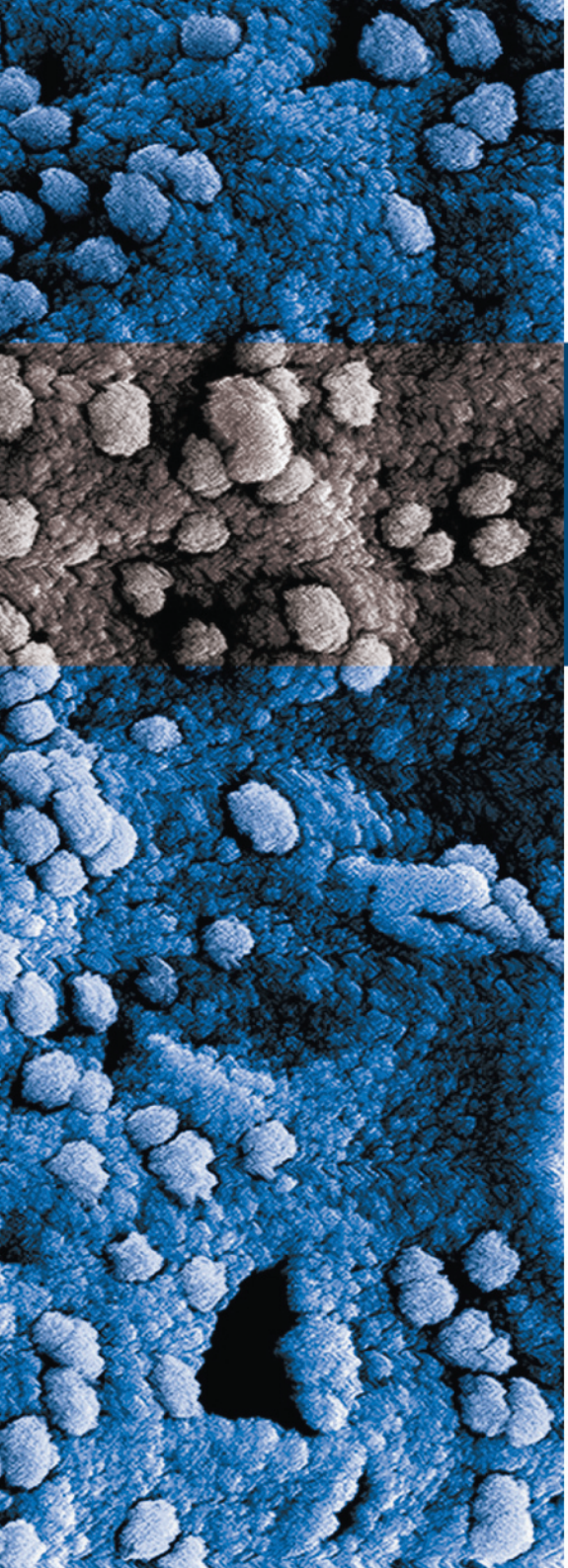
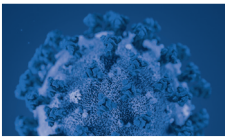


DNA-TECHNOLOGY

SARS-CoV-2/ SARS-CoV

Questions and answers regarding laboratory topics on COVID-19

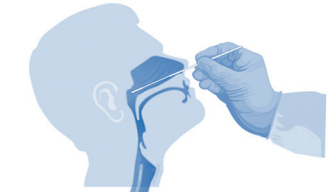
Contents

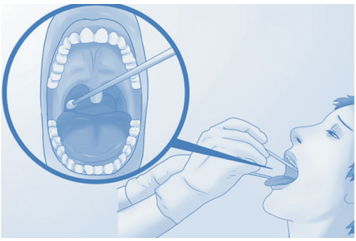


How to collect swabs correctly? Should the specimens be combined in a single tube?

According to WHO's interim guidance from March 19th, 2020 "Laboratory testing for coronavirus disease (COVID-19) in suspected human cases" and CDC's "Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19" "a nasopharyngeal (NP) specimen or an oropharyngeal (OP) specimen collected by trained healthcare personnel are acceptable depending on the authorized SARS-CoV-2 viral test used. If both NP and OP specimens are collected, they should be combined in a single tube to maximize test sensitivity".

