Quantitative cell array screening to identify regulators of gene expression

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Abstract
In the last decade or so, advances in genome-scale technologies have allowed systematic and detailed analysis of gene function. The experimental accessibility of budding yeast makes it a test-bed for technology development and application of new functional genomic tools and resources that pave the way for comparable efforts in higher eukaryotes. In this article, we review advances in reporter screening technology to discover trans-acting regulators of promoters (or cis-elements) of interest in the context of a novel functional genomics approach called Reporter Synthetic Genetic Array (R-SGA) analysis. We anticipate that this methodology will enable researchers to collect quantitative data on hundreds of gene expression pathways in an effort to better understand transcriptional regulatory networks.

Keywords: gene expression; reporter gene; Saccharomyces cerevisiae; cell cycle; histone gene

INTRODUCTION
Proper control of gene expression is vital for virtually all aspects of cellular function. In many cases, aberrant gene expression is apparent in various diseased states, including many types of cancers [1]. Exploration of mechanisms of transcriptional control in Saccharomyces cerevisiae has led to major discoveries on how gene expression is regulated, which are generally applicable in higher organisms because of the high degree of conservation of the transcriptional machinery [2]. Furthermore, the relatively straightforward genetics that can be employed in yeast make it an excellent model organism for testing and validating new technologies [3].

Perhaps one of the most groundbreaking technological advances was the development of DNA microarrays, which revolutionized the gene expression field. Generation of large compendiums of gene expression profiles and simple clustering algorithms have elucidated mechanisms of gene function and have even been successful in predicting drug targets [4]. Major applications of gene expression profiling have also been seen in cancer biology, exemplified by one study that characterized breast tumours based on observed expression profiles [5].

In addition to expression profiling, high resolution DNA microarray platforms have been used to probe other contributors to transcriptional control, including chromatin organization, epigenetic marks, and protein–DNA interactions on a genome-wide scale [6–12]. In particular, huge efforts have been made to characterize the transcriptional regulatory network in yeast by charting genome–wide localization of known DNA-binding proteins using the ChIP–chip (Chromatin Immunoprecipitation–chip) methodology [13, 14]. ChIP–chip experiments have also been performed with sensitive antibodies that recognize specific chromatin marks to allow analysis of the genome–wide distribution of histone modifications [11, 15]. These and other studies have described thousands of regulator-promoter interactions, have begun to reveal the epigenetic landscape of the genome and have established general rules governing promoter regulation by DNA-binding proteins. In other work, the sequence motifs recognized by DNA-binding proteins have been...
systematically explored using protein-binding microarrays (PBM) [9, 16]. In these experiments, purified proteins are incubated with high-density custom-designed microarrays carrying all 10-mer sequence variants to obtain thorough binding-site measurements for any transcription factor of interest. The PBM method has been productively used to identify sequence motifs for many DNA-binding proteins from both yeast and mouse [8, 10, 17, 18]. Finally, genome-wide maps of nucleosome occupancy on DNA in vivo have been constructed for yeast [6, 12], which set the stage for studies characterizing the effect of gene mutations on chromatin organization throughout the genome [8].

The methods summarized above have produced enormous advances in our understanding of transcriptional control. However, it is clear that much remains to be learned about transcriptional regulatory networks, even in well studied organisms like yeast, where many genes remain unannotated [19]. Also, approaches such as ChIP-chip demand prior knowledge of gene function to choose proteins to tag with the purpose of understanding their roles in transcriptional regulation at all promoters. A powerful complementary approach to scan the genome for trans-acting regulators of promoters of interest involves the use of sensitive promoter-reporter genes. In this article, we review both classical and contemporary genetic screening approaches that are applied to discover the components of transcriptional regulatory pathways. This overview will set the stage for discussion of a novel screening approach we recently developed to quantitatively assess the effect genetic perturbations have on reporter genes.

CLASSICAL REPORTER SCREENS

Reporter genes are constructed by fusing a promoter or any cis-regulatory element of interest to a gene that provides an easily assayable readout of the activity of that particular promoter (or cis element). One such reporter gene that has been widely employed is the lacZ gene from E. coli that encodes the enzyme β-galactosidase [20, 21]. When a promoter-lacZ reporter gene is introduced into a particular cell, the activity of the promoter can be assayed by plating cells on medium containing X-gal. When β-galactosidase is produced, the substrate X-gal is cleaved which results in the formation of blue colonies. If the promoter is inactive in a particular cell, lacZ expression is turned off and colonies appear white [20, 21]. Reporter technology can be combined with forward genetic screens to isolate mutants that cause differential expression of the reporter gene, which elucidates candidate regulators of the promoter being analyzed (Figure 1). Randomly mutagenized cells that result in increased or decreased reporter activity are isolated and the gene mutation characterized to discover the gene responsible for controlling the promoter driving lacZ expression.

