

**GENE EXPRESSION IN
LYMPHOBLASTOID CELL LINES IN A
HIGH DENSITY MULTIPLEX FAMILY**

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Microarray screening of lymphocyte gene expression differences in a multiplex schizophrenia pedigree

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Agonal Events That May Negatively Affect mRNA Quality And Expression Patterns

1. Head Injury
2. Multiple Organ Failure
3. Hypoxia
4. Coma
5. Rapidity of Death
6. pH

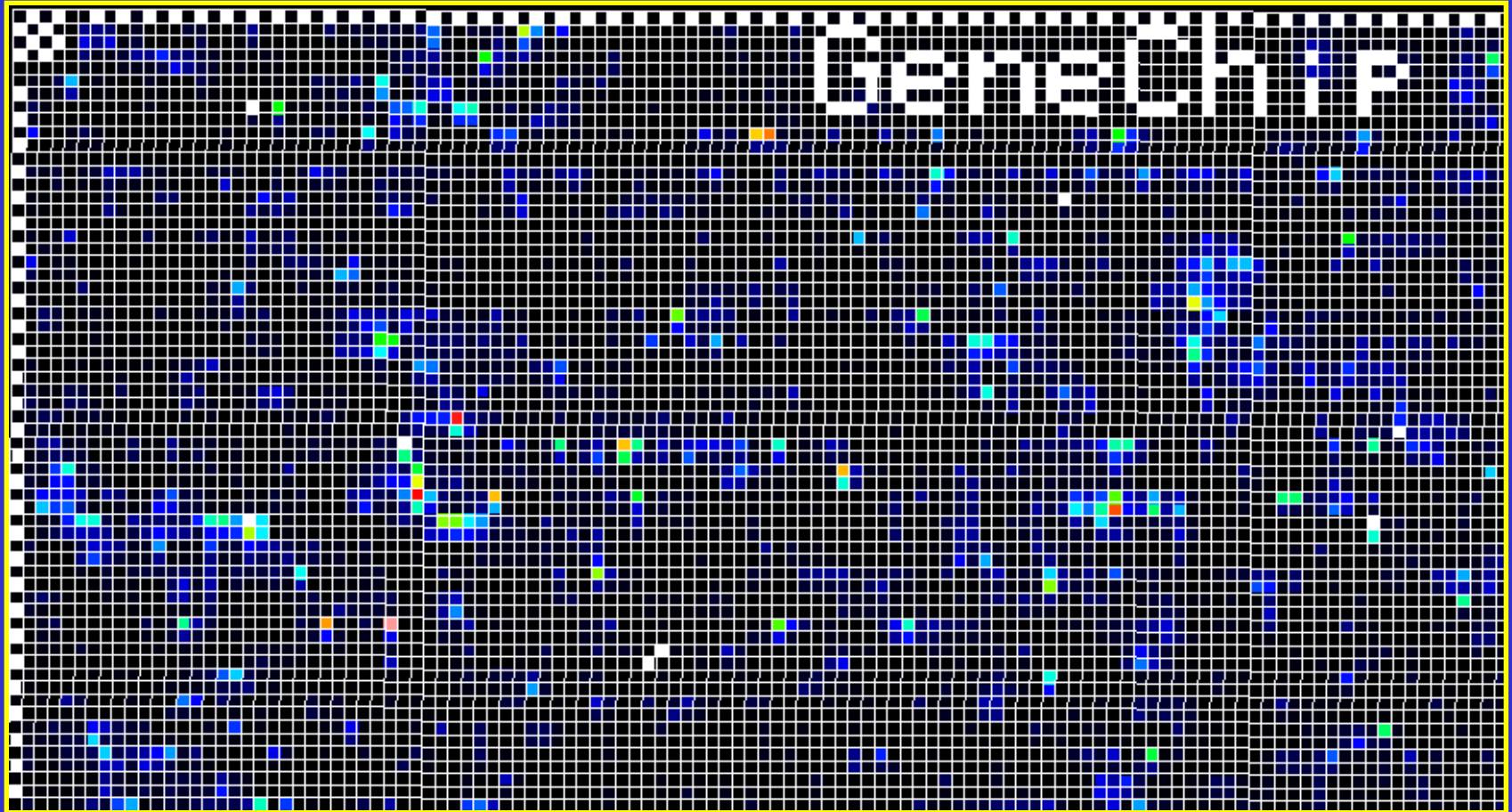
Advantage to cell lines

- Less agonal artifacts
- Medication wash out
- Easier and more abundant access
- Can be used in experimental research

