Table 1: Comparison to other DNA assembly platforms

<table>
<thead>
<tr>
<th>Efficiency of</th>
<th>Single T.U. Testing</th>
<th>Scarless</th>
<th>Flexible Delivery</th>
<th>Existing Parts Repositories</th>
<th>Network Variants</th>
<th>No PCR Mutation Risk</th>
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<tbody>
<tr>
<td>Gibson</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<td>Golden-Gate</td>
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<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
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<td>ATCC Toolkit</td>
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<td>Yes</td>
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</table>

Figure 2. Generation of a single T.U comprising four different fluorescent protein genes via Gateway® reaction. A common URA selection marker was combined with GFP to form a network with constitutive AC120 and YFP to induce expression. Individual TUs were assembled from borosilicate tubes containing yeast colonies in the order.

Figure 3. Single-stage LR product verification. Four plasmids were simultaneously assembled in vivo using 4-way LR recombination functionality embedded in the fluorescent protein chassis. Plasmids were transferred into yeast with W303-A background prior to assembly. All promoters are normalized using calibrated beads and geometric means. Error bars correspond to n=3 biological replicates.

Figure 4. Two-stage assembly LR and Gibson Assembly® product characterization. Yellow and lacZ transcriptional units were formed into 3 networks differing in backbone: a low-copy, high-copy, or HO-locus integrating plasmid. The assays were performed after 14 hours under G418 selection to ensure the measurements is at the steady-state.

Figure 5. Context-dependent characterization of constitutive promoters. A) Schematic outline of four TUs that were constructed to ensure the measurement is at the steady-state. B) Expression profiles of the four TUs under regulation of P101-VP64/GFP and P101-Renilla/Renilla and with varying metabolic loads (increased repression needs).

Figure 6. DOX-inducible circuit and characterization. A) Schematic diagram of DOX-inducible circuit. The TUs were assembled with constitutive expression of YFP, inducible expression of YFP and KanMX selection. B) DOX response of DOX on the dox-dependent YFP expression. All promoters are normalized using calibrated beads and geometric means. Error bars correspond to n=3 biological replicates.

Figure 7. Characterization regulatory promoters. A-B) Similar to construction of the PhlF inducible systems, we created a set of promoters with 8 different context-dependent promoters with varying regulation needs. C-D) The response of DAPG on YFP expression under regulation of PphlF-CYC1min/PhlF-VP64 and PphlF-mKate, respectively. All promoters are normalized using calibrated beads and geometric means. Error bars correspond to n=3 biological replicates.

Figure 8. Transcriptional regulator characterization. A) Schematic of three TUs that were assembled with 8 different context-dependent promoters with varying regulation needs. B) The response of DAPG on YFP expression under regulation of PphlF-CYC1min/PhlF-VP64 and PphlF-mKate, respectively. All promoters are normalized using calibrated beads and geometric means. Error bars correspond to n=3 biological replicates.

Characterization of DOX-inducible promoter systems

Conclusions

- We created a toolkit that encompasses 47 plasmids and is readily expandable to be used in creating genetic networks with up to 8 individual transcriptional units in less than 5 days with only a workload of 1-3 hours per day.
- The toolkit includes a promoter collection that spans 2-logs of constitutive protein expression and novel inducible systems that span 11-fold expression change.
- The toolkit includes set of transcriptional regulators capable of ~40-fold change differences in regulation.