Making and Using a cDNA-Based Microarray for *Drosophila*

Tim Westwood
Department of Zoology, University of Toronto
and the Canadian *Drosophila* Microarray Centre

Montreal Microarray Symposium
March 18-19, 2004

Topics

Part A- The Canadian *Drosophila* Microarray Centre

1. CDMC staff and funding
2. Current products and services
3. Future products and services
4. CDMC bioinformatics tools
5. Microarray experiment analysis

Part B Using the CDMC Microarray

1. RNA labelling and hybridization
2. Experimental design
3. Analysis parameters
4. Results

The Canadian Drosophila Micoarray Centre (CDMC)

- Located at University of Toronto at Mississauga
- Funded by CIHR and NSERC
- Over 40 labs across Canada, the U.S. and India have used the CDMC
- Director: Tim Westwood (Dept. Zoology, U. Toronto)
- Co-directors: Howard Lipshitz (Dev. Biol. Program, Hosp. for Sick Children)

  and Henry Krause (Dept. of Medical Res., Best Inst., U. Toronto)
- Current employees: Jianming Pei,Scott Neal, Huafen Li

Original Goals of the CDMC

1. Manufacture a *Drosophila* DNA microarray and distribute to Canadian researchers for a nominal fee

2. Establish a DNA Microarray Experimental Service Facility to Analyze Samples on the *Drosophila* microarrays

2. Current Products and Services

A. *Drosophila* 12K version 1 Microarray

cDNA

- *Dm* Gene Collections 1 & 2
  - BDGP
  - 5928 clones (DGC1)
  - 5530 clones (DGC2)
- NIH Testis Library
  - J. Andrews and B. Oliver
  - 5000+ clones
  - ~400 non-redundant to DGC1 and 2

Other Amplicons

- Genomic Amplicons
  - PI Gene Request Lists (Non-redundant with DGC 1 & 2)
  - ~500 genes
- Control Genes
  - Bacterial genes
    - *B. subtilis*
  - Fusion tag sequences
    - GFP, LacZ, Gal4, etc.
  - Pooled Amplicons (MSP)

B. Quality Control Image Check of Microarray Batches

C. “Downloads”

- Protocols for RNA preparation, labelling and hybridization
- Microarray support files: Quantarray and Axon gal files for array analysis; gene look-up tables

D. Experimental Services

1. Microarray scanning
2. RNA labelling, hybridization and scanning
3. RNA amplification service
4. Custom Arraying

E. Data Analysis

1. Experiment Consultation
2. Microarray Experiment Analysis

F. Berkeley *Drosophila* Genome Project DGC 1.0 and 2.0 Clones

CDMC Website

www.flyarrays.com

3. Future Products and Services

A. "Full Transcriptome" Microarrays

Long oligos (Summer 2004?)

(International Drosophila Microarray Consortium standard)

- approximately 14,000-17,000 genes

B. Chromatin IPs (Fall 2004?)

- "ChIP on Chip" assays

C. CGH (Fall 2004?)

Chromatin Genomic Hybridization

4. Bioinformatics and Database Mining

Current CDMC tools:

A. Flydb

- A fast, stripped-down database containing info from: (1). CDMC 12K1, 7K2 and 7K3 microarray gene layouts, (2). DGC1.0 and 2.0 clone data, (3). Flybase data, (4). Unigene data from NCBI, and (5). Gadfly release 3.1, and 3.0.

B. Gbrowse

- This CDMC graphic annotation database includes: Gadfly releases 3.1, 3 and 2. By uploading your microarray data in gene feature format (gff), you can browse your array results based on the Gadfly annotation.

3. Mosquito: Flydb

- A cross species database which hosts the mosquito (*Anopheles gambiae*) genes homologous to the genes from *Drosophila melanogaster*.

4. Cross:Flydb

- A cross species database which hosts the genes homologous to the genes from *Drosophila melanogaster.*

5. Microarray Data Aligned to Chromosomes

- “Expression ratios” mapped to chromosome ideograms
- Useful for finding “expression domains” (Re: Spellman and Rubin. 2002. Evidence for large domains of similarly expressed genes in the *Drosophila* genome. J. Biology 1:5.)

5. Microarray Experiment Analysis

A. Experiment Consultation

- Help design what samples should be compared, how many replicates to do, etc.

