

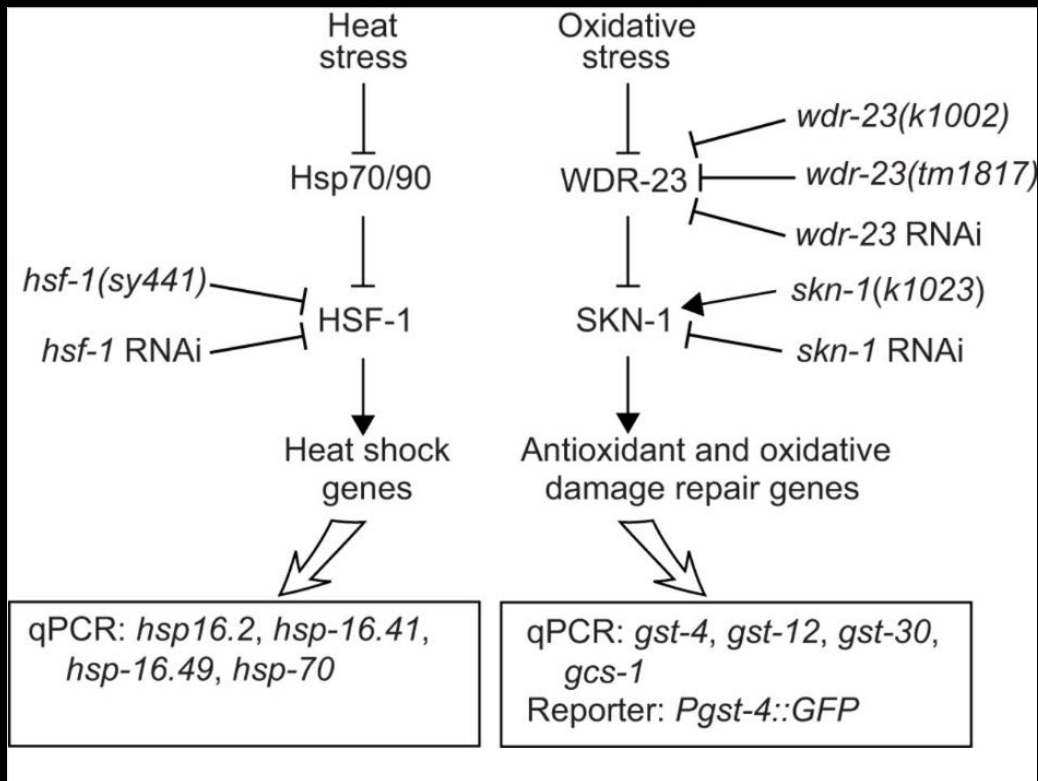
Finding the Shortest-Lived Worm

Analysis of transcription factor mutations in Caenorhabditis elegans.

