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

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SUPPORTING INFORMATION

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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 (gaataagggtaatacaca) (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 \textit{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 \textit{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 \textit{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$_{LC2}$ linearized by inverse PCR with primers FAB6/7.

The plasmids for DoE pFABs1 ($P_{regC}/P_{enzA}/\text{RBS}_{\text{out}}$ pattern at levels $-1/-1/+1$) to pFABs9 ($P_{regC}/P_{enzA}/\text{RBS}_{\text{out}}$ 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 ($P_{regC}/P_{enzA}/\text{RBS}_{\text{out}}$ pattern at levels $+1/+1/+0.81$), pFABsG19 ($P_{regC}/P_{enzA}/\text{RBS}_{\text{out}}$ pattern at levels $+1/+1/+0.89$) and pFABsG21 ($P_{regC}/P_{enzA}/\text{RBS}_{\text{out}}$ 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$_{\text{out}}$ 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 (AGGAGGAAAAAA) 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 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 $R^2 = 0.986$, $P = 1.2 \times 10^{-11}$, ON $R^2 = 0.988$, $P = 6.8 \times 10^{-12}$, ON/ OFF $R^2 = 0.95$, $P = 1.6 \times 10^{-08}$. 

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).

*Sphingobium Operator* : (19 bp)  IR2: ATGCTATGGCTTATAGCAT
Supplementary Figure 4. Full factorial DoE model for FAB.

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

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

| Construct | Trial | $P_{\text{reg}}$ | $P_{\text{out}}$ | RBS$_{\text{out}}$ | 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.

| Term                | Contrast | Lenth t-Ratio | GFP OFF (log10) Individual p-value | Simultaneous p-Value | Contrast | Lenth t-Ratio | GFP OFF (log10) Individual p-value | Simultaneous p-Value | Contrast | Lenth t-Ratio | GFP OFF (log10) 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            | 15.8659  | 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          | 0.001411157                        | 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.

| Term          | Estimate  | Std Error | t Ratio | Prob>|t| | Estimate  | Std Error | t Ratio | Prob>|t| | Estimate  | Std Error | t Ratio | Prob>|t| |
|---------------|-----------|-----------|---------|--------|-----------|-----------|---------|--------|-----------|-----------|-----------|---------|--------|
| Interceptor   | 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 $P_{\text{reg}}$ controlling pcaV.

| Construct | Set     | $P_{\text{reg}}$ | $P_{\text{out}}$ | RBS$_{\text{out}}$ | 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 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 $P_{\text{reg}}$ controlling $pcaV$.

| Construct | $P_{\text{reg}}$ | $P_{\text{out}}$ | RBS$_{\text{out}}$ | 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\text{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 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 |
|-------------------|------------|-------------|
| $P_{\text{araBAD}}$/AraC | 178.5 ±12.2 | 36.3 ± 1.3 |
| $P_{\text{pv}}$/PcaV | 224.5 ± 2.9 | 219.2 ± 12.0 |
| $P_{\text{lac}}$/LacI/T7RNAP | 363.2 ± 25.6 | 84.0 ± 1.0 |
| $P_{\text{rhaBAD}}$/RhaS | 27.4 ± 1.8 | 2.9 ± 0.2 |

