

Supplementary Figures and Tables

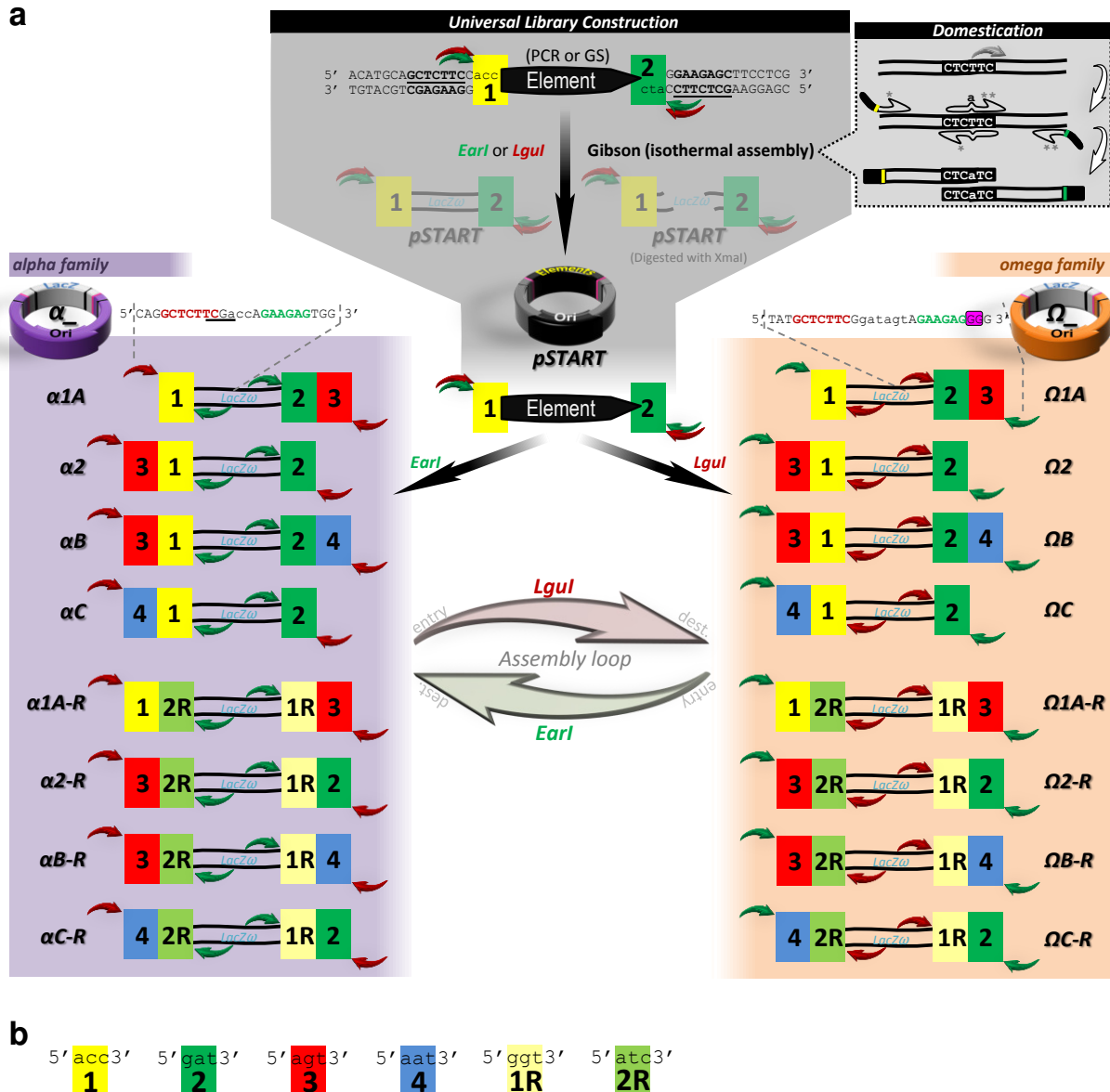
“An innovative platform for quick and flexible joining of assorted DNA fragments”

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Supplementary Figure 1

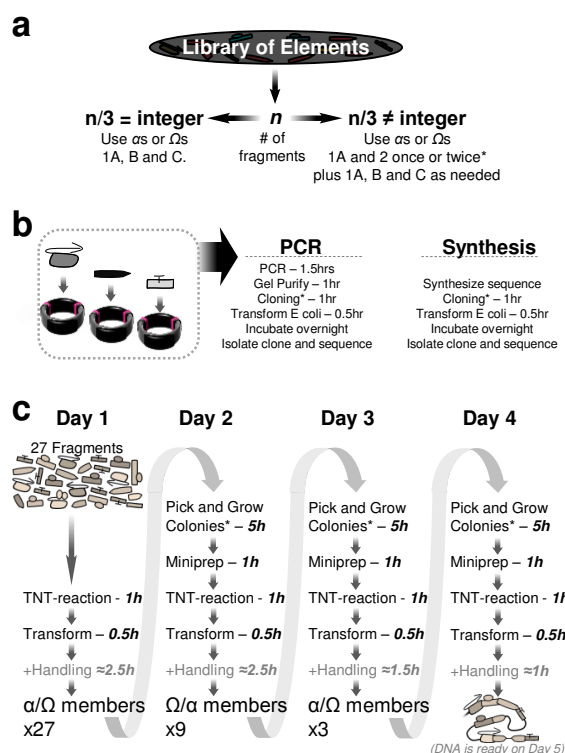


Supplementary Figure 1 – TNT-cloning system technical details

(a) Details of the library construction in the pSTART vector including the optional domestication process (top) along with the α family of vectors (purple, left) and Ω family of vectors (orange, right). Fragments of interest (element) can be produced by gene synthesis (“GS”) or amplified by PCR using the sequence shown (plus the three nucleotide code for signatures 1 and 2) to be inserted in the pSTART vector by either restriction enzymes (*EarI*/*LglI*) or “Gibson” isothermal assembly (which requires previous linearization of pSTART, as shown for *XmaI*). If extrusion of 5’CTCTTC3’ sites within the fragment is desired, overlapping oligos carrying a point mutation (e.g., T>A) can be used for amplification (asterisks denotes pair of primers to be used) and directly used for Gibson assembly with the linearized pSTART

(Domestication, top right). pSTART carries signatures 1 (yellow) and 2 (green) used to transfer the fragment from the library to any member of either α family (using Earl, green arrow) or Ω family (using Lgul, red arrow). Versions “R” in both families were created to allow fragment reorientation (sense or anti-sense insertion). Signatures 3 (red) and 4 (blue) will be used to join two (only signature 3) or three (both signatures 3 and 4) fragments together (Fig. 2b). Elements are also transferred from Ω s to α s using Earl and from α s to Ω s using Lgul. The enzyme setup and the selection markers employed allow for one-pot reactions, which uses up to three plasmids carrying the “inserts” (entry) plus one destination (dest.) vector (either α or Ω member) and for multiple rounds of cloning alternating between α and Ω families (Assembly loop). If constructs are done in *E. coli* T7Express (NEB), the reporter LacZ ω allow for white/“blue” screening (using either “5-Bromo-4-chloro-3-indolyl β -D-galactopyranoside” or “5-Bromo-3-indolyl β -D-galactopyranoside” but not “8-Hydroxyquinoline- β -D-galactopyranoside”, data not shown). Sequences at the top show the M.TaqI site (underlined, TCGA) in α members (absent in pSTART and Ω members). At the right side, the point mutation created upstream of Earl site to minimize Lgul promiscuity is circled with pink background (see also Supplementary Fig. 7). Both, Earl and Lgul sites match the arrow colors green and red, respectively. **(b)** Detailed sequence of each signature shown from 5' to 3'. Note that signatures 1R and 2R are the antisense of signatures 1 and 2, respectively. Signatures were chosen based on GC content, Adenine positioning to create M.TaqI site and to avoid stop codons as well as internal starting codons in case multiple CDS are to be joined.

Supplementary Figure 2



Supplementary Figure 2 –Multi-gene assembling using the TNT-cloning system.

(a) Ideogram: once cloned in the library, the number of fragments will define the vectors to be used and the number of cloning rounds necessary. If n number of fragments is divisible by 3 use only 1A, B and C versions. If $n-1$ or $n-2$ gives a number divisible by 3, versions 1A and 2 will be used twice or once, respectively, along with versions 1A, B and C as needed (asterisks). **(b)** Timeline with detailed description of each step during library construction into pSTART. Genes are either amplified or synthesized. If domestication is necessary, only one PCR is required upon use of Gibson isothermal assembly for cloning (asterisk; see also Supplementary Fig. 1). For multiple fragments assembly may be necessary to perform a regular TNT-reaction (asterisk, 50 cycles of '45sec 34 C, 4.5min 16 C'). **(c)** Timeline in days with detailed breakdown for hours necessary to accomplish each step outlined. Colonies can be picked, diluted in 10 μ l of water, inoculated (5 μ l) and simultaneously checked by colony PCR (1 μ l), so, only positive clones are used for mini-prep and cloning (asterisk). TNT-reaction shown is for destination vectors linearized in advance (otherwise, a regular TNT-reaction of 4.5 h is necessary for binary/tertiary assembling). Growing and transformation times are shown for fast growing strains as Mach1TM-T1^R or T7Express. If non-domesticated fragments are present and the *BlindSpot* protocol is chosen, it has to be included separately (1-12 h). Constructs expected to be larger than 4 kb may require an extra hour for digestion and agarose gel analysis after mini-prep instead of colony PCR.

