Microarray studies in a calanoid copepod, *Calanus finmarchicus*

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David Towle – Colleague Extraordinaire

1941-2011

“Everything that I have learned about molecular biology has been from David.” - Cedomil Lucu

Research Goals

- Identify genes that could serve as good biomarkers
- Gain insight into the physiological processes that are being regulated in response to the environment
- Recognize/predict population changes

Functional Genomics Approaches

- Techniques to quantify expression levels for individual mRNAs

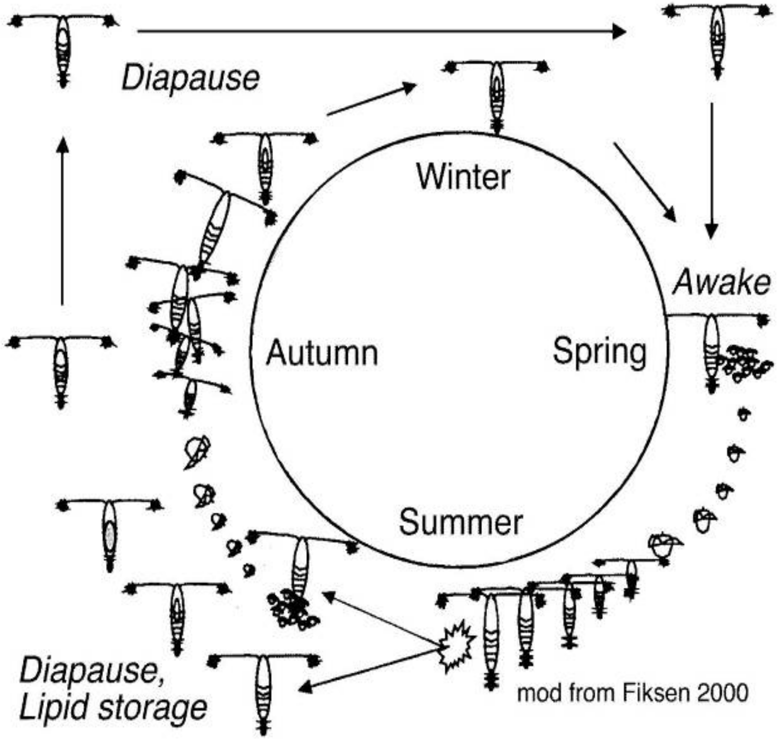
- Quantitative real-time PCR, Microarray, NextGen sequencing


Micrograph: Pat Hassett

Calanus finmarchicus

- Ecologically important
- Concern about population distributions with climate change

Life cycle of *C. finmarchicus* showing possible variations (after Fiksen, 2000)