The following inducers were used: L-arabinose for $P_{\text{araBAD}}$/AraC; PCA for $P_{\text{pv}}$/PcaV; IPTG for $P_{\text{lac}}$/LacI/T7RNAP; and L-mannose for $P_{\text{rhaBAD}}$/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 | $P_{\text{regC}}$ | $P_{\text{enzA}}$ | RBS$_{\text{out}}$ | OFF      | ON        |
|-----------|-------|-------------------|-------------------|-------------------|-----------|-----------|
| pFABs1    | 1     | -1                | -1                | 1                 | 14966     | 15030.4   | 14469.1   | 102558   | 93161.8  | 893772.3 |
| pFABs2    | 2     | -1                | 0                 | 1                 | 8262.1    | 7285.6    | 7940.3    | 91256.4  | 86897.7  | 794597.5 |
| pFABs3    | 3     | -1                | 1                 | 1                 | 33529.8   | 333274.8  | 33699.2   | 90905.3  | 94533.2  | 295824.9 |
| pFABs4    | 4     | 0                 | -1                | 1                 | 6846.6    | 6608.7    | 6492.1    | 89215.4  | 87395.5  | 590105.1 |
| pFABs5    | 5     | 0                 | 0                 | 1                 | 6769.1    | 6876.2    | 6683.6    | 88137.9  | 86719.3  | 389006.7 |
| pFABs6    | 6     | 0                 | 1                 | 1                 | 6552.8    | 6517      | 6039.9    | 88549.2  | 89600.4  | 488144.6 |
| pFABs7    | 7     | 1                 | -1                | 1                 | 2076.8    | 2164.6    | 2180.1    | 79578.6  | 85037.8  | 884313.2 |
| pFABs8    | 8     | 1                 | 0                 | 1                 | 1971.8    | 2042.2    | 1868.6    | 75845.8  | 78895.3  | 376475.9 |
| pFABs9    | 9     | 1                 | 1                 | 1                 | 1356.1    | 1396.5    | 1545.8    | 71633.6  | 77672.7  | 775372.2 |
**Supplementary Table 11.** Tuning the FAB for optimal dynamic range by varying the level of RBS$_{out}$ controlling the sfGFP output.

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

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.
| Primer name | Sequence |
|-------------|----------|
| AB 09       | GCGGCCGCGTCGTGACTGGGAAAA |
| AB 10       | GGCCTAGGCGGCCTCCTGTGTGAATTG |
| AB 11       | AGCGGATAACAATTCACACAGGA |
| AB 12       | CGCCAGGGTTTTCCCACTGCA |
| AB 15       | AACAAATTTCACACAGGAGGCAGGCCTAGGCTGCTTTTTATTATACGTTTGCTCCCATACCCG |
| AB 18       | CCAGGGTTTTCCACGTACAGACGCGCCGCTTTATTTATACAGTTTCCATCCATACCAGG |
| AB 27       | ATGAGCAAAAGGTGAAGAAGAAGAATGTTTAC |
| AB 28       | ATGGTTTCTAAAGGTGAAGAAGAAGAAGAATGTTTAC |
| AB 29       | GCTCGGATCCACTAGTAGAGG |
| AB 30       | CGGTACCACGTGCAATCAT |
| AB 34       | GAAAGTACGTCAGCCCAGAG |
| AB 39       | CCAAATGCGACGCAATCAC |
| AB 40       | GTTATCTGCGAGCCGAAAG |
| AB 61       | GAATCCAGAAAAGCAGCCCAT |
| AB 94       | AGTCAACACTCCTTTTGATAAAATTTTGCATGC |
| AB 95       | GCATGCAAATATTATCAAAAGAGTG |
| AB 100      | GGAATTCCCATATGTTTTATCTCCTACTAGTTTATTTTGACACCAGAACCACACAATCGGTAATTG |
| AB 101      | GGAGGATATTCATATAGACCATGATTGCATAGCAGGATACAAATTTTCACACAGGA |
| AB 114      | CATGCAAATTTATCAAAAAGAGTGTGTTGACTATACCTACAGTGCCTGACTATGATACTTTAAGAGGTTNNTATTACCATATGAGCA |
| AB 115      | CATGCAAATTTATCAAAAAGAGGTAATTTACCATATGAGGTTNNTATTACCATATGAGCA |
| AB 127      | GTTTAACCTTTGAAATAAGGAGGTAATACAAAATGGCAGCAGCTTTGATCTGGCAAC |
| AB 128      | TTGTATTACCTCCTTTATTTCAAAAGGTAAAC |
| AB 129      | CCATCGGAAGCCTGTGGTATG |
| AB 130      | GAATTCAGGACCTGCACAGCC |
| AB 142      | CATGCAAATTTATCAAAAAGAGTGTGTTGACTATACCTACAGTGCCTGACTATGATACTTTAAGAGGTTNNTATTACCATATGAGCA |
| AB 143      | CATGCAAATTTATCAAAAAGAGGTAATTTACCATATGAGGTTNNTATTACCATATGAGCA |
AAGGTGAAGAAGCTTTACCG

