I WANT TO LIVE FOREVER...

2A. GENES AND MUTATIONS

You are a researcher in the LifeSpan project. Like all of the other students in the class, you are conducting research on ageing. Your specific research is on the role played in ageing by genes and mutations. Ultimately you will be presenting your results to the rest of the class. The following assignments will help you to study the results, and to prepare your presentation.

Have fun!

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De Praktijk

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Assignment 1. Gene regulation in C. elegans

1. Watch the following video:
Daryl Shanley 3 – Gene regulation in C. elegans

Caenorhabditis elegans is a nematode. Because they are relatively simple organisms with such short lifespans, these small worms make excellent test subjects. By activating and deactivating certain genes in *C. elegans* in the laboratory, the role played by the genes can be examined.

It has been shown that mutations in one or more of the worm's genes can have enormous effects on its lifespan, particularly mutations in those genes that code for *transcription factors*. Transcription factors are proteins that influence the expression of other genes, by binding to a *promoter* (a piece of DNA located just in front of a gene) which activates or represses gene expression. This process is called *gene regulation*.

2. The diagram below shows how transcription factors work. Label the following elements in the diagram: *DNA*, *protein*, *mRNA*, *promoter* and *transcription factor*.

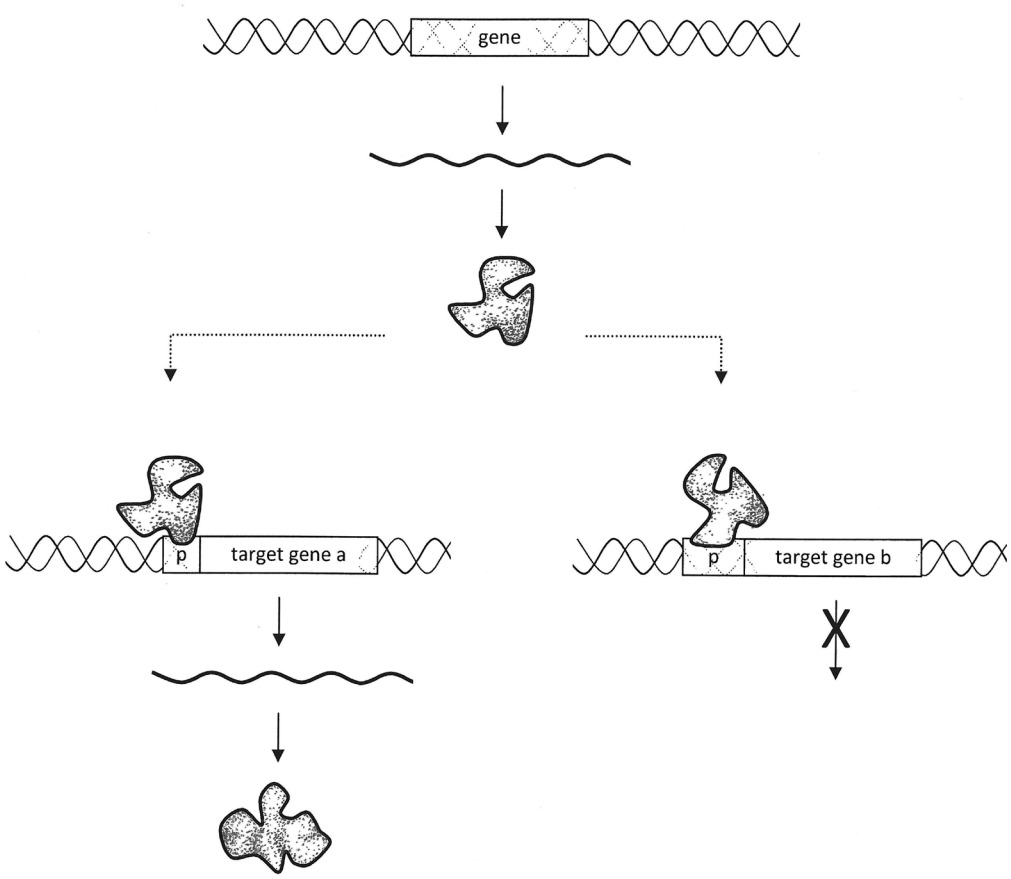

Figure 1. How transcription factors work. Diagram: C. Geraedts.

3. *C. elegans* has a transcription factor called DAF-16. In some long-living mutants of *C. elegans* the concentration of DAF-16 in the cells is higher than normal. Research has shown that DAF-16 affects the expression of dozens of genes. For each of the genes below, indicate whether they contribute to maintenance or reproduction. Also indicate whether DAF-16 activates or represses gene expression (cross out answers that do not apply).