Microarray Development

High quality total RNA

Normalized cDNA library
Invitrogen

Expressed sequence tags database
Clustering and annotation of ESTs

1000 genes targeted
Probes: unique 50-mer sequences

Customized microarray spotted

Microarray Target Genes

1. mRNAs from genes representing a wide range of biological processes

1. Analyze target genes using Bioinformatics tools:
   a. Gene ontology
   b. Pathway annotations

Gene Ontology Project

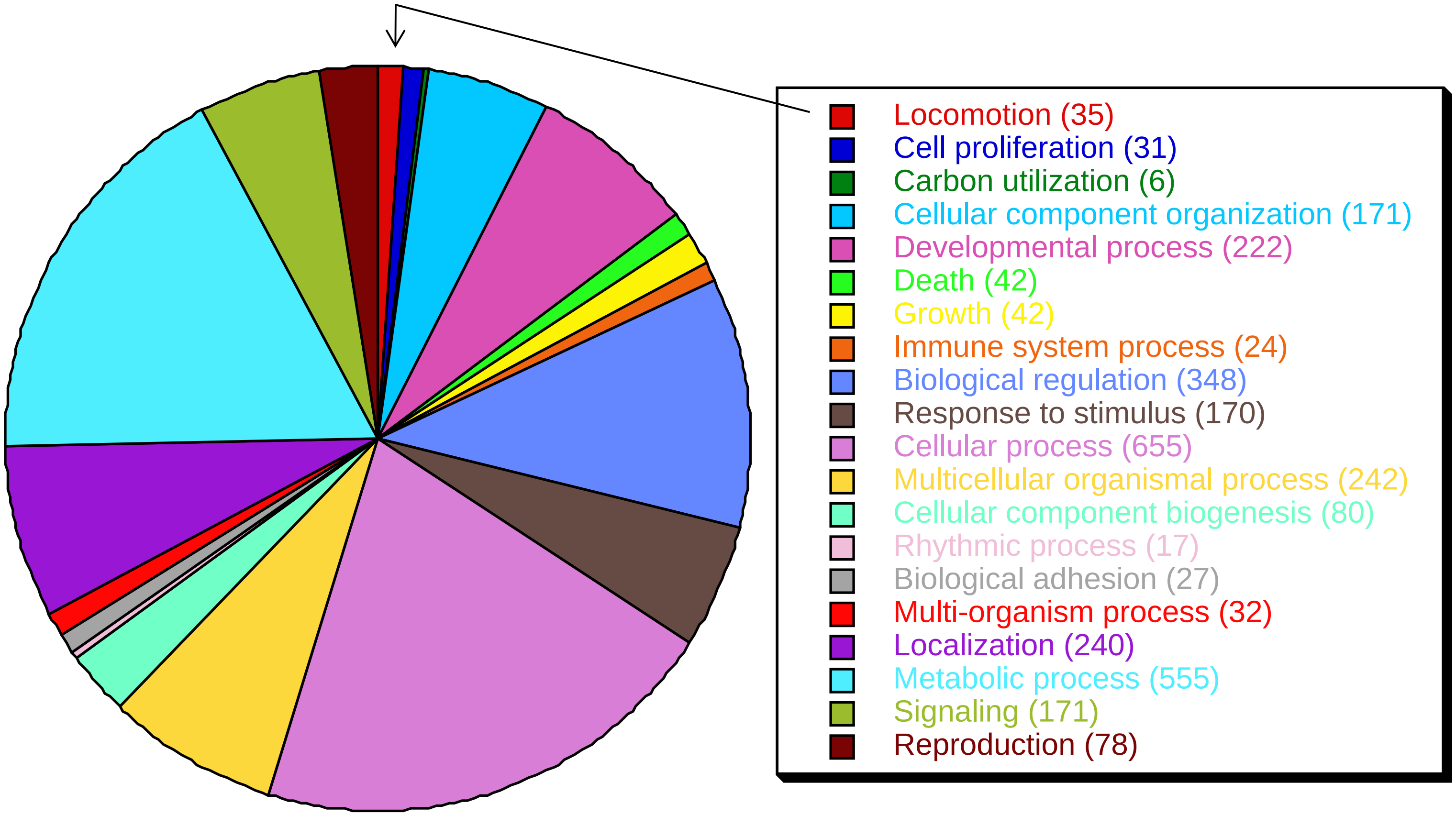
The need for consistent and common language to describe gene products in different databases through:

1. Development and maintenance of ontologies
2. Annotation of gene products
3. Development of tools to create, maintain and use ontologies

