4
3
5
2
6
1
JOINT INSTITUTE FOR NUCLEAR RESEARCH
26/07/2019

LABORATORY OF

NUCLEAR PROBLEMS

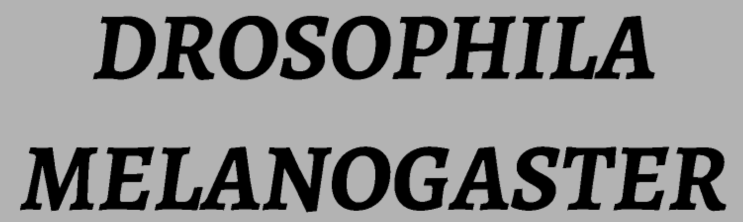
PCR-assay of intragenic DNA lesions induced by ionizing radiation at the vestigial gene of Drosophila melanogaster

Practical approach Leader: *Kristina P. Afanasyeva*

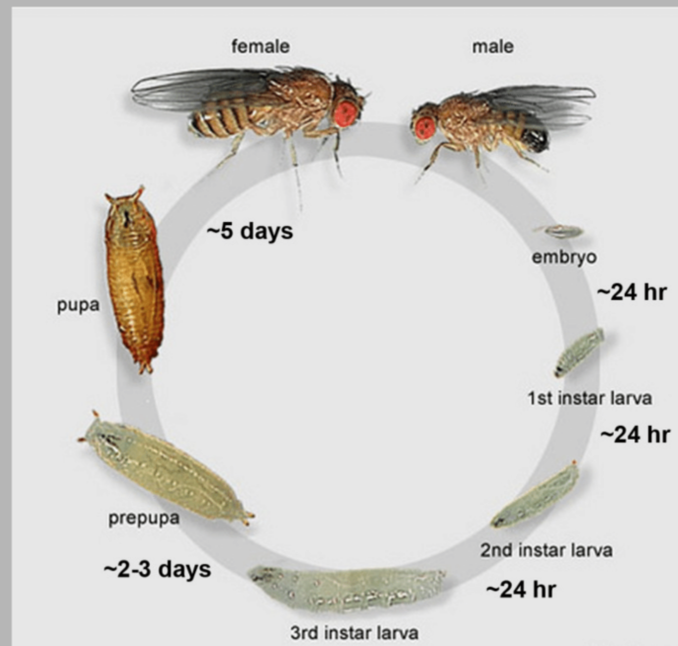
Overall Leader: *Igor D. Alexandrov*

Biricioiu Maria-Roxana,
West University of Timisoara
Romania

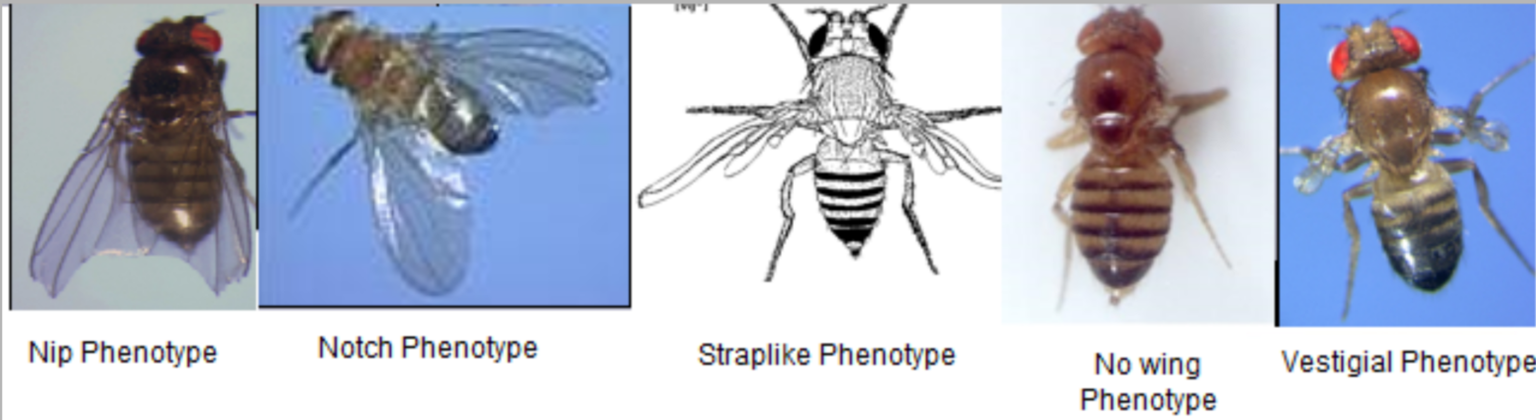
4
3
5
2
6
1
JOINT INSTITUTE FOR NUCLEAR
RESEARCH
26/07/2019


