BELARUS

Transfer of DNA-Barcoding Technology for the Inventory and Monitoring of Rare and Endangered Plant Species in Belarus and other Central and Eastern European Countries

(project proposal was submitted to BBI in 2016 and approved by BBI project council in 2017)

Minsk, 2018

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species.

Баркоди́рование ДНК (ДНК-штрихкодирование, генетический баркодинг, ДНК-баркодинг, англ. DNA barcoding) — метод молекулярной идентификации, который позволяет по коротким генетическим маркерам в ДНК определять принадлежность организма к определённому таксону.

The most commonly used barcode region for animals and protists is a segment of approximately 600 base pairs of the mitochondrial gene cytochrome oxidase I (COI or COX1).

Наиболее часто используемым локусом генетического баркодинга для животных является участок митохондриального гена цитохромоксидазы I из примерно 600 пар нуклеотидов.

This differs in the case of fungi, where part of Internal Transcribed Spacer 2 (ITS2) between rRNA genes is used, and again in plants, where multiple regions are used.

Для грибов чаще всего используются локусы ITS2 (внутреннего транскрибируемого локуса), находящиеся между генами рРНК, а у растений используются также некоторые мультилокусы.

Applications include, for example, identifying plant leaves even when flowers or fruit are not available, identifying insect larvae (which may have fewer diagnostic characters than adults and are frequently less well-known), identifying the diet of an animal, based on its stomach contents or faeces and identifying products in commerce (for example, herbal supplements, wood, or skins and other animal parts).

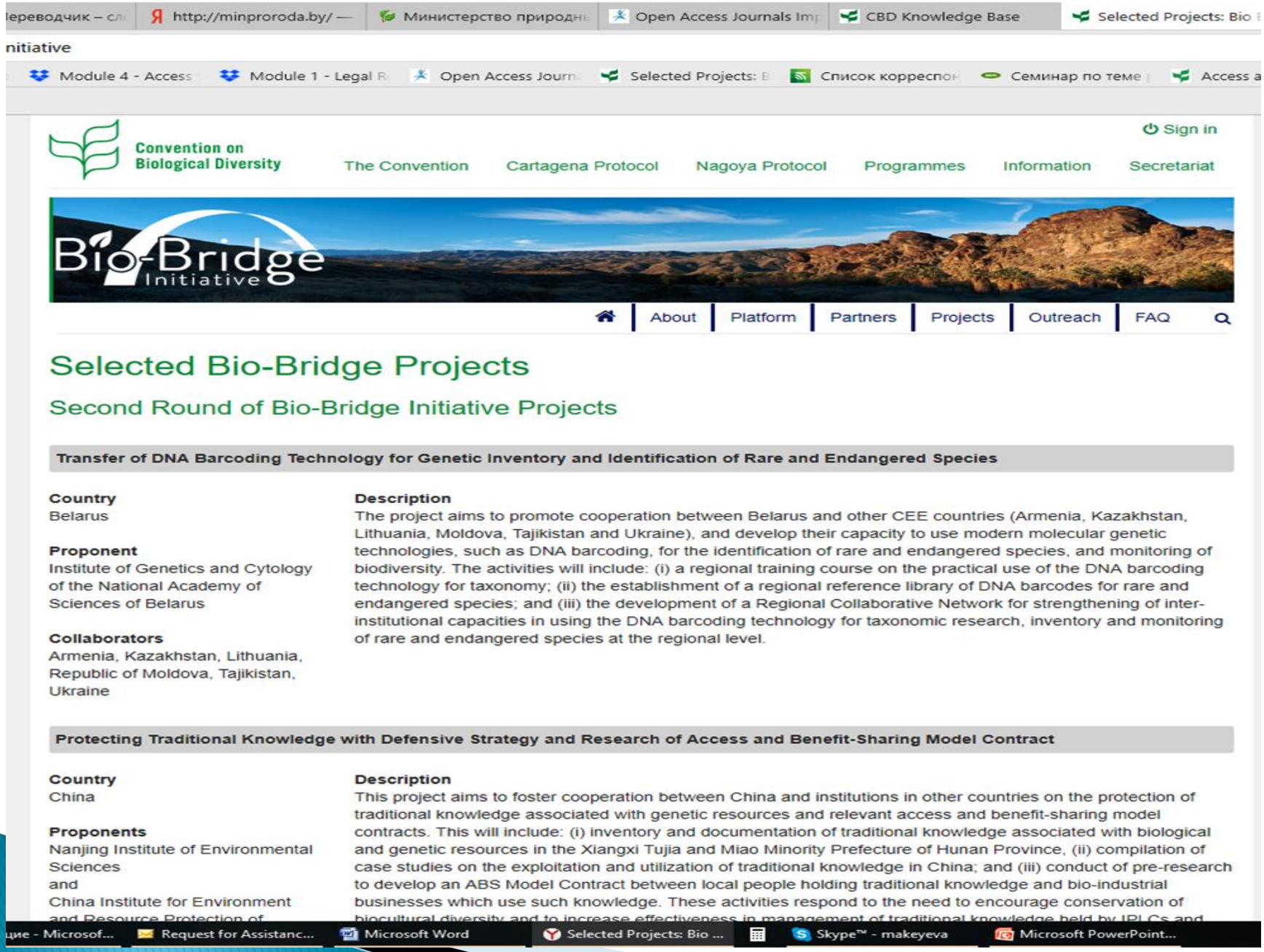
Применение ДНК-баркодирования распространяется на такие задачи, как, например, идентификация растения только по его листьям (к примеру, если недоступны цветки или плоды), идентификация личинок насекомых (которые могут иметь меньше диагностических признаков, чем взрослые особи, и часто менее изучены), определение рациона питания животных по содержанию желудка или фекалиям, и многое другое.