AB 144 CATGCAAATTTATAACAAAGAGTTAAGATATATGCACTGACTATTATGTTTA
GTTTCAATCTCAGTGCCCTGACTATTTATACCTTTAAGAGATATGTAACGTAGTGCTACATAGATGAGCAA
AGGTTGAAGAAACTTTACCG

AB 145 CATGCAAATTTATAACAAAGAGTTAAGATATATGCACTGACTATTATGTTTA
GTTTCAATCTCAGTGCCCTGACTATTTTAACTTTAAGATAGTCATATACATATGAGCA
AAGGTTGAAGAAACTTTACCG

AB 146 CCACACCGGGTTTCCCTCTAC
AB 147 CATAGACCTAGGGCAGCAGA
AB 148 AAAATTATTGATAGAGGGAACGTTTGTTGATCTCCTGTAATATTANNTACGAGCCTTA
TGCTAGGCCCCTAAAGCTTTATCCAGCAACCACCTCATTGAGATCCAGAGCTAGAGGA
CGAC
AB 159 GCCCATATGATATCTCCTCTTTCTAAG
AB 160 TGTTAATTAAGTGCCGCTTCC
AB 161 GCTTAGGAAACCCAACTTAAATACATTATTTATACGTTCATCATCCATACCATG
AB 162 CATGGTATATCTCCTCTTTAATAGTTAAC
AB 163 CTGGTTTGGCGGATGAGAGA
AB 164 GCTGAAAATCTTCTCTCATCCGCCAAAACAGTTATTTATACGTTCATCCATACCATG
G
AB 165 TCAACCCGGTGCAACTGC
AB 166 CTGAAAATCTTCTCTCATCCGCCAAAACAGTTATTTATACGTTCATCCATACCATG
G
AB 167 TCAACCCGGTGCAACTGC
AB 168 CATATGTATACACCCTCTTAAAGTTAAA
AB 169 GGCAAAAAACATTATCCAGAACG
AB 170 TTTTAACTTTAAGAAGGAGAGATAACCAGCAAAAGGATGGAAGAATGTTTTAC
AB 171 CTGGTTTGGCGGATGAGAGA
AB 172 CATATGTATACACCCTCTTAAAGTTAAA
AB 173 GCTGAAAATCTTCTCTCATCCGCCAAAACAGTTATTTATACGTTCATCCATACCATG
G
AB 174 TCAACCCGGTGCAACTGC
AB 175 GCGCCGATTCATTAATGCAGCTGACGCAATTAATGTAAGTTAGCT
AB 176 GATGATTTTCTCGGTACCGCATGTAACAAAGCCCGAAAGGAAG
AB 177 AGCTTCCTTTCGGCCTTTGTTACATGCGGTACCGAGAAATCATC
AB 178 CTTCCGATGGCTGCCTGACGCCAGTAGTAGGTTGAGGCCGTT
AB 179 TCAACGGCCTCAACCTACTACTGGCGTCAGGCAGCCATCGGA
AB 180 AGCTAACTTACATTAATTGCGTCAGCTGCATTAATGAATCGGCCAAC
AB 181 ATGAGCAAAGGTGAAGAACTTTACCG
AB 182 CTCCCGTTCTGGATAATGTTTTTTGCC
AB 183 CTTTGAAATAAGGAGGTAATACAAATGGCCGTTGAAGCGGTTCGTAC
AB 184 GCCCATATGATATCTCCTCTTTCTAAG
AB 185 TTGCAAAAAATCTTCTCTCATCCGCCAAAACAGTTATTTATACGTTCATCCATACCATG
G
AB 186 TCAACCCGGTGCAACTGC
AB 187 CATATGTATACACCCTCTTAAAGTTAAA
21
FAB 10  GGCAAAAAACATTATCCAGAACGGGAGTGCGCC
FAB 11  GCACTCCGGTTCTGGAATAATGTTTTTTGCCCCACAGCTAAACACCACGTGC
FAB 12  GATCATCCTGACGCATACGCTTACCCATTTGATATTACCTCTTTATTTCAAGGTTA
FAB 13  TAACCTTTAATGAGAGGTACATAAACATGTTGGAACGTATGCTGATGAGATGC
