



Gene Networks

Reversed Engineering (data-driven) of Gene (Regulatory) Networks from Expression Data



Gene Networks

Introduction

- When a comprehensive gene expression data set representing a large number of perturbations is made available, the reversed engineering of gene regulatory networks becomes a logical step towards the elucidation of biological pathways of interest.
- While developmental (ie. Time series) experiments provide the ideal framework, Basso et al (2005; Nature Genetics, 37:382) showed that, with the right mathematical approach, a large number of perturbations can also do the trick.
- Barabasi & Oltvai (2004) Network Biology: Understanding the cell's functional organization. Nature Review Genetics 5:101.

"Network theory offers unforeseen possibilities to understand the cell's internal organization and evolution, fundamentally altering our view of cell biology".



Gene Networks

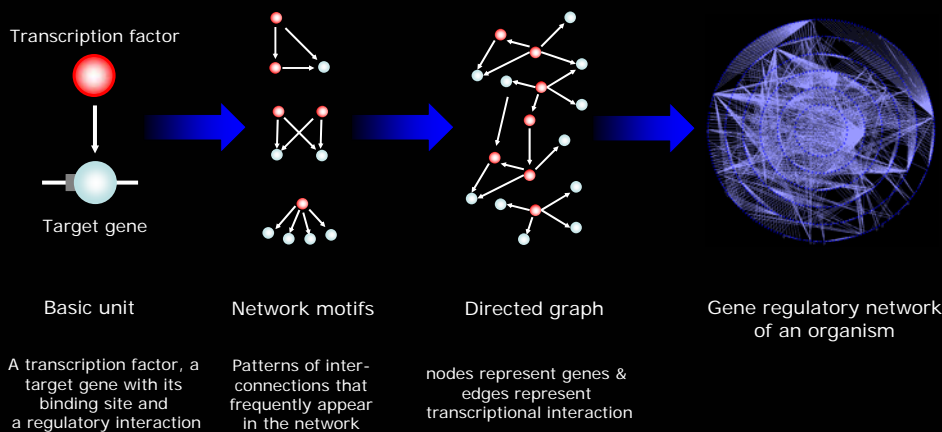
Justification and Concepts

- Networks contain small repeating patterns of interconnections, called network **Motifs**.
- Basic network motifs include:
 - (1) Feed forward; (2) Single input;
 - (3) Multiple input; (4) A combination of the above.
- Think of motifs as simple building blocks of complex networks.
- Much of a cell's activity is organised as a network of interacting **Modules**: Sets of genes co-regulated to respond to different conditions.
- Think of Modules as clusters, i.e., genes being highly connected within a cluster but sparsely (if at all) connected across modules.
- Understanding this organisation is crucial for understanding cellular responses to internal and external signals.
- Once a network is build, both its
 - (1) **Mathematical** → **Scale-free, power-law distribution of its connectivity**
 - and
 - (2) **Biological** → **Targets via essays**
Effects via knock-outs



Gene Networks

Organisation of the gene regulatory network



Source: M. Madan-Babu
MRC Laboratory of Molecular Biology, Cambridge



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Motifs

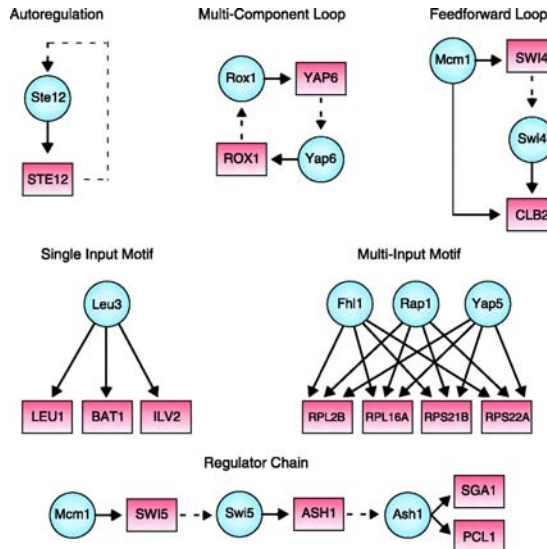
MORE TERMINOLOGY:

Nodes are Genes.

Connections (or edges or vertices or links) are interactions.

Directed interactions (ie. having a regulatory nature) involve a Transcription Factor and its Target(s).

In the main, we'll deal with gene co-expression networks (...a way to explore the correlation matrix).



Lee et al. (2002). "Transcriptional regulatory networks in Saccharomyces cerevisiae." Science 298:799-804.



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Connectivity Rules

Does this map tell you which cities are important?

This one does!



The nodes with the largest number of links (connections) are most important!

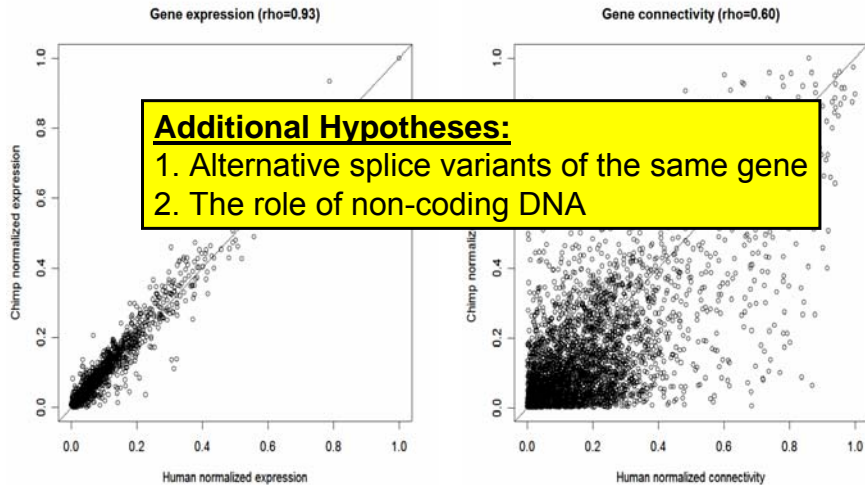


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Connectivity Rules

Chimp vs Human

Gene expression is more strongly preserved than gene connectivity.
Hypothesis: Molecular wiring makes us human



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Network Measures

- ➡ **Degree** (or **Connectivity**) of a node, k , is the number of links (edges) this node has.
- ➡ The **Degree Distribution**, $P(k)$, is the probability that a selected node has exactly k links. Networks are classified by their degree distribution.
- ➡ The **Clustering coefficient**, $C(k) = 2k/(N(N-1))$, measures the amount of cohesiveness, the tendency of nodes to form clusters or modules.
Note 1: the maximum number of connections is $N(N-1)/2$ (ie. Number of off-diagonals in the R matrix), in which case $C(k) = 1.0$.
Note 2: For many networks, $C(k) \sim k^{-1}$ which is an indication of a network hierarchical character (more on this later).
Note 3: For a single node i , $C(k_i) = 2n_i/(k_i(k_i-1))$, where n_i is the number of links connecting the k_i neighbours of node i with each other and $k_i(k_i-1)/2$ is the total number of triangles that would pass through node i should all of its neighbours be connected with each other.
- ➡ The **Path Length** = Links we need to pass to travel between two nodes. The mean path length, l tells us the average shortest pass between all pairs of nodes and offers a measure of overall navigability.

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Three Types of Networks According to their Connectivity Structure

1. Random Network
2. Scale-Free Network
3. Hierarchical Network

NB: Biological networks are reported to be **Scale-Free**



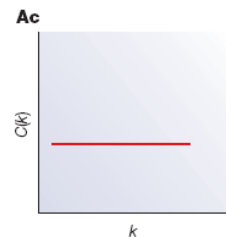
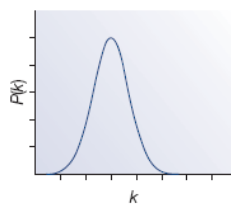
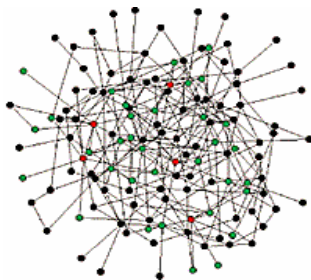
Random Networks

Each pair of nodes is connected with probability p , creating a graph with approximately $p N(N-1)/2$ randomly placed links.