Some disadvantages

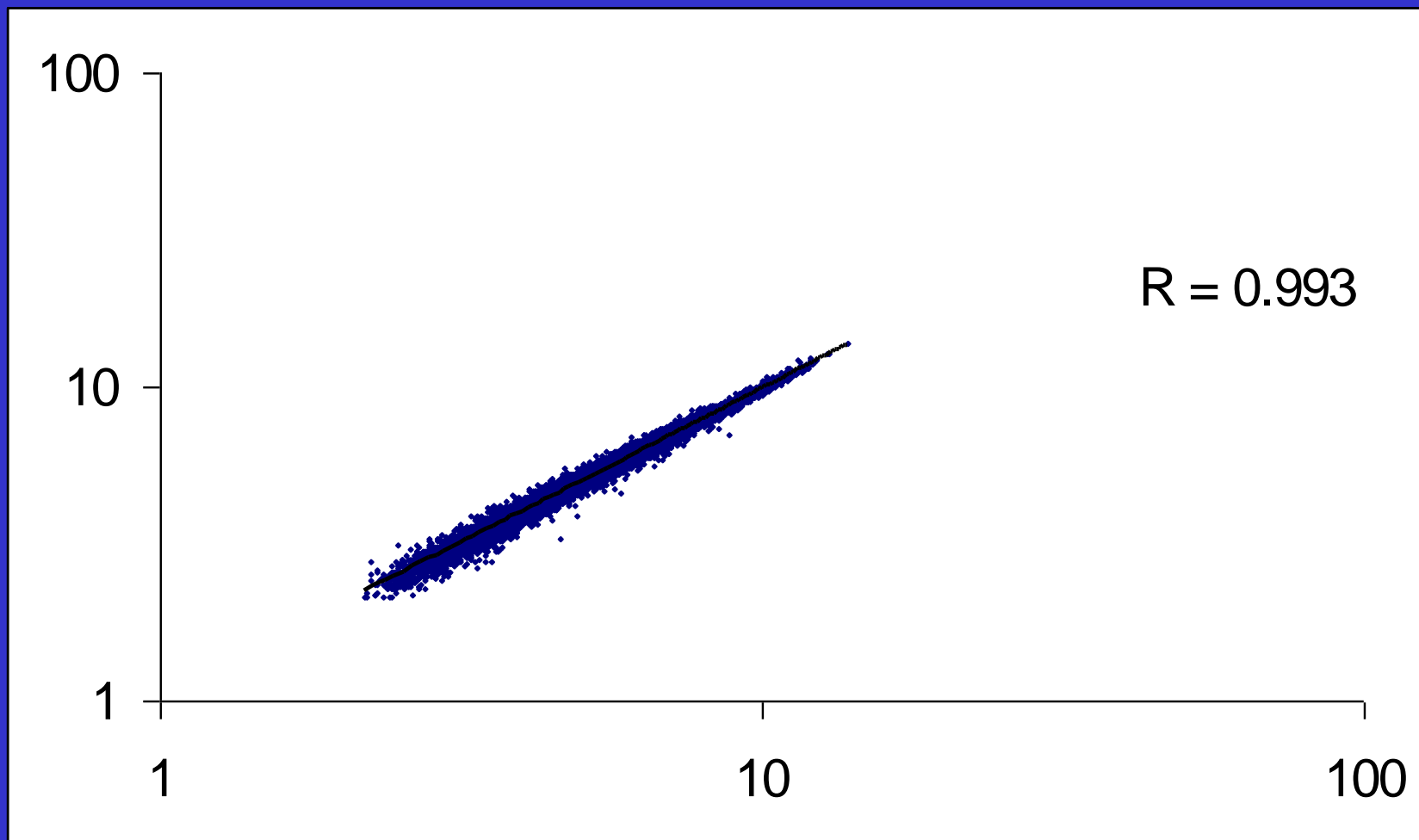
Not related to brain tissue, usually immortalized which might alter gene transcription machinery.

Oligonucleotide Microarray



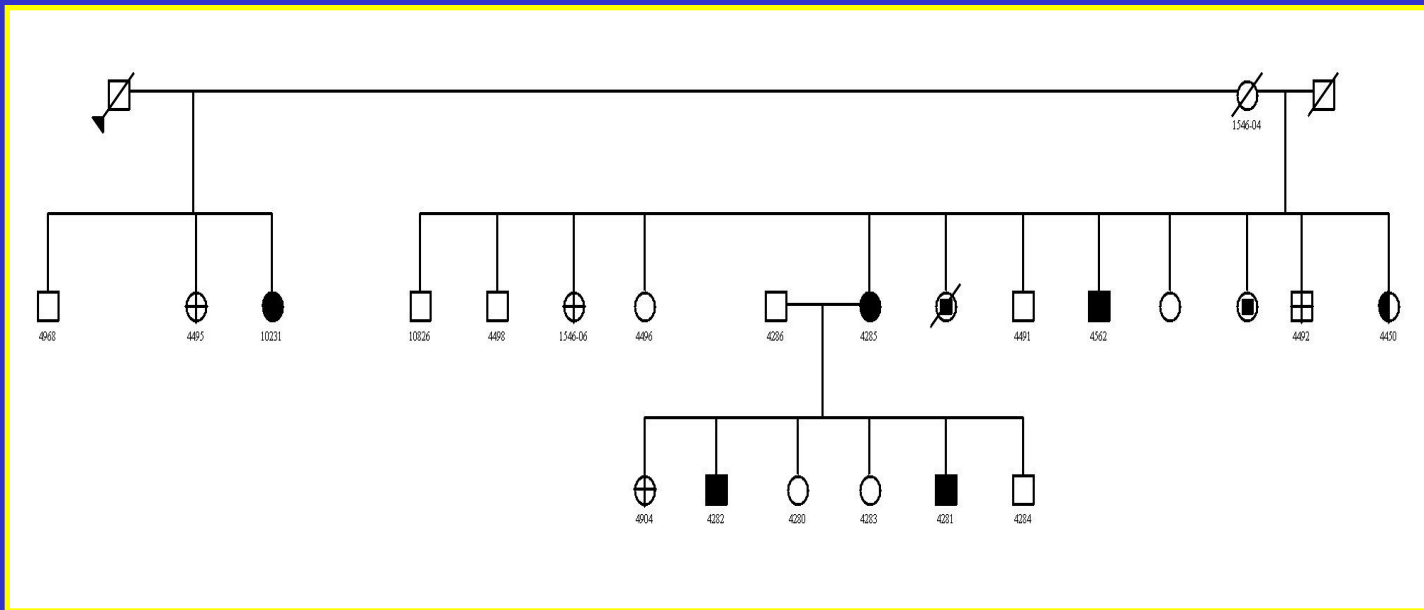
Robust Multi-Array Average (RMA)

Log₂ Expression (Anterior
Cingulate - Duplicate)



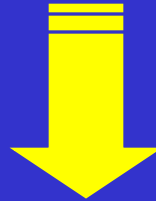
Log₂ Expression (Anterior Cingulate)

Pedigree of High Density Family With Schizophrenia



Differential Expression In Lymphocytes

- Expression differences by group t-test
- Gene Detection --Present vs absent calls by subject

