Institute of Genetics and Cytology
NAS of Belarus

Genetic resources – conservation and use in scientific research. Practices.

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GENETIC RESOURCES FOR SUSTAINABLE DEVELOPMENT

BIODIVERSITY AND GENETIC RESOURCES

Past two and half decades are characterized by a rapid increase in the use of biological (genetic) resources in various areas of production activities.

Genetic resources have become not only of commercial interest, but also a reason for the increased "bioprospecting" and "biopiracy".

The latter entailed the adoption of the Convention on Biological Diversity

with a view of *conserving* of biological diversity, the *sustainable use of its components and the fair and equitable sharing of benefits arising from the utilization of genetic resources* by providing the required access to them and the transfer of appropriate technologies, taking into account all rights to such resources and technologies, as well as due financing of this activity.

In line with the Convention on Biological Diversity:

"genetic material" means any material of plant, animal, microbial or other origin containing heredity units (any material carriers of genetic information, including individual genes and their combinations, DNA fragments, RNA samples, and etc.)

"genetic resources" – the genetic material of intrinsic or potential value.

Pursuant to the international law norms, natural resources belong to the state and shall be alienated by representatives of other states only by authorization and on a reimbursable basis with due regard to the interests of indigenous people living in the territory of genetic resources' withdrawal.

Compensation forms for accessed genetic resources:

- **Tangible benefits**
- **Intangible benefits – information support, extended education, co-authorship in publications, patent applications, leasing, provided scientific and technical literature resources, devices, reagents, techniques, and etc.**

Nagoya Protocol to the Convention on Biological Diversity

The Protocol sets the legal framework to ensure greater certainty and transparency in the interaction of countries supplying genetic resources and biotechnology and the countries that use them.

In May 2014, the Republic of Belarus acceded to the Nagoya Protocol to the Convention on Biolological Diversity.

http://abs.igc.by

The National Coordination Centre on Access to Genetic Resources and Benefit-sharing (the Institute of Genetics and Cytology, NAS of Belarus)

BIODIVERSITY & GENETIC RESOURCES

In line with Article 2 of the Nagoya Protocol, "the use of genetic resources" means research and development using genetic material of actual or potential value, including through the use of biotechnology.

Therefore, those organizations that have their own collections of living objects (whole organisms, tissues, cells) or DNA Banks, as well as those that use living organisms in the production process, are subject to the Nagoya Protocol.

Genetic Resources' Holders in Belarus

- **National parks and nature reserves. Conserved genetic resources – wild species of flora and fauna**

- **Scientific Institutions of the National Academy of Sciences of Belarus. Conserved genetic resources – collections of living plants, collections of tree species of plants and fungi, herbarium, collections of seeds and cell cultures, selection and breeding farms for agricultural animals, DNA Bank.**



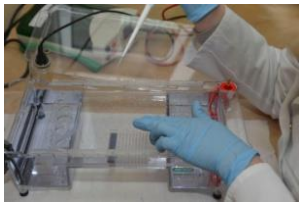
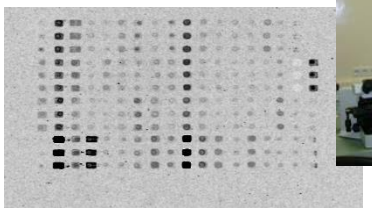


Institute of Genetics and Cytology, the National Academy of sciences of Belarus

- In the form of **living organisms** – collections of cultivated plant varieties (tomato, pepper, fizalis, wheat, triticale, flax, soybean, sunflower, potato)
- In the form of **DNA collections** – the Republican DNA Bank of a human, plants, animals and microorganisms





Republican DNA Bank of a human, plants animals and microorganisms

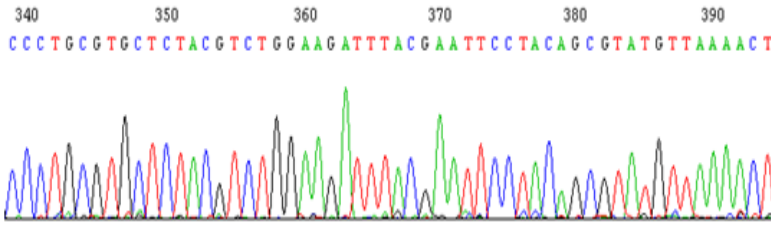
established in 2013
In 2016, acquired the National Heritage status
(the Resolution of the Council of Ministers of the Republic of Belarus August 13, 2016 No. 629)

