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Rapid Synthesis of Defined Eukaryotic Promoter Libraries

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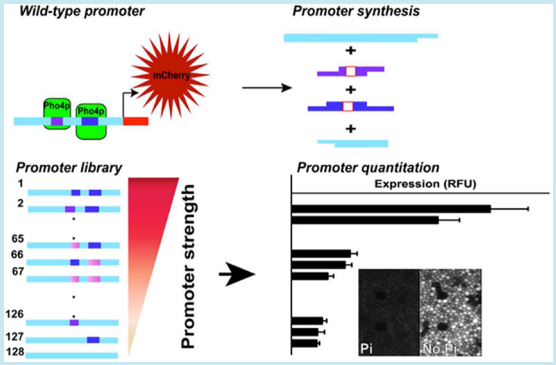
Supporting Information

ABSTRACT: Current gene synthesis methods allow the generation of long segments of dsDNA. We show that these techniques can be used to create synthetic regulatory elements and describe a method for the creation of completely defined, synthetic variants of the *PHO5* promoter from the budding yeast *Saccharomyces cerevisae*. Overall, 128 promoters were assembled by high-temperature ligation, cloned into plasmids by isothermal assembly, maintained in *E. coli*, and consequently transformed into yeast by homologous recombination. Synthesis errors occurred at frequencies comparable to or lower than those achieved with current gene synthesis methods. The promoter synthesis method reported here is robust, fast, and readily accessible. Synthetically engineered promoter libraries will be useful tools for dissecting the intricacies of promoter input-output functions and may serve as tunable components for synthetic genetic networks.



KEYWORDS: *synthetic biology, promoter synthesis, promoter architecture, gene regulation, PHO5, synthetic promoters*

Gene synthesis, or more broadly the synthesis of long dsDNA from smaller ssDNA components, has recently become an important tool in synthetic biology, genetic, and genome engineering.[1] With respect to efficiency and fidelity, these methods work best for the assembly of segments or subassemblies of DNA roughly 1 kb or smaller in size.[2] When parallelized and coupled with other assembly techniques, it is possible to build entire genomes[3] or carry out high-throughput gene synthesis.[4−6] Such feats have been made possible by the maturation of gene synthesis protocols, which use ligation or polymerase-based methods for assembly.[2,7] By careful design of components and the use of high-fidelity or mismatch-cleaving enzymes, error rates of ∼0.1% (or 1 error per kb) have been achieved.[2]

Libraries of native promoters can yield useful information on the rules governing gene regulation, an approach that has recently been used for yeast ribosomal protein genes.[8] Random assembly or modification of promoter components has also been used to study promoter architecture.[9−12] However, the scope of such studies is limited by the inherent randomness of assembled promoters, allowing only broad inferences on promoter architecture and gene regulation to be derived.[13,14] Such an approach has nonetheless evaluated the contribution of each nucleotide in a specific promoter to its function,[9−11] revealed the role of low-affinity TF-DNA interactions in gene regulation[15] and the modularity of core promoter elements,[16] and demonstrated the ability of TFs to switch function depending on environmental conditions.[17] However, synthetic promoters constructed this way can introduce levels of complexity that make it difficult to interpret the resulting data.

Defined synthetic promoter libraries allow the study of gene regulation in a systematic manner. By engineering promoters it is possible to systematically investigate how individual regulatory elements contribute to the behavior of the promoter as a whole. A defined synthetic promoter can be modular, allowing the insertion, duplication, removal, or displacement of regulatory elements with no loss of combinatorial flexibility. More specifically, modifying the context of a given regulatory element, its initial accessibility to binding proteins, or its distance from the transcription start site can provide insight into rules governing promoter architecture. While large libraries of modified short promoters can be directly created by oligonucleotide synthesis or PCR,[18,19] similar-sized libraries of entire eukaryotic promoters require different protocols that can be efficiently integrated into large-scale workflows.

We have developed a method requiring neither specialized equipment nor reagents for generating large libraries of defined eukaryotic promoters and generated a total of 128 promoter variants modifying both Pho4 binding sites in the *PHO5* promoter. The entire workflow requires 13−16 days for one batch of promoters to be chromosomally integrated and fully sequence verified. When induced, promoters from the library were found to be fully functional. The cost per promoter variant compares favorably to commercial site-directed mutagenesis kits, and the cost of individual promoters decreases with increased library sizes.

