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# Exploring the transcriptional landscape of plant circadian rhythms using genome tiling arrays

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# Abstract

**Background**

Organisms are able to anticipate changes in the daily environment with an internal oscillator know as the circadian clock. Transcription is an important mechanism in maintaining these oscillations. Here we explore, using whole genome tiling arrays, the extent of rhythmic expression patterns genome wide, with an unbiased analysis of coding and noncoding regions of the Arabidopsis genome.

**Results**

As in previous studies, we detected a circadian rhythm for approximately 25% of the protein coding genes in the genome. With an unbiased interrogation of the genome, extensive rhythmic introns were detected predominantly in phase with adjacent rhythmic exons creating a transcript that if translated would be expected to produce a truncated protein. In some cases such as the MYB transcription factor *PHOSPATE STARVATION RESPONSE1*, an intron was found to exhibit a circadian rhythm while the remainder of the transcript was otherwise arrhythmic. In addition to several known non-coding transcripts including miRNA, trans-acting siRNA, and snoRNA, greater than one thousand intergenic regions were detected as circadian clock regulated, many of which have no predicted function, either coding or non-coding. Nearly 7% of the protein coding genes produced rhythmic antisense transcripts, often for genes whose sense strand was not similarly rhythmic.

**Conclusions**

This study revealed widespread circadian clock regulation of the Arabidopsis genome extending well beyond the protein coding transcripts measured to date. This suggests a greater level of structural and temporal dynamics than previously known.

## Background

Many organisms exhibit cyclic changes in physiology and behavior in accordance with predictable changes in their daily environment. Namely, this is caused by shifts in temperature and light intensity owing to transitioning exposure to the sun caused by Earth's rotation. In addition to reacting directly to external stimuli, many organisms time their behavior in anticipation of periodic changes in the environment. Such circadian rhythms are believed to be adaptive and indeed has been demonstrated in both prokaryotic and eukaryotic photosynthetic organisms [1, 2]. The endogenous timing mechanism known as circadian clocks is widespread across life and is primarily based on interlocking transcriptional feedback loops and regulated protein turnover [3].

Circadian clock regulation of transcription in plants appears to be extensive and many pathways governing processes such as photosynthesis, cold acclimation, and cell wall dynamics, for example, exhibit circadian rhythms at multiple levels [4-6]. Estimates of the extent of circadian clock regulation are primarily derived from the use of high-density oligonucleotide arrays with features that mostly correspond to the 3' end of genes annotated as protein coding (e.g. [4-6]). Recently, there has been a flourish of transcript mapping using genome tiling arrays capable of measuring nearly all nonredundant sequences in the genome, far beyond the capability of previous studies [7-9]. In excess of the number of protein coding transcripts, noncoding RNAs (ncRNA), which include natural antisense transcripts (NAT), appear to be a large component of the remarkably complex transcriptome in all organisms examined to date: *Arabidopsis*, *C. elegans*, *Chlamydomonas*, *Drosophila*, *E. coli*, human, rice, and yeast [10-24]. Aside from hybridization based detection systems, sequencing

approaches such as serial analysis of gene expression (SAGE), massively parallel signature sequencing (MPSS), and directional cDNA cloning and sequencing have confirmed widespread existence of these transcripts in plants and other species [25-27]. It is not difficult to fathom the existence of numerous and sundry ncRNA. There are several classes of long studied ncRNA such as transfer (tRNA), ribosomal (rRNA), and small nuclear (snRNA) in addition to the more recently discovered small nucleolar (snoRNA), micro (miRNA), and short interfering RNA (siRNA) [28]. Nevertheless, the existence of these specific forms does not explain the excessive ncRNA measured by tiling arrays. This suggests a complex RNA regulatory network akin to that revealed through the study of X chromosome silencing, for example [29].

Tiling array experiments have done little to characterize large-scale transcriptional activity beyond to say it exists. Here, we explore circadian clock controlled transcriptional regulation in *Arabidopsis* using high-density oligonucleotide tiling arrays. In addition to protein coding genes and intergenic regions, we measured circadian regulation of introns, as well as clock-regulated NATs.

## Results and Discussion

**Tiling array characteristics and performance**. The Affymetrix *Arabidopsis* tiling arrays each contain 1,683,620 unique 25-mer oligonucleotide features. One array is comprised of the forward or Watson strand and the other the reverse or Crick strand. The *Arabidopsis* Information Resource Version 7 (TAIR7) genome annotation includes a total of 32,041 genes, among them, 27,029 are considered to be protein coding [30]. Nearly 95% (25,677) of the protein coding genes have at least two

corresponding exon array features as do 74% (2,863) of the transposons and pseudogenes (Table 1). Due to their small size and sequence redundancy within gene families; only 202 of the 1,123 annotated ncRNA have at least two corresponding array features of those 62 are miRNA. Labeled cRNA was prepared from twelve samples collected during a two day circadian time course at 4hr resolution. Samples were independently hybridized to each array as previously described [4]. Spectral analysis was used to test for a circadian rhythm in the hybridization intensity of each feature across the two day time course. Rather than treat each feature as an independent experiment, a sliding window approach was used to exploit the redundant signal in neighboring features (see Materials and Methods). As a test of the capabilities of the tiling arrays, RNA time course, and spectral analysis, we specifically looked at the expression of fourteen circadian clock associated genes: *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), *GIGANTEA* (*GI*), *TIMING OF CAB2 EXPRESSION1* (*TOC1*), *PSEUDO RESPONSE REGULATOR3, 5, 7,* and *9* (*PRR3, 5, 7,* and *9*), *LOV KELCH PROTEIN2* (*LKP2*), *LUX ARRHYTHMO (LUX), EARLY FLOWERING3* and *4* (*ELF3* and *ELF4)*, *FLAVIN-BINDING*, *KELCH REPEAT*, *F-BOX 1* (*FKF1*) and *ZEITLUPE* (*ZTL*) (Fig 1-4) [31]. Here we plot the results of the spectral analysis of the expression level time course for individual features on the array. Each of these genes had at least two exon features that satisfied the $p < 0.005$ cut off as well as a phase [see Additional data files #1 and #2] similar to that reported previously. Two clock genes with weak rhythms at the transcriptional level, *LKP2* [32] and *ZTL,* exhibited the expected behavior (Fig 3). A clock gene that does not cycle at the transcriptional level, *TIME FOR COFFEE* [33], was similarly found not to exhibit circadian regulation (Fig 4C). In addition to these consistencies we compared the tiling array

dataset with a similarly produced two-day time course [GEO:GSE8365, [34] hybridized to the Affymetrix ATH1 gene array. The spectral analysis for each gene on the gene array was plotted against all of the features for that transcript on the tilling array. While comparison between these platforms should be interpreted cautiously, there was strong accord between data sets for significance in rhythmicity as well as circadian phase [see Additional data file #3]. At the genome level, 24.4% of the protein coding genes were circadian clock regulated (<0.05% FDR), that is to say, the transcript exhibited a rhythmic 24hr period over a two-day time course [see Table S3 in Additional data file #4]. This result is well within the range of recent reports [35, 36] that used the *Arabidopsis* ATH1 array. In these study, greater than 75% of the protein coding transcripts assayed were found to cycle when driven by various conditions of photocycles and/or thermocycles or under constant conditions. While all phases were represented, there was an increase in frequency of genes with peak expression just prior to dawn and dusk suggesting an important role of the circadian clock in anticipating the transitions between day and night (Fig 6A). These data can also be queried and visualized at the Arabidopsis Cyclome Expression Database [37].

**Circadian clock regulation of introns**. Unlike the design of the *Arabidopsis* ATGenome1 and ATH1 arrays, where features quantify hybridization of the sense strand transcript of the protein coding regions, AtTILE1 features also correspond to 597,856 intergenic and 301,733 intronic loci on each strand. Interestingly, these features capably detected 499 transcripts with rhythmic introns [Table S4 in Additional data file #4]. In cases where cycling introns were observed in genes with cycling exons (n=213), the introns frequently had a similar phase to the coding regions of the transcript (Fig 6B). Unlike an alternatively spliced exon, introns are

nonsense sequences and their inclusion tends to introduce a translational stop as in the examples of *ELF3* (Fig 1B) and *CONSTANS LIKE2* (*COL2*) (Fig 4D). Transcripts of these genes were transcriptionally verified for an exon and intron using quantitative PCR of reverse transcriptase amplified cDNA (QRT-PCR) of an experimentally independent time course [see Additional data file #5]. For both genes (*ELF3* [GenBank:AY136385 and Y11994]; *COL2* [GenBank:L81119 and L81120]), a cDNA of both splice forms, with and without the detected cycling intron, has been captured and sequenced. By assaying RNA from pooled whole seedlings with an oligonucleotide array platform, it is not clear if both variants occur in the same cell or tissue types or if they are simply immature transcripts sampled prior to complete processing. Hybridization intensities of individual features do suggest the intron variant of *COL2*, for example, is present in appreciable quantities [see Additional data file #5]. If so, this presents somewhat of a conundrum. For example, mutations in *ELF3* can cause a rather dramatic effect on flowering time and circadian rhythms in *Arabidopsis* [38] and curiously, inclusion of the second intron as we observed could produce a protein similar to that of the *elf3-1* mutant [39]. In a number of instances, introns exhibited a phase differing from the coding region of the transcript by greater than four hours (Fig 6B).

