



## Supplementary Materials for **Amplifying Genetic Logic Gates**

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**Other Supplementary Material for this manuscript includes the following:**  
available at [www.sciencemag.org/cgi/content/full/science.1232758/DC1](http://www.sciencemag.org/cgi/content/full/science.1232758/DC1)

Movie S1

## Materials and Methods

### 1. Molecular biology

Coding sequences for Bxb1 and TP901 integrases were synthesized by DNA 2.0 (Menlo Park, CA, USA). Logic elements were synthesized by IDT (Carlsbad, USA). Plasmids and parts encoding pBAD/AraC ((30), iGEM registry accession number: BBa\_I0500), superfolder GFP ((31), iGEM registry accession number: BBa\_I746916) and pTetO promoter ((32), iGEM registry accession number: Bba\_R0040), and terminators B0015 (iGEM registry accession number: B0015) and J61048 (iGEM registry accession number: J61048) were obtained from the iGEM Registry of Standard Biological Parts (<http://partsregistry.org>).

We chose terminator B0015 as it has already been measured as (i) having a strong forward termination efficiency (T.E. ~98%) and (ii) having asymmetric termination efficiencies between its forward and its reverse orientations (reported reverse T.E. between ~30% and ~60%). No reverse efficiency information was available for J61048 but it was measured as having a strong forward T.E. (~98%) and our experiments show that it is an asymmetric terminator; see [http://partsregistry.org/Part:BBa\\_B0015](http://partsregistry.org/Part:BBa_B0015) and [http://partsregistry.org/Part:BBa\\_J61048](http://partsregistry.org/Part:BBa_J61048)) for more information. Gates were cloned within a pSB4A5 low copy plasmid ((33) pSC101 origin, 5 to 10 copies, iGEM registry accession number: pSB4A5), the low copy phagemid pWSK29 ((34), kindly provided by Prof. Sydney A. Kurshner, University of Georgia, USA, GenBank accession number: AF016889.1), or the CRIM integration vector (35).

All cloning PCR reactions were performed using the platinum Hi-Fi PCR supermix (Invitrogen, USA), using a 1 min. extension time per kilobase. Primers were purchased from IDT. All DNA assembly reactions were performed via Gibson one step isothermal assembly (36). Plasmid maps are shown in Appendix 3. Primers sequences are given in Appendix 4. DNA sequences have been deposited in Genbank (accession numbers: KC529324-KC529332).

### **1.1. Dual controller plasmid**

The RAD module G8-C1 ((5), J64100 plasmid, ColE1 origin of replication, 50-70 copies, chloramphenicol resistance) containing the Bxb1 integrase under the control of pTET and Bxb1 RDF plus integrase under the control of pBAD was PCR amplified with primers JB424-425, removing the Bxb1 RDF/integrase cassette. The TP901 integrase was PCR amplified using primers JB422-423. This PCR product was ligated downstream of pBAD via Gibson assembly to generate the dual controller plasmid and sequence verified.

### **1.2. Gate plasmids**

#### *XOR*

The pSB4A5 backbone containing the RBS-GFP-terminator cassette was PCR amplified using primers JB431 and JB434 to generate an open backbone with a 3' end BioBrick prefix on a 5' end GFP cassette. The XOR gate was amplified by PCR using primers G1004 (fwd BioBrick prefix) and JB435. XOR was ligated by Gibson assembly between the BioBrick prefix and the GFP cassette to generate pSB4A5\_XOR\_GFP.

#### *AND*

To generate pSB4A5\_AND\_GFP, the pSB4A5\_XOR\_GFP backbone was PCR amplified using primers JB459 and JB460 to remove the XOR logic register while conserving the input promoter, part of the TP901 recombination site, and the GFP cassette. The AND gate was PCR amplified with primers JB457 and JB458 and the two fragments were assembled and sequence verified.

#### *NAND*

To generate pSB4A5\_NAND\_GFP, the pSB4A5\_XOR\_GFP backbone was PCR amplified using primers JB470 and JB468 to remove the XOR logic register while conserving the input promoter, part of the TP901 recombination site, and the GFP cassette. The NAND gate was PCR amplified with primers JB457 and JB458 and the two fragments were assembled and sequence verified.

#### *OR*

For building pSB4A5\_OR\_GFP, the pSB4A5\_AND\_GFP backbone was PCR amplified using primers JB470 and JB 468 to remove the AND logic register while conserving the input promoter, part of the TP901 recombination site, and the GFP cassette. The OR gate was PCR amplified with primers JB466 and JB473 and the two fragments were assembled and sequence verified.

### *NOR*

For building pSB4A5\_NOR\_GFP, the pSB4A5\_AND\_GFP backbone was PCR amplified using primers JB469 and JB 468 to remove the AND logic register while conserving the input promoter, part of the TP901 recombination site, and the GFP cassette. The NOR gate was PCR amplified with primers JB466 and JB483 and the two fragments were assembled and sequence verified.

### *XNOR*

For building pSB4A5\_XNOR\_GFP, the pSB4A5\_XOR\_GFP backbone was PCR amplified using primers JB470 and JB468 to remove the XOR logic register while conserving the input promoter, part of the TP901 recombination site, and the GFP cassette. The XNOR gate was PCR amplified with primers JB472 and JB473 and the two fragments were assembled and sequence verified.

*pIT3\_Phi21\_AND\_GFP, piT3\_Phi21\_AND\_GFP (plasmid for chromosomal integration).*

The pIT3\_phi21\_Kan vector was PCR amplified using primers JB518 and JB519. pSB4A5\_AND\_GFP and pSB4A5\_XNOR\_GFP were PCR amplified using primers JB520 and JB521, and the two fragments were assembled and sequence verified.

*pWSK29mod\_AND (plasmid for M13 packaging).*

To clone the gates into the phagemid pWSK29 (34), the phagemid backbone was amplified by PCR using primers JB551 and JB552, eliminating the lacZalpha fragment and adding the BioBrick prefix and suffix in a single PCR reaction. The input promoter, logic element, and GFP sequences from pSB4A5\_AND\_GFP were amplified in a second PCR reaction using primers JB549 and JB550. The two DNA strands were ligated together via Gibson assembly and sequence verified.

## **1.3. Measurement plasmids**

### *Dual controller pTET\_GFP*

The dual controller plasmid was PCR amplified with primers JB510 and JB511, removing the Bxb1 integrase gene and its RBS. The GFP cassette (RBS plus GFP) was PCR amplified from pSB4A5\_AND\_GFP by using primers JB508 and JB509. This PCR product was ligated downstream of pTET in the above vector via Gibson assembly to generate a dual controller measurement plasmid in which Bxb1 is replaced by the GFP cassette used in the logic gates.

### *Dual controller pBAD\_GFP*

The dual controller plasmid was PCR amplified with primers JB514 and JB515, removing the TP901 integrase gene and its RBS. The GFP cassette was PCR amplified from pSB4A5\_AND\_GFP by using primers JB512 and JB513. This PCR product was ligated downstream of pTET in the above vector via Gibson assembly to generate a dual controller measurement plasmid in which TP901 is replaced by the GFP cassette used in the logic gates.

## **2. Cell Culture, data collection, and analysis.**

Plasmids were transformed via heat-shock in chemically competent *E. coli* DH5alphaZ1 (32) and plated on LB agar plates containing the appropriate antibiotics. For main Figures 2-4 the controller plasmid was co-transformed with the pSB4A5\_Gate plasmid. For the sequential input logic experiment, the controller plasmid was transformed in cells containing a Phi21 chromosomally integrated logic gate and harboring a kanamycin resistance cassette. Antibiotics were used at the following concentrations: carbenicillin (25ug/ml), kanamycin (30ug/ml) and chloramphenicol (25ug/ml) (all from Sigma). Cells containing chromosomally integrated gates were grown with 5ug/ml of kanamycin.

For each experiment, and unless otherwise stated, after overnight incubation of the plate, three colonies were separately inoculated in Azure Hi-Def media (Teknova, Hollister, USA) with glycerol (0.4%, from Fisher Scientific) added as a carbon source and appropriate antibiotics and grown for approximatively 18 hours to obtain starter cultures. L-arabinose (ara) was obtained from Calbiochem. Anhydrotetracycline (aTc) was obtained from Sigma.

### **2.1. Buffer gate operation**

Bxb1 integrase and excisionase were controlled via aTc and arabinose inducible promoters, respectively, on pSB3k1 plasmid (p15A origin; 15-20 copies). DNA data register was on pSB4A5 plasmid ((32), pSC101 origin; 5 copies; Genbank:JQ929581). The experiment was performed in *E. coli* DH5alpha (tetR-) cultured at 37C in supplemented M9 medium [M9 salt (Sigma), 1 mM thiamine hydrochloride(Sigma), 0.2% casamino acid (Across Organics), 0.1 M MgSO4 (EMD reagents), 0.5 M CaCl2 (Sigma) with glycerol (0.4%, Fisher Scientific) added as a carbon sources], 25 ug/ml carbenicillin and 50 ug/ml kanamycin. L-arabinose was used at a final concentration 0.1% w/v concentration. For each duty cycle, cultures were diluted 1:1000 in media with inducer, grown overnight, then diluted 1:1000 in media without inducer and grown over night. Results are presented in figure S3.

## ***2.2. Dual control signal transfer functions.***

To generate the dual control signal transfer functions presented in Figure 2, cells from the overnight starter culture were diluted 1:5000, induced with different concentrations and combinations of inducers, and grown in Azure Hi-Def media for 18H at 37C. Just before measurement, the resulting saturated cultures were diluted 1:20 in PBS. Samples were then analyzed on a Wallac Victor3 multi-well fluorimeter (Perkin Elmer). Absorbance at 600 and (AB600) and GFP intensity were measured (600 nm absorbance filter, 0.1 second counting time for AB; 485 nm excitation filter, 525 nm emission filter for GFP). Media background was subtracted from AB600 and GFP values, and AB600 values obtained from the plate reader were converted to optical density (OD) by using the equation :  $OD_{600} = ((AB_{600} - 0.06) * 3.11) - 0.0158$ ; obtained from calibration (see also: [http://openwetware.org/wiki/Endy:Victor3\\_absorbance\\_labels](http://openwetware.org/wiki/Endy:Victor3_absorbance_labels) for more details). Average OD600 in the measured samples were between ~0.2-0.3, and we did not observe correlations between OD and GFP levels. For each well, the GFP value was divided by the OD600 value to correct for differences in cell density. GFP/OD were plotted using the MATLAB imagesc function. Numerical values and standard deviations for OD and GFP measurements are available in appendices 1 and 2 of the SOM.

## ***2.3. Flow cytometry***

Flow cytometry acquisition was performed at the Stanford Shared FACS Facility (SSFF) using a BD-Bioscience-LSR II cytometer (BD-Bioscience, San Jose, CA) coupled with a high-throughput sampler. 30,000 cells were collected for each data point. All acquisitions were performed using the same machine and settings. Just before measurement, cultures were diluted 1:100 in ice cold PBS. Flow cytometry data were analyzed using the FlowJo software (Treestar inc., Ashland, USA). Figure S5 details the different analyses that were performed.

For single cell measurements in Figure 2, arabinose was used at a final 1% w/v concentration and aTc was used at a final concentration of 200 ng/ml. Measurements of the percentage of cells flipping were performed using a common fluorescence intensity threshold for all gates as represented by the red line.

#### ***2.4. Transfer functions and switching range in response to individual controllers.***

Transfer functions for individual integrases were measured using cells containing the dual controller plasmid and a Bxb1 or a TP901 BP register encoded on pSB4A5 ((5) and plasmid map K).

For experiments presented in Figures 3 and 4, cells from the starter cultures were diluted 1:5000 in different inducers concentrations and grown for 20 hours at 30C. For AND and NAND gates, measurement with one inducer were done in the presence of saturating concentration of the other. For each experimental replicate, the measurement plasmids pTET-GFP and pBAD-GFP were run in parallel.

For Figure 4, gate-specific thresholds were defined for each logic function. Histograms displaying fluorescence intensities of GFP were generated and the cell population in the ON state was gated. We used the gate specific gating (fig. S7) to quantify the fraction of cells in ON state, from which we determined the gates switching ranges (fig. S8). To quantify fluorescence intensity, we measured the median fluorescence intensity of the whole population using FlowJo.

#### ***2.5. Digitization and digital error rate calculation***

For Figure 3CD, controllers and logic gates were gated manually to minimize the probability of scoring a cell in the OFF state as ON, and a cell in the ON state as OFF. We added the two numbers to obtain a digitization error rate.

#### ***2.6. Fold changes calculations for gates and promoters.***

Whole population median fluorescence intensities for different gates were extracted from flow-cytometry data at various inducer concentrations (fig. S8). For Figure 4, all fluorescence intensity values were normalized for each gate and controllers to equal a value of 1 in the absence of inducer. As such, normalized values correspond to the fold change in fluorescence intensity versus a base fluorescence intensity for each gate or controller. For each experiment the control signal vectors used to quantify controller activity were run in parallel with the gates. Calculations of fold change were made independently for each experiment. Experiments were then averaged, standard deviation calculated, and data plotted using MATLAB (Mathworks, Natic, USA). Results are the average of three independent experiments +/- SD. Non-normalized plots of the GFP values corresponding to the respective gate outputs versus GFP levels corresponding to the control signals are provided in figure S10.

### ***2.5. Single-cell dynamic ranges and fold changes measurements.***

The dynamic ranges of logic gate outputs and of the control signals were measured across a common gate switching range, using the cytometry gating method presented in figure S7. Single cell fluorescence intensity of the OFF state for AND, OR and XOR was determined by measuring the median fluorescence intensity for the fraction of the cell population gated for the OFF state at the *lower* inducer concentration of the switching range (0.2 ng/ml for pTET and 1E-4% for pBAD). Single cell fluorescence intensity of the OFF state for NAND, NOR and XNOR was determined a similar manner but at the *higher* inducer concentration of the switching range (5 ng/ml for pTET and 1E-2% for pBAD). Single cell fluorescence intensity of the ON state for AND, OR and XOR was determined by measuring the median fluorescence intensity for the fraction of cell population gated for the ON state, at the *higher* inducer concentration of the switching range. Single cell fluorescence intensity of the ON state for NAND, NOR and XNOR was determined in a similar manner but at the the *lower* inducer concentration of the switching range. Results are presented in figure S9.

### ***2.6. Sequential logic experiment.***

Logic gates used in the sequential logic experiment were integrated into *E. coli* DH5alphaZ1 chromosome (32) using a modified version of the CRIM system (34), Sherwin, St-Pierre unpublished results), using the phi21 integrase integration sites. Sequential input logic experiments were performed at 37C with 1% w/v arabinose and 200 ng/ml aTc. For storage mode, induced cultures were washed and diluted 1:2000 in media without inducer, in order to achieve about 10 generations per day ( $\log_2 2000 = 10.96$ ). Results are presented in figure S11.

### ***2.7. Cell-cell communication of DNA encoding intermediate logic states***

All experiments for phage-based logic messaging were conducted in LB liquid culture using *E. coli* strain DH5alphaZ1, F<sup>+</sup>. This F<sup>+</sup> strain was created by mating DH5alphaZ1 with XL1-Blue. For all experiments, infected sender cells were prepared by co-transforming chemically-competent cells with M13K07 phagemid in addition to the messaging phagemid, pWSK29mod\_AND. Receiver cells were prepared by transforming chemically-competent cells with the dual-controller plasmid via heat shock. Overnight cultures of sender and receiver cells were diluted 100x into fresh media with appropriate antibiotics (sender cells were grown with 50 µg/mL kanamycin and 50 µg/mL carbenicillin; receiver cells were grown with 10 µg/mL tetracycline and 25 µg/mL chloramphenicol). The diluted cultures were returned to log phase ( $OD_{600} \approx 0.7$ ) by incubation with shaking at 37C. From these log phase cultures we prepared co-cultures containing a 50x dilution of both sender and receiver cells in a total of 5 mL fresh media without added antibiotic. Co-cultures were incubated at 37C with shaking for 5 hours and then diluted 100x into fresh media containing 50 µg/mL carbenicillin and 25 µg/mL chloramphenicol, in one of four induction states: (1) no



inducers; (2) anhydrous tetracycline only; (3) arabinose only; and, (4) both anhydrous tetracycline and arabinose. Inducer levels were set at 0.1% w/v arabinose and 200ng/ml anhydrous tetracycline. These cultures were grown in 96-well plate format at 37C for 16 hours. For each messaging phagemid, three colonies were tested, and inducer states for each colony were tested in triplicate. After incubation, a 200  $\mu$ l aliquot of each culture was transferred into a flat-bottomed 96-well plate and OD<sub>600</sub> and GFP fluorescence of each culture was measured on a Wallac Victor3 multi-well fluorimeter (Perkin Elmer). Results are presented in figure S12.