Apfeld Lab

Julian Stanley, July 2016

Different Pathways Regulate Aging

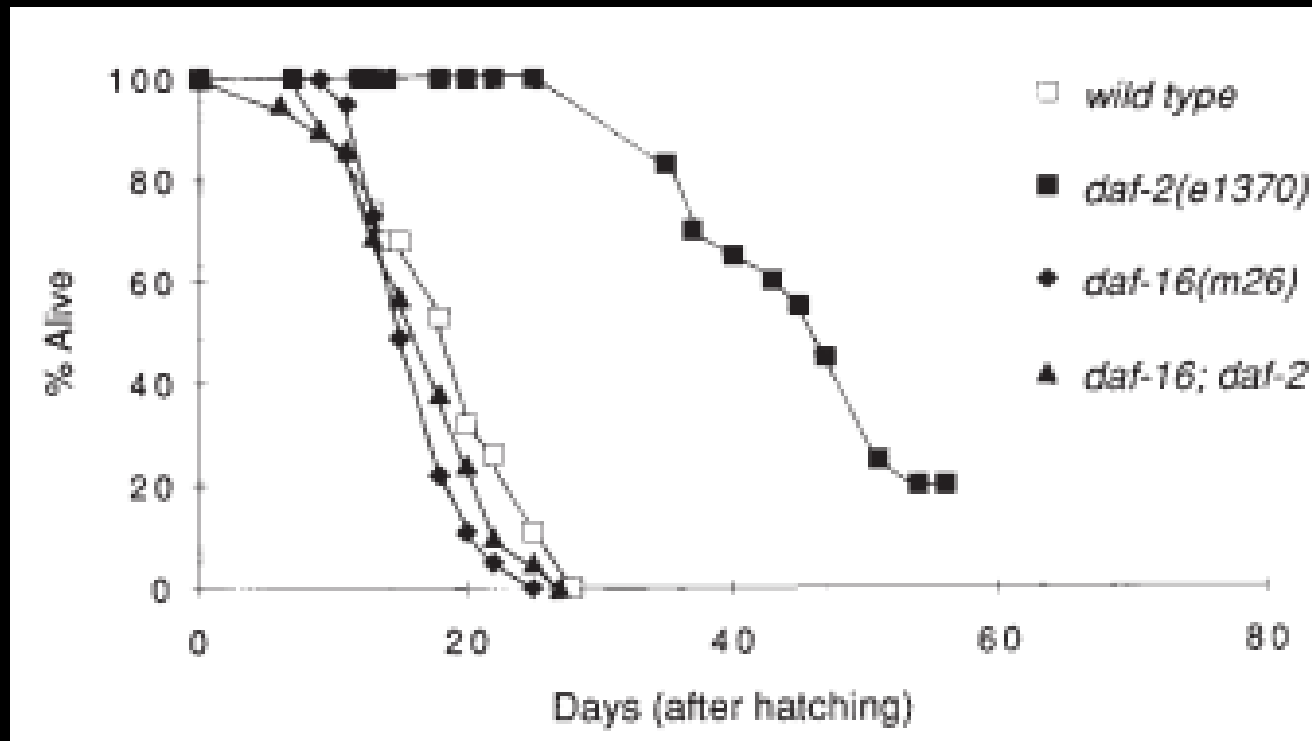


Some of pathways:

