Slide 1>>

CNS Development

- Diverse array of cell and tissue types
- Intrinsic and extrinsic mechanisms governing:
  - Specification of cell types
  - Proliferation and maturation of cell lineages
  - Cell migration and axon extension
  - Synaptic targeting and synapse formation
  - Plasticity
  - Regeneration and repair

Some Big Picture Questions

- What makes one neuron different from another? Can this be best defined by the genes these cells express?
- How might differences in gene expression define and dictate different functions of one brain region from another (and also within a region)?
- How does gene expression change over developmental time? Can we identify programs of gene expression?

Development of the CNS

Spatial patterns of gene expression:

the olfactory bulb

Temporal patterns of gene expression:

the developing cerebellum

Gene Expression Profiling in the Mouse Olfactory Bulb

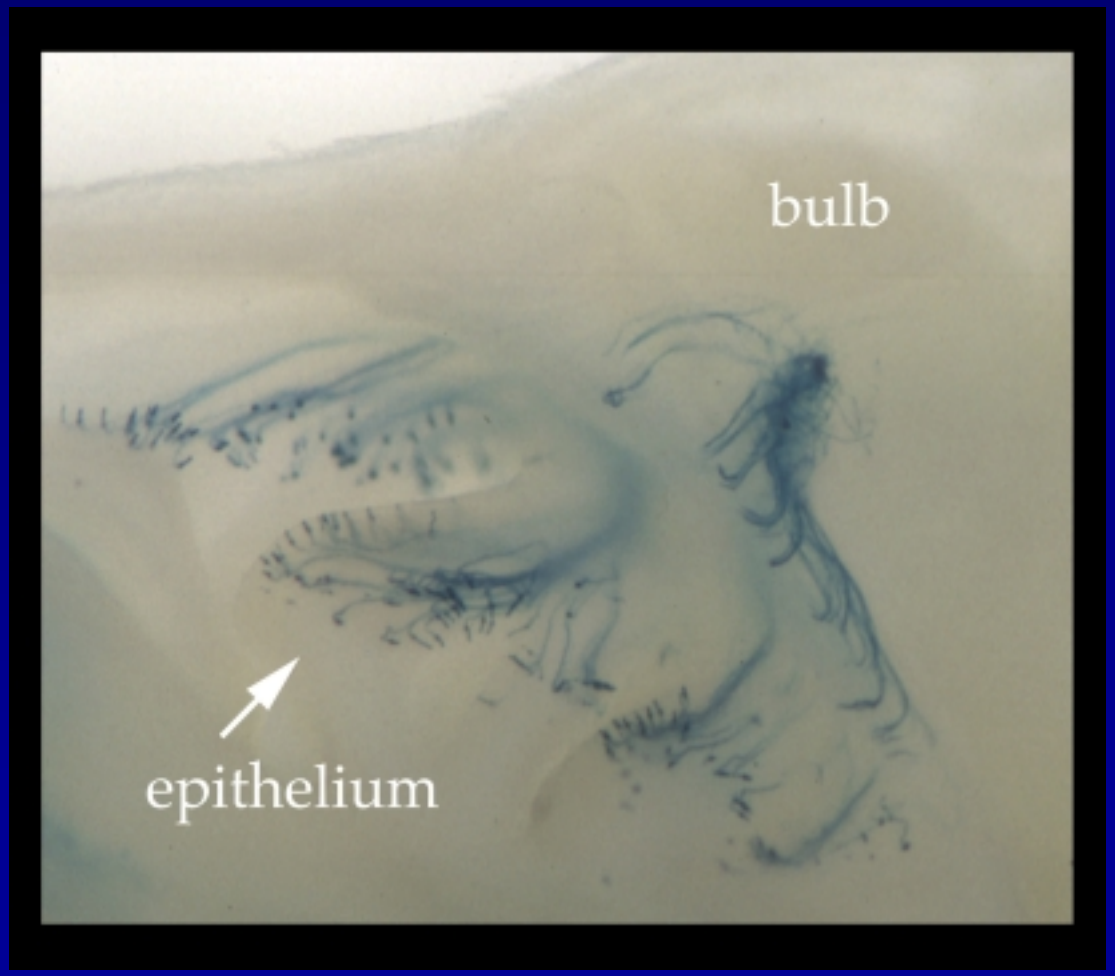
David Lin
Jean Yang
Terry Speed

Hardwiring of the Vertebrate Olfactory System

—Expression of a specific odorant receptor gene by an olfactory neuron

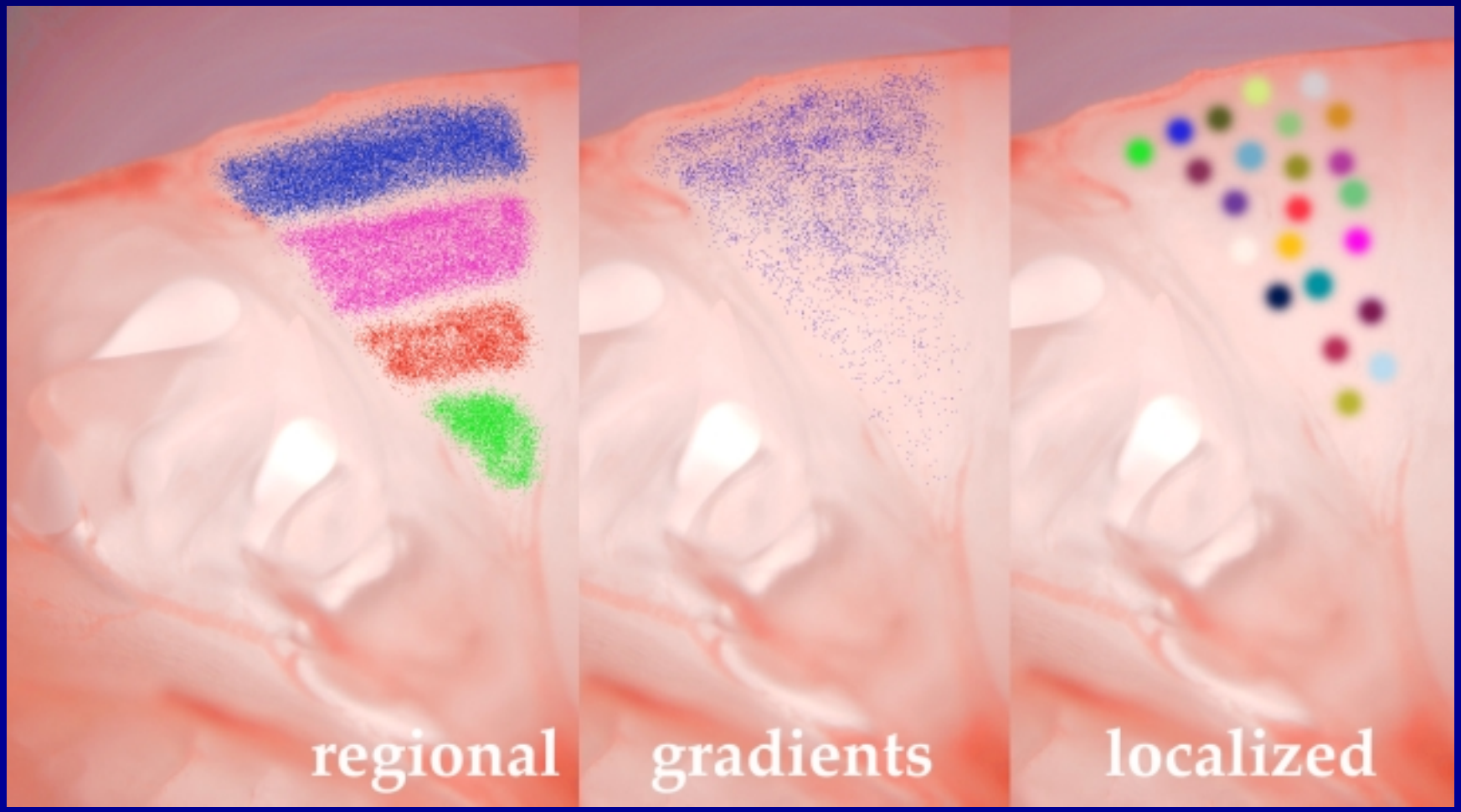
—Targeting and convergence of like axons to specific glomeruli in the olfactory bulb

P2-IRES-tau::lacZ



Hypothesis:

Molecules showing spatially restricted patterns of expression in the olfactory bulb are responsible for establishing patterns of afferent innervation.


regional
gradients
localized

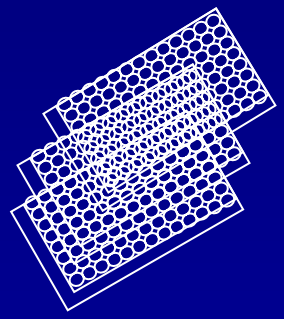
Are there genes with spatially restricted expression patterns within the olfactory bulb?

DNA Microarray Analysis

- "Genome wide" analysis of gene expression
- High throughput, sensitive, quantitative
- An unbiased approach (more or less…)
- "A vs. B" comparisons
- Identification of patterns of gene expression and genetic networks
  - Changes in gene expression during development of CNS structures
  - Spatial patterns of gene expression in the CNS

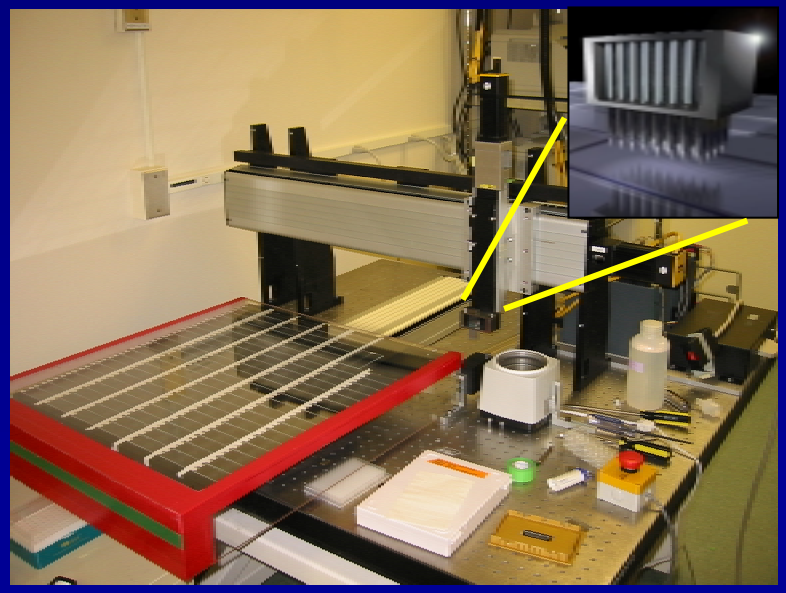
Fabrication of “Spotted Arrays”

~19,000 cDNAs

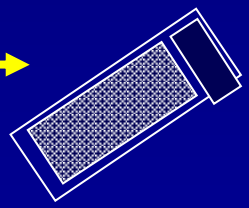


PCR amplification and precipitation

Printing



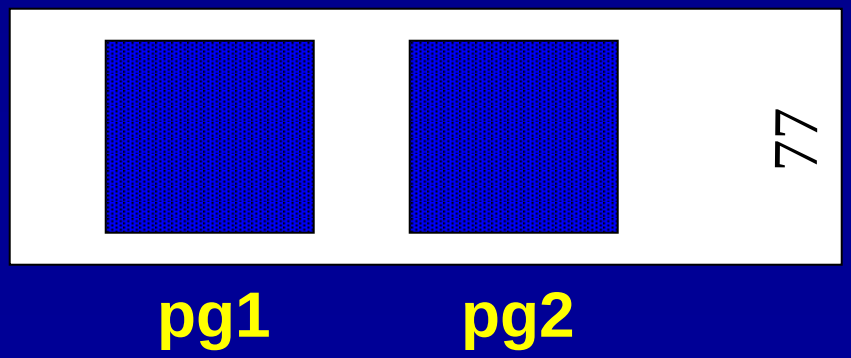
Slides



Riken Release 1 Cloneset - ~19,000 mouse cDNAs plus “usual suspect” genes

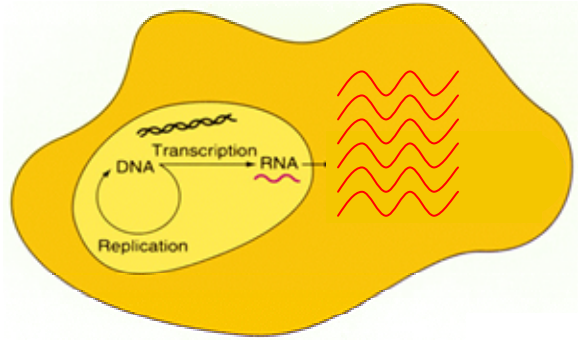
Layout of the cDNA Microarrays

- Sequence verified, normalized mouse cDNAs
  - RIKEN Release 1 Cloneset
- 19,200 spots in two print groups of 9,600 each
  - 4 x 4 grid, each with 25 x24 spots
  - Controls on the first 2 rows of each grid.

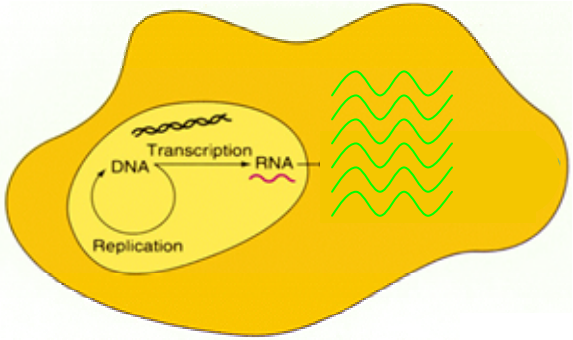


Expression Profiling with DNA Microarrays

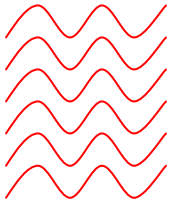
Cell "A"



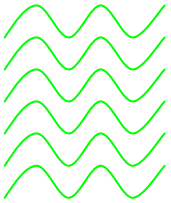
Cell "B"



