Annex 3.

Form of opening (renewal) for Project / Sub-project of LRIP

APPROVED

JINR DIRECTOR

_____ /_____

"____" _____ 202 г.

PROJECT PROPOSAL FORM

1. General information on the research project

1.1 Theme code 04-2-1132-2017/2023

1.2 Project/LRIP subproject code (for extended projects)

1.3 Laboratory Dzhelepov Laboratory of nuclear problems

1.4 Scientific field Life science

1.5 Title of the project The molecular genetics of radiation-induced changes at the gene and genome level in *Drosophila melanogaster*. PROJECT RADIOGENE

1.6 Project leader Afanasyeva Kristina

1.7 Scientific leader Alexandrov Igor

1.8 Project deputy leader Rusakovich Artem

2 Scientific case and project organization

2.1 Annotation

Currently, we are conducting research on the study of de novo inherited genetic changes in the offspring of irradiated *Drosophila melanogaster* parents. In the new five-year period, we plan to continue and expand the data of the research. For this purpose, molecular genetic experiments will be carried out in the following areas:

1) Analysis of inherited gene changes induced by Co60 γ-rays and neutrons in germ cells of *D. melanogaster* male parents (PCR and Sanger sequencing methods);
2) Whole genome sequencing of the offspring of the γ-irradiated ***D. melanogaster*** males on the MGI platform and bioinformatic analysis of the sequencing results;
3) Whole genome sequencing of the offspring of the γ-irradiated ***Mus musculus*** males on the MGI platform and bioinformatic analysis of the sequencing results;
4) Whole genome sequencing of the offspring of *D. melanogaster* males irradiated with new types of ionizing radiation: a) high-energy electrons (5 and 100 MeV, LINAC-200 accelerator, DLNP); b) light and heavy ions (NICA complex, LHEP);
5) Analysis of the expression of *D. melanogaster* mutant genes to establish the relationship between the nature of DNA damage and gene activity.

New data from areas 1-4 will be of great fundamental and applied importance for predicting the genetic consequences of radiation exposure at the individual and population levels. Also, these data can be a scientific basis for predicting the genetic consequences of exposure to various types of ionizing radiation in humans.

2.2 Scientific case

2.2.1 The aims of the project

1. Study of *de novo* genetic changes in the offspring of irradiated parents (*Drosophila* and mice)
2. Analysis of the expression of *D. melanogaster* mutant genes to establish the relationship between the nature of DNA damage and gene activity.

2.2.2 Relevance and scientific novelty

Modern ideas about de novo genetic changes in the offspring of irradiated parents are based mainly on the data of classical radiation genetics of Drosophila and mice. This data characterizes the frequency of inherited gene mutations in the germ cells of parents after exposure to rare ionizing radiation [1–4]. It is these data that became the scientific basis for the first assessments of the genetic hazard of rare ionizing radiation in the induction of such mutations [5]. However, the question of the nature of DNA changes in such mutations remained unknown. The latest results of a comprehensive genetic, cytogenetic, and molecular analysis of radiation-induced mutations of individual genes in Drosophila have shown their complex genetic nature [6]. Molecular analysis of gene radiation mutations by PCR and Sanger sequencing revealed a complex spectrum of DNA changes for different genes and types of radiation (γ-quanta and neutrons). Further studies in this area will reveal the general patterns and gene-specific features of radiation mutagenesis in *D. melanogaster* germ cells. However, inherited gene mutations are only one part of a complex spectrum of genetic changes induced in germ cells. Therefore, it does not give a complete picture of the nature and extent of de novo genetic changes received by offspring from irradiated parents.