RESULTS AND DISCUSSION

Construction of Synthetic Promoters. We tiled the native *PHO5* promoter into overlapping oligo pairs to create a

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modular synthetic promoter allowing individual regulatory components to be independently assembled (Figure 1, Supplementary Table S1). We chose 90 bp long oligos to minimize synthesis costs. Given that the promoter has high AT content, it was also necessary to have sufficiently long overlaps allowing them to anneal at high temperatures. While constructing the exposed-site variant library, we found it necessary to change the length of the left arm to accommodate site variants and minimize oligo synthesis expenses. In all cases, the overlaps between oligo pairs had melting points of at least 58 °C (Supplementary Table S2), which set a lower limit to the ligation temperature.

We found high-temperature, single-step ligation to be a simple yet robust promoter assembly method. While a 4-h ligation was sufficient to yield assembled promoter upon amplification, ligation was normally allowed to run overnight to maximize yield. Our choice of ligase was based on the fact that 9°N ligase preferentially ligates long overlaps (12 bases; New England Biolabs, personal communication). With the reaction conditions described here we were consistently able to amplify 1.5−2 pmol of full-length promoter. As our synthetic promoters are derived from a native promoter, constraints on the assembly scheme imposed by the promoter sequence made us forego normalization of the melting temperature of overlaps between segments, which is commonly used to improve assembly efficiency. However, promoter synthesis was efficient and robust without this precaution.

We maintained each library in *E. coli* to ensure a stable copy of each construct from which DNA for transformation into yeast could be readily generated. Gibson assembly consistently yielded sufficient quantities of plasmid for transformation.[20] Changing neither the promoter-to-plasmid backbone ratio nor assembly time significantly impacted the yield or quality of assembled plasmid. In contrast to ligation, Gibson assembly carries little to no risk of the plasmid backbone religating and contaminating the transformants, in addition to allowing a scarless fusion of promoter and reporter sequences. Finally, the exonuclease used in a Gibson assembly digests any secondary ligation products, thus eliminating the need for gel purification of full-length promoter assemblies prior to cloning.

Fidelity of Promoter Assembly. It was our intention to create a library of *PHO5* promoters containing all possible variants of the nucleosomal and exposed Pho4 E-box half-sites. We constructed 128 independently varied half-sites and maintained them in *E. coli* and budding yeast. At each step, we verified the library sequences to determine the presence and frequency of assembly errors. We considered a promoter to be functional if it contained no more than 1% errors (indels, mutations, and ambiguous sequencing base-calls) in regions outside known regulatory elements. The quality and coverage of our promoter libraries are listed in Table 1. The absence of errors in identical locations suggested that no errors arose from oligonucleotide synthesis.

***E. coli* Library Quality.** We successfully cloned all variants of each Pho4 site into *E.coli*, with each promoter having at least 2-fold coverage. All promoters had ∼1.6 errors per kilobase and 84% of the promoters (108 out of 128) were perfect (Table 2, Figure 2a and c). The remaining promoters had mostly single-base deletions. However, we were able to retrieve clones we considered to be functional for each imperfect promoter variant. The majority of promoter synthesis errors were deletions, with single insertions, mutations, and ambiguous base-calls from sequencing making up the remainder of errors.