Table 1. Genes whose expression is influenced by DAF-16 (in C. elegans). From: Murphy et al. (2003).

gene	encodes	maintenance or reproduction?	activated or repressed by DAF-16?
hsp-16.1	a heat shock protein, a protein that is activated by exposure to (excessively) high temperatures	maintenance/ reproduction	activated/ repressed
lys-7	a lysozyme, a protein that breaks down bacterial cell walls	maintenance/ reproduction	activated/ repressed
vit-2	vitellogenin, a protein necessary for the creation of egg cells	maintenance/ reproduction	activated/ repressed
sod-3	superoxide dismutase, an enzyme that removes free radicals from cells (free radicals are highly reactive atoms that can damage all kinds of molecules)	maintenance/ reproduction	activated/ repressed

Figure 2. C. elegans
source: Wikipedia / Bob Goldstein

Assignment 2. Biobank

1. Watch the following video:
Andres Metspalu 1 – Genetic variation in humans

Andres Metspalu conducts research into the relationship between human genes and life expectancy. Are there certain genes in the human genome that encode 'long life'? To answer this question, an enormous database has been created in Estonia that contains data on the DNA of thousands of people. Comparable research is being conducted in the Netherlands by scientists at the University Medical Centre in Leiden. Table 2 below shows a small sample from the biobank being used in the 'Leiden Long-Life Study'. Of course, in reality there are hundreds of thousands of genetic markers (DNA segments) being researched.

Table 2. Biobank data from 7 people in the Leiden Long-Life Study. For each genetic marker, the table indicates whether a person is a carrier of the most common genetic variant (0) or an alternative variant (1).

	age	marker 1	marker 2	marker 3	marker 4	marker 5
donor 1739 (M)	89	1	0	0	0	1
donor 1740 (V)	56	1	0	0	0	0
donor 1741 (M)	92	0	1	0	0	1
donor 1742 (V)	59	1	0	1	0	1
donor 1743 (M)	61	1	0	0	0	0
donor 1744 (V)	95	0	0	0	0	1
donor 1745 (V)	92	1	0	0	0	1
etc.						

2. Most genes come in different varieties, often involving differences in one or several nucleotides. A genetic marker is a location within a gene where the nucleotide sequence is variable. What is another name for the different variants of one specific gene?

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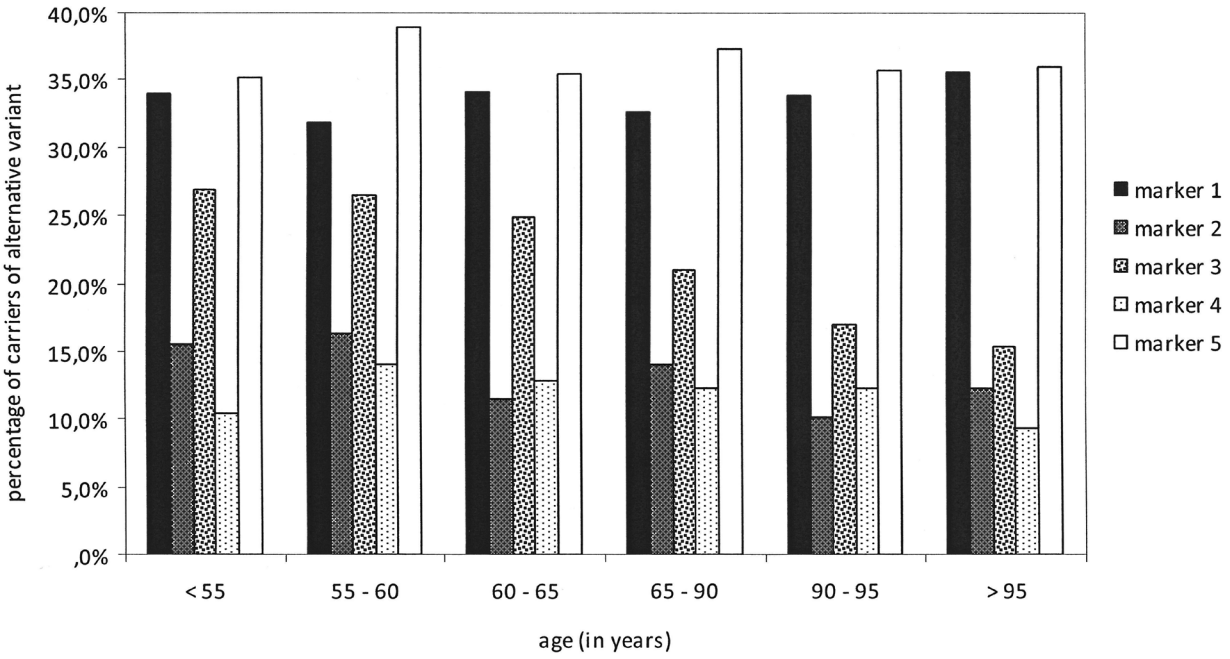

Figure 3. The percentage of carriers of the alternative variant of five different markers, arranged according to age group. Based on research by Rudi Westendorp and Eline Slagboom (Leiden University Medical Centre).