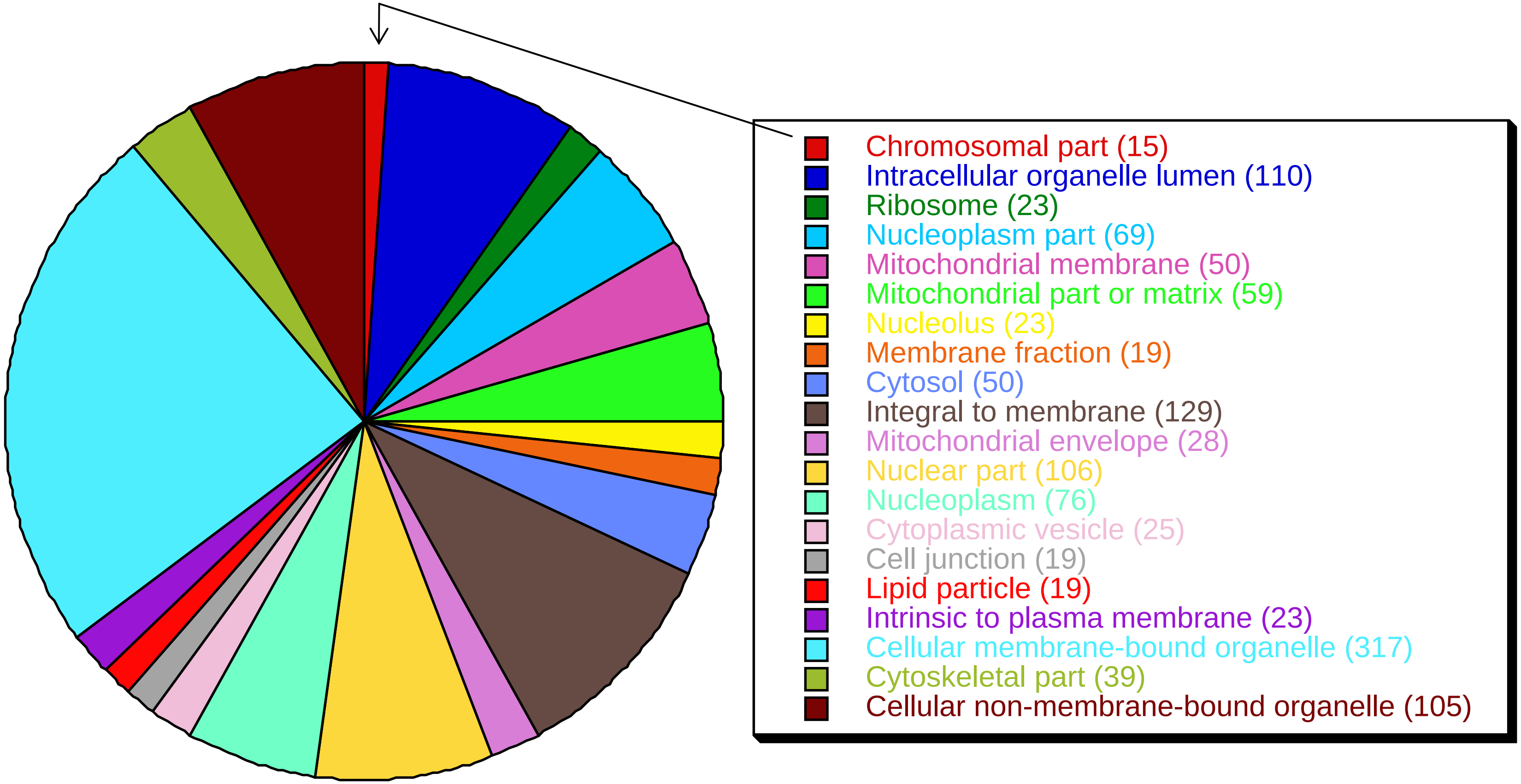
Provides:

Classification of gene products into **biological processes**, **cellular components** and **molecular functions**

