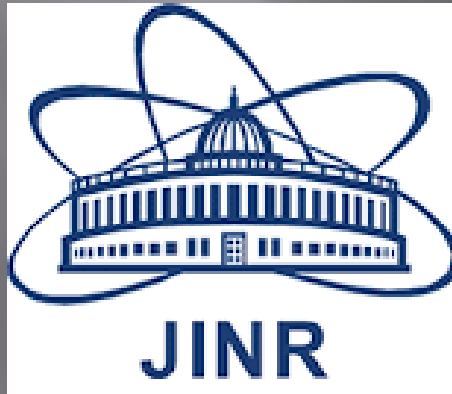


PCR assay of the molecular alteration at vestigial genes of *Drosophila melanogaster* produced spontaneously or by radiation

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Content

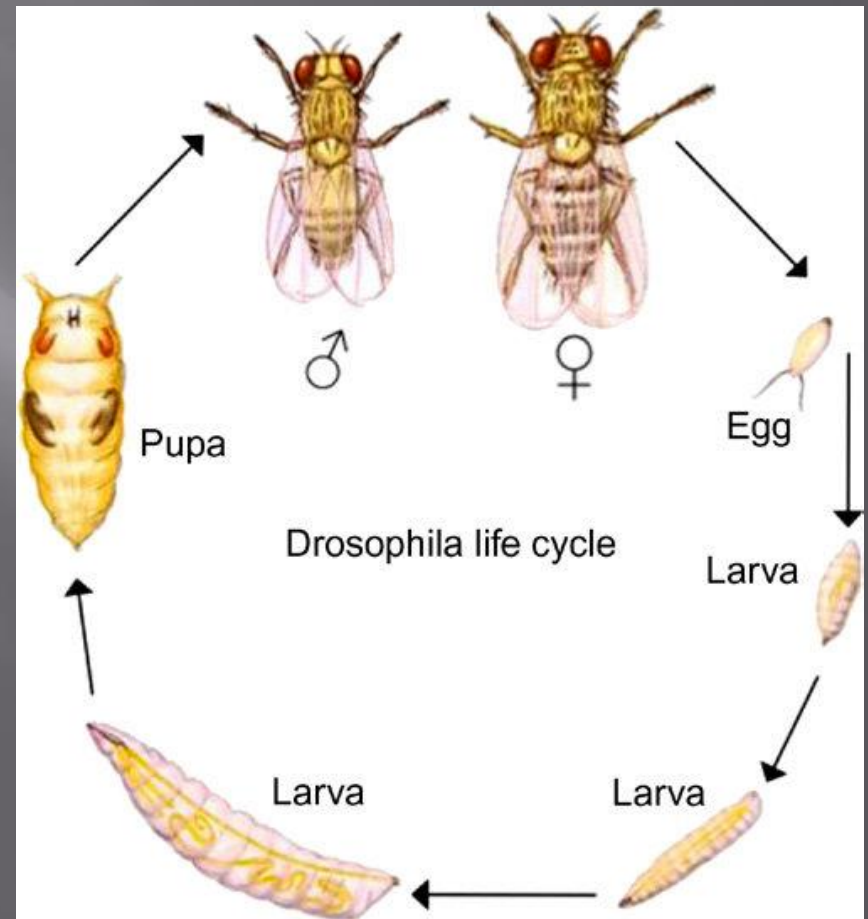
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Aim of project

- Investigation of mutation induced by γ -rays and neutrons
- Comparing spontaneous mutations with those induced by radiation
- Learning the structure of the gene to detect the location of different damages in wild type and mutants

Why Drosophila

- The insect has relatively short life;
- The promiscuous nature and high rates of reproduction of Drosophila;
- Has common principal DNA structure with humans;
- Permits the study of heritable gene mutation



