Supporting Information

Rewritable Digital Data Storage in Live Cells via Engineered Control of Recombination Directionality

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Supplementary Methods

Molecular Biology

Plasmids were constructed using standard BioBrick™ (1) or Gibson assembly (2). Coding sequences for BioBrick™ versions of Bxb1 integrase and excisionase and for LR and BP DNA register were synthesized by DNA 2.0 (Menlo Park, CA, USA). Plasmids and parts encoding P_{BAD}/AraC (ref. 33 of main text) (BBa_I0500), Superfolder GFP (3) (BBa_I746916), and the $P_{Ltet-O1}$ (ref. 32 of main text) (BBa_R0040), were obtained from the MIT Registry of Standard Biological Parts (http://partsregistry.org). For the decoupled reset circuit, Bxb1 integrase was cloned on pSB4A5 plasmid (pSC101 origin, 5-10 copies (4) while the excisionase was cloned on J64100 plasmid (regulated ColE1; 50-70 copies). Sequences are available via GENBANK accession numbers JQ929581 to JQ929585 and via the MIT Registry of Standard Biological Parts.

Site specific chromosomal integration

The DNA registers in BP and LR states were integrated into *E. coli* DH5alphaZ1 chromosome (Ref. 32 of main text) using a modified version of the CRIM system (5; St-Pierre F., Cui L., Endy D., and Shearwin K., manuscript in preparation), using phages

HK022 (Fig. 2*B,* Fig. S3) or Phi80 integrases integration sites (Fig. 2*A*, *E*, *F*, *G*; Fig. 3, Fig. 4, Fig S2, S9 and S11). Both integration sites are around the same chromosomal location (25 minutes (5))

Architecture of the DNA data register

The DNA data register in BP and LR states consist of a constitutive promoter (BBa_J23119) flanked by BP or LR recombination sites positioned in opposite orientation, resulting in DNA inversion when recombined (Figs 1 & 2 and Figs S1E and S1F). A Rrnp T1 terminator (BBa_J61048) was added in reverse orientation upstream of the promoter to prevent transcriptional read-through in the opposite orientation, so that in each state, only one fluorescent protein is visibly expressed. On each side of the recombination target, we cloned superfolder GFP and mKate2 (6) under translational control of measured strong RBSs (BIOFAB pilot C-dog project http://biofab.org/data). DNA data registers were first cloned on pSB4A5 low copy plasmid then integrated on the chromosome as described in the material and methods section.

Details on the set and reset circuits and alternate reset architectures

To build the set circuit presented in Fig 2A, Bxb1 integrase was cloned downstream of the PBAD/AraC promoter (BBa_I0500) on pSB3K1 plasmid bearing a p15A origin of replication (15-20 copies). This version of the integrase has a 6-His-tag which we found to stabilize the protein. Therefore, a weak RBS (BBa_B0031) and a LAA ssrA tag was added to reduce the basal expression of the enzyme. For the reset circuit, PBAD controls expression of a polycistron encoding excisionase with a strong RBS designed using the RBS Calculator (7) to have a target Translation Initiation Rate (TIR) of 50000, followed by Bxb1 integrase with a GTG start codon to decrease its TIR (8) and increase the excisionase to integrase ratio. This construct was cloned in a plasmid with a regulated ColE1 origin of replication (J64100, 50-70 copies). We found that another way to match the correct excisionase to integrase ratio was to have a different copy number of each gene, by cloning them on different plasmids with different compatible origins of replication (Fig. S2*A*). We cloned PBAD-Bxb1 integrase (with no 6-His tag and no Ssra tag) on a pSC101 plasmid pSB4A5 (5-10 copies), and transformed it in cells containing the DNA data register in LR state along with pBad-RBS50000-Xis construct cloned in J64100 (excisionase only). We found that cells were able to flip from LR to BP with approximately 85% efficiency (Fig. S2*B*).

Cell culture and circuits operating conditions

Plasmids were transformed in chemically competent *E. Coli* DH5alphaZ1 and plated on LB agar plates containing the appropriate antibiotics. The set circuit presented in Fig 2*B* was transformed in cells containing HK022 integrated BP DNA register bearing

a chloramphenicol resistance cassette. The polycistronic reset circuit, the decoupled reset circuit, and all S/R RAD modules were transformed in cells containing Phi80-integrated BP or LR DNA register bearing a kanamycin resistance cassette. Cells containing the chromosomal DNA data register were grown with an additional 5μg/ml of kanamycin or chloramphenicol depending on the integrated cassette.

Data collection and analysis

Flow cytometry analysis was performed at the Stanford Shared Facs Facility (SSFF). The use of 2 channels increased the resolution of the measurements and was important to analyze the intermediate state resulting from bidirectional flipping. For figures 4*B* and 4*C* of the main text, data were gated by forward and side scatter, and forward scatter vs GFP was plotted.

Generation of a degenerated library of ssrA tagged reset integrases and library screening

We built a screening vector (Fig. S1*C*) containing the excisionase fused to an AAK Ssra tag under the control of the arabinose promoter. A $P_{LtetO-1}$ promoter followed by HindIII and NsiI sites allows for cloning and screening of Atc controlled set circuits. The excisionase gene is followed by AscI and the BioBrick™ suffix SpeI and PstI sites, allowing for cloning of a reset integrase.

The last residues of the Ssra tag of the reset integrase were randomized using a reduced 12 amino-acids alphabet (9) (ndt codons: Phe, Leu, Ile, Val, Tyr, His, Asn, Asp, Cys, Arg, Ser, Gly), therefore reducing the library size to 144 variants with no stop codons while conserving an equal representation of each type of amino-acids. The library was built by amplifying the Bxb1 integrase gene with forward primers containing B0031 RBS and a GTG start codon and reverse primers containing the randomized ssrA tag, and cloning the PCR library into the screening vector in between the AscI and SpeI sites. Ligations were transformed into DH5alphaZ1 cells containing the chromosomal LR DNA register. 384 clones, corresponding to almost 95% coverage of the library (10) were inoculated in supplemented M9 in 96 deep-well plates and grown for 18H at 37C. Cells were then diluted 1:200 in M9 + 0.5% arabinose and grown for another 18H, then analyzed by flow cytometry on a LSRII cytometer equipped with a plate reader. Clones that were showing higher flipping toward the BP state were diluted 1:2000 in M9 without inducer and grown again for 18H, after what they were analyzed by flow cytometry. One of the working reset circuits isolated, termed “C1”, was chosen and combined with “G8” set generator to obtain the G8-C1 RAD module.

Computational design of RBSs using the RBS calculator and screening

We designed 14 different RBSs, using the RBS calculator (7) (https://salis.psu.edu/software/), with Translation Initiation Rate (TIR) values ranging from 1, 5, 10, 20, 50, 100 and 200. We designed 2 different RBSs for each TIR values. Cells were grown without or with Anhydrotetracycline to test for leakiness and capacity to be induced, respectively. The results are presented in Figure S4. DNA inversion was monitored by cloning Gemini (11) a bifunctional reporter containing the alpha-fragment and GFP, downstream of the invertible promoter. Therefore, recombinase activity can be monitored by beta-galactosidase activity. For beta-gal. plate assay, X-Gal was used at a final concentration of 70 µg/mL concentration and IPTG at a 80uM final concentration.

RBS randomization and set circuits screening

The Bxb1 integrase gene was PCR amplified using primers containing a RBS with a randomized Shine-Delgarno sequence and a GTG start codon (cggctttcacac**NNNNNN**gctagc**GTG)** (11), cloned into the screening vector downstream of the $P_{LtetO-1}$ promoter (HindIII/NsiI). Clones were inoculated in supplemented M9 in 96 deep well plates and grown for 18H at 37°C, diluted 1:200 in SM9 containing 20ng/ml Atc, and analyzed by flow cytometry. Screening 288 clones was sufficient to obtain 5 different working set circuits. We picked the circuit termed "G8", which was a good compromise between low basal expression (and thus low spontaneous flipping) and efficient switching.

Mathematical modeling

We used ordinary differential equation (ODE) to describe the dynamical behaviors of RAD modules (Fig. 1*D*, 3*A*, Fig S6, S7, S8) and S/R latches (Fig. S10). ODEs keep track of the levels of recombinases and total DNA register in each state. ODEs were numerically solved using MATLAB © ODE23 solver. We assume that all binding–unbinding reactions are at quasi-steady-states. The quasi-steady-states are updated every time step of ODEs by using MATLAB © fsolve function. For the stochastic simulation (Fig. 2*D*), we only keep track of the number of DNA registers in each state and fluorescent reporters. The stochastic simulation is implemented in MATLAB using Gillespie algorithm.

4. Kinetic model of RAD modules

Integrase-excisionase based RAD module model

The model consists of three components: integrase (I), excisionase (X) and DNA register (D). BP to LR recombination is catalyzed by an integrase tetramer (a complex of integrase dimer binding to attB and attP sites). LR to BP recombination is catalyzed by an integrase-excisionase complex. integrase-excisionase stoichiometry in the complex for Bxb1 is unknown but is assumed to be 1:1. As shown schematically in Fig 1*C*, the dynamics of total register in an LR state, D_{LRtot}, can be written as:

$$\frac{d[D_{LRtot}]}{dt} = -k_c[D_{LR}I_4X_4] + k_c[D_{BP}I_4],$$

when k_c is an inversion rate constant.

Previous biochemical studies also indicated that: 1) Bxb1 integrase can form a dimer in solution, 2) integrase-excisionase cannot form complex in solution, 3) integrase dimer and integrase excisionase complex can bind to all four possible recombination sites (attB, attP, attL and attR). We assume that all complexes are at quasi-steady-state relative to integrase-excisionase production, degradation and DNA recombination. The quasi-steady-state concentration of each complex can be written as:

$$[DI_2X_m] = \frac{[D][I]^2[X]^m}{K_{di}K_i K_{dix}^m}, m = 0, 1 \text{ or } 2,$$

$$[DI_4X_m] = \frac{[D][I]^4[X]^m}{K_{di}^2 K_i^2 K_{dix}^m}, m = 0, 1, 2, 3 \text{ or } 4$$

when K_i , K_{di} and K_{dix} are dissociation equilibrium constants of integrase-integrase dimer, of integrase dimer-recombination site complex and of integrase-excision complex on a recombination site, respectively.

Note that in the default model we assume that the $[DI_4X]$, $[DI_4X_2]$ and $[DI_4X_3]$ are inactive and that integrase-excisionase cannot form a complex without an att site. For the model used for generating Figure S8A-vi, we assume that $[DI_4X]$, $[DI_4X_2]$ and $[DI_4X_3]$ can undergo bidirectional inversion. In other words,

$$\frac{d[D_{LRtot}]}{dt} = -k_c[D_{LR}I_4X_4] + k_c[D_{BP}I_4] - k_c[D_{LR}I_4X_m] + k_c[D_{BP}I_4X_m], \quad m = 1, 2 \text{ or } 3.$$

For the model used for generating Fig. S8A-vii, we assume that there also exist the following integrase-excisionase complexes in cytosol,

$$[I_2X_m] = \frac{[I]^2[X]^m}{K_i K_{dix}^m}, m = 1 \text{ or } 2.$$

Dual recombinase RAD module model

The dual recombinase RAD module model (Fig. S10A) consists of three components: recombinase-1 (R1), recombinase-2 (R2) and DNA register (D). BP to LR and LR to BP recombination are catalyzed by R1 tetramer and R2 tetramer, respectively. We assume that R1 and R2 bind to an att site as dimer; only one dimer can bind to each att site at a time; $R1_2$ and $R2_2$ can bind to both states of the register. Putting together, the system is governed by the following system of equations.

$$\frac{d[D_{LRtot}]}{dt} = -k_c[D_{LR}(R1)_4] + k_c[D_{BP}(R2)_4],$$

$$[D(R1)_2] = \frac{[D][R1]^2}{K_{di}K_i}, \qquad [D(R2)_2] = \frac{[D][R2]^2}{K_{di}K_i},$$

$$[D(R1)_4] = \frac{[D][R1]^4}{K_{di}^2K_i^2}, \qquad [D(R2)_2] = \frac{[D][R2]^4}{K_{di}^2K_i^2},$$

$$[D(R1)_2(R2)_2] = \frac{[D][R1]^2[R2]^2}{K_{di}^2K_i^2},$$

where k_c is an inversion rate constant and K_i , and K_{di} are dissociation equilibrium constants of recombinase dimer and recombinase dimer-recombination site, respectively.

Mutual-inhibition S/R latch model

Binary state could be stored epigenetically using a bistable gene regulator network, for example, a system of two mutually repressing genes (Ref. 2 of the main text). The network can be set and reset simply by adding external inducers (IPTG, aTc, heat shock, etc.) that can inactivate one of the two repressors, allowing the other repressor to express.

In order to make this system modular with respect to inputs, i.e., capable of storing arbitrary transcriptional input signal, one could have an extra copy of each repressor gene driven by external transcriptional input signal (Fig S10B). This way, a pulse of generic transcriptional signal can switch the system state by temporary increasing the production of one repressor over the other. Such input modularization has been demonstrated, albeit for only a set circuit (12).

The mutual inhibition S/R latch model presented here ((Fig S10*B*) consists of repressor R1 and R2 mutually repressing each other expression and an extra copy of R1 and R2 driven by set input and reset input, respectively. Assuming that repressors bind to their cognate operator sites as tetramer (as we assume for recombinases), the dynamics of R1 and R2 concentrations can be written as:

$$\frac{d[R1]}{dt} = \alpha_{set} + \frac{\alpha K_d^4}{K_d^4 + [R2]^4} - \gamma [R1],$$

$$\frac{d[R2]}{dt} = \alpha_{reset} + \frac{\alpha K_d^4}{K_d^4 + [R1]^4} - \gamma [R2],$$

where α_{set} and α_{reset} are production rate of R1 and R2 from input set and input reset, respectively; α is the maximal production rate of R1 and R2 from their repressible promoters. K_d is a dissociation equilibrium constant between each repressor and its cognate repressible promoter. Υ is a degradation rate of each repressor.

Parameter non-dimensionalization and efficiency measurements

While exact kinetic parameters are unknown, we can still understand the general features of the latch behaviors by nondimensionalizing all concentration and time units, here, in term of K_i and k_c^{-1} respectively. Our default parameter set has $K_{di} = K_{dix} = 1\ K_i$; degradation rate constants of integrase and excisionase, Υ_i and Υ_x, equal to 1 k_c; the default expression scaling for integrase and excisionase, β_i and β_e, are equal to 1 $K_i k_c$. Basal integrase or excisionase production rates are $0.1\,\beta_i$ and $0.1\,\beta_e$, respectively; induced excisionase production rate during reset and induced integrase production rate during set or reset are $10\,\beta_e$ and $10\,\beta_i$, respectively (i.e., fold change, F_c, from basal production = 100). Total DNA register concentration is 1 (in K_i unit).

For a mutual inhibition S/R latch, we have repressor binding dissociation equilibrium constant , K_d = 1; basal and induced production of each repressor from set and reset promoter = 0.1 $K_{i2}k_c$ and 10 $K_{i2}k_c$, respectively. The maximal production of each repressor from the repressible promoter is 10 $K_{i2}k_c$. "Set (reset) efficiency" is defined as the fraction of the register that can be switched from BP to LR (LR to BP) state after inducing with a set (reset) pulse for 200 k_c^{-1} and also can hold state for at least 200 k_c^{-1} after the pulse ends. "set-reset efficiency" is defined as the product of set and reset efficiency. For a mutual inhibition SR-latch, we use the ratio between [R1] and [R1]+[R2] instead of [BP] fraction.

We studied how the efficiency of the latch changes as we tune basal integrase and excisionase (or R1 and R2) concentrations while keeping the fold change constant. In Fig

3, we scale steady-state concentrations of Int and Xis by tuning Υ_i and Υ_x. For our default modeling parameters and conditions, scaling proportionally scaling basal and induced production also gives similar results.

General features of RAD module operable range and modes of failure

One could think of a RAD module as a device with two elements: state storage and input interface elements. The state storage element allows the latch to maintain the state; this includes a DNA register of our DNA inversion RAD module or a bistable mutual inhibition circuit of a mutual inhibition S/R latch. A DNA register encodes a state in a DNA sequence which is naturally maintained and replicated inside living cells; a mutual inhibition circuit encodes a state as repressor concentration which can be maintained through a feedback loop. The input interface element allows external inputs (transcriptional signals for examples presented here) to perturb and change the state of the state storage element. This includes the integrase-excisionase genes (or dual recombinases) for our DNA inversion RAD module or extra copies of repressor genes driven by inputs, for a mutual inhibition S/R latch.

A challenge in implementing a RAD module is to properly "map" the dynamic ranges of the external input of interest, via the input interface element, to the state phase of the state storage element. In this study, such mapping is represented as the expression scaling parameter β. Physically, β is proportional to translation rate and inverse proportional to protein degradation rate. When the scaling parameter for integrase, β_i, is large, even low transcriptional set or reset signal is mapped to high integrase concentration (the upper part of Fig. 1*D*). If β_i is too large, even basal transcriptional signal set or reset signal results in too much integrase for the RAD module to hold the state. On the contrary, if β_i is too small, transcriptional signal during a set or reset will not give enough integrase for the RAD module to set or reset. The same idea holds true for a mutual inhibition S/R latch: scaling parameter for the repressor from input interface element must be low enough to allow the state storage element to remain bistable in the absence of an input pulse and high enough to cause the loss of bistability in the presence of an input pulse.

Another challenge for implementing a RAD module is to optimize two antagonizing mechanisms, the set and the reset mechanisms, within the same chassis. Optimal conditions for resetting of the integrase-excisionase based S/R latch, for example, having a stable and efficiently translating excisionase would be likely to have so high excisionase basal expression that can interfere with the setting mechanism.

In general, the size of the operable range with respect to the scaling parameter will be proportional to the fold changes between the basal input level and the input pulse. If the fold change is small, one needs to precisely match the basal input level to state

storage regime and the induced input level to the state switching regime. Thus, the operable range will be narrow (Fig. S8*A*, iv).

Features of integrase-excisionase based RAD modules operable range

Let's first consider switching efficiencies from BP to LR state during a long set pulse and from LR to BP state during a long reset pulse (Fig 3*A*, top row). Both set and reset operable ranges have a lower bound of integrase expression level corresponding to the induced integrase level that is "enough" for efficient recombination. For a set, at the limit of low excisionase expression, the rate of BP to LR recombination is governed by the level of $[DI_4]$, which can be switched to LR state, relative to $[D]$ and $[DI_2]$ which cannot:

$$\frac{[DI_4]}{[D] + [DI_2]} = \frac{\frac{[I]^4}{K_{di}^2}}{1 + \frac{[I]^2}{K_{di}}},$$

$$[I_{tot}] - 4 * [D_{tot}] < [I] < [I_{tot}],$$

*all concentrations are normalized with respect to K_i.

