DNA-TECHNOLOGY

CATALOG EQUIPMENT

CATALOG EQUIPMENT

Moscow
2017

CONTENTS

ABBREVIATIONS

HIV	Human immunodeficiency virus
GE	Genome equivalent
DNA	Deoxyribonucleic acid
dATP	Deoxyadenosine triphosphate
dGTP	Deoxyguanosine triphosphate
dNTP	Deoxynucleotide triphosphate
dTTP	Deoxythymidine triphosphate
dCTP	Deoxycytidine triphosphate
SIC	Sample intake control
cDNA	Complementary DNA
RT	Reverse transcriptase
RT-PCR	Reverse transcription polymerase chain reaction
PCR	Polymerase chain reaction
Real-time PCR	Real-time polymerase chain reaction
FLASH	Fluorescent Amplification-based Specific Hybridization
IVD	*In vitro* diagnostics
qPCR	Quantitative real-time polymerase chain reaction
Rt	Real-time
RUO	Research Use Only

NOTE! **Information contained in this catalog may not be consistent with the latest version of specifications for the specified product**

INTRODUCTION

Dear colleagues and friends,

Our company, DNA-Technology LLC, in 2013 celebrated her 20th year anniversary of successful activities in the area of development of unique PCR-based diagnostics technologies. We have been able to design and deploy the following projects into medical practice:

1993: Tercyc – multichannel thermocycler

The technical specifications of the device are unique in its class. The device is characterized by high reliability, compactness, control flexibility and low cost, which makes it very popular in Russia and CIS countries. Development and deployment of Tercyc in laboratory practice made PCR analysis more accessible to many research and clinical diagnostic laboratories. There are currently over 5000 devices in use in Russia and CIS countries.

2001: FLASH technology

FLASH is a unique technology that had no counterparts in the world at the time of development. The technology is used for detection of fluorescently-labeled probes at the end of thermal cycling. This technology includes both reagents and unique fluorescence detector "Gene", all of which allows to solve important challenges faced by clinical laboratories – reducing contamination risk. As a result, the work of laboratory doctors and clinical pathologists is significantly simplified, while the requirements for PCR laboratories are reduced.

FLASH technology is a very powerful impetus for emergence of new PCR labs and increase in the number of PCR analysis in clinical practice.

2005: Detecting thermocycler DT-322.

DT-322 is Russia's first detecting thermocycler for **real-time PCR analysis**. This device has made available to clinical laboratories quantitative PCR studies that are necessary for diagnosis and monitoring of treatment of such socially significant diseases as AIDS, hepatitis B and C.

2007: Detecting thermocycler DT-96 (DT*prime*).

DT-96 is the first Russian-made detecting thermocycler with four channels and 96 holes. DT-96 was the parent of a series of devices that brought Russian-made medical PCR devices to world standards. Introduction of DT-96 led to creation of fully automated PCR systems with the use of Russian-made devices.

As of today, over 1000 DT detecting thermocyclers are used in clinical and research laboratories.

2008-2009: Reagent kit Femoflor®.

World's first developed and patented FEMOFLOR® Real-Time PCR Kit for study of the the female urogenital microbiom composition.

Clinicians received an analytically accurate and technological instrument for qualitative and quantitative analysis of complex microbial communities. As of today, over 1,000,000 patients have been examined, where FEMOFLOR® Real-Time PCR Kit was used as the primary instrument for diagnostics of dysbiosis of various etiology.

2010-2011: Reagent kits for detection of genetic polymorphisms associated with the risk of multifactorial diseases using real-time PCR.

Studying the human genetics, namely predispositions to various multifactorial diseases, is now becoming the most urgent focus of PCR use. In the human genome nowadays, up to 10 million SNPs (single nucleotide polymorphisms – small changes in the genome that affect 1-2 neighboring polymorphisms) are identified. Single nucleotide polymorphisms, as well as larger genetic disorders (deletions and chromosomal aberrations) are a risk factor for many diseases and cause metabolic defficiency of various substances.

2016: "Androflor®" and "Androflor®Screen" – Kits for male urogenital tract research. This unique technology allows diagnosing of male urogenital tract infection-inflammatory diseases and provides significant medical data in cases of non-apparent or asymptomatic course of infection as well as at equivalent research of couples experiencing reproductive problems.

Fetal gender" and "Fetal RHD Genotyping" – Kits for noninvasive prenatal molecular-genetic diagnosis. These kits allow to analyze fetal DNA starting from 8-10th week of gestational. The obtained molecular data helps to prevent birth of diseased children in families with burdened familial history and to prevent development of Rh-conflict associated diseases. The realized approach, allowing identification of genotypically Rh positive patients, is the first of a kind for national market.

"ImmunoQuantex" – Kit for innate immunity genes expression profiling, intended for evaluation of local inflammation of female lower reproductive tract.

«Prix Galien Russia 2016» – DNA-Technology is award winner in the category «The best Russian product».

New concept – complex evaluation of vaginal flora state and local inflammation of female reproductive tract **"Femoflor®"** + **"ImmunoQuantex"**) with use of molecular-genetic analysis allows early and very-early stage diagnosis of reproductive diseases, allows to move the accent from treatment to prevention and minimize risk of relapse.

We are grateful to you that throughout these years you have stayed close by, supported us in our undertakings, applied to us with questions and suggestions, taught us and been taught by us, improved PCR-technology, invited us to conferences and took part in our events. We hope that our future cooperation will be even more productive and eventful.

D.Y. Trofimov,
CEO, DNA-Technology

ABOUT US

DNA-Technology Group has been designing and deploying high-tech equipment and reagents for **polymerase chain reaction (PCR)** analysis since 1993.

Our team brings together leading experts in the field of molecular biology, immunogenetics, medicine, thermodynamics, optics, electronics and programming. This has enabled us preserve our high research and technical potential and ensure high quality and control standards at all production stages.

Our production base meets all the medical equipment standards practiced by global companies. Our innovations are recognized through various documents: we have been licensed by the Federal Service on Surveillance in Healthcare and Social Development; we have obtained a certificate certifying that our quality management system for production of medical equipment and reagents for laboratory diagnostics complies with GOST ISO 9001 – 2001 (9001:2000), and quality management certificates (ISO 13485:2003 and 9001:2008). The Company's products are also CE marked.

Our main areas of activities are:

- Development of unified technological solutions for PCR laboratories – from laboratory plan and equipment and reagent supply to promotion of the widest range of research, training of laboratory staff, interpretation of results and collaboration with clinicians;
- Development and production of high-tech equipment for PCR-based diagnostics and research;
- Production of a wide range of reagents for the needs of clinical bacteriology, virology and gene diagnostics;
- Production of a wide range of reagents for detection of the DNA of infectious agents of crops and diagnosis of genetically modified organisms (GMOs);
- Provision of service support;
- Collaboration with clinicians.

Our product range includes basic equipment and devices for PCR laboratories:

- Detecting thermocyclers for real-time PCR analysis (DT devices);
- Tercyc thermocycler for polymerase chain reaction with subsequent result detection via electrophoresis or end point (FLASH format);
- Fluorescence detectors for PCR analysis with FLASH result detection (Gene devices);
- Thermostats;
- Power supplies (Elf devices);
- PCR cabinets.

The company has established a strong research base that features highly sensitive and specific reagent kits for PCR studies, such as:

- Detection of viral and bacterial infections:
  - Hepatitis and HIV;
  - Urogenital infections;
  - Herpesvirus infections;
  - Human papillomavirus infections;
  - Respiratory tract infections;
  - Especially dangerous and natural focal infections;
  - Other infections;
- Analysis of dysbiotic states of the urogenital tract;
- Identification of GMOs;
- Genotyping of humans and microorganisms.

Our team at DNA-Technology has developed a unique and unparalleled (in the Russian market) technology for detection of hereditary diseases and gene diagnostics of predisposition to a number of diseases causing multisystemic pathology, cancer, metabolic disorders and immune system disorders.

Our highly-skilled experts and well-equipped laboratory facilities enable us to train laboratory diagnostics specialists in advanced molecular genetic techniques and carry out advisory activities on PCR studies and subsequent interpretation of results.

TECHNOLOGIES

I. PCR: MODERN CLINICAL LABORATORY DIAGNOSTIC METHOD

Polymerase chain reaction (PCR) is the most effective molecular diagnostics method widely used in *in vitro* laboratory tests. PCR is involved in up to 80 % of laboratory tests in the EU. Over 40 million PCR analyses are conducted yearly in Russia.

1. PCR STRATEGY

PCR is an experimental method of molecular biology. It allows to perform a significant increase of even small concentrations of certain fragments of nucleic acid (DNA/RNA) obtained when collecting and extracting DNA/RNA from a biological material (probe).

Application of PCR is based on in vitro amplification of DNA fragments with fully or partially known sequence.