Cell sample

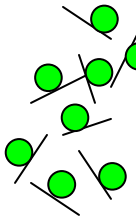


Isolate mRNA



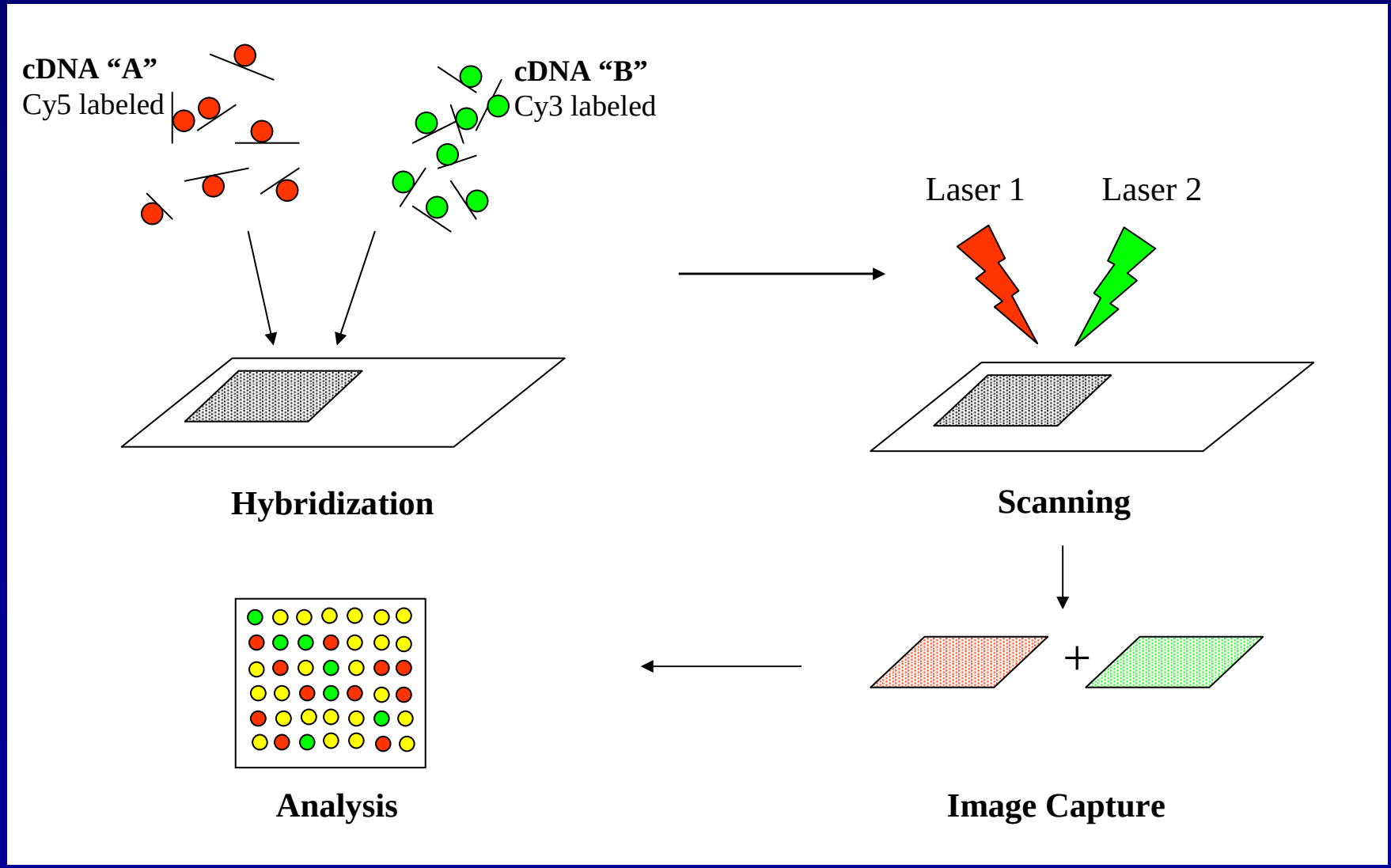
cDNA "A"
Cy5 labeled

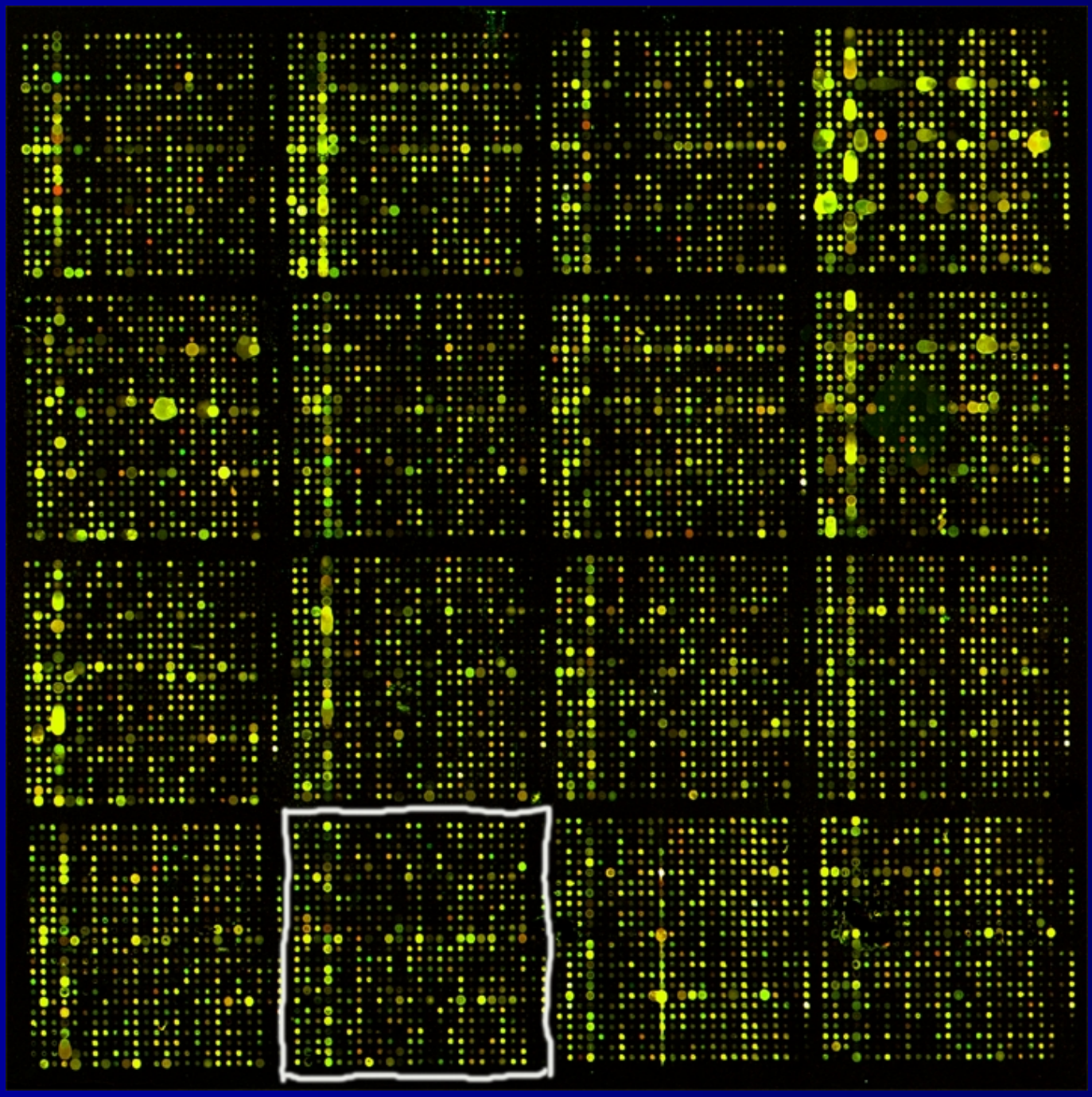
Synthesize fluorescently-labeled cDNA

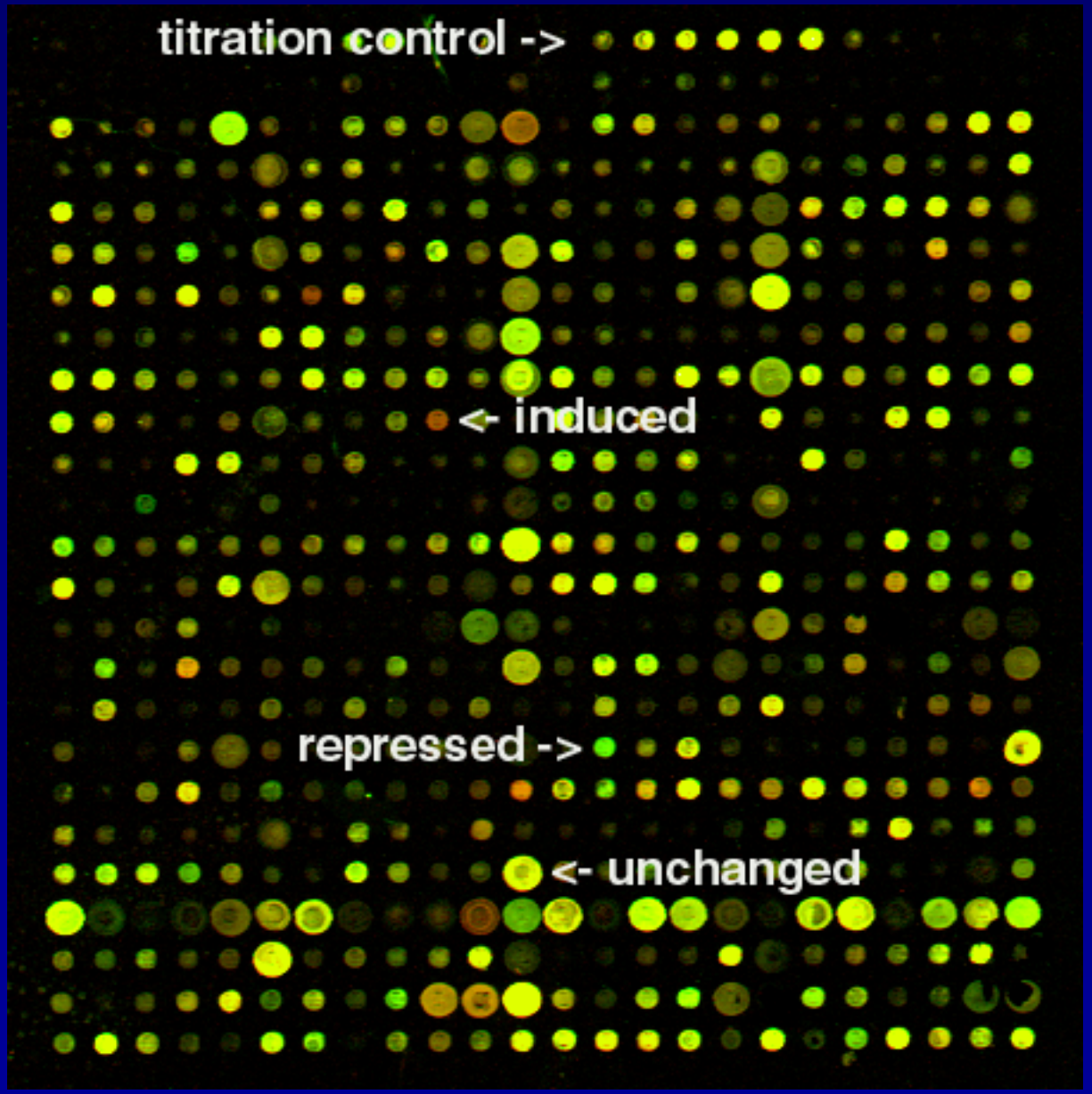


cDNA "B"
Cy3 labeled

Expression Profiling with DNA Microarrays






titration control ->
<- induced
repressed ->
<- unchanged

Quantitation of signals

M

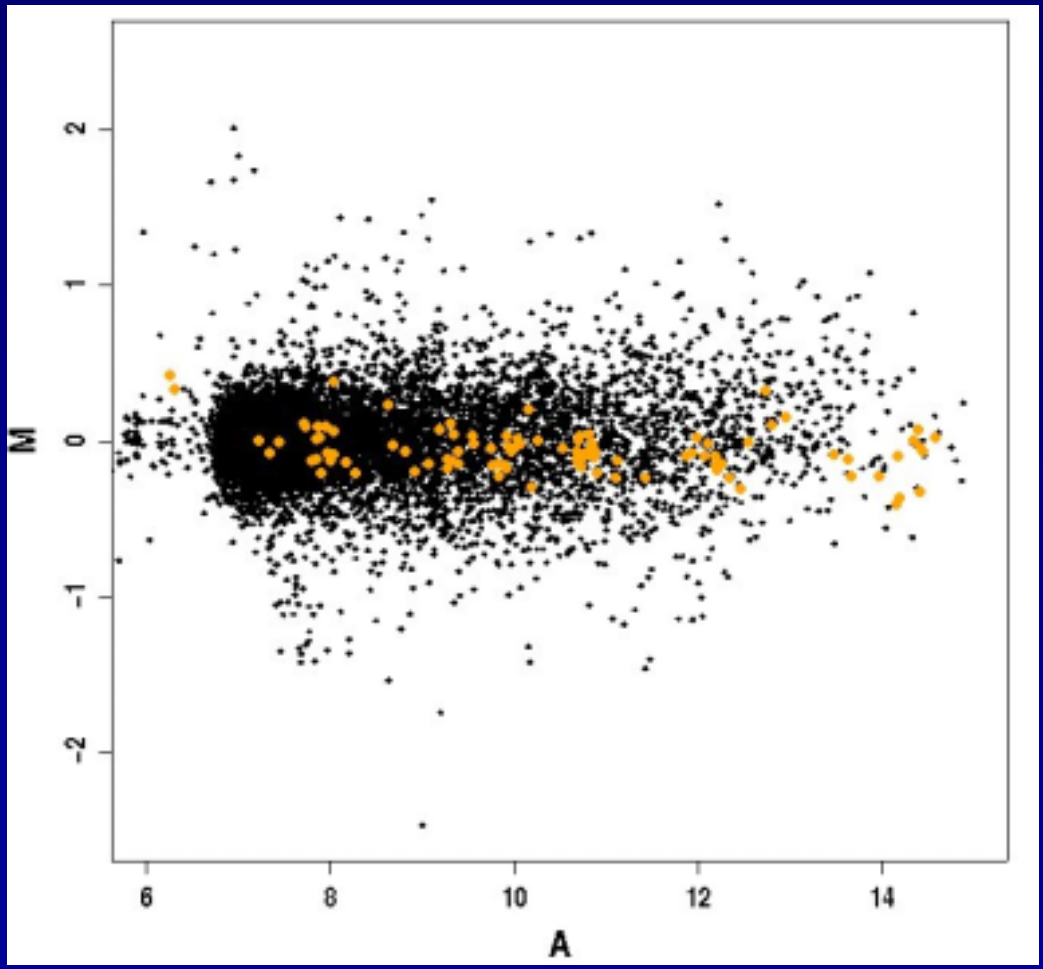
Log_2 (Red/ Green)

vs.

A

$[\log_2 R + \log_2 G]/2$

(average intensity)



"M *vs.* A" plot

Back to the biological question...

Are there genes with spatially restricted expression patterns within the olfactory bulb?

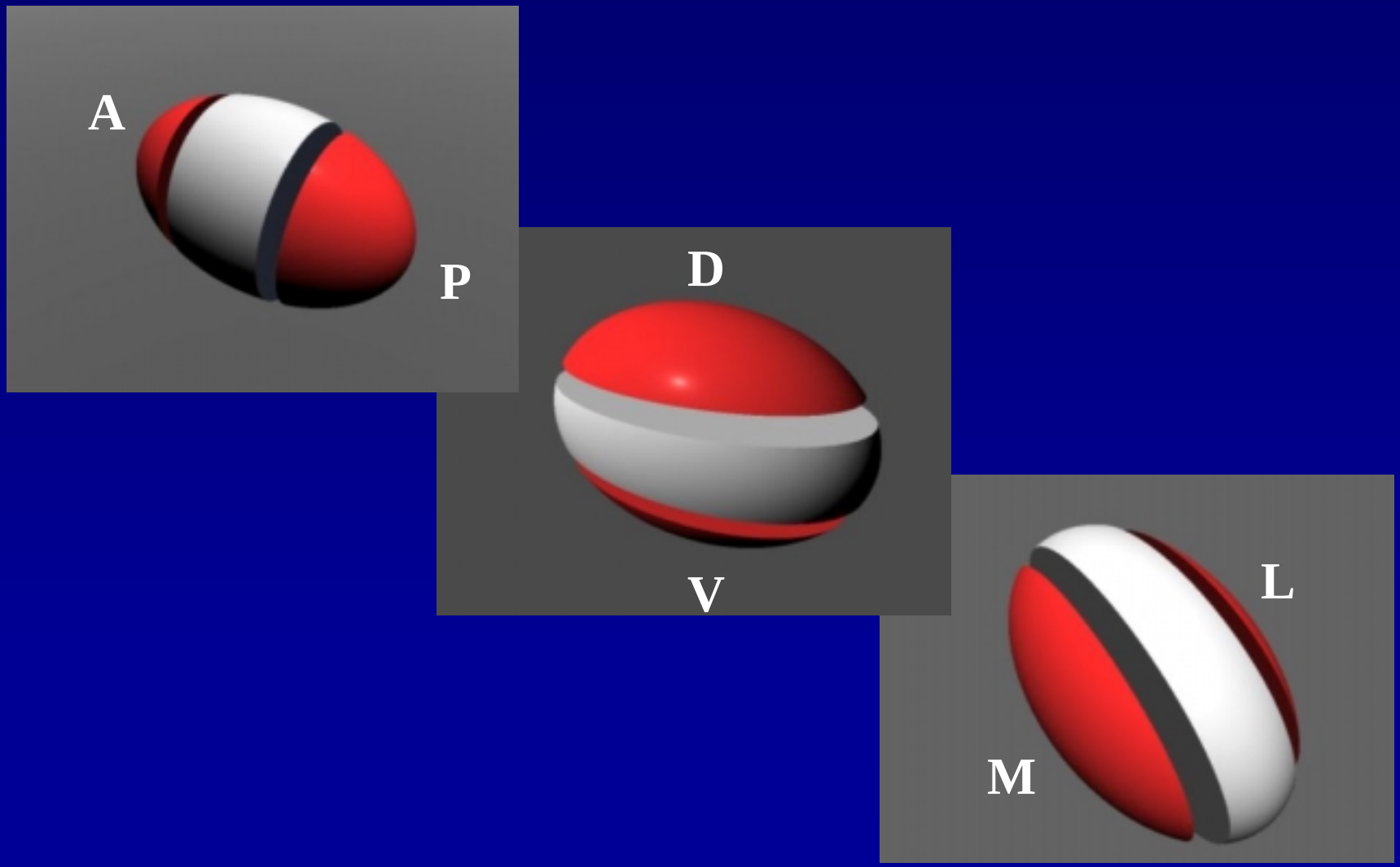
Design: Preparation of Samples for Microarray Analysis

—Isolation of RNA from spatially defined regions of neonatal mouse olfactory bulb

—Amplification of mRNA with T7 RNA polymerase (linearity => preservation of representation)

—Hybridization to cDNA microarrays

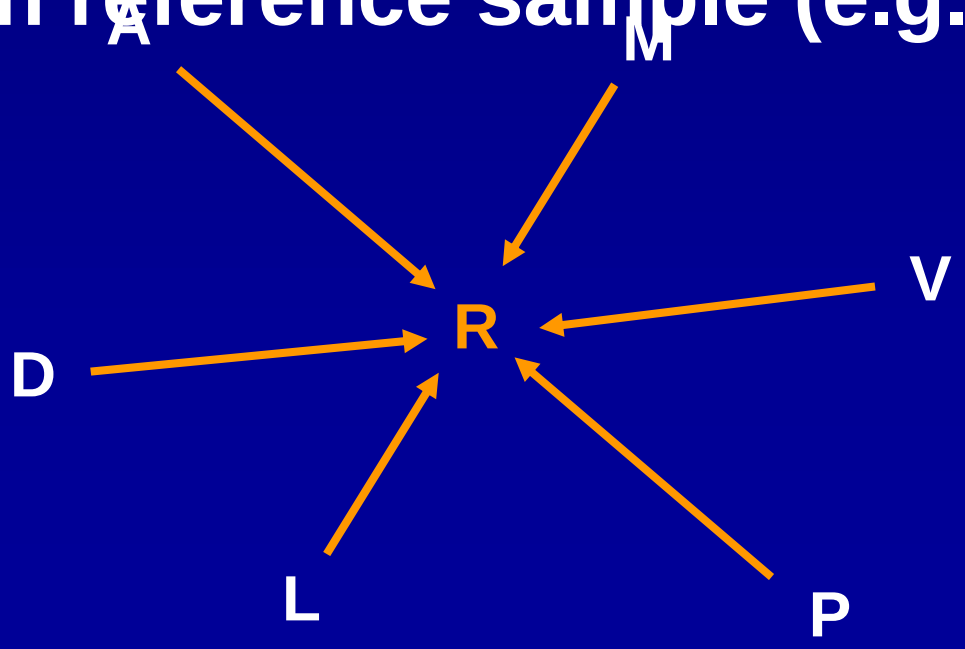
Design: How We Sliced Up the Bulb



Design: Two Ways to Do the Comparisons

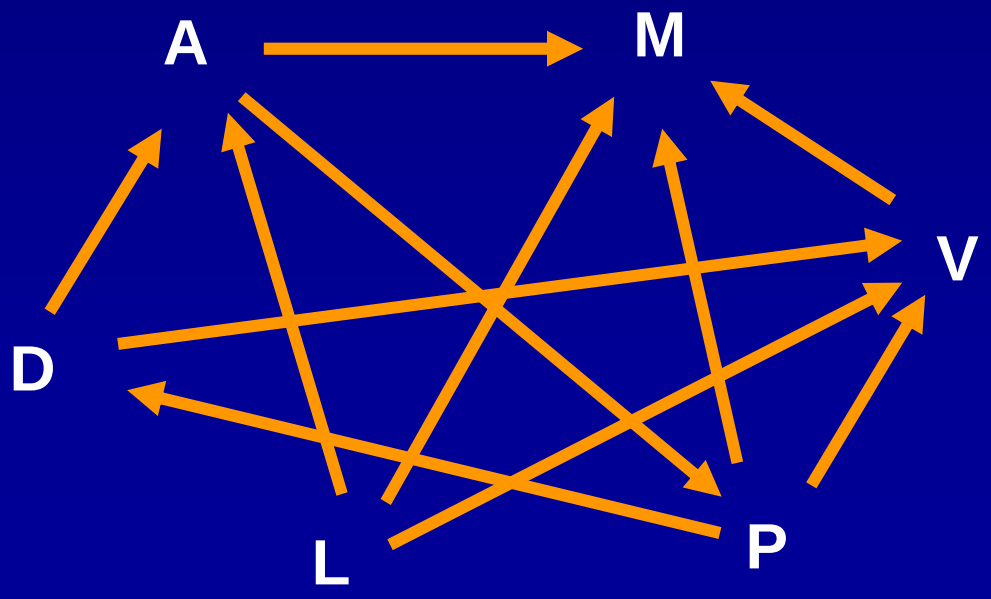
Goal: 3-D representation of gene expression

Design 1: Compare all samples to a common reference sample (e.g., whole bulb)



Design: The Other Way...

Design 2: Multiple direct comparisons between different samples (no common reference)



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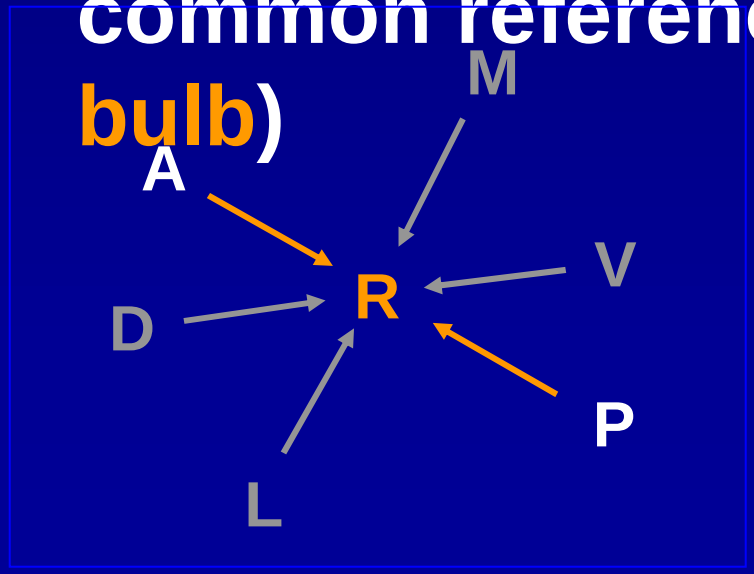
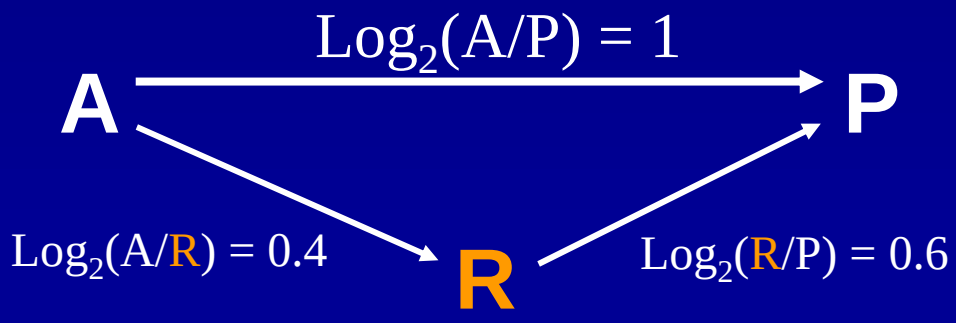
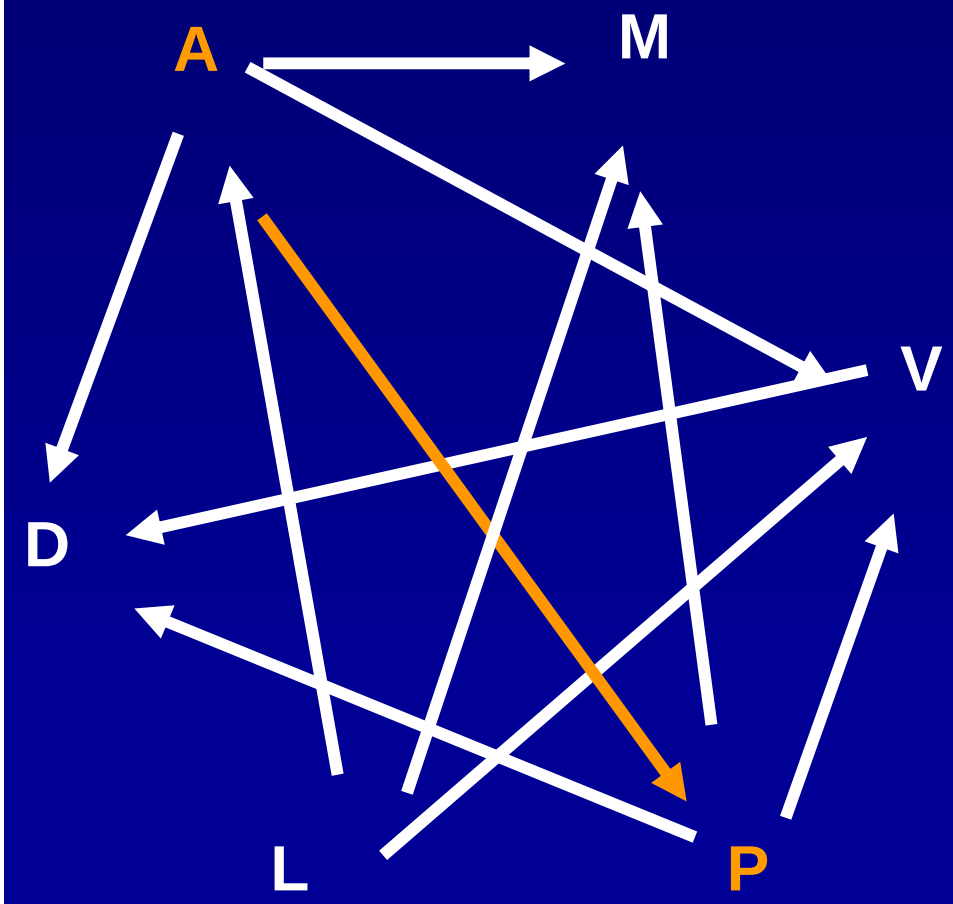


For example:



An Important Aspect of Our Design

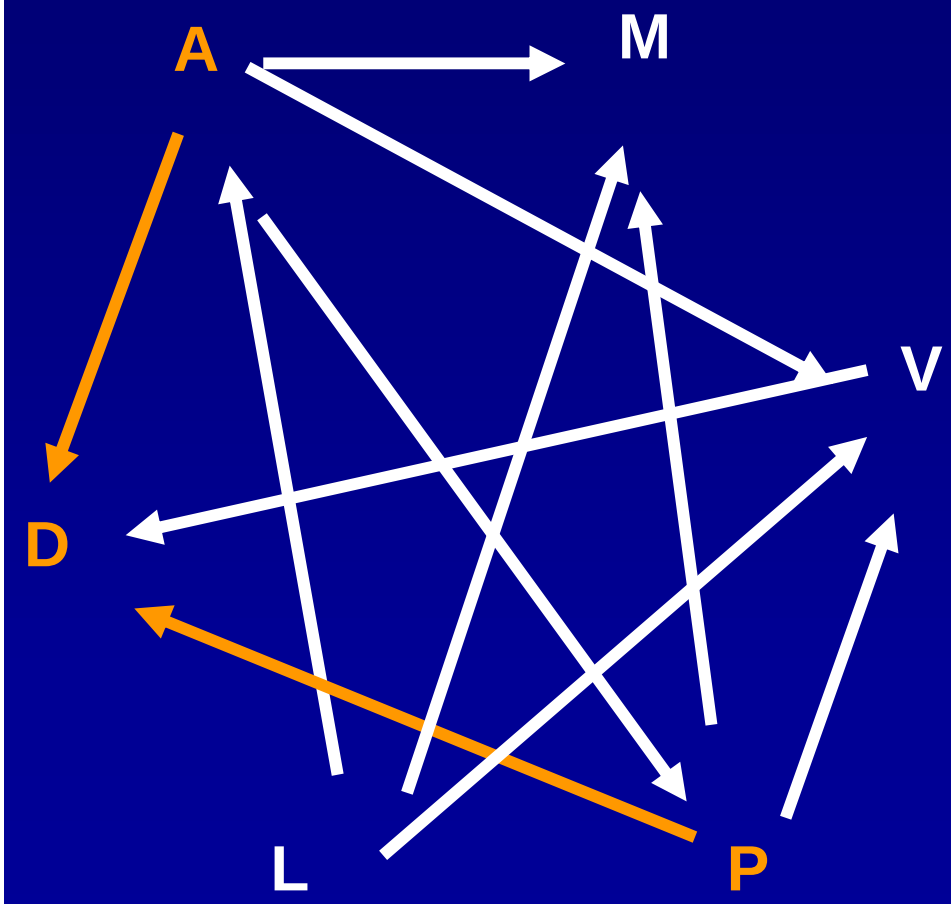


Different ways of estimating the same <u>contrast</u>:

e.g. A compared to P

Direct = A-P

An Important Aspect of Our Design



Different ways of estimating the same <u>contrast</u>:

e.g. A compared to P

Direct = A-P

Indirect = A-D - (P-D) or

A-M - (P-M) or

A-V - (P-V)

Two Advantages of Our Design:

