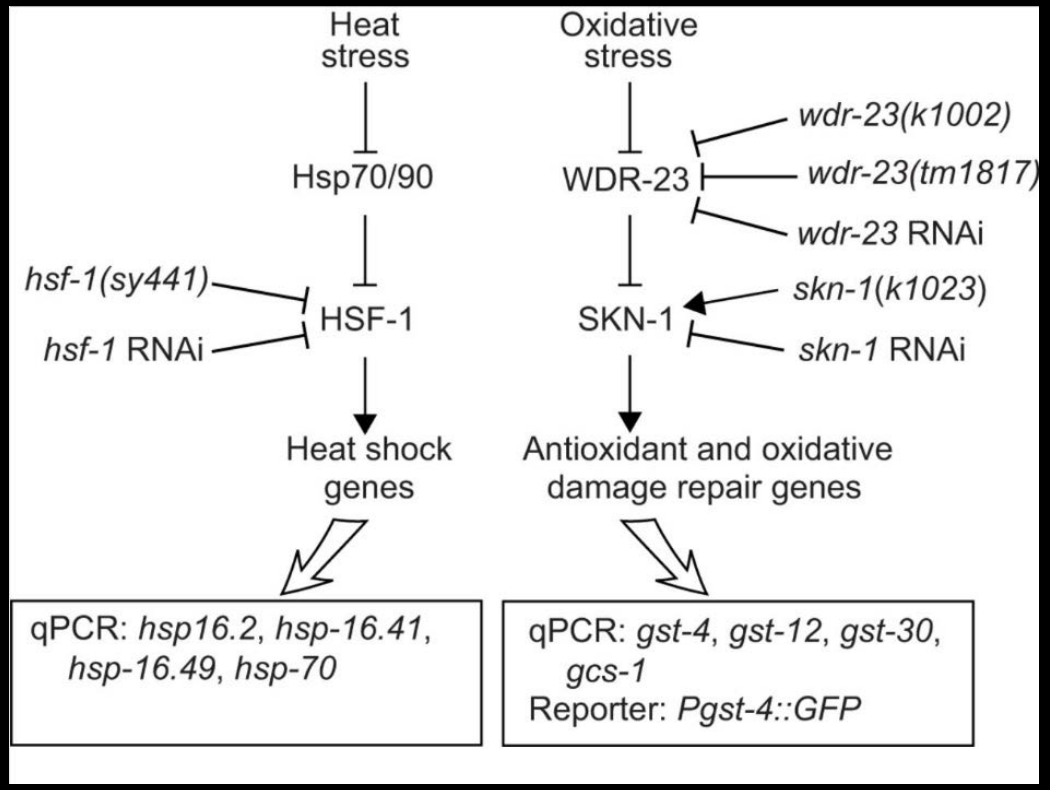
Finding the Shortest-Lived Worm

Analysis of transcription factor mutations in Caenorhabditis elegans.

