Tradict enables compressive sampling and high fidelity reconstruction of the eukaryotic transciptome

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**Abstract**

**Introduction**

As the critical determinant of the proteome and therefore cellular status, the transcriptome represents a key node of regulation for all life1. Transcriptional control is managed by a finely tuned network of transcription factors that integrate environmental and developmental cues in order to actuate the appropriate responses in gene expression2–4. Importantly, the transcriptomic state space is constrained. Pareto optimality suggests that no gene expression profile or phenotype can be optimal for all tasks, and consequently, that some expression profiles or phenotypes must come at the expense of others5,6. Furthermore, across all major studied kingdoms of life, metabolic networks demonstrate remarkably conserved scale-free properties that are topologically characterized by a small minority of highly connected regulatory nodes that link the remaining majority of sparsely connected nodes to the network7–9. These theories suggest that the effective dimension of the transcriptome should be far less than the total number of genes it contains. If true to a large enough extent, it may be possible to faithfully compress and prospectively reconstruct the entire transcriptomes using only a small, carefully chosen subset of it.

Indeed, previous studies have exploited this reduced dimensionality to perform gene expression imputation for microarray data for missing or corrupted values10–12. Others have extended these intuitions to predict expression from probe sets containing a few hundred genes13,14. However, prediction accuracies have been modest and usually limited to 4,000 target probes/genes. Recently, several studies examined the transcriptomic information recoverable by shallow sequencing especially as it applies to single-cell experiments15–18. Jaitin *et al.* (2014) and Pollen *et al.* (2014) demonstrated only tens of thousands of reads are required per cell to learn and classify cell types *ab initio*16,18. Heimberg *et al.* (2016) extended these intuitions and showed the major principal components of a typically sequenced mouse bulk or single-cell expression dataset are estimatable with little error at even 1% of the depth15. However, though these approaches advance the notion of strategic transcriptome undersampling, they only recover broad transcriptional states and are restricted to measuring only the most abundant genes. During sample preparation -- arguably the most expensive cost of a multiplexed sequencing experiment -- shallow sequencing based approaches still utilize protocols meant for sampling the entire transcriptome and therein consume more resources than necessary. Furthermore, given expression of even the most abundant genes is highly skewed, sequencing effort is wastefully distributed compared to an approach that chooses which genes to measure more wisely. Finally, it is still not clear from sample sizes and biological contexts previously studied whether the low dimensionality of the transcriptome may be leveraged unconditionally (or nearly so) across organism and application.

In this work, we introduce Tradict (transcriptome predict), a novel, robust-to-noise, and probabilistically sound algorithm for inferring the expression of all transcriptional programs and genes in the transcriptome using only the expression measurements of a single, context-independent, machine-learned subset of 100 marker genes. Using a transcriptionally representative sampling of over 23,000 publicly available, transcriptome-wide RNA-Seq datasets for *Arabidopsis thaliana* and *Mus musculus*, we train Tradict to prospectively reconstruct gene expression, and to predict, to near perfection, the expression of a comprehensive, but quickly interpretable collection of transcriptional programs. Our work demonstrates, for the first time, the development and large-scale application of a multivariate count/non-negative data model, and highlights the power of directly modeling the expression of transcriptional programs in a supervised manner. We believe Tradict, coupled with targeted RNA sequencing, can improve the time and cost of performing large forward genetic, breeding, or chemogenomic screens, accurately profiling single-cells, and performing gene expression based clinical diagnostics.

