We used this protocol to build a series of codon optimized BFP expressing vectors in the context of the EMS initiative from Gordon and Betty Moore foundation. A complete list of files corresponding to the DNA parts and vectors used in this study can be found at our Drive and zenodo database.
DNA) or existing vectors; or by fully synthesising (e.g. gblocks from IDT) DNA fragments of interest.

For PCRRed parts, reactions were performed with Phusion® High-Fidelity DNA Polymerase following these conditions:

**Thermocycling**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>98°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>(98°C x 10 sec + 60°C x 30 sec + 72°C x 2 min) x 35 cycles</td>
<td></td>
</tr>
<tr>
<td>72°C</td>
<td>10 min</td>
</tr>
<tr>
<td>4°C</td>
<td></td>
</tr>
</tbody>
</table>

**PCR mix:**

- 1ul primer forward Gibson
- 1ul primer reverse Gibson
- 1.33ul template (concentration: 1ng/ul)
- 8ul Buffer HF 5X
- 0.8ul dNTPs (10uM)
- 0.4ul Phusion® High-Fidelity DNA Polymerase (2U/ul)
- 27.46 ul H2O

Commercially synthesized DNA fragments were ordered containing the 20-30bp overhangs used for Gibson assembly into the level 0 part vector.

**Gibson assembly step for the creation of level 0 part vector**

Desired PCR products were identified by gel electrophoresis and extracted with the purification kit Wizard SV Gel & PCR Clean-Up System (Promega). DNA concentration were corroborated with a Take3™ Micro-Volume Synergy™ HTX before performing Gibson reactions. The volumes used for each part were calculated by eq 1, 2 and 3.

**Eq.1**

\[
X \ [\text{pMol/ul}] = \frac{\text{Concentration} [\text{ng/ul}] \times 1000}{650 \ [\text{gr/mol}] \times \text{Length} [\text{bp}]}
\]

**Eq.2**

\[
Vol_{ratio \ i} = \frac{X}{\text{pmol}_{req}}
\]

Volume ratio [ul] = pmol/required pmol
where required pmol is:
- 0.01 for parts >2500 bp
- 0.03 for parts >200 bp and <2500 bp
- 0.05 for parts <200 bp
Volume to be added was calculated as:

\[ V_{\text{ol piece i}} = \frac{V_{\text{ol total}} \cdot V_{\text{ol ratio i}}}{\sum_{i=1}^{n} V_{\text{ol ratio i}}} \]

1.5µl of parts were combined with 4.5µl of Gibson Master Mix in ice. This reaction was incubated at 50°C for 1 hour.

Reagents:
Gibson Mix (1.33X):
- 100 µl 5X Isothermal Buffer
- 2 µl T5 Exonuclease (1 U/µl)
- 6.25 µl Phusion® High-Fidelity DNA Polymerase (2U/µl)
- 50 µl Taq DNA ligase (40U/µl)
- 216.75 µl H2O
Store at -20°C.

5X Isothermal Buffer:
- 25% PEG-8000
- 500 mM Tris-HCl pH 7.5
- 50 mM MgCl2
- 50 mM DTT
- 1 mM dATP
- 1 mM dTTP
- 1 mM dCTP
- 1 mM dGTP
- 5 mM NAD
- H2O
Store at -80°C

Transformation of Level 0 donor vectors

These reactions were transformed into chemically competent TOP10 cells prepared by the OOW protocol (http://www.openwetware.org/wiki/TOP10_chemically_competent_cells), minipreped and sequenced. These level 0 parts were stored as vectors for the following Golden Gate reactions.

Golden Gate assembly of promoters and fluorescent proteins combinations

Final combinatorial assemblies of promoters, CDSes and terminators here described were produced by Golden Gate. For this, a general acceptor vector was created that accepts level 0 parts for the assembly of transcriptional units (e.g. promoter-CDS-terminator of interest).
The assembly was performed as follows:
(37°C x 3 min + 16°C x 5 min) * 40 cycles.
50°C x 5 min.
80°C x 10 min
4°C

3µl of these reactions were used to transform TOP10 Escherichia coli cells following the step described above.
Positive colonies were identified by colony PCR and sequenced.