B. Microarray Experiment Analysis

Loading of array data into CDMC database

- Support for GeneTraffic DUO (CDMC arrays) and UNO (Affymetrix arrays) (Iobion Informatics)
- Help with the posting of microarray data to the public databases (e.g. CDMC, Geo (NCBI), ArrayExpress)

GeneTraffic (Iobion Informatics)

- MIAME (minimal information about a microarray experiment) compliant database and analysis package
- Allows one to upload their microarray experiments into the database and perform numerous types of analyses including: data normalization; comparison of multiple replicates of the same conditions (with statistics- SAM, volcano plots, T-tests, F-tests, PCA); find up- and down-regulated genes; perform gene clustering, etc.
- The database and analysis software will be accessible over the internet using Microsoft Internet Explorer 6 on a PC computer.

The CDMC Microarray Database

It will contain:

(1) Publicly available microarray data

(e.g. CDMC SL2 and Kc cell data; Kevin White/Stanford developmental time course; Paul Spellman/BDGP assorted Affymetrix data)

(2) “Client data”

- Data will remain confidential until publication

Part B Using the CDMC Array

Projects we are doing:

- Cell line expression patterns (SL2 and Kc167 cells)
- Heat Shock Response
- Ecdysone regulated genes

Cell Line Gene Expression Patterns (SL2 and Kc167 cells)

- RNA isolation
  - Amount of RNA required
- Direct labelling protocol
  - Potential for dye bias
- Data analysis
  - Number of replicates required

This has been published…

Scott J. Neal, Meredith L. Gibson, Anthony K.-C. So and J. Timothy Westwood. Construction of a cDNA-based microarray for *Drosophila melanogaster*: A comparison of gene transcription profiles from SL2 and Kc167 cells.

Genome 46, 879-892 (2003)

- Can request free reprints from the CDMC website (www.flyarrays.com)

The Heat Shock Response

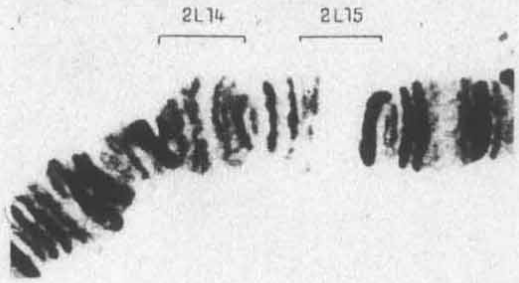


Fig. 1. The 2L 14 and 15 regions of salivary gland chromosome of *D. buscki* larvae reared at 25°C about 15 h before pupation.

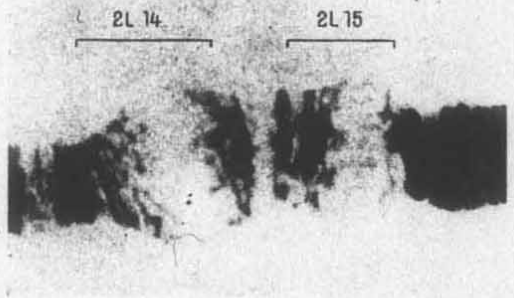


Fig. 2. The same regions as in Figure 1 after a thermal shock of 30 min at 30°C. Larvae near to pupation.