DROSOPHILA
MELANOGASTER

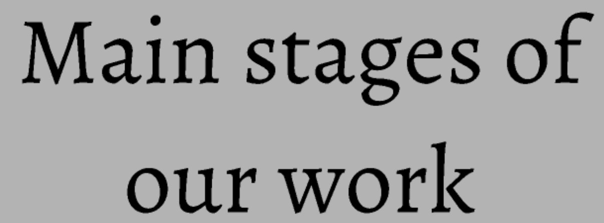
Drosophila melanogaster is commonly known as the vinegar fly.
One of the best model organism in genetics and radiobiology because:
-It has a short life cycle (about 10 days at room temperature) so several generations can be studied within a few weeks.
-It has only four pairs of chromosomes



Vestigial gene is charged with normal development of wings. Mutation of this gene conducts to reduction of wings in various degrees.



4
3
5
2
6
1
JOINT INSTITUTE FOR NUCLEAR
RESEARCH
26/07/2019



DNA isolation

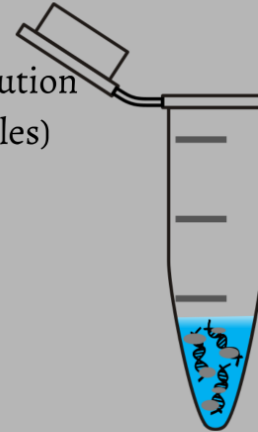
PCR

ELECTROPHORESIS

DNA isolation

We isolated DNA with two methods:

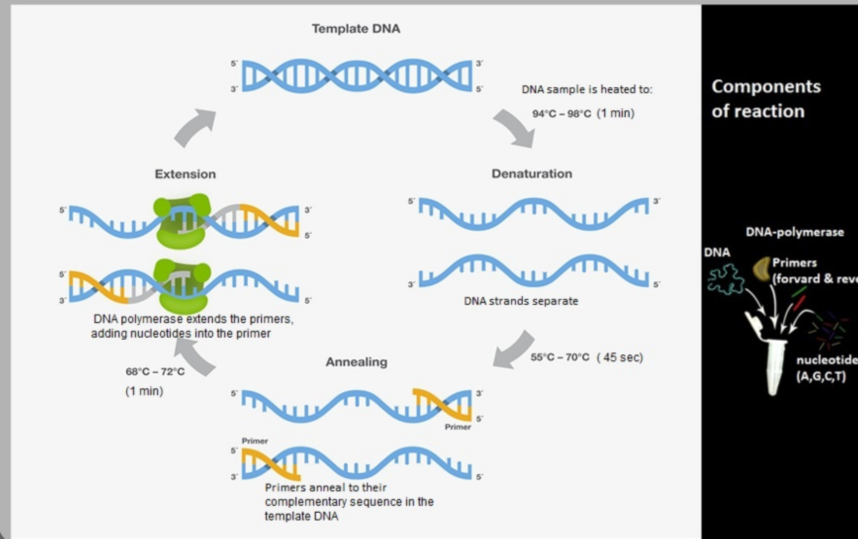
I)
- Cell lysis
- DNA absorption using Silica Solution (Nucleo S)
- DNA purification using Buffer solution
- DNA extraction (from silica particles) using Extra gene



