Appendix

Appendix A: Construction of promoter-RBS components

The promoter allows the RNA-polymerase molecules to latch upon a DNA strand and initializes the transcription of downstream gene to mRNA, and the RBS allows the ribosomes to bind itself to perform the translation of mRNA. In this study, we suppose the effects of promoter on transcription and RBS on translation is integrated as a lumped reaction because the half-life of mRNA is shorter than the half-life of corresponding protein (typically, the half-life of mRNA of *E. coli* is 6.8 min [A1]). This consideration is similar to the results of previous literature [A2] despite the fact that there are a variety of strengths of both promoters and RBSs that could be combined to allow synthetic biologists to achieve a variety of transcription and translation strengths. Therefore, the combination of promoter and RBS is considered as a promoter-RBS component to regulate the expression of downstream gene. Meanwhile, the produced protein will be degraded by the corresponding protease and diluted by the cell growth. The protein expression driven by promoter-RBS component can be modeled as follows

$$\dot{x}(t) = P(P_u, P_l, TF, I) - (k + \gamma_c)x(t)$$  \hspace{1cm} (A1)

where $x(t)$ denotes the concentration of protein, $P(P_u, P_l, TF, I)$ denotes the promoter-RBS regulation function of the maximum and minimum promoter-RBS strengths $P_u$ and $P_l$ from the corresponding components, $TF$ denotes transcription factor concentration and $I$ denotes inducer concentration. $k$ and $\gamma_c$ denote the dilution rate due to cell growth and degradation rate of corresponding protein, respectively.

There are three types of promoter-RBS components with three different promoter-RBS regulated functions i.e. constitutive, repressor and activator-regulated functions, respectively. The constitutive promoter-RBS component $C_i$ without the impact by any TF can express its downstream gene continually at the maximum expression of the
component. So the promoter-RBS regulation function of constitutive promoter-RBS components \( i^{th} \) can be represented by

\[
P_c(P_{u,i}, 0, 0, 0) = P_{u,i}
\]

with the maximum strength \( P_{u,i} \). Therefore, the protein expression of the constitutive promoter-RBS component set \( C_i \) in equation (A1) is modified as follows.

\[
\dot{x}(t) = P_{u,i} - (k + \gamma_s)x(t) , \quad i = 1,...,C
\]  

(A2)

where \( x(t) \) is the concentration of target protein, the constitutive promoter-RBS regulation function only contains the maximum strength \( P_{u,i} \), \( k \) is the dilution rate due to cell growth and \( \gamma_s \) is the degradation rate due to the cleavage of target protein \( x(t) \) by protease. \( i \) denotes the \( i^{th} \) constitutive promoter-RBS component of component set \( C_i \).

The repressor-regulated promoter-RBS component \( R_j \) can be bound with free repressor to regulate its protein expression. The repressor-regulated promoter-RBS component, \( R_j \), has the minimum and maximum strengths \( P_{r,j} \) and \( P_{u,j} \). The concentration of free repressor, \( x_r^*(x_r,I_r) \), is regulated through the binding reaction by external inducer \( I_r \) as follows:

\[
x_r^*(x_r,I_r) = \frac{x_r}{1 + \frac{I_r}{K_{I_r}}}
\]

(A3)

where \( x_r \) denotes the total repressor concentration including free and inducer-bound repressor, \( I_r \) denotes inducer concentration for regulating \( x_r^*(x_r,I_r) \) and \( K_{I_r} \) denotes the inducer-repressor dissociation rate. The promoter-RBS regulation function
of repressor-regulated promoter-RBS component $R_j$ can be represented as follows

$$P_r(P_{u,j}, P_{l,j}, x_r, I_r) = P_{i,j} + \frac{P_{u,j} - P_{l,j}}{1 + \left(\frac{x_r^*(x_r, I_r)}{K_r}\right)^{n_r}}$$

Therefore, the protein expression of repressor-regulation promoter-RBS component in (A1) is modified as the follows.

$$\dot{x}(t) = P_{i,j} + \frac{P_{u,j} - P_{l,j}}{1 + \left(\frac{x_r^*(x_r, I_r)}{K_r}\right)^{n_r}} - (k + \gamma_x) x(t), \quad j = 1, \ldots, R$$

(A4)

where $K_r$ and $n_r$ denote the repressor-promoter binding affinity and binding cooperativity between the regulatory protein and the repressor-regulated promoter-RBS component, respectively; $P_{u,j}$ and $P_{l,j}$ denote the maximum and minimum strengths of repressor-regulated promoter-RBS of the $j^{th}$ component set, respectively. $j$ denotes the $j^{th}$ repressor-regulated promoter-RBS component of component set $R_j$.

