

CHANGES IN GENE EXPRESSION RELATED TO DECLINE OF THE AGING IMMUNE SYSTEM

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Oregon State University

Student: Jenny Tran
Mentor: Adrian F. Gombart, Ph.D.

My project involves...

Studying age-related changes in gene expression in the bone marrow of aging mice

Background

- Healthy Aging
 - ▣ Balance of gene expression
- “Inflamm-aging” (1)

1. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000 Jun;908:244-54.

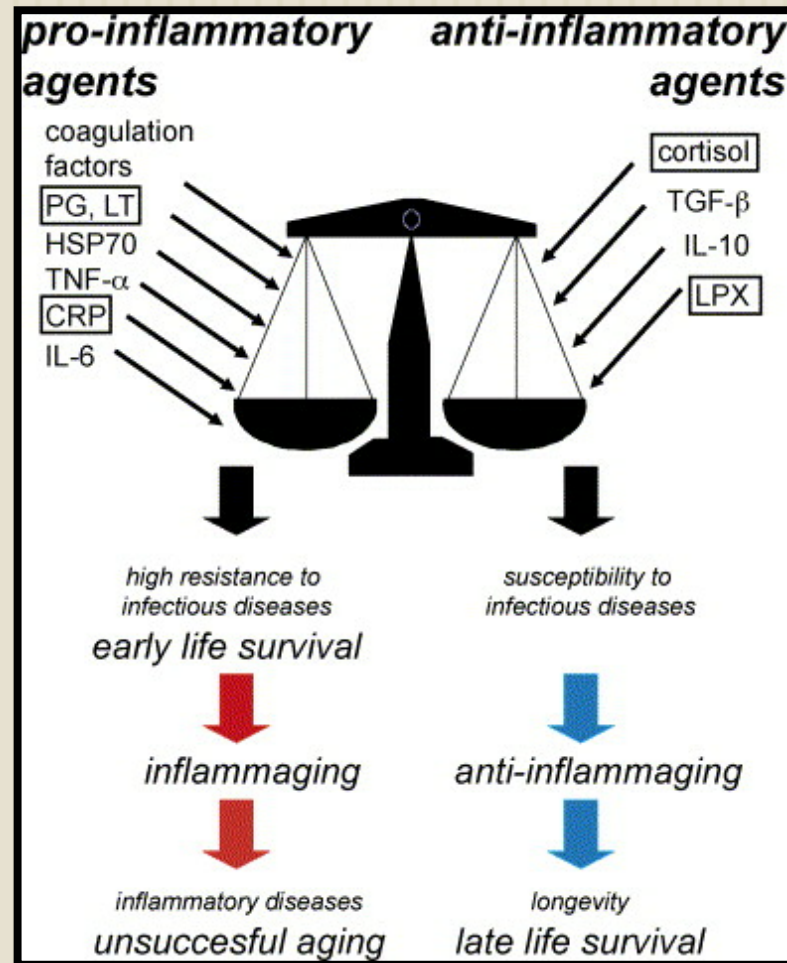
Inflamm-aging

- Low-level inflammatory status/ up-regulation of pro-inflammatory cytokines
- Decline in adaptive immunity
 - ▣ Highly specialized systemic cells and processes that attack pathogens
- Over-active innate immune system
 - ▣ First line of defense
 - ▣ Macrophages and neutrophils (Bone Marrow)

Importance of inflamm-aging

- Contribution to the development of chronic diseases
 - ▣ Infections
 - ▣ Cardiovascular Disease
 - ▣ Neurological Disease

Example: Factors



Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia M, Invidia L, Celani L, Scurti M, Cevenini E, Castellani G, Salvioli S. **Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans.** Mech Ageing Dev. 2007 Jan;128(1):92-105. Epub 2006 Nov 20.