## **2.8. Measurement of recombination kinetics.**

### *Gate switching kinetics via plate reader measurements*

Three colonies containing the AND or the XOR gate with the dual controller plasmid or containing the pTET or pBAD measurement plasmid only were inoculated in Azure Hi-Def media and grown overnight. On the next day, these starter cultures were diluted 1:100 and grown in Azure Hi-Def media until they reach 0.3 OD. We transferred 200  $\mu$ l from each cultures into a well on a flat-bottomed 96 well plate, with different combinations of inducers: for XOR, (i) no inducers, (ii) arabinose at 0.1% w/v, (iii) aTc at 200ng/ml, and for AND, (i) no inducers, (ii) arabinose plus aTc at the previous concentrations. Measurement plasmids were run in parallel with the same inducer concentrations. We incubated the plate in a Wallac Victor3 multiwell fluorimeter at 37C and assayed the samples with an automatically repeating protocol of absorbance measurements (600 nm absorbance filter, 0.1 second counting time), fluorescence measurements (485 nm excitation filter, 525 nm emission filter, 0.1 second measurement time), and shaking (10 minutes interval between measurements with linear type). Data were plotted using a custom MATLAB script (figure S13).

### *Recombination kinetics measured via flow cytometry.*

Cells were co-transformed with: (i) a pSB4A5-BP\_GFP plasmid (containing a data register with Bxb1 or TP901 BP sites flanking a constitutive promoter and producing GFP upon inversion, plasmid maps L and M) and (ii) pBAD-Bxb1-Set Generator ((5), Genbank:JQ929583) or pBAD-TP901 (same as previous but Bxb1 gene replaced by TP901, plasmid map J). Colonies were inoculated overnight and the day after, starter cultures were diluted in 30ml flasks in supplemented M9 media and grown at 37C. When OD reached 0.4, cells were split in two cultures, one which was induced with 0.1% arabinose, the other being left un-induced. At each time-point, a 1 ml aliquot was taken and immediately fixed by addition of paraformaldehyde (PFA) (Electron Microscopy Sciences #15714-S) to a final concentration of 1% followed by storage at 4C. The non-induced cells were also fixed in a similar manner. Samples were diluted 1:100 in PBS prior to flow cytometry (fig. S13C).

### *Minimum control signal duration experiments*

Starter culture from cells containing pBAD-Bxb1-Set-Generator (JQ929583) and the pSB4A5\_BP register (JQ929581) were diluted in 30ml flasks in supplemented M9 media and grown at 37C. When OD reached 0.4, cells were split into two cultures, one which was induced with 0.1% arabinose, the other left un-induced. Five minutes before each time point, a 1ml aliquot was taken and cells were immediately spun and washed twice with fresh media that did not contain arabinose. Washed cells were diluted 1:1000 in fresh media and grown overnight at 37C. Samples were diluted 1:100 in PBS prior to being analyzed flow cytometry (fig. S13D).

### *Single cell time lapse microscopy*

Cells containing the pBAD-Bxb1-Set-Generator ((5), JQ929583) and the pSB4A5\_BP-register (JQ929581) were grown to exponential phase in supplemented M9 (OD~0.3) and spread on M9/glycerol 2% agarose pads supplemented with 0.5% arabinose to induce expression of the Bxb1 integrase cassette. We immediately incubated the cells in a microscope heating chamber set to 37C, taking phase contrast, GFP and RFP fluorescence images every 5 min at a 100X magnification (see Supplementary Movie 1).

## **3. Phenomenological model of Boolean integrase logic gates**

We fit the response curves of individual DNA registers (with inducer concentration as an input and probability of being in LR state as an output) to Hill functions below (fig. S4).

For the arabinose inducible TP901 DNA register,

$$LR_{TP} = f(ara) = \frac{ara^2}{ara^2 + 0.003^2} \quad (A).$$

For the aTc inducible Bxb1 DNA register,

$$LR_{BX} = g(ATc) = \frac{ATc^2}{ATc^2 + 1.5^2} \quad (B).$$

For all two-input logic gates, we assume no cross talk between Bxb1 and TP901 integrase. Thus, the probability of recombining Bxb1 attB/attP is independent of TP901 integrase inducer level, and vice versa.

An AND gate is in an ON state when both Bxb1 and TP901 DNA register are in an LR state. Under no-cross talk assumption, the probability of having an AND gate in an ON state is thus the product of the probability of having the Bxb1 DNA register in a LR state and the

$$ON_{AND} = AB.$$

probability of having the TP901 DNA register in an LR state, i.e., Similarly, a NOR gate is in an ON state when both Bxb1 and TP901 sites are in BP state. Thus, the probability of having a NOR gate in an ON state is thus the product of the probability of having the Bxb1 latch in a BP state and the probability of having the TP901 DNA register in a BP state, i.e.,

$$ON_{NOR} = (1 - A)(1 - B).$$

An OR gate is in an ON state when either Bxb1 or TP901 DNA registers are in LR state. Thus, the probability of having an OR gate in an ON state is determined by the DNA register, Bxb1 or TP901, that is more likely to be in an LR state, i.e.,

$$ON_{OR} = \max(A, B).$$

Similarly, a NAND gate is in an ON state when either Bxb1 or TP901 sites are in BP state. Thus, the probability of having a NAND gate in an ON state is determined by the DNA register, Bxb1 or TP901, that is more likely to be in a BP state, i.e.,

$$ON_{NAND} = \max((1 - A), (1 - B)).$$

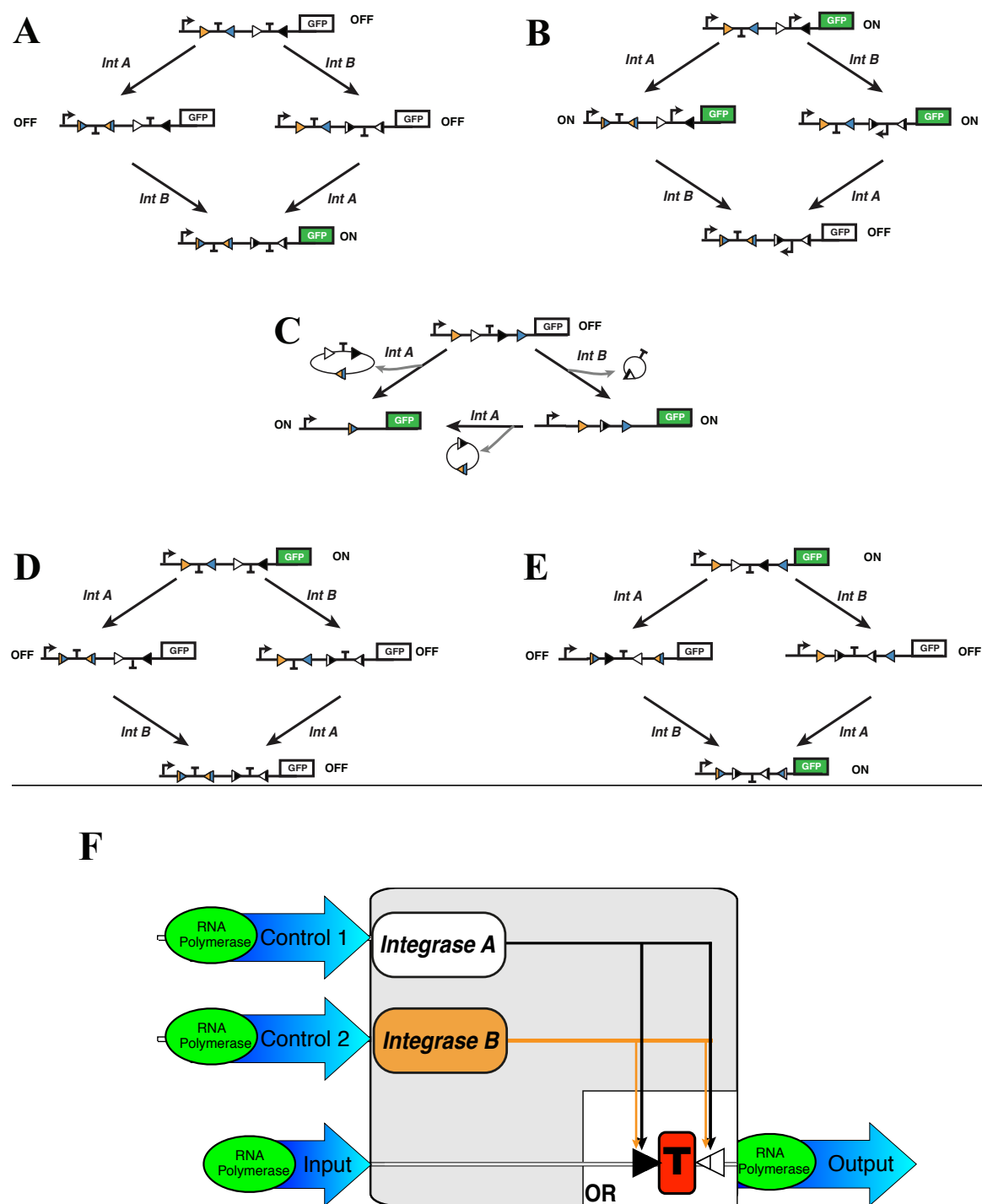
An XOR gate is in an ON state in two possible scenarios. The first scenario is when Bxb1 DNA register is in a BP state and TP901 DNA register is in an LR state. Under non-crosstalk assumption, the probability of this scenario is simply the product of the probability of having a BP state Bxb1 DNA register and the probability of an LR state TP901 DNA register, i.e.,  $A(1-B)$ . The second scenario is when Bxb1 DNA register is in an LR state and that Bxb1 DNA register is in a BP state. Under non-crosstalk assumption, this probability would be  $(1-A)B$ . In total, the probability of being in either scenarios is:

$$ON_{XOR} = \max(A(1 - B), (1 - A)B).$$

Similarly, an XNOR gate is in an ON state in two possible scenarios: when both Bxb1 and TP901 DNA registers are in LR state or when both DNA register are in BP state. Given non-crosstalk assumption, the former has probability  $AB$  while the latter has probability  $(1-A)(1-B)$ . Thus, the probability of having ON XNOR becomes:

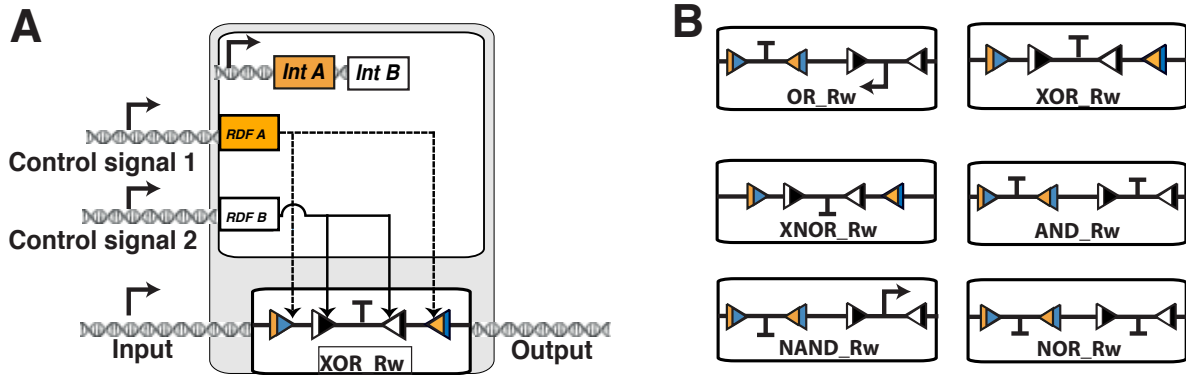
$$ON_{XNOR} = \max(AB, (1 - A)(1 - B)).$$

**fig. S1:**



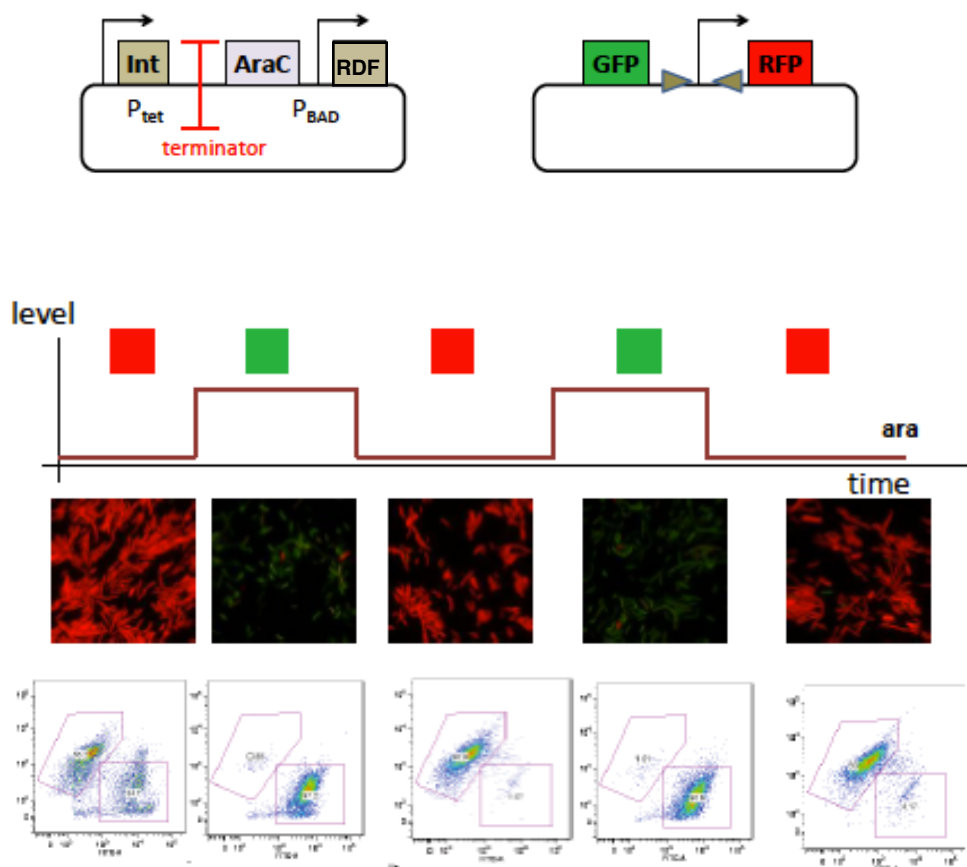
**figure S1: Detailed gates state diagram: (A) AND, (B) NAND, (C) OR, (D) NOR, and (E) XNOR.** Logic gates consist of: (i) a constitutive input promoter (P7-modular promoters library (44)) (ii) a logic element made of a specific arrangement of recombination sites and transcription regulatory sequences controlling the flow of RNA polymerase through the gate. For AND and NOR gates, we used a double terminator (BBa\_B0015) flanked by TP901 target sites (blue and orange) and a Rnp T1 terminator (BBa\_J61048) flanked by Bxb1 target sites (black and white). For NAND gate, B0015 is flanked by TP901 sites and P7 promoter is flanked by Bxb1 sites. For OR, XOR and XNOR, we used the B0015 terminator. (iii) finally downstream of the logic element we cloned superfolder GFP (31)BBa\_I746916) under translational control of a measured strong RBS (JBEI-RBS, Biofab pilot C-dog project <http://biofab.org/data>) to record gate outputs **(F)** An alternative architecture OR gate in which two copies of the same integrase are controlled by two distinct signals.