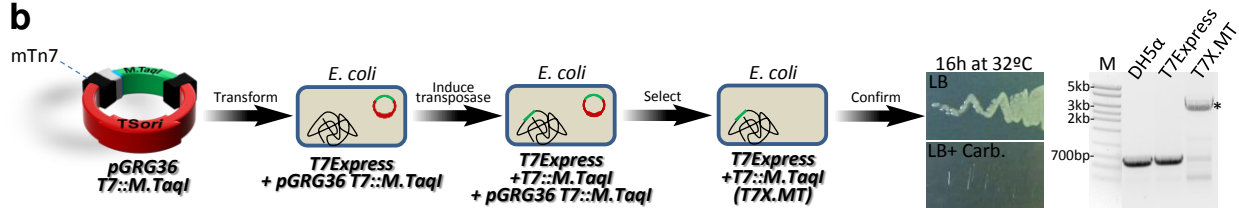
Supplementary Figure 3

a

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b

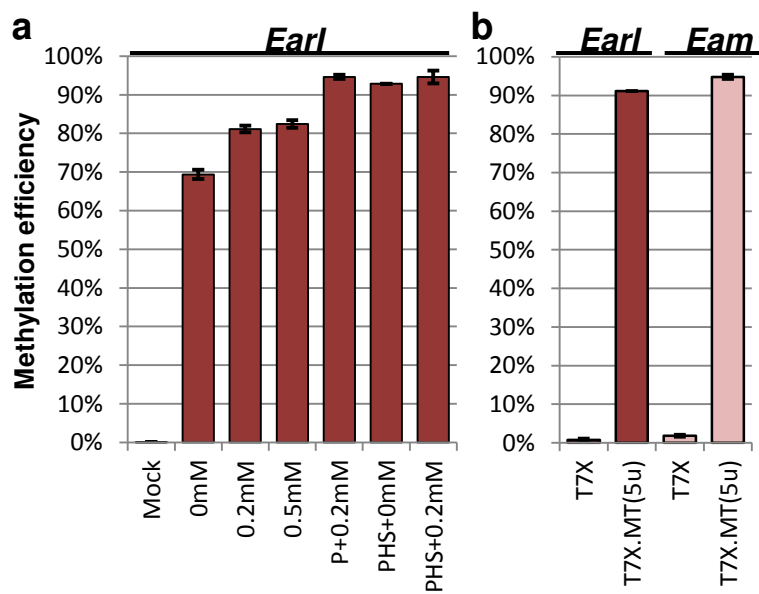


Supplementary Figure 3 – M.TaqI gene cluster synthesized (1884 bp) and graphical representation of its insertion into the T7Express (NEB) genome.

(a) M.TaqI gene was codon optimized for *E. coli* as shown (green highlight). T7 elements, promoter (light blue highlight) and terminator (dark blue highlight) plus the *lac*Operator (yellow highlight) and the overlap region for cloning in pGRG36 (dark red) at the NotI site (underlined) are shown. Ribosome binding site is bold/italic (AAGGAG), light gray is a secondary expression cassette and lower case is a linker sequence. Cloning region is flanked by the terminal repeats of Tn7 (mTn7) at the pGRG36 vector³⁶. **(b)** Graphical representation for construction of T7X.MT as described in McKenzie and Craig³⁶. Briefly, the synthetic fragment shown in “a” was cloned in pGRG36 using the Gibson assembly strategy, transformed in the T7Express (NEB) strain and selected in carbenicillin plates at 30-32°C due to a thermosensitive replication origin (TSori). Five single colonies were picked and inoculated in liquid LB media in the presence of 0.1% L-arabinose to induce the transposases overnight. Cultures were diluted and plated at 42°C overnight (to block plasmid replication and allow growth of transgenes). Single colonies were again grown in liquid media and re-streaked in LB plates. Carbenicillin sensitivity tests were conducted (16 h growth at 32°C) and one sensitive strain (defined as T7X.MT) had the insertion into the genome confirmed by PCR. In the absence of a Tn7 insertion, the genomic primers will yield a ≈678 bp product (DH5α and T7Express, controls). In the presence of the Tn7insertion this product will

increase and final size should be larger than 2562 bp but smaller than 3466 bp (asterisk), depending on the strain used for engineering. The strain shown was used for our methylation tests (see Fig. 3) and hosts the TNT-system vectors. We choose the T7X strain because of its specific genotype that allows stable expression of exogenous proteins (deficient in proteases *Lon* and *OmpT*) and its ability in not restricting methylated DNA (*McrA*⁻, *McrBC*⁻, *EcoBrm*⁻, *Mrr*⁻). We found the M.TaqI expression significantly impairs *E. coli* growth if incubation temperature goes over 42°C or IPTG concentration goes over 0.5 mM (data not shown).

Supplementary Figure 4



Supplementary Figure 4 – Methylation efficiency of T7X.MT at different conditions.

E. coli strains T7Express (mock) or T7X.MT were transformed with the M.Test vector, plated and two colonies were picked for overnight grown (≈ 18 h) in liquid LB media. Digested vector had the agarose gel bands quantified and expressed as percentage: $1 - [\text{digested} / (\text{digested} + \text{linearized})]$ in each tube. **(a)** Conditions shown are: IPTG concentration during the liquid growth (0 mM, 0.2 mM or 0.5 mM); presence (0.3 mM) or absence of IPTG in the plate during transformation with M.Test plasmid (*P*); presence or absence of a heat shock treatment for 1 h at 50°C right before DNA extraction (*HS*). **(b)** Stability of methylated DNA after 11 weeks subjected to both Earl (5 U) and Eam1104I (5 U) digestion. New replicates of P+0.2 mM were used ($n=3$). Note the minimal difference between freshly prepared DNA (shown in **A**) and 11-weeks-old DNA (5.9% SE \pm 1.0) to Earl inhibition and the similar inhibition of Eam1104I to the same DNA.

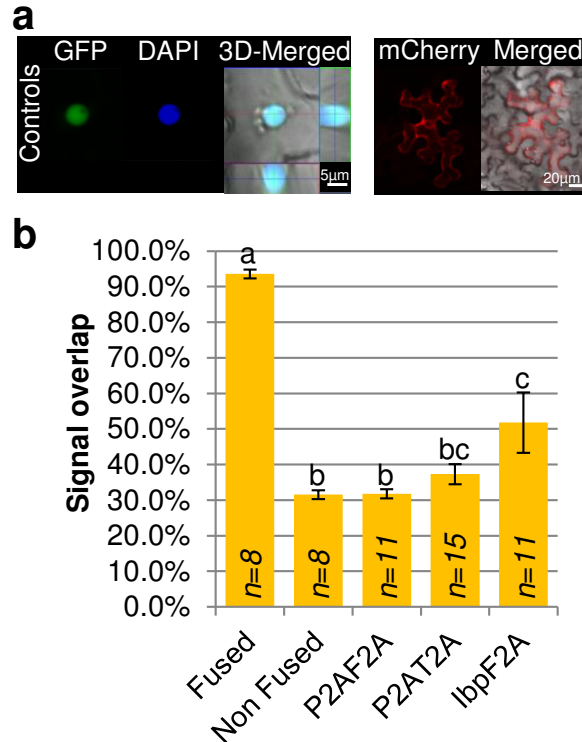
Supplementary Figure 5

	Sites	Type IIS				
		Methylase	BspQI	Lgul	SapI	EarI
Expected	$\begin{array}{c} \text{GAGCTCTTC} \\ \text{CTCGAGAAG} \end{array}$	M.SacI	?	?	?	?
	$\begin{array}{c} \text{tGCCTTCG} \\ \text{aCGAGAAG} \end{array}$	M.SssI	100%*	100%	100%	50%
	$\begin{array}{c} \text{GCTCTCGA} \\ \text{CGAGAAGCT} \end{array}$	M.TaqI	100%*	?	0%*	0%
Observed	$\begin{array}{c} \text{GAGCTCTTC} \\ \text{CTCGAGAAG} \end{array}$	M.SacI	≈20%	≈35%	≈15%	≈20%
	$\begin{array}{c} \text{tGCCTTCG} \\ \text{aCGAGAAG} \end{array}$	M.SssI (I)	≈100%	≈100%	≈60%	≈75%
	$\begin{array}{c} \text{CGCTTTC} \\ \text{GCGAGAAG} \end{array}$	M.SssI (II)	≈100%	≈100%	≈30%	≈100%
	$\begin{array}{c} \text{GCTCTCGA} \\ \text{CGAGAAGCT} \end{array}$	M.TaqI	≈90%	<0.1%	<0.1%	<0.1%

Supplementary Figure 5 – Methylation sensitivity chart for BspQI, Lgul, SapI and EarI enzymes.

Top- Sensitivity chart extracted from REBASE ²¹. Asterisks represent testing using synthetic oligonucleotides and question mark represents unavailable data. *Bottom-* Sensitivity observed on our assays. In our hands, M.SssI partially inhibited SapI activity. At least two replicates were performed for each pair of enzyme-methylase. Evaluation was performed using the same template used for EarI assays (see Fig. 3). Digestion ran for 1hour at 37°C (except for BspQI, where 50°C were used) using 5 U of each enzyme (except SapI, where 10 U were used) in 20 µl reaction volume. Amount of DNA was around 400 ng (PCR product) and 1 µg (plasmid DNA). Overlapped sites between methylase binding site and the *type IIS* enzymes are highlighted in black/bold. Nucleotides modified are indicated by a CH₃ branch.

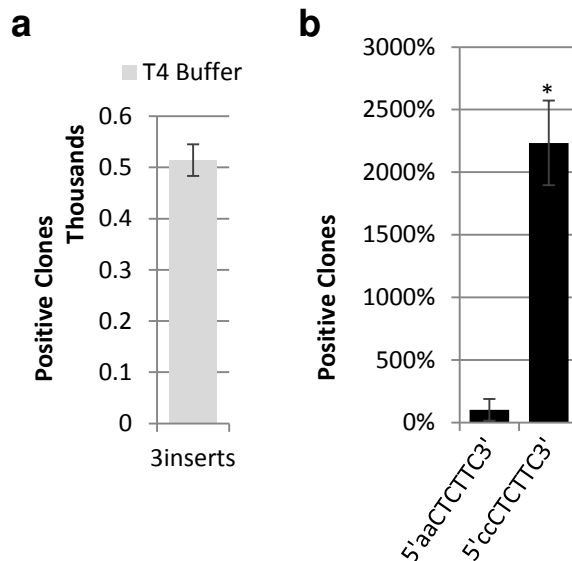
Supplementary Figure 6



Supplementary Figure 6 – Confocal image of controls and assessment of enhanced split efficiency of P2A clusters.

(a) Representative confocal images of *35S::NLS-GFP-NLS-Term* (GFP control) localized in the nucleus and *35S::tag-PmCherry-Term* (PmCherry control) localized in the karyoteca and plasma membrane. Signals were captured at a reduced window spectrum for GFP (493-556 nm) and mCherry (578-650 nm). 3D-merge shows 12 stacked images over 6 μm . For 4',6-diamidino-2-phenylindole (DAPI) images, leaf tissue (0.5 cm^2) were fixed in formalin+0.2% Triton-X for 30 min and stained (1.25 $\mu\text{g}/\text{ml}$) for 15 min right before analysis, washed in PBS and mounted in water. Scale bars are indicated for each panel. Plants were grown on standard greenhouse conditions and infiltration was performed as described previously³⁶. (b) At least one hundred cells from confocal images were visually scored for mCherry and GFP fluorescence separation in each P2A cluster constructs and 8-15 images taken had each channel plotted through a 10-18 μm section of a nucleus (Fig. 4c). Graph area for each channel were measured using ImageJ and the raw signal overlap $[(\text{PmCherry}/\text{GFP}) * 100]$ is shown. Split efficiency was then calculated related to the Non Fused control: *P2AF2A* (99.7% SE \pm 1.2, n=11) and *P2AT2A* (94.2% SE \pm 2.8, n=15) showed the best sub-cellular separation of signals, against the partial separation observed for *lbpF2A* (79.7% SE \pm 8.4, n=11). Holm analysis, $p < 0.01$ (a-b) and $p < 0.05$ (b-c).

