

Lab Meeting

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7th Feb 2018

Background

Project

Examine the effect of regeneration on the molecular age profile of *Parhyale* limbs

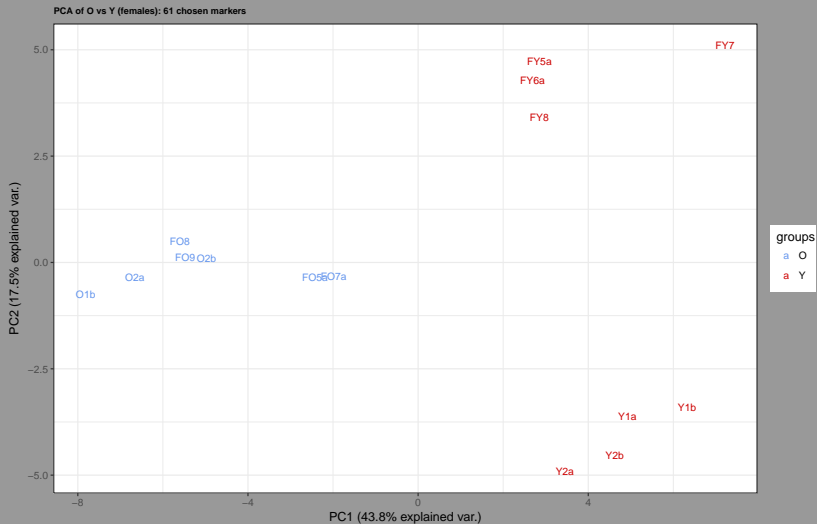
Designing codeset

- *Nanostring as method to quantify gene expression
- *200 genes in codeset
- 195 genes chosen on the basis of differential expression analysis
- 5 control genes: do not vary in expression between conditions

Initial test of aging separation



Using subset of chosen markers



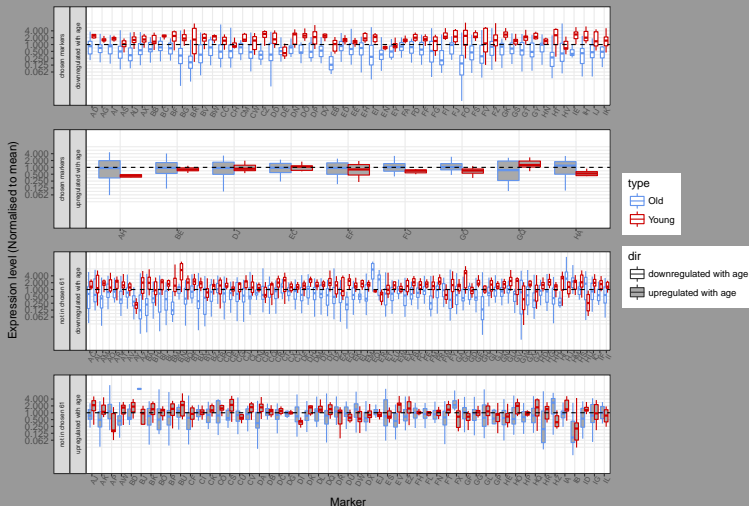
New dataset

Add new female samples



Variation amongst markers

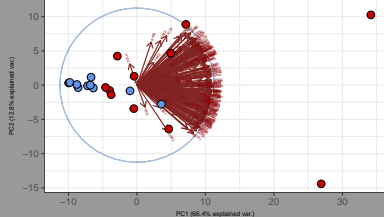
Old vs Young in females, both chosen and other markers



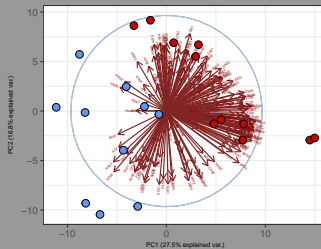
Is this due to normalisation?

How does normalisation affect the PCA?