For the first time, advances in DNA and IT technologies open up the possibility of studying the genetic consequences in the offspring of irradiated parents at the DNA level of the whole genome. Research in the area of radiation genomics is new and the results of the first studies on mice showed that acute X-rays irradiation (3Gy, LD_{50}) several times increases the frequency of inherited structural changes in the genome DNA, such as large deletions, duplications (Copy number variants - CNVs), and also smaller insertions and deletions (indels). [7]. Since the data obtained in the Drosophila-mouse system

are decisive for extrapolation to humans, genomic studies on these types of animal organisms under the same experimental conditions are of particular relevance.

The first results of our pilot experiment on *Drosophila* on whole genome sequencing and bioinformatics analysis have already shown that almost all of the 9 studied offspring of γ-irradiated males (40 Gy) had the same multiple structural changes in DNA as in mice Fig. 1 [8]. Further expansion of the already begun genomic studies on Drosophila with the inclusion in the analysis of a new object - the mouse, and with the involvement of new types of rare and dense ionizing radiation are the main directions of research planned for a five-year period.

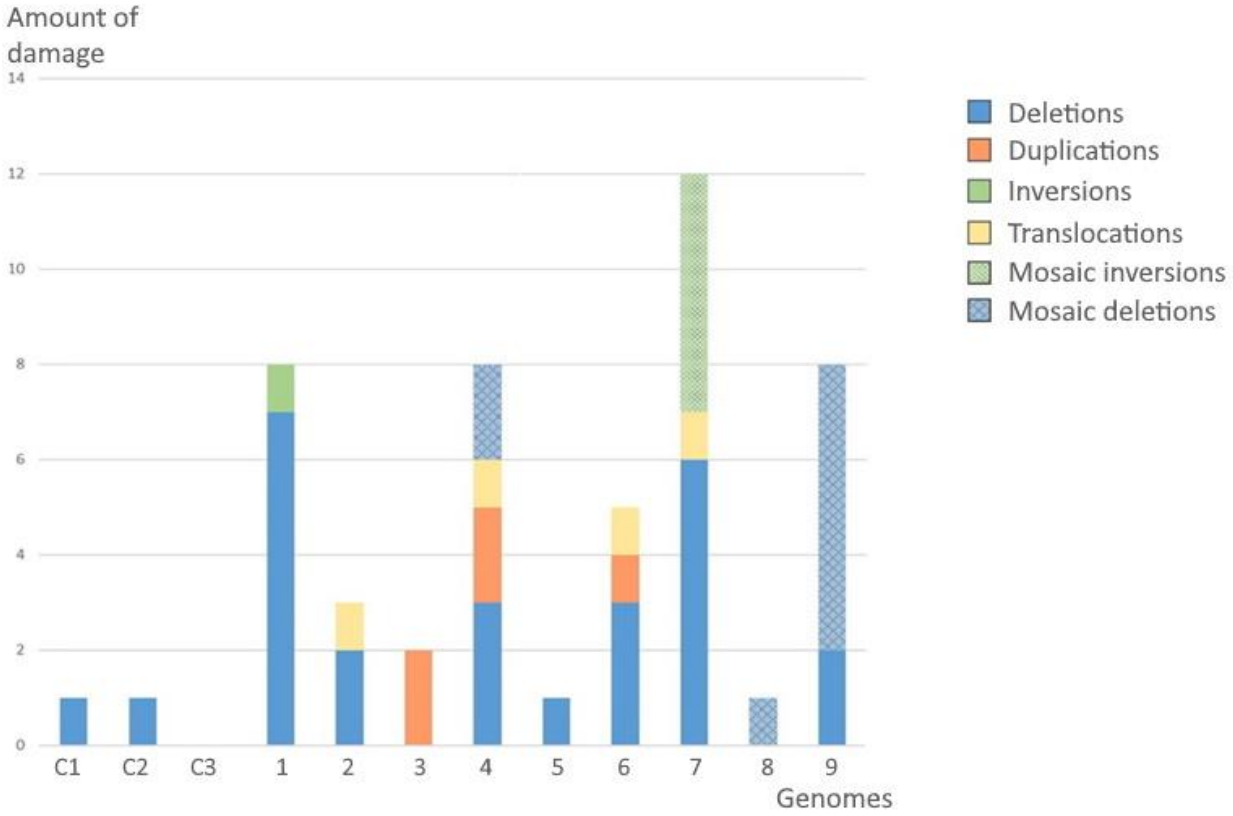

Fig. 1. Number and types of DNA changes in the genomes of offspring from control (K1-K3) and γ-irradiated (40Gy, Co^{60}) *D. melanogaster* males.