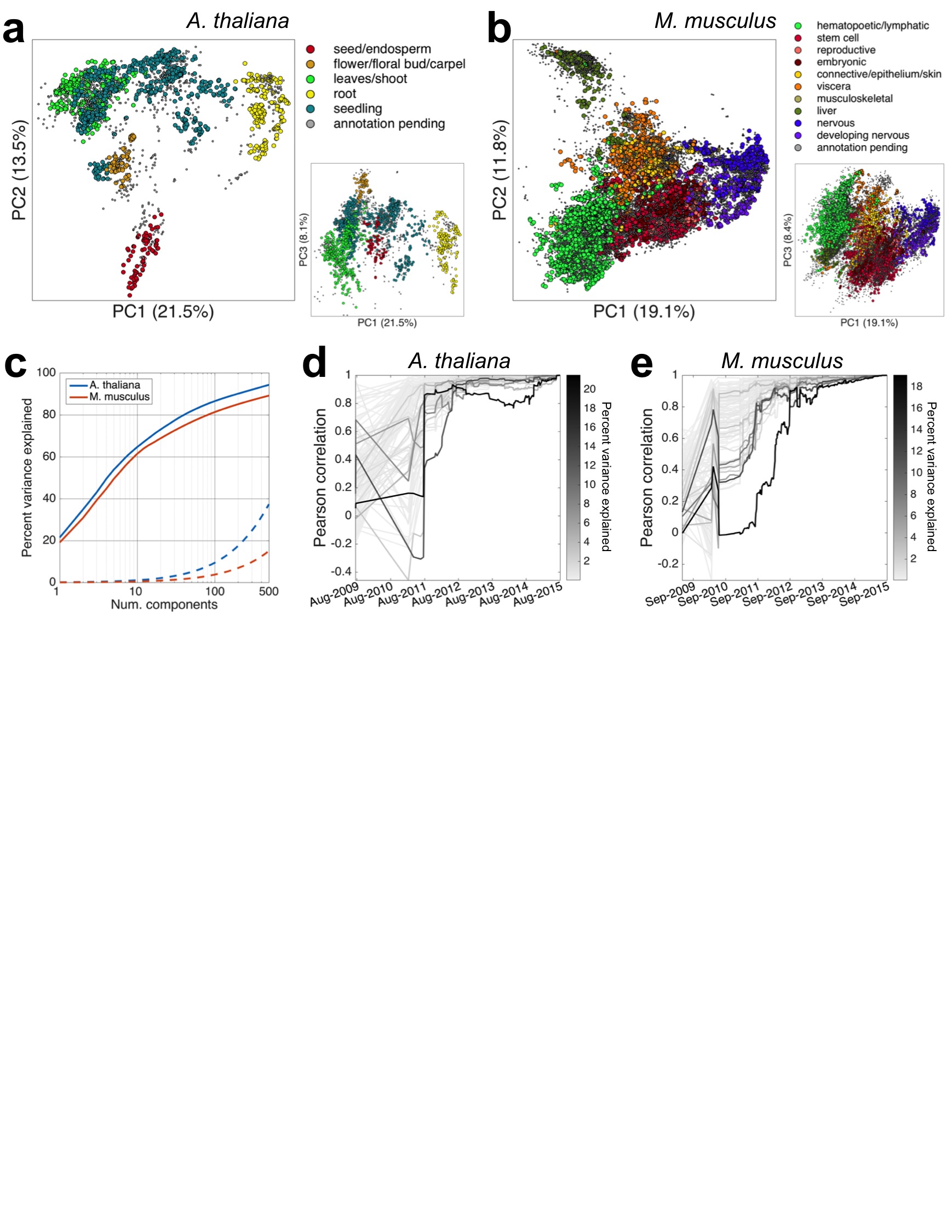
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Figure 1. The primary drivers of variation in our training transcriptome collection are developmental stage and tissue. a) *A. thaliana*, b) *M. musculus*.

**Results**

**Assembly of a comprehensive training collection of transcriptomes**

We attempted to download all available Illumina sequenced publicly deposited RNA-Seq samples (transcriptomes) on NCBI’s Sequence Read Archive (SRA). Among samples with at least 4 million reads, we successfully downloaded and quantified the raw sequence data of 3,621 and 27,450 transcriptomes for *A. thaliana* and *M. musculus,* respectively. After stringent quality filtering, we retained 2,597 (71.7%) and 20,847 (76.0%) transcriptomes comprising 225 and 732 unique SRA submissions for *A. thaliana* and *M. musculus*, respectively. An SRA ‘submission’ consists of multiple, experimentally linked samples submitted concurrently by an individual or lab. We defined 21,277 (*A. thaliana*) and 21,176 (*M. musculus)* measurable genes with reproducibly detectable expression given our tolerated minimum sequencing depth and mapping rates. See Supplemental Information “Materials and Methods” for further information regarding data acquisition, transcript quantification, quality filtering, and expression filtering. We hereafter refer to the collection of quality and expression filtered transcriptomes as our *training transcriptome collection*.