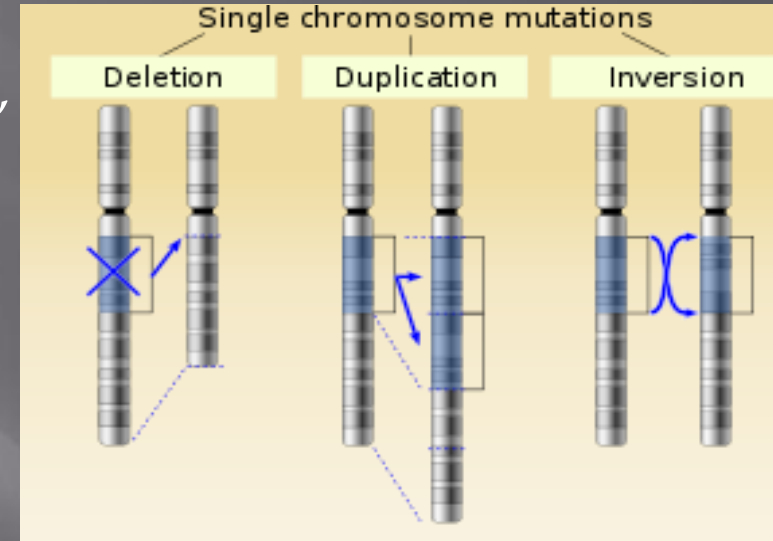
Effects of radiation

γ-rays ^{60}Co

- a source of radiation : “Gamma-cell-220”
- dose: 5-40 Gy
- N=5,7 Gy/min
- LET: 0.3 keV/micron

Monoenergetic neutrons

- a source of radiation : reactor BR-10, Obninsk
- dose: 2,5–20 Gy
- N=2,6 Gy/min
- LET: 78 keV/micron



Point mutations

Substitution

CTGGAG
CTGGGG

Insertion

CTGGAG
CTGGTGGAG

Deletion

CTGGAG
CTAG

damage of DNA → mutations

Visible effects of mutations



Picture 1. Wild phenotype of Drosophila melanogaster

Picture 2. Phenotypes of vestigial, black, cinnabar, yellow, white

All of the studied flies are from next filial generations of irradiated subjects.

METHODS



1 DNA Isolation

2 Polymerase chain reaction

3 Gel Electrophoresis

1. DNA Isolation

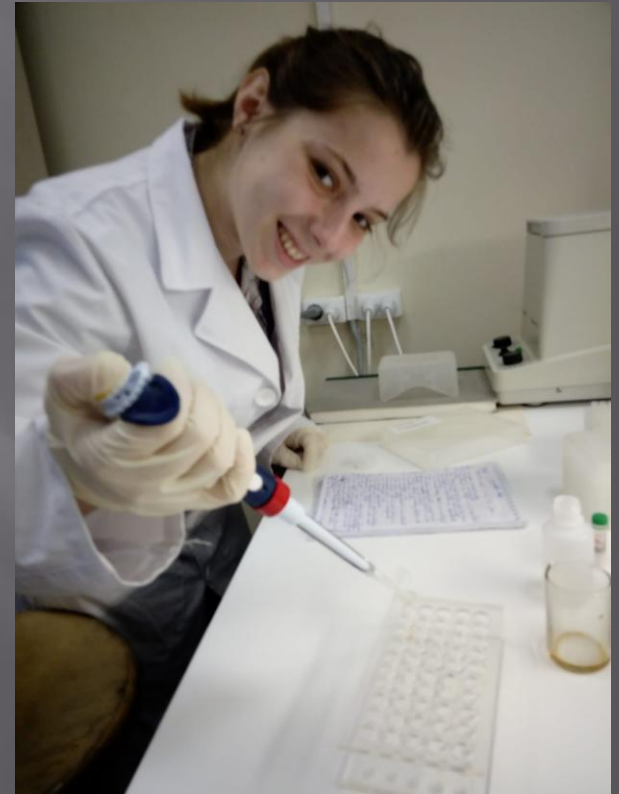
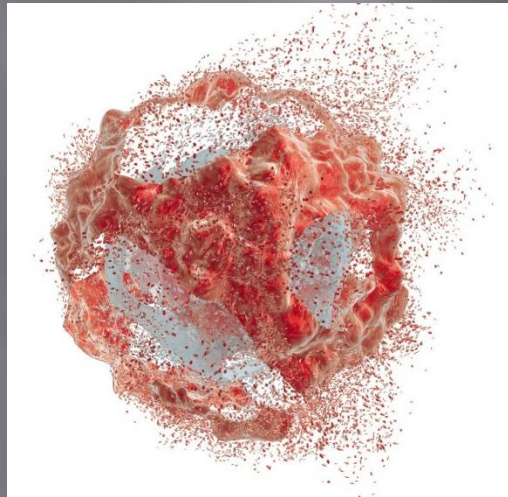
Lysis of cell