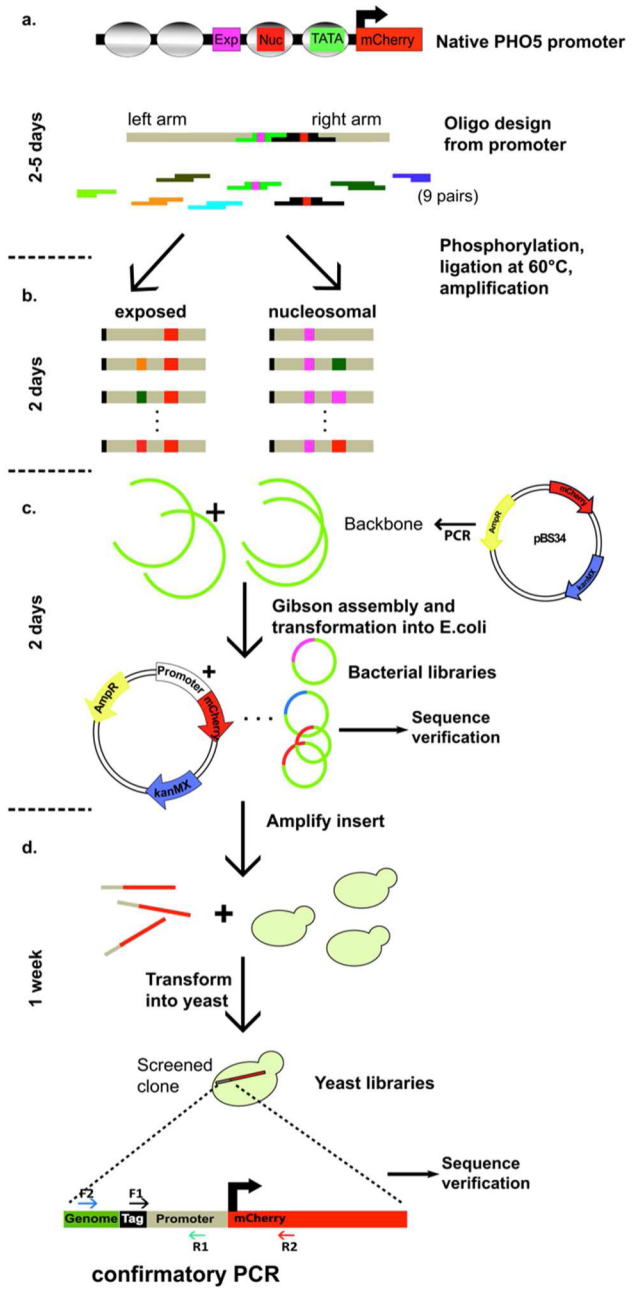

Figure 1. Promoter synthesis and library generation. (a) The *PHO5* promoter sequence is tiled into 9 component oligo pairs, keeping the Pho4 sites ("Exp" and "Nuc") on separate pairs. Each promoter variant is assembled from phosphorylated oligo pairs as described in Methods. (b) Each promoter library is ligated at 60 °C and then amplified for cloning. (c) Each promoter variant is cloned into a plasmid containing mCherry and a yeast selection marker using Gibson assembly. Successful transformants are confirmed by colony PCR, and their promoter sequences are subsequently confirmed. (d) Promoters with a correct sequence, along with the reporter and marker, are amplified from the plasmid and transformed into yeast. G418-resistant clones exhibiting lysine auxotrophy are checked for correct integration of the construct by nested colony PCR. The products of primers F1 and R1 confirm the presence of the synthetic promoter, whereas the products of F2 and R1 confirm its integration. Clones giving positive products for both primer pairs have their entire promoters amplified using primers F2 and R2 for sequence verification. The entire assembly process from oligo pairs to yeast strain takes 10−14 days for a batch of promoters.

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Table 1. Promoter Library

PHO5 promoter site modified	host	promoters with the correct site	clones with the correct site	perfect promoters	clones with perfect promoter
nucleosomal	*E.coli*	64	152	54	86
	yeast	62	84	37	44
exposed	*E.coli*	64	155	54	91
	yeast	62	93	45	67

All errors were distributed randomly across the promoters (Supplementary Figure S2a and c).

Yeast Library Quality. A total of 124 out of 128 synthetic promoters, amounting to 62 variants of each Pho4 site, were transformed into yeast by homologous recombination. While the average error rate was lower than that of the *E. coli* library, the errors were spread out over more promoters. Nonetheless, over 50% and 70% of nucleosomal- and exposed-site promoters, respectively, were error-free (Figure 2b and d). Errors in nucleosomal-site promoters were mostly ambiguous base-calls, whereas those in exposed-site promoters were single-base deletions. Unlike the bacterial library, the majority of sequence errors in the yeast library were ambiguous base-calls from sequencing reads, followed by single-base deletions. Most of these ambiguous base-calls were located either in or near repetitive stretches of the promoter or near the beginning of the sequencing read, while other errors were randomly distributed over the promoter (Supplementary Figures S2b and S2d).

***In Vivo* Functionality of Synthetic Promoters.** To test the functionality of our promoter library, we measured the induction of 12 synthetic promoters whose modified nucleosomal and exposed sites cover the entire range of Pho4 affinities (Figure 3a and b).[21,22] It has previously been shown that the induction kinetics of Pho4-regulated promoters during Pi starvation are well described by a time-dependent Hill function.[23] This was also the case for our synthetic promoters (Supplementary Table S3). As expected, F_{max}, the Hill fit parameter related to the final induction level, was dependent on the affinity of the modified nucleosomal and exposed Pho4 sites (Supplementary Figure S3a and b). On the other hand the time to half-maximal induction, $t_{1/2}$ (used as a measure of the time to induction), depended only upon the affinity of the exposed Pho4 site (Supplementary Figure S3d).