In order to assess the quality and comprehensiveness of our training collection, we performed a deep characterization of the expression spaced spanned by these transcriptomes. We found that the transcriptome of both organisms was highly compressible and that the primary drivers of variation were tissue and developmental stage (Figure 1a-b, Figure S1), with many significant, biologically realistic trends within each cluster (Supplemental Note 1). We additionally examined the distribution of submissions across the expression space, compared inter-submission variability within and between tissues, inspected expression correlations among genes with well-established regulatory relationships, and assessed the evolution of the expression space across time. These investigations revealed our training collection is of high and reproducible technical quality, reflective of known biology, and stable (Supplemental Note 1, Figures S2-S4). Given additionally the diversity of tissues, genetic perturbations, and environmental stimuli represented in the SRA, these results, taken together, suggest that our training collection is an accurate and representative sampling of the transcriptomic state space that is of experimental interest for both organisms.

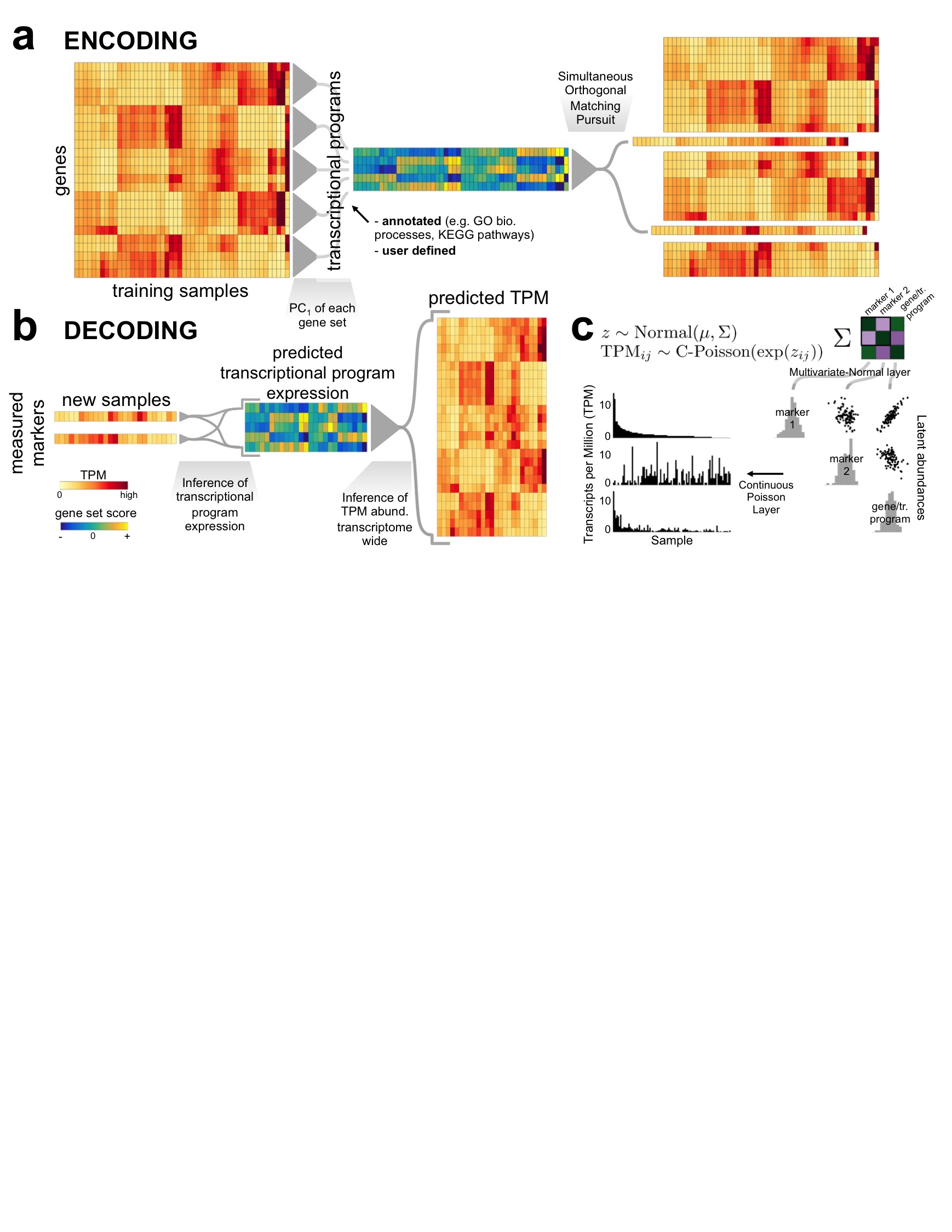


Figure 2. Tradict’s algorithmic workflow. a) During encoding the transcriptome is first quantitatively summarized in terms of a collection of a few hundred, biologically comprehensive transcriptional programs. These are then decomposed into a subset of marker genes using an adaptation of the Simultaneous Orthogonal Matching Pursuit algorithm, and a Multivariate Normal Continuous-Poisson hierarchical model is used as a predictive model to capture covariance relationships between markers, transcriptional programs, and non-markers. b) During decoding, Tradict predicts the expression of transcriptional programs and all genes in the transcriptome using only expression measurements of the marker genes. c) The Multivariate Normal Continuous-Poisson hierarchy enables Tradict to efficiently model statistical coupling between the non-negative expression measurements typical of sequencing. This is done by assuming that associated with each observed, noisy TPM measurement, there is an unmeasured, denoised latent abundance the logarithm of which comes from a Multivariate Normal distribution over all genes and transcriptional programs.

