Development of high-performance whole cell biosensors aided by statistical modelling

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SUPPORTING INFORMATION

Contents

Additional methods - Molecular cloning

The PCA biosensor was assembled by isothermal assembly from the following fragments: pSEVA131 linearized by inverse PCR (primers AB9/10); *mCherry* amplified with primers AB15/28 from a synthetic gene (GeneArt); *sfGFP* amplified with primers AB18/27 from a synthetic gene (GeneArt), and synthetic DNA (IDT) incorporating the ProB promoter (1) fused to a strong RBS (gaaataaggaggtaatacaa) (2), the P_{PV} promoter (3) fused to the G10 RBS (4) and a 150 bp spacer (5) to yield the template plasmid (p131B). Promoter (P_{reg}-lib and P_{out}-lib) and RBS (RBS_{out}-lib) libraries were generated by linearising p131B by inverse PCR with primers AB27/94 (for P_{out}-lib and RBS_{out}-lib) and AB146/147 (for P_{reg}-lib) and inserting the following degenerate ssDNA oligonucleotides via isothermal assembly: for P_{out}-lib oligo AB115, for RBS_{out}-lib oligo AB114, and for P_{reg}-lib oligo AB148 (Supplementary Table 12). The library members were designated p131B-BX for P_{reg}-lib, p131B-GX for RBS_{out}-lib, and p131-VX for P_{out}-lib, with X denoting the clone number, which was assigned based on subsequent screening and rank order of expression output.

Constructs corresponding to the DoE Definitive Screening Design table (Table 1) were generated in three stages. Firstly, *mCherry* was replaced with *pcaV* using *in vivo* assembly, using the selected library plasmids (those with P_{reg} at level -1, 0 and +1) linearized by inverse PCR with primers AB10/128, and *pcaV* amplified from pPv-Pcav (p44-pcaV) (3) with primers AB96/127, to yield pD2 (P_{reg}/P_{out}/RBS_{out} pattern at level 0/+1/+1), pD7 (P_{reg}/P_{out}/RBS_{out} at levels +1/+1/+1), and p131C-B20 (P_{reg}/P_{out}/RBS_{out} pattern at level -1/+1/+1). Secondly, these plasmids were again linearized by inverse PCR with primers AB27/94 and the oligos AB142, AB143, AB144 and AB145 (corresponding P_{out}/RBS_{out} patterns at level 0/-1, 0/0, -1/-1 and -1/0, respectively), were inserted by isothermal assembly to create plasmids pD1 (P_{reg}/P_{out}/RBS_{out} pattern at level 0/0/0), pD3 (P_{reg}/P_{out}/RBS_{out} pattern at level -1/-1/-1), pD4 (P_{reg}/P_{out}/RBS_{out} pattern at level +1/-1/0), pD6 (P_{reg}/P_{out}/RBS_{out} pattern at level 0/-1/-1), and pD8 (P_{reg}/P_{out}/RBS_{out} pattern at level +1/0/-1). Next, the final set of DoE constructs were made by *in vivo assembly* using selected plasmids from the P_{out}-lib and RBS_{out}-lib libraries linearized by inverse PCR with primers AB10/130, and P_{reg}-*pcaV* amplified with primers AB11/129 from pD2, pD7 and p131C-B20. This yielded plasmids pD5 (P_{reg}/P_{out}/RBS_{out} pattern at level -1/+1/0), pD9 (P_{reg}/P_{out}/RBS_{out} pattern at level +1/-1/+1), pD10 (P_{reg}/P_{out}/RBS_{out} pattern at level -1/0/+1), pD11 (P_{reg}/P_{out}/RBS_{out} pattern at level +1/+1/-1), pD12 (P_{reg}/P_{out}/RBS_{out} pattern at level -1/-1/+1), and pD13 (P_{reg}/P_{out}/RBS_{out} pattern at level -1/+1/-1). Validation constructs for modelling of the DoE dataset were created with *in vivo* assembly using selected members of the P_{reg}

library linearized by inverse PCR (primers AB10/128) and *pcaV* amplified by PCR (primers AB96/127) from p44-pcaV. The pKIKO set of vectors (6) was used to make genomic insertions of different PAB variants. The PAB was amplified from selected DoE plasmids by PCR with primers AB101/102 and inserted via *in vivo* assembly into pKIKOarsBKm that had been linearized by inverse PCR with primers AB29/30.

For the ferulic acid biosensor (FAB) designs, the pFABsP vector was constructed by isothermal assembly, using (i) pET28a (Novagen) served as a backbone and linearized by PCR with primers FAB1/2 to remove *lacI* and the T7 promoter; (ii) the chimeric P_{LC} promoter-operator (7) and the G10 RBS were incorporated into the forward primer of the FAB3/4 pair and used to amplify *sfGFP* from a synthetic gene (IDT) and (iii) the FerC transcription factor and FerA enzyme (7) amplified with primers FAB5/6 from p15FABs to yield pFABsP. The new strong promoter-operator P_{LC2} (Supplementary Figure 3) was synthesised as a gBlock (IDT) and exchanged with the P_{LC} promoter by isothermal assembly using pFABsP_{LC} linearized by inverse PCR with primers FAB6/7.