Quite unexpected, 286 genes that showed no evidence of rhythmic expression of coding regions contained an intron exhibiting circadian rhythmcity [see Table S5 Additional data file #4]. This form of alternative splicing or "gated intron inclusion" could result in altered protein function that occurs at a specific time of day. For example, the fifth intron of *PHOSPHATE STARVATION RESPONSE1* (*PSR1*, Fig 5A) cycles with peak expression in the late afternoon and this was confirmed by

QRT-PCR using a second experimental time course [see Additional data file #5]. Under these circumstances, the complete message was constitutively or at least arrhythmically expressed. Perhaps, the point of peak rhythmic expression of the intron is a circadian clock regulated occurrence of intron inclusion where the transcribed protein is truncated. This phenomenon is not difficult to reconcile with what is known about the *Arabidopsis* genome. Among the protein coding transcripts, nearly 15% have an annotated splice variant [30], which is appreciably smaller than that of mammalian genomes [40, 41]. In addition to the distinction in overall proportion of splice variant genes, intron inclusion is a lesser form in mammals but the most prevalent in *Arabidopsis* with at least 8% of protein coding genes exhibiting intron inclusion [42, 43]. Considering that the vast proportion of the genome is diurnally and circadian regulated, including many RNA binding proteins, the occurrence of circadian gated intron inclusion is not inexplicable [35, 44]. However, the exact mechanism for any one of these events and their biological relevance is not well understood. In a number of instances, introns were observed exhibiting a peak phase of expression four to twelve hours different from the coding region of a transcript (Fig 6B).

**Circadian clock regulation of ncRNA.** Certain ncRNA known as miRNA fold back and form imperfect dsRNA that are processed by the Dicer and RNaseIII-like families to create ~22bp fragments [45]. In plants, transcripts with exact homology to the mature miRNA are targeted for post-transcriptional regulation. Many miRNA are responsible for silencing transcription factors associated with growth and development and their expression is often tightly regulated both developmentally and spatially [46-48]. Although the AtTILE1 arrays are capable of distinguishing only a

fairly small proportion of the 114 annotated miRNA in the *Arabidopsis* genome, several were found to cycle in one week old seedlings. Our protocol amplified and is assumed to detect polyadenylated transcripts only, and in the case of the miRNA loci, some relatively large cycling premature transcripts were observed. Two miRNA in particular, *MIR160B* and *MIR167D* [see Additional data file #5] target several members of the *AUXIN RESPONSE FACTOR* (*ARF*) family which bind to the auxin response elements (TGTCTC) in promoters of early auxin response genes [49]. *MIR160B* targets *ARF10*, *ARF16*, and *ARF17* which are all believed to be involved in germination and post-germination stages of growth [50, 51]. *MIR167D* targets *ARF6* and *ARF8* which are involved in male and female reproductive development [51, 52]. Two other clearly cycling miRNA are *MIR158A* with no known target and *MIR157A* which targets several members of the *SQUAMOSA BINDING PROTEIN* family: *SPL3*, *SPL4*, and *SPL5*. Interestingly, the target *SPL*s and *ARF*s were not found to be circadian regulated. We speculate that for such a pattern to occur, the target must be expressed constitutively and only in cell types with rhythmic target miRNA expression. Otherwise, the signal from cells where miRNA are not expressed may obscure a rhythmic signal caused by miRNA expression in other cells. Additionally, the relationship between target degradation and miRNA concentration would need to be somewhat linear; where as in practice it is more qualitative, requiring a certain threshold of accumulation prior to detectable degradation [53]. Therefore, the absence of a reciprocal expression pattern of the target transcripts does not rule out a specific function behind the circadian behavior of the miRNA.

The well-described complexity of *AFR* transcript regulation is also influenced from trans-acting small interfering RNA (ta-siRNA), namely *TAS3* [54-57]. Dicer

processing of the primary *TAS* transcripts is triggered by miRNA-guided cleavage. In the case of *TAS3*, *MIR390* directed cleavage results in a 21bp dsRNA with posttranscriptional properties similar to miRNA [58]. While both *MIR390A* (At2g38325) and *MIR390B* (At5g58465) were reliably detected by the AtTILE1 arrays, neither was found to exhibit a circadian rhythm [see Additional data files #1 and #2]. On the other hand, the abundance of the primary *TAS3* transcript is clearly circadian clock regulated, a pattern confirmed in two independent time courses [see Additional data file #5]. While transcript abundance of *TAS3* and possibly *TAS2* [see Additional data files #1 and #2] is clearly clock regulated, a functional ncRNA will only arise with the coincidence of the initiating miRNA. This scenario explains a mechanism for very specific regulation of *ARF* transcript degradation possibly dependent on both internal and external cues [59].

While few small nucleolar RNAs (snoRNAs) were detected by the arrays, one such ncRNA, *snoRNA77* (At5g10572), cycled with a peak expression in the late evening (data not shown). This class of snoRNA is believed to target certain transcripts for chemical modification, namely 2′-O-methylation [60]. Circadian clock regulation of these transcripts suggests that this form of transcriptional modification could be, in part, circadian regulated as well. However, behavior of this transcript was arrhythmic when measured using QRT-PCR of two independent time courses (data not shown). The irreproducibility could be due to a false positive in the tilling array data and analysis, QRT-PCR data, or due to experimental differences between time courses.

**Circadian clock regulation of natural antisense transcripts.** Perhaps one of the more uniquely revealing aspects of a genome tiling array is the ability to differentiate

probe strandedness. Indeed, rhythmic natural antisense transcripts (NATs) were detected for 7% (n=1,712) of the protein coding genes detected by the arrays (Table S4). Among them were the core clock associated MYB transcription factors *LHY* and *CCA1*, and the *PSEUDO RESPONSE REGULATORS* (*TOC1*, *PRR3*, *5*, *7*, and *9*) (Fig 1-4). On the other hand, no NATs were observed for *GI*, *LUX*, or *ELF3*. Among the aforementioned rhythmic NATs, all of them exhibited a similar time of peak expression as the sense transcript. Overall, the majority of the rhythmic NATs overlapped with circadian regulated sense transcripts with a similar phase of expression (Fig 6D). The expected outcome of NAT expression based on functional characterization and expression pattern of the *Neurospora* core clock gene *FREQUENCY* [61] is inverse expression of the complementary transcript. This leaves in question the potential role of the circadian regulated NATs we detected with similar expression to their corresponding sense transcripts. The use of reverse transcriptase to generate the array probe has been shown to generate artifacts in the form of fragments antisense to coding sequences presumably derived from self priming or mispriming by other fragments[62, 63]. This bias, if real, would have to be sequence specific, or it would be ubiquitous across genes, which we do not see. Considering splice junctions are not palindromic, NATs spliced in a similar fashion to sense transcripts, and exhibiting nearly identical expression patterns, are generally artifacts. At the same time, extensive anti-correlated expression of *cis*-NAT pairs resulting in subsequent siRNA has been observed in *Arabidopsis*, but this is only a trend and many do not adhere to this rule [27, 64, 65]. As with miRNA, observations at the whole genome level without genetic experimentation might not resolve a complex relationship between sense and antisense pairs. However, consistent with the detection of rhythmic introns in otherwise arrhythmic genes, we detected 813

instances of rhythmic *cis*-NATs with an arrhythmic corresponding sense strand transcript [see Tables S6 in Additional data file #4]. In these examples, there was obviously no anti-correlated sense strand pattern resolved, and the absence of a circadian-regulated coding transcript argues against the NATs as experimental artifacts, as does the nearly 8,000 NATs detected by Stolc et al. [66] that exhibited greater hybridization intensity on the antisense strand than the sense strand in *Arabidopsis* cell cultures. The overall phase distribution of the NATs, regardless of sense strand cycling, was clearly distinct from the coding transcript phase distribution mentioned earlier (Fig 6A). Rather than an overrepresentation of rhythmic transcripts just prior to dawn and dusk, NATs as with rhythmic sense strand introns (Fig 6C), are enriched towards the morning.

**Circadian clock regulation of intergenic regions.** Numerous regions (n=1,052) not annotated as expressed portions of the genome in TAIR7 exhibited circadian behavior [see Tables S7 in Additional data file #4]. These areas consist of several different classes. The first are simple annotation errors, where the array hybridization implies a larger transcript than that found in the annotation. Criteria to identify this type is that they are immediately adjacent features to the annotated transcript with a similar phase of expression such as *PRR3* and *FKF1* which have three and two cycling intergenic features that would extend the annotation of the 3' end by at least 147bp each (Fig 2D and 4A). A second class of cycling intergenic regions has supportive expressed sequence tag (EST) evidence that is not incorporated into the formal annotation. These include protein coding transcripts as well as ncRNAs [67]. Perhaps the most interesting regions are those with scant or no support from ESTs or previous tiling array efforts [14, 66]. For example, a region of at least 350bp on chromosome 5

(6,839,029 bp to 6,839,383 bp) is rhythmic, and a coding or functional non-coding transcript is not evident (Fig 5D).

