Proposal -2022

1. Title of Project:

   PCR and sequencing as tools for detecting intragenic changes induced by ionizing radiation in the *vestigial* gene of *Drosophila melanogaster*.

2. Project Leader:

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3. Project Summary:

   3.1. Goal of Project:

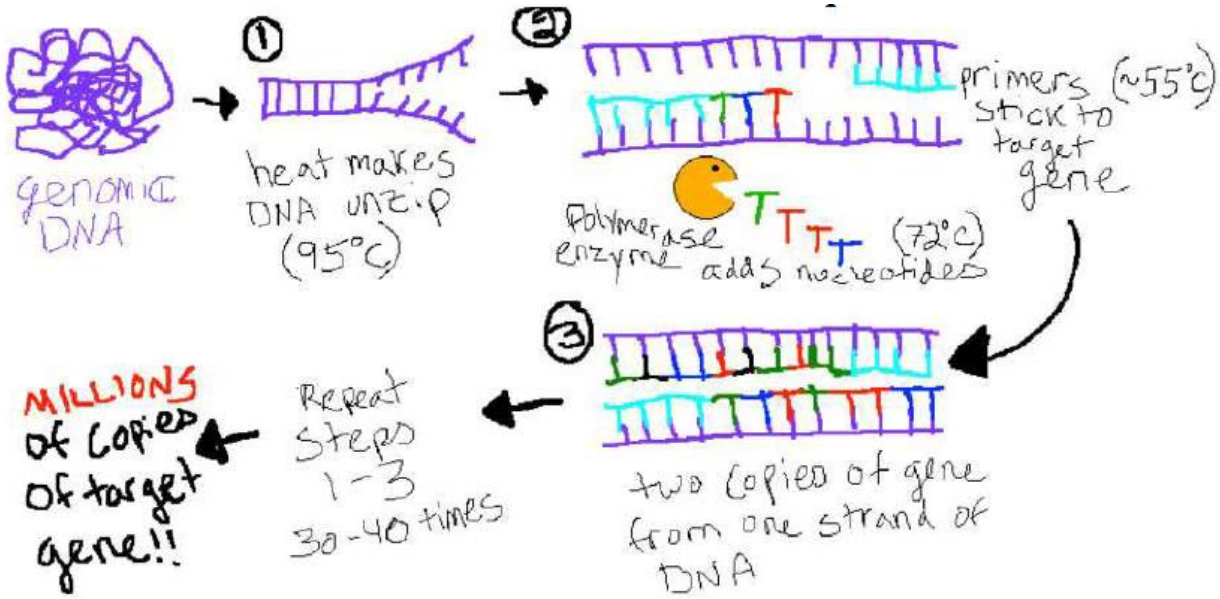
To clarify the nature and location of DNA alterations induced by γ-rays and neutrons at the *vestigial* gene of *Drosophila melanogaster*.

   3.2. Background and Topicality of Project:

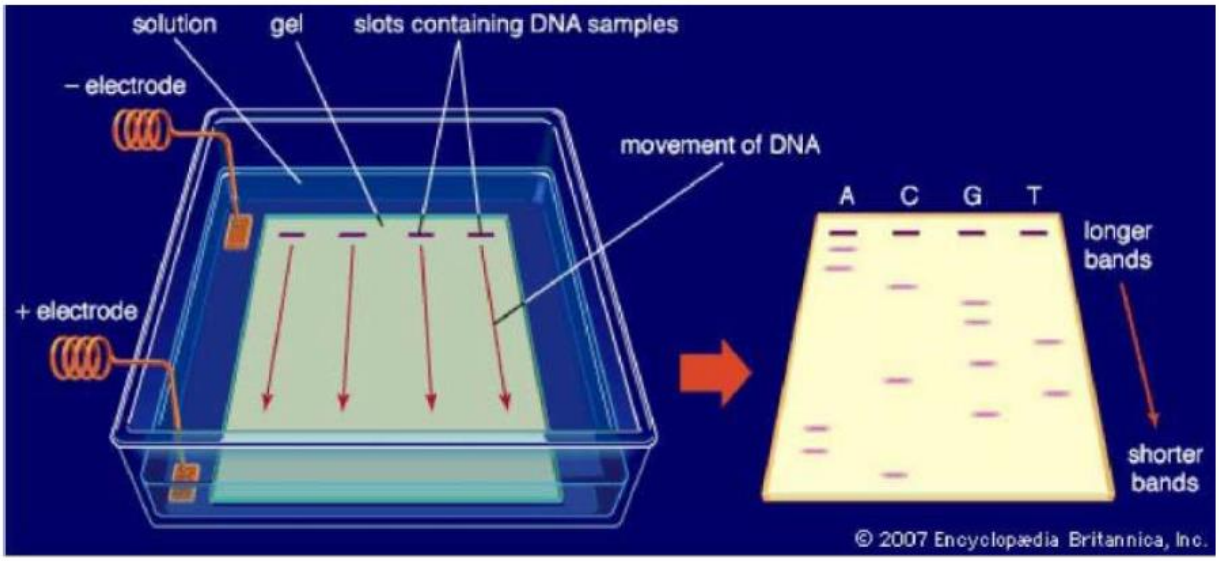
A large body of experimental data shows that deletions of the greater part or a whole gene in mammalian somatic cells in vitro or in vivo are mainly induced by different quality radiation (for example, see [1, 2]).

But no wide molecular analysis of gene mutations induced by γ-rays and neutrons in animal germ cells was performed so far. In the meantime, a better knowledge of the molecular nature of heritable gene mutations is of great fundamental and applied significance being the scientific basis for assessment of the potential genetic risks of radiations for progeny and populations. To study this issue and to detect the mode of intragenic distribution of radiation-induced DNA alterations, a random sample of γ-rays and neutrons induced heritable mutations at the *vestigial* gene of *Drosophila melanogaster* and the polymerase chain reaction (PCR) and sequencing techniques for analysis of DNA structure are suggested to use.

Scheme of the PCR technique



Scheme of agarose gel electrophorese



3.3. Research Program:

(1) Acquainting with the collection of radiation-induced *Drosophila melanogaster* mutants (*yellow*, *cinnabar*, *black*, *white*, *vestigial* genes);

(2) Introducing to functions and molecular structure of the *vestigial* gene;

(3) The theory of molecular genetics and principal steps of DNA isolation;

(4) Isolation of wild-type and mutant genomic DNA by several ways;

(5) Assessment of quality and concentration of DNA

(6) The theory of PCR (basic protocols and optimization strategies)

(7) Optimization of PCR for fragments under study;

(8) PCR in practice;

(9) Performance of agarose gel electrophorese for visualization and documentation resulting products of PCR;

(10) Analysis and discussion of results;

(11) Cleansing and quality control of samples. Sample preparation for sequencing;

(12) Principle of Sanger sequencing analysis. The easy way.

3.4. Expected Results:

For the first time to identify the DNA alterations at mutants irradiated by γ-rays and neutrons.

You will get new skills in:

(1) DNA isolating

(2) PCR

(3) Agarose gel electrophorese

(4) Cleansing of DNA samples

(5) Sequence analysis

References

1. Rothkamm K., Gunasekara K. et all. ‘Radiation -induced HPRT mutations resulting from misrejoined DNA double -strand breaks’. Radiation Res. 2008, 169, p. 639-648

2. Mognato M., Ferraro P., et all. “Analysis of mutational effect at the HPRT locus in human G0 phase lymphocytes irradiate in vitro with –rays” Mutational research, 2001, 474(1-2) p.147-158.