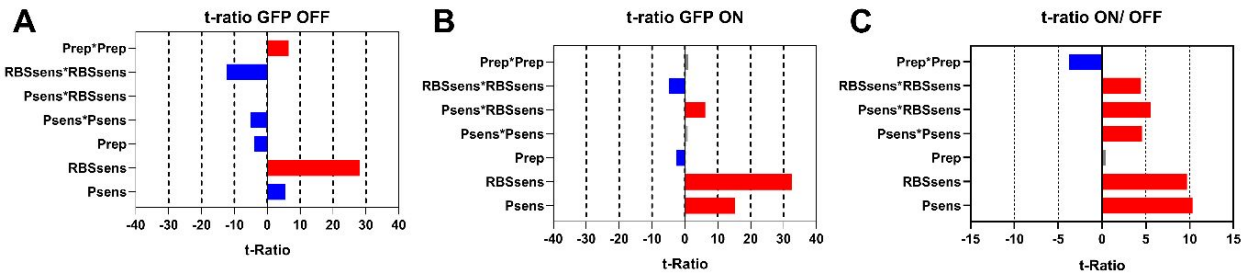
The plasmids for DoE pFABs1 (P_{regC} /P_{enzA}/RBS_{out} pattern at levels -1/-1/+1) to pFABs9 (P_{regC} /P_{enzA}/RBS_{out} pattern at levels +1/+1/+1) were generated using the pFABsP_{LC2} as backbone. The constructs were made by isothermal assembly using four PCR products as parts: (i) The backbone with ferA_p28 (ColE1)_P_{LC2}_G10_sfGFP was linearized from pFABsP_{LC2} with primers FAB9/10, (ii) Promoters corresponding to levels -1, 0 and +1 from the P_{reg} library (B20, B10 and B12, respectively) amplified with primers FAB11/12 to be placed upstream to *ferC* (renamed as P_{regC} promoters), (iii) *ferC* amplified with primers FAB13/14 from p15ferCA (7), (iv) Promoters corresponding to levels -1, 0 and +1 from the P_{reg} library (B20, B10 and B12, respectively) amplified with primers FAB15/16 to be placed upstream of *ferA* (renamed as P_{enzA} promoters) . Plasmids lacking *ferC* (pFABsPLC2 FerC KO) or *ferA* (pFABsPLC2 FerA KO) were made by linearizing and reassembling pFABsP_{LC2} by inverse PCR (FAB17/18 and FAB19/20, respectively).

The plasmids for the second iteration pFABsG12 (P_{regC} /P_{enzA}/RBS_{out} pattern at levels +1/+1/+0.81), pFABsG19 (P_{regC} /P_{enzA}/RBS_{out} pattern at levels +1/+1/+0.89) and pFABsG21 (P_{regC} /P_{enzA}/RBS_{out} pattern at levels +1/+1/+0.94) were generated using pFABs9 as backbone. Forward primers (FAB21, FAB22, FAB23) were designed with the sequences from the RBS_{out} library corresponding to levels 0.81, 0.89 and 0.94. A reverse primer (FAB24) with overlapping nucleotides to the forward primers was designed. Inverse PCR of pFABs9 with these primers followed by isothermal assembly was carried out to insert the new RBS sequences.

The *pcaK* gene from *Pseudomonas putida* was synthetized (IDT) with codon-optimsation for expression in *E. coli* with a short translational initiation region (AGGAGGAAAAAAA) at the 5' of the start codon. The gene was inserted downstream of *pcaV* via *in vivo* assembly into plasmid p131C-B10, linearised by PCR with primers AB10/167, to create p131C-B10-pcaK. The extender plasmid p261-lacI-pcaK, contains the p15A origin and a kanamycin selection marker, and was assembled by isothermal assembly from the following fragments: (i) the pSEVA261 backbone and linearized by inverse PCR with primers AB9/10; (ii) the *lacI* gene amplified by PCR from pET44 with primers AB197/198; (iii) the P_{pv} promoter, G10 RBS (set at level 0) and a 150 bp spacer amplified by PCR with primers AB195/196; and (iv) synthetic DNA (IDT) consisting of the P_{LlacO1} promoter (8) and G10 RBS (level -1). The additional combinatorial RBS constructs were constructed by isothermal assembly using ssDNA oligonucleotides (IDT) (AB 303-309) into the p261-lacI-pcaK backbone, linearized by inverse PCR with primers AB 301/302.

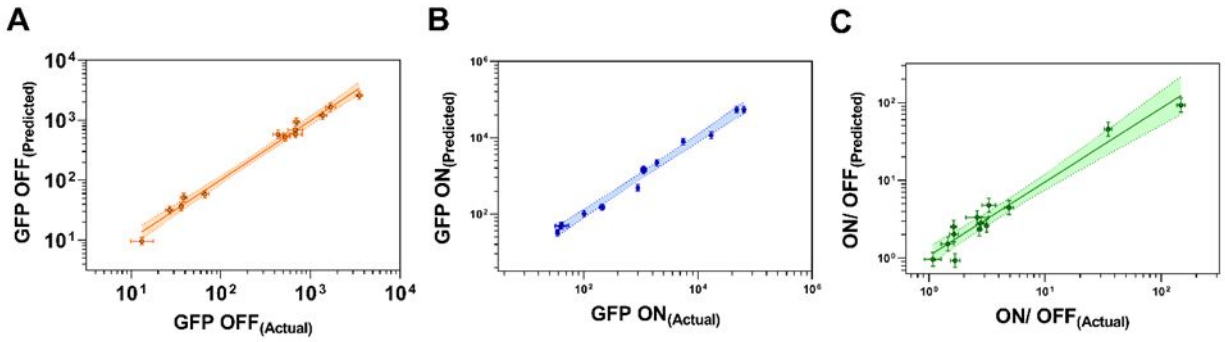
Benchmarking plasmids were constructed by *in vivo* assembly. pET44 and pBAD were linearised by inverse PCR with primers AB159/160 and AB163/164, respectively. *sfGFP* was amplified from p131B with primers AB161/162 for insertion into pET44 and AB165/166 for insertion into pBAD. pCK302 was a gift from John Heap (Addgene plasmid #87768).

SUPPLEMENTARY FIGURES



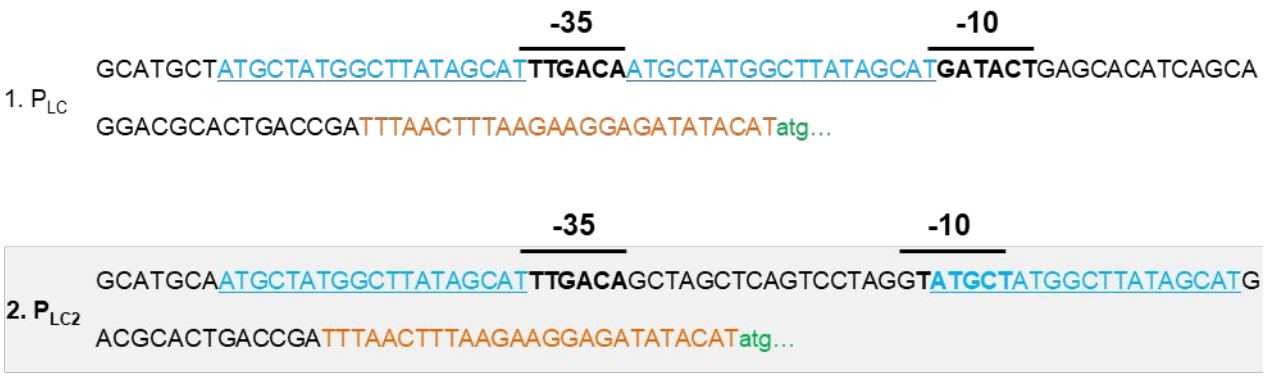
Supplementary Figure 1. Factor screening and selection

