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Д.В. Сокольский атындағы «Жанармай,  
катализ және электрохимия институты» АҚ

# Х А Б А Р Л А Р Ы

## ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК  
РЕСПУБЛИКИ КАЗАХСТАН  
АО «Институт топлива, катализа и  
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## NEWS

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## RAW MATERIALS IDENTIFICATION AND MANUFACTURED PRODUCTS AUTHENTICATION TECHNOLOGIES

**Abstract.** DNA technologies for the raw grapes identification and the authentication of wine products made from them are popular scientific and practical direction with the prospect of introducing developments into the quality management system. The molecular genetic approaches development to the technical grape varieties' genetic identification and wines DNA authentication produced from them was the aim of this work. Sample preparation of experimental wines was carried out by exposure in a mixture with a precipitant and a co-precipitant, followed by wine debris precipitation. The nucleic acids extraction from wine debris was carried out with the same commercial kit as from the pulp of a mature grape fruit, but with an additional lysis solution modification. The obtained results indicated the selected conditions effectiveness for sample preparation, DNA isolation and PCR for amplification of the analyzed *Vitis vinifera* L. *UFGT*-gene loci. The described strategy for the raw materials geno-identification and the manufactured products authentication is based on the polymorphic positions of diagnostically significant *UFGT*-gene-associated groups analysis, interpreted by sequencing the corresponding PCR product.

**Key words:** grapes, wine, variety, *Vitis vinifera* L, sample preparation, extraction, DNA, identification, authentication, gene, *UFGT*, SNP, PCR, sequencing.

**Introduction.** The development of DNA technologies for the raw grapes identification and the wine products made from them authentication is a popular area of molecular genetic research methods, whose promising developments can be introduced into the quality management system to ensure traceability of the entire product life cycle and counterfeit products monitoring [1-6].

Wines DNA authentication is a technological process of verifying their authenticity by geno-identification of the main plant component (technical grape varieties) through molecular genetic analysis of the extracted of *Vitis vinifera* L. nucleic acids. At the same time, the yield of isolated from wine debris DNA is significantly reduced in the vinification process up to its complete absence by wine aging [7-11].

Among genetic markers multitude, SNP-markers have a high identification potential, which are suitable for the raw materials genetic identification [12-14] and wines DNA authentication [8, 15, 16] due to discrimination of individual genotypes of *Vitis vinifera* L. and identification of combined genotypes groups, including single-varietal and blended wines in the analysis of fragmented nucleic acid of low quality and yield.

The research purpose is the molecular genetic approaches development to the genetic identification of technical grape varieties and DNA authentication of wines produced from them.

**Material and research methods.** The work was carried out on the basis of the Intersectoral Scientific and Technical Center for Monitoring the Quality of Food Products of the All-Russian Scientific Research Institute of Brewing, Beverage and Wine Industry – Branch of V.M. Gorbatov Federal Research Center for Food Systems of RAS.

Research objects: technical grape varieties and experimental young wines produced from them.

Sample preparation of technical grape varieties was carried out by extracting 50 mg of the ripe fruit pulp and placing the selected sample in an Eppendorf test tube.

Sample preparation of experimental wines was carried out by exposure in a mixture with a precipitant (isopropanol) and a co-precipitant (polyvinylpyrrolidone), followed by centrifugal wine debris precipitation.

When using only a precipitant [17], they were poured into 15 ml Falcon tubes, each 8 ml of the test wine samples. Then 6 ml of cold isopropanol was added, smoothly stirring the mixture until

homogeneous state by manually test tube shaking the. The tubes were kept with tightly closed lids at a temperature of -20 °C for 1-14 days in a freezer. After the exposure, the tubes were centrifuged in a CM-6M centrifuge (Elmi, Latvia) at 3000 rpm for 1 hour. The supernatant was decanted off, and the precipitate was dried by keeping the tubes upside down under a filter paper layer.