The connectivity degree follows a Poisson distribution: Nodes that deviate from the average are rare and decreases exponentially.

The clustering coefficient is independent of a node's degree of connectivity, so it appears as a horizontal line.

Mean shortest path is $l \sim \log(N)$ indicating that most nodes are connected by a short path (*Small World Property*).





Gene Networks

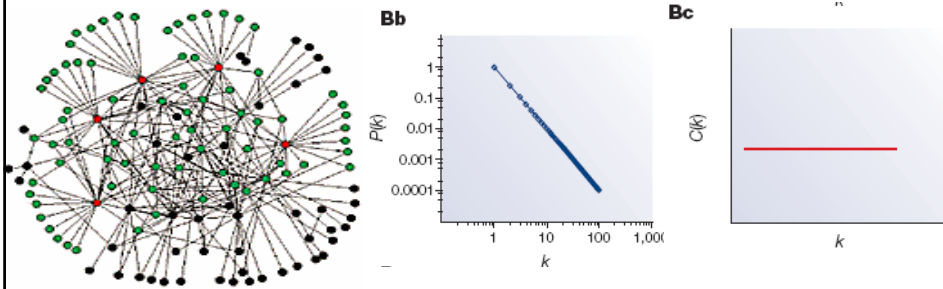
Scale-Free (Power Law) Networks

Most nodes are poorly while a few are highly connected (**Hubs**).

The degree distribution approximates a power law: $P(k) \sim k^{-\gamma}$, where γ is the degree exponent (Straight line in a Log-Log plot).

The smaller the γ , the more important is the role of the Hubs. Most biological networks have $2 < \gamma < 3$. For $\gamma > 3$, Hubs are irrelevant and the network behaves like a random network.

The mean shortest path length is proportional to $\log(\log(N))$ (ie. Much shorter than Small World Property).



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Gene Networks

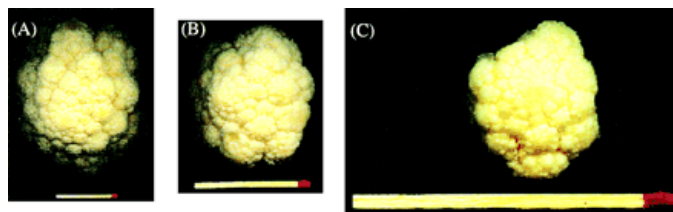
Scale-Free (Power Law) Networks (cont'ed)

Line Random Networks, $C(k)$ is independent of k (straight line)

Scale-Free networks are invariant to changes in scale. Any function of $P(k)$ remains unchanged within a multiplicative factor $P(ak) = b P(k)$.

This implies that scale-free networks are self-similar, i.e. any part of the network is statistically similar to the whole network and parameters are assumed to be independent of the system size.

Think of a cauliflower:



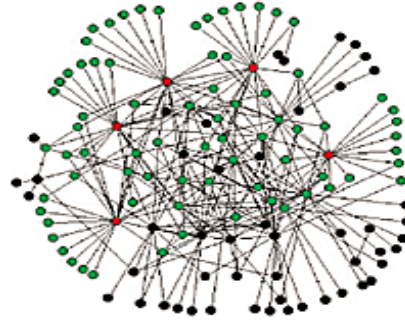
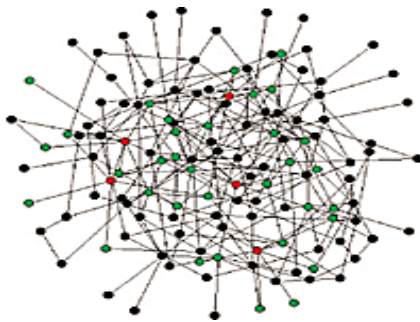
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Random vs Scale-Free Networks

- In the random network, the five nodes with the most links (in red) are connected to only 27% of all nodes (green). In the scale-free network, the five most connected nodes (red) are connected to 60% of all nodes (green)



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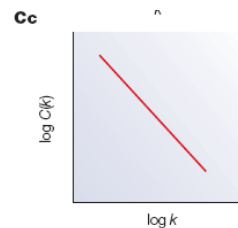
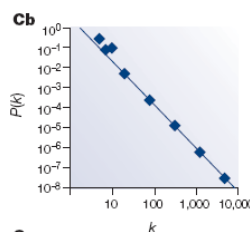
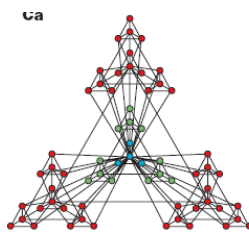
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Hierarchical Networks

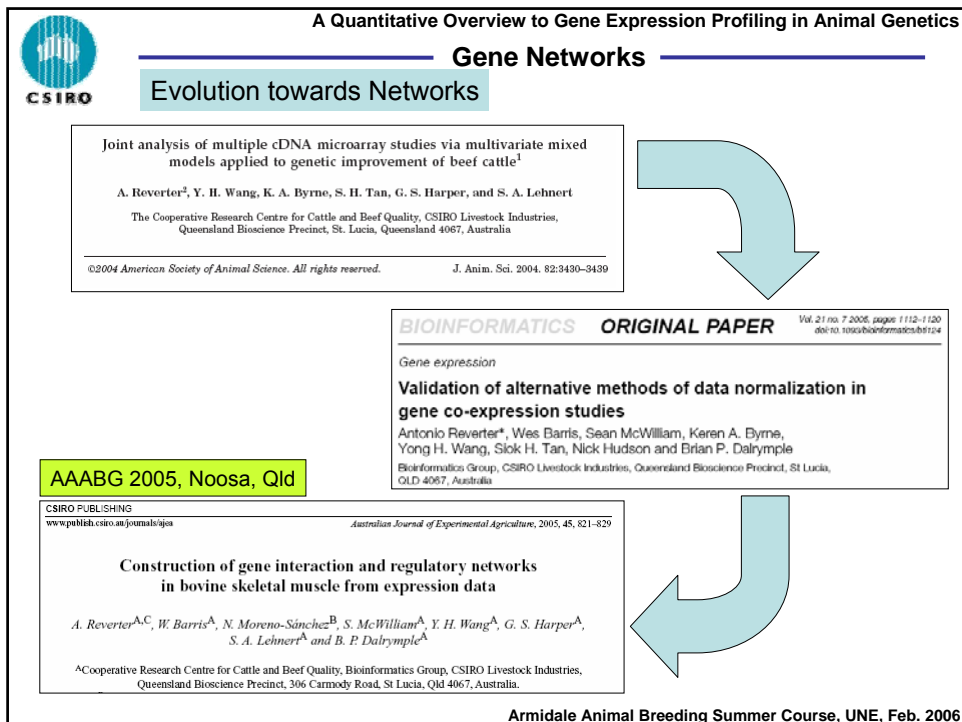
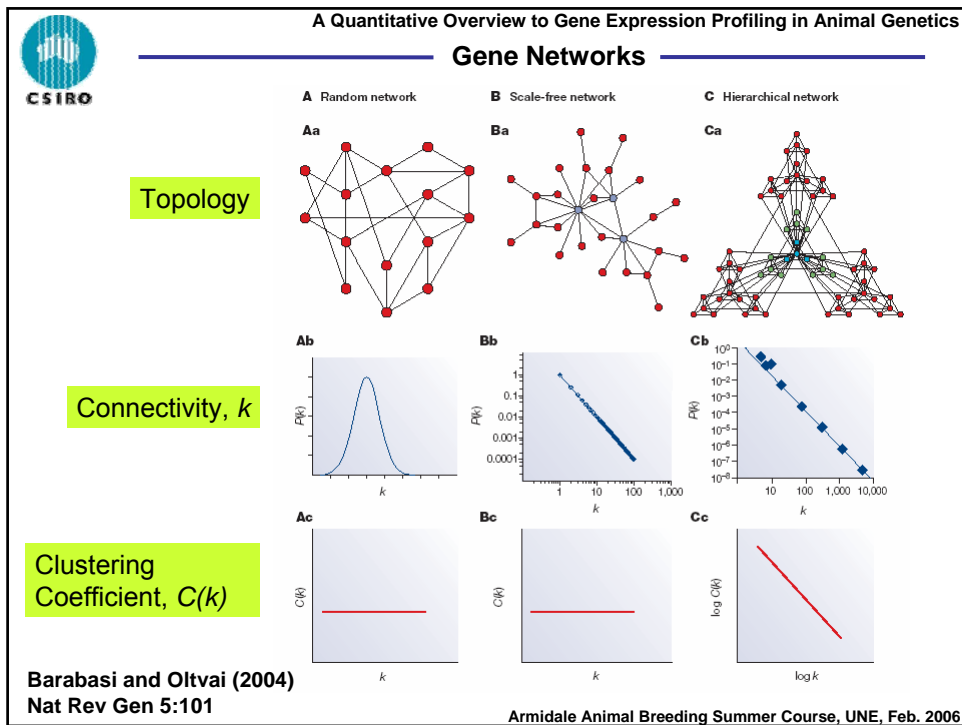
To accommodate modularity, clusters combine in an iterative manner, generating a hierarchical network.