**fig. S2:**



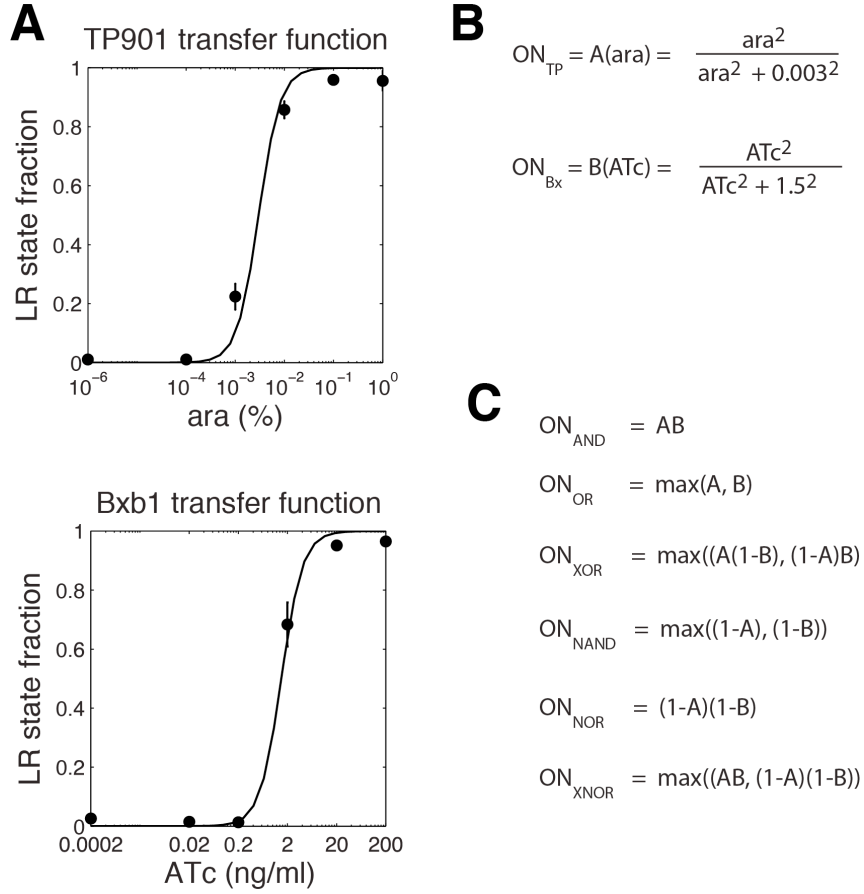
**figure S2: Rewritable Boolean integrase logic gates.** (A) A constitutive promoter expresses both integrases, Int A and Int B. Control signals drive expression of recombination directionality factors (RDFs); BP sites used in permanent gates are replaced by LR sites recognized by integrase/RDFs complexes, producing rewritable (Rw) logic gates (see Bonnet et al., 2012 for details (5)). (B) Rewritable logic element schematics for all gates presented in the main text.

**fig. S3:**



**figure S3: Demonstration of a rewritable buffer gate. (A)** Schematic of the constructs used in this experiment. Integrase expression is under the control of the pTET promoter, while RDF expression is controlled by pBAD. The target plasmid contains a promoter flanked by BP sites. The BP state expresses GFP while the LR state expresses RFP. **(B)** Rewritable Buffer gate operation. Cells were exposed to pulses of arabinose (overnight cultures) to vary expression of the RDF while integrase expression was made constitutive by using a DH5alpha strain lacking the Tet repressor. Expression of the RDF drives cells to the BP state with near-completion efficiency. In the absence of arabinose (overnight cultures), cells relax back to the LR state via integrase mediated recombination. The system can be cycled multiple times.

**Fig S4:**

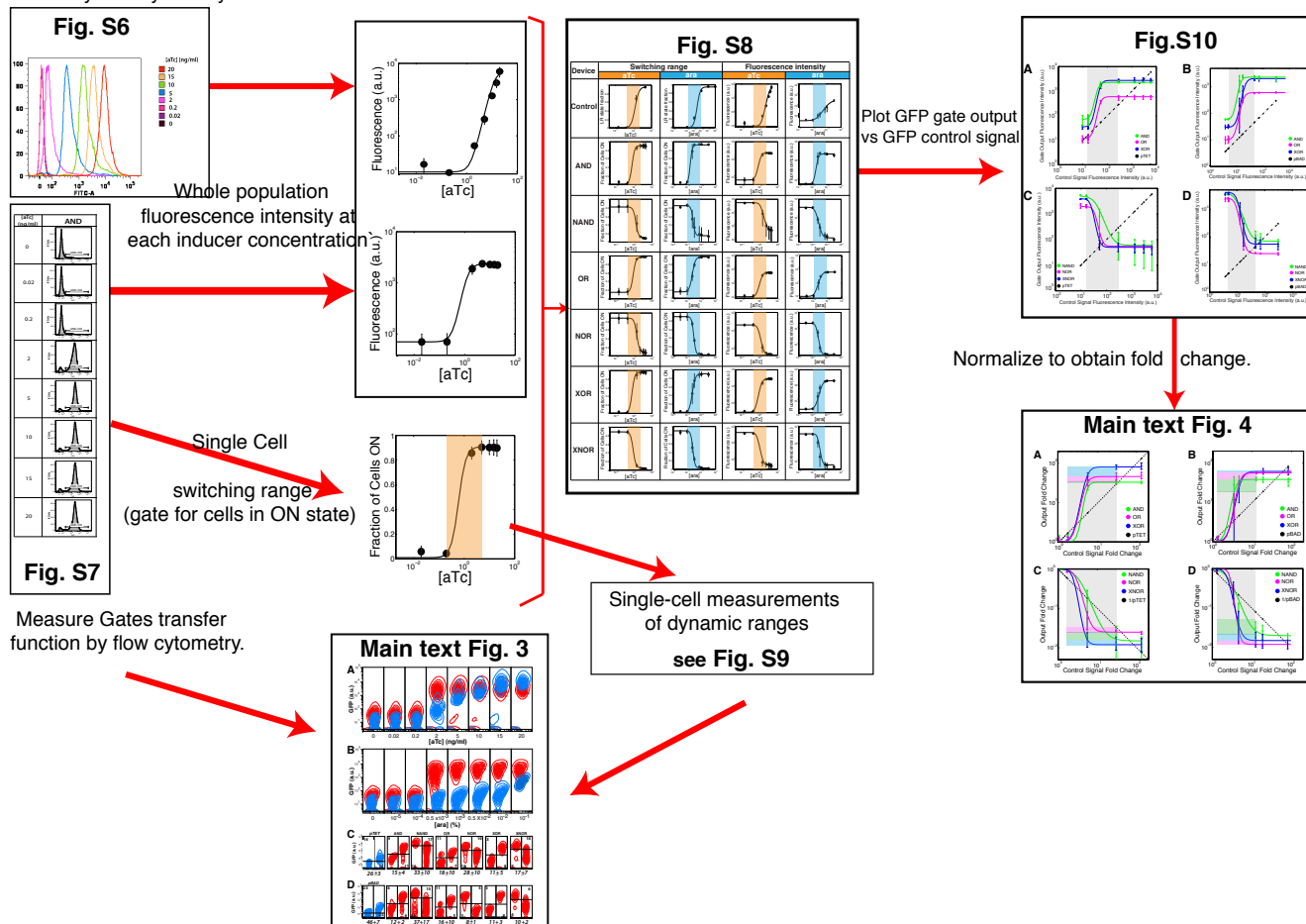


**figure S4: Abstracted models for logic element switching.** (A) Experimentally measured transfer functions for the TP901-1 and Bxb1 integrases. The fraction of cells flipping from a BP to LR state was measured as a function of arabinose (ara) or anhydrotetracycline (aTc) concentrations (B) Hill equation parameters for each individual integrase were obtained by fitting experimental data. (C) The equations obtained in (B) were combined into gate-specific functions for each distinct Boolean operation and used to predict two controller transfer functions shown in Main Figure 2.



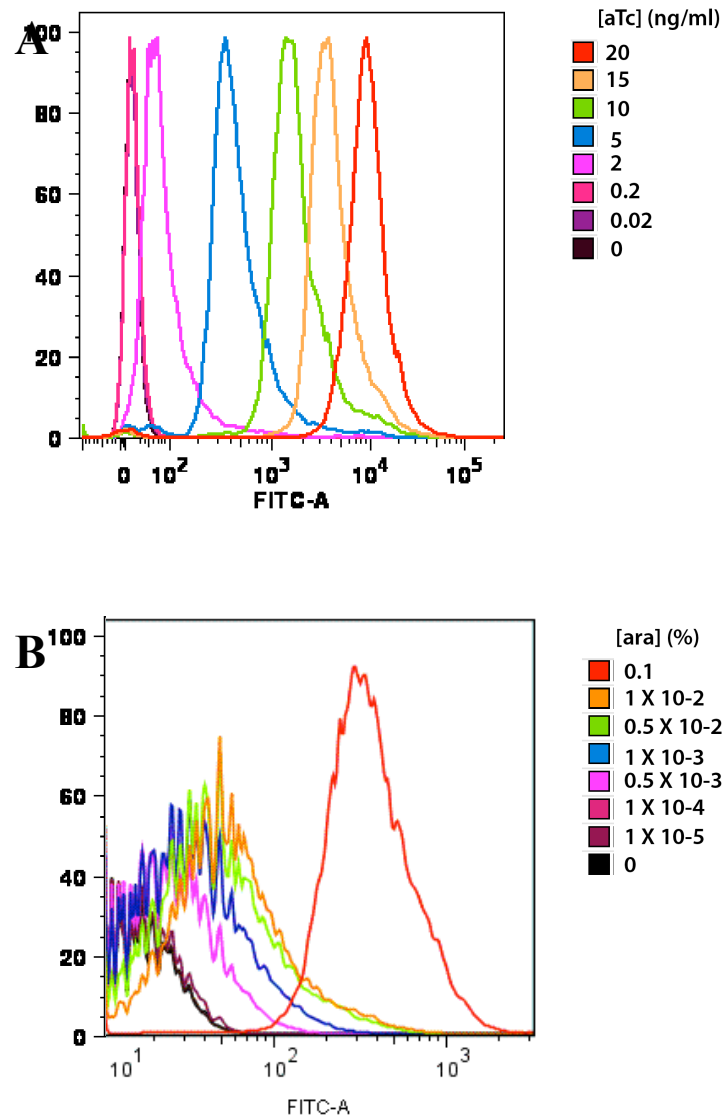
**fig. S5**

Measure Control Signals transfer function by flow cytometry.



**figure S5:** Schema describing how transfer functions, dynamic ranges, and fold changes were measured and processed to produce various figures. After measuring transfer functions for both control signals (fig. S6) and the logic gates by flow cytometry (fig. S7), whole population fluorescence intensity was measured at each inducer concentration. An example is depicted here with an AND gate and the pTET controller. The resulting transfer functions were plotted with single cell switching transfer functions in fig. S8. The flow cytometry data were used to determine the digital error rate for the gates and the promoters (Figure 3, main text). The raw, whole population GFP output from the gates was then plotted against the GFP output of the control signal at each inducer concentration (fig. S10). These data were normalized and plotted to obtain the relative gate output fold change versus control signal fold changes presented in Main Figure 4. Single cell measurements of dynamic ranges and fold changes are displayed in more detail in fig. S9.

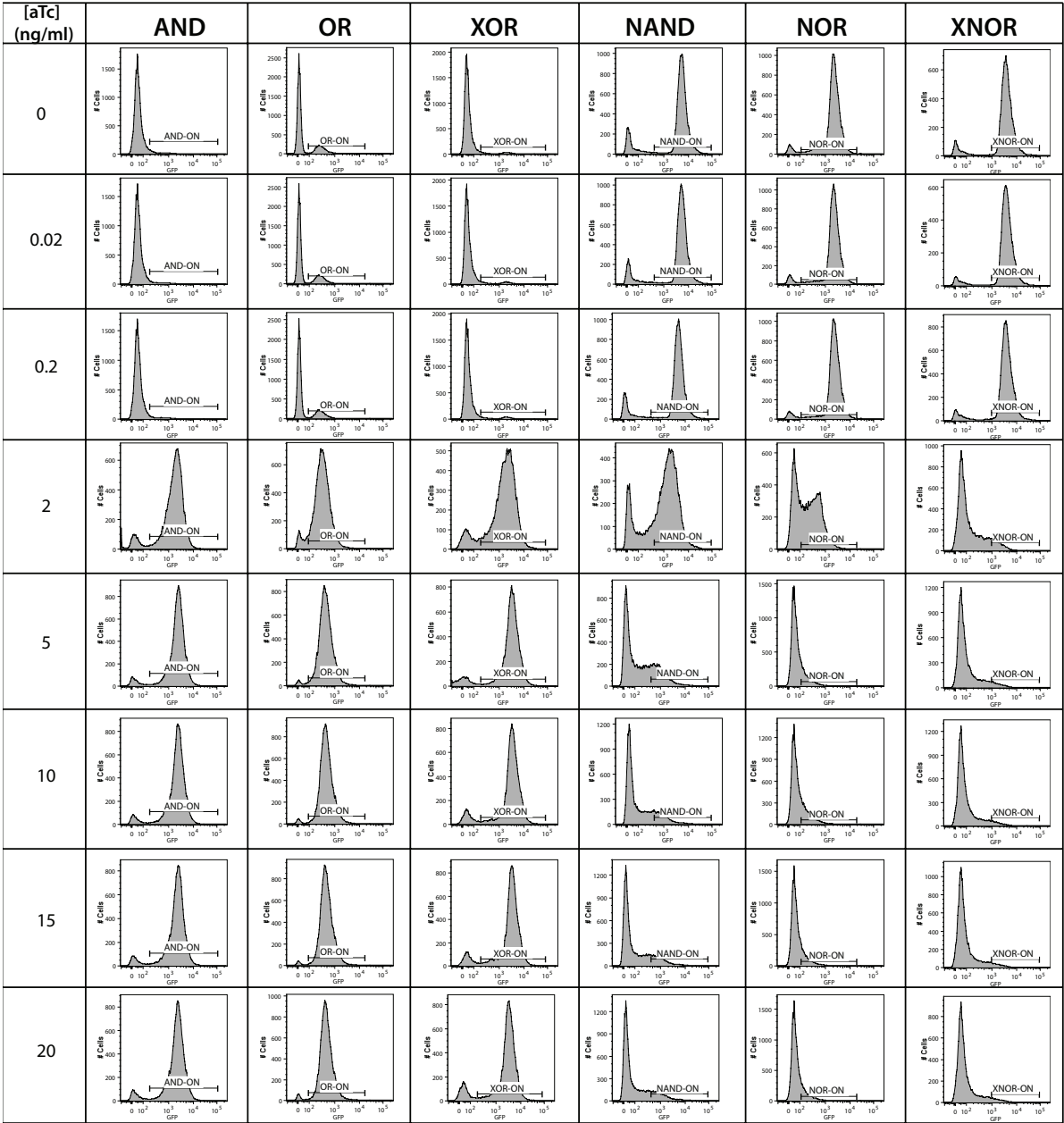
**fig. S6**



**figure S6:** Raw flow cytometry data for the control signals transfer functions presented in Fig. 3 and fig. S8. (A) pTET and (B) pBAD, using the measurement plasmids (plasmid maps, B-C).