To implement this strategy, a reaction mixture is required, which should contain the following components:

- *Primers:* artificially synthesized oligonucleotides, usually with 15 to 30 nucleotides that are complementary to the corresponding regions of the DNA target sequence. They play a key role in formation of amplification reaction products.
- *Taq-polymerase*: thermostable enzyme that completes the construction of the 3'-end of the second DNA strand according to the complementarity principle.
- *A mixture of deoxynucleotide triphosphate (dNTP):*
  - Deoxyadenosine triphosphate (dATP);
  - Deoxyguanosine triphosphate (dGTP);
  - Deoxycytidine triphosphate (dCTP);
  - Deoxythymidine triphosphate (dTTP).

It is a 'construction material' used by Taq-polymerase for DNA strand synthesis.

- *Buffer:* a mixture of cations and anions in a certain concentration, providing optimal reaction conditions and a stable pH.
- *Sample analyzed:* a control sample prepared, which is introduced into the reaction mixture and may contain the tested DNA, e.g., DNA of microorganisms serving as a target for subsequent multiple copying. If the DNA target sequence is absent, the specific amplification product is not formed.

Amplification process consists of three phases:

1. *Denaturation:* DNA transition from double-stranded to single-stranded form at breakage of hydrogen bonds between complementary base pairs at high temperatures (Fig. 1).

2. *Annealing:* attachment of primers to single-stranded DNA target sequence. Reagent producers choose primers in such a way as to ensure that the primers limit the tested fragment and are complementary to opposite DNA strands.

3. *Elongation (synthesis).* After annealing of primers, Taq-polymerase begins complete construction of the second DNA strand from 3'-end of the primer.

The reaction mixture temperature is brought to optimum for Taq-polymerase, which, with maximum efficiency, begins synthesis of the second DNA strand from the 3'-end of the primer, bound to the DNA template, and moves towards 5'-end from the 3'-end (Fig. 2).

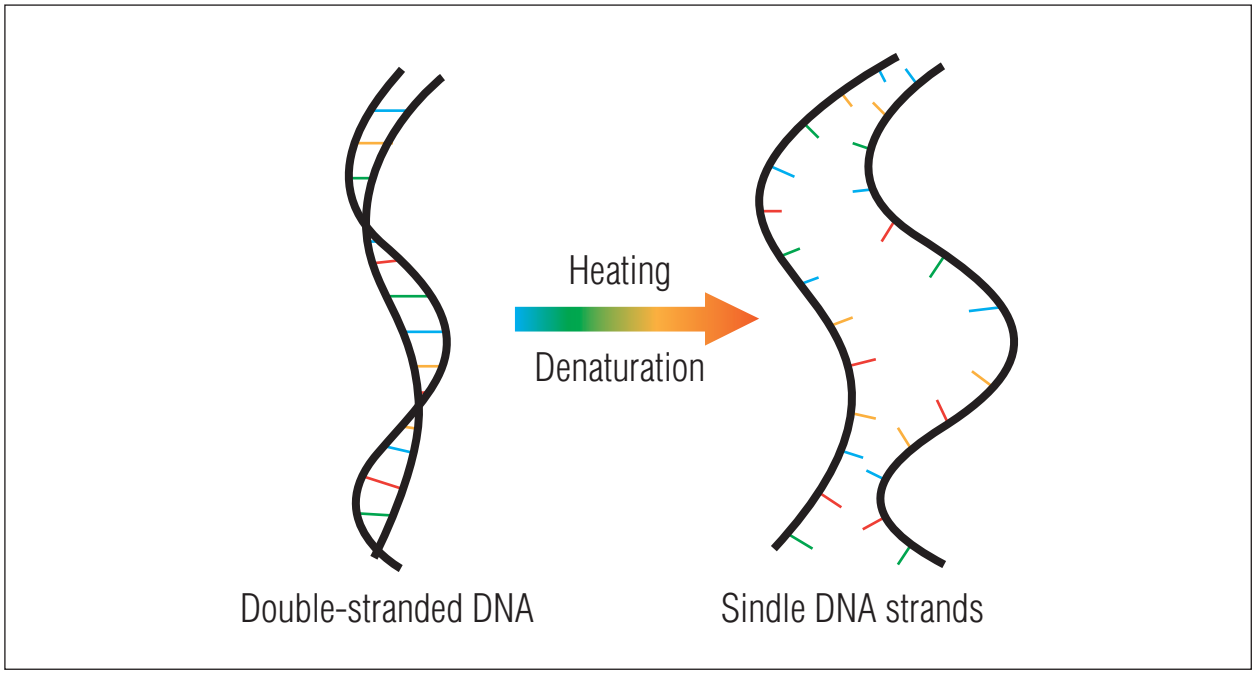


Fig. 1. DNA denaturation

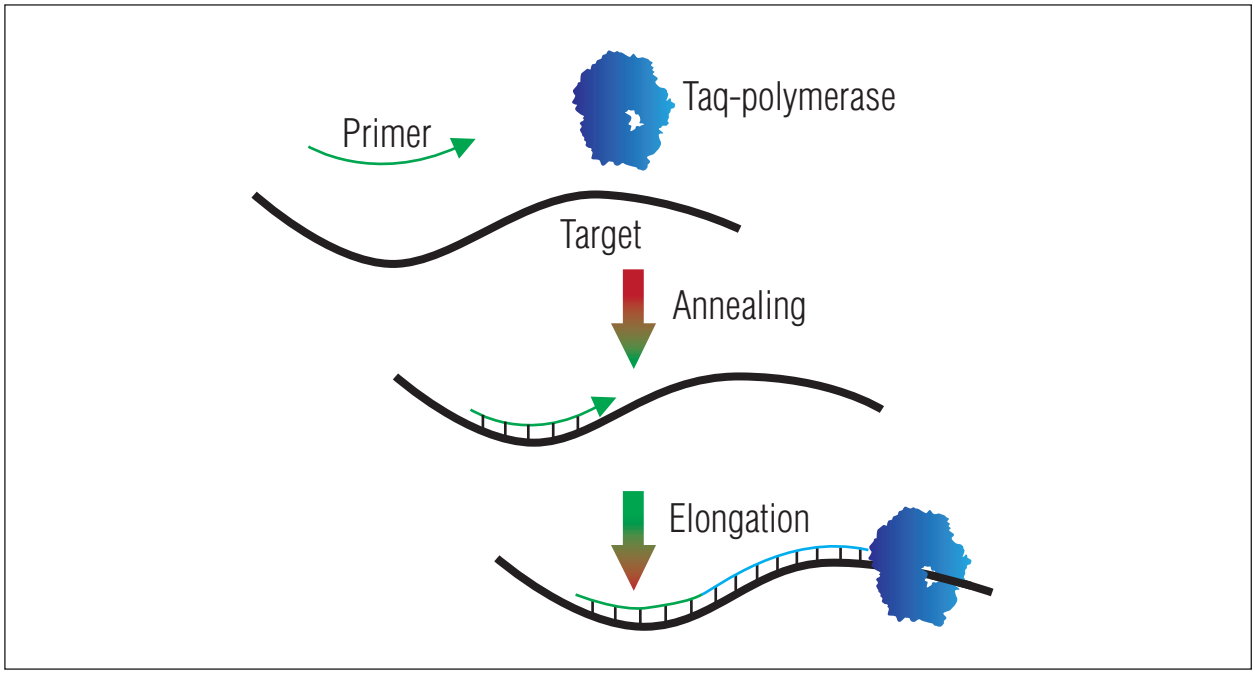


Fig. 2. Annealing of primer (P) and elongation

The thermal amplification cycle is repeated many times – 30 times or more (Fig. 3).

