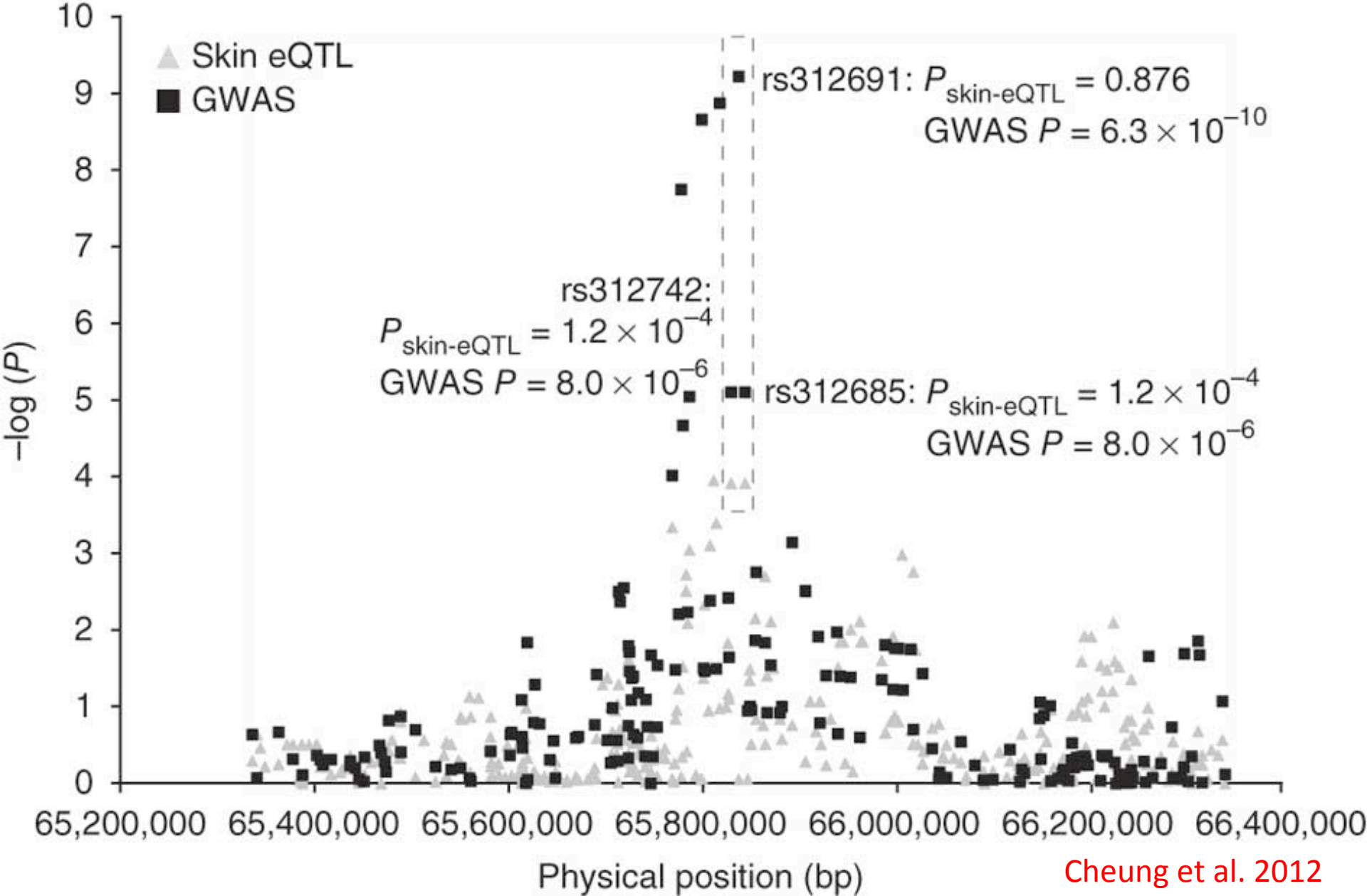
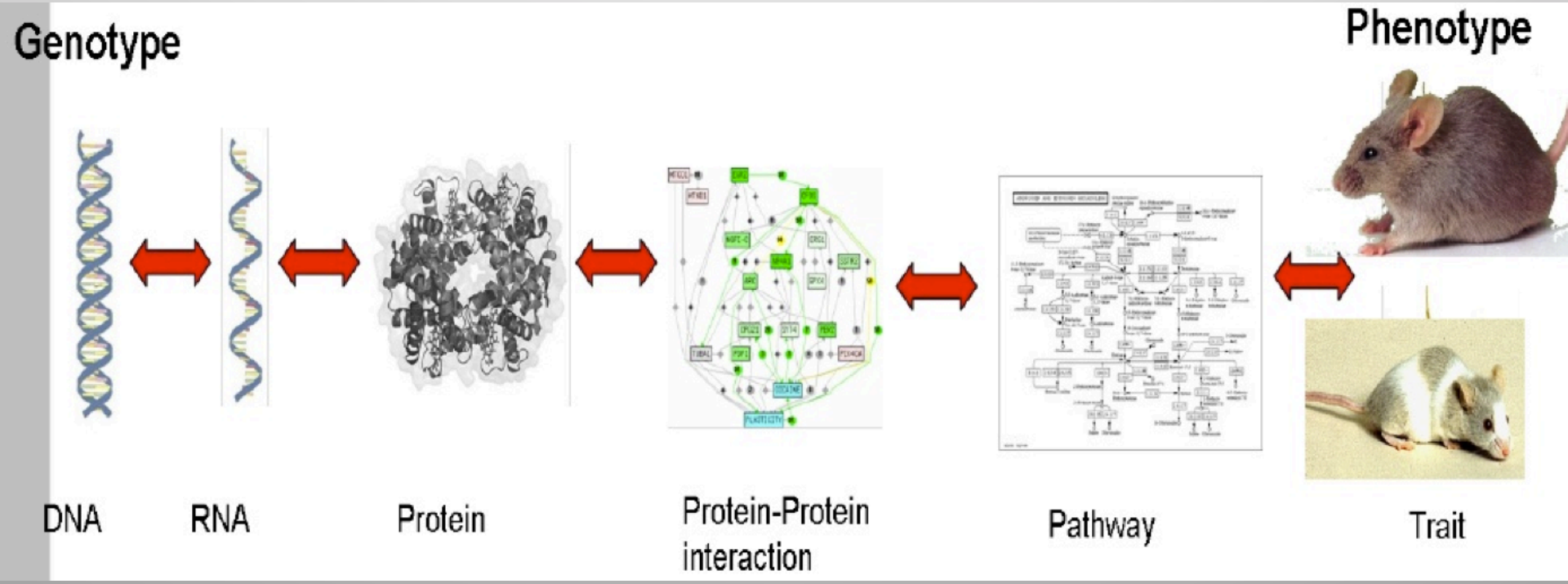
Gene expression in population genomics