—Incorporates direct comparisons which reduce the variance of each measurement

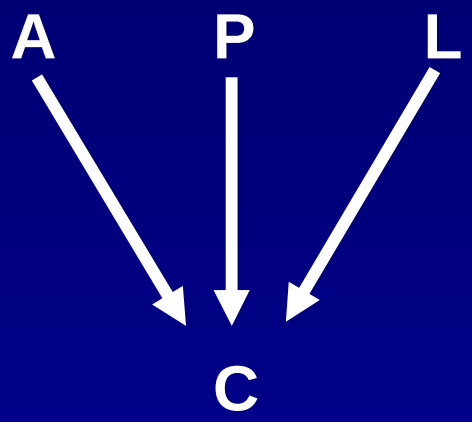
—For a given number of hybridizations, increased number of total measurements (direct & indirect) for a specific contrast

Direct and indirect measurements can be combined and averaged for each specific contrast (multiple regression analysis)

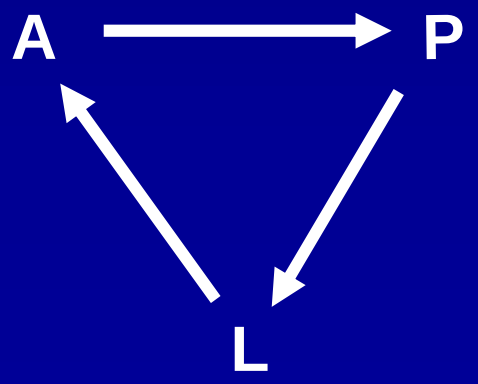
=> *increased precision*

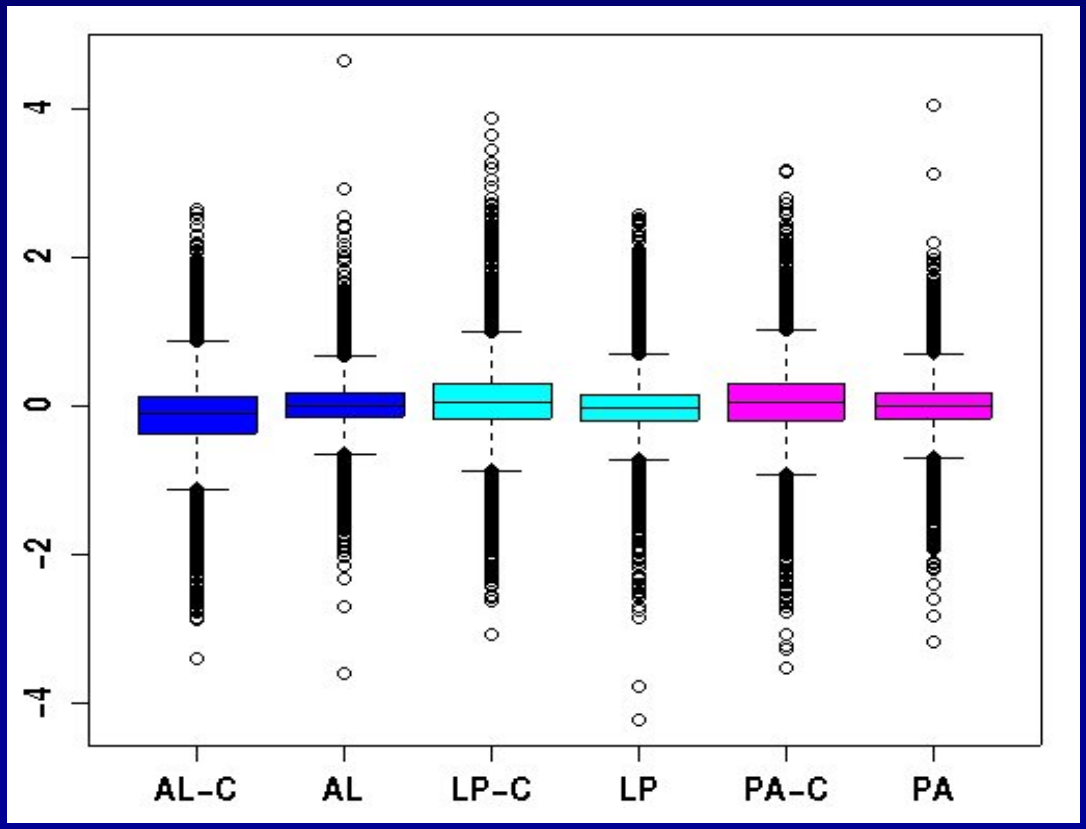
Common Reference vs. Direct Comparisons

Design A:



Design B:

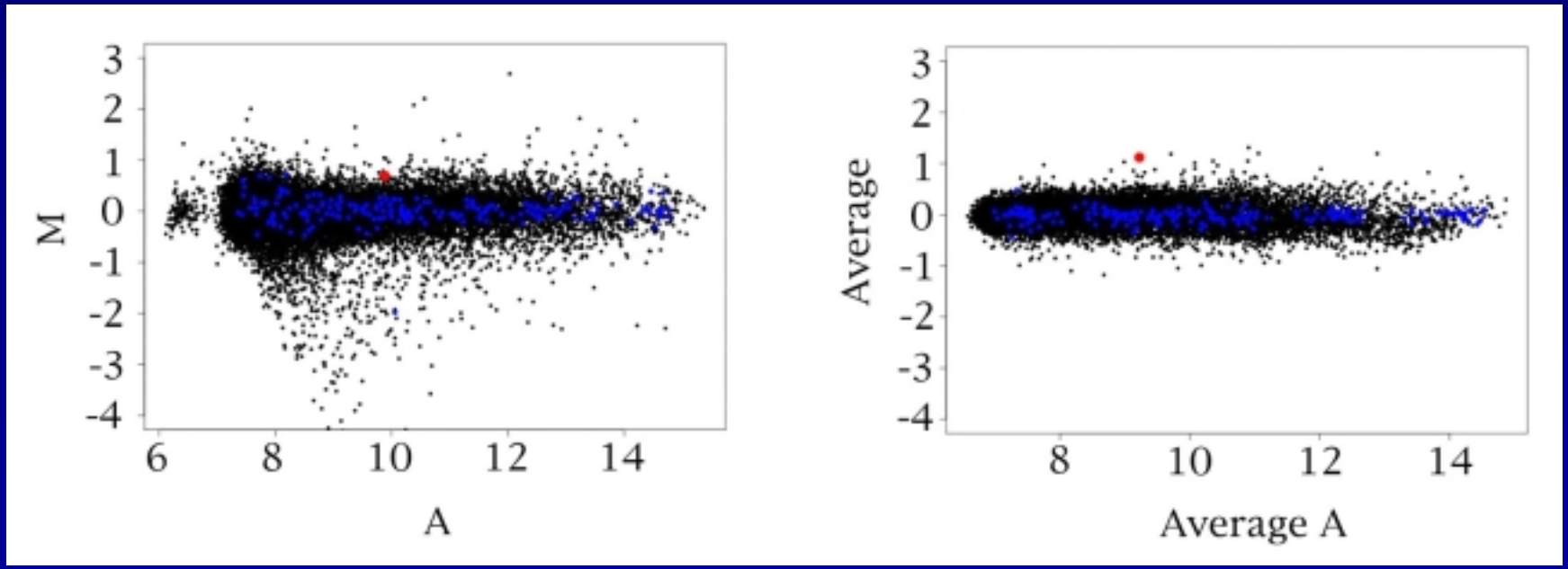




A benefit of replication...

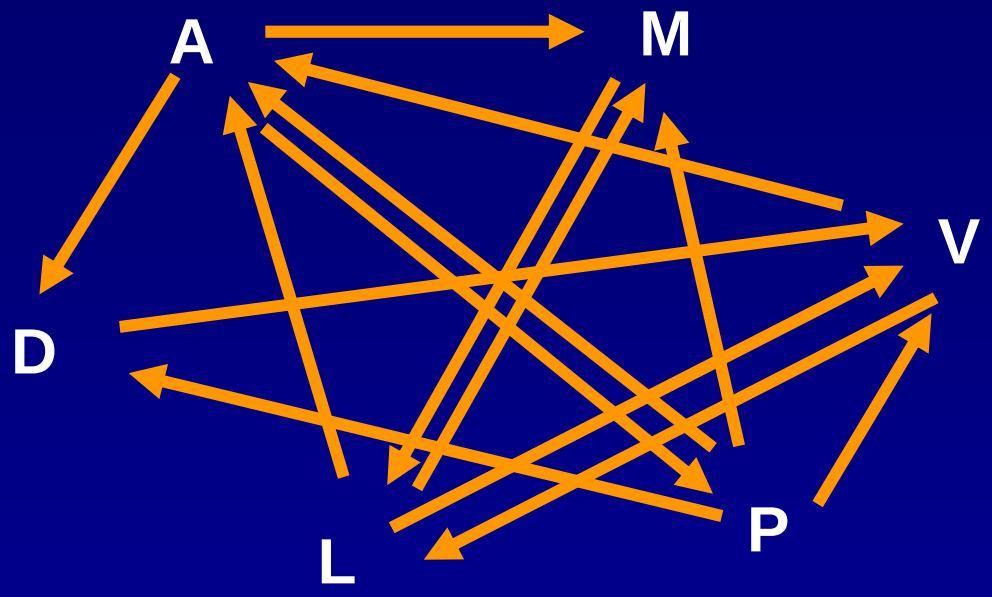
$M = \log_2(P/A)$

Contrast PA (n=4)



- 15228

The Olfactory Bulb Experiments



Samples: tissues from different regions of the olfactory bulb.

Question 1: can we detect differences between different regions?
(contrasts)

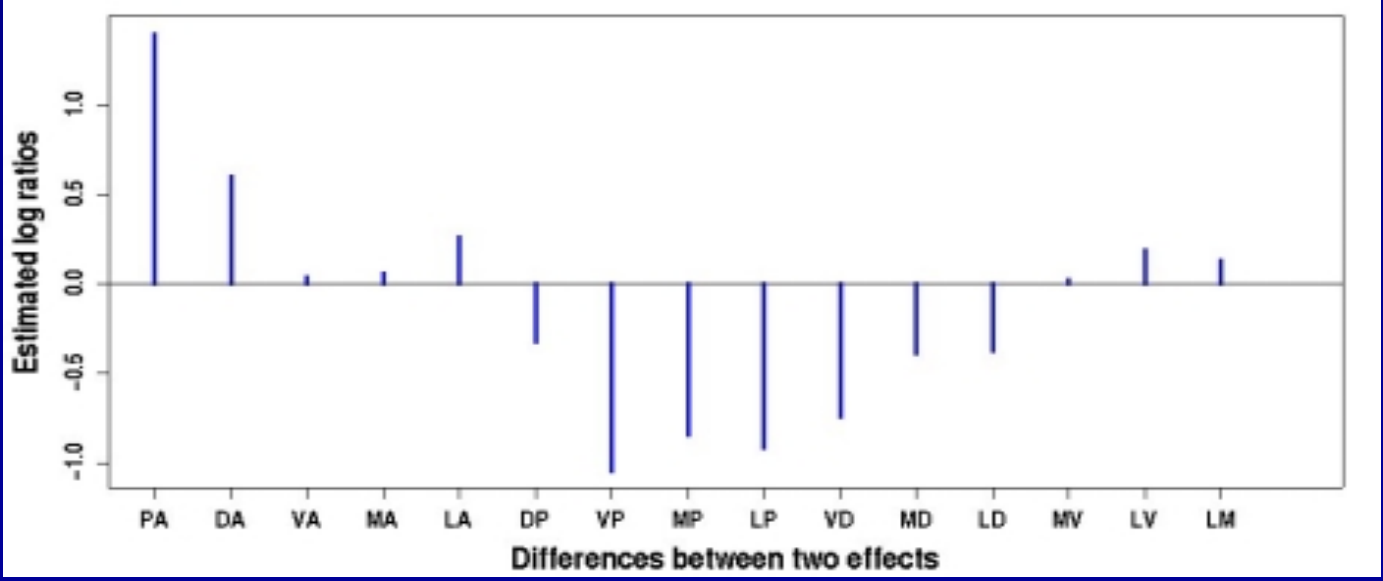
Question 2: can we identify genes with common restrictions across regions?
(patterns)

Contrasts & Patterns

As we can estimate all 15 different pairwise comparisons…

For every gene we thus have a profile based on the 15 pairwise comparisons.

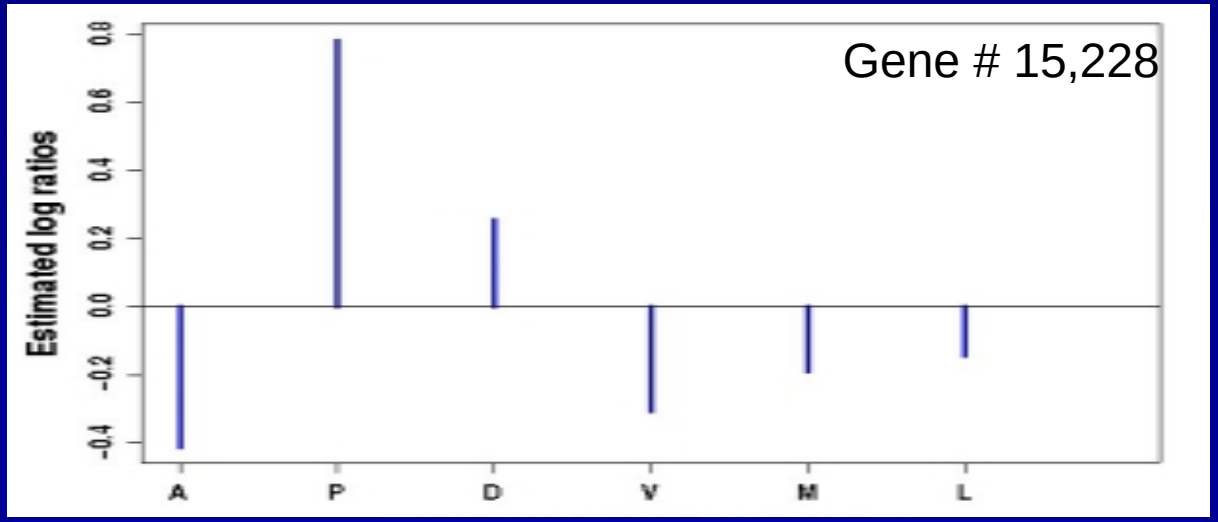
e.g., Gene #15,228



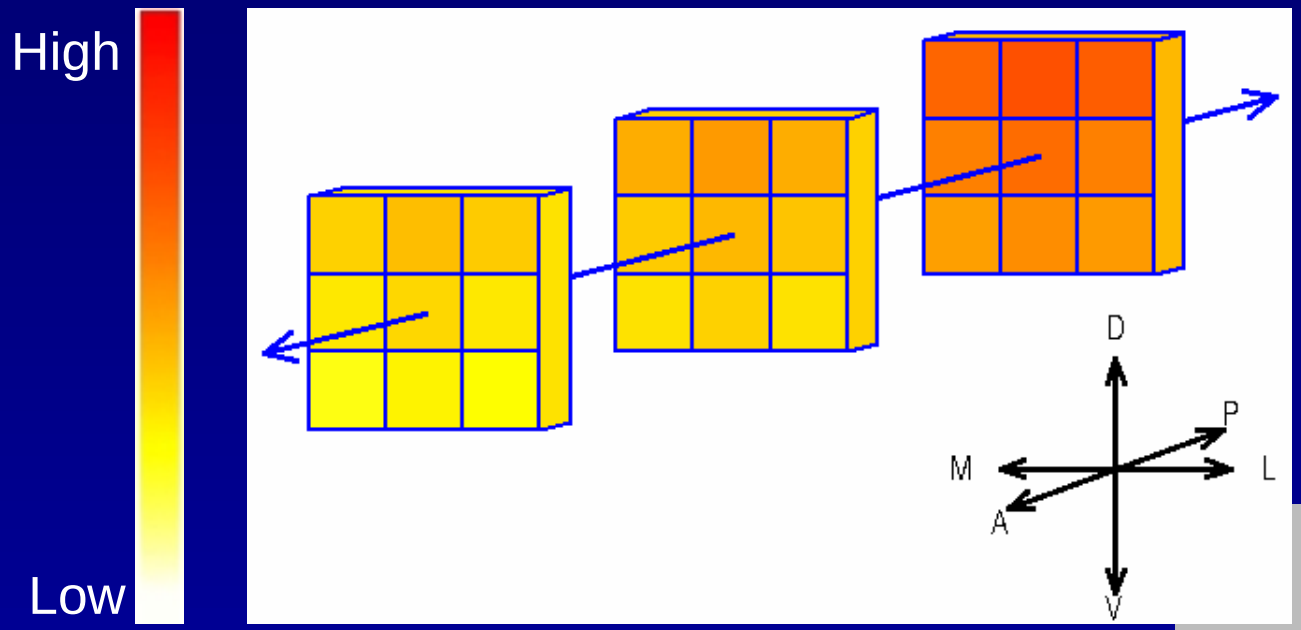
Back to Reality:
The Bulb as a 3-Dimensional Structure

From the 15 pairwise comparisons we can estimate the contrasts of the original 6 samples (A, P, D, V, M, L) and a "whole-bulb reference" computed *in silico*.

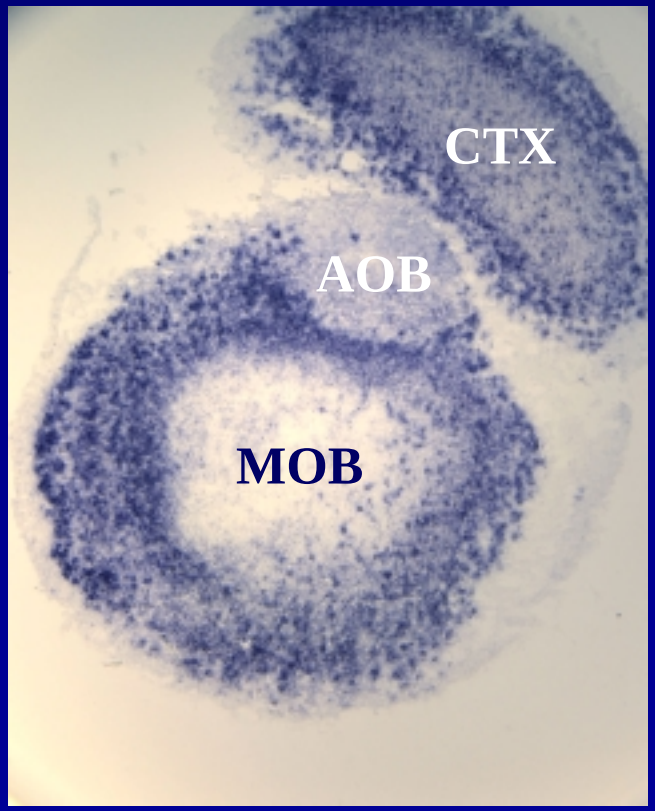
=> 3-D representation of gene expression in the bulb



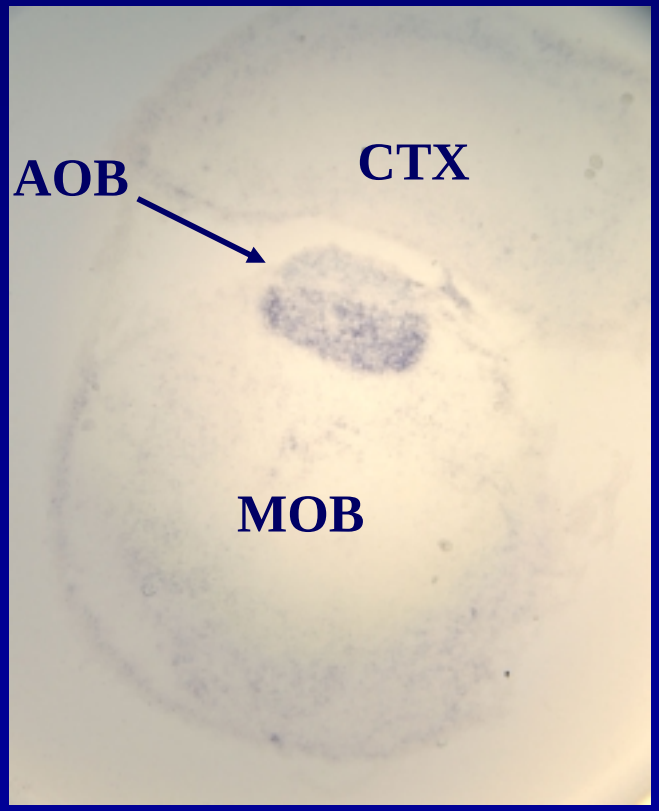
Reconstruction of the Bulb as a Cube: Expression of Gene # 15,228



Validation of Gene # 15,228 Expression Pattern by RNA *In Situ* Hybridization



gluR



#15,228

Patterns, More Globally...

Can we identify genes with common restrictions across regions?

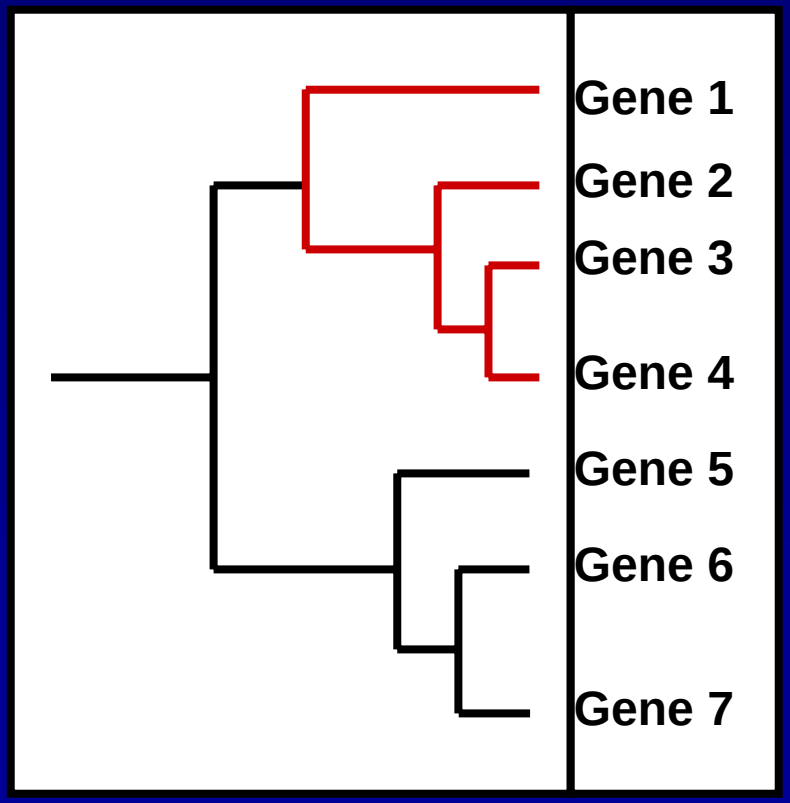
=> use spatial patterns to help identify "interesting" genes...