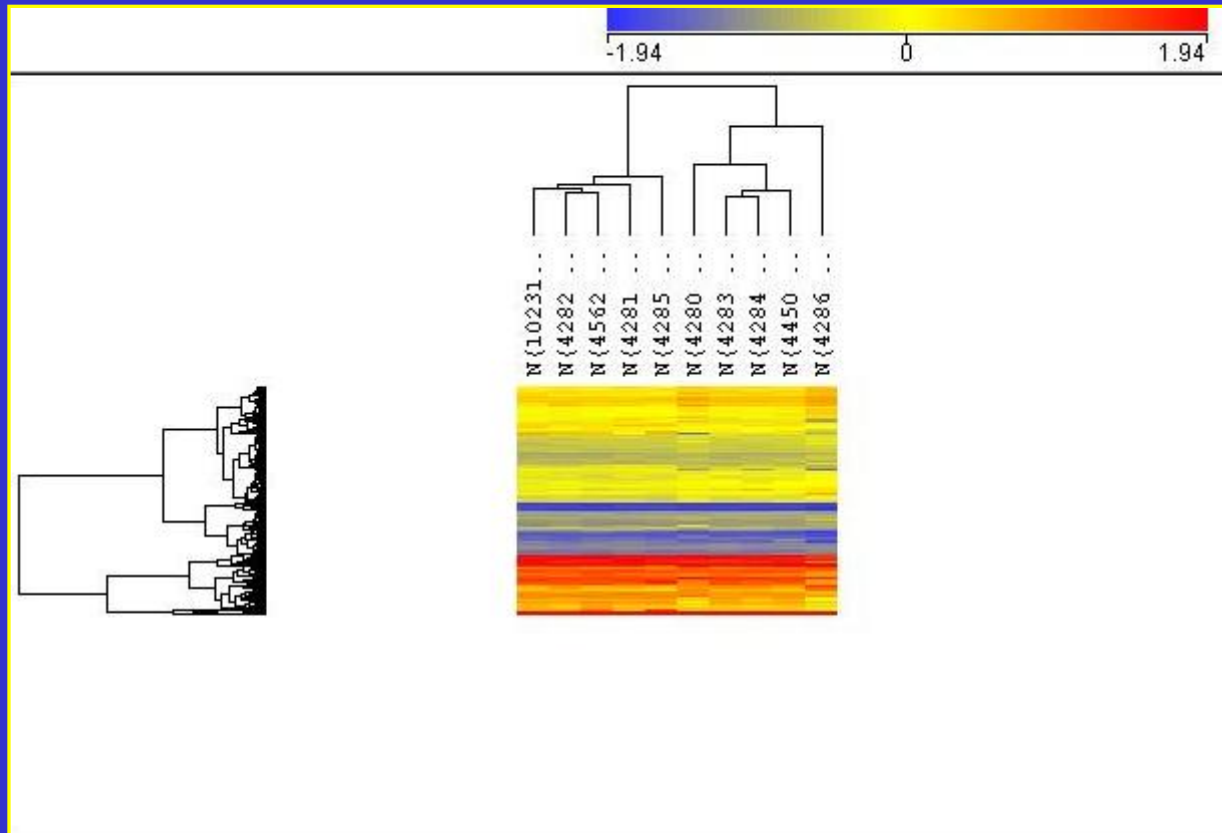


Underlying regulatory mechanisms

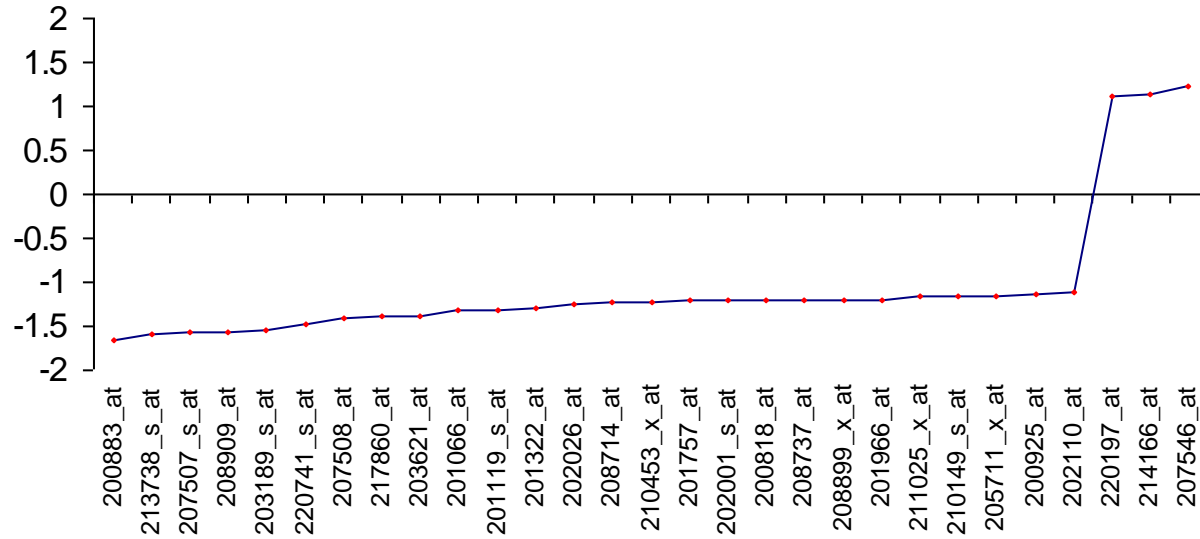
T-test Analysis of Schizophrenia vs Control Lymphocyte in Family-Results of Permutative Analysis of 22,283 Probesets

Result Summary						
.....	P.All	P.<0.05	P.<0.02	P.<0.01	P.<0.005	P.<0.001
FC >1.1	6806	1837	896	589	276	276
FC >1.5	222	90	44	31	11	11
FC >2	25	14	8	6	3	3
FC >3	3	3	3	2	1	1
Expected by Chance		1114	445	222	111	22