The hierarchical network model seamlessly integrates a scale-free topology with an inherent modular structure by generating a network that has a power-law degree distribution with degree exponent $\gamma = 1 + \ln 4 / \ln 3 = 2.26$.

The most important signature of hierarchical modularity is the scaling of the clustering coefficient, which follows $C(k) \sim k^{-1}$ a straight line of slope -1 on a log-log plot.



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Background

1. Lists of DE are being reported for a variety of questions
2. A “*These Go*” (Disco?) analysis is suboptimal
3. A Gene Ontology analysis is a (minimum) must
4. Pairs of genes showing co-expression are likely to belong to the same pathway
5. Genes regulated by the same transcription factor show higher than average co-expression
6. Hence, the trend to work on reversed engineering reconstruction of Gene Regulatory Networks (GRN)
7. Basso et al., 2005, Nat Genet, 37:382

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Background (cont'd)

8. The Beef CRC has explored a (reasonably) large number of perturbations
9. A method, anchored in MME, has been devised to jointly analyse seemingly independent experiments (JAS, 2004, 82:3430) and to compute co-expression measurements (Bioinformatics, 2005, 21:1112).

Objective

To resort to the above-mentioned data and method to reversed engineer a gene regulatory network for Bovine skeletal muscle

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Validation of alternative methods of data normalization in gene co-expression studies. *Bioinformatics* 2005, 21:1112

A. Reverter, W. Barris, S.M. McWilliam, K.A. Byrne, Y.H. Wang, S.H. Tan, N. Hudson, and B.P. Dalrymple

Experiment ^a	Hybs.	Cond.	Signals ^b		
			N	Mean	STD
1. Two breeds by two diets	7	4	193,175	7.61	2.99
2. Three diets	14	3	361,320	8.52	2.84
3. Two diets at three ages	24	6	801,807	11.60	1.89
4. Two breeds at three ages	18	6	459,978	8.34	3.28
5. Two fat treatments at two ages	15	4	418,817	7.04	3.31

Expression of each clone (gene) across 23 conditions



Gene Networks

Building the Network

- Step 1 → Select Muscle-specific genes (MSG) from the entire SAGE database
- Step 2 → Identify which MSG from Step 1 were surveyed in the Beef CRC studies
- Step 3 → Iteratively extract genes with co-expression > |0.75| with genes from Step 2
- Step 4 → Identify potential Transcription Factors
- Step 5 → Build the entire network keeping track of emerging modules within the network
- Step 6 → Assess the genomic functionality by significance analysis of gene ontologies



A Quantitative Overview to Gene Expression Profiling in Animal Genetics

Gene Networks

The screenshot shows the CGAP website interface. At the top, there's a navigation bar with tabs for Genes, Chromosomes, Tissues, SAGE Genie, RNAi, Pathways, and Tools. Below this, the main content area is titled 'The CANCER GENOME ANATOMY PROJECT'. It includes a brief description of the project's goal, a list of resources (Genes, Chromosomes, Tissues, SAGE Genie, RNAi, Pathways, Tools), and a 'New Initiatives' section. The 'Genes' resource is highlighted, showing information about gene information, clone resources, and transcriptome analysis.

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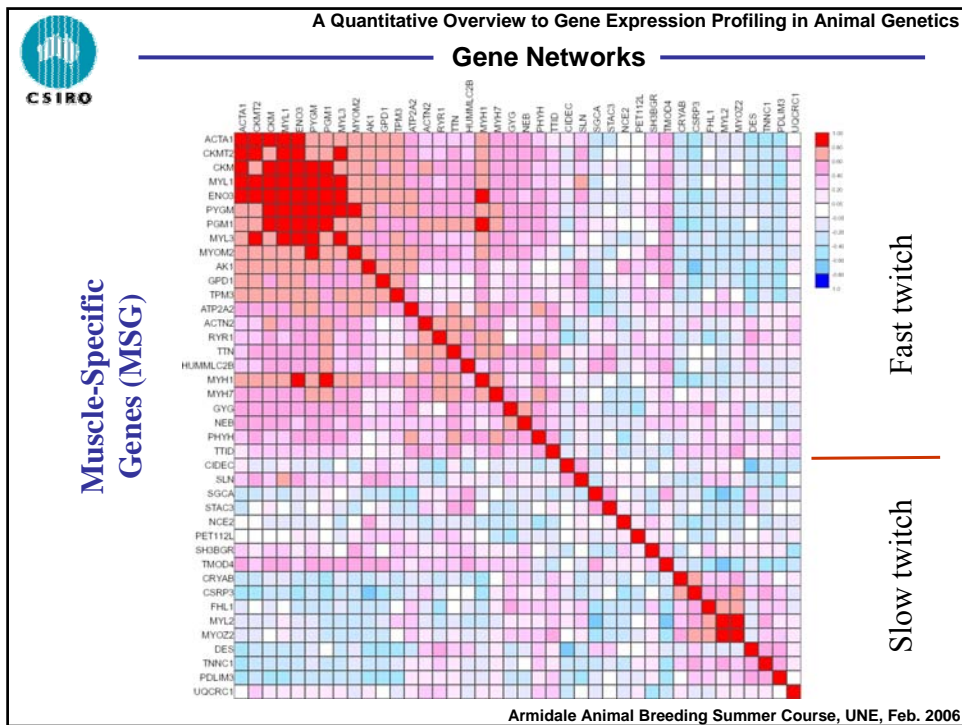
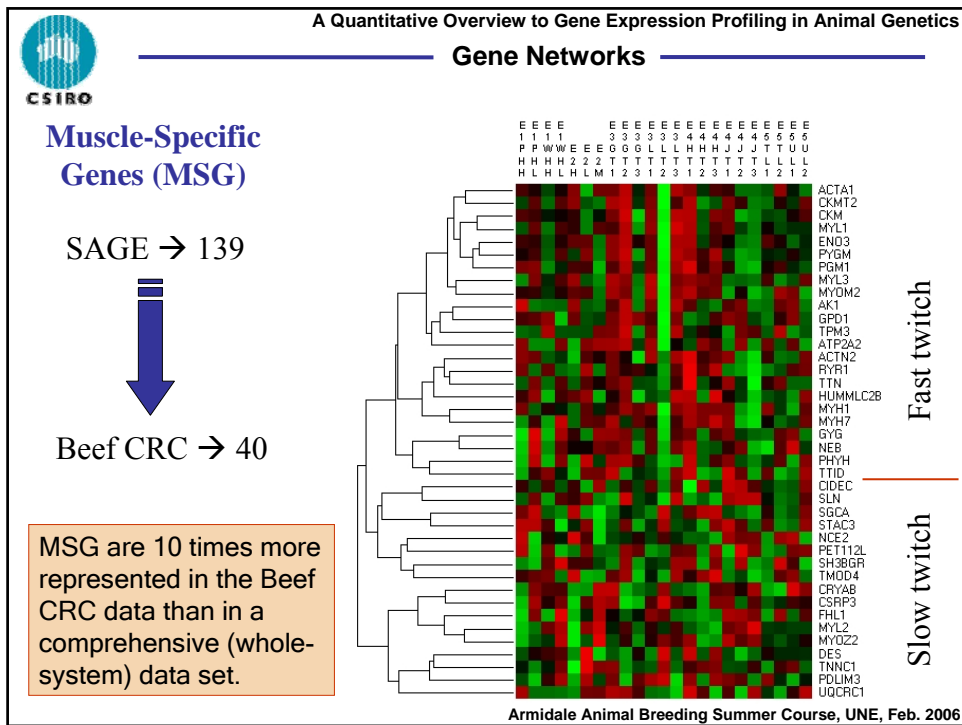
A Quantitative Overview to Gene Expression Profiling in Animal Genetics