(**A-C**) Lenth t-ratio of each factor, (A) OFF, (B) ON, and (C) ON/ OFF, showing those factors deemed important by the JMP factor screening platform. The t-ratio is derived from the PSE (OFF PSE = 38.2239, ON PSE = 206.038, ON/ OFF PSE = 0.71191) and is used to assess factor importance. The colour of the bar indicates the predicted effect of this factor on the indicated response (blue – negative, red – positive). Factors deemed significant at the 0.1 confidence level are deemed significant and were included in the model.



Supplementary Figure 2. Least Squares model performance

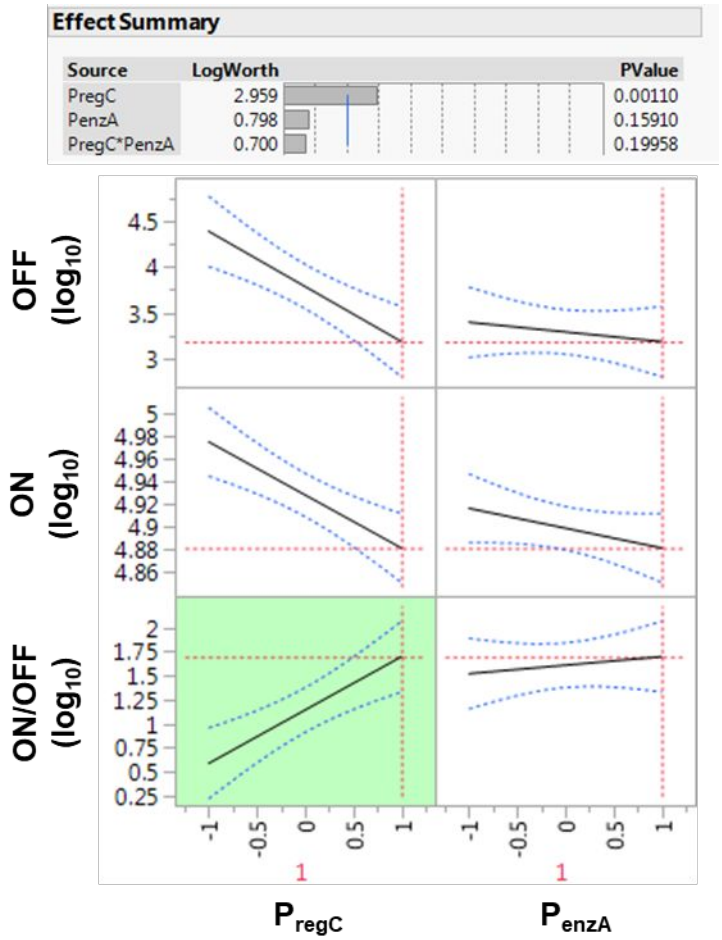
Actual versus predicted plots showing the performance of the Least Squares regression model in predicting (A) OFF, (B) ON and (C) ON/OFF. The model shows good prediction of all three responses. OFF R^2 = 0.986, P = 1.2 x 10^{-11}, ON R^2 = 0.988, P = 6.8 x 10^{-12}, ON/ OFF R^2 = 0.95, P = 1.6 x 10^{-08}.



***Sphingobium* Operator :** (19 bp) **IR2:** ATGCTATGGCTTATAGCAT

Supplementary Figure 3. Reengineering of the promoter-operator for the FA Biosensor.

The original promoter-operators P_{LC} (1) and new reengineered P_{LC2} (2) sequences downstream to a 5' prime region and the Rogers G10 RBS (orange) followed by a sfGFP gene are shown. The IR2 palindromic DNA operator sequence from *Sphingobium* (light blue) is also shown. The promoter P_{LC2} was designed replacing the -35 region of the Phage lambda promoter (P_L) for IR2 and fusing it with the spacer sequence of the strong constitutive promoter from the Anderson's library (BBa_J23119).



Supplementary Figure 4. Full factorial DoE model for FAB.

Standard least squares regression (SLSR) model of the DoE dataset. Effect summary of P_{regC}, P_{enzA} and P_{regC}*P_{enzA} for OFF, ON and ON/OFF showing significative effect of P_{regC} ($P<0.05$) for the performance. Model prediction of P_{regC} and P_{enzA} for OFF, ON and ON/OFF showing positive linear effect of P_{regC} levels for ON/OFF (green framed square).

SUPPLEMENTARY TABLES

Supplementary Table 1. Raw data for the PAB definitive screening design.

Construct	Trial	P_{reg}	P_{out}	RBS_{out}	OFF			ON		
pD1	1	0	0	0	612.7	578.4	590.6	1013.8	1046.0	1046.6
pD2	2	0	1	1	398.5	394.3	400.9	61768.3	61213.1	63230.6
pD3	3	-1	-1	-1	28.3	29.6	28.8	41.8	44.3	51.0
pD4	4	1	-1	0	481.0	481.0	477.5	843.2	867.3	870.9
pD5	5	-1	1	0	1533.6	1593.5	1502.7	5430.4	5621.1	5587.0
pD6	6	0	-1	-1	21.0	14.1	13.8	34.0	42.1	31.8
pD7	7	1	1	1	1247.3	1322.5	1276.7	45791.4	46571.6	49052.4
pD8	8	1	0	-1	36.3	40.2	46.4	46.5	51.9	50.8
pD9	9	1	-1	1	593.7	602.0	630.9	1035.5	1037.7	1025.5
pD10	10	-1	0	1	3252.4	3255.1	3407.2	17056.6	17312.8	17266.8
pD11	11	1	1	-1	32.6	37.9	42.5	96.9	101.9	101.2
pD12	12	-1	-1	1	645.4	650.4	683.4	1753.2	1969.2	1801.8
pD13	13	-1	1	-1	84.2	64.5	67.1	220.5	246.6	212.7

Supplementary Table 2. Definitive screening design factor screening.