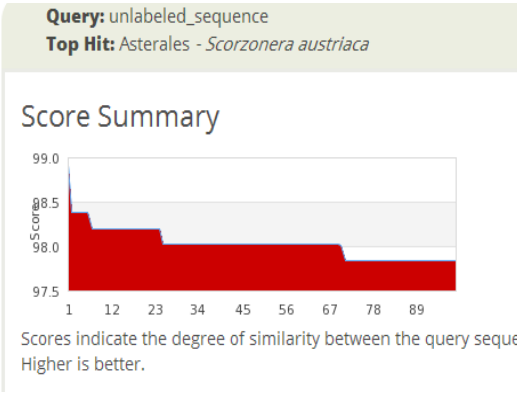
to ensure the preservation of unique DNA and biological material collections of the Institute of Genetics and Cytology, NAS of Belarus

Head of DNA Bank: A.V. Kilchevsky
Academician, NAS of Belarus

- **The DNA Bank provides conditions for developing of biotechnologies in our country, scientific work in the framework of large-scale, multidisciplinary research, both within the country and for cooperation as part of international research projects and programs**

- **It allows to carry out the DNA-inventory of Belarus's genetic resources**





Forms of DNA Samples' Depositing (Storage) in a Bank

One of the storage forms – for scientific purposes

- **Used by depositors for their own needs**
- **To exchange DNA samples between the Institutions' Laboratories of the Republic of Belarus and other countries involved in molecular-genetic research**

ИНСТИТУТ ГЕНЕТИКИ И ЦИТОЛОГИИ НАН БЕЛАРУСИ
Республиканский банк ДНК человека, животных, растений и микроорганизмов

УТВЕРЖДАЮ
Зам. директора Института генетики и цитологии НАН Беларуси
Е.А. Сычёва
20 февраля 2018г.

Акт
выдачи образцов ДНК
из хранилищ Республиканского банка ДНК человека, животных, растений и микроорганизмов

Настоящий акт составлен в том, что из хранилищ Республиканского банка ДНК человека, животных, растений и микроорганизмов выдаются образцы биологического материала для лаборатории молекулярных основ стабильности генома Института генетики и цитологии НАН Беларуси в количестве 159 образцов ДНК

по профилю: «Банк ДНК (Ювенильный идиопатический артрит (ЮИА), суставный синдром, системная красная волчанка, спондилоартрит, гемофилический васкулит, контроль.)»

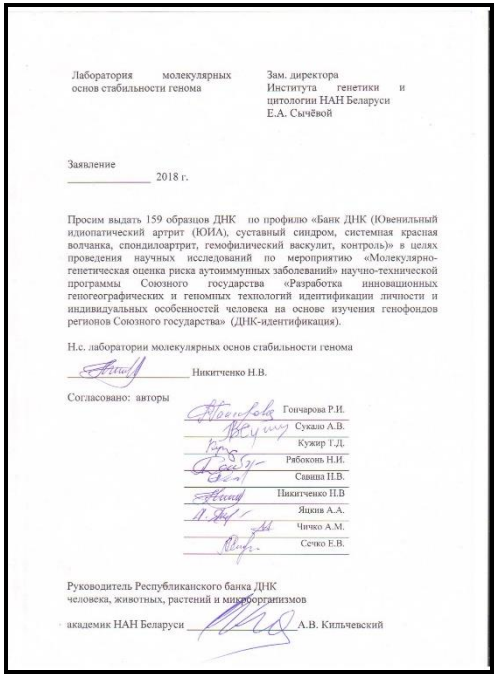
форма депонирования для научных целей

для выполнения работ по мероприятию «Молекулярно-генетическая оценка риска аутоиммунных заболеваний» научно-технической программы Союзного государства «Разработка инновационных геногеографических и геномных технологий идентификации личности и индивидуальных особенностей человека на основе изучения генофондов регионов Союзного государства» (ДНК-идентификация).

Руководитель Республиканского банка ДНК человека, животных, растений и микроорганизмов
академик НАН Беларуси А.В. Кильчевский

Образцы передал:
н.с. лаборатории экологической генетики и биотехнологии
Н.В. Савина

Образцы принял:
н.с. лаборатории молекулярных основ стабильности генома
Н.В.Никитченко



Лаборатория молекулярных основ стабильности генома

Зам. директора Института генетики и цитологии НАН Беларуси Е.А. Сычёвой

Заявление
2018 г.

Просим выдать 159 образцов ДНК по профилю «Банк ДНК (Ювенильный идиопатический артрит (ЮИА), суставный синдром, системная красная волчанка, спондилоартрит, гемофилический васкулит, контроль)» в целях проведения научных исследований по мероприятию «Молекулярно-генетическая оценка риска аутоиммунных заболеваний» научно-технической программы Союзного государства «Разработка инновационных геногеографических и геномных технологий идентификации личности и индивидуальных особенностей человека на основе изучения генофондов регионов Союзного государства» (ДНК-идентификация).