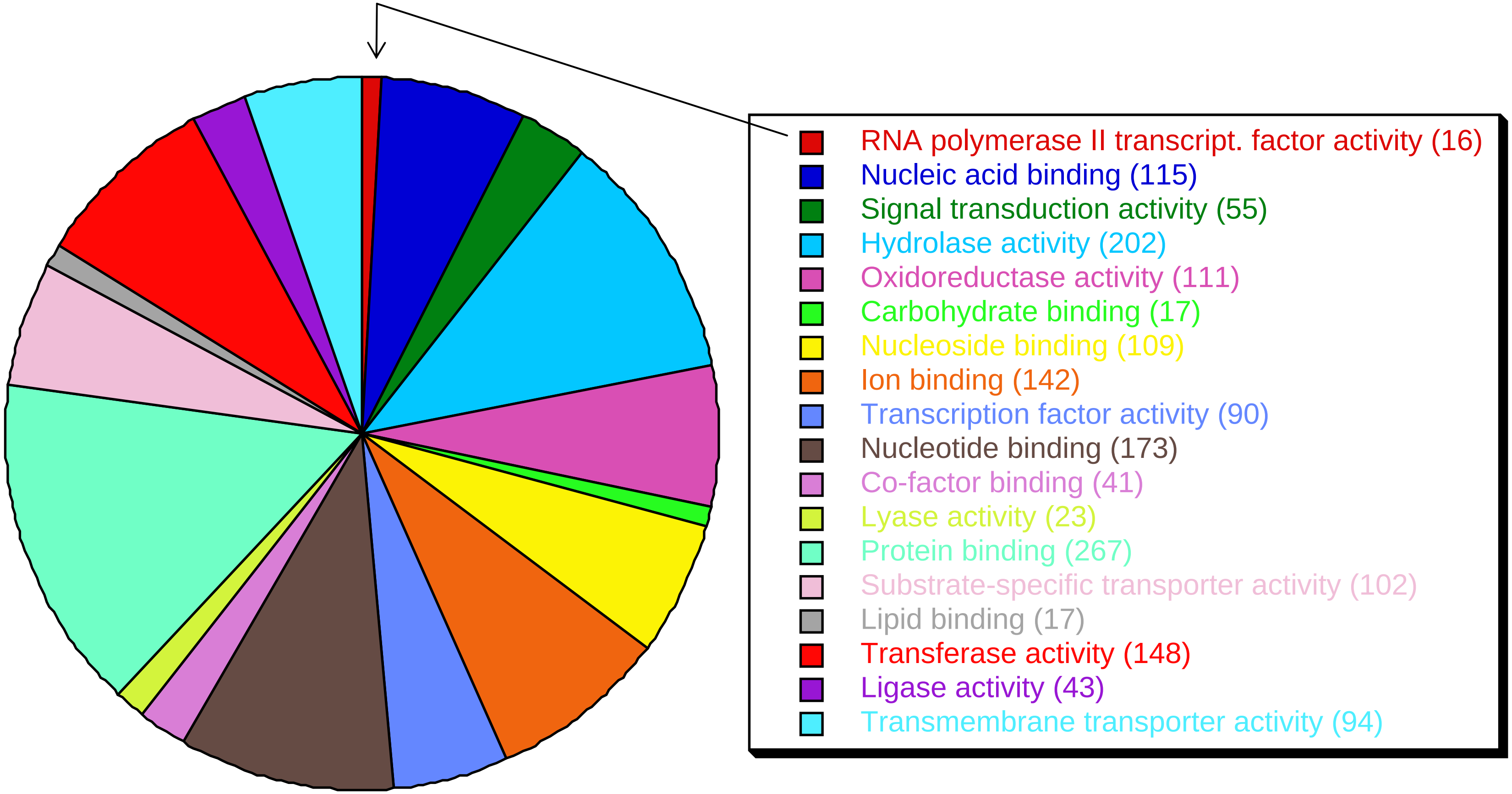
Gene Ontology – Microarray Probes
Blast2GO - Biological Process



Gene Ontology – Microarray Probes
Blast2GO – Cellular Component



Gene Ontology – Microarray Probes
Blast2GO – Molecular Function



Example: Citrate Synthase

CFX00003-2

Biological Process

- Cellular carbohydrate metabolic process (GO:0044262)
- Response to stress (GO:0006950)
- Tricarboxylic acid cycle (GO:0006099)
- Glyoxylate metabolic process (GO:0046487)

Cellular Component

- Mitochondrial matrix (GO:0005759)

Molecular Function

- Citrate (Si)-synthase activity (GO:0005759)