Apfeld Lab

Julian Stanley, July 2016

Different Pathways Regulate Aging

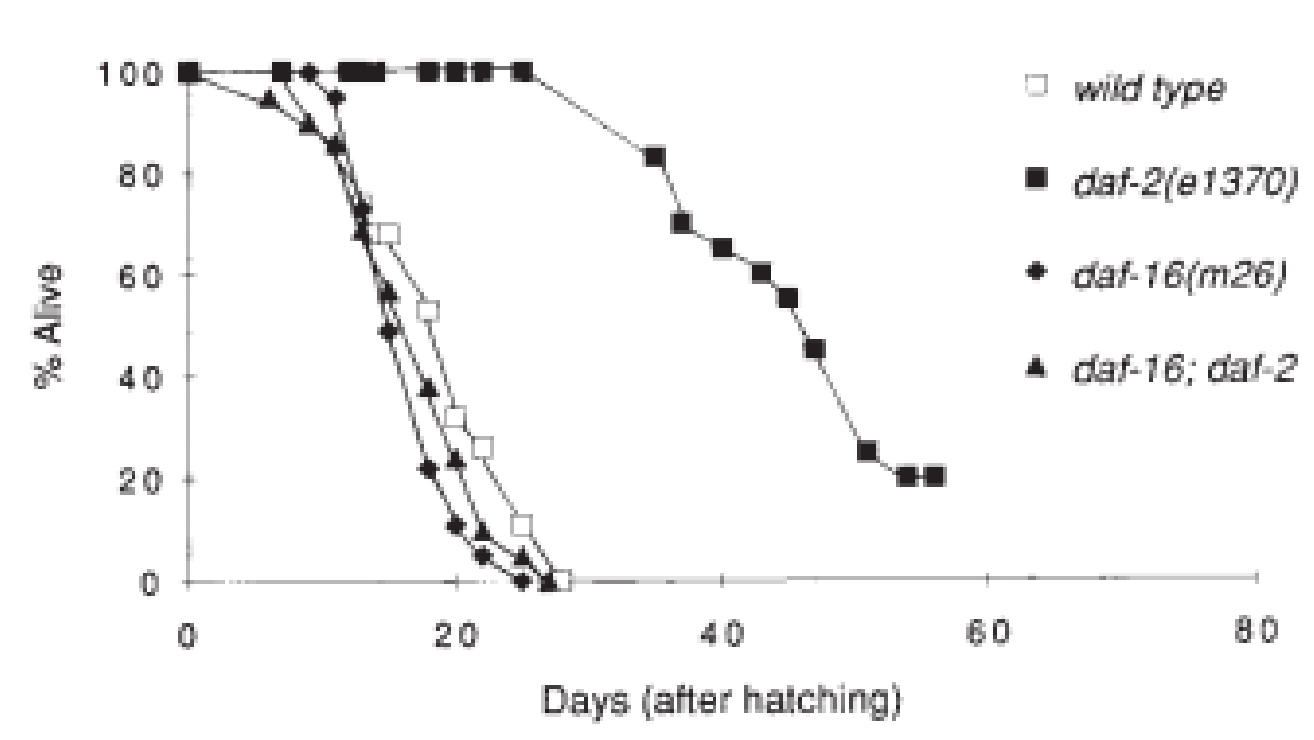


Crombie et al. 2016

Some of pathways:

- Heat shock
- TOR signaling
- SKN-1 stress response
- TFEB/HLH-30
- Unfolded protein response
- Insulin/IGF-1 signaling

daf-16/FOXO is Highly Conserved and Well-Studied

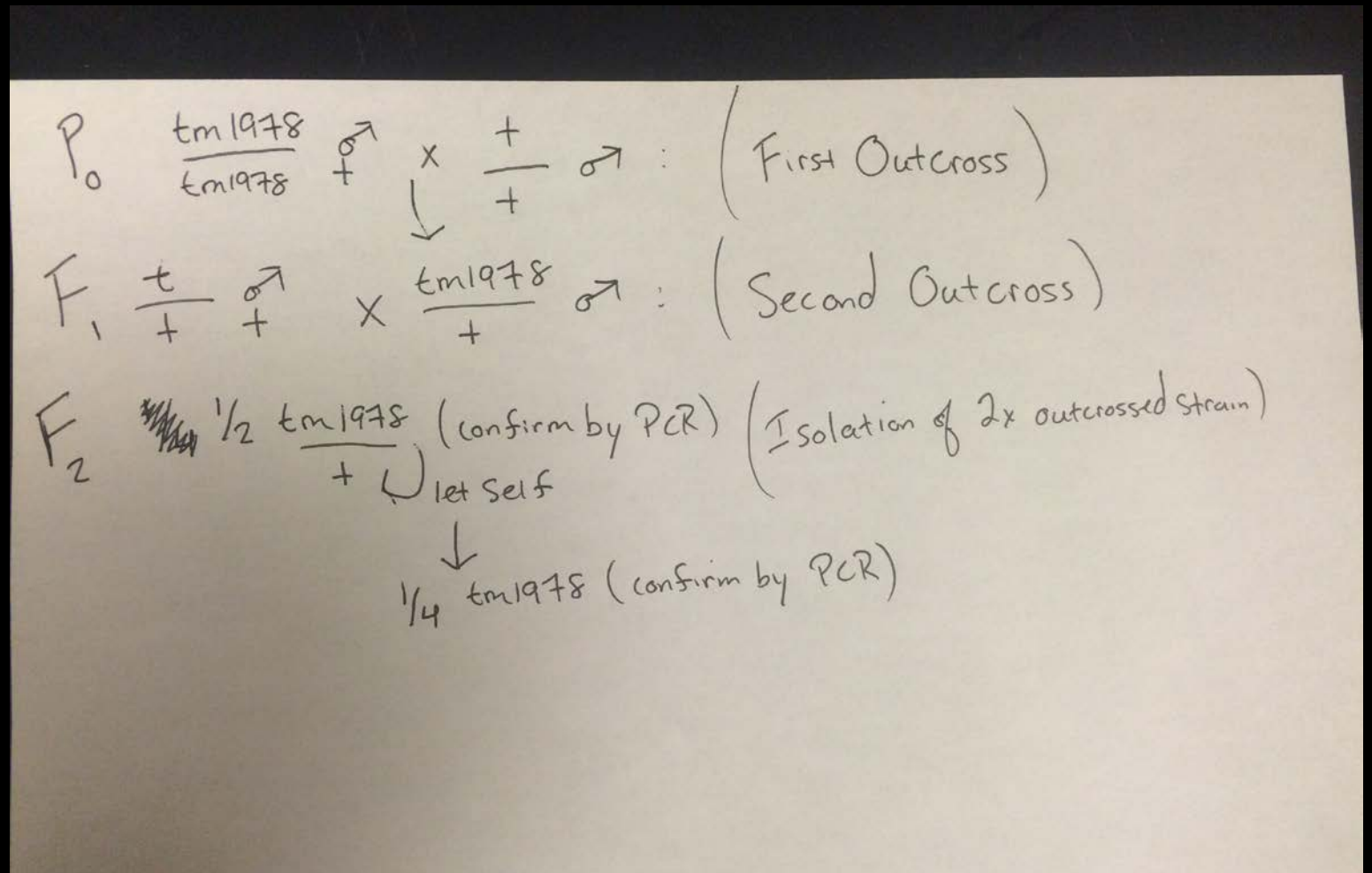


Kenyon 1993

Goal: Combine *daf-16* with Other Transcription Factor Mutants

- Identified mutations:
  - *daf-16 (mu86) I → Null*
  - *daf-12 (rh61rh411) X → Null*
  - *hsf-1 (sy441) I → Non-Null*
  - *nhr-49 (nr2041) I → Null*
  - *xbp-1 (tm2482) III → Null*
  - *skn-1 (zu135) IV → Presumed Null, Maternal Lethal*
  - *hlh-30 (tm1978) IV → Null*
  - *sbp-1 (ep79) III → Non-Null (lethal)*
  - *atfs-1 (gk3094) V → Null*
  - *daf-3 (mgDf90) X → Null*

