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ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН
АО «Институт топлива, катализа и
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Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабарлары. Химия және технология сериясы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Webof Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабарлары. Химия және технология сериясы Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді химиялық ғылымдар бойынша контентке адалдығымызды білдіреді.

НАН РК сообщает, что научный журнал «Известия НАН РК. Серия химии и технологий» был принят для индексирования в Emerging Sources Citation Index, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Известия НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному контенту по химическим наукам для нашего сообщества.

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TECHNOLOGY OF *BOVINE LEUKEMIA VIRUS* GENODIAGNOSTICS IN CATTLE, IN PRODUCED RAW MATERIALS AND PRODUCTS

Abstract. The most important task of the dairy cattle industry is to obtain high quality raw milk. To achieve it, a set of measures is required, including aimed at increasing the biological safety of produced raw materials. The aim of the study was to create a scientific and methodological basis for the *Bovine leukemia virus* (BLV) gene diagnostics in a combined format of pathogen indication and identification. This required updating the strategy of BLV PCR-RFLP genotyping, consistent with its phylogenetic classification, taking into account the growing knowledge about the genetic diversity of 11 genotypes of the studied viral pathogen. When staging nested PCR, oligonucleotide primers were used, which initiate at the final stage of the reaction the production of a 444 bp *env*-gene fragment of the pathogen. Five restriction endonucleases were used in PCR-RFLP BLV genotyping of: *Pvu*II*, Ssp*I, *Asu*HPI*, Hae*III*, and Bst*X2I. As a result of verification of the developed *Bovine leukemia virus* method for gene identification with an updated genotyping strategy, a technical result was obtained, expressed in the ability to identify all 11 BLV genotypes discovered to date by interpreting the generated 58 genotype-associated combinations of PCR-RFLP profiles.

Key words: *Bovine leukemia virus*, BLV, cattle, milk, PCR, RFLP, sequencing, gene diagnostics, genotyping, env-gene.

Introduction. Obtaining high-quality raw milk is the most important task for the dairy farming industry, including for the production of functional and gerodietetic food products. To achieve it, a set of measures is required, including aimed at increasing the biological safety of the produced raw materials, which ultimately affects the products quality [1,2].

Bovine leukemia virus is a chronic infectious disease of a tumor nature, causing significant economic damage to the dairy farming industry, including due to products shortage and a decrease in its quality. The causative agent of the disease itself – the *Bovine leukemia virus* (BLV) can also be detected in milk raw materials obtained from infected cows [3,4].

It is believed that most of the traditionally used types of technological milk processing, mostly heat treatment, inactivate the virus, but do not destroy the genome. At the same time, the assortment of dairy products includes a number of products with gentle process parameters that do not have a lethal effect on the virus [5,6].

Currently, to eradicate BLV, early pathogen gene diagnostics is introduced into the system of antiepizootic measures, followed by the infected animal's removal from the herd. In this regard, the pathogen gene identification is of great importance [7,8].

Molecular genetic research methods make it possible to assess the genetic diversity of BLV, and are the most informative approaches to its gene identification, both when using phylogenetic analysis of sequenced nucleotide DNA sequences of the provirus, and PCR-RFLP analysis in accordance with the phylogenetic pathogen classification [9,10].

Investigations of the last decade have identified 11 BLV genotypes, however, the features of genotype-associated pathogenesis have not been studied [11-17].

The aim of the study is to create a scientific and methodological basis for the BLV gene diagnostics in a combined format of indication and identification of the infectious agent. To achieve the goal, the current research task was formulated:

- update the strategy of PCR-RFLP-genotyping of BLV, consistent with its phylogenetic classification, taking into account the growing knowledge about the genetic diversity of eleven known genotypes of the studied viral pathogen.

Material and research methods. The work was carried out on the basis of the Laboratory of Canned Milk, the Laboratory of Standardization, Metrology and Patent-Licensing Work of the All-Russian Dairy Research Institute.