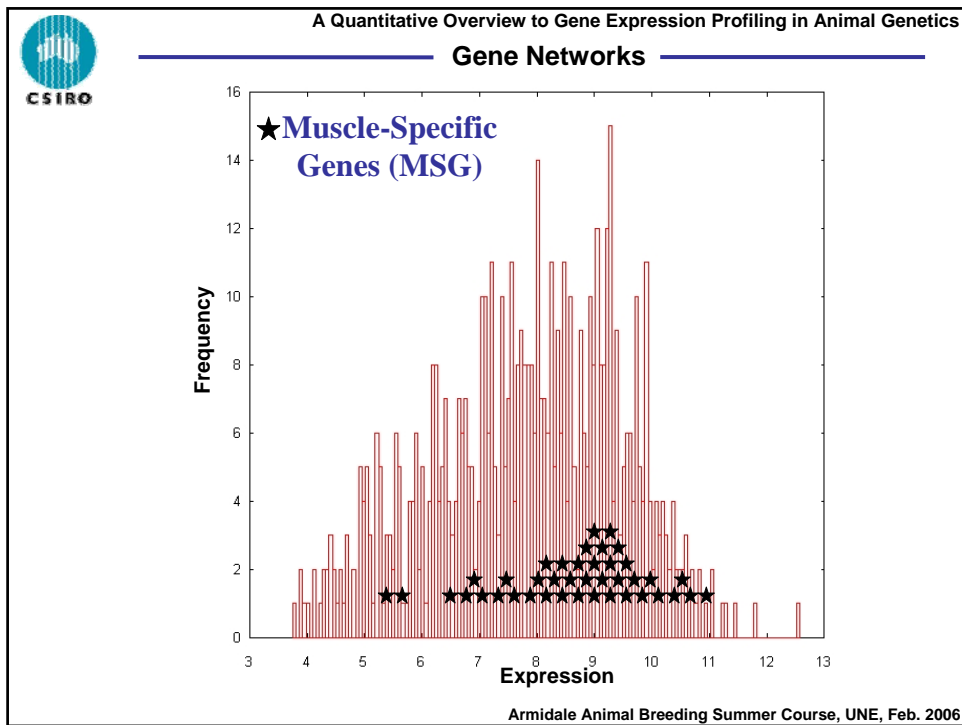
Gene Networks

NORMAL				CANCER			
Tissue	L	Genes	Extr.G	Tissue	L	Genes	Extr.G
Brain	10	16,123	1	Brain	79	17,925	0
Breast	10	14,684	7	Breast	32	16,847	0
Colon	2	8,388	29	Colon	6	13,888	3
Kidney	1	6,265	31	Kidney	3	11,543	23
Liver	2	10,938	113	Liver	3	11,995	13
Lung	3	10,936	30	Lung	6	11,881	15
Ovary	2	9,557	16	Ovary	6	13,121	12
Pancreas	3	8,634	82	Pancreas	6	12,073	22
Peritoneum	1	7,661	27	Peritoneum	1	6,306	41
Placenta	2	11,447	45	Placenta	1	8,863	34
Prostate	4	12,180	6	Prostate	11	14,313	19
Skin	1	5,687	32	Skin	3	7,853	239
Stomach	2	6,576	49	Stomach	4	12,594	8
Thyroid	1	10,232	13	Thyroid	2	12,617	14
Vascular	2	9,901	7	Vascular	2	10,606	9
White BC	1	5,048	9	White BC	3	10,245	61
Lymph	1	11,002	146	Cartilage	8	15,147	7
Leukocytes	1	5,360	62	Fibroblast	1	4,343	79
Bone	3	10,954	22				
Heart	1	7,962	55				
Muscle	2	7,588	84				
Retina	4	13,881	20				
Spinal C	1	8,176	19				

➔ 139 Muscle-Specific Genes

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A Quantitative Overview to Gene Expression Profiling in Animal Genetics

Gene Networks

Building the Network

Step 3 → Iteratively extract genes with co-expression > |0.75| with genes from Step 2

Step 4 → Identify potential Transcription Factors

↓

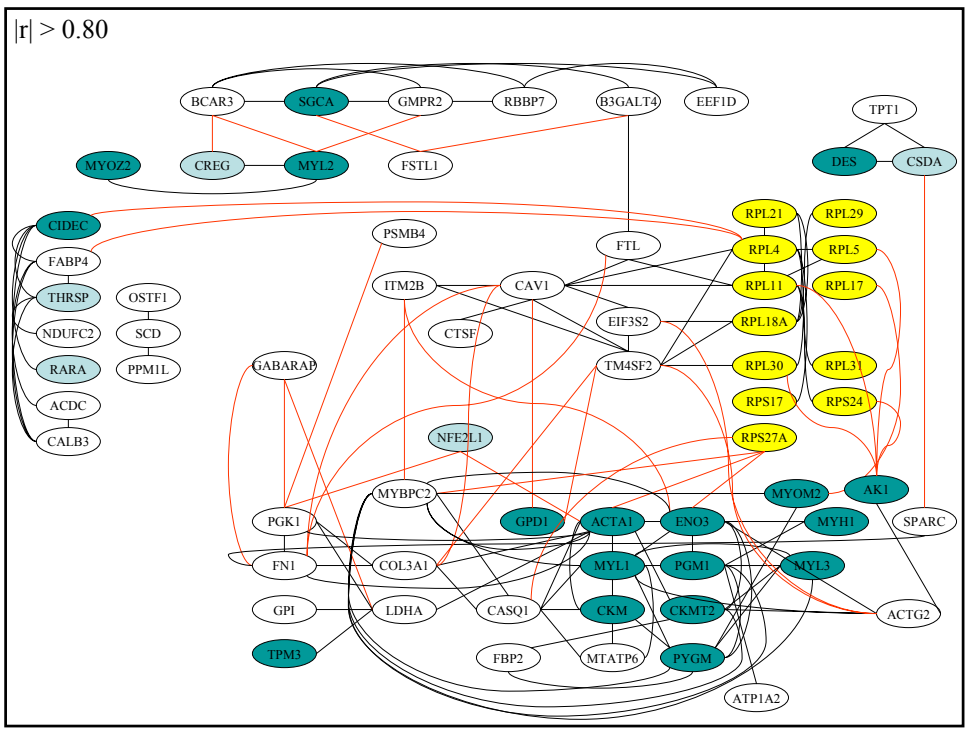
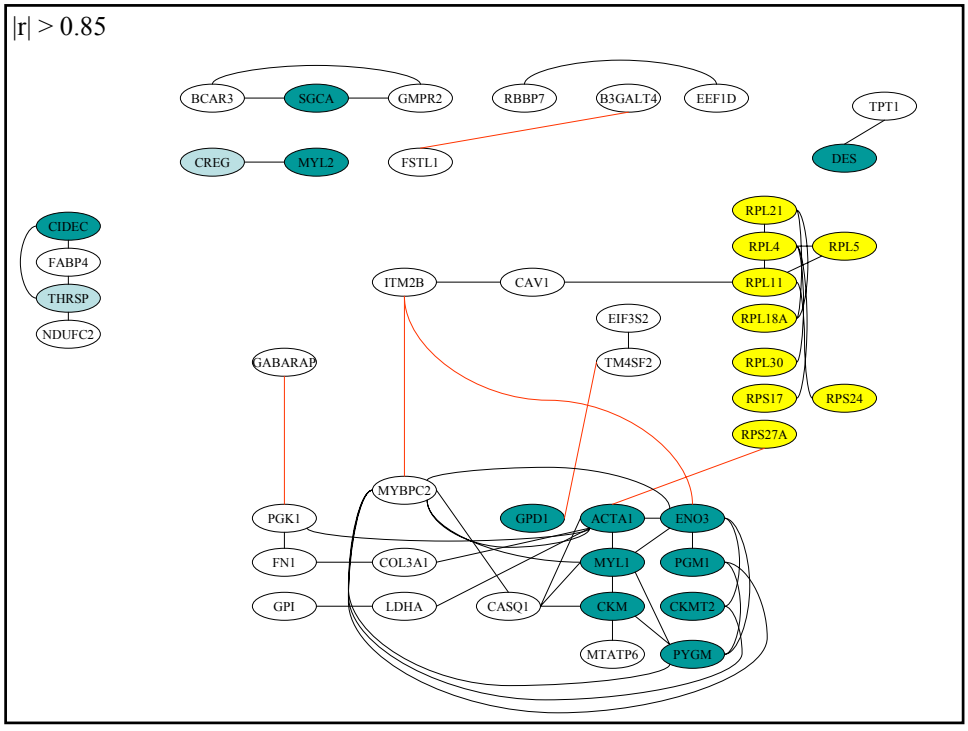
40 MSG → 102 Total Genes → 7 Transcription Factors