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

Crombie et al. 2016

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

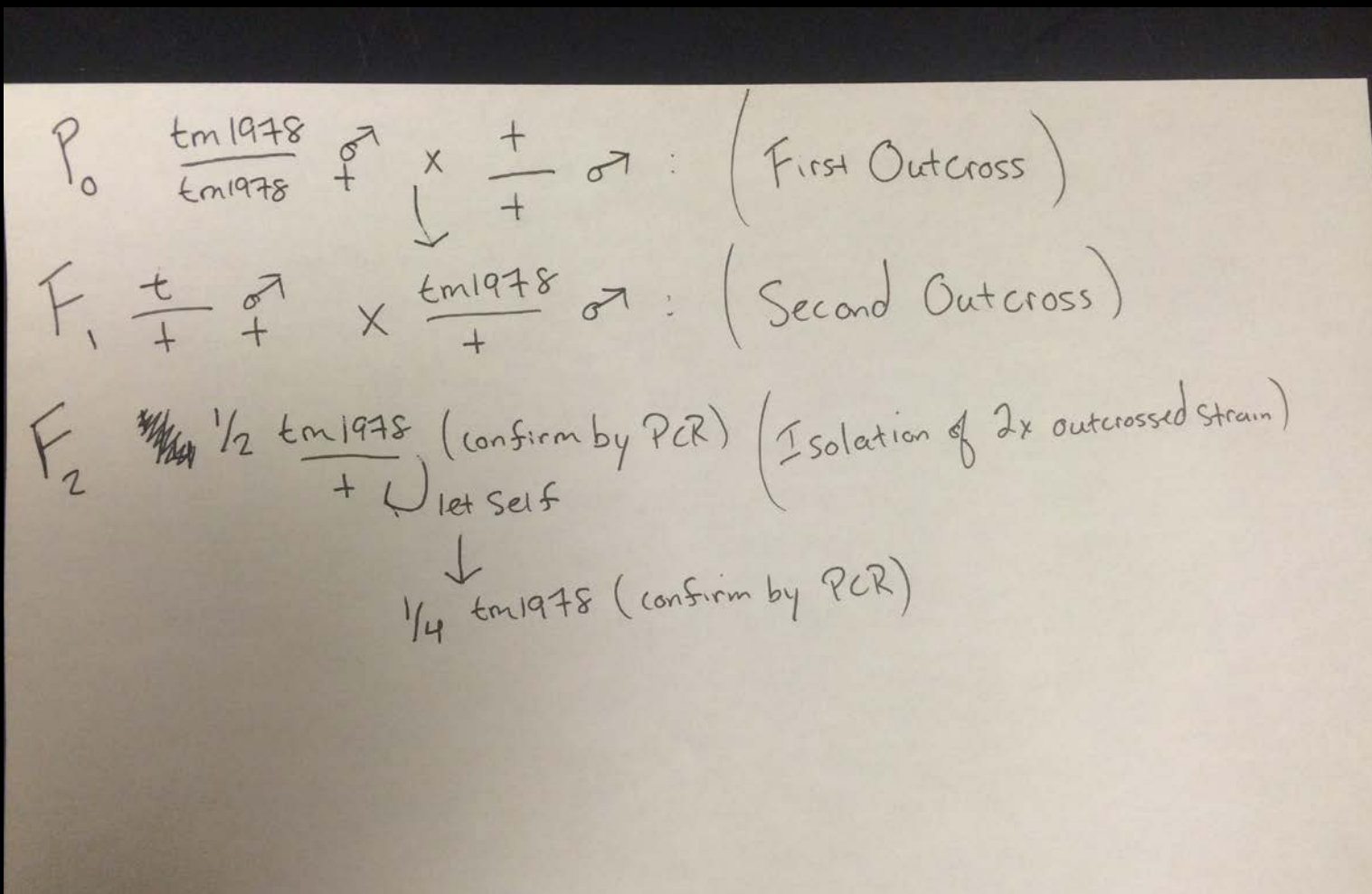


Kenyon 1993

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

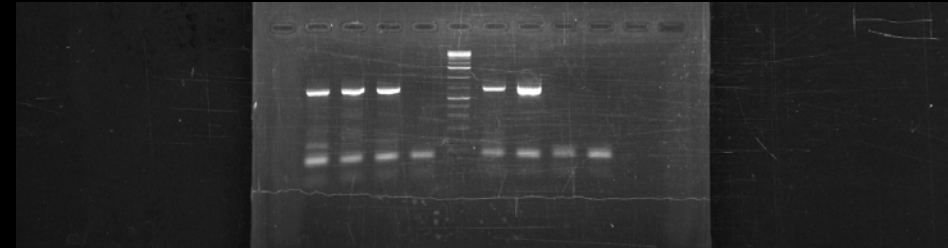
Not- Outcrossed

sbp-1 (ep79)

atfs-1 (gk3094)