If $[I_{tot}]$ during set is small compared to the square root of K_{di} then the ratio between $[DI_4]$ and $[D] + [DI_2]$ become small and thus setting is inefficient. Therefore, we would expect that the integrase expression lower bound for set to scale with a square root of K_{di}. For a reset, interestingly, the lower bound of integrase expression is somewhat lower than that for set. This is because excisionase binding could help pushing the complex equilibrium toward $[DI_4X_4]$ complex.

Excisionase determines directionality of state switching. Too high basal excisionase expression will break a set. On the contrary, too low induce excisionase expression will break a reset. Let's consider how much excisionase expression scaling will allow both efficient set and reset. The net BP to LR and LR to BP recombination rates depend on the relative amount of the active recombination complex, $[DI_4]$ and $[DI_4X_4]$, which, in turn, depends on excisionase level:

$$\frac{[DI_4]}{[DI_4X_4]} = \frac{K_{dix}^4}{[X]^4}$$

$$[X_{tot}] - 4 * [D_{tot}] < [X] < [X_{tot}]$$

If basal [X] during set is significantly larger than K_{dix}, $[DI_4]/[DI_4X_4]$ will become very small and set will not work. In Fig. 3*A*, basal excisionase level is $0.1\beta_e$ so we would expect to see the upper bound of excisionase level for set operable range at $0.1\beta_e \sim K_{dix}$.

On the contrary, if induced [X] during reset is significantly smaller than, $[DI_4]/[DI_4X_4]$ will become very large and reset will not work. In Fig. 3*A*, induced excisionase level is $10\beta_e$ so we would expect to see the lower bound of excisionase level for reset operable range at $10\beta_e \sim K_{dix}$. Note that since excisionase can only bind to integrase on the recombination site but not free integrase, no matter how much integrase we express, the lower limit of [X] will not go below $[X_{tot}] - 4*[D_{tot}]$. Therefore, the upper bound of excisionase expression for a set operable range and the lower bound of excisionase expression for reset operable range will be almost independent of integrase level. However if we have large amount of $[D_{tot}]$ or allow integrase-excisionase binding in cytosol, the [X] will also depend on how much integrase we have and this lower bound will increase as integrase level increases (Fig. S8A, v and vii).

Now, consider switching efficiency after the input pulse is gone (Fig 3*A*, 2nd to 4th rows). Reset operable range has an additional bound: the upper bound of integrase production which is not enough for causing spontaneous BP to LR recombination at the end of the reset pulse (Fig. S6). Reset becomes inefficient if $[I_{tot}] > [X_{tot}]$ during the reset pulse because, at the end of the pulse, excisionase will disappear first and thus left over integrase will drive BP state register back to LR state again. Note that this upper bound is not simply a straight line $[I_{tot}] = [X_{tot}]$; once there is enough basal excisionase to bind to all integrases on att sites, BP to LR switching become very inefficient, regardless of how much integrase we have in the system. Therefore, this upper bound sharply rises asymptotically toward the excisionase upper bound of the set operable range described above. When $[D_{tot}]$ is large or Int-Xis can form complex in the cytosol, this the upper bound becomes a diagonal line $[I_{tot}]=[X_{tot}]$.

The set operable range after an input pulse remains almost the same as during an input pulse. The only difference is a small notch locating approximately where $[I_{tot}]= [X_{tot}]$ line cross the excisionase upper bound of the set operable range (Fig. S8). At this point, there is enough basal integrase expression and basal excisionase expression to cause LR to BP switching in the absence of a pulse.

The sharp boundary between operable and non-operable regions of the set or the reset arises from the fact that four integrase monomers act cooperatively to catalyze BP to LR recombination and four excisionase monomers act cooperatively to enable LR to BP recombination. Cooperative effect is particularly strong because we assume inactive intermediate complex (a DNA register with integrase tetramer and 1-3 excisionase monomers can neither undergo BP to LR nor LR to BP recombination). If we instead assume that these intermediate complexes can undergo both BP to LR and LR to BP recombination, then the boundary of the operable range become smoother (Fig. S8A, vi).

Operable range features of S/R latches with alternative mechanisms

Similar to integrase-excisionase RAD module based S/R latch, operable ranges of dual recombinase RAD module based S/R latch or mutual inhibition S/R latch with respect to input element expression scaling are constrained by (Fig. S10): 1) the expression scaling lower bound that is large enough to allow state change during a pulse, and 2) the expression scaling upper bound that small enough to not allow spontaneous state switching in the absence of an input pulse. Operable range size, i.e., the distance between the lower and the upper expression scaling bounds is approximately the fold changes between the induced and the basal input levels.

Rectangular operable range shape results from the fact that the set and the reset mechanism for the dual recombinase RAD module (or for the mutual inhibition S/R latch) do not directly interacting with each other and that there is no loss of operable regime due to stoichiometry mismatch. Note that for mutual inhibition S/R latch, there is a sharp transition between efficient and inefficient set or reset operable range due to bistability of the system.

Simulated operable range with respect to DNA register and integrase-excisionase gene copy numbers

Simulated RAD operable range can recapitulate experimentally observed dependence between the copy number of DNA register, the copy number of integrase-excisionase genes and S/R latch efficiency (Fig S3*A* and S3*B*). Specifically, increasing the copy number of DNA register relative to the copy number of integrase excisionase genes decreases resetting efficiency. Parameter setting used in the simulation shown here is the same as that of the default parameter setting except for that excisionase production rate during reset is reduced to only half of integrase production rate. At the limit of high DNA register copy number, the amount of integrase-DNA register complexes approaches the total amount of integrase. If total integrase outnumbers total excisionase, there are too many integrase-DNA register complexes for excisionase to bind to and thus BP to LR recombination cannot be suppressed completely. At the limit of low DNA register copy number, the amount of integrase-DNA register is limited by the number of total DNA register. Thus, although total excisionase is less than total integrase, there could still be enough excisionase to bind to all integrase-DNA register complexes, allowing for complete suppression of BP to LR recombination and thus efficient resetting. If we change the model assumption to allow for integrase-excisionase complex formation in the absence of DNA register, reset efficiency is no longer sensitive to the copy number of DNA register (Fig S3*C*). Therefore, the fact that reset efficiency is sensitive to DNA register copy number supports a prior finding that excisionase only binds to integrase-recombination site complex but not to free integrase (Ref. 27 of main text).

Note that varying DNA register and integrase-excisionase gene copy number also has other side effects. Lowering integrase-excisionase copy number relative to DNA register copy number means that we reduce the amount of enzyme (integrase or integrase-excisionase complex) relative to the amount substrate (DNA register). Thus, state switching, both set and reset, will need more time to complete which could explain why both set and reset become inefficient (Fig S3*B* and S3*C*, lower right corner). On the contrary, increasing integrase-excisionase gene copy number results in higher basal expression levels of integrase and excisionase, leading to spontaneous state switching. Thus, state storage becomes inefficient (Fig S3*B* and S3*C*, bottom row, upper left corner).

Stochastic simulation of bidirectional DNA inversion

In Fig. 3*B* of the main text, we present the failure mode of a RAD module in which each cell in the population expressing both GFP and RFP during an input pulse and then splitting into two populations of cells expressing either GFP or RFP after the pulse. We could explain this observation using a stochastic simulation of bidirectional DNA inversion. The model consists of a single copy DNA register which can be in either state 0, expressing GFP, or state 1, expressing RFP. We simulate the scenario in which the net propensity for inverting from state 0 to state 1 and from state 1 to state 0 are equal. This scenario corresponds to the region between the set and the reset regime in Fig. 1*D*. We also assume that the degradation propensity of the both reporters is ten times slower than the inversion propensity (We expected that in our experimental system reporter kinetics is slow because GFP and RFP are stable). During an input pulse, bidirectional inversion occurs so fast relative reporter kinetics that cells appear to have both reporters expressing although its DNA register can be in either state 0 or state 1 at any given moment. After an input pulse, DNA inversion stops and half of the population will stochastically end up in state 0 and the other half in state 1.

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Supporting Figures

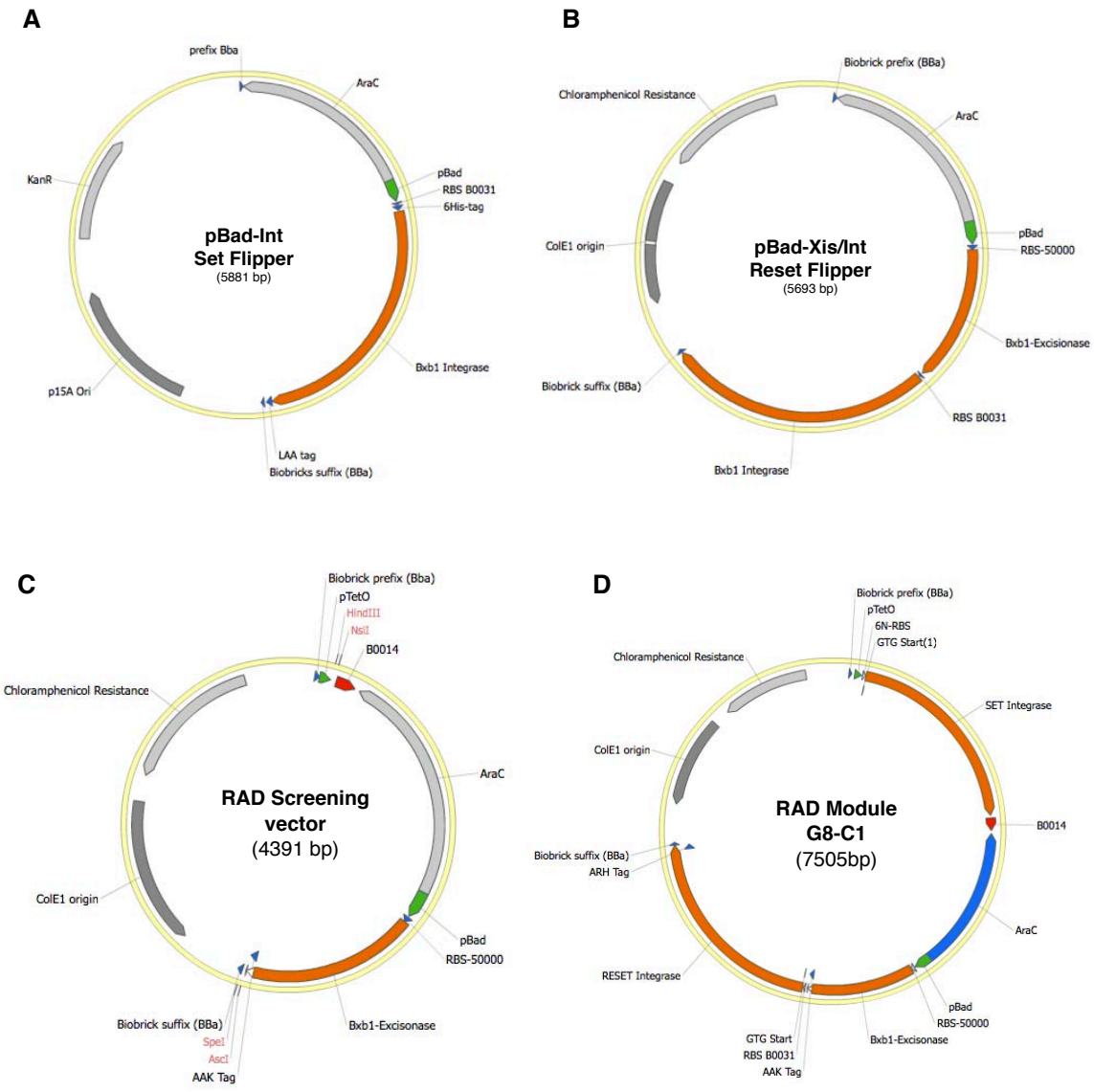
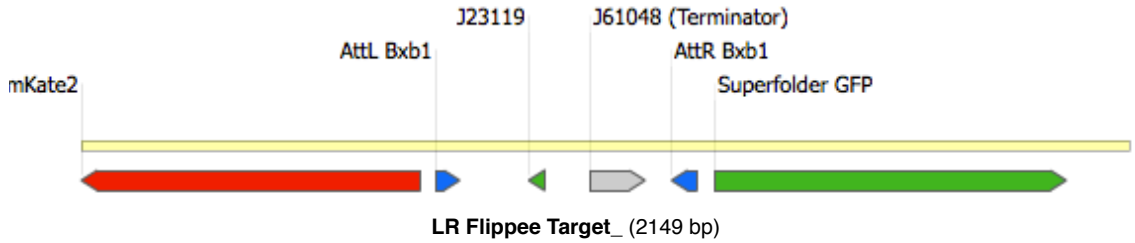


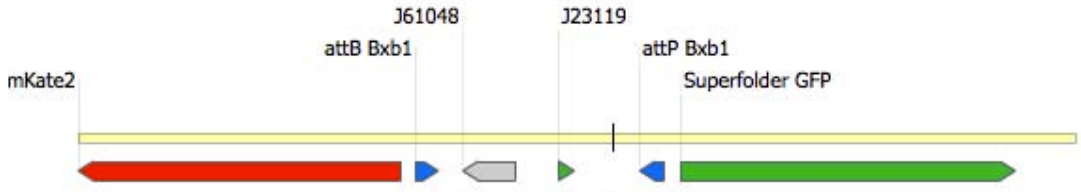
Figure S1: Maps of the principal constructs used in this study.
A, The PBAD-Int set flipper where Bxb1 integrase was cloned downstream of the PBAD/AraC promoter (BBa_I0500) on pSB3K1 plasmid bearing a p15A origin of replication (15-20 copies). **B**, The PBAD-Xis/Int reset flipper circuit. **C**, The screening vector and **D**, The RAD module depicted in Fig. 4*A*.

E



LR Flippee Target_ (2149 bp)

F



BP Flippee Target_ (2149 bp)

Figure S1 (continued): E and **F**: Detailed architecture of the DNA data register in state 0 (BP) and state 1 (LR) (see SI text). Maps were generated using the JBEI (Joint Bio-Energy Institute , Emeryville, CA) online vector editor (http://j5.jbei.org/VectorEditor/VectorEditor.html).

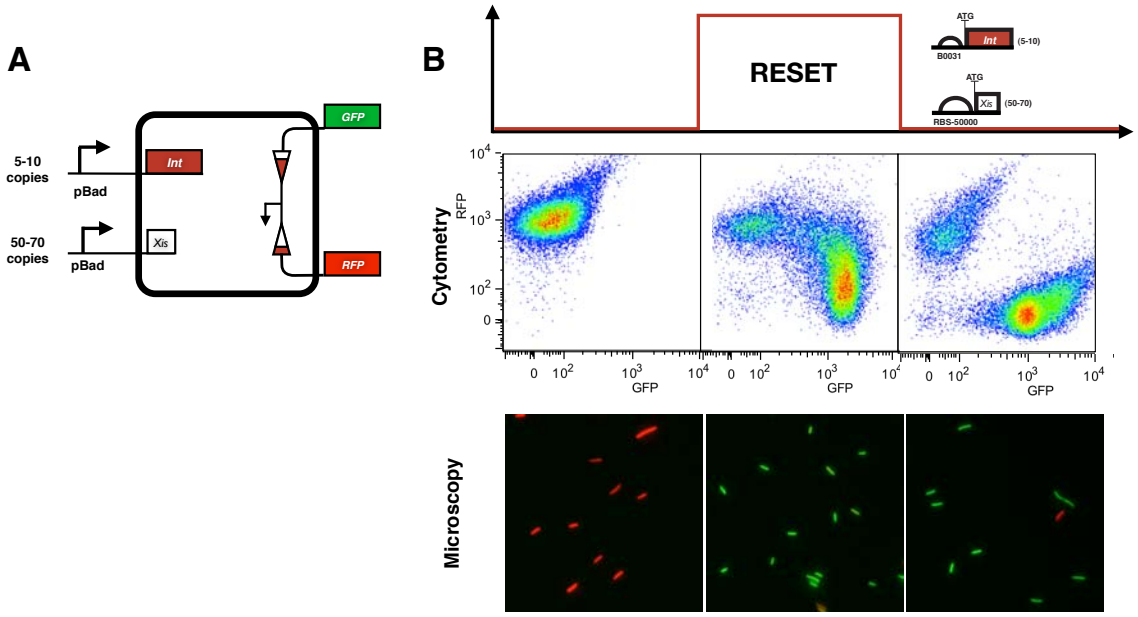


Figure S2: Alternate architecture for a reset circuit.
A, Schematic diagram of the decoupled reset circuit where integrase is expressed from a low-copy plasmid while excisionase is expressed from a medium-copy plasmid. **B**, Cells bearing the chromosomal LR DNA register were transformed with both plasmids encoding integrase and excisionase, pulsed with arabinose and analyzed by flow cytometry. Cells relaxed to the BP state after induction with approximatively 85% efficiency.

