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DEVELOPMENT OF SIMPLE DIAGNOSTIC KIT FOR QUICK DETECTION DNA OF AFRICAN SWINE FEVER VIRUS

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From 2012 year in Ukraine emerged very difficult situation with regard to African Swine Fever (ASF). Scientists from State Science-Control Institute of Biotechnology and Strains of Microorganisms developed highly sensitive, highly specific and not very expensive simple diagnostic kit for quick detection DNA of ASF Virus. Diagnostic kit is developed in two variants on test system that differ in the technique of DNA isolation. Using this kit possible not only carry out rapid identification of the causative agent in acute forms of the disease, but also to ensure control of imported products during the quarantine of measures, necessary to detect early signs of disease.
Keywords: AFRICAN SWINE FEVER, DIAGNOSTIC KIT, POLYMERASE CHAIN REACTION.

African swine fever (ASF) is especially dangerous viral disease, which provokes severe economic losses and for which no vaccine is currently available [1–3]. Last years in Ukraine emerged very difficult situation with regard to ASF [4]. However ASF problem - it's not just a question of the security of Ukraine, but also the European Union as a whole [5].

Effective modern method of early diagnosis of ASF is a polymerase chain reaction (PCR). That's why by scientists of our institute developed, tested and registered in the established order the test kit for the diagnosis of ASF for molecular genetic techniques.

Materials and methods. Development of a diagnostic test kit for PCR was carried out with the recommendations of OIE. Testing of specificity and sensitivity was performed on the sample, the total DNA isolated from pigs that died from ASF and of the recombinant plasmids carrying the gene fragment of the main ASF virus capsid protein. As a negative control were used materials from healthy domestic pigs. Activity and specificity of the means of has been assessed comparing with similar imported commercial test kits.

Results and discussion. Simple diagnostic kit based on the classical PCR variant has been developed in SSCIBSM. It is aimed on quick detection of ACFV DNA (amplification of conservative regions B646L gene (VP72) (Fig. 1), protein is the main component of the viral capsid) (Cobbold and Wileman, 1998) [6] in biological materials and environment (Fig. 2).

We modified the 3'-ends of the oligonucleotide primers recomended by OIE (Fig. 3, 4) for this gene and this gave the possibility to increase their annealing temperature by 5 degrees and as a result to improve reaction specificity (Fig.5, 6).

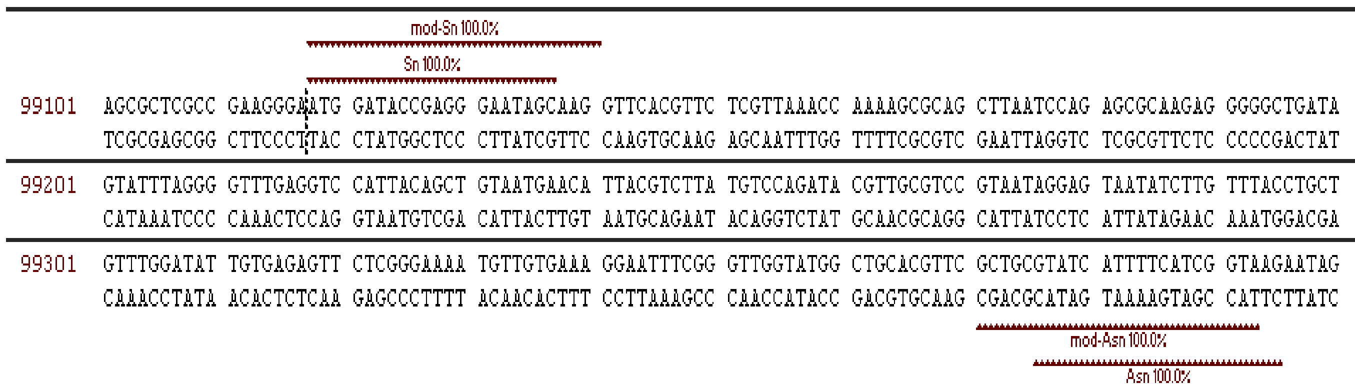

Fig. 1. The comparative position of modified 3'-END primers on the nucleotide sequence of the gene B646L