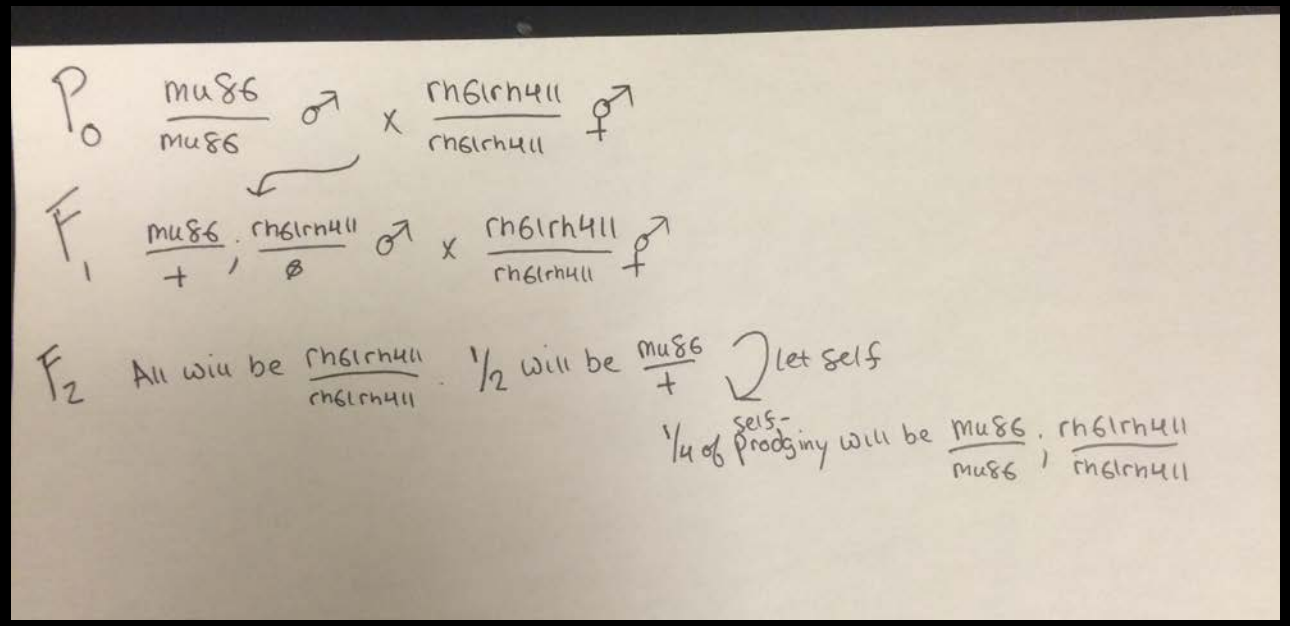
Strains will be 6x Outcrossed to Eliminate Background Mutations

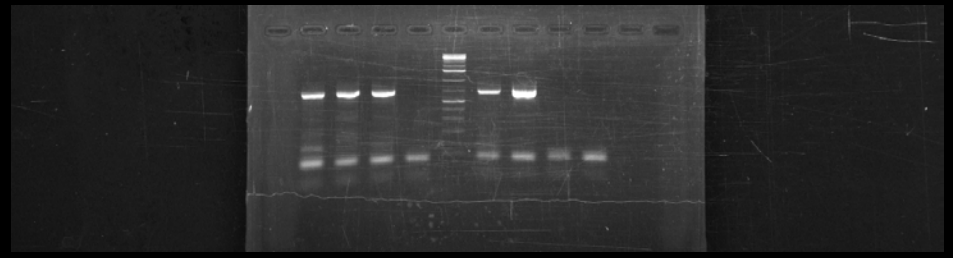


Half of Identified Strains are Outcrossed

Outcrossed	*daf-16 (mu86)*
	daf-12 (rh41rh411)
	nhr-49 (nr2041)
	hsf-1 (sy441)
	skn-1 (zu135)
Outcrossing In Process	*hlh-30 (tm1978)* → *5X outcrossed*
	nhr-49 (nr2041) → *4X outcrossed*
	xbp-1 (tm2482) → *A Struggle*
Not-Outcrossed	*sbp-1 (ep79)*
	atfs-1 (gk3094)