A promoter is expected to be functional even if it contains a few errors, so long as these errors lie outside of regulatory elements. Induction experiments on perfect and error-containing clones of the same promoter found no difference in induction kinetics (Figure 3c). Indeed, deletions as large as 5 bases in a non-regulatory region have no effect on a promoter's output (Figure 3d). On the other hand, we found that errors in or near known or predicted regulatory elements do affect promoter induction characteristics and can do so with unexpected results. The decrease in induction for promoter D3E (Figure 3e) can be attributed to deletions in a known binding region for the co-regulator Pho2[24] and a predicted Swi5 binding site.[25] The effect of defects in G4N is harder to explain as the insertion near the TATA box is expected to neither improve nor weaken it with regard to the consensus sequence.[26] This mutation nonetheless increased induction by nearly 2-fold (Figure 3f).

De novo synthesis of gene-sized dsDNA is a powerful tool in synthetic biology and genomics research. The method described here allows the rapid creation of defined synthetic promoters, which in turn permit the systematic exploration of the structure–function relationship of eukaryotic promoters. We have taken an existing, well-studied promoter from yeast and developed a workflow to create libraries of defined variants, using a straightforward but effective promoter synthesis protocol. To ensure that promoter assembly and amplification was carried out at the highest fidelity possible, we used a thermostable ligase that would not ligate short mis-annealed overlaps and a polymerase with low error rate. When compared to existing gene synthesis methods, our method yielded error rates comparable to the best of these methods[4,27−33] (Supplementary Table S4). While single-step ligation is one of the oldest assembly methods,[34] our work shows that it can assemble DNA with a fidelity matching state-of-the-art techniques. As most errors encountered seem to appear during transformation into *E. coli*, it is unclear whether post-assembly error correction would yield better results.

To identify the origin of sequence errors found after cloning, we asked whether single-base errors occurred at breakpoints between component oligos. We measured the distance of deletions in the bacterial library from the nearest breakpoints and found that they were no closer to breakpoints than randomly chosen locations on the promoter (Supplementary Figure S4). It is possible, however, that errors cluster near short component oligos with lower annealing temperatures, as may be the case for the oligo pair containing the exchangeable exposed site. The preponderance of deletions in gene synthesis errors could be attributed to the use of a proofreading polymerase, as has previously been reported.[33]

A time estimate for creating a synthetic promoter library consisting of 50 members is provided in Supplementary Table S5. Cloning into *E. coli* did not significantly increase the time taken to construct the library, as the rate-limiting step is the integration of the library into yeast by homologous recombination. If used in conjunction with robotic handling, the time to create a library could be considerably reduced.

The size of our 800 bp synthetic promoters is near the limit at which a DNA fragment can be synthesized in a single step with an acceptable error rate. Single-step syntheses beyond 1 kb are inefficient due to either imbalances in component oligo

Table 2. Promoter Library Sequence Errors

modified Pho4 site	host	bases sequenced	deletions per kb	insertions per kb	mutations per kb	ambiguous base calls per kb	total errors per kb
nucleosomal	*E. coli*	123866	1.17	0.09	0.073	0.27	1.6
	yeast	67192	0.104	0.045	0.104	0.67	0.91
exposed	*E. coli*	121399	1.64	0.016	0.066	0.01	1.67
	yeast	75170	0.2	0.027	0.013	0.17	0.41
both	***E. coli***	**245265**					**1.64**
	yeast	**142362**					**0.65**

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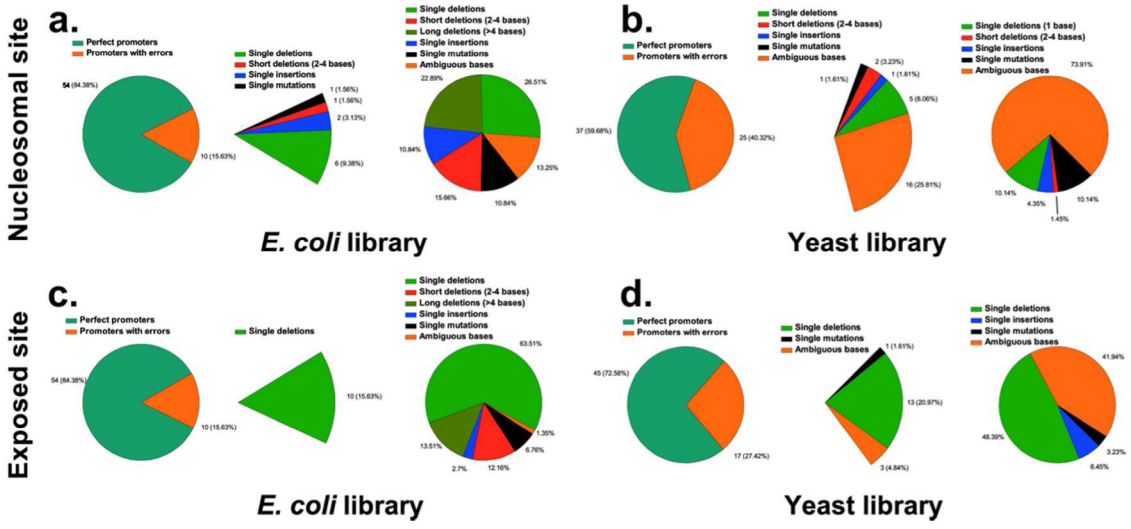