DNA isolation from whole canned cattle blood, milk and dairy products was carried out by commercial kits "DNA-sorb B" and "DNA-sorb S-M" (Central Research Institute of Epidemiology, Rospotrebnadzor, Ministry of Health of the Russian Federation).

When staging nested PCR with extracted samples of BLV proviral DNA, we used “external” (“env5032” and “env5608”) and “internal” (“env5099” and “env5521”) primers, initiating at the final stage of the reaction the production of a fragment of the *env*-gene of the pathogen length of 444 bp [18].

Corresponding restriction endonucleases used in PCR-RFLP genotyping of BLV, consistent with its phylogenetic classification: *Pvu*II, *Ssp*I, *Asu*HPI (*Hph*I isoschizomer), *Hae*III, *Bst*X2I (*Bst*YI isoschizomer).

For PCR-RFLP modeling, NEBcutter v.2.0 software was used.

To detect the obtained results of PCR and PCR-RFLP analysis, horizontal electrophoresis in 2.5% agarose gel with TBE buffer (pH 8.0) containing ethidium bromide was used, followed by electrophoregrams examination in a UV-transilluminator (λ=310 nm). The sizes of generated DNA fragments were estimated by comparison with standard DNA molecular weight markers (“SibEnzyme OOO” (Limited Liability Company)).

Sequencing of PCR amplification products of the *env*-gene locus of detected isolates of the BLV provirus was carried out on an ABI PRISM 3100 genetic capillary analyzer (sequencer) (Applied Biosystems, USA) using “internal” oligonucleotide primers “env5099” and “env5521” as sequential. Alignment of the sequenced sequences of the *env*-gene locus of the BLV provirus isolates with the corresponding nucleotide sequences of the reference BLV isolates previously deposited in GenBank was carried out using the BLAST and MEGA-4 programs with subsequent phylogenetic analysis.

Results and discussion. As part of the current task, we interpreted the *env*-PCR-RFLP profiles of 520 BLV isolates generated during the analysis of restriction mappings of the *env*-gene locus by 5 restriction enzymes, which actually reflect the strategy of PCR-RFLP BLV genotyping in accordance with its phylogenetic classification, whose data are presented in the summary table.

Updated strategy for PCR-RFLP BLV genotyping

G	Isolate	GenBank A/N	PCR product (bp)	RFLP-fragments (bp)					C	N
				*Pvu*II	*Ssp*I	*Hph*I	*Hae*III	*Bst*YI		
1	AL-63	FJ808571	444	444	399/45	224/220	198/94/87/32/27/6	198/128/118	1	56
1	Cow 527	AF007764	444	444	399/45	224/220	285/94/32/27/6	198/128/118	2	8
1	23	U87873	444	444	399/45	224/220	312/94/32/6	198/128/118	3	1
1	AL-2106	FJ808578	444	444	399/45	224/220	198/94/87/32/27/6	246/198	4	42
1	UruC06II	FM955558	444	444	399/45	224/220	285/94/32/27/6	246/198	5	1
1	VdM	M35239	444	444	399/45	224/181/39	198/94/87/32/27/6	316/128	6	1
1	Kurdistan	EU266062	444	444	399/45	220/196/28	198/119/94/27/6	198/128/118	7	1
2	AL-164	FJ808574	444	280/164	399/45	224/220	198/94/87/32/27/6	198/128/118	8	34
2	PL-4960	FJ808590	444	280/164	399/45	224/220	198/87/49/45/32/27/6	198/128/118	9	1
2	ARGSF8	AF485773	444	280/164	399/45	444	198/94/87/32/27/6	198/128/118	10	1
2	AL-1453	FJ808577	444	280/164	444	224/220	198/94/87/32/27/6	198/128/118	11	1