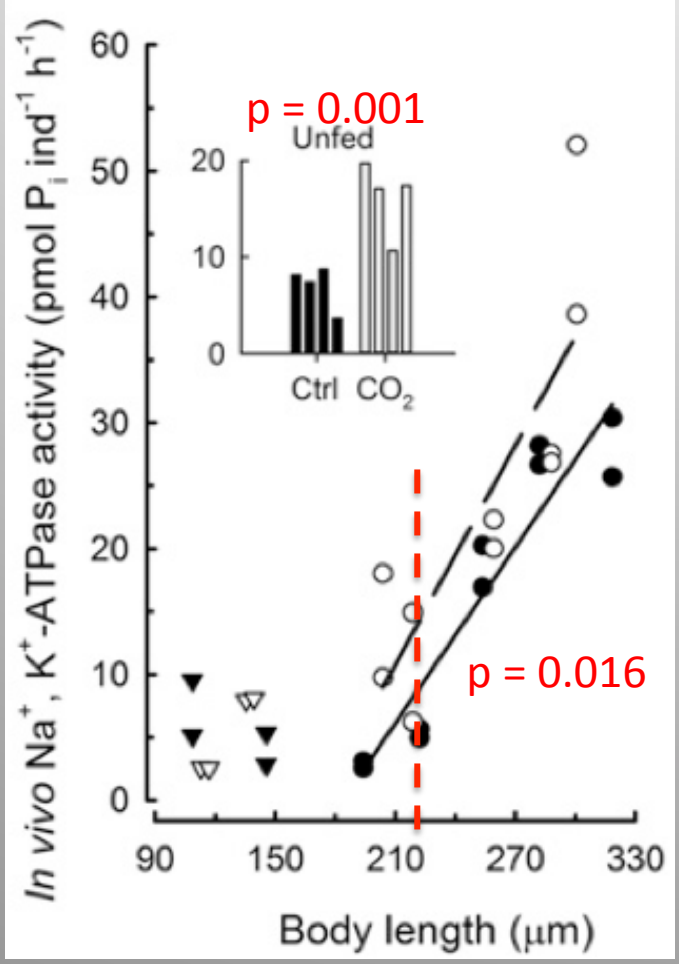


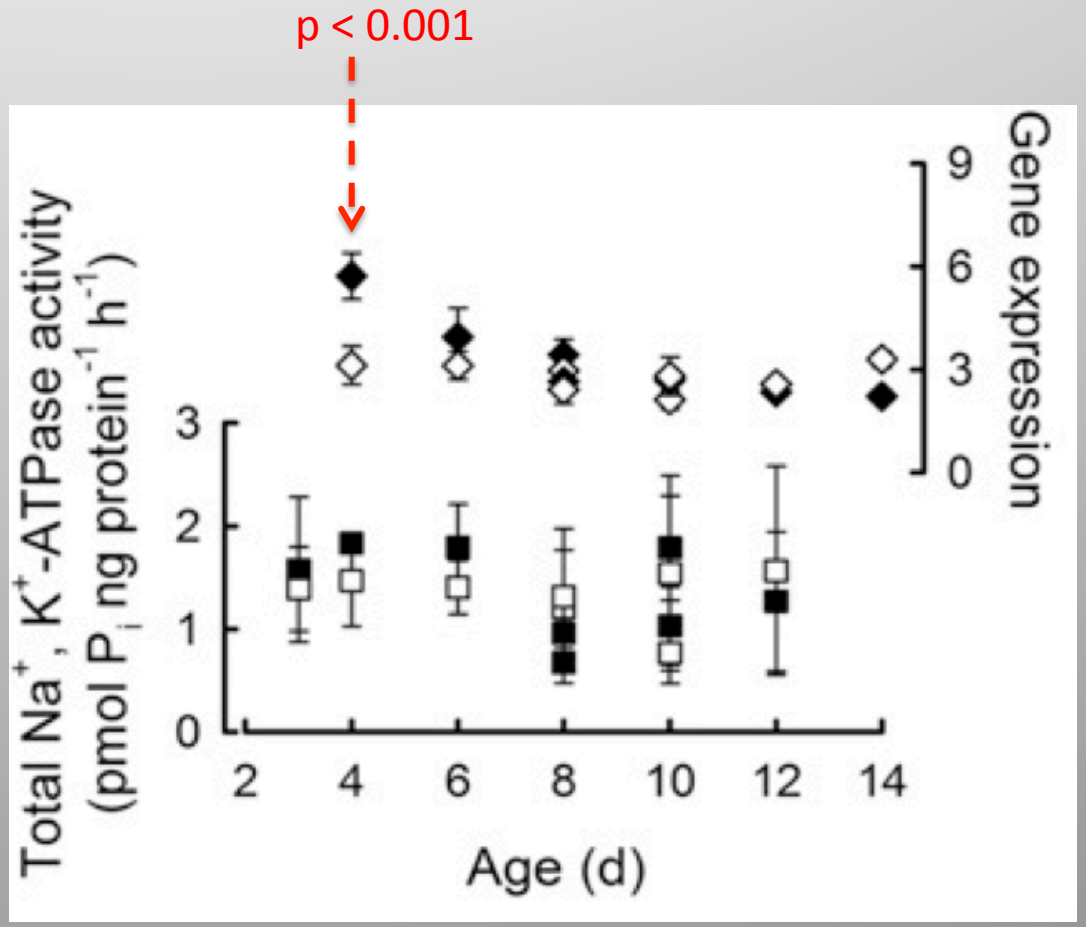
Cheung et al. 2012

Gene expression is a phenotype



But with a complex relationship with cellular function

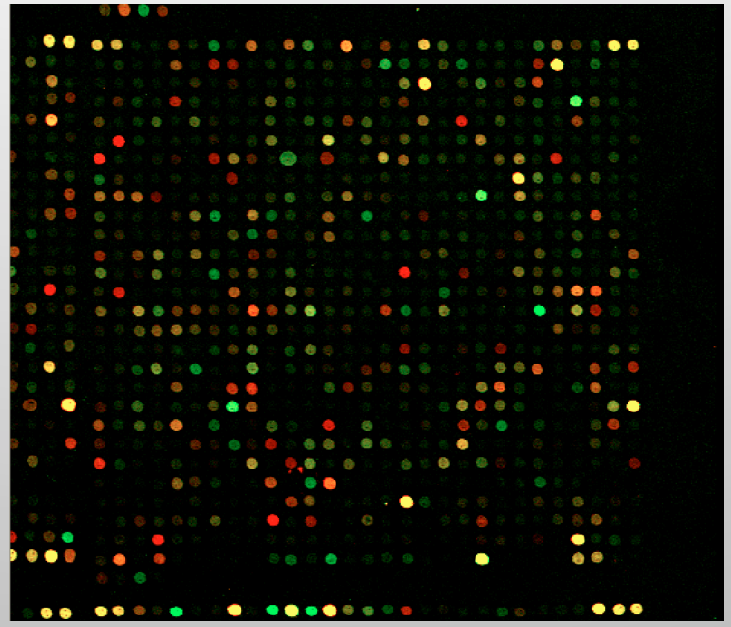




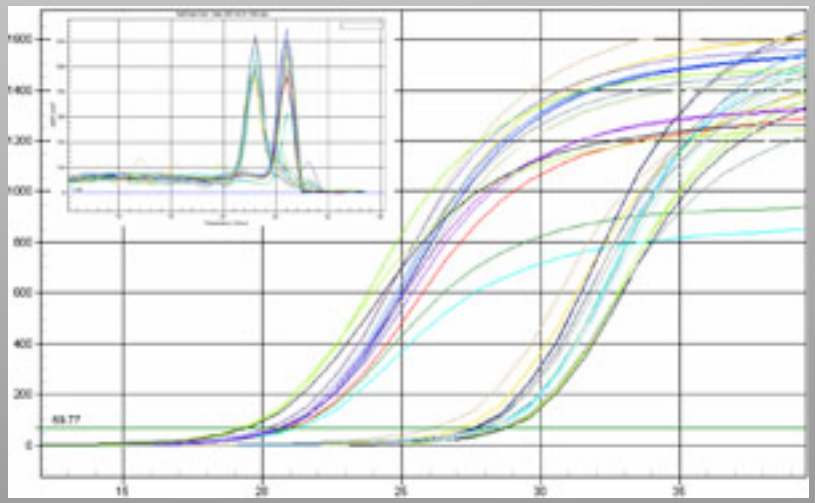