Н.с. лаборатории молекулярных основ стабильности генома
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Use of DNA collections of a human, plants and animals for scientific research

STATE PROGRAM

"Science-based technologies and machinery"

Subprogram 4 "Mobilization and rational use of plant genetic resources of the National Bank for breeding and enriching the cultivated and natural flora of Belarus"

2016-2020

Target "Creating the genetically marked collection of grain, vegetable and industrial crops for including in breeding programs and the National Bank of Plant Genetic Resources of the Republic of Belarus, designing of an interactive electronic database to monitor the use of plant genetic resources"

Comparative genomics. Solanaceous crops

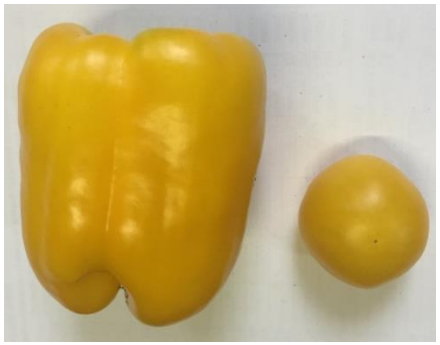
Based on the phylogenetic proximity of Solanaceous crops, the research is underway:

- Search for ortholog genes by economically valuable traits in pepper, eggplant, *Physalis* using the markers developed for tomato
- Identifying allelic polymorphism of genes
- Comparing phenotypic manifestation and functions of ortholog genes







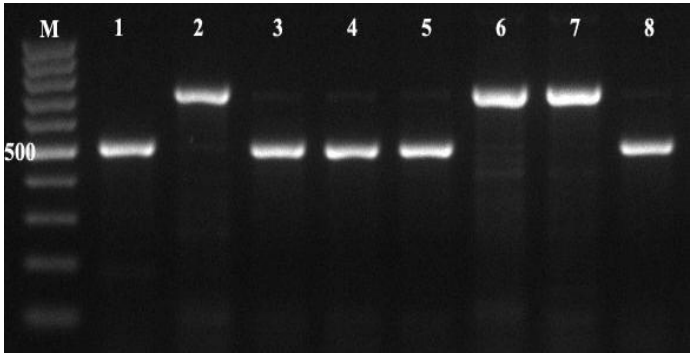


Genotyping of varietal gene pool of soft wheat

Grain quality associated genes	**•Grain hardness genes *PinA-D1* and *PinB-D1b*** **•Genes of reserve seed proteins – glutenins *Glu-A1, Glu-B1* and *Glu-D1*** **•Preharvest seed germination gene *Vp-1*** **•Presence of 1BL.1RS rye translocation**
Grain mass associated genes	**•Cell wall invertase gene*TaCwi-A1*** **•*TaGW2* gene that affects the mass of a thousand grains** **•Sucrose synthase gene 2 *TaSus2***
Grain height associated genes	**•Dwarf genes *Rht1*, *Rht2* and *Rht8***
Genes associated with the plant development type	**•*Ppd-D1* photoperiodism resistance gene** **•Springness/cold resistance genes *Vrn-A1, Vrn-B1 и Vrn-D1***
Genes associated with environmental adaptability	**•Stress-associated protein family genes*TaSap-A1***

DNA-marking for wheat resistance to fungal diseases

A collection of **50 DNA markers** linked to **50 wheat genes resistant** to powdery mildew, brown, stem and yellow rust formed *Lr1, Lr9, Lr10, Lr19/Sr25, Lr20/Sr15/Pm1, Lr21, Lr22a, Lr24/Sr24, Lr25/Pm7, Lr26/Sr31/Yr9/Pm8, Lr28, Lr29, Lr34/Yr18/Pm38, Lr35/Sr39, Lr37/Sr38/Yr17, Lr42, Lr47, Sr22, Sr26, Sr1RSAmigo, Sr2, Sr36, Sr40, Sr44, Sr45, Yr5, Yr10, Yr26, Pm3 (Pm3a, Pm3b, Pm3c, Pm3d, Pm3e, Pm3f, Pm3g), Pm4 and Pm17*.



Results of electrophoresis separation of amplification products in a 1.5% agarose gel developed with 2 pairs of SCAR markers, SCS265512 and SCS253736, to the *Lr19* brown rust resistance gene.
Well 1 – the isogenic line of soft wheat Thatcher/7**Thinopyrum elongatum* (Tc+*Lr19*) (positive control).