• lysis buffer

Absorption of
DNA

Washing DNA

DNA extraction
from silica solution



1. DNA Isolation

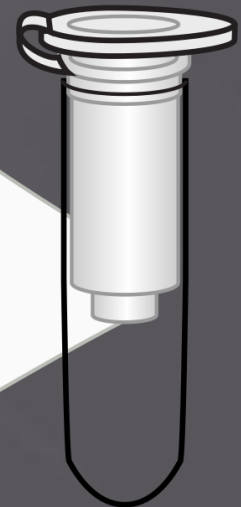
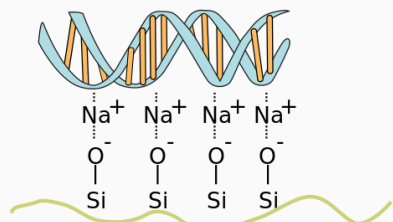
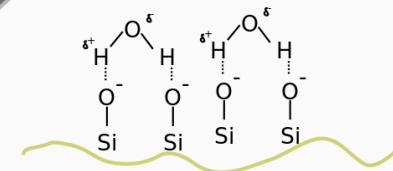
Lysis of cell

Absorption of DNA

Washing DNA

DNA extraction from silica solution

• silica solution (Nucleo S)



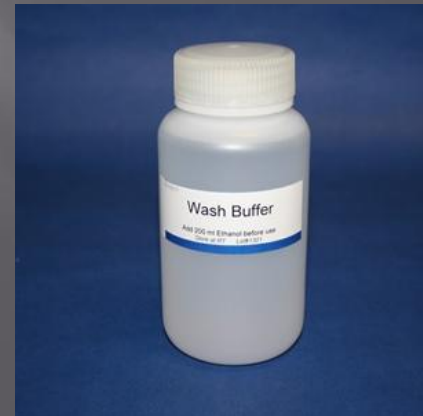
1. DNA Isolation

Lysis of cell

Absorption of
DNA

Washing DNA • solution buffer

DNA extraction