	GFP OFF (log10)				GFP OFF (log10)				GFP OFF (log10)			
Term	**Contrast**	**Lenth t-Ratio**	**Individual p-value**	**Simultaneous p-Value**	**Contrast**	**Lenth t-Ratio**	**Individual p-value**	**Simultaneous p-Value**	**Contrast**	**Lenth t-Ratio**	**Individual p-value**	**Simultaneous p-Value**
Prep	-293.405	-11.57	1.5E-09	1.60341E-05	2214.7	8.74	0.000118247	0.000708798	2.3319	2.33	0.033105519	0.408426961
Prep*Prep	223.873	8.83	3.0372E-06	0.000473797	-2015.4	-7.95	0.000183189	0.001025964	-13.298	-13.3	4E-10	2.53E-08
Prep*Psens	-182.802	-7.21	0.000132789	0.002035138	10014.2	39.5	< 1.0E-25	< 1.0E-25	15.9431	15.95	<8.5E-15	<3.2E-11
Psens	145.343	5.73	0.000783357	0.007750242	-996.1	-3.93	0.004264757	0.059177616	-5.1029	-5.11	0.00108595	0.014827773
Psens*Psens	-278.853	-11	8.8E-09	0.000038746	1974.3	7.79	0.000196565	0.001084874	7.439	7.44	0.000206546	0.002076476
RBSsens	575.014	22.68	< 1.0E-25	<1.8E-16	11588.6	45.71	< 1.0E-25	< 1.0E-25	15.8569	15.86	<1.1E-14	<4.0E-11
RBSsens*Prep	-210.003	-8.28	1.10021E-05	0.000640039	-767.3	-3.03	0.01308755	0.183798057	-7.932	-7.94	0.000151893	0.001130195
RBSsens*Prep*Prep	312.489	12.32	1E-10	4.7395E-06	10695.3	42.19	< 1.0E-25	< 1.0E-25	17.3039	17.31	<6.2E-17	<6.6E-13
RBSsens*Prep*Psens	-305.415	-12.05	3E-10	7.5076E-06	-1970.9	-7.77	0.000197683	0.001089795	-13.7655	-13.77	1E-10	8.5E-09
RBSsens*Psens	228.542	9.01	1.9361E-06	0.000452371	2303.5	9.09	3.96331E-05	0.000462061	6.8437	6.85	0.000295195	0.003063257
RBSsens*RBSsens	-116.524	-4.6	0.00141157	0.025293161	4548.2	17.94	<3.2E-16	0.0000001	6.6929	6.7	0.000413395	0.003446297
RBSsens*RBSsens*Prep	41.775	1.65	0.103189081	0.853679627	3330.2	13.14	5.8E-09	0.000132891	10.3242	10.33	9.099E-07	1.16639E-05

Supplementary Table 3. Parameter estimates for standard least squares model.

	GFP OFF (log10)				GFP ON (log10)				GFP ON/OFF (log10)			
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	2.840341	0.071618	39.66	<.0001	3.144749	0.091299	34.44	<.0001	0.304408	0.097342	3.13	0.0058
Psens	0.144009	0.026528	5.43	<.0001	0.516504	0.033819	15.27	<.0001	0.372495	0.036057	10.33	<.0001
RBSsens	0.748324	0.026528	28.21	<.0001	1.097718	0.033819	32.46	<.0001	0.349394	0.036057	9.69	<.0001
Prep	-0.10328	0.026528	-3.89	0.0011	-0.08711	0.033819	-2.58	0.019	0.01617	0.036057	0.45	0.6592
Psens*Psens	-0.28425	0.056222	-5.06	<.0001	0.063164	0.071673	0.88	0.3898	0.347418	0.076417	4.55	0.0003
Psens*RBSsens	0.011279	0.033663	0.34	0.7415	0.266777	0.042913	6.22	<.0001	0.255498	0.045754	5.58	<.0001
RBSsens*RBSsens	-0.69105	0.056222	-12.29	<.0001	-0.3521	0.071673	-4.91	0.0001	0.338954	0.076417	4.44	0.0003
Prep*Prep	0.413538	0.063683	6.49	<.0001	0.086261	0.081184	1.06	0.302	-0.32728	0.086558	-3.78	0.0014

Supplementary Table 4. Tuning the PAB for optimal performance by varying the level of P_{reg} controlling *pcaV*.

Construct	Set	P_{reg}	P_{out}	RBS_{out}	OFF	ON	ON/OFF
p131C-B20	validation	-1.00	1	1	14705.3 ± 430.2	69296.2 ± 407.9	4.7 ± 0.15
p131C-B9	validation	-0.56	1	1	1418.5 ± 43.9	66255.0 ± 1099.0	46.7 ± 1.6
p131C-B3	validation	-0.28	1	1	816.9 ± 14.5	62160.1 ± 984.2	76.1 ± 0.57
pD2	training	0	1	1	397.9 ± 3.4	62070.6 ± 1042.1	156.0 ± 1.5
p131C-B10	validation	0.14	1	1	187.3 ± 0.7	51858.5 ± 507.6	276.8 ± 3.34
p131C-B6	validation	0.36	1	1	170.8 ± 1.6	39229.3 ± 796.1	229.7 ± 6.79
p131C-B18	validation	0.67	1	1	338.2 ± 7.2	20486.1 ± 166.1	60.6 ± 1.23
pD7	training	1	1	1	1282.1 ± 37.9	47138.5 ± 1702.8	36.8 ± 1.6

OFF and ON measurements were made in the absence or presence of 1 mM PCA, respectively. The values for OFF, ON and OFF/ON indicate the mean of three biological replicates with ± denoting the standard deviation of those replicates.