2.2.3 Approaches

The approach of the planned studies is a comparative analysis:

1. Analysis of inherited DNA changes in different genes under the action of γ-radiation and neutrons (the first area of work);
2. Analysis of genomic changes in the offspring of *Drosophila* males and mice irradiated with Co^{60} γ-quanta (second and third areas of work);
3. Analysis of genomic changes in the offspring of *Drosophila* males irradiated with different types of radiation with different LET (fourth area);
4. Comparative analysis of expression of radiation alleles of the studied genes with a known pattern of inherited DNA changes (fifth area).

2.2.4 Methods and expected results

In accordance with the idea of the work in the first area, the following methods will be used:

- isolation of genomic DNA from biological material using reagent kits;
- design of primers for polymerase chain reaction (PCR) and their synthesis;
- PCR with standard and long protocol
- purification and Sanger sequencing of the obtained DNA fragments of the gene

- analysis of sequencing results using Sequence scanner v1.0 and Ugene software.

As a result, it is expected to obtain new data which characterize the nature and gene map distribution of inherited DNA changes induced by γ-radiation and neutrons. The obtained data will also make it possible to compare the spectra of γ- and neutron-induced DNA changes and to evaluate the genetic efficiency of densely ionizing neutrons compared to rare ionizing γ-rays in inducing these changes. At the same time, these results can become a scientific basis for a comparative assessment of the genetic hazard of the two studied types of radiation during the induction of inherited gene mutations.

In the process of multi-stage genomic research in the second - fourth areas, it is planned:

- Carrying out experiments on irradiation of *Drosophila* males with γ-rays at doses of 1, 5, 10, 20, 40 Gy (LD_5-LD_{85}) and male mice at a dose of 3 Gy (LD_{50}) (source of γ-rays Co^{60} 17165, FLNR).
- Carrying out preliminary experiments on *Drosophila* with sources of accelerated electrons and ions to determine LD_{50} after exposure to these types of radiation (LINAC-200 DLNP, NICA complex, LHEP).
- Carrying out experiments on irradiation of *Drosophila* males with accelerated electrons and ions in established doses with LD_{50}.
-The next stage of work after irradiation in all cases will be the crossing of irradiated males with females to obtain the first generation. They will be used as material for subsequent molecular genetic analysis of their genome (20-30 offspring in each experiment).
- Isolation of total genomic DNA from individual offspring by the phenol-chloroform extraction.
- New generation sequencing of offsprings genomes on the MGITech or Illumina platform (Kurchatov Genomic Center).
- Bioinformatic analysis of the results of whole genome sequencing (Kurchatov Genomic Center).

The expected results in the second direction will allow for the first time to obtain a full range of changes in the DNA of the genome, which may include: base substitutions, large deletions and duplications, indels, intra- and inter-chromosomal exchanges. At the same time, these data will make it possible to establish the nature of dose dependences for genomic changes after exposure to rare-ionizing γ-rays (as standard) and to assess the possibility of extrapolating effects to lower doses, which are of interest for human radiation genetics.

New data in the third area will expand our understanding of the effect of rare ionizing radiation at the level of the mouse genome. The mouse is an essential element in the Drosophila-mouse model system, which is important for extrapolation to humans.

The results of the fourth area will for the first time make it possible to obtain data on the features of genome changes after exposure to ionizing radiation with fundamentally different mechanisms of interaction with matter compared to γ-radiation.