Hypothesis

Age-related changes in gene expression in the bone marrow lead to over-activity of the innate immune cells in the aging immune system

Prediction

Changes will either cause down- or up-regulation of genes that are directly involved with innate immune function and inflammation

Significance of Findings

- Gene Expression
- Mechanism

Samples

- RNA
 - ▣ Bone Marrow
- “Black 6”
- Ages
 - ▣ 3 months
 - ▣ 25.5 months



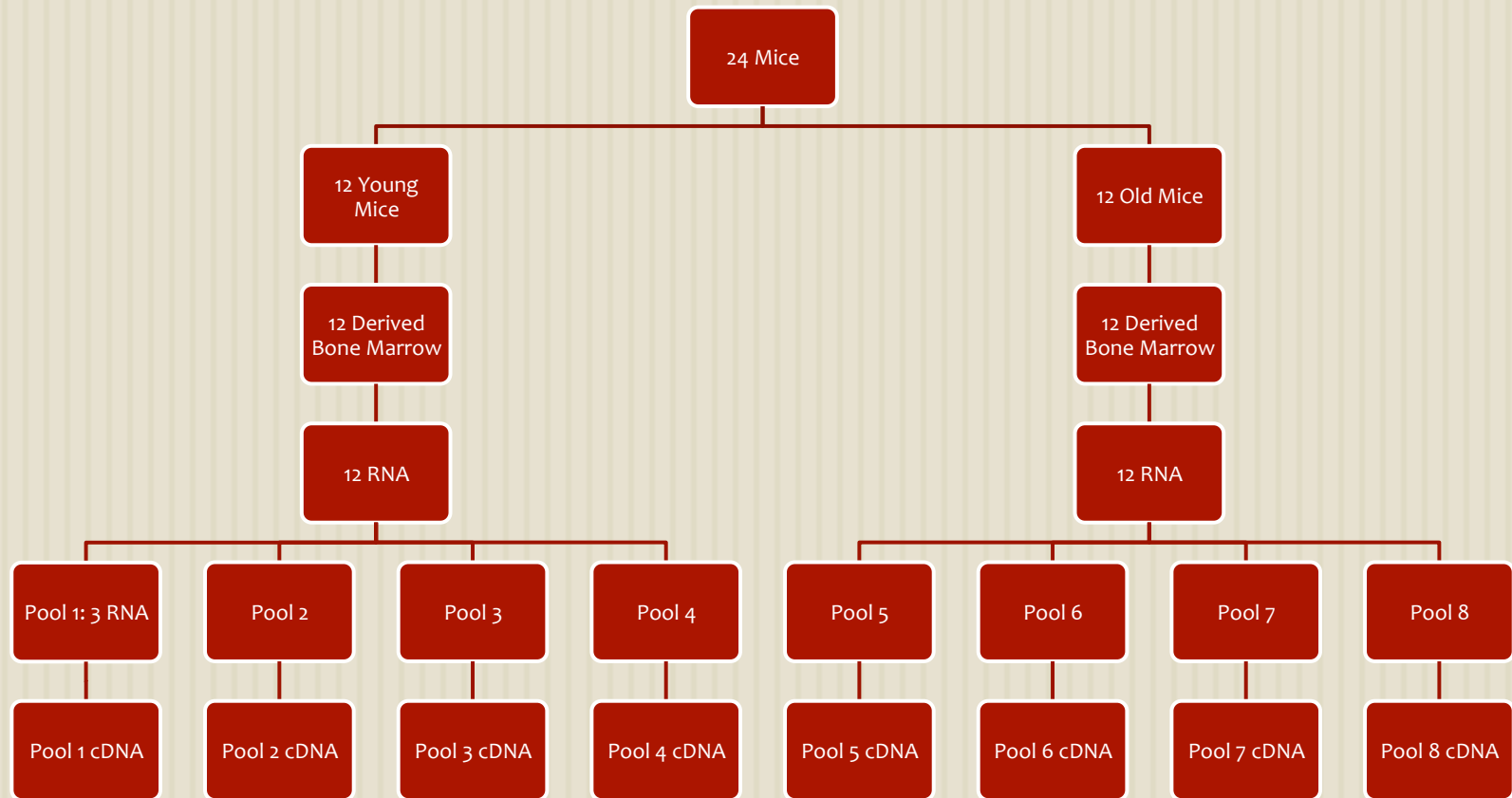
Groups

Samples	Pool #	Age
545, 546B, 547	1	3 months
548B, 549, 550B	2	3 months
535, 536B, 537	3	3 months
538B, 539, 540B	4	3 months
559, 560B, 561	5	25.5 months
562B, 563, 564B	6	25.5 months
553, 554B, 555	7	25.5 months
556B, 557, 558B	8	25.5 months

Overview: Methods

- ① Synthesize complementary DNA (cDNA) from RNA
- ② DNA microarray analysis
- ③ Computer analysis of genes

