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SYNTHESIS OF PRIMERS AND DEVELOPMENT OF SIGNIFICANT GENES OF B.1.1.7 (ALPHA) VARIANT OF THE SARS-COV-2 VIRUS

Abstract. The threat of human infection with coronavirus became known back in the 60s of the last century. Since then, zoonotic coronavirus infection has spread among people and there were two major epidemics at the beginning of the 21st century. The rate of human infection with coronavirus infection spread rapidly and caused serious concern in the world. Coronavirus infection has undergone various genetic changes over time, as a result, in 2019, in Wuhan, China, coronavirus infection spread among people. Due to the widespread spread of coronavirus infection in the world, *WHO* declared a pandemic on March 11, 2020. Coronavirus infection *SARS-CoV-2* has been registered in about 200 countries around the world and has undergone various genetic changes over time. Due to genetic changes, a new strain of coronavirus infection appeared in the *UK* in the autumn of 2020. To determine the genetic changes of coronavirus infection and to study them, 65 pairs of primers were developed in the Collective Use Laboratory to obtain the complete nucleotide sequence of the *SARS-CoV-2* virus. Specific primers have been developed for the development of significant genes of the *SARS-CoV-2* virus. The developed primers allow us to obtain complete information about the genes of *SARS-CoV-2* viruses.

The presented primers can be used for identification and development of the complete genome of the *SARS-CoV-2* virus

Keywords: *SARS-CoV-2*, mutation, PCR.

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ПРАЙМЕРЛЕР СИНТЕЗІ ЖӘНЕ SARS-COV-2 ВИРУСЫ B.1.1.7 (АЛЬФА) НҰСҚАСЫНЫҢ МАҢЫЗДЫ ГЕНДЕРДІН ӨҢДЕУ

Аннотация. Адамның коронавирус жұқтыру қауіпі өткен ғасырдың 60-жылдарында белгілі болды. Содан бері зоонозды коронавирустық инфекция адамдарға таралып, 21 ғасырдың басында екі үлкен індет болды. Адамдардың коронавирустық инфекцияны жұқтыру көрсеткіші тез таралды және әлемде үлкен алаңдаушылық тудырды. Коронавирустық инфекция уақыт өте келе әртүрлі генетикалық өзгерістерге ұшырады, нәтижесінде 2019 жылы Ухань қ. ҚХР коронавирустық инфекция адамдар арасында таралды. Әлемде

коронавирустық инфекцияның кең таралуына байланысты ДДСҰ 2020 жылдың 11 наурызында пандемия жариялады. *SARS-CoV-2* коронавирустық инфекциясы әлемнің 200-ге жуық елінде тіркелген және уақыт өте келе әртүрлі генетикалық өзгерістерге ұшырады. Генетикалық өзгерістерге байланысты 2020 жылдың күзінде Ұлыбританияда коронавирустық инфекцияның жаңа штаммы пайда болды. Коронавирустық инфекцияның генетикалық өзгеруін анықтау және оларды ажырату үшін біздің зертханада геномдық толық сиквенс жасау үшін 65 жұп праймер жасалынды. *SARS-CoV-2* вирусының маңызды гендерін өңдеуге арналған арнайы праймерлер жасалды. Әзірленген праймерлер *SARS-CoV-2* вирустарының гендері туралы толық ақпарат алуға мүмкіндік береді.

Ұсынылған праймерлерді *SARS-CoV-2* вирусының толық геномын анықтау және идентификациялау үшін пайдалануға болады

Кілт сөздер: *SARS-CoV-2*, мутация, ПТР.

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СИНТЕЗ ПРАЙМЕРОВ И НАРАБОТКА ЗНАЧИМЫХ ГЕНОВ B.1.1.7 (АЛЬФА) ВАРИАНТА ВИРУСА SARS-COV-2