SAY14 is a *daf-16;daf-12* double mutant

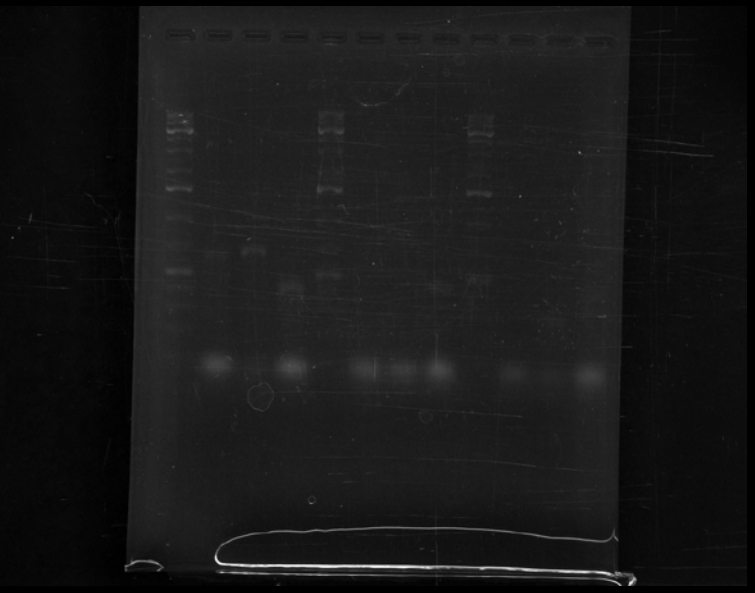




Crosses begun on March 18th

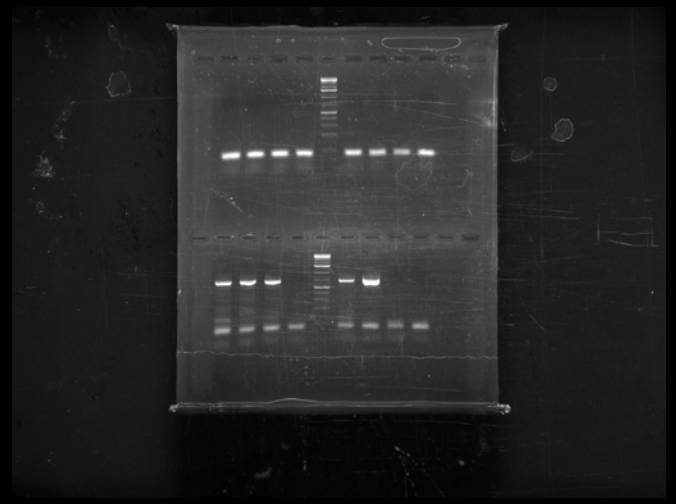