Two approaches:

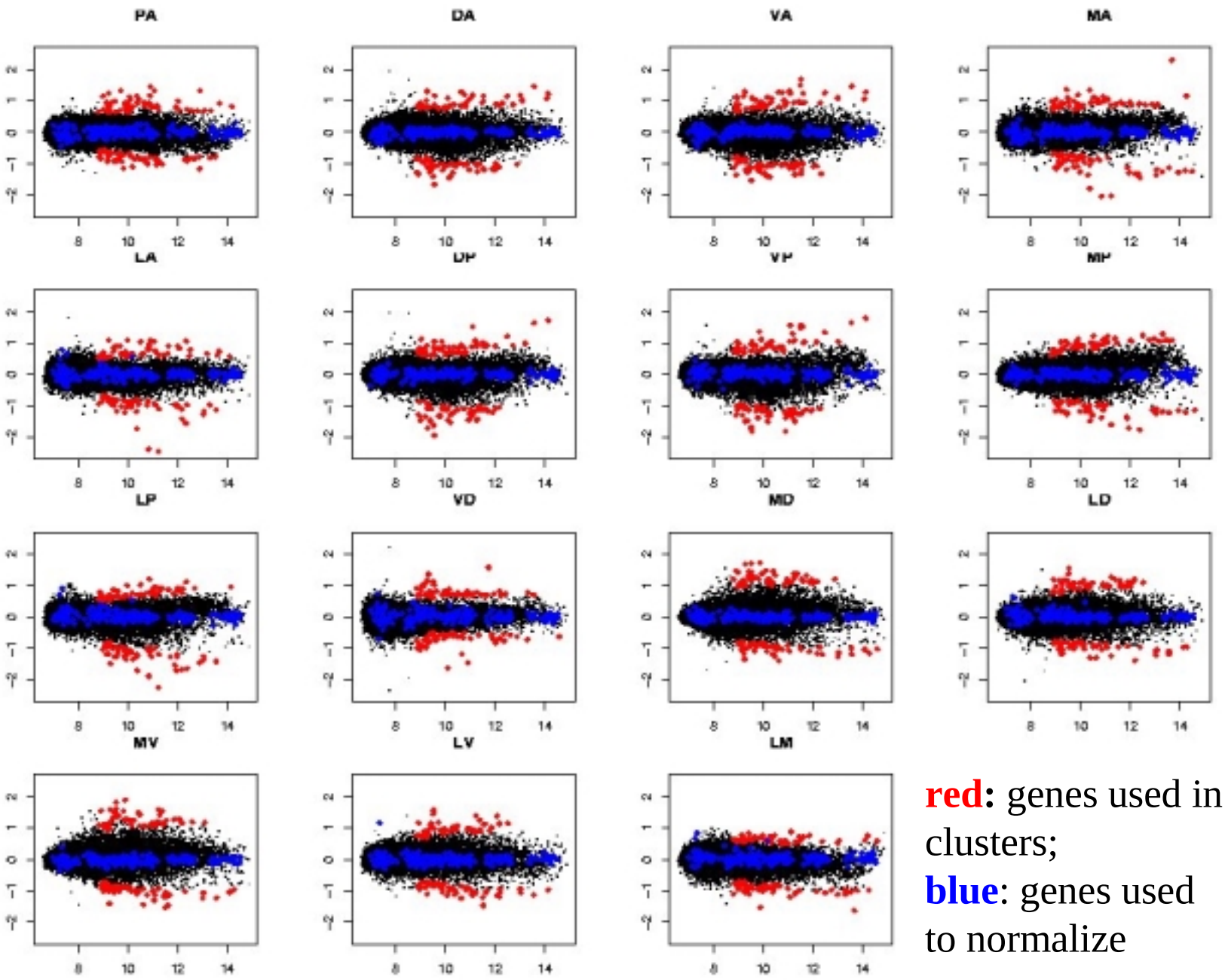
1. Determine whether any genes fit specific, predefined patterns.
2. Perform cluster analysis - see what patterns emerge.

Hierarchical Clustering: A Tool for Gene Discovery

- Genes on adjacent branches have similar profiles
- Euclidean distance (modified)

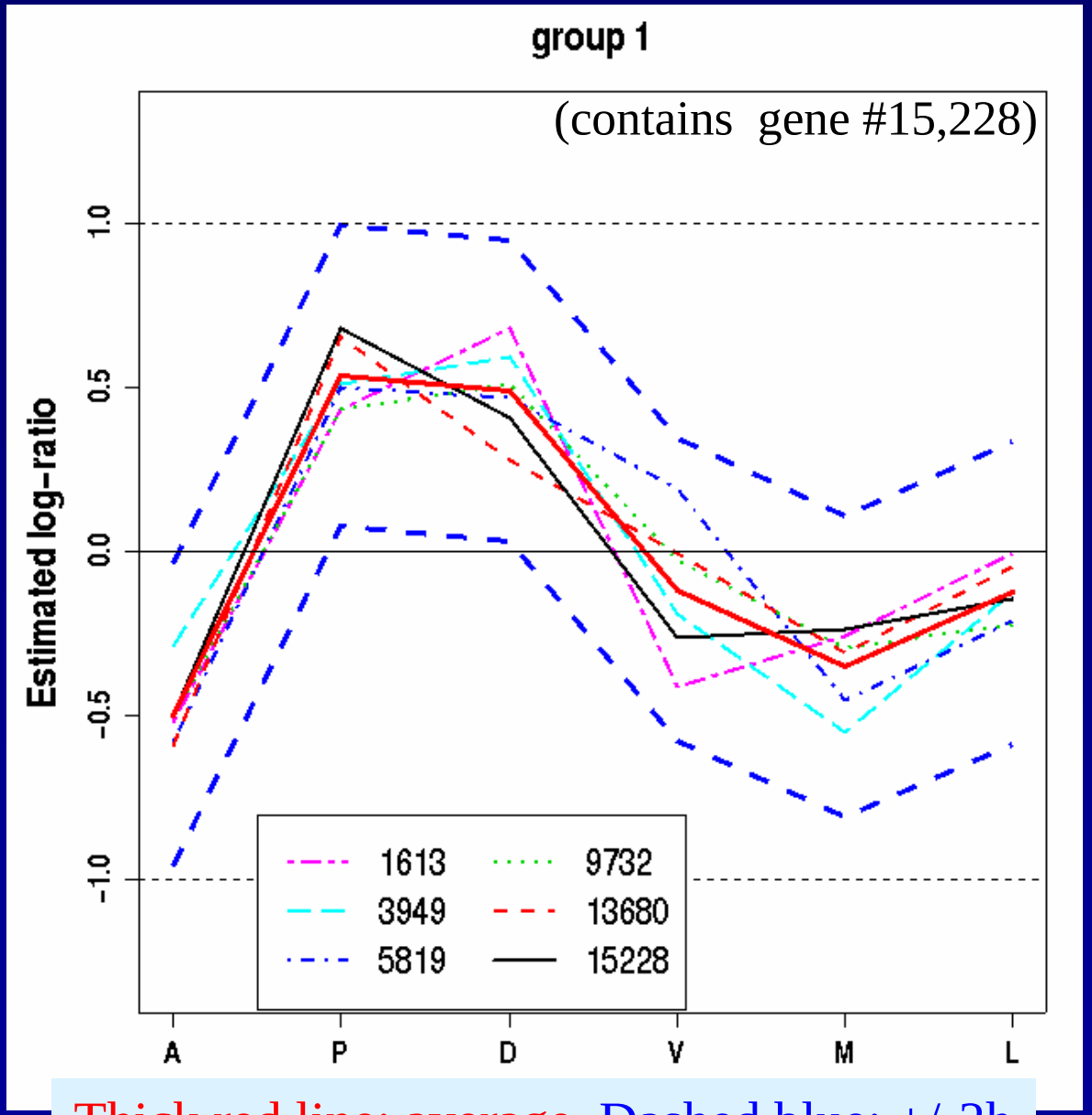


Pairwise Comparisons: Values vs. Average Intensity



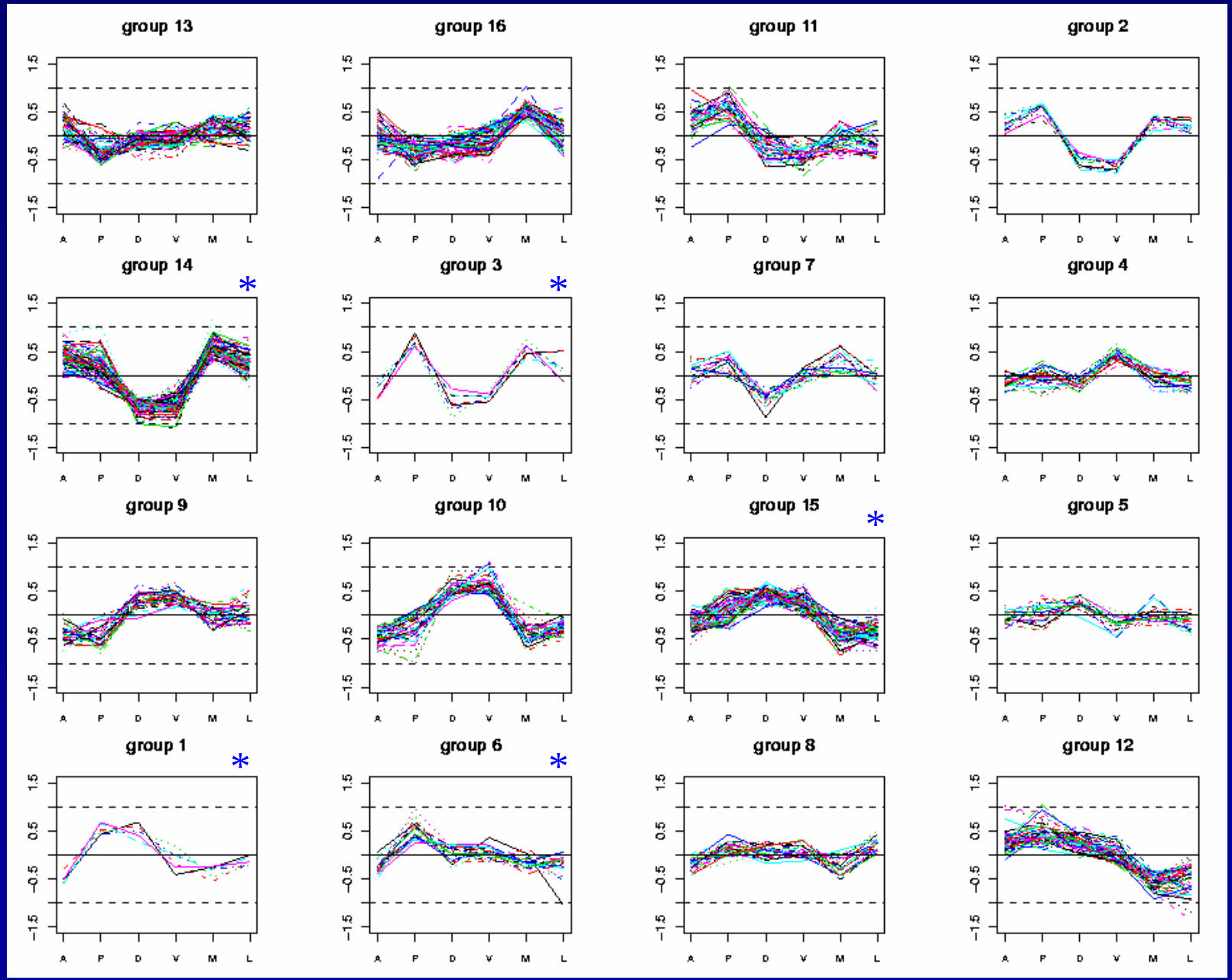
red: genes used in clusters;
blue: genes used to normalize

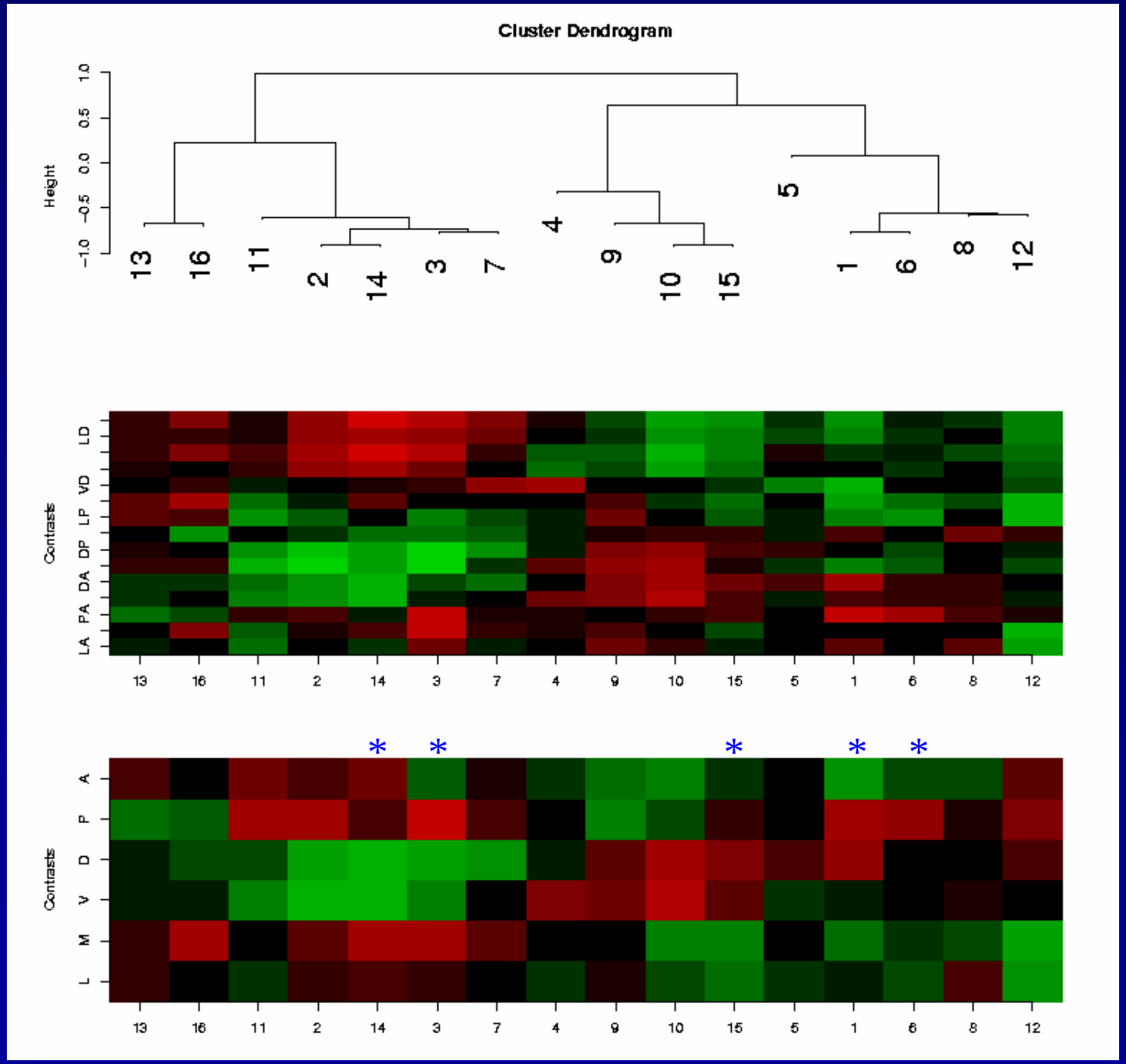
One Cluster (6 point representation)



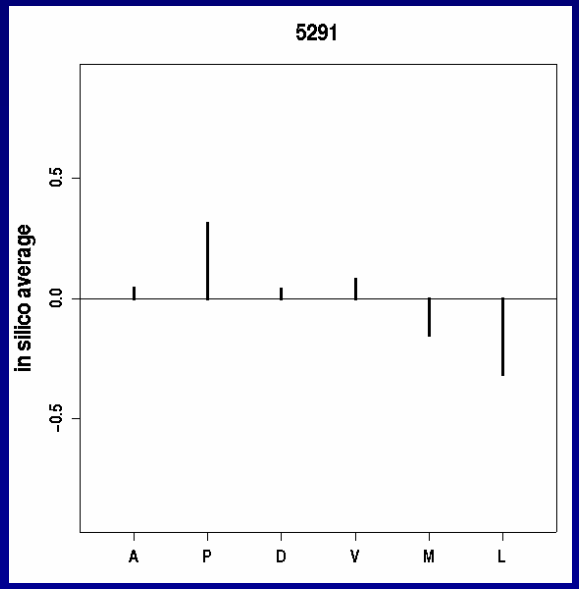
Thick red line: average Dashed blue: +/-2h

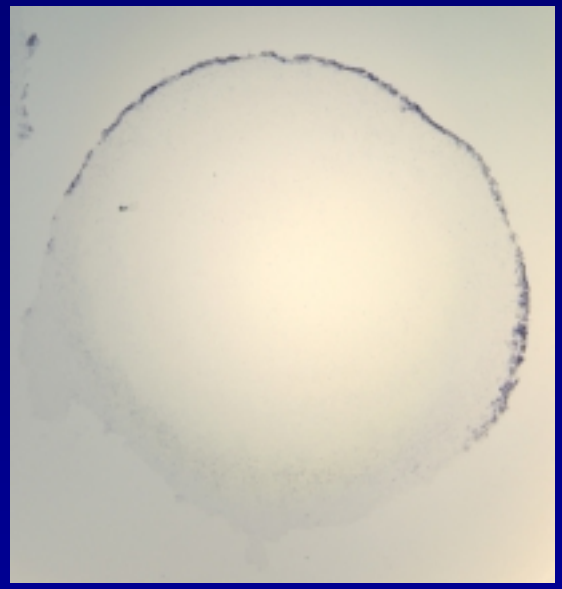
16 Clusters Systematically Arranged (6 point representation)



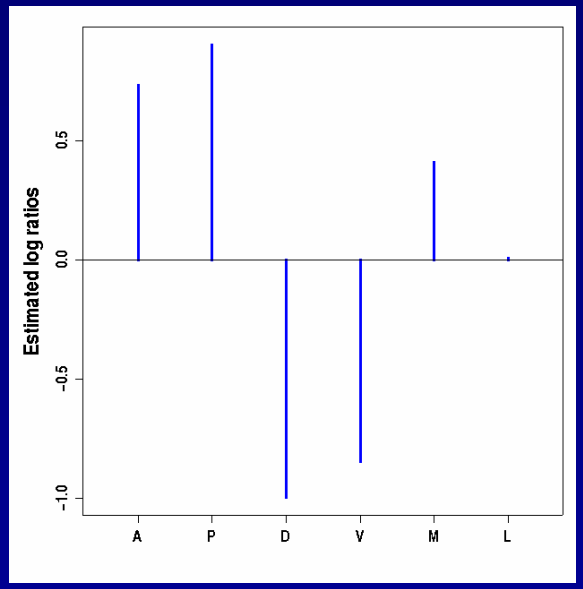

Cluster Dendrogram
Height
1.0
0.5
0.0
−0.5
−1.0
13
16
11
2
14
3
7
4
9
10
15
5
1
6
8
12
Contrasts
LD
VD
LP
DP
DA
PA
LA
13
16
11
2
14
3
7
4
9
10
15
5
1
6
8
12
*
*
*
*
*
Contrasts
A
P
D
V
M
L
13
16
11
2
14
3
7
4
9
10
15
5
1
6
8
12

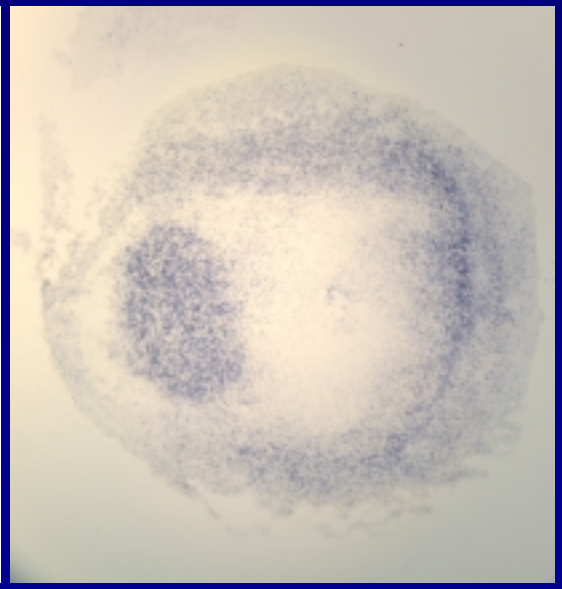
5,291
(group 6)



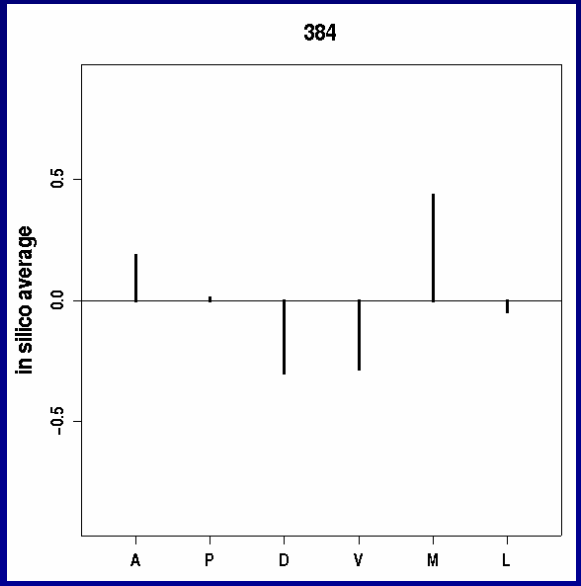


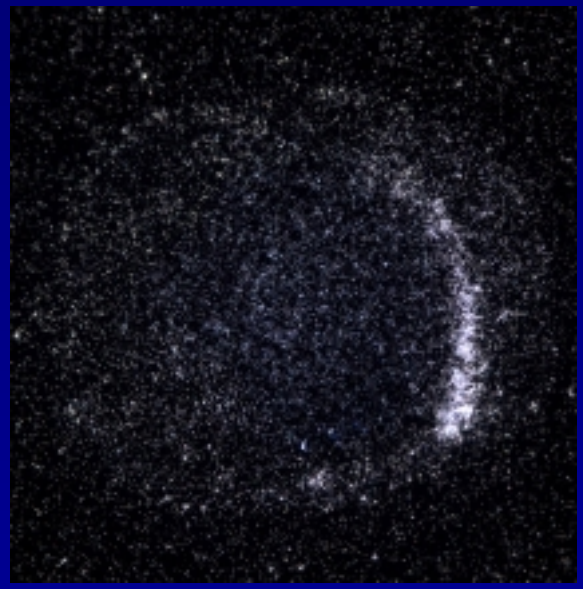
8,496
(group 14)



