# A Comprehensive Yeast Tool Kit with Novel Synthetic Regulation Systems for Gene Circuit Construction and Delivery

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## Background and Summary

Complex sophisticated behavior within cells manifests from multiple regulatory networks, in which transcriptional factors (TFs) regulate gene expression, while binding to their cognate operator sequences. Here, we present a framework for building gene circuits and present a set of well-characterized DNA parts for use in *Saccharomyces cerevisiae.* For the assembly of novel gene circuits, we used Gateway® recombination and Gibson Assembly® methods; hierarchical assembly of gene circuits comprising multiple transcriptional units was mediated through unique 45 bp sequences. The entire procedure of building a gene circuit from more than 10 basic parts took less than 5 days with only a workload of 1-3 hours per day. Characterization of various promoters and the rules of network design were evaluated by analyzing fluorescent protein expression (GFP and YFP) via flow cytometry. We also characterized a diverse set of promoters consisting of constitutive promoters, native inducible promoters, synthetic inducible promoters, synthetic promoters regulated by activators, and synthetic promoters regulated by repressors. Promoter expression was measured in molecules of equivalent fluorescein (MEFL) units with the following results: (1) constitutive promoters demonstrated a wide range of strengths (up to a 100-fold difference), (2) the new inducible systems enabled an 11-fold change in expression, and (3) the activators/repressors showed a maximum 35-fold and 45-fold change of expression, respectively. In conclusion, this study demonstrates the feasibility of quickly and easily constructing gene circuits for delivery into *S. cerevisiae*; the utility of a fully characterized set of diverse promoters, activators, and repressors; and the applicability of this system in constructing large-scale gene circuit libraries with reliable gene expression and designing logic operations for a complex network in *S. cerevisiae*.

## Toolkit Development

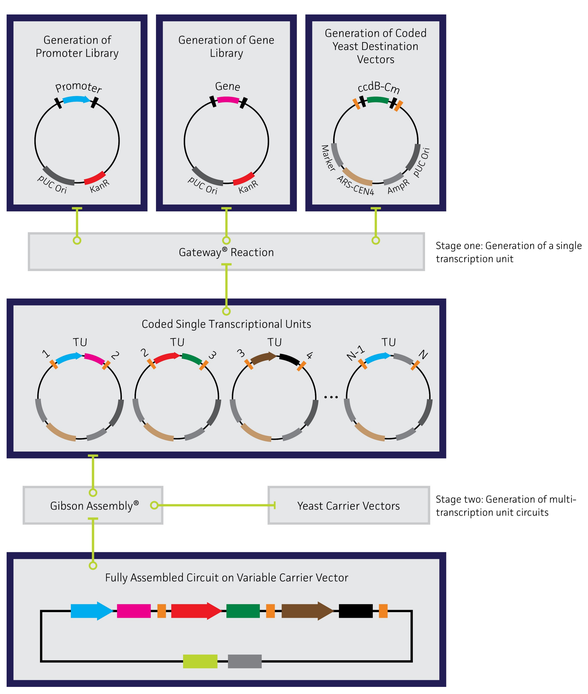


**Figure 1. Two-stage assembly system for eukaryotic circuit construction and delivery.** We created a two-step platform utilizing Gateway recombination-based cloning and Gibson Assembly to permit circuit construction. To build a network, the following steps can be used: (1) design the network (*i.e.*, required genes, expression levels, and regulation); (2) acquire the appropriate promoters/genes from the ATCC Yeast Toolkit, plasmid repositories, or through cloning; (3) assemble single transcriptional units (TUs) via Gateway recombination; and (4) form complete multi-TU networks by combining individual TUs with a carrier vector (high-copy, low-copy, or site-specific integration) and adapter fragment via Gibson Assembly.

## Characterization of Constitutive Promoters

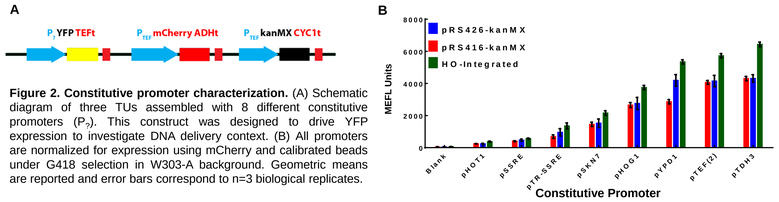


**Figure 2. Constitutive promoter characterization.** (A) Schematic diagram of three TUs assembled with 8 different constitutive promoters ($P_2$). This construct was designed to drive YFP expression to investigate DNA delivery context. (B) All promoters are normalized for expression using mCherry and calibrated beads under G418 selection in W303-A background. Geometric means are reported and error bars correspond to n=3 biological replicates.