from silica solution



1. DNA Isolation

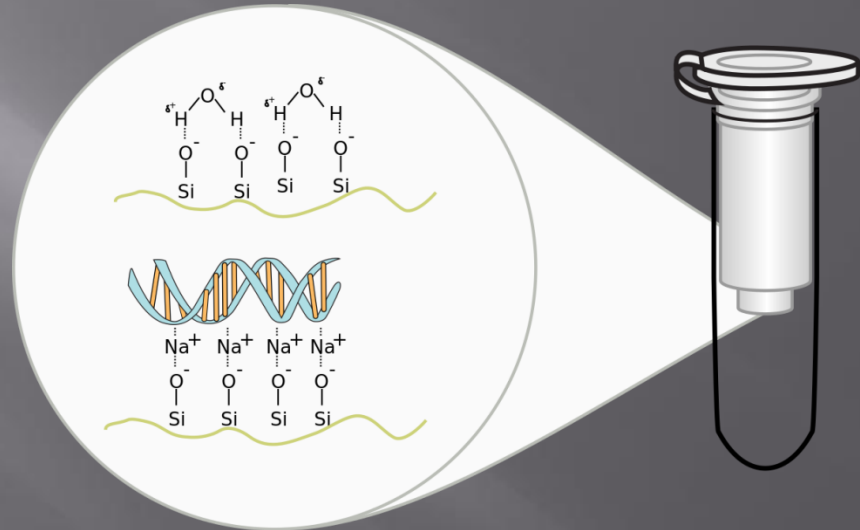
Lysis of cell

Absorption of
DNA

Washing DNA

DNA extraction
from silica solution

• ExtraGene™

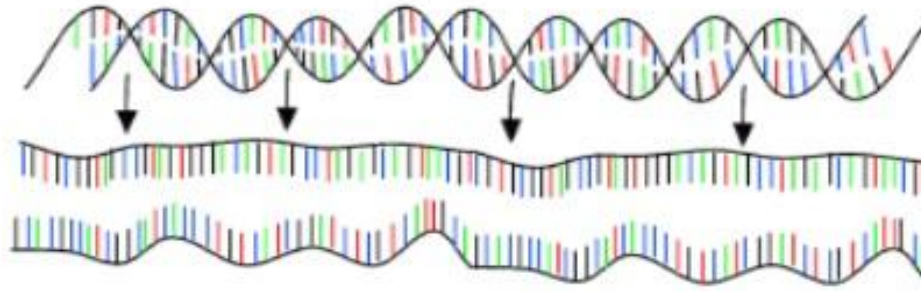


PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :

Step 1 : denaturation

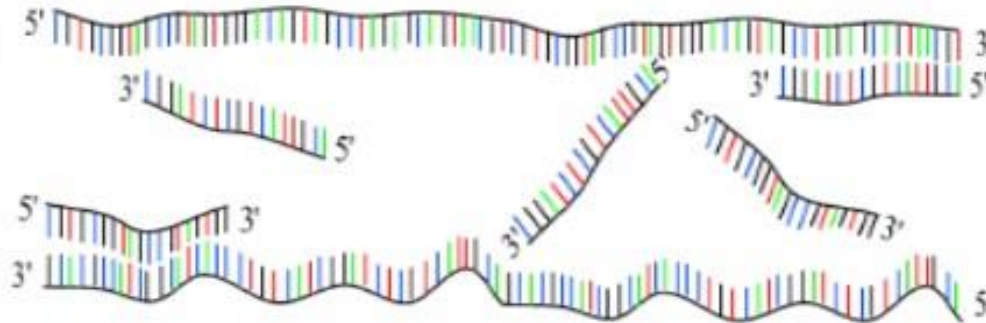
1 minut 94 °C



Step 2 : annealing

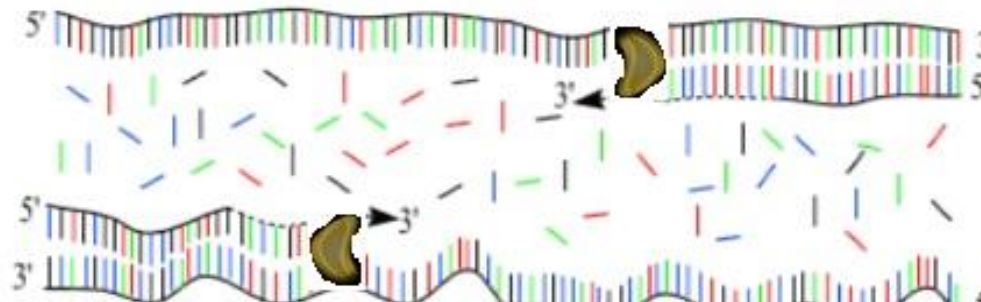
45 seconds 54 °C

forward and reverse primers !!!