Figure 2. Synthetic promoter library quality. The promoter library charts are sorted vertically according by modified site (a,c) and horizontally by host (b,d). The first pie chart in each panel shows the number of perfect promoters per variant, the exploded slice shows the errors of the imperfect promoters, and the third chart shows the error distribution for all clones with sequence information. Further details can be found in Tables 1 and 2.

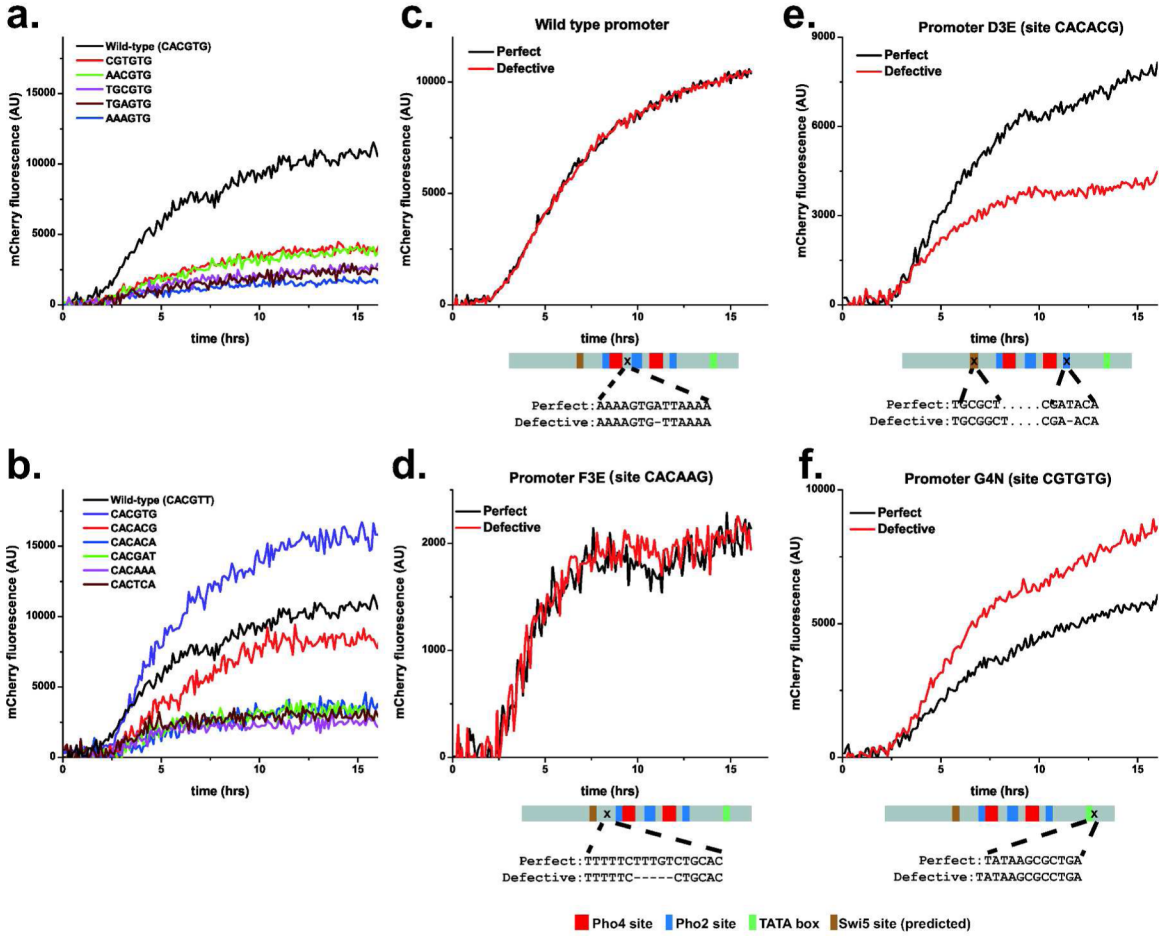

Figure 3. Characterization of synthetic promoter functionality and the effect of sequence errors on promoter activity. Induction curves for six variants of the nucleosomal (a) and exposed (b) Pho4 sites (including the wild-type *PHO5* promoter) under phosphate starvation. (c,d) The presence of deletions or insertions in non-regulatory regions in a promoter has no effect on induction behavior. (e,f) Errors in or near known regulatory regions do affect promoter activity. (e) Deletions in a Pho2 binding site decrease induction levels significantly, and an insertion near the TATA box (f) increases induction. Promoter maps below each graph indicate regulatory sites of interest and the presence of errors in defective promoters marked by "x".

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concentrations[3] or increased sequence errors.[29] However, using Gibson or PCR-based assembly, larger constructs can be assembled from subassembled components,[20] with essentially no upper size limit.[35] Defined synthetic promoters of several kilobases in size could thus be generated and may in turn be useful for deciphering promoter and enhancer function in higher eukaryotes. Finally, libraries consisting of hundreds to thousands of defined synthetic promoters can be readily generated using the approach described here. The main limitations are genomic integration and validation of the integrated promoter constructs. As discussed above, automated robotic platforms may be used to increase throughput on this level, and the use of plasmids instead of genomic integration would also drastically simplify the approach. Although genomic integration of promoter constructs is highly preferable if the promoters are to be used to understand promoter input-output fine-structures, plasmids may suffice in certain instances.[12]

While best suited for studying derivatives of a native promoter, synthetic promoter library construction offers a number of advantages over site-directed mutagenesis. The latter typically offers the ability to modify a few bases, and only a few commercially available kits allow the simultaneous modification of several bases. Creating a synthetic promoter can introduce as many sequence modifications as needed with high efficiency. Supplementary Table S6 provides the cost of creating a single promoter from our library and maintaining it in *E. coli* and compares it against the price for a single mutagenesis reaction from a set of commercially available site-directed mutagenesis kits. The comparison shows that the cost of one such reaction (36.03 CHF) compares favorably with the price range of commercial mutagenesis kits (32−50 CHF not counting primer and sequencing costs).