Table continuation

3	USCA-1	EF065647	444	444	399/45	444	285/94/32/21/6/6	198/128/96/22	12	1
3	USCA-2	EF065648	444	444	399/45	444	285/94/32/27/6	198/128/96/22	13	2
3	JPFU	EF065650	444	444	399/45	444	285/94/32/27/6	198/128/118	14	1
4	BG	EF065638	444	280/164	399/45	224/220	198/94/87/32/27/6	444	15	115
4	3	U87872	444	444	399/45	224/220	198/94/87/32/27/6	444	16	1
4	1S-c16	JQ353652	444	280/164	399/45	444	198/94/87/32/27/6	444	17	16
4	N023	KC867149	444	280.164	399.45	224.220	198/94/87/32/27/6	253/191	18	1
4	1_BY	HQ902258	444	280/164	444	224/220	198/94/87/32/27/6	444	19	7
4	N034	KC886611	444	280/164	399/45	224/220	198/121/87/32/6	444	20	1
4	1S-c9	JQ353640	444	280/164	399/45	224/220	198/119/94/27/6	444	21	1
4	NK11	JQ686117	444	280/164	399/45	224/220	285/94/32/27/6	444	22	6
4	1S-c10	JQ353650	444	280/164	399/45	220/145/79	198/94/87/32/27/6	444	23	1
5	CRAS-1	EF065635	444	280/164	399/45	224/181/39	198/94/87/32/27/6	316/128	24	8
5	CRGC	EF065639	444	280/164	399/45	224/181/39	285/94/32/27/6	316/128	25	1
5	CRLC-1	EF065655	444	280/164	444	224/181/39	198/94/87/32/27/6	316/128	26	2
6	PL-1238	FJ808582	444	444	399/45	224/220	285/94/32/27/6	316/128	27	7
6	151	AY185360	444	444	399/45	224/220	198/94/87/32/27/6	316/128	28	40
6	GS3	MF574055	444	444	399/45	444	198/94/87/32/27/6	316/128	29	11
6	SC2	MF574060	444	444	399/45	224/220	198/94/87/32/27/6	242/128/74	30	1
6	QH1	MF574057	444	444	213/186/45	444	198/94/87/32/21/6/6	316/128	31	1
6	Pucallpa-7	LC075552	444	444	399/45	444	198/94/87/32/27/6	316/79/49	32	1
6	Paraguay-96	LC075556	444	444	399/45	444	198/121/87/32/6	316/128	33	1
7	N28	HM102356	444	444	444	224/137/83	198/94/87/32/27/6	294/128/22	34	7
7	176	AY515276	444	444	444	224/137/83	198/94/87/32/27/6	316/128	35	53
7	I2	S83530	444	444	444	224/220	285/94/32/27/6	316/128	36	1
7	14	AY515274	444	444	444	145/137/83/79	198/94/87/32/27/6	316/128	37	1
7	30	DQ059417	444	444	444	444	198/87/49/45/32/27/6	316/128	38	1
7	3S	JF720351	444	280/164	444	224/137/83	198/94/87/32/27/6	316/128	39	3
7	4T-c19	JQ353655	444	444	399/45	224/137/83	198/94/87/32/27/6	316/128	40	3
7	1S-c4	JQ353651	444	444	444	224/137/83	198/94/87/32/27/6	316/79/49	41	1
7	NK17	JQ686120	444	444	444	224/137/83	198/87/49/45/32/27/6	316/128	42	2
7	4S	JF720352	444	444	444	224/137/83	198/119/94/27/6	316/128	43	1
7	1S-c6	JQ353633	444	444	444	224/137/83	198/121/87/32/6	316/128	44	1
7	4T-c11	JQ353656	444	444	444	224/137/83	285/94/32/27/6	316/128	45	1
7	N067	KC886618	444	444	444	224/137/44/39	198/94/87/32/27/6	316/128	46	1
7	1S-c1	JQ353649	444	444	444	224/220	198/94/87/32/27/6	444	47	2
8	M1/ELG_Cro/08	GU724606	444	444	399/45	224/220	225/94/87/32/6	198/128/118	48	13
8	N174	JF713455	444	444	399/45	224/220	225/94/87/32/6	316/128	49	4
8	ELG_Cro/VRA/09	JN990072	444	444	444	224/220	225/94/87/32/6	198/128/118	50	2
8	4-6	HM563764	444	444	399/45	224/137/83	225/94/87/32/6	198/128/118	51	1
8	MKC2137	JQ675759	444	444	399/45	444	225/94/87/32/6	198/128/118	52	1
9	Monetro-1	LC075563	444	444	399/45	224/171/49	285/94/32/27/6	198/128/118	53	19
9	Portachello-20	LC075567	444	444	399/45	224/171/49	285/94/32/27/6	246/198	54	3
10	Pa51-A3	KU233547	444	444	399/45	224/220	198/94/81/32/27/6/6	444	55	12
10	ML45-B3	KU233540	444	444	399/45	224/220	279/94/32/27/6/6	444	56	11
10	L1	LC154066	444	444	444	224/220	198/94/81/32/27/6/6	444	57	1
11	E101	KU764746	444	444	444	224/220	285/94/32/27/6	444	58	2