II)
- Fly Crushing Buffer
- Acetate with pH 5.5
- Chloroform
- Isopropanol
- 70% Ethanol

PCR-
Polymerase Chain Reaction

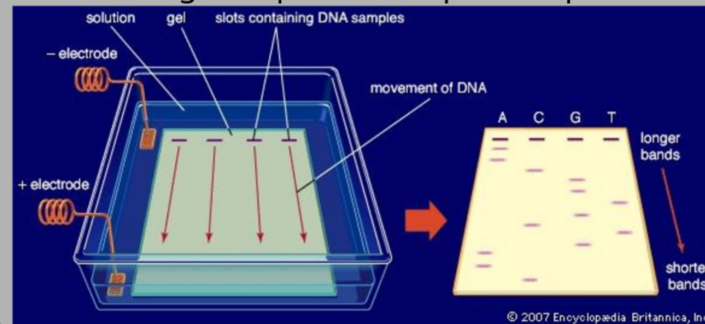
PCR is a technique to make many copies of a particular section of DNA



Electrophoresis in agarose gel

Agarose gel 1%:
-0,7g Agarose
-70 ml Buffer TBE(Tris,Boric acid,EDTA)
-Colorant – ethidium bromide
Conditions:100V, 30 min
Visualisation: UV

Since DNA has negative charge, the electrodes make the DNA run from the negative pole to the positive pole.

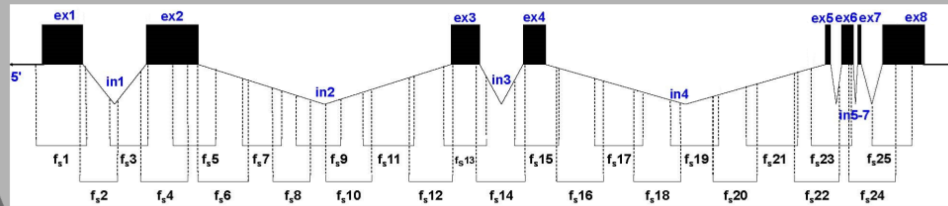


4
3
5
2
6
1
JOINT INSTITUTE FOR NUCLEAR RESEARCH
26/07/2019

The goal of our researches:

-To study vestigial gene (25 fragments) for all mutants for understanding if some deletion is in the mutant gene

-To study the size and localization of deletions

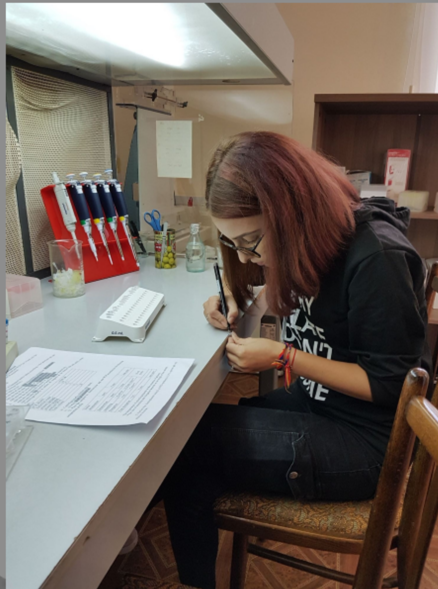


Gene vg= 14 754 bp (8 ex+7 in)

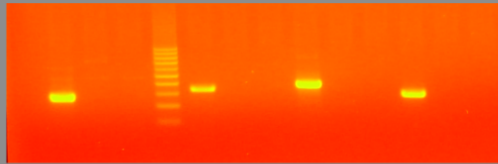
We isolated DNA several times with different numbers of flyes, even with one fly. We used both methods and we also measure the quality of DNA to compare them.