Аннотация. Угроза заражения человека коронавирусом стала известна еще в 60-х годах прошлого века. С тех пор зоонозная коронавирусная инфекция распространилась среди людей и в начале 21-го века было две крупные эпидемии. Показатель заражения людей коронавирусной инфекцией быстро распространился и вызвал серьезную обеспокоенность в мире. Коронавирусная инфекция со временем претерпела различные генетические изменения, в результате в 2019 году в городе Ухань, КНР коронавирусная инфекция распространилась среди людей. Из-за широкого распространения коронавирусной инфекции в мире ВОЗ объявила пандемию 11 марта 2020 года. Коронавирусная инфекция *SARS-CoV-2* зарегистрирована около в 200 странах мира и со временем претерпела различные генетические изменения. Из-за генетических изменений в Великобритании осенью 2020 года появился новый штамм коронавирусной инфекции. Для определения генетических изменений коронавирусной инфекции и для их различения, в нашей лаборатории было разработано 65 пар праймеров для получения полного геномного сиквенса. Разработаны специальные праймеры для наработки значимых генов вируса *SARS-CoV*-2. Разработанные праймеры позволяют получить полную информацию о генах вирусов *SARS-CoV*-2.

Представленные праймеры можно использовать в целях идентификации и наработки полного генома вируса *SARS-CoV*-2.

Ключевые слова: *SARS-CoV-2*, мутация, ПЦР.

Introduction. Coronaviruses are a zoonotic and well-studied group of single-stranded RNA viruses of the Coronaviridae family. Coronaviruses (family Coronaviridae) are round enveloped virions with a diameter of 80-220 nm, which contain a single-stranded (+) RNA genome with a size of about 26-32 tn [1].

Since the late 1960s, coronaviruses have been detected in people with mild cold-related symptoms as a causative agent of respiratory diseases [1,2]. Currently, seven different strains

of coronavirus have been described. Of these, 4 coronavirus infections occur in the upper respiratory tract and are characterized by mild symptoms, the remaining 3 coronavirus infections infect the lower respiratory tract and affect a person in severe form, which include: *SARS-CoV* (*Severe acute respiratory syndrome*), *MERS-CoV* (*Middle East respiratory syndrome*) and *SARS-CoV-2* (*COVID-19*) [3].

In December 2019, a new coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was discovered in Wuhan, China and caused an epidemic in this area [4]. Due to the spread of coronavirus infection in the world on March 11, 2020, the World Health Organization (*WHO*) declared *COVID-19* a pandemic [5]. As of January 20, a total of 332617707 confirmed cases were registered, resulting in 5551314 deaths. According to *WHO* data, a total of 1169932 cases, 18338 cases with a fatal outcome have been registered in Kazakhstan to date [6].

All viruses, including *SARS-CoV-2* causing *COVID-19*, undergo genetic changes over time. One of the strains that have undergone genetic changes of *COVID-19* is considered to be variant *Alpha B.1.1.7* of the *SARS-CoV-2* virus [7]. On May 31, 2021, WHO officially announced the renaming of the British strain to "*alpha*" [8, 9]. According to *WHO*, the *Alpha* coronavirus strain appeared in the *UK* in September last year. The alpha variant of the coronavirus has an unusually large number of genetic changes, especially in the *Spike* protein that the virus uses when attaching to cells [10]. Three of these mutations have potential biological meanings. These mutations in *Spike* protein include *N501Y, P681H* and deletions *H69-V70, Y144/145* [11].

Timely accurate diagnosis of infectious diseases occupies an important place in molecular genetic research. In this study, we present a detailed method for developing the complete genome of the *Alpha* strain of the *SARS-CoV-2* coronavirus.

Materials and methods. Clinical samples were obtained from the branch of the Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring of the RSE at the National Center for Public Health of the Ministry of Health of the Republic of Kazakhstan.

Extraction of nucleic acids. Viral RNA was extracted from 140 µl of the sample using *QIAamp Viral mini kits* (*Qiagen, Germany*) in accordance with the manufacturer's instructions.

Synthesize first – strand cDNA. The reaction was carried out in a *Mastercycler X50s* thermocycler. RT-PCR was performed as follows: 5 µl of extracted *RNA* was amplified using *SuperScript VILO cDNA Synthesis Kit* and the *RT-PCR* program was performed under the conditions: 25°C for 10 min; 42°C for 60 min; 85°C for 5 min.

PCR analysis. Amplification was performed in a Mastercycler *X50s* thermocycler using a *Platinum™ SuperFi™ PCR Master Mix (Invitrogen ™)*. The master mix of 25 µl, 2X *Platinum ™ SuperFi ™ PCR Master Mix*– 12,5 µl, 10 *µM forward primer* – 1,25 *µl,* 10 *µM reverse primer* – 1,25 *µl*, *5X SuperFi ™ GC Enhancer* – 5 µl, H_2O – 2 µl, *cDNA* – 3 *µl*. *PCR* was performed under the conditions given in Table 1.

