Exercise in forward genetics part I

Regulation of lifespan in the Nematode *C. elegans*

The Nematode *C. elegans* is an excellent model to identify and study genes determining the lifespan of animals. There exist many different *C. elegans* strains, which are either short lived with an average lifespan of only 10 days or long lived with an average lifespan of up to 30 days.

1. You want to use these long and short lived strains to identify *quantitative trait loci* (QTLs) regulating the lifespan of *C. elegans*. Describe in a few sentences the experiments you need to perform to find QTLs determining the lifespan of *C. elegans* and to identify the *individual genes* responsible for the differences in lifespan.
2. Through this QTL analysis, you have identified five genes called *age-1* to *age-5*. Using reverse genetics, you have generated *loss-of-function* ("knock-out") mutations in each of the five *age* genes in the standard *C. elegans* Bristol background. You have measured the lifespan of the individual *age* mutants. These are the results:

genotype	average lifespan ± standard deviation (days)
+/+ (wild-type *C.e.* Bristol)	18 ±2
age- 1(lf)/+	17 ±3
age- 1(lf)/age- 1(lf)	29 ±3
age- 2(lf)/+	19 ±2
age- 2(lf)/age- 2(lf)	27 ±5
age- 3(lf)/+	18 ±3
age- 3(lf)/age-3(lf)	12 ±3
age- 4(lf)/+	18 ±2
age- 4(lf)/age- 4(lf)	27 ±4
age- 5(lf)/+	20 ±3
age- 5(lf)/age- 5(lf)	11 ±2

1. line of two different strands (long and short life span), cross them, then isolate them with long short life span. Doesn't make a difference, but it is more comfortable to have lines, that are alive longer. —> Establish line with long life span.
Need to know the markers (genetic differences)= SNP's. Should know as many as possible.
Vorteil an C. Elegans, dass sie homophroditen sind. Then QTL analysis, use molecular markes, then identify the genes (already done in this question).
Knock out one after the other (e.g. reverse genetics) until we see a change of life style.
What if too many genes for knock out?
—> Interrogation Lines! (small piece)

(a) Which of these genes five inhibit ageing and which genes promote ageing?

1. 2. and 4. promote aging.
5. and 3. (WT-Version) ages faster
Mutations are all recessive, now we can make a conclusion. But we need to know this.

You then perform epistasis analysis by measuring the lifespan of all the double mutant combinations. These are the results: (The numbers indicate the average life span ±std.dev. of the respective double mutants.)

gene1/gene2	*age-1(lf)/age-1(lf)*	*age-2(lf)/age-2(lf)*	*age-3(lf)/age-3(lf)*	*age-4(lf)/age-4(lf)*	*age-5(lf)/age-5(lf)*
age-1(lf)/age-1(lf)		28±4	11±3	30±4	19±3
age-2(lf)/age-2(lf)	28±4		13±4	29±3	18±2
age-3(lf)/age-3(lf)	11±3	13±4		10±4	11±3
age-4(lf)/age-4(lf)	30±4	29±3	10±4		28±3
age-5(lf)/age-5(lf)	19±3	18±2	12±2	28±3	

(b) Draw a genetic model based the results of the epistasis analysis. Indicate the sign (positive or negative) of each genetic interaction (for example use → for positive and ⊣ for negative regulation). Be sure to include the outcome (aging) at the end of your pathway. Discuss the different scenarios that are possible based on the available data.

(c) Design a synthetic forward screen to identify additional genes (i.e. *age-6*, *age-7* etc) regulating the lifespan of *C. elegans*. What phenotype can you select for to identify new regulators of lifespan?

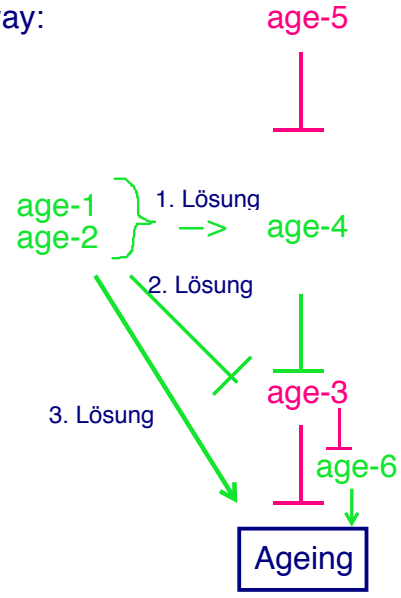
b) age-3 —I
age-5 —I

age-1 —>
age-2 —>
age-4 —>

age-3 always wins! it is epistatic over all muations!
age-3 and age-5 behave very differently.
age-3 is last in the pathway because it is epistatic

if no age-4, age-5 becomes more active,
if there is no age-4, it doesn't matter if there is an age-5.

Possible Pathway:



für age-1 und für age-2 gibt es 3 mögliche Lösungen
Können nicht entscheiden, ob age-1 und age-2 zusammen wirken oder getrennt.

If we just look at age-4, it is long lived, age-4 is epistiatic to age-5 (because together they are still long lived)

c) Use age-3, is epistatic to everything else, can we find anything that lives longer than age-3? Age-3 loss-of-function mutations, short lived.
Phenotype: long lived. Alles was unter age-3 ist und länger lebt, wäre besser