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

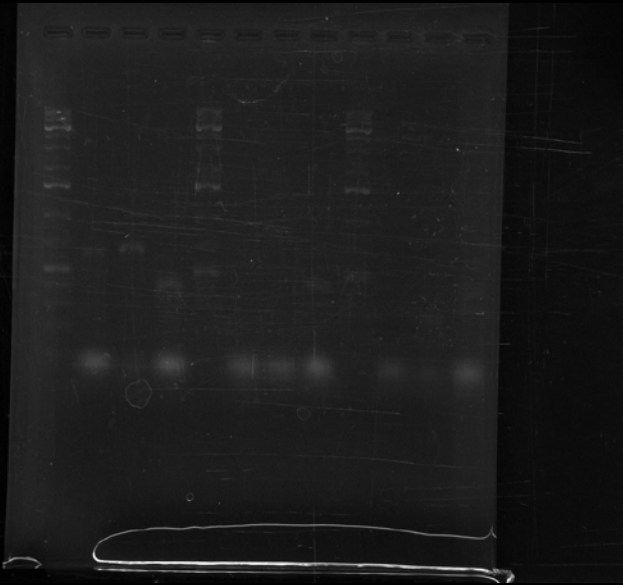
P_0 $\frac{mu86}{mu86} \sigma \times \frac{rh61rh411}{rh61rh411} \phi$
 F_1 $\frac{mu86}{+} ; \frac{rh61rh411}{\emptyset} \sigma \times \frac{rh61rh411}{rh61rh411} \phi$
 F_2 All will be $\frac{rh61rh411}{rh61rh411}$ $\frac{1}{2}$ will be $\frac{mu86}{+}$ \hookrightarrow let self
 $\frac{1}{4}$ of progeny will be $\frac{mu86}{mu86} ; \frac{rh61rh411}{rh61rh411}$