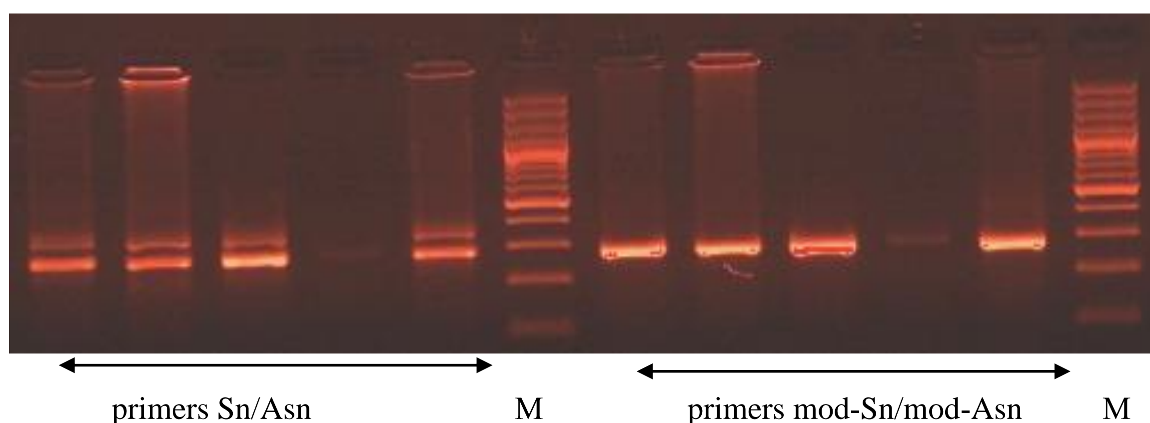

Fig. 2. Results of analysis the samples of pathological materials to source (OIE) and modified primers in PCR

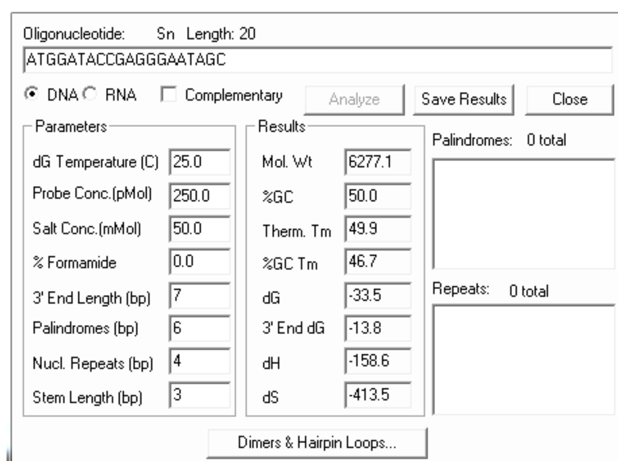

Fig. 3

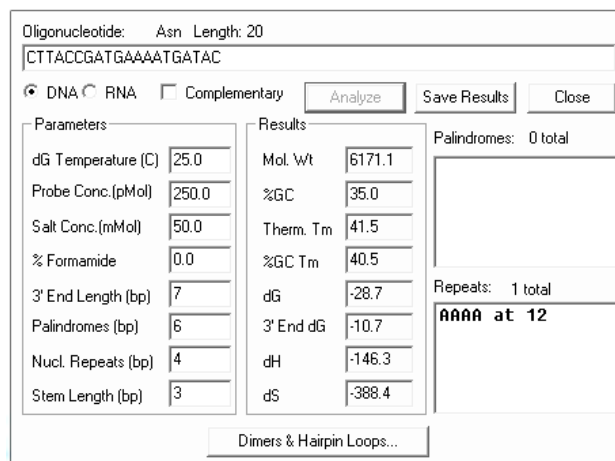

Fig. 4

Oligonucleotide: mod-Sn Length: 23
ATGGATACCGAGGGAATAGCAAG
DNA RNA Complementary Analyze Save Results Close

Parameters		Results	
dG Temperature (C)	25.0	Mol. Wt	7232.7
Probe Conc.(pMol)	250.0	%GC	47.8
Salt Conc.(mMol)	50.0	Therm. Tm	55.3
% Formamide	0.0	%GC Tm	50.2
3' End Length (bp)	7	dG	-39.0
Palindromes (bp)	6	3' End dG	-12.7
Nucl. Repeats (bp)	4	dH	-181.3
Stem Length (bp)	3	dS	-471.2

Palindromes: 0 total
Repeats: 0 total
Dimers & Hairpin Loops...