## Characterization of Two Novel Small Molecule Induction Systems

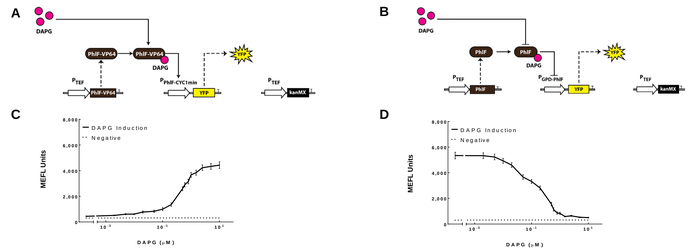


**Figure 3. DAPG-inducible circuit characterization.** Two small-molecule inducible systems were created. These systems involve the PhlF repressor (a TetR homlog), which binds to the small molecule 2,4-diacetylphloroglucinol (DAPG). (A) By fusing PhlF operator sites upstream of the CYC1min promoter in tandem with a PhlF-VP64 fusion protein, or (B) fusing PhlF operator sites downstream of the constitutively active GPD promoter in tandem with PhlF expression, we were able to make two inducible systems. (C & D) The dose response of DAPG on YFP expression under regulation of $P_{PhlF\text{-}CYC1min}$/PhlF-VP64 and $P_{GPD\text{-}phlF}$/PhlF, respectively, was evaluated.

## Characterization of Transcriptional Regulators

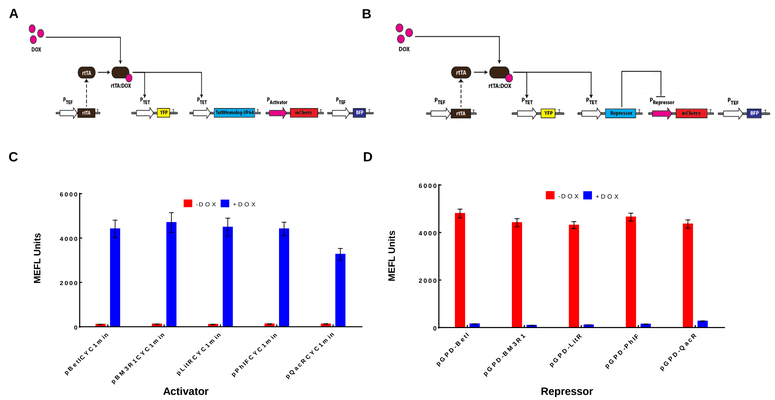


**Figure 4. Transcriptional regulator characterization.** (A & B) Similar to the construction of the PhlF inducible systems, a set of promoters were created with TetR homolog binding sites in conjunction with expression of their TetR-VP64 or TetR homologs, respectively. (C & D) In the presence of DOX, rtTA binds to the pTET promoter, creating expression of both YFP and the regulator of interest. The regulator can then bind its cognate promoter, modulating mCherry expression. Expression was measured and normalized to MEFLs.

## ATCC® Synthetic Biology Yeast Toolkit

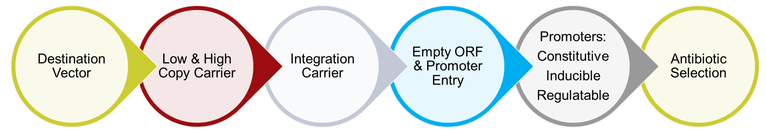


**Table 1.** ATCC Synthetic Biology Solutions

| Product Name | Product Description* |
|---|---|
| ATCC Basic Yeast Synthetic Biology Tool Kit (ATCC® SB-2000™) | The basic tool kit comprises 4 destination vectors (SB-1000™ , SB-1001™ , SB-1002™ , and SB-1003™), 3 carrier vectors (SB-1004™, SB-1005™, and SB-1024™), 2 empty entry vectors of the Gateway system (SB-1013™ and SB-1014™), a Kanamycin/G418-resistant gene (SB-1006™), 4 yeast constitutive promoters (SB-1008™, SB-1035™, SB-1041™, and SB-1105™), and an inducible promoter (SB-1046™). |
| Regulator and promoter pairs | Pairs include a synthetic promoter and its cognate regulator based on the tetracycline (Tet) inducible system and a TetR homolog (BetI, BM3R1, LitR, PhlF, and QacR). |
| Individual components | Individual components from the ATCC Basic Yeast Synthetic Biology Tool Kit, including destination vectors, carrier vectors, entry vectors, a Kanamycin/G418-resistant gene, promoters, and regulators. |

*For more information on the tool kit and individual parts, visit ATCC online at www.atcc.org/SyntheticBiology

## Comparison to Other DNA Assembly Platforms

| | Gateway | Gibson Assembly | Golden-Gate | Synthesis | ATCC Tool kit |
|---|---|---|---|---|---|
| **Efficiency of *in vitro* steps** | Yes | Maybe | Size limits | Maybe | Yes |
| **No PCR mutation risk** | Yes | Maybe | Maybe | Yes | Yes |
| **Network variants** | N/A (1-T.U.) | Yes | Yes | $ Limited | At ‘parts’ level |
| **Existing parts repositories** | Yes | No | Limited | N/A | Yes |
| **Flexible delivery** | N/A (1-T.U.) | Yes | Yes | Yes | Yes |
| **Large (>6 TUs)** | No | Yes | Hierarchical | Yes | Yes |
| **Scarless** | No | Yes | Yes | Yes | No |
| **Single TU testing** | Yes | Maybe | Yes | Maybe | Yes |

## Conclusions

- ATCC created a tool kit that encompasses 42 plasmids and is readily expandable for use in creating genetic networks with up to 8 individual transcriptional units. This process takes less than 5 days with a workload of only 1-3 hours per day.
- Tool kit includes a promoter collection that spans 2-log decades of constitutive protein expression and novel inducible systems that span 11-fold expression change.
- Toolkit includes set of transcriptional regulators capable of ~40-fold change differences in regulation.