When using a precipitant and a co-precipitant, it was poured into 1.5 ml Eppendorf tubes with 10 mg of polyvinylpyrrolidone K30, 900 µl of isopropanol, previously poured into them, the mixture was stirred until homogeneous state on a Microspin FV-2400 vortex centrifuge (Biosan, Latvia). Then, 100 µl of the test red wines samples were added, stirring the mixture until homogeneous state by smooth manual shaking of the test tube. The tubes with tightly closed lids were kept at room temperature for 10 minutes to 14 days. After exposure, the tubes were centrifuged in a CM-50 microcentrifuge (Elmi, Latvia) at 15,000 rpm for 10 min. The supernatant was disposed of using an FTA-1 aspirator with a trapping flask (Biosan, Latvia).

The extraction of nucleic acids from the grape fruit pulp was carried out with the commercial set "DNA-sorb-S-M" (Central Research Institute of Epidemiology, Russia), and from the precipitated young wine debris - with the same set, but with an additional modification of the lysis solution by adding 4 µl of mercaptoethanol and 100 µl of proteinase K of initial concentration 10 mg/ml.

PCR with isolated DNA samples was performed with a commercial Encyclo Plus PCR kit ("Evrogen ZAO" (Close Joint-stock Company), Russia), which included the necessary components of the reaction mixture (sterile water, buffer system, a mixture of dNTP and Encyclo polymerases).

The list of oligonucleotide primers [14, 15] and thermocycling modes on the Tertsik amplifier (DNA-technology, Russia) are presented in table 1.

Table 1 – Primer kits and thermal cycling modes

Name and sequence of oligonucleotide primers	Thermal cycling modes	PCR-product
Vv1-Fwd: 5'-GCAATGTAATATCAAGTCC-3' Vv1-Rev: 5'-TTTCTTTCTTTGAGCCATT-3'	×1: 95 °C – 300 s, ×40: 95 °C – 30 s, 58 °C – 30 s, 72 °C – 30 s. ×1: 72 °C – 300 s	705 bp
Vv3-Fwd: 5'-AGCAGAGATGGGGGTGGCTT-3' Vv3-Rev: 5'-AGCAGGTAACACCTGAA-3'	×1: 95 °C – 300 s, ×40: 95 °C – 30 s, 58 °C – 30 s, 72 °C – 30 s. ×1: 72 °C – 300 s	119 bp
UFGT-F1: 5'-CTTGGCTGCCGTTTGGA-3' UFGT-R1: 5'-AGGTAAAACACCTGAAACT-3'	×1: 95 °C – 300 s, ×40: 95 °C – 10 s, 58 °C – 10 s, 72 °C – 10 s. ×1: 72 °C – 300 s	99 bp

Electrophoretic detection of PCR products in a SE2 horizontal electrophoresis chamber (Helikon, Russia) was carried out in 2.5% agarose gel with 1×TAE buffer stained with ethidium bromide, followed by result visualization in a UV-transilluminator (Vilber Lourmat, France).

Amplicons of the analyzed *UFGT*-gene locus were sequenced on an ABI PRISM 3100 genetic analyzer (Applied Biosystems, USA) with their subsequent alignment in BLAST with the corresponding partial nucleotide sequences of *Vitis vinifera* L.

**Results and discussion.** The initial stage of technical grape varieties sample preparation, which consisted in extracting and transferring the ripe fruits pulp into test tubes for further DNA extraction procedure, was a balanced approach to the selection of biomaterial from studied raw material.

At the same time, the *Vitis vinifera* L. nucleic acids samples isolated from the raw grapes sample-prepared material by the commercial kit "DNA-sorb-S-M" ensured the achievement of positive PCR results, expressed in the amplification of specific amplicons of the analyzed gene loci.

The electrophoretic results pattern of the *Vitis vinifera* L. *UFGT*-gene loci PCR amplification initiated with sets of well-known primers is shown in figure 1.