**Tradict - algorithm overview**

Given a training sample of transcriptomes, Tradict encodes the transcriptome into a single subset of globally representative *marker* genes and learns their predictive relationship to the expression of a comprehensive collection of transcriptional programs (e.g. pathways, biological processes) and to the rest of the transcriptome. Tradict’s key innovation lies in using a Multivariate Normal Continuous-Poisson (MVN-CP) hierarchical model to model marker latent abundances -- rather than their measured, noisy abundances -- jointly with the expression of transcriptional programs, and ultimately, the latent abundances of the remaining non-marker genes in the transcriptome. In so doing, Tradict is able to 1) efficiently capture covariance structure within the non-negative, right-skewed output typical of sequencing experiments, and 2) perform robust inference of gene set and non-marker expression even in the face of extreme noise.

Figure 2 illustrates Tradict’s general workflow. Estimates of expression are noisy, especially for low to moderately expressed genes. Given samples are often explored unevenly and that the *a priori* abundance of each gene differs, the level of noise in a gene’s measured expression for a given sample varies, but it is estimatable. Therefore, during training, Tradict first learns the log-latent, denoised abundances for each gene in every sample in the training collection using the lag transformation19. It then collapses this latent transcriptome into a collection of predefined, globally comprehensive collection of *transcriptional programs* that represent the major processes and pathways of the cell related growth, development, and response to the environment. In this work, we focus on creating a Gene Ontology derived panel of transcriptional programs, in which the first principal component of all genes contained within an appropriately sized and representative GO term is used to define an accordingly named transcriptional program20,21. The expression values of these programs are then encoded using an adapted version of the Simultaneous Orthogonal Matching Pursuit into a small subset of marker genes selected from the transcriptome22,23. Tradict finally stores the mean and covariance relationships between the log-latent expression of the selected markers, the transcriptional programs, and the log-latent expression of the remaining non-marker genes at the Multivariate Normal layer of the underlying MVN-CP hierarchical model for use in future decoding (Figure 2a).