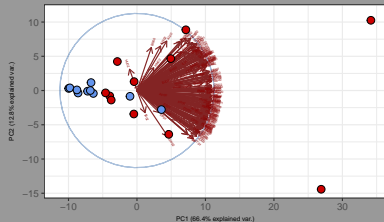
PCA of O vs Y: no normalisation (195 markers)



PCA of O vs Y: full normalisation (195 markers)

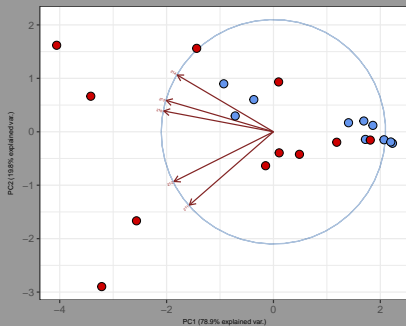


PCA of O vs Y: normalisation by internal controls only (195 markers)

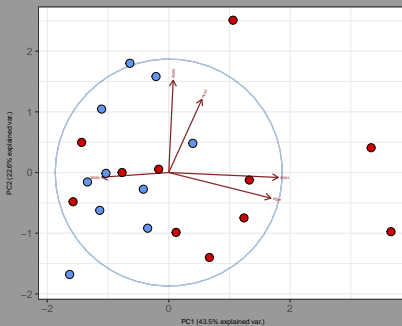


How do the housekeeping genes behave?

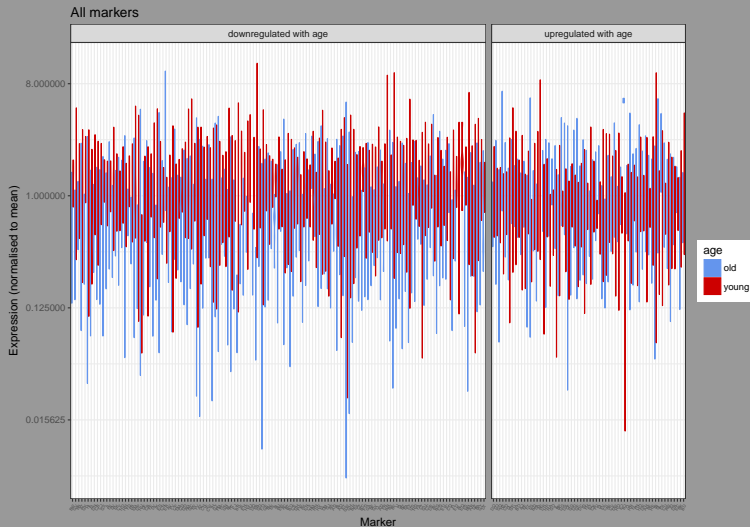
PCA of old vs young using housekeeping genes values (raw)



PCA of old vs young using housekeeping genes values (normalised)