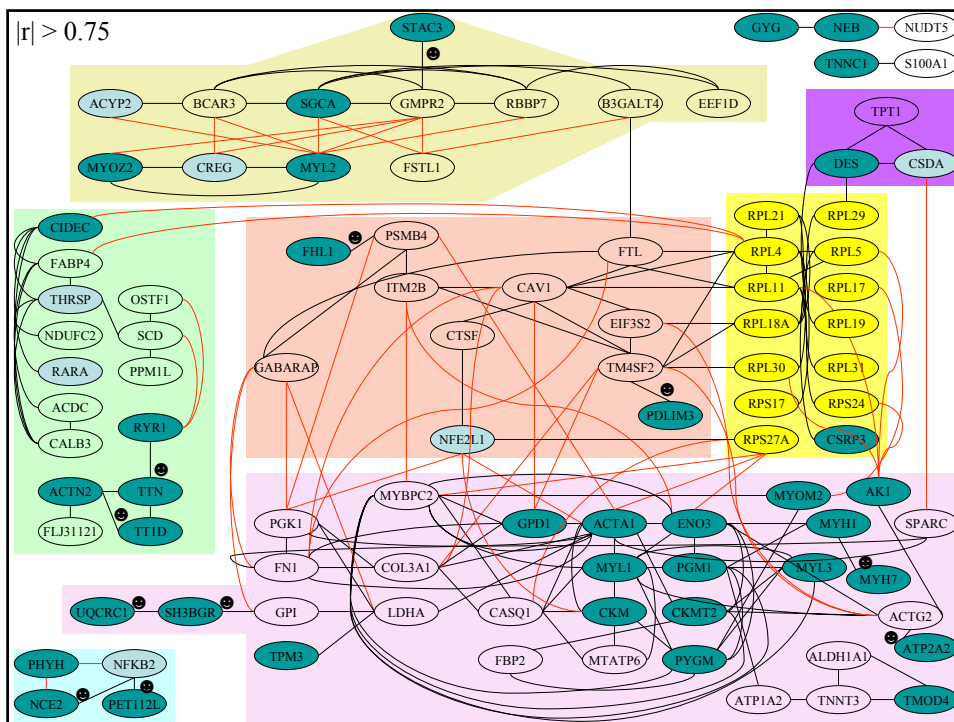
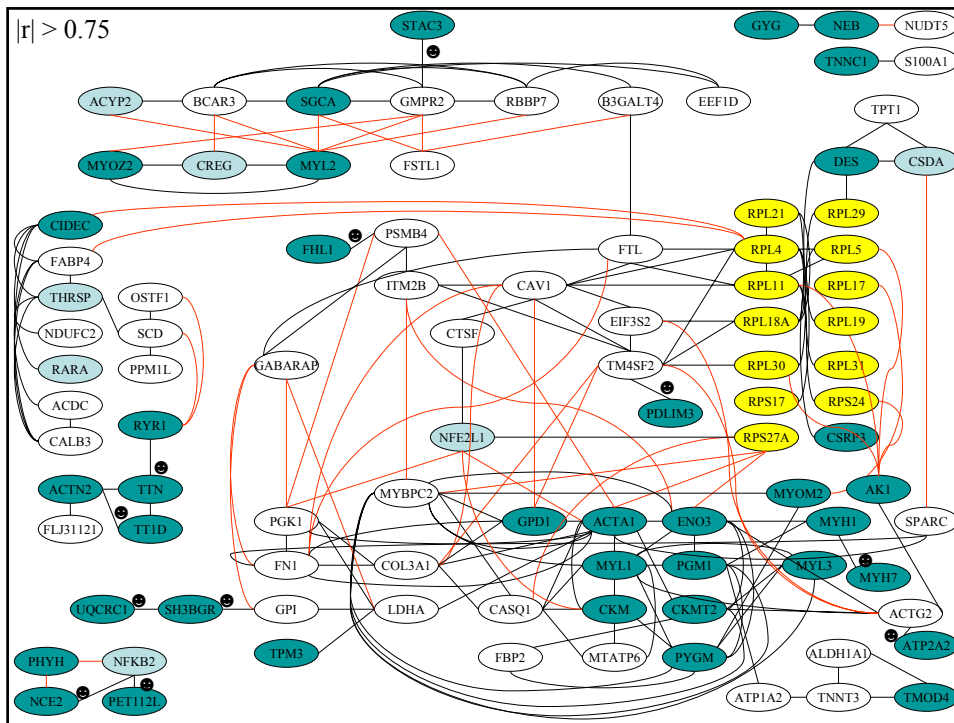
Table 2. Transcription factors

Gene	Description	Clones	Avg	Pct
ACYP2	Acylphosphatase 2, muscle type	1	9.92	91.9
CREG	Cellular repressor of E1A genes	1	11.21	99.3
CSDA	Cold shock domain protein A	11	10.69	98.0
NFE2L1	Nuclear factor (erythroid derived 2)	2	8.04	52.7
NFKB2	Nuclear factor of kappa light enhancer in B cells	1	6.02	15.7
RARA	Retinoic acid receptor alpha	1	6.23	24.3
THRSP	Thyroid hormone responsive (Spot 14 homolog)	1	8.57	63.8

Step 5 → Build the entire network keeping track of emerging modules within the network

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CSIRO

Functional Annotations

Step 6 → Assess the genomic functionality by significance analysis of the ontologies

$$P(z, n, t, x) = \frac{\binom{t}{z} \binom{n-t}{x-z}}{\binom{n}{x}},$$

n = genes on the microarray ($n = 624$)
 x = genes in the gene network ($x = 102$)
 t = genes in the GO of interest in the entire data
 z = genes from the GO of interest in the network

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Functional Annotations

Module 6

- Cytoskeleton
- Transferase activity, glycosyl
- Protein biosynthesis

Module 4

- Muscle contraction

Module 5

- Structural of muscle
- Smooth endoplasmic reticulum
- Sarcomere
- Carbohydrate metabolism
- Fatty acid biosynthesis
- Energy pathways