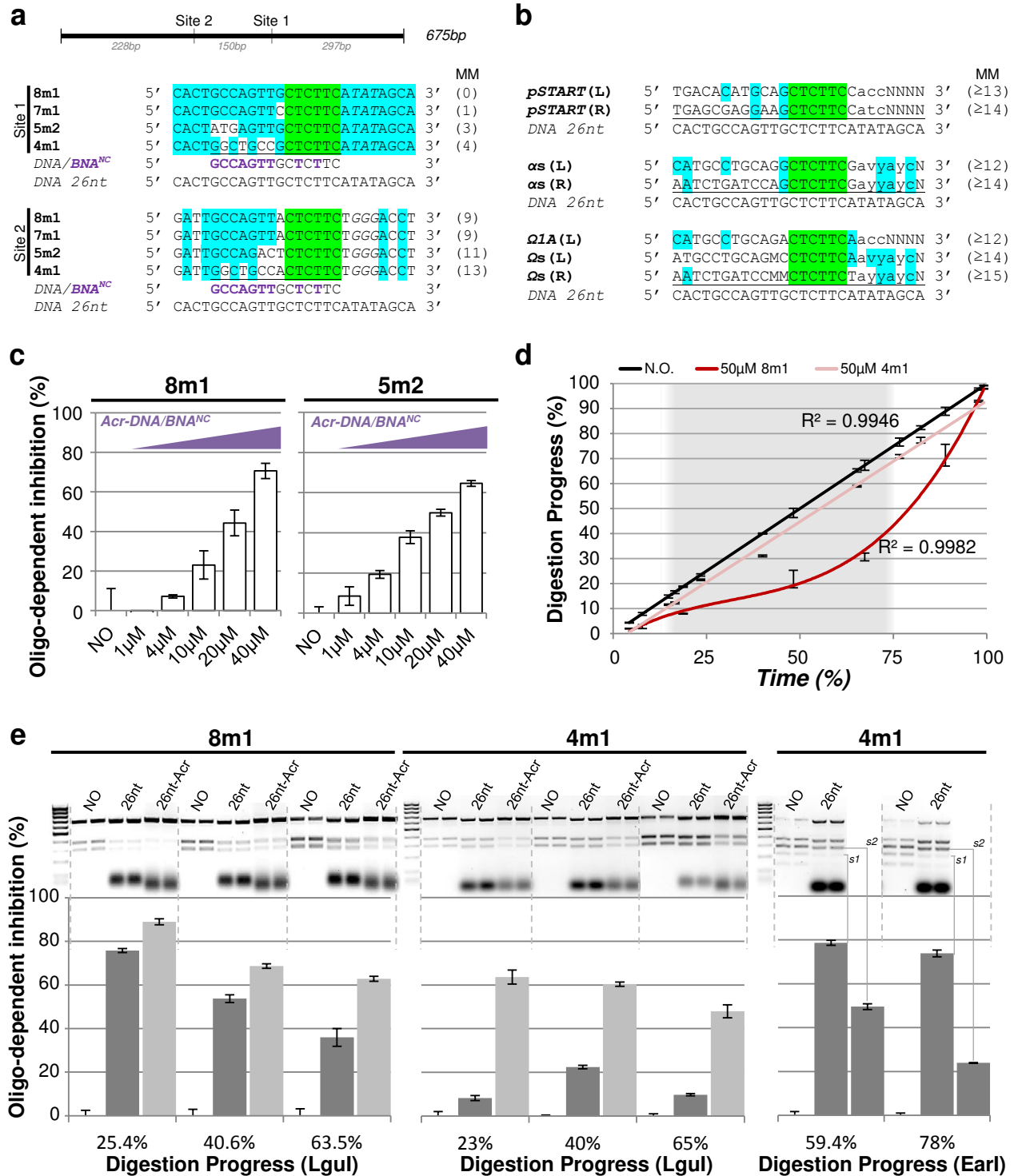
Supplementary Figure 7



Supplementary Figure 7 – Cloning efficiency of T4DNA Ligase buffer for 3 elements cloning and two different versions of Ω vector with upstream point mutations.

(a) Three fragments assembly from both ways reactions (α 's to Ω 's and vice-versa) using T4 DNA ligase buffer (T4 Buffer) using the GoldenBraid (50 cycles of: 37°C 2min, 16°C 5min) reaction. Error bars are from 3 independent cloning reactions. Number of clones shown are positive clones confirmed by colony PCR ($16 < n < 32$). Same lot of competent cells was used here and for Fig. 4e. **(b)** Optimized nucleotide sequence upstream of Earl site to minimize unspecific cleavage by LgI. Most clones obtained from tertiary assembly reactions were unspecific transfer of one fragment from $\alpha 1A$ to $\Omega 1A$, which represents unspecific cleavage of LgI at the Earl site exposing the signature number 3 of $\Omega 1A$ rather than signature number 2 for proper cloning of the final tertiary construct (data not shown; see also Supplementary Fig. 1). Therefore, three new point mutations were tested at the 5' end of Earl site. The best result is shown here, for sequence 5'ccCTCTTC3' where more than 2000% increase in cloning efficiency were observed compared to the previous Ω version 5'aaCTCTTC3'. This sequence were implemented in all Ω members at sites that bear 2 signatures side by side and represent the final set of Ω plasmids (Supplementary Fig. 1). Error bars are standard error for 4 biological replicates. Bonferroni analysis are $p < 0.05$ (*) and $p < 0.01$ (**, letters).

Supplementary Figure 8



Supplementary Figure 8 – Oligo-dependent inhibition of digestion at 5'(G)CTCTTC3' sites.

(a) DNA templates studied with nomenclature, sequence and number of mismatches (MM) related to the 14nt DNA/BNA^{NC} (purple) and 26-nt DNA oligonucleotides. PCR product (675 bp) was used here.

Restriction sites for Lgul and Earl as well as the distance between them are indicated at sites 1 and 2. 5'CTCTTC3' sequence and matches to the oligo are indicated in green and blue, respectively. **(b)** TNT-plasmids showing the 5'CTCTTC3' flanking sequences aligned to the 26-nt DNA oligo. All the α and Ω members (except Ω 1A) share the same sequence within each family at the left (L) or at the right (R) side of the LacZw reporter, respectively. Number of mismatches is also indicated. V = A, C or G, M = A or C, Y = C or T, N = any. **(c)** Oligo-dependent inhibition of digestion at site 1 using the templates 8m1 (200 ng) and 5m2 (200 ng) during increasing amounts of 14-nt DNA/BNA^{NC} linked to acridine²⁸ (Acr-DNA/BNA^{NC}). Oligos were incubated for 6 h at 37°C in Tris-Acetate buffer (50 mM pH 7.0, 20 mM NaCl, 10 mM MgCl₂) in 5 μ l before being subjected to digestion (1 U Lgul, 25 min at 34°C) in 10 μ l final volume. Reaction was stopped and gel bands quantified and plotted as percentage of the control without oligo (N.O.). Digestion progress is the ratio of digested/(digested+non-digested) bands in each tube. Values when present are expressed as percentage of N.O. tube. Digestion progress in these panels was 19.7% (SE \pm 2.2). Oligo amount is indicated. **(d)** Digestion progression curve in the absence (N.O.) or presence of the 26-nt DNA oligo (50 μ M) in two different templates: 8m1 (0 mismatch, 180 ng) and 4m1 (4 mismatches, 180 ng). Oligo and templates were incubated for 45°C-12°C (every 3°C, 1 h each) in 4 μ l Tris-HCl buffer (50 mM pH 5.8, 75 mM NaCl, 10 mM MgCl₂, 2 mM DTT) before being subjected to digestion (1.5 U Lgul, 2-30 min at 25°C) in 10 μ l final volume (completed with Tris-HCl 50 mM pH 6.3, 10 mM MgCl₂, 2mM DTT); 5, 10 and 15 min in these conditions corresponds to 24.4% (SE \pm 0.6), 40.3% (SE \pm 0.6) and 62.0% (SE \pm 2.1) digestion progression, respectively. Digestion is fairly linear in the N.O. tube in the range of 2 min (14.8% SE \pm 0.6) to 20 min (76.6% SE \pm 1.6) ($R^2=0.9967$), gray background. R^2 values shown are for linear (4m1, 0.9946) and polynomial (8m1, 0.9982) trends. **(e)** Oligo-dependent inhibition of digestion at sites 1 (Lgul and Earl) and 2 (Earl only) using the templates 8m1 (180ng) and 4m1 (180ng). The DNA oligos 26 nt and 26 nt fused to acridine (26nt-Acr) (always 50 μ M) were incubated with each template as in **d**. Reaction was stopped and gel bands (shown) quantified and plotted as percentage of the control without oligo (N.O.) as in **c**. Earl activity on each site, s1 (4 mismatches) and s2 (13 mismatches), was evaluated independently (right panel). The 4m1, 5m2, 7m1 and 8m1 templates were originally cloned in pSTART from three GBlocks: two universal GBlocks (TFOsynt_Uni5' and TFOsynt_Uni3') along with its respective central fragment TFOsynt_4m1, TFOsynt_5m2, TFOsynt_7m1, TFOsynt_8m1 (Supplementary Table 3).

Supplementary Table 1 - Type IIS enzymes available on Rebase (Roberts et al., 2015, Nuc. Acid. Res.)

<i>Enzymes</i>	<i>Recognition Sequence</i>	<i>Suppliers</i>	<i>Reach</i>	<i>Reach</i>	<i>Extension*</i>	<i>Recognition Sequence</i>
Bco5I	CTCTTC (1/4)	-	1	4	3 nt 5'	5' CTCTTCN^
Bco116I	CTCTTC (1/4)	-	1	4	3 nt 5'	5' CTCTTCN^
BcoKI	CTCTTC (1/4)	-	1	4	3 nt 5'	5' CTCTTCN^
BsaXI	(9/12) ACNNNNNCTCC (10/7)	N	10	7	3 nt 3'	5' ACNNNNNCTCCNNNNNNNNNN^
BsaXI	GGAGNNNNNGT (10/7)	N	12	9	3 nt 3'	5' GGAGNNNNNGTNNNNNNNNNN^
BseZI	CTCTTC (1/4)	-	1	4	3 nt 5'	5' CTCTTCN^
BspQI	GCTCTTC (1/4)	N	1	4	3 nt 5'	5' GCTCTTCN^
BssIMI	GGGTC (-3/0)	-	-3	0	3 nt 5'	5' GG^GTC
Bst6I	CTCTTC (1/4)	IV	1	4	3 nt 5'	5' CTCTTCN^
Bsu6I	CTCTTC (1/4)	-	1	4	3 nt 5'	5' CTCTTCN^
CatHI	CTCTTC (1/4)	-	1	4	3 nt 5'	5' CTCTTCN^
Eam1104I	CTCTTC (1/4)	B	1	4	3 nt 5'	5' CTCTTCN^
EarI	CTCTTC (1/4)	N	1	4	3 nt 5'	5' CTCTTCN^
Ksp632I	CTCTTC (1/4)	-	1	4	3 nt 5'	5' CTCTTCN^
Lgul	GCTCTTC (1/4)	B	1	4	3 nt 5'	5' GCTCTTCN^
PciSI	GCTCTTC (1/4)	I	1	4	3 nt 5'	5' GCTCTTCN^
RleAI	CCCACA (12/9)	-	12	9	3 nt 3'	5' CCCACANNNNNNNNNN^
SapI	GCTCTTC (1/4)	N	1	4	3 nt 5'	5' GCTCTTCN^
SimI	GGGTC (-3/0)	-	-3	0	3 nt 5'	5' GG^GTC
VpaK32I	GCTCTTC (1/4)	-	1	4	3 nt 5'	5' GCTCTTCN^

*Filter was applied to exhibit " 3 nt 3' " and " 3 nt 5' ".