Fig. 5

Oligonucleotide: mod-Asn Length: 23
TACCGATGAAAATGATACGCAGC
DNA RNA Complementary Analyze Save Results Close

Parameters		Results	
dG Temperature (C)	25.0	Mol. Wt	7127.7
Probe Conc.(pMol)	250.0	%GC	43.5
Salt Conc.(mMol)	50.0	Therm. Tm	55.8
% Formamide	0.0	%GC Tm	48.4
3' End Length (bp)	7	dG	-38.6
Palindromes (bp)	6	3' End dG	-15.7
Nucl. Repeats (bp)	4	dH	-177.1
Stem Length (bp)	3	dS	-458.5

Palindromes: 0 total
Repeats: 1 total
AAAA at 10
Dimers & Hairpin Loops...

Fig. 6

Comparative characteristics of oligonucleotide primers the recommended by the OIE (Fig. 3, 4) and modified (Fig. 5, 6).

Sequences of the modified primers: mod-Sn 5'-ATGGATACCGAGGGAATAGCAAG-3 and mod-Asn 5'-TACCGATGAAAATGATACGCAGC-3'. DNA from recombinant plasmid pB646 is used as positive control.

Diagnostic kit is developed in two variants on test system that differ in the technique of DNA isolation. In version A, DNA isolation is performed with the use of sorbent, which has to be precipitated by centrifugation. In variant B DNA isolation is performed with the use of Ukrainian magnetide nanosorbent with saturation magnetization 37 ($A \cdot m^2/kg$), it can be precipitated in special magnetic holder (support, stand) without centrifugation. Such variant of DNA isolation not only minimizes the risk of contamination of the surrounding surface of the test material, but also can be used in the field.

Established that developed by us test system is specific (100 %) and sensitive enough and not inferior in quality research world standards.

CONCLUSIONS

Have been developed highly sensitive, highly specific and not very expensive diagnostic test kit for the diagnosis of ASF for PCR. Using this kit possible not only carry out rapid identification of the causative agent in acute forms of the disease, but also to ensure control of imported products during the quarantine of measures, necessary to detect early signs of disease.

Implementation of the developed diagnostic means in practice, we hope, will contribute to improve epizootic situation with regard to ASF in Ukraine.

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РОЗРОБКА ПРОСТОГО ДІАГНОСТИЧНОГО НАБОРУ ДЛЯ ШВИДКОГО ВИЗНАЧЕННЯ ДНК ВІРУСУ АФРИКАНСЬКОЇ ЧУМИ СВИНЕЙ

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А Н О Т А Ц І Я

Із 2012 року в Україні виникла дуже складна ситуація щодо африканської чуми свиней (АЧС). Вчені Державного науково-контрольного інституту біотехнології і штамів мікроорганізмів розробили високочутливий, вельми специфічний, не дуже високовартісний простий у виконанні діагностичний набір для швидкого виявлення ДНК вірусу АЧС. Діагностичний набір розроблений у двох варіантах, які відрізняються за методикою виділення ДНК. За допомогою цього набору можливо не тільки проводити швидку ідентифікацію збудника при гострих формах захворювання, а й забезпечити контроль імпортної продукції під час карантинних заходів, що необхідні для виявлення ранніх ознак захворювання.

Ключові слова: АФРИКАНСЬКА ЧУМА СВИНЕЙ, ДІАГНОСТИЧНИЙ НАБІР, ПОЛІМЕРАЗНА ЛАНЦЮГОВА РЕАКЦІЯ.

РАЗРАБОТКА ПРОСТОГО ДИАГНОСТИЧЕСКОГО НАБОРА ДЛЯ БЫСТРОГО ОБНАРУЖЕНИЯ ДНК ВИРУСА АФРИКАНСКОЙ ЧУМЫ СВИНЕЙ

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С 2012 года в Украине возникла очень сложная ситуация по африканской чуме свиней (АЧС). Ученые Государственного научно-контрольного института биотехнологии и штаммов микроорганизмов разработали высокочувствительный, весьма специфичный, не слишком дорогой и простой в исполнении диагностический набор для быстрого выявления ДНК вируса АЧС. Диагностический набор разработан в двух вариантах, которые отличаются по методике выделения ДНК. С помощью этого набора можно не только проводить быструю идентификацию возбудителя при острых формах заболевания, но и обеспечить контроль импортной продукции при карантинных мероприятиях, которые необходимы для раннего выявления заболевания.

Ключевые слова: АФРИКАНСКАЯ ЧУМА СВИНЕЙ, ДИАГНОСТИЧЕСКИЙ НАБОР, ЦЕПНАЯ ПОЛИМЕРАЗНАЯ РЕАКЦІЯ.

R E F E R E N C E S

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