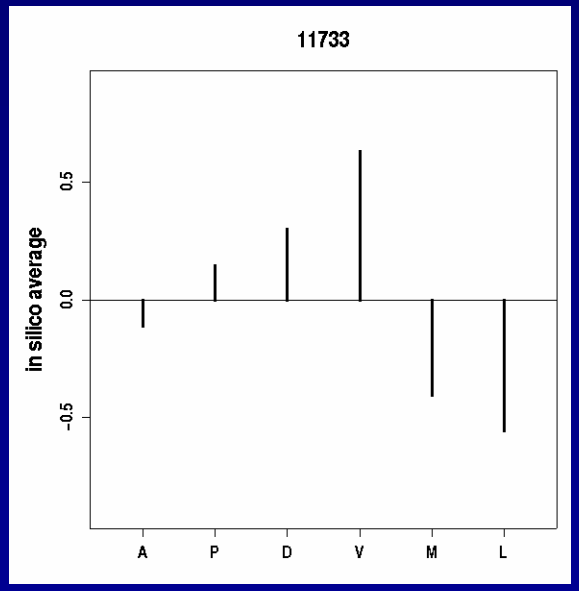


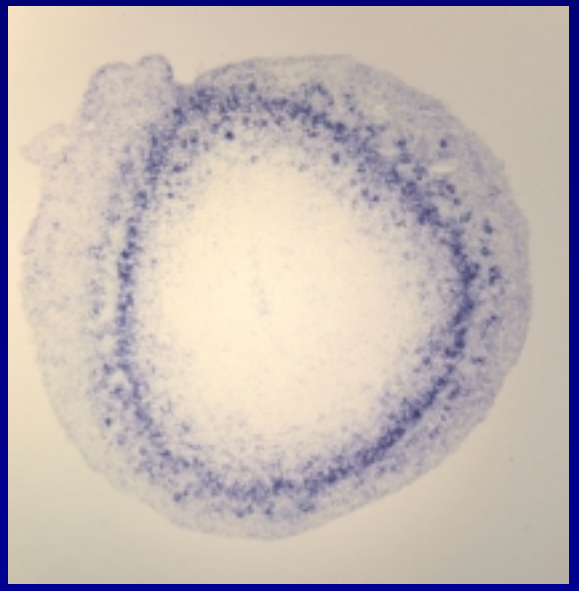
384
(group 3)





11,733
(group 15)



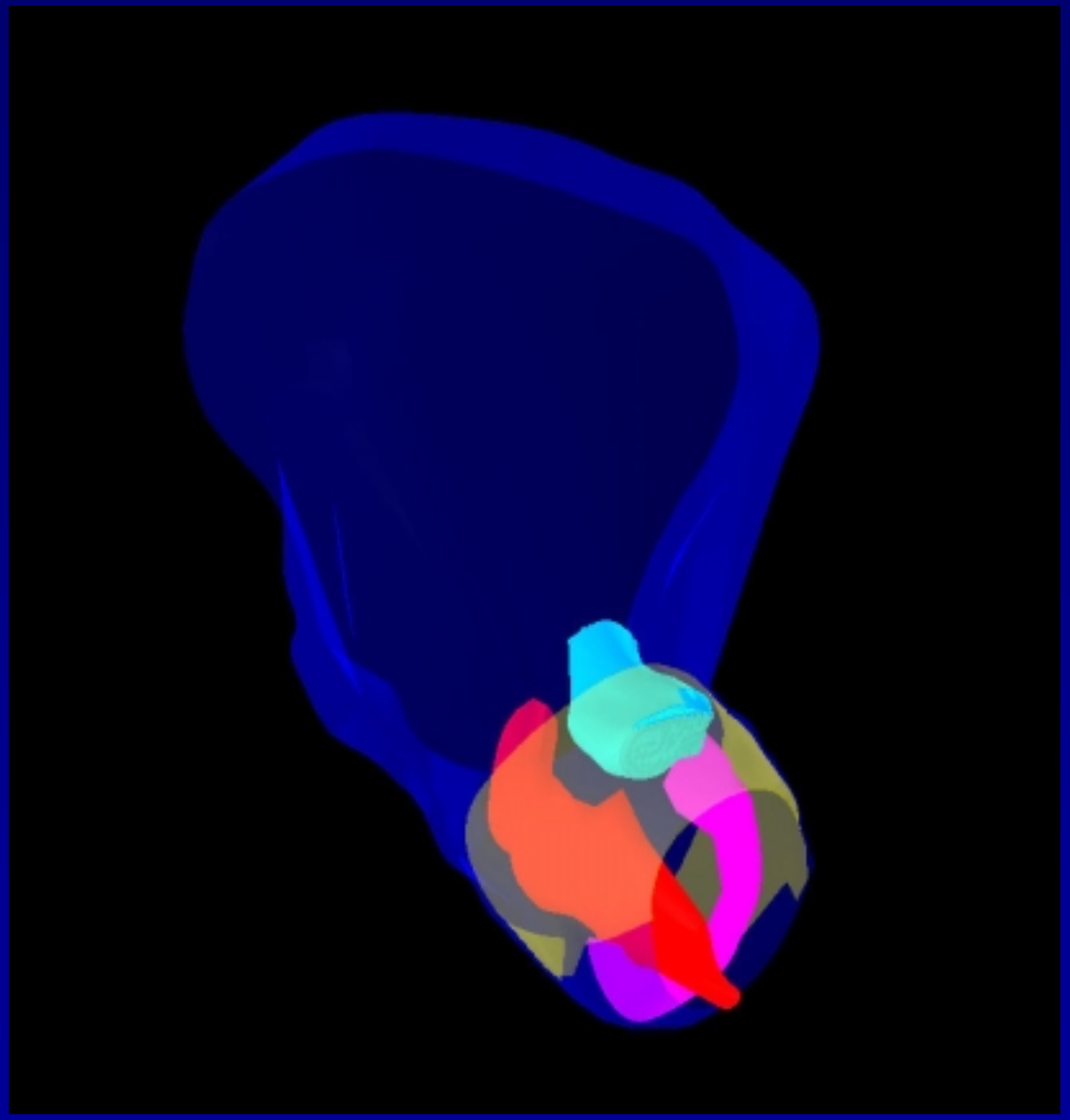


15,228
(jagged)

5,291
(D2 synthase)

8,496
(cadherin-11)

384
(NT-3)



Summary

Identification of a spatial map of gene expression in the olfactory bulb

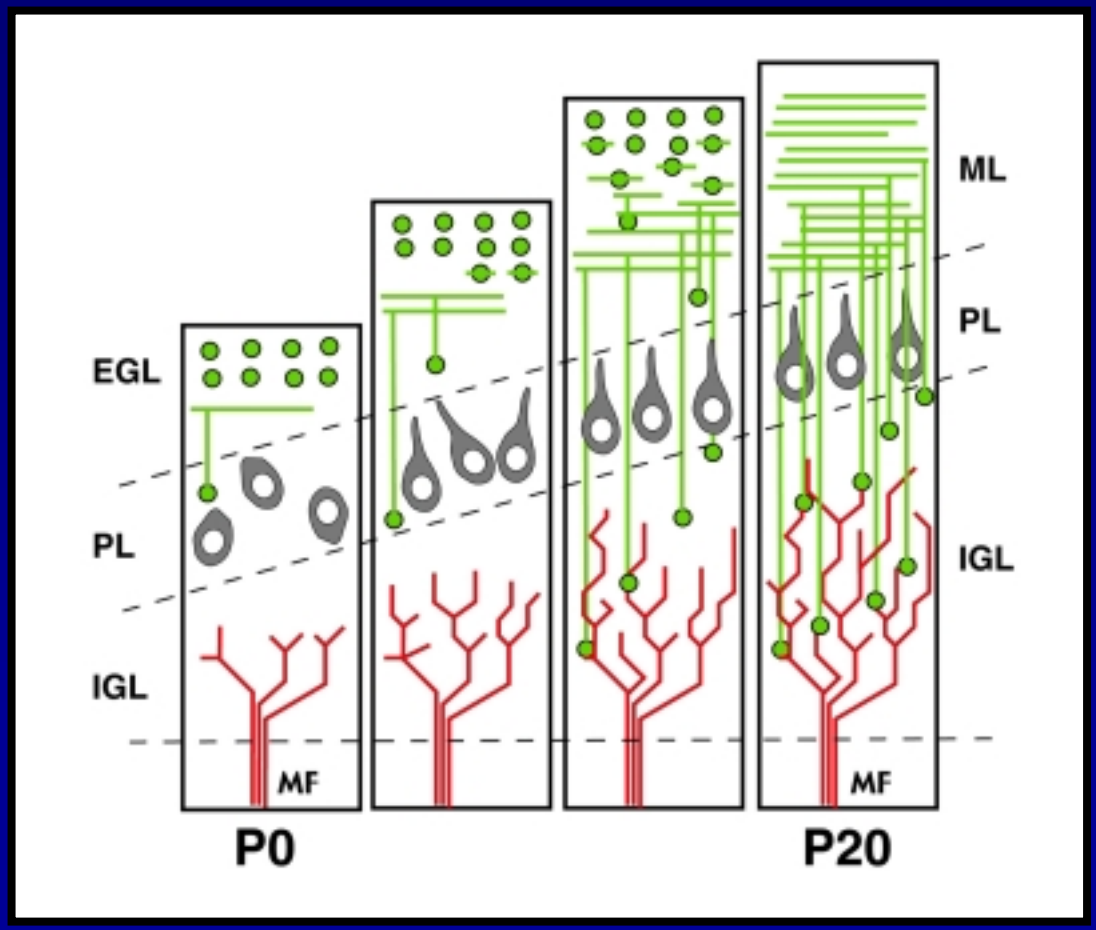
- **Regression analysis of microarray data allows 3-D reconstruction of gene expression patterns in the bulb**
- **Clustering identifies groups of genes showing similar spatial patterns of expression**
- **Subtle differences detected by our methods**
- **Validation by *in situ* hybridizations**

- ***Refine analysis with more experiments, finer dissections***
- ***Functional validation of interesting candidates***
- ***Comparison to other areas of the nervous system...***

Temporal Analysis of Gene Expression during CNS Development

Elva Diaz
Yangchao Ge
Jean Yang
Terry Speed

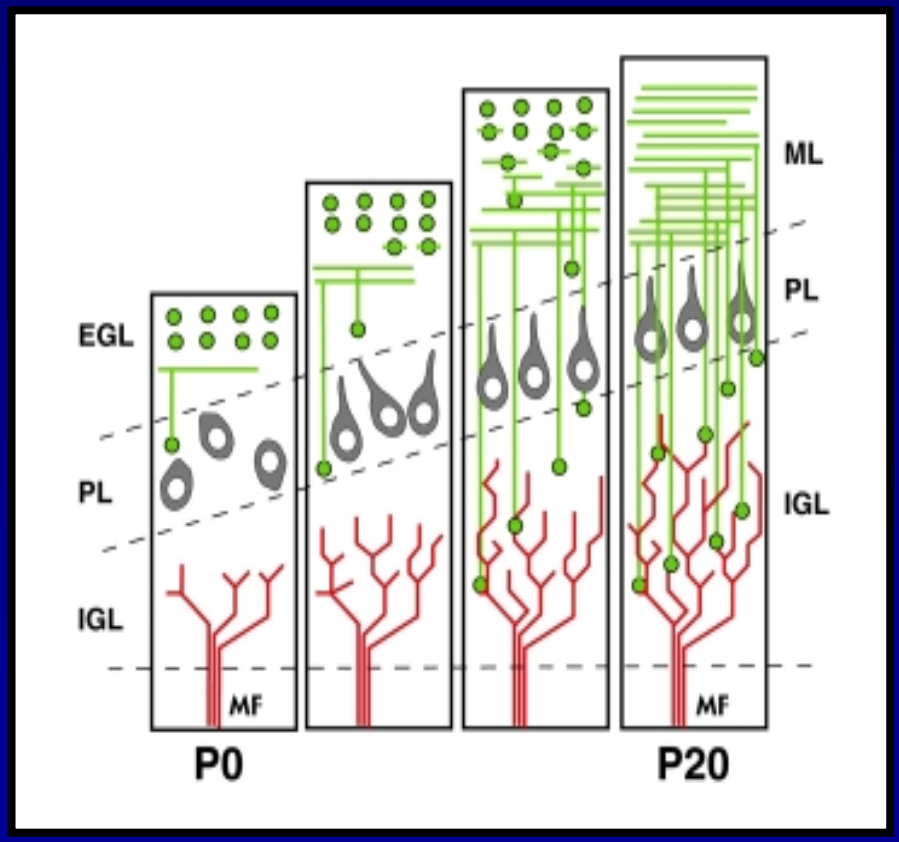
Cerebellar Development



Expression Profiling

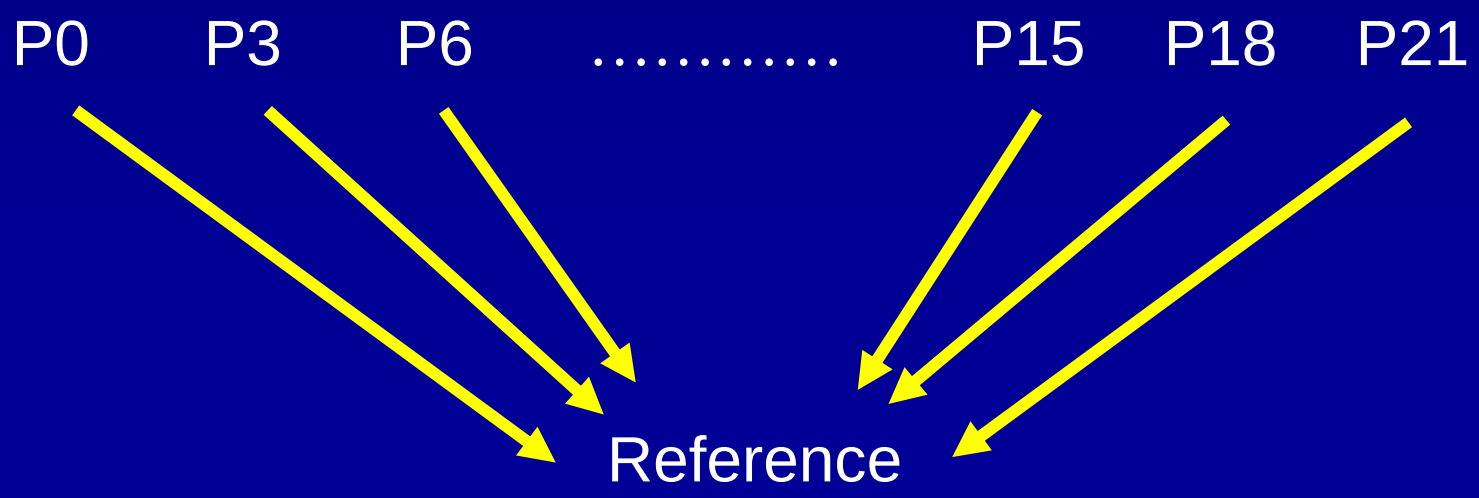
Can we identify cell- and stage-specific programs of gene expression by DNA microarray analysis?

Focus on granule cells



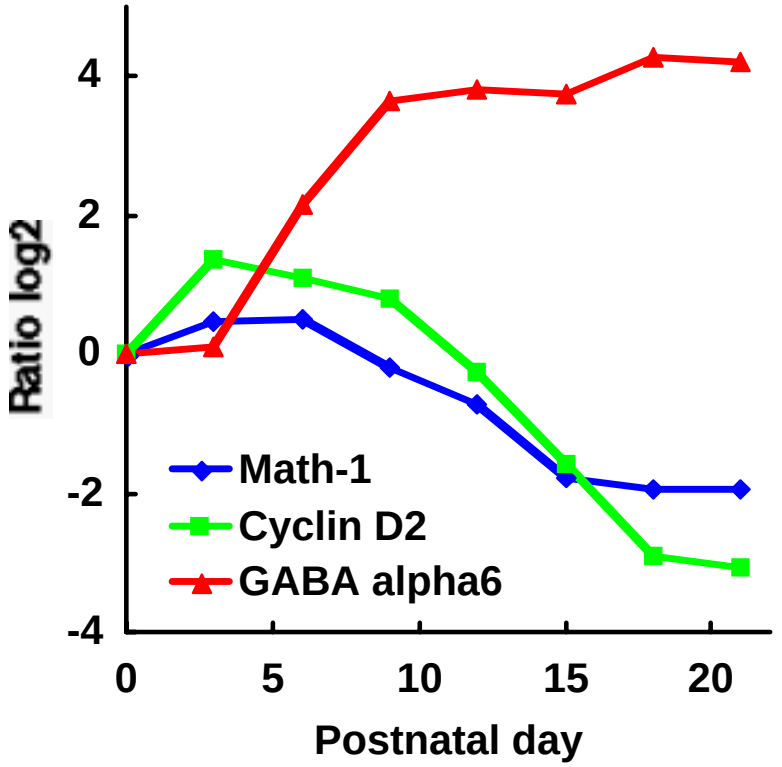
Design: Time Course

- —Isolate RNA from cerebellum
- —Reference: pool of all time points
- —Relative to initial time point



Known Marker Genes

- **Math-1:** granule cell specification
- **Cyclin D2:** granule cell precursors
- **GABA α6:** mature granule neurons
- ***Can we generalize to identify programs of gene expression?***



Hierarchical Clustering

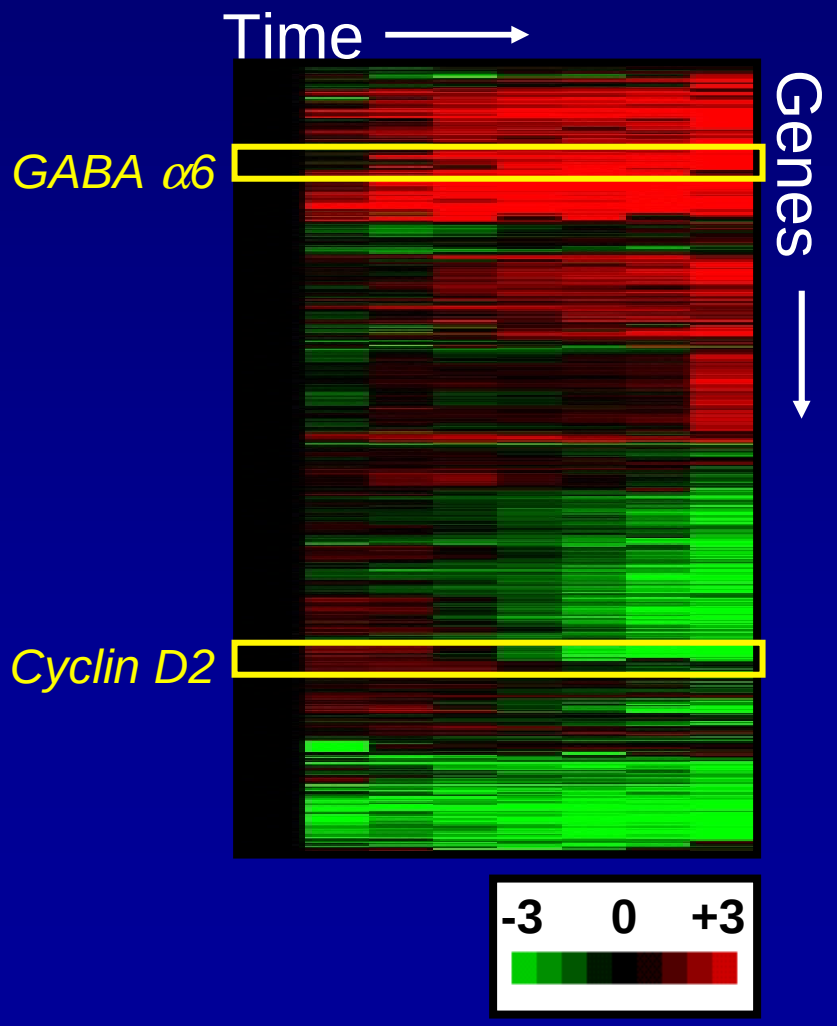
Hypothesis: genes with similar expression profiles will define distinct cell types and stages of neuronal differentiation

=> *Carry out hierarchical clustering to group genes based on their expression profiles*

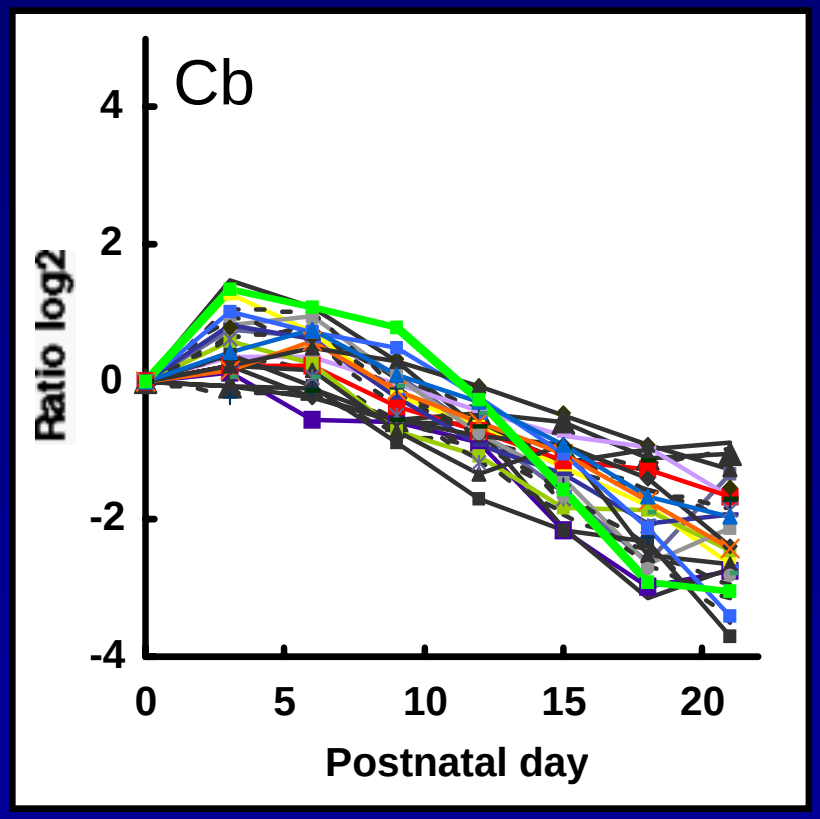
Cerebellum Developmental Time Course

—**Marker genes help define cellular origins of gene clusters**

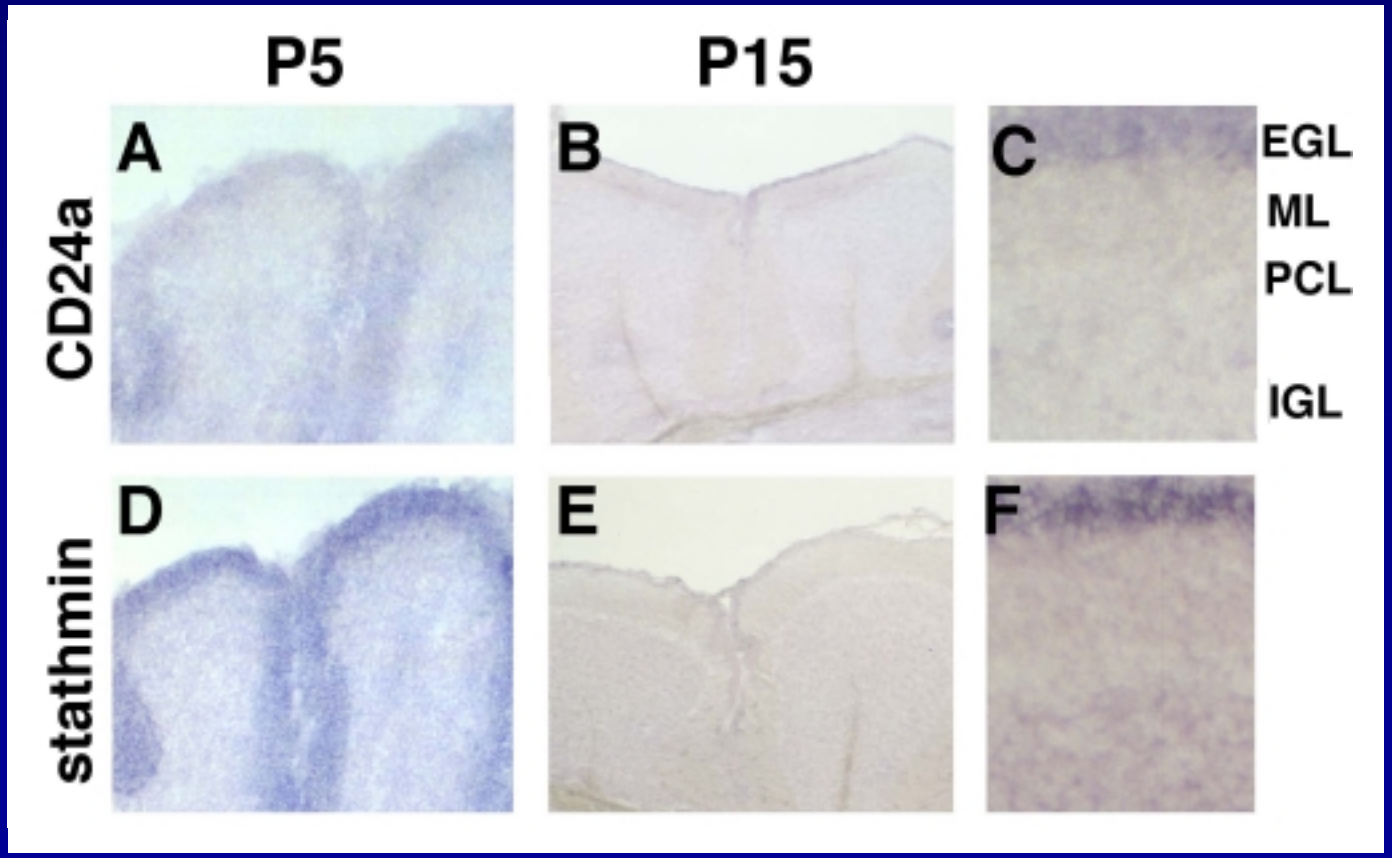
- ***GABA α6:*** **granule cells**
- ***Cyclin D2:*** **proliferating granule cell precursors**