Recent high-throughput studies of eukaryotic promoters based on random assembly of constructs or short defined promoters and *a posteriori* determination of expression levels and sequencing provide valuable insights into the general design principles of promoters.[9,10,12] To decipher the precise contribution of transcription factor binding sites to promoter output, it is necessary to retain a wild type promoter background, which is possible in our synthetic approach. Furthermore, it is possible to generate synthetically defined promoter libraries for fine-tuned and well-characterized promoter constructs, which will be valuable for the optimization of synthetic metabolic networks. Overall, "promoter-bashing" approaches[9−12] and the generation of defined full-length synthetic promoters are orthogonal and contribute to our understanding of how promoters integrate transcription factor binding events to regulate expression levels.

In summary, we developed a robust method for generating synthetic promoter libraries and demonstrated their utility as tools for studying gene regulation. The ability to quickly and robustly generate hundreds of eukaryotic promoter variants, integrate them into the genome, and measure their output using a reporter gene will be useful in deciphering the rules governing gene regulation. Finally, defined and well-characterized promoter libraries will be valuable components for building synthetic genetic networks.

METHODS

Synthetic Promoter Design. Our promoter library consists of defined synthetic derivatives of the yeast *PHO5* promoter. The *PHO5* promoter regulates the synthesis of an acid phosphatase during inorganic phosphate (Pi) starvation and is one of the best-studied eukaryotic promoters. This promoter is regulated by the transcription factor Pho4, which is thought to bind to two sites (Figure 1a and Supplementary Figure S1): an initially exposed, low-affinity site and a high-affinity site covered by nucleosomes.[36,37] The presence of exposed and nucleosomal Pho4 sites allows independent control of *PHO5* induction thresholds and expression levels.[23]

Each synthetic promoter was designed to consist of interchangeable pieces containing the nucleosomal and exposed Pho4 sites flanked by two constant arms (Figure 1a). Pho4 binding sites are 10-mers centered on a symmetric, hexameric E-box.[21] The library described in this paper consisted of variants of either site's E-box (Figure 1b).