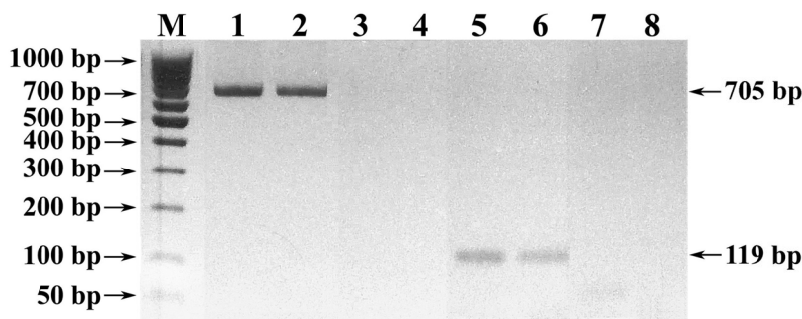


Figure 1 – Electropherogram of the PCR amplification results of the *Vitis vinifera* L. *UFGT*-gene loci

Notations: M) DNA markers 100 bp + 50 bp (SibEnzyme). 1-4) Amplified with Vv1-Fwd and Vv1-Rev primers PCR tests of DNA samples extracted from the ripe grape pulp and precipitated debris of wine material: 1) the ripe grape pulp of Cabernet Sauvignon variety; 2) the ripe grape pulp of Chardonnay variety; 3) the precipitated debris of Cabernet Sauvignon wine material; 4) the precipitated debris of Chardonnay wine material; 5-8) Amplified with Vv3-Fwd and Vv3-Rev primers PCR tests of DNA samples extracted from the ripe grape pulp and precipitated debris of wine material: 5) the ripe grape pulp of Cabernet Sauvignon variety; 6) the ripe grape pulp of Chardonnay variety; 7) the precipitated debris of Cabernet Sauvignon wine material; 8) the precipitated debris of Chardonnay wine material.

Thus, in PCR tests of DNA samples extracted from the industrial grape varieties pulp using a set of DNA-sorb-S-M, specific amplicons were generated, initiated with Vv1-Fwd and Vv1-Rev (705 bp) [14] and Vv3-Fwd and Vv3-Rev (119 bp) [15] primers, respectively. At the same time, in PCR tests of DNA samples isolated from precipitated wine debris, by a similar extraction method, specific PCR products were not amplified (figure 1).

Thus, the obtained results indicated the effectiveness of the selected conditions for sample preparation, DNA isolation and PCR for amplification of the analyzed *Vitis vinifera* L. *UFGT*-gene loci in relation to the studied raw grape samples, but not to the wine materials produced from them.

Subsequent extraction of nucleic acids from sample-prepared wine materials using the DNA-sorb-S-M kit modified with supplemental application of mercaptoethanol and proteinase K to the lysis solution led to a satisfactory PCR result in conjunction with the UFGT-F1 and UFGT-R1 primers that we designed (figure 2).

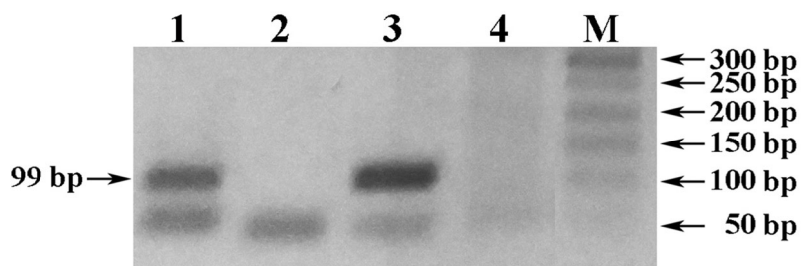


Figure 2 – Electropherogram of the PCR amplification result of the *Vitis vinifera* L. *UFGT*-gene locus with UFGT-F1 and UFGT-R1 primers

Notations: 1-3) PCR tests of DNA sample extracted from the precipitated Cabernet Sauvignon wine material debris: 1) 3  $\mu$ l of DNA; 2) 4  $\mu$ l of DNA; 3) 5  $\mu$ l of DNA added to the PCR reaction mixture; 4) Negative control of DNA extraction. M) DNA length marker 50+ bp DNA Ladder (Evrogen).