Pan et al. 2015 PNAS

Traditional gene expression assays

- Microarrays



- qPCR



INNOVATION

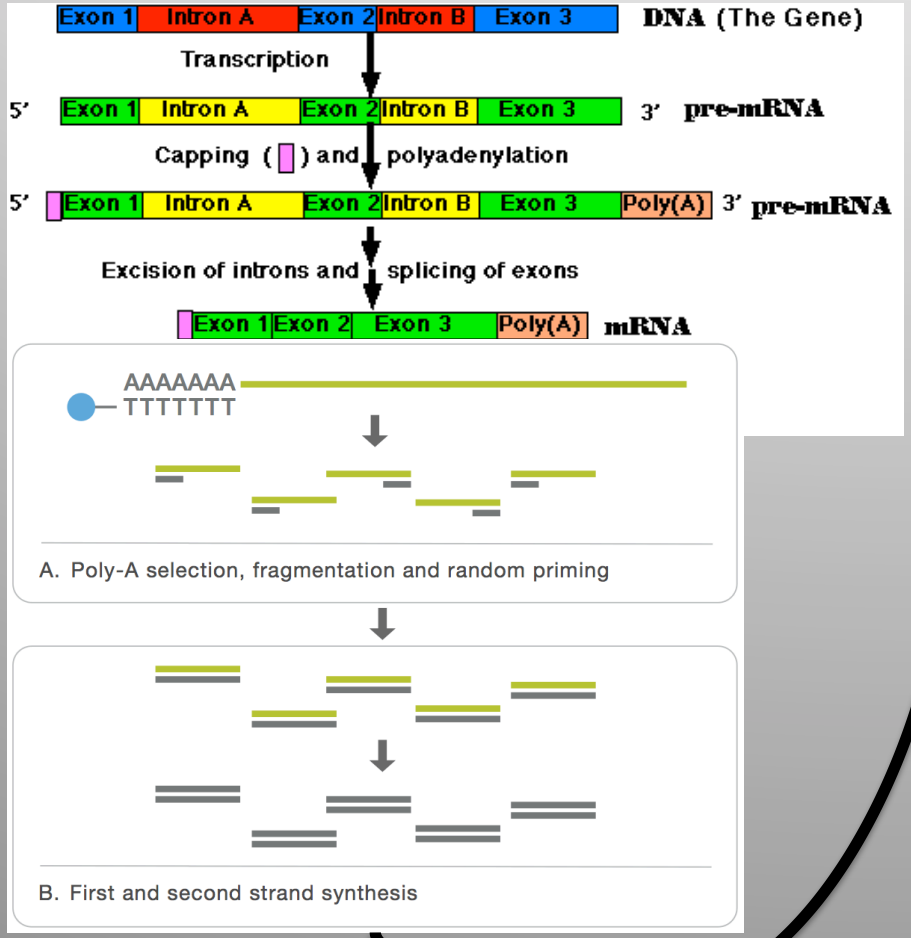
RNA-Seq: a revolutionary tool for transcriptomics

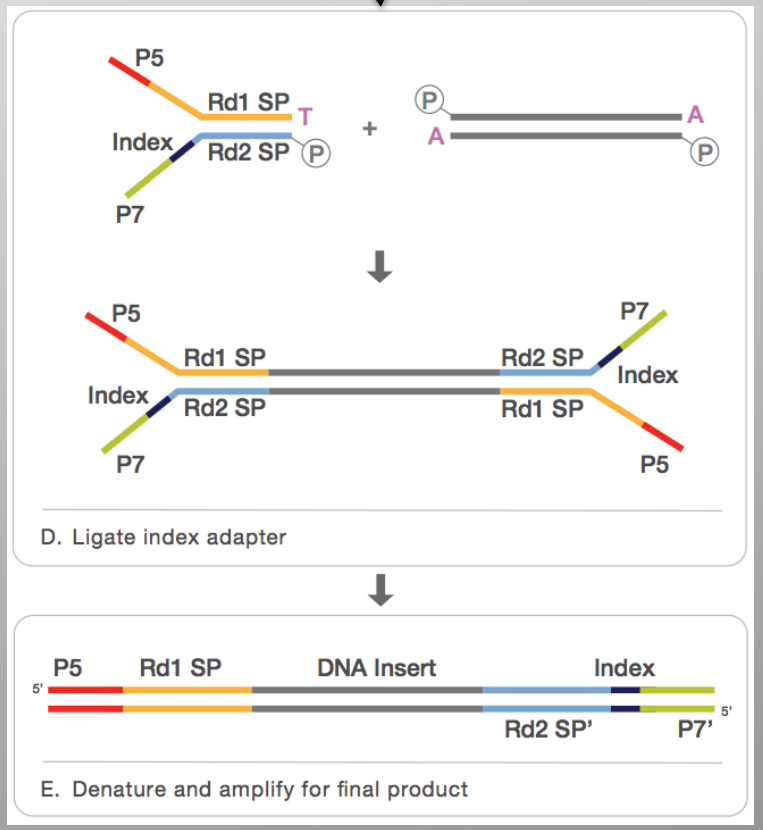
Zhong Wang, Mark Gerstein and Michael Snyder