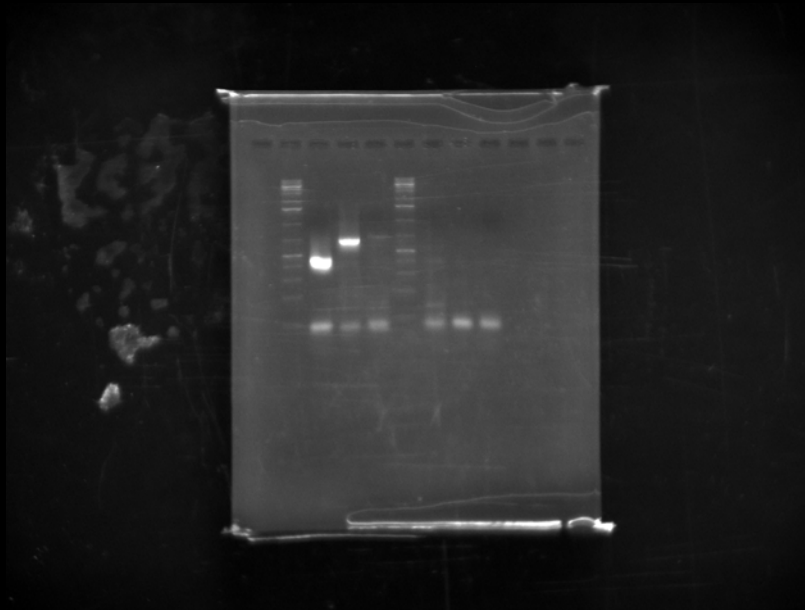
Crosses begun on March 18th

Final gel dated June 23rd

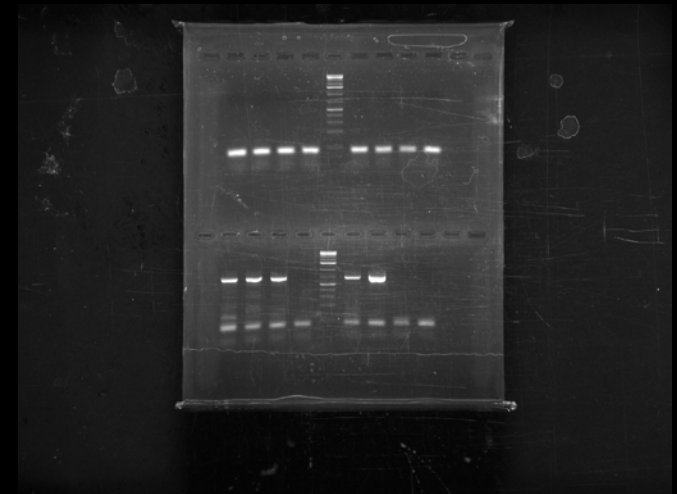
***daf-16* Genotyping Finally Works**



April

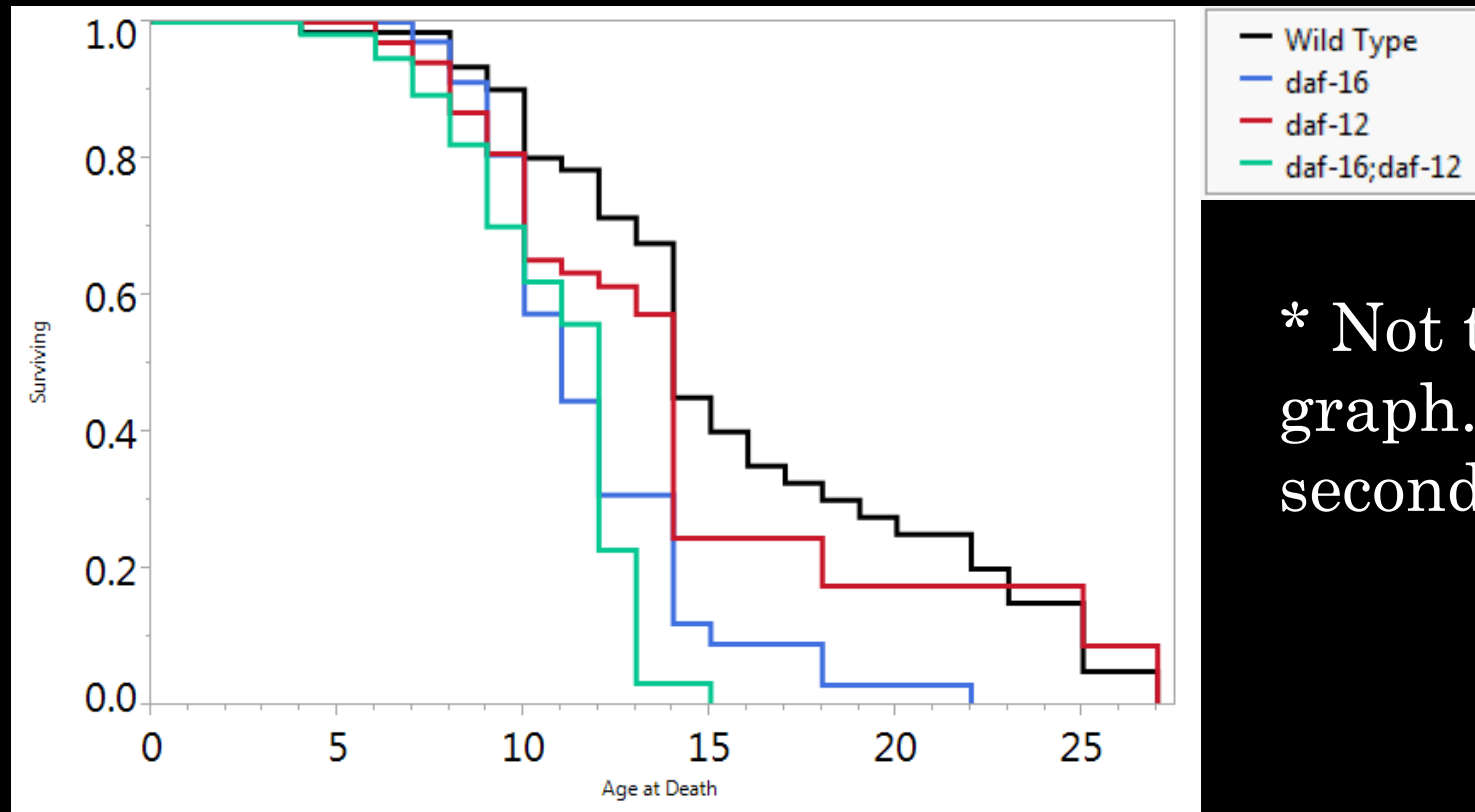


May



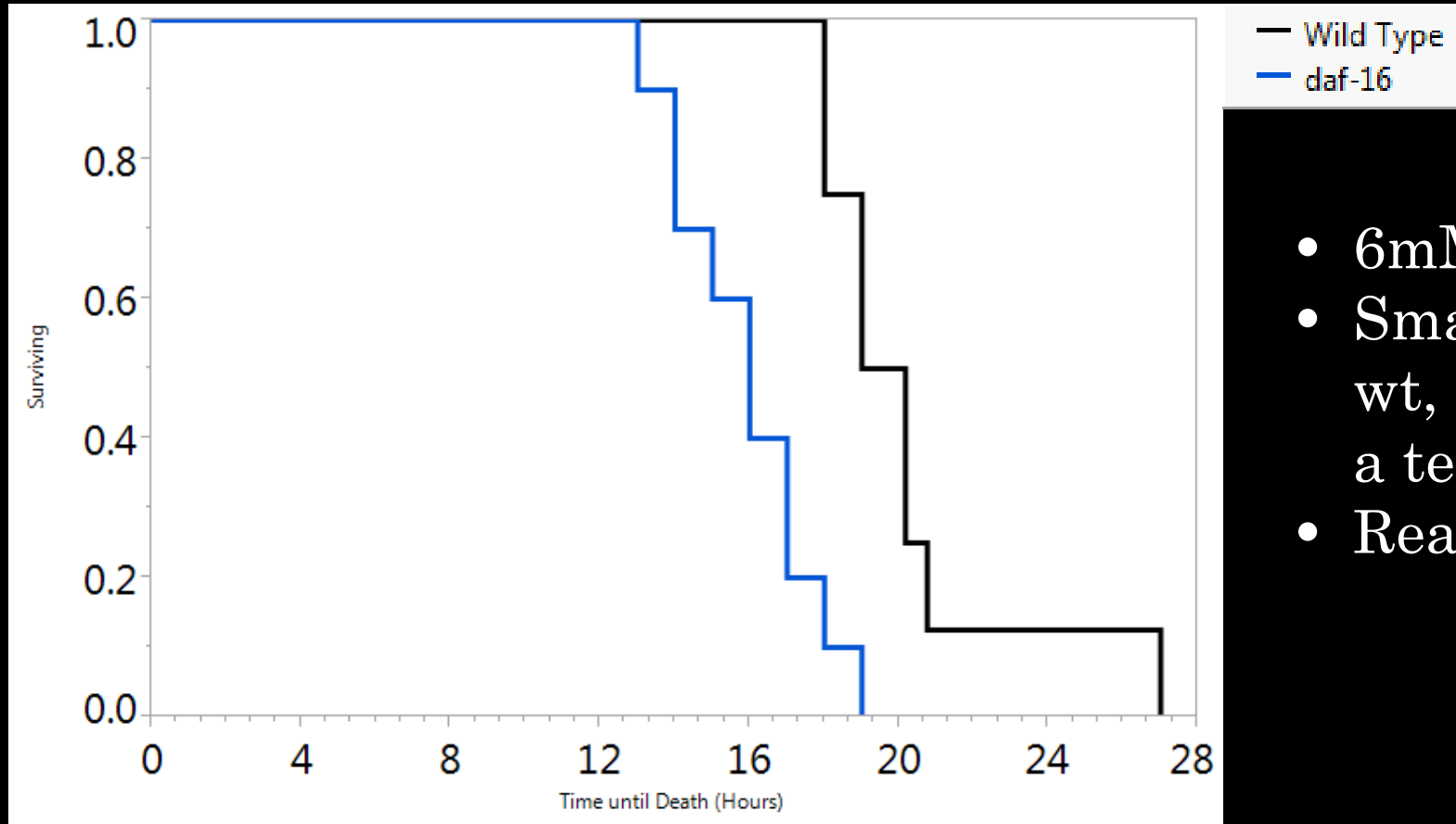
June

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

P₀ $\frac{mu86}{+}$ ♂ x $\frac{Skn-1}{NT1}$ ♂ $\left(\frac{NT1}{NT1} \text{ is lethal, } \frac{NT1}{+} \text{ is unc} \right)$

F₁ $\left[\frac{1}{2} \frac{mu86}{+}, \frac{1}{2} \frac{+}{+} \right]; \frac{Skn-1}{+}$ (if non-unc) ♂ x $\frac{Skn-1}{NT1}$ ♂

F₂ will be $\frac{1}{8} \frac{mu86}{+}$ or $\frac{3}{4} \frac{+}{+}$ and, if unc, $\frac{Skn-1}{NT1}$ or $\frac{+}{NT1}$.