The results of the fourth area will for the first time make it possible to obtain data on changes in the genome after the action of ionizing radiation with fundamentally different mechanisms of interaction with matter compared to γ-rays.

It is planned to use the following methods and equipment to carry out work in the fifth area:

- Total RNA isolation from mutants selected for analysis using commercial kits (Evrogen) and the necessary equipment: a) UVC/T-AR PCR box), b) microcentrifuge with cooling, c) individual set of microdispenser pipettes;
- obtaining cDNA based on isolated RNA using reverse transcription with a BioRad T100 Thermal Cycler equipment;
- Design of primers for cDNA of three studied genes using software;
- Carrying out cDNA amplification using the obtained primers and commercial kits of reagents (Evrogen) with an assessment of the activity of expression of mutant alleles of genes, using a DNA real time amplifier CFx96 Touch Bio-Rad.

Expected results in the fifth area: data will be obtained that will characterize the transcriptional activity of the studied mutant alleles of the genes. As a next step, the dependence of transcriptional activity on the type and location of gene DNA damage at different stages of ontogeny, including adults, will be established.

2.2.5 Risks

For the planned project, a big risk of non-fulfillment of certain parts of the project is the underfunding of the project at one stage or another.

References

1. Alexander ML Mutation rates at specific autosomal loci in the mature and immature germ cell of *Drosophila melanogaster*. Genetics, 1954, v39, pp409-428
2. Muller H.J. Advances in radiation mutagenesis through studies on Drosophila. In Bugher J.C.: "Progress in Nuclear energy Series VI" New York, Pergamon Press, 1959, pp 146-160/
3. Russel W.L. Comparision of X-ray-induced mutation rates in Drosophila and mice. Am Nat, 1956, V90, pp 67-80
4. Searle A.G. Mutation induction in mice. In let JT, Adler HI, Zelle M: Advances in Dadiation biology" New York, Academic press, 1974, pp 131-207.
5. Neel J.V. Reappraisal of studies concerning the genetic effects of the radiation of humans, mice, and drosophila. Enviromental and molecular mutagenesis, 1998, v.31, pp 4-10.
6. Alexandrov ID, Alexandrova MV, Afanasyeva KP The Nature of Radiation-induced Inherited Recessive Gene Mutations in *Drosophila melanogaster*, Arch Mol Biol Genet. 2021 V1, Issue 1, pp12-19.
7. Adeolu B. Adewoye, Sarah J. Lindsay, Yuri E. Dubrova The genome-wide effects of ionizing radiation on mutation induction in the mammalian germline NATURE COMMUNICATIONS | 6:6684
8. Afanasyeva K.P., Alexandrov I.D. et al., "Genomic changes in the offspring of Drosophila melanogaster males irradiated with Co^{60} γ-rays". Scientific publication "Sakharov's Readings 2022: Environmental Problems of the 21st Century", Proceedings of the International Scientific Conference May 19-20, Minsk, p. 328.