The promoter was taken to be the 800 bp sequence upstream of the *PHO5* open reading frame, (chrII:430946-431745, minus strand) and the sequence was taken from the June 2008 build of the *S. cerevisae* genome available at the UCSC Genome Browser (http://genome.ucsc.edu/). We tiled the native promoter into 90 bp oligonucleotide pairs (Supplementary Table S1) with 5′ and 3′ overhangs 30 bp long (Figure 1a). We normalized the overlap between oligo pairs with regards to length rather than melting temperature due to constraints imposed by the promoter. Melting temperatures of the overlaps were calculated using the Oligo Analysis Tools found on the Operon Web site (http://www.operon.com/technical/toolkit.aspx), and each oligo was checked for secondary structure at the ligation temperature using the mFold server.[38] The oligos making up the constant arms were ordered from IDT (Leuven, Belgium), and the Pho4 site variant oligos were ordered from Invitrogen (Carlsbad, USA).

Promoter Assembly and Amplification. Aliquots (300 pmol) of each oligo pair were phosphorylated overnight at 37 °C using 10U T4 polynucleotide kinase (NEB) in the provided buffer (70 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT at pH 7.6) supplemented with 1 mM ATP, followed by heat inactivation for 20 min at 65 °C. The phosphorylated oligos were directly used for promoter assembly without further purification. For assembly, 3 pmol of each oligo pair forming the flanking arms was mixed with an equimolar amount of oligo pairs containing the Pho4 site variant, and a one-pot ligation was carried out in a volume of 15 μL using 9°N ligase (NEB), with a final oligo concentration of 200 nM in 1x ligase buffer (10 mM Tris-HCl, 0.6 mM ATP, 2.5 mM $MgCl_2$, 2.5 mM DTT, 0.1% Triton X-100 at pH 7.5). The mixture was heated to 95 °C for 6 min, cooled to 60 °C at a rate of 0.1 °C/min and incubated overnight (typically 14 h) at 60 °C. The 9°N ligase was chosen for its ability to preferentially ligate long overlaps and thus avoid mis-ligation. The ligation temperature was chosen to be close to but lower than the average melting point of the overlaps between oligo pairs (63 °C, Supplementary Table S2). A 4 fmol portion of the assembled promoter was used as a template for amplification. A typical PCR reaction was carried out with Phusion 2x Master Mix with HF buffer (Finnzymes) and 200 nM concentration of each primer in a volume of 40 μL. The forward primer incorporates a 30 bp synthetic sequence upstream of the promoter to label the promoter as "synthetic" and to track its integration in yeast. The promoter was amplified using 30 cycles of 10 s at 98 °C, 20 s at 65 °C, and 15 s at 72 °C, followed by a final extension at 72 °C for 6 min (Figure 4a).

Plasmid Assembly and Cloning. Each promoter was cloned into plasmid pBS34 (obtained from the Yeast Resource Center, University of Washington) directly upstream of the