## Conclusions

Numerous forms of ncRNA are well known to be an integral part of genomes, yet many of these transcripts, described here and by others, detected by tiling arrays in several organisms fail to qualify as a functionally characterized ncRNA type [8]. Genome-wide transcription studies have forced a new paradigm of genome organization where most of the genome is expressed, yet often with an unknown function (e.g., [68]). In addition to documenting the existence of such transcripts, we have described a very specific rhythmic expression behaviour that is likely controlled by only a small number of genes making up the *Arabidopsis* circadian clock [31]. The patterns within this study alone strongly suggest these are meaningful expression patterns. For example, antisense transcripts often exhibited very different expression patterns from sense strand transcripts. Also, genes classified as pseudogenes/transposons are severely underrepresented among circadian regulated transcripts, both on sense and antisense strands. Thus, mechanisms of clock regulation were either not maintained with loss of gene function or did not spontaneously occur suggesting that the novel rhythmic transcription described within is functional.

# Materials and methods

### Plant materials and sample preparation

Seedlings of *Arabidopsis thaliana* accession Col-0 were grown on MS media (supplemented with 2% D-glucose and solidified with 1% agar) 7 days in 12 h light:12 h dark cycles under white fluorescent bulbs at 100 μmol $m^{-2}$ $s^{-1}$ before release to constant light and temperature. Samples were collected every 4 h beginning at the time of lights on, ZT0. RNA was extracted by using the Qiagen (Valencia, CA) RNeasy Plant Mini Kit. Labeled cRNA probe was synthesized according to standard Affymetrix (Santa Clara, CA) protocol.

### Array design and annotation

We used high-density oligonucleotide GeneChip® *Arabidopsis* Tiling 1.0R and 1.0F arrays. Each array is comprised of more than 3.2 million 25bp perfect match features along with corresponding mismatch features of either the Watson (1.0F) or Crick (1.0R) sequence stand. On average, each probe was spaced every 35bp of genome sequence. As previously described [69] perfect match probes from *Arabidopsis* Tiling 1.0F array were megablasted against *Arabidopsis* genome release version 7 (TAIR7) [30] including mitochondria and chloroplast sequences with word size >=8 and E-value <= 0.01. Single perfect matches, without a $2^{nd}$ partial match of >18/25 bp were selected giving a total of 1,683,620 unique features. These were mapped to annotated mRNAs as intron, exon, inter-genic region, or flanking probes which span an annotated boundary. Background correction and quantile normalization was performed separately on the forward and reverse strand arrays using the affy

Bioconductor package in R according to Bolstad et al. [70]. The Affymetrix AtTILE1 Genechip data (.CEL files) have been deposited at The Gene Expression Omnibus [GEO:GSE13814].

**Fourier/spectral analysis**

Hybridization efficiencies of oligonucleotide probes on tiling arrays vary considerably and some probes tend to be unresponsive. Thus, to avoid spurious decrease of signal in the spectral analysis from poorly responsive probes, we filtered out probes which are lowly expressed (mean < 3) and furthermore show very little variation (standard deviation < 0.25) across the time series leaving a total of 1,609,258 features between both the forward and reverse strand arrays. The twelve measurements for each probes were standardized and Fourier analysis was used to evaluate the RNA expression pattern over the two-day time course [71]. To exploit redundancy of features we grouped all probes for the same exon based on the TAIR7 genome annotation [30], or applied 200 bp windows centered on each intronic or intergenic probe position while stopping at exon boundaries. We then computed the 24-h spectral power F24 from the average of the standardized probes within a group, following Wijnen et al. [71]. To assess the significance of these F24 scores, we built empirical null distributions that take into account the number of probes (weight) that went into the calculation of the spectral power. The family of null distributions was calibrated from the distribution of scores of all probes annotated as intergenic. We parametrized these distributions as exponential functions which gave excellent fits [see Additional data file #6]. The p-values for all features were then computed from the fitted distributions. The labeling method which used oligo dT for first strand amplification of the RNA produces a 3' biased probes; therefore, any annotation unit with at least two features

satisfying $p < 0.005$ was considered circadian regulated. For Fig. 2 the phases for genes were computed from the circular averages of the phase in individual exons using CIRCSTAT [72].

## Abbreviations

Natural antisense transcripts (NATs); The Arabidopsis Information Resource (TAIR); Quantitative reverse transcriptase PCR (QRT-PCR)

## Authors' contributions

SPH and SAK conceived the study. SPH and JMG carried out the experiments. FN, TQ, JOB, and SPH analyzed the data. SPH, FN, JOB, and SAK drafted the manuscript. JOB, HC, and JRE and carried out the array annotation and web interface support. All authors read and approved the final manuscript.

## Additional data files

The following additional data are available with the online version of this paper. Additional data files 1 and 2 are tables listing the spectral analysis of each microarray time course. Additional data file 3 is figure comparing the spectral analysis of a gene array time course with the tiling array time course. Additional data file 4 is a series of tables extracted from the spectral analysis. Additional data file 5 is a series of figures demonstrating experimental verification of observations made with the tiling arrays. Additional data file 6 is a figure of the distributions of the exponential functions from the spectral analysis.

-3 provide supplemental tables. Additional data files 4-6 provide supplemental figures.

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# References


**1.** Woelfle M, Ouyang Y, Phanvijhitsiri K, Johnson C: **The adaptive value of circadian clocks: an experimental assessment in cyanobacteria.** *Curr Biol* 2004, 14:1481 - 1486.

**2.** Dodd A, Salathia N, Hall A, Kevei E, Toth R, Nagy F, Hibberd J, Millar A, Webb A: **Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage.** *Science* 2005, 309:630 - 633.

**3.** Young MW, Kay SA: **Time zones: a comparative genetics of circadian clocks.** *Nat Rev Genet* 2001, 2:702-715.

**4.** Harmer S, Hogenesch J, Straume M, Chang H, Han B, Zhu T, Wang X, Kreps J, Kay S: **Orchestrated transcription of key pathways in Arabidopsis by the circadian clock.** *Science* 2000, 290:2110 - 2113.

**5.** Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB: **Coordinated transcription of key pathways in the mouse by the circadian clock.** *Cell* 2002, 109:307-320.

**6.** Wijnen H, Naef F, Boothroyd C, Claridge-Chang A, Young MW: **Control of daily transcript oscillations in *Drosophila* by light and the circadian clock.** *PLoS Genet* 2006, 2:e39.

**7.** Mockler TC, Chan S, Sundaresan A, Chen H, Jacobsen SE, Ecker JR: **Applications of DNA tiling arrays for whole-genome analysis.** *Genomics* 2005, 85:1-15.

**8.** Willingham AT, Gingeras TR: **TUF love for "junk" DNA.** *Cell* 2006, 125:1215-1220.

**9.** Johnson JM, Edwards S, Shoemaker D, Schadt EE: **Dark matter in the genome: evidence of widespread transcription detected by microarray tiling experiments.** *Trends Genet* 2005, 21:93-102.

**10.** Manak JR, Dike S, Sementchenko V, Kapranov P, Biemar F, Long J, Cheng J, Bell I, Ghosh S, Piccolboni A, Gingeras TR: **Biological function of unannotated transcription during the early development of *Drosophila melanogaster*.** *Nat Genet* 2006, 38:1151-1158.

**11.** He H, Wang J, Liu T, Liu XS, Li T, Wang Y, Qian Z, Zheng H, Zhu X, Wu T, Shi B, Deng W, Zhou W, Skogerbo G, Chen R: **Mapping the *C. elegans* noncoding transcriptome with a whole-genome tiling microarray.** *Genome Res* 2007, 17:1471-1477.

**12.** Selinger DW, Cheung KJ, Mei R, Johansson EM, Richmond CS, Blattner FR, Lockhart DJ, Church GM: **RNA expression analysis using a 30 base pair resolution *Escherichia coli* genome array.** *Nat Biotechnol* 2000, 18:1262-1268.

**13.** Shoemaker D, Schadt E, Armour C, He Y, Garrett-Engele P, McDonagh P, Loerch P, Leonardson A, Lum P, Cavet G: **Experimental annotation of the human genome using microarray technology.** *Nature* 2001, 409:922 - 927.

**14.** Yamada K, Lim J, Dale J, Chen H: **Empirical analysis of transcriptional activity in the Arabidopsis genome.** *Science* 2003, 302:842.

**15.** Li L, Wang X, Stolc V, Li X, Zhang D, Su N, Tongprasit W, Li S, Cheng Z, Wang J, Deng XW: **Genome-wide transcription analyses in rice using tiling microarrays.** *Nat Genet* 2006, 38:124-129.

**16.** Stolc V, Gauhar Z, Mason C, Halasz G, van Batenburg MF, Rifkin SA, Hua S, Herreman T, Tongprasit W, Barbano PE, Bussemaker HJ, White KP: **A gene expression map for the euchromatic genome of *Drosophila melanogaster*.** *Science* 2004, 306:655-660.

**17.** Cawley S, Bekiranov S, Ng H, Kapranov P, Sekinger E, Kampa D, Piccolboni A, Sementchenko V, Cheng J, Williams A: **Unbiased mapping of transcription factor binding sites along human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs.** *Cell* 2004, 116:499 - 509.

**18.** Tjaden B, Saxena R, Stolyar S, Haynor D, Kolker E, Rosenow C: **Transcriptome analysis of *Escherichia coli* using high-density oligonucleotide probe arrays.** *Nucleic Acids Res* 2002, 30:3732 - 3738.