Example: Cytochrome Oxidase subunit I

CFX04627_1

Biological Process

- Aerobic respiration (GO:0009060)
- Electron transport (GO:0006118)
- Mitochondrial electron transport (GO:0006123)
- Proton transport (GO:0015992)

Cellular Component

- Mitochondrial inner membrane (GO:0005743)
- Integral to membrane (GO:0016021)
- Respiratory chain complex IV (GO:0045277)

Molecular Function

- Electron carrier activity (GO:0009055)
- Heme binding (GO:0020037)
- Cytochrome-c oxidase activity (GO:004129)

Kyoto Encyclopedia of Genes and Genomes (KEGG)

Resource for deciphering the genome through a collection of databases on genomes, enzymatic pathways and biological chemicals.

It connects known information on molecular networks.
PATHWAY mapping uses large-scale datasets in genomics, transcriptomics, proteomics, and metabolomics to map molecular interactions and reaction networks.

http://www.genome.ad.jp/kegg/

KEGG Pathway Annotation

Example: Fatty Acid Metabolism

Enzyme	Gene
enoyl-CoA hydratase	CFX02954_1
acyl-CoA oxidase	CFX02263_1
aldehyde dehydrogenase (NAD+)	CFX01002_1
long-chain-enoyl-CoA hydratase	CFX02954_1
long-chain-3-hydroxyacyl-CoA dehydrogenase	CFX02954_1
3-hydroxyacyl-CoA dehydrogenase	CFX02954_1, CFX02200_1
acetyl-CoA C-acyltransferase	CFX02954_1, CFX00268_1
glutaryl-CoA dehydrogenase	CFX01016_1
acyl-CoA dehydrogenase	CFX02803_1, CFX02569_1, CFX02263_1, CFX00958_1

KEGG Pathway Annotation

Example: Galactose metabolism

Enzyme	Gene
galactokinase	CFX00838_1
hexokinase	CFX04635_1, CFX04005_1, CFX03763_1
6-phosphofructokinase	CFX01084_1

Example: Fructose and mannose metabolism

Enzyme	Gene
hexokinase	CFX04635_1, CFX04005_1, CFX03763_1
mannose-1-phosphate guanylyltransferase	CFX01563_1
6-phosphofructokinase	CFX01084_1