1837 Genes Significant Following 1000 Rounds of Permutation. Expression level shown as z-scores



Fold Change Oxidative Phosphorylation System



Gene Detection Method-Present and Absent Confidence Level

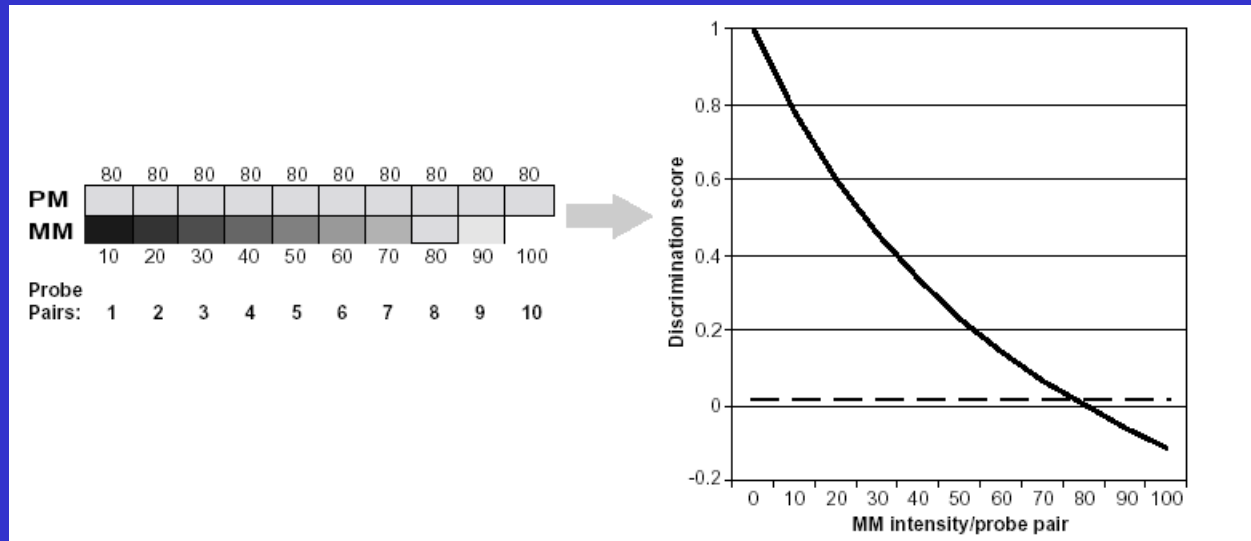
This analysis generates a **Detection p-value** which is evaluated against user-definable cut-offs to determine the **Detection** call. This call indicates whether a transcript is reliably detected (Present) or not detected (Absent). Additionally, a **signal** value is calculated which assigns a relative measure of abundance to the transcript.

	Stat Pairs	Stat Pairs Used	Signal	Detection	Detection p-value
37984_s_at	16	16	92.2	P	0.000219
32102_at	16	16	59.5	P	0.000218
37900_at	16	16	72.6	P	0.000219
31697_s_at	16	16	664.2	P	0.000219
40567_at	16	16	502.3	P	0.000219
35808_at	16	16	212.6	P	0.000219
34819_at	16	16	143.0	P	0.000219
35787_at	16	16	295.7	P	0.000219
35758_at	16	16	301.0	P	0.000219
34817_s_at	16	16	339.6	P	0.000219
34644_at	16	16	723.9	P	0.000219
34608_at	16	16	3313.0	P	0.000219

Figure 1. Data analysis output (.CHP file) for a Single Array Analysis includes Stat Pairs, Stat Pairs Used, Signal, Detection, and the Detection p-value.

$$R = (PM - MM) / (PM + MM)$$

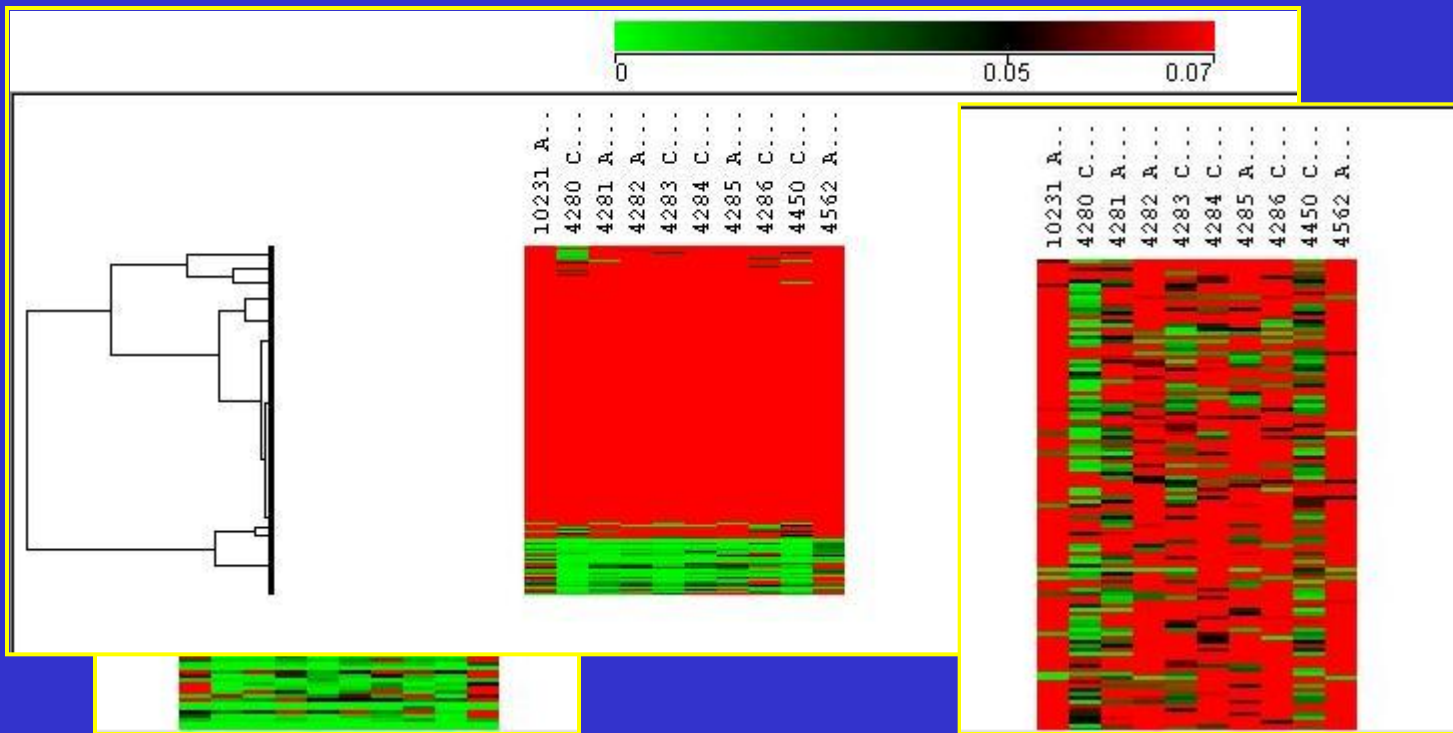
- Based on 11 Perfect Match (PM) and 11 Mismatch (MM) probes within each of 22,283 probesets.



