Proposal -2017

1. Title of Project: “PCR-assay of intragenic DNA lesions induced by ionizing radiation at the *vestigial* gene of *Drosophila melanogaster*”

2. Project Leaders:
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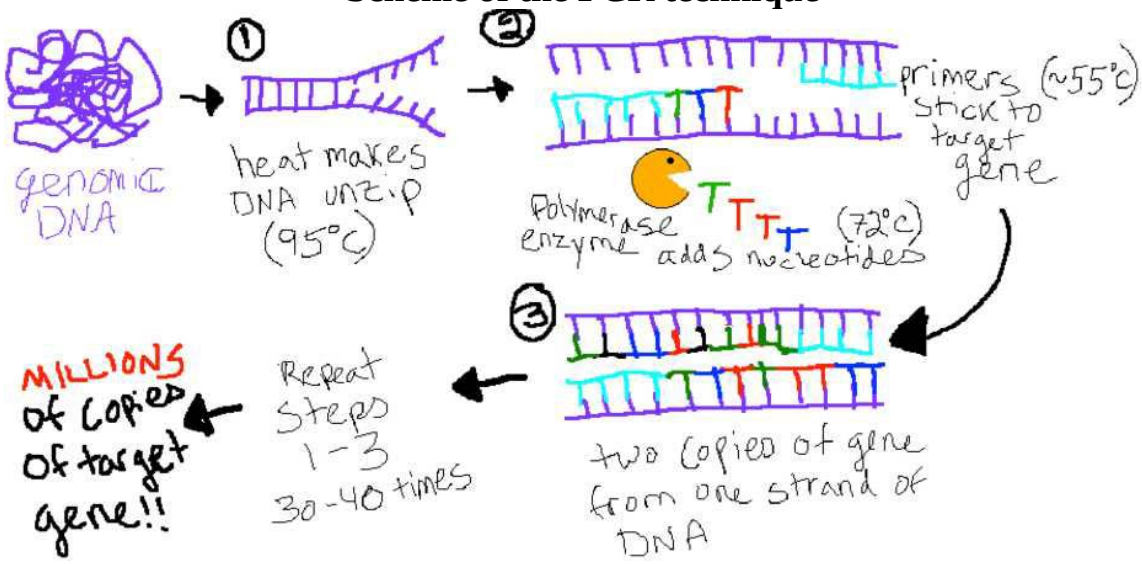
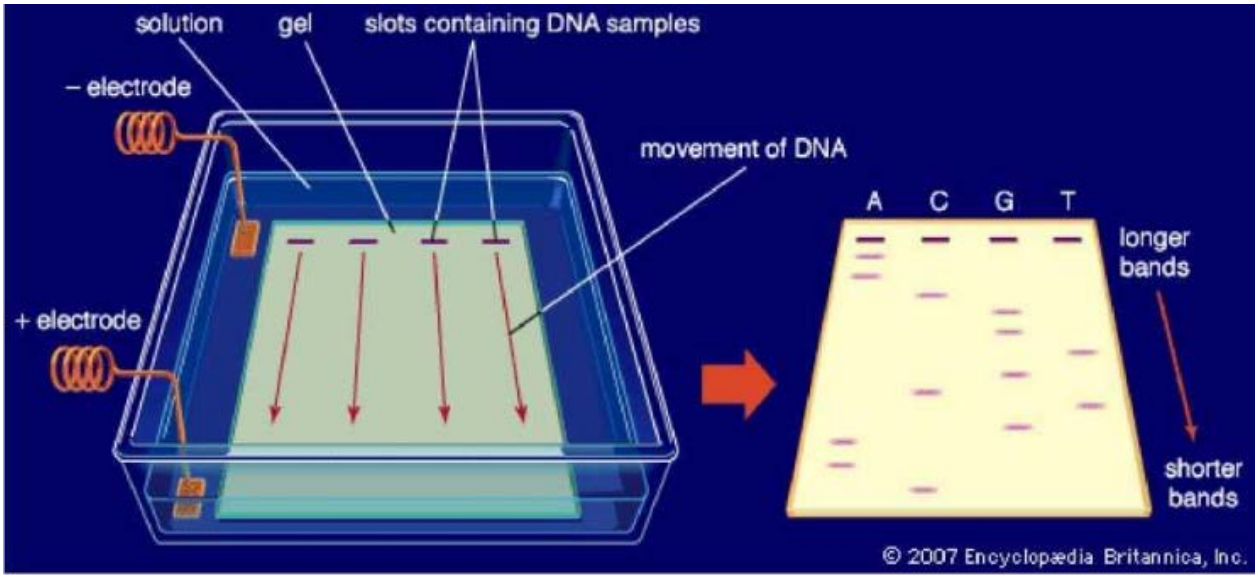
3. Project Summary:
3.1. Goal of Project:
The goal of the Project is to detect the nature and location of DNA alterations induced by γ-rays and neutrons at the introns of *vestigial* gene of *Drosophila melanogaster*.

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) technique for analysis of DNA structure are suggested to use.

Scheme of the PCR technique



Scheme of agarose gel electrophorese



3.2. Research Program:

The PCR - assay of quality and frequency patterns of intragenic DNA alterations should include the following research steps:

(i) We show you our collection of radiation-induced *Drosophila melanogaster* mutants (*yellow*, *cinnabar*, *black*, *white*, *vestigial* genes);
(ii) Introducing to functions and molecular structure of the *vestigial* gene;
(iii) The theory of molecular genetics and principal steps of DNA isolating
(iv) Isolation of wild-type and mutant genomic DNA as template;
(v) The theory of PCR (basic protocols and optimization strategies)
(vi) Optimization of PCR for fragments under study;
(vii) Performance of PCR -assay;
(viii) Performance of agarose gel electrophorese for visualization and documentation resulting products of PCR;
(ix) Assessment of quality and frequency patterns of DNA alterations observed for the gene under study after action of γ-rays.

3.3. Expected Results:

The new results expected are following:

(i) For the first time to identify the quality and frequency patterns of DNA alterations observed;
(ii) To detect the intragenic distribution of different DNA alterations relative to the exon-intron structure of the gene under study

You will get a new skills in:

(i) DNA isolating
(ii) PCR
(iii) Agarose gel electrophorese

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.