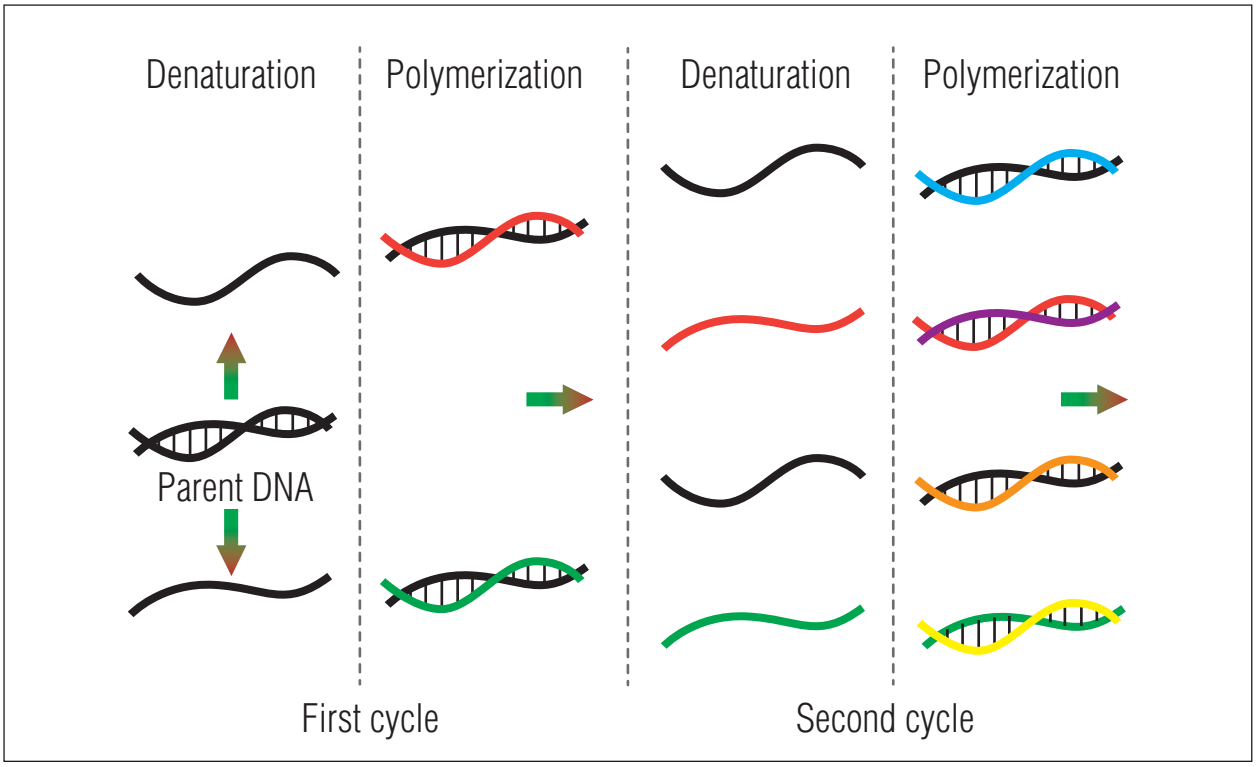


Fig. 3 PCR phases

The number of synthesized copies of DNA fragment is doubled in each cycle. Specific amplification products accumulate in geometric progression. Then, its effectiveness lessens critically – the *plateau effect*.

For ease of amplification detection or control of amplification effectiveness, additional components may be added to the reaction mixture, such as:

- *DNA-probes – artificially synthesized small-size oligonucleotides (about 30 nucleotides), complementary to specific amplicons (reaction products). The presence of isotope or fluorescent labels in a probe allows detection of reaction products.*

While implementing internal quality control for laboratory tests, panels certified for the presence of analyte (amount of analyte) produced by manufacturers of commercial kits or inter-laboratory certified samples containing and not containing the nucleic acid of specific pathogens in various concentrations that are stable under storage conditions can be used.

According to **ISO 22174:2005** *"Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – General requirements and definitions"* and **ISO 24276:2006** *"Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – General requirements and definitions"*, several types of control samples are used at different PCR phases (Table 1)

Table 1. Types of controls used in PCR

№	Name of the control	Characteristics of the control	Rol of the control
1.	**Positive process control (Positive extraction control)**	Sample spiked with the target organism, which should be treated in the same way as the test sample	Demonstration that the nuclelc acid extraction procedure has been done in the way that would allow the extractin of the targen DNA from the matrix of the sample
2.	**Negative process control**	Targen pathogen-free sample of the food matrix which is run together all stages of the analytical process	Demonstration the absence of contaminating nucleic acid during the analytical process
3.	**Negative extraction control (Extraction blank control)**	Control carried out through all steps of the DNA extraction procedure in the absence of test sample	Demonstration the absence of contaminating nucleic acid during the extraction procedure. This control is not necessary when the negative process control is performed
4.	**Internal amplification control**	DNA added ti each reaction in a defined amount or copy number which serves as an internal control for amplification	The absence of false negative results can be demonstrated
5.	**External amplification control**	Control DNA added to an aliquot of the extracted nucleic acid in s defined amount or copy number which serves as a control for amplification in a separate reaction.	The absence of false negative results can be demonstrated
6.	**Positive PCR control (DNA target control)**	Reaction containing the target DNA ib a defined amount or copy number	By the use of this control the proper acting of the PCR reagents can be demonstrared
7.	**Negative PCR control (DNA target control)**	Reaction performed with DNA-free water without any PCR inhibitors	The use of this control confirns that the results of analysis of the test samples which do not contain the target sequence are negative
8.	**PCR reagent control (Blank)**	Contains all of the amplification reagents except template DNA extracted from the test sample. Instead of template DNA a corresponding volume of nucleic acid free water is added to the reaction	This control is applied to demonstrate the absence of contaminating nucleic acid in the reagents
9.	**Environment control (Premise control)**	A tube containing the master mix left open in the PCR setup room or in other working area to detect possible contaminating DNA in the environment	It is used to identify the sources of contamination and the contaminated working area. It has to be done in certain intervals as part of the quality assurance program of the laboratory
10.	**Standard concentration**	Three to four samples containing serial 10-fold dilutions of a known number of target DNA copies in a range above the detection limit.	Control of quantative PCR reactions

Implementation of these provisions is possible during testing of internal controls, use of positive control samples (K+) and during testing of special controls.

- *Internal controls* represent any control DNA sample not similar to the DNA of the target microorganism. β-globin gene is sometimes used to detect infectious agents. DNA regions, homologous to primers that are included in the reagent kit are added to the ends of β-globin gene via genetic engineering.

If an internal control DNA is introduced to the reaction mixture together with the test sample, then, regardless of whether a microorganism is present or not in the biological sample, the internal control will lead to formation of amplicons differing by length from specific fragments amplified from DNA template during the PCR.

The presence of internal control amplicons in the reaction mixture shows that amplification reaction has taken place and that there are no inhibitors. If amplicons of the required size and internal control amplicons are not formed, it can be concluded that a problem occurred during PCR, such as presence of undesired impurities in the sample and/or defects in the reaction technology. In any case, the reaction result should be considered invalid.

- *Positive Control:* is needed for internal laboratory quality control of tests. For this purpose, a control DNA sample containing primer-annealing sites is used. For example, the DNA of the target microorganism or cloned specific parts of its genome. Nonspecific amplicons differ in size from fragments that result from amplification with control DNA sample. They can be both larger and smaller than the positive control. In the worst case, they may be of the same size and read as positive in electrophoresis.

To control the specificity of the resulting amplification product, one can use hybridization probes labeled with fluorescent markers or radioisotopes and interacting with DNA in accordance with the same principles as primers.

It is especially important to use control samples in the following cases:

- Change of reagent series;
- Change or recalibration of thermocycler;
- Change of DNA extraction system;
- If it is not possible to identify a genotype automatically using a software program (for DT thermocyclers).

- *Special controls* can estimate the amplification efficiency and control the specificity of the results obtained, as well as carry out quantitative DNA analysis.

Special control include the following:

- DNA ladders;
- Background control;
- Standards and calibrators;
- Sample intake control (SIC).

DNA ladders are used to detect PCR results using gel electrophoresis. Standards (labels) are double-stranded DNA fragments of strictly-defined length, which are used to identify and characterize bands obtained in the gel and to evaluate the specificity of analysis results.

Background control is the most essential using hybridization method in the amplification process, since the device registers the specific and background fluorescence simultaneously. The value of the background fluorescence depends on: properties of labeled probes; concentration of individual components of the reaction mixture; mode and duration of storage; plastic used; characteristics of the registering equipment.

Analysis of the target value signal from amplicons over background fluorescence and noise during real-time PCR allows to set a threshold fluorescence value. It is the same for all samples analyzed together and is carried out automatically, without requiring additional manipulation on preparation of background samples. In conducting FLASH analysis, separate background test tubes are introduced.

Standards and calibrators are most often used in PCR quantitative analysis. This type of control involves construction of a calibration curve in coordinates with a DNA standard dilution series from which substrate concentration are found in experimental samples.

The accuracy of the method depends on how close the conditions of DNA standard series (primarily amplification efficiency) are to the conditions of PCR experimental samples.

In cases where it is required to estimate the «absolute» number of substrate, selecting standards for the calibration curve is a difficult task.

To determine the number of DNA templates in real-time PCR, the following versions of standards exist:

- Purified real-time PCR product;
- Recombinant DNA;
- Recombinant RNA followed by reverse transcription;
- Synthetic oligonucleotide containing amplified sequence.

Standards and calibrators enable you to determine DNA concentration in two variants (e.g., when analyzing for the presence of pathogenic microorganisms in a probe):

- Number of genomic equivalents of microbial cells per unit volume of clinical specimen (GE/ml), which reflects the absolute concentration of these microorganisms in the clinical specimen;
- Calculation of the ratio of the number of genomes to the genomes of human cells. For this purpose, human DNA calibrators can be present in the PCR mixture along with calibrators of the microorganism DNA. The relative concentrations of microorganism DNA to human DNA obtained in this way may reflect the density of contamination by the microorganisms.