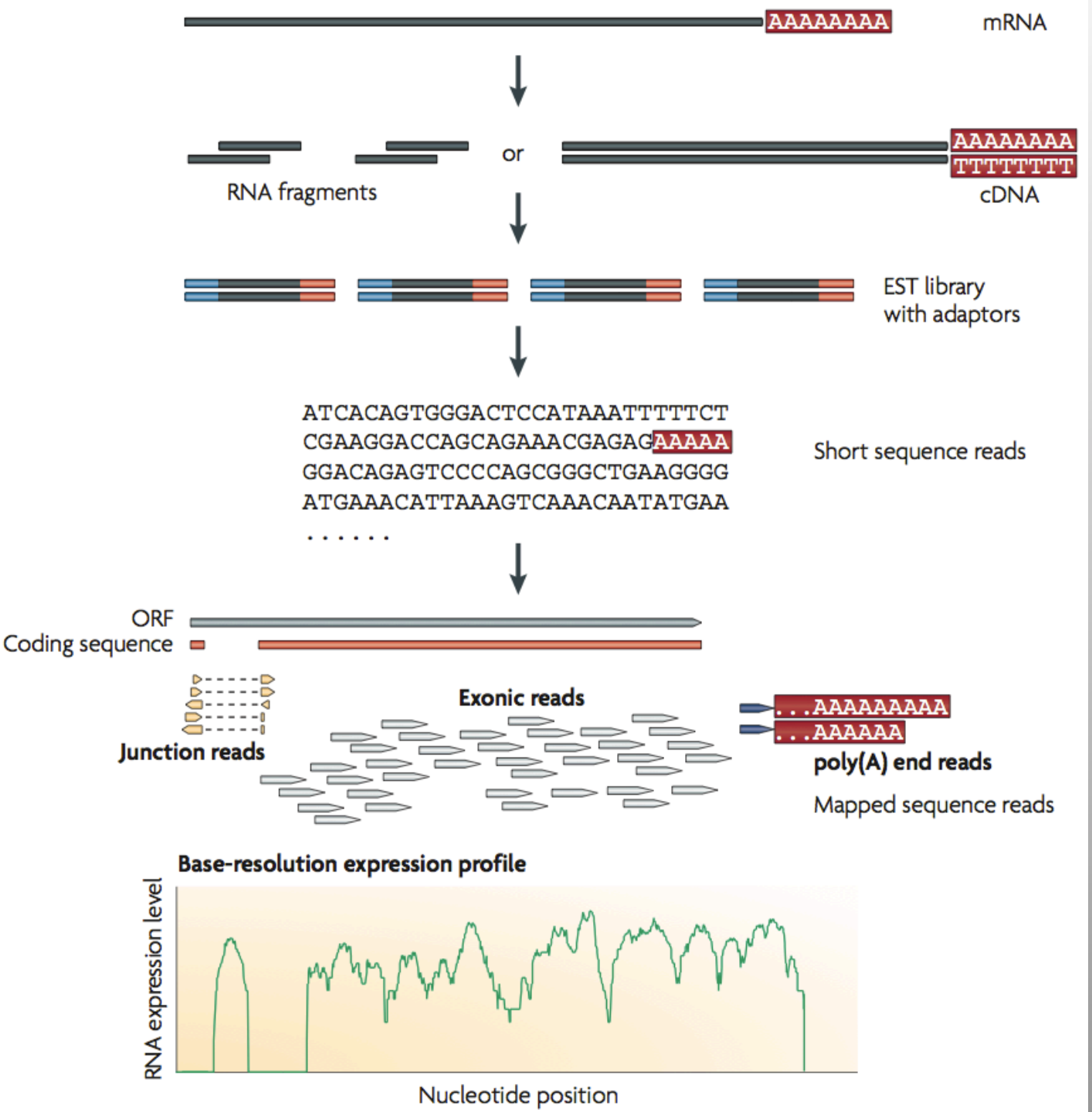
Abstract | RNA-Seq is a recently developed approach to transcriptome profiling that uses deep-sequencing technologies. Studies using this method have already altered our view of the extent and complexity of eukaryotic transcriptomes. RNA-Seq also provides a far more precise measurement of levels of transcripts and their isoforms than other methods. This article describes the RNA-Seq approach, the challenges associated with its application, and the advances made so far in characterizing several eukaryote transcriptomes.

What is RNA-Seq?

Reduced Representation Libraries – sequencing ONLY mature mRNA







Wang et al. 2009

Why focus on mRNA?

Assembly and annotation of whole genomes is HARD – repeat regions, polymorphisms, gene prediction.

Higher coverage & reduced complexity by creating reduced representation libraries (RAD-tags, GBS, RNA-Seq)

By focusing on mRNA, we know that all sequenced material belong to an expressed gene – Functional information (plus, it simplifies annotation considerably)

RNA-Seq provides both gene expression profiles AND nucleotide sequences at the same time.

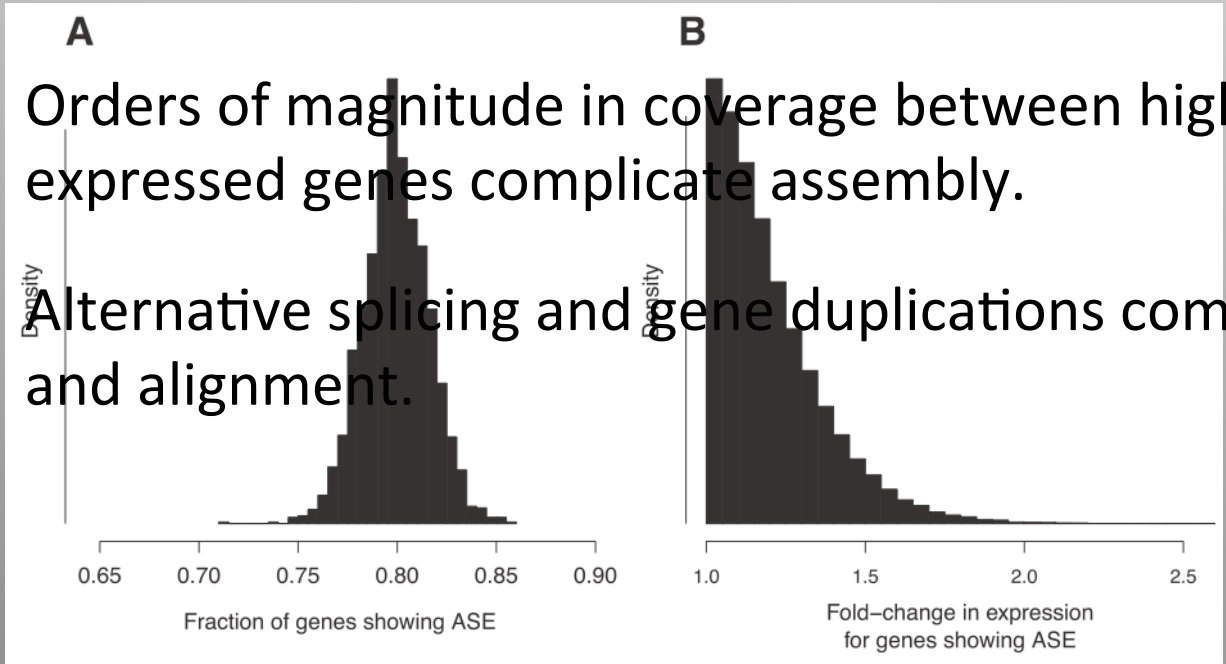
Ozsolak & Milos 2011. *Nature Reviews Genetics*

Issues with RNA-Seq

mRNA degradation

Some functional information is located in regulatory regions - will be missed by mRNA sequencing (e.g. 41 % entirely in non-coding regions in Sticklebacks (Jones et al)).

Allele-specific expression might bias gene expression profiles and genotype assignments, unless taken into account.



Orders of magnitude in coverage between highly/lowly expressed genes complicate assembly.

Alternative splicing and gene duplications complicate assembly and alignment.

Skelly et al. 2011

Experimental design

RNA-Seq has the potential to answer many questions – if the experimental design is good

WHAT QUESTION DO YOU WANT TO ANSWER? –
Different considerations depending on the goal of the project.

For gene expression analyses – Common gardening, reciprocal transplants.

For nucleotide sequence analysis – population structure, sampling location, amino acid sequence.

Auer & Doerge 2010. *Genetics*

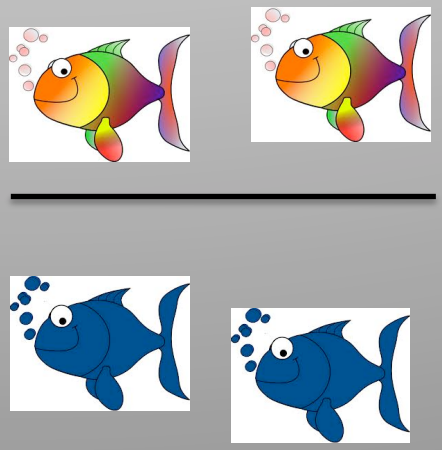
Common Garden / Reciprocal transplants

Gene expression is HIGHLY plastic in time and space

Plastic effects can remain after several generations in the same environment (e.g. Torrens et al. 2008)

In order to study adaptation from gene expression, long-term transplants are necessary.