Supplementary Table 2. List of fragments relevant for this work and their respective vectors.

<i>Vector</i>	<i>Inserts</i>
pSTART	d35S_h-h, PmCherry, Lumio, RGR gene, P2A, T2A, Cas9*, F2A, lbp, GFP, 35SProm, 35STerm, NLS, NosProm, rGUS, HCC (Hig-CodA, see methods), Kan-ORF, 8m1*, 7m1*, 5m2*, 4m1*, 8m2*, CircRep
α 1A	NosProm, d35S_h-h, NLS, 35SProm, RGR gene, GFP cluster, 35SProm-L-PmCherry, CircRep-8m1-8m1*
α 1AR	GUS, HCC cluster
α 2	35STerm, GFP, RGR gene, nGFP-L-Term
α 2R	35SProm, Kan-ORF
α B	Kan-ORF, 35STerm, GFP, Lumio, P2A-T2A, P2A-F2A, Lumio, RGR gene**, lbp-F2A
α BR	GFP, 8m2*
α C	35STerm, P2A-F2A, NLS, PmCherry, RGR gene, nGFP-L-Term, nGFP-L-Term, 8m2*
α CR	35SProm
Ω 1A	PmCherry cluster, P2A, lbp, P2A-T2A cluster, nGFP, 35SProm, 35SProm-L-PmCherry, PmCherry cluster, 8m1*, 7m1*, CircRep
Ω 1AR	35STerm
Ω 2	35SProm-GUS, T2A, F2A
Ω B	P2A-F2A cluster, nGFP, Lumio, 8m1*
Ω C	lbp-F2A cluster, PmCherry, 35STerm, 8m1*
Ω CR	35SProm

*Fragments were either partially or not domesticated.

**RGR gene, ribozyme-gRNA-ribozyme⁴⁰

Supplementary Table 3. Primers (green), Gblocks (blue) and GeneSynthesis (purple) used in this study and their respective name, length(bp) and sequence.

<i>Name</i>	<i>Length</i>	<i>Sequence</i>
pUPD-FW1	32	GCTCTCCACCCCGGGGCTGGCTTAACTATGC
pUPD-RW1	27	TGGCGTAATAGCGAGGAGGCCCGCACC
pUPD-FW2	27	GGTGCGGGCCTCCTCGCTATTACGCCA
pUPD-RW2	31	CTCTAGAGGATCCCTGTTACCGAGCTCGAA
pUPD-FW3	31	TTCGAGCTCGGTACCAGGGGATCCTCTAGAG
pUPD-RW3	35	GCTCTCCATCCCGGGCGCCAATACGCAAACCGC
pUPD-FW4	34	CCCGGGATGGAAGAGCTTCTCGCTCACTGACTC
pUPD-RW4	41	GCTTCAATAATATTGAAAAAGGAGGAGTATGAGTATTCAAC
pUPD-FW5	41	GTTGAATACTCATACTCTCTTTTCAATATTATTGAAGC
pUPD-RW5	33	GCCCCGGGGTGAAGAGCTGCATGTGTGAGAGG
pUPD-RW3.1	27	CAGCTATGACCATGATTACGGATTAC
FW_adap	67	CTCTCCACCCCGGGGCTGGCTTAACTATGCGGCATCAGAGCTTATTTTTGACACCAGACCAACTGG
pUPD-FW3.1	31	CGTAATCATGGTCATAGCTGTTTCTGTGTG
pUPD-RW5	33	GCCCCGGGGTGAAGAGCTGCATGTGTGAGAGG
KStrat2_TNT-Fw	26	CATTACAGCTTACGAACCGAACGAGG
KStrat2_TNT-Rw	21	GCAGCGAGTCAGTGAGCGAGG
Kan_to_O-FW2	25	GGAATTTATGCCGCTTCCGACCATC
KStrat2_TOP-Rw	21	CCTCGCTCACTGACTCGCTGC
KStrat2_TOP-FW	37	TCGGTTCGTAAGCTGTAATGTTCTGGCAGCTCTGGC
Kan_to_O-RW1	25	GATGGTCGGAAGCGGCATAAATTCC
$\alpha\Omega$ Vector_FW	24	TGGATCAGATTGTGCTTTCCCGCC
$\alpha\Omega$ Vector_RW	24	CTGCAGGCATGCAGCTCGAATTAT
aO_vector-nested-RW	27	GCTCGAATTATCGATCATGAGCGGAGA
aO_vector-nested-FW	30	GTTTCCCGCTTCAGTTAAACTATCAGTG
PCR2_to_ α Vector-Fw	24	GCTGCATGCCTGCAGGCTCTTCA
PCR2_to_ α Vector-Rw	38	GGG AAA CGA CAA TCT GATCCA GCT CTT CGA
α 1A-Fw	55	GCAGGCTCTTCGACCAGAAGAGT GGC TTA ACT ATG CGG CAT CAG AGC
α 1A-Rw	63	GATCCA GCT CTT CGA CTA TCT GAA GAG T ATA CGC AAA CCG CCT CTC CC
α 2-Fw	58	GCAGGCTCTTCGAGTACCAGAAGAGT GGC TTA ACT ATG CGG CAT CAG AGC
α 2-Rw	58	GATCCAGCT CTT CGA TCT GAA GAG T ATA CGC AAA CCG CCT CTC CC
α B-Fw	58	GCAGGCTCTTCGAGTACCAGAAGAGT GGC TTA ACT ATG CGG CAT CAG AGC
α B-Rw	62	GATCCAGCT CTT CGA TTA TCT GAA GAG T ATA CGC AAA CCG CCT CTC CC
α C-Fw	58	GCAGGCTCTTCGAATACCAGAAGAGT GGC TTA ACT ATG CGG CAT CAG AGC
α C-Rw	58	GATCCAGCT CTT CGA TCT GAA GAG T ATA CGC AAA CCG CCT CTC CC
α 1R-Fw	58	GCAGGCTCTTCGACCATCAGAAGAGT GGC TTA ACT ATG CGG CAT CAG AGC
α 1R-Rw	62	GATCCAGCT CTT CGA CTA CCT GAA GAG T ATA CGC AAA CCG CCT CTC CC
α 2R-Fw	58	GCAGGCTCTTCGAGTATCAGAAGAGT GGC TTA ACT ATG CGG CAT CAG AGC
α 2R-Rw	62	GATCCAGCT CTT CGA TCA CCT GAA GAG T ATA CGC AAA CCG CCT CTC CC
PCR2_to_ Ω Vector-Fw	22	GCTGCATGCCTGCAGACTCTTC
PCR2_to_ Ω Vector-Rw	34	GGG AAA CGA CAA TCT GATCCA ACTCTTC
Ω 1A-Fw	56	GCAG ACTCTTCAACCCGAAGAGC GGC TTA ACT ATG CGG CAT CAG AGC
Ω 1A-Rw	63	GATCCA ACT CTT CTA CTA TCC GAA GAG C ATA CGC AAA CCG CCT CTC CC
Ω 2-Fw	59	GCAG ACTCTTCAAGTACCCGAAGAGC GGC TTA ACT ATG CGG CAT CAG AGC
Ω 2-Rw	59	GATCCA ACT CTT CTA TCC GAA GAG C ATA CGC AAA CCG CCT CTC CC
Ω B-Fw	59	GCAG ACTCTTCAAGTACCCGAAGAGC GGC TTA ACT ATG CGG CAT CAG AGC