The activator-regulated promoter-RBS component $A_m$ have the maximum and minimum strengths $P_{u,m}$ and $P_{l,m}$, respectively. The concentration of inducer-bound activator, $x_a^*(x_a, I_a)$, is regulated through the binding reaction by external inducer $I_a$ as follows

$$x_a^*(x_a, I_a) = \frac{x_a \cdot I_a}{I_a + K_{I_a}}$$

(A5)

where $x_a$ denotes the total activator concentration including free and inducer-bound activator, $I_a$ denotes inducer concentration for regulating the activator activity $x_a^*(x_a, I_a)$, and $K_{I_a}$ denotes the inducer-activator dissociation rate. The regulatory
strength of activator-regulated promoter-RBS component $A_m$ can be represented by a promoter-RBS regulation function as follows [A3]

$$P_a(P_{a,m}, P_{l,m}, x_a, I_a) = P_{l,m} + \frac{(P_{a,m} - P_{l,m}) \cdot \left(x_a^*(x_a, I_a)\right)^{n_a}}{\left(x_a^*(x_a, I_a)\right)^{n_a} + K_a^{n_a}}$$

Therefore, the protein expression of activator-regulated promoter-RBS component $m^{th}$ in (A1) is modified as follows

$$\dot{x}(t) = P_{l,m} + \frac{(P_{a,m} - P_{l,m}) \cdot \left(x_a^*(x_a, I_a)\right)^{n_a}}{\left(x_a^*(x_a, I_a)\right)^{n_a} + K_a^{n_a}} - (k + \gamma_a)x(t), \quad m = 1, ..., A \quad (A6)$$

where $K_a$ and $n_a$ denote the inducer binding affinity and binding cooperativity between regulatory protein and activator-regulated promoter-RBS component, respectively; $P_{a,m}$ and $P_{l,m}$ denote the maximum and minimum activator-regulated promoter-RBS strengths of activator-regulated promoter-RBS component of the $m^{th}$ component set, respectively. $m$ denotes the $m^{th}$ activator-regulated promoter-RBS component of component set $A_m$.

We use the steady state GFP values to characterize promoter-RBS components by means of the nonlinear least square estimation method [A4]. The identification results are listed in Tables A1, A2 and A3, respectively.


Table A1 The constitutive promoter-RBS library.

<table>
<thead>
<tr>
<th>Index</th>
<th>BioBrick component</th>
<th>$P_u$</th>
</tr>
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<tbody>
<tr>
<td>$C_1$</td>
<td>J23101-B0031</td>
<td>94.520</td>
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<tr>
<td>$C_2$</td>
<td>J23101-B0032</td>
<td>65.892</td>
</tr>
<tr>
<td>$C_3$</td>
<td>J23101-B0034</td>
<td>237.000</td>
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<td>$C_4$</td>
<td>J23105-B0031</td>
<td>14.493</td>
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<td>$C_5$</td>
<td>J23105-B0032</td>
<td>6.512</td>
</tr>
<tr>
<td>$C_6$</td>
<td>J23105-B0034</td>
<td>28.586</td>
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<tr>
<td>$C_7$</td>
<td>J23106-B0031</td>
<td>22.011</td>
</tr>
<tr>
<td>$C_8$</td>
<td>J23106-B0032</td>
<td>12.193</td>
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<tr>
<td>$C_9$</td>
<td>J23106-B0034</td>
<td>58.781</td>
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Table A2 The repressor-regulated promoter-RBS library.

<table>
<thead>
<tr>
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<th>BioBrick component</th>
<th>$P_u$</th>
<th>$P_l$</th>
<th>Parameters</th>
</tr>
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<tbody>
<tr>
<td>$R_{T1}$</td>
<td>R0040-B0031</td>
<td>11.965</td>
<td>3.031</td>
<td>$K_{atc} = 6.794 \text{ ng/ml}$</td>
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<tr>
<td>$R_{T2}$</td>
<td>R0040-B0032</td>
<td>8.740</td>
<td>3.381</td>
<td>$K_{Tor} = 45.130 \text{ nM}$</td>
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<td>$R_{T3}$</td>
<td>R0040-B0034</td>
<td>162.557</td>
<td>4.837</td>
<td>$n_{Tor} = 2$</td>
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Table A3 The activator-regulated promoter-RBS library.

<table>
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<tr>
<th>Index</th>
<th>BioBrick component</th>
<th>$P_u$</th>
<th>$P_l$</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>A\textsubscript{L1}</td>
<td>R0062-B0032</td>
<td>360.761</td>
<td>7.970</td>
<td>$K_{AHL} = 4.541\text{nM}$</td>
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<td></td>
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<td>$K_{LuxR} = 55.964\text{nM}$</td>
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<tr>
<td>A\textsubscript{L2}</td>
<td>R0062-B0034</td>
<td>460.353</td>
<td>8.978</td>
<td>$n_{LuxR} = 1$</td>
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