This primers set for PCR amplification of the *Vitis vinifera* L. *UFGT*-gene locus by PCR differs from the prototype [15] in that other oligonucleotide sequences are used that initiate the production of specific PCR product with a length of 99 bp with localization in the flanked region (404-502 nt, GenBank A/N: DQ513314) 5 polymorphic positions interpreted by sequencing or HRM analysis during genotyping of grape varieties and DNA authentication of the wine materials produced from them.

In this case, the PCR sensitivity and reproducibility with the designed primers set (UFGT-F1 and UFGT-R1) turned out to be higher than the prototype (Vv3-Fwd and Vv3-Rev) (figure 3).

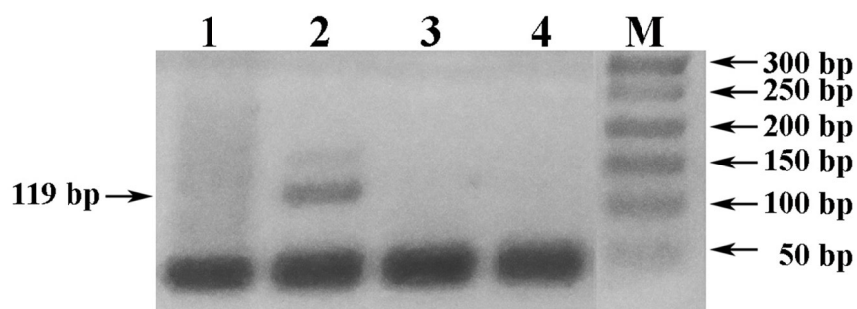


Figure 3 – Electropherogram of the PCR amplification result of the *Vitis vinifera* L. *UFGT*-gene locus with Vv3-Fwd and Vv3-Rev primers

Notations: 1-3) PCR tests of DNA sample extracted from the precipitated Cabernet Sauvignon wine material debris; 1) 3 µl of DNA; 2) 4 µl of DNA; 3) 5 µl of DNA added to the PCR reaction mixture. 4) Negative control of DNA extraction. M) DNA length marker 50+ bp DNA Ladder (Evrogen).

However, it should be noted that the efficiency of the well-known Vv3-Fwd and Vv3-Rev primers, initiating the PCR product amplification with a length of 119 bp, was previously shown in DNA extraction by the Pereira method [17], which is characterized by a relatively high nucleic acids yield isolated from precipitated wine debris, in comparison with other methods [18-21].

The technical grape varieties grouping and breakdown according to the analyzed polymorphic positions profile of the *UFGT*-gene locus is one of the ways of their gene identification and DNA authentication of wines produced from them.

Bioinformatic analysis of nucleotide sequences of the *Vitis vinifera* L. *UFGT*-gene locus allows to distribute technical grape varieties by breaking down into gene-associated groups (table 2).

Table 2 – Polymorphic positions of the *UFGT*-gene locus of *Vitis vinifera* L.