**19.** David L, Huber W, Granovskaia M, Toedling J, Palm CJ, Bofkin L, Jones T, Davis RW, Steinmetz LM: **A high-resolution map of transcription in the yeast genome.** *Proc Natl Acad Sci USA* 2006, 103:5320-5325.

**20.** Stolc V, Samanta MP, Tongprasit W, Marshall WF: **Genome-wide transcriptional analysis of flagellar regeneration in *Chlamydomonas reinhardtii* identifies orthologs of ciliary disease genes.** *Proc Natl Acad Sci USA* 2005, 102:3703-3707.

**21.** Kampa D, Cheng J, Kapranov P, Yamanaka M, Brubaker S, Cawley S, Drenkow J, Piccolboni A, Bekiranov S, Helt G: **Novel RNAs identified from an in-depth analysis of the transcriptome of human chromosomes 21 and 22.** *Genome Res* 2004, 14:331 - 342.

**22.** Rinn J, Euskirchen G, Bertone P, Martone R, Luscombe N, Hartman S, Harrison P, Nelson F, Miller P, Gerstein M: **The transcriptional activity of human chromosome 22.** *Genes Dev* 2003, 17:529 - 540.

**23**. Schadt E, Edwards S, GuhaThakurta D, Holder D, Ying L, Svetnik V, Leonardson A, Hart K, Russell A, Li G, Cavet G, Castle J, McDonagh P, Kan Z, Chen R, Kasarskis A, Margarint M, Caceres R, Johnson J, Armour C, Garrett-Engele P, Tsinoremas N, Shoemaker D: **A comprehensive transcript index of the human genome generated using microarrays and computational approaches.** *Genome Biol* 2004, 5:R73.

**24.** Li L, Wang X, Sasidharan R, Stolc V, Deng W, He H, Korbel J, Chen X, Tongprasit W, Ronald P, Chen R, Gerstein M, Wang Deng X: **Global identification and characterization of transcriptionally active regions in the rice genome.** *PLoS ONE* 2007, 2:e294.

**25.** Robinson SJ, Cram DJ, Lewis CT, Parkin IAP: **Maximizing the efficacy of SAGE analysis identifies novel transcripts in *Arabidopsis*.** *Plant Physiol* 2004, 136:3223-3233.

**26.** Meyers BC, Vu TH, Tej SS, Ghazal H, Matvienko M, Agrawal V, Ning J, Haudenschild CD: **Analysis of the transcriptional complexity of *Arabidopsis thaliana* by massively parallel signature sequencing.** *Nat Biotech* 2004, 22:1006-1011.

**27.** Wang X, Gaasterland T, Chua N: **Genome-wide prediction and identification of cis-natural antisense transcripts in *Arabidopsis thaliana*.** *Genome Biol* 2005, 6.

**28.** Mattick J, Makunin I: **Non-coding RNA.** *Hum Mol Genet* 2006, 15:R17 - R29.

**29.** Maxfield Boumil R, Lee JT: **Forty years of decoding the silence in X-chromosome inactivation.** *Hum Mol Genet* 2001, 10:2225-2232.

**30.** Swarbreck D, Wilks C, Lamesch P, Berardini T, Garcia-Hernandez M, Foerster H, Li D, Meyer T, Muller R, Ploetz L, Radenbaugh A, Singh S, Swing V, Tissier C, Zhang P, Huala E: The Arabidopsis Information Resource (TAIR): **gene structure and function annotation.** *Nucleic Acids Res* 2008, 36:D1009 - D1014.

**31.** Gardner M, Hubbard K, Hotta C, Dodd A, Webb A: **How plants tell the time.** *Biochemical J* 2006, 397:15 - 24.

**32.** Schultz TF, Kiyosue T, Yanovsky M, Wada M, Kay SA: **A role for LKP2 in the circadian clock of *Arabidopsis*.** *Plant Cell* 2001, 13:2659-2670.

**33.** Ding Z, Millar AJ, Davis AM, Davis SJ: ***TIME FOR COFFEE*** **encodes a nuclear regulator in the *Arabidopsis thaliana* circadian clock.** *Plant Cell* 2007, 19:1522-1536.

**34.** Covington M, Harmer S: **The circadian clock regulates auxin signaling and responses in Arabidopsis.** *PLoS Biol* 2007, 5:e222.

**35.** Michael TP, Mockler TC, Breton G, McEntee C, Byer A, Trout JD, Hazen SP, Shen R, Priest HD, Sullivan CM, Givan SA, Yanovsky M, Hong F, Kay SA, Chory J: **Network discovery pipeline elucidates conserved time-of-day-specific cis-regulatory modules.** *PLoS Genet* 2008, 4:e14.

**36.** Covington M, Maloof J, Straume M, Kay S, Harmer S: **Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development.** *Genome Biol* 2008, 9:R130.

**37.** **Arabidopsis Cyclome Expression Database [http://signal.salk.edu/cgi-bin/cyclome]**

**38.** Hicks KA, Millar AJ, Carre IA, Somers DE, Straume M, Meeks-Wagner DR, Kay SA: **Conditional circadian dysfunction of the *Arabidopsis early-flowering 3* mutant.** *Science* 1996, 274:790-792.

**39.** Hicks KA, Albertson TM, Wagner DR: ***EARLY FLOWERING3*** **encodes a novel protein that regulates circadian clock function and flowering in *Arabidopsis*.** *Plant Cell* 2001, 13:1281-1292.

**40.** The FANTOM Consortium, Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T, Lenhard B, Wells C, Kodzius R, Shimokawa K, Bajic VB, Brenner SE, Batalov S, Forrest ARR, Zavolan M, Davis MJ, Wilming LG, Aidinis V, Allen JE, Ambesi-Impiombato A, Apweiler R, Aturaliya RN, Bailey TL, Bansal M, Baxter L, Beisel KW, Bersano T *et al*: **The transcriptional landscape of the mammalian genome.** *Science* 2005, 309:1559-1563.

**41.** Nagasaki H, Arita M, Nishizawa T, Suwa M, Gotoh O: **Species-specific variation of alternative splicing and transcriptional initiation in six eukaryotes.** *Gene* 2005, 364:53-62.

**42.** Ner-Gaon H, Fluhr R: **Whole-genome microarray in *Arabidopsis* facilitates global analysis of retained introns.** *DNA Res* 2006, 13:111-121.

**43.** Ner-Gaon H, Halachmi R, Savaldi-Goldstein S, Rubin E, Ophir R, Fluhr R: **Intron retention is a major phenomenon in alternative splicing in *Arabidopsis*.** *Plant J* 2004, 39:877-885.

**44.** Staiger D: **RNA-binding proteins and circadian rhythms in *Arabidopsis thaliana*.** *Phil Trans R Soc B* 2001, 356:1755-1759.

**45.** Chapman EJ, Carrington JC: **Specialization and evolution of endogenous small RNA pathways.** *Nat Rev Genet* 2007, 8:884-896.

**46.** Valoczi A, Varallyay E, Kauppinen S, Burgyan J, Havelda Z: **Spatio-temporal accumulation of microRNAs is highly coordinated in developing plant tissues.** *Plant J* 2006, 47:140-151.

**47.** Xie Z, Allen E, Fahlgren N, Calamar A, Givan SA, Carrington JC: **Expression of Arabidopsis MIRNA Genes.** *Plant Physiol* 2005, 138:2145-2154.

**48.** Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP: **Prediction of Plant MicroRNA Targets.** *Cell* 2002, 110:513-520.

**49.** Teale WD, Paponov IA, Palme K: **Auxin in action: signalling, transport and the control of plant growth and development.** *Nat Rev Mol Cell Biol* 2006, 7:847-859.

**50.** Liu P-P, Montgomery T, Fahlgren N, Kasschau K, Nonogaki H, Carrington J: **Repression of *AUXIN RESPONSE FACTOR10* by *microRNA160* is critical for seed germination and post-germination stages.** *Plant J* 2007, 52:133-146.

**51.** Jones-Rhoades MW, Bartel DP: **Computational Identification of Plant MicroRNAs and Their Targets, Including a Stress-Induced miRNA.** *Molecular Cell* 2004, 14:787-799.

**52.** Wu M-F, Tian Q, Reed JW: **Arabidopsis *microRNA167* controls patterns of *ARF6* and *ARF8* expression, and regulates both female and male reproduction.** *Development* 2006, 133:4211-4218.

**53.** Levine E, Zhang Z, Kuhlman T, Hwa T: **Quantitative characteristics of gene regulation by small RNA.** *PLoS Biology* 2007, 5:e229.

**54.** Williams L, Carles CC, Osmont KS, Fletcher JC: **A database analysis method identifies an endogenous trans-acting short-interfering RNA that targets the *Arabidopsis ARF2*, *ARF3*, and *ARF4* genes.** *Proc Natl Acad Sci USA* 2005, 102:9703-9708.

**55.** Garcia D, Collier SA, Byrne ME, Martienssen RA: **Specification of leaf polarity in *Arabidopsis* via the trans-acting siRNA pathway.** *Curr Biol* 2006, 16:933-938.