Supplementary Table 5. Comparison of the original and optimised PAB.

PCA biosensor	OFF	ON	ON/OFF	EC_{50} (μM)
original	7.5 ± 8.0	3121.2 ± 88.4	417.4 ± 95.9	537
p131C-B10	186.5 ± 5.6	97099.3 ± 612.4	521.1 ± 18.9	897

The titration was carried out with a PCA concentration ranging from to 3.9 to 4000 μM. OFF and ON measurements were made in the absence or presence of 4 mM PCA, respectively. The values for OFF, ON and OFF/ON indicate the mean of three biological replicates with ± denoting the standard deviation of those replicates.

Supplementary Table 6. Tuning the chromosome-integrated PAB for optimal performance by varying the level of P_{reg} controlling *pcaV*.

Construct	P_{reg}	P_{out}	RBS_{out}	OFF	ON	ON/OFF
pDK-B9	-0.56	1	1	6562.6 ± 62.4	7163.7 ± 38.4	1.09 ± 0.02
pDK-B20	0.00	1	1	2826.0 ± 92.8	7066.3 ± 43.8	2.50 ± 0.09
pDK-B10	0.14	1	1	2543.5 ± 16.6	6841.8 ± 97.9	2.69 ± 0.05
pDK-B6	0.36	1	1	688.7 ± 24.3	6628.1 ± 83.8	9.63 ± 0.37
pDK-B17	0.53	1	1	357.5 ± 2.8	7071.9 ± 87.4	19.78 ± 0.25
pDK-B15	0.61	1	1	155.9 ± 3.0	6677.9 ± 191.8	42.85 ± 1.53
pDK-B18	0.67	1	1	205.6 ± 6.9	7200.8 ± 135.6	35.05 ± 1.29
pDK-B23	0.77	1	1	264.1 ± 2.1	6721.3 ± 140.4	25.44 ± 0.35
pDK-B16	0.94	1	1	284.2 ± 9.6	6910.2 ± 135.6	24.34 ± 1.23
pDK-B12	1.00	1	1	5759.6 ± 117.7	6956.9 ± 71.4	1.21 ± 0.03

OFF and ON measurements were made in the absence or presence of 1 mM PCA, respectively. The values for OFF, ON and OFF/ON indicate the mean of three biological replicates with ± denoting the standard deviation of those replicates.

Supplementary Table 7. Comparison of the sensitivity of the PAB to PCA with and without the PcaK transporter.

PCA biosensor	OFF	ON	ON/OFF	EC_{50} (μM)
p131C-B10	164.1 ± 4.5	72521.7 ± 1656.3	442.1 ± 13.2	557
p131C-B10-pcaK	359.7 ± 11.7	68864.9 ± 1133.7	191.6 ± 6.4	0.335

The titration was carried out with a PCA concentration ranging from to 0.0038 to 4000 μM. OFF and ON measurements were made in the absence or presence of 4 mM PCA, respectively. The values for OFF, ON and OFF/ON indicate the mean of three biological replicates with ± denoting the standard deviation of those replicates.

Supplementary Table 8. Assessment of the performance of dose-response extender variants.

Dose-response extender	OFF	ON	ON/OFF	Hill coefficient	EC50 (μM)	DRLR
reporter only	181.5 ± 10.3	81004.9 ± 356.6	447.2 ± 24.1	0.980 ± 0.041	281.8 ± 24.5	88.7
PcaK_-1_LacI_-1	302.7 ± 27.3	104898.5 ± 1063.7	348.3 ± 29.9	1.65± 0.33	1.73 ± 0.19	14. 4
PcaK_-1_LacI_0	210.2 ± 42.2	79030.1 ± 22808.3	374.3 ± 79	0.926 ± 0.29	11.5 ± 4.9	117.8
PcaK_0_LacI_-1	417.0 ± 51.8	105094.9 ± 4218.8	254.2± 3	1.58 ± 0.14	0.948 ± 0.067	16. 3
PcaK_0_LacI_0	263.1 ± 12.4	99438.9 ± 2299.6	378.6 ± 23.4	1.42 ± 0.089	1.94 ± 0.09	22.1
PcaK_1_LacI_-1	382 ± 15.2	105063.1 ± 5121.3	275.3 ± 16.0	1.79 ± 0.4	0.354 ± 0.02	11.7
PcaK_1_LacI_0	170.6 ± 6.8	102360.6 ± 155.3	600.6 ± 24.8	1.69 ± 0.12	1.65 ± 0.059	13.6

The titration was carried out with a PCA concentration ranging from to 0.0128 to 1000 μM. OFF and ON measurements were made in the absence or presence of 1 mM PCA, respectively. The values for OFF, ON and OFF/ON indicate the mean of three biological replicates with ± denoting the standard deviation of those replicates.

Supplementary Table 9. Comparison of dynamic range of the PAB against popular expression systems.

Expression system	ON/OFF 3 h	ON/OFF 24 h
P_{araBAD}/AraC	178.5 ±12.2	36.3 ± 1.3
P_{pv}/PcaV	224.5 ± 2.9	219.2 ± 12.0
P_{lac}/LacI/T7RNAP	363.2 ± 25.6	84.0 ± 1.0
P_{rhaBAD}/RhaS	27.4 ± 1.8	2.9 ± 0.2

The following inducers were used: L-arabinose for P_{araBAD}/AraC; PCA for P_{pv}/PcaV; IPTG for P_{lac}/LacI/T7RNAP; and L-mannose for P_{rhaBAD}/RhaS. Titrations were carried out with inducers at concentrations ranging from to 3.9 to 4000 μM. OFF and ON measurements were made in the absence or presence of 4 mM of inducer, respectively. The values for OFF/ON indicate the mean of three biological replicates with ± denoting the standard deviation of those replicates.

Supplementary Table 10. Raw data for the FAB full factorial design.