An excellent example of early applications of this approach is the effort to learn how the HO gene in S. cerevisiae is regulated. The HO gene encodes an endonuclease that is involved in mating type switching. HO transcription occurs only in haploid MATα or MATα cells but not in a/α diploid cells [22]. Furthermore, HO expression is cell cycle-regulated with transcripts peaking in late G1 and HO expression is restricted to the mother cell and is not seen in the newly formed daughter cell [23]. A forward genetic screen was employed to identify trans-acting factors that control expression of a HO promoter-lacZ reporter gene [24]. Five new SWI genes were identified that caused a defect in HO promoter-lacZ transcription [24], adding to the five already known [25]. Further dissection of the HO upstream regulatory sequence (URS) revealed that a short motif repeated throughout the URS, now called the Swi4-6-dependent cell cycle box (SCB) element [CACGA]4 is sufficient to confer cell cycle regulation of the HO gene [24]. To characterize which SWI mutants specifically act through the [CACGA]4 motif in the HO promoter, a [CACGA]4-lacZ reporter gene was introduced into the 10 SWI mutants. Only deletion of SWI3, SWI4 and SWI6 caused a defect in [CACGA]4-lacZ expression, suggesting the proteins encoded by these genes are acting specifically through the SCB sequence while other activators like SWI1, 2 and 5 regulate the promoter independently of [CACGA]4 [24]. Further work revealed that Swi4 and Swi6 directly interact through their C-terminal regions to form a heterodimeric transcription factor called SBF and that Swi4 is responsible for directly binding DNA [26, 27].

Reporter gene screens have become a standard tool in transcriptional analysis. As described above, forward genetic screening typically involves random mutagenesis of strains harbouring the reporter gene of interest but limitations to this approach exist.

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First, random mutagenesis is normally not saturating for the genome nor is it truly random. Second, a large mutant library must be constructed to ensure high coverage of the genome. Third, significant follow-up work is required to characterize mutants. Fourth, these types of reporter screens provide only qualitative measures of gene expression. To combat many of these problems, we and others have sought to make use of the sophisticated functional genomic tools and resources in yeast to facilitate high-throughput genetic manipulation. Below, we first summarize the genomic methods and reagents required for systematic genetic screens in yeast. We then describe methods that take advantage of these tools for carrying out systematic reverse genetic screens to identify regulators of a promoter of interest.

**CONTEMPORARY REPORTER SCREENS**

**The yeast deletion array and the synthetic genetic array (SGA) approach**

Since genomes of a variety of organisms are fully sequenced, researchers have knowledge of all predicted open reading frames (ORFs). These gene models have allowed the creation of genome-wide resources where most genes are deleted or can be systematically perturbed. RNA interference (RNAi) is typically used to knockdown gene function by...
reducing levels of messenger RNA (mRNA). In *Caenorhabditis elegans*, *Drosophila melanogaster*, human and mouse, genome-wide RNAi libraries exist to reduce expression of each gene [28–30].

In budding yeast, more directed approaches have been used to generate strain collections where each full length gene is deleted. Here, researchers take advantage of the high intrinsic rate of recombination to replace each gene with a dominant antibiotic resistance cassette. The first construction of a complete gene–deletion library was reported in budding yeast, *S. cerevisiae*, where a kanamycin resistance cassette (KanMX) with flanking regions homologous to each yeast ORF was targeted to replace each coding sequence [31]. Strategies have been used to create gene–disruption libraries in other organisms like *Schizosaccharomyces pombe* (available from BiONEER), *E. coli* [32] and *Cryptococcus* sp. [33].