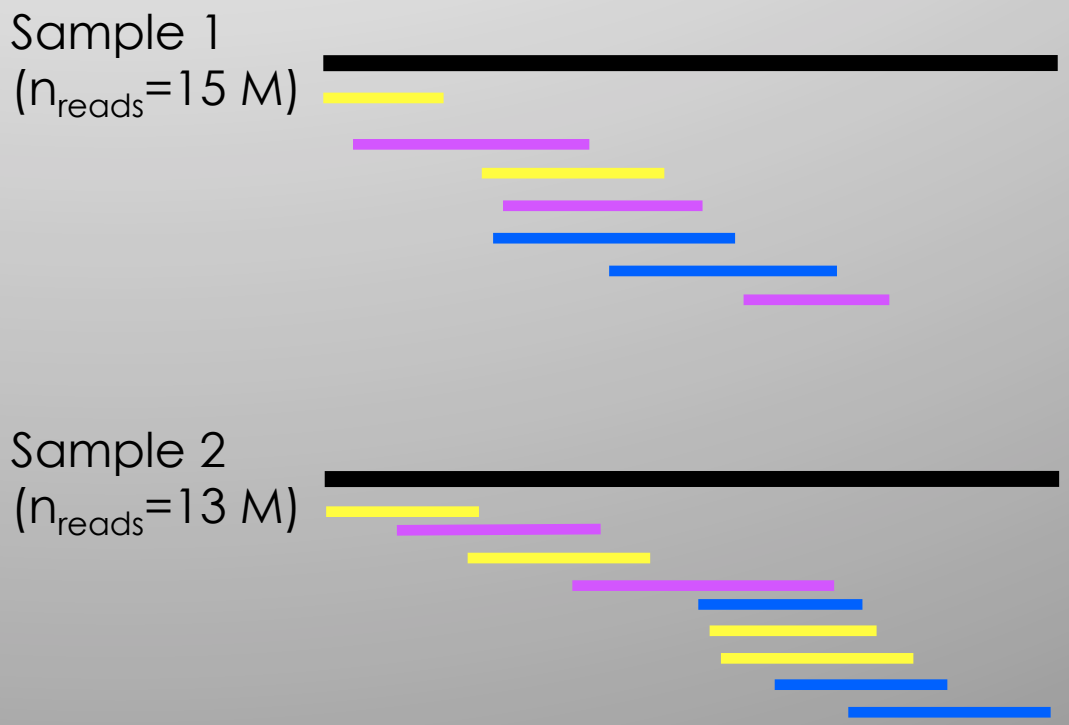
Reciprocal transplant:



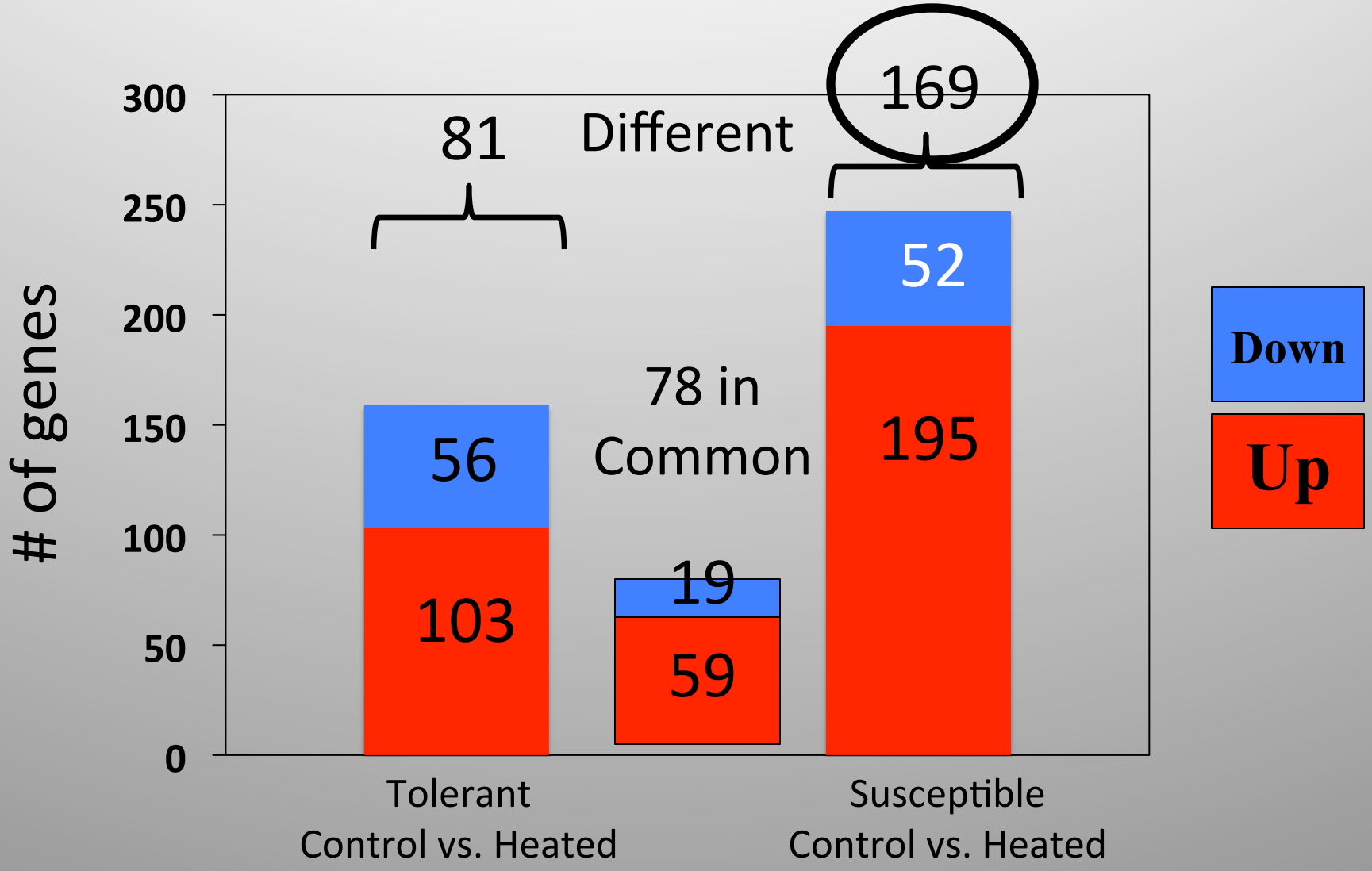
Gene expression from RNA-Seq data

- By comparing the fraction of reads mapping to a certain contig across samples, we can study differential expression patterns
- We first count reads, then normalize and test for significant differences
- Corrections for multiple tests are critical!

Example: is this gene differentially expressed?