ΩB-Rw	63	GATCCA ACT CTT CTA TTA TCC GAA GAG C ATA CGC AAA CCG CCT CTC CC
ΩC-Fw	59	GCAG ACTCTTCAAATACCCGAAGAGC GGC TTA ACT ATG CGG CAT CAG AGC
ΩC-Rw	59	GATCCA ACT CTT CTA TCC GAA GAG C ATA CGC AAA CCG CCT CTC CC
Ω1R-Fw	59	GCAG ACTCTTCAACCATCCGAAGAGC GGC TTA ACT ATG CGG CAT CAG AGC
Ω1R-Rw	63	GATCCA ACT CTT CTA CTA CCC GAA GAG C ATA CGC AAA CCG CCT CTC CC
Ω2R-Fw	59	GCAG ACTCTTCAAGTATCCGAAGAGC GGC TTA ACT ATG CGG CAT CAG AGC
Ω2R-Rw	63	GATCCA ACT CTT CTA TCA CCC GAA GAG C ATA CGC AAA CCG CCT CTC CC
Earl-FW1	31	GTTTTTCATTACCGACGAGATCGAGGCGGAG
Earl-FW2	31	GCGCACAGCCGACGAGCTGCAAAAAG
Earl-FW3	31	CAGGCGCTCTTACGCTTCCTCGCTC
Earl-FW4	31	TTCGCCGCCAAGTTCTTCAGCAATATC
Earl-FW5	31	CGAGCCCCTGATGTTCTTCGTCCAG
Earl-RW1	31	CTCCGCCTCGATCTCGTCGGTAATGAAAAAC
Earl-RW2	31	CTTTTTCGAGCTCGTCGGCTGTGCGC
Earl-RW3	31	GAGCGAGGAAGCGTAAGAGCGCCTG
Earl-RW4	31	GATATTGCTGAAGAACTTGCGCGCGAA
Earl-RW5	31	CTGGACGAAGAACATCAGGGGCTCG
TNT-αΩ-seqRW	22	CTCTTAGGTTTACCCGCCAATA
TNT-αΩ-seqFW	22	AACGTGACTCCCTTAATTCTCC
pUPD_adap_met.test-FW	43	CTCTTCGACCCCGGGGC TGGCTTAACTATGCGGCATCAGAGC
pUPD-RW5-M_Test	33	GCCCCGGGGTGAAGAGCTGCATGTGTAGAGG
pUPD-seqFW	20	GCCACCTGACGTCTAAGAAA
pUPD-seqRW	25	CCTGATTCTGTGGATAACCGTATTA
TNT-Lumio_FW	72	ACATGCAGCTCTCCACCGGTGCTGGTGGTTGCTGCCCTGGTTGCTGCGGTGGTGATGGAAGAGCTTCCTCG
TNT-Lumio_RW	72	CGAGGAAGCTCTTCCATCACCACCGCAGCAACCAGGGCAGCAACCACCAGCACCGGTGGAAGAGCTGCATGT
TNT-GFP_FW	35	ACATGCAGCTCTTCCACCGTGAGCAAGGGCGAGGA
TNT-GFP_RW	38	CGAGGAAGCTCTTCCATCCTTGTACAGCTCGTCCATGC
TNT-NLS_FW	66	ACATGCAGCTCTTCCACCCCTAAGAAGAAGCGTAAGGTCGAGGACCCTGATGGAAGAGCTTCCTCG
TNT-NLS_RW	66	CGAGGAAGCTCTTCCATCAGGGTCTCGACCTTACGCTTCTTCTTAGGGGTGGAAGAGCTGCATGT
TNT-P2A_FW	93	ACATGCAGCTCTTCCACCGTACCAACTTCTCTCTCCTCAAGCAGGCTGGTGACGTCGAGGAGAACCTGGTCTGATGGAA GAGCTTCCTCG
TNT-P2A_RW	93	CGAGGAAGCTCTTCCATCAGGACCAGGGTCTCTCGACGTCACCAGCCTGCTTGGAGAGAGAGAAGTTGGTAGCGGTGG AAGAGCTGCATGT
TNT-T2A_FW	96	ACATGCAGCTCTTCCACCCGTGCTGAGGGTCTGTTCTCTCTCACCTGCGGTGACGTCGAGGAGAACCTGGTCTGAT GGAAGAGCTTCCTCG
TNT-T2A_RW	96	CGAGGAAGCTCTTCCATCAGGACCAGGGTCTCTCGACGTCACCAGGTCGAGGAGAGAACACGACCCTCAGCACGGG TGGAAGAGCTGCATGT
TNT-IbP_FW	90	ACATGCAGCTCTTCCACCCCTTGTCTAACGCTGCTGACGAGGTCGCTACCCTGAGGACGTCGAGCCTGGTATGGAAGA GCTTCCTCG
TNT-IbP_RW	90	CGAGGAAGCTCTTCCATCACCAGGCTCGACGCTCCTCAGGGGTAGCGACCTCGTCAGCAGCGTTAGAGCAAGGGGTGGAAG AGCTGCATGT
TNT-PmCherry_FW1	37	ACATGCAGCTCTTCCACCATGGCAAAGGATGTGGAAG
TNT-PmCherry_RW1	31	GATGTATAAGAATAGGAGAGTGGCTACGAAC
TNT-PmCherry_FW2	31	GTTCGTAGCCACTCTCTATTCTTATACATC
TNT-PmCherry_RW2	37	CGAGGAAGCTCTTCCATCAGATCTGTACAGCTCGTCC
TNT-35SPro_FW	40	ACATGCAGCTCTTCCACCCACAACATACGAGCCGGAAGCA
TNT-35SPro_RW	42	CGAGGAAGCTCTTCCATCCATGGCTATCGTTCTGTAATGGTG
TNT-35STerm_FW	45	ACATGCAGCTCTTCCACCTAAGTAGCTGAATCCCGCGCCATGCT

TNT-35STerm_RW	37	CGAGGAAGCTCTTCCATCTCGGGCTAGGCCGACGTC
TNT-F2A_FW	156	ACATGCAGCTCTCCACCCTCTCGCTATCCACCCTACCGAGGCTCGTACAAGCAGAAGATCGTCTGCTCTGTCAAGCAGACCCTCAACTTCGACCTCTCAAGCTCGCTGGTGACGTCGAGTCTAACCTGGTCTGATGGAAGAGCTTCCTCG
TNT-F2A_RW	156	CGAGGAAGCTCTTCCATCAGGACCAGGGTTAGACTCGACGTCACCAGCGAGCTTGAGGAGGTGGAAGTTGAGGGTCTGCTTGACAGGAGCGACGATCTTCTGCTTGACGAGCTCGGTAGGGTGGATAGCGAGGAGGGTGAAGAGCTGCATGT
TNT-Cas9-FW1	42	ACATGCAGCTCTCCACCATGGATTACAAGGATGATGATGAT
TNT-Cas9-RW1	35	GAA TCG AAA AGA AGT GCA CCG ATA AGG
TNT-Cas9-FW2	27	CCTTATCGGTGCACTTCTTTTCGATTC
TNT-Cas9-RW2	33	ACT CGT AAA GAA GTG AGT GCT TTG G
TNT-Cas9-FW3	25	CCAAAGCACTCACTTCTTTACGAGT
TNT-Cas9-RW3	30	TGC TCG TGA AGT GAA TCT CCC TG
TNT-Cas9-FW4	23	CAGGGAGATTCACCTCACGAGCA
TNT-Cas9-RW4	31	CTT AGA TGG AAG TGC AAG CTC GTT
TNT-Cas9-FW5	24	AACGAGCTTGCACTTCCATCTAAG
TNT-Cas9-RW5	45	CGAGGAAGCTCTTCCATCTTT ATG CCT GCA GGT CGC GAG
Ω2-lefCC-FW	51	CGAGCTGCATGCCTGCAGCCCTCTTCAAGTACCCGAAGAGCGGCTTAACTA
Ω2-lefCC-RW	51	TAGTTAAGCCGCTCTTCGGGTACTTGAAGAGGGCTGCAGGCATGCAGCTCG
ΩC-lefCC-FW	51	CGAGCTGCATGCCTGCAGCCCTCTTCAAATACCCGAAGAGCGGCTTAACTA
ΩC-lefCC-RW	51	TAGTTAAGCCGCTCTTCGGGTATTTGAAGAGGGCTGCAGGCATGCAGCTCG
Ω1R-lefCC-FW	51	CGAGCTGCATGCCTGCAGCCCTCTTCAACCATCCGAAGAGCGGCTTAACTA
Ω1R-lefCC-RW	51	TAGTTAAGCCGCTCTTCGGATGGTTGAAGAGGGCTGCAGGCATGCAGCTCG
Ω2R-lefCC-FW	51	CGAGCTGCATGCCTGCAGCCCTCTTCAAGTATCCGAAGAGCGGCTTAACTA
Ω2R-lefCC-RW	51	TAGTTAAGCCGCTCTTCGGATACTTGAAGAGGGCTGCAGGCATGCAGCTCG
ΩB-rigCC-FW	84	GTTTGCGTATGCTCTTCGGATAATAGAAGAGGGGGATCAGATTGTCGTTTCCCGCCTTCAGTTTAAACTATCAGTGTGTTGACAG
ΩB-rigCC-RW	84	CTGTCAAACACTGATAGTTTAAACTGAAGGCGGGAAACGACAATCTGATCCCCCTCTTCTATTATCCGAAGAGCATAACGCAAAC
Ω1R-rigCC-FW	84	GTTTGCGTATGCTCTTCGGGTAGTAGAAGAGGGGGATCAGATTGTCGTTTCCCGCCTTCAGTTTAAACTATCAGTGTGTTGACAG
Ω1R-rigCC-RW	84	CTGTCAAACACTGATAGTTTAAACTGAAGGCGGGAAACGACAATCTGATCCCCCTCTTCTACTACCCGAAGAGCATAACGCAAAC
Ω2R-rigCC-FW	84	GTTTGCGTATGCTCTTCGGGTGATAGAAGAGGGGGATCAGATTGTCGTTTCCCGCCTTCAGTTTAAACTATCAGTGTGTTGACAG
Ω2R-rigCC-RW	84	CTGTCAAACACTGATAGTTTAAACTGAAGGCGGGAAACGACAATCTGATCCCCCTCTTCTATACCCGAAGAGCATAACGCAAAC
rGUS-FW1	39	ACATGCAGCTCTCCACCATGTTACGTCCTGTAGAAACC
rGUS-RW1	29	CGA GCA TCT CCT CAG CGT AAG G
rGUS-FW2	29	CCT TAC GCT GAG GAG ATG CTC G
rGUS-RW2	29	TGA CTG CCT CCT CGC TGT ACA G
rGUS-FW3	29	CTG TAC AGC GAG GAG GCA GTC A
rGUS-RW3	30	ACA CTG ATA CTC CTC ACT CCA CA
rGUS-FW4	30	TGT GGA GTG AGG AGT ATC AGT GT
rGUS-RW4	47	CGAGGAAGCTCTTCCATCTTG TTT GCC TCC CTG CTG CGG T
Hig-CodA-FW1	39	ACATGCAGCTCTCCACCATGAAAAAGCCTGAACCTCACC
Hig-CodA-RW1	29	CAC AGC CCC TCC TCG CCT GGT A
Hig-CodA-FW2	29	TAC CAG GCG AGG AGG GGC TGT G
Hig-CodA-RW2	29	CGT AAC GCC TCC TCC AGC AAC G
Hig-CodA-Fw3	29	CGT TGC TGG AGG AGG CGT TAC G
Hig-CodA-RW3	42	CGAGGAAGCTCTTCCATCACGTTTGAATCGATGGCTTCTGG