Genotype for *daf-16* and waiten future generations.

$\frac{Skn-1}{NT1} \rightarrow \frac{(wt)}{Skn-1}; \frac{(unc)}{Skn-1} \text{ or } \frac{(dead)}{NT1}$
 ↓
 Dead
 eggs

$\frac{+}{NT1} \rightarrow \frac{(wt)}{+}; \frac{(unc)}{+}; \frac{(dead)}{NT1}$
 ↓
 only
 wildtype

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

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

$$\frac{sy441}{sy441} \times \frac{mu86}{+} \rightarrow$$

↓
 $\frac{1}{2} \frac{mu86}{+} \frac{+}{+}$ (genotype to confirm)
 $\frac{+}{+} \frac{sy441}{+}$ let self

	$mu86; +$	$+; sy441$	$+; +$	$mu86; sy441$
$mu86; +$	m/m $+/+$	$m/+$ $sy/+$	$m/+$ $+/+$	m/m $sy/+$
$+; sy441$	$m/+$ $sy/+$	$+/+$ sy/sy	$+/+$ $sy/+$	$m/+$ sy/sy
$+; +$	$m/+$ $+/+$	$+/+$ $sy/+$	$+/+$ $+/+$	$m/+$ $sy/+$
$mu86; sy441$	m/m $sy/+$	$m/+$ sy/sy	$m/+$ $sy/+$	m/m sy/sy

$mu86/mu86$ genotype:

$\frac{mu86}{mu86}; \frac{+}{+}$ (non-Rec)

$\frac{mu86}{mu86}; \frac{sy}{+}$ (Single Rec)

$\frac{mu86}{mu86}; \frac{sy}{sy}$ (Double Rec)

So look for a copy of
 $sy441$ to find a
 recombinant

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

Wrapping Up

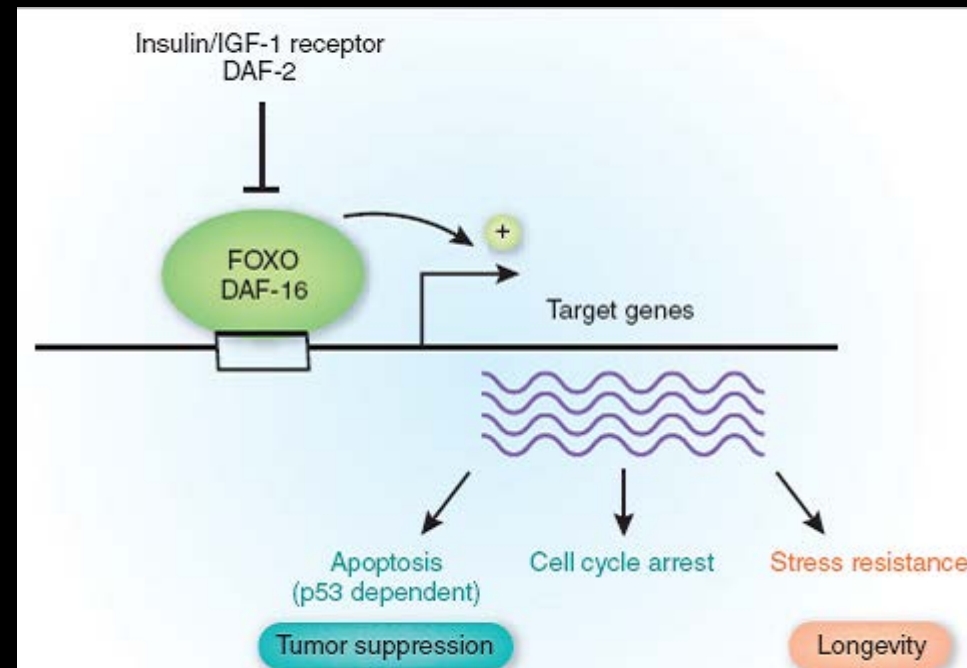


HETEROZYGOATS

Just allele uneven.