Sample intake control is a key point in determining the quality of the sample taken for study. This approach helps to eliminate errors in the pre-analytical phase during the study of a biological material that contains human cells, and to avoid obtaining inaccurate, false-positive or false-negative PCR results. Besides, it can be used to estimate the amount of human genomic DNA.

Thus, there exists a range of approaches that achieve reliable results and help control PCR quality and efficiency and optimize laboratory work.

Precautions when handling the PCR reagent kits

- The reagent kit is intended for use in in vitro diagnosis;
- The position of screw caps of tubes should not be interchanged to avoid contamination;
- Only clean filter tips and dispensers for positive control samples should be used;
- The tube caps should be closed tightly after using reagents;
- Don't use after expiry date;
- Don't use with the reagent kits of other manufacturers.

2. TECHNOLOGIES

DNA-Technology offers reagent kits for molecular genetic tests using various technological approaches. Each technology has its own characteristics and area of use. The laboratory equipment and set up will largely depend on the technology platform chosen. Many reagent kits are available in certain variants for different technology platforms.

PCRs can be classified into two depending on the reaction phase at which results are detected:

- *End-point PCR:* the reaction results are measured at the end of the amplification process.
- *Real-time PCR:* a specific DNA sequence in a sample is determined in real time after each amplification cycle.

PCRs can be classified into two depending on the detection method:

- PCR with electrophoretic detection;
- PCR with fluorescence detection.

2.1. Real-time PCR

Today, real-time PCR is the most effective method for solving molecular genetic problems in the diagnosis of infectious diseases, study of complex biological systems, such as urogenital and gastrointestinal tract biocenoses, and analysis of genetic polymorphisms.

Real-time PCR allows to:

- Increase reaction efficiency;
- Reduce the risk of formation of non-specific products;
- Make it possible to carry out both qualitative and quantitative analysis of tested regions of DNA/RNA molecule.

When creating reagent kits for real-time PCR, DNA-Technology implements the *TaqMan* technology (splitting of the 5'-end tag using 5' exonuclease activity of the polymerase).

DNA probes, which consist of a fluorescent label at the 5' position, fluorescence quencher at 3' position and a phosphate group at 3' position, are added to the reaction mixture. These probes have a landing site within the amplified region. The quencher absorbs the radiation emitted by the fluorescent label, while the phosphate group at 3' position blocks the polymerase (Fig. 4).

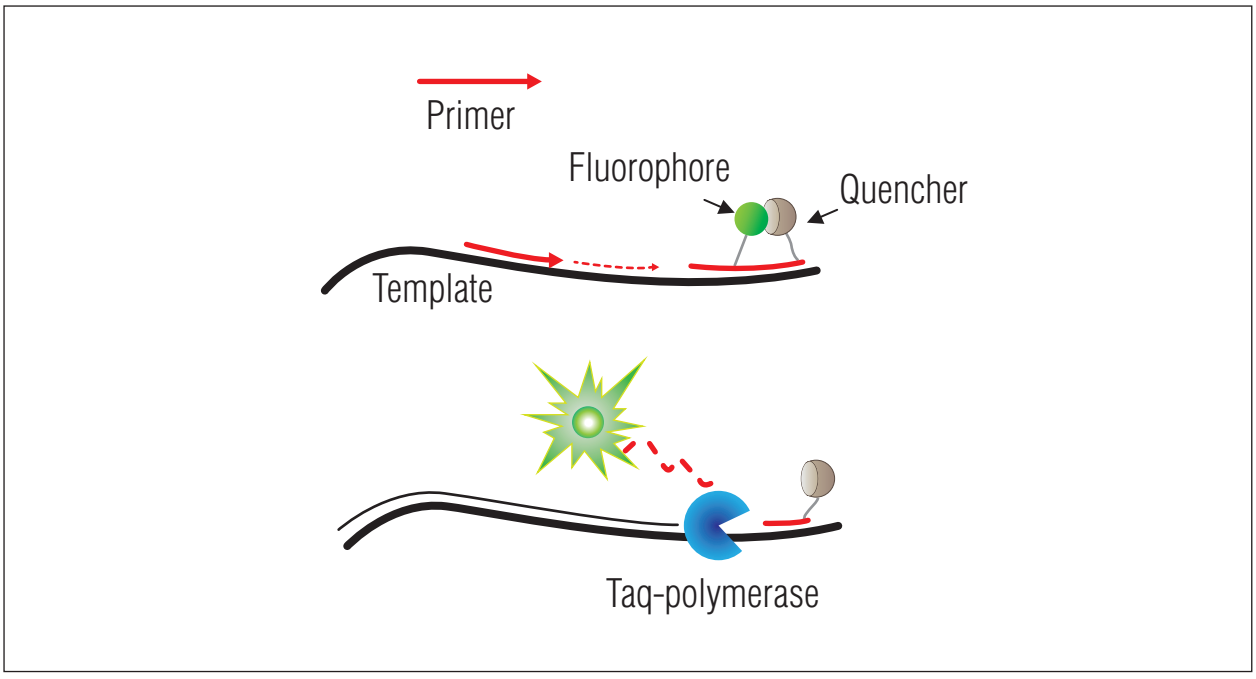


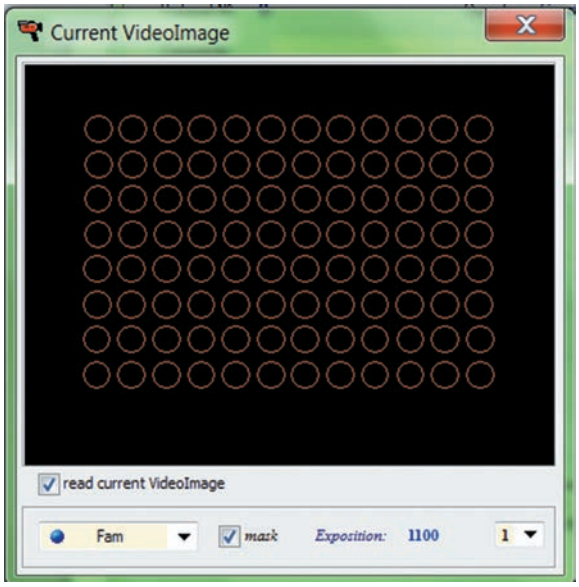
Fig. 4. Splitting the 5'end tag

In polymerase chain reaction, DNA probe is attached to the complementary DNA strand during primer annealing. Here, the greater the amplification products are produced during PCR, the more probe molecules will hybridize to complementary PCR fragments. During elongation phase, polymerase synthesizes the complementary DNA strand and upon reaching the probe, begins to degrade due to the presence of 5'-exonuclease activity.

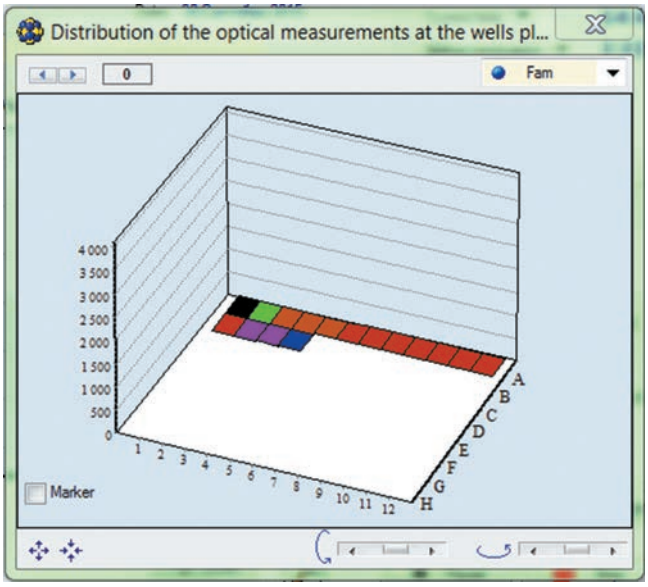
High signal/noise ratio and high sensitivity are the advantages of this approach.

Special DNA thermocyclers with an optical unit are used for real-time analysis. This allows to detect fluorescence within the reaction tube at each reaction cycle, for example, DT devices manufactured by DNA-Technology (DT*prime*, DT*lite*) (Fig. 5).

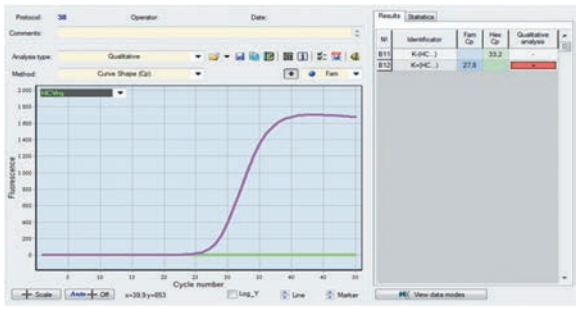
A.