Characterizing the library of yeast deletion strains revealed that ~20% of all 6000 yeast genes are essential for haploid viability, only about half of which were previously known [31]. This remarkable result revealed that extensive redundancy exists in the genome that buffers the consequence of single gene deletions. This observation fuelled development of a high-throughput method for genetic manipulation of the yeast deletion library termed the synthetic genetic array (SGA) approach [34, 35]. Initially, this approach was used to combine a query mutation of choice with each yeast deletion strain to create an output array of double mutants where arrayed colonies could be scored for a growth defect that is more severe than each single gene deletion (so called synthetic lethal or synthetic sick interactions). The SGA method has been described in detail elsewhere [36]. Key features of the SGA approach are: (1) it is automated by use of robotics to replicate arrays of yeast colonies onto different selection media; (2) since yeast deletion mutants are arrayed, the position of each yeast mutant is known; (3) yeast of *MATα* mating type with a marked allele ‘A’ are mated to the array of *MATα* yeast deletion mutants to produce diploid yeast strains which are then sporulated and (4) *MATα* meiotic progeny with the marked query allele ‘A’ combined with each arrayed deletion mutant are preferentially selected because they contain a *STE2* promoter driving expression of an auxotrophic marker gene that is only expressed in *MATα* cells, allowing them to grow on media lacking the appropriate amino acid [34, 35]. The net result of these features is that arrays of yeast mutants harbouring a query mutation (such as a deletion allele) or reporter gene of interest (see below) can be rapidly produced through a series of simple pinning steps.

Combining automated genetics with reporter genes

To combat many of the problems associated with classical forward genetic screens (described above), we recently described methods for carrying out systematic reverse genetic screens to identify regulators of a promoter of interest using array-based functional genomic tools and resources in yeast [37]. The method involves use of the SGA procedure to introduce a reporter gene of interest from an otherwise wild-type query strain into the ordered array of ~4500 known yeast deletion mutants. We first applied the method with two types of reporter genes, which are based on a colourimetric assay or auxotrophy. In one case, the activity of a promoter fused to *lacZ* is assessed in each yeast deletion mutant by replica pinning the entire array onto medium containing the substrate X-gal (see above). As noted earlier, on this medium, deletion of genes encoding repressors of the promoter driving *lacZ* expression will result in higher levels of *lacZ* transcription and thus greater β-galactosidase activity leading to very blue colonies. If the deleted gene encodes an activator of the promoter, *lacZ* transcription will be reduced leading to a white colony colour.

A second reporter gene is based on *HIS3* auxotrophy. Cells that cannot produce histidine because they lack the *HIS3* gene must be grown on media supplemented with histidine. In this case, a reporter gene harbouring a promoter fused to *HIS3* is introduced into the yeast deletion array using the SGA methodology and deletion of genes that allow growth on medium lacking histidine are scored as repressors of that promoter [37]. This type of screen is useful only if the promoter driving *HIS3* is weak enough so that a growth defect is seen when cells are grown on medium lacking histidine or a mutation is made so that the promoter driving *HIS3* is inactivated.

The *HIS3* reporter gene approach was used to screen for new regulators of the SCB element, which is bound by the transcription factor SBF (see above). In this case, an SCB-*HIS3* reporter gene was introduced into a strain with a *CLN3* deletion [38]. The absence of the cyclin *CLN3* prevents activation
of SCB-dependent transcription, thus cells cannot grow in the absence of histidine because of the failure of the SCB element to drive transcription of the HIS3 gene. The SGA methodology was used to combine the reporter gene and marked CLN3 deletion allele with each yeast deletion mutant. The resulting array of yeast mutants was screened on media lacking histidine and a new repressor of G1 transcription was discovered. Deletion of WHI5 resulted in growth of colonies because the absence of Whi5 relieved repression of the SCB element (caused by the CLN3 mutation) and allowed HIS3 transcription [38]. Follow up experiments revealed that Whi5 association with SBF to allow expression of late G1 genes resulted in growth of colonies because the absence of Whi5 relieved repression of the SCB element to drive transcription of the HIS3 gene. The SGA methodology was used to combine the reporter gene and marked CLN3 deletion allele with each yeast deletion mutant. The resulting array of yeast mutants was screened on media lacking histidine and a new repressor of G1 transcription was discovered. Deletion of WHI5 resulted in growth of colonies because the absence of Whi5 relieved repression of the SCB element (caused by the CLN3 mutation) and allowed HIS3 transcription [38]. This study defined a pathway in yeast analogous to the Rb-E2F pathway in mammalian cells, which is often targeted in many types of tumours.

Although these types of reporter screens have proven useful, they are generally not quantifiable making them difficult to employ in large-scale studies, which led us to develop our next generation R-SGA screening platform based on the use of fluorescent proteins. The recent engineering of fluorescent proteins in a variety of different colours across non-overlapping spectral classes has made multi-colour cell biological experiments feasible [39, 40]. Additionally, fluorescent proteins are easy to detect with the correct optics and fluorescent signals can be rapidly quantified, making them useful markers of a variety of cellular events.