The use of nucleotide sequence variations to investigate evolutionary relationships is not a new concept.

Carl Woese used sequence differences in ribosomal RNA (rRNA) to discover archaea, which in turn led to the redrawing of the evolutionary tree, and molecular markers (e.g., allozymes, rDNA, and mtDNA sequences) have been successfully used in molecular systematics for decades.

DNA-barcoding provides a standardised method for this process via the use of a short DNA sequence from a particular region of the genome to provide a 'barcode' for identifying species.

In 2003, Paul D.N. Hebert from the University of Guelph, Ontario, Canada, proposed the compilation of a public library of DNA barcodes that would be linked to named specimens. This library provideы a new master key for identifying species, one whose power will rise with increased taxon coverage and with faster, cheaper sequencing".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Selected Bio-Bridge Projects
Second Round of Bio-Bridge Initiative Projects
Transfer of DNA Barcoding Technology for Genetic Inventory and Identification of Rare and Endangered Species
Country
Belarus
Proponent
Institute of Genetics and Cytology of the National Academy of Sciences of Belarus
Collaborators
Armenia, Kazakhstan, Lithuania, Republic of Moldova, Tajikistan, Ukraine
Description
The project aims to promote cooperation between Belarus and other CEE countries (Armenia, Kazakhstan, Lithuania, Moldova, Tajikistan and Ukraine), and develop their capacity to use modern molecular genetic technologies, such as DNA barcoding, for the identification of rare and endangered species, and monitoring of biodiversity. The activities will include: (i) a regional training course on the practical use of the DNA barcoding technology for taxonomy; (ii) the establishment of a regional reference library of DNA barcodes for rare and endangered species; and (iii) the development of a Regional Collaborative Network for strengthening of inter-institutional capacities in using the DNA barcoding technology for taxonomic research, inventory and monitoring of rare and endangered species at the regional level.
Protecting Traditional Knowledge with Defensive Strategy and Research of Access and Benefit-Sharing Model Contract
Country
China
Proponents
Nanjing Institute of Environmental Sciences
and
China Institute for Environment
Description
This project aims to foster cooperation between China and institutions in other countries on the protection of traditional knowledge associated with genetic resources and relevant access and benefit-sharing model contracts. This will include: (i) inventory and documentation of traditional knowledge associated with biological and genetic resources in the Xiangxi Tujia and Miao Minority Prefecture of Hunan Province, (ii) compilation of case studies on the exploitation and utilization of traditional knowledge in China; and (iii) conduct of pre-research to develop an ABS Model Contract between local people holding traditional knowledge and bio-industrial businesses which use such knowledge. These activities respond to the need to encourage conservation of
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PROJECT OBJECTIVES