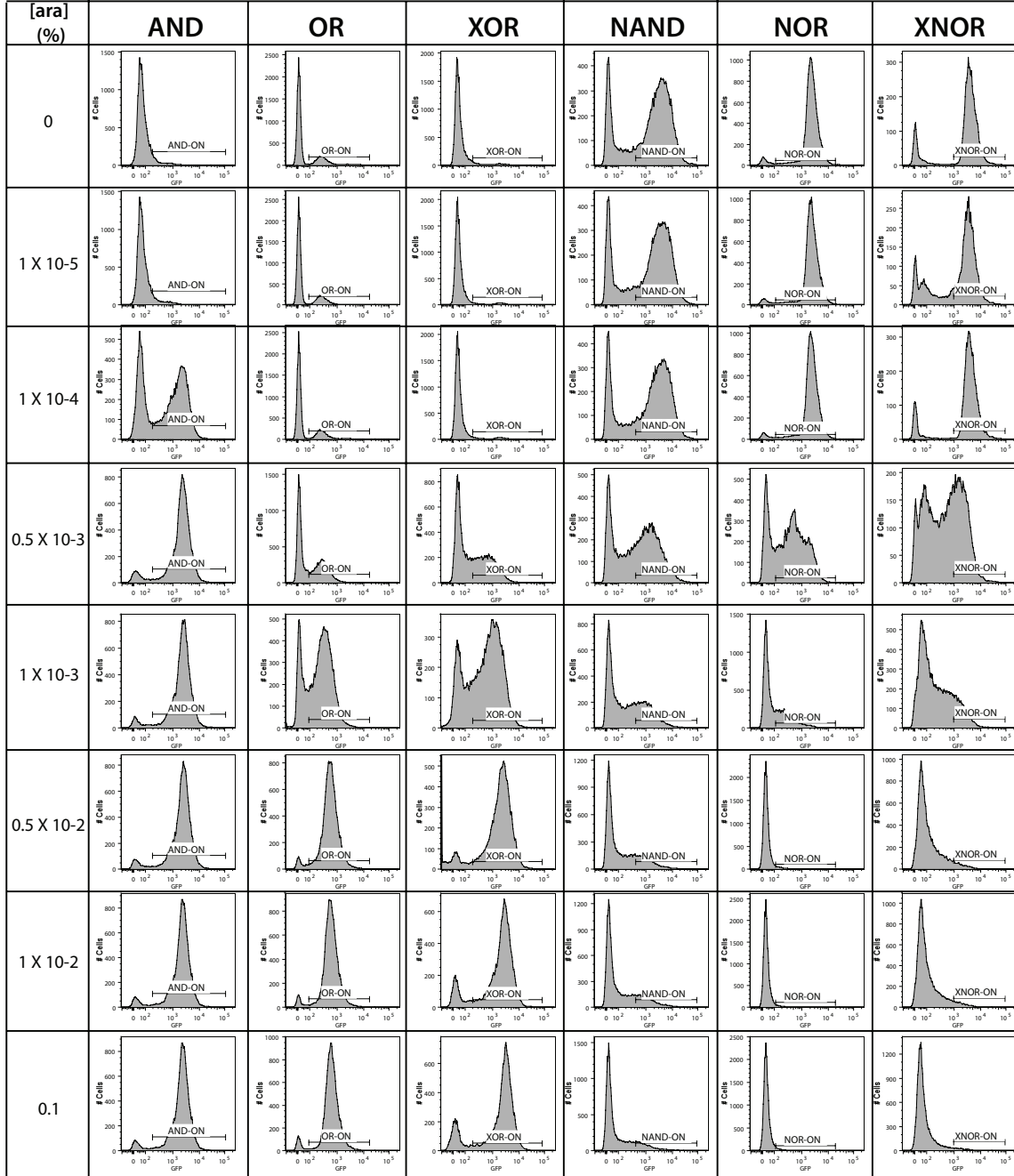
**figure S7**

**A**

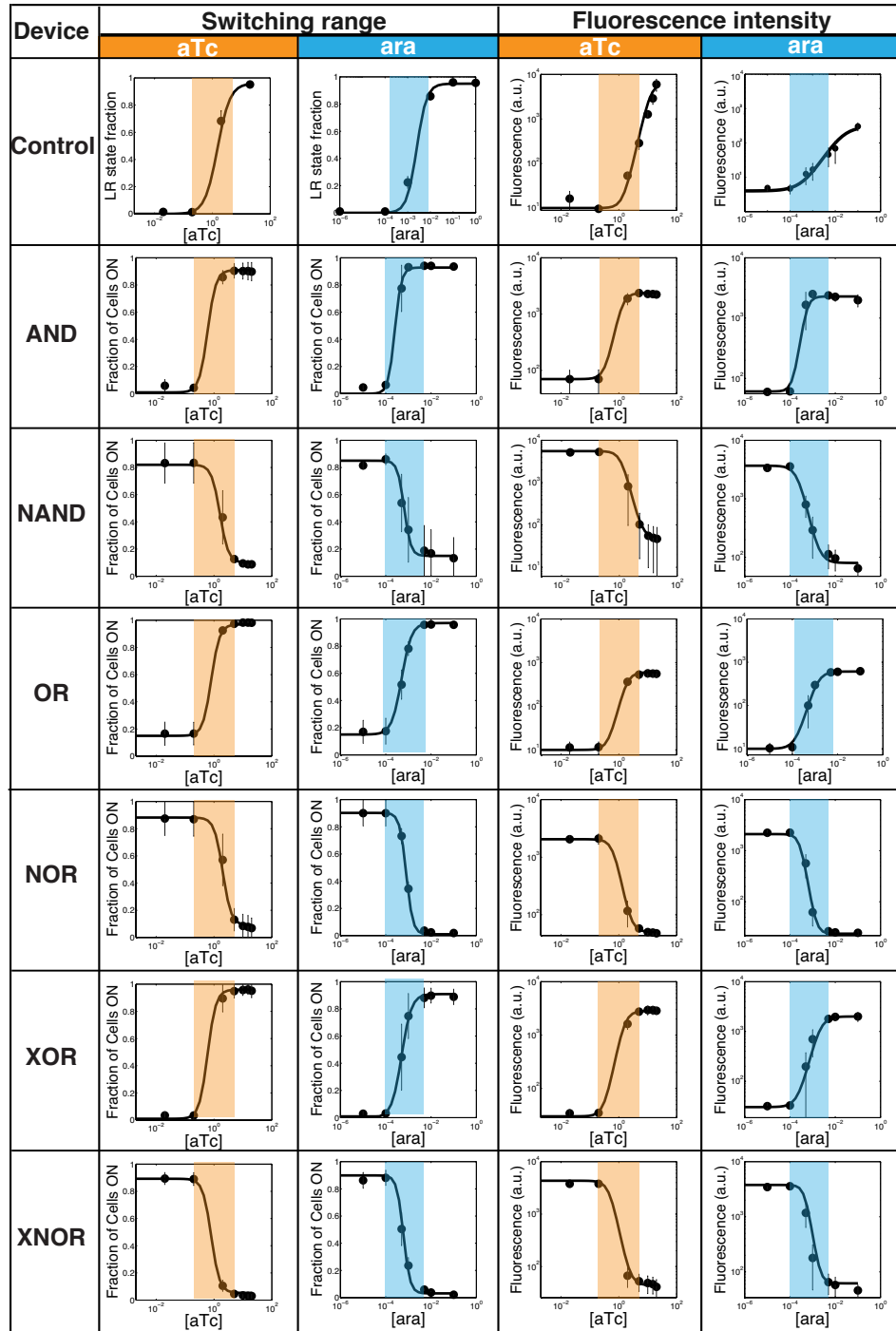


**figure S7 (continued)**

**B**

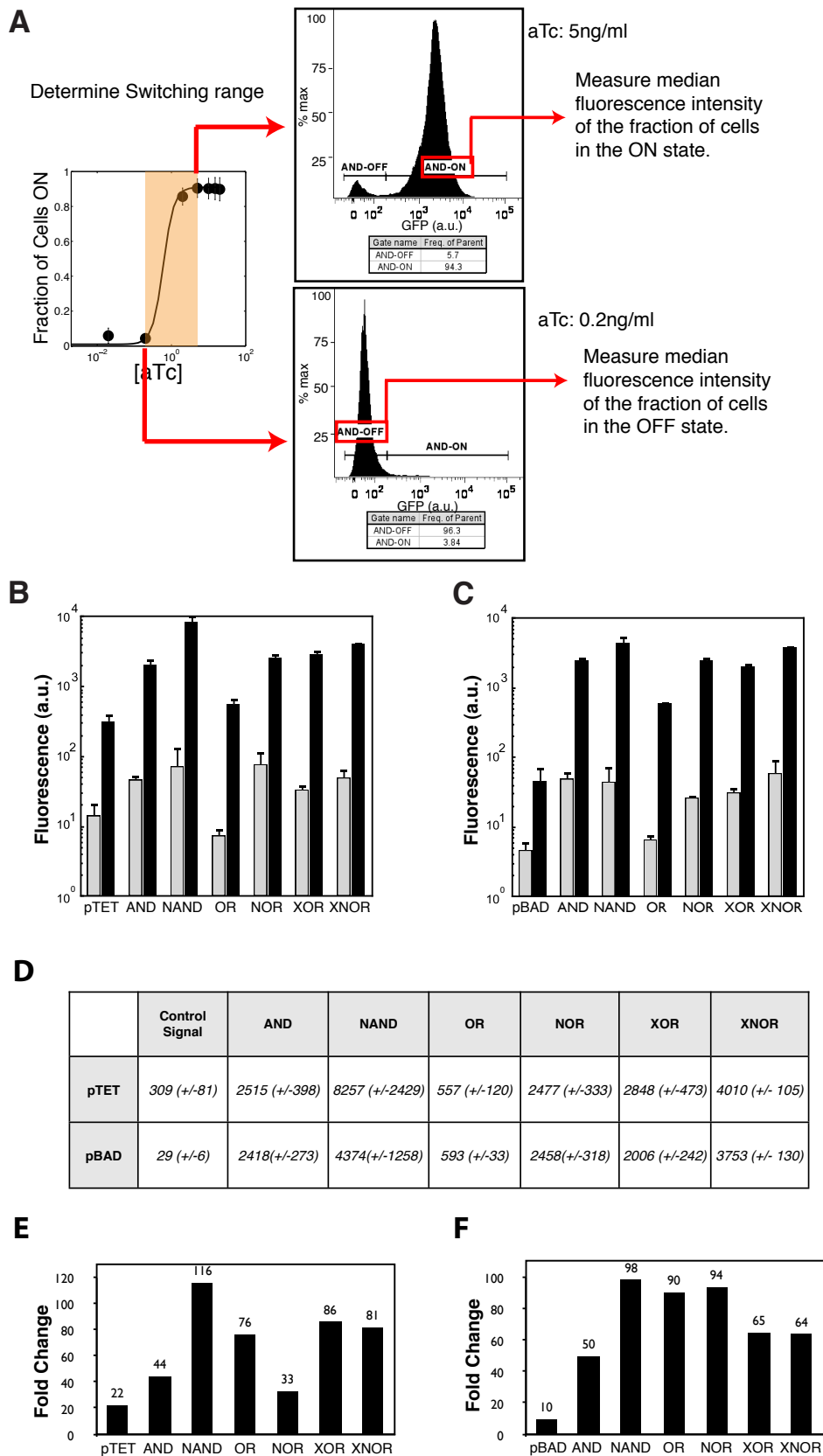


**figure S7:** Example of raw flow cytometry data and the gating method used to generate the data presented in fig. S8, S9, S10 and Main Text Fig. 4. (A) Response of gates to aTc regulated controller. (B) Response of gates to arabinose regulated controller. For the AND and NAND gates, measurement for a particular inducer was done in the presence of saturating concentrations of the other inducer.

**fig. S8****figure S8: Quantitative measurement of gates response to individual control signals.**

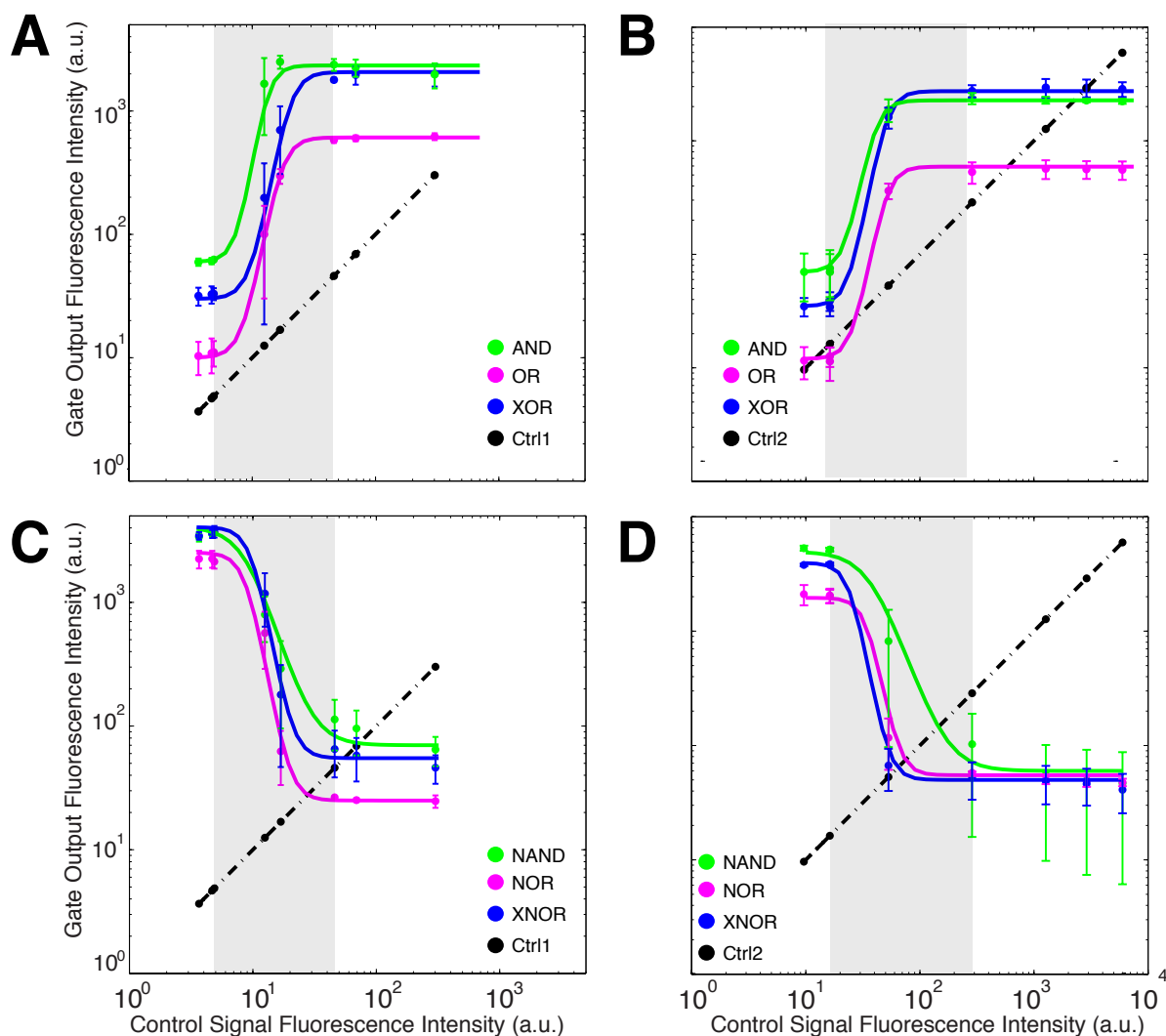
The common control switching range (shaded areas) was measured as the range of inducer concentration required to switch between less than 10% and more than 80% of cells in an ON state. The “control” graphs in the switching range section represent the transfer functions of individual Bxb1 (aTc) and TP901 integrase (ara) buffer gates (see also fig. S4). The “control” graphs in the fluorescence intensity section represent the transfer functions of individual pTET (aTc) and pBAD (ara) promoters performed using the measurement plasmids. Variation in fluorescence intensity in response to varying concentrations of individual inducers for each gate was plotted as the median intensity of the whole population at each inducer concentration. Results are the average of three independent experiments +/- SD.

fig. S9



**figure S9: (previous page) Gate dynamic ranges and control signal amplification. (A)** Method for determining the gate dynamic ranges at the single cell level: example with the AND gate response to aTc. The dynamic ranges of the logic gates and of the control signals were measured across the common gate switching ranges, using the cytometry gating method presented in fig. S7. Single cell fluorescence intensity of the OFF state for AND, OR and XOR was determined by measuring the median fluorescence intensity for the fraction of the cell population gated for the OFF state at the *lower* inducer concentration of the switching range (0.2 ng/ml for pTET and 1E-4% for pBAD). Single cell fluorescence intensity of the OFF state for NAND, NOR and XNOR was determined a similar manner but at the *higher* inducer concentration of the switching range (5 ng/ml for pTET and 1E-2% for pBAD). Single cell fluorescence intensity of the ON state for AND, OR and XOR was determined by measuring the median fluorescence intensity for the fraction of cell population gated for the ON state, at the *higher* inducer concentration of the switching range. Single cell fluorescence intensity of the ON state for NAND, NOR and XNOR was determined in a similar manner but at the the *lower* inducer concentration of the switching range. **(B)** Dynamic range of the gates in response to pTET and **(C)** Dynamic range of the gates in response to pBAD. Gray bars: OFF state. Black bars: ON state. Results are the average of 3 independent experiments +/- SD. **(D)** Differences in fluorescence intensity (arbitrary units) between the ON and OFF state for each gate, compared to the control signals, in response to individual inducers. Values are presented +/- SD. **(E-F)** Absolute values of the fold change in logic gate outputs between the ON and OFF states in response to pTET (E) or pBAD (F), determined from data in (B) and (C).