B.



C.



Qualitative analysis

Number of the hole	Identificator of the tube	Cp, Fam	Cp, Hex	Result
B11	K– (HCVrq)		33.2	–
B12	K+ (HCVrq)	27.8		+

Fig. 5. Analysis of optical measurement by DT*prime* device

A – Fluorescence in Fam channel

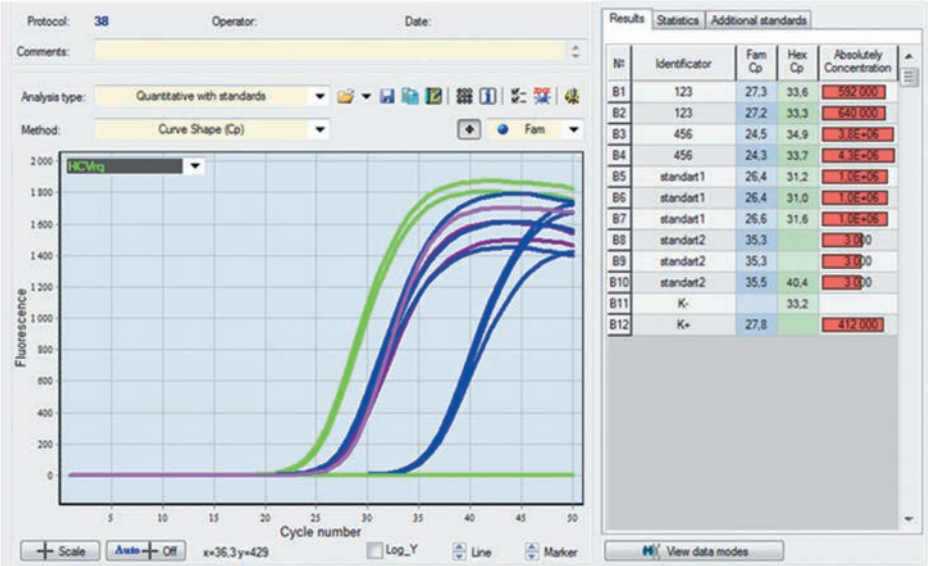
B – Data of optical measurements in Fam channel in selected tubes

C – Analysis of optical measurement results

This technology has been successfully used in creation of reagent kits *for qualitative and quantitative analysis*, and for *analysis of gene expression*.

Quantitative analysis of samples most commonly uses the format, where each series of experiments is accompanied by testing of a reference sample with known concentration (number of copies).

A.



B. Qualitative analysis

Number of the hole	Identificator of the tube	Cp, Fam	Cp, Hex	Concentration, copies/ml
B1	123 (HCVrq)	27.3	33.6	592 000
B2	123 (HCVrq)	27.2	33.3	640 000
B3	456 (HCVrq)	24.5	34.9	3.8E+06
B4	456 (HCVrq)	24.3	33.7	4.3E+06
B5	standart1 (HCVrq)	26.4	31.2	1.0E+06
B6	standart1 (HCVrq)	26.4	31.0	1.0E+06
B7	standart1 (HCVrq)	26.6	31.6	1.0E+06
B8	standart2 (HCVrq)	35.3		3 000
B9	standart2 (HCVrq)	35.3		3 000
B10	standart2 (HCVrq)	35.5	40.4	3 000
B11	K– (HCVrq)		33.2	
B12	K+ (HCVrq)	27.8		412 000

Fig. 6. Results of optical measurement analysis (Hepatitis C virus quantitative PCR Kit)

A – Analysis of optical measurements (channel Fam)

B – Analysis result report

Comparing the kinetics of amplification product accumulation in the experimental and control samples allows to estimate DNA concentration in the range of dilutions of control DNA samples (Fig. 7).

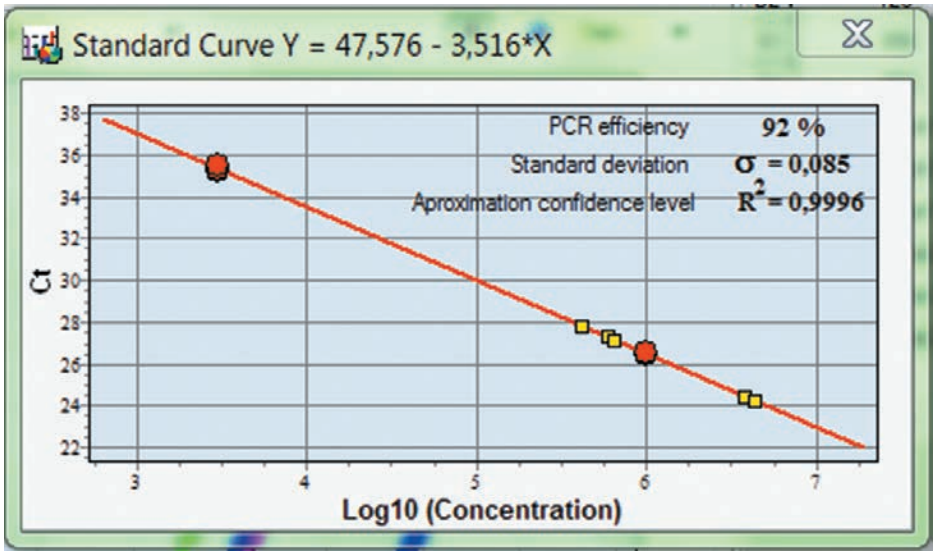


Fig. 7. Standard curve*

**Note*. The program for DT devices manufactured by DNA-Technology draws a straight line Y=A*x+B using calibration sample points ◆, with known concentrations and value Ct (or Cp). Using the Log10 concentration found, the program calculates the concentration of the tested samples ■.

It should be noted that to perform quantitative PCR analysis, it is recommended to use control DNA samples with high purity since the presence of undesirable impurities (inhibitors) reduces the amplification efficiency of the study and control DNA.

Calibrated internal controls are used to control the accuracy of quantitative analysis. In some cases, there may be DNA loss at the extraction phase, which can lead to significant distortion in the real amount of DNA in the sample. To control such losses, an internal control, whose amount is determined together with the DNA amount of the infectious agent, is introduced in the sample before sample processing.

The scope of classical PCR is expanding significantly:

- Multiplex (multiprimer) PCR;
- Reverse transcription PCR (RT-PCR);
- Analysis of melting curves.

Multiplex (multiprimer) PCR – simultaneous amplification of two or more DNA sequences in a single tube.

Reverse transcription polymerase chain reaction (RT-PCR) is used to identify a known RNA sequence. In the first phase, single-stranded DNA molecule (cDNA) is synthesized from RNA using *reverse transcriptase*. In the second phase, the resulting cDNA is introduced as a template into the standard PCR.

The possibility of using RNA as a target for PCR significantly expands the range of application of this method, for example, the genomes of many viruses (hepatitis C, influenza viruses, HIV, etc.) are represented with RNA.

Real-time PCR is often combined with RT-PCR to measure the amount of mRNA. This helps to estimate the level of gene expression in a particular tissue.

Melting curve analysis for systems with DNA probes makes it possible to distinguish point mutations located within the binding domains of the DNA template and probe. Presence of such mutations can lead to a change in the melting temperature of the probe and changes in the melting curve graph.

In a study to identify single nucleotide substitutions, as well as single deletions and insertions, it is possible to detect one of the three genotype variants: *homozygotes with original sequence, heterozygotes with point mutation and homozygotes with point mutation*.

In implementing the approach of identifying ***single nucleotide polymorphisms*** (SNPs), there are a number of technical peculiarities capable of affecting the quality of result. The first commercial kits were designed for analyzing polymorphisms. There were systems with allele-specific primers and subsequent result detection via electrophoresis. High degree of result interpretation subjectivity, as well as possible contamination risk are the disadvantages of these systems.

The next step in this direction was the use of SYBR-GREEN intercalating dyes. In terms of quality of results obtained, it should be noted that the use of dyes of this group much more often leads to detection of not only a specific sequence, but also non-specific amplification products (e.g. dimers) in comparison with similar indicators when using fluorescent probes.

Moreover, test systems with intercalating dyes involve the use of two test tubes for identification of one SNP. This reduces the throughput of the laboratory. Another shortcoming is that it is difficult to automatically interpret the results obtained using intercalating dyes.

DNA-Technology has developed a unique technology for identification of single nucleotide substitutions using PCR with melting curve analysis. This technology makes it possible to accurately and efficiently differentiate genotypes obtained, automatically interpret melting curve graphs and conduct "one SNP-in one tube" analysis.