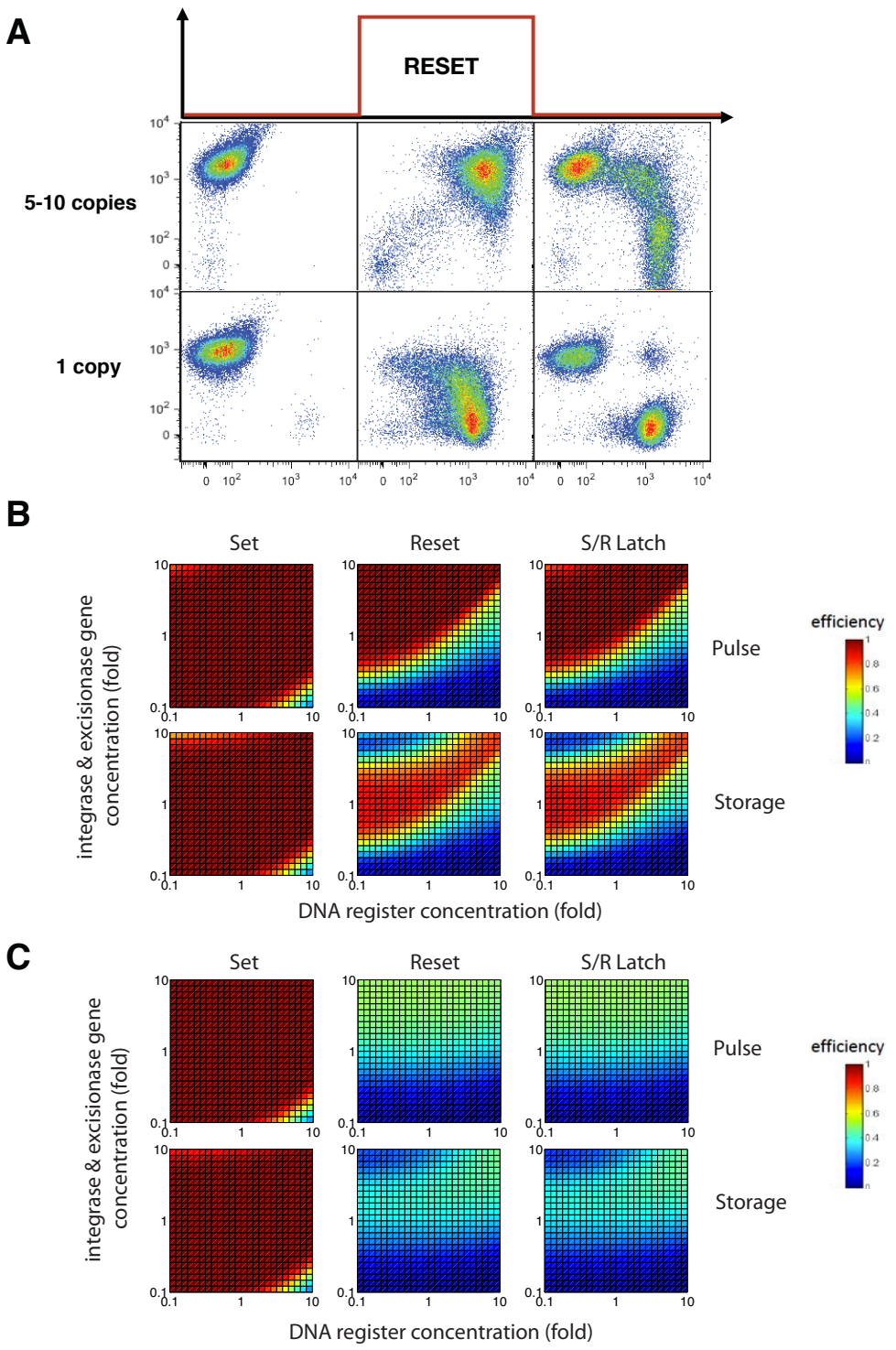


Figure S3: Influence of register copy number on recombination efficiency and consequences for integrase-excisionase mechanism**. A,** Influence of copy number of the DNA register on the efficiency of integrase-excisionase mediated recombination. The bi-directional reset generator form Fig2*C* was transformed in cells containing the DNA data register in the LR state on a pSC101 plasmid

(upper panel) or integrated in the chromosome (lower panel), and cells were pulsed with arabinose. When the register was on the chromosome, cells were driven toward the BP state more efficiently during induction, and the recombination efficiency of the Int/Xis reaction for LR to BP reaction was higher after inducer removal. Part of the cells with a DNA register on a plasmid stayed in the intermediate state after inducer removal, due to the presence of various copies of the DNA register in different states within the same cell. These data support the biochemical studies suggesting that excisionase interacts preferentially with DNA-bound integrase (Ref. 27 of main text) as we assumed in our kinetic model. **B, C,** Simulated operable range of set, reset, and set/reset function, during and after a pulse, with respect to variations in register copy number and flipper copy number, using 2 different mechanistic assumptions for integrase-excisonase recombination. in **B,** we assume that excisionase interacts with DNA bound integrase only. in **C,** we assume that integrase and excisionase can interact in the cytosol (see SI text for details).

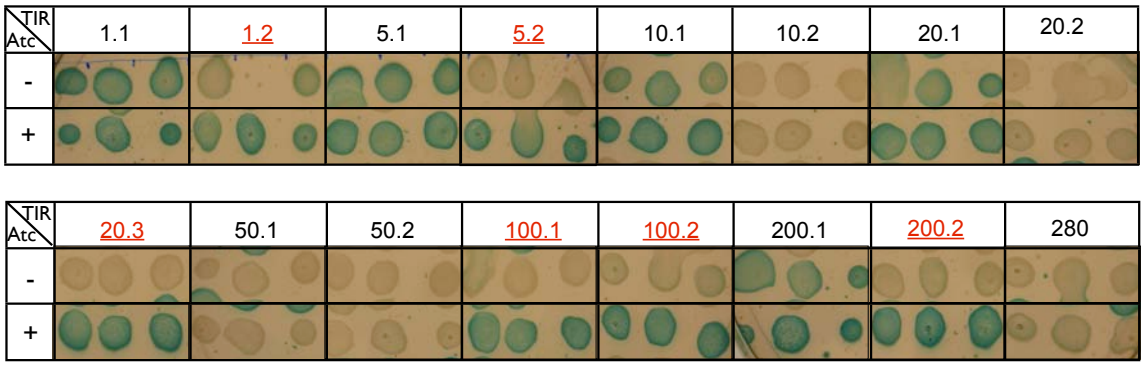


Figure S4: Screening of different RBS designed using the RBS calculator (7). Constructs were tested using a DNA register that expresses gemini (10) when flipped. Cells were co-transformed with the target and the different RBS variants constructs. Cells were grown with or without Anhydrotetracycline (Atc) to test for leakiness and capacity to be induced, respectively. Cultures were spotted after induction on plates supplemented with X-Gal plus IPTG and beta-galactosidase activity was observed. Target TIR (Translation Initiation Rate) values entered in the forward engineering mode of the RBS calculator are indicated on top rows. Constructs displaying the required characteristics (non-leaky and inducible) are highlighted in red. Many designs expected to exhibit the same quantitative performance produced qualitatively different results. Such differences are likely due to three coupled factors: i) because the recombinase enzyme is very active, a small level of spontaneous basal expression can switch the target DNA, ii) we used the RBS calculator to target extremely low TIRs, likely operating near a limit of the algorithm in terms of its design range, iii) regardless, as published, the calculator is expected to only have a ~47% chance of producing a specific designed RBS that produces a TIR to within a factor-of-two target range.

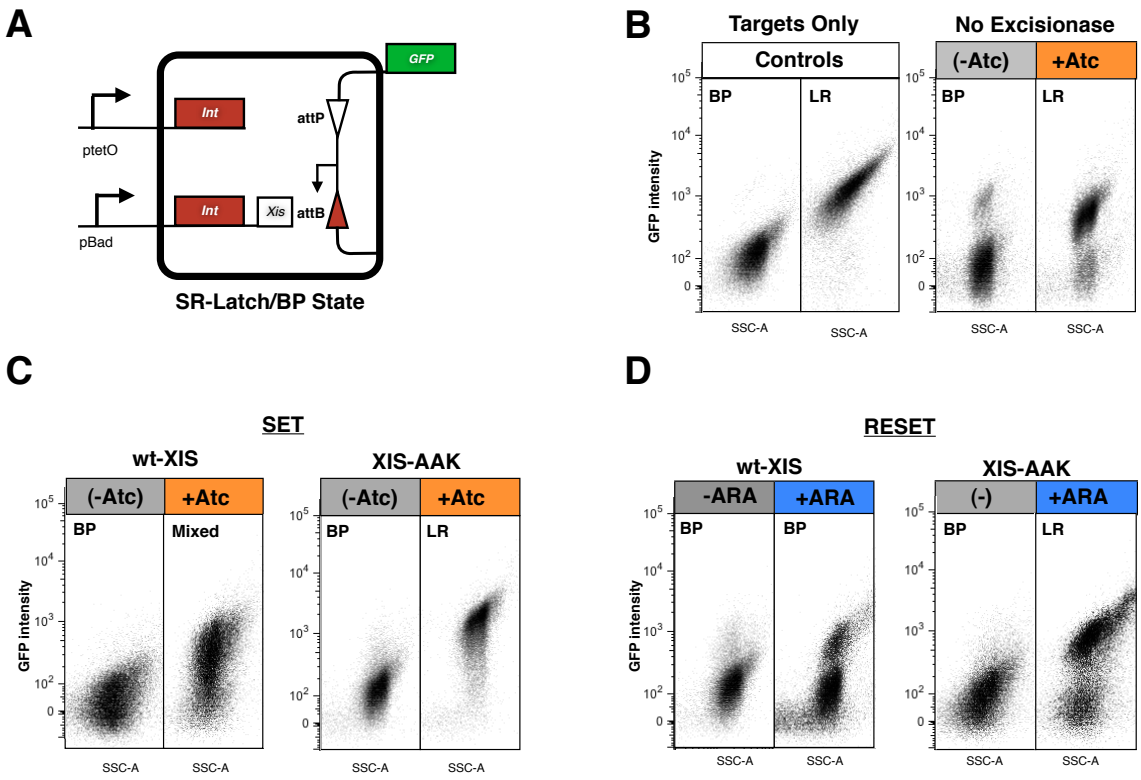


Figure S5: Effect of the down-regulation of excisionase on set and reset functions. **A**, schematic representation of the RAD module used for this particular experiment. Here the DNA data register has only one output, GFP, in the LR state. The $P_{Ltet\text{-}O\text{-}1}$ promoter controls the set integrase while the P_{BAD} promoter controls a polycistron expressing integrase and excisionase. Both set and reset circuits are cloned on pSB3K1 plasmid (p15A origin, 15-20 copies). **B**, control experiments. *Left panel*: BP and LR Target constructs on pSB4A5 low-copy plasmid (5-10 copies), showing the 2 states of the system (low or high GFP for BP and LR, respectively). *Right panel*: a RAD module with no copy of the excisionase was transformed in cells containing the DNA data register in the BP state on pSB4A5 and the set generator was induced with Atc. Cells flipped to the LR state as monitored by GFP expression. **C**, reduction of interference by down regulation of excisionase basal levels. While inducing the set led to entry into an intermediate state in presence of the wild type excisionase (wt-Xis, left panel), addition of a AAK ssrA tag to the excisionase restored the set function (Xis-AAK, right panel). **D**, down-regulation of excisionase breaks the reset circuit. Arabinose induction of a reset generator containing wt-excisionase (wt-Xis, left panel) shows little flipping from BP to LR, due to excisionase mediated inhibition of integrase activity toward the BP DNA register. However, a reset generator in which the excisionase is down-regulated with an AAK ssrA tag (Xis-AAK, right panel) acts as a set generator and can flip from BP to LR due to stoichiometry mismatch and higher integrase levels.

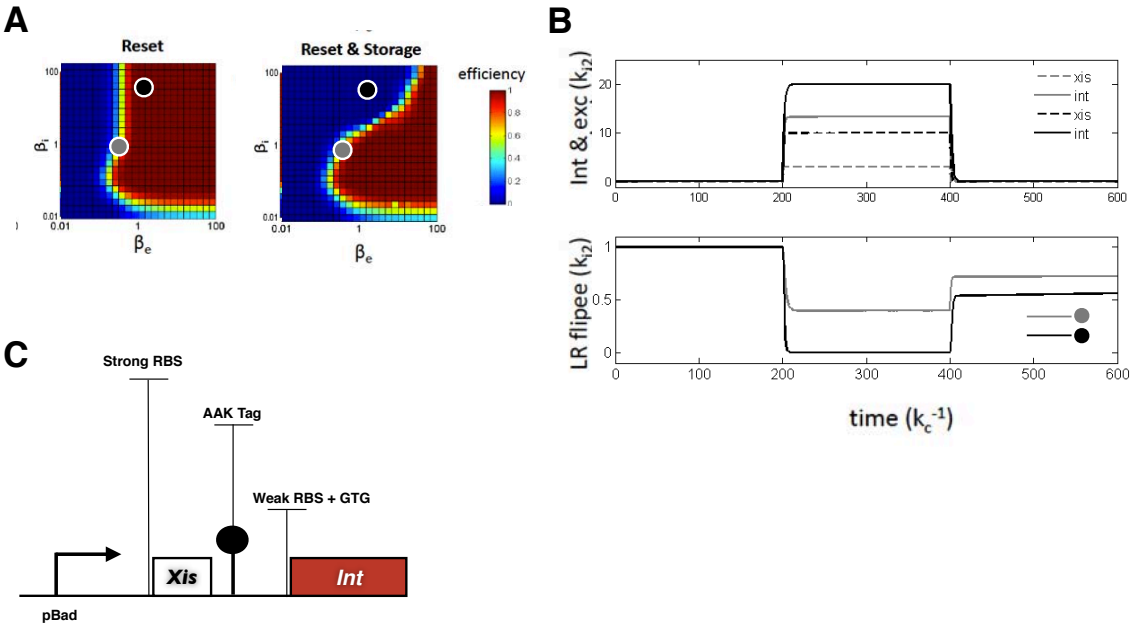


Figure S6: Example of an efficient reset generator setting-back after the end of a pulse. **A,** Kinetic model based simulation of the reset efficiencies during and after the pulse for different expression scaling. A gray dot marks an integrase–excisionase expression scales that keeps the latch in an intermediate state both during and after a RESET pulse; a black dot marks an expression scale that causes the latch to revert back to LR state after a reset pulse. **B**, Time-course simulation showing resetting failures during and after a RESET pulse. Gray and black lines are simulated using integrase and excisionase expression scaling parameters as marked with gray and black dots in (**A**). **C,** Schematic representation of the particular construct used in Fig 3*C* of the main text, in which excisionase has a strong RBS and AAK degradation tag while integrase has a very weak RBS (BBa_B0033) followed by a GTG start codon. Therefore, even if the stoichiometry between the two proteins is correct during the pulse, the degradation rate of excisionase is higher than for integrase, resulting in entry into the set regime after the pulse.

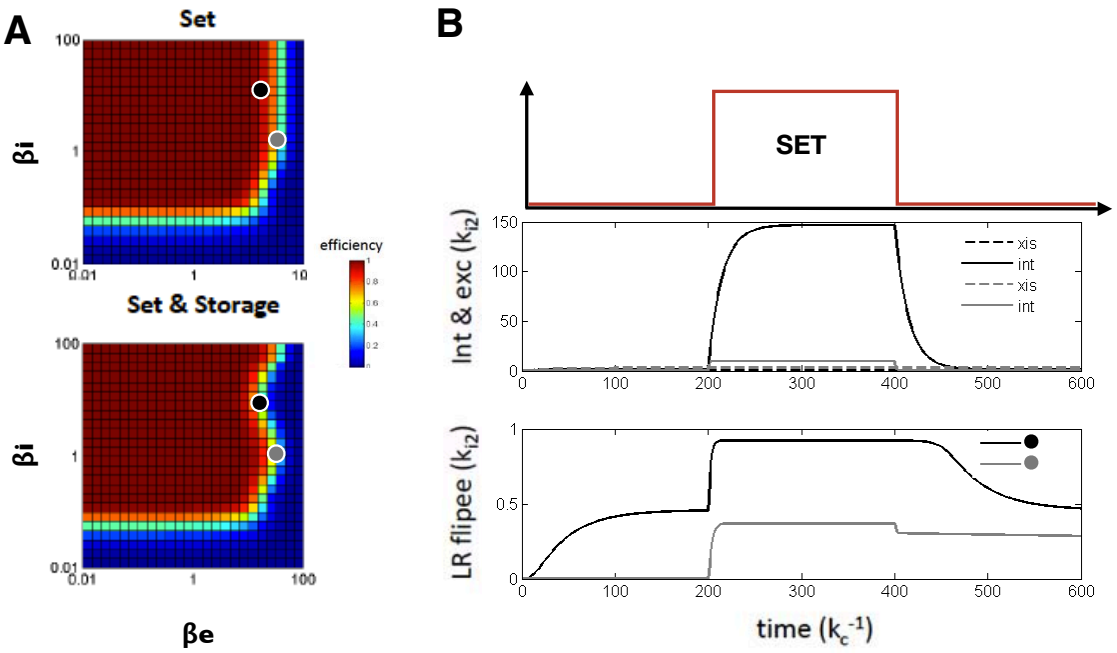


Figure S7: Example of a set circuit resetting back after the end of a pulse. **A,** Kinetic model based simulation of the reset efficiencies during and after the pulse for different expression scaling. A gray dot marks an integrase–excisionase expression scales that keep the latch in an intermediate state both during and after a set pulse; a black dot marks an expression scales that cause the latch to revert back to BP state after a SET pulse. **B**, Time-course simulation showing setting failures during and after a RESET pulse. Gray and black lines are simulated using integrase and excisionase expression scaling parameters as marked with gray and black dots in (**A**). Note that for the black line simulated condition, the basal expression levels of integrase and excisionase are both high enough so that the DNA register quickly reaches an intermediate BP-LR state even before an input pulse arrives.

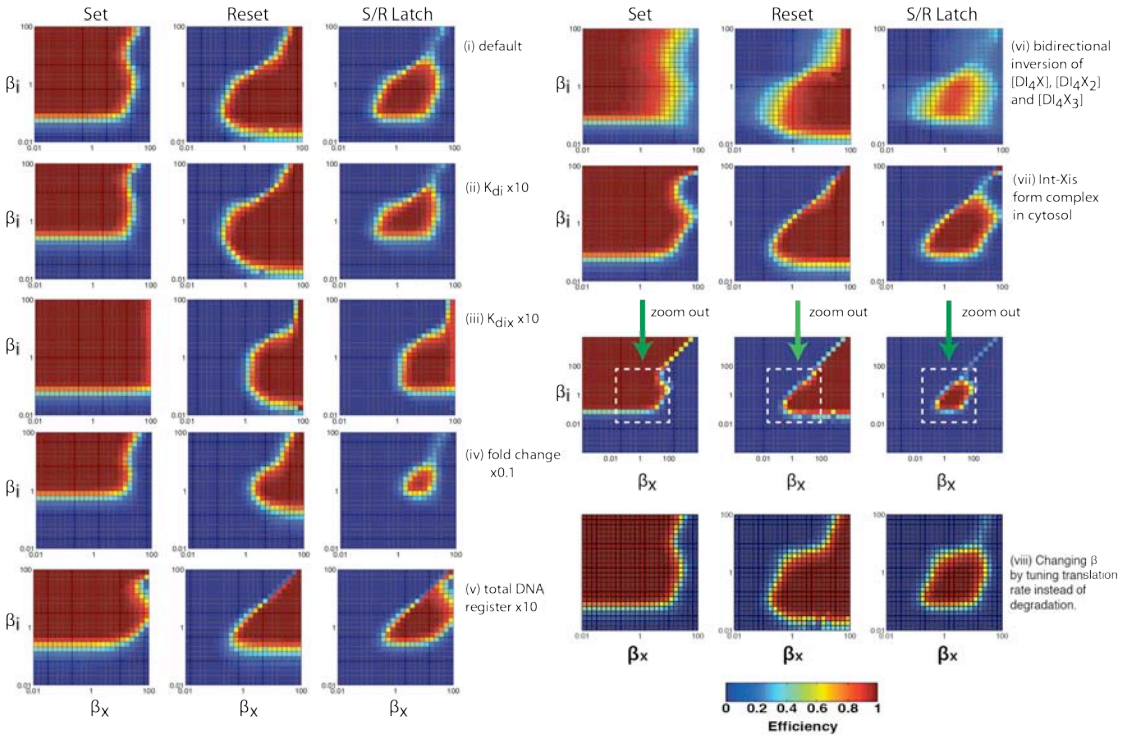


Figure S8: Detailed parameter sensitivity analysis of DNA inversion RAD module operable range. **(i),** Kinetic model based simulation of the set, reset and SR-latch efficiencies after the pulse for different expression scaling and kinetic parameters. The integrase lower bound of the operable range scales with integrase-flipper dissociation constant **(ii)**, the excisionase upper bound of the set operable range and the excisionase lower bound of the reset operable range scale with integrase-excisionase dissociation constant **(iii)**, the excisionase lower bound of the reset operable range and the integrase lower bound of the set and the reset operable range scale with the fold change between induced and basal expression **(iv)** increasing the amount of DNA register changes the excisionase upper bound of set operable range and the excisionase lower bound of reset operable range. With higher DNA register concentration, these bounds become more sensitive to integrase level even at high excisionase level (**v**), allowing incomplete integrase-excisionase complex to catalyze bidirectional recombination broadens the parameter ranges for partially switching latch **(vi)**, allowing integrase–excisionase to form complexes in the cytosol changes the set and the reset circuits operable range in a similar way to increasing DNA register concentration **(vii),** under this assumption, the set operable range can be extended to arbitrarily high excisionase level by increasing integrase level. **(viii)**, Changing the scaling factor ß by altering proteins translation rates instead of proteins degradation rates does not give a qualitatively different operable range in our default scenario.