Final gel dated June 23rd

daf-16 Genotyping Finally Works



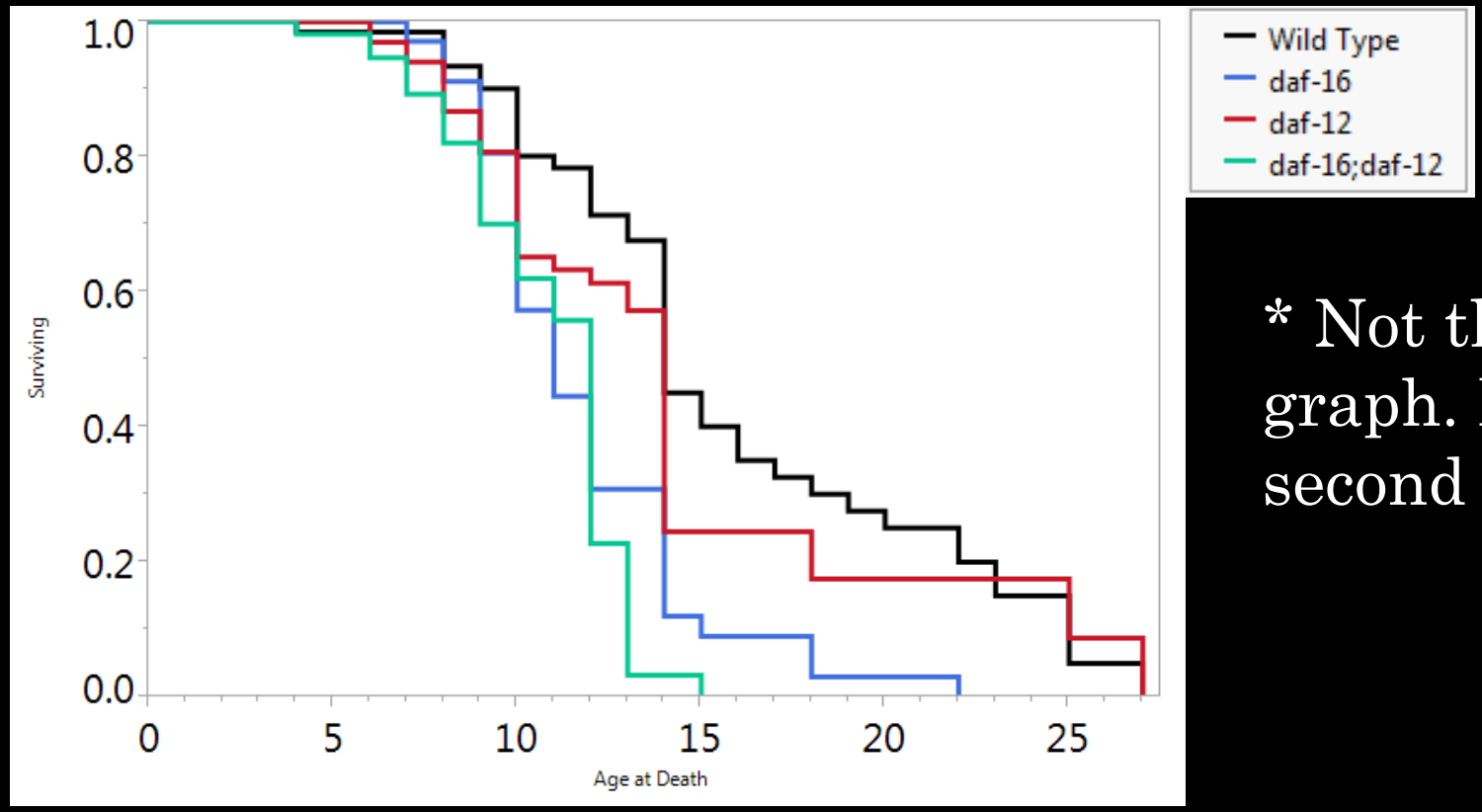
April

May



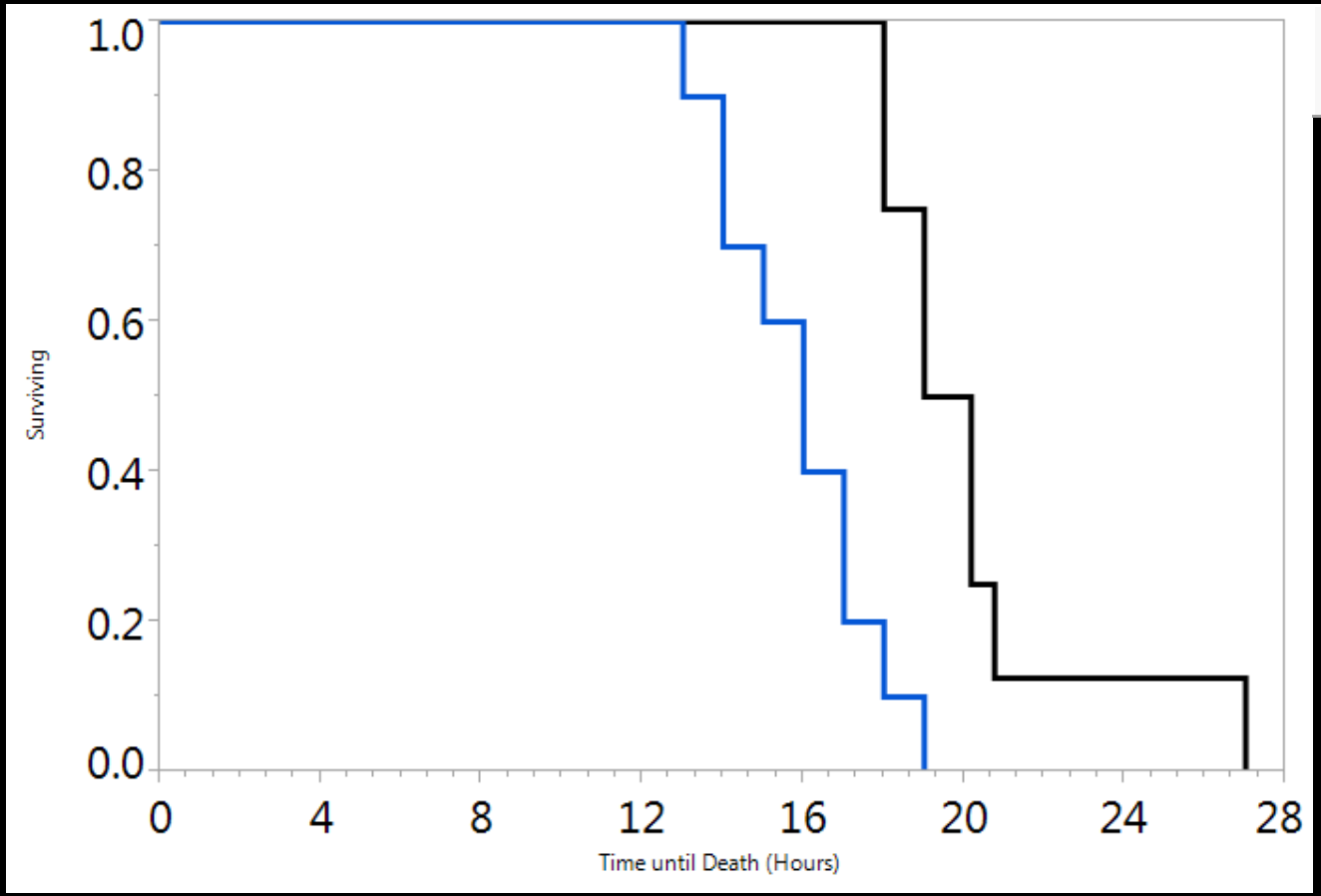