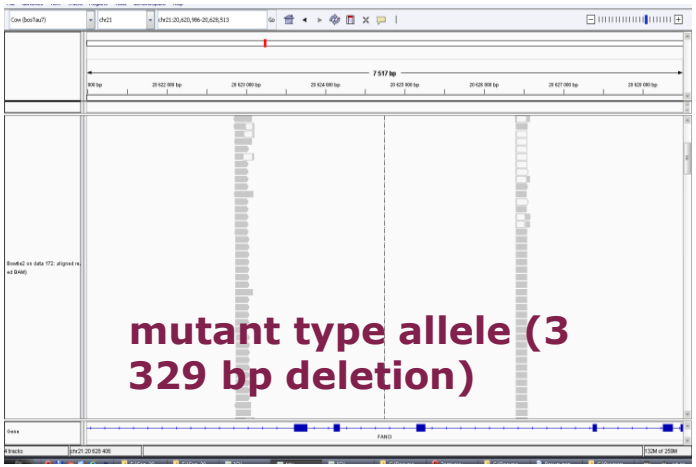
A collection of more than **500 isogenic** wheat **lines** and varieties with the known fungal disease resistance genes formed.

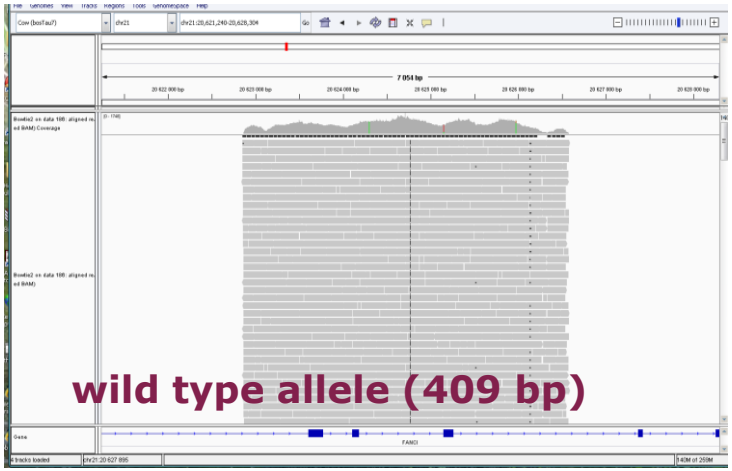
New sources of wheat resistance to fungal diseases identified and their donor properties characterized.

NGS sequencing using Illumina MiSeq

The FANCI gene fragment was sequenced, the presence of a mutation in it determines the development of a genetically conditioned defect of Brachyspina (low fecundity) in cattle.

The alleles of mutant (3 329 bp deletion) and wild types of the *FANCI* gene were identified.





The nucleotide sequence of PCR products corresponds to the reference nucleotide sequence AC_000178.1 (GenBank), which confirms the method specificity and the reliability of results.

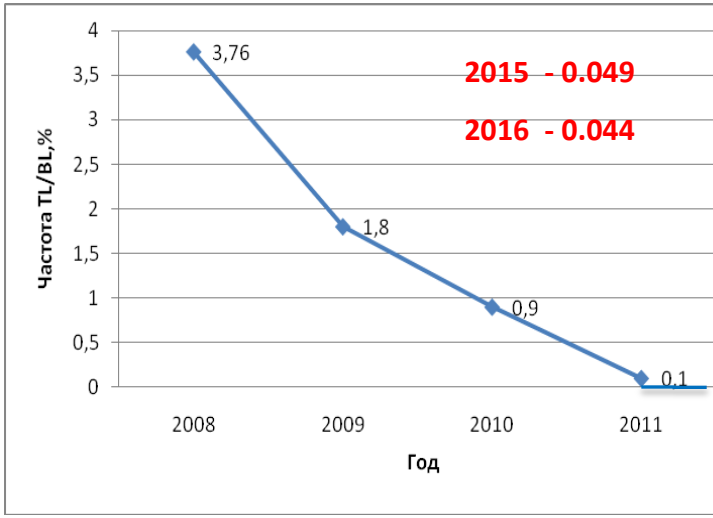
A DNA technology for detecting a genetic defect in cattle of the Holstein breed, which determines Brachyspina syndrome (BY), developed.

DNA-diagnosis for the carriage of farm animals' hereditary diseases

In accordance with the Law of the Republic of Belarus "On pedigree work in animal breeding", all highly productive pedigree animals should be subjected to genetic examination, including by the DNA-markers to genetically determined diseases.

DNA-diagnostics of hidden carriers of hereditary cattle diseases:

- ❖Hereditary immunodeficiency (BLAD-syndrome)
- ❖ Early abortion of embryos (DUMPS)
- ❖ Complex vertebral malformation (CVM)
- ❖ Blood coagulation factor deficiency (FXID)
- ❖ Bone deformation – brachyspina (BY)
- ❖Urea biosynthesis disturbance – citrullinemia (BC)



Monitoring the mutant genotypes' frequency causing the development of immunodeficiency state in cattle in Belarus

2008-2016

It allows to perform the strict genetic control of breeding (pedigree) animals, **to identify hidden carriers of a mutant allele and to control the mutation spread within the population.**

Fertility Haplotypes in Cattle

In the Holstein breed, **10 fertility haplotypes** (HCD, HH0, HH1, HH2, HH3, HH4, HH5, HHB, HHC, HHD) are currently registered, affecting the percentage of successful inseminations (from the pregnancy start), and/or **associated with embryonic and early postembryonic mortality** at various stages and occurring at a frequency of 0.01 to 2.95% (*Larkin D.M., 2012*).