**56.** Adenot X, Elmayan T, Lauressergues D, Boutet S, BouchÈ N, Gasciolli V, Vaucheret H: **DRB4-Dependent TAS3 trans-Acting siRNAs Control Leaf Morphology through AGO7.** *Curr Biol* 2006, 16:927-932.

**57.** Fahlgren N, Montgomery TA, Howell MD, Allen E, Dvorak SK, Alexander AL, Carrington JC: **Regulation of *AUXIN RESPONSE FACTOR3* by *TAS3* ta-siRNA affects developmental timing and patterning in *Arabidopsis*.** *Current Biology* 2006, 16:939-944.

**58.** Montgomery TA, Howell MD, Cuperus JT, Li D, Hansen JE, Alexander AL, Chapman EJ, Fahlgren N, Allen E, Carrington JC: **Specificity of *ARGONAUTE7-miR390* interaction and dual functionality in *TAS3* trans-acting siRNA formation.** *Cell* 2008, 133:128-141.

**59.** Nozue K, Covington M, Duek P, Lorrain S, Fankhauser C, Harmer S, Maloof J: **Rhythmic growth explained by coincidence between internal and external cues.** *Nature* 2007, 448:358 - 361.

**60.** Kiss TS: **Small nucleolar RNAs: an abundant group of noncoding RNAs with diverse cellular functions.** *Cell* 2002, 109:145-148.

**61.** Kramer C, Loros JJ, Dunlap JC, Crosthwaite SK: **Role for antisense RNA in regulating circadian clock function in *Neurospora crassa*.** *Nature* 2003, 421:948-952.

**62.** Perocchi F, Xu Z, Clauder-Munster S, Steinmetz LM: **Antisense artifacts in transcriptome microarray experiments are resolved by actinomycin D.** *Nucl Acids Res* 2007:gkm683.

**63.** Wu J, Du J, Rozowsky J, Zhang Z, Urban A, Euskirchen G, Weissman S, Gerstein M, Snyder M: **Systematic analysis of transcribed loci in ENCODE regions using RACE sequencing reveals extensive transcription in the human genome.** *Genome Biol* 2008, 9:R3.

**64.** Jin H, Vacic V, Girke T, Lonardi S, Zhu J-K: **Small RNAs and the regulation of cis-natural antisense transcripts in *Arabidopsis*.** *BMC Molecular Biology* 2008, 9:6.

**65.** Henz S, Cumbie J, Kasschau K, Lohmann J, Carrington J, Weigel D, Schmid M: **Distinct expression patterns of natural antisense transcripts in *Arabidopsis*.** *Plant Physiol* 2007, 144:1247 - 1255.

**66.** Stolc V, Samanta MP, Tongprasit W, Sethi H, Liang S, Nelson DC, Hegeman A, Nelson C, Rancour D, Bednarek S, Ulrich EL, Zhao Q, Wrobel RL, Newman CS, Fox BG, Phillips GN,

Markley JL, Sussman MR: **Identification of transcribed sequences in *Arabidopsis thaliana* by using high-resolution genome tiling arrays.** *Proc Natl Acad Sci USA* 2005, 102:4453-4458.

**67.** Riano-Pachon D, Dreyer I, Mueller-Roeber B: **Orphan transcripts in *Arabidopsis thaliana*: identification of several hundred previously unrecognized genes.** *Plant J* 2005, 43:205-212.

**68.** Kapranov P, Willingham A, Gingeras T: **Genome-wide transcription and the implications for genomic organization.** *Nat Rev Genet* 2007, 8:413 - 423.

**69.** Zhang X, Shiu S, Cal A, Borevitz JO: **Global analysis of genetic, epigenetic and transcriptional polymorphisms in *Arabidopsis thaliana* using whole genome tiling arrays.** *PLoS Genetics* 2008, 4:e1000032.

**70.** Bolstad BM, Irizarry RA, Astrand M, Speed TP: **A comparison of normalization methods for high density oligonucleotide array data based on variance and bias.** *Bioinformatics* 2003, 19:185-193.

**71.** Wijnen H, Naef F, Young MW, Michael WY: **Molecular and statistical tools for circadian transcript profiling.** In: *Methods in Enzymology.* vol. Volume 393: Academic Press; 2005: 341-365.

**72.** Nicholas J: CIRCSTAT: **Stata modules to calculate circular statistics.** *Boston College Department of Economics* 1998:S362501.

**Figure 1 - The *Arabidopsis* tiling arrays portray several interesting classes of circadian behavior in the genome.**

Each symbol is a feature on the tilling array showing location in the genome (x-axis) and significance of the spectral analysis (y-axis) for (A) *LUX ARRHYTHMO* (B) *CIRCADIAN CLOCK ASSOCIATED1*, (C) *LATE ELONGATED HYPOCOTYL*, and (D) *EARLY FLOWERING3*. The top half of each panel displays the Watson strand and the bottom half the Crick strand. Individual features that exceed the FDR 5% p-value threshold (-) are considered to have a circadian rhythm.

**Figure 2 - The *Arabidopsis* tiling arrays portray several interesting classes of circadian behavior in the genome.**

Each symbol is a feature on the tilling array showing location in the genome (x-axis) and significance of the spectral analysis (y-axis) for (A) *EARLY FLOWERING4* (B) *TIMING OF CAB2 EXPRESSION1*, (C) *PSEUDO RESPONSE REGULATOR5*, and (D) *PSEUDO RESPONSE REGULATOR3*. The top half of each panel displays the Watson strand and the bottom half the Crick strand. Individual features that exceed the FDR 5% p-value threshold (-) are considered to have a circadian rhythm.

**Figure 3 - The *Arabidopsis* tiling arrays portray several interesting classes of circadian behavior in the genome.**

Each symbol is a feature on the tilling array showing location in the genome (x-axis) and significance of the spectral analysis (y-axis) for (A) *PSEUDO RESPONSE REGULATOR7* (B) *PSEUDO RESPONSE REGULATOR9*, (C) *LOV KELCH PROTEIN2*, and (D) *ZEITLUPE*. The top half of each panel displays the Watson strand and the bottom half the Crick strand. Individual features that exceed the FDR 5% p-value threshold (-) are considered to have a circadian rhythm.

**Figure 4 - The *Arabidopsis* tiling arrays portray several interesting classes of circadian behavior in the genome.**

Each symbol is a feature on the tilling array showing location in the genome (x-axis) and significance of the spectral analysis (y-axis) for (A) *FLAVIN-BINDING KELCH DOMAIN F BOX PROTEIN1* (B) *GIGANTEA*, (C) *TIME FOR COFFEE*, and (D) *CONSTANS LIKE2*. The top half of each panel displays the Watson strand and the bottom half the Crick strand. Individual features that exceed the FDR 5% p-value threshold (-) are considered to have a circadian rhythm.

**Figure 5 - The *Arabidopsis* tiling arrays portray several interesting classes of circadian behavior in the genome.**

Each symbol is a feature on the tilling array showing location in the genome (x-axis) and significance of the spectral analysis (y-axis) for (A) *PHOSPHATE STARVATION RESPONSE1* (B) *MIR167*, (C) *TRANS-ACTING siRNA3*, and (D) transfrag-5-6839029. The top half of each panel displays the Watson strand and the bottom half the Crick strand. Individual features that exceed the FDR 5% p-value threshold (-) are considered to have a circadian rhythm.

**Figure 6 - Different types of transcripts and transcription units have variable phase distributions across the day as well as within a locus.**

(A) Relative phase frequency distribution of cycling sense and antisense transcript phase. (B) Scatter plot of the expression phases of loci with both sense and antisense strand cycling transcripts. (C) Relative phase frequency distribution of cycling sense strand and antisense strand introns and intergenic transcript phase. (D) Scatter plot of the expression phases of transcripts and their cycling introns.

**Table 1. *Arabidopsis* genome and AtTILE1 array annotation data. Annotation units receiving consideration had at least two unique corresponding array features. Values in parenthesis are the number of transcripts with a single corresponding feature.**

| Annotation | TAIR7* | AtTILE1 | CCGs** |
|---|---|---|---|
| Protein coding | 27,029 | 25,677 | 6,269 |
| pseudogenes or TE | 3,889 | 2,863 | 81 |
| Non-coding RNAs | 1,123 | | |
| Micro RNA | 114 | 62(30) | 6 |
| Small nucleolar RNA | 71 | 17(29) | 1 |
| Small nuclear RNA | 13 | 0 | nd |
| Pre-transfer RNA | 631 | 2(129) | 0 |
| Ribosomal RNA | 4 | 0 | nd |
| Other | 221 | 121(29) | 15 |
| Total | 32,041 | | 6.372 |

*The *Arabidopsis* Information Resource (TAIR) version 7 genome annotation [30].
**Circadian clock regulated genes