Construct	Trial	P_{regC}	P_{enzA}	RBS_{out}	OFF			ON		
pFABs1	1	-1	-1	1	14966	15030.4	14469.1	102558	93161.8	93772.3
pFABs2	2	-1	0	1	8262.1	7285.6	7940.3	91256.4	86897.7	94597.5
pFABs3	3	-1	1	1	33529.8	33274.8	33699.2	90905.3	94533.2	95824.9
pFABs4	4	0	-1	1	6846.6	6608.7	6492.1	89215.4	87395.5	90105.1
pFABs5	5	0	0	1	6769.1	6876.2	6683.6	88137.9	86719.3	89006.7
pFABs6	6	0	1	1	6552.8	6517	6039.9	88549.2	89600.4	88144.6
pFABs7	7	1	-1	1	2076.8	2164.6	2180.1	79578.6	85037.8	84313.2
pFABs8	8	1	0	1	1971.8	2042.2	1868.6	75845.8	78895.3	76475.9
pFABs9	9	1	1	1	1356.1	1396.5	1545.8	71633.6	77672.7	75372.2

Supplementary Table 11. Tuning the FAB for optimal dynamic range by varying the level of RBS_{out} controlling the sfGFP output.

Construct	P_{regC}	P_{enzA}	RBS_{out}	OFF	ON	ON/OFF
pFABs9	1	1	1	83845.3 ± 2968.9	1378.4 ± 13.7	60.8 ± 2.0
pFABsG21	1	1	0.94	76569.2 ± 2157.5	1018.2 ± 33.8	75.2 ± 0.8
pFABsG19	1	1	0.89	62005.7 ± 2732.2	666.2 ± 20.4	93.1 ± 4.0
pFABsG12	1	1	0.81	30783.2 ± 1224.7	261.5 ± 11.3	117.7 ± 8.4

OFF and ON measurements were made in the absence or presence of 1 mM FA, respectively. The values for OFF, ON and OFF/ON indicate the mean of three biological replicates with ± denoting the standard deviation of those replicates.

Supplementary Table 12. Primer used in this study.

Primer name	Sequence
AB 09	GCGGCCGCGTCGTGACTGGGAAAA
AB 10	GGCCTAGGCGGCCTCCTGTGTGAAATTG
AB 11	AGCGGATAACAATTTCACACAGGA
AB 12	CGCCAGGGTTTTCCCAGTCA
AB 15	AACAATTTCACACAGGAGGCCGCCTAGGCCTTATTTATACAGTTCGTCCATACCGC
AB 18	CCAGGGTTTTCCCAGTCACGACGCGGCCGCTTATTTATACAGTTCATCCATACCATGGG
AB 27	ATGAGCAAAGGTGAAGAACTGTTTAC
AB 28	ATGGTTTCTAAAGGTGAAGAAGAC
AB 29	GCTCGGATCCACTAGTAGAGG
AB 30	CGGTACCGCATGCAATCAT
AB 34	GAAAGTACGTGCAGCCAGAG
AB 39	CCAAATCGCAGCCAATCACA
AB 40	GGTTATCTGGCAGCCGAAAG
AB 61	GAATCCAGAAAAGCGGCCAT
AB 94	AGTCAACACTCTTTTTGATAAATTTTGCATGC
AB 95	GCATGCAAAATTTATCAAAAAGAGTG
AB 100	GGAATTCCATATGTTTTATCCTCTACTAGTTTATTTTTGACACCAGACCAACTGGTAATG
AB 101	GGAGGATATTCATATAGACCATGATTGCATAGCGGATAACAATTTCACACAGGA
AB 114	CATGCAAAATTTATCAAAAAGAGTGTTGACTATACTCAGTGCCCTGACTATGATACTTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGANNNNNNTATACATATGAGCAAAGGTGAAGAACTGTTTACCG
AB 115	CATGCAAAATTTATCAAAAAGAGTGTTNANNATACTCAGTGCCCTGACTATNATNNTTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGAAGGAGATATACATATGAGCAAAGGTGAAGAACTGTTTACCG
AB 127	GTTTAACTTTGAAATAAGGAGGTAATACAAATGGCAGCAGTTGATCTGGCAAC
AB 128	TTGTATTACCTCCTTATTTCAAAGTTAAAC
AB 129	CCATCGGAAGCTGTGGTATG
AB 130	GATTTACGACCTGCACAGCC
AB 142	CATGCAAAATTTATCAAAAAGAGTGTTCATGATACTCAGTGCCCTGACTATAATGATTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGATAGTCATATACATATGAGCAAAGGTGAAGAACTGTTTACCG
AB 143	CATGCAAAATTTATCAAAAAGAGTGTTCATGATACTCAGTGCCCTGACTATAATGATTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGAAGGGTGTATACATATGAGCA

AAGGTGAAGAACTGTTTACCG

AB 144 CATGCAAAATTTATCAAAAAGAGTGTTAAAGATACTCAGTGCCCTGACTATTATGTTTA GATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGATAGTCATATACATATGAGCAA AGGTGAAGAACTGTTTACCG

AB 145 CATGCAAAATTTATCAAAAAGAGTGTTAAAGATACTCAGTGCCCTGACTATTATGTTTA GATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGAAGGGTGTATACATATGAGCA AAGGTGAAGAACTGTTTACCG

AB 146 CCACAACGGTTTCCCTCTAC

AB 147 CATAGACCTAGGGCAGCAGA

AB 148 AAAATTATTTGTAGAGGGAAACCGTTGTGGTCTCCCTGAATATANNNTACGAGCCTTA TGCATGCCCGTAAAGTTATCCAGCAACCACTCATAGACCTAGGGCAGCAGATAGGGA CGAC

AB 159 GCCCATATGTATATCTCCTTCTTAAAG

AB 160 TGTTAATTAAGTTGGGCGTTCC

AB 161 GTTTAACTTTAAGAAGGAGATATACATATGAGCAAAGGTGAAGAACTGTTTAC

AB 162 GCCTAGGAACGCCCAACTTAATTAACATTATTTATACAGTTCATCCATACCATGGG

AB 163 CATGGTATATCTCCTTCTTAAAGTTAAAC

AB 164 CTGTTTTGGCGGATGAGAGA

AB 165 TTTTGTTTAACTTTAAGAAGGAGATATACCATGAGCAAAGGTGAAGAACTGTTTAC

AB 166 CTGAAAATCTTCTCTCATCCGCCAAAACAGTTATTTATACAGTTCATCCATACCATGG G

AB 167 TCAACCCGGTGCAACTGC

AB 195 CATATGTATACACCCTTCTTAAAGTTAAA

AB 196 GGCAAAAAACATTATCCAGAACG

AB 197 TTTAACTTTAAGAAGGGTGTATACATATGGTGAAACCAGTAACGTTATACGATG

AB 198 CCAGGGTTTTCCCAGTCACGACGCGGCCGCTCACTGCCCGCTTTCCAG

AB 199 ACGTCTAAGCTTTTACCTACAGAATTTTGCGCCTGATTCATATGTATATGACTATCTTA AAGTTAAAGGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAAT GTCAATTGTTATCCGCTCACAATTCTCGAGGGCAAAAAACATTATCCAGAACGGGAGT GCGCC