3. Look at Figure 3. Which marker shows the greatest correlation to age? Are carriers of the alternative variant more or less likely to live longer? Cross out answers that do not apply.

correlation with age is the highest for marker 1 / 2 / 3 / 4 / 5

carriers of the alternative variant are more / less *likely to live longer*

4. Genes have been identified in *C. elegans* that are capable of increasing the lifespan of an individual by a factor of five. Such genes have not (yet) been identified in humans. What reason can you think of to explain this?

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5. Imagine that in the future it will be possible to make a complete analysis of your chances of developing all kinds of hereditary (and other) diseases. They will also be able to estimate your individual life expectancy. Give one argument for why you would like to know the results of this kind of examination, and also for why you would not.

I would like to know the results, because...

.....

.....

I would not like to know the results, because...

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.....

Assignment 3. Mutations

All of the cells in your body contain the same DNA. However, DNA is a large and complex molecule. Over time, defects inevitably appear in the order of the nucleotides in the DNA. These defects are called 'mutations'. Mutations sometimes occur spontaneously, but their frequency can increase considerably under the influence of radiation, chemicals or viruses.

1. The game *I want to make babies!* presented you with a number of causes of mutations. Some of these are presented again in the list below. Note the letters of the causes under the proper category.

spontaneous mutations: .....

mutagenic radiation: .....

mutagenic chemicals: .....

viruses: .....

Causes of mutations

a. *depurination* – Every day, hydrolysis spontaneously causes thousands of purines (A and G) to split away from the DNA strand. This produces 'holes' in the rungs of DNA.

b. *heat* – Short-term exposure to heat (heat shock) causes all kinds of hydrolytic reactions, damaging the DNA.

c. *human papilloma virus* - Human papilloma virus (HPV), which causes diseases such as cervical cancer, can alter gene expression.

d. *solvents* – Benzene (a solvent once used in paints and other agents) can cause mutations if inhaled.

e. *radioactivity* – The energy released by radioactive decay can damage DNA in a variety of ways.

f. *replication errors* – Incorrect bases are inserted during DNA replication, prior to cell division. Sometimes too many or too few bases are inserted.

g. *cigarette smoke* – Cigarette smoke contains polycyclic aromatics, nitrosamines and other substances that can cause a range of possible mutations.

h. *free radicals* – Metabolism produces a constant stream of oxygen atoms with an unpaired electron in the outermost shell. These free radicals oxidise the nitrogenous bases in DNA.

i. *sunlight* – The ultraviolet (UV) rays in sunlight cause two adjacent pyrimidines (C or T) to bind together. This severs the bonds with the bases on the opposite side.

It is estimated that under normal conditions, hundreds of thousands of mutations take place within a single cell every day. A large number of these mutations are quickly repaired again – to do this, cells possess a range of DNA repair proteins.

Imagine that the following two mutations occur in a DNA strand (see Figure 2). On the left there is a nitrogen base missing; on the right, the wrong base has been inserted. Both mutations will be repaired.

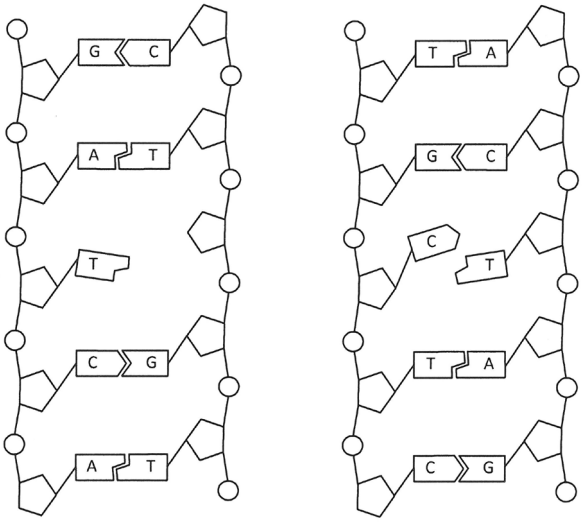

Figure 4. Two point mutations. Diagram: C. Geraedts.