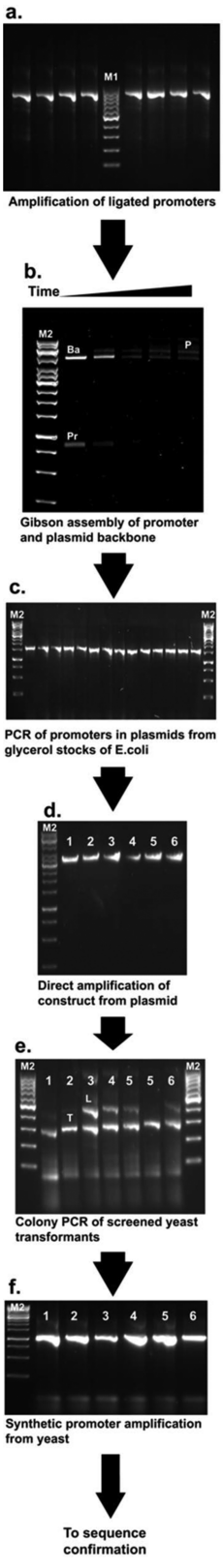

Figure 4. Representative synthetic promoter products at each step of library assembly. (a) Amplification of eight synthetic promoters with modified nucleosomal sites. The added synthetic tag and overlap sequences for Gibson assembly increase the product's length from 800 bp to over 900 bp. (b) Time course of a typical Gibson assembly of promoter (Pr) and backbone (Ba) to product (P). (c) Colony PCR of promoters transformed into *E. coli*. The PCR product corresponds to the promoter-mCherry construct. (d) Direct amplification of promoter-mCherry-kanMX cassettes from glycerol stocks. (e) Colony PCR of G418-resistant, Lys^- colonies transformed into yeast. A successful nested PCR yields two products positive for the correct synthetic promoter (T) and integration (L). (f) Direct amplification of the synthetic promoter for sequence verification from yeast. PCR-positive colonies from panel (e) were selected for this final amplification. Markers used in the gels were either the Fermentas GeneRuler 100 bp (M1) or 1 kb (M2) ladder.

mCherry gene (Figure 1c) using one-step, isothermal Gibson assembly.[20] We chose mCherry as our reporter because of its short maturation time[39] and the low autofluorescence of yeast in its emission range. For the purpose of isothermal assembly, each promoter has the first 25 bases of the mCherry gene added to its 3′ end during amplification, and the linearized plasmid backbone in turn bears the promoter's 30 bp synthetic tag. In brief, 5 μL of backbone and promoter were added to 15 μL of Gibson reaction mixture containing 0.75 U/mL T5 exonuclease, 25 U/mL Phusion polymerase, and 4 U/mL Taq DNA ligase in 100 mM Tris-HCl, 5 mM $MgCl_2$, 10 mM DTT, 1 mM NAD, 5% PEG, and 200 μM concentration of each dNTP. The reactions were carried out for 1 h using a promoter-to-backbone ratio of 2:1 in a volume of 15 μL at 50 °C (Figure 4b). A 2 μL portion of the assembly mix was directly transformed into DH5α *E. coli* cells made competent by the $CaCl_2$ method.

Three clones from each transformation were screened for the promoter-containing plasmid by colony PCR. Colony PCR was carried out using primers flanking mCherry in the pBS34 backbone; the presence of a promoter-containing plasmid would yield a product of ∼1.6 kb, roughly twice the size of mCherry (Figure 4c). Once transformed, the promoter library was sent for sequencing using a sequencing primer 72 bp downstream of the mCherry start codon.

Library Transformation into Yeast. Promoter constructs with mCherry and the kanMX6 marker from the plasmid were directly amplified from each clone's glycerol stock for transformation into yeast by homologous recombination at the LYS2 locus (Figure 4d); 40 bp sequence insertion tags were added during amplification. The amplified constructs were transformed into yeast strain BY4741 (MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) by the lithium acetate/PEG method.[40] Colonies resistant to 350 μg/mL G418 were screened for lysine auxotrophy to verify integration at the LYS2 locus. We picked up to three colonies per strain for final confirmation by colony PCR.

We used semi-nested colony PCR to confirm correct insertion of the synthetic promoter at the LYS2 locus by using two forward primers, one targeting ∼100 bp upstream of the insertion site and the second targeting the synthetic tag (Figure 1d). Clones with both positive colony PCR products (Figure 4e, lanes 3−5) were archived, and their synthetic promoters were reamplified from yeast for sequence verification (Figure 4f). All primers used in promoter assembly, amplification, and colony PCR are listed in Supplementary Table S7.

Predicting Pho4 Affinity to Engineered Regulatory Elements. We calculated the probability of Pho4 binding to a 24 bp region centered on the Pho4 E-box using a simple *in silico* model. Binding probabilities (P_{occ}) were calculated from

measured Pho4 binding energies to sites on a 12 bp long sliding window.[21,41]

Kinetic Induction Measurements. Strains were grown in YPD supplemented with 10 mM Pi and 200 μg/μL G418 at 30 °C for 26 h, then diluted 30-fold in synthetic complete medium with 10 mM Pi, and allowed to re-enter log phase. Cells were washed twice in Pi-free synthetic medium and diluted to a starting OD of 0.1−0.2 in Pi-free medium. mCherry fluorescence (587 nm excitation and 610 nm emission, 9 nm bandwidth) was measured every 6 min for 16 h on a plate reader (BioTek SynergyMx) and normalized to cell number by dividing by the optical density. Time-dependent Hill functions were fit to the normalized induction curve.

■ ASSOCIATED CONTENT

Supporting Information

Supplementary tables, figures, and references. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

S.J.M. and A.S.R. designed the library content; A.S.R. designed the synthetic promoter structure and carried out the experiments and data analysis; both authors wrote the manuscript.

Notes

The authors declare no competing financial interest.

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