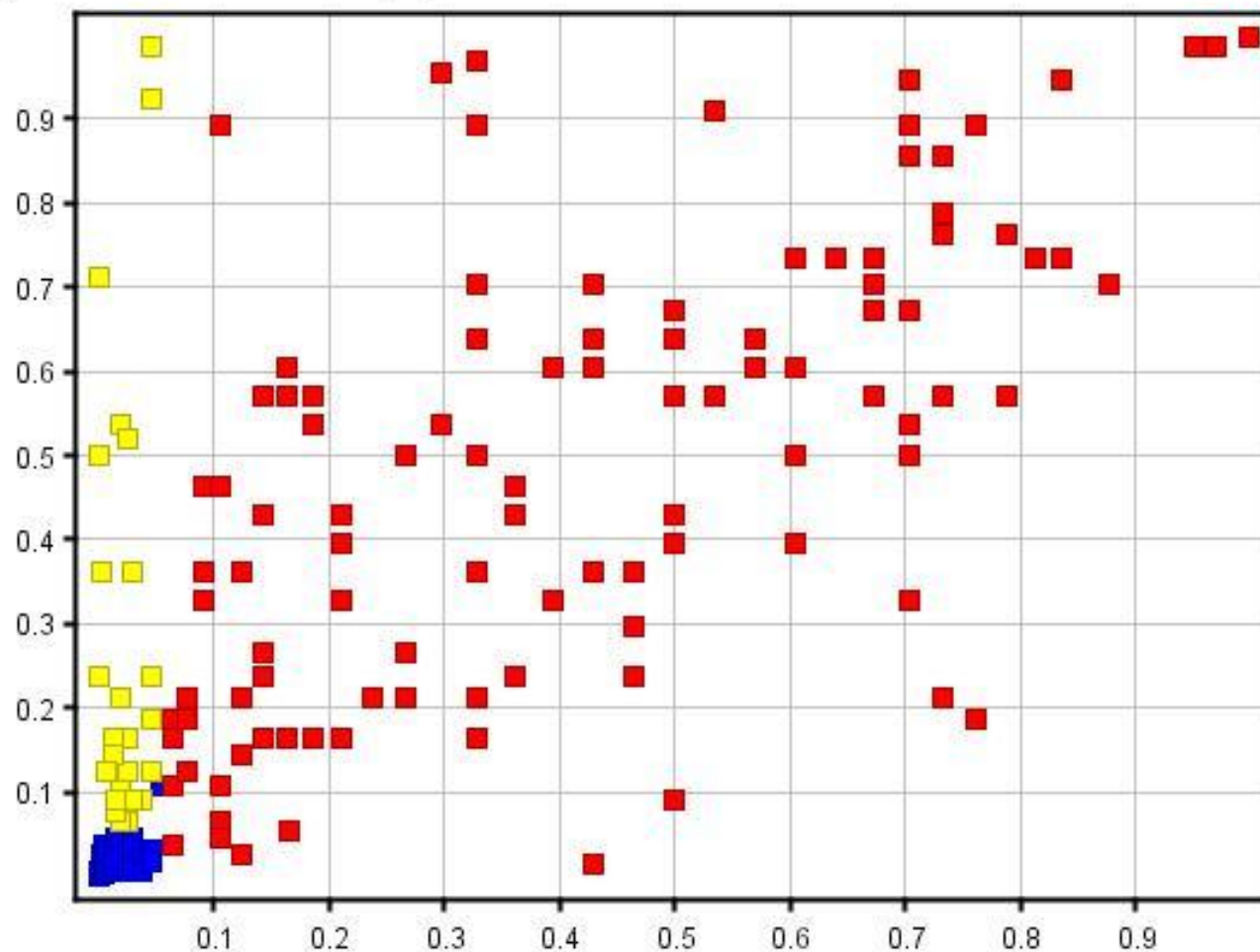
The next step toward the calculation of a Detection p -value is the comparison of each Discrimination score to the user-definable threshold τ . τ is a small positive number that can be adjusted to increase or decrease sensitivity and/or specificity of the analysis (default value = 0.015). The One-sided Wilcoxon's Signed Rank test is the statistical method employed to generate the Detection p -value. It assigns each probe pair a rank based on how far the probe pair Discrimination score is from τ .

Detection Present (green)