Prospectively, only the expression of these marker genes needs to be measured and the expression of transcriptional programs and/or the rest of the transcriptome can be inferred as needed. During this process of decoding, Tradict first utilizes an iterative Bayesian updating algorithm to learn the log-latent abundances associated with each measured maker for every sample. Though a simply a consequence of proper inference of our model, this denoising step adds considerable robustness to Tradict’s predictions. Tradict then uses the covariance relationships learned during training to formulate a prediction for the expression of transcriptional programs and the most likely expression values for all remaining non-marker genes (Figure 2b).

**Tradict prospectively predicts unseen transcriptomes and transcriptional programs with robust accuracy**

[Error increases in decreasing mean expression]

An SRA submission consists of multiple, experimentally linked samples submitted concurrently by an individual or lab. Consequently, high *intra*-submission prospective prediction accuracy is most indicative a method’s performance.

To more completely understand Tradict’s prospective predictive performance, we performed 20-fold cross validation on the training transcriptome collections for both *A. thaliana* and *M. musculus* and evaluated Pearson correlation coefficients (PCC) between predicted and actual expression for each fold when the remaining 95% of folds were used for training. To make this experiment as reflective of reality as possible, folds were divided by submission so that samples from the same set of experiments would not appear both in training and test sets. Because submissions to the SRA span a broad array of biological contexts, the total biological signal contained in the test set exceeds that of what would be expected for typical application, which in turn would lead to overly optimistic estimates of prediction accuracy. To therefore evaluate *intra-submission* accuracy, PCC calculations were performed on ‘submission-adjusted’ expression values in which each submission’s mean expression was subtracted from the expression values of all associated samples.

Figures 3c and 3e illustrate that the reconstruction performance for transcriptional programs in both organisms is strikingly accurate across all collected submissions. Quantitatively speaking, the average intra-submission PCCs for transcriptional programs are 0.94 and 0.93 for *A. thaliana* and *M. musculus*, respectively. This is despite lower, but still accurate prediction performance on gene expression (Figures 3d and 3f). Intuitively, this because transcriptional programs are measured as as linear combinations of the log-latent TPMs of the genes that comprise them, effectively smoothing over the orthogonal noise present in each gene’s expression prediction. Prediction accuracy was negatively correlated with mean expression both of genes and transcriptional programs, consistent with the findings of Donner *et al.* 2012.