The project aims to promote cooperation between Belarus and other CEE countries (Armenia, Kazakhstan, Lithuania, Moldova, Tajikistan and Ukraine), and develop their capacity to use modern molecular genetic technologies, such as DNA barcoding, for the identification of rare and endangered plant species, and monitoring of biodiversity.

The activities will include:

(i) a regional training course on the practical use of the DNA barcoding technology for taxonomy;

(ii) (ii) the establishment of a regional reference library of DNA barcodes for plant rare and endangered species; and

(iii) (iii) the development of a Regional Collaborative Network for strengthening of inter-institutional capacities in using the DNA-barcoding technology for taxonomic research, inventory and monitoring of rare and endangered plant species at the regional level.

TIMEFRAME-BASED IMPLEMENTATION PLAN

•***March – May 2018:***

Project registration at the Ministry of Economy of the Republic Belarus.

•***April-May 2018:***

Selection of plant samples for DNA-barcoding analysis.

Preparation of a model of the Regional Reference Library of DNA Barcodes as a special unit of the Republican DNA Bank to be used by the partner countries to facilitate their compliance with ABS regulations under the Nagoya Protocol to the Convention on Biological Diversity.

Collection of the known taxonomic and genetic information related to those specimens by use of national and international information resources.

Working out a Regional Collaborative Network on the use of the DNA barcoding technology in the region (start-up stage).

TIMEFRAME-BASED IMPLEMENTATION PLAN

•*June 2018:*

DNA-barcoding training course execution.

Entering information into the Regional Reference Library of DNA Barcodes.

Development of a project proposal for a follow-up cooperation to be submitted for funding from other sources.

• *July 2018:*

• Preparation and submission of the final project reports (practical results and financial report).

Republican DNA Bank of a human, animals, plants and microorganisms

► **Gathering, storing and systemizing of information on genetic material collections**

► **Collecting and storing rare and endangered species of Belarus' plants and animals**

► **Organizing effective collaboration with other DNA Banks and genetic material collections to join the International Network of DNA Banks to exchange samples, technologies and information**

► **Coordinating and developing cooperative links with related institutions to carry out joint scientific research by the directions demanded in the real sector of economy. Effective integrating of scientific developments into practical use.**

Shareable Core Facilities GENOME

Automatic Station for Disruption and Homogenization of Biological Samples *TissueLyser II* (Qiagen, Germany). Designed for homogenization from 1 to 48 biological samples in 1.2 or 2 ml microtubes by shaking with solid balls. Homogenized samples can be used to isolate biomolecules, including DNA, RNA and proteins.

System for Droplet Digital PCR QX200™ destined for droplet digital PCR of nucleic acids to analyze the expression of single genes, to detect and assess pathogens, to determine gene copy numbers (CNV) and viral load, to detect GMOs, to work with microRNAs and to validate and quantify NGS Libraries. In addition to working with Taq-man PCR, the possibility of using EvaGreen intercalating dye is realized. About 20 000 drops formed from 20 μl of the reaction mixture are analyzed. The amplification reaction format – nanoliter drops.

The format determines the accuracy of quantitative analysis of sequence-specific sequences. The system allows to perform a reverse transcription (RT) reaction and droplet digital PCR with samples in one reaction mixture to reduce the risk of rare transcripts' loss in the transfer of a sample from one tube to another.

Genetic Analyzers 3500 (Applied Biosystems).