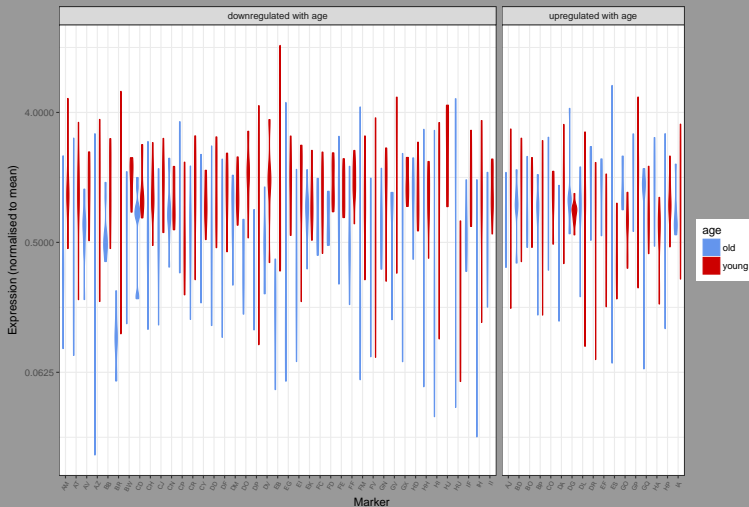


Further optimisation of marker set



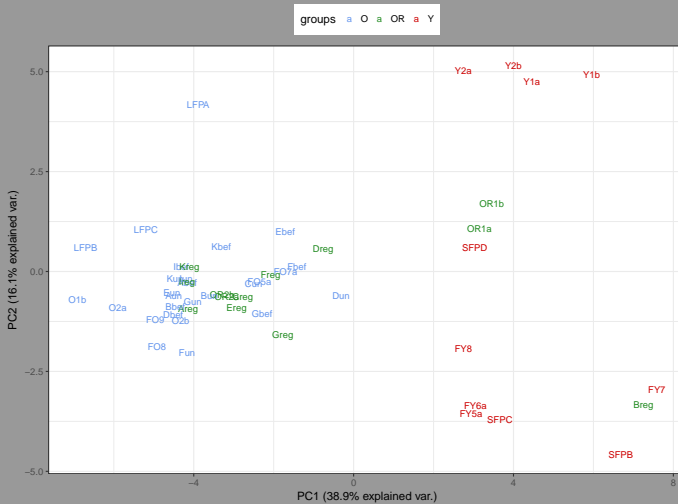
Chosen markers

New optimised set: 58 markers



On PCA

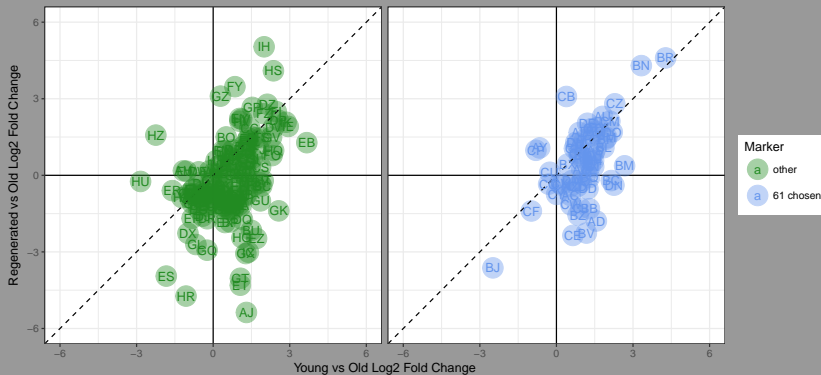
PCA Old vs Young + regenerated projected



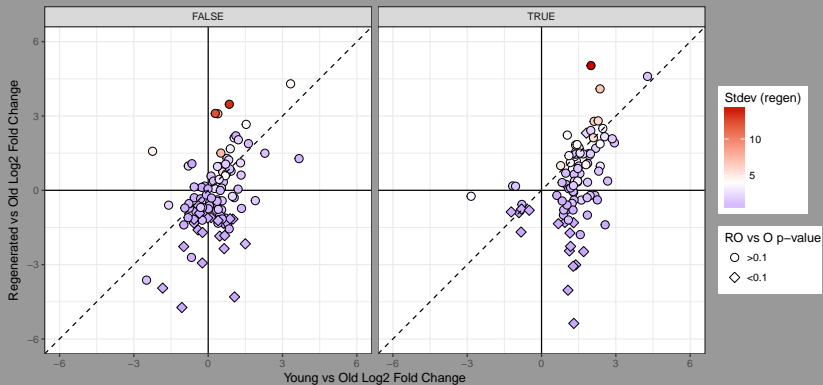
per marker



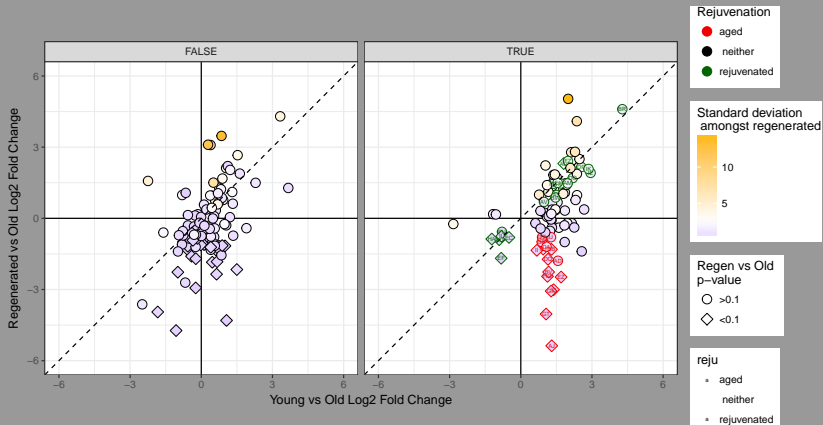
Find rejuvenating markers



Reliable rejuvenation?