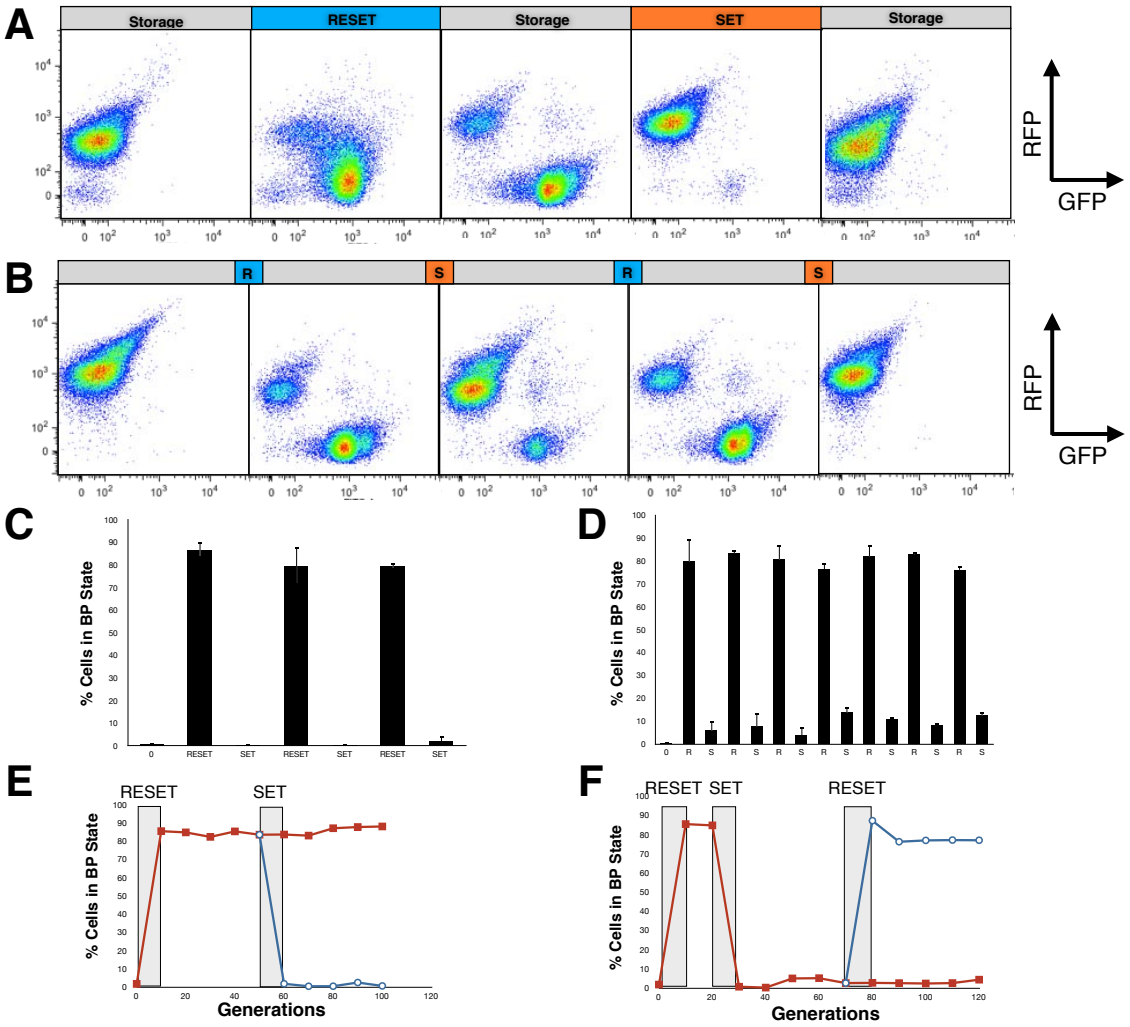


Figure S9: Detailed data for the RAD module operation cycles. **A** and **B**: Typical RFP/GFP plots for switching and storage cycle for long and short pulses, respectively. **C**, Quantification of switching plus storage efficiency of the RAD module for long input cycles. **D**, Quantification of switching plus storage efficiency of the RAD module for short input cycles. (**C)** and (**D)**: error bars are the SD of 2 experiments performed in triplicates **E**, long term storage of the BP state in the context of the RAD module. Cells in the LR state were RESET with arabinose, switched and stored the BP state. After 40 generation, cells were SET back with Atc (blue line) or grown without inducer up to 100 generations of storage (red line). **F**, long term storage of the LR state in the context of the RAD module. After one cycle of RESET with arabinose and SET with Atc, cells were grown for 40 generations, RESET with arabinose or grown without inducer up to 100 generations. In Both (**E**) and (**F**), switching plus storage efficiency was comparable with the initial efficiencies.

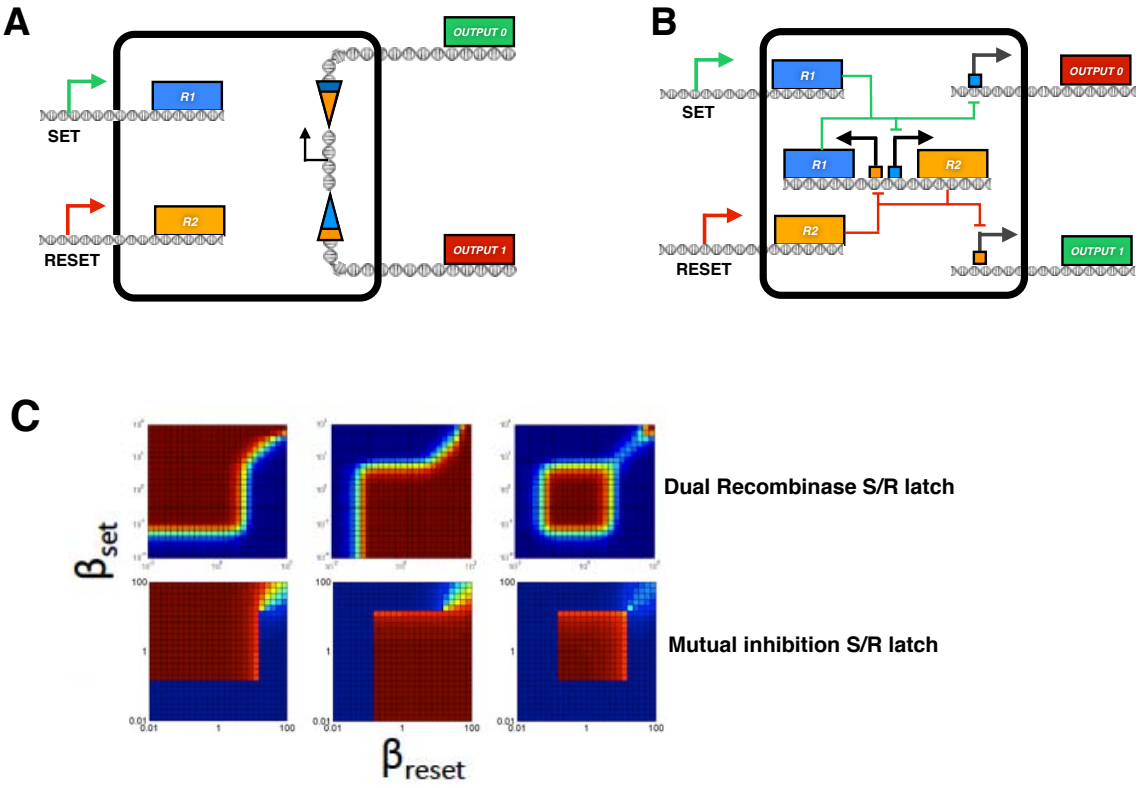


Figure S10: Comparison of operable ranges of S/R latches using alternative mechanisms. **A,** Schematic diagram of an hypothetic S/R latch based on a DNA inversion RAD module whose DNA register can be inverted and reverted by two different recombinase, Rec1 and Rec2 respectively. **B**, Schematic diagram of a mutual inhibition S/R-latch. A pair of mutually repressed genes functions as a bistable switch; an additional copy of each gene, driven by SET or RESET inputs, is necessary to couple arbitrary transcriptional signals to the state of the bistable switch. **C**, Kinetic model based simulation of the set, reset and SR-latch. Note that the operable range for set and reset circuits are symmetric and there is no efficiency loss due to stoichiometry mis-match as in the case of integrase-excisionase based DNA inversion latch (see SI text).

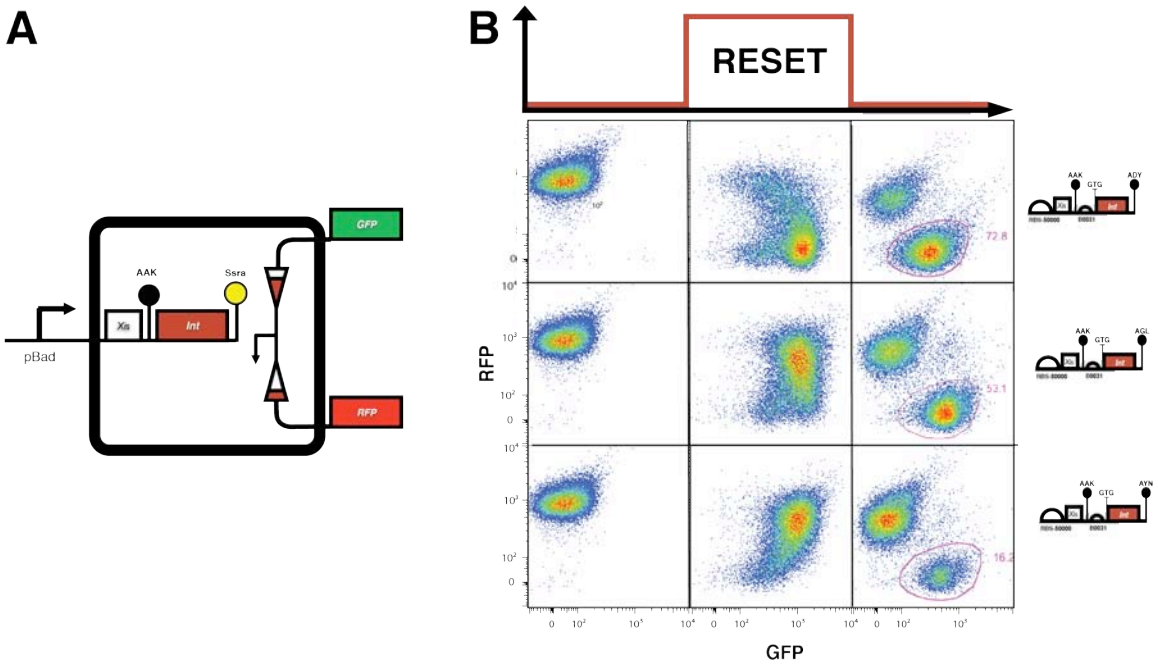
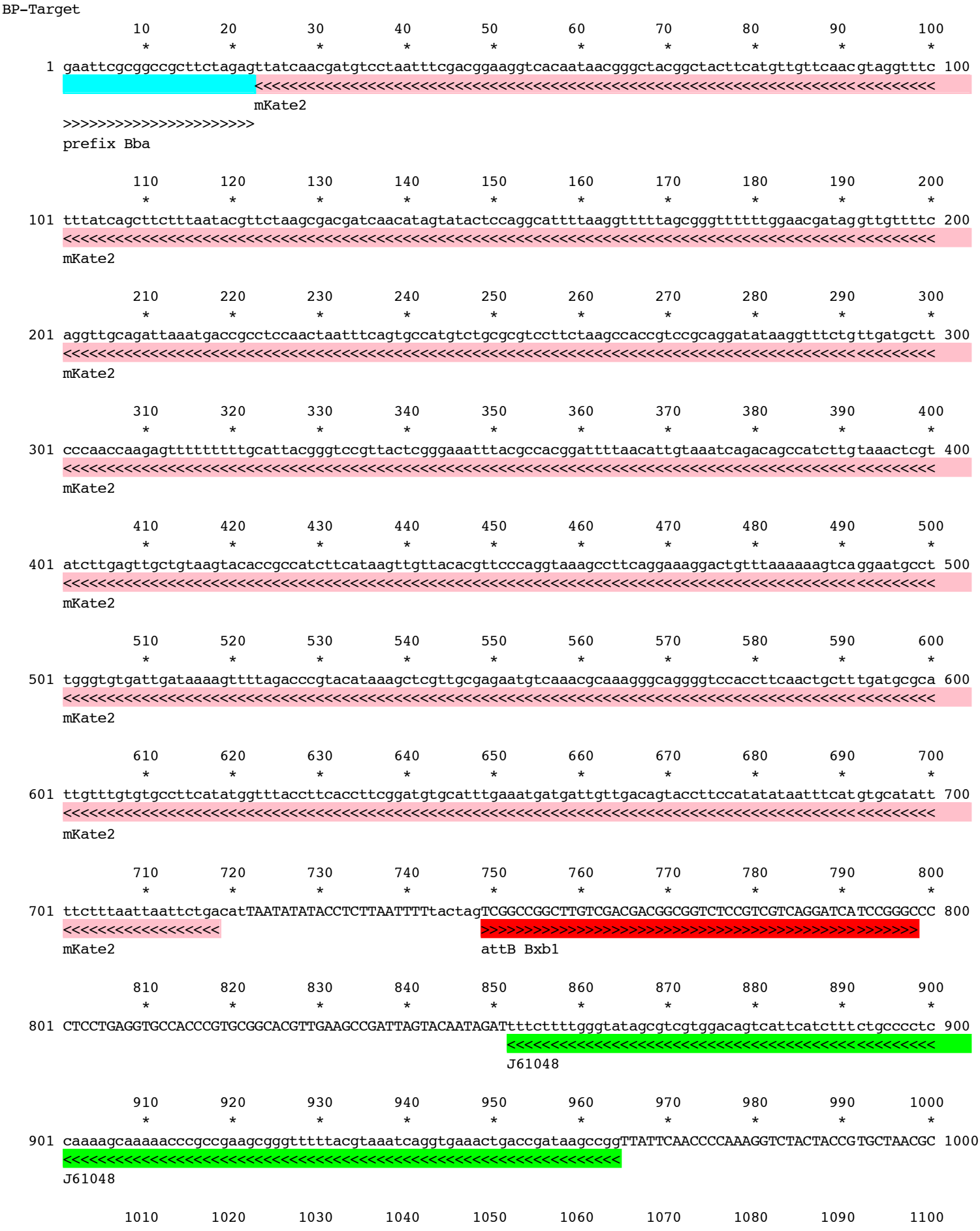


Figure S11: Tuning the reset-specific integrase degradation rate allows for different bias in the outcome of integrase-excisionase mediated recombination. **A**, Schematic representation of the system used in this experiment. Only the integrase ssrA degradation tag changes. These constructs are alternate resets elements obtained during the screen for a functional reset using a destabilized excisionase (see SI text). **B**, Three resets elements using different ssrA sequences for the integrase display different proportions of cells in the BP state after pulse (pink: gate used to measure the percentage of cells in BP along with the actual value). Note also that: i) the intermediate state is observable during induction even at single copy number of the DNA register, as simulated by the stochastic model in Fig 2*D*. ii) differences in bias toward BP during induction are also observed. In front of each row is depicted a schematic representation of the actual reset element with relevant sequence information.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J61048
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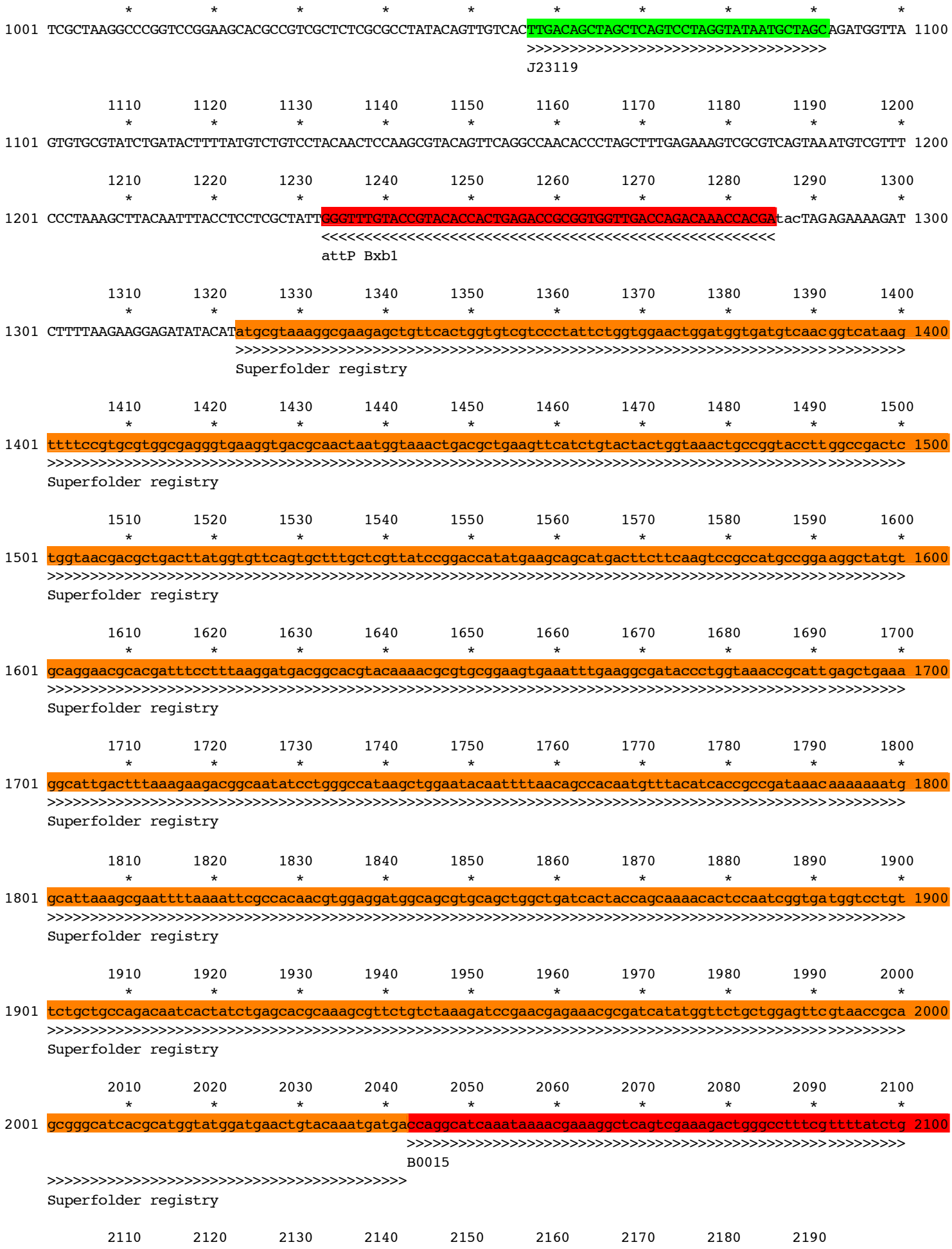
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attP Bxb1
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Superfolder registry
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Superfolder registry
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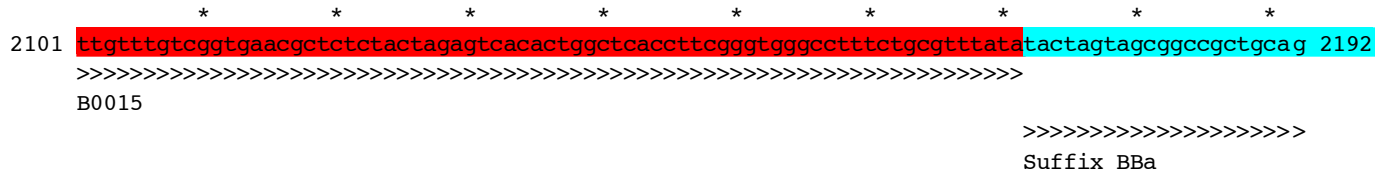
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Suffix BBa

