Modelling Transcriptional Networks in Plant Senescence

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Abstract

During natural senescence of Arabidopsis thaliana, a number of phenotypical changes can be seen as the plant tries to reabsorb nutrients from ageing leaves. Very little, however, is known about the gene interactions during senescence. A large quantity of highly replicated microarray timecourse data has been collected from leaves of Arabidopsis which will make it possible to quantify these interactions. MAANOVA makes it possible to analyse this microarray data and obtain a normalised data set over the timecourse which contains no undesirable variability. Variational Bayes State Space Modelling can then identify likely between gene interactions from select lists of genes. The result will be a network map within which some genes act as hubs. These genes can be verified by experimental testing, using over and under expressor mutants. Using these methods will allow us to develop global models for transcriptional networks in the senescing leaf to elucidate the crosstalk between signalling pathways.

1. The Experiment

- Warwick HRI have collected a large quantity of data from a highly replicated complex time course series analysed on 2-dye CATMA microarrays produced on site.
- By developing an alternative system to analyse this data (MAANOVA), the most can be made of the 2-dyes.
- Leaf 7 of Arabidopsis thaliana plants kept in 16 hour long day conditions was tagged with loose cotton soon after emergence.
- 4 samples (entire of leaf 7) were collected morning and evening every other day for 20 days following the initial 19 days of growth.
- Each sample is present 4 times across the experiment (twice tagged with Cy3 and twice tagged with Cy5).

2. Aims

- Produce an analysis pipeline for 2-dye arrays.
- Find genes involved in plant senescence.
- Identify interactions between them.
- Produce and verify accurate network models of these genes.

3. Planning

- Use MAANOVA to test quality and normalise data and to select genes with significant expression changes over time.
- Use SplineCluster to find similar expression profiles between genes.
- Use Variational Bayes State Space Modelling to quantify interactions.

4. Why MAANOVA?

- Most systems of microarray analysis work best with reference samples and one-dye arrays.
- To harness the power of two-dye arrays, the relationship between samples on a slide need to be considered, whereas most systems take a ratio of the dyes, losing valuable data.
- MAANOVA uses the experimental design to find links between samples and keep expression data separate.

5. SplineCluster

- Significantly changing genes are clustered using SplineCluster.
- SplineCluster has been used regularly in the past and provides excellent results.
- Where genes lie in the same cluster, they are likely to be co-regulated and as such are selected for network modelling together.

6. VBSSM

- Network modelling is performed on selected clusters.
- Known senescence related genes are also included.
- VBSSM identifies likely interactions between genes.
- A package called Cytoscape displays these visually.

7. Results

- Genes identified by MAANOVA are showing interesting expression patterns such as that shown to the left.
- These genes are producing believable networks such as that shown below.

8. Future Work

- Further genes will be selected from clusters to find interactions between them.
- The interaction maps will identify which genes are the controlling factors during senescence.
- These hubs will be tested using under and over expressor mutants.
- Expression of downstream genes can be checked using techniques such as qPCR.
- This information will provide priors for further models of VBSSM.

References

- Kerr, M., Martin, M., Churchill, G., Analysis of Variance for Gene Expression Microarray Data, 2000, The Jackson Laboratory, Maine, USA.