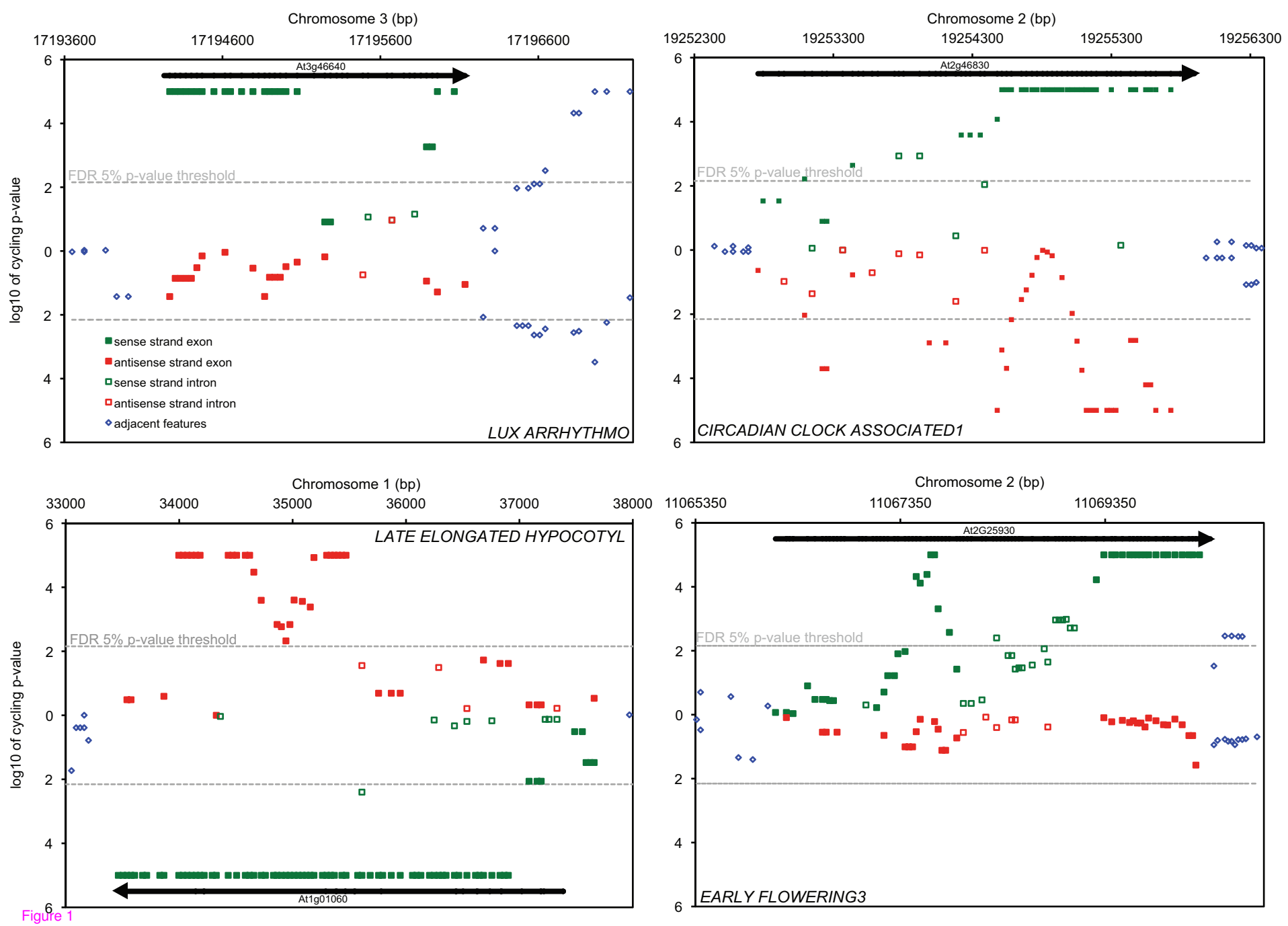
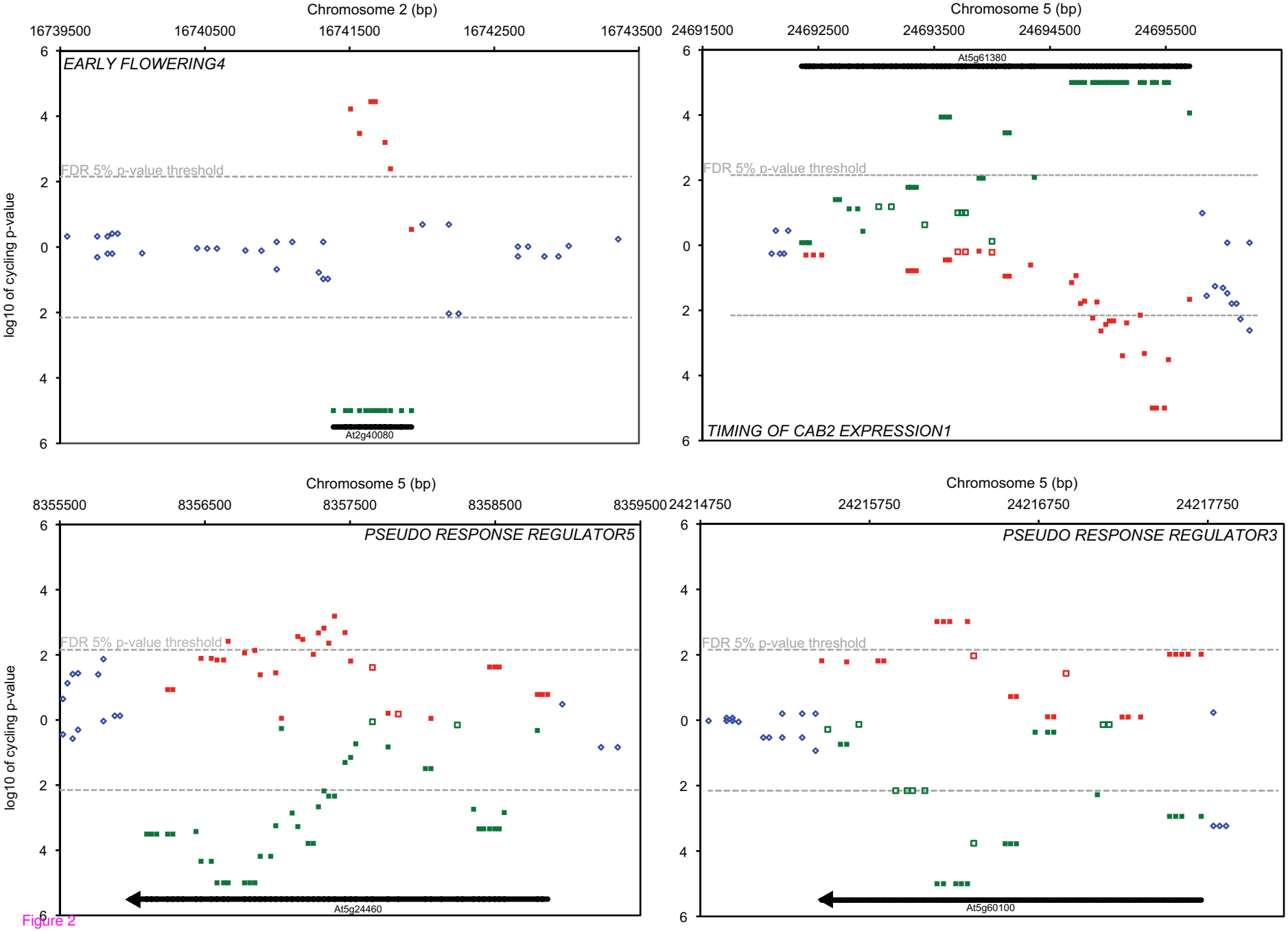

Chromosome 3 (bp)
17193600
17194600
17195600
17196600
At3g46640
FDR 5% p-value threshold
log10 of cycling p-value
sense strand exon
antisense strand exon
sense strand intron
antisense strand intron
adjacent features
LUX ARRHYTHMO
Chromosome 2 (bp)
19252300
19253300
19254300
19255300
19256300
At2g46830
FDR 5% p-value threshold
CIRCADIAN CLOCK ASSOCIATED1
Chromosome 1 (bp)
33000
34000
35000
36000
37000
38000
LATE ELONGATED HYPOCOTYL
FDR 5% p-value threshold
log10 of cycling p-value
At1g01060
Chromosome 2 (bp)
11065350
11067350
11069350
At2G25930
FDR 5% p-value threshold
EARLY FLOWERING3

Figure 1


Chromosome 2 (bp)
16739500
16740500
16741500
16742500
16743500
EARLY FLOWERING4
FDR 5% p-value threshold
At2g40080
log10 of cycling p-value
Chromosome 5 (bp)
24691500
24692500
24693500
24694500
24695500
At5g61380
FDR 5% p-value threshold
TIMING OF CAB2 EXPRESSION1
Chromosome 5 (bp)
8355500
8356500
8357500
8358500
8359500
PSEUDO RESPONSE REGULATOR5
FDR 5% p-value threshold
At5g24460
log10 of cycling p-value
Chromosome 5 (bp)
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24215750
24216750
24217750
PSEUDO RESPONSE REGULATOR3
FDR 5% p-value threshold
At5g60100