Technical grape variety	Polymorphic positions (INDEL*/SNPs)																
	205	207	220	238	240	257	264	265	272	309	366	375	424*	425	442	459	483
Chardonnay	R	S	S	G	C	A	G	C	T	G	T	Y	-	G	C	C	G
Touriga Brasileira	G	G	G	G	C	A	G	C	T	G	T	T	-	G	C	C	G
Gouveio	G	G	S	G	M	A	G	C	T	G	T	Y	-	G	C	C	G
Donzelinho Tinto	G	G	C	R	M	R	K	C	T	G	T	Y	-	G	C	C	G
Tinta Francisca	G	G	S	R	C	R	K	C	T	R	T	Y	-	G	C	C	S
Alicante Bouschet	G	G	S	R	C	R	K	C	T	R	T	Y	-	G	C	C	S
Tinta Amarela	G	G	S	R	C	R	K	C	T	R	T	Y	-	G	C	C	S
Côdega do Larinho	G	G	S	R	C	R	K	C	T	R	T	Y	-	G	C	C	S
Fernão Pires	A	C	C	G	C	A	G	M	C	A	T	C	-	G	C	T	G
Tinta Roriz	A	C	C	G	C	A	G	M	C	A	T	C	-	G	C	T	G
Malvasia Fina	A	C	C	G	C	A	G	A	C	A	T	C	-	G	C	T	G
Tinto Cão	R	S	C	R	C	R	K	C	Y	R	T	Y	-	G	C	Y	G
Pinot Noir	G	G	S	S	C	R	K	C	T	G	T	T	-	G	C	Y	G
Moscatel Galego	R	S	S	G	C	A	G	C	Y	R	T	Y	-	G	C	Y	G
Merlot	G	G	G	G	C	A	G	C	T	W	W	C	-	K	C	C	C
Tinta Barroca	G	G	G	G	C	A	G	C	T	A	T	C	-	K	C	C	C
Touriga Nacional	G	G	G	G	C	A	G	C	T	R	T	Y	-	K	C	C	S
Touriga Franca	G	G	S	R	C	R	K	C	T	R	T	Y	-	K	C	C	S

Technical grape variety	Polymorphic positions (INDEL*/SNPs)																
	525	555	560	562	598	600	617	619	636	663	685	762	789	816	841	843	850
Viosinho	R	S	S	G	C	A	G	C	Y	A	T	C	-	G	C	Y	S
Rufete	R	S	C	R	C	A	G	M	C	A	T	C	-	G	M	Y	G
Cabernet Sauvignon	R	S	S	G	C	A	G	C	Y	A	T	C	T	G	C	Y	S
Sousão	R	S	S	G	C	A	G	C	Y	A	T	C	T	G	C	T	S
Cannonao	G	G	S	G	C	A	G	C	T	R	T	C	-	G	C	C	C
Parda	G	G	C	A	C	R	K	C	Y	R	T	Y	-	G	M	C	G
Chardonnay	A	M	A	G	A	T	C	A	C	C	A	C	C	T	R	A	A
Touriga Brasileira	A	A	A	G	A	T	C	A	C	C	A	C	C	T	A	A	A
Gouveio	A	M	R	G	A	Y	M	R	Y	Y	A	C	C	T	R	A	A
Donzelinho Tinto	A	C	G	R	A	Y	A	G	T	T	A	Y	M	Y	G	A	W
Tinta Francisca	A	C	R	R	A	T	M	R	Y	Y	A	Y	M	Y	G	W	W
Alicante Bouschet	A	C	R	R	A	T	M	R	Y	Y	A	Y	M	Y	G	W	W
Tinta Amarela	A	C	R	R	A	T	M	R	Y	Y	A	Y	M	Y	G	W	W
Côdega do Larinho	A	C	R	R	A	T	M	R	Y	Y	A	Y	M	Y	G	W	W
Fernão Pires	T	C	A	G	T	T	C	A	C	C	A	C	C	Y	G	A	A
Tinta Roriz	T	C	A	G	W	T	C	A	C	C	A	C	C	Y	G	A	A
Malvasia Fina	T	C	A	G	W	T	C	A	C	C	A	C	C	Y	G	A	A
Tinto Cão	W	C	R	R	A	T	M	R	Y	Y	A	Y	M	C	G	A	W
Pinot Noir	W	M	A	G	A	T	C	A	C	C	W	C	C	T	S	W	A
Moscatel Galego	W	M	A	G	A	T	C	A	C	C	A	C	C	T	R	A	A
Merlot	A	C	A	G	A	T	C	A	C	C	A	C	C	T	G	T	A
Tinta Barroca	A	C	A	G	A	T	C	A	C	C	A	C	C	T	G	T	A
Touriga Nacional	A	M	A	G	A	T	C	A	C	C	A	C	C	T	R	W	A
Touriga Franca	A	C	R	R	A	T	M	R	Y	Y	A	Y	M	Y	G	W	W
Viosinho	W	C	A	G	A	T	C	A	C	C	A	C	C	Y	G	W	A
Rufete	W	C	A	G	W	T	C	A	C	C	A	C	C	T	G	A	A
Cabernet Sauvignon	W	C	A	G	A	T	C	A	C	C	A	C	C	Y	G	W	A
Sousão	W	C	A	G	A	T	C	A	C	C	A	C	C	Y	G	W	A
Cannonao	A	C	R	R	A	T	M	R	Y	Y	A	C	C	T	G	W	A
Parda	A	C	R	G	A	T	M	R	Y	Y	A	C	M	Y	G	A	A