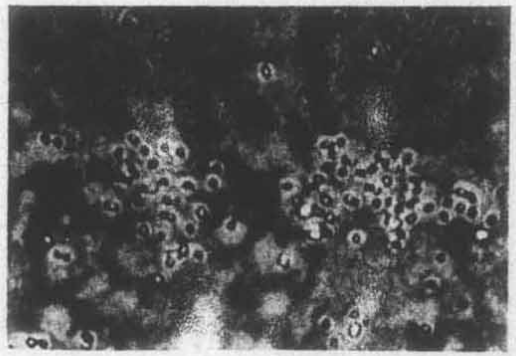
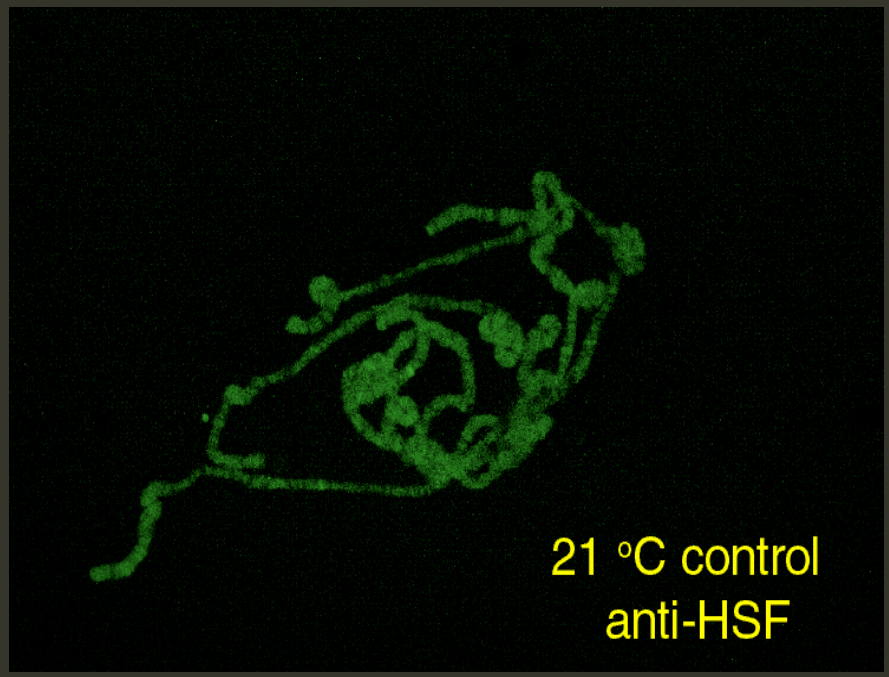
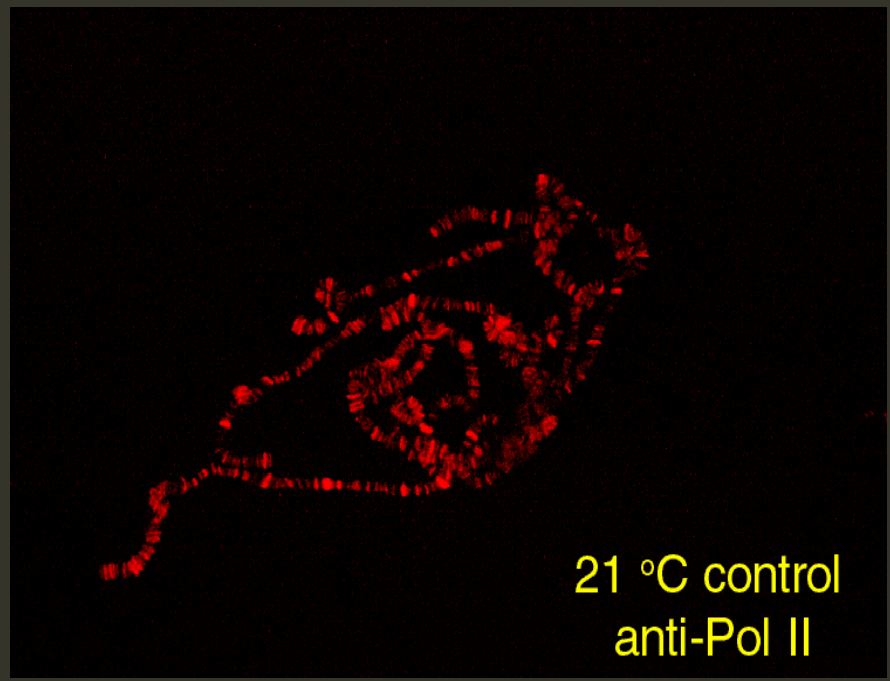


Fig. 3. The induced puffs after 10 min of incubation in 2.5 mm^3 of a saline solution (0.17 mC/ml) of tritiated cytidine (Schwarz, s.a. 1 C/m*M*). 4 days exposition. Stripping films Kodak AR. 10.