Designations: **G** – genotype. **C** – combination. **N** – number of analyzed BLV isolates with an established combination of PCR-RFLP profiles.

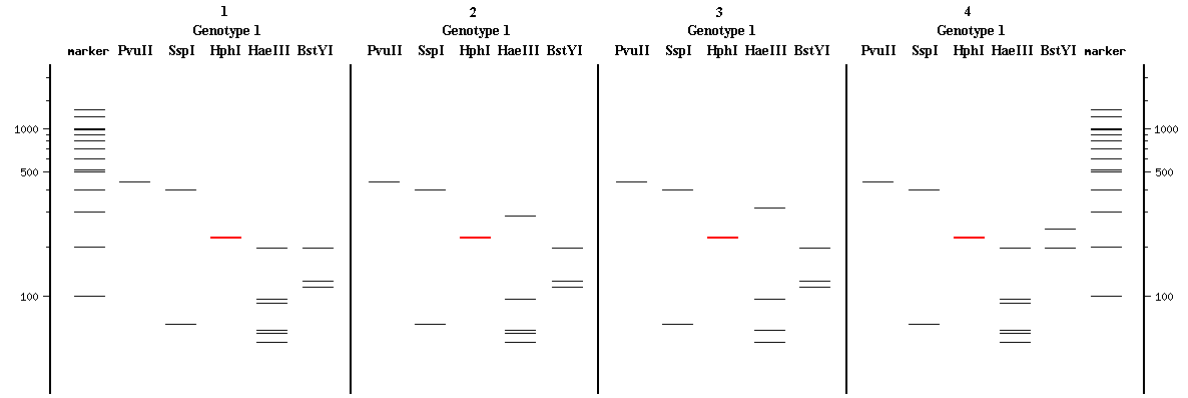

Figure 1 – *In silico* modeling of restriction patterns for 5 restriction enzymes