2.3 Estimated completion date

Stages of work	Content of works	Artist of research work
2024	1. Work on PCR analysis of structural intragenic changes in γ- and neutron-induced mutants of the autosomal vestigial gene and the sex-linked white gene.Проведение экспериментальных работ на *D. melanogaster* по определению LD_{50} для пучка ускоренных электронов с энергиями 5 и 100 МэВ (Установка ЛИНАК-200, ЛЯП). 2. Conducting experimental work on *D. melanogaster* to determine LD_{50} for a beam of accelerated electrons with energies of 5 and 100 MeV (LINAC-200, DLNP). 3. Irradiation of *D. melanogaster* males with beams of accelerated electrons with energies of 5 and 100 MeV at doses equivalent in LD_{50}, established earlier. 4. Carrying out genetic work to obtain first-generation offspring from electron-irradiated males of *D. melanogaster* 5. Carrying out molecular work to isolate genomic DNA from individuals of the first generation. 6. New generation sequencing of genomic DNA of control and experimental offsprings. Sample size: 10 control and 30 irradiated genomes. 7. Bioinformatic analysis of sequencing results with identification of the types of DNA changes that have occurred 8. Statistical analysis of the obtained results.	JINR, DLNP FLNR LHEP
2025	1. Conducting genomic sequencing of the offsprings of *Drosophila* males irradiated with γ-rays at a dose of 10 Gy (source of γ-rays Co^{60} FLNR). Sample size: 10 control and 20 irradiated genomes. The stages of the experiment are similar to 2024 year, points 4-8. 2. Carrying out genomic sequencing of the offsprings of linear mice's male irradiated with γ-quanta at a dose of 3Gy, LD_{50}. Sample size: 6 control, 6 irradiated genomes. The stages of the experiment are similar to 2024 year, points 4-8.	JINR, DLNP JINR, FLNR JINR, LHEP
2026 г.	1. Conducting genomic sequencing of the offsprings of *Drosophila* males irradiated with γ-rays at doses of 1 and 40 Gy (source of γ-rays Co^{60} FLNR). Sample size: 10 control and 30 irradiated genomes. The stages of the experiment are similar to 2024 year, points 4-8. 2. Carrying out experimental work on D. melanogaster to determine LD50 for accelerated ions (NICA complex, FLNR).	JINR, DLNP JINR, FLNR
2027	1. Conducting genomic sequencing of the offsprings of *Drosophila* males irradiated with γ-rays at a dose of 5 Gy (source of γ-rays Co^{60} FLNR). Sample size: 10 control and 20 irradiated genomes. The stages of the experiment are similar to 2024 year, points 4-8. 2. Conducting genomic sequencing of the offsprings of *Drosophila* males irradiated with heavy ions (NICA complex). Sample size: 10 control and 20 irradiated genomes. The stages of the experiment are similar to 2024 year, points 4-8. 3. Preparatory work for the analysis of the expression of radiation-induced mutant alleles of the studied genes	JINR, DLNP JINR, FLNR
2028	1. Selection of mutant alleles of the gene under study (black, cinnabar, vestigial genes) to analyze their expression; 2. Isolation of total RNA from mutants selected for analysis using commercial kits and the necessary equipment: a) UVC/T-AR PCR box,	JINR, DLNP

	b) microcentrifuge with cooling, c) individual kit pipet-current-microdispensers 3. Obtaining cDNA from isolated RNA by reverse transcription using a Bio-Rad T100 Thermal Cycler 4. Design of primers for cDNA of the three studied genes and their synthesis. 5. Carrying out amplification of cDNA using the obtained primers and commercial kits of reagents using a DNA real time amplifier CFx96 Touch Bio-Rad with an assessment of the level of expression of mutant alleles of genes	

2.4 Participating JINR laboratories
FLNR (source of γ-rays Co^{60})
LHEP (source of ions, complex NICA, Ph.D. Belov O.)

2.4.1 MICC resource requirements

Computing resources	**Distribution by year**				
	1st year	2nd year	3rd year	4th year	5th year
Data storage (TB) - EOS - Tapes	-	-	-	-	-
Tier 1 (CPU core hours)	-	-	-	-	-
Tier 2 (CPU core hours)	-	-	-	-	-
SC Govorun (CPU core hours) - CPU - GPU	-	-	-	-	-
Clouds (CPU cores)	-	-	-	-	-

2.5. Participating countries, scientific and educational organizations

Organization	**Country**	**City**	**Participants**	**Type of agreement**

2.6. Key partners *(those collaborators whose financial, infrastructural participation is substantial for the implementation of the research program. An example is JINR's participation in the LHC experiments at CERN).*

3. Manpower

3.1. Manpower needs in the first year of implementation

№№ n/a	Category of personnel	JINR staff, amount of FTE	JINR Associated Personnel, amount of FTE
1.	research scientists	4	-
2.	engineers	2	-
3.	specialists	3	-
4.	office workers	-	-
5.	technicians	-	-
	Total:	**9**	