The fluorescent protein-based R-SGA system [41] includes a query strain that harbours any promoter of interest fused to GFP on a plasmid as well as an integrated control promoter fused to RFP (Figure 2). The query strain is crossed to the collection of ~4500 viable haploid yeast deletion mutants using the SGA platform, resulting in an output array in which each deletion mutant contains both reporter genes. By constructing such an output colony array, the effect of each yeast deletion mutant on reporter gene activity is easily assessed by scanning both GFP and RFP fluorescence intensities directly from colonies arrayed on agar plates using a scanning fluorimager. After quantifying these data, the GFP intensity captured from each colony can be standardized to the RFP signal from that same colony to identify deletion mutants causing differential GFP expression. It is expected that deletion of a putative activator will result in a decreased GFP:RFP ratio while deletion of a putative repressor will result in an increased GFP:RFP ratio. This methodology allows a quantitative and unbiased survey of the genome to identify both direct regulators and upstream signals and pathways that impinge upon a promoter of interest.

The R-SGA approach was applied to study transcription of histone gene expression [42], a group of genes whose transcripts are tightly cell-cycle-regulated during S-phase [43]. We fused the promoter of the histone H2A gene (HTA1) to GFP and carried out a R-SGA screen to identify HTA1-promoter regulators [42]. This screen identified the known trans-acting regulatory proteins (Hir1, Hir2, Hir3 and Hpc3 or HIR) that repress HTA1 transcription by acting through a negative (NEG) regulatory site in the promoter [44-49]. The screen also led to the discovery of another histone chaperone protein, Rtt106, which was required for repression of HTA1 transcription. Follow-up studies revealed that Rtt106 functions in a pathway with the known repressors (Asf1 and HIR) to limit expression of the HIR-regulated histone genes to S-phase of the cell cycle [42]. Repression by Rtt106 appears to be controlled through the NEG sequence in HIR-regulated histone gene promoters since HIR1 is required for Rtt106 recruitment to a region of the promoter that encompasses the NEG site and previous work has shown that the NEG sequence is required for Hir1-mediated cell cycle regulation of histone genes [48].

Other large-scale approaches were carried out to further characterize the molecular defect caused by deletion of histone chaperones on gene promoters and expression. Genome-wide nucleosome occupancy experiments [6] carried out in rtt106Δ and hir1Δ mutants revealed that histone promoters are generally nucleosome-free (except the HTA2-HTB2 promoter which is regulated independent of the HIR complex) [42]. Other promoters were also identified in both of these mutants that appear to be depleted of nucleosomes indicating that HIR and Rtt106 dictate regions of repressive chromatin at other promoters in addition to those that drive expression of histone genes. A number of promoters unique to each mutant are also nucleosome-depleted indicating that other pathways likely exist where HIR and Rtt106 assemble nucleosomes at promoters independently of each other. In future work, genome-wide localization studies using ChIP-seq on TAP-tagged versions of Rtt106 and Hir1 will
be important for determining the promoter regions bound by these proteins. These data coupled with nucleosome occupancy data already generated and gene expression profiling should elucidate the collaborative and independent roles these proteins are playing at promoters throughout the genome.

The functional genomics screen carried out to identify specific regulators of the \textit{HTA1} promoter, revealed that deletion of \textit{YTA7} caused decreased GFP expression compared to the RFP control gene [42], although previous work suggested a role for Yta7 is proper repression [50]. Yta7 is a bromodomain-containing protein that acts as a barrier protein on chromatin at the \textit{HMR} locus in yeast [51]. Detailed analysis of Yta7 function at the \textit{HTA1} promoter revealed that it localizes to both the NEG-containing region of the promoter as well as the coding region. When \textit{YTA7} is deleted, Rtt106-TAP spreads laterally across the promoter region and into the \textit{HTA1} ORF, where it is normally not present, revealing a role for Yta7 in properly localizing Rtt106 to the histone promoter. It is likely that the

\begin{figure}
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\caption{Fluorescent protein based R-SGA screen to identify regulators of a promoter-reporter gene of interest. A query strain harbouring an experimental test promoter fused to GFP along with a control promoter under independent regulation fused to RFP is introduced into the yeast deletion array [41]. After appropriate selection, an output array of haploid yeast strains with both reporter genes combined with each yeast deletion mutant is produced. GFP and RFP fluorescence intensities are assayed directly from colonies arrayed on agar plates using a scanning fluorimeter. Subsequent intensities are quantified to determine deletion mutants causing differential reporter gene expression.}
\end{figure}
lateral spread of Rtt106 into the coding region is propagating a repressive chromatin structure or is interfering with RNA polymerase II elongation which explains the HTA1 transcriptional defect observed in the YTA7 deletion strain.