Overview: Methods

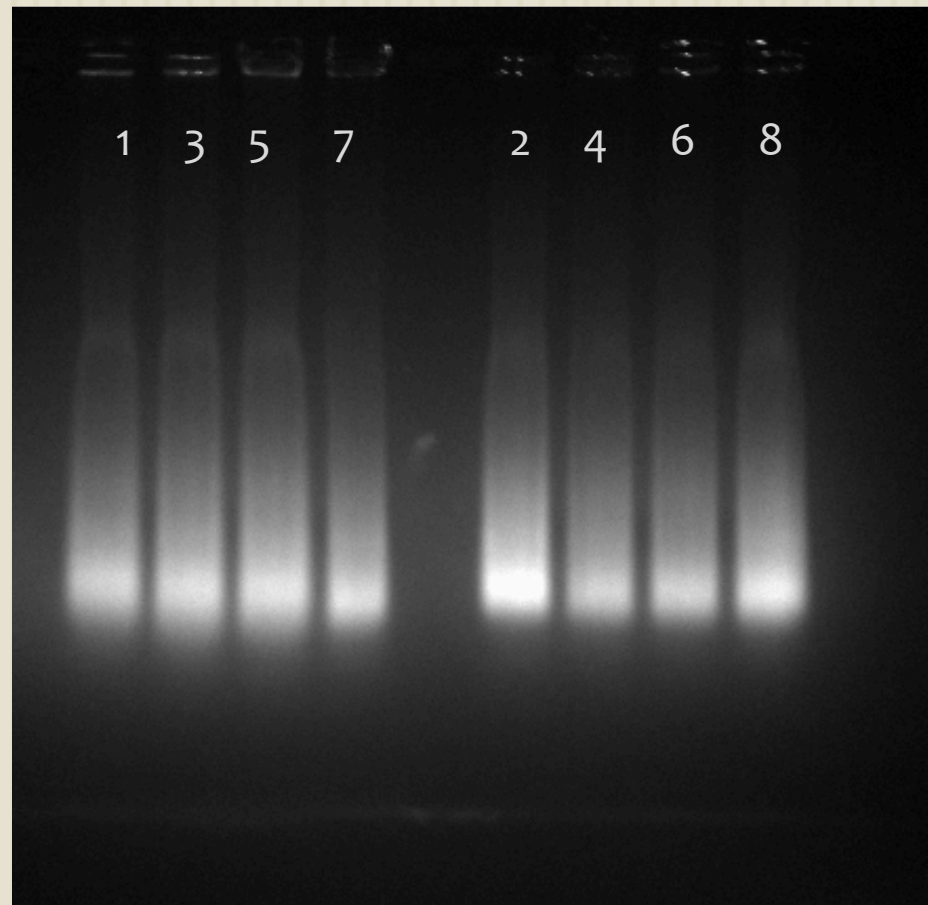


All then labeled, hybridized, and analyzed!

Synthesis of cDNA

- Make complementary DNA (cDNA) from pooled RNA
 - ▣ Clean-up
- Labeled using Klenow Fragment, exo-
 - ▣ Large fragment of DNA polymerase I
 - ▣ Cy3 Dye

