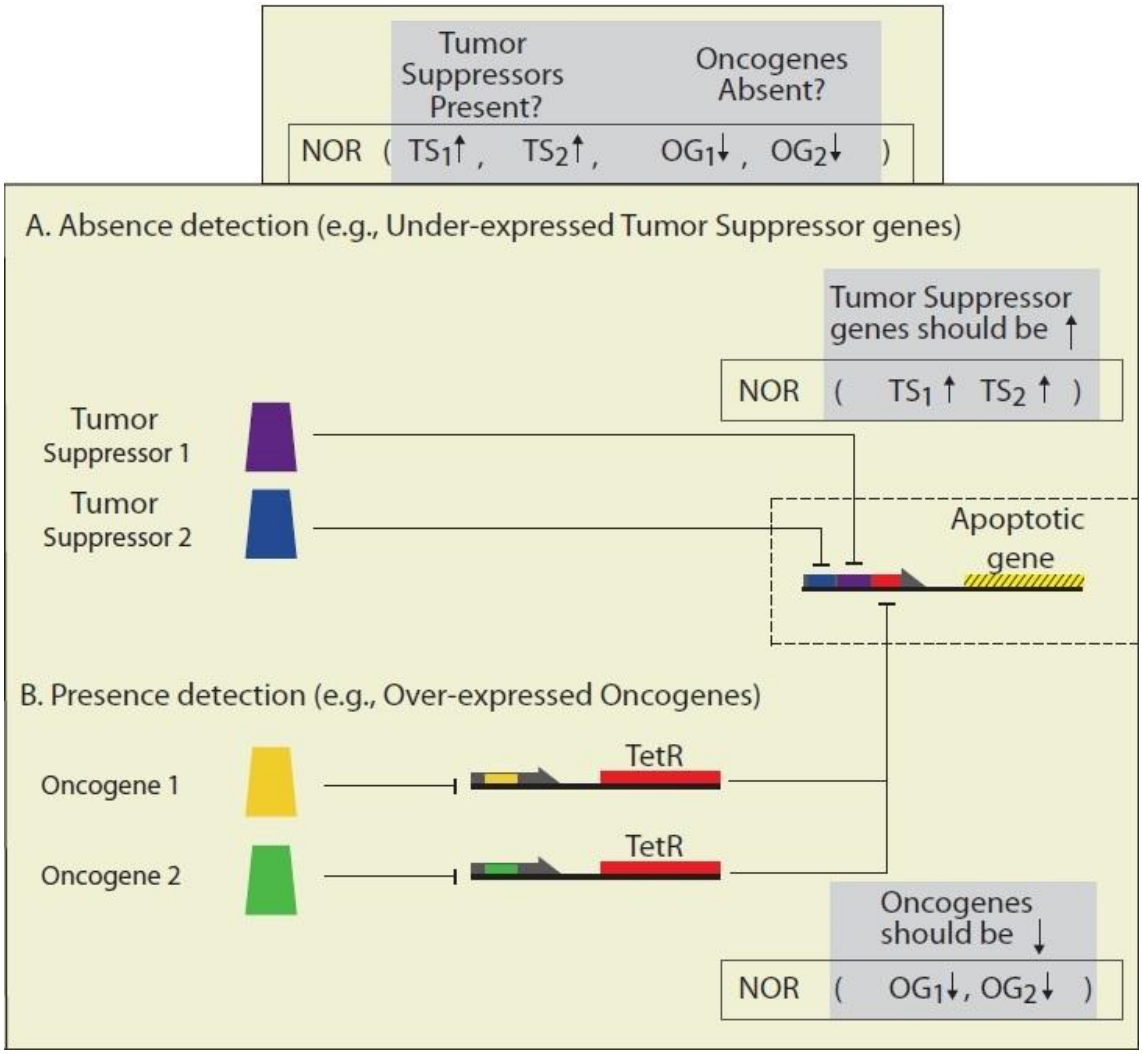
Upper right panel, TetR expressing bacteria. To counteract TetR and check for potential unwanted cross-talks, four different condition were tested – by adding to the medium nothing, IPTG, aTc or IPTG and aTc (IPTG should counteract LacI solely, while

aTc should counteract TetR solely). As can be seen, only when TetR was active, *i.e.*, no aTc was present in the medium, TetR repressed the expression of the λ-Repressor which in turn did not repress its downstream promoter which in turn expressed GFP (first and second bars). The fifth condition tested the addition of the plasmid carrying a promoter incorporating the Lac Operator controlling the expression of the λ-Repressor. Addition of the plasmid should result the GFP's quenching – given that the strain does not express LacI, λ-Repressor's expression is enabled. λ-Repressor will in turn repress the expression of the GFP.