The results described above reinforce the need for carrying out quantitative and unbiased reporter screens to identify new avenues of research leading to discoveries on how genes are regulated at the level of transcription. By screening an array of almost all viable yeast deletion mutants for an effect on a GFP reporter gene relative to an RFP control gene, virtually all known regulators of histone HTA1 transcription were identified along with new regulators that could be placed in a common pathway using traditional genetic and biochemical approaches. Carefully selected promoters can be screened using this approach to understand transcriptional regulatory networks that underlie specific cellular processes. For example, in our work, we are screening a panel of ~30 cell-cycle-regulated promoters that are controlled by known cell cycle transcription factor pathways in an effort to describe a detailed interplay of direct and upstream regulators that control cell cycle transcription (P.K. and B.J.A., unpublished data).

THE FUTURE OF CONTEMPORARY REPORTER SCREENS
Screening other arrays of yeast alleles
The screening approach we describe here makes use of a collection of yeast deletion mutants. However, because ~1000 genes are essential [31], the effect that mutation of these genes causes on reporter genes cannot be scored in the present screening format. One way to identify regulatory pathways controlled by essential genes is to examine the consequence of gene overexpression on a reporter gene of interest. An array of yeast strains where each yeast colony overexpresses a different yeast protein has been constructed and is useful for these types of experiments [52]. Each colony on the overexpression array harbours a high-copy plasmid where the GAL1/10 promoter, which is induced in the presence of galactose, drives expression of a different ORF [52]. This array can be manipulated using the SGA methodology to combine GFP and RFP reporter genes with overexpression of each ORF when grown in the presence of galactose. In addition to analysis of essential genes, opposite effects to gene-deletions may result such that increased GFP:RFP would indicate the overexpressed gene is an activator of the promoter while decreased GFP:RFP would identify putative repressors.

Previous work revealed that deletion of many transcription factors results in little effect on target genes examined by gene expression microarrays, likely because the transcription factor is inactive under the conditions tested [53]. However, overexpression in many cases results in a gene expression pattern above noise indicating that artificially activating expression of these proteins bypasses the need for specific activating conditions and can be used to identify target genes of transcription factors [53]. Similarly, by overexpressing genes it may be possible to identify regulators of a promoter that may not normally be active in the conditions tested. Thus, overexpression screens are advantageous because they: (1) allow analysis of essential genes; (2) allow identification of regulatory proteins that are only expressed under specific conditions and (3) provide complementary data to deletion screens.

Analysis of essential gene function on reporter genes can also be examined more directly by screening other available yeast arrays of essential genes including: (1) arrays of tetracycline-repressible alleles [54, 55], (2) temperature sensitive strains and (3) alleles which contain a disruption in the 3′ untranslated region (UTR) of essential genes to knockdown gene expression (called the decreased abundance of mRNA by perturbation or DAmP strains) [56].

Increasing throughput of reporter-gene analysis using pooled screens in yeast and higher eukaryotes
Above, we described the development of a systematic approach to study gene regulation by combining fluorescent reporter genes, the SGA method and a simple assay for detecting fluorescence from yeast colonies arrayed on agar plates. Although this approach has proven useful for studying gene regulatory pathways in yeast, the plate-based colony assay is generally not adaptable to higher eukaryotes. To address this issue, promoter-reporter construct screening methods could be adapted to screen pooled cultures for any organism for which appropriate reagents exist.

One remarkable feature of the yeast deletion collection (and now other gene disruption libraries) is that each knockout cassette is flanked by unique sequences that are used as strain identifiers or
molecular barcodes and all mutants contain a universal sequence that allows a single primer set to amplify each unique barcode [31]. If the entire collection is pooled into a single culture and treated with a particular condition (for example drug treatment), deletion strains that are sensitive to treatment are under-represented in the population. DNA is prepared from the pooled culture, PCR-amplified with the universal primer set and hybridized to a microarray that contains oligonucleotide probes homologous to each molecular barcode. Positions on the microarray that no longer show a signal after hybridization identify mutants sensitive to the particular treatment. This 'bar-code' strategy has been used to quantitatively monitor the deletion collection for strains that show a fitness defect when grown in rich-media or under various conditions [57].