The table below shows 34 polymorphic positions of the *Vitis vinifera* L. *UFGT*-gene locus (33 SNP and 1 INDEL), flanked by the Vv1-Fwd and Vv1-Rev primers, the interpretation of which allows the geno-identification of technical grape varieties by establishing their belonging to a certain gene-associated group by sequencing or HRM analysis of the amplified PCR product with a length of 705 bp.

The generated table is based mainly on the previously presented information [14], which allowed the authors to discriminate 18 different grapes genotypes from 22 studied technical varieties, supplemented by us with two more genotypes (varieties Cannonao and Parda) as reference.

In total, this identification strategy is characterized by the presence of 21 diagnostically significant *UFGT*-gene-associated groups.

With regard to DNA authentication of wine material, the most valuable is the analysis of 5 polymorphic positions of the *Vitis vinifera* L. *UFGT*-gene locus (1 INDEL - 424 nt, 4 SNPs - 425, 442, 459, and 483 nt), flanked as well-known (Vv3-Fwd and Vv3-Rev) [15], and a set of primers designed by us (*UFGT*-F1 and *UFGT*-R1).

In total, this DNA authentication strategy is already characterized by the presence of 12 diagnostically significant *UFGT*-gene-associated groups, which are also identified by sequencing or HRM analysis of the corresponding PCR product.

**Conclusion.** The described strategy of raw grapes geno-identification and DNA-authentication of wine material, based on the detection and interpretation of polymorphic positions (SNP/INDEL) of diagnostically significant *UFGT*-gene-associated groups by sequencing or HRM analysis, is achieved in a complex of selected conditions for sample preparation, nucleic acid isolation and PCR for amplification of the corresponding gene loci. The practical significance of the work is aimed at introducing developments into the quality management system of raw materials and products based on the standards being developed for the wine industry.

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## **ШИКЗАТТЫ ИДЕНТИФИКАЦИЯЛАУ ЖӘНЕ ӨНДІРЕЛТІН ӨНІМДІ АУТЕНТИФИКАЦИЯЛАУ ТЕХНОЛОГИЯСЫ**

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## **ТЕХНОЛОГИИ ИДЕНТИФИКАЦИИ СЫРЬЯ И АУТЕНТИФИКАЦИИ ПРОИЗВОДИМОЙ ПРОДУКЦИИ**