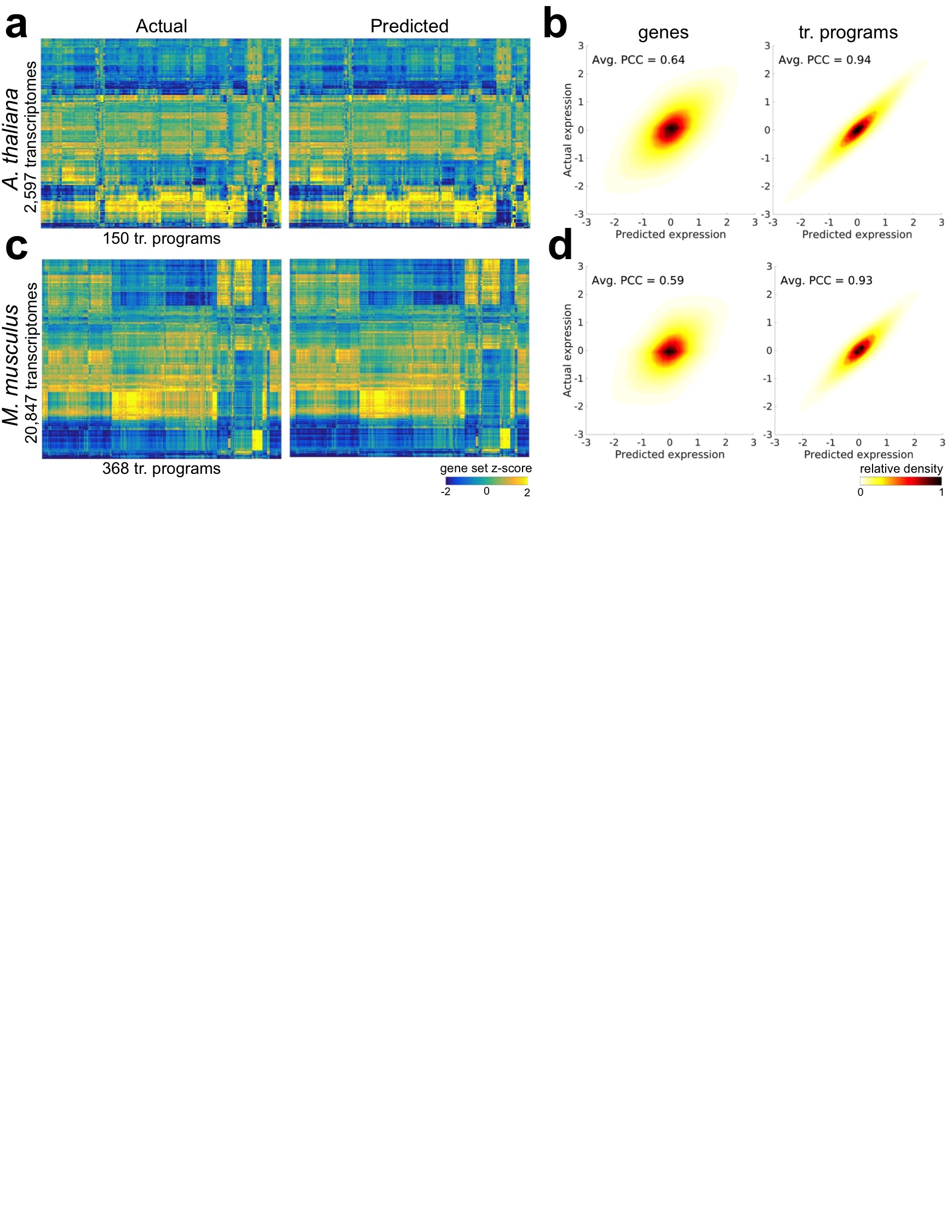


Figure 3. Tradict prospectively predicts unseen transcriptomes with robust accuracy. c-f) Tradict’s prospective prediction accuracy during 20-fold cross validation of the entire training collection for both organisms. c) Heatmaps illustrating test-set reconstruction performance of all transcriptional programs for *A. thaliana*. d) Density plots of predicted vs. actual test-set expression for all genes (left) and transcriptional programs (right) for *A. thaliana*, after controlling for inter-sumbission biological signal. The intra-submission expression of each gene and transcriptional program was z-score transformed to make their expression dynamics comprarable. e & f) Same as c & d, but for *M. musculus*.

Need marker lists in supplemental

**Case studies reveal the power of predicting and studying predefined transcriptional programs**

To better contextualize how Tradict may be applied in practice, we focused on two case studies related to innate immune signaling -- one in *A. thaliana*, and the other in *M. musculus*. We trained Tradict on our full collection of training transcriptomes for each organism to produce two organism-specific Tradict models. Each was based on the selection of 100 markers that we assert are globally representative, and context-independent. The case study samples do not, of course, appear in the collection of training transcriptomes.