Module 3

- Nucleus
- Integral to plasma membrane
- Protein biosynthesis

Module 1

- Protein biosynthesis
- Intracellular
- Ribosome
- Structural of ribosome

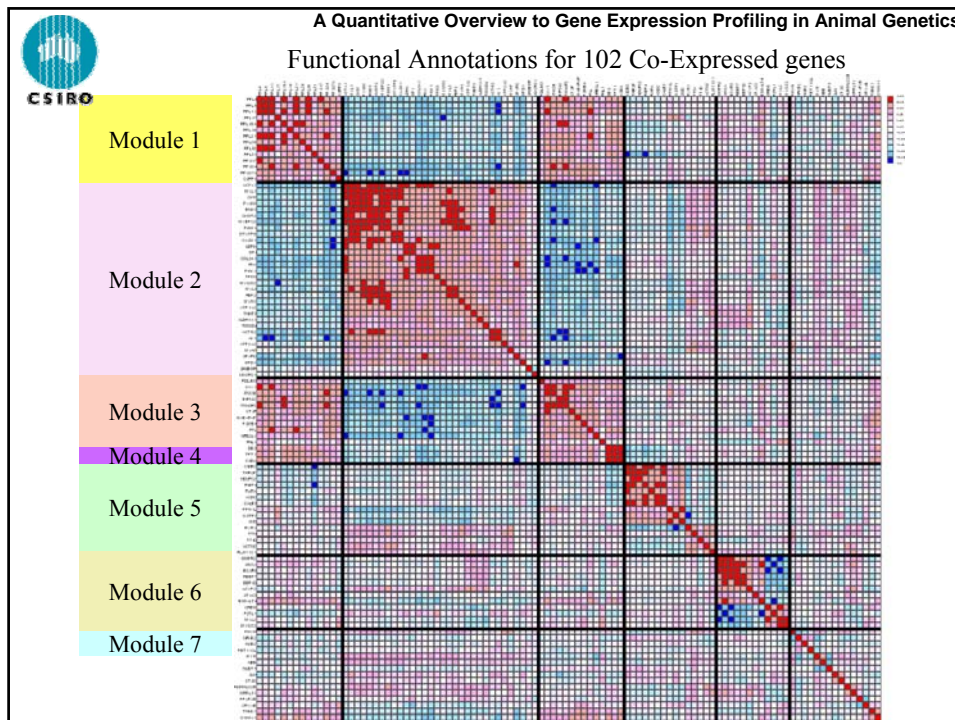
Module 2

- Glycolysis
- Muscle development
- Actin binding
- Striated muscle thick filament
- Transferase activity, phosphorus
- Creatine kinase activity
- Tropomyosin binding
- Myosin
- Magnesium ion binding

Module 7

- Neurogenesis
- Protein biosynthesis

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A Quantitative Overview to Gene Expression Profiling in Animal Genetics

Gene Networks

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Conclusions

A gene interaction and regulatory network has been proposed that:

1. Goes beyond the standard description of DE genes
2. Increases our understanding of bovine skeletal muscle growth and development
3. ... or at least, provides for new hypotheses to be postulated

Concerning issues

1. Limited number of genes and TF (MYOG, MYF6)
2. Limited number of perturbations (see McWilliam et al. 2005, AAABG Poster)

.....to be addressed in forthcoming work

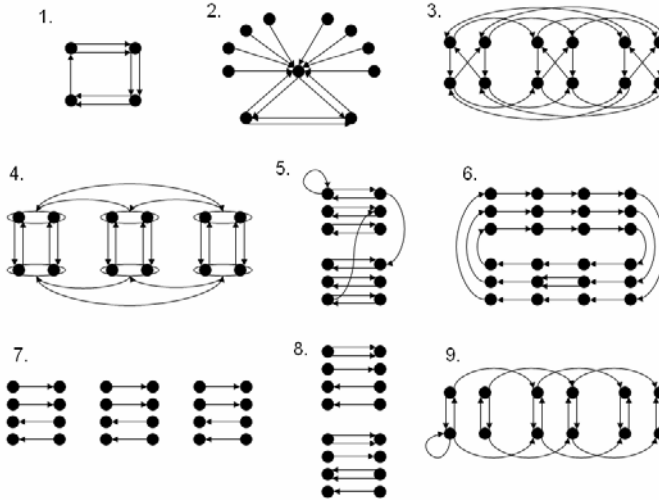
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Gene Networks

...Growing bigger

From 5 to 9 Experiments
From 78 to 147 Microarrays
From 23 to 47 Conditions



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Gene Networks

...Growing bigger

From 5 to 9 Experiments
From 78 to 147 Microarrays
From 23 to 47 Conditions

General statistics:

traits	# rec.	min.	max.	avg.	std.
grafton	189194	.00000	15.99850	7.61883	2.99228
diets	353643	.00000	15.99840	8.53072	2.84115
keren	790590	.00000	15.99860	11.55905	1.92068
japox	451115	.00000	15.99870	8.35423	3.28001
adipo	410683	.00000	15.99840	7.08354	3.31402
foetal	837888	.00000	15.99910	11.52842	2.64171
vita	278220	.00000	15.99550	6.57997	2.89477
jers	263575	.00000	15.99530	6.77378	2.98053
japox2	712653	.00000	15.99930	10.39026	2.72805

Nine-Variate Mixed-Model (1,762,338 Eqs, 81 Components):

$$Y \sim MVN \left[X\beta, G\Sigma_g G^T + A\Sigma_a A^T + D\Sigma_d D^T + V\Sigma_v V^T + \Sigma_e \right]$$

Log₂ Intens CGroup Gene (G) GxArray GxDye GxVariety Error
(clones)

NB: 7,898 Clones representing 822 Genes

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Gene Networks

...Growing bigger

Nine-Variate Mixed-Model (1,762,338 Eqs, 81 Components):

$$Y \sim MVN[X\beta, G\Sigma_g G^T + A\Sigma_a A^T + D\Sigma_d D^T + V\Sigma_v V^T + \Sigma_e]$$

these are the corresponding ratios:

gene	grafton	diets	keren	japox	adipo	foetal	gxp1	grafton		
	.826	.900	.335	.872	.480	.317		.008		
		.825	.402	.895	.520	.368	gxp2	diets		japox2
			.860	.317	.138	.881		.017		.268
				.669	.728	.288	gxp3	keren		.346
					.765	.159		.023		.887
						.616	gxp4	japox		.249
								.019		.103
							gxp5	adipo		.791
								.005		.046
										.059
							gxp6	foetal		.856
								.023		
							gxp7	vita		
								.005		
							gxp8	jers		
								.002		
							gxp9	japox2		
								.004		
Resid.dat5	adipo									
	.049									
Resid.dat6	foetal									
	.041									
Resid.dat7	vita									
	.157									
Resid.dat8	jers									
	.149									
Resid.dat9	japox2									
	.031									

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Gene Networks

Identification of Significant Associations

- Measure co-expression by a similarity $s(i,j)$ in $[0,1]$ e.g. absolute value of the Pearson correlation coefficient.
- Define an adjacency matrix as $A(i,j)$ using an Adjacency Function, $AF(s(i,j))$
- AF is a monotonic function from $[0,1]$ onto $[0,1]$
- Here we consider 2 classes of AF s:
 - **Threshold**: $AF(s)=I(s>\tau)$; τ being the threshold that applies across all correlations.
 - **DPI**: Data Processing Information Index applied to each trio of genes.

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Gene Networks

Identification of Significant Associations

- 2 classes of Adjacency Functions:
 - **Threshold**: $AF(s) = I(s > \tau)$; τ being the threshold that applies across all correlations.
 - Cross-Validation: $\tau > 0.75 \rightarrow FDR < 1\%$
 - **DPI**: Data Processing Information Index applied to each trio of genes in (x, y, z) :
 - If $s = |r_{xy}| > |r_{xz}|(1-\epsilon)$
 - and $s = |r_{xy}| > |r_{yz}|(1-\epsilon)$
 - then the link between genes x and y is established in the network.