In identification of single nucleotide substitutions, PCR with primers common to both DNA sequence variants is initially performed, and then the reaction mixture temperature is lowered for hybridization of the template obtained with oligonucleotide probes (Fig. 8).

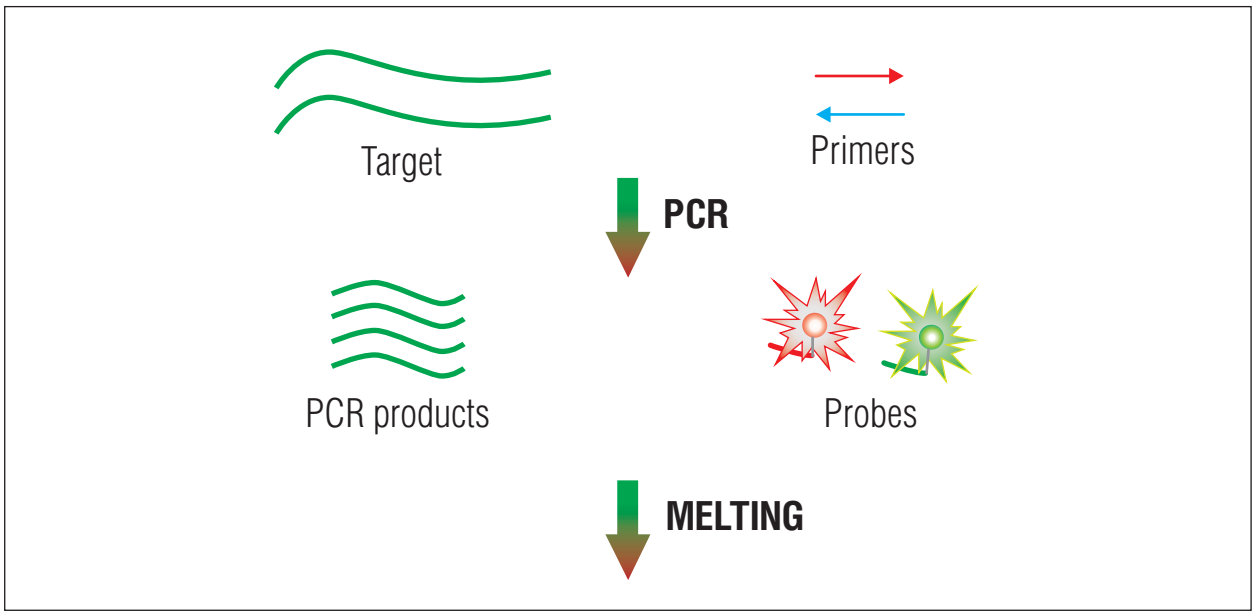


Fig. 8. Variants of sequences formed during amplification

To implement a «hot start» without using paraffin wax, without preventing non-specific primer annealing in the initial PCR phases and without increasing the sensitivity of test systems in general, DNA-Technology offers the use of Taq-polymerase, blocked by specific antibodies.

In order to determine the sequence variant, the original version of the *kissing probe* method (or *resonance energy transfer*) was developed, which is based on the use of two types of oligonucleotides (probes) that hybridize to the template at low temperature in close proximity of each other. One of the oligonucleotides is labeled with a fluorescent donor, the other – with an acceptor (quencher) (Fig. 9).

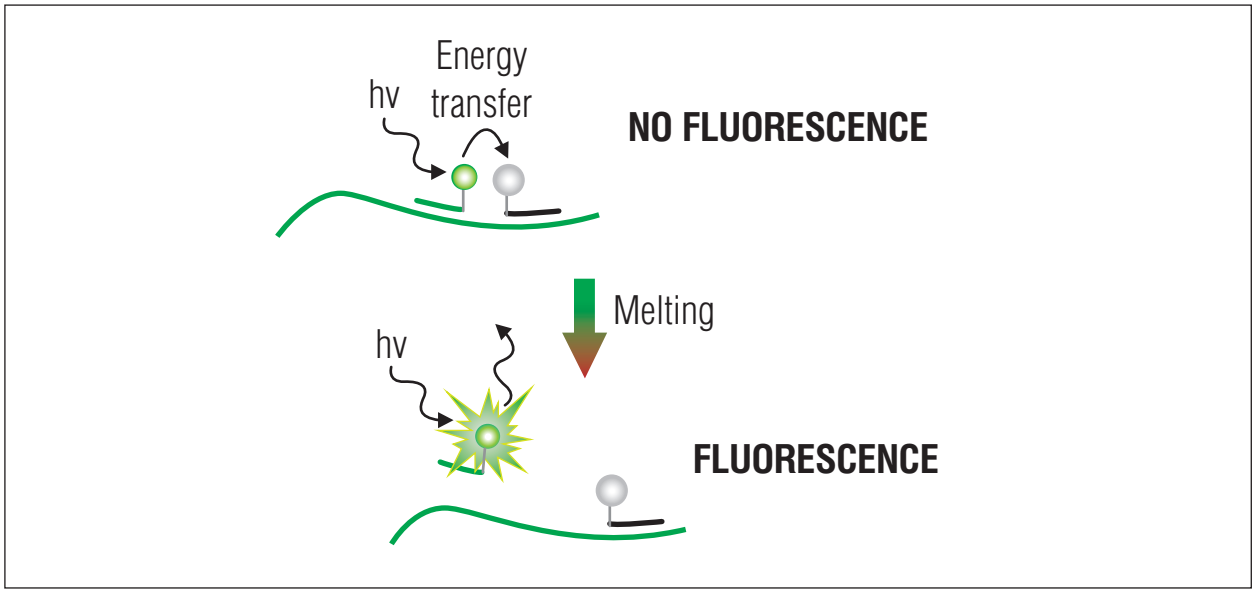


Fig. 9. Application of two probes with resonance energy transfer

Melting of double-stranded DNA formed by hybridization of DNA fragments and oligonucleotide probes is used to identify the nucleotide sequence of the analyzed sample. Duplex melting occurs under gradual increase in the reaction mixture temperature. In order to implement this approach, *sequence-specific typing oligonucleotides* and one common oligonucleotide (refractory with fluorescence quencher) are used. To improve the typing reliability (this is especially important in clinical trials), simultaneous hybridization with two alternative typing probes labeled with *different fluorophores* is used. This allows to detect both variants in *one tube* (Fig. 10.)

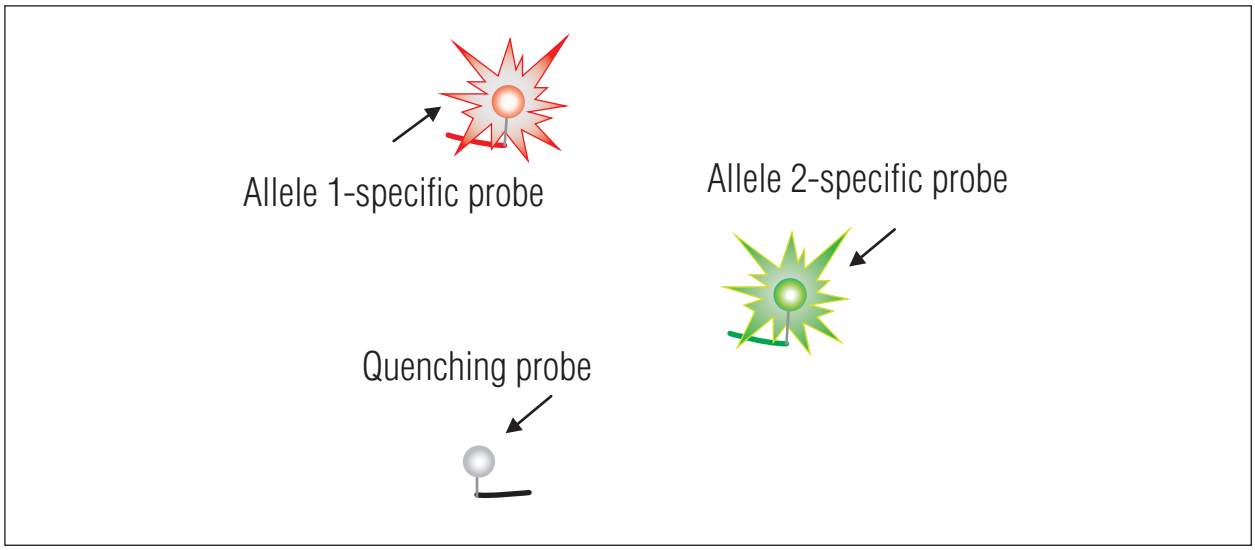


Fig. 10. A mixture of oligonucleotide probes

The genotype is identified by measuring the fluorescence level at temperature denaturation of oligonucleotide duplexes and derived templates. This measurement is carried out in real time and melting curves are the results.