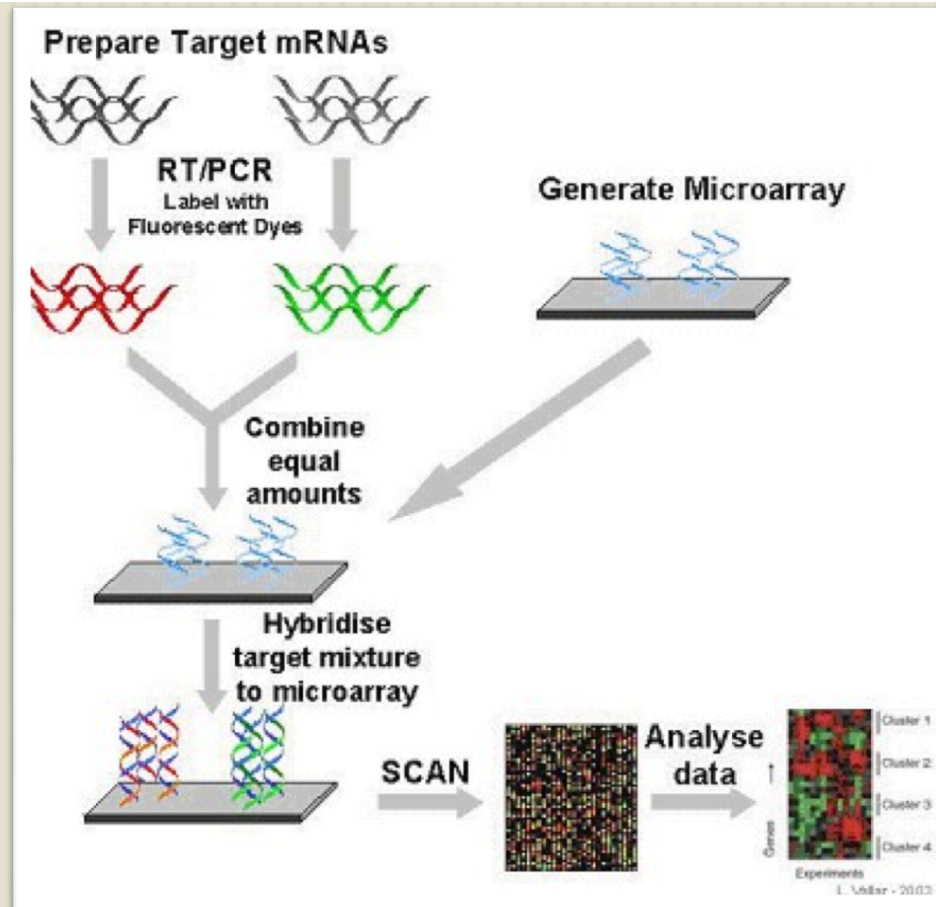
Verification: Labeling



Next step

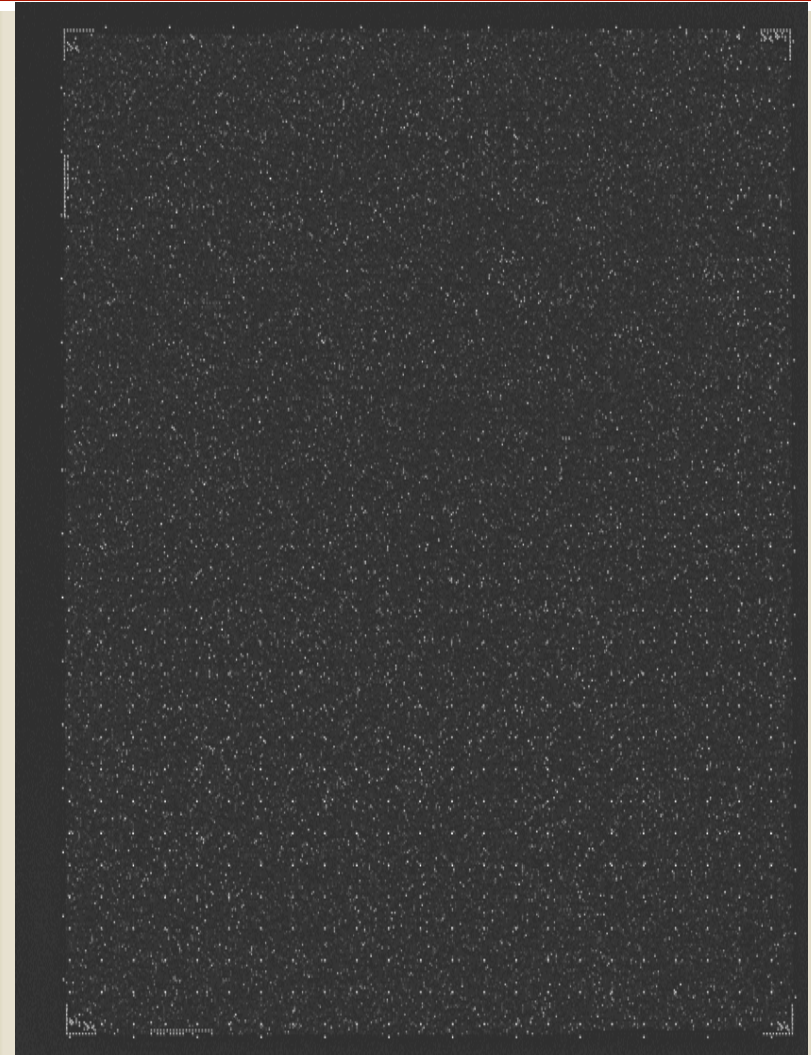
- Hybridization
- Use in DNA microarray

Overview of DNA Microarray



DNA Microarray Analysis

- Collection of microscopic DNA spots
 - ▣ Glass microscope slide
- Primary focus:
 - ▣ Genes functioning in immunity and inflammation