2. The mutation on the left is repaired by the enzyme Pol β (a DNA polymerase). Pol β automatically inserts the correct nitrogenous base where it is missing. How does Pol β 'know' which base to insert?

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3. The mutation on the right may cause the order of bases in the repaired DNA to differ from the original order. Explain.

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4. Cancer develops due to a range of mutations in the tumour cells. In many tumours, the mutations occur in the genes encoding the proteins for DNA damage-repair. Explain why it is often mutations in these genes in particular that are associated with cancer.

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Assignment 4. The LifeSpan conference

At scientific conferences, researchers from a range of universities come together to share information and discuss research results. You are going to do this too. To conclude this assignment you will hold a kind of scientific conference, in which you will give a short presentation on the subjects you have studied thus far. You will also write a short proposal for further research.

The presentation will consist of three sections:

A. Genes and ageing in C. elegans

Use the diagram on page 10 to explain the following:

- which genes are active in long-living mutants of *C. elegans* (tip: the genes in the diagram are also listed in the table on page 3),
- how DAF-16 regulates the expression of other genes,
- that long-living mutants invest more energy in repairing damage.

B. Genes and ageing in humans

Use the diagram on page 11 to explain the following:

- what a biobank is,
- that certain genetic markers play a role in lifespan,
- that no human genes have as yet been found that have an influence on lifespan as large as that in *C. elegans*.