Destined to determine the nucleotide sequence of DNA fragments to perform SSR, LOH, SNP, MLPA, AFLP and t-RFLP tests. It allows to perform de novo sequencing and re-sequencing of small genomes up to 1 000 bp. The number of capillaries – 8. The number of dyes – 6. The ability to read labels: TAMRA, ROX, R6G, FAM, LIZ. Sequencing speed is 700 bps with the accuracy of 98.5% (less than 40 min.).

MiSeq System (Illumina, Inc, США)

The MiSeq system facilitates research with a wide range of sequencing applications. Allows to solve specialized tasks, such as investigation of genome target regions, metagenomics, sequencing of small genomes, estimation of gene group expression, amplicon sequencing and HLA typing. A new generation of MiSeq reagents allows to achieve a performance of 15 billion basepairs and 25 million of individual mappings, while the mapping length is 2 × 300 bp.

DNA Bar-coding Technology in Belarus: Perspectives and Needs

☞ **Biological diversity of the Republic of Belarus is represented by 500 animal species and 14 000 plant species, including 4100 of higher plants (1400 aboriginal species), 442 of bryophytes, 669 of lichens and more than 9 000 species of lower pants (algae and fungi). About 50 wild plants of aboriginal species have vanished over the last 100 years. 93,1% of Belarus territory is covered by flora, including 30% of the forest area;**

☞ There are **50 unique protected areas in Belarus, including 5 National Parks and one Biosphere Preservation supported by UNESCO;**

☞ **Conservation of biological diversity is supported by the Belarus Government. The National Herbarium, the Bank of Plant Genetic Resources, the Republican DNA Bank and other collections are funded from the State budget;**

☞ The Republican DNA Bank is recognized by the Council of Ministers of the Republic of Belarus as National Heritage;

☞ **DNA-barcoding technique is taken as a basic approach to the species identification and genetic resources' inventory. In Belarus, access to genetic resources is regulated by the Nagoya Protocol to the Convention on Biological Diversity.**

The Institute of Genetics and Cytology initiated use of a DNA-barcoding technique for taxonomy, including research in rare and endangered species. A plant DNA collection of 35 species (33 species among them belong to rare and endangered species) was first created at the Republican DNA Bank. The biological material was collected without removal of the whole plant from the places of its growing (two National Parks: Narochansky and Belovezhskaya Puscha).

To conduct identification of plant genus, species and sub-species taxonomic categories, the following plastid (rbcl, matK and psbA-trnH) and nuclear (ITS2) markers were used.

The DNA-barcoding technique used for the Dáphne cneórum (волчеягодник боровой) plant genome analysis revealed the following mistake made by the taxonomist: the comparison of nucleotide sequences received for the Dáphne cneórum sample with the NCBI database resulted in the identification of that sample as Daphne mezerum (волчник смерельный, волчье лыко).

We consider that result as evidence that a DNA-barcoding technique should be included in the list of taxonomic tools used for the taxonomic identification of biological objects.

Training support in that area for our specialist is very important.

The Institute of Genetics and Cytology, NAS of Belarus, will hold DNA-barcoding training courses jointly with the Scientific and Practical Centre for Bioresources.

As agreed with our organizations, staff members of the Canadian Center of DNA-barcoding, University of Guelph, where DNA-barcoding was developed and is implemented worldwide, and Nina Voronova (BSU Associate Professor, PhD) and Tatiana Lipinskaya, PhD (Scientific and Practical Center for Bioresources), experienced in this technology use, have been invited as trainers.

Technical support will be provided by the Republican DNA Bank staff and of the Shareable Core Facilities GENOME.

So, our goals are as follows:

to organize DNA-barcoding training,

develop National Reference Libraries of plant and animal DNA codes,

establish a Regional DNA-barcoding Network for Collaboration and Information Exchange and develop of a project proposal for a follow-up cooperation to be submitted for funding from other sources.

I am sure we will achieve all our goals due to support of the Bio-Bridge Initiative.

Thank you for your attention!

СПАСИБО ЗА ВНИМАНИЕ!