Choose most reliable



Rejuvenating Markers

Only those with: Ratio of YvO I2fc / RO vs O I2fc > 0.6 Stdev
between regenerated <3

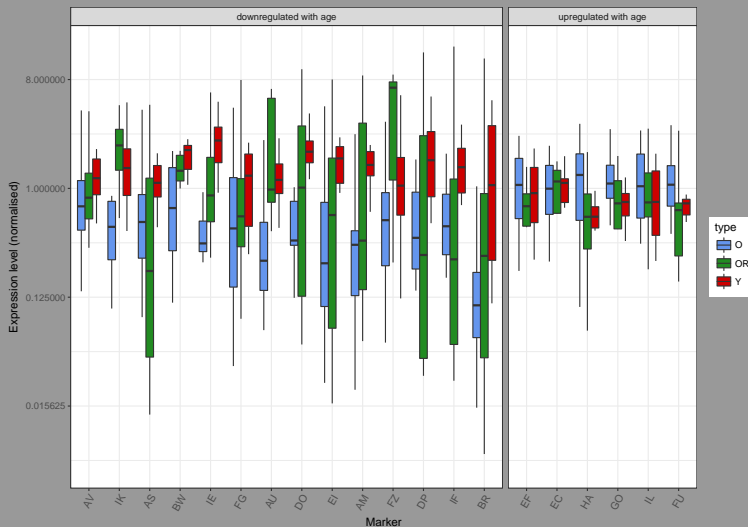
```
## [1] "AM: lipase 3 [Tribolium castaneum]"
## [2] "AS: -"
## [3] "AU: mucin 91C, isoform B [Drosophila melanogaster]"
## [4] "AV: -"
## [5] "BR: Polyprotein of retroviral origin, partial [Daphnia]"
## [6] "BW: GLT25_DROME RecName: Full=Glycosyltransferase"
## [7] "D0: IP02041p, partial [Drosophila melanogaster]"
## [8] "DP: CG6696 [Drosophila melanogaster]"
## [9] "EC: hypothetical protein DAPPUDRAFT_260927 [Daphnia]"
## [10] "EF: -"
```


Rejuvenating Markers

Only those with: Ratio of YvO I2fc / RO vs O I2fc > 0.6 Stdev
between regenerated < 3

```
## [1] "EI: cubilin [Tribolium castaneum]"
## [2] "FG: Membrane-spanning 4-domains subfamily A member"
## [3] "FU: Fibulin 1 and, partial [Daphnia magna]"
## [4] "FZ: CG6763, isoform B [Drosophila melanogaster]"
## [5] "G0: AF487537_1 cytochrome P450 CYP6P2 [Anopheles gambiae]"
## [6] "HA: -"
## [7] "IE: -"
## [8] "IF: Cuticular protein analogous to peritrophins 1-4"
## [9] "IK: Signal-transducer and activator of transcription 1"
## [10] "IL: Transmembrane protease serine [Daphnia magna]"
```

Rejuvenating Markers



Extra-aged Markers

Only those with: Ratio of YvO l2fc / RO vs O l2fc < -0.6 Stdev
between regenerated <3

```
## [1] "AD: Zinc carboxypeptidase [Daphnia_magna]"
## [2] "AJ: protein expanded [Tribolium castaneum]"
## [3] "BB: -"
## [4] "BV: Pro-resilin [Daphnia magna]"
## [5] "CJ: RNA-directed DNA polymerase from transposon BS"
## [6] "DA: melanization protease 1, isoform A [Drosophila"
## [7] "DQ: Secreted protein [Daphnia magna]"
## [8] "DT: -"
## [9] "EZ: Carbohydrate sulfotransferase 11-like Protein"
## [10] "FV: Fibulin 1 and, partial [Daphnia magna]"
```

Extra-aged Markers

Only those with: Ratio of YvO l2fc / RO vs O l2fc < -0.6 Stdev
between regenerated < 3

```
## [1] "GE: neyo, isoform B [Drosophila melanogaster]"
## [2] "GN: Aquaporin AQPAn.G [Daphnia magna]"
## [3] "GT: Pre-B-cell leukemia transcription factor, putat
## [4] "GX: -"
## [5] "HG: -"
## [6] "IC: Uncharacterized protein APZ42_032971 [Daphnia m
## [7] "II: -"
## [8] "IJ: GH01154p [Drosophila melanogaster]"
```

Extra-aged Markers