Table 1 – Procedure details

Initial denaturation	95°C- 30 сек	1
Denature	95°C- 10 сек	35
Anneal	55°C- 30 сек	
Extend	72°C- 30 сек	
Final extension	72°C- 5 мин	1
	4°C	∞

The following oligonucleotide primers were used for *PCR* (Table 2):

Table 2 – Developed primers for sequencing the complete genome of the *Alpha* strain of the *SARS – CoV-2* coronavirus.

№	Gene	Position	Sequence (5'->3')
1	ORF1a	16-36	Forward primer CTTCCCAGGTAACAAACCAAC
		639-620	Reverse primer TTACGAAGAAGAACCTTGCG
2		111-130	Forward primer TTAGTGCACTCACGCAGTAT
		842-823	Reverse primer GGCCACAGAAGTTGTTATCG
3		767-786	Forward primer GGTGTTACCCGTGAACTCAT
		1467-1448	Reverse primer CCACCCTTACGAAGAATGGT
4		1405-1424	Forward primer TGAGCATAGTCTTGCCGAAT
		2076-2057	Reverse primer CCACCTGTAATGTAGGCCAT
5		1905-1924	Forward primer TTTTCTCCCGCACTCTTGAA
		2643-2624	Reverse primer ATTTCGAGCAACATAAGCCC
6		2589-2608	Forward primer AAGCTCCATTGGTTGGTACA
		3262-3243	Reverse primer TGTCTGATTGTCCTCACTGC
7		2872-2891	Forward primer TGAGTTCGCCTGTGTTGTGG
		3622-3603	Reverse primer GCCGACAACATGAAGACAGT
8		3429-3450	Forward primer CAGTGGTTGTTAATGCAGCCAA
		4200-4180	Reverse primer ATTTCAGTAGTGCCACCAGCC
9		3724-3744	Forward primer TTTTGGTGCTGACCCTATACA
		4401-4382	Reverse primer AGCATTTCTCGCAAATTCCA
10		4281-4300	Forward primer TAGAGGAGGCAAAGACAGTG
		4909-4890	Reverse primer ACCATCTAGGTGGAATGTGG
11		4773-4792	Forward primer AAACCATCTCACTTGCTGGT
		5522-5503	Reverse primer TACACACCACGTTCAAGACT
12		5378-5398	Forward primer GGTGAAGCTGCTAACTTTTGT
		6038-6019	Reverse primer AGCTTGCGTTTGGATATGGT
13		5811-5832	Forward primer ACGGTGCTTTACTTACAAAGTC
		6558-6539	Reverse primer GCAGCCATTAGATCTGTGTG
14		6372-6391	Forward primer TGGATAATCTTGCCTGCGAA
		7119-7100	Reverse primer GAACCAGTACAGTAGGTTGC
15		7005-7024	Forward primer CCGCTGCTTTAGGTGTTTTA
		7691-7672	Reverse primer GTAGTGACAAGTCTCTCGCA
16		7456-7475	Forward primer TGTGCATGTTGTAGACGGTT
		8196-8177	Reverse primer GAATCAACAAACCCTTGCCG
17		8013-8032	Forward primer GTGCGGAAGTTGCAGTTAAA
		8715-8696	Reverse primer GTGACACCACCATCAATAGC
18		8534-8554	Forward primer AAGATAGCACTTAAGGGTGGT
		9142-9123	Reverse primer GCCATCCATGAGCACATAAC
19		8808-8827	Forward primer ACAAAGCTTGCCCATTGATT
		9427-9407	Reverse primer AGCTACAATACCACCAGCTAC
20		9305-9325	Forward primer TTACCAGGAGTTTTCTGTGGT
		10037-10018	Reverse primer TGATAGAGGTTTGTGGTGGT
21		9647-9667	Forward primer TGGATGGTTATGTTCACACCT
		10350-10331	Reverse primer GGTGTCTTAGGATTGGCTGT
22		10169-10188	Forward primer CCAAGACATGTGATCTGCAC
		10851-10831	Reverse primer GCACACATATCTAAAACGGCA
23		10666-10685	Forward primer TTTAGCTTGGTTGTACGCTG
		11388-11369	Reverse primer ACTCTCCTAGCACCATCATC
24		11226-11245	Forward primer ATATGCCTGCTAGTTGGGTG
		11948-11929	Reverse primer GTAACTGGACACATTGAGCC
25		11792-11811	Forward primer AAATTGTTGGGTGTTGGTGG
		12438-12419	Reverse primer GGAACACAACCATCTCTTGC
26		12130-12149	Forward primer AGCTTTTGCTACTGCTCAAG
		12799-12780	Reverse primer ACCTCCCTTTGTTGTGTTGT
27		12460-12479	Forward primer AACAGCAGCCAAACTAATGG
		13181-13162	Reverse primer GACCAGTACCAGTGTGTGTA
28		12891-12910	Forward primer TGGAACCACCTTGTAGGTTT
		13542-13523	Reverse primer AGCCCTGTATACGACATCAG