Find a criterion for estimating tolerance parameter ϵ



Gene Networks

Identification of Significant Associations

If $s = |r_{xy}| > |r_{xz}|(1-\epsilon)$ and $s = |r_{xy}| > |r_{yz}|(1-\epsilon)$
 then the link between genes x and y is established in the network.

Find a criterion for estimating tolerance parameter ϵ

Options:

- A) FIND ϵ THAT RESULTS IN APPROXIMATE SCALE-FREE TOPOLOGY** (Basso et al. 2005. Nature Gen 37:382).
- B) FIND ϵ THAT RESULTS IN THE HIGHEST MEAN NUMBER OF CONNECTIONS** (Partial Correlation Coefficients: de la Fuente et al. 2004. Bioinformatics 20:3565).

Criterion **A** is motivated by the finding that most metabolic networks have been found to exhibit a scale-free topology

Criterion **B** leads to high power for detecting modules (clusters of genes) and hub genes.



Gene Networks

Identification of Significant Associations

My (educated?) Option: A Combination of both

The Partial Correlation between x and y given z is the correlation between x and y that is independent of z

$$r_{xy.z} = \frac{r_{xy} - r_{xz} r_{yz}}{\sqrt{(1-r_{xz}^2)(1-r_{yz}^2)}}$$

For every trio of genes in x, y and z (having 92,231,140 combinations of 822 genes taking 3 at a time), we computed the (first-order) partial correlation coefficients in $r_{xy.z}$, $r_{xz.y}$ and $r_{yz.x}$.

Then, the average ratio of partial to direct (or zeroth-order) correlation was computed as follows:

$$(1-\epsilon) = \frac{1}{3}(r_{xy.z}/r_{xy} + r_{xz.y}/r_{xz} + r_{yz.x}/r_{yz}).$$

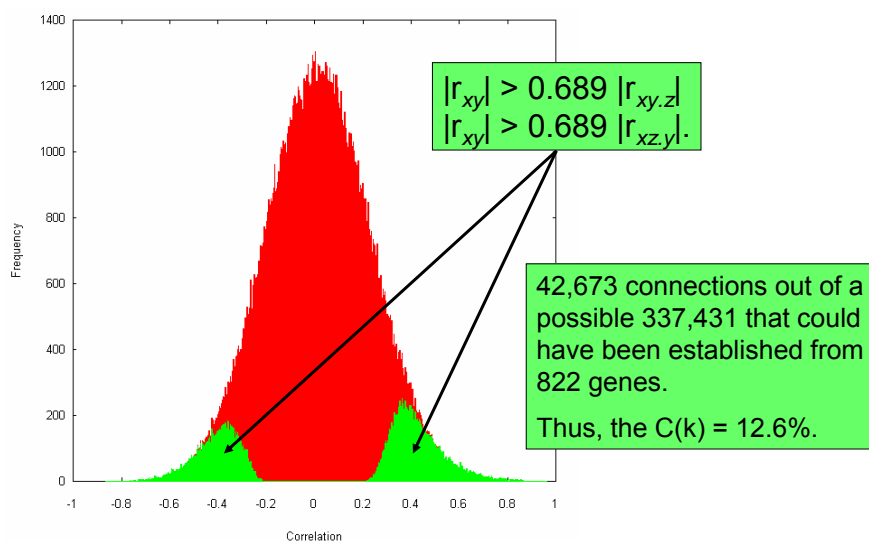
This tolerance level equated to 0.689 and the association between genes x and y was set to zero if $|r_{xy}| \leq 0.689 |r_{xy.z}|$ and $|r_{xy}| \leq 0.689 |r_{xz.y}|$.

Otherwise, the association was assessed as significant and the connection between the pair of genes established in the reconstruction of the network.



Gene Networks

Identification of Significant Associations

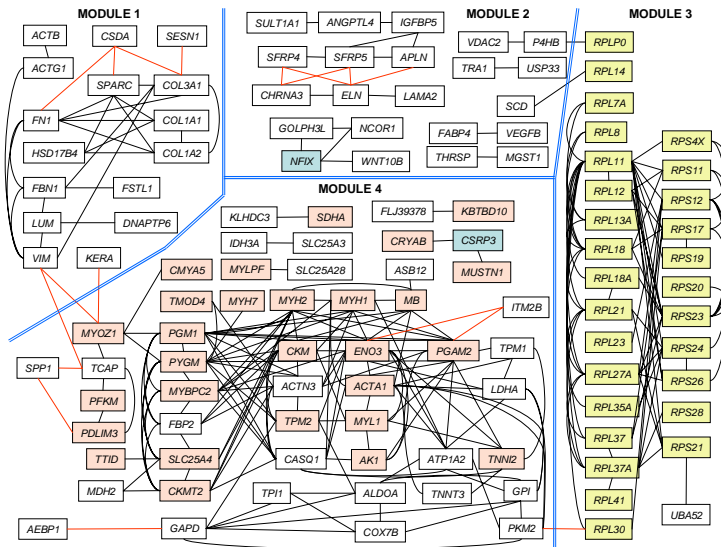




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Gene Networks

123 Genes linked by 312 $|r| > 0.75$ (FDR < 1%)



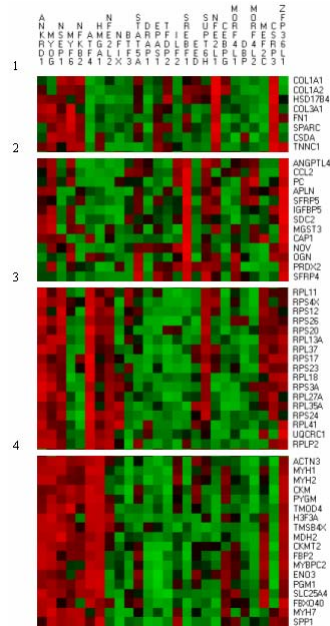
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A Quantitative Overview to Gene Expression Profiling in Animal Genetics

Gene Networks

Validation #1



Hierarchical clustering of correlation coefficients between genes (rows) and transcription factors (TF; columns) reveals modules that comprise clusters affected by biologically meaningful TF.

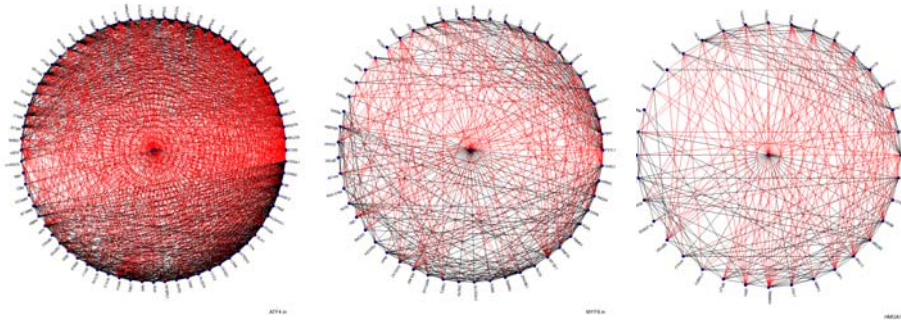
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Gene Networks

Validation #2

A given gene was allocated to a TF-Hub if the $|r|$ between the gene and this TF was bigger than the $|r|$ between the same gene and any other TF.