FAB 14  GATAGGGGACGACGTGTTAGGTCTGCTGAATAAAGGAAAGGCAGCTCTTC
FAB 15  GAAGACTGGGCTTTTCGTTATTCTAGACACAGCTAAACACCACGTGCACCTATC
FAB 16  CTGAGGACGAACACCAGCTTCAACGGGCCATTTGGTTACCTCCTTTATTTCAAAAGTTA
                     AC
FAB 17  TCTAGACCATCGAATGGAAGCAAAAAACCTTCGCG
FAB 18  GGCAAAAAACATTATCCGAGACGGGAGTGCGCC
FAB 19  GGCCGATTCTTTAATGCACGCTGACGCAATTAATGTAAGTTAGCT
FAB 20  GTTCTGGTCCACATCACCATCAC
FAB 21  GATTTAACCTTTAAGACTTTTGTTATACATATGAGCAAAAGGTAAGA
FAB 22  GATTTAACCTTTAAGACTTTTGTTATACATATGAGCAAAAGGTAAGA
FAB 23  GATTTAACCTTTAAGACTTTTGTTATACATATGAGCAAAAGGTAAGA
FAB 24  CTTAAGATATTTACCGTCATCAGTGATGATGCG
AB 301  ATGGTAAGACGTTATACGATGTCG
AB 302  ATGAAATCGGCAAGCAATTTTACGATGATGTCG
AB 303  GCTTTTACCTACAGAATTTTGCGCTGATATCCTTACATATGTAACCCCTTTTTTTAAAGTT
GTCAGTGTTCTCTGTCATCTCTGCTATCTCTGCTACACTATCAGCTGACGCGCTT
TTATCCGCCTCAAAACATAGTTGTCAGTTCATGTTCTTATCCCTACACTACAGCTGACGCGCTT
GAGCGACACAAATTTAATGCTGAGCTTATACAGCTGACGCGCTTACACTACAGCTGACGCGCTT
TGCTGGCCTGAGCGCAAGCAATTTATGCTGAGCTTATACAGCTGACGCGCTTACACTACAGCTGACGCGCTT
ACGCATGCAAAATTTATCAGATTTTGTTATACAGCTGACGCGCTTACACTACAGCTGACGCGCTT
CTTAGATTCTACATCTAGTGCCCTCAATATTTTAACTTTAAAGGGTGTATACATATGG
TGAAACACCTGACGTGACATACGATGTCG
AB 304  GCTTTTACCTACAGAATTTTGCGCTGATATCCTTACATATGTAACCCCTTTTTTTAAAGTT
GTCAGTGTTCTCTGTCATCTCTGCTATCTCTGCTACACTATCAGCTGACGCGCTT
TTATCCGCCTCAAAACATAGTTGTCAGTTCATGTTCTTATCCCTACACTACAGCTGACGCGCTT
GAGCGACACAAATTTAATGCTGAGCTTATACAGCTGACGCGCTTACACTACAGCTGACGCGCTT
TGCTGGCCTGAGCGCAAGCAATTTATGCTGAGCTTATACAGCTGACGCGCTTACACTACAGCTGACGCGCTT
ACGCATGCAAAATTTATCAGATTTTGTTATACAGCTGACGCGCTTACACTACAGCTGACGCGCTT
CTTAGATTCTACATCTAGTGCCCTCAATATTTTAACTTTAAAGGGTGTATACATATGG
TGAAACACCTGACGTGACATACGATGTCG
AB 305  GCTTTTACCTACAGAATTTTGCGCTGATATCCTTACATATGTAACCCCTTTTTTTAAAGTT
GTCAGTGTTCTCTGTCATCTCTGCTATCTCTGCTACACTATCAGCTGACGCGCTT
TTATCCGCCTCAAAACATAGTTGTCAGTTCATGTTCTTATCCCTACACTACAGCTGACGCGCTT
GAGCGACACAAATTTAATGCTGAGCTTATACAGCTGACGCGCTTACACTACAGCTGACGCGCTT
TGCTGGCCTGAGCGCAAGCAATTTATGCTGAGCTTATACAGCTGACGCGCTTACACTACAGCTGACGCGCTT