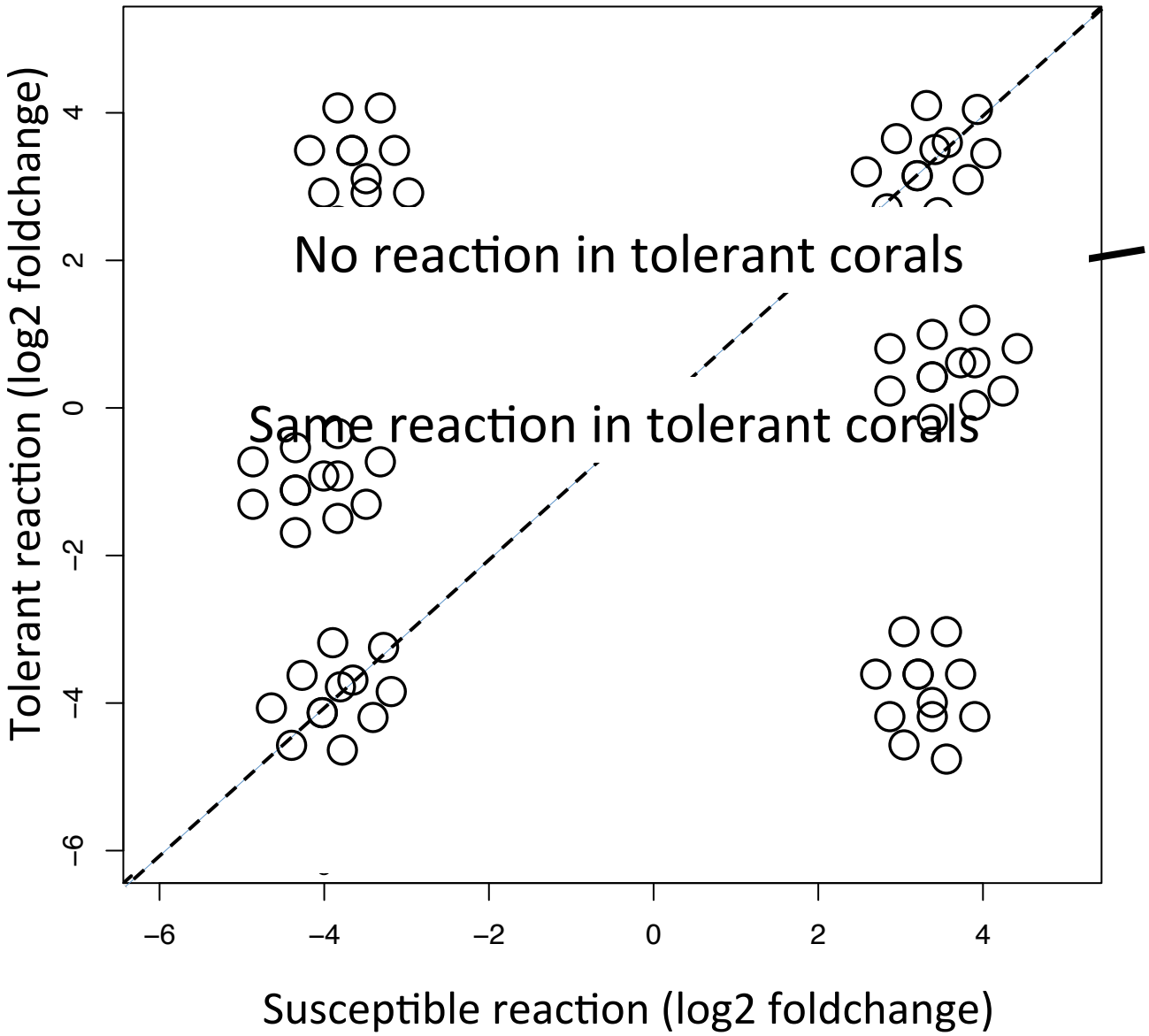


Coral genes responding to temperature

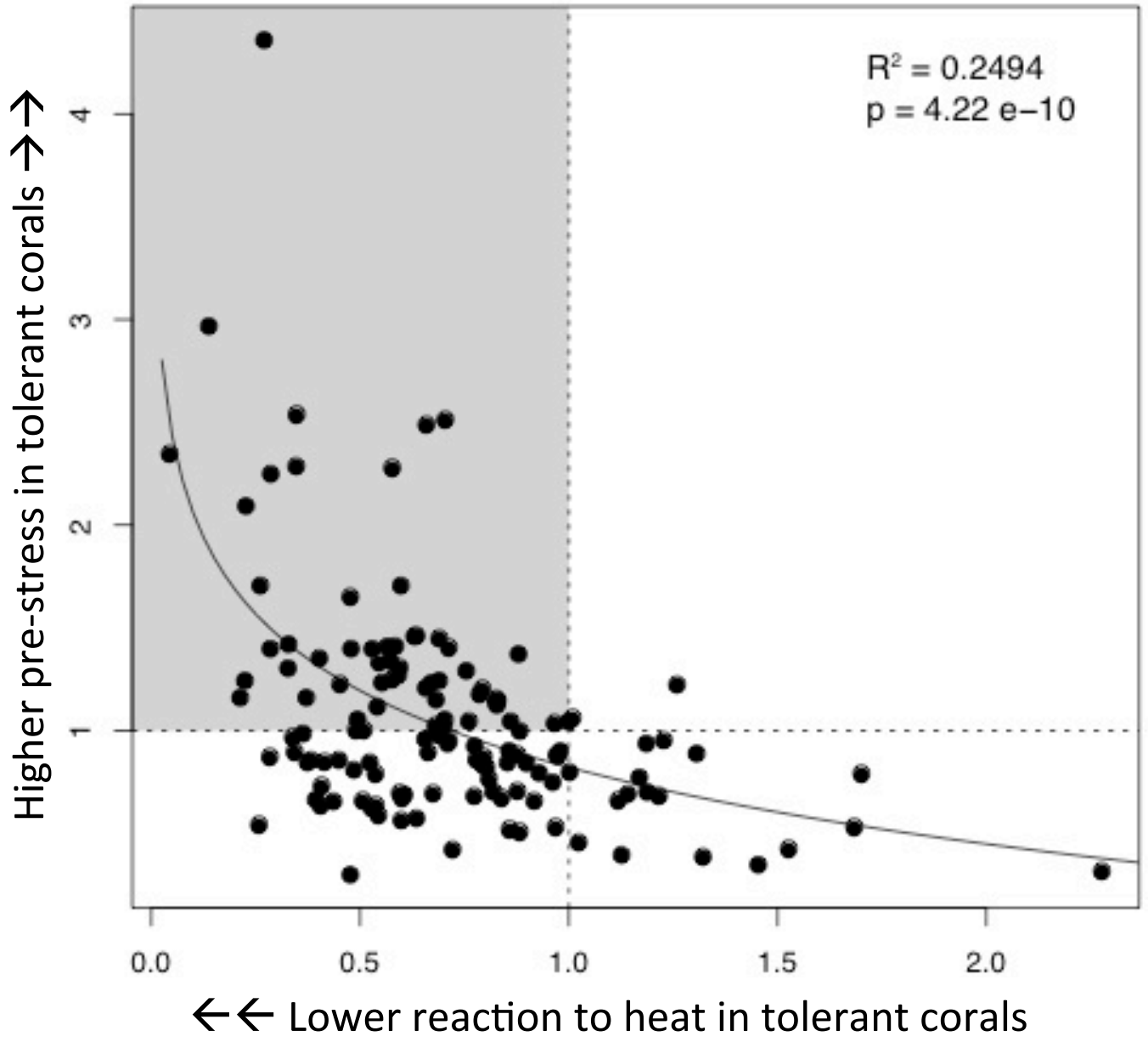


Barshis *et al.* 2013 PNAS

Reaction in tolerant corals?