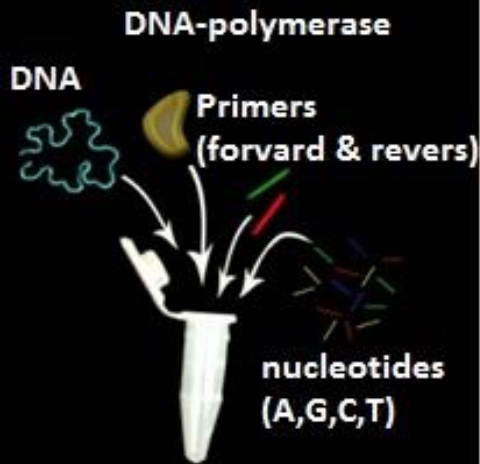


Step 3 : extension

2 minutes 72 °C
only dNTP's

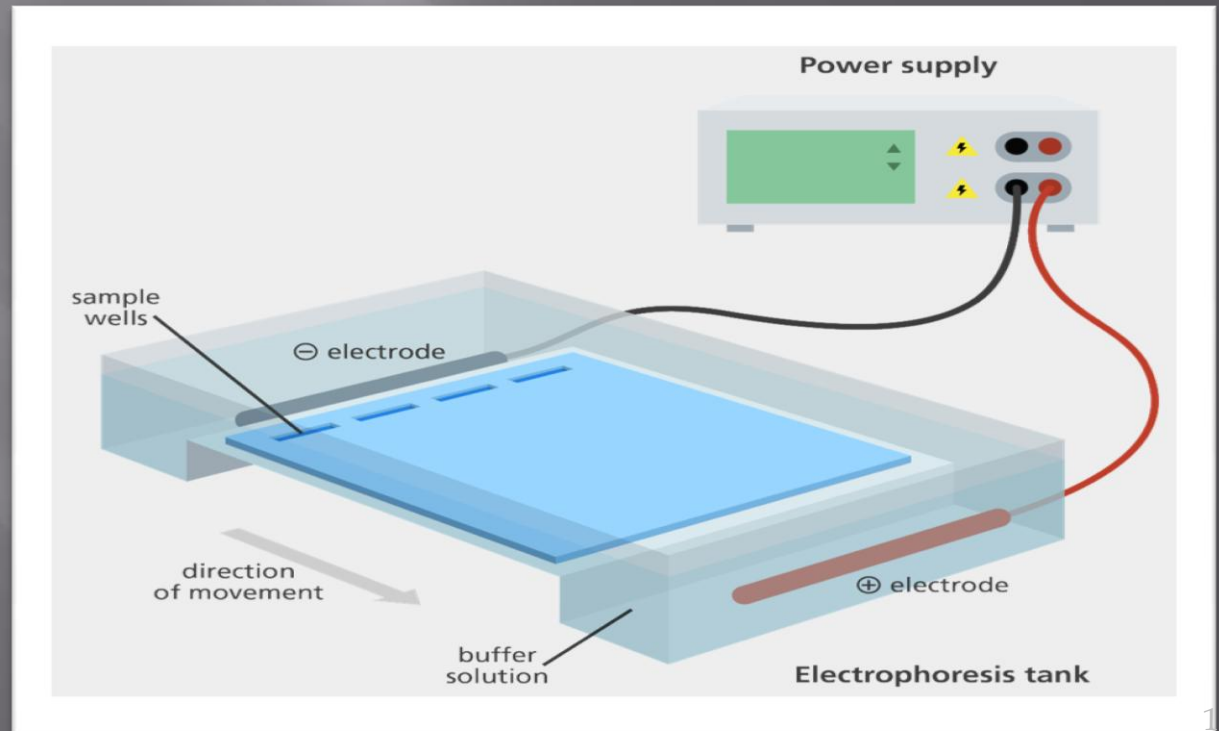


Components of reaction



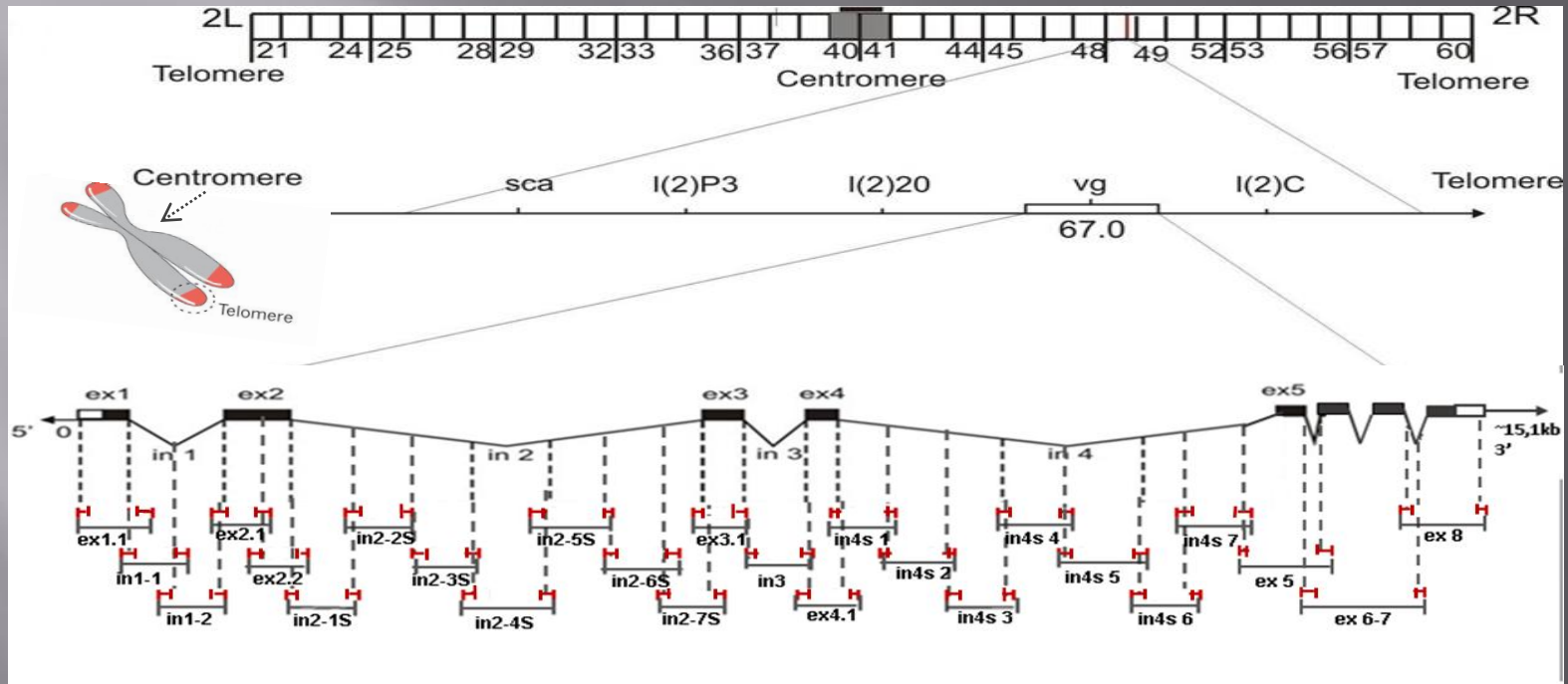
3. Gel Electrophoresis

- DNA has negative charge