29	*ORF1b*	13341-13360	*Forward primer ACCCTGTGGGTTTTACACTT*
		14046-14027	*Reverse primer AACAATACCAGCATTTCGCA*
30		13963-13982	*Forward primer TACGCCAACTTAGGTGAACG*
		14601-14582	*Reverse primer TAGATTACCAGAAGCAGCGT*
31		14478-14497	*Forward primer CCACTTCAGAGAGCTAGGTG*
		15190-15171	*Reverse primer CTCTAGTGGCGGCTATTGAT*
32		14913-14932	*Forward primer CCAAGTCATCGTCAACAACC*
		15556-15537	*Reverse primer CATTAACATTGGCCGTGACA*
33		15372-15391	*Forward primer GTGTTGTAGCTTGTCACACC*
		16030-16011	*Reverse primer TAGCTAAAGACACGAACCGT*
34		15834-15853	*Forward primer ATGTTGGACTGAGACTGACC*
		16502-16483	*Reverse primer ACTTGTCCATTAGCACACAA*
35		16374-16393	*Forward primer TCCGTATGTTTGCAATGCTC*
		17085-17066	*Reverse primer TGGTCCCTGGAGTGTAGAAT*
36		16743-16762	*Forward primer TGGTAAACCTAGACCACCAC*
		17492-17473	*Reverse primer GGTTCTAGTGTGCCCTTAGT*
37		17386-17405	*Forward primer TTGAGTGTTGTCAATGCCAG*
		18043-18024	*Reverse primer CAGCTTGTAAAGTTGCCACA*
38		17832-17851	*Forward primer TGTTGATTCATCACAGGGCT*
		18463-18444	*Reverse primer GCGGTGGTTTAGCACTAACT*
39		18314-18333	*Forward primer AGGGGTGTCATGCTACTAGA*
		18917-18898	*Reverse primer GTCCAGTCAACACGCTTAAC*
40		18695-18714	*Forward primer CTGCTTCAGACACTTATGCC*
		19334-19315	*Reverse primer TCAAAAGCTGGTGTGTGGAA*
41		19088-19107	*Forward primer TCTATGATGCACAGCCTTGT*
		19799-19780	*Reverse primer TTAGCCCAAAGCTCAAATGC*
42		19466-19485	*Forward primer GTTGCAATTTAGGTGGTGCT*
		20095-20076	*Reverse primer GTTTGGGACCTACAGATGGT*
43		19840-19859	*Forward primer AATTTGGGTGTGGACATTGC*
		20482-20464	*Reverse primer ATGAACCTGTTTGCGCATC*
44		20077-20096	*Forward primer CCATCTGTAGGTCCCAAACA*
		20676-20657	*Reverse primer TTGCCACGCTTGACTAGATT*
45		20456-20475	*Forward primer TCATAACAGATGCGCAAACA*
		20166-21147	*Reverse primer TTATAGCCACGGAACCTCCA*
46		20907-20927	*Forward primer TGTTTTAAGACAGTGGTTGCC*
		21644-21625	*Reverse primer ATGCAGGGGGTAATTGAGTT*
47	*S*	21300-21319	*Forward primer ACCACGCGAACAAATAGATG*
		21921-21901	*Reverse primer ACAATAAGTAGGGACTGGGTC*
48		21765-21785	*Forward primer TACATGTCTCTGGGACCAATG*
		22440-22421	*Reverse primer AGTGCACAGTCTACAGCATC*
49		22345-22364	*Forward primer TGCTGCAGCTTATTATGTGG*
		23006-22987	*Reverse primer CATTACAAGGTGTGCTACCG*
50		22849-22868	*Forward primer TACAGGCTGCGTTATAGCTT*
		23465-23446	*Reverse primer CACGCCAAGTAGGAGTAAGT*
51		23193-23212	*Forward primer ATGGTTTAACAGGCACAGGT*
		23914-23894	*Reverse primer TTGTGCAAAAACTTCTTGGGT*
52		23498-23517	*Forward primer CGTGCAGGCTGTTTAATAGG*
		24226-24207	*Reverse primer AAAGGTCCAACCAGAAGTGA*
53		23851-23870	*Forward primer AAACCGTGCTTTAACTGGAA*
		24571-24552	*Reverse primer ACTTTGAAGTCTGCCTGTGA*
54		24189-24208	*Forward primer CACTGTTAGCGGGTACAATC*
		24853-24833	*Reverse primer TGAAACAAAGACACCTTCACG*
55	*ORF3a*	24756-24775	*Forward primer TGACTTATGTCCCTGCACAA*
		25487-25468	*Reverse primer GTAGCGCGAACAAAATCTGA*
56		24925-24945	*Forward primer TGTGTCTGGTAACTGTGATGT*
		25611-25592	*Reverse primer GAGTGCTAGTTGCCATCTCT*
57		25493-25512	*Forward primer CGATACCGATACAAGCCTCA*
		26147-26128	*Reverse primer GAACCGTCGATTGTGTGAAT*
58		25790-25809	*Forward primer GCCGTTCCAAAAACCCATTA*
		26533-26514	*Reverse primer GAACCTGCCATGGCTAAAAT*