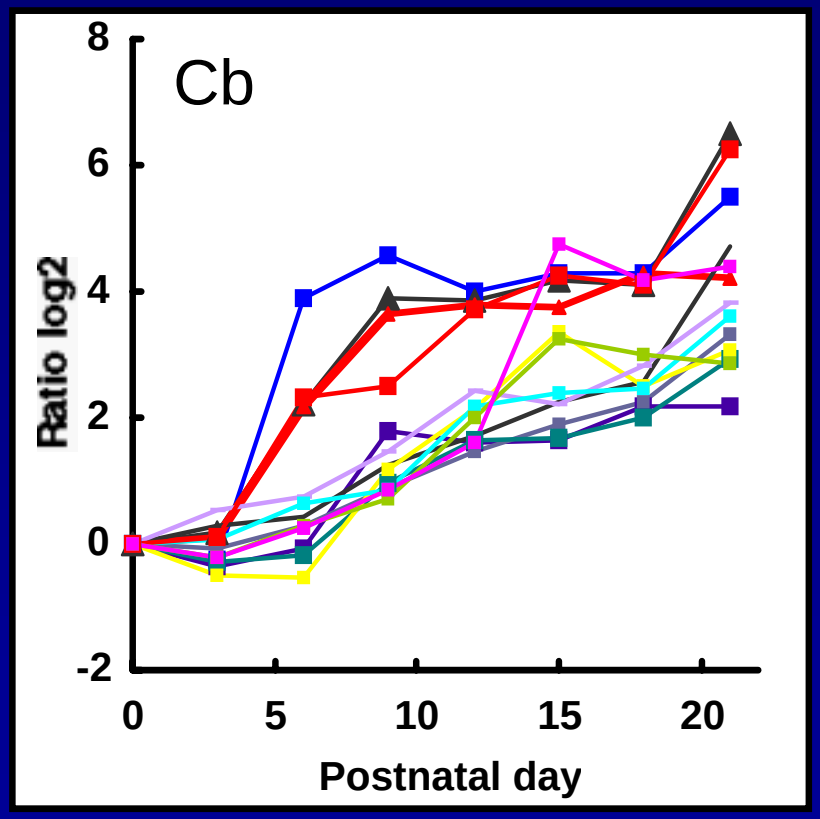
Cyclin D2 Cluster



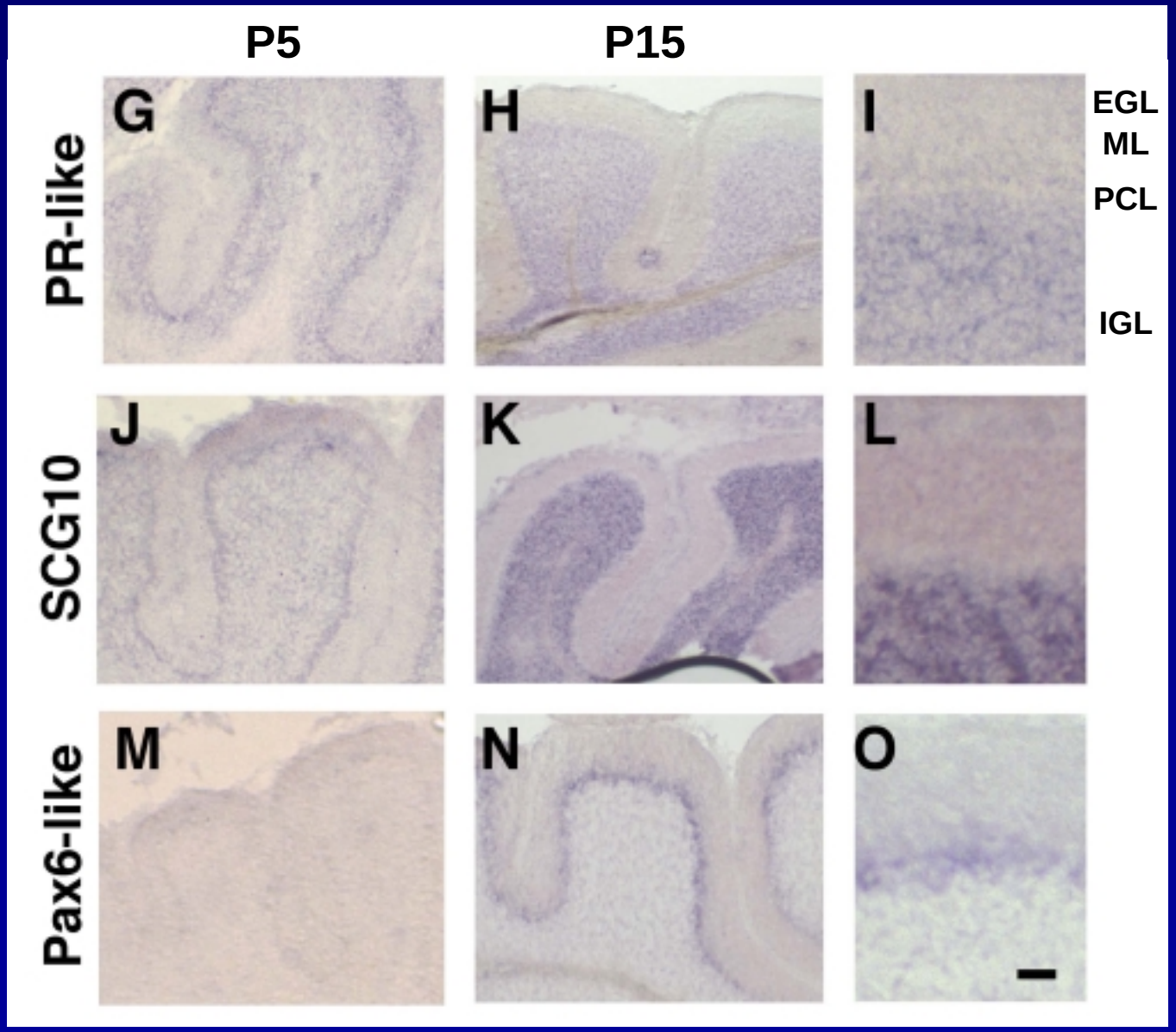
Cyclin D2 Cluster



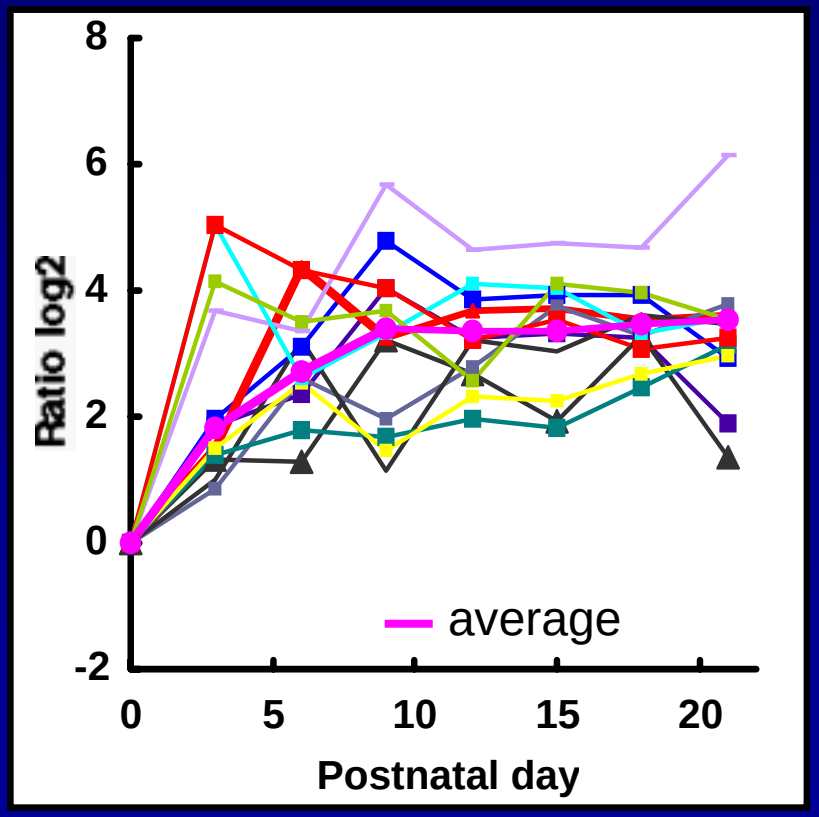
GABA α6 Cluster

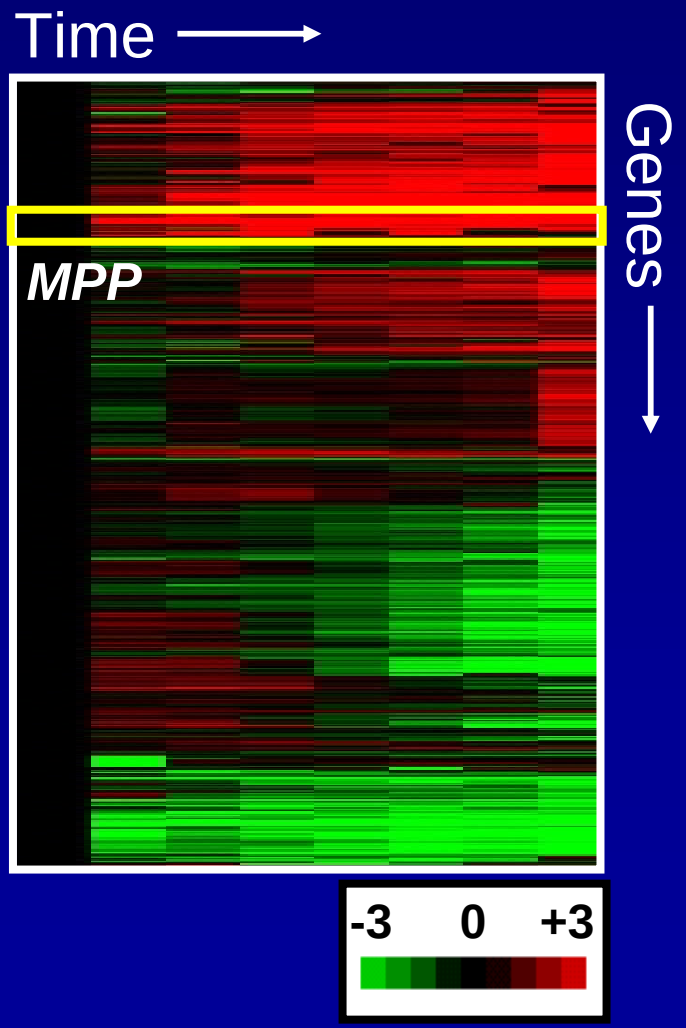


GABA α6 cluster

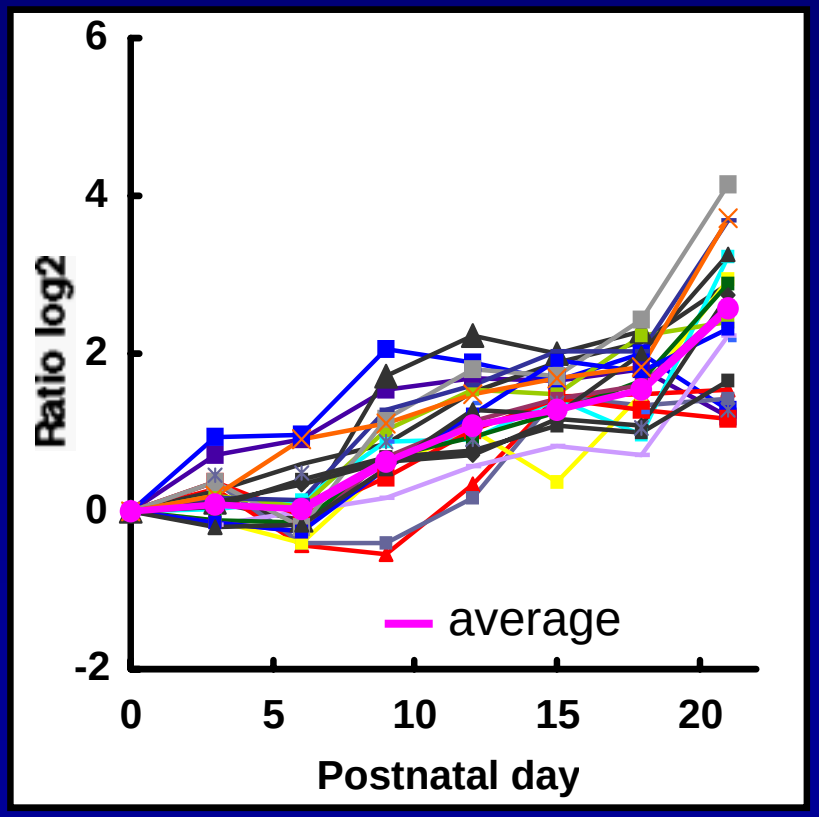


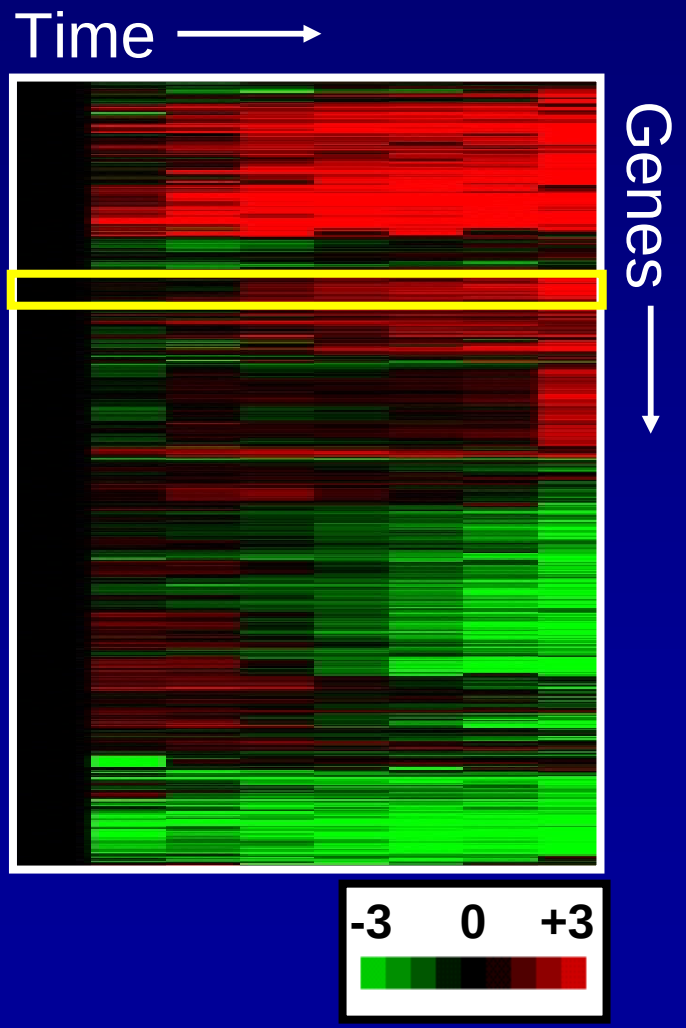
Glial Cluster





Neuronal Cluster



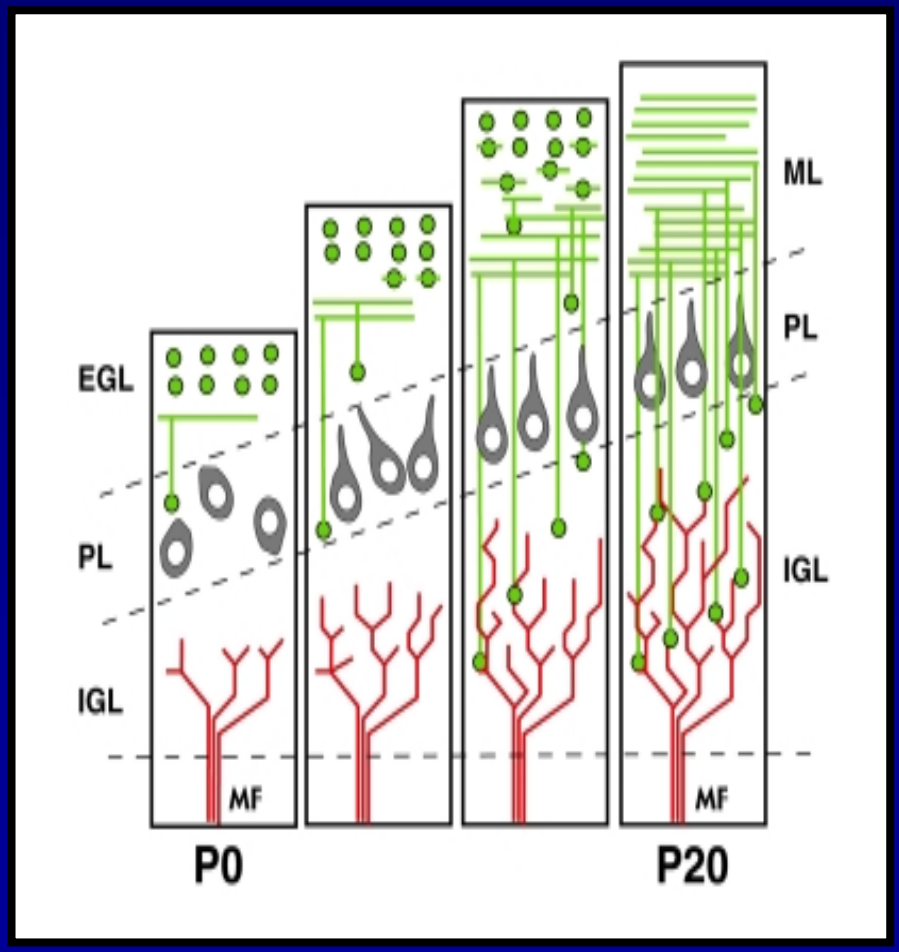


So far…

- —Validation of microarray expression profiles by *in situ* hybridizations -> clusters represent distinct cell types and stages of differentiation
- —Prediction: Expression profiles will be altered in mutant mouse lines with defects in cerebellum development
  - – Additional means of validation
  - – Identify effects of cell-cell interactions on gene expression

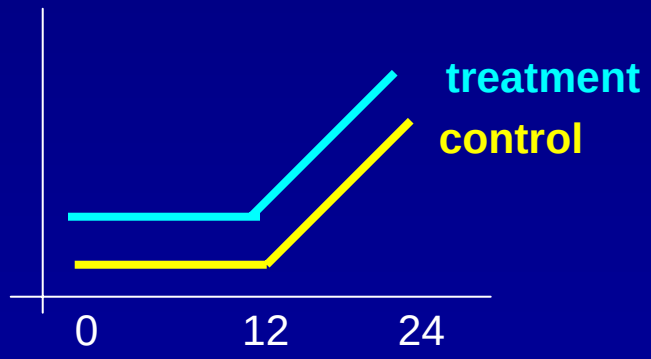
Weaver Mice

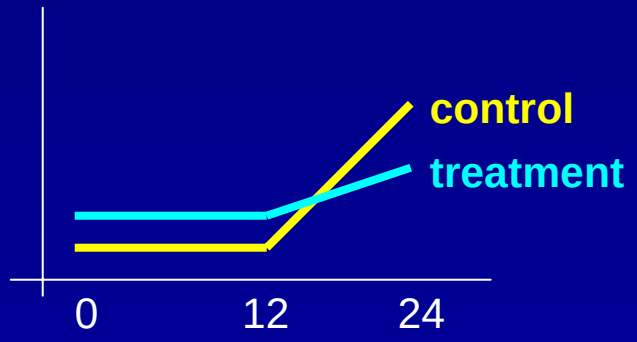
- —Mutation in potassium channel (Kcnj6) gene
- —Granule cells fail to migrate and die
- —Profiled @ P1, P11, P21



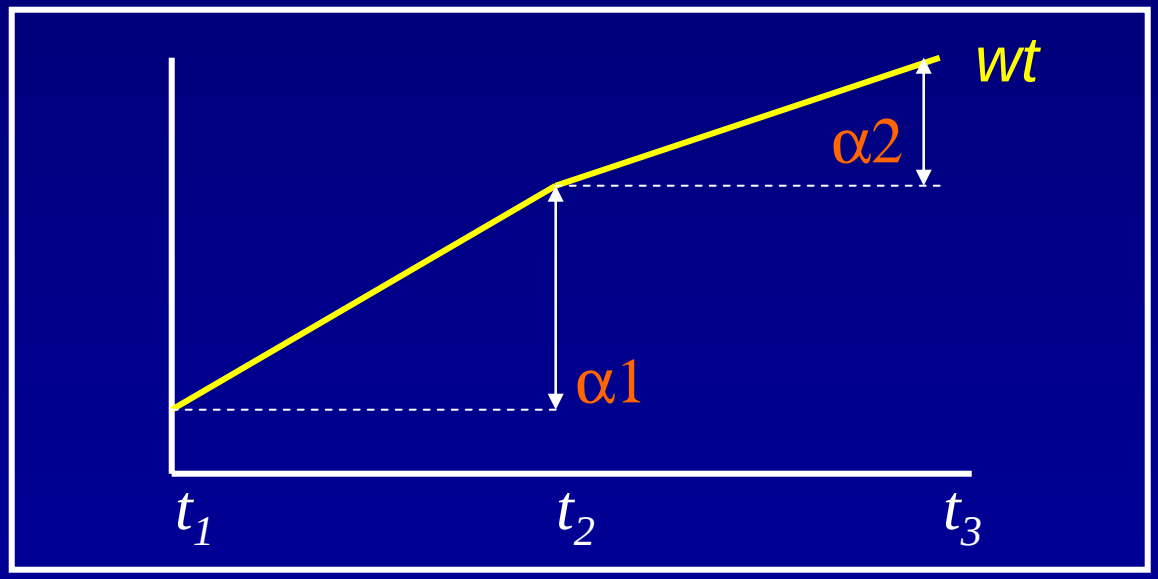
Mutant vs. Wild Type Comparisons

Interested in cases where there are differences (interactions) between treatment and control over time - how to quantitate these differences?