Lower left & right panels, LacI & TetR expressing bacteria. Left panel. To counteract LacI and/or TetR, four different conditions were tested – by adding to the medium nothing, IPTG, aTc or IPTG and aTc (IPTG should counteract LacI solely, while aTc should counteract TetR solely). As can be seen, when either LacI or TetR, or both were counteracted by either the addition of IPTG and/or aTc, λ- Repressor was expressed which in turn repressed its downstream promoter controlling the expression of the GFP (first, second and third bars). **Right panel.** Kinetic results.



Supplementary Figure S3. Scalability of a NOR-gate VS. AND-gate based systems. Corresponding colors represent a TF with its corresponding promoter **a)** AND-gate based systems, such as systems based on the two-hybrid system[12] (which use activator TFs), require co-interaction of pairs of inputs through iterative sub-computations. **b)** In a NOR-gate based system, following a design such as the one we have suggested, inputs directly control the output. The addition of new inputs does not require new intermediate proteins or interactions, enabling simple scalability. c) AND-gate based systems accept as input the activating TFs controlling the expression of the analyzed proteins. Panel c, depicts a NOR-based design that by utilizing a signal inverter, is equivalent to the design depicted in panel a. in terms of the input it accepts and the corresponding output. In both designs, if and only if all activating TFs are present, the final output will be expressed, only that the NOR-gate design spares the co-interactions required by the AND-gate's design.



Supplementary Figure S4. Expression based selective induction of apoptosis. High levels of tumor suppressors and low levels of oncogenes are characteristic of healthy normal cells. In the NOR-based circuit presented, it suffices for one tumor suppressor or oncogenes to be normally expressed to suppress the suicide gene. In other words, for the apoptosis inducing gene to be expressed, all tumor suppressors should be suppressed and all oncogenes over-expressed.

Origin of replication	Resistance marker	Promoter	Expression unit	Designation of vector (example)
colE1 (E)	Ampicillin (1)	$P_{LtetO-1}$ (1)	MCS	pZE11MCS
p15A (A)	Kanamycin (2)	$P_{LlacO-1}$ (2)	luc	pZA22luc
pSC101 (S)	Chloramphenicol (3)	$P_{A1lacO-1}$ (3)	MCS	pZS33MCS
pSC101* (S*)	Spectinomycin (4)	$P_{lac/ara-1}$ (4)	luc	pZS*44luc
	Tetracycline (5)	$P_{N25tetO-1}$ (5)	luc	pZE55luc

Supplementary Table 1. pZ Expression System and its nomenclature.

Name	Plasmid original name	restricted out	Insert	Resistance	Promoter	Protein
A→O	pZS12-GFP (supplied generously by Uri Alon)			Ampicillin	$P_{LlacO-1}$ (Lac Operator)	GFP
B→O	pZE21-GFP (supplied generously by Uri Alon)			Kanamycin	$P_{LtetO-1}$ (Tet Operator)	GFP
A B→O	pZE21-GFP	Promoter restricted out	Synthetic insert TetO -35, LacO, -10, TetO sequences	Kanamycin	$P_{LtetO-1}$ (Tet Operator)+ $P_{LlacO-1}$ (Lac Operator) + $P_{LtetO-1}$ (Tet Operator)	GFP
C→O				chloramphenicol	pR (LambdaPromoter)	GFP

A→C	pZE12-cI (supplied generously by Michael Elowitz)			Ampicillin	$P_{LlacO-1}$ (Lac Operator)	Lambda Repressor-cI
B→C	pZS21-cI (supplied generously by Michael Elowitz)			Kanamycin	$P_{LtetO-1}$ (Tet Operator)	Lambda Repressor-cI
A B→C	A B→O	GFP restricted out	Lambda Repressor-cI PCRed from pZE12-cI	Kanamycin	$P_{LtetO-1}$ (Tet Operator)+ $P_{LlacO-1}$ (Lac Operator) + $P_{LtetO-1}$ (Tet Operator)	Lambda Repressor-cI

Supplementary Table 2. Plasmids used in our paper and their nomenclature.