ACGCATGCAAAATTATCAAAAAGAGTGGTTGACTATATCTCAGTGCCCTGACTATGATA
CTTAGATTACATACATCGTGCCCTGACTATTATTTTTAAGGGGAGGTGATACATATGG
TGAACCAGTAACGGTTATACGATGTCG

AB 306
GCTTTTACCTACAGAAATTTCGGCCCTGATTTCATATGTATACCCCTTCTTTAAAAAGTTAAA
GGTCAGTGCGTGCTCITCGAGTGTGGCTGAGAGATCTTCCCAATATGTAATTTG
TTATCCGCTCACAATTCTCAGGGGAAAAACATATTACAGAAAGGGGAGGTGCCCTT
GAGCGACAGAATTATGCAGTATTACGGACTGACGAGCCATACACAGCTTCCCA
TGGCTGCCTGCAGCGGAAAGTGGCTGACGGGTGCAGTATGATAAGCTTGCAA
ACGCATGCAAAATTATCAAAAAGAGTGGTTGACTATATCTCAGTGCCCTGACTATGATA
CTTAGATTACATACATCGTGCCCTGACTATTATTTTTAAGGGGAGGTGATACATATGG
TGAACCAGTAACGGTTATACGATGTCG

AB 307
GCTTTTACCTACAGAAATTTCGGCCCTGATTTCATATGTATATCCTTCTTTAAAAAGTTAAA
GGTCAGTGCGTGCTCITCGAGTGTGGCTGAGAGATCTTCCCAATATGTAATTTG
TTATCCGCTCACAATTCTCAGGGGAAAAACATATTACAGAAAGGGGAGGTGCCCTT
GAGCGACAGAATTATGCAGTATTACGGACTGACGAGCCATACACAGCTTCCCA
TGGCTGCCTGCAGCGGAAAGTGGCTGACGGGTGCAGTATGATAAGCTTGCAA
ACGCATGCAAAATTATCAAAAAGAGTGGTTGACTATATCTCAGTGCCCTGACTATGATA
CTTAGATTACATACATCGTGCCCTGACTATTATTTTTAAGGGGAGGTGATACATATGG
TGAACCAGTAACGGTTATACGATGTCG

AB 308
GCTTTTACCTACAGAAATTTCGGCCCTGATTTCATATGTATATCCTTTCTTTAAAAAGTTAAA
GGTCAGTGCGTGCTCITCGAGTGTGGCTGAGAGATCTTCCCAATATGTAATTTG
TTATCCGCTCACAATTCTCAGGGGAAAAACATATTACAGAAAGGGGAGGTGCCCTT
GAGCGACAGAATTATGCAGTATTACGGACTGACGAGCCATACACAGCTTCCCA
TGGCTGCCTGCAGCGGAAAGTGGCTGACGGGTGCAGTATGATAAGCTTGCAA
ACGCATGCAAAATTATCAAAAAGAGTGGTTGACTATATCTCAGTGCCCTGACTATGATA
CTTAGATTACATACATCGTGCCCTGACTATTATTTTTAAGGGGAGGTGATACATATGG
TGAACCAGTAACGGTTATACGATGTCG