Work is underway to monitor hidden mutation carriers associated with cattle fertility in Belarus

Distribution of animals by the identified genetic abnormalities associated with fertility in the cattle population of the Republic of Belarus (*Mikhailova M.E. et al 2018*)

Fertility haplotype, gene	The number of the studied animals	Identified carriers			
				including	
		%	n	cows, heads	bulls, heads
HH1, gene *APAF1*	**104**	2,88	3	1	2
HH3, gene *SMC2*	**325**	3,38	11	9	2
HH4, gene *GART*	**324**	1,23	4	4	-
HH5, gene *TFB1M*	**409**	2,69	11	7	4
HCD, gene APOB	**320**	1,25	4	-	4
HHO (BY)	**334**	1,9	8	5	3
HHB (BLAD)	**417**	0,48	2	2	-
HHC (CVM)	**417**	2,88	12	10	2
HHD (DUMPS)	**409**	-	-	-	-

GENETIC DIVERSITY OF BELARUSIAN AND POLISH POPULATIONS OF THE EUROPEAN BISON (*Bison bonasus*)

Objective: To assess the genetic diversity of the Belarusian and Polish populations of the European bison **by microsatellite loci and *DRB3 and DQB* genes' polymorphism of the main histocompatibility complex** for conservation and rational use of this species.

Microsatellite loci polymorphism of the European bison of the Belarusian and Polish populations

Polish population			Belarusian population	
Allele	Frequency (%)	Locus	Allele	Frequency (%)
-	-	ETH3	119	6.6
125	46,3		125	45.6
129	53,7		129	47.8
250	62,9	SPS115	250	14.2
254	7,5		254	25.0
258	29,6		258	60.8
-	-	TGLA122	132	6.5
144	75,9		144	83.7
164	24,1		164	9.8
-	-	BM2113	121	33.4
-	-		125	20.6
-	-		129	10.8
130	7,4		-	-
132	92,6		133	35.2
153	51.8	ETH225	153	47.8
155	48.2		155	52.2
181	16,7	BM1824	181	34.7
183	83,3		183	65.3
213	29,6	ETH10	213	25.0
215	2.0		215	14.1
217	62,9		217	54.4
221	5,5		221	6.5
153	75,9	TGLA53	153	60.0
155	24,1		155	40.0
112	12,9	TGLA126	112	13.0
116	59,3		116	66.3
122	27,8		120	20.7
100,0	192	INRA23	192	100,0

(Mikhailova M., Medvedeva Y. 2012)

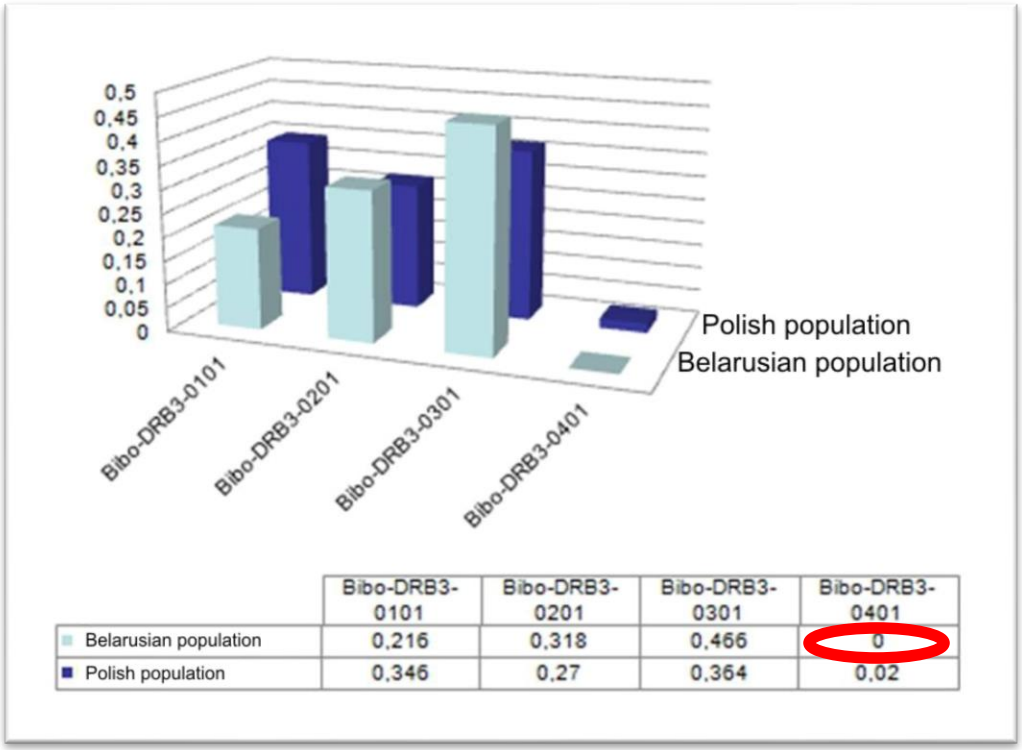
DNA collection

- **30 samples of the Belavezha line**
- **120 samples of the Belavezha-Caucasus line (Belarus)**