59	*E*	26200-26219	*Forward primer ACTACTAGCGTGCCTTTGTA*
		26922-26903	*Reverse primer GAAGCGGTCTGGTCAGAATA*
60	*M*	26795-26814	*Forward primer GTGGCTCAGCTACTTCATTG*
		27530-27511	*Reverse primer AATGGTGAATTGCCCTCGTA*
61	*ORF6*	27426-27445	*Forward primer ACTCGCTACTTGTGAGCTTT*
		28096-28077	*Reverse primer TTAGAACCAGCCTCATCCAC*
62	*ORF7a*	27874-27893	*Forward primer CTTGTCACGCCTAAACGAAC*
		28528-28509	*Reverse primer GCCAATTTGGTCATCTGGAC*
63	*ORF8*	28288-28307	*Forward primer ACCCCAAAATCAGCGAAATG*
		29015-28996	*Reverse primer TAGTGACAGTTTGGCCTTGT*
64	*N*	28825-28844	*Forward primer TCGTTCCTCATCACGTAGTC*
		29465-29446	*Reverse primer CAGCAGGAAGAAGAGTCACA*
65	*ORF10*	29126-29145	*Forward primer AATTTTGGGGACCAGGAACT*
		29786-29767	*Reverse primer CAGCTCTCCCTAGCATTGTT*

Horizontal gel electrophoresis was performed in 1.5% agarose solution (*Sigma, USA*), stained with ethidium bromide, in a tris-acetate buffer at a voltage of 100 *V / cm* of gel length, for 30 minutes with further detection on a *MiniBIS Pro transilluminator (Israel)*. Visualization and documentation of the results of gel electrophoresis was carried out using the "*GelCapture*" program.

Results and discussion. This study is based on the use of special primers covering the entire genome of the Alpha strain of the *SARS – CoV-2* coronavirus to develop amplified fragments of the nucleotide sequence. To achieve the goal of the work, 65 pairs of specific primers were developed (Table 2) in accordance with the *GenBank* reference sequence: *MN908947.3* [12]. The location of the *SARS – CoV-2* coronavirus genes is shown below (Figure 1).