LR Target


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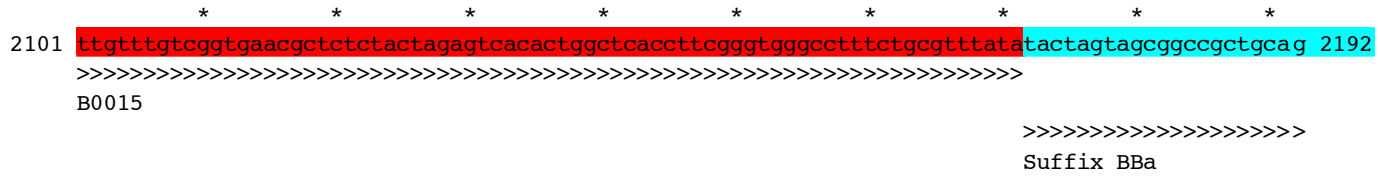
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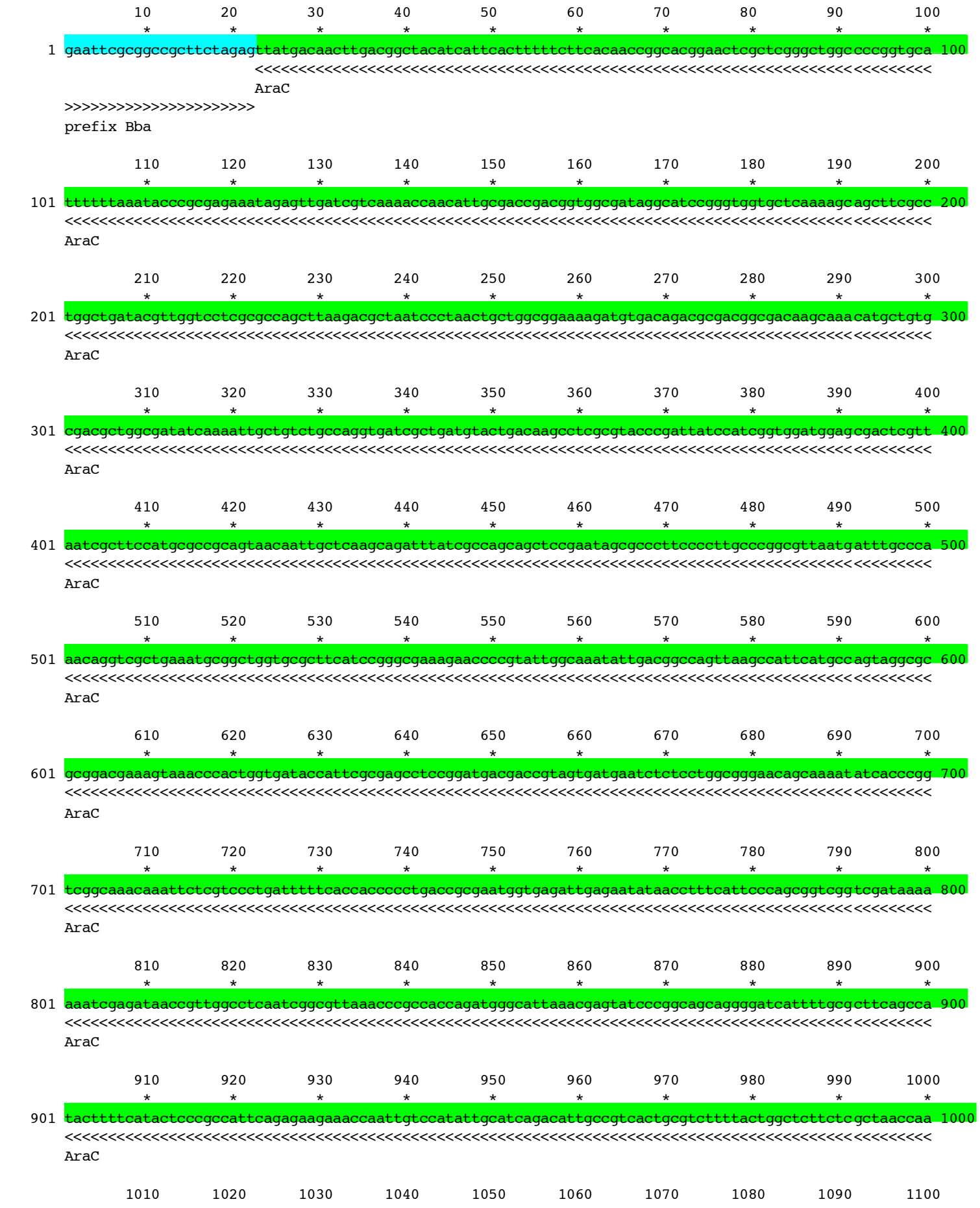

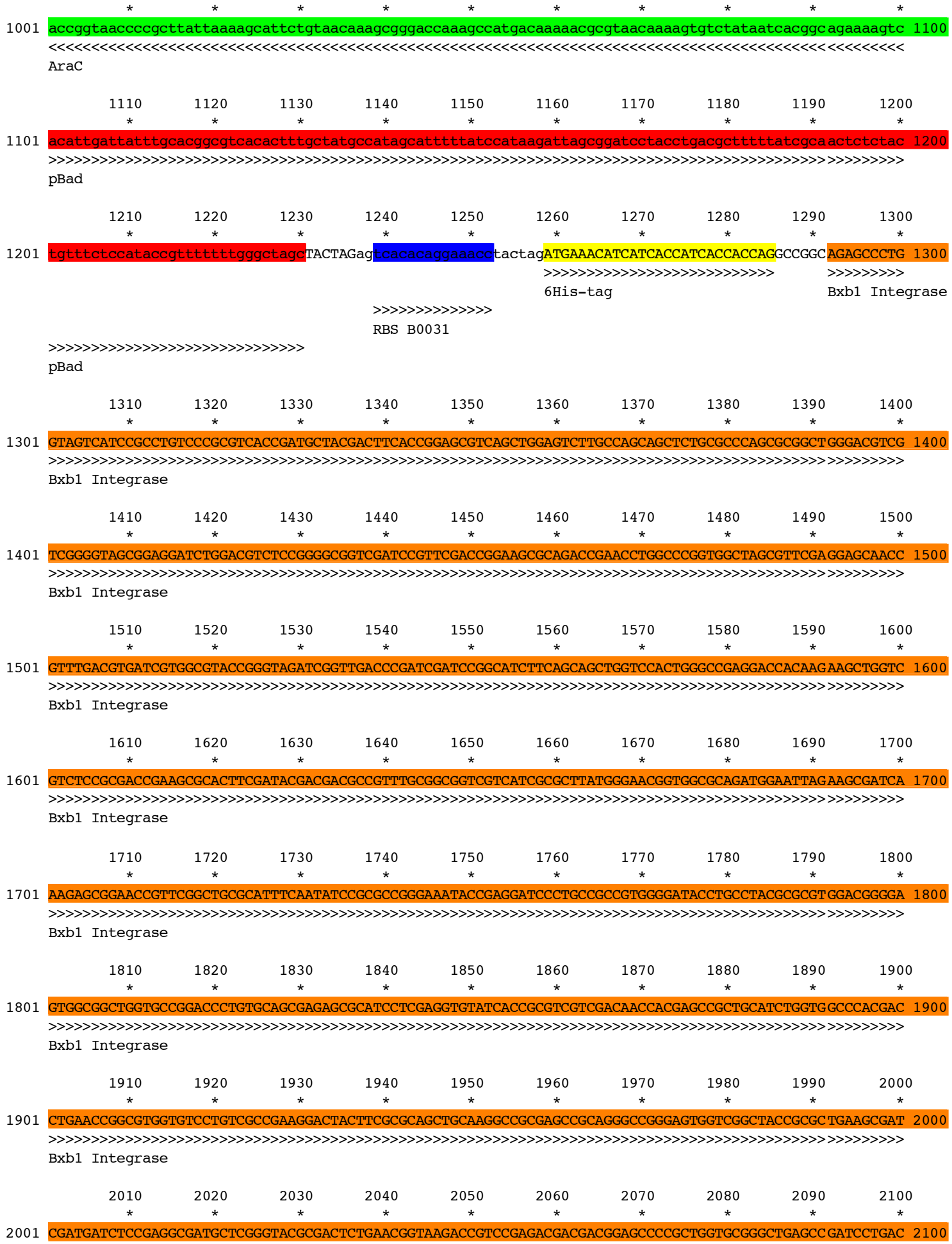
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AraC
1101 acattgattatttgcacggcgtcacactttgctatgccatagcatttttatccataagattagcggatcctacctgacgctttttatcgcaactctctac 1200
pBad
1201 tgtttctccataccgtttttttgggctagcTACTAGagtcacacaggaaacctactagATGAAACATCATCACCATCACCACCAGGCCGGCAGAGCCCTG 1300
6His-tag
Bxb1 Integrase
RBS B0031
pBad
1301 GTAGTCATCCGCCTGTCCCGCGTCACCGATGCTACGACTTCACCGGAGCGTCAGCTGGAGTCTTGCCAGCAGCTCTGCGCCCAGCGCGGCTGGGACGTCG 1400
Bxb1 Integrase
1401 TCGGGGTAGCGGAGGATCTGGACGTCTCCGGGGCGGTCGATCCGTTCGACCGGAAGCGCAGACCGAACCTGGCCCGGTGGCTAGCGTTCGAGGAGCAACC 1500
Bxb1 Integrase
1501 GTTTGACGTGATCGTGGCGTACCGGGTAGATCGGTTGACCCGATCGATCCGGCATCTTCAGCAGCTGGTCCACTGGGCCGAGGACCACAAGAAGCTGGTC 1600
Bxb1 Integrase
1601 GTCTCCGCGACCGAAGCGCACTTCGATACGACGACGCCGTTTGCGGCGGTCGTCATCGCGCTTATGGGAACGGTGGCGCAGATGGAATTAGAAGCGATCA 1700
Bxb1 Integrase
1701 AAGAGCGGAACCGTTCGGCTGCGCATTTCAATATCCGCGCCGGGAAATACCGAGGATCCCTGCCGCCGTGGGGATACCTGCCTACGCGCGTGGACGGGGA 1800
Bxb1 Integrase
1801 GTGGCGGCTGGTGCCGGACCCTGTGCAGCGAGAGCGCATCCTCGAGGTGTATCACCGCGTCGTCGACAACCACGAGCCGCTGCATCTGGTGGCCCACGAC 1900
Bxb1 Integrase
1901 CTGAACCGGCGTGGTGTCCTGTCGCCGAAGGACTACTTCGCGCAGCTGCAAGGCCGCGAGCCGCAGGGCCGGGAGTGGTCGGCTACCGCGCTGAAGCGAT 2000
Bxb1 Integrase
2001 CGATGATCTCCGAGGCGATGCTCGGGTACGCGACTCTGAACGGTAAGACCGTCCGAGACGACGACGGAGCCCCGCTGGTGCGGGCTGAGCCGATCCTGAC 2100

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     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

            2110      2120      2130      2140      2150      2160      2170      2180      2190      2200
              *         *         *         *         *         *         *         *         *         *
2101 CCGTGAGCAGCTGGAGGCGCTGCGCGCCGAGCTCGTGAAGACCTCCCGGGCGAAGCCCGCGGTGTCTACCCCGTCGCTGCTGCTGCGGGTGTTGTTCTGC 2200
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

            2210      2220      2230      2240      2250      2260      2270      2280      2290      2300
              *         *         *         *         *         *         *         *         *         *
2201 GCGGTGTGCGGGGAGCCCGCGTACAAGTTCGCCGGGGGAGGACGTAAGCACCCGCGCTACCGCTGCCGCTCGATGGGGTTCCCGAAGCACTGCGGGAACG 2300
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

            2310      2320      2330      2340      2350      2360      2370      2380      2390      2400
              *         *         *         *         *         *         *         *         *         *
2301 GCACGGTGGCGATGGCCGAGTGGGACGCGTTCTGCGAGGAGCAGGTACTGGATCTGCTCGGGGACGCGGAGCGTCTGGAGAAAGTCTGGGTAGCGGGCTC 2400
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

            2410      2420      2430      2440      2450      2460      2470      2480      2490      2500
              *         *         *         *         *         *         *         *         *         *
2401 GGACTCCGCGGTCGAACTCGCGGAGGTGAACGCGGAGCTGGTGGACCTGACGTCGCTGATCGGCTCCCCGGCCTACCGGGCGGGCTCTCCGCAGCGAGAA 2500
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

            2510      2520      2530      2540      2550      2560      2570      2580      2590      2600
              *         *         *         *         *         *         *         *         *         *
2501 GCACTGGATGCCCGTATTGCGGCGCTGGCCGCGCGGCAAGAGGAGCTGGAGGGCCTGGAGGCTCGCCCGTCTGGCTGGGAGTGGCGCGAGACCGGGCAGC 2600
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

            2610      2620      2630      2640      2650      2660      2670      2680      2690      2700
              *         *         *         *         *         *         *         *         *         *
2601 GGTTCGGGGACTGGTGGCGGGAGCAGGACACCGCGGCAAAGAACACCTGGCTTCGGTCGATGAACGTTCGGCTGACGTTCGACGTCCGCGGCGGGCTGAC 2700
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

            2710      2720      2730      2740      2750      2760      2770      2780      2790      2800
              *         *         *         *         *         *         *         *         *         *
2701 TCGCACGATCGACTTCGGGGATCTTCAGGAGTACGAGCAGCATCTCAGGCTCGGCAGCGTGGTCGAACGGCTACACACCGGGATGTCGACCGGcGCTGCT 2800
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase
                                                                                                   >>>>>>
                                                                                                   LAA tag

            2810      2820      2830      2840      2850      2860      2870      2880      2890      2900
              *         *         *         *         *         *         *         *         *         *
2801 AACGACGAAAACTACGCTCTGGCTGCTTAAACCGGTTAAtactagtagcggccgctgcagcaatggcaacaacgttgcgcaaactattaactggcgaact 2900
     >>>>>>>>>>>>>>>>>>>>>>>>>>>            >>>>>>>>>>>>>>>>>>>>>
     LAA tag                                Biobricks suffix (BBa)

            2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
              *         *         *         *         *         *         *         *         *         *
2901 acttactctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttatt 3000

            3010      3020      3030      3040      3050      3060      3070      3080      3090      3100
              *         *         *         *         *         *         *         *         *         *
3001 gctgataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacgg 3100

            3110      3120      3130      3140      3150      3160      3170      3180      3190      3200
              *         *         *         *         *         *         *         *         *         *
3101 ggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaactgtcagaccaagtttactcatatat 3200

            3210      3220      3230      3240      3250      3260      3270      3280      3290      3300
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          *        *        *        *        *        *        *        *        *        *
3201 actttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatccttttgataatctcatgaccaaaatcccttaacgtgagttttcg 3300

        3310     3320     3330     3340     3350     3360     3370     3380     3390     3400
          *        *        *        *        *        *        *        *        *        *
3301 ttccactgagcgtcagacccccttaataagatgatcttcttgagatcgttttggtctgcgcgtaatctcttgctctgaaaacgaaaaaaccgccttgcagg 3400
                                     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
                                     p15A Ori

        3410     3420     3430     3440     3450     3460     3470     3480     3490     3500
          *        *        *        *        *        *        *        *        *        *
3401 gcggtttttcgaaggttctctgagctaccaactctttgaaccgaggtaactggcttggaggagcgcagtcaccaaaacttgtcctttcagtttagcctta 3500
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     p15A Ori

        3510     3520     3530     3540     3550     3560     3570     3580     3590     3600
          *        *        *        *        *        *        *        *        *        *
3501 accggcgcatgacttcaagactaactcctctaaatcaattaccagtggctgctgccagtggtgcttttgcatgtctttccgggttggactcaagacgata 3600
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     p15A Ori

        3610     3620     3630     3640     3650     3660     3670     3680     3690     3700
          *        *        *        *        *        *        *        *        *        *
3601 gttaccggataaggcgcagcggtcggactgaacgggggttcgtgcatacagtccagcttggagcgaactgcctacccggaactgagtgtcaggcgtgga 3700
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     p15A Ori

        3710     3720     3730     3740     3750     3760     3770     3780     3790     3800
          *        *        *        *        *        *        *        *        *        *
3701 atgagacaaacgcggccataacagcggaatgacaccggtaaaccgaaaggcaggaacaggagagcgcacgagggagccgccaggggaaacgcctggtat 3800
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     p15A Ori

        3810     3820     3830     3840     3850     3860     3870     3880     3890     3900
          *        *        *        *        *        *        *        *        *        *
3801 ctttatagtcctgtcgggtttcgccaccactgatttgagcgtcagatttcgtgatgcttgtcaggggggcggagcctatggaaaaacggctttgccgcgg 3900
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     p15A Ori

        3910     3920     3930     3940     3950     3960     3970     3980     3990     4000
          *        *        *        *        *        *        *        *        *        *
3901 ccctctcacttccctgttaagtatcttcctggcatcttccaggaaatctccgccccgttcgtaagccatttccgctcgccgcagtcgaacgaccgagcgt 4000
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     p15A Ori