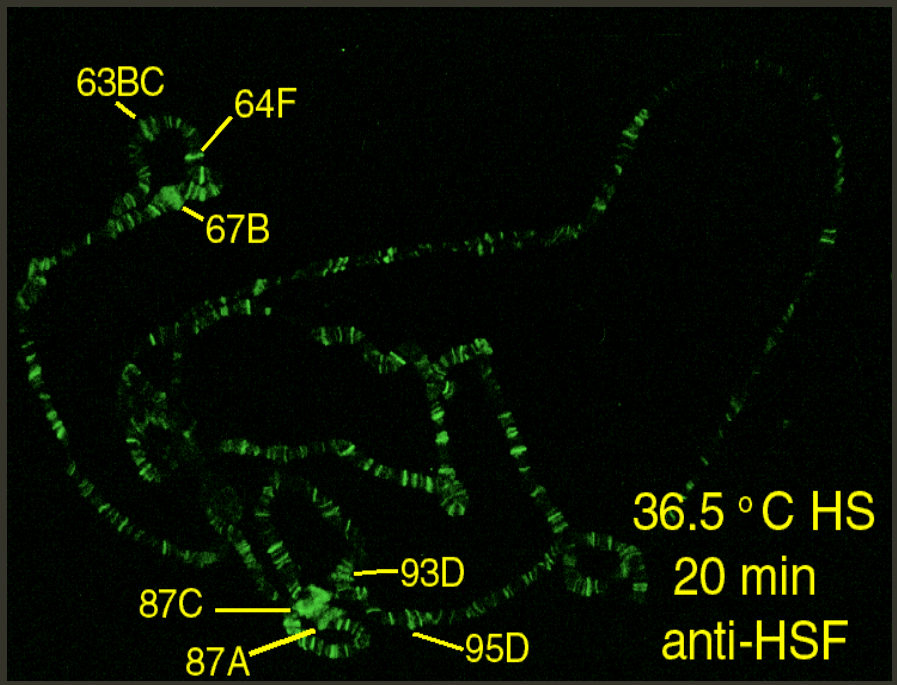
Ritossa (1962) Experientia XVII, 571-573

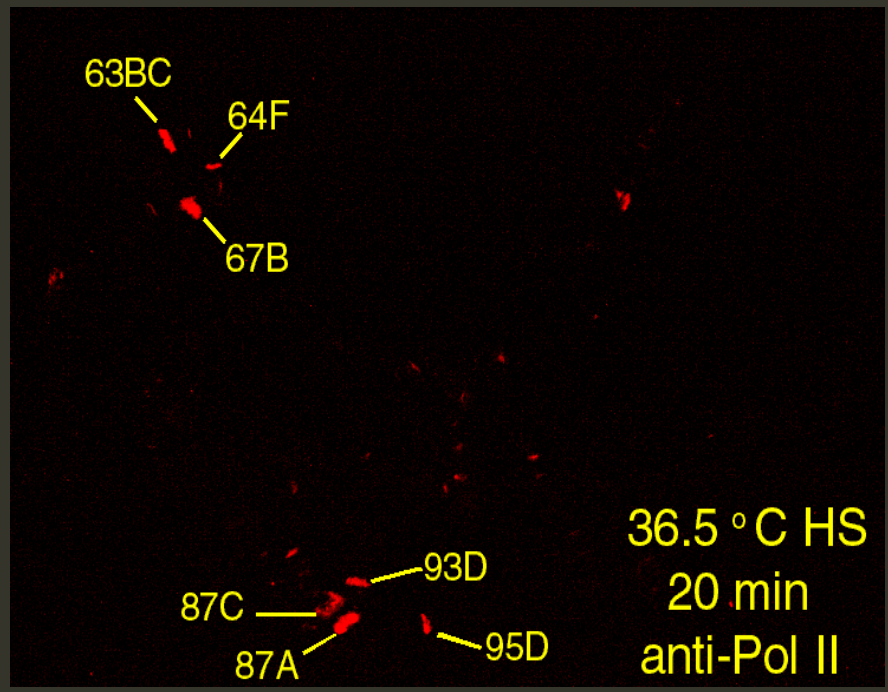
RNA polymerase is found at hundreds of sites at normal temperatures