NP and OP specimens are collected with sterile swabs, which should be placed immediately into a sterile transport tube after collection (taking into account the recommendations of the manufacturer of test kits/reagents). NP and OP specimens are collected with different swabs.





NASOPHARYNGEAL SPECIMENS

Use only synthetic fiber swabs with plastic shafts. Gently and slowly insert a swab 2-3 cm into the nostril parallel to the palate until inferior nasal concha. Then the swab inserts into inferior nasal meatus under the inferior nasal concha. Gently rub and roll the swab and slowly remove it.

In practice, specimens collected from oropharynx are more common. The patient's compliance with the rules for preparing for the study and sufficient number of cells from the surface of tonsils, palatine arches and the posterior oropharyngeal wall are the main conditions for appropriate specimens.

How long and how should specimens, collected to saline and transport medium, be stored?

Specimens that are delivered to the laboratory can be stored and shipped in a transport medium for storage and transportation of respiratory swabs (the manufacturer of the transport medium indicates storage conditions in the instruction) or saline (if the specimens are delivered to the laboratory within 24 hours after the collection).

The following recommendations are determined by the instruction for use for the specimen transport medium “STOR-F” (“DNA-Technology TS” LLC):

- to store specimens collected in the transport medium “STOR-F” at 4°C for up to 7 days after collection;
- it is allowed to transport and store specimens collected in the transport medium “STOR-F” at 18-25°C for up to 48 hours after collection.

Are urine and stool specimens appropriate for SARS-CoV-2 viral testing? How to work with them?

Testing urine for the presence of SARS-CoV-2 is impractical due to the low concentration of the virus in this specimen type (Chan VW et al. A systematic review on COVID-19: urological manifestations, viral RNA detection and special considerations in urological conditions. World J Urol. 2020; 10.1007/s00345-020-03246-4).

Stool can be used as additional clinical specimen in adults. In newborns, the novel coronavirus can be detected in the following specimen types: nasopharyngeal washes, sputum, bronchoalveolar lavage, whole blood and stool (Methodical recommendations of the Ministry of Health of the Russian Federation "Organization of medical care for pregnant women, women in labor, postpartum women and newborns with new coronavirus COVID-19 infection"). Stool testing can be performed in the presence of gastrointestinal symptoms, as well as in the presence of COVID-19 symptoms with negative OP and NP swabs.

To isolate viral RNA from stool, you can use a nucleic acid extraction kit ("PREP-NA"/"PREP-NA-PLUS"), produced by "DNA-Technology, Research & Production" LLC, Russia.

What are extraction kits compatible with the SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit, produced by “DNA-Technology”?

We recommend using the kits validated for RNA extraction and further study with the SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR Detection Kit:

- “PREP-NA” Extraction Kit, produced by “DNA-Technology, Research & Production” LLC, Russia.
- AmpliSens® RIBO-prep, produced by InterLabService Ltd.

Now we have a new registered significantly shortened version of sample preparation – “PREP-NA-S” DNA/RNA Extraction Kit. It was developed specifically for work with the “SARS-CoV-2 / SARS-CoV” kit.

How to suspect contamination and prevent it?

In practice, there are several sources of contamination in PCR laboratory. We will analyze each of them.

Contamination between specimens during the preanalytical phase (1) and during aliquoting of the primary specimens (2) are critical for the laboratory performing the study, since the contamination is not reduced by reanalysis of the primary specimens.

Contamination during sample preparation (3) can happen even when experienced medical laboratory scientists work. The main difficulties you can face are the difficult test procedure, the presence of low- and high-copy number specimens in one assay setup can lead to cross-contamination. Therefore, it is necessary to have separate work stations and strictly follow the guidelines to prevent cross-contamination – use filter tips, tubes with snap-on cap, and often change gloves.

Contamination during addition of nucleic acid to the reaction mixture (4) and amplicon contamination (5) occur rarely and are mainly associated with inaccurate work of a laboratory assistant. If this happens, it is recommended to identify and reduce the source of contamination, the following decontamination and cleaning procedures should be completed in accordance with internal or local regulations (Appendix 2). Where a workspace has been affected by a contamination incident, and the contamination may still present an issue, processing of material within the affected workspace shall cease until it has been subject to the decontamination regimes.