Example: Microarray

$$Y_1 + Y_2$$



$$Y_3 + Y_4$$



$$Y_1 + Y_2 + Y_3 + Y_4$$

$$O_1 + O_2$$



$$O_3 + O_4$$



$$O_1 + O_2 + O_3 + O_4$$

Results

- Analyzed total: 44,170 genes
- Of the total: 148 genes
 - ▣ Significantly different between young and old
- Control
 - ▣ Young mice

Gene Name	Fold-change	Parametric p-value
Col1a1	0.14	< 1e-07
Col1a2	0.32	2.40E-06
Bglap-rs1	0.38	1.70E-06
Fos	0.38	0.0014724
Col1a1	0.39	3.00E-07
Vpreb3	0.39	2.75E-05
N/A	0.41	3.00E-07
Cpm	0.45	1.20E-06
Cpm	0.46	3.20E-06
My14	0.47	5.61E-05
Fam129c	0.48	2.00E-07
Sparc	0.5	0.0001561
Bach2	0.51	5.00E-06
Vpreb2	0.51	9.60E-06
Chst10	0.52	8.04E-05
Akap12	0.52	0.0001151
Bach2	0.53	5.00E-06
Cecr2	0.53	0.000292
Cecr2	0.53	0.0006091
Gam129c	0.54	7.20E-06
Gfra1	0.54	0.0001327
Sparc	0.54	0.0002048
Egfl6	0.57	3.71E-05
Kifc1	0.58	0.0013927
Cecr2	0.61	0.0018144
Kifc1	0.62	0.0023379
Gm2344	0.63	0.0009404
epor	0.64	0.0022494
Cdr2	0.65	0.0013642
N/A	1.45	0.0024971
N/a	1.48	0.0021086
Vcan	1.5	0.0017379
N/A	1.5	0.0018616
Il13ra1	1.51	0.0009845
Gm10586	1.52	0.0020763
cd244	1.52	0.002485
Cald1	1.55	0.0006677
N/A	1.55	0.0007242
Iy6a	1.55	0.002406
N/A	1.58	0.0005513
Ces3	1.6	0.0008228
N/A	1.61	0.0007128
C1qa	1.61	0.001245
Pavr	1.62	0.0004471
Themis	1.62	0.001269
Slamf1	1.63	0.0002085
timp3	1.63	0.0021668
N/A	1.65	0.0018711
Esm1	1.66	0.0010509
ptrf	1.66	0.002272
lax1	1.67	0.0001081
Rab27b	1.67	0.0006895
Axl	1.67	0.0013663
enpp1	1.67	0.002278
Gucy1b3	1.68	0.0001152
Ptx3	1.68	0.0008365
N/A	1.69	0.0002489
N/a	1.69	0.000551
Ccnd2	1.7	6.80E-05
Gimap4	1.7	0.0013672
Gm2635	1.71	0.0001402
Mmrn1	1.71	0.000955
Cald1	1.71	0.0018171
N/A	1.72	0.0001768
Nbea	1.75	7.33E-05
Cd3g	1.77	0.0001082
Selp	1.77	0.0002817