FAB 1 GGCCGATTCATTAATGCAGCTGACGCAATTAATGTAAGTTAGCT

FAB 2 GATGATTTCTCGGTACCGCATGTAACAAAGCCCGAAAGGAAG

FAB 3 AGCTTCCTTTCGGGCTTTGTTACATGCGGTACCGAGAAATCATC

FAB 4 CTTCCGATGGCTGCCTGACGCCAGTAGTAGGTTGAGGCCGTT

FAB 5 TCAACGGCCTCAACCTACTACTGGCGTCAGGCAGCCATCGGA

FAB 6 AGCTAACTTACATTAATTGCGTCAGCTGCATTAATGAATCGGCCAAC

FAB 7 ATGAGCAAAGGTGAAGAACTGTTTACCG

FAB 8 CTCCCGTTCTGGATAATGTTTTTTGCC

FAB 9 CTTTGAAATAAGGAGGTAATACAAATGGCCGTTGAAGCCGGTGTTCGTC

FAB 10 GGCAAAAAACATTATCCAGAACGGGAGTGCGCC

FAB 11 GCACTCCCGTTCTGGATAATGTTTTTTGCCCACAGCTAACACCACGTC

FAB 12 GATCATCCTGACGCATACGTTCACCCATTTGTATTACCTCCTTATTTCAAAGTTA

FAB 13 TAACTTTGAAATAAGGAGGTAATACAAATGGGTGAACGTATGCGTCAGGATGATC

FAB 14 GATAGGGACGACGTGGTGTTAGCTGTGTCTAGAATAAAACGAAAGGCCCAGTCTTC

FAB 15 GAAGACTGGGCCTTTCGTTTTATTCTAGACACAGCTAACACCACGTCGTCCCTATC

FAB 16 CTGAGGACGAACACCGGCTTCAACGGCCATTTGTATTACCTCCTTATTTCAAAGTTAA
AC

FAB 17 TCTAGACCATCGAATGGTGCAAAACCTTTCGCG

FAB 18 GGCAAAAAACATTATCCAGAACGGGAGTGCGCC

FAB 19 GGCCGATTCATTAATGCAGCTGACGCAATTAATGTAAGTTAGCT

FAB 20 GTTACTGGTTTCACATTCACCACCC

FAB 21 GATTTAACTTTAAGACTTTGGTATACATATGAGCAAAGGTGAAGAACT

FAB 22 GATTTAACTTTAAGAGGCTTATATACATATGAGCAAAGGTGAAGAACT

FAB 23 GATTTAACTTTAAGAGGGAGGTATACATATGAGCAAAGGTGAAGAACT

FAB 24 CTTAAAGTTAAATCGGTCAGTGCGTCATGC

AB 301 ATGGTGAAACCAGTAACGTTATACGATGTCG

AB 302 ATGAATCAGGCGCAAAATTCTGTAGGTAAAAGC

AB 303 GCTTTTACCTACAGAATTTTGCGCCTGATTCATATGTATACACCCTTCTTAAAGTTAAA
GGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTG
TTATCCGCTCACAATTCTCGAGGGCAAAAAACATTATCCAGAACGGGAGTGCGCCTT
GAGCGACACGAATTATGCAGTGATTTACGACCTGCACAGCCATACCACAGCTTCCGA
TGGCTGCCTGACGCCAGAAGCATTGGTGCACCGTGCAGTCGATGATAAGCTGTCAA
ACGCATGCAAAATTTATCAAAAAGAGTGTTGACTATACTCAGTGCCCTGACTATGATA
CTTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGAAGGGTGTATACATATGG
TGAAACCAGTAACGTTATACGATGTCG

AB 304 GCTTTTACCTACAGAATTTTGCGCCTGATTCATATGTATATGACTATCTTAAAGTTAAA
GGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTG
TTATCCGCTCACAATTCTCGAGGGCAAAAAACATTATCCAGAACGGGAGTGCGCCTT
GAGCGACACGAATTATGCAGTGATTTACGACCTGCACAGCCATACCACAGCTTCCGA
TGGCTGCCTGACGCCAGAAGCATTGGTGCACCGTGCAGTCGATGATAAGCTGTCAA
ACGCATGCAAAATTTATCAAAAAGAGTGTTGACTATACTCAGTGCCCTGACTATGATA
CTTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGAAGGGTGTATACATATGG
TGAAACCAGTAACGTTATACGATGTCG

AB 305 GCTTTTACCTACAGAATTTTGCGCCTGATTCATATGTATATCTCCTTCTTAAAGTTAAA
GGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTG
TTATCCGCTCACAATTCTCGAGGGCAAAAAACATTATCCAGAACGGGAGTGCGCCTT
GAGCGACACGAATTATGCAGTGATTTACGACCTGCACAGCCATACCACAGCTTCCGA
TGGCTGCCTGACGCCAGAAGCATTGGTGCACCGTGCAGTCGATGATAAGCTGTCAA

ACGCATGCAAAATTTATCAAAAAGAGTGTTGACTATACTCAGTGCCCTGACTATGATA
CTTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGAAGGGTGTATACATATGG
TGAAACCAGTAACGTTATACGATGTCG