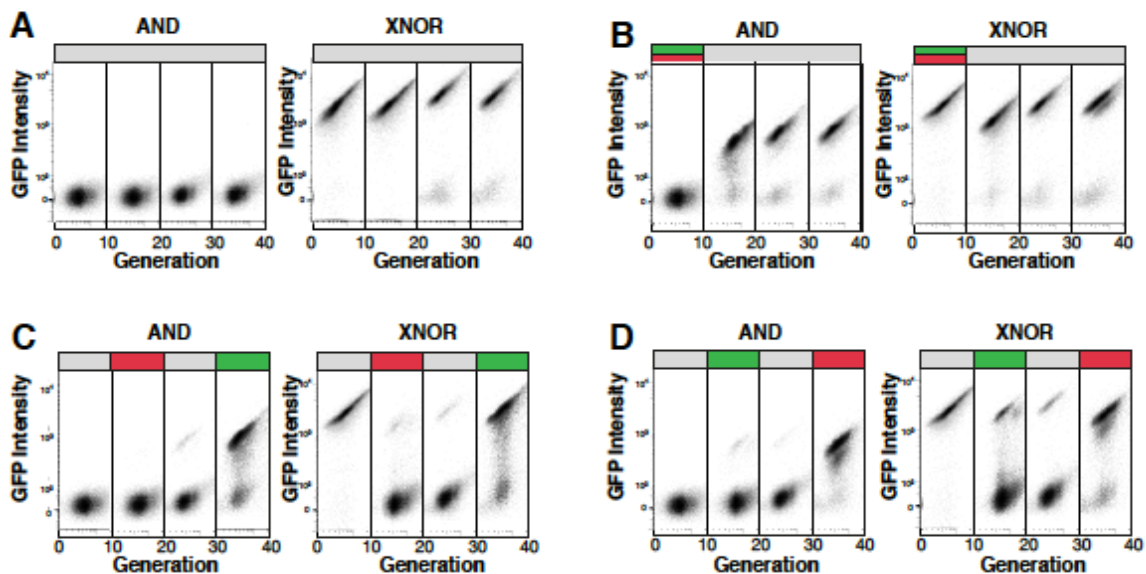
**fig. S10:**



**figure S10: Population average, raw GFP values for gate outputs as a function of GFP levels from control signals.** Response of amplifier gates to (A) increasing arabinose-mediated expression of TP901-1 integrase and (B) increasing aTc-mediated expression of Bxb1 integrase. The raw Green Fluorescent Protein (GFP) intensity produced by the gates (colored lines, as noted) is plotted against the raw GFP intensity driven by the integrase expression controllers (dashed straight line). The common switching range is highlighted by the gray box. Response of inverting amplifier gates: (C) as in (A), and (D) as in (B).

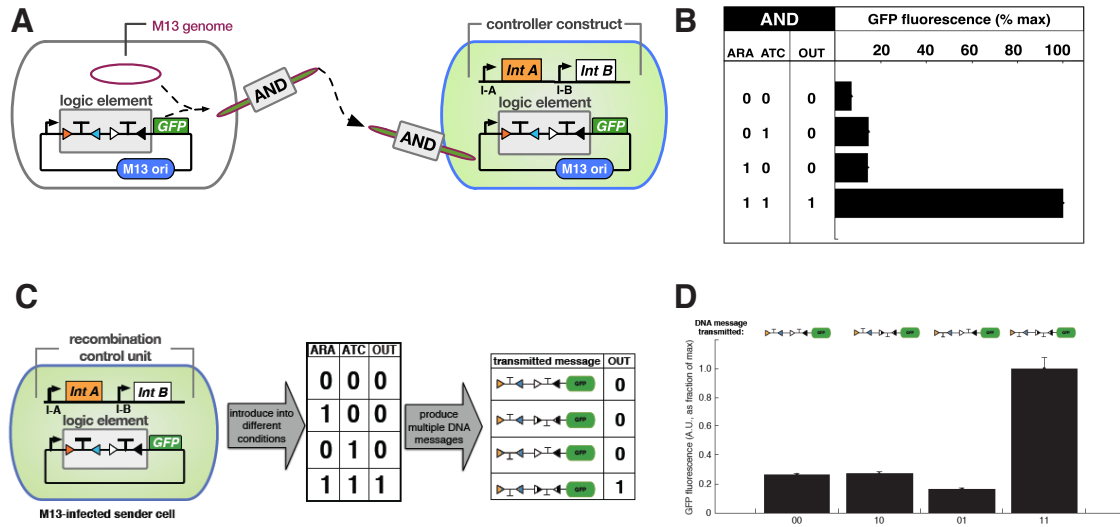


**fig. S11**



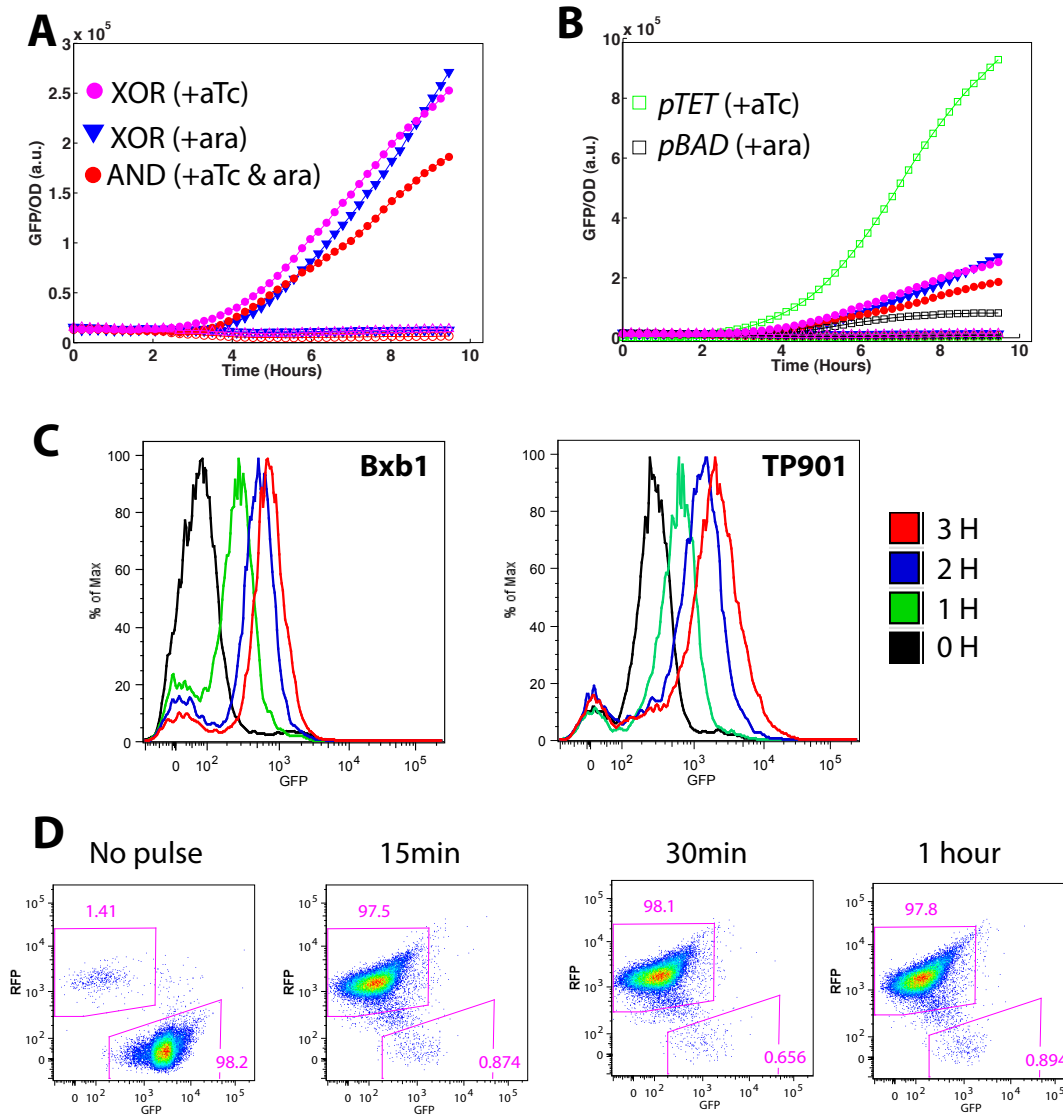
**figure S11: Sequential input logic and multi-generation logic registers.** (A) Cells containing AND or XNOR logic gates grown for 40 generations with no control signals. Single cell gate output distributions were observed every 10 generations via flow cytometry (forward scatter, x-axis; GFP intensity, y-axis). (B) Cells as before exposed to a pulse of both arabinose (red bar) and aTc (green bar) control signals immediately after culture inoculation followed by no further control signals. (C) Cells as before exposed to time-separated pulses control inputs starting at generation 10. (D) As in (C) but with the relative timing of control inputs reversed.

**fig. S12**



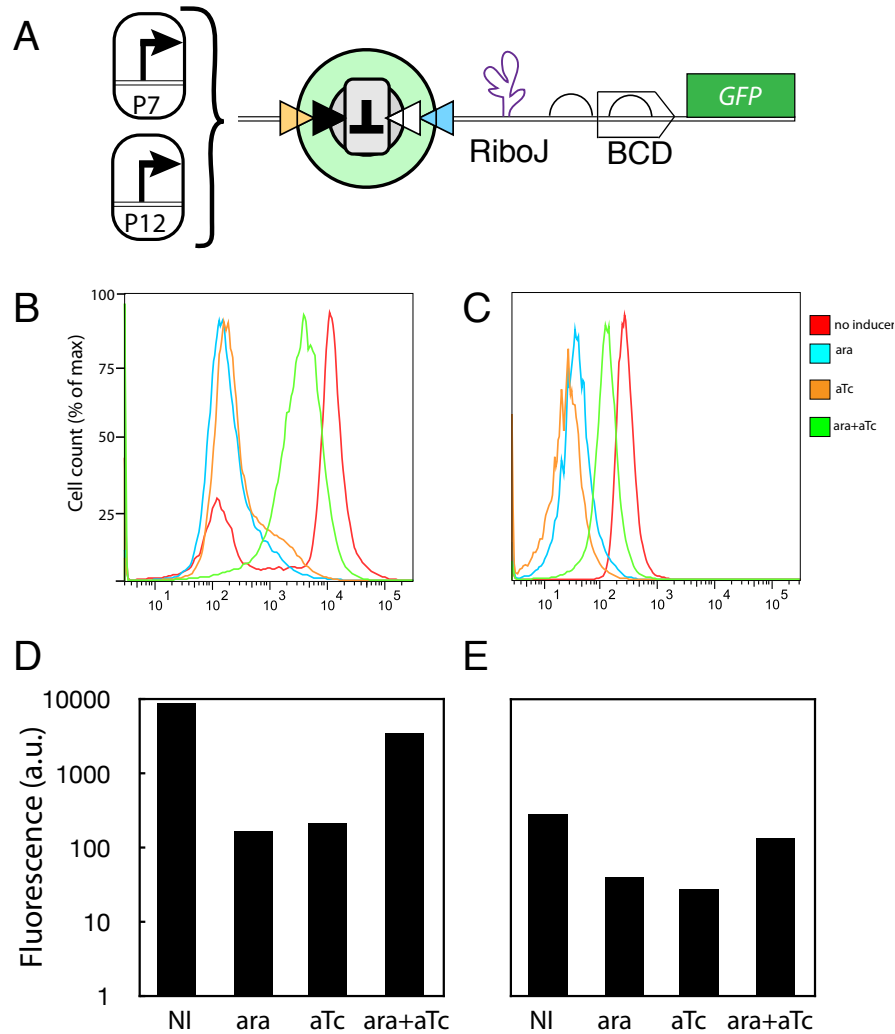
**figure S12: Cell-cell communication of programmable DNA logic.** (A) Sender cells encoding a Boolean integrase AND gate within a bacteriophage M13 messaging phagemid were co-cultured with receiver cells encoding a recombination control element under the control of exogenous arabinose and aTc induction. Sender cells package the AND gate within M13 phage particles that are secreted into the media and transfect receiver cells. (B) AND gate performance within receiver cells. Bulk fluorescence was measurement by platereader (triplicate experiments). (C) Sender cells encoding a Boolean integrase AND gate within a bacteriophage M13 messaging phagemid were transformed with the recombination control element and exposed to different combination of arabinose and aTc to generate the four possible states of the AND gate. (D) Conservation of DNA states during DNA messaging. Bulk fluorescence from receiver cells co-cultured with senders containing AND gate in different intermediate states was measured by plate-reader (triplicate experiments). All co-cultures were incubated for 5 hours without antibiotic selection followed by 16 hours of outgrowth under conditions selecting for message transmission.

**fig. S13**



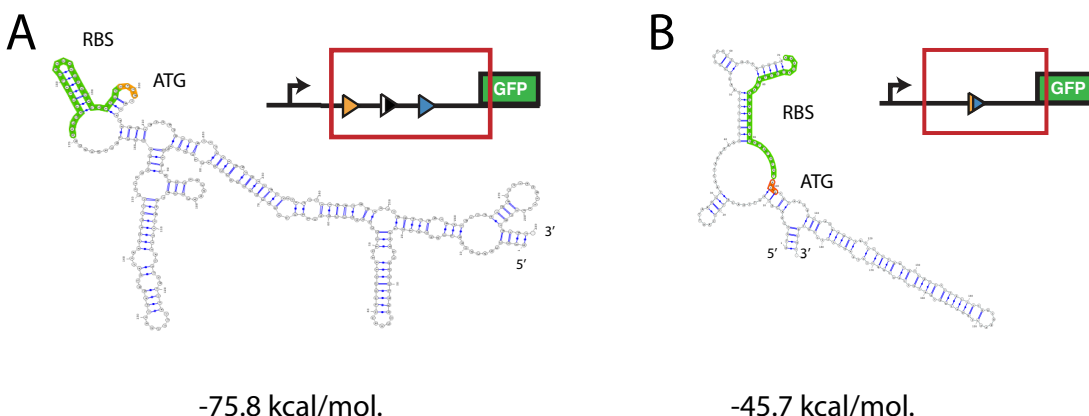
**figure S13: Kinetic measurement of gates operation and individual recombinases properties.** (A) Plate reader measurement of XOR and AND gate induction kinetics, as indicated. Curves of the same color with open symbol are the same samples but uninduced. An increase in output fluorescence is detectable starting between 3 and 4 hours. (B) As in (A) but with control signal promoters plotted on the same graph (squares). Note that control signals were induced with max. concentration of inducers. (C) Flow cytometry measurements of recombination kinetics of Bxb1 and TP901 both under the control of the pBAD promoter ((5), plasmid map *J*, respectively) using a target plasmid expressing GFP upon DNA inversion (plasmid maps L and M). After 1 hour, an homogenous increase in the cell population fluorescence is detectable. (D) Minimal input time assays for pBAD-Bxb1-Set-Generator (5). Cells were incubated with arabinose for the indicated time, washed, diluted 1:1000 and grown overnight before being assayed by flow cytometry. A 15 min. inducer pulse is sufficient to flip more than 95% of the cell population. See also Supplementary Movie 1.