Ctsc	1.77	0.0008356
gimap4	1.77	0.0024679
Igk-V21-4	1.78	0.0006529
Gm7592	1.79	0.0002503
Syn3	1.79	0.0015402
N/A	1.82	0.0002272
Esm1	1.83	0.0001855
Tmem176a	1.84	0.0008167
Csprs	1.85	9.33E-05
Sdc1	1.87	0.0002408
Sel1l	1.89	0.0006599
Prss23	1.89	0.0009959
N/A	1.89	0.0013992
Txndc5	1.91	0.0002446
Gm2619	1.99	0.0003069
N/A	1.99	0.0006265
Cldn7	2.01	0.0016364
Igh-6	2.02	2.40E-06
Gm7582	2.03	8.60E-06
Txndc5	2.03	9.87E-05
A530040E14Rik	2.04	2.00E-06
Txndc5	2.04	5.80E-06
N/A	2.07	3.20E-06
N/A	2.07	2.64E-05
N/A	2.09	4.00E-06
Cd5l	2.09	2.94E-05
Igh-6	2.1	1.60E-06
Txndc5	2.11	7.70E-06
C1qc	2.13	0.0014348
Igh	2.13	0.0019605
Hepacam2	2.16	5.21E-05
Derl3	2.16	0.0001574
Derl3	2.2	1.77E-05
Csprs	2.21	1.00E-07
Igh	2.25	0.0007656
Derl3	2.26	2.93E-05
Sdc1	2.26	7.31E-05
Igfbp5	2.27	3.00E-07
Prdm1	2.32	2.04E-05
Igfbp5	2.33	9.17E-05
Notch4	2.36	1.04E-05
Spon1	2.39	7.10E-05
Apol11a	2.42	0.0008554
Igh	2.43	0.001876
Igh	2.49	0.0004875
Igi	2.55	2.90E-06
chst1	2.57	0.0001027
Igh	2.59	0.0001198
Epcam	2.62	0.0002235
Igh	2.7	7.66E-05
Igh	2.81	1.49E-05
Chst1	2.87	1.41E-05
Gpnmb	2.93	2.10E-05
Igh	2.94	4.37E-05
Igh	2.95	0.0002999
Igh	3	0.0001376
Igh	3.07	6.43E-05
Igh	3.09	0.0002236
Igh	3.1	2.77E-05
N/A	3.1	4.04E-05
Igh	3.16	2.07E-05
Igh-VJ558	3.22	7.36E-05
Igh	3.24	5.00E-06
Igh-VJ558	3.29	3.31E-05
Csn3	3.48	9.80E-06
Igh	3.48	5.93E-05
Ighg	3.51	7.59E-05
Igh	3.57	1.49E-05
Loc677563	3.6	3.75E-05
Igh	3.63	8.00E-06

Igh	3.68	5.39E-05
Igh	3.7	3.25E-05
Igh	3.81	1.01E-05
Igh	3.81	4.50E-05
Igh	3.93	1.24E-05
Igh	4	8.78E-05
N/A	4.03	3.00E-07
Igh	4.23	1.04E-05
Ighg	5.37	4.10E-06
Epcam	5.49	3.00E-07
Igh	7.67	0.0006964

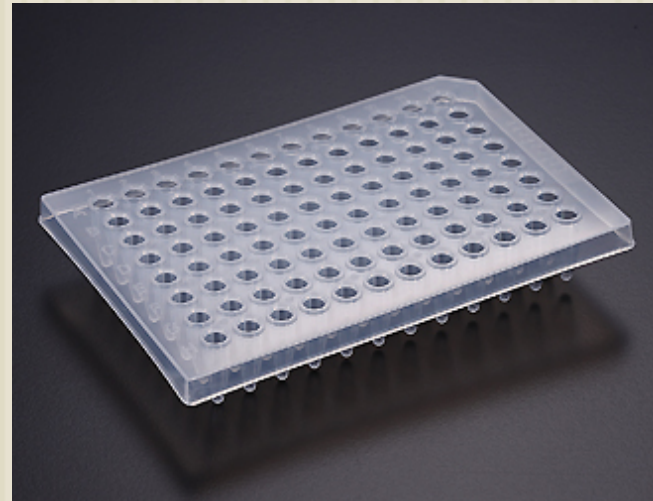
List of
Genes!

A closer look at a few important genes...

Gene Name	Fold-change	Parametric p-value	Description
Epcam	5.49	3.00E-07	Mus musculus tumor-associated calcium signal transducer 1, mRNA
Gpnmb	2.93	2.10E-05	Mus musculus glycoprotein (transmembrane) nmb, mRNA
Cd5l	2.09	2.94E-05	Mus musculus CD5 antigen-like, mRNA
Ctsc	1.77	0.0008356	Mus musculus bone marrow macrophage cDNA
Slamf1	1.63	0.0002085	Mus musculus signaling lymphocytic activation molecule family member 1, mRNA
Ly6a	1.55	0.002406	Mouse T-cell activating protein (TAP) mRNA, complete cds
Gm2344	0.63	0.0009404	Mus musculus similar to CD79A antigen (immunoglobulin-associated alpha)
Bach2	0.51	5.00E-06	Mus musculus BTB and CNC homology 2 (Bach2), transcript variant 1, mRNA
Fam129c	0.48	2.00E-07	Mus Musculus adult male corpora quadrigemina cDNA

Future Work

- Confirmation
 - ▣ RT-PCR



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