Absent (red) HGU133A Hierarchical
Cluster



Y-Axis: 4562 A.CEL_pVal_call

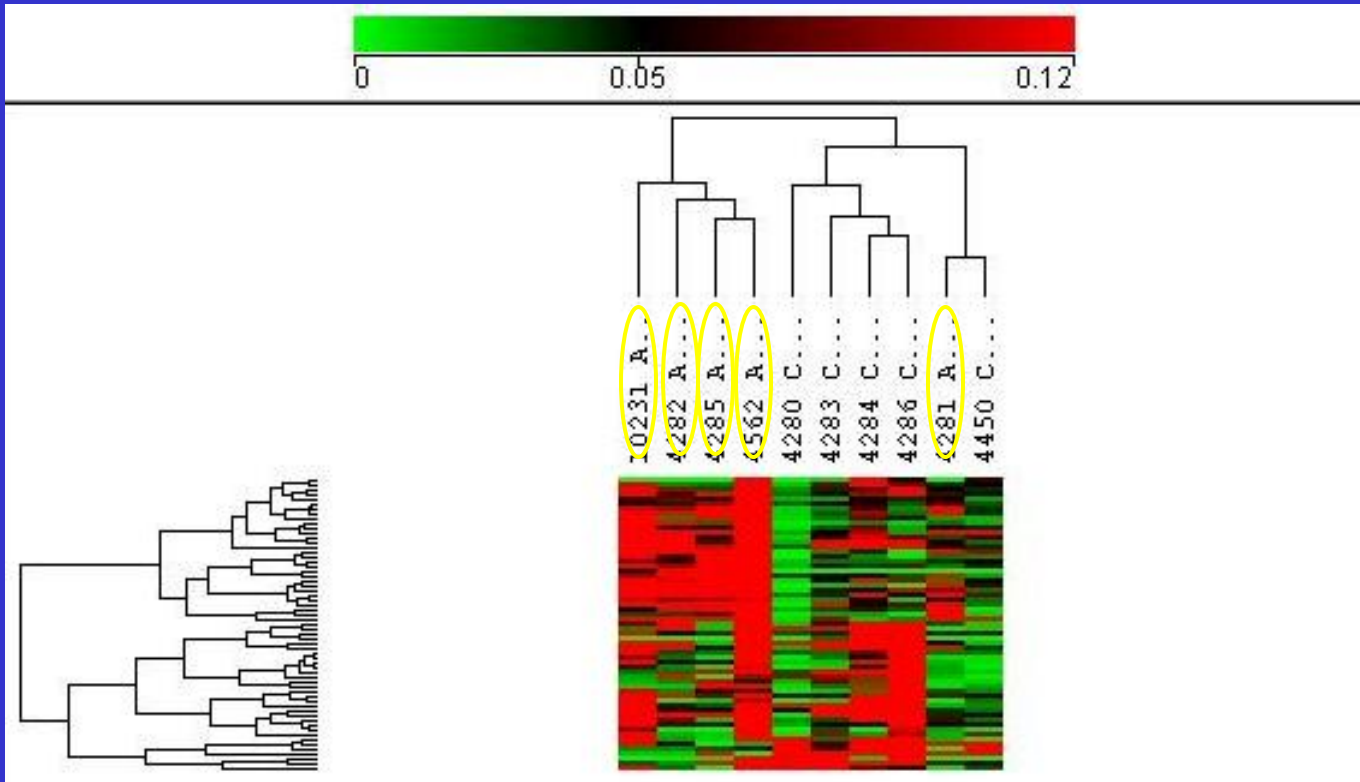


X-Axis: 10231 A.CEL_pVal_call

Mitochondrion (present absent values lymphocytes)

Gene Identifiers	Gene Name	Gene Symbol
38	acetyl-Coenzyme A acetyltransferase 1 (acetoacetyl Coenzyme A thiolase)	ACAT1
204	adenylate kinase 2	AK2
211	aminolevulinate, delta-, synthase 1	ALAS1
9556	chromosome 14 open reading frame 2	C14orf2
1329	cytochrome c oxidase subunit Vb	COX5B
1349	cytochrome c oxidase subunit VIIIb	COX7B
1371	coproporphyrinogen oxidase (coproporphyrin, harderoporphyria)	CPO
2235	ferrochelatase (protoporphyrin)	FECH
3030	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	HADHA
3032	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit	HADHB
3033	L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain	HADHSC
29796	ubiquinol-cytochrome c reductase complex (7.2 kD)	HSPC051
10989	inner membrane protein, mitochondrial (mitofilin)	IMMT
56922	methylcrotonoyl-Coenzyme A carboxylase 1 (alpha)	MCCC1
84263	hypothetical protein MGC10940	MGC10940
4297	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	MLL
10240	mitochondrial ribosomal protein S31	MRPS31
4515	mature T-cell proliferation 1	MTCP1
4705	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10, 42kDa	NDUFA10
4700	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa	NDUFA6
4720	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)	NDUFS2
4725	NADH dehydrogenase (ubiquinone) Fe-S protein 5, 15kDa (NADH-coenzyme Q reductase)	NDUFS5
4727	NADH dehydrogenase (ubiquinone) Fe-S protein 7, 20kDa (NADH-coenzyme Q reductase)	NDUFS7
4723	NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa	NDUFV1
23530	nicotinamide nucleotide transhydrogenase	NNT
23530	nicotinamide nucleotide transhydrogenase	NNT
5018	oxidase (cytochrome c) assembly 1-like	OXA1L
6392	succinate dehydrogenase complex, subunit D, integral membrane protein	SDHD
5250	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3	SLC25A3
58472	sulfide quinone reductase-like (yeast)	SQRDL
8802	succinate-CoA ligase, GDP-forming, alpha subunit	SUCLG1

Significant t-test, present absent calls
variable across sample.



Clustering present absent calls, shows affected family members
cluster together.