Figure 2

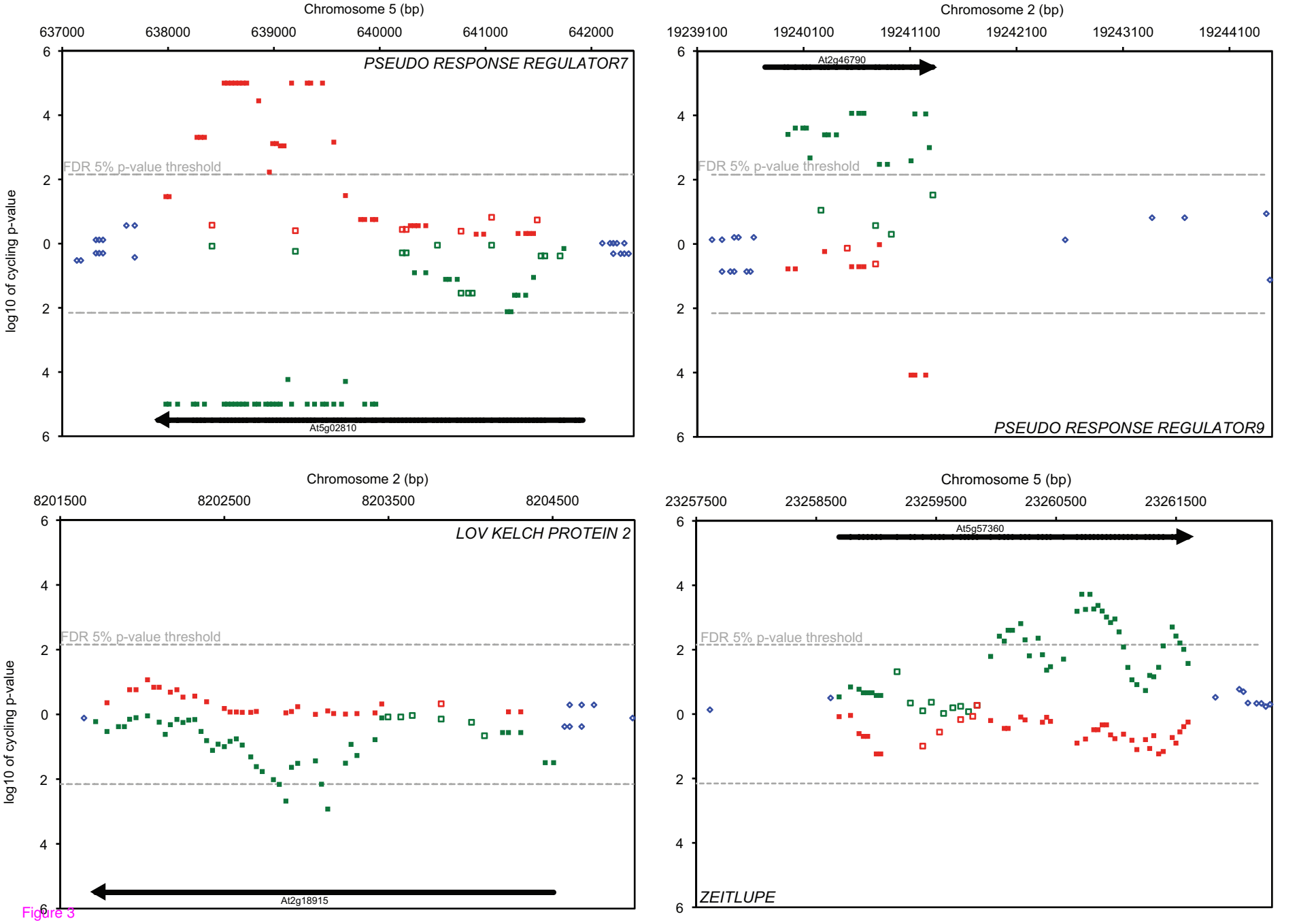
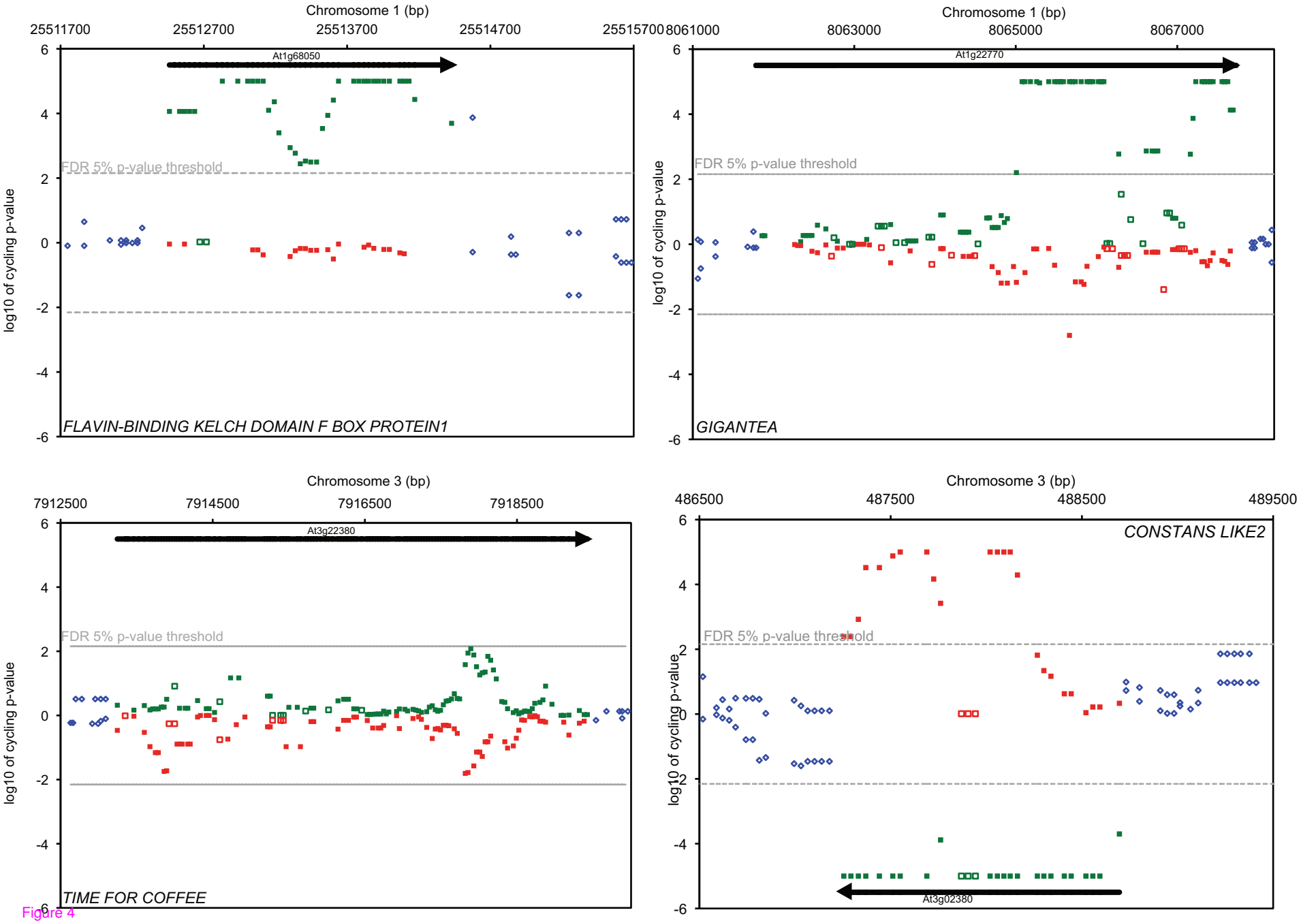

Chromosome 5 (bp)
637000
638000
639000
640000
641000
642000
PSEUDO RESPONSE REGULATOR7
FDR 5% p-value threshold
log10 of cycling p-value
At5g02810
Chromosome 2 (bp)
19239100
19240100
19241100
19242100
19243100
19244100
At2g46790
FDR 5% p-value threshold
PSEUDO RESPONSE REGULATOR9
Chromosome 2 (bp)
8201500
8202500
8203500
8204500
LOV KELCH PROTEIN 2
FDR 5% p-value threshold
log10 of cycling p-value
At2g18915
Chromosome 5 (bp)
23257500
23258500
23259500
23260500
23261500
At5g57360
FDR 5% p-value threshold
ZEITLUPE