June

daf-16;daf-12 Seems to Live as Short as *daf-16*



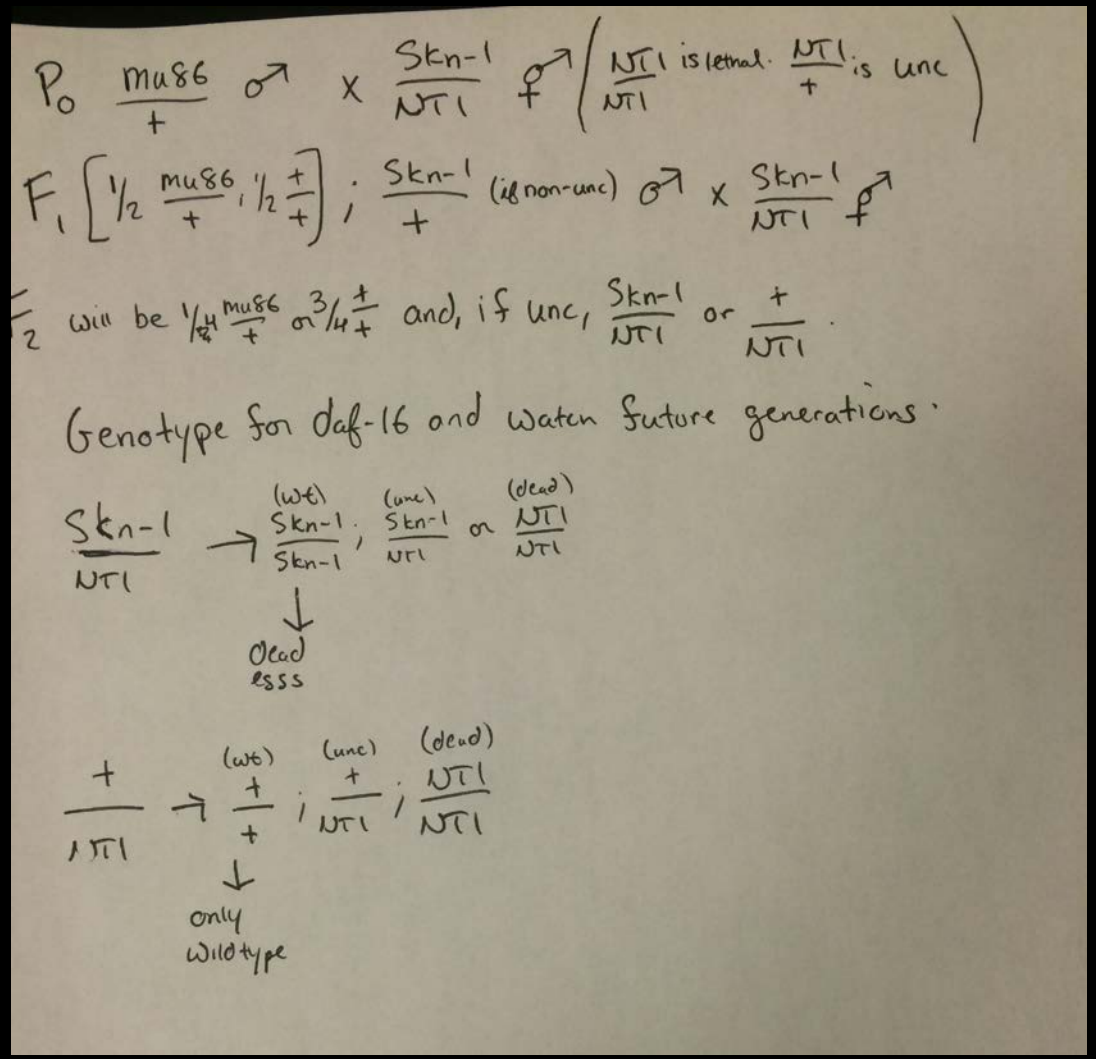
* Not the prettiest graph. Running second replicate now