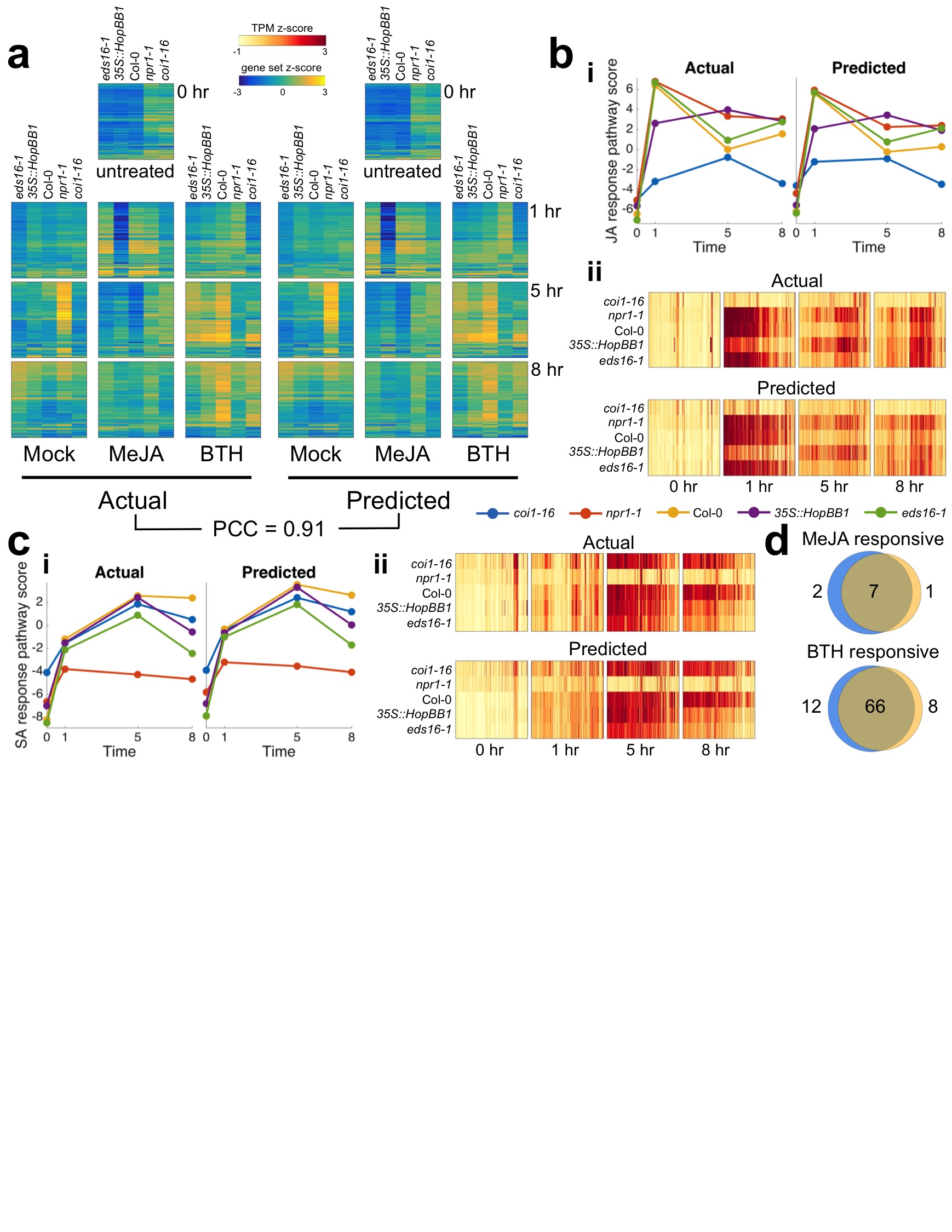


Figure 4. Tradict accurately predicts transcriptional responses across time in response to hormone perturbation in an *A. thaliana* innate immune signaling dataset. After being trained on the full *A. thaliana* training transcriptome collection, the selected set of 100 globally representative and context-independent markers were used to predict the expression of transcriptional programs and all genes for the transcriptomes presented in Yang *et al.* (2016). a) Actual vs. predicted heatmaps for the expression of all 150 transcriptional programs in *A. thaliana* across genotype, time, and hormone treatment. b) Predicted vs. actual expression of i) the JA response transcriptional program, and ii) the genes involved in the JA response program. c i-ii) Same as b, but for the SA response transcriptional program. d) Hypothesis free, differential transcriptional program expression analysis as performed on the actual expression of transcriptional programs vs those predicted by Tradict. Blue circles represent the actual and orange represent the predicted. All heatmaps are clustered in the same order across time, treatment, genotype, and between predicted and actual.