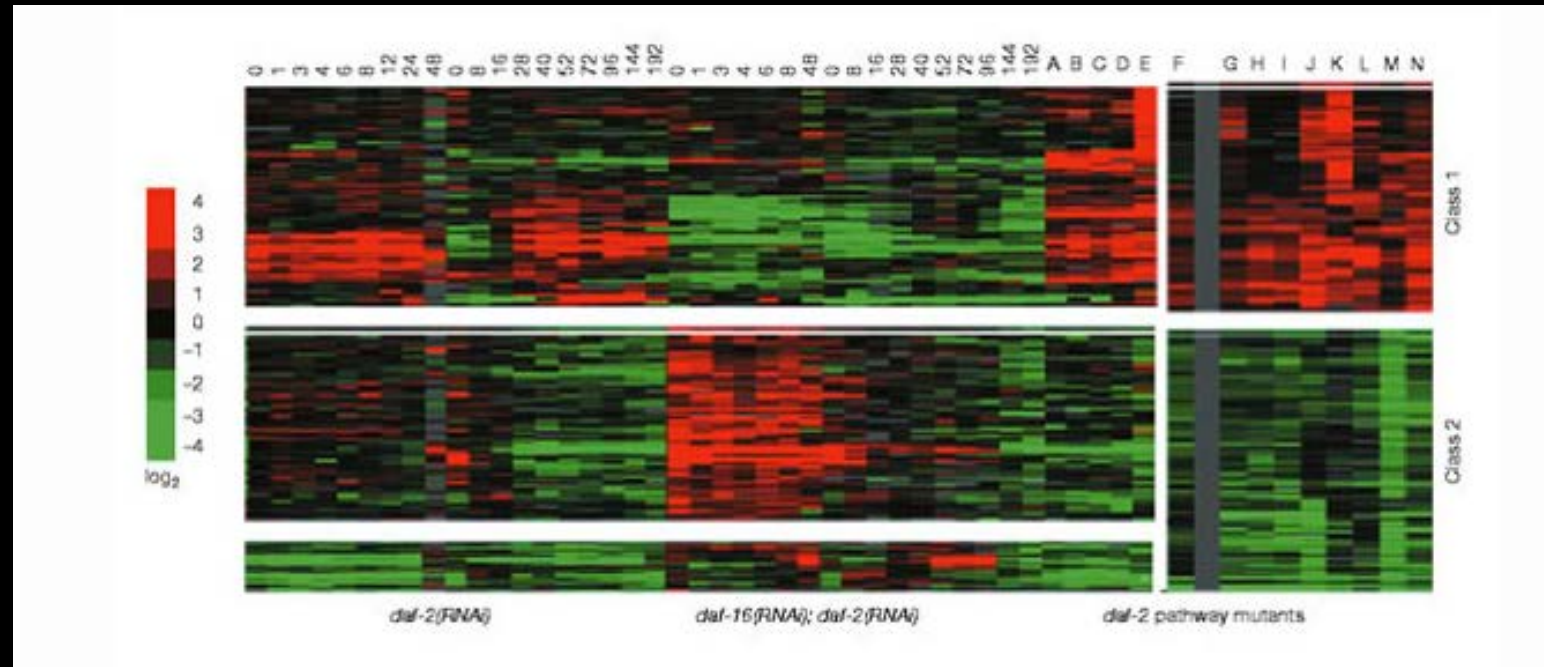
- Questions?

Transcription factors have clear targets



Brunet 2007

Transcription factors give clear follow-up experiments



Murphy et al. 2003