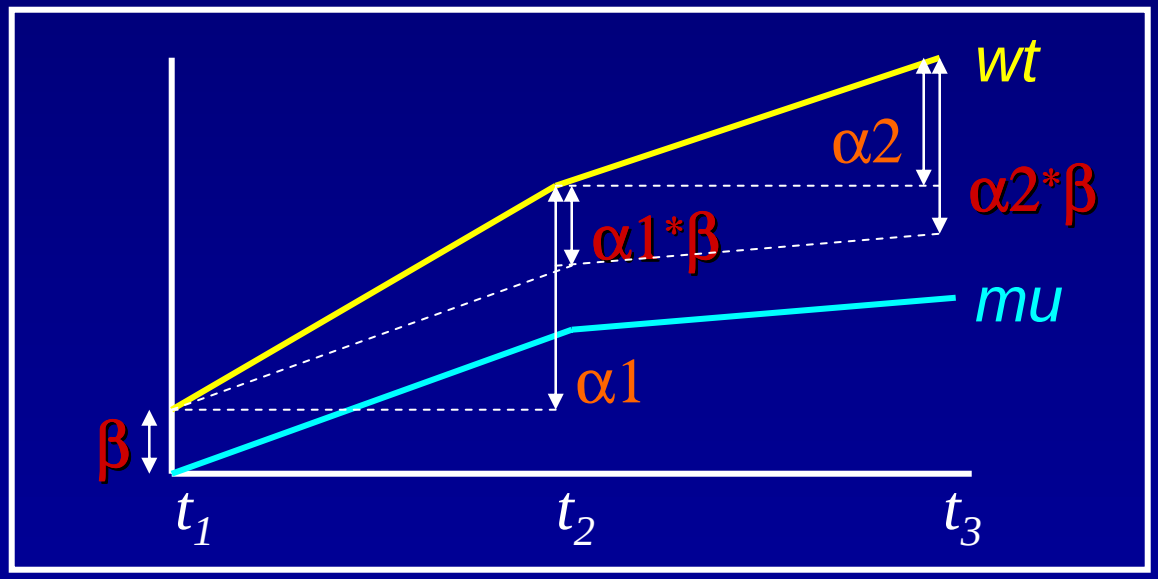




Factorial Design

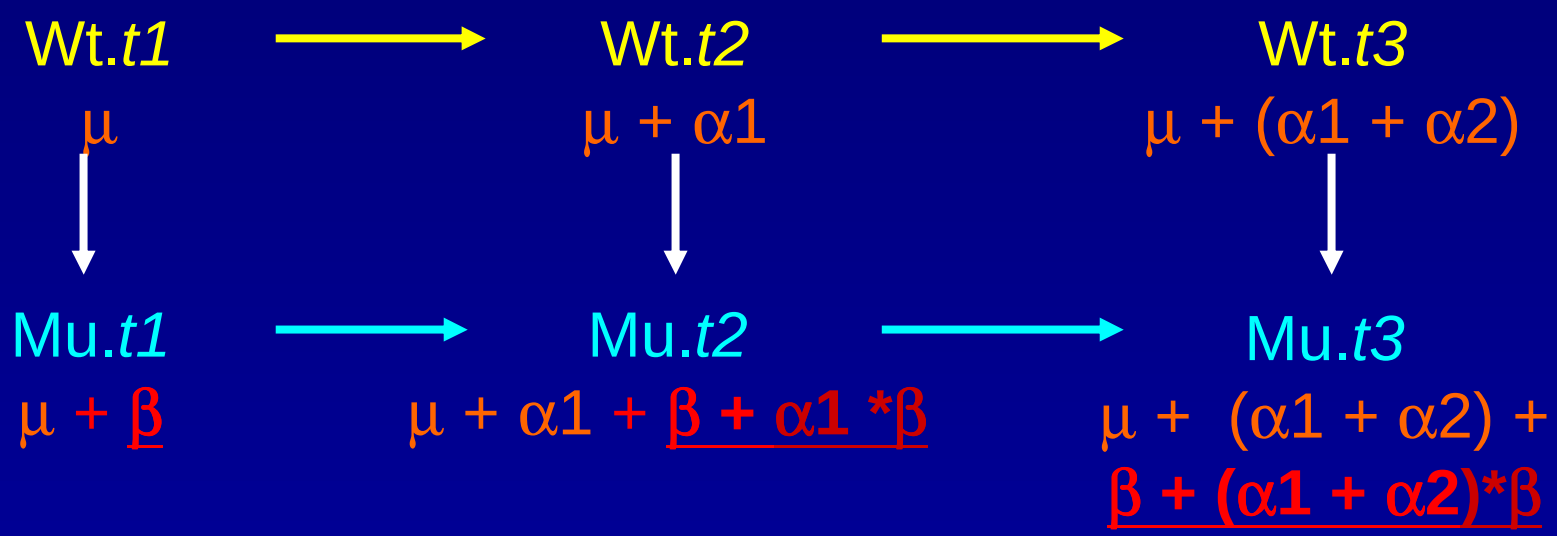


Factorial Design



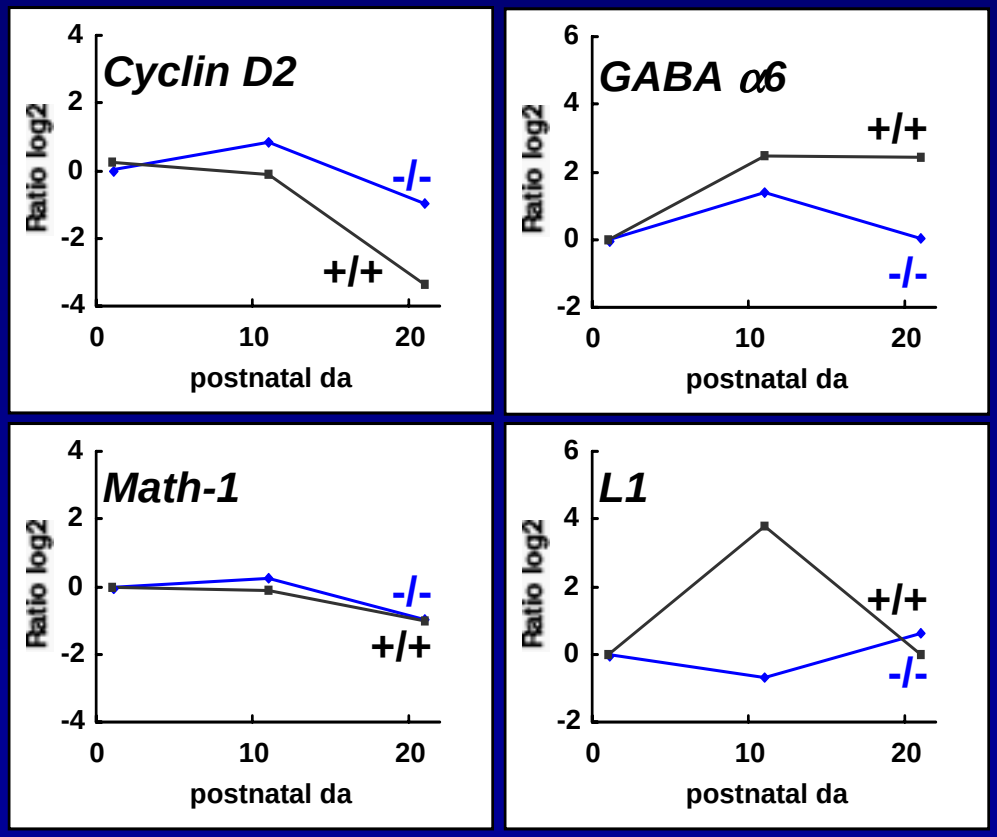
(a 2 x 3 factorial design)

Factorial Design

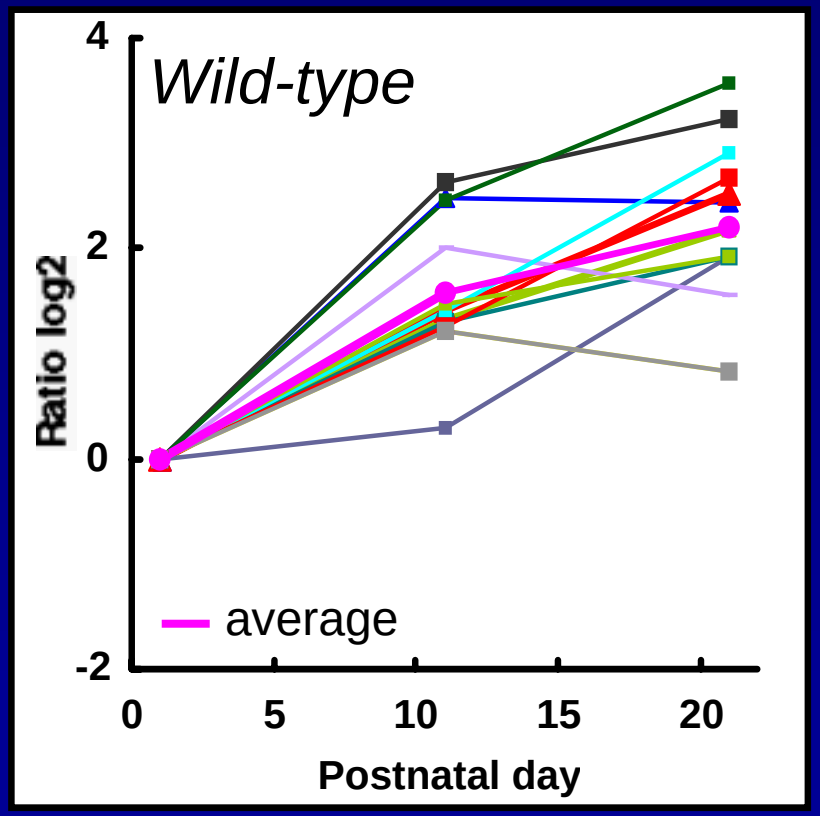


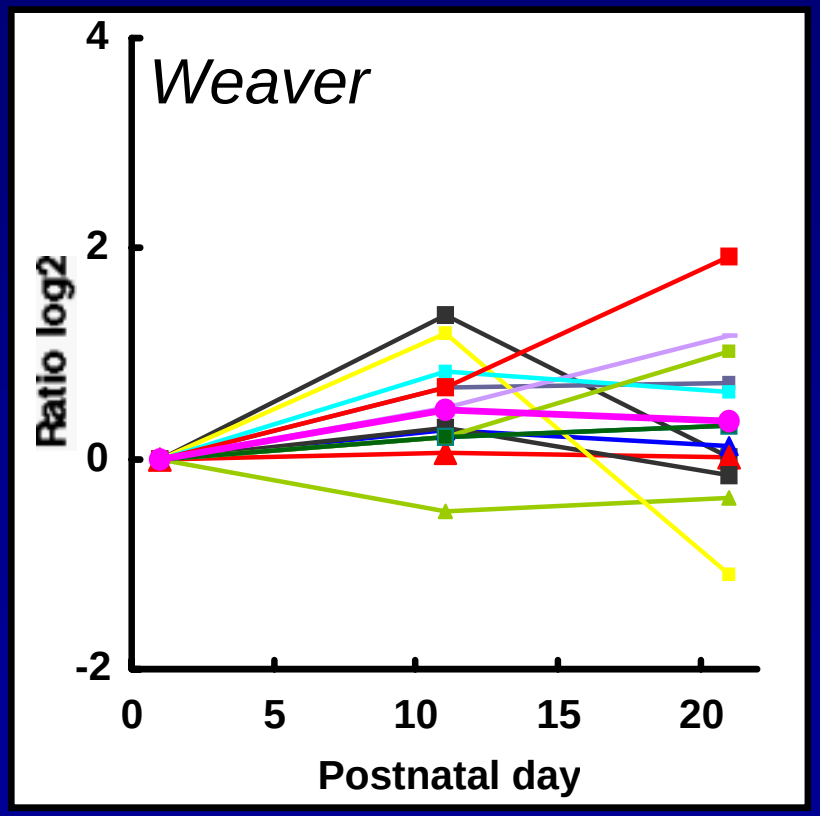
Assess genes and clusters based on interactions…

Control Genes

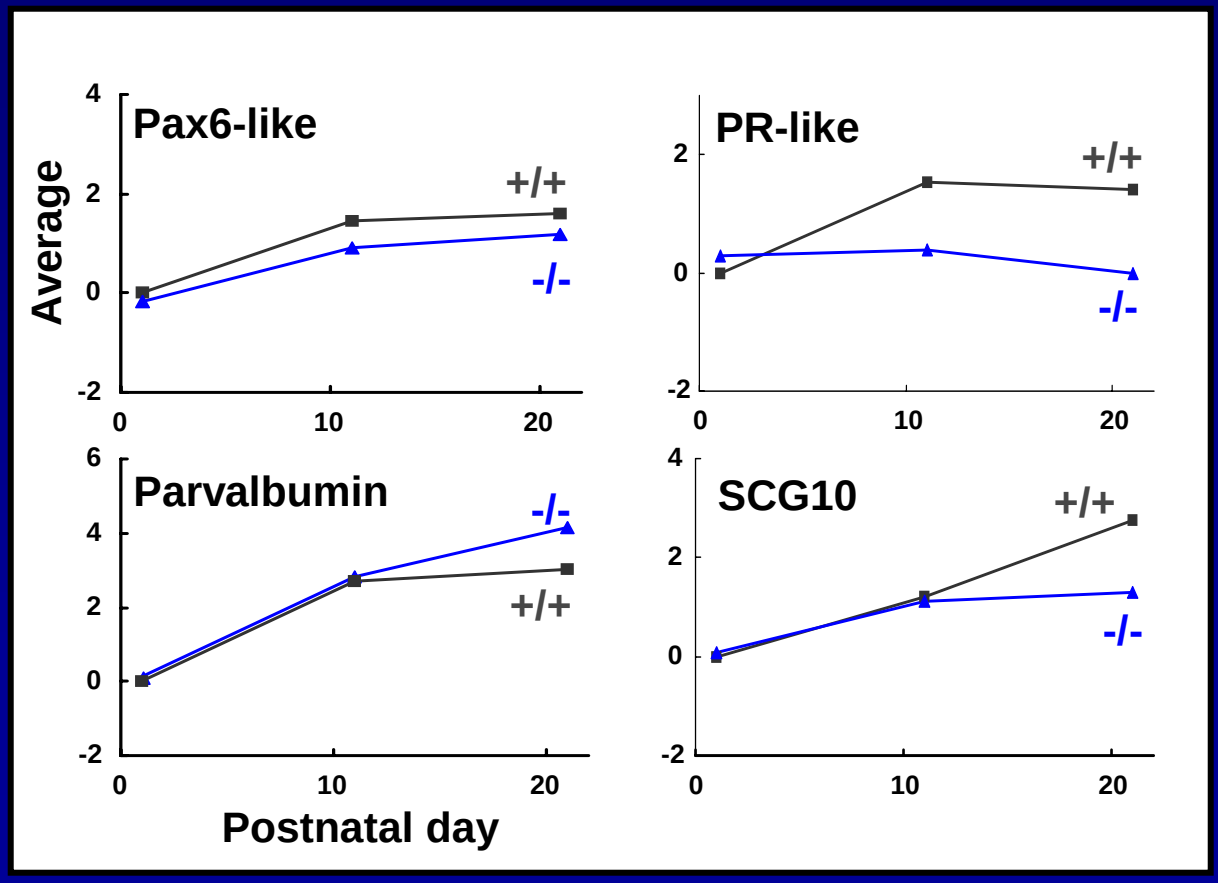


GABA α6 Cluster

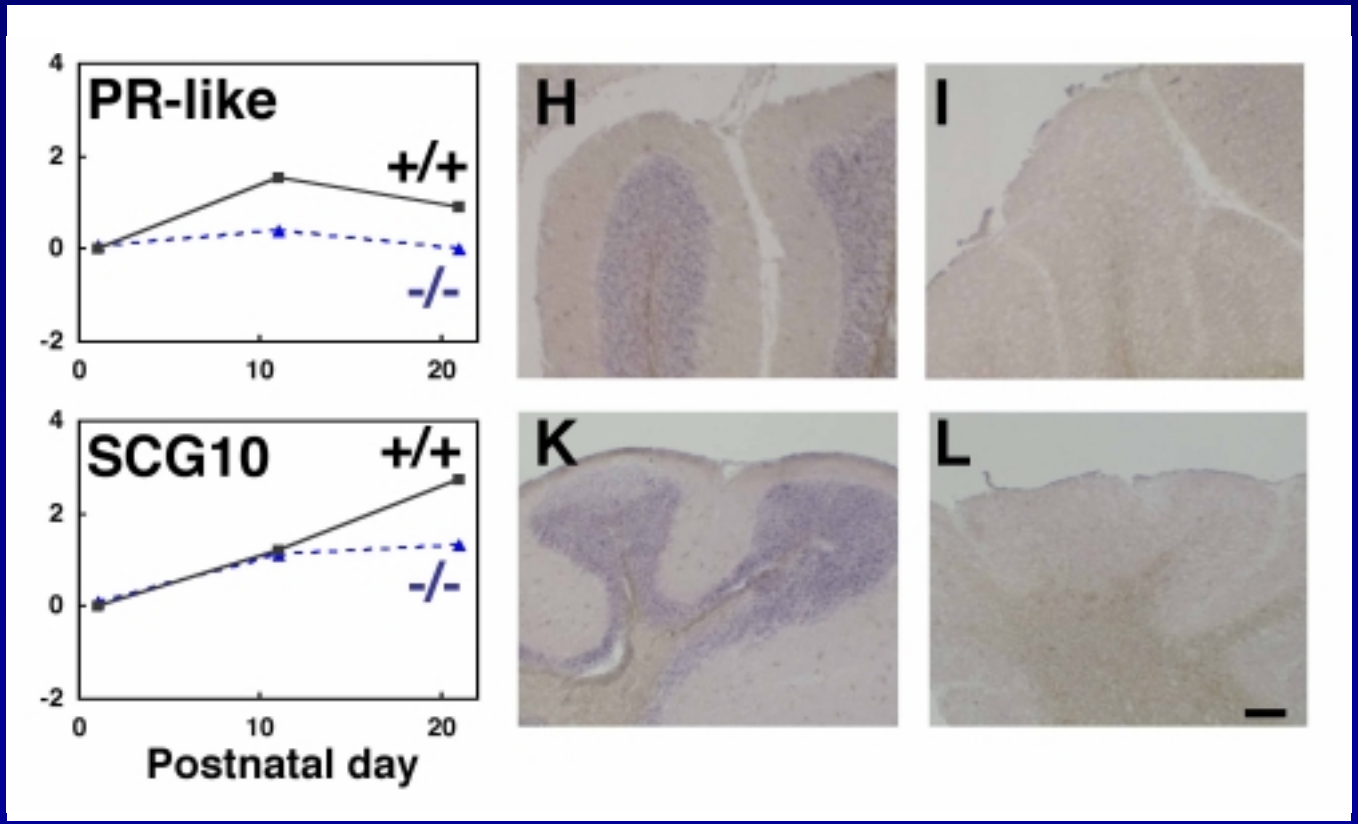




Granule Cell Profiles are Altered in *weaver* Mice



In Situ Hybridization



Summary

—**Clusters represent distinct cell types and stages of differentiation in the cerebellum**

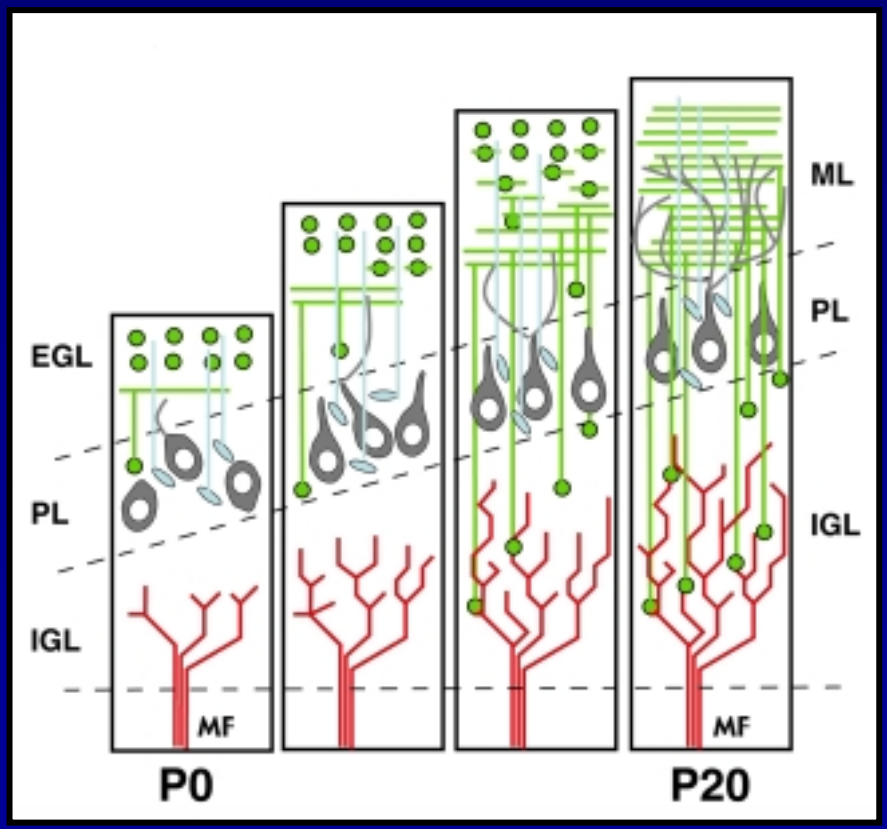
- **Genes within a cluster have similar expression patterns by *in situ* hybridization**