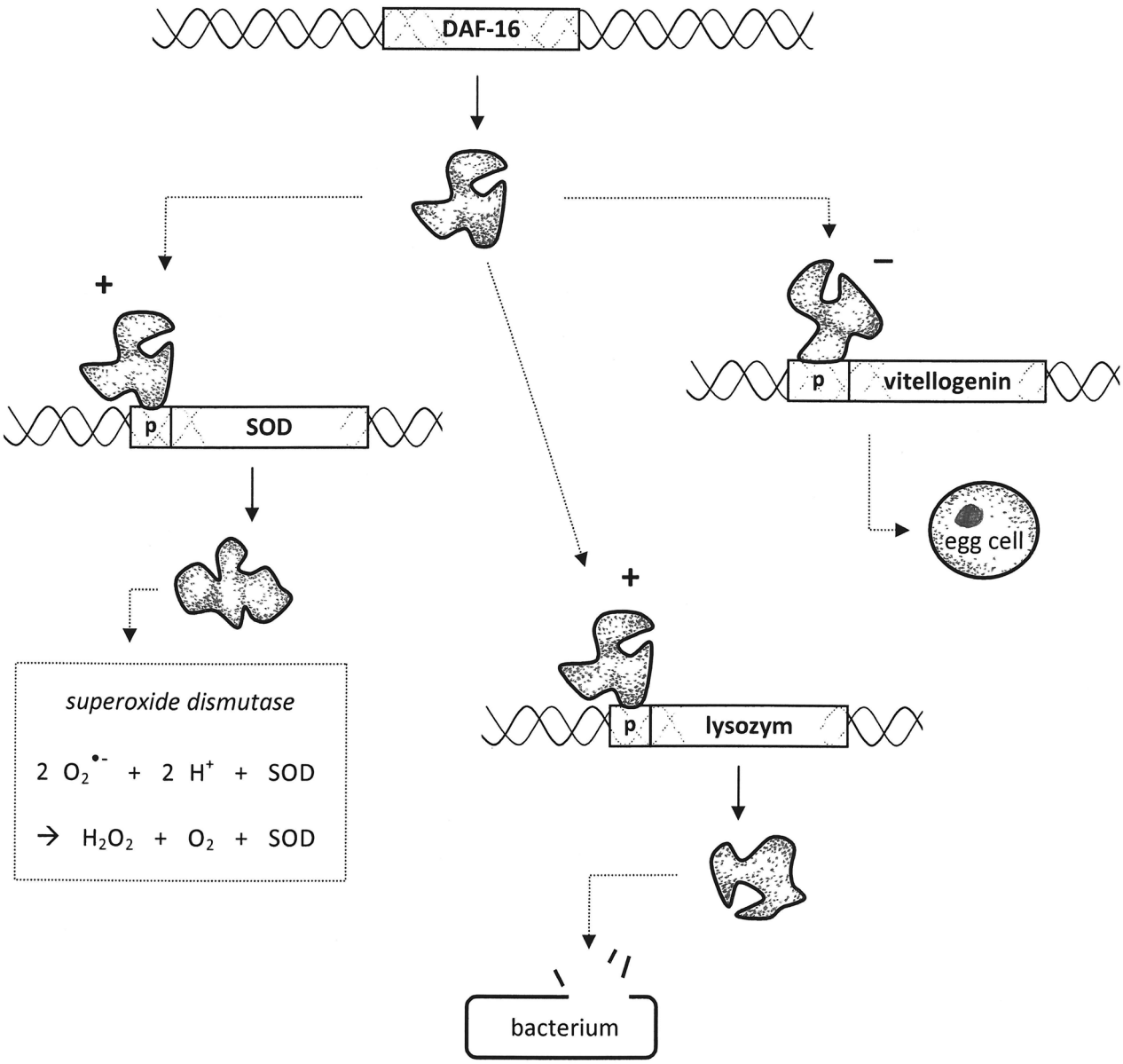
C. Proposal for further research

You wish to calculate how much influence genes have on lifespan. For your research, you will use data from several pairs of identical twins and their partners. Briefly describe the steps you will take to answer your research question (see the template on page 12).

Preparation
PowerPoint slides have already been made for you, containing the images on pages 10 and 11. Use these copies to make your notes. Write your research proposal using the template on page 12. Make a rough copy first if necessary.

The conference
Using your PowerPoint slides, give a short presentation on what you have learned. You may refer to your notes if you wish. Also present your research proposal to the rest of the class. The teacher will determine whose turn it is.

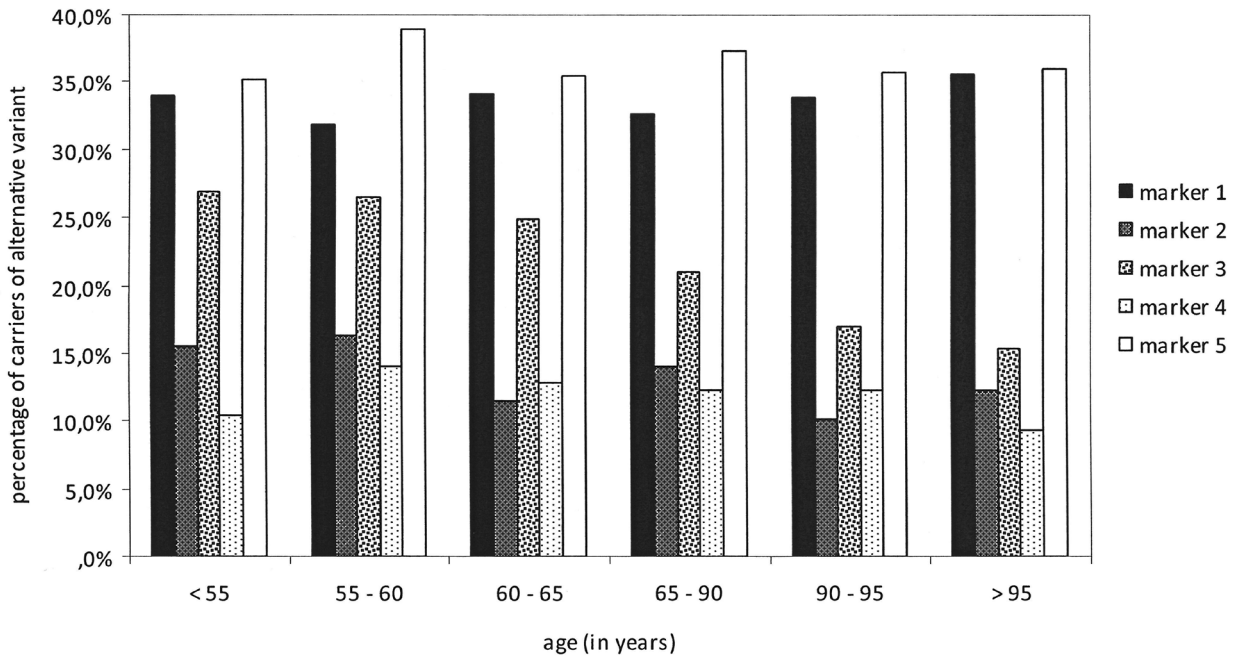
A. Regulation by DAF-16



B. Biobank (fragment)

	age	marker 1	marker 2	marker 3	marker 4	marker 5
donor 1739 (M)	89	1	0	0	0	1
donor 1740 (V)	56	1	0	0	0	0
donor 1741 (M)	92	0	1	0	0	1
donor 1742 (V)	59	1	0	1	0	1
donor 1743 (M)	61	1	0	0	0	0
donor 1744 (V)	95	0	0	0	0	1
donor 1745 (V)	92	1	0	0	0	1
etc.						

B. Correlation between five markers and age



C. Research proposal

Title of research project
Introduction (approx. 50 words)
Research question
Hypothesis
Research plan (including materials and methods)