- Microsatellite analysis showed that, despite the common origin and high similarity in the Belarusian and Polish populations of the European bison, **different breeding principles led to obvious differences in the genetic structure**

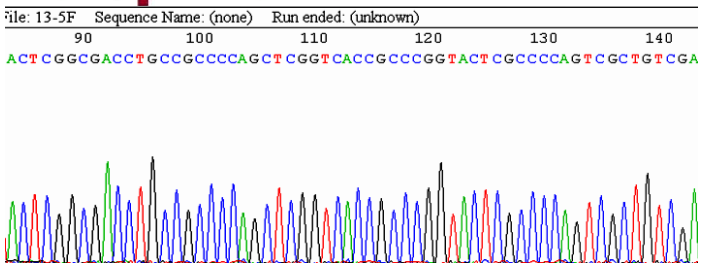
- The presence of certain alleles of microsatellite loci confirms the **hybrid origin of the Belarusian livestock**

Search for rare allelic variants of the *DRB3* gene involved in the immunity development



	Bibo-DRB3-0101	Bibo-DRB3-0201	Bibo-DRB3-0301	Bibo-DRB3-0401
Belarusian population	0,216	0,318	0,466	0
Polish population	0,346	0,27	0,364	0,02

Comparison of *DRB3* gene allele frequencies of the main histocompatibility complex in the Belarusian (Mikhailova M., Medvedeva Y. 2013) and Polish populations (Radwan J. 2007)



A fragment of the *DRB3* gene exon sequence II

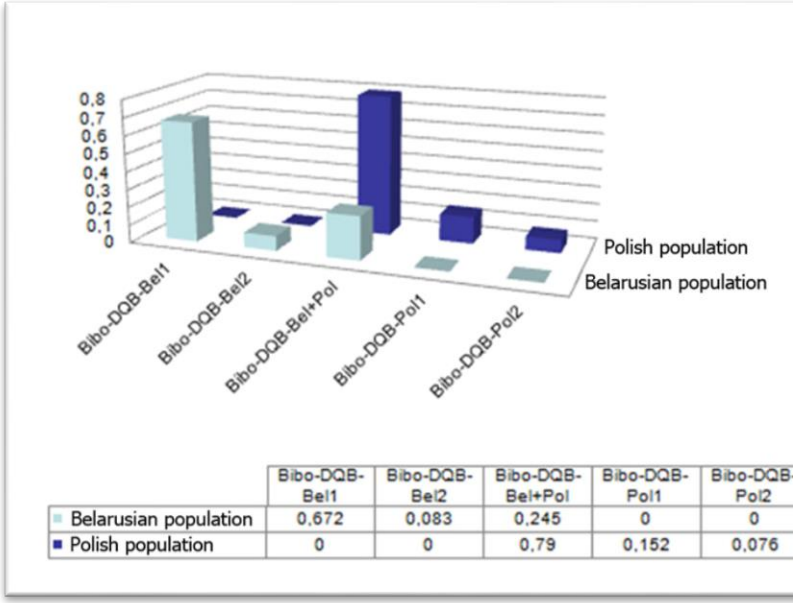
The absence of the Bibo-DRB3*0401 major histocompatibility complex allele in the Belarusian bison population is shown.

Study of the *DQB* gene polymorphism in the immunity development

5 allelic variants of the *DQB* gene of the major histocompatibility complex in the European bison identified.

The genetic structure difference in the Belarusian population of the European bison is shown by the frequency of allelic ***DQB*** gene variants' occurrence of the major histocompatibility complex.

The presence in the Polish population of the **unique allelic variants** of the Bibo-DQB-Pol2 and Bibo-DQB-Pol3 gene identified, making them **valuable** for the increased genetic diversity of the Belarusian population.