Change “JA response pathway score” to “JA response”

**Tradict accurately predicts temporal transcriptomic expression patterns for a diverse panel of immune signaling mutants under different hormone perturbations -** The hormones salicylic acid (SA) and jasmonic acid (JA) play a major, antagonistic regulatory role in the activation of plant defense responses to pathogens. Yang *et al.* (2016) investigate the effect of a transgenically expressed bacterial effector, HopBB1, on JA signaling in *A. thaliana*24*.* In their study, they perform a time course experiment, treating plants with MeJA (a JA response inducer), BTH (an SA mimic and SA response inducer), or mock buffer and monitoring the transcriptome of bulk seedlings at 0 hr, 1 hr, 5 hr, and 8 hr post treatment. Importantly, their experiments include several immune signaling mutants with differing degrees of response efficiency to MeJA and BTH treatment. Among other findings, they conclude that HopBB1 enhances the JA response, thereby repressing the SA response and facilitating pathogen infection.

We asked to what extent reduced sampling of the transcriptome and application of Tradict could quantitatively recapitulate the findings of Yang *et al.* (2016). Given Tradict’s near perfect accuracy on predicting the expression of transcriptional programs, we took a top down, but hypothesis driven approach to our analysis which first examined the expression of transcriptional programs. Figure 4a illustrates the actual and predicted expression all transcriptional programs in *A. thaliana* as a function of time and treatment. Here, Tradict delivers a slightly below-average performance, but is able to reconstruct the expression of all transcriptional programs with an average PCC of 0.91.

Recall the genes participating in each of our transcriptional programs are pre-defined, in this work, by a carefully chosen, interpretable, but maximally representative GO biological processes. Therefore, given the goals of this study, we next examined the expression of the “response to jasmonic acid” and “response to salicylic acid” transcriptional programs. Figure 4b shows the expression behavior for the “response to jasmonic acid” transcriptional program across all the genotypes and time points upon MeJA treatment. More specifically, part (i) shows that the predicted expression and actual expression are qualitatively and quantitatively in agreement, both in magnitude and rank across the different genotypes. For example, as expected, *coi1-16*, which cannot sense JA, does not respond to the MeJA stimulus, while wildtype Col-0 does. However, even more subtle expression dynamics are captured by Tradict’s predictions. For example, *eds16-1* and *npr1-1 --* slightly and strongly impaired SA responders, respectively -- are slightly and strongly hyper-responsive to MeJA, respectively -- just as expected from the JA-SA antagonism. Furthermore, as demonstrated in Yang *et al.* (2016), the *35S::HopBB1* transgenic line demonstrates a prolonged and sustained JA response for both the actual and predicted expression for this transcriptional program. Part (ii) of Figure 4b illustrates the expression of all the MeJA responsive genes in this transcriptional program. Again Tradict’s predictions are in agreement with actuality, achieving a PCC of 0.72, and it’s visually clear that the expression magnitude of these genes positively correlates with the registered expression magnitude of the “response to jasmonic acid” transcriptional program. Figure 4c parts (i) and (ii) are presented in the same light as Figure 4b, but are instead illustrated for the SA response transcriptional program and constituent genes under BTH treatment. Again predictions match actuality, and the observed trends make biological sense [REF].

In order to illustrate Tradict’s use in hypothesis-free investigation, we performed a differential transcriptional program expression analysis for transcriptional programs affected by MeJA or BTH treatment (Figure 4d, see Methods). Differentially expressed transcriptional programs based on Tradict’s predictions versus actual measurements were highly concordant and biologically reasonable. Transcriptional programs differentially expressed with respect to MeJA treatment included “response to bacterium,” “defense response to fungus”, “response to wounding,” and “response to jasmonic acid” as expected. Transcriptional programs differentially expressed with respect to BTH treatment included various abiotic stress responses, “defense response to fungus”, “response to jasmonic acid” (via antagonism), and “response to salicylic acid,” again, as expected.

**Discussion**

[pending]

**Conclusion**

[pending]

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