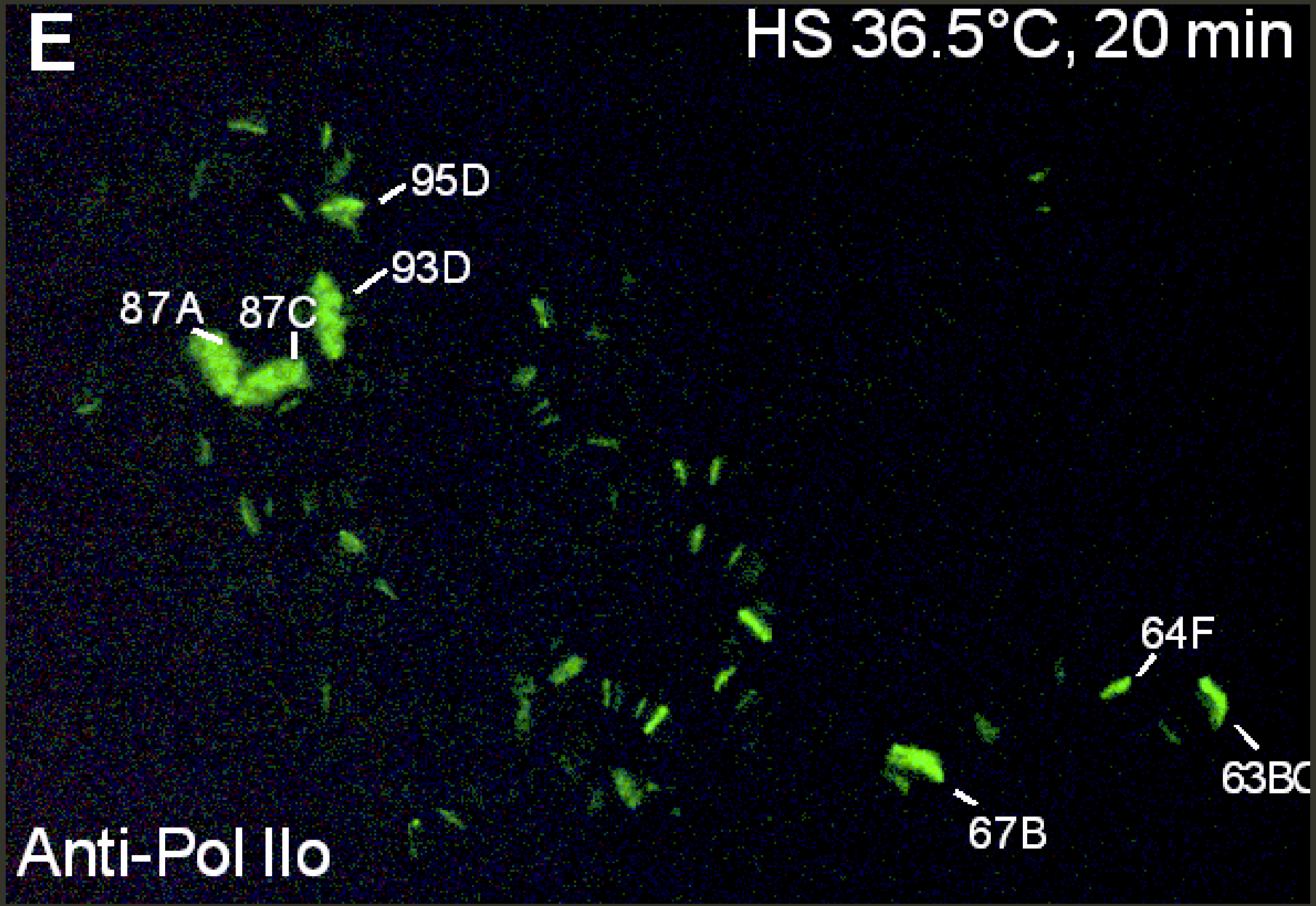




Heat shock causes Pol II to redistribute to the heat shock gene loci






E
HS 36.5°C, 20 min
95D
93D
87A
87C
64F
63BC
67B
Anti-Pol IIo

Heat Regulated Genes in SL2 Cells

n = 20 arrays

35 candidate genes upregulated, 7 downregulated

(8 more genes that are less reproducibly affected)