- separation of DNA fragments on 1% agarose gel
- visualisation: ethidium bromide + UV



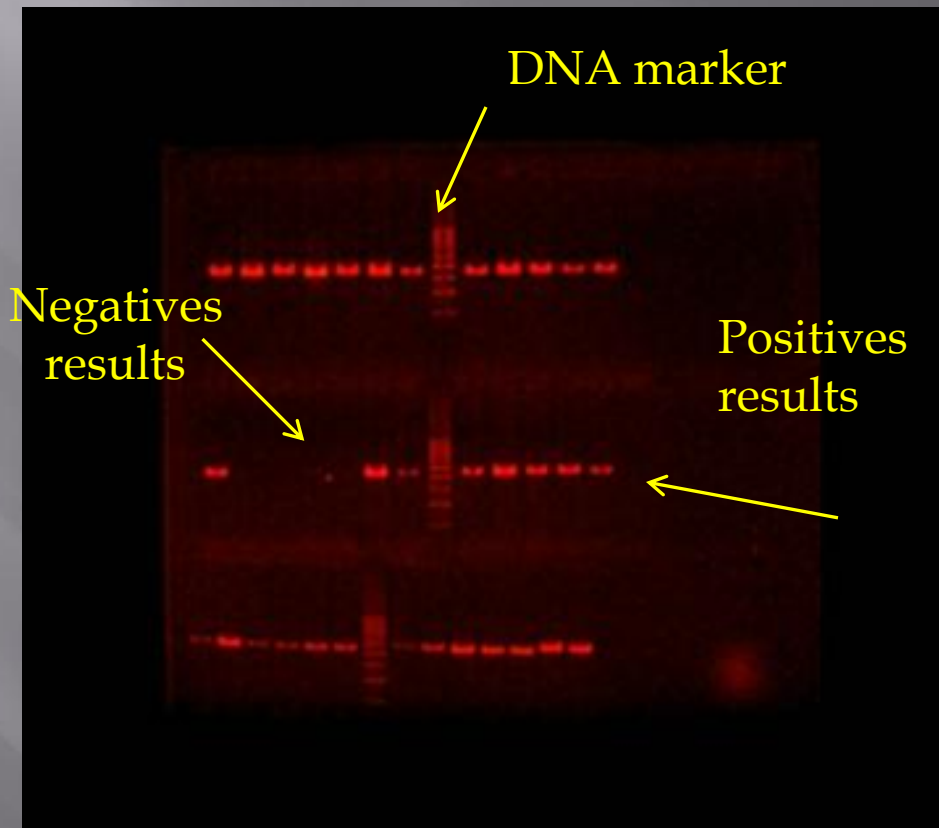
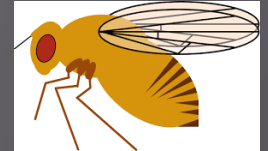
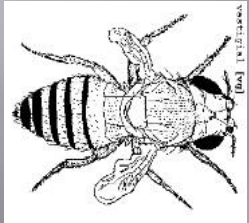
Scheme of vestigial gene