Practical laboratory aspects of SARS-COV-2

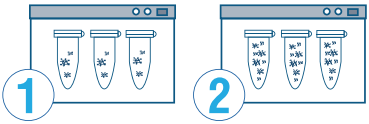
ATTENTION! The range of SARS-CoV-2 viral load can vary widely from very low values (10^4 or less copies/mL) in the biomaterial of asymptomatic carriers and patients in the recovery stage to extremely high values (more than 10^9 copies/mL) in the biomaterial of patients with a clinical picture of acute viral pneumonia.

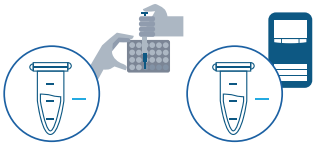
In this regard, when performing research in a clinical laboratory, the risk of cross contamination between samples at all stages of work is a serious danger, especially during aliquoting and RNA extraction. Cross-contamination with high-copy biomaterial can lead to sporadic false positive results.

To prevent cross-contamination of the biological material in the laboratory, the following rules are recommended:

1. It is necessary to conduct a visual assessment of the incoming biomaterial and cull test tubes with broken integrity;

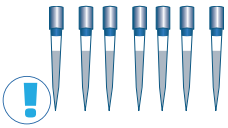
2. If possible, it is recommended to analyze specimens of patients with symptoms of acute infection separately from the rest of the samples. It is desirable to work with the supposed high-copy samples in a separate box or after working with the supposed low-copy samples;





3. It is necessary to use negative control samples, starting from the stage of RNA extraction in each protocol;

4. Use tips with aerosol filters at all stages of the assay;



5. Strictly follow the assay procedure, open the Eppendorf test tubes with tweezers (do not touch inside the tube cap by the gloved hand); when applying reagents, do not touch inside the test tube by the tip (if this happened, immediately replace the tip).

Compliance with recommendations will allow obtaining correct results when using a highly sensitive RT-PCR method, both when working with high-copy samples during differential diagnosis, and when examining persons in close contact with someone who has COVID-19, as well as when conducting screening to prevent further spread of infection.

Risk of contamination with amplification products (amplicons) when using the kits produced by “DNA-Technology” is minimized - tubes with PCR-mix are sealed beneath a layer of paraffin, which is an additional protection factor. At the end of the amplification program, the amplicons are beneath a layer of paraffin that prevents contamination even if the tube accidentally opens.

However, in case of accidents (for example, you dropped a test tube after PCR, stepped on it, picked up a broken test tube with hands, opened tubes for disinfection etc.), the risk of contamination of a PCR laboratory with amplicons remains.

Taking into account the importance of obtaining the correct test result for SARS-CoV-2, we recommend reviewing and analyzing the protocols manually, in addition to automatic protocol. When analyzing the completed PCR protocol, special attention should be paid to low-copy samples. Based on our experience, the number of samples with a truly low viral load ranges from 5% to 20%, depending on the population surveyed. A truly low viral load can be observed in people who have been in close contact with someone who has COVID-19, in patients in the first days after infection and during the period of convalescence. Contamination should be suspected when a large number of low- (Cp 37-40) and high-copy samples are presented in the protocol at the same time.

False negative and false positive results: how to answer doctors' questions?

Different specialists can interpret the meaning of "false negative result" differently. In the laboratory «false negative result» is defined as a negative sample, which contains the genetic material of the virus. In this case, an incorrect result of a PCR study may be the result of non-compliance with the test's procedure. A false-negative result may occur if a specimen is improperly collected, transported or handled. This problem is extremely urgent, because of widespread testing and increased workload among laboratory workers during a pandemic.

In addition, when the test results obtained in different laboratories do not match, we can talk about "false negative results". In these cases, it is necessary to pay attention to the analytical sensitivity (detection limit) of the kit on which the studies were performed. This characteristic significantly affects the test result, especially for low-copy samples (less than 10^3 copies/ml). The sensitivity of the "SARS-CoV-2 / SARS-CoV" kit produced by DNA-Technology is 500 copies/ml in the sample, while a significant part of the kits approved for laboratory diagnosis of COVID-19 have an analytical sensitivity of 10^3 copies/ml (for more details, see question 8).