**fig S14**



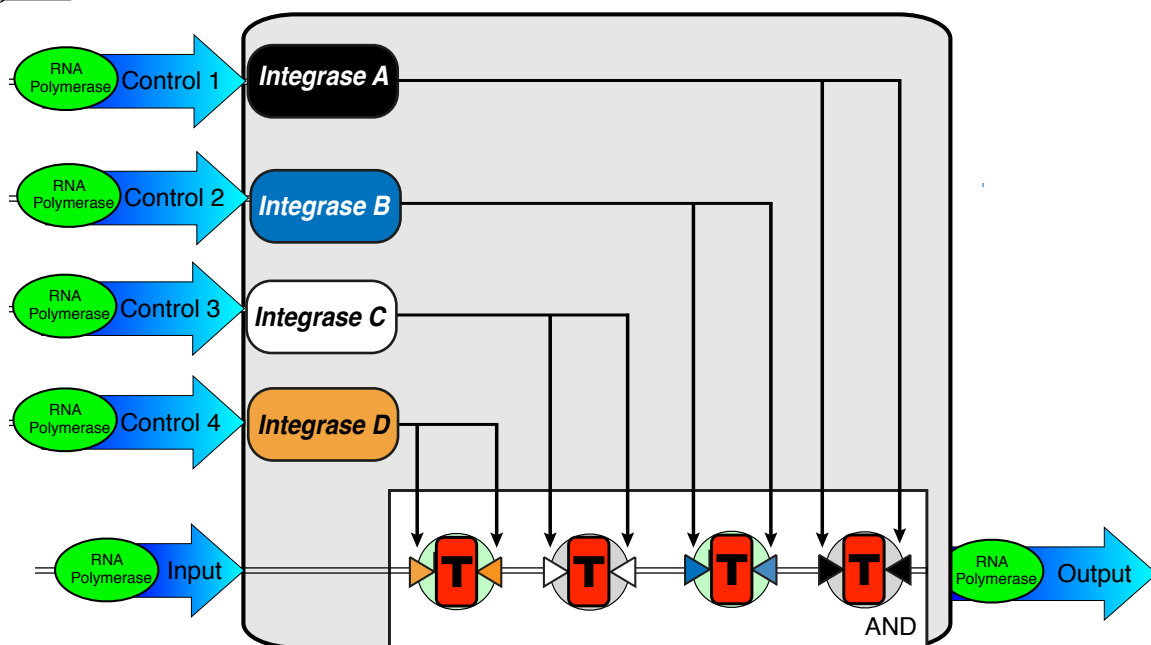
**figure S14: Gate output can be tuned by only changing the input promoter. (A)** A XNOR gate version using a RiboJ-BCD 5'UTR was used (24, 44). 2 promoters from the BIOFAB collection were used as input signal: P7 and P12, in which P12 is ~6 times weaker than P7 (44). **(B), (C)** Cells containing the dual-controller plasmid and the P7-XNOR (B) or P12-XNOR (C) were grown in different inducers combinations in azure media for 16 hours at 37 C (aTc: 200ng/ml, ara: 0.1% w/v). **(D), (E)** Comparison of whole population median fluorescence intensity between P7-XNOR (D) and P12-XNOR (E).

**fig. S15**



**figure S15:** Example of predicted structural differences in mRNA encoded by the Boolean integrase logic gates in different states. **(A)** The OR gate 5'UTR after Bxb1 mediated terminator excision. **(B)** The OR gate 5'UTR after TP901 mediated terminator excision. The RBS is highlighted in green and the start codon in orange. Note the difference in free energy between the two predicted mRNA folds due to a different 5'UTR region. Difference in 5'UTR regions are well known to influence downstream CDS expression through changes in mRNA stability and/or in translation initiation efficiencies. Such issues have recently been addressed by different groups (24, 27), and could likely be reapplied to engineer a second generation of Boolean integrase logic gates. The RNA structure models include the gate sequences plus the first 90 nucleotides of the GFP coding sequence. Structures were calculated using the rnafold software (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>) using the free energy minimization algorithm and built using the Varna applet (<http://varna.lri.fr>).

**fig. S16**



**figure S16: Design for a four control signal Boolean integrase AND gate.** Four independent integrases control the inversion of four asymmetric terminators serially positioned along the DNA strand. All four terminators must be inverted to obtain a high output from the gate.

**Appendix 1:** Numerical values and standard deviations (SD) of OD600 for the plate reader measurements used to develop the experimental dual control plots presented in Main Text Figure 2.

AND\_OD\_avg =

0.2952	0.29313	0.2444	0.26617	0.24544	0.2527
0.32112	0.26721	0.26721	0.28483	0.25995	0.25166
0.2444	0.25684	0.28172	0.28691	0.2838	0.27343
0.30453	0.28898	0.30868	0.31179	0.29313	0.30557
0.28276	0.27758	0.28794	0.30142	0.29416	0.25477
0.3149	0.28691	0.29313	0.29935	0.31697	0.31801

AND\_OD\_SD =

0.011213	0.070668	0.058542	0.057036	0.032469	0.028897
0.052441	0.078122	0.050051	0.058871	0.0089778	0.01616
0.08643	0.085303	0.099196	0.062921	0.056095	0.057598
0.047878	0.035048	0.037492	0.057794	0.037492	0.070531
0.063126	0.058871	0.022498	0.029668	0.031461	0.045813
0.0762	0.028219	0.064515	0.017129	0.032469	0.08088

OR\_OD\_avg =

0.24337	0.27654	0.25166	0.28483	0.22989	0.21641
0.27239	0.30971	0.27965	0.26514	0.31075	0.27965
0.26825	0.3066	0.27965	0.26617	0.31801	0.24959
0.30246	0.30453	0.32215	0.29416	0.34911	0.28691
0.24544	0.26514	0.27654	0.27965	0.25892	0.22263
0.22678	0.28276	0.28587	0.27136	0.28069	0.26721

OR\_OD\_SD =

0.062304	0.052318	0.05909	0.048414	0.035323	0.027172
0.073486	0.031152	0.027642	0.033254	0.09392	0.082927
0.070942	0.043577	0.04828	0.027172	0.068796	0.032667
0.057036	0.038968	0.014364	0.012569	0.027875	0.042339
0.06842	0.025138	0.037835	0.037707	0.028729	0.059525
0.04937	0.035048	0.026572	0.010922	0.023549	0.035866

XOR\_OD\_avg =

0.32112	0.30246	0.32112	0.31801	0.29416	0.26306
0.28794	0.2952	0.31075	0.29624	0.29727	0.29105
0.34703	0.33667	0.33356	0.32837	0.32215	0.28898
0.3066	0.33045	0.33874	0.3377	0.32215	0.29624
0.30764	0.29209	0.31593	0.28898	0.29105	0.2838

0.30764	0.31904	0.32112	0.3066	0.30142	0.30971
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XOR\_OD\_SD =

0.072091	0.07162	0.025329	0.052257	0.031152	0.014024
0.032667	0.057598	0.051761	0.015959	0.049598	0.065997
0.053627	0.038636	0.0089778	0.023549	0.012948	0.029992
0.028219	0.021844	0.020394	0.020707	0.027172	0.026082
0.0311	0.026572	0.018223	0.011213	0.014024	0.027172
0.036666	0.021844	0.029559	0.047608	0.045175	0.012948

NAND\_OD\_avg =

0.22678	0.21434	0.23767	0.233	0.22367	0.21279
0.21434	0.22056	0.24855	0.247	0.28121	0.247
0.21745	0.21901	0.23766	0.25633	0.26099	0.23145
0.19723	0.21279	0.24855	0.26254	0.23456	0.29053
0.2159	0.20967	0.22523	0.25321	0.25477	0.24389
0.247	0.22989	0.25477	0.26255	0.25788	0.22989

NAND\_OD\_SD =

0.017593	0.0087964	0.02419	0.021991	0.026389	0.02419
0.035186	0.0043982	0.0087964	0.019792	0.032987	0.019792
0.057177	0.010996	0.0021991	0.032987	0.026389	0.010996
0.063774	0.028588	0.0043982	0.019792	0.010996	0.059376
0.050579	0.050579	0.050579	0.0021991	0.026389	0.041783
0.07257	0.04838	0.04838	0.0065973	0.0043982	0.0043982

NOR\_OD\_avg =

0.32008	0.28794	0.29313	0.30868	0.27239	0.23507
0.30038	0.31179	0.32734	0.32112	0.28587	0.29002
0.32112	0.3066	0.31179	0.27654	0.27447	0.30971
0.30453	0.30038	0.31179	0.28794	0.28794	0.29313
0.29831	0.28172	0.26721	0.24959	0.2641	0.24855
0.32423	0.3149	0.27758	0.2838	0.29935	0.2838

NOR\_OD\_SD =

0.035323	0.034956	0.036046	0.068231	0.028729	0.025138
0.045318	0.090796	0.08283	0.079432	0.070646	0.074662
0.041995	0.051323	0.054343	0.02348	0.033544	0.074662
0.042755	0.0529	0.042906	0.0095012	0.025329	0.0472
0.032469	0.038636	0.017316	0.011774	0.044092	0.041141
0.042906	0.045848	0.017129	0.017129	0.038005	0.069981



XNOR\_OD\_avg =

0.34185	0.32786	0.29831	0.33563	0.21279	0.23611
0.31231	0.31853	0.31231	0.31853	0.28587	0.25477
0.3263	0.35274	0.3403	0.33719	0.27499	0.26099
0.31697	0.31075	0.33252	0.33408	0.32008	0.29675
0.28743	0.30142	0.33874	0.33874	0.31697	0.26876
0.32786	0.32475	0.33874	0.3403	0.31853	0.29831

XNOR\_OD\_SD =

0.013195	0.015394	0.013195	0.070371	0.054978	0.0043982
0.0065973	0.010996	0.0021991	0.0065973	0.026389	0.013195
0.017593	0.041783	0.07257	0.032987	0.015394	0.0087964
0.017593	0.013195	0.026389	0.02419	0.04838	0.010996
0.015394	0.021991	0.021991	0	0.0043982	0.028588
0.028588	0.0065973	0.0043982	0.010996	0.041783	0.026389

**Appendix 2:** Numerical values and standard deviations (SD) of GFP intensity for the plate reader measurements used to develop the experimental dual control plots presented in Main Text Figure 2.

AND (GFP/OD) =

6621.1	8575.9	1.0367e+05	1.0927e+05	1.0937e+05	1.0294e+05
4078.9	18631	71900	97169	80649	79889
3479.4	8456.5	28417	35453	34520	40759
2111.9	2263.7	3546.1	5005.6	6666.5	8689
2221.4	2738.1	3374.8	4660.8	5010.3	6291.7
1969.2	2048.8	3026.1	3799.8	5160.3	5874.4

AND\_SD =

2688	1600.5	8525.9	8184.9	17931	8309.5
1179.6	21720	19069	11112	6196.5	5488.7
1171.4	10385	38506	29545	40672	42772
263.85	530.17	979.56	1501.2	3854.8	5920.1
841.87	922.36	1165	653.36	2349.6	1634.8
854.91	897.99	1180.5	940.3	1705.8	1332.4

OR (GFP/OD) =

39938	47332	64088	83729	1.1154e+05	1.1312e+05
33099	36746	52548	77566	85242	98759
8435.8	9451.9	26018	59341	66276	91239
2673.4	4148.6	17917	47868	66654	85734
3148.5	5276.5	21179	54346	79187	1.0449e+05
2920.7	3850.1	14412	54534	79039	85024

OR\_ SD =

4873.6	13239	14718	12554	21445	21805
7304.2	10000	9260	16367	13526	26485
9554.4	10515	20228	23393	13834	24729
483.65	2225.6	10600	12353	18921	22619
739.05	2641.1	23702	19340	23030	36954
1182.6	1486.5	8108.8	10712	32239	21952

XOR (GFP/OD) =

97466	1.0226e+05	52447	15657	13593	10328
75920	77761	70149	27781	21113	16108
7954.6	5352.8	17672	70960	80904	72932
4147.9	4663.9	16668	77085	89253	85172
4505	5787.3	15807	85069	97891	1.0085e+05
4037.6	5327.6	12599	81244	96891	92228

XOR\_ SD =

35049	44059	21845	6323.7	5868.3	3617
36867	39567	22730	9760.7	9445.3	6278.6
5233.9	1277	1736	20814	29706	18116
1387.2	1036.8	4438.2	9494.6	21447	7486.7
648.29	1452.6	7253.8	11676	27915	15739
254.85	854.22	3828.4	11282	20726	13049

NAND(GFP/OD) =

3.3622e+05	3.5218e+05	2.1329e+05	99729	77936	76817
3.3431e+05	3.8059e+05	2.574e+05	1.542e+05	84090	78804
5.8208e+05	6.6315e+05	4.9814e+05	3.9412e+05	3.6318e+05	3.5761e+05
7.0418e+05	7.3472e+05	6.4686e+05	6.1739e+05	7.4259e+05	5.9855e+05
6.7389e+05	7.3897e+05	6.1321e+05	5.4242e+05	5.6473e+05	5.4577e+05
6.3287e+05	7.1337e+05	5.8596e+05	5.8903e+05	6.2565e+05	6.3932e+05

NAND\_ SD =

58801	40712	1.1441e+05	39770	36329	39146
86648	1.3163e+05	91815	76721	40564	46346
1.5715e+05	1.4243e+05	1.9812e+05	1.4619e+05	2.5546e+05	2.3957e+05
11368	97694	89480	21825	97664	79543
3273.4	18821	5406	4501.8	14415	13101
58803	31336	29521	98789	65661	1.1022e+05

NOR (GFP/OD) =

18883	19054	11205	3658.5	3529.3	4716.2
32665	48612	15497	4784.6	3928.2	3658
1.0911e+05	1.0393e+05	76423	15116	8233.9	6713.4
1.6275e+05	1.4517e+05	87396	13267	8495.8	6864.7
1.6248e+05	1.438e+05	87920	13810	9034.6	6665.9
1.5628e+05	1.6229e+05	89103	15830	8766.5	6246.7

NOR\_ SD =

4311.6	4422.1	4066.9	1182.8	158.34	547.51
12313	45431	8761.2	1977.9	1662.2	557.77
64785	34878	48756	11804	5249.6	1110.4
13802	17338	42360	9421.4	3580.9	690.96
10277	29142	55526	9707.1	4266.7	1000.3
16376	9311	61068	11941	2942.6	1025.6

XNOR (GFP/OD) =

86907	1.068e+05	3.8816e+05	6.8936e+05	6.9736e+05	5.9656e+05
85863	1.2738e+05	4.1851e+05	5.5173e+05	5.8615e+05	5.2688e+05
2.004e+05	2.8565e+05	2.8234e+05	3.0046e+05	4.4083e+05	3.0108e+05
5.577e+05	5.877e+05	1.0098e+05	25070	24521	18461
6.3308e+05	7.2948e+05	1.0264e+05	28348	23541	19690