D18-1P	85.4 ng/µl
D32-1P	77.8 ng/µl
Or-2P	877 ng/µl
1F-1P	20 ng/µl
1F-2P	18.3 ng/µl
Vg 1	58 ng/µl
Vg 7	31.45 ng/µl

We prepared the samples for all the 25 fragments with one control sample, two types of vestigial gene mutation and one negative control. After that, we put the samples to amplificator.



After PCR we prepared our agarose gel for electrophoresis and then we put our samples in the gel to see if our fragments are present or not.



4
3
5
2
6
1
JOINT INSTITUTE FOR NUCLEAR RESEARCH
26/07/2019

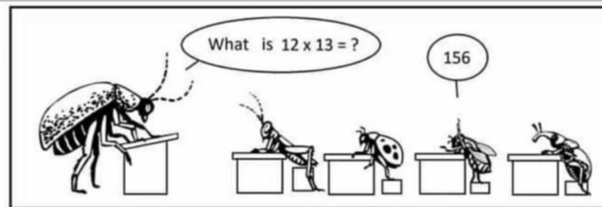
CONCLUSIONS

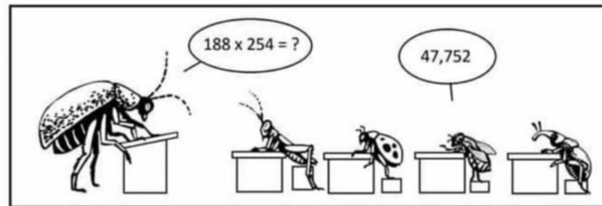
- The second method of DNA isolation gives us a bigger quality of DNA
- We found that exist some deletion in the last 4 fragments of vestigial gene 1.

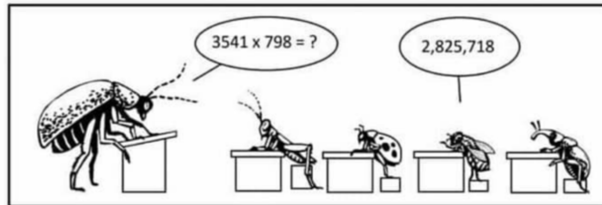
№	name of line	sorse of radiation, dose	fragment of the vestigial gene																							
			ex1.1 (845b)	in1-1 (630b)	in1-2 (636b)	ex2.1 (795b)	ex2.2 (718b)	in2-1S (844b)	in2-2S (656b)	in2-3S (624b)	in2-4S (751b)	in2-5S (771b)	in2-6S (852b)	in2-7S (726b)	ex3.1 (711b)	in3 (812b)	ex4.1 (739b)	in4s1 (787b)	in4s2 (810b)	in4s3 (786b)	in4s4 (802b)	in4s5 (736b)	in4s6 (858b)	in4s7 (650b)	ex5 (670b)	ex6-7 (783b)	ex8 (664b)
			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
1	C-1	--	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	vg1	γ, 40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
3	vg7	γ, 40	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
4	Neg.C	--	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

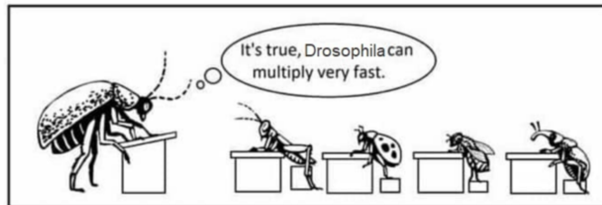
- I have new skils in DNA isolation with two methods, in PCR and electrophoresis in agarose gel.

4
3
5
2
6
1
JOINT INSTITUTE FOR NUCLEAR RESEARCH
26/07/2019









THANK YOU!

4
3
5
2
6
1
JOINT INSTITUTE FOR NUCLEAR
RESEARCH
26/07/2019