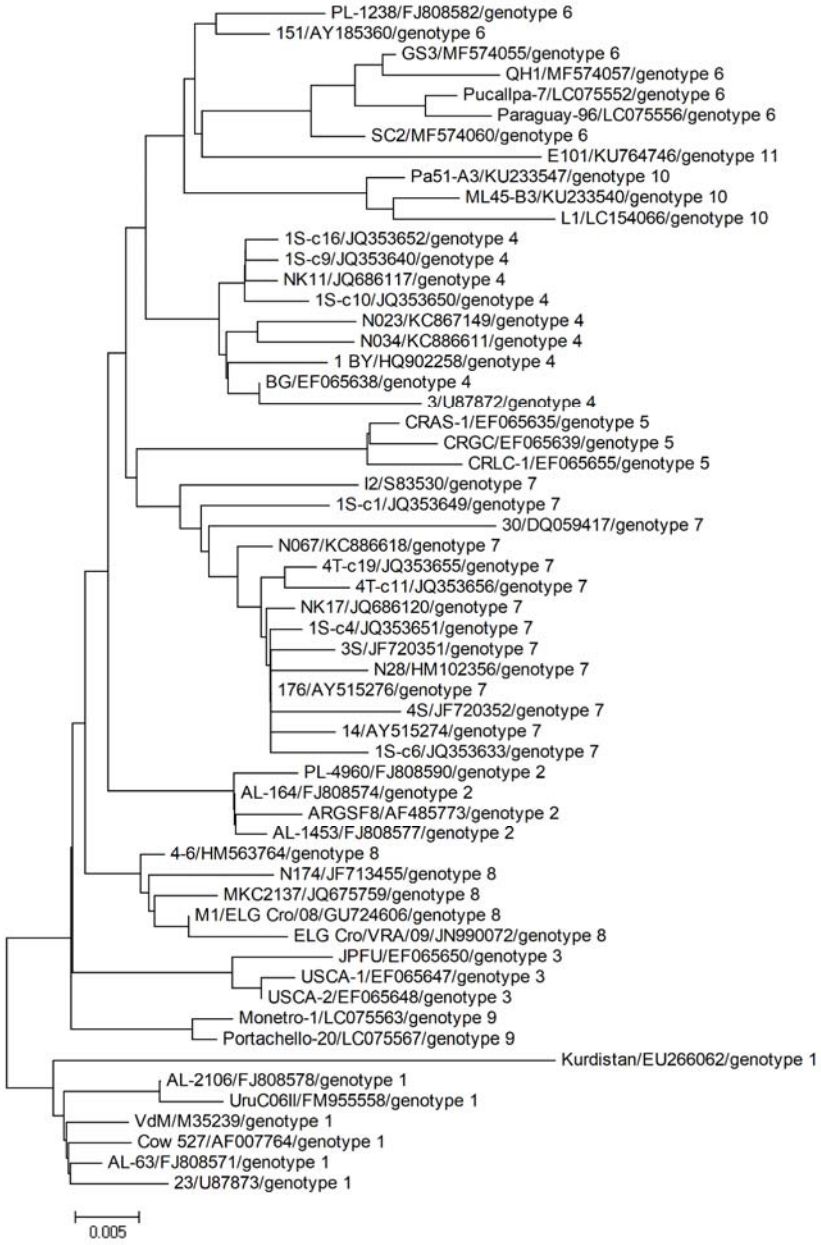

Figure 2 – Phylogram of 58 reference isolates of 11 open BLV genotypes, built on the basis of phylogenetic analysis of the *env*-gene locus [MEGA-4: NJ algorithm, 400 nt, 58 seq.]

Our updated strategy of PCR-RFLP BLV genotyping is consistent with its phylogenetic classification, making it possible to identify all eleven currently known genotypes of the viral pathogen under study.

An illustrative example of *in silico* modeling of restriction patterns for five restriction endonucleases is shown in figure 1, which reflects 4 of 58 genotype-specific combinations of *env*-PCR-RFLP BLV profiles.

The reliability of restriction patterns *in silico* modeling was substantiated by the data obtained as a result of alignment and restriction mapping of the DNA sequences of the *env*-gene fragment of the reference isolates of the known BLV genotypes amplified with the oligonucleotide primers "env5099"+ +"env5521".

The consistency of the improved strategy of PCR-RFLP BLV genotyping with its phylogenetic classification is substantiated, including by phylogenetic analysis of the *env*-gene fragment of 58 reference isolates of eleven open genotypes of the pathogen (figure 2), generating 58 genotype-associated combinations of PCR-RFLP profiles.

An illustrative example of the PCR-RFLP BLV genotyping strategy implementation in accordance with its phylogenetic classification is shown in figure 3.