Preliminary Testing of Microarray

1 Adult females under experimental conditions: High food vs. low food conditions for 8 days

2 CV summer morphotypes: Lipid-rich vs. lipid-poor

Micrograph: Pat Hassett

Microarray Hybridization Protocol

RNA extraction

cDNA labeled with fluorescent probes

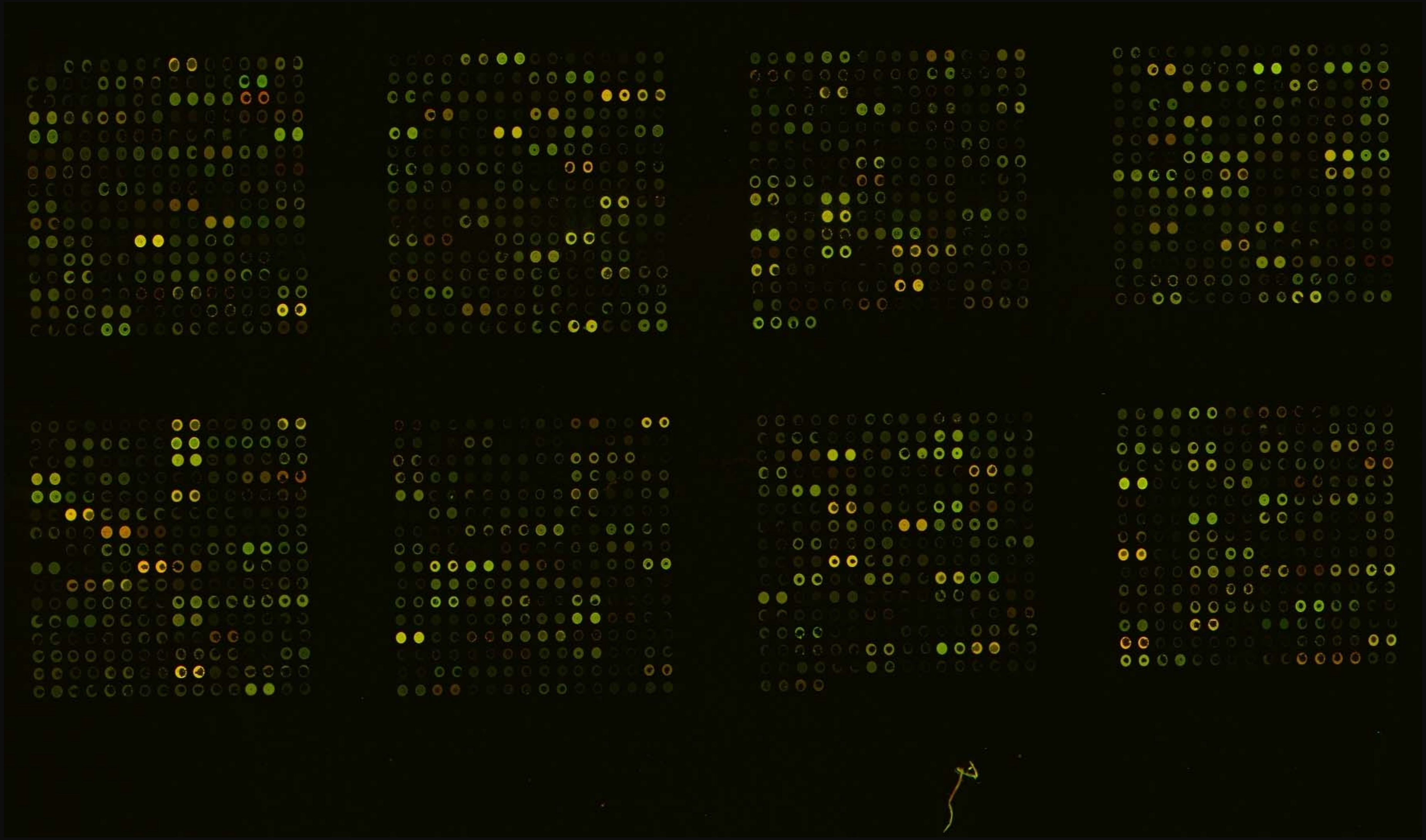
Combine equal amounts of cDNA from control and treatment