25 fragments of vestigial gene

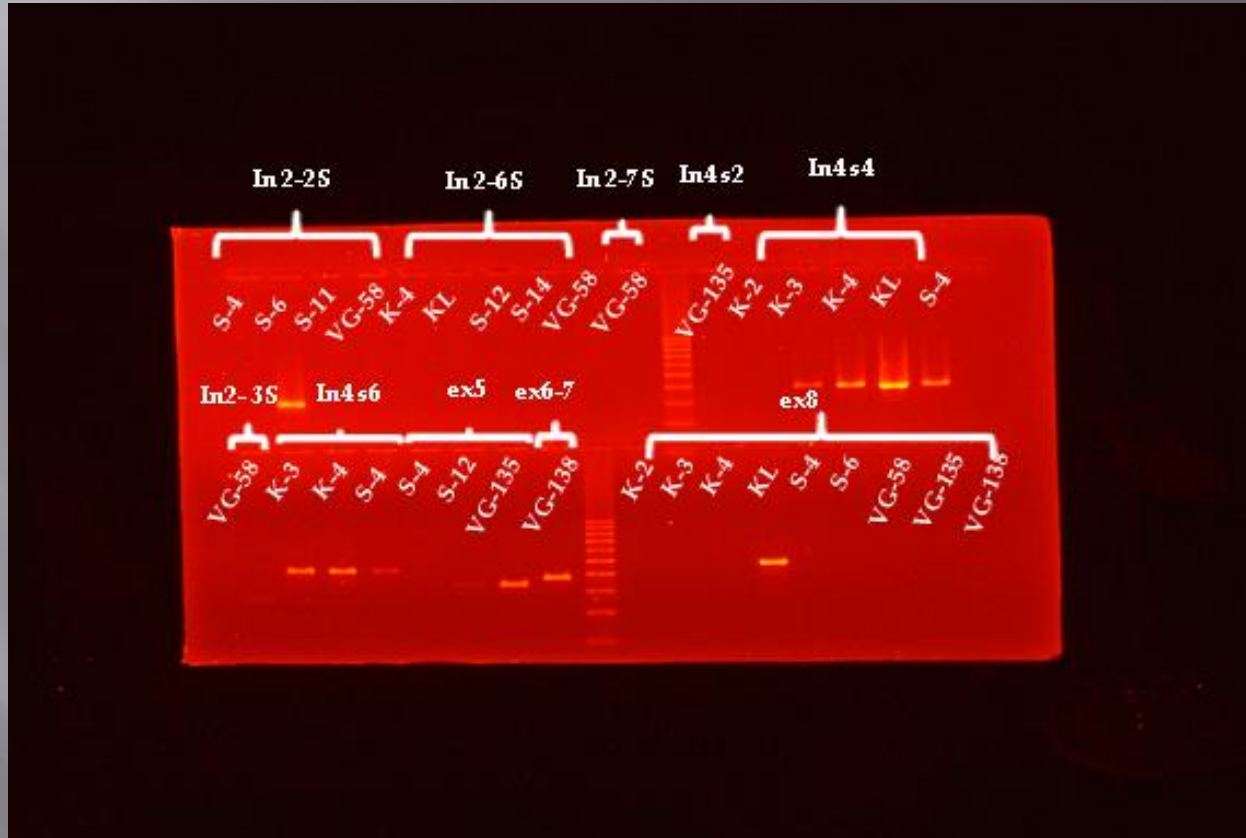


12 lines: wild, spontaneous and irradiated
We can find the fragment where a mutation occurred

Visualization of Electrophoresis Using UV Light



Visualization of Electrophoresis Using UV Light



- Different fragments with different size
 - Our resolution 50 base pair



Measurement and results

- more than 300

name of line	source of radiation, dose	fragment of the vestigial gene																								
		ex1.1 [45b]	int-1 [80b]	int-2 [60b]	ex2.1 [94b]	ex2.2 [17b]	int-15 [44b]	int-25 [56b]	int-35 [24b]	int-45 [30b]	int-55 [71b]	int-65 [32b]	int-75 [26b]	ex3.1 [10b]	int8 [12b]	ex4.1 [32b]	int4.1 [27b]	int4.2 [10b]	int4.3 [26b]	int4.4 [6b]	int4.5 [26b]	int4.6 [36b]	int4.7 [36b]	ex5 [10b]	ex6-7 [22b]	ex8 [20b]
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
K-2	..	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	-
K-3	..	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
K-4	..	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
KL	..	+	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+
S-4	spontaneous	+	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-
S-6	spontaneous	+	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
S-11	spontaneous	+	-	+	+	-	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
S-12	spontaneous	+	-	+	+	-	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+
S-14	spontaneous	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
vg 58	y, 40	+	+	-	+	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-
vg 135	n, 10	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-
vg 138	n, 10	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
K**	..	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Gold rule: we repeat all our negative results!



We have one fragment mutation



two fragments mutations



three or more mutations



Measurement and results