At the time of DNA duplex melting at increased temperature, fluorescence increases, enabling to identify the presence of single nucleotide substitutions. If the analyzed fragment has no mutations, the fragment-probe duplex will melt at the same temperature (Fig. 11).

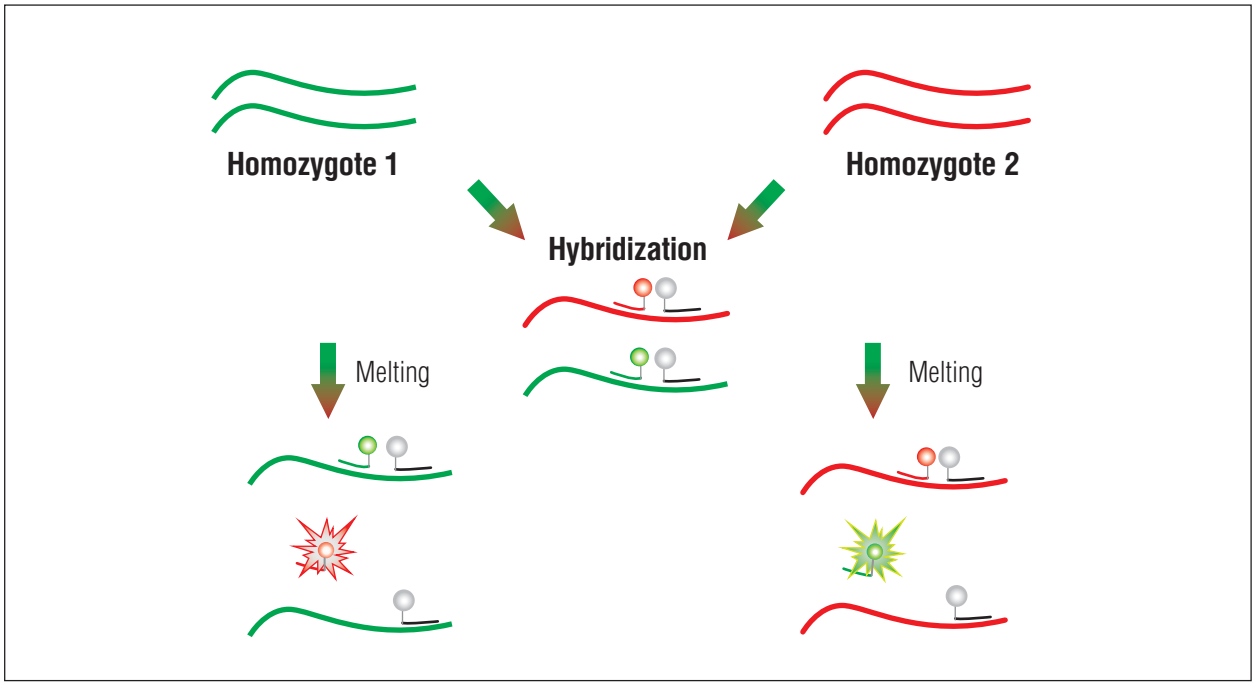


Fig. 11. Hybridization (duplex formation) and subsequent melting of complementary complex not carrying point mutation (genotype 1 – homozygote)

If for the analyzed fragment, mutation is in the same place as that of the probe, the fragment-probe duplex will be stable and melt similarly, but at a higher or lower temperature. It is defined by the ration of GC and AT pairs in the sequence.

If it is a heterozygote, then hybridization forms a significant amount of duplexes both with partial and with full complementarity. This results to characteristic double peaks on the melting curves (Fig. 12).

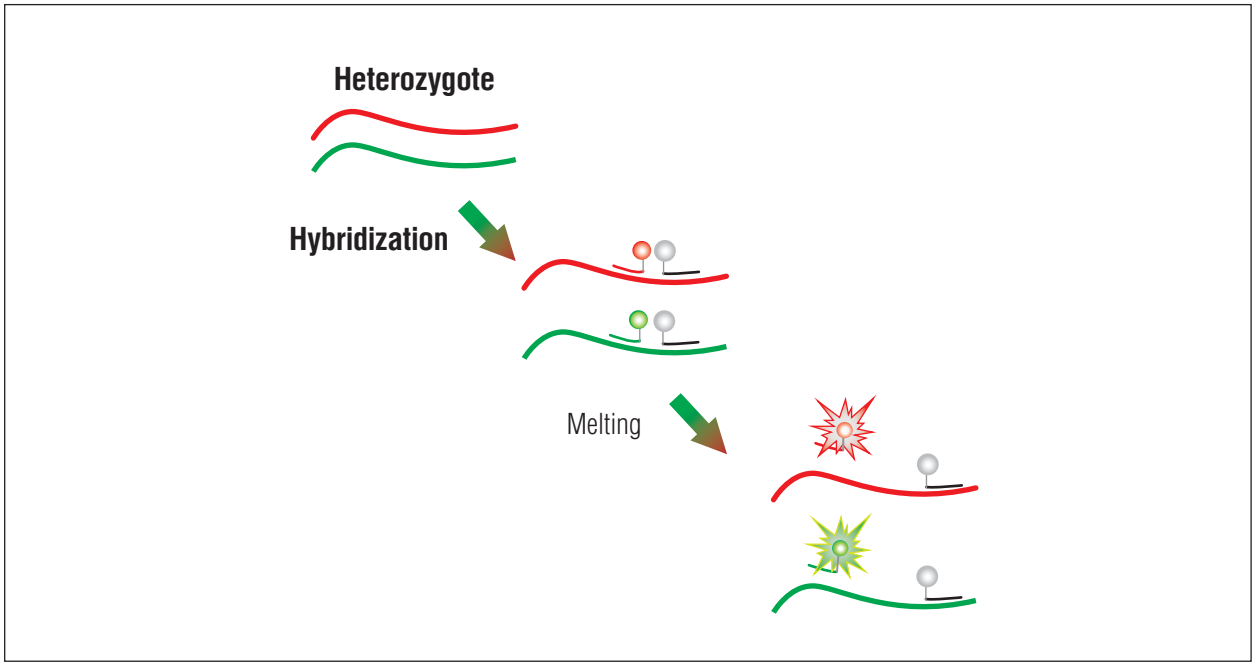


Fig. 12. Hybridization (duplex formation) and subsequent melting of *partially* and *fully* complementary complexes (genotype 2 – heterozygote)

The above variant of kissing probe method, due to its accuracy, is very popular in laboratory practice and scientific research, especially for SNP analysis by PCR.

In view of the importance of analysis of polymorphisms in clinical practice, the main directions in improving the PCR method were: automation of data processing and result delivery and improvement in the accuracy and stability of the system. Here, it is worth noting the PCR with HRM (high resolution melting), which is offered by some manufacturers of reagent kits and equipment for PCR diagnostics.

At the heart of this approach lies heteroduplex analysis using interpolating dyes and subsequent melting of amplicons in the same tube. Genotyping is carried out automatically on the basis of analysis of curve forms, using specialized software. The disadvantage of this approach is that it is difficult to interpret curve forms for different genotypes, which is why mathematical methods are needed for analysis of results obtained.

Besides, the melting temperature difference for various genotypes should be 0.1 °C (in cases where the difference should exist) to make the system less robust, not allowing for reliable automatic genotyping and leaving no opportunity for visual result interpretation.

Fig. 11 shows the results of analysis of individuals with different genotypes by *kissing probe* method using reagent kits from DNA-Technology (Fig. 13).