From the clinician's point of view, obvious symptoms of acute respiratory infection or pneumonia during the COVID-19 pandemic in the absence of a positive test for COVID-19 can be considered as a false negative result. However, do not forget that the symptoms of a respiratory infection are nonspecific and SARS-CoV-2 is not the only cause, but also other viruses or bacteria (for more details, see question 9).

What to do if positive results obtained with DNA-Technology kits are not confirmed in the reference laboratory?

Currently, this problem exists; there is no single solution yet. For a prompt response and collection analytics, please report inconsistencies in the results to the DNA-Technology employees.

However, what are the reasons of inconsistencies? The detection limit of the "SARS-CoV-2 / SARS-CoV" kit by DNA-Technology is 500 copies/ml in the sample in case of using "PREP-NA" DNA/RNA Extraction Kit. In other words, samples containing 500 or more viral particles in 1.0 ml will be "POSITIVE" with a probability no less than 95%. The detection of SARS-CoV-2 RNA in the samples containing less than 500 viral particles in 1.0 ml is still possible due to high analytical sensitivity of the kit (for such samples, Cp on the Fam, Rox, Cy5 channels is usually more than 36), a positive test result is reliable. Keep in mind that when conducting repeated or parallel testing on the same low-copy sample, not all results will be positive. This is due to the likely distribution of single RNA/DNA copies in the test tubes.

A significant part of the COVID-19 PCR kits approved for in vitro diagnostics have an analytical sensitivity of 1000 copies/ml. This may be one of the reasons why in case of low-copy samples, positive results are not confirmed by reference laboratories.

Another reason that can lead to discrepancy between the results is a possible decrease in the virus concentration below reliably determined values, which can be critical for low-copy samples during their transportation to the reference laboratory and storage.

To avoid discrepancy with the reference laboratory, it is necessary to confirm the primary positive result by repeating the test and to ensure that there is no contamination. We recommend starting the study with the stage of RNA extraction, for which it is imperative to aliquot the samples and keep aliquots of positive samples for the reference laboratories.

Is it necessary to diagnose other respiratory viruses besides SARS-CoV-2? Whom? When?

According to WHO's interim guidance "Clinical management of COVID-19", both upper respiratory tract and low respiratory tract specimens can be tested for other respiratory viruses, such as influenza A and B (including zoonotic influenza A), respiratory syncytial virus, parainfluenza viruses, rhinoviruses, adenoviruses, enteroviruses (e.g. EVD68), human metapneumovirus and endemic human coronaviruses (i.e. HKU1, OC43, NL63, and 229E). LRT specimens can also be tested for bacterial pathogens, including Legionella pneumophila. In malaria-endemic areas, patients with fever should be tested for the presence of malaria or other co-infections with validated rapid diagnostic tests (RDTs) or thick and thin blood films. In endemic settings arbovirus infection (dengue/chikungunya) should also be considered in the differential diagnosis of undifferentiated febrile illness, particularly when thrombocytopenia is present".

The research can be carried out with the use of "DNA-Technology" kits –"SARS COV2 Influenza", "Influenza A&B virus", "Influenza A virus", "Influenza B virus", "Acute viral respiratory infections complex".

In practice, due to the laboratory workload, testing is carried out only for SARS-CoV-2, without testing for other respiratory infections. Sometimes this leads to erroneous conclusions about the low sensitivity and specificity of PCR diagnostics.

Is it possible to store the extracted RNA?

Storage conditions of the extracted RNA depend on the way of extraction. All the conditions are detailed in the user manuals of the extraction kits.

If it is necessary, the resulting RNA preparation can be stored for a short period (30 min – 2h) at temperatures from +2°C to +8°C before reverse transcription. For longer storage RNA preparation should be frozen.

How many samples can be performed in one run?

The minimum number of samples in one sample preparation is 2 (clinical specimen and negative control sample). In this case, the amplification procedure will include 3 samples – the clinical specimen, negative control sample from the nucleic acid extraction stage, and the positive control sample (added only to RT-PCR). If there are many samples in the laboratory, then it is convenient to carry out sample preparation in batches according to the number of wells in a high-speed centrifuge, e.g. 24. This maximum will be 23 clinical specimens and 1 negative control sample. If cross-contamination is suspected in the laboratory, it is recommended to increase the number of negative controls, starting with the aliquot stage of the primary specimens.