**Аннотация.** Развитие ДНК-технологий идентификации винограда-сырья и аутентификации произведенной из нее винодельческой продукции является востребованным направлением молекулярно-генетических методов исследования, чьи перспективные разработки могут быть внедрены в систему менеджмента качества с обеспечением прослеживаемости всего жизненного цикла продукта и мониторинга фальсифицированной продукции. ДНК-аутентификация вин – технологический процесс проверки их подлинности геноидентификацией основного растительного компонента (технических сортов винограда) посредством молекулярно-генетического анализа экстрагируемых нуклеиновых кислот *Vitis vinifera* L. При этом выход выделенной из винного дегриза ДНК значительного сокращается в процессе винификации вплоть до полного ее отсутствия по мере старения вина. Цель исследований – разработка молекулярно-генетических подходов к геноидентификации технических сортов винограда и ДНК-аутентификации производимых из них вин. Работа выполнена на базе Межотраслевого научно-технического центра мониторинга качества пищевых продуктов Всероссийского научно-исследовательского института пивоваренной, безалкогольной и винодельческой промышленности – филиала Федерального научного центра пищевых систем им. В.М. Горбатова РАН. Объекты исследований: технические сорта винограда и произведенные из них экспериментальные молодые вина. Пробоподготовку технических сортов винограда проводили извлечением 50 мг мякоти зрелого плода и помещением отобранного образца в пробирку типа Эппендорф. Пробоподготовку экспериментальных вин осуществляли экспозицией в смеси с осадителем (изопропанол) и соосадителем (поливинилпирролидон) с последующим центробежным осаждением винного дегриза. Экстракцию нуклеиновых кислот из мякоти плода винограда осуществляли коммерческим набором «ДНК-сорб-С-М» (ЦНИИ Эпидемиологии, Россия), а из осаденного дегриза молодого вина – этим же набором, но с дополнительной модификацией лизирующего раствора добавлением 4 мкл меркаптоэтанола и 100 мкл протеиназы К исходной концентрации 10 мг/мл. Постановку ПЦР с образцами выделенной ДНК выполняли коммерческим набором реактивов «Encyclo Plus PCR kit» (ЗАО «Евроген», Россия), включающего необходимые компоненты реакционной смеси (стерильная вода, буферная система, смесь dNTP и полимераз Encyclo). Образцы нуклеиновых кислот *Vitis vinifera* L, выделенные из пробоподготовленного материала винограда-сырья коммерческим набором «ДНК-сорб-С-М», обеспечивали достижение положительных результатов ПЦР, выраженных в амплификации специфичных ампликонов анализируемых локусов гена. Последующая экстракция нуклеиновых кислот из пробоподготовленных виноматериалов набором «ДНК-сорб-С-М» в модификации с дополнительным внесением в лизирующий раствор меркаптоэтанола и протеиназы К, приводила к удовлетворительному результату ПЦР в связке со сконструированными нами праймерами UFGT-F1 и UFGT-R1. Данный набор праймеров для амплификации локуса *UFGT*-гена *Vitis vinifera* L. методом ПЦР, отличается от прототипа тем, что используются другие последовательности олигонуклеотидов, иницирующих наработку специфичного ПЦР-продукта длиной 99 bp с локализацией во фланкируемом регионе (404-502 nt, GenBank A/N: DQ513314) 5 полиморфных позиций, интерпретируемых секвенированием или HRM-анализом при геноидентификации сортов винограда и ДНК-аутентификации производимых из них виноматериалов. Группирование и разбивка технических сортов винограда по профилю анализируемых полиморфных позиций локуса *UFGT*-гена является одним из способов их

геноидентификации и ДНК-аутентификации производимых из них вин. Биоинформационный анализ нуклеотидных последовательностей локуса *UFGT*-гена *Vitis vinifera* L. позволяет распределить технические сорта винограда разбивкой на ген-ассоциированные группы. Описанная стратегия геноидентификации винограда-сырья и ДНК-аутентификации виноматериала, базирующаяся на детекции и интерпретации полиморфных позиций (SNP/INDEL) диагностически значимых *UFGT*-ген-ассоциированных групп секвенированием или HRM-анализом, достигается в комплексе подобранных условий пробоподготовки, выделения нуклеиновых кислот и постановки ПЦР для амплификации соответствующих локусов гена. Практическая значимость работы направлена на внедрение разработок в систему менеджмента качества сырья и продукции на основе разрабатываемых стандартов для винодельческой промышленности.

**Ключевые слова:** виноград, вино, сорт, *Vitis vinifera* L, пробоподготовка, экстракция, ДНК, идентификация, аутентификация, ген, *UFGT*, SNP, ПЦР, секвенирование.

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