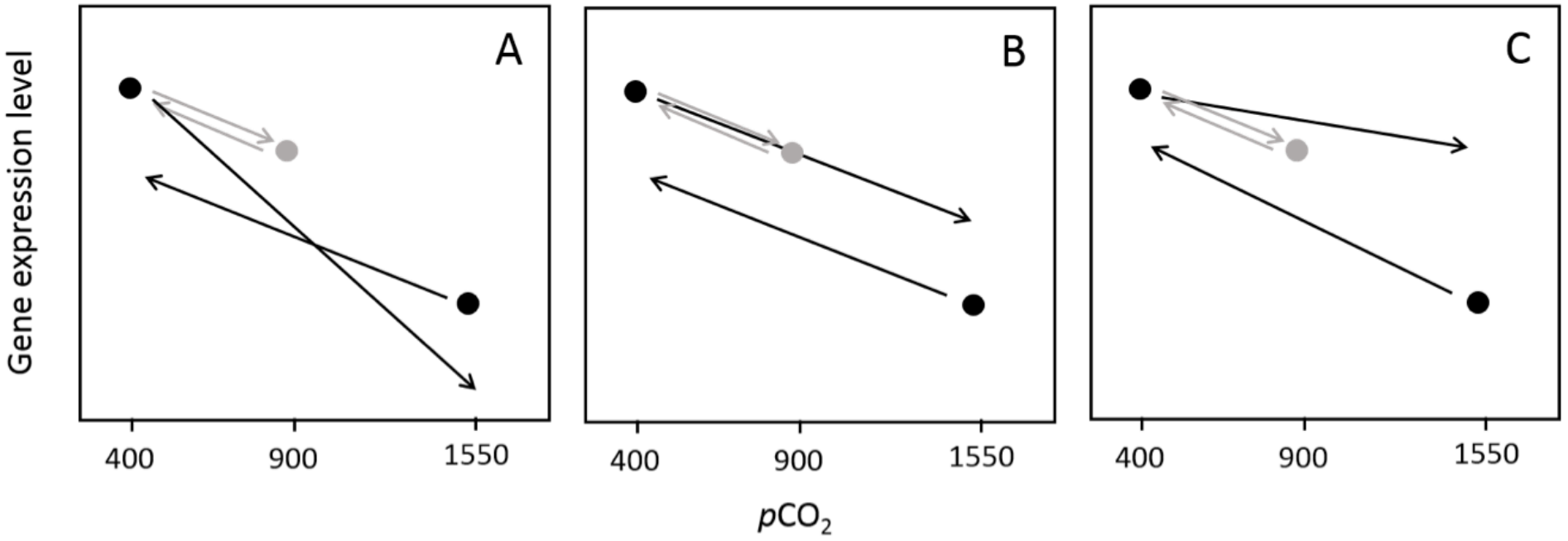
Constitutively on in tolerant corals



Barshis *et al.* 2013 PNAS

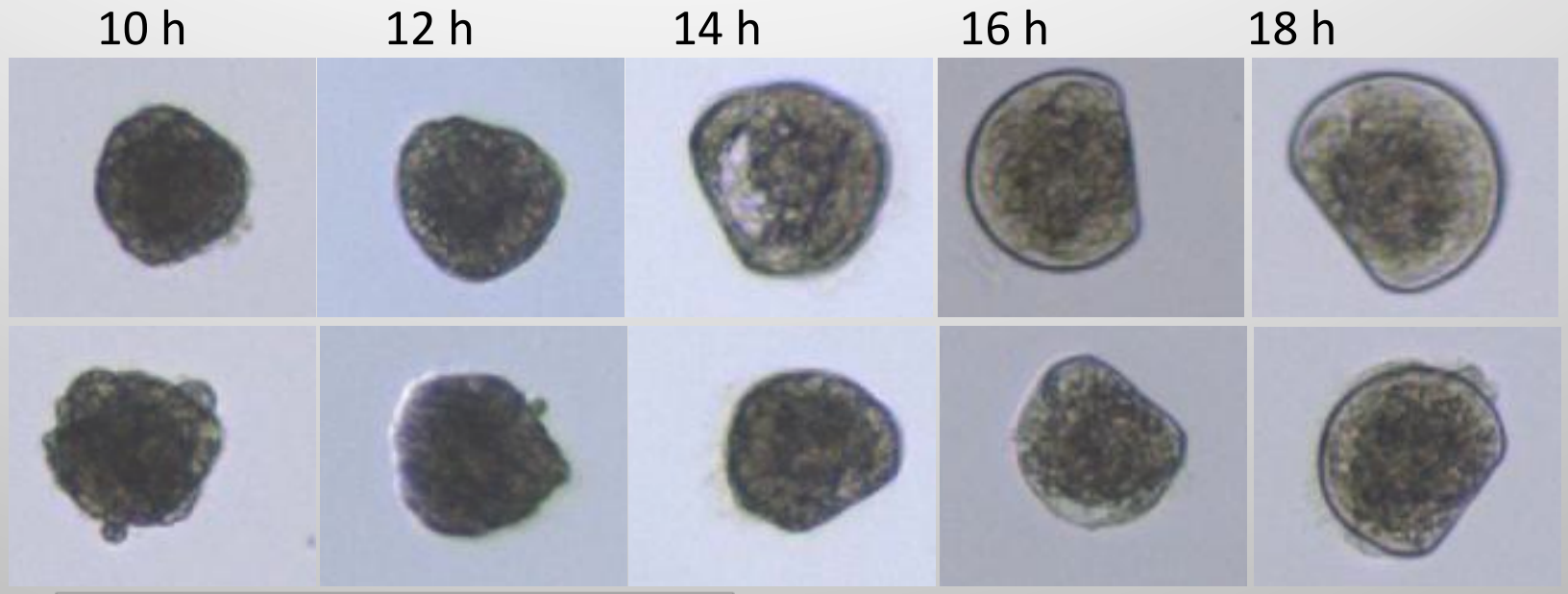
Regression analyses

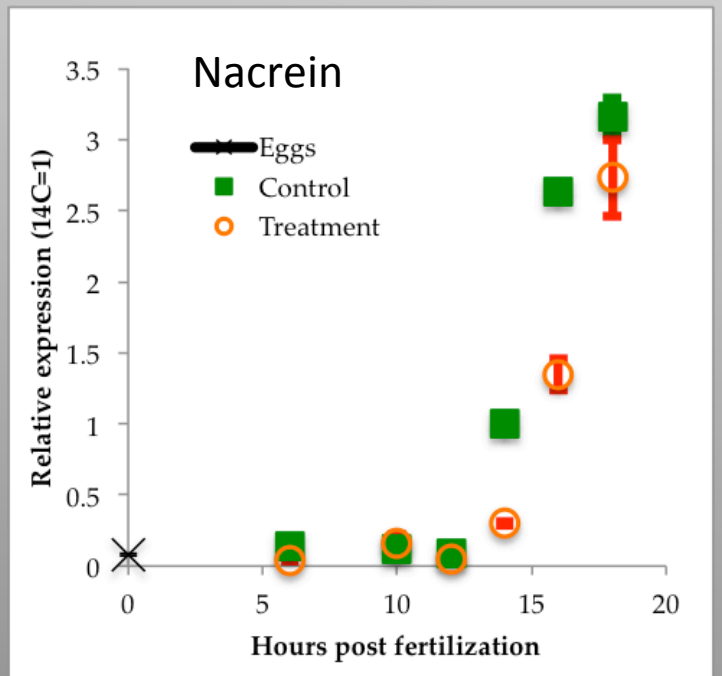
Testing gene expression against patterns predicted by physiological data