Luc+_pUPD_FW1	42	ACATGCAGCTCTCCACCATGGAAGATGCCAAAAACATAAAG
Luc+_pUPD_RW1	27	GGG CGT ATC TTT TCA TAG CCT
Luc+_pUPD_FW2	27	AGG CTA TGA AAA GAT ACG CCC
Luc+_pUPD_RW2	33	AAG AAT TGA AGT GAG TTT TCA CTG C
Luc+_pUPD_FW3	33	GCA GTG AAA ACT CAC TTC AAT TCT T
Luc+_pUPD_RW3	34	CCT CAG AAA CAG TTC TTC TTC AAA TC
Luc+_pUPD_FW4	34	GAT TTG AAG AAG AAC TGT TTC TGA GG
CircRep-FW	39	ACATGCAGCTCTCCACCGAGGGCGGTCCGCTGCCTTTT
CircRep-RW	39	CGAGGAAGCTCTTCCATCCCTTGTTCCTGGCGGCAGT
TaqI-Fw	29	AAC CGT CTA TCA GGG CGA TGG C
TaqI-Rw	29	GGC TTT CCA CTT CCC CGA AAC C
TaqI-Fw1.1	27	CGCAAGCTTGGATCGAAGAGCTCTTAG
TaqI-Rw1.1	27	CTAAGAGCTCTTCGATCCAAGCTTGCG
15ntW-H.TFOs1	15	AATTTGTCGCTTCTC
22ntW-H.TFOs1	21	AGCCAGAATTTGTCGCTTCTC
15ntRvH.TFOs1	15	CTCTTCGCTGTTTAA
22ntRvH.TFOs1	22	CTCTTCGCTGTTTAAAGACCGAT
15ntW-H.TFOs2	15	GTTCACGTCTTCTC
22ntW-H.TFOs2	22	CCGGCGTGTTCACGTCTTCTC
15ntRvH.TFOs2	15	CTCTTCTGTCACTTG
22ntRvH.TFOs2	22	CTCTTCTGTCACTTGTGCGGCC
26RvH.DNAsyn8mDW	26	CACTGCCAGTTGCTCTTCATATAGCA
26RvH.DNAsyn8mDW-Acridine 3'	26	CACTGCCAGTTGCTCTTCATATAGCA-Acridine
5'-Acridine-DNA _(n) /BNA ^{NC} _(n+)	14	Acridine-G+C+A+G+T+T+GCT+CT+TC
TFOsynt_Uni5'	225	ACATGCAGCTCTCCACCAAACCTATAACAGGGAACATAATTAGGACTAAAGAAGATTCAACGTACATTGATCTGACACA GTAGATTTAGTTGTCTCTGTACATACAGTATCTAGGATTATTCAACGAAAACAATATCAATTTCTCTACAGAAACCAAC GGCCAGTACTCTTTTGGCCATAAAAAGACCGTAACCCTAATTGTACACTGAGAATCTAACG
TFOsynt_Uni3'	316	TAGCAGATGCTACGATCTGTACGAACTGAGAAGTCTATTTGCTTTTGTGATTACGGAATATGCTGAATTCCTGCACGAATT CATGTGCGCTGTAAAGCAGAATATGGAGAGAAAGTGTGGTTCAGGTGAGCCATAGGATACTCTTAAGAATATGATT GTTGTACAGAACTACGATAAAAAGATGTCGGGAATTAATATCATACTCATCTTTTTCAGTTTGAAGATTTTGCAAACCAAT GCGTTTGACCTTTTGTCTAAGTACAGTGATAGCTTTCTGCCACTTGTGTATCGATGGAAGAGCTTCTCTCG
TFOsynt_8m1	210	GTCACACTGAGAATCTAACGATTGCCAGTTACTCTTCTGGGACCTACGACGAAGGATGACTCCGTCACGTTCTTCTCACT GTTTGACAATAAGCTCCAATTTTCAGACTTTTCATTTCAAACCTGTGGGTCTCATTTTCTCTGGCCTATATAAATCCAATATC CTCCACTGCCAGTTGCTCTTCATATAGCAGATGCTACGATCTGT
TFOsynt_8m2	210	GTCACACTGAGAATCTAACGATTAGTCCCGCTCTTCTGGGACCTACGACGAAGGATGACTCCGTCACGTTCTTCTCACT GTTTGACAATAAGCTCCAATTTTCAGACTTTTCATTTCAAACCTGTGGGTCTCATTTTCTCTGGCCTATATAAATCCAATATC CTCCACTGCCAGTTGCTCTTCATATAGCAGATGCTACGATCTGT
TFOsynt_7m1	210	GTCACACTGAGAATCTAACGATTGCCAGTTACTCTTCTGGGACCTACGACGAAGGATGACTCCGTCACGTTCTTCTCACT GTTTGACAATAAGCTCCAATTTTCAGACTTTTCATTTCAAACCTGTGGGTCTCATTTTCTCTGGCCTATATAAATCCAATATC CTCCACTGCCAGTTCTCTTCATATAGCAGATGCTACGATCTGT
TFOsynt_5m2	210	GTCACACTGAGAATCTAACGATTGCCAGACTCTTCTGGGACCTACGACGAAGGATGACTCCGTCACGTTCTTCTCACT GTTTGACAATAAGCTCCAATTTTCAGACTTTTCATTTCAAACCTGTGGGTCTCATTTTCTCTGGCCTATATAAATCCAATATC CTCCACTATGAGTTGCTCTTCATATAGCAGATGCTACGATCTGT

TFOsynt_4m1

210

GTCACACTGAGAATCTAACGATTGGCTGCCACTTCTGGGACCTACGACGAAGGATGACTCCGTCCACGTTCTTCTCACT
GTTTGACAATAAGCTCCAATTTTCAGACTTTTCATTTCAAACCTGTGGGTCTCATTTCTCTGGCCTATATAAATCCACTATC
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1960

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CAACATGAAATCGCTGATTTGTGTAGTCGGTTTATGCAGCAACGAGACGTACGGAAAATGCCGCTCATCGCCACATAT
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CAGGTCAAATTCAG

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AGCGCAGACCGTTTTGCTCGGGAAGACGTACGGGGTATACATGTCTGACAATGGCAGATCCCAGCGGTCAAACAGGCG
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GCTTCTGGTGCCGAAACCAGGCAAAGCGCCATTCGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGC
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ATCCCCATATGGAACCGTCGATATTCAGCCATGTGCCTTCTCCGCGTGACGAGATGGCGATGGCTGGTTCCATCAGTT
GCTGTTGACTGTAGCGGCTGATGTTGAACTGGAAGTCGCCGCGCCACTGGTGTGGGCCATAATTCAATTCGCGCTCCCGC
AGCGCAGACCGTTTTTCGCTCGGGAAGACGTACGGGGTATACATGTCTGACAATGGCAGATCCCAGCGGTCAAACAGGGC
GCAGTAAGGCGGTGGGATAGTTTTCTGGGCCCTAATCCGAGCCAGTTTACCCGCTCTGCTACCTGCGCCAGCTGGCAG
TTCAGGCCAATCCGCGCCGATGCGGTGTATCGCTCGCCACTTCAACATCAACGGTAATCGCCATTTGACCACTACCATCAA
TCCGGTAGGTTTTCCGGCTGATAAATAAGGTTTTCCCTGATGCTGCCACGCGTGAGCGGTGTAATCAGCACCGCATCAGC
AAGTGTATCTGCCGTGCACTGCAACAACGCTGCTTCGGCCTGGTAAT

omegaCR-gb right

718

AATGCGCTCAGGTCAAATTCAGACGGCAAACGACTGTCTGGCCGTAACCGACCCAGCGCCCGTTGCACCACAGATGAAAC
GCCGAGTTAACGCCATCAAAAATAATTCGCGTCTGGCCTTCTGTAGCCAGCTTTCATCAACATTAATGTGAGCGAGTAAC
AACCCGTGGATTCTCCGTGGGAACAAACGGCGGATTGACCGTAATGGGATAGGTCACGTTGGTGTAGATGGGCGCATCG
TAACCGTGATCTGCCAGTTTGAGGGGACGACGACAGTATCGGCCTCAGGAAGATCGCACTCCAGCCAGCTTCCGGCACC
GCTTCTGGTGCCGAAACCAGGCAAAGCGCCATTCGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGC
CTCGACGGCCAGTGAATCCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTTGTTATCCGCTCACAATCCACACAACATA
CGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCATATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTACTGCC
GCTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACCGCGGGGAGAGGGGTTTGCATGCTC
TTCGGGTGATAGAAGAGGGGGATCAGATTGTCGTTCCCGCCTCAGTTTAAACTATCAGTGTGGACAG

RGR gene

247

ACATGCAGCTCTCCaccGGGTTACTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCTAACCCAAGGGGTGACAAGC
GTTTTAGAGTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAGTGGCACCGGAGTCGGTGCTTTTG
GCCGGCATGTTCCAGCCTCCTCGCTGGCCCGGCTGGCAACATGCTTCGGCATGGCAATGGGACgatGGAAGAGCTTC
CTCG

M.Sacl cluster

1924

TCAGATCCCGGGTCAATAGCATTCTACCAATAAAAAACGCCGGCGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTA
CTGGATCTATCAACAGGAGTCCAAGCGCGCTTTTTTACCTCTAAAAGTTAAACAAAATTATTCTAGAGGGAACCGTTGTGGAATTGTGA
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AAAATATAGCGCAAACTAGGATAAATATCGCGCGGTCTCATCTATGTTACTAGATCAGGCTGCGACCCGGCGGTACCGGATCGCTCGACCTCGAGCATAAA

Cas9* (CRISPR associated protein 9)

4755

lbp (Impatiens balsamina peptide)

54

CCTTGTCTAACGCTGCTGACGAGGTCGCTACCCCTGAGGACGTGACGCTGGT

GFP (Green fluorescent protein)

714

GTGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTGCCATCTGGTGCAGCTGGACGGCAGCTAAACGGCCACAAGTTCAAGGCTGCGGCGGCGGAGGAGGCGGTTGCGTATTGGCTAGAGCAGCTTGCCAA
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35SProm (35SPromoter sense)