AB 309
GCTTTTACCTACAGAAATTTCGGCCCTGATTTCATATGTATATCCTTTCTTTAAAAAGTTAAA
GGTCAGTGCGTGCTCITCGAGTGTGGCTGAGAGATCTTCCCAATATGTAATTTG
TTATCCGCTCACAATTCTCAGGGGAAAAACATATTACAGAAAGGGGAGGTGCCCTT
GAGCGACAGAATTATGCAGTATTACGGACTGACGAGCCATACACAGCTTCCCA
TGGCTGCCTGCAGCGGAAAGTGGCTGACGGGTGCAGTATGATAAGCTTGCAA
ACGCATGCAAAATTATCAAAAAGAGTGGTTGACTATATCTCAGTGCCCTGACTATGATA
CTTAGATTACATACATCGTGCCCTGACTATTATTTTTAAGGGGAGGTGATACATATGG
TGAACCAGTAACGGTTATACGATGTCG
### 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; \(P_{\text{lac/LacI/T7RNAP}}\); \(oriV\) (pBR322), Am\(^r\) | Merck              |
| pET44a                 | Expression vector; \(P_{\text{lac/LacI/T7RNAP}}\); \(oriV\) (pBR322), Am\(^r\) | Merck              |
| pBAD                   | Expression vector; \(P_{\text{araBAD/AraC}}\); \(oriV\) (pBR322), Am\(^r\) | ThermoFisher       |
| pCK302                 | sfGFP expression vector; \(P_{\text{rhaBAD/RhaS}}\); \(oriV\) (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\(^b\)         | \(P_{\text{reg}}\)-library vectors; \(oriV\) (pBBR1), Am\(^r\) | This study         |
| p131B-GX\(^b\)         | RBS\(_{\text{out}}\)-library vectors; \(oriV\) (pBBR1), Am\(^r\) | This study         |
| p131-VX\(^b\)          | \(P_{\text{out}}\)-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; \(P_{\text{lac/LacI/T7RNAP}}\); \(oriV\) (pBR322), Am\(^r\) | This study         |
| pBAD-sfGFP             | sfGFP expression vector; \(P_{\text{araBAD/AraC}}\); \(oriV\) (pBR322), Am\(^r\) | This study         |
| pFABsP\(_{LC}\)        | FA biosensor vector promoter variant \(PLC\); \(oriV\) (pBR322), Km\(^r\) | This study         |
| pFABsP\(_{LC2}\)       | FA biosensor vector promoter variant \(PLC2\); \(oriV\) (pBR322), Km\(^r\) | This study         |
| pFABsP\(_{LC2}\)\(_{\Delta}\text{FerC}\) KO | FA biosensor vector promoter variant \(PLC2\) \(_{\Delta}\text{ferC}\); \(oriV\) (pBR322), Km\(^r\) | This study         |
| pFABsP\(_{LC2}\)\(_{\Delta}\text{FerA}\) KO | FA biosensor vector promoter variant \(PLC2\) \(_{\Delta}\text{ferA}\); \(oriV\) (pBR322), Km\(^r\) | This study         |
| pFABsX (DOE)           | DoE FA biosensor vectors; \(oriV\) (pBR322), Km\(^r\)            | (10)               |
| pFABsG12               | DoE FA biosensor variant RBS\(_{\text{out}}\) at 0.81; \(oriV\) (pBR322), Km\(^r\) | This study         |
| pFABsG19               | DoE FA biosensor variant RBS\(_{\text{out}}\) at 0.89; \(oriV\) (pBR322), Km\(^r\) | This study         |
| pFABsG21               | DoE FA biosensor variant RBS\(_{\text{out}}\) at 0.94; \(oriV\) (pBR322), Km\(^r\) | This study         |
| p261Lac[X]\(_{\text{PcaK[X]}}\) \(_{\text{PcaK[X]}}\) 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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