        4010     4020     4030     4040     4050     4060     4070     4080     4090     4100
          *        *        *        *        *        *        *        *        *        *
4001 agcgagtcagtgagcgaggaagcggaatatatcctgtatcacatattctgctgacgcaccggtgcagccttttttctcctgccacatgaagcacttcact 4100
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     p15A Ori

        4110     4120     4130     4140     4150     4160     4170     4180     4190     4200
          *        *        *        *        *        *        *        *        *        *
4101 gacaccctcatcagtgccaacatagtaagccagtatacactccgctagcgctgaggtctgcctcgtgaagaaggtgttgctgactcataccaggcctgaa 4200
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     p15A Ori

        4210     4220     4230     4240     4250     4260     4270     4280     4290     4300
          *        *        *        *        *        *        *        *        *        *
4201 tcgccccatcatccagccagaaagtgagggagccacggttgatgagagctttgttgtaggtggaccagttggtgattttgaacttttgctttgccacgga 4300

        4310     4320     4330     4340     4350     4360     4370     4380     4390     4400
          *        *        *        *        *        *        *        *        *        *
4301 acggtctgcgttgtcgggaagatgcgtgatctgatccttcaactcagcaaaagttcgatttattcaacaaagccacgttgtgtctcaaaatctctgatgt 4400

        4410     4420     4430     4440     4450     4460     4470     4480     4490     4500
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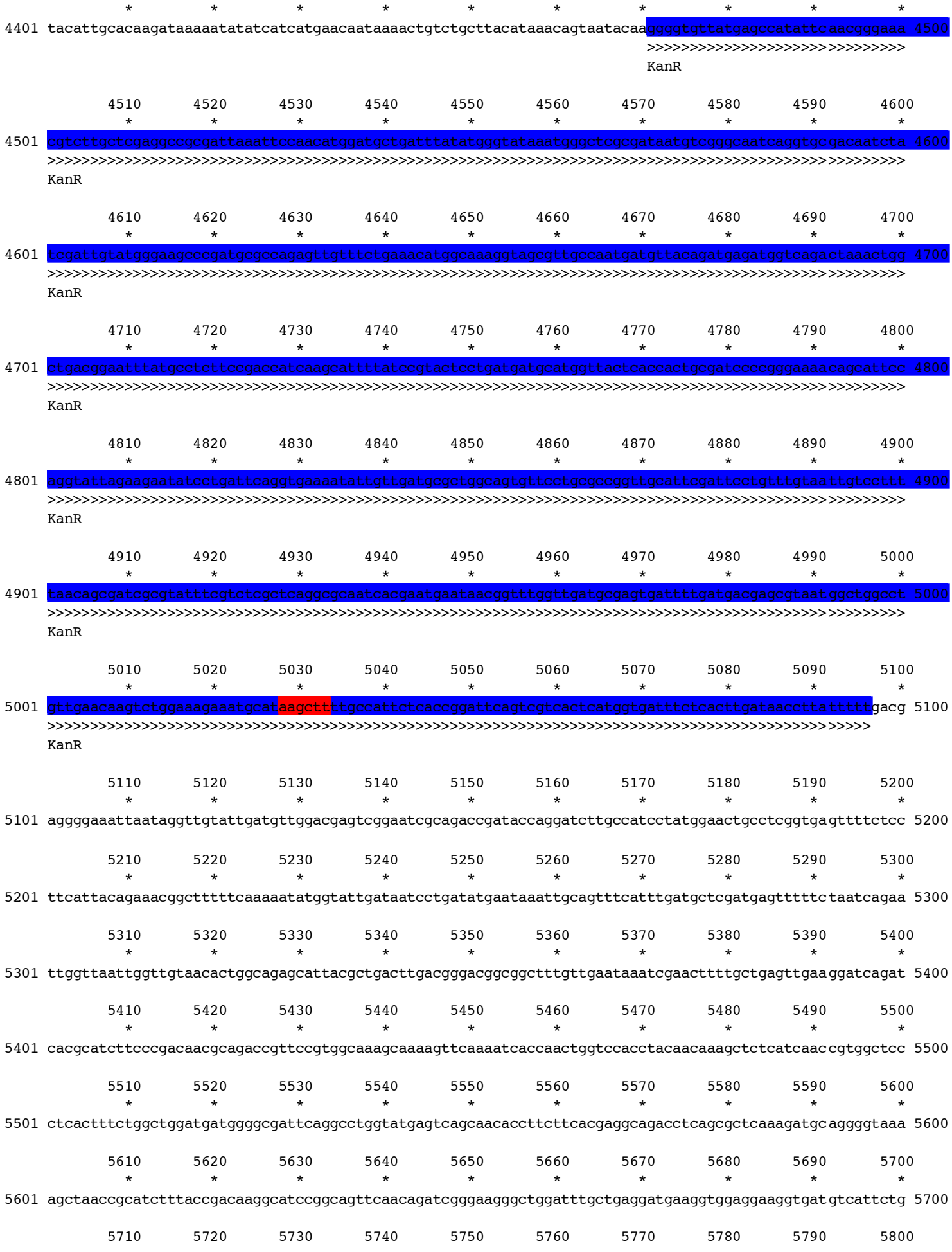
4401 tacattgcacaagataaaaatatatcatcatgaacaataaaactgtctgcttacataaacagtaatacaaggggtgttatgagccatattcaacgggaaa 4500
KanR
4501 cgtcttgctcgaggccgcgattaaattccaacatggatgctgatttatatgggtataaatgggctcgcgataatgtcgggcaatcaggtgcgacaatcta 4600
KanR
4601 tcgattgtatgggaagcccgatgcgccagagttgtttctgaaacatggcaaaggtagcgttgccaatgatgttacagatgagatggtcagactaaactgg 4700
KanR
4701 ctgacggaatttatgcctcttccgaccatcaagcattttatccgtactcctgatgatgcatggttactcaccactgcgatccccgggaaaacagcattcc 4800
KanR
4801 aggtattagaagaatatcctgattcaggtgaaaatattgttgatgcgctggcagtgttcctgcgccggttgcattcgattcctgtttgtaattgtccttt 4900
KanR
4901 taacagcgatcgcgtatttcgtctcgctcaggcgcaatcacgaatgaataacggtttggttgatgcgagtgattttgatgacgagcgtaatggctggcct 5000
KanR
5001 gttgaacaagtctggaaagaaatgcataagcttttgccattctcaccggattcagtcgtcactcatggtgatttctcacttgataaccttatttttgacg 5100
KanR
5101 aggggaaattaataggttgtattgatgttggacgagtcggaatcgcagaccgataccaggatcttgccatcctatggaactgcctcggtgagttttctcc 5200
5201 ttcattacagaaacggctttttcaaaaatatggtattgataatcctgatatgaataaattgcagtttcatttgatgctcgatgagtttttctaatcagaa 5300
5301 ttggttaattggttgtaacactggcagagcattacgctgacttgacgggacggcggctttgttgaataaatcgaacttttgctgagttgaaggatcagat 5400
5401 cacgcatcttcccgacaacgcagaccgttccgtggcaaagcaaaagttcaaaatcaccaactggtccacctacaacaaagctctcatcaaccgtggctcc 5500
5501 ctcactttctggctggatgatggggcgattcaggcctggtatgagtcagcaacaccttcttcacgaggcagacctcagcgctcaaagatgcaggggtaaa 5600
5601 agctaaccgcatctttaccgacaaggcatccggcagttcaacagatcgggaagggctggatttgctgaggatgaaggtggaggaaggtgatgtcattctg 5700

```
             *        *        *        *        *        *        *        *        *        *
5701 gtgaagaagctcgaccgtcttggccgcgacaccgccgacatgatccaactgataaaagagtttgatgctcagggtgtagcggttcggtttattgacgacg 5800

            5810      5820      5830      5840      5850      5860      5870      5880
              *        *        *        *        *        *        *        *
5801 ggatcagtaccgacggtgatatggggcaaatggtggtcaccatcctgtcggctgtggcacaggctgaacgccggaggatcc 5881
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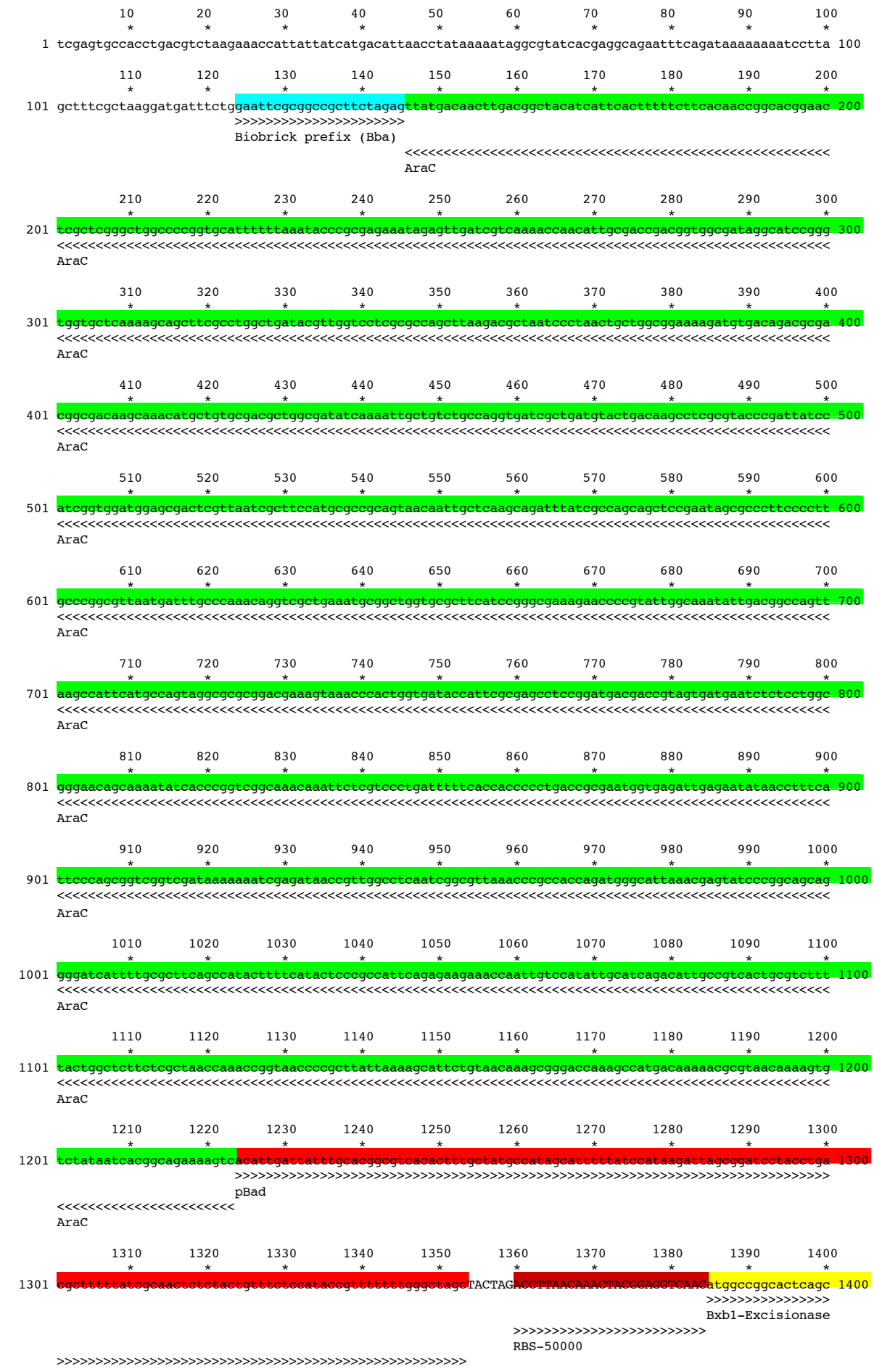
pBad-Reset-Circuit



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     pBad

           1410      1420      1430      1440      1450      1460      1470      1480      1490      1500
              *         *         *         *         *         *         *         *         *         *
1401 gtatcgtctttctacccgatactcagttgcctttcgaggcgcgcaaagagatgcaagcggtcatccgcttcatcggggatgtccagccgtacggcgtggt 1500
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1-Excisionase

           1510      1520      1530      1540      1550      1560      1570      1580      1590      1600
              *         *         *         *         *         *         *         *         *         *
1501 acatatcggtgacgtcctagacctgccgcagccctcgcgctggaaccggggaaccaagggcgagttcgagggctcggtgtaccgcgacgcggactacgcc 1600
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1-Excisionase

           1610      1620      1630      1640      1650      1660      1670      1680      1690      1700
              *         *         *         *         *         *         *         *         *         *
1601 aagaagaacctgatggagccgctgcgcaaggtctacgacggctggatcgggatgcacgagggcaaccacgatctgcgagcccgcgagtacctggccaaga 1700
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1-Excisionase

           1710      1720      1730      1740      1750      1760      1770      1780      1790      1800
              *         *         *         *         *         *         *         *         *         *
1701 acgcaccggccctggagggtacgcacgctttcgacatcgacgtgctgctcgacttcgacgggttcggtgtggagctgctgcctgacttctacgacatcgc 1800
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1-Excisionase

           1810      1820      1830      1840      1850      1860      1870      1880      1890      1900
              *         *         *         *         *         *         *         *         *         *
1801 tccgggctggatctccactcacgggcacatgggcaagatgacgctatcccagatcgccggatcgacagcgctcaacggtgccaagaagttcggcaagtcc 1900
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1-Excisionase

           1910      1920      1930      1940      1950      1960      1970      1980      1990      2000
              *         *         *         *         *         *         *         *         *         *
1901 gtggtctgcggccacacgcaccggcaggctgtcgtctcgcactcgttcgggtacggcggctcggtgcgcaagaccgtcaccggcatggaagtcgggcacc 2000
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1-Excisionase

           2010      2020      2030      2040      2050      2060      2070      2080      2090      2100
              *         *         *         *         *         *         *         *         *         *
2001 tgatggacatgaagaaggccaactatctaaagggcggagccgggaactggcagatgggcttcgggatgctcacggtcgacggcaagcatgtcaaggctga 2100
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1-Excisionase

           2110      2120      2130      2140      2150      2160      2170      2180      2190      2200
              *         *         *         *         *         *         *         *         *         *
2101 gatcgtcccgatcctgggaggcaagttcaccgttgacggccaggtctgggaagtcaccggtTAAtactagtcacacaggaaacctactagGTGAGAGCCC 2200
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>         >>>>>>>>>>>>>>      >>>>>>>>>>
     Bxb1-Excisionase                                                      RBS B0031           Bxb1 Integrase

           2210      2220      2230      2240      2250      2260      2270      2280      2290      2300
              *         *         *         *         *         *         *         *         *         *
2201 TGGTAGTCATCCGCCTGTCCCGCGTCACCGATGCTACGACTTCACCGGAGCGTCAGCTGGAGTCTTGCCAGCAGCTCTGCGCCCAGCGCGGCTGGGACGT 2300
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

           2310      2320      2330      2340      2350      2360      2370      2380      2390      2400
              *         *         *         *         *         *         *         *         *         *
2301 CGTCGGGGTAGCGGAGGATCTGGACGTCTCCGGGGCGGTCGATCCGTTCGACCGGAAGCGCAGACCGAACCTGGCCCGGTGGCTAGCGTTCGAGGAGCAA 2400
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

           2410      2420      2430      2440      2450      2460      2470      2480      2490      2500
              *         *         *         *         *         *         *         *         *         *
2401 CCGTTTGACGTGATCGTGGCGTACCGGGTAGATCGGTTGACCCGATCGATCCGGCATCTTCAGCAGCTGGTCCACTGGGCCGAGGACCACAAGAAGCTGG 2500
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

           2510      2520      2530      2540      2550      2560      2570      2580      2590      2600
              *         *         *         *         *         *         *         *         *         *
2501 TCGTCTCCGCGACCGAAGCGCACTTCGATACGACGACGCCGTTTGCGGCGGTCGTCATCGCGCTTATGGGAACGGTGGCGCAGATGGAATTAGAAGCGAT 2600
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

           2610      2620      2630      2640      2650      2660      2670      2680      2690      2700
              *         *         *         *         *         *         *         *         *         *
2601 CAAAGAGCGGAACCGTTCGGCTGCGCATTTCAATATCCGCGCCGGGAAATACCGAGGATCCCTGCCGCCGTGGGGATACCTGCCTACGCGCGTGGACGGG 2700
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

           2710      2720      2730      2740      2750      2760      2770      2780      2790      2800
              *         *         *         *         *         *         *         *         *         *
2701 GAGTGGCGGCTGGTGCCGGACCCTGTGCAGCGAGAGCGCATCCTCGAGGTGTATCACCGCGTCGTCGACAACCACGAGCCGCTGCATCTGGTGGCCCACG 2800
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

           2810      2820      2830      2840      2850      2860      2870      2880      2890      2900
              *         *         *         *         *         *         *         *         *         *
2801 ACCTGAACCGGCGTGGTGTCCTGTCGCCGAAGGACTACTTCGCGCAGCTGCAAGGCCGCGAGCCGCAGGGCCGGGAGTGGTCGGCTACCGCGCTGAAGCG 2900
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase
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```
        2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
          *          *          *          *          *          *          *          *          *          *
2901 ATCGATGATCTCCGAGGCGATGCTCGGGTACGCGACTCTGAACGGTAAGACCGTCCGAGACGACGACGGAGCCCCGCTGGTGCGGGCTGAGCCGATCCTG 3000
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

        3010       3020       3030       3040       3050       3060       3070       3080       3090       3100
          *          *          *          *          *          *          *          *          *          *
3001 ACCCGTGAGCAGCTGGAGGCGCTGCGCGCCGAGCTCGTGAAGACCTCCCGGGCGAAGCCCGCGGTGTCTACCCCGTCGCTGCTGCTGCGGGTGTTGTTCT 3100
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

        3110       3120       3130       3140       3150       3160       3170       3180       3190       3200
          *          *          *          *          *          *          *          *          *          *
3101 GCGCGGTGTGCGGGGAGCCCGCGTACAAGTTCGCCGGGGGAGGACGTAAGCACCCGCGCTACCGCTGCCGCTCGATGGGGTTCCCGAAGCACTGCGGGAA 3200
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

        3210       3220       3230       3240       3250       3260       3270       3280       3290       3300
          *          *          *          *          *          *          *          *          *          *
3201 CGGCACGGTGGCGATGGCCGAGTGGGACGCGTTCTGCGAGGAGCAGGTACTGGATCTGCTCGGGGACGCGGAGCGTCTGGAGAAAGTCTGGGTAGCGGGC 3300
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

        3310       3320       3330       3340       3350       3360       3370       3380       3390       3400
          *          *          *          *          *          *          *          *          *          *
3301 TCGGACTCCGCGGTCGAACTCGCGGAGGTGAACGCGGAGCTGGTGGACCTGACGTCGCTGATCGGCTCCCCGGCCTACCGGGCGGGCTCTCCGCAGCGAG 3400
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

        3410       3420       3430       3440       3450       3460       3470       3480       3490       3500
          *          *          *          *          *          *          *          *          *          *
3401 AAGCACTGGATGCCCGTATTGCGGCGCTGGCCGCGCGGCAAGAGGAGCTGGAGGGCCTGGAGGCTCGCCCGTCTGGCTGGGAGTGGCGCGAGACCGGGCA 3500
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

        3510       3520       3530       3540       3550       3560       3570       3580       3590       3600
          *          *          *          *          *          *          *          *          *          *
3501 GCGGTTCGGGGACTGGTGGCGGGAGCAGGACACCGCGGCAAAGAACACCTGGCTTCGGTCGATGAACGTTCGGCTGACGTTCGACGTCCGCGGCGGGCTG 3600
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

        3610       3620       3630       3640       3650       3660       3670       3680       3690       3700
          *          *          *          *          *          *          *          *          *          *
3601 ACTCGCACGATCGACTTCGGGGATCTTCAGGAGTACGAGCAGCATCTCAGGCTCGGCAGCGTGGTCGAACGGCTACACACCGGGATGTCGtaaTACTAGT 3700
                                                                                                >>>>>>>
                                                                                                Biobrick suffix (BBa)
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Bxb1 Integrase

        3710       3720       3730       3740       3750       3760       3770       3780       3790       3800
          *          *          *          *          *          *          *          *          *          *
3701 AGCGGCCGCTGCAGtccggcaaaaaaacgggcaaggtgtcaccaccctgccctttttctttaaaaccgaaaagattacttcgcgttatgcaggcttcctc 3800
     >>>>>>>>>>>>>>>
     Biobrick suffix (BBa)

        3810       3820       3830       3840       3850       3860       3870       3880       3890       3900
          *          *          *          *          *          *          *          *          *          *
3801 gctcactgactcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatcctagggcgttcggctgcggcgagcggtatc 3900

        3910       3920       3930       3940       3950       3960       3970       3980       3990       4000
          *          *          *          *          *          *          *          *          *          *
3901 agctcactcaaaggcggtaatacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaa 4000

        4010       4020       4030       4040       4050       4060       4070       4080       4090       4100
          *          *          *          *          *          *          *          *          *          *
4001 aaggccgcgttgctggcgtttttccataggctccgcccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactat 4100
       <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
       ColE1 origin

        4110       4120       4130       4140       4150       4160       4170       4180       4190       4200
          *          *          *          *          *          *          *          *          *          *
4101 aaagataccaggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttcgggaag 4200
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     ColE1 origin

        4210       4220       4230       4240       4250       4260       4270       4280       4290       4300
          *          *          *          *          *          *          *          *          *          *
4201 cgtggcgctttctcaatgctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgttcagcccgac 4300
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     ColE1 origin

        4310       4320       4330       4340       4350       4360       4370       4380       4390       4400
          *          *          *          *          *          *          *          *          *          *
4301 cgctgcgccttatccggtaactatcgtcttgagtccaatgccacctgacgtctaagaacccggtaagacacgacttatcgccactggcagcagccactgg 4400
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     ColE1 origin

        4410       4420       4430       4440       4450       4460       4470       4480       4490       4500
          *          *          *          *          *          *          *          *          *          *
```

```
4401 taacaggattagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgc 4500
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     ColE1 origin

            4510      4520      4530      4540      4550      4560      4570      4580      4590      4600
              *         *         *         *         *         *         *         *         *         *
4501 gctctgctgaagccagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggtggtttttttgtttgcaagcagc 4600
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     ColE1 origin

            4610      4620      4630      4640      4650      4660      4670      4680      4690      4700
              *         *         *         *         *         *         *         *         *         *
4601 agattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttctacggggtctgacgctcagtggaacgaaaactcacgttaagggattttggt 4700
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     ColE1 origin

            4710      4720      4730      4740      4750      4760      4770      4780      4790      4800
              *         *         *         *         *         *         *         *         *         *
4701 catgacttgtgcttggattctcaccaataaaaaacgcccggcggcaaccgagcgttctgaacaaatccagatggagttctgaggtcattactggatctat 4800
     <<<<<
     ColE1 origin

            4810      4820      4830      4840      4850      4860      4870      4880      4890      4900
              *         *         *         *         *         *         *         *         *         *
4801 caacaggagtccaagcgagctcgatatcaaattacgccccgccctgccactcatcgcagtactgttgtaattcattaagcattctgccgacatggaagcc 4900
                           <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
                           Chloramphenicol Resistance

            4910      4920      4930      4940      4950      4960      4970      4980      4990      5000
              *         *         *         *         *         *         *         *         *         *
4901 atcacagacggcatgatgaacctgaatcgccagcggcatcagcaccttgtcgccttgcgtataatatttgcccatggtgaaaacgggggcgaagaagttg 5000
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     Chloramphenicol Resistance

            5010      5020      5030      5040      5050      5060      5070      5080      5090      5100
              *         *         *         *         *         *         *         *         *         *
5001 tccatattggccacgtttaaatcaaaactggtgaaactcacccagggattggctgagacgaaaaacatattctcaataaaccctttagggaaataggcca 5100
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     Chloramphenicol Resistance

            5110      5120      5130      5140      5150      5160      5170      5180      5190      5200
              *         *         *         *         *         *         *         *         *         *
5101 ggttttcaccgtaacacgccacatcttgcgaatatatgtgtagaaactgccggaaatcgtcgtggtattcactccagagcgatgaaaacgtttcagtttg 5200
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     Chloramphenicol Resistance

            5210      5220      5230      5240      5250      5260      5270      5280      5290      5300
              *         *         *         *         *         *         *         *         *         *
5201 ctcatggaaaacggtgtaacaagggtgaacactatcccatatcaccagctcaccgtctttcattgccatacgaaattccggatgagcattcatcaggcgg 5300
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     Chloramphenicol Resistance

            5310      5320      5330      5340      5350      5360      5370      5380      5390      5400
              *         *         *         *         *         *         *         *         *         *
5301 gcaagaatgtgaataaaggccggataaaacttgtgcttatttttctttacggtctttaaaaaggccgtaatatccagctgaacggtctggttataggtac 5400
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     Chloramphenicol Resistance

            5410      5420      5430      5440      5450      5460      5470      5480      5490      5500
              *         *         *         *         *         *         *         *         *         *
5401 attgagcaactgactgaaatgcctcaaaatgttctttacgatgccattgggatatatcaacggtggtatatccagtgatttttttctccatttagcttc 5500
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     Chloramphenicol Resistance

            5510      5520      5530      5540      5550      5560      5570      5580      5590      5600
              *         *         *         *         *         *         *         *         *         *
5501 cttagctcctgaaaatctcgataactcaaaaaatacgcccggtagtgatcttatttcattatggtgaaagttggaacctcttacgtgccgatcaacgtct 5600

            5610      5620      5630      5640      5650      5660      5670      5680      5690
              *         *         *         *         *         *         *         *         *
5601 cattttcgccagatatcgacgtctaagaaaccattattatcatgacattaacctataaaaataggcgtatcacgaggccctttcgtcttcacc 5693
```