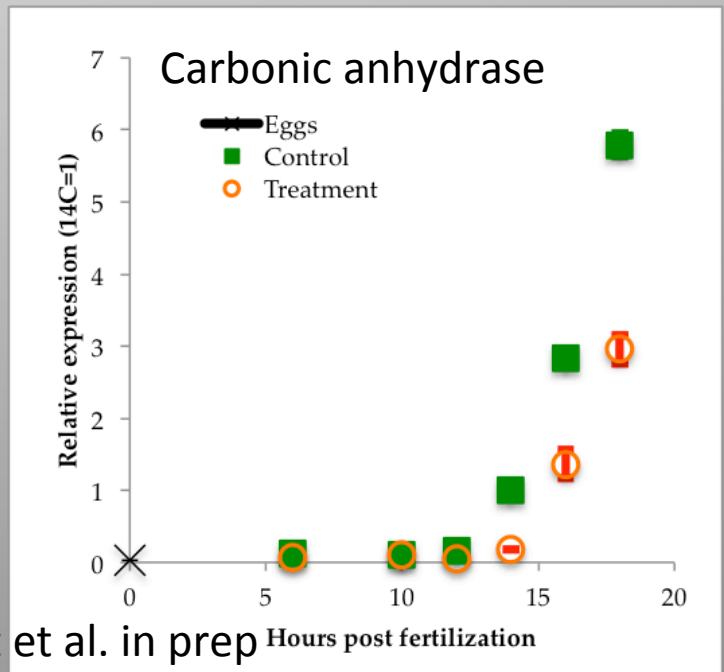


De Wit et al. 2015 Evol Appl.

Regression analyses







De Wit et al. in prep

Functional Enrichment

By comparing the gene expression changes within a certain function to those of the whole dataset, we can see if any functions are *enriched* for gene expression changes.

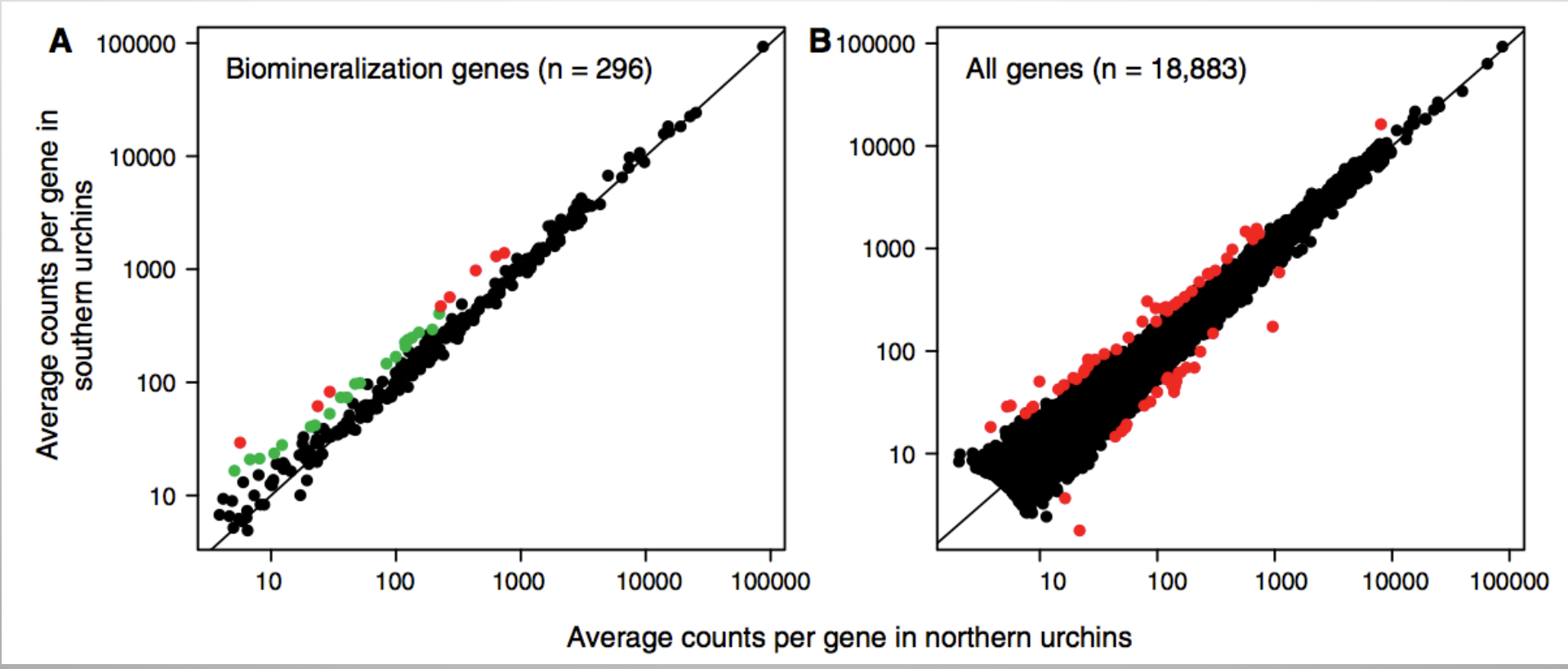
Example:

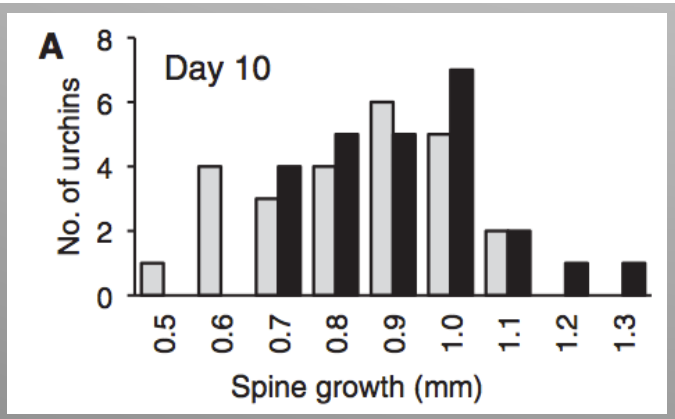
All genes
1200/24000

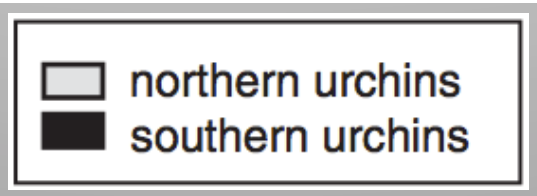
Immune genes
12/35

Useful tool for testing for non-random distribution of functions in a list of genes of interest. Provides evidence for biological significance.

Functional Enrichment







Pespeni, Barney & Palumbi 2012