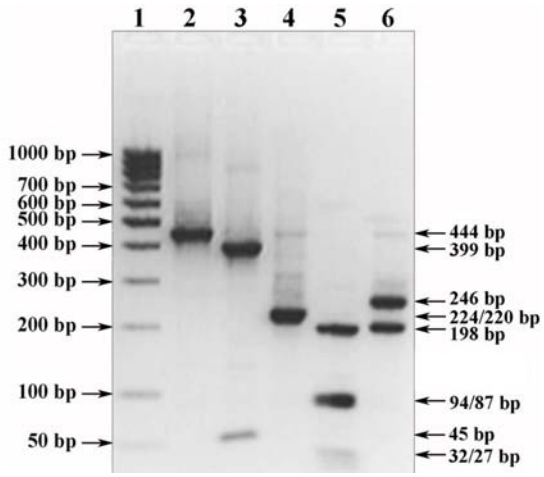

Figure 3 – Electropherogram of PCR-RFLP profiles of the 1st BLV genotype (updated genotyping strategy)
Keys: 1) DNA markers 100 bp + 50 bp (SibEnzyme). 2-6) PCR-RFLP-profile of the BLV provirus isolate "N-4" (G1, C4): 2) *Pvu*II-RFLP (444 bp); 3) *Ssp*I-RFLP (399/45 bp); 4) *Hph*I-RFLP (224/220 bp); 5) *Hae*III-RFLP (198/94/87/32 / 27.6 bp); 6) *Bst*YI-RFLP (246/198 bp). G - genotype. C - combination.

PCR-RFLP-profile of the BLV provirus isolate "N-4" (figure 3, tracks 2-6) characterizes the 4th combination (C4) of the *env*-PCR-RFLP profile of the 1st BLV genotype (G1), which includes at least 42 identified representatives of the viral pathogen under study (table).

Conclusion. Thus, as a result of verification of the developed *Bovine leukemia virus* gene identification method with an updated strategy of PCR-RFLP BLV genotyping, consistent with its phylogenetic classification, a technical result was obtained, expressed in the ability to identify all 11 viral pathogen genotypes discovered to date by interpreting the generated 58 genotype-associated combinations of PCR-RFLP profiles. The proposed technology of BLV gene diagnostics is implemented in a combined format of causative agent of cattle infection indication and identification, in the produced raw materials and manufactured products.

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ІРІ ҚАРА МАЛДАҒЫ, ШИКІЗАТТАҒЫ ЖӘНЕ ӨНДІРІЛГЕН ӨНІМДЕГІ *BOVINE LEUKEMIA VIRUS* ГЕНОДИАГНОСТИКА ТЕХНОЛОГИЯСЫ

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ТЕХНОЛОГИЯ ГЕНОДИАГНОСТИКИ *BOVINE LEUKEMIA VIRUS* У КРУПНОГО РОГАТОГО СКОТА, В ВЫРАБАТЫВАЕМОМ СЫРЬЕ И ПРОИЗВОДИМОЙ ПРОДУКЦИИ

Аннотация. Важнейшей задачей молочного скотоводства является получение высококачественного сырого молока. Для ее достижения необходим комплекс мер, в том числе направленных на повышение биологической безопасности производимого сырья.

Целью исследования явилось создание научно-методической основы генодиагностики вируса лейкоза крупного рогатого скота (БЛВ) в комбинированном формате индикации и идентификации возбудителя. Это потребовало актуализации стратегии генотипирования BLV PCR-RFLP в соответствии с ее филогенетической классификацией с учетом растущих знаний о генетическом разнообразии 11 генотипов изучаемого вирусного патогена. При постановке вложенной ПЦР использовали олигонуклеотидные праймеры, которые инициируют на заключительной стадии реакции продуцирование фрагмента гена env 444 bp патогена. Пять рестрикции были использованы в ПЦР-ПДРФ-генотипирования БЛВ: PvuII, Ссли, AsuHPI, рестриктазами haeiii, и BstX2I. В результате верификации разработанного метода идентификации генов вируса лейкоза крупного рогатого скота с обновленной стратегией генотипирования был получен технический результат, выражающийся в возможности идентификации всех 11 обнаруженных на сегодняшний день генотипов BLV путем интерпретации сгенерированных 58 генотип-ассоциированных комбинаций профилей ПЦР-РФЛП.

Ключевые слова: *Bovine leukemia virus*, BLV, крупный рогатый скот, молоко, ПЦР, ПДРФ, секвенирование, генодиагностика, генотипирование, *env*-ген.

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