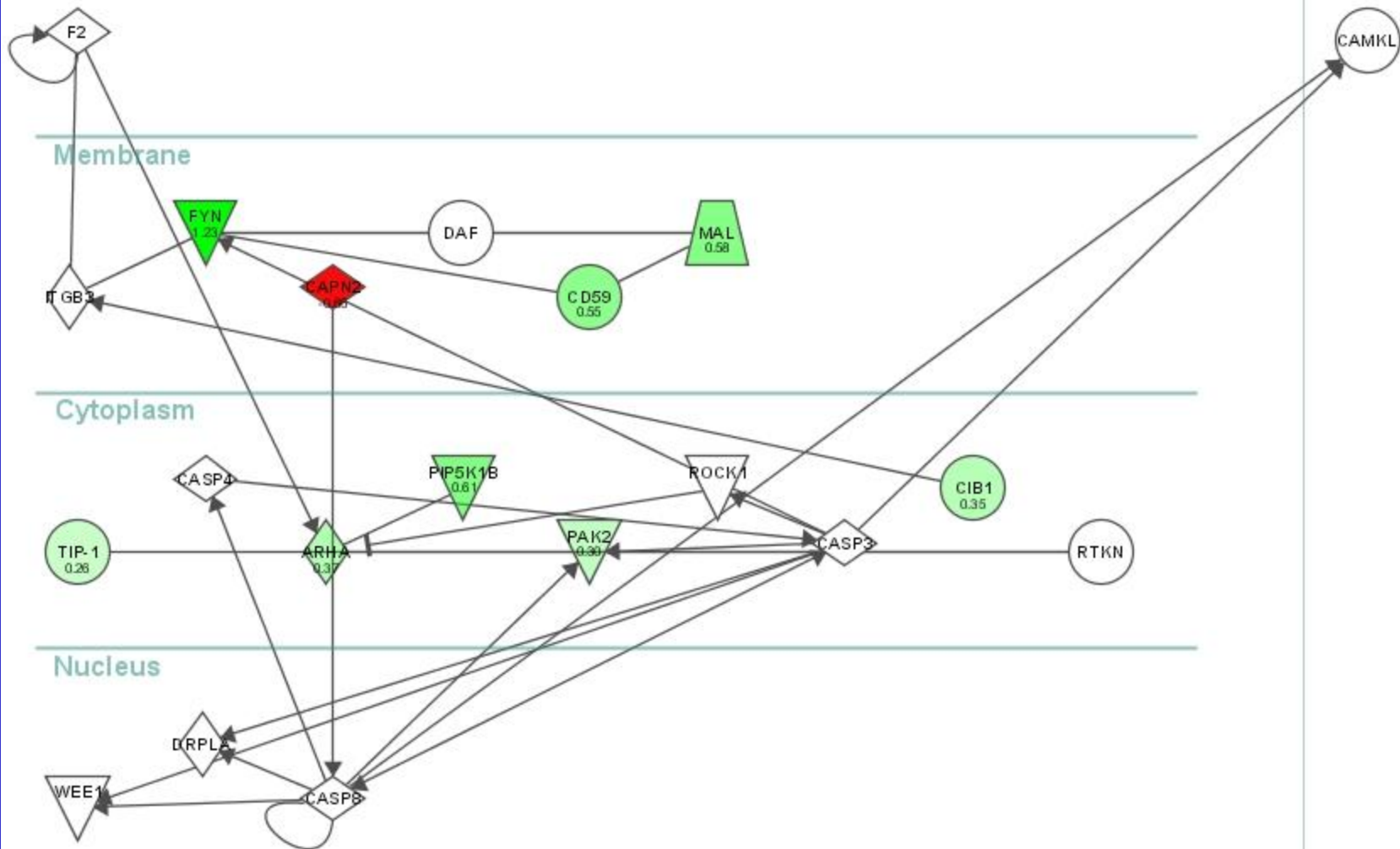
Extracellular

Unknown

Membrane

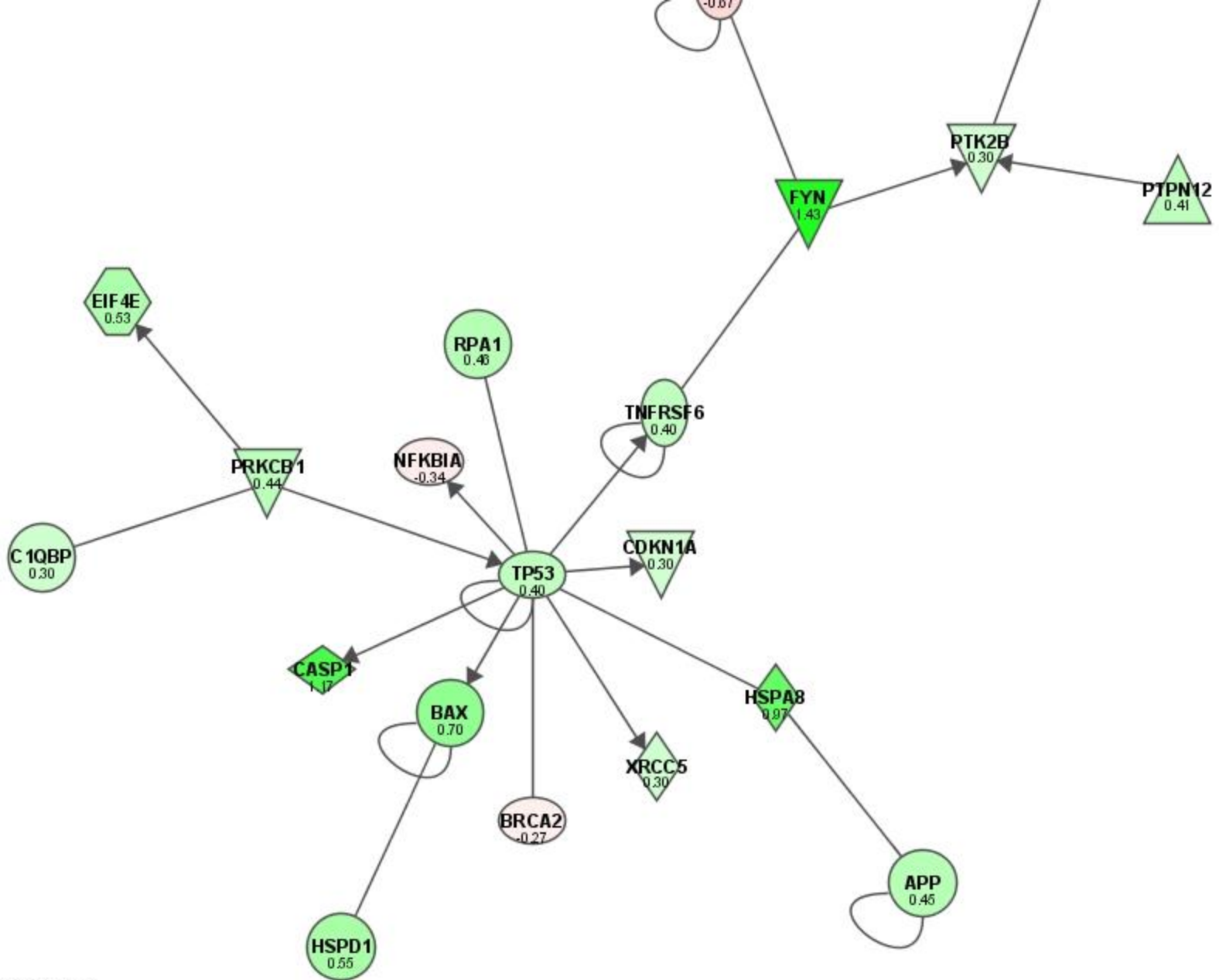
Cytoplasm

Nucleus



(c) 2003, Ingenuity Systems

1a lymph dlpc overlap



(c) 2003, Ingenuity Systems

T fc lymphs 1

Chromosomal Location	Gene Symbol	Gene Name
1p34.2-p33	NDUFS5	NADH dehydrogenase (ubiquinone) Fe-S protein 5, 15kDa (NADH-coenzyme Q reductase)
1p36.1-p35	SDHB	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)
1q23	NDUFS2	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)
2cen-q13	COX5B	cytochrome c oxidase subunit Vb
2q31.2	ATP5G3	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3
2q31.2	ATP5G3	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3
2q37.3	NDUFA10	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10, 42kDa
3q27.1	NDUFB5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, 16kDa
4q25	PPA2	inorganic pyrophosphatase 2
7q33-q34	ATP6V0A1	ATPase, H+ transporting, lysosomal V0 subunit G isoform 1
8q24.3	CYC1	cytochrome c-1
9q33.1	ATP6V1G1	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 1
10q22-q23	ATP5C1	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1
11q12-q13	COX8	cytochrome c oxidase subunit VIII
11q13	NDUFS8	NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase)
11q13	NDUFV1	NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa
11q23	SDHD	succinate dehydrogenase complex, subunit D, integral membrane protein
11q23	ATP5L	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit g
12p13-qter	ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide
12q24.2	COX6A1	cytochrome c oxidase subunit VIa polypeptide 1
13q34	ATP4B	ATPase, H+/K+ exchanging, beta polypeptide
14q	ATP6V1D	ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D
16p12	UQCRC2	ubiquinol-cytochrome c reductase core protein II
17q25	ATP5H	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d
18q12-q21	ATP5A1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, cardiac muscle
19q12-q13.1	UQCRCF1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
21q22.11	ATP5O	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein)
22q13.2-q13.31	NDUFA6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa
Xq13.3	COX7B	cytochrome c oxidase subunit VIIb

Gene Ontology—1837 Significant Genes Lymphocytes