Hybridize overnight

Read spot intensities

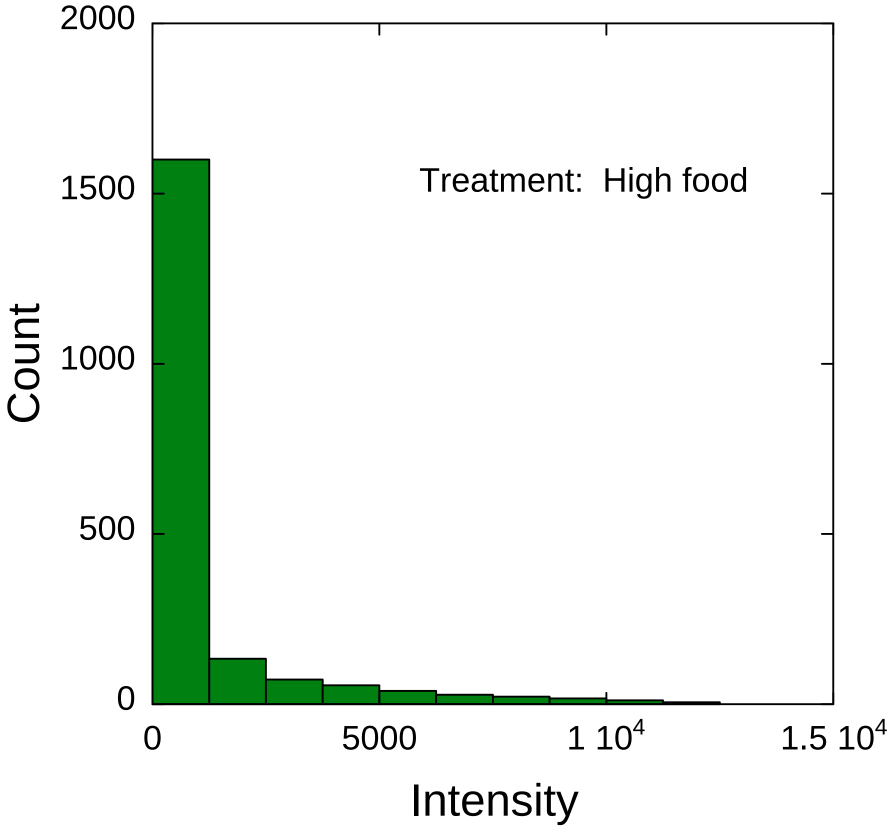
Data analysis: Acuity, Excel, R

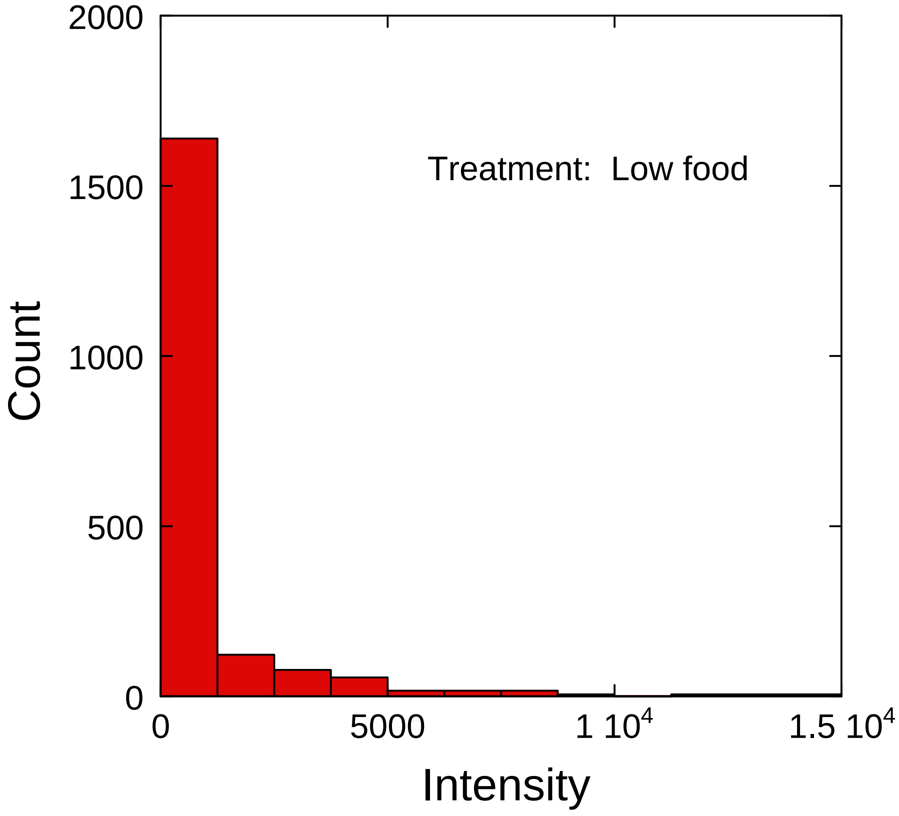
Calanus finmarchicus
Microarray Image



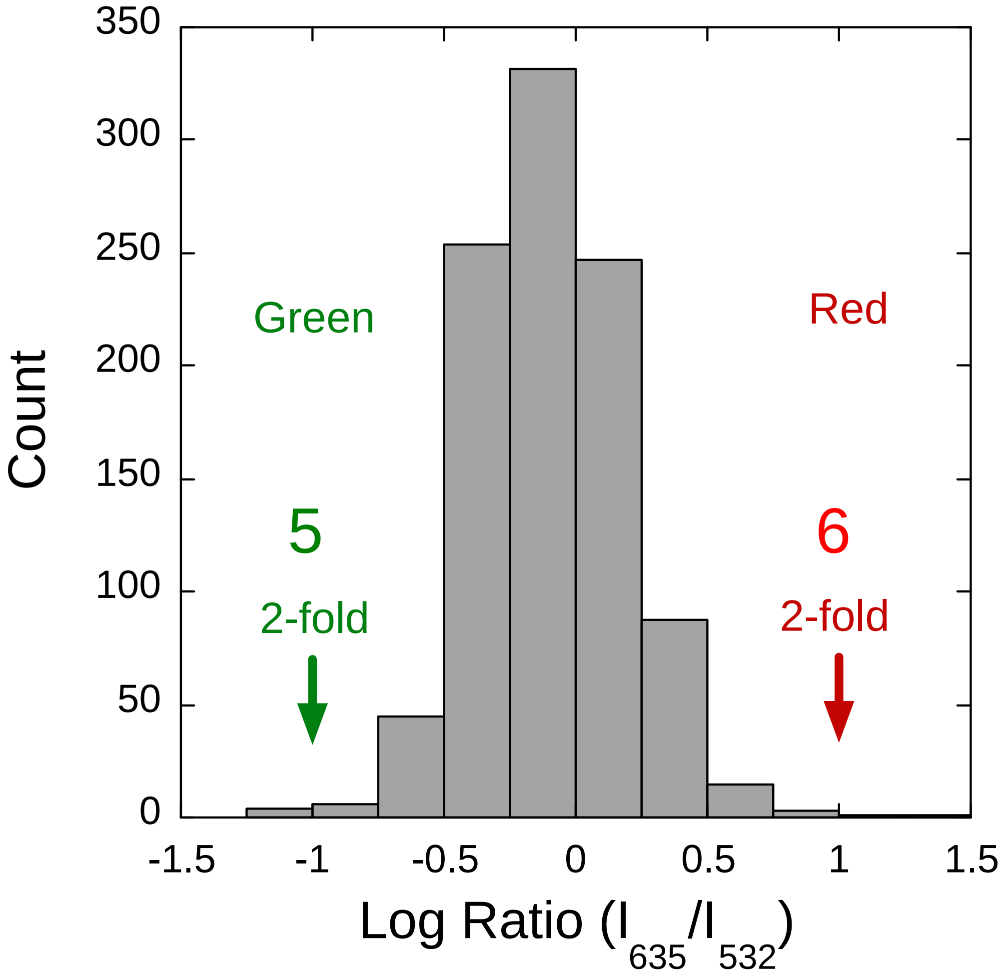
Microarray Results: Spot Intensities
Adult Females - High vs. Low Food

Normalized





Relative Gene Expression:
Log Ratio of Intensities



Examples of Regulated Genes in Adult Females under Low Food

Contig ID	Upregulated Low Food	Annotation
CFX01549	2x	Branched chain keto acid dehydrogenase Gluconeogenesis/Glycolysis
CFX01803	2x	Translation elongation factor g Regulation of translational regulation
CFX00403	2x	Fasciculation and elongation protein zeta-2 Axon cargo transport
CFX01407	1.5x	AMP-activated protein kinase Signal transduction

P < 0.01, Mann-Whitney U

Summary

- Gene expression used as a proxy for physiology

- Simultaneous screening of many physiological processes

- Stepping stone to even more powerful techniques
    - Selection of target genes for more detailed analysis
    - Analysis of a larger number of genes

Mahalo

- Andy Christie
- Dan Hartline
- Lisa Crockett
- Ann Castelfranco
- Gabor Mocz

- Chris Smith
- Andrew Peterson

- Maria Voznesensky
- Kelly Baehre
- Lisa Oliveira

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