—**Average profiles of specific clusters are altered in *weaver* mice**

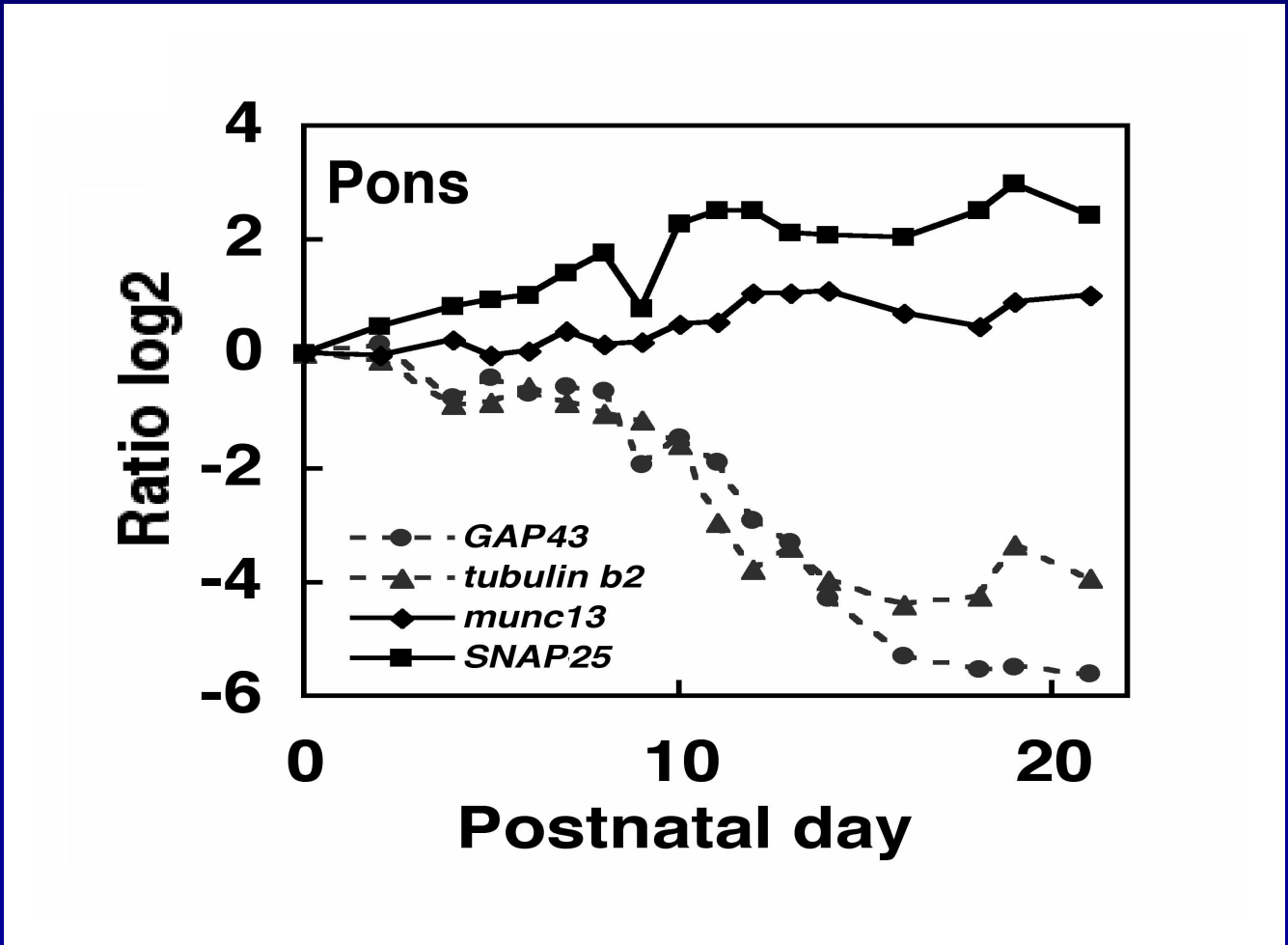
=>Clustering expression profiles identifies distinct developmental stages of specific cell lineages within a complex tissue

Gene Expression in Pontine Nuclei

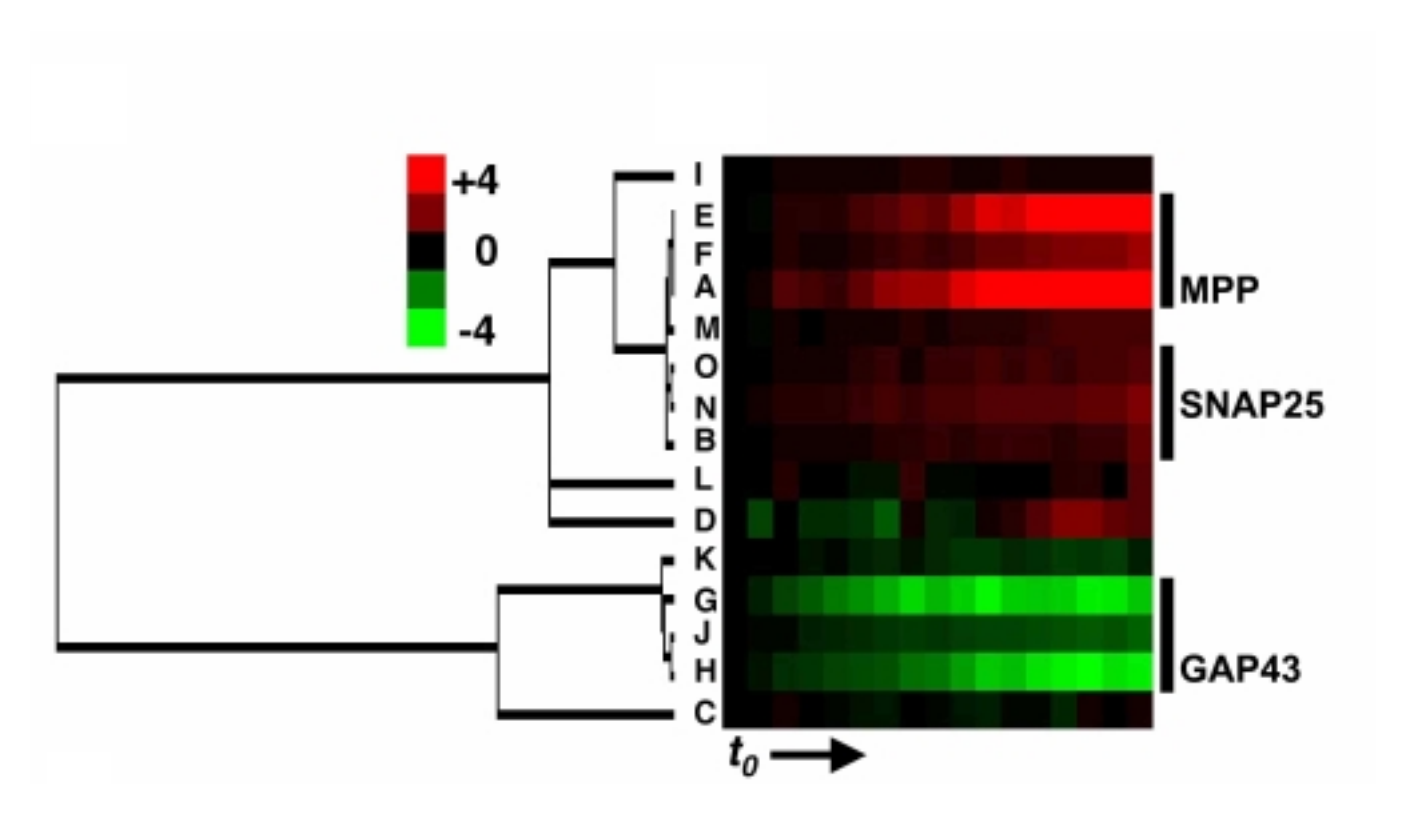
- —Synapse formation between granule cells and pontine mossy fibers
- —Identify target-dependent gene expression profiles using *weaver* and *lurcher* mice



Behavior of Known Genes Suggests "Axon Outgrowth" and "Synaptic" Programs

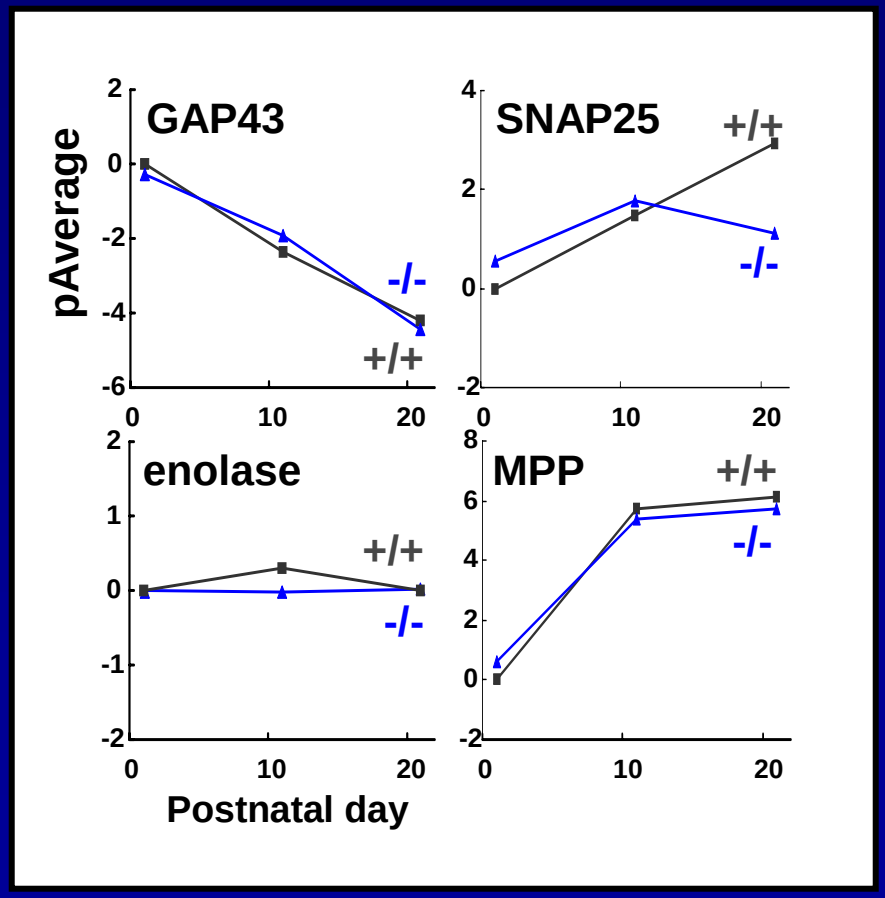


Developmental Profile in the Pons

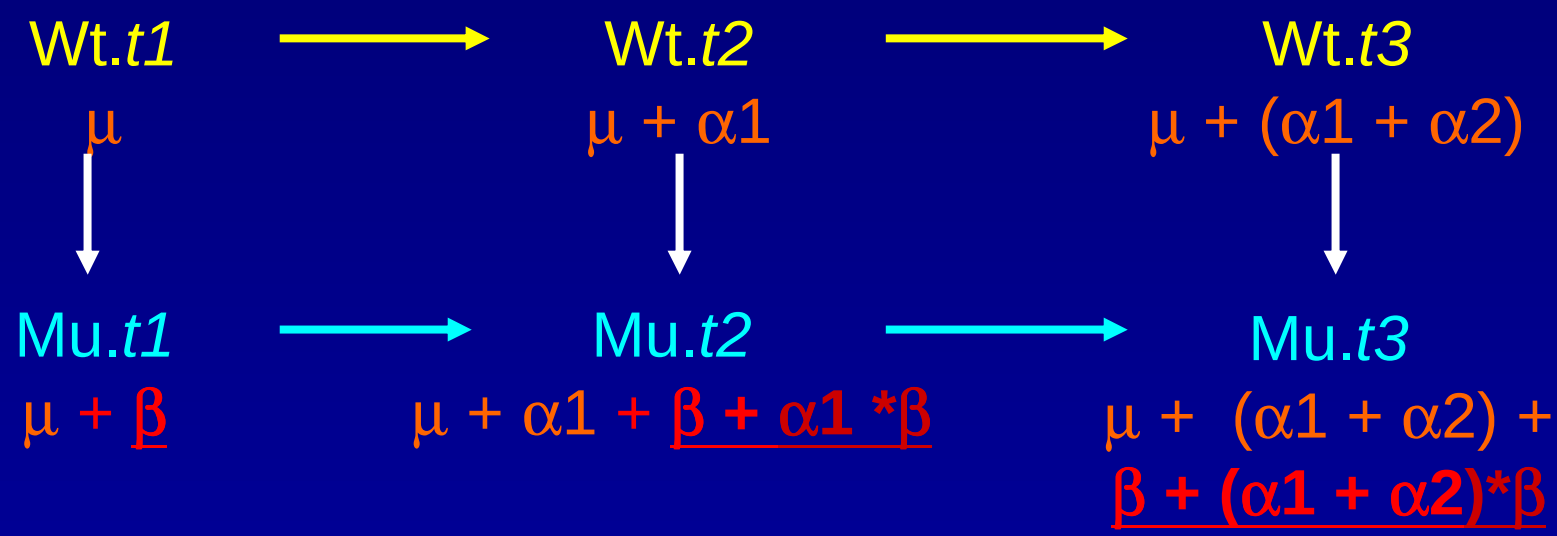


Target-Dependent Gene Expression in Pontine Nuclei

- —Functional switch between axon growth and synapse formation
- —Selective effect of *weaver* mutation on gene expression

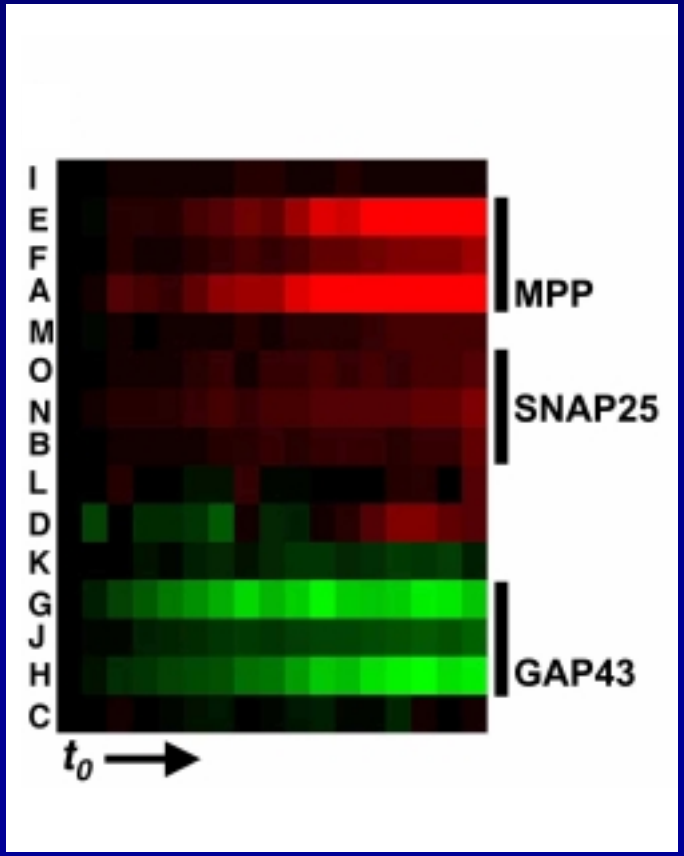


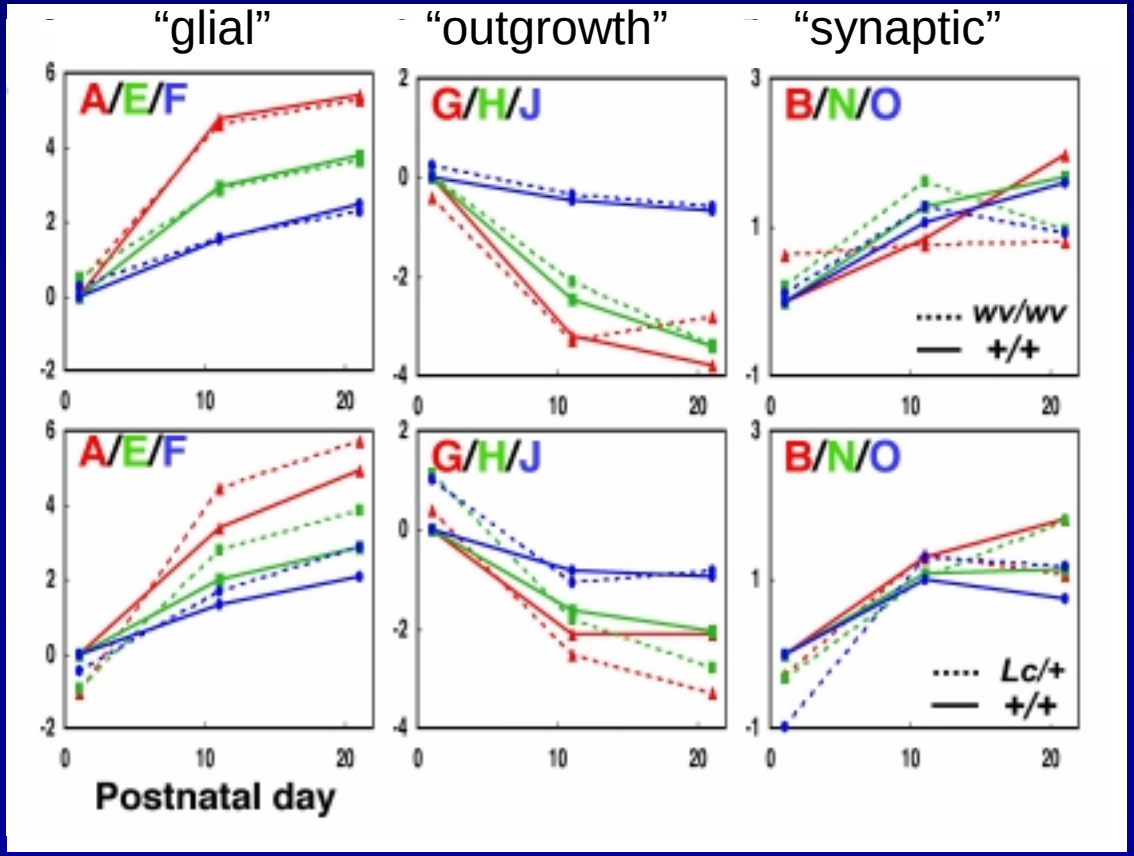
Mutant Analysis to Dissect Progams of Gene Expression in the Pons



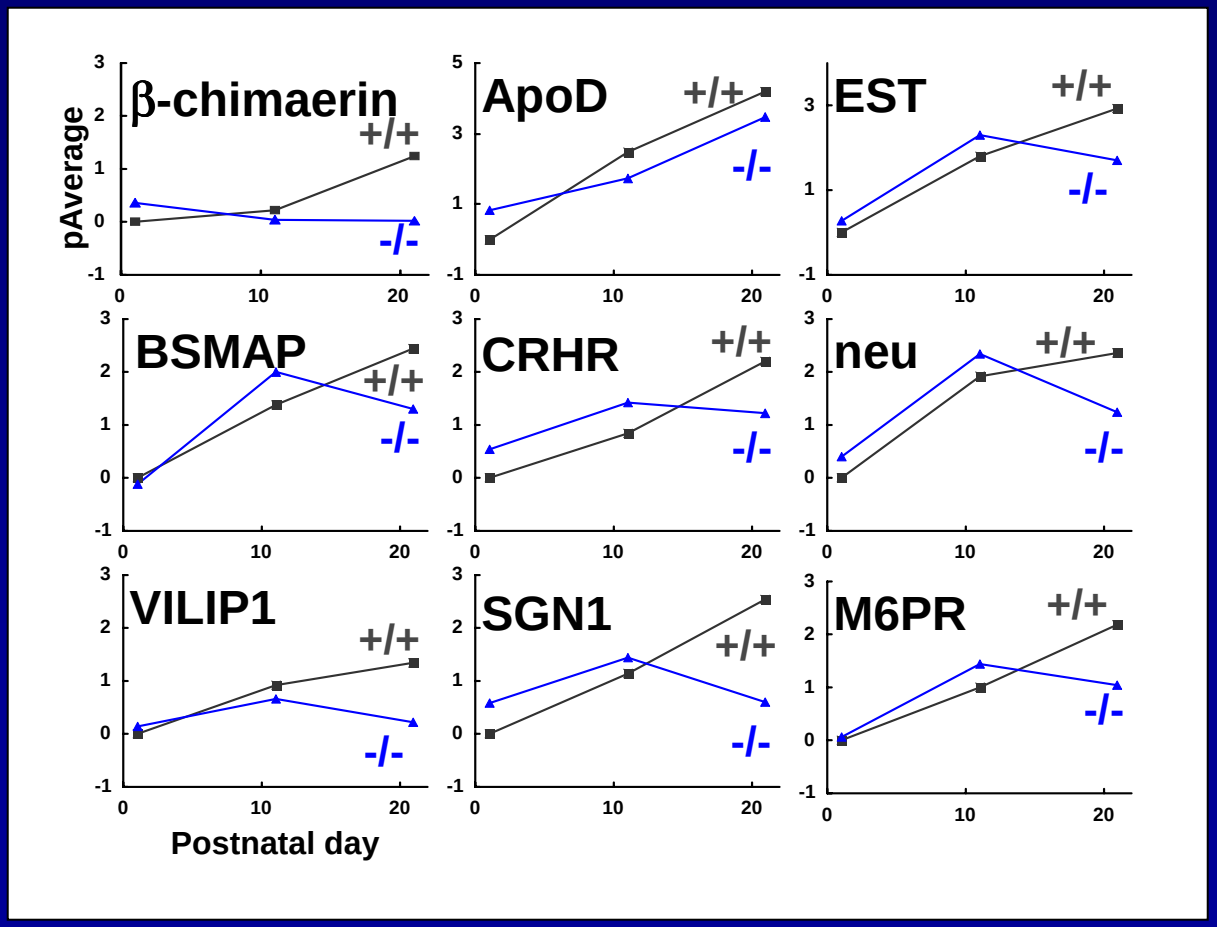
Assess clusters based on average interactions…
Rank genes within clusters based on interactions…

Differential Effects of *weaver* and *lurcher* Mutations on Pontine Gene Expression





Candidate Target-Dependent Genes in the Pontine Nuclei



Summary

—**Gene expression in pontine nuclei:**

- **Gene expression changes correlate with different phases of development**
- **Analysis of *weaver* mice identifies target-dependent programs of gene expression**
- ***Lurcher* gene expression profile suggests that pontine gene expression is responsive to cues in target environment during axon outgrowth**
- **Comparison of *weaver* and *lurcher* profiles suggests that axon outgrowth and synaptic gene expression programs can be decoupled**

=>Ability to identify patterns of gene expression dependent on cell-cell interactions

Analysis of Gene Expression in Space and in Time

- —Statistical modeling of microarray data to elucidate complex gene expression patterns
- —Clustering allows the elucidation of cell-specific programs of gene expression
- —Mutant analysis: a means of validation and refinement, also potentially useful in identifying cell-cell interactions
- —Future studies: single cell profiling, additional mutant mouse models, functional validation of most interesting candidates…

Acknowledgments

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Yangchao Ge

Terry Speed

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