11 (up) are known hs genes

15 genes have no clearly defined function

~75 % of genes map to loci where HSF is known to bind on polytene chromosomes

Functions of Differentially Expressed Genes

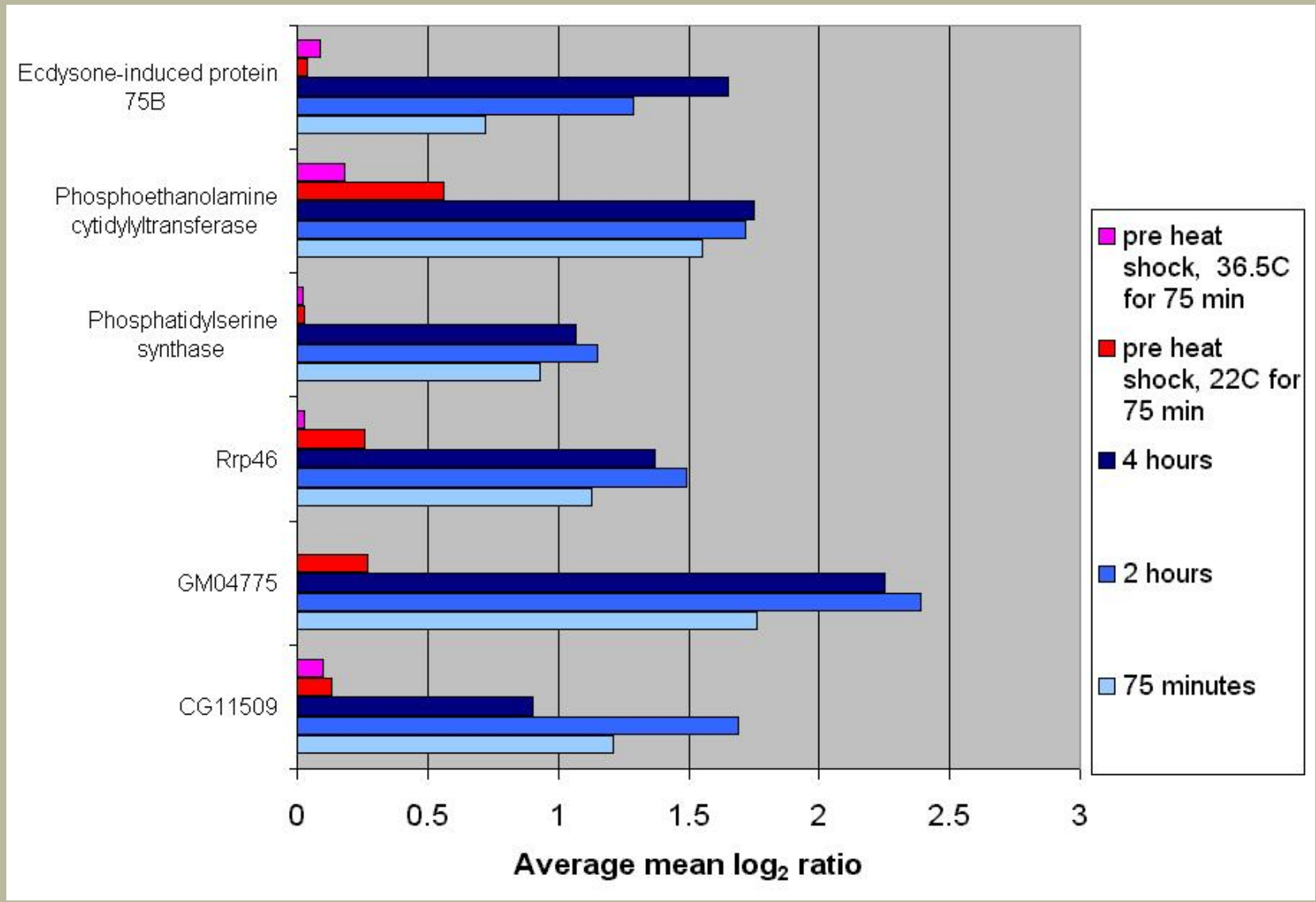
Up

- 3 transcription factors
- ubiquitin-specific protease
- ABC transporter
- adenylyl cyclase
- G protein
- DAG kinase
- actin binding
- RNA binding

Down

- V-SNARE
- H^+/oligopeptide symporter

Genes repressed in cells treated with 15 minute heat shock followed by ponasterone A treatment



Acknowledgements

The CDMC Staff

Jianming Pei, Huafen Li, Meredith Gibson, Tony So and Scott Neal

Westwood Lab

Scott Neal, Amy Kehoe

Clone Sources

BDGP; B. Oliver and J. Andrews (NIH)

Technical Advice

Neil Winegarden and Eric Ho (UHN, Toronto)

Daniel Tessier, Tracy Rigby (BRI, NRC Montreal)

Companies

Iobion Informatics, Perkin Elmer

Funding Agencies

CIHR and NSERC