Oxidation Assays are in Progress



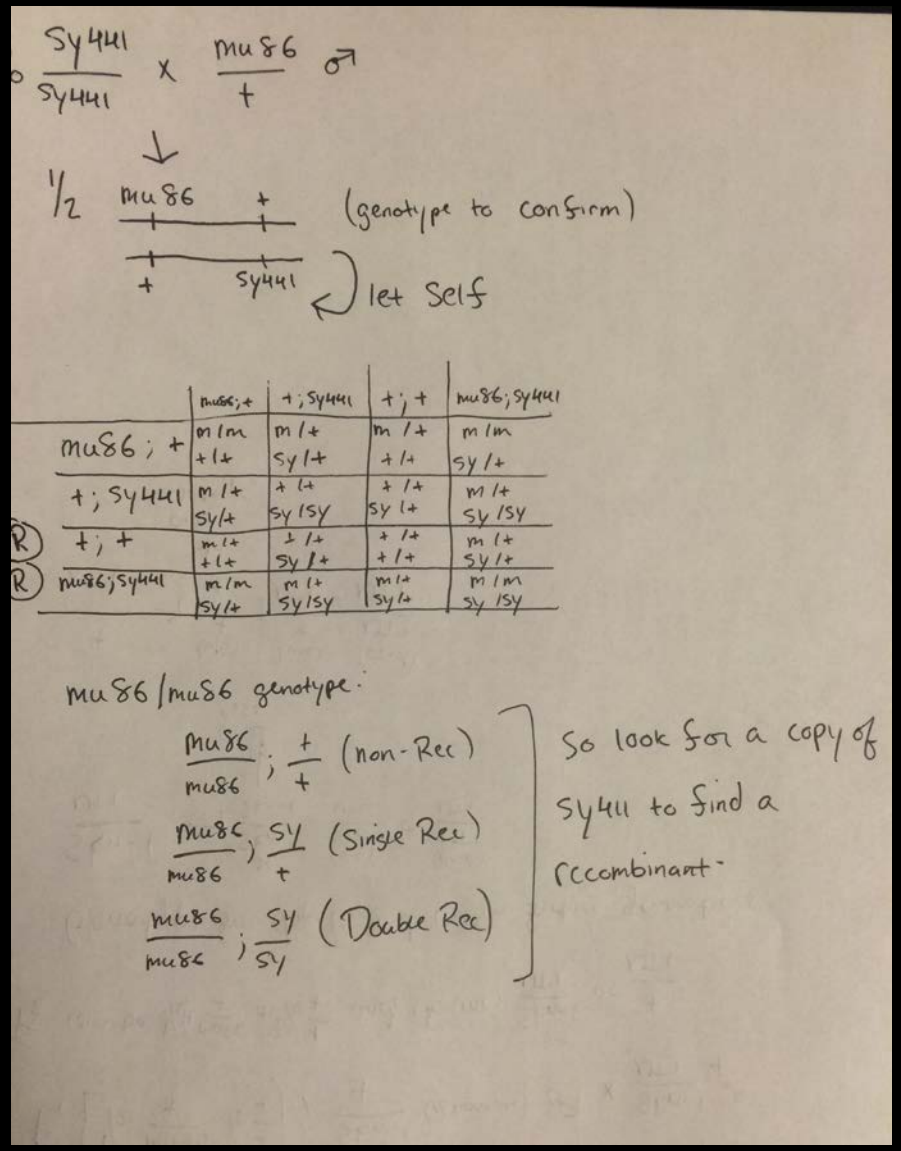
- 6mM T-BuOOH
- Small sample (8 wt, 10 *daf-16*) as a test
- Ready to scale up

Creating a *daf-16;skn-1* Double Mutant



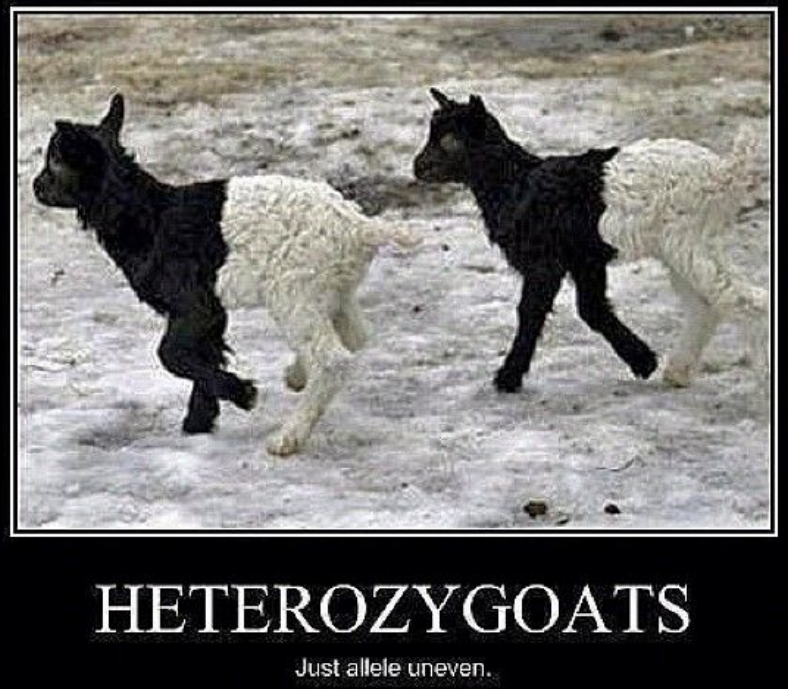
- Maternal lethal
- Only requires *daf-16* genotype!