796 Genes
26 Transcription Factors Hubs



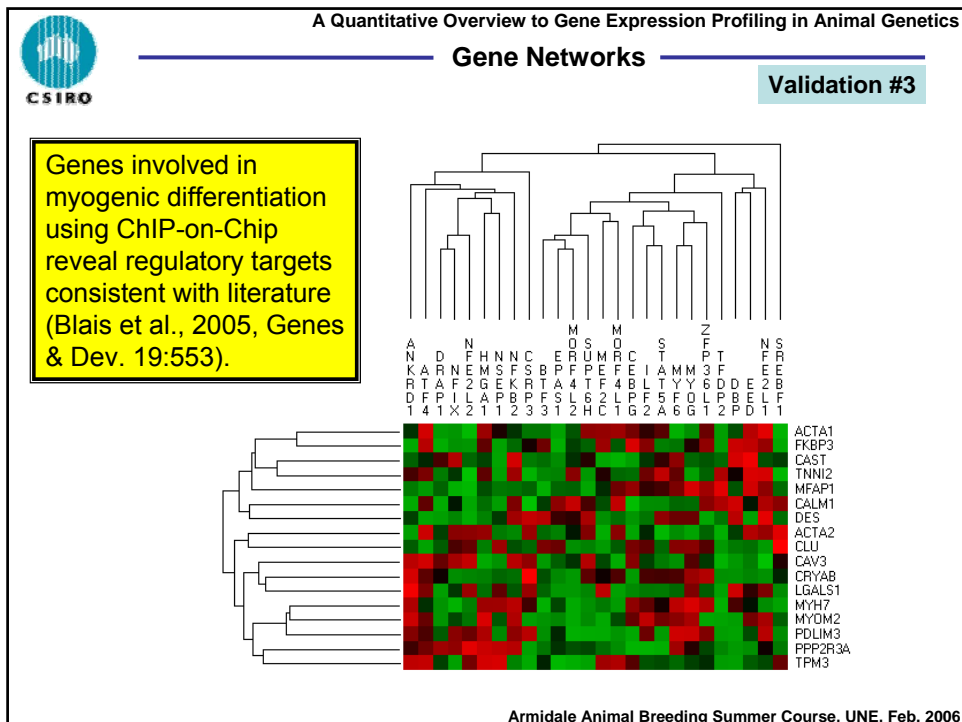
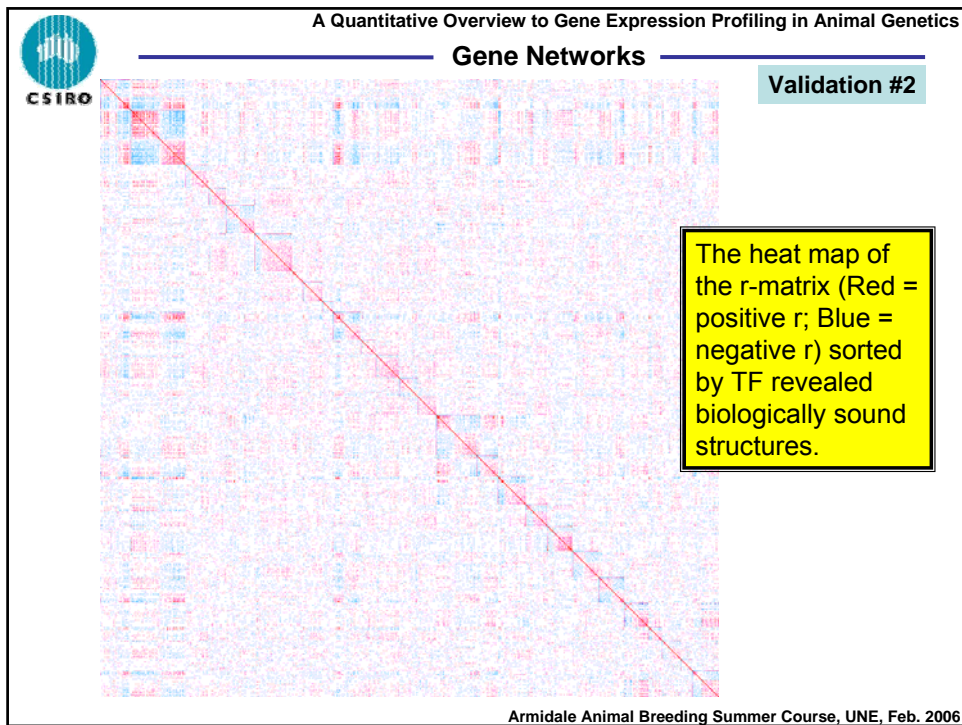
Gene Networks

Validation #2

A given gene was allocated to a TF-Hub if the $|r|$ between the gene and this TF was bigger than the $|r|$ between the same gene and any other TF.

TF	<i>g</i>	MSG	<i>k</i>	<i>c(k)</i>	<i>r</i>	<i>Pr</i>	<i>P_{MSG}</i>
<i>ANKRD1</i>	40	7	264	0.34	0.483	0.0037	0.0282
<i>ATF4</i>	73	11	1,279	0.49	0.545	0.0000	0.0191
<i>CSRP3</i>	38	8	238	0.34	0.438	0.1811	0.0083
<i>HMGAI</i>	37	6	247	0.37	0.474	0.0106	0.0500
<i>MYF6</i>	54	6	426	0.30	0.459	0.0108	0.1271
<i>MYOG</i>	28	2	140	0.37	0.482	0.0160	0.2697
<i>NFIX</i>	33	2	219	0.41	0.488	0.0033	0.2484
<i>NFKB2</i>	35	0	190	0.32	0.438	0.1887	0.0542
<i>SREBF1</i>	32	0	185	0.37	0.483	0.0082	0.0699
<i>ZFP36LI</i>	36	3	205	0.32	0.474	0.0122	0.2290

NB: Entire Network, $C(k) = 12.6\%$





Gene expression profiling of muscle tissue in Brahman steers during nutritional restriction¹

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ABSTRACT: Expression profiling using microarrays allows for the detailed characterization of the gene networks that regulate an animal's response to environmental stresses. During nutritional restriction, processes such as protein turnover, connective tissue remodeling, and muscle atrophy take place in the skeletal muscle of the animal. These processes and their regulation are of interest in the context of managing livestock for optimal production efficiency and product quality. Here we expand on recent research applying complementary DNA (cDNA) microarray technology to the study of the effect of nutritional restriction on bovine skeletal muscle. Using a custom cDNA microarray of 9,274 probes from cattle muscle and s.c. fat libraries, we examined the differential gene expression profile of

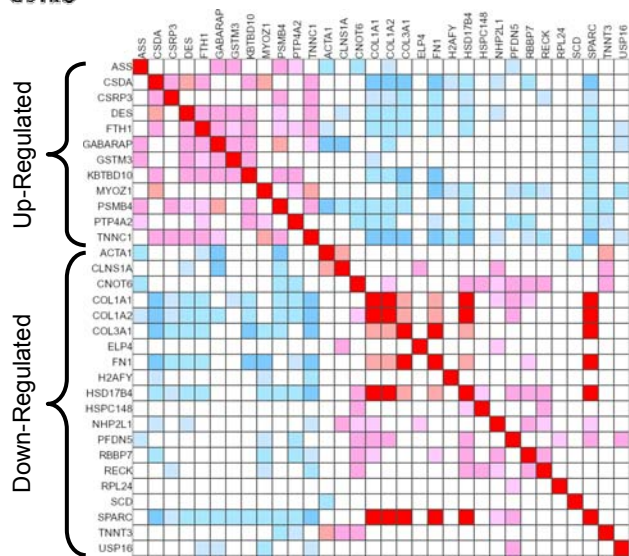
the LM from 10 Brahman steers under three different dietary treatments. The statistical approach was based on mixed-model ANOVA and model-based clustering of the BLUP solutions for the gene x diet interaction effect. From the results, we defined a transcript profile of 156 differentially expressed array elements between the weight loss and weight gain diet substrates. After sequence and annotation analyses, the 57 upregulated elements represented 29 unique genes, and the 99 downregulated elements represented 28 unique genes. Most of these co-regulated genes cluster into groups with distinct biological function related to protein turnover and cytoskeletal metabolism and contribute to our mechanistic understanding of the processes associated with remodeling of muscle tissue in response to nutritional stress.

Key Words: Beef, Complementary DNA, Gene Expression, Nutrition

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J. Anim. Sci. 2005. 83:1-12

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Genes involved in food deprivation have a r structure consistent with their effect (up- or down-regulation).

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