Since molecular barcodes allow identification of deletion mutants in a mixed population, it should be possible to combine a pooling strategy with fluorescent reporter genes and fluorescence activated cell sorting (FACS) (Figure 3) to explore transcriptional

Figure 3: Reporter screening using barcoded gene disruption libraries and FACS. A pooled culture of cells with a promoter-GFP reporter gene combined with each yeast deletion mutant is subject to FACS to physically sort cells based on the intensity of GFP signal. Mutants present in the brightest and dimmest sub-populations after sorting are identified by hybridization to a barcode microarray and normalized to the hybridization signal from the unsorted population, which defines the mutants present in the initial pooled culture.
regulatory networks. For yeast screens, a promoter-GFP reporter gene can be introduced into the collection of deletion mutants using the SGA approach or by directly transforming the reporter plasmid into a pooled culture of all viable deletion strains. The population of cells is then pooled and grown in appropriate selection media and subject to FACS so that the brightest cells in the population are sorted into one sub-population and the dimmest cells are sorted into a different sub-population. Flow cytometry has been used to make precise measurements of GFP-tagged proteins from single cells, which indicates the feasibility of the approach described here for detecting fine cell-to-cell changes in GFP expression [58]. The brightest cells expressing high levels of GFP likely contain deletion mutants that are required to repress the promoter of interest while the dimmest cells likely contain activators of the promoter. To identify mutants present in each population, DNA prepared from each pool is hybridized to a barcode microarray and compared to hybridization signal from an unsorted population. This type of approach could be used to determine how the promoter-reporter gene responds to different environmental conditions or drug treatments (particularly when drugs are scarce) in each mutant background and should aid in discovery of new pathways of gene regulation.

In human cells and other eukaryotes, barcoded RNAi libraries exist to perturb expression of each gene [28]. Reporter genes of interest could be stably expressed in cell lines of choice and combined with the RNAi library. Similar to the above experiment, FACS cell sorting could then be used to physically sort bright and dim cells from the population and RNAi molecular barcodes detected to determine which gene is targeted. Pooled RNAi barcode screens have been carried out previously on a genome-scale and identified a number of genes required for cell proliferation in different tumour types [59, 60]. The FACS-based reporter-gene screens described in this article should prove useful for studying transcriptional regulatory pathways that are perturbed in cancer cell types.

CONCLUDING REMARKS
The advent of DNA microarrays has revolutionized the gene expression field and has led to major discoveries on regulator-gene interactions. Parallel transcript profiling, genome–wide analysis of transcription factor localization or histone modifications on chromatin, maps of nucleosome occupancy and analysis of DNA sequences bound by regulatory proteins are all powerful array-based approaches that have enabled detailed analysis of mechanisms that control how the genome is regulated. Now that next generation sequencing applications are becoming commonplace, gene expression regulatory pathways are being examined with unprecedented resolution. It is clear that even though an enormous amount of data has been generated using microarray-based platforms, there remain many more regulatory pathways to be discovered, even in well studied organisms like S. cerevisiae. Here, we reviewed genetic approaches that exploit reporter genes and yeast functional genomics to link protein function to transcriptional regulation. As-proof-of-principle, we discussed the results of recent genome-wide screens that identified regulators of histone gene transcription that had previously been mysterious.

In addition, we also discussed adaptations to systematic reporter gene methods that will allow more detailed analysis of transcription factor pathways by increasing the spectrum of genetic perturbations accessible to screening, including gene overexpression and alleles of essential genes. We also considered the prospect of carrying out fluorescent protein-based reporter screens in higher eukaryotes by combining reporter genes with barcoded RNAi libraries and FACS. Although we focus our attention on reporter screens in this review, for complete elucidation and characterization of transcription factor pathways, no one approach should be considered superior. Instead a combined approach utilizing the spectrum of functional genomic techniques and tools should be carried out to fully realize the goal of defining all pathways that control gene expression.

Key Points

- The R-SGA screening approach allows quantitative analysis of the effect deletion mutants cause on a promoter-reporter gene of interest.
- The R-SGA approach was applied to study histone gene regulation, revealing that histone chaperone proteins are required for nucleosome assembly at the promoter and that the repressor Rsc106 is properly localized at the HTA1 promoter because of the presence of Yta7.
- Future screens in higher eukaryotes may be employed by combining fluorescent reporter genes, barcoded RNAi libraries and FACS.
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Quantitative cell array screening