Creating a *daf-16;hsf-1* Double Mutant



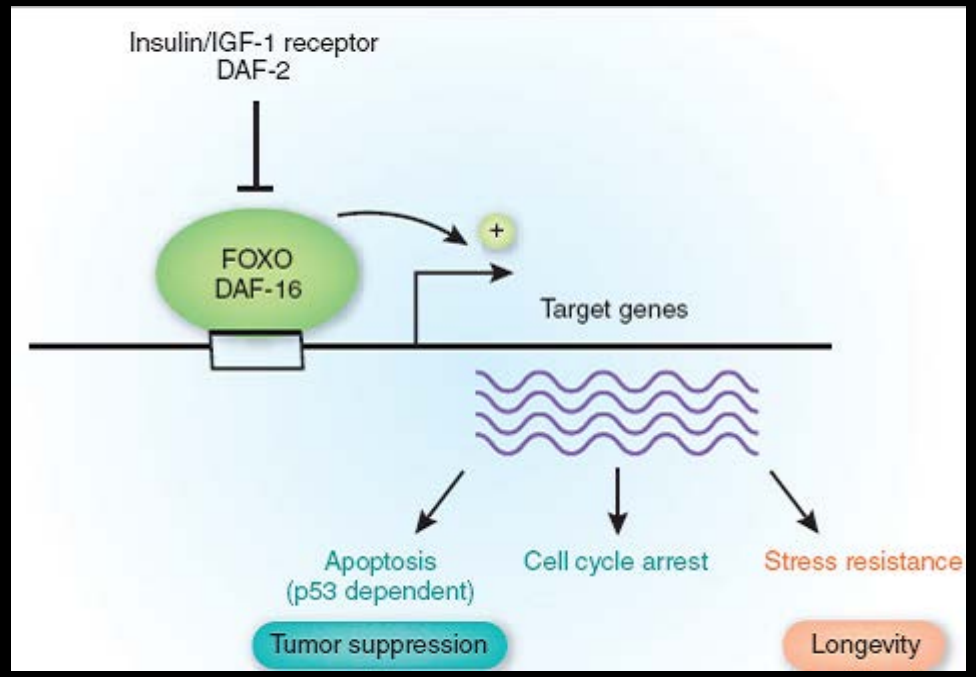
- Recombination 7.93% of the time
- Will follow a similar scheme for *daf-16;nhr-49*, which has ~4% recombination.

Wrapping Up



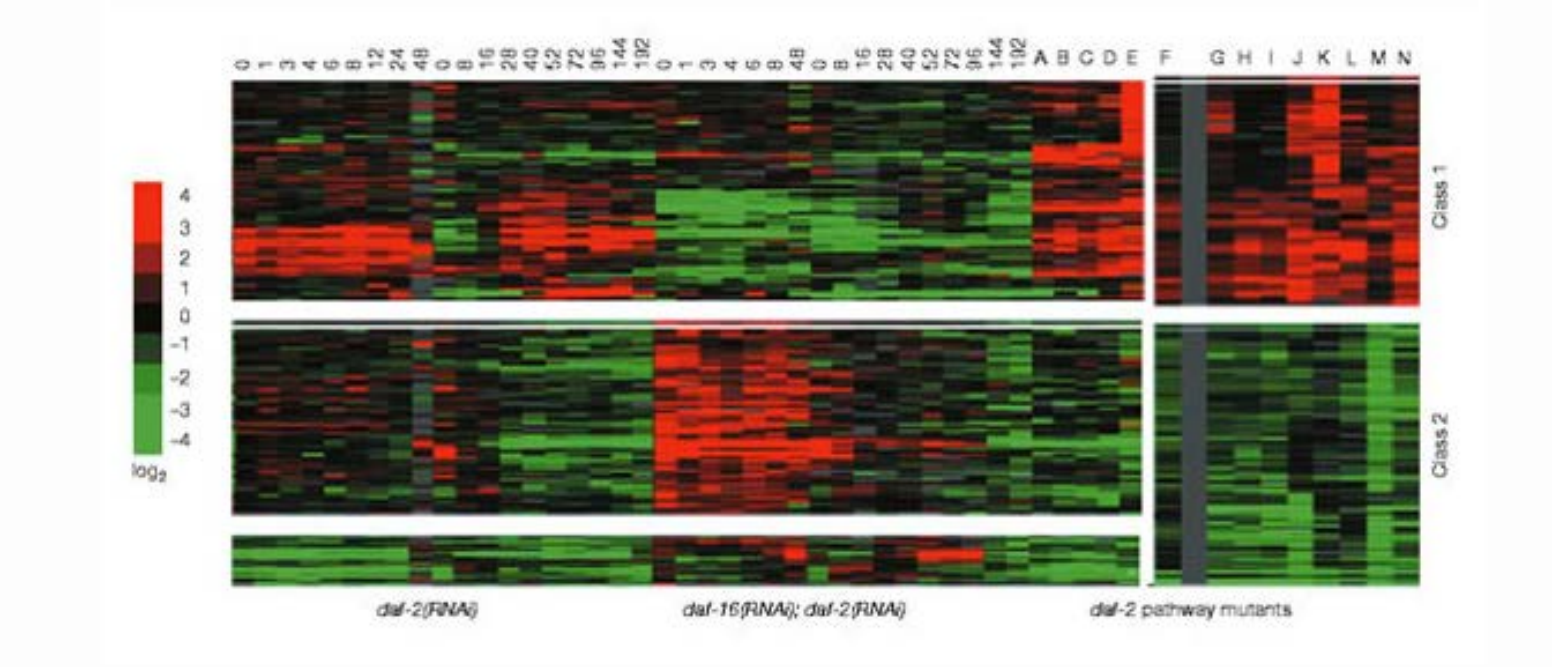
- Questions?

Transcription factors have clear targets



Brunet 2007

Transcription factors give clear follow-up experiments



Murphy et al. 2003