Mitochondrial Related Changes--

Antipsychotic drug treatment

pH and Agonal Factor changes in the Brain

Antidepressants

Aging

Neurodegenerative disorder

This is a co-regulated pathway conserved across yeast, *Drosophila*, *C. elegans*, and *H. sapiens*.

Compare Dysregulated Expression In Lymphocyte and DLPFC- Schizophrenia

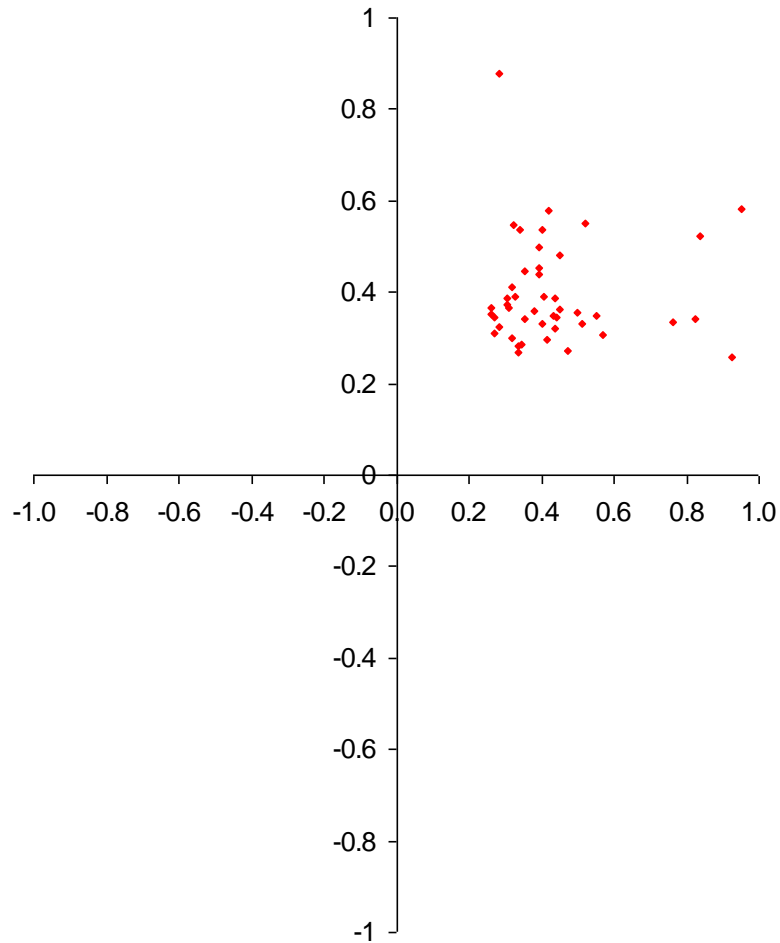
	DLPFC Lymphocyte common	DLPFC Sz Top Bottom 500	Lymphocyte Sz
Probesets in Common	4032	1000	
Locus Link in Common	3700	922	365
Unique Genes In Common	2875	780	350

Compare Dysregulated Expression In Lymphocyte and DLPFC- Schizophrenia

	DLPFC Lymphocyte common	DLPFC Sz Top Bottom 500	Lymphocyte Sz, MDD, BPD
Probesets in Common	4032		
Locus Link in Common	3700		
Unique Genes In Common	2875		350 + 165 + 401 = 916

Schizophrenia—Comparison of DLPFC and Lymphocyte

- What genes were common?
- In the top 500 from each analysis, expect 22 false positives.
- Found an overlap of 43 genes: 26 up and 17 down genes.
- Immune category significant for each comparison, and for the overlap.



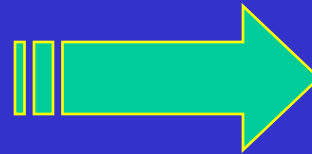
Overlaps with DLPFC
(fold change) and T-
test and lymphocyte
(fold change and T-
test)

Brain And Lymphocyte
Number of Common Probesets In Controls
1 Fold Change

DLPFC U133A (n = 8)



Unique Locus
Links



2,875 Unique
Known Genes In
Common

Control Lymphocyte U133A (n = 9)

Summary

- Preliminary investigation with Neuroarray indicates that lymphocyte gene expression has some overlap with brain gene expression.
- In a high density pedigree with schizophrenia, we found that gene expression differences for NPY1R and GNAO1 was also within the linkage region.
- Full scale gene expression studies of brain and lymphocytes with over 35,000 transcripts is planned to follow-up on these results.
- We are currently scanning these genes for mutations that occur in the NPY1R gene.

Conclusions

Lymphocytes measure some brain related genes

Changes in lymphocytes are found in co-regulated pathways

Family members with schizophrenia show decreased oxphos in culture, not related to drug or environmental events, but perhaps genetic.

Absent/present calls for genes may give some underlying genetic control information.

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