Is it always necessary to use negative controls for every 10th sample?

11

The purpose of "negative controls for every 10th sample" is to control cross-contamination at the stages of aliquoting, sample preparation and RT-PCR. It is necessary to perform the same manipulations with negative control samples as with real clinical specimens and process them together. For example, 9 clinical specimens, 10th sample – C-, 9 clinical specimens, 10th sample – C-. This approach will help to detect contamination in time and exclude false positive results.

Do I need to use a special tube opener for Eppendorf tubes?

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Instead of tweezers, you can use special devices to open caps of the tubes, e.g. microcentrifuge tube opener (SSI, USA, cost – $ 0.6). Although there is an opinion that such openers are inconvenient.

When and why is it necessary to analyze specimens of patients from different groups separately?

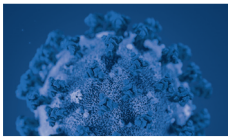
It is necessary, if a laboratory works with high-copy and negative samples at the same time. This helps to reduce the risk of cross-contamination and increase the accuracy of the test result.

Is it possible to use reagents from other kits for SARS-CoV-2 testing, e.g. “Acute viral respiratory infections complex”?

No. The “SARS-CoV-2/SARS-CoV” kit is a one-tube RT-PCR system, so the reverse transcription and subsequent PCR occur in one tube using the Taq/RT enzyme and other reagents.

In “Acute viral respiratory infections complex” kit, the reverse transcription and PCR steps take place in separate test tubes, different enzymes are involved at every step – reverse transcriptase and Taq polymerase, respectively.

Appendix 1



Calculation of the amount of reagents for preparing a mixture of RT-PCR buffer and Enzyme Taq/RT when working with the “SARS-CoV-2 / SARS-CoV” kit

The calculation of the amount of the buffer and enzyme for the preparation of the mixture is made according to the total amount of:

- clinical specimens (RNA from samples) in the RT-PCR setting;
- negative control samples (there may be more than one in the RT-PCR protocol);
- and one positive control sample.

In order to avoid a lack of volume of the buffer and enzyme, the mixture is prepared with a margin of one tube according to the formula:

15.0 x (N+1) µL of RT-PCR-buffer;
0.5 x (N+1) µL of Enzyme Taq/RT,
where N is a number of amplification tubes, including samples to be tested, negative and positive control samples.

Number of amplification tubes **(N)**	RT-PCR-buffer (µL) **(N + 1) × 15**
	Enzyme Taq/RT (µL) **(N + 1) × 0,5**

Attention!

- It is necessary to use positive control sample (C+) and negative control sample (C-) in each protocol.
- It is not recommended to perform less than 8 samples (6 clinical specimens, one positive control sample and one negative control sample) in one run.

Number of clinical specimens per run	Number of amplification tubes per run (including C+ and C-)	Volume of RT-PCR-buffer (including 1 extra tube), µL	Volume of Enzyme Taq/ RT (including 1 extra tube), µL	Maximum number of tests «SARS-CoV-2/ SARS-CoV» (96 tests)
6	8	135	4,5	12
10	12	195	6,5	8
22	24	375	12,5	4
44	48	735	24,5	2
94	96	1455	48,5	1

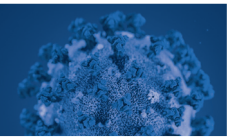
Table of reagent consumption for preparing a mixture of RT-PCR buffer and Enzyme Taq/RT for the total number of samples

Sample, pcs	RT-PCR-buffer, µL	Enzyme Taq/RT, µL
1	15	0,5
2	30	1
3	45	1,5
4	60	2
5	75	2,5
6	90	3
7	105	3,5
8	120	4
9	135	4,5
10	150	5
11	165	5,5
12	180	6
13	195	6,5
14	210	7
15	225	7,5
16	240	8
17	255	8,5
18	270	9
19	285	9,5
20	300	10
21	315	11
22	330	11
23	345	12
24	360	12
25	375	13

Sample, pcs	RT-PCR-buffer, µL	Enzyme Taq/RT, µL
26	390	13
27	405	14
28	420	14
29	435	15
30	450	15
31	465	16
32	480	16
33	495	17
34	510	17
35	525	18
36	540	18
37	555	19
38	570	19
39	585	20
40	600	20
41	615	21
42	630	21
43	645	22
44	660	22
45	675	23
46	690	23
47	705	24
48	720	24
49	735	25
50	750	25