3.2. Available manpower

3.2.1. JINR staff

No.	Category of personnel	Full name	Division	Position	Amount of FTE
1.	research scientists	Alexandrov I.D.	DLNP OP	Chief researcher	1
		Alexandrova M.V.	DLNP OP	Senior researcher	1
		Afanasyeva K.P.	DLNP OP	Researcher	1
		Rusakovich A.N.	DLNP OP	Junior researcher	1
2.	engineers	Korablinova S.V.	DLNP OP	engineer	1
		Rusakovich A.E.	DLNP OP	engineer	1
3.	specialists	Korobina L.N.	DLNP OP	specialist	1
		Harchenko N.E.	DLNP OP	specialist	1
		Solodilova O.P.	DLNP OP	specialist	1
4.	technicians				
	Total:	**9**			

3.2.2. JINR associated personnel

No.	Category of personnel	Partner organization	Amount of FTE
1.	research scientists	-	-
2.	engineers	-	-
3.	specialists	-	-
4.	technicians	-	-
	Total:	-	-

4. Financing

4.1 Total estimated cost of the project/LRIP subproject

246,3 thousand dollars

4.2 Extra funding sources

Project (LRIP subproject) Leader _____/_____/

Date of submission of the project (LRIP subproject) to the Chief Scientific Secretary: _____
Date of decision of the laboratory's STC: _____ document number: _____

Year of the project (LRIP subproject) start: _____

(for extended projects) – Project start year: _____

Proposed schedule and resource request for the Project / LRIP subproject

Expenditures, resources, funding sources			Cost (thousands of US dollars)/ Resource requirements	Cost/Resources, distribution by years				
				1st year	2nd year	3rd year	4th year	5th year
		International cooperation	25	5	5	5	5	5
		Materials	16,3	7,3	2	2	2	3
		Equipment, Third-party company services	59				12,4	46,6
		Commissioning						
		R&D contracts with other research organizations	146	34	62	24	24	2
		Software purchasing						
		Design/construction						
		Service costs (*planned in case of direct project affiliation)*						
Resources required	**Standard hours**	Resources						
		– the amount of FTE,		9	10	10	10	10
		– accelerator/installation,						
		– reactor,…						
Sources of funding	**JINR Budget**	JINR budget *(budget items)*	246,3	46,3	69	31	43,4	56,6
	Extra fudning (supplementary estimates)	Contributions by partners Funds under contracts with customers Other sources of funding						

Project (LRIP subproject) Leader_____/_____/

Laboratory Economist _____/_____/

APPROVAL SHEET FOR PROJECT / LRIP SUBPROJECT

TITLE OF THE PROJECT/LRIP SUBPROJECT

SHORT DESIGNATION OF THE PROJECT / SUBPROJECT OF THE LRIP

PROJECT/LRIP SUBPROJECT CODE

THEME / LRIP CODE

NAME OF THE PROJECT/ LRIP SUBPROJECT LEADER

AGREED

JINR VICE-DIRECTOR	_____ SIGNATURE	_____ NAME	_____ DATE
CHIEF SCIENTIFIC SECRETARY	_____ SIGNATURE	_____ NAME	_____ DATE
CHIEF ENGINEER	_____ SIGNATURE	_____ NAME	_____ DATE
LABORATORY DIRECTOR	_____ SIGNATURE	_____ NAME	_____ DATE
CHIEF LABORATORY ENGINEER	_____ SIGNATURE	_____ NAME	_____ DATE
LABORATORY SCIENTIFIC SECRETARY THEME / LRIP LEADER	_____ SIGNATURE	_____ NAME	_____ DATE
PROJECT / LRIP SUBPROJECT LEADER	_____ SIGNATURE	_____ NAME	_____ DATE

APPROVED BY THE PAC

_____ SIGNATURE _____ NAME _____ DATE