	Bibo-DQB-Bel1	Bibo-DQB-Bel2	Bibo-DQB-Bel+Pol	Bibo-DQB-Pol1	Bibo-DQB-Pol2
Belarusian population	0,672	0,083	0,245	0	0
Polish population	0	0	0,79	0,152	0,076

Comparison of the DQB gene allele frequencies of the major histocompatibility complex in the Belarusian and Polish populations of the European bison
(Mikhailova M., Medvedeva Y. 2015)

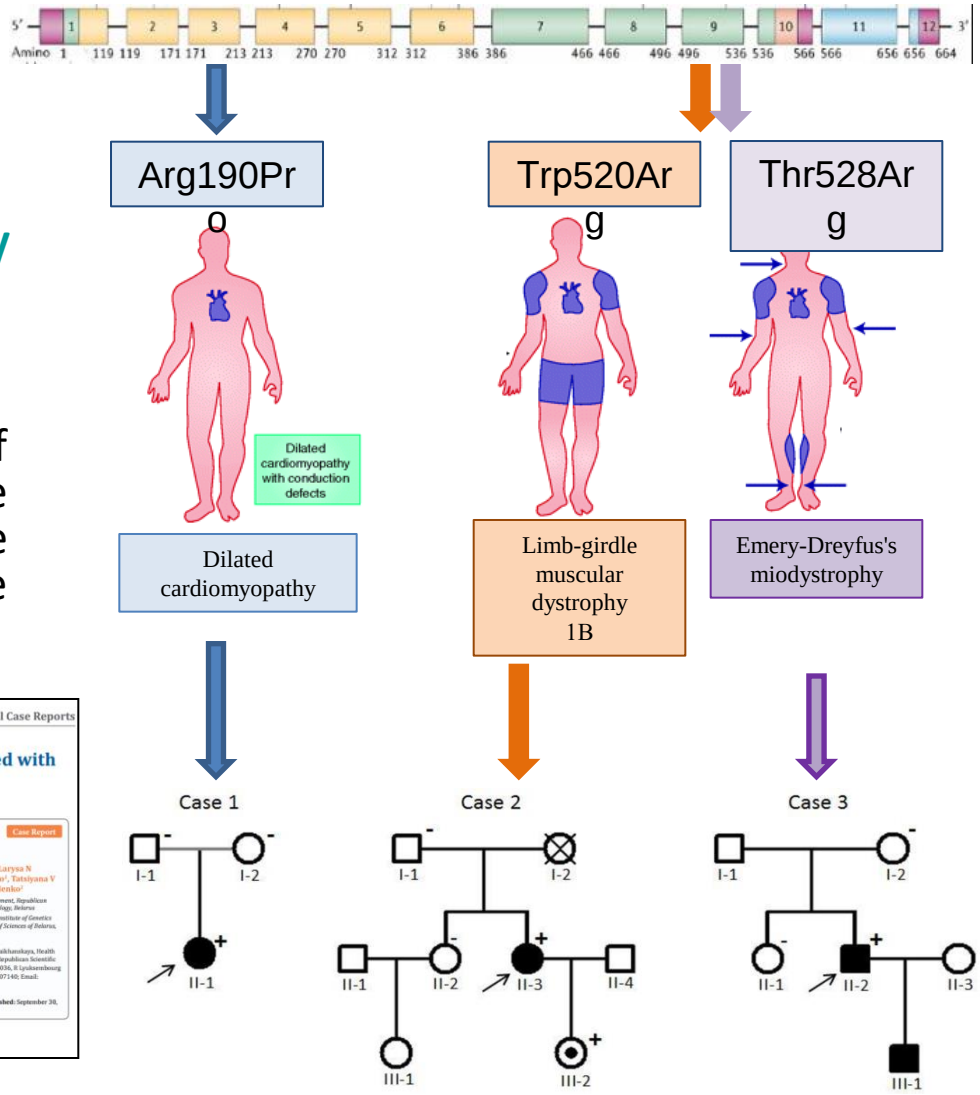
Identification of individuals carrying rare allelic variants of microsatellite loci and the major histocompatibility complex genes will contribute to the increased genetic diversity and the involvement of unique genes and alleles in the breeding process and this will undoubtedly allow to increase the species viability.

Illumina MiSeq-based NGS sequencing

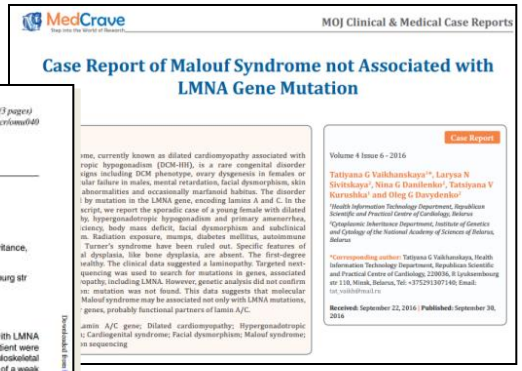
Using modern sequencing technologies, mutation carriers were found in the *LMNA* gene associated with the development of dilated cardiomyopathy and associated life-threatening syndromes.