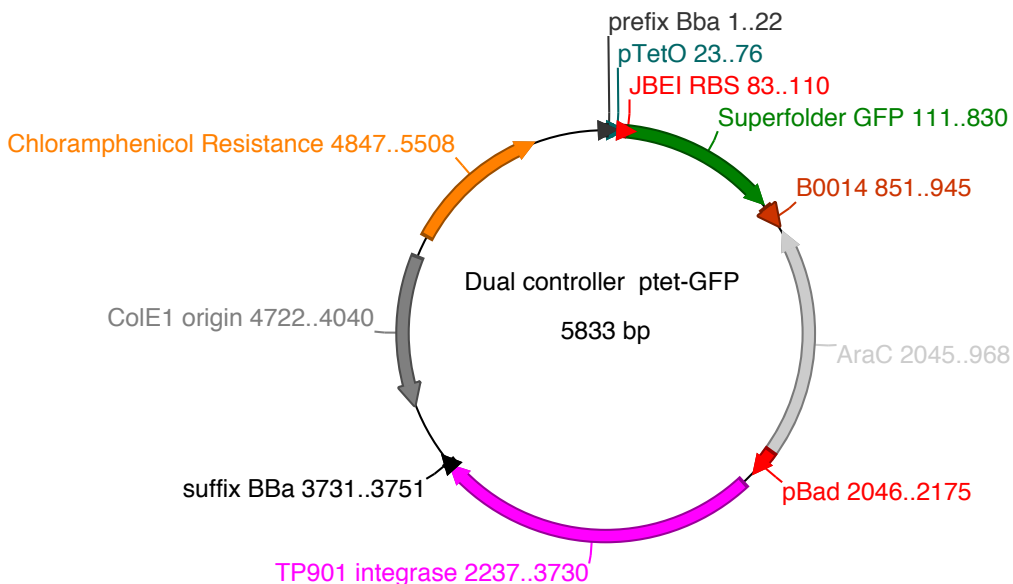
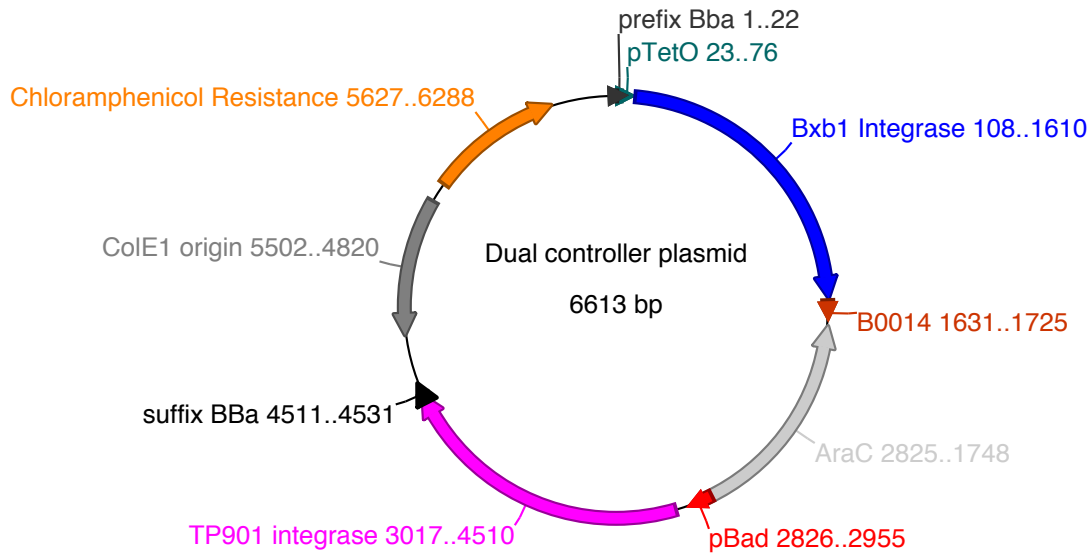
6.1967e+05	5.8398e+05	1.3152e+05	22111	20845	17337
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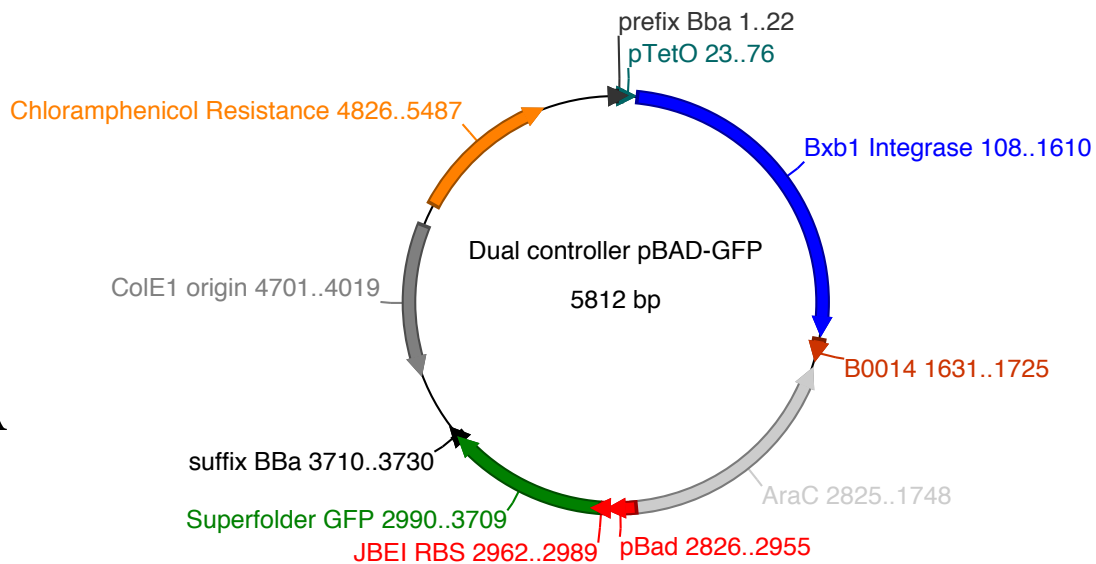
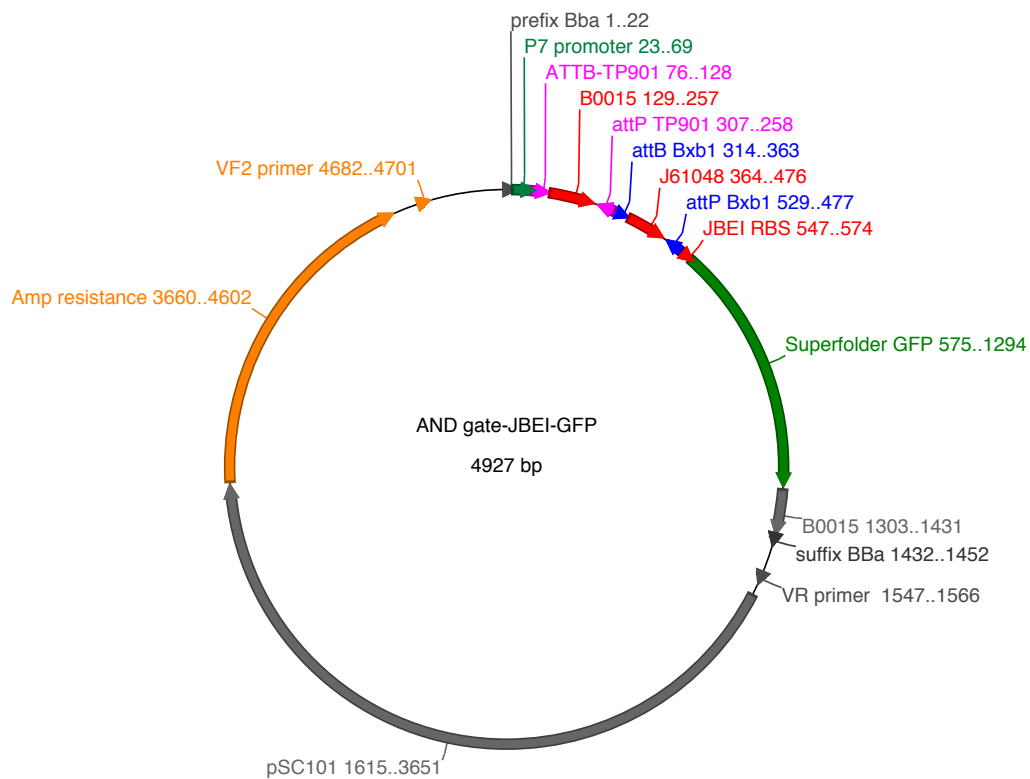
XNOR\_ SD =

5991.7	16845	1.7166e+05	66185	1.7336e+05	18301
1931.9	7204.5	1.9091e+05	99003	6245	21524
83127	1.6112e+05	5505.6	57425	63250	1.4369e+05
1.3611e+05	1.0429e+05	26607	12750	1746.5	9115.8
1.6676e+05	62539	62778	7720.9	2331.4	6337.6
1.923e+05	1.8354e+05	29286	12972	8343.6	9764.8

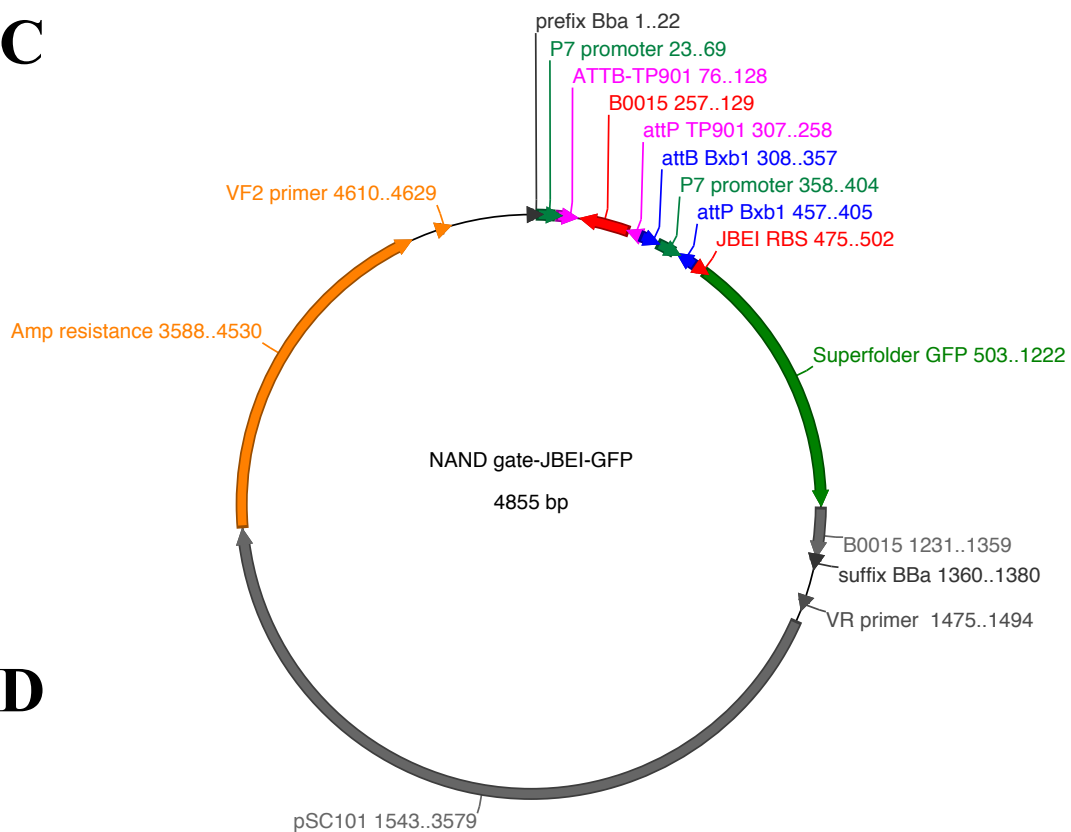
### Appendix 3: Plasmids Maps

(A) The dual controller plasmid with pBad-TP901 and pTET-Bxb1. (B) The measurement plasmid where the Bxb1 integrase is replaced by the superfolder-GFP cassette (C) The measurement plasmid where the TP901 integrase is replaced by the superfolder-GFP cassette. (D) to (I) the different logic gates plasmids. (J) the pBAD-TP901-Set-Generator (K) The TP901 BP register with RFP and GFP outputs. (L) and (M) the Bxb1 and TP901 BP register expressing GFP upon flipping.