Figure 3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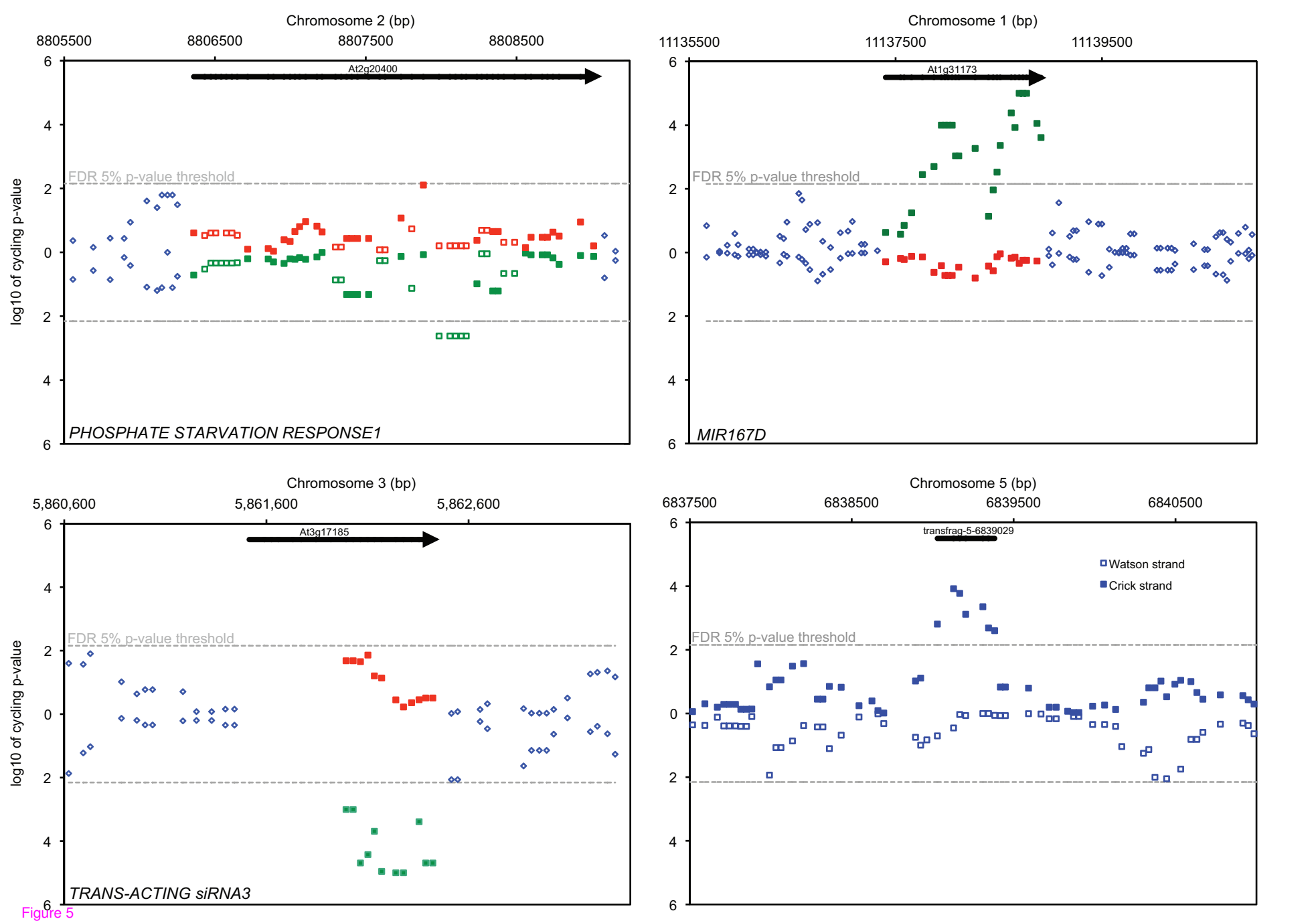
25512700
25513700
25514700
25515700
At1g68050
FDR 5% p-value threshold
log10 of cycling p-value
FLAVIN-BINDING KELCH DOMAIN F BOX PROTEIN1
Chromosome 1 (bp)
8061000
8063000
8065000
8067000
At1g22770
FDR 5% p-value threshold
log10 of cycling p-value
GIGANTEA
Chromosome 3 (bp)
7912500
7914500
7916500
7918500
At3g22380
FDR 5% p-value threshold
log10 of cycling p-value
TIME FOR COFFEE
Chromosome 3 (bp)
486500
487500
488500
489500
CONSTANS LIKE2
FDR 5% p-value threshold
log10 of cycling p-value
At3g02380

Figure 4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8806500
8807500
8808500
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log10 of cycling p-value
PHOSPHATE STARVATION RESPONSE1
Chromosome 1 (bp)
11135500
11137500
11139500
At1g31173
FDR 5% p-value threshold
MIR167D
Chromosome 3 (bp)
5,860,600
5,861,600
5,862,600
At3g17185
FDR 5% p-value threshold
log10 of cycling p-value
TRANS-ACTING siRNA3
Chromosome 5 (bp)
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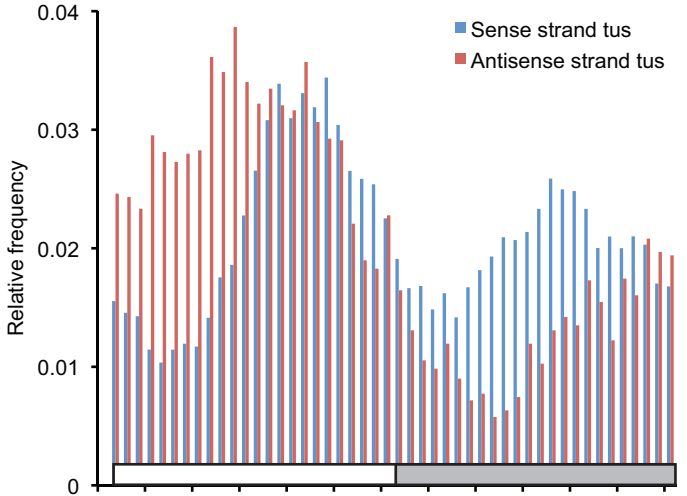
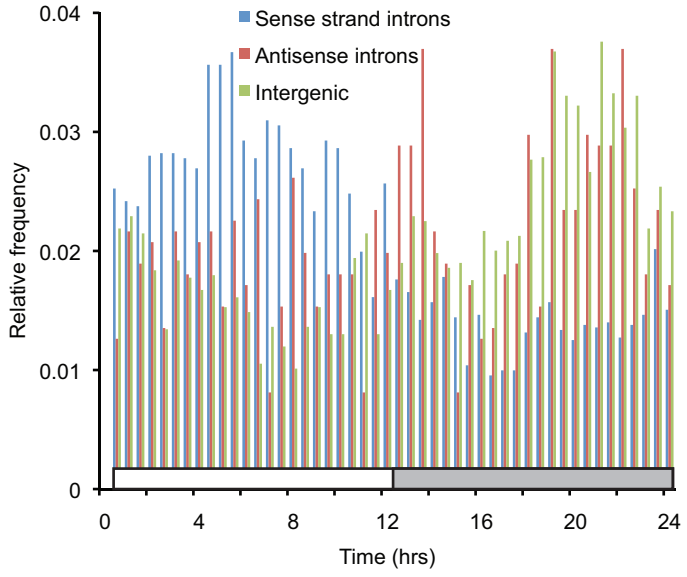
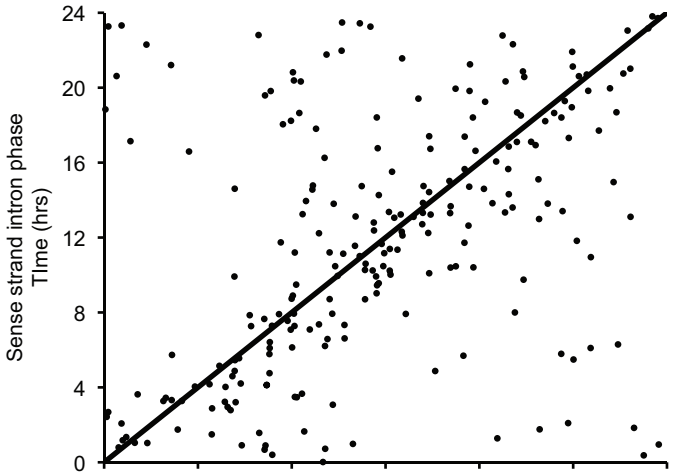
6838500
6839500
6840500
transfrag-5-6839029
Watson strand
Crick strand
FDR 5% p-value threshold

Figure 5


Sense strand tus
Antisense strand tus
Relative frequency
0.04
0.03
0.02
0.01
0


Sense strand introns
Antisense introns
Intergenic
Relative frequency
0.04
0.03
0.02
0.01
0
0
4
8
12
16
20
24
Time (hrs)


Sense strand intron phase
TIme (hrs)
24
20
16
12
8
4
0

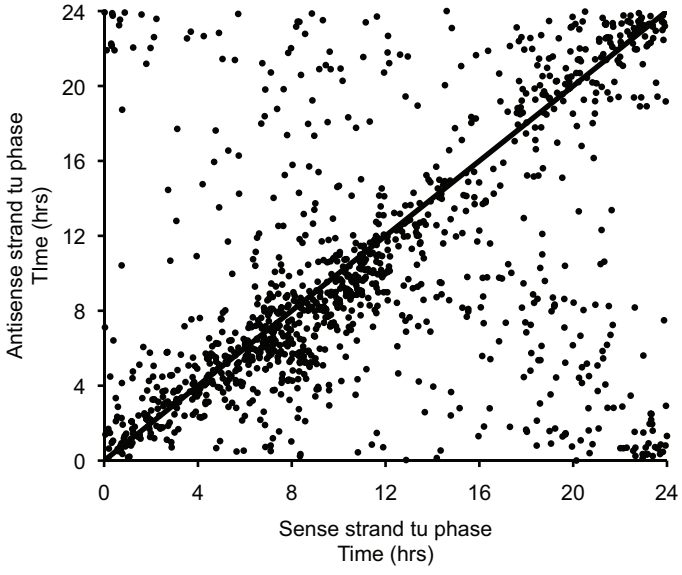

Antisense strand tu phase
TIme (hrs)
24
20
16
12
8
4
0
0
4
8
12
16
20
24
Sense strand tu phase
Time (hrs)

Figure 6

**Additional files provided with this submission:**

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