1099

CACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATGCGTTGCGCTCACTGCCGCTT
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<p>35STerm (35S Terminator sense)</p>	<p>291</p>	<p>TAAGTAGCTGAATCCCAGGCCATGCTAGAGTCCGCAAAAATCACCAGTCTCTCTACAAAATCTATCTCTCTATTTTTCTCCAGAATAATGTG TGAGTAGTTCCAGATAAGGGAATTAGGGTTCTTATAGGGTTTCGCTCATGTGTTGAGCATATAAGAAACCCTTAGTAGTATTGATTGTTGTA AATACTTCTATCAATAAAATTTCTAATTCTAAAACAAAATCCAGTGACCTGCAAGCATGCGACGCTCGGGCTAGCCCGAGACGCTCGGGCCTAG CCCGA</p>
<p>NLS (Nuclear localization signal)</p>	<p>30</p>	<p>CCTAAGAAGAAGCGTAAGGTCGAGGACCCT</p>
<p>NosProm (Nos Promoter sense)</p>	<p>321</p>	<p>TGATCATGAGCGGAGAATTAAGGGAGTACGTTATGACCCCCCGGATGACGCGGGACAAGCCGTTTTACGTTTGGAACTGACAGAACCGCCAA CGATTGAAGGAGCCACTCAGCCGCGGTTTTCTGGAGTTAATGAGCTAAGCACATACGTCAGAAACCATATTGCGCGTTCAAAGTGCCTAA GGTCACTATCAGCTAGCAAAATTTCTTGTCAAAAATGCTCCACTGACGTTCCATAAATCCCTCGGTATCAATTAGAGTCTCATATTTACTCTC AATCCAAATAATCTGCACCGGATCTGGATCGTTTCG</p>
<p>rGUS (β-glucuronidase)</p>	<p>1806</p>	<p>ATGTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAATCTGACGGCCTGTGGGCATTAGTCTGGATCGCGAAAATGTTGGAATTGATC AGCGTTGGTGGGAAAGCGGTTACAAGAAAGCCGGCAATTTGCTGTGCCAGGCAGTTTTAACGATCAGTTCCGCGATGACAGATTCGTAATTA TGCGGGCAACGCTGTGTATCAGCGGAAGTCTTTATACCGAAAGTTGGCAGGCCAGCGTATCGTGTGCGTTTCGATGCGGTCCTACTCATT GGCAAAAGTGTGGTCAATAATCAGGAAGTGTAGGAGCATCAGGGCGGCTATACGCCATTTGAAGCCGATGTCACGCGCTATGTTATTGGCGGG AAAAGTGTACGTATCACCGTTTGTGTGAACAACGAACTGAAGTGGCAGACTATCCCGCGGGAATGGTGATTACCGACGAAAACCGCAAGAAA AAGCAGTCTTACTTCCATGATTTCTTAACTATGCCGGAATCCATCGCAGCTAATGCTCTACACCAGCCGAACACCTGGGTGGACGATATCAC CGTGGTGACGCATGTCGCGCAAGACTGTAACCACCGCTGTTGACTGGCAGGTGGTGGCCAATGGTGATGTCAGCGTTGAAGTGCCTGATGC GGATCAACAGGTGGTTGCAACTGGACAAGGCACTAGCGGACTTTGCAAGTGGTGAATCCGACCTCTGGCAACCAGGTTGAAGTTATCTCTAT GAAGTGTGCGTACAGCCAAAAGCCAGACAGAGTGTGATATCACCCTTCGCGTCCGATCCGCTCAGTGGCAGTGAAGGGCCAAACAGTTC CTGATTAACCACAACCGTTACTTTACTGCTTGTGCTGATGAAGATGCGGACTTACGTGGCAAAGGATTCGATAACGTCGATGGTGCA CGACCACGATTAATGGACTGGATTGGGGCAACTCTACCTACCTCGCATTACCTTACGCTGAGGAGATGCTCGACTGGCAGATGAACAT GGCATCGTGGTATTGATGAACTGCTGCTGTCGGCTTTAACCTCTCTTAGGCATTGGTTTTCAAGCGGGCAACAGCCGAAAGAACTGTACA GCGAGGAGGCGAGTCAACGGGGAAACTCAGCAAGCGCACTTACAGGCGATTAAGAGCTGATAGCGCGTGACAAAAACCCCAAGCGTGGTG ATGTGGAGTATGCCAACGAACCGGATACCCGTCGCAAGTGCACGGGAATATTTGCCACTGGCGGAAGCAACGCTAACTCGACCCGACG CGTCCGATCACCTGCGTCAATGTAATGTTCTGCGACGCTCACACCGATAACCATCAGCGATCTCTTTGATGTGCTGTGCGCTGAACCGTTATTACGG ATGGTATGTCAAAGCGCGATTGGAAACGGCAGAGAAGTACTGGAAAAGAACTTCTGGCTGGCAGGAGAACTGCATCAGCCGATTAT CATCACCGAATACGGCGTGGATACGTTAGCCGGGCTGCACTCAATGTACACCGACATGTGGAGTGAAGGATATCAGTGTGATGGCTGGATAT GTATCACCGCTCTTTGATCGCGTACGCGCGTGTGCGGTGAACAGGTATGGAATTTCCGCGATTTTGCACCTCGCAAGGCATATTGCGCGTTG GCGGTAAACAAGAAAGGGATCTTCACTCGCGACCGCAAAACCGAAGTCCGCGGCTTTTCTGTGCAAAAACGCTGGACTGGCATGAATTCGGTG AAAAACCGCAGCAGGGAGGCAAAACA</p>
<p>HCC (Hygromycin resistance and CodA genes fused by F2A viral protein)</p>	<p>2390</p>	<p>CCACCATGAAAAGCCTGAAGTACCCGCGAGCTGTGCGAGAAGTTTCTGATCGAAAAGTTCGACAGCGTCTCCGACCTGATGCGACTCTCGGAGGGCGAAGATCTC GTGTTTCAGCTTCGATGAGGAGGGCGTGGATATGCTGCGGGTAAATAGCTGCGCGGATGGTTTCTACAAAAGATCGTTATGTTATCGGCACCTTTCATCGGCCG GCTCCCGATTCCGGAAGTCTTGACATTGGGGAGTTTAGCGAGAGCCTGACCTATTGCATCTCCCGCGTGACAGGGTGCACGTTGCAAGACCTGCCTGAAACCGAA CTGCCCGCTGTTTACAACCGGTGCGGAGGCTATGGATGCGATCGCTGCGCGGATCTTAGCCAGACGAGCGGGTTCCGGCCATTCCGACCGCAAGGAATCGGTCAA TACACTACATGGCGTATTTATATGCGGATTGCTGATCCCATGATATCATCGGCAACTGTGATGGACGACACCGCTCAGTGCCTCGTCCGCGCAGGCTCTCGATG AGCTGATGCTTTGGGCGGAGGACTGCCCGAAGTCCGGACCTCGTGACGCGGATTTGGCTCCAACAATGTCTGACGGACAATGGCCGATAACAGCGGTCATTG ACTGGAGCGAGGCGATGTTCCGGGATTCCCAATACGAGGTCGCAACATCTTCTCTGAGGCGCGTGGTTGGCTGTATGGAGCAGCAGACGCGCTACTTCAGCGGGA GGCATCCGAGCTTGCAGGATCGCCAGACTCCGGCGTATATGCTCCGATTGGTCTTGACCAACTCTATCAGAGCTTGGTTGACGGCAATTTGATGATGACGCTT GGCGCAGGGTGCATGCGACGCAATCGTCCGATCCGGAGCCGGGACTGTCGGCGTACACAAATCGCCGCAAGCGCGGCTGTTGACCGGATGGCTGTGTAGAA TACTCGCCGATAGTGGAAACCGACGCCAGCACTCGTCCGAGGGCAAGAAAGCGGCGCAGTGAAGCAGACCCCTAAATTTGATCTTTTGAAGCTCCTGCTGGTATG TTGAGTCTAATCCAGTCTGGATCATGTGAATAACGCTTTACAACAATTAATAACGCCGGTTACCAGGCGAGGAGGGGCTGGCAGATTATCTGCAAGGACG GAAAATCAGCGCATTGATGCGCAATCCGGCGTATGCCATACTGAAAACAGCCTGGATGTCGAACAAGGTTAGTTATACCGCCGTTTGGAGCCACATATTC CCTGGACACCAGCAAAACCGCGCAACCGAAGTGAATCAGTCCGCGCAGCTGTTGAAAGCATTGAACGCTGGGCGGAGCGCAAGCGTTAATTAACCATGACGA TGTGAAAACAACCGCATGGCAACCGCTGAAATGGCAGATTGCCAACGGCATTACGATGTGCGTACCCATGTCGATGTTTCGGATGCAACGCTAACTGCGCTGAAAGC AATGCTGGAAGTGAAGCAGGAAGTCCGCGGTTGATGATCTGCAAACTGTCGCTTCCCTCAGGAAGGGATTTTGTGATCTCCAACGGTGAAGCGTTGCTGGAGGA GGCGTTACGCTTAGGGGCGAGATGATGTTGGGGGATTCGCAATTTGAAATTTACCCTGAAATACGCGTGGAGTGCCTGATATAAACTTCCGCTTGGCGCAAAATA CAGCCGCTCATCGACTTCACTGTGATGAGATCGATGACGAGCAGTCCGCTTTGTGCAAAACCGTTGCTGCCGACCAATGAAGGCATGGGCGCGGAGTCA CGCCAGCCACACCGCAATGCACTCTATAACGGGGCGTATACCTCAGCGCTTCCGCTTGTGAAAATGCGGGTATTAACCTTGTGCCAACCCGCTGGTCAATA TTATCTGCAAGGACGTTTCGATACGATATCAAAACGTCGCGGCATCAGCGCGTTAAAGAGATGCTGGAGTCCGCGATTAACTGCTGTTGGTCAAGTATGATGCT CGATCCGTTGATCCGCTGGGAACGGCAATATGCTGCAAGTGTGATATGGGCTGCATGTTTGCAGTTGATGGGCTACGGGAGATTAACGATGGCCTGAATTT AATCACCCACACAGCGCAAGGACGTTGAAATTTGCAAGGATTACGGCATTCCGCGGAAACAGCGCAACCTGATTAATCTGCGCGGCAAAATGGGTTGATGCGCT GCGCGTCAAGTTCCGCTGATGTTTCCGTCAGTGGCGCAAGGTTGATGCCAGCACACAACCGGCACAAACCCGCTATATCTGGAGCAGCCAGGACATCGATTA CAAACGT</p>