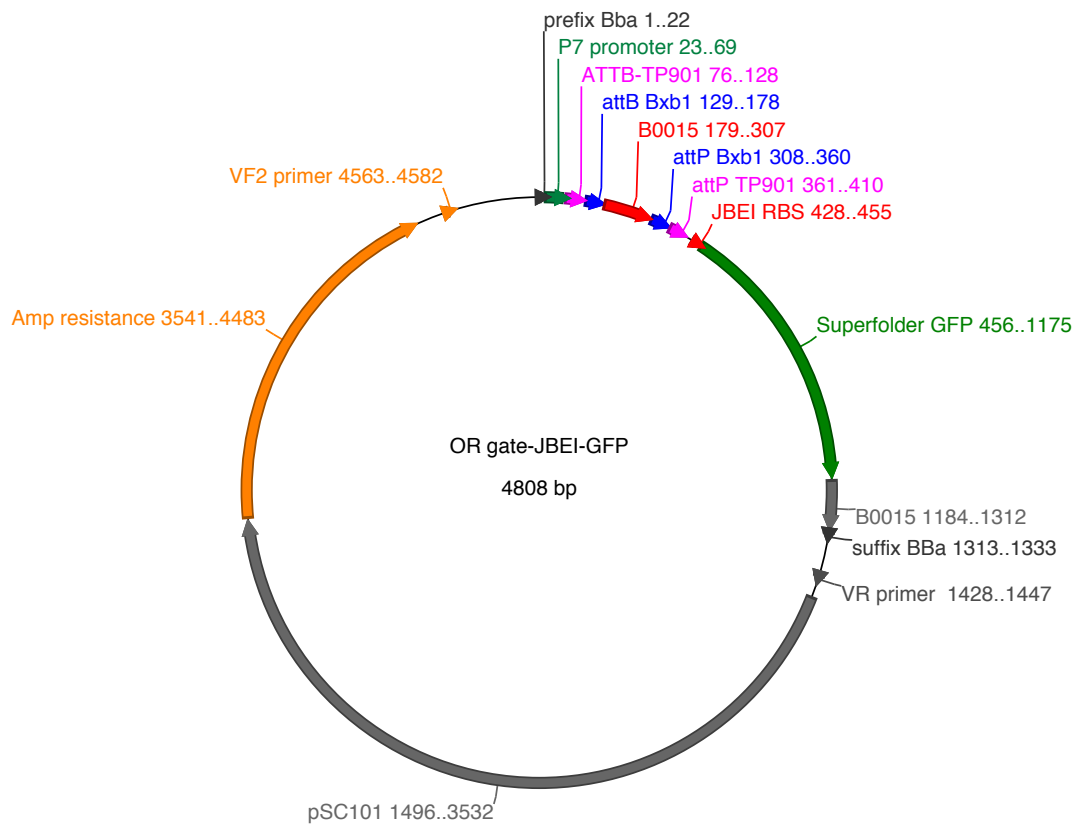


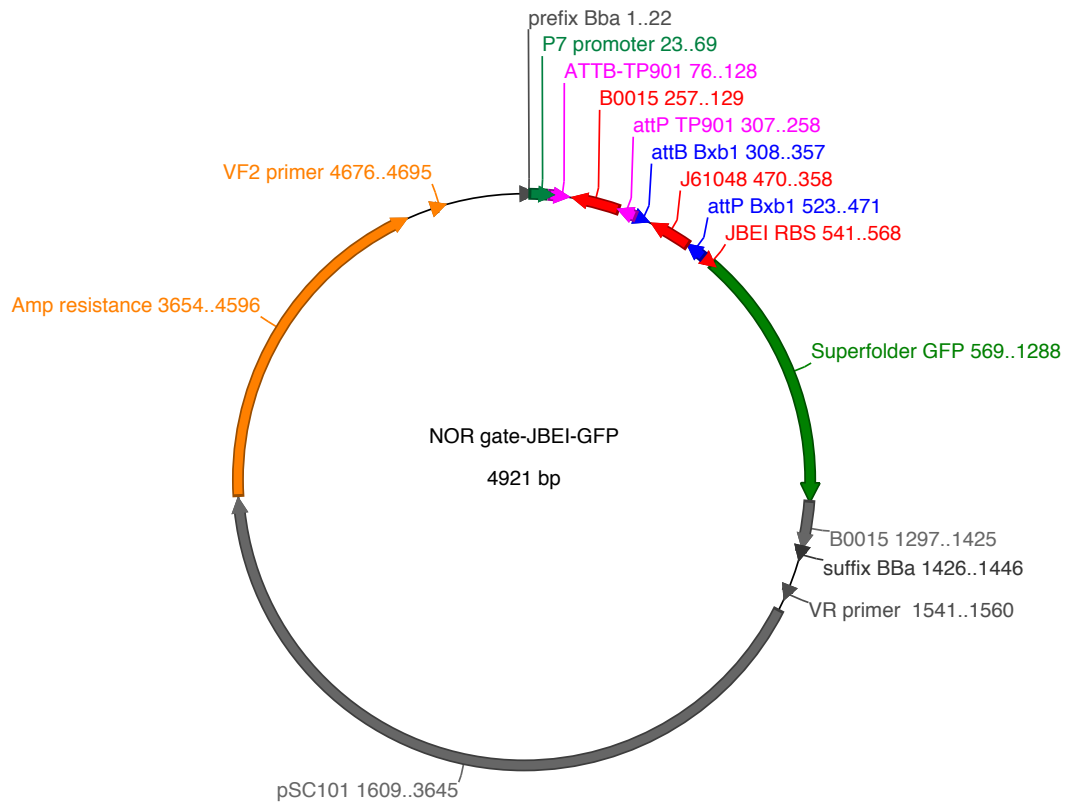
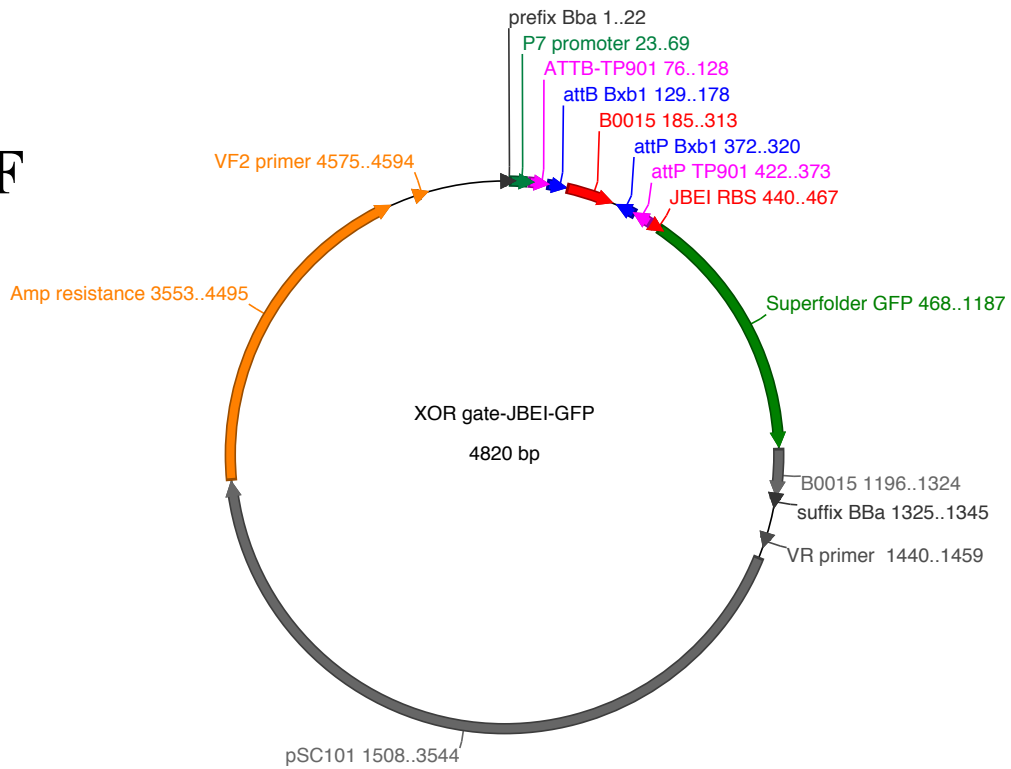
**A****B**

C



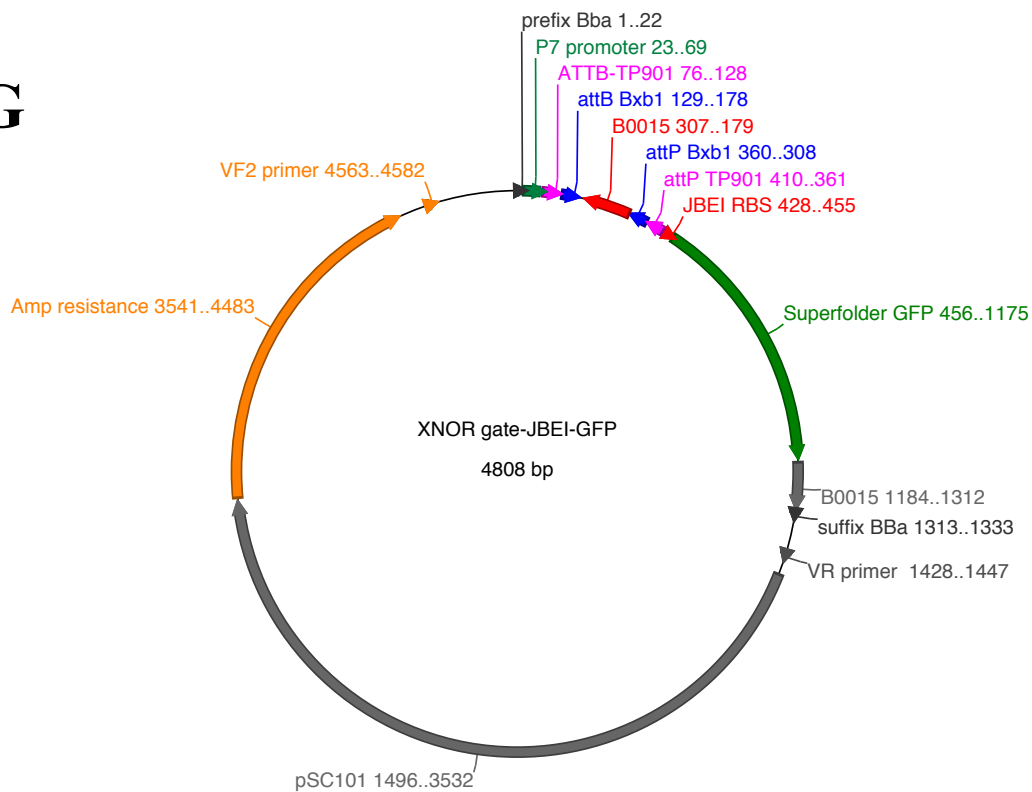
D



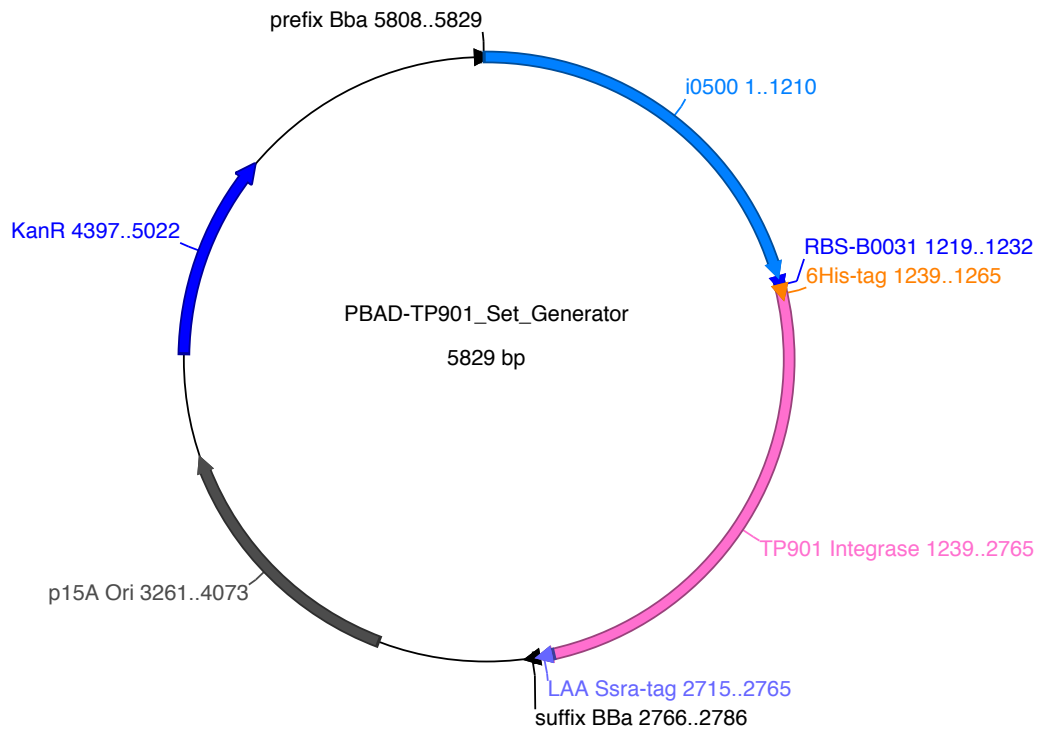
**E****F**



G

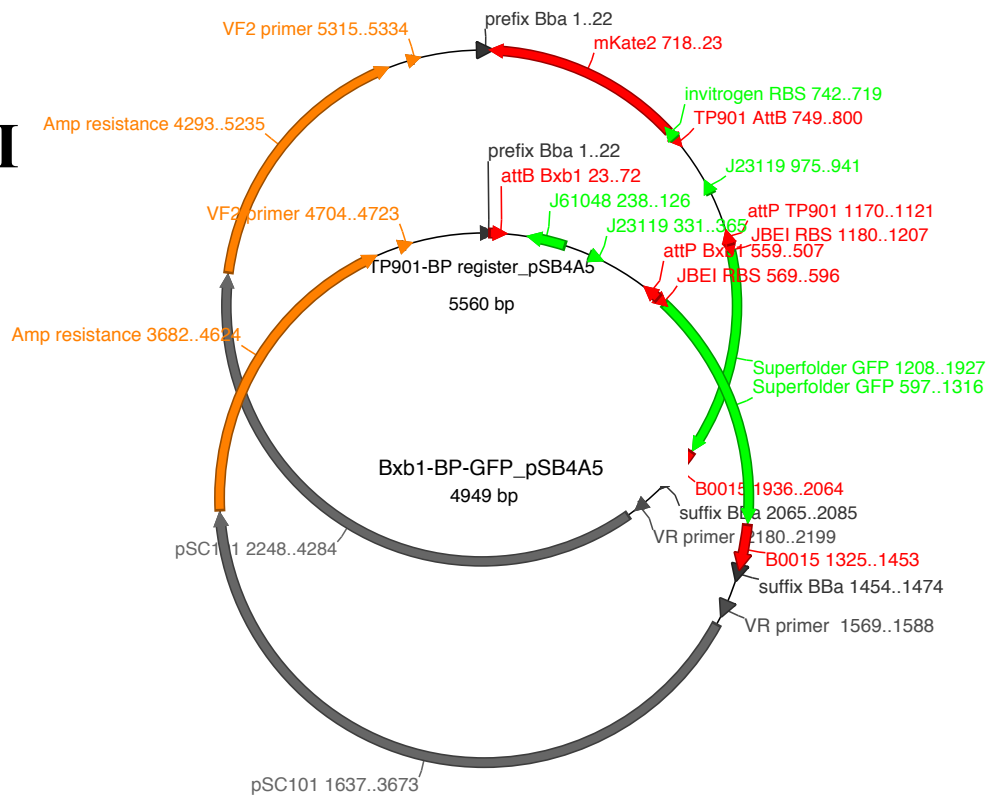


H

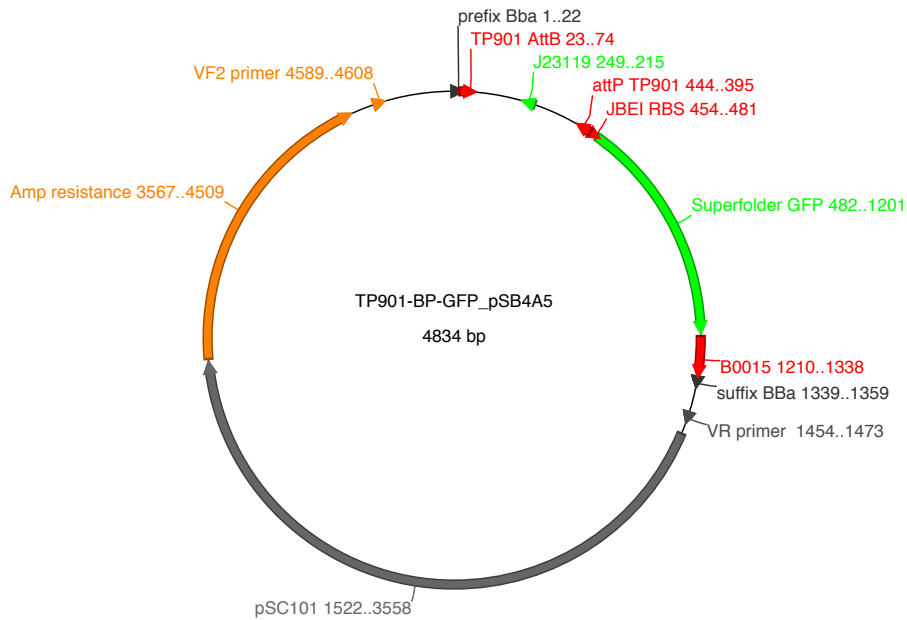


M

I



J



#### Appendix 4: Sequences of primers used in this study.

G1004	gtttcttcgaattcgcggccgcttctag
JB-422	acattgattatttgcacggcgctcac
JB-423	CTGCAGCGGCCGCTACTAGTATTAAGCAGCCAGAGCGTAGTTTTTCG
JB-424	gctaattcttatggataaaaaatgctatggcatagc
JB-425	CGAAAACCTACGCTCTGGCTGCTTAATACTAGTAGCGGCCGCTGCAG
JB-431	aAAGCAAATAAATTTTTTctctagaagcggccgcgaattc
JB-434	GCCTacTAGAGAAAAGATCTTTTAAGAAGGAGATATACAT
JB-435	ATGTATATCTCCTTCTTAAAAGATCTTTTCTCTAgtAGGCGCGCCgcgagtttttatttc
JB-457	TAACATCTCAATCAAGGTAAATGCTTTTTTGCT
JB-458	ATGTATATCTCCTTCTTAAAAGATCTTTTCTTAGTAGG
JB-459	GGCGCGCCTACTAGGAAAAG
JB-460	GCAAAAAAAGCAAAAAGCATTTACCTTGATTG
JB-466	TAACATCTCAATCAAGGTAAATGCTTTTTTGCTTTTTTGC
JB-468	GCAAAAAAAGCAAAAAGCATTTACCTTGATTGAGATGTTA
JB-469	TGAGACCGCGGTGGTTGACC
JB-470	TACTAGAGAAAAGATCTTTTAAGAAGGAGATATACATATGCG
JB-471	CATATGTATATCTCCTTCTTAAAAGATCTTTTCTctagtaTCGTGGTTTGTCTGGTCAACC
JB-472	CATATGTATATCTCCTTCTTAAAAGATCTTTTCTctagtagcgagtttttatttcgtttatttcaattaaggtaac
JB-473	CATATGTATATCTCCTTCTTAAAAGATCTTTTCTctagtaaaaggagtttttagttaccttaattgaaataaacgaaataaaa
JB-483	ctagtaTCGTGGTTTGTCTGGTCAACCACCGCGGTCTCAGTGGTGTACGGTACAAACCCcgg
JB-508	AGAGATACTGAGCACAAAGCTTAAAGATCTTTTAAGAAGGAGATATACATatgcgtaaagg
JB-509	gtgaTTATTAATGCATgcggccgctcatcatttgtacagttcatccataccatg
JB-510	CTCCTTCTTAAAAGATCTTTAAGCTTGTGCTCAGTATCTCTATCACTG
JB-511	actgtacaaatgatgagcggccgcATGCATTAATAAtcac
JB-512	tttttttgggctagcTACTAGAAAGATCTTTTAAGAAGGAGATATACATatgcgtaaagg
JB-513	CTGCAGCGGCCGCTACTAGTAtcatcatttgtacagttcatccataccatgc
JB-514	gcatATGTATATCTCCTTCTTAAAAGATCTTTCTAGTAgttagcccaaaaaaacgggatg
JB-515	tgaactgtacaaatgatgaTACTAGTAGCGGCCGCTGCAG
JB-518	GGCATGCCTCGAGATGCATG
JB-519	AGGATCCCCGGGTACCGAGC
JB-520	TTGGGGATCGGAATTCGAGCTCGGTACCCGGGGATCCTgaattcgcggccgcttctagag
JB-521	TCAGTTTAGGTTAGGCGCCATGCATCTCGAGGCATGCCctgcagcggccgctactagta
JB-549	ccattcgcattcaggctgcgcaactgttgggaagggcggaattcgcggccgcttctagag
JB-550	gagtgagctgataccgctcgccgcagccgaacgaccgagctgcagcggccgctactagta
JB-551	cgctacagggcgcgctc
JB-552	ccctgattctgtggataaccgt

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