Sample, pcs	RT-PCR-buffer, µL	Enzyme Taq/RT, µL
51	765	26
52	780	26
53	795	27
54	810	27
55	825	28
56	840	28
57	855	29
58	870	29
59	885	30
60	900	30
61	915	31
62	930	31
63	945	32
64	960	32
65	975	33
66	990	33
67	1005	34
68	1020	34
69	1035	35
70	1050	35
71	1065	36
72	1080	36
73	1095	37
74	1110	37
75	1125	38

Sample, pcs	RT-PCR-buffer, µL	Enzyme Taq/RT, µL
76	1140	38
77	1155	39
78	1170	39
79	1185	40
80	1200	40
81	1215	41
82	1230	41
83	1245	42
84	1260	42
85	1275	43
86	1290	43
87	1305	44
88	1320	44
89	1335	45
90	1350	45
91	1365	46
92	1380	46
93	1395	47
94	1410	47
95	1425	48
96	1440	48
97	1455	49
98	1470	49
99	1485	50
100	1500	50



Appendix 2

Basic decontamination procedure of a PCR laboratory.

1. Employees carrying out decontamination procedure should be provided with gowns (preferably disposable), hats, disposable shoe covers and gloves, disposable rags, containers for preparing the sanitizing solutions.

2. Laboratory workers disinfect the zone of the laboratory, where they work.

3. Different cleaning equipment is used for each zone; it should be disinfected with appropriate disinfectants after the use.

4. Each zone of the laboratory is divided into areas, for example:
   - area 1 – biosafety cabinet and equipment inside it;
   - area 2 – external surfaces of the biosafety cabinet;
   - area 3 – storage cabinets;
   - area 4 – sample and reagent refrigerators;
   - area 5 – equipment outside the biosafety cabinet;
   - area 6 – room surfaces (walls, windows, radiators, ceiling, doors, etc.);
   - area 7 – floor.

5. Disinfection is carried out from one area to another. Use different rags for each area. Prepare disinfectants before disinfecting.

6. The surfaces are first treated with a washing solution to remove grease; the remaining detergent is removed with a rag moistened with water.

7. Apply the bleach solution to surfaces for 30 minutes. The remaining detergent is removed with a rag moistened with water.

8. The wet surfaces are disinfected with ultraviolet radiation for 45 minutes.

9. Repeat steps 7 and 8.

10. Upon completion of decontamination procedure, collect surface samples by swabbing for nucleic acids and (or) amplicons, taking into account the length of pathogen-specific amplified fragments of the nucleic acids, which are specified in the kit instruction.

11. Use separate sterile stick swabs, moistened in 0.9% sodium chloride solution or TE buffer (10 mM Tris, 1 mM EDTA) and wipe the working surfaces of the equipment, furniture, door handles, jambs, telephones with rotating movements within 10–15 sec. Pay special attention to the common areas (canteen, bathroom, etc.). After taking the swabs, replace them into 1.5 ml tubes with 300-400 µl of TE-buffer or 0.9% sodium chloride solution, rotate for 10–15 sec, avoiding splashing the solution, squeeze the swab on the wall of the test tube to eliminate excess liquid, then remove it. The suspensions are vortexed for 1 min. To set up the amplification reaction, use the required volume of liquid in accordance with the kit instruction. After receiving the results, a protocol is drawn up.

12. If samples are positive for tested pathogens, repeat the decontamination procedure.

13. Contaminated consumables (test tubes, tips, reagents, etc.) and contaminated surface swabs (except for the source material) are sterilized in an autoclave.

14. Cases of contamination are documented in a special journal with an indication of decontamination procedure and the results of internal laboratory control.

15. It is not allowed to carry out amplification in the laboratory before the completion of decontamination regimes.

Notes:

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DNA-TECHNOLOGY

CONTACT OFFICE:
DNA-Technology LLC, 125 Zh, Varshavskoye Highway, bld. 6, Moscow, Russia.
Phone / Fax: +7 (495) 640-17-71, www.dna-technology.com, info@dna-technology.com

CUSTOMER SUPPORT:
8 800 200-75-15 (free call from within Russia), hotline@dna-technology.ru