Kan-ORF (Kanamycin resistance gene CDS)	792	<p>ATGATTGAACAAGATGGATTGCACGCAGGTTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGC TCTGATGCCCGCTGTTCCGGCTGTACGCGCAGGGGCGCCCGTTCTTTTGTCAAGACCGACTGTCCGGTGCCTGAATGAACTGCAGGACG AGGCAGCGCGGCTATCGTGGTGGCCACGACGGGCGTTCCTTGGCAGCTGTGCTCGACGTTGCTACTGAAGCGGGAAGGGACTGGTGTCTAT TGGGCGAAGTGCCGGGGCAGGATCTCTGTCTACCTTGTCTCTGCGGAGAAAGTATCCATCATGGCTGATGCAATGCCGCGGCTGCATAC GCTTGATCCGGCTACCTGCCATTGACACCAAGCGAAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCCGGTCTTGTGATCAGGAT GATCTGGACGAGGAACATCAGGGGCTCGGCCAGCGAAGTGTTCGCCAGGCTCAAGGCGCGCATGCCGACGCGGAGGATCTCGTGTGACC CATGGCGATGCCTGCTTCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTATCGACTGTGCCGCGCTGGGTGTGGCGGACCGCTATC AGGACATAGCGTTGGCTACCCGTGATATTGCTGAGGAACTTGGCGGCGAATGGGCTGACCGCTTCTCGTGTCTTACGGTATCGCCGCTCCCGA TTGCGAGCGCATCGCCTTCTATCGCCTTCTGACGAGTCTTTC</p>
8m1* (synthetic DNA)	675	<p>AAACTATAACAGGGAAGTATAATTAGGACTAAAGAAGATTCAACGTACATTGATCTGACACAGTAGATTTAGTTGTCTCTTGTACATACACAGT ATCTAGGATTATTCAACGAAAACAATATCAATTGTCTCTACAGAAACCAACGGCCAGTACTCTTTTGCCTAAAAAGACCGTAACCTAATTGTCA CACTGAGAATCTAACGATTGCCAGTACTCTTCTGGGACCTACGACGAAGGATGACTCCGTCACGTTCTTCTTCACTGTTTGACAATAAGCTCCA ATTTTCAGACTTTTCATTTCAAACCTTGTGGGTCTCATTTTCTCTGGCCTATATAAATCCACTATCCTCCACTGCCAGTTGCTCTTCATATAGCAGAT GCTACGATCTGTCAGCACTGAGAAGTCTATTTGCTTTTGTGATTGAGAAATGCTGAATTCCTGCACGAATTCATGTGCGCTGTAAGCAGAA CTATGGAGAGAAAAGTGTGGTTCAGGTGAGCCATAGGATACTCTTAAAGAACTATGATTGTTGTCAGAACTACGATAAAAAGATGTCCGGAATT AATATCATACTCATCTTTTCAGTTTGAAGATTTTGCAAACCAATGCGTTTGACCTTTTGTCTAAGTACAGTGATAGCTTTCTGCCACTTGTG TATC</p>
8m2* (synthetic DNA)	675	<p>AAACTATAACAGGGAAGTATAATTAGGACTAAAGAAGATTCAACGTACATTGATCTGACACAGTAGATTTAGTTGTCTCTTGTACATACACAGT ATCTAGGATTATTCAACGAAAACAATATCAATTGTCTCTACAGAAACCAACGGCCAGTACTCTTTTGCCTAAAAAGACCGTAACCTAATTGTCA CACTGAGAATCTAACGATTAGTCCCGCTCTTCTGGGACCTACGACGAAGGATGACTCCGTCACGTTCTTCTTCACTGTTTGACAATAAGCTCC AATTTTCAGACTTTTCATTTCAAACCTTGTGGGTCTCATTTTCTCTGGCCTATATAAATCCACTATCCTCCACTGCCAGTTGCTCTTCATATAGCAGA TGCTACGATCTGTCAGCACTGAGAAGTCTATTTGCTTTTGTGATTGAGAAATGCTGAATTCCTGCACGAATTCATGTGCGCTGTAAGCAGAA ACTATGGAGAGAAAAGTGTGGTTCAGGTGAGCCATAGGATACTCTTAAAGAACTATGATTGTTGTCAGAACTACGATAAAAAGATGTCCGGAATT TAATATCATACTCATCTTTTCAGTTTGAAGATTTTGCAAACCAATGCGTTTGACCTTTTGTCTAAGTACAGTGATAGCTTTCTGCCACTTGTG GTATC</p>
7m1* (synthetic DNA)	675	<p>AAACTATAACAGGGAAGTATAATTAGGACTAAAGAAGATTCAACGTACATTGATCTGACACAGTAGATTTAGTTGTCTCTTGTACATACACAGT ATCTAGGATTATTCAACGAAAACAATATCAATTGTCTCTACAGAAACCAACGGCCAGTACTCTTTTGCCTAAAAAGACCGTAACCTAATTGTCA CACTGAGAATCTAACGATTGCCAGTACTCTTCTGGGACCTACGACGAAGGATGACTCCGTCACGTTCTTCTTCACTGTTTGACAATAAGCTCCA ATTTTCAGACTTTTCATTTCAAACCTTGTGGGTCTCATTTTCTCTGGCCTATATAAATCCACTATCCTCCACTGCCAGTTCTCTTCATATAGCAGAT GCTACGATCTGTCAGCACTGAGAAGTCTATTTGCTTTTGTGATTGAGAAATGCTGAATTCCTGCACGAATTCATGTGCGCTGTAAGCAGAA CTATGGAGAGAAAAGTGTGGTTCAGGTGAGCCATAGGATACTCTTAAAGAACTATGATTGTTGTCAGAACTACGATAAAAAGATGTCCGGAATT AATATCATACTCATCTTTTCAGTTTGAAGATTTTGCAAACCAATGCGTTTGACCTTTTGTCTAAGTACAGTGATAGCTTTCTGCCACTTGTG TATC</p>
5m2* (synthetic DNA)	675	<p>AAACTATAACAGGGAAGTATAATTAGGACTAAAGAAGATTCAACGTACATTGATCTGACACAGTAGATTTAGTTGTCTCTTGTACATACACAGT ATCTAGGATTATTCAACGAAAACAATATCAATTGTCTCTACAGAAACCAACGGCCAGTACTCTTTTGCCTAAAAAGACCGTAACCTAATTGTCA CACTGAGAATCTAACGATTGCCAGTACTCTTCTGGGACCTACGACGAAGGATGACTCCGTCACGTTCTTCTTCACTGTTTGACAATAAGCTCCA ATTTTCAGACTTTTCATTTCAAACCTTGTGGGTCTCATTTTCTCTGGCCTATATAAATCCACTATCCTCCACTATGAGTTGCTCTTCATATAGCAGAT GCTACGATCTGTCAGCACTGAGAAGTCTATTTGCTTTTGTGATTGAGAAATGCTGAATTCCTGCACGAATTCATGTGCGCTGTAAGCAGAA CTATGGAGAGAAAAGTGTGGTTCAGGTGAGCCATAGGATACTCTTAAAGAACTATGATTGTTGTCAGAACTACGATAAAAAGATGTCCGGAATT AATATCATACTCATCTTTTCAGTTTGAAGATTTTGCAAACCAATGCGTTTGACCTTTTGTCTAAGTACAGTGATAGCTTTCTGCCACTTGTG TATC</p>
4m1* (synthetic DNA)	675	<p>AAACTATAACAGGGAAGTATAATTAGGACTAAAGAAGATTCAACGTACATTGATCTGACACAGTAGATTTAGTTGTCTCTTGTACATACACAGT ATCTAGGATTATTCAACGAAAACAATATCAATTGTCTCTACAGAAACCAACGGCCAGTACTCTTTTGCCTAAAAAGACCGTAACCTAATTGTCA CACTGAGAATCTAACGATTGGCTGCCACTCTTCTGGGACCTACGACGAAGGATGACTCCGTCACGTTCTTCTTCACTGTTTGACAATAAGCTCC AATTTTCAGACTTTTCATTTCAAACCTTGTGGGTCTCATTTTCTCTGGCCTATATAAATCCACTATCCTCCACTGGCTGCCGCTCTTCATATAGCAG ATGCTACGATCTGTCAGCACTGAGAAGTCTATTTGCTTTTGTGATTGAGAAATGCTGAATTCCTGCACGAATTCATGTGCGCTGTAAGCAG AACTATGGAGAGAAAAGTGTGGTTCAGGTGAGCCATAGGATACTCTTAAAGAACTATGATTGTTGTCAGAACTACGATAAAAAGATGTCCGGA TTAATATCATACTCATCTTTTCAGTTTGAAGATTTTGCAAACCAATGCGTTTGACCTTTTGTCTAAGTACAGTGATAGCTTTCTGCCACTTGT GTATC</p>

CircRep (synthetic
DNA)

777

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GAGGGCGGTCCGCTGCCTTTTGCATTGACATCCTGGCCCCGTGCTGTATGTACGGCTCTAAGACCTTCATTAACACGTGAGCGGTATCCCGGA  
TTACTTTAAAGAGTCCTTTCCAGAGGGCTTCACTTGGGAACGTACCCAGATTTTTGAGGACGGTGGTGTCTGACCCGCGACCAAGACACCAGCC  
TGGAAAGGTAATTGCCTGATCTATAAAGTGAAGTTCTGGGTACCAATTTCCCGGCGAATGGTCCGGTGATGCAAAAGAAAACCGGGGTTGGG  
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CTCGAGGGTCTACCCCAAGGGCGACACCCCTAATTAGCCCGGGCGAAAGGCCAGTCTTCGACTGAGCCTTTCGTTTTATTGATGCCTGGC  
AGTTCCTACTCTCGCATGGGGAGTCCCACACTACCATCGGCGCTACGGCGTTTCACTTCTGAGTTCGGCATGGGGTCAGGTGGGACCACCGC  
GCTACTGCCGCCAGGCAACAAGG
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Except for Cas9*, coding sequences have no STOP codons and Promoters end with ATG (d35S_h-h require a second ATG to be included if both directions are to be used for ORFs).

Supplementary Table 5 - Vendor, catalog number and specific reagents used in this study.

<i>Vendor</i>	<i>Cat. No.</i>	<i>Product Description</i>
Fisher Scientific	FERER0231	10U/μL, 300U Thermo Scientific Eam1104I (EarI)
Sigma	B2904-100MG	5-Bromo-3-indolyl β-D-galactopyranoside
Sigma	B4252-1G	5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside
Sigma	B4252-1G	5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside
Santa Cruz Biotechnology	sc-281503	8-Hydroxyquinoline-b-D-galactopyranoside
NEB	P0756S	Adenosine 5'-Triphosphate (ATP)
NEB	R0712L	BspQI
Qiagen	19063	Buffer QG (250 ml)
Life Technologies	10177-012	Carbenicillin, Disodium Salt
NEB	M0226S	CpG Methyltransferase M.SssI
GenScript	C01577-1	dATP (100 mM)
GenScript	C01579-1	dCTP (100 mM)
GenScript	C01578-1	dGTP (100 mM)
Epoch Life Science Inc	1920-250	DNA purification colums
GenScript	C01580-1	dTTP (100 mM)
NEB	R0528L	EarI
5PRIME	2300010	FastPlasmid Mini Kit
FroggaBio	DF300	Gel/PCR DNA Fragments Extraction Kit
Sigma	I6758-1G	IPTG
Life Technologies	11815-032	Kanamycin Sulfate
Sigma	A3256-25G	L-(+)-Arabinose
Thermo Scientific	# ER1932	Lgu I
NEB	B7024S	Gel Loading Dye, Purple (6x)
LABREPCO	11608031	Micro Disposable Electroporation Chambers
NEB	B9007S	NAD
Sigma	N7878-25G	Nitrofurantoin
NEB	R3193S	NcoI-HF
NEB	R3189S	NotI-HF
Sigma	435406	PEG-PPG-PEG (PPG)
addgene	Plasmid 16666	pGRG36 (Plasmid DNA)
Thermo Scientific	F-530L	Phusion High-Fidelity DNA Polymerase (2 U/μL)
NEB	R0560S	PmeI
Sigma	76293	Polyethylene glycol solution
FroggaBio	DFL100	Presto™ Max Gel/PCR Kit
NEB	R3140S	PstI-HF
Thermo Scientific	EN0531	RNAse
NEB	R3138S	Sall-HF
NEB	R0569S	SapI
Sigma	85555-5G	Spectinomycin Dihydrochloride
NEB	M0202L	T4 DNA Ligase (NEB)
Epicentre	T5E4111K	T5 exonuclease (Epicentre)
NEB	C2566I	T7 Express Competent E. coli (High Efficiency)
Qiagen	201203	Taq DNA Polymerase
NEB	M0219S	TaqI Methyltransferase
NEB	R0180S	XmaI