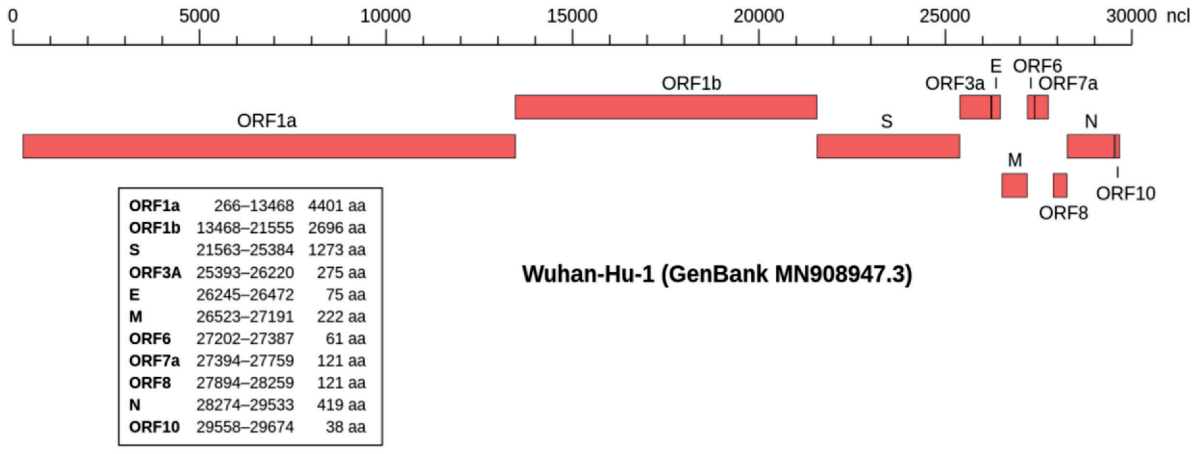

Figure 1 – Organization of the *SARS-CoV-2* coronavirus genome
(https://www.ncbi.nlm.nih.gov/nuccore/MN908947)

The *SARS-CoV-2* coronavirus genes were developed using specific primers. The developed *PCR* products are shown in Figures 2 and 3.

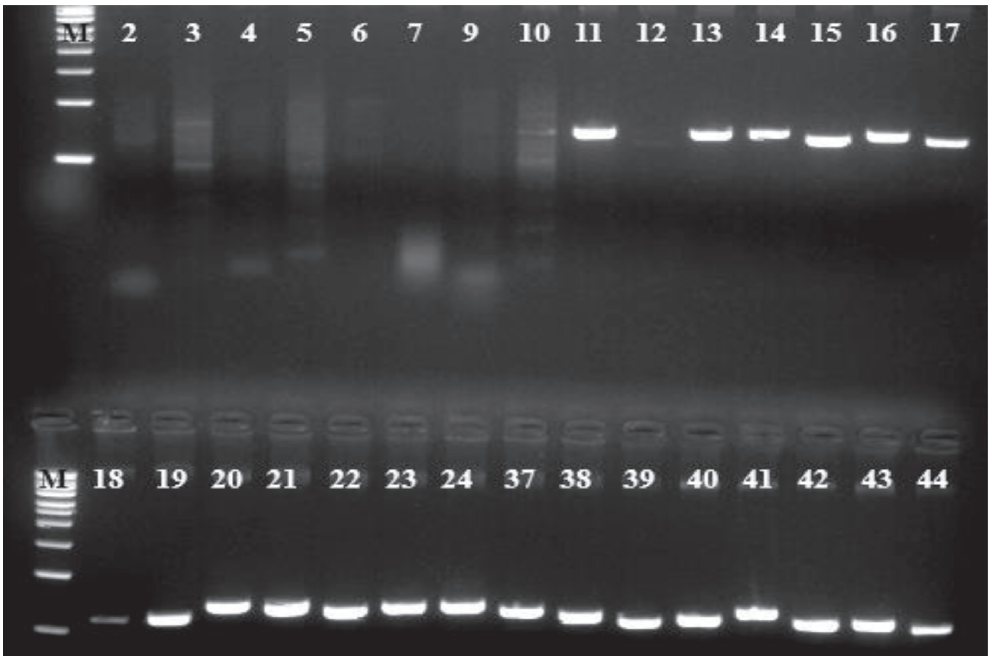

Figure 2 – Electrophoregram of *PCR* products of fragments of the *ORF1a* and *ORF1b* genes of the *Alpha* strain of the *SARS-CoV-2* coronavirus