DNA collection – 160 samples

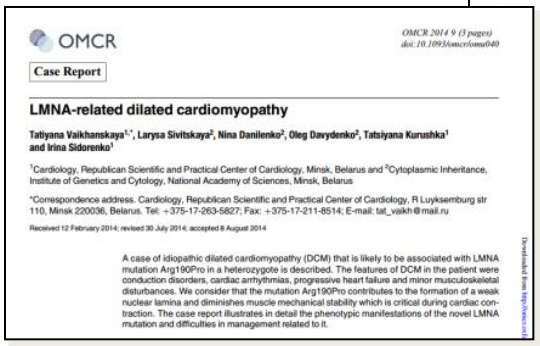
For the first time ever, the Arg190Pro mutation of the *LMNA* gene is described in the human genome. Its phenotypic manifestation was studied and pathogenicity was identified.



The asymptomatic carriers (children) of mutations in the patients' families were determined. This allows to carry out the therapeutic correction of unfavorable disease outcomes.


MedCrave
MOJ Clinical & Medical Case Reports

Case Report of Malouf Syndrome not Associated with LMNA Gene Mutation


OMCR

Case Report

LMNA-related dilated cardiomyopathy

Tatiyana Vaikhanskaya[1,*], Larysa Sivitskaya[2], Nina Danilenko[2], Oleg Davydenko[2], Tatsiyana Kurushka[1] and Irina Sidorenko[1]

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A case of idiopathic dilated cardiomyopathy (DCM) that is likely to be associated with LMNA mutation Arg190Pro in a heterozygote is described. The features of DCM in the patient were conduction disorders, cardiac arrhythmias, progressive heart failure and minor musculoskeletal disturbances. We consider that the mutation Arg190Pro contributes to the formation of a weak nuclear lamina and diminishes muscle mechanical stability which is critical during cardiac contraction. The case report illustrates in detail the phenotypic manifestations of the novel LMNA mutation and difficulties in management related to it.

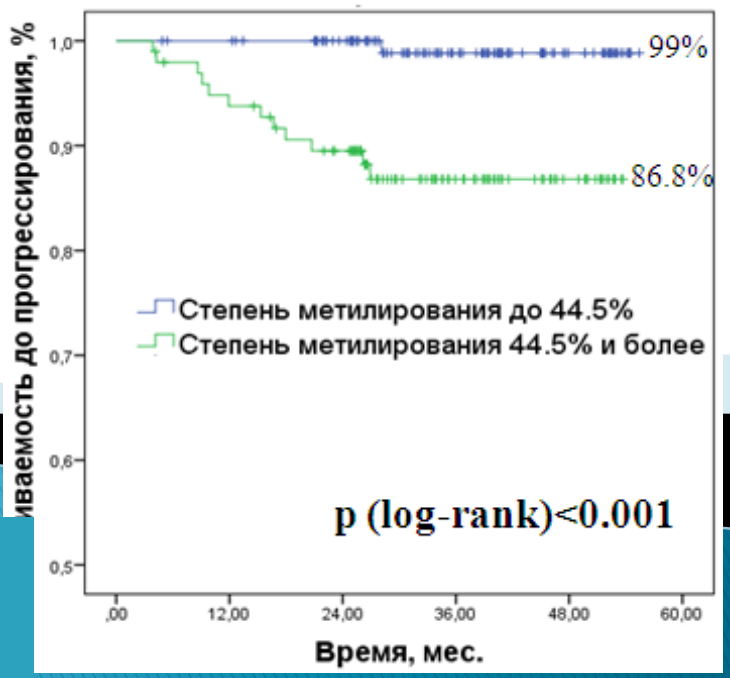
Evaluation of the epigenetic variability effect on the clinical course of bladder cancer

DNA collection – 380 samples

- Qualitative and quantitative analysis on the methylation of the *RUNX3, TBX4, HOXA9, SOX1* gene promoter regions using methyl-specific PCR and Ms-SNuPE techniques carried out

- A statistically significant relationship between the *RUNX3*, *TBX4* and *SOX1* gene hypermethylation with pathomorphologic indices of disease aggressiveness is shown: muscular invasion, low differentiation degree, large tumor size

- **It was established that epigenetic changes in the *RUNX3* and *TBX4* genes are independent from the clinical parameters of risk factors that cause the bladder cancer development**



The three-year survival rate prior to progression
at a low methylation degree of *TBX4* – 99%
at a high methylation degree of *TBX4* – 86,8%

DNA-testing in sports

More than 500 representatives of 30 Olympic and National Teams of Belarus tested

DNA Bank of elite athletes established

DNA-testing programmes developed:

- **To select sport beginners (by athletic talent genes)**
- **For sports profiling**
- **To adjust the training process**
- **To choose medicobiologic support for athletes**

DNA-testing contributes to the enhanced sports selection, the optimized training process and the adjusted biomedical support of athletes and this ultimately contributes to the enhanced effectiveness and the strengthened athlete's potential realization.

Thank you for your attention!

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