RAD Module G8-C1

```
         10        20        30        40        50        60        70        80        90       100
          *         *         *         *         *         *         *         *         *         *
  1 tcgagtgccacctgacgtctaagaaaccattattatcatgacattaacctataaaaataggcgtatcacgaggcagaatttcagataaaaaaaatcctta 100

        110       120       130       140       150       160       170       180       190       200
          *         *         *         *         *         *         *         *         *         *
101 gctttcgctaaggatgatttctgGAATTCGCGGCCGCTTCTAGAGTCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCACA 200
                           >>>>>>>>>>>>>>>>>>>>>>
                           prefix Bba
                                                 >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
                                                 pTetO

        210       220       230       240       250       260       270       280       290       300
          *         *         *         *         *         *         *         *         *         *
201 AGCTTGCGGCTTTCACACTTGGGGGCTAGCGTGAGAGCCCTGGTAGTCATCCGCCTGTCCCGCGTCACCGATGCTACGACTTCACCGGAGCGTCAGCTGG 300
                              >>>
                              GTG Start
                              >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
                              SET Integrase
         >>>>>>>>>>>>>>>>>>>>>>>>>
         6N-RBS

        310       320       330       340       350       360       370       380       390       400
          *         *         *         *         *         *         *         *         *         *
301 AGTCTTGCCAGCAGCTCTGCGCCCAGCGCGGCTGGGACGTCGTCGGGGTAGCGGAGGATCTGGACGTCTCCGGGGCGGTCGATCCGTTCGACCGGAAGCG 400
    >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
    SET Integrase

        410       420       430       440       450       460       470       480       490       500
          *         *         *         *         *         *         *         *         *         *
401 CAGACCGAACCTGGCCCGGTGGCTAGCGTTCGAGGAGCAACCGTTTGACGTGATCGTGGCGTACCGGGTAGATCGGTTGACCCGATCGATCCGGCATCTT 500
    >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
    SET Integrase

        510       520       530       540       550       560       570       580       590       600
          *         *         *         *         *         *         *         *         *         *
501 CAGCAGCTGGTCCACTGGGCCGAGGACCACAAGAAGCTGGTCGTCTCCGCGACCGAAGCGCACTTCGATACGACGACGCCGTTTGCGGCGGTCGTCATCG 600
    >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
    SET Integrase

        610       620       630       640       650       660       670       680       690       700
          *         *         *         *         *         *         *         *         *         *
601 CGCTTATGGGAACGGTGGCGCAGATGGAATTAGAAGCGATCAAAGAGCGGAACCGTTCGGCTGCGCATTTCAATATCCGCGCCGGGAAATACCGAGGATC 700
    >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
    SET Integrase

        710       720       730       740       750       760       770       780       790       800
          *         *         *         *         *         *         *         *         *         *
701 CCTGCCGCCGTGGGATACCTGCCTACGCGCGTGGACGGGGAGTGGCGGCTGGTGCCGGACCCTGTGCAGCGAGAGCGCATCCTCGAGGTGTATCACCGC 800
    >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
    SET Integrase

        810       820       830       840       850       860       870       880       890       900
          *         *         *         *         *         *         *         *         *         *
801 GTCGTCGACAACCACGAGCCGCTGCATCTGGTGGCCCACGACCTGAACCGGCGTGGTGTCCTGTCGCCGAAGGACTACTTCGCGCAGCTGCAAGGCCGCG 900
    >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
    SET Integrase

        910       920       930       940       950       960       970       980       990      1000
          *         *         *         *         *         *         *         *         *         *
901 AGCCGCAGGGCCGGGAGTGGTCGGCTACCGCGCTGAAGCGATCGATGATCTCCGAGGCGATGCTCGGGTACGCGACTCTGAACGGTAAGACCGTCCGAGA 1000
    >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
    SET Integrase
```


1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
1001 CGACGACGGAGCCCCGCTGGTGCGGGCTGAGCCGATCCTGACCCGTGAGCAGCTGGAGGCGCTGCGCGCCGAGCTCGTGAAGACCTCCCGGGCGAAGCCC 1100
SET Integrase
1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
1101 GCGGTGTCTACCCCGTCGCTGCTGCTGCGGGTGTTGTTCTGCGCGGTGTGCGGGGAGCCCGCGTACAAGTTCGCCGGGGGAGGACGTAAGCACCCGCGCT 1200
SET Integrase
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
1201 ACCGCTGCCGCTCGATGGGGTTCCCGAAGCACTGCGGGAACGGCACGGTGGCGATGGCCGAGTGGGACGCGTTCTGCGAGGAGCAGGTACTGGATCTGCT 1300
SET Integrase
1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
1301 CGGGGACGCGGAGCGTCTGGAGAAAGTCTGGGTAGCGGGCTCGGACTCCGCGGTCGAACTCGCGGAGGTGAACGCGGAGCTGGTGGACCTGACGTCGCTG 1400
SET Integrase
1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
1401 ATCGGCTCCCCGGCCTACCGGGCGGGCTCTCCGCAGCGAGAAGCACTGGATGCCCGTATTGCGGCGCTGGCCGCGCGGCAAGAGGAGCTGGAGGGCCTGG 1500
SET Integrase
1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
1501 AGGCTCGCCCGTCTGGCTGGGAGTGGCGCGAGACCGGGCAGCGGTTCGGGGACTGGTGGCGGGAGCAGGACACCGCGGCAAAGAACACCTGGCTTCGGTC 1600
SET Integrase
1610 1620 1630 1640 1650 1660 1670 1680 1690 1700
1601 GATGAACGTTCGGCTGACGTTCGACGTCCGCGGCGGGCTGACTCGCACGATCGACTTCGGGGATCTTCAGGAGTACGAGCAGCATCTCAGGCTCGGCAGC 1700
SET Integrase
1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
1701 GTGGTCGAACGGCTACACACCGGGATGTCGtaagcggccgcATGCATTAATAAtcacactggctcaccttcgggtgggcctttctgcgtttatatactag 1800
B0014
SET Integrase
1810 1820 1830 1840 1850 1860 1870 1880 1890 1900
1801 agagagaatataaaaagccagattattaatccggctttttttattatttCCTGCAGGgcggccgcCATATGttatgacaacttgacggctacatcattcac 1900
B0014
AraC
1910 1920 1930 1940 1950 1960 1970 1980 1990 2000
1901 tttttcttcacaaccggcacggaactcgctcgggctggccccggtgcatttttttaaatacccgcgagaaatagagttgatcgtcaaaaccaacattgcga 2000
AraC
2010 2020 2030 2040 2050 2060 2070 2080 2090 2100
2001 ccgacggtggcgataggcatccgggtggtgctcaaaagcagcttcgcctggctgatacgttggtcctcgcgccagcttaagacgctaatccctaactgct 2100

```
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     AraC

              2110      2120      2130      2140      2150      2160      2170      2180      2190      2200
                 *         *         *         *         *         *         *         *         *         *
2101 ggcggaaaagatgtgacagacgcgacggcgacaagcaaacatgctgtgcgacgctggcgatatcaaaattgctgtctgccaggtgatcgctgatgtactg 2200
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     AraC

              2210      2220      2230      2240      2250      2260      2270      2280      2290      2300
                 *         *         *         *         *         *         *         *         *         *
2201 acaagcctcgcgtacccgattatccatcggtggatggagcgactcgttaatcgcttccatgcgccgcagtaacaattgctcaagcagatttatcgccagc 2300
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     AraC

              2310      2320      2330      2340      2350      2360      2370      2380      2390      2400
                 *         *         *         *         *         *         *         *         *         *
2301 agctccgaatagcgcccttccccttgcccggcgttaatgatttgcccaaacaggtcgctgaaatgcggctggtgcgcttcatccgggcgaaagaaccccg 2400
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     AraC

              2410      2420      2430      2440      2450      2460      2470      2480      2490      2500
                 *         *         *         *         *         *         *         *         *         *
2401 tattggcaaatattgacggccagttaagccattcatgccagtaggcgcgcggacgaaagtaaacccactggtgataccattcgcgagcctccggatgacg 2500
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     AraC

              2510      2520      2530      2540      2550      2560      2570      2580      2590      2600
                 *         *         *         *         *         *         *         *         *         *
2501 accgtagtgatgaatctctcctggcgggaacagcaaaatatcacccggtcggcaaacaaattctcgtccctgatttttcaccaccccctgaccgcgaatg 2600
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     AraC

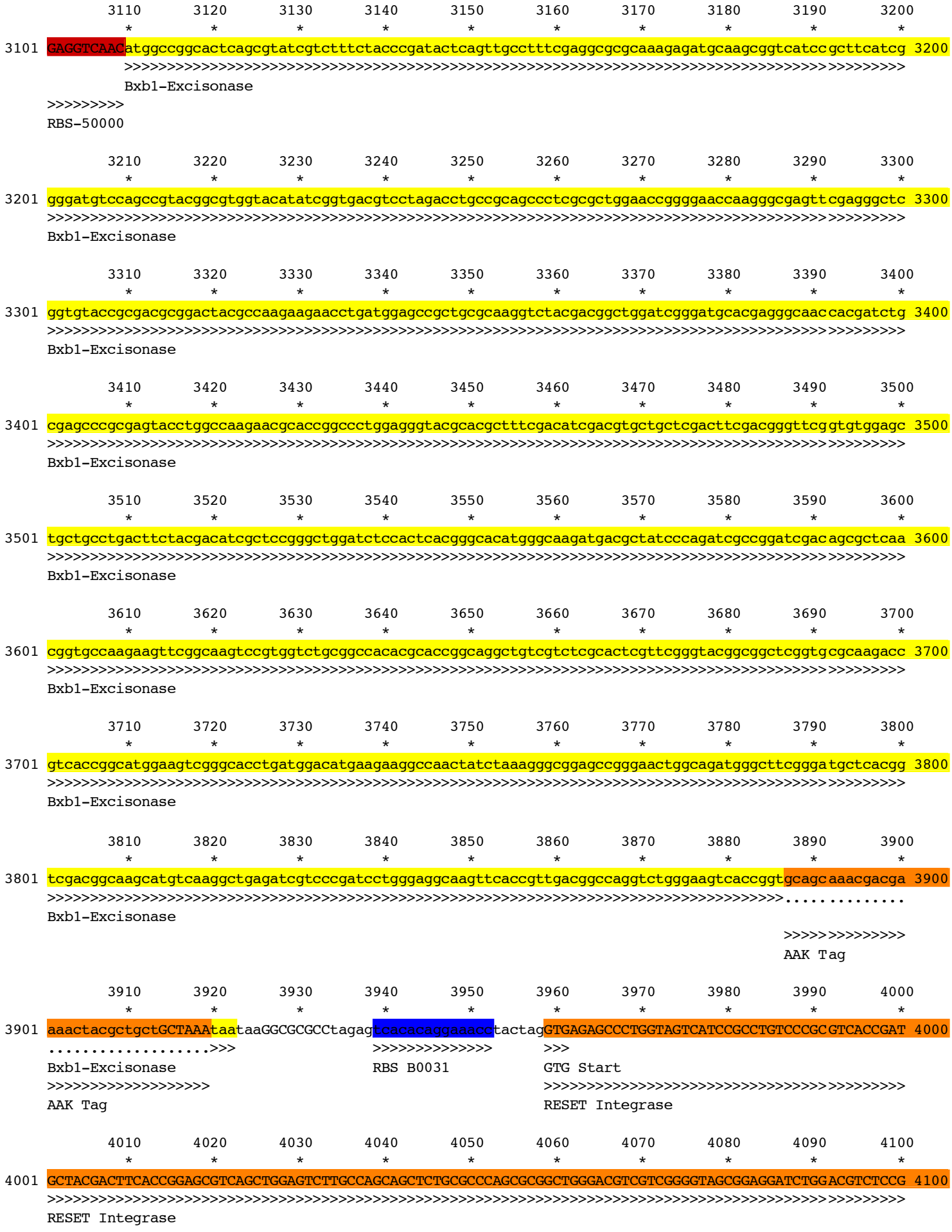
              2610      2620      2630      2640      2650      2660      2670      2680      2690      2700
                 *         *         *         *         *         *         *         *         *         *
2601 gtgagattgagaatataacctttcattcccagcggtcggtcgataaaaaaatcgagataaccgttggcctcaatcggcgttaaacccgccaccagatggg 2700
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     AraC

              2710      2720      2730      2740      2750      2760      2770      2780      2790      2800
                 *         *         *         *         *         *         *         *         *         *
2701 cattaaacgagtatcccggcagcaggggatcattttgcgcttcagccatacttttcatactcccgccattcagagaagaaaccaattgtccatattgcat 2800
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     AraC

              2810      2820      2830      2840      2850      2860      2870      2880      2890      2900
                 *         *         *         *         *         *         *         *         *         *
2801 cagacattgccgtcactgcgtcttttactggctcttctcgctaaccaaaccggtaaccccgcttattaaaagcattctgtaacaaagcgggaccaaagcc 2900
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     AraC

              2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
                 *         *         *         *         *         *         *         *         *         *
2901 atgacaaaaacgcgtaacaaaagtgtctataatcacggcagaaaagtcacattgattatttgcacggcgtcacactttgctatgccatagcatttttatc 3000
                                                     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
                                                     pBad
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     AraC

              3010      3020      3030      3040      3050      3060      3070      3080      3090      3100
                 *         *         *         *         *         *         *         *         *         *
3001 cataagattagcggatcctacctgacgctttttatcgcaactctctactgtttctccataccgtttttttgggctagcTACTAGACCTTAACAAACTACG 3100
                                                                                     >>>>>>>>>>>>>>>>
                                                                                     RBS-500 00
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     pBad
```