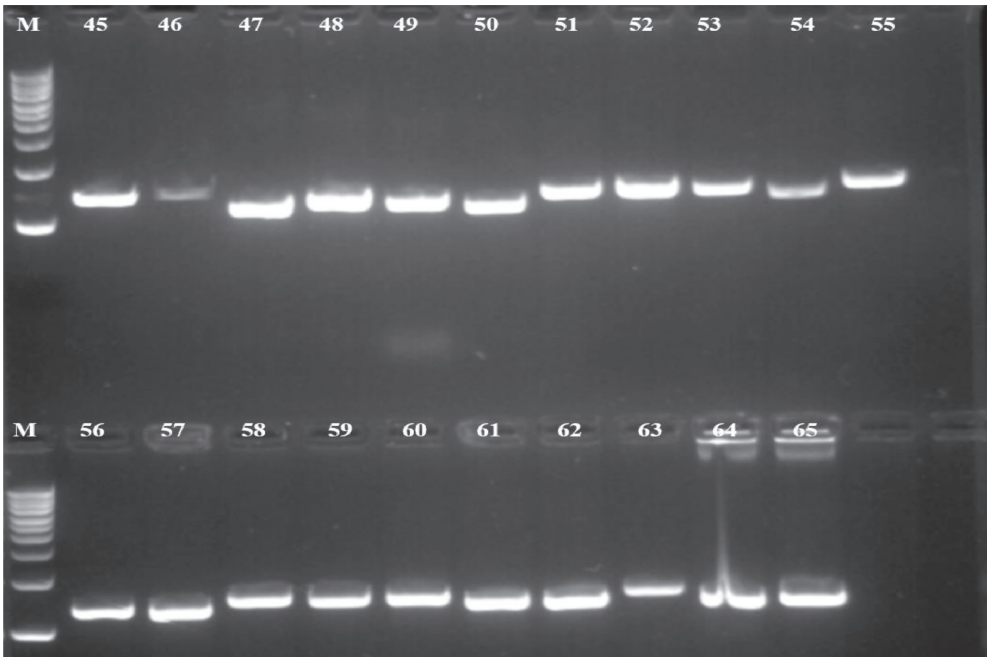

Figure 3 – Electrophoregram of *PCR* products of fragments of genes *S, ORF3a, E, M, ORF6, ORF7a, ORF8, N and ORF10* of the *Alpha* strain of the *SARS-CoV-2* coronavirus

As can be seen from Figures 2 and 3, *PCR* products of all the genes of the SARS-CoV-2 virus were developed as *ORF1a, ORF1b, ORF3a, E, M, ORF6, ORF7a*, *ORF8, N* and *ORF10*. As a result of the studies in Figure 2, they were amplified with primers (Tab.2) the *ORF1a* and *ORF1b* genes of the Alpha strain of the *SARS*-CoV-2 coronavirus. Figure 3 shows the accumulated PCR products of the genes *S, ORF3a, E, M, ORF6, ORF7a, ORF8, N* and *ORF10* of the Alpha strain

of the *SARS-CoV-2* coronavirus. As a result of the work carried out, PCR products of the above-mentioned genes of the *Alpha* variant of the *SARS-CoV-2* coronavirus have been developed, which are prepared for full-genome sequencing.

Conclusion. Coronaviruses are a well-studied group of single-stranded (+) *RNA* viruses of the Coronaviridae family. The facts of human infection with coronavirus have become known since the 60s of the last century. Before the global *SARS-CoV-2* pandemic, there was an epidemic of 2 coronavirus cases worldwide: *SARS-CoV* (severe acute respiratory syndrome) and *MERS-CoV (Middle East respiratory syndrome)*. According to WHO, a total of 332617707 cases, 5551314 cases of death have been registered in the world today.

The molecular genetic study of the *MERS-CoV-2* coronavirus is an important measure for assessing the prevalence of the disease and analyzing genomic mutations. As a result of such studies, a new strain of coronavirus was identified in the *UK* in the autumn of 2020. Depending on the place of origin, this strain has been called the British strain. On May 31, 2021, *WHO* assigned the name "alpha" to this strain. Using *PCR*, the genes of the Alpha strain of the *MERS-CoV-2* coronavirus were developed using specially developed primers. In the future, as a result of these works, full-genome sequencing of the Alpha strain of the *SARS-CoV-2* coronavirus is expected.

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