name of line	source of radiation, dose	fragment of the vestigial gene																								
		ex1.1 [B47b]	ins-1 [K50b]	ins-2 [K00b]	ex2.1 [P94b]	ex2.2 [P17b]	in2-15 [B44b]	in2-25 [K36b]	in2-35 [K24b]	in2-45 [P30]	in2-55 [P71b]	in2-65 [K32b]	in2-75 [P26b]	ex3.1 [P10b]	in3 [K12b]	ex4.1 [P33b]	in4.1 [P27b]	in4.2 [K10b]	in4.3 [P26b]	in4.4 [K06b]	in4.5 [P26b]	in4.6 [K36b]	in4.7 [P35b]	ex5 [B01b]	ex6-7 [P22b]	ex2 [K20b]
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
K-2	..	+	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-	
K-3	..	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	
K-4	..	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	

Control K 2-4: normal phenotype but several mutations!



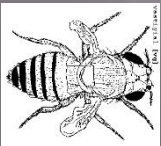
Constructive line KL: all mutations are presented (black, cinnabar, vestigial, yellow and white)

4	KL	..	+	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+
5	S-4	sponte nious	+	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+
6	S-6	sponte nious	+	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
7	S-11	sponte nious	+	-	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
8	S-12	sponte nious	+	-	+	+	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+
9	S-14	sponte nious	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+

Spontaneous mutations

1	vg 58	γ, 40	+	+	-	+	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+	-
2	vg 138	n, 10	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-
3	vg 138	n, 10	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-

γ-rays and neutron irradiated



Conclusions

- By PCR and electrophoresis we found the specific fragments in our gene where mutation appeared
- In order to precisely tell which mutations have been responsible for the changes in the phenotype of drosophila, sequencing is needed

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THANK YOU FOR YOUR
ATTENTION!