3110 3120 3130 3140 3150 3160 3170 3180 3190 3200
3101 GAGGTCAACatggccggcactcagcgtatcgtctttctacccgatactcagttgcctttcgaggcgcgcaaagagatgcaagcggtcatccgcttcatcg 3200
Bxb1-Excisonase
RBS-50000
3210 3220 3230 3240 3250 3260 3270 3280 3290 3300
3201 gggatgtccagccgtacggcgtggtacatatcggtgacgtcctagacctgccgcagccctcgcgctggaaccggggaaccaagggcgagttcgagggctc 3300
Bxb1-Excisonase
3310 3320 3330 3340 3350 3360 3370 3380 3390 3400
3301 ggtgtaccgcgacgcggactacgccaagaagaacctgatggagccgctgcgcaaggtctacgacggctggatcgggatgcacgagggcaaccacgatctg 3400
Bxb1-Excisonase
3410 3420 3430 3440 3450 3460 3470 3480 3490 3500
3401 cgagcccgcgagtacctggccaagaacgcaccggccctggagggtacgcacgctttcgacatcgacgtgctgctcgacttcgacgggttcggtgtggagc 3500
Bxb1-Excisonase
3510 3520 3530 3540 3550 3560 3570 3580 3590 3600
3501 tgctgcctgacttctacgacatcgctccgggctggatctccactcacgggcacatgggcaagatgacgctatcccagatcgccggatcgacagcgctcaa 3600
Bxb1-Excisonase
3610 3620 3630 3640 3650 3660 3670 3680 3690 3700
3601 cggtgccaagaagttcggcaagtccgtggtctgcggccacacgcaccggcaggctgtcgtctcgcactcgttcgggtacggcggctcggtgcgcaagacc 3700
Bxb1-Excisonase
3710 3720 3730 3740 3750 3760 3770 3780 3790 3800
3701 gtcaccggcatggaagtcgggcacctgatggacatgaagaaggccaactatctaaagggcggagccgggaactggcagatgggcttcgggatgctcacgg 3800
Bxb1-Excisonase
3810 3820 3830 3840 3850 3860 3870 3880 3890 3900
3801 tcgacggcaagcatgtcaaggctgagatcgtcccgatcctgggaggcaagttcaccgttgacggccaggtctgggaagtcaccggtgcagcaaacgacga 3900
Bxb1-Excisonase
AAK Tag
3910 3920 3930 3940 3950 3960 3970 3980 3990 4000
3901 aaactacgctgctGCTAAAtaataaGGCGCGCCtagagtcacacaggaaacctactagGTGAGAGCCCTGGTAGTCATCCGCCTGTCCCGCGTCACCGAT 4000
Bxb1-Excisonase
RBS B0031
GTG Start
AAK Tag
RESET Integrase
4010 4020 4030 4040 4050 4060 4070 4080 4090 4100
4001 GCTACGACTTCACCGGAGCGTCAGCTGGAGTCTTGCCAGCAGCTCTGCGCCCAGCGCGGCTGGGACGTCGTCGGGGTAGCGGAGGATCTGGACGTCTCCG 4100
RESET Integrase

```
        4110       4120       4130       4140       4150       4160       4170       4180       4190       4200
          *          *          *          *          *          *          *          *          *          *
4101 GGGCGGTCGATCCGTTCGACCGGAAGCGCAGACCGAACCTGGCCCGGTGGCTAGCGTTCGAGGAGCAACCGTTTGACGTGATCGTGGCGTACCGGGTAGA 4200
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        4210       4220       4230       4240       4250       4260       4270       4280       4290       4300
          *          *          *          *          *          *          *          *          *          *
4201 TCGGTTGACCCGATCGATCCGGCATCTTCAGCAGCTGGTCCACTGGGCCGAGGACCACAAGAAGCTGGTCGTCTCCGCGACCGAAGCGCACTTCGATACG 4300
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        4310       4320       4330       4340       4350       4360       4370       4380       4390       4400
          *          *          *          *          *          *          *          *          *          *
4301 ACGACGCCGTTTGCGGCGGTCGTCATCGCGCTTATGGGAACGGTGGCGCAGATGGAATTAGAAGCGATCAAAGAGCGGAACCGTTCGGCTGCGCATTTCA 4400
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        4410       4420       4430       4440       4450       4460       4470       4480       4490       4500
          *          *          *          *          *          *          *          *          *          *
4401 ATATCCGCGCCGGGAAATACCGAGGATCCCTGCCGCCGTGGGGATACCTGCCTACGCGCGTGGACGGGGAGTGGCGGCTGGTGCCGGACCCTGTGCAGCG 4500
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        4510       4520       4530       4540       4550       4560       4570       4580       4590       4600
          *          *          *          *          *          *          *          *          *          *
4501 AGAGCGCATCCTCGAGGTGTATCACCGCGTCGTCGACAACCACGAGCCGCTGCATCTGGTGGCCCACGACCTGAACCGGCGTGGTGTCCTGTCGCCGAAG 4600
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        4610       4620       4630       4640       4650       4660       4670       4680       4690       4700
          *          *          *          *          *          *          *          *          *          *
4601 GACTACTTCGCGCAGCTGCAAGGCCGCGAGCCGCAGGGCCGGGAGTGGTCGGCTACCGCGCTGAAGCGATCGATGATCTCCGAGGCGATGCTCGGGTACG 4700
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        4710       4720       4730       4740       4750       4760       4770       4780       4790       4800
          *          *          *          *          *          *          *          *          *          *
4701 CGACTCTGAACGGTAAGACCGTCCGAGACGACGACGGAGCCCCGCTGGTGCGGGCTGAGCCGATCCTGACCCGTGAGCAGCTGGAGGCGCTGCGCGCCGA 4800
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        4810       4820       4830       4840       4850       4860       4870       4880       4890       4900
          *          *          *          *          *          *          *          *          *          *
4801 GCTCGTGAAGACCTCCCGGGCGAAGCCCGCGGTGTCTACCCCGTCGCTGCTGCTGCGGGTGTTGTTCTGCGCGGTGTGCGGGGAGCCCGCGTACAAGTTC 4900
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        4910       4920       4930       4940       4950       4960       4970       4980       4990       5000
          *          *          *          *          *          *          *          *          *          *
4901 GCCGGGGGAGGACGTAAGCACCCGCGCTACCGCTGCCGCTCGATGGGGTTCCCGAAGCACTGCGGGAACGGCACGGTGGCGATGGCCGAGTGGGACGCGT 5000
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        5010       5020       5030       5040       5050       5060       5070       5080       5090       5100
          *          *          *          *          *          *          *          *          *          *
5001 TCTGCGAGGAGCAGGTACTGGATCTGCTCGGGGACGCGGAGCGTCTGGAGAAAGTCTGGGTAGCGGGCTCGGACTCCGCGGTCGAACTCGCGGAGGTGAA 5100
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        5110       5120       5130       5140       5150       5160       5170       5180       5190       5200
          *          *          *          *          *          *          *          *          *          *
5101 CGCGGAGCTGGTGGACCTGACGTCGCTGATCGGCTCCCCGGCCTACCGGGCGGGCTCTCCGCAGCGAGAAGCACTGGATGCCCGTATTGCGGCGCTGGCC 5200
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase
```

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        5210       5220       5230       5240       5250       5260       5270       5280       5290       5300
          *          *          *          *          *          *          *          *          *          *
5201 GCGCGGCAAGAGGAGCTGGAGGGCCTGGAGGCTCGCCCGTCTGGCTGGGAGTGGCGCGAGACCGGGCAGCGGTTCGGGGACTGGTGGCGGGAGCAGGACA 5300
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        5310       5320       5330       5340       5350       5360       5370       5380       5390       5400
          *          *          *          *          *          *          *          *          *          *
5301 CCGCGGCAAAGAACACCTGGCTTCGGTCGATGAACGTTCGGCTGACGTTCGACGTCCGCGGCGGGCTGACTCGCACGATCGACTTCGGGGATCTTCAGGA 5400
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        5410       5420       5430       5440       5450       5460       5470       5480       5490       5500
          *          *          *          *          *          *          *          *          *          *
5401 GTACGAGCAGCATCTCAGGCTCGGCAGCGTGGTCGAACGGCTACACACCGGGATGTCGACCGGCGCAGCAAACGACGAAAACTACGCTGCTCGTCATTAA 5500
                                                                >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
                                                                ARH Tag
     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     RESET Integrase

        5510       5520       5530       5540       5550       5560       5570       5580       5590       5600
          *          *          *          *          *          *          *          *          *          *
5501 TAATACTAGTAGCGGCCGCTGCAGtccggcaaaaaaacgggcaaggtgtcaccaccctgccctttttctttaaaaccgaaaagattacttcgcgttatgc 5600
        >>>>>>>>>>>>>>>>>>>>
        suffix BBa
     >>>
     ARH Tag
     >>>
     RESET Integrase

        5610       5620       5630       5640       5650       5660       5670       5680       5690       5700
          *          *          *          *          *          *          *          *          *          *
5601 aggcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatcctagggcgttcggctgcggc 5700

        5710       5720       5730       5740       5750       5760       5770       5780       5790       5800
          *          *          *          *          *          *          *          *          *          *
5701 gagcggtatcagctcactcaaaggcggtaatacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccag 5800

        5810       5820       5830       5840       5850       5860       5870       5880       5890       5900
          *          *          *          *          *          *          *          *          *          *
5801 gaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccg 5900
             <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
             ColE1 origin

        5910       5920       5930       5940       5950       5960       5970       5980       5990       6000
          *          *          *          *          *          *          *          *          *          *
5901 acaggactataaagataccaggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcc 6000
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     ColE1 origin

        6010       6020       6030       6040       6050       6060       6070       6080       6090       6100
          *          *          *          *          *          *          *          *          *          *
6001 cttcgggaagcgtggcgctttctcaatgctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgt 6100
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     ColE1 origin

        6110       6120       6130       6140       6150       6160       6170       6180       6190       6200
          *          *          *          *          *          *          *          *          *          *
6101 tcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagccactggtaacaggatt 6200
     <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
     ColE1 origin

        6210       6220       6230       6240       6250       6260       6270       6280       6290       6300
          *          *          *          *          *          *          *          *          *          *
6201 agcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctgctga 6300
```

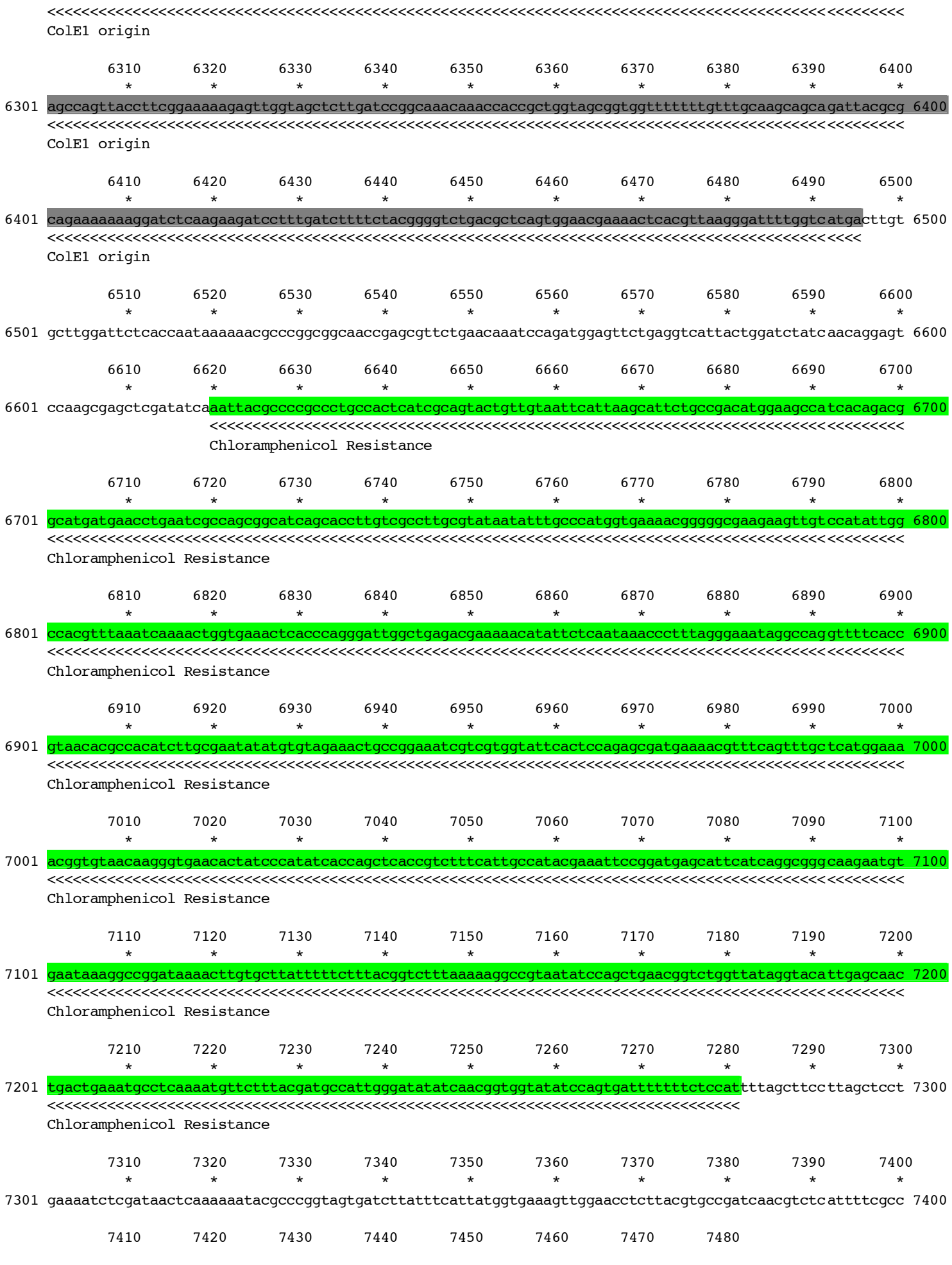
ColE1 origin
6310 6320 6330 6340 6350 6360 6370 6380 6390 6400
6301 agccagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggtggttttttgtttgcaagcagcagattacgcg 6400
ColE1 origin
6410 6420 6430 6440 6450 6460 6470 6480 6490 6500
6401 cagaaaaaaaggatctcaagaagatcctttgatcttttctacggggtctgacgctcagtggaacgaaaactcacgttaagggattttggtcatgacttgt 6500
ColE1 origin
6510 6520 6530 6540 6550 6560 6570 6580 6590 6600
6501 gcttggattctcaccaataaaaaacgcccggcggcaaccgagcgttctgaacaaatccagatggagttctgaggtcattactggatctatcaacaggagt 6600
6610 6620 6630 6640 6650 6660 6670 6680 6690 6700
6601 ccaagcgagctcgatatcaaattacgccccgccctgccactcatcgcagtactgttgtaattcattaagcattctgccgacatggaagccatcacagacg 6700
Chloramphenicol Resistance
6710 6720 6730 6740 6750 6760 6770 6780 6790 6800
6701 gcatgatgaacctgaatcgccagcggcatcagcaccttgtcgccttgcgtataatatttgcccatggtgaaaacgggggcgaagaagttgtccatattgg 6800
Chloramphenicol Resistance
6810 6820 6830 6840 6850 6860 6870 6880 6890 6900
6801 ccacgtttaaatcaaaactggtgaaactcacccagggattggctgagacgaaaaacatattctcaataaaccctttagggaaataggccaggttttcacc 6900
Chloramphenicol Resistance
6910 6920 6930 6940 6950 6960 6970 6980 6990 7000
6901 gtaacacgccacatcttgcgaatatatgtgtagaaactgccggaaatcgtcgtggtattcactccagagcgatgaaaacgtttcagtttgctcatggaaa 7000
Chloramphenicol Resistance
7010 7020 7030 7040 7050 7060 7070 7080 7090 7100
7001 acggtgtaacaagggtgaacactatcccatatcaccagctcaccgtctttcattgccatacgaaattccggatgagcattcatcaggcgggcaagaatgt 7100
Chloramphenicol Resistance
7110 7120 7130 7140 7150 7160 7170 7180 7190 7200
7101 gaataaaggccggataaaacttgtgcttatttttctttacggtctttaaaaaggccgtaatatccagctgaacggtctggttataggtacattgagcaac 7200
Chloramphenicol Resistance
7210 7220 7230 7240 7250 7260 7270 7280 7290 7300
7201 tgactgaaatgcctcaaaatgttctttacgatgccattgggatatatcaacggtggtatatccagtgatttttttctccatttagcttccttagctcct 7300
Chloramphenicol Resistance
7310 7320 7330 7340 7350 7360 7370 7380 7390 7400
7301 gaaaatctcgataactcaaaaaatacgcccggtagtgatcttatttcattatggtgaaagttggaacctcttacgtgccgatcaacgtctcattttcgcc 7400
7410 7420 7430 7440 7450 7460 7470 7480

```
            *        *        *        *        *        *        *        *
7401 agatatcgacgtctaagaaaccattattatcatgacattaacctataaaaataggcgtatcacgaggccctttcgtcttcacc 7483
```