AB 306 GCTTTTACCTACAGAATTTTGCGCCTGATTCATATGTATACACCCTTCTTAAAGTTAAA
GGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTG
TTATCCGCTCACAATTCTCGAGGGCAAAAAACATTATCCAGAACGGGAGTGCGCCTT
GAGCGACACGAATTATGCAGTGATTTACGACCTGCACAGCCATACCACAGCTTCCGA
TGGCTGCCTGACGCCAGAAGCATTGGTGCACCGTGCAGTCGATGATAAGCTGTCAA
ACGCATGCAAAATTTATCAAAAAGAGTGTTGACTATACTCAGTGCCCTGACTATGATA
CTTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGATAGTCATATACATATGG
TGAAACCAGTAACGTTATACGATGTCG

AB 307 GCTTTTACCTACAGAATTTTGCGCCTGATTCATATGTATACACCCTTCTTAAAGTTAAA
GGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTG
TTATCCGCTCACAATTCTCGAGGGCAAAAAACATTATCCAGAACGGGAGTGCGCCTT
GAGCGACACGAATTATGCAGTGATTTACGACCTGCACAGCCATACCACAGCTTCCGA
TGGCTGCCTGACGCCAGAAGCATTGGTGCACCGTGCAGTCGATGATAAGCTGTCAA
ACGCATGCAAAATTTATCAAAAAGAGTGTTGACTATACTCAGTGCCCTGACTATGATA
CTTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGAAGGAGATATACATATGG
TGAAACCAGTAACGTTATACGATGTCG

AB 308 GCTTTTACCTACAGAATTTTGCGCCTGATTCATATGTATATGACTATCTTAAAGTTAAA
GGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTG
TTATCCGCTCACAATTCTCGAGGGCAAAAAACATTATCCAGAACGGGAGTGCGCCTT
GAGCGACACGAATTATGCAGTGATTTACGACCTGCACAGCCATACCACAGCTTCCGA
TGGCTGCCTGACGCCAGAAGCATTGGTGCACCGTGCAGTCGATGATAAGCTGTCAA
ACGCATGCAAAATTTATCAAAAAGAGTGTTGACTATACTCAGTGCCCTGACTATGATA
CTTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGATAGTCATATACATATGG
TGAAACCAGTAACGTTATACGATGTCG

AB 309 GCTTTTACCTACAGAATTTTGCGCCTGATTCATATGTATATCTCCTTCTTAAAGTTAAA
GGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAATGTCAATTG
TTATCCGCTCACAATTCTCGAGGGCAAAAAACATTATCCAGAACGGGAGTGCGCCTT
GAGCGACACGAATTATGCAGTGATTTACGACCTGCACAGCCATACCACAGCTTCCGA
TGGCTGCCTGACGCCAGAAGCATTGGTGCACCGTGCAGTCGATGATAAGCTGTCAA
ACGCATGCAAAATTTATCAAAAAGAGTGTTGACTATACTCAGTGCCCTGACTATGATA
CTTAGATTCATACTCAGTGCCCTGACTATTTTAACTTTAAGATAGTCATATACATATGG
TGAAACCAGTAACGTTATACGATGTCG

Supplementary Table 13. Plasmid used or constructed in this study

Plasmid name	Relevant characteristics[a]	Source or reference
pSEVA131	Cloning vector; *oriV* (pBBR1), Am^r	(9)
pSEVA 261	Cloning vector; *oriV* (p15A), Km^r	(9)
pET28a	Expression vector; P_{lac}/LacI/T7RNAP; *oriV* (pBR322), Km^r	Merck
pET44a	Expression vector; P_{lac}/LacI/T7RNAP; *oriV* (pBR322), Am^r	Merck
pBAD	Expression vector; P_{araBAD}/AraC; *oriV* (pBR322), Am^r	ThermoFisher
pCK302	sfGFP expression vector; P_{rhaBAD}/RhaS; *oriV* (pBR322), Am^r	(10)
pKIKOarsBKm	Integration vector; *oriV* (RK6), Am^r, Km^r	(6)
p131B	Template vector with *mCherry* and *sfGFP*; *oriV* (pBBR1), Am^r	This study
p131B-BX[b]	P_{reg}-library vectors; *oriV* (pBBR1), Am^r	This study
p131B-GX[b]	RBS_{out}-library vectors; *oriV* (pBBR1), Am^r	This study
p131-VX[b]	P_{out}- library vectors; *oriV* (pBBR1), Am^r	This study
pDX	DoE PCA biosensor vectors; *oriV* (pBBR1), Am^r	This study
p131CB-X[b]	DoE PCA biosensor validation vectors; *oriV* (pBBR1), Am^r	This study
pDK-BX	DoE PCA biosensor integration vectors; *oriV* (pBBR1), Am^r	This study
pET44-sfGFP	sfGFP expression vector; P_{lac}/LacI/T7RNAP; *oriV* (pBR322), Am^r	This study
pBAD-sfGFP	sfGFP expression vector; P_{araBAD}/AraC; *oriV* (pBR322), Am^r	This study
$pFABsP_{LC}$	FA biosensor vector promoter variant P_{LC} ; *oriV* (pBR322), Km^r	This study
$pFABsP_{LC2}$	FA biosensor vector promoter variant P_{LC2} ; *oriV* (pBR322), Km^r	This study
$pFABsP_{LC2}$ FerC KO	FA biosensor vector promoter variant P_{LC2} Δ*ferC*; *oriV* (pBR322), Km^r	This study
$pFABsP_{LC2}$ FerA KO	FA biosensor vector promoter variant P_{LC2} Δ*ferA*; *oriV* (pBR322), Km^r	This study
pFABsX (DOE)	DoE FA biosensor vectors; *oriV* (pBR322), Km^r	This study
pFABsG12	DoE FA biosensor variant RBS_{out} at 0.81; *oriV* (pBR322), Km^r	This study
pFABsG19	DoE FA biosensor variant RBS_{out} at 0.89; *oriV* (pBR322), Km^r	This study
pFABsG21	DoE FA biosensor variant RBS_{out} at 0.94; *oriV* (pBR322), Km^r	This study
p261LacI[X]_PcaK[X][b]	DoE PCA biosensor extender vectors; *oriV* (p15A), Km^r	This study

a. Antibiotic markers: Am^r, ampicillin; Km^r, kanamycin
b. For these plasmids X denotes a library member or DoE variant

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