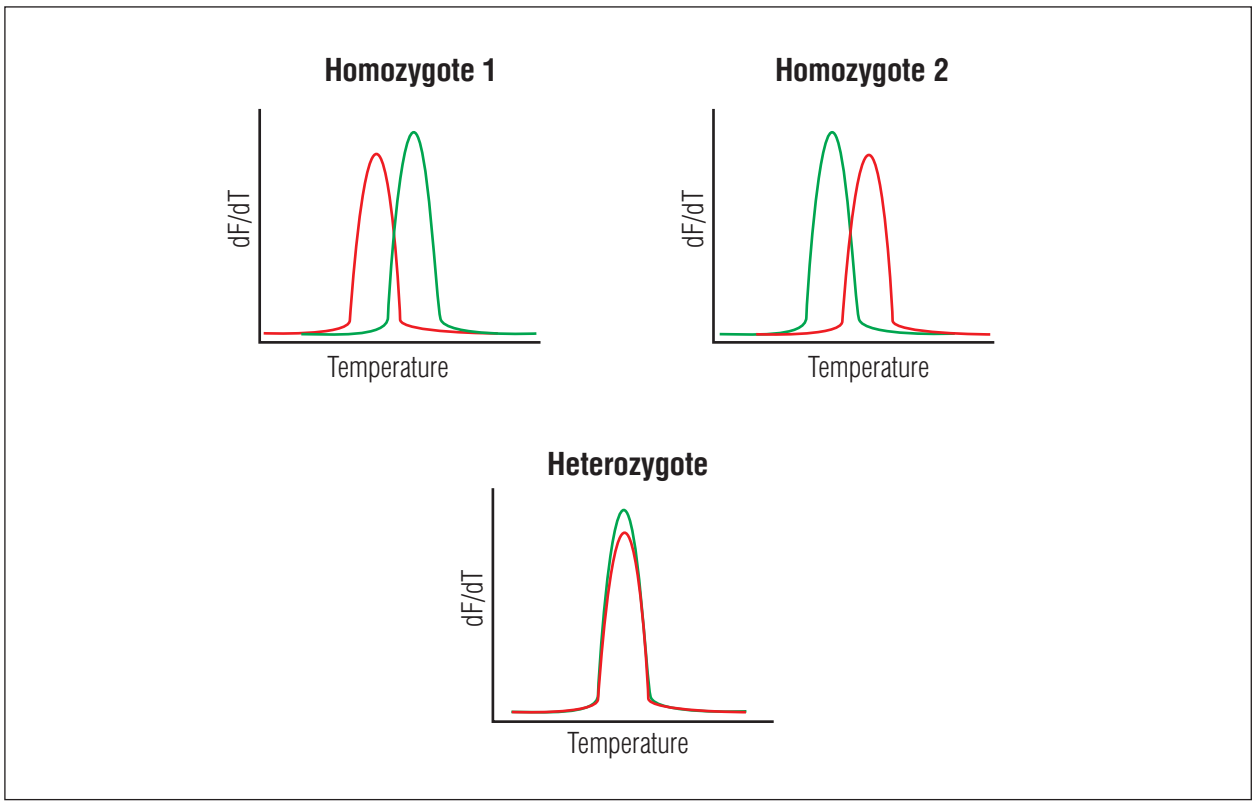


Fig. 13. Melting curves typical for genotyping by *kissing probe* method

The technology from DNA-Technology make it possible to minimize interpretation error due to determination of temperature difference for allelic variants of at least 4-5 °C. This provides maximum stability and reproducibility of results. The melting temperature of oligonucleotide probes is determined using a single device – e.g., DT detecting thermocycler manufactured by DNA-Technology.

EQUIPMENT

II. PCR EQUIPMENT

3. PCR EQUIPMENT

DNA-Technology produces the main hardware for all PCR phases (sample processing, amplification, detection) and offers complex solutions for equipment of PCR laboratory taking into account the results analysis format selected.

3.1. DT devices

DT devices are ***open platform*** for a wide range of real-time PCR studies both in routine practice of clinical diagnostic laboratories and in solving scientific research tasks. The set of innovative technical characteristics of devices and flexible software configuration provide high performance and comfortable information environment.

DT devices ensure implementation of ***qualitative and quantitative analyses***. They make it possible to conduct ***complex multiparameter tests*** and ***genetic studies*** in the shortest possible time with maximum reproducibility.

Existence of various versions of DT devices makes PCR method available both for low-throughput laboratories and for handling large amount of samples. Moreover, devices can be integrated into a laboratory information system and ***a single complex can be created for laboratory test automation***.

DESIGN FEATURES

- ***Motorized thermal unit:*** a thermal unit with automatic electric drive. It's easy to handle both by the lab assistant and for conducting work under an automated complex (Fig. 14).

A.

B.

Fig.14. The motorized thermal unit of Dt*prime* devices
A. M version (96-well template)
B. X1 version (384-well template)

- The thermal unit design ensures accurate positioning of spare parts with the reaction mixture in the optical scheme
- The thermal unit design ensures uniform heating of the reaction mixture in each well
- The thermal unit design prevents spontaneous opening of caps and possible contamination
- The thermal unit design ensures high efficiency of the hot cap
- The thermal unit template, made of aluminum alloy, ensures high rate of heating/cooling of the reaction mixture. The template is highly corrosion resistant. The shape of the template wells is optimized for heat transfer to the ***large-volume (up to 50 mcl)*** mixture samples.
- ***Forced heating/cooling mode*** is a unique solution based on the law of change of temperature of the thermal unit template, taking into account delay in the reaction mixture temperature in the test tube.

The reaction speed, while maintaining accuracy of temperature maintenance in reagent optimum operation mode at each PCR phase, is achieved by precise mathematical modeling of the influence of the template temperature on the reaction mixture temperature, depending on its volume. To achieve the target temperature of the mixture in the shortest possible time, the template temperature should be higher than this value by about 2 °C (Fig.15)

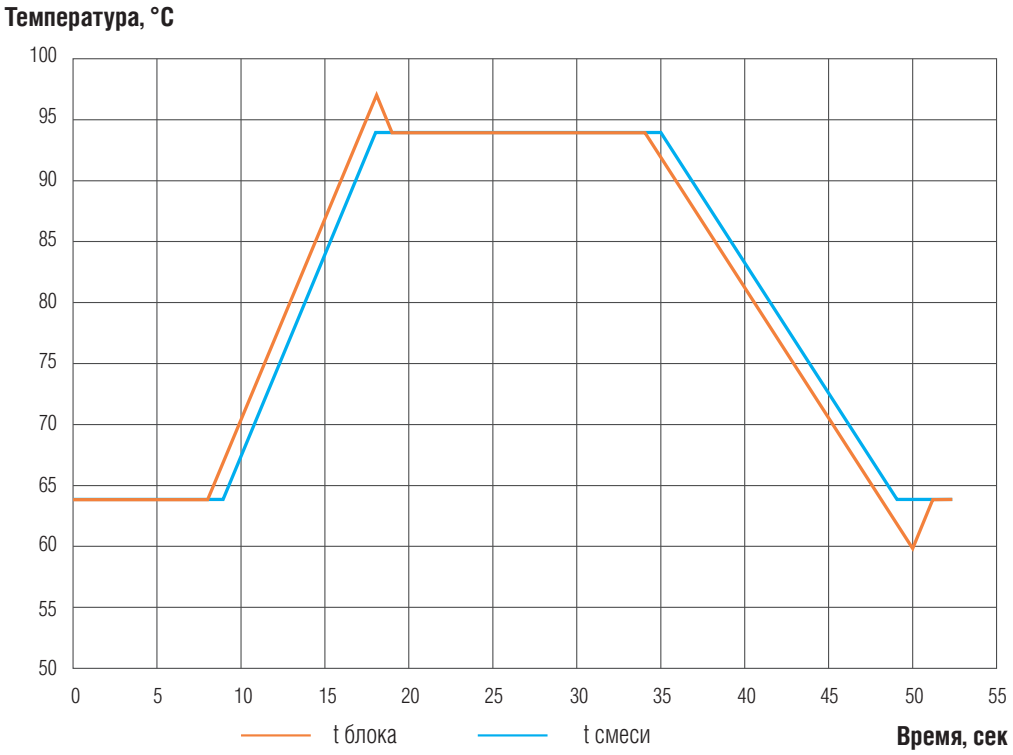


Fig. 15. Forcing the heating/cooling temperature in the thermal unit template

- ***Ability to work with a wide range of spare parts*** – the template design is a cellular structure with reinforcing ribs, which is based on 48 wells (DT*lite*) or 96 wells (DT*prime*) under standard PCR 200-mcl tubes with steps of a standard 48-well or 96-well plate (Fig.16)

Fig. 16. Types of DT device spare parts

- ***Unique lightweight template*** for DT*prime* devices in X1 version (384-well template) and DTlite in L1 version (192-well template). The profiling (a design feature) of the template of the above devices significantly reduces the weight of the template, increases the heating/cooling speed, while maintaining high temperature uniformity throughout the template. Reduction of transition time from one temperature shelf to the other increases PCR efficiency by 30 % (Fig. 17)

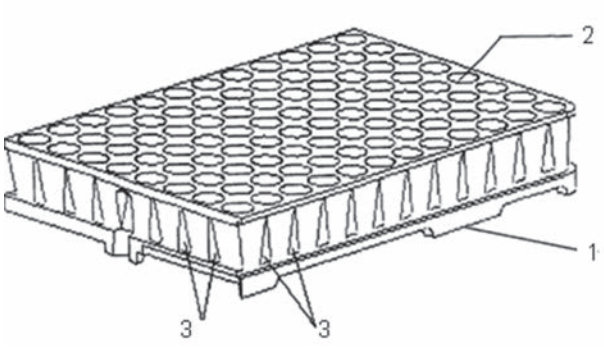


Fig. 17. Structure of lightweight template
1 – template, 2 – wells,
3 – grooves to make the design light

- A feature of the DT*prime* thermal unit in X1 version is its ***built-in barcode scanner*** that provides control over the correctness of installation of the reactive dies in accordance with laboratory information system data. All DT*prime* versions can be optionally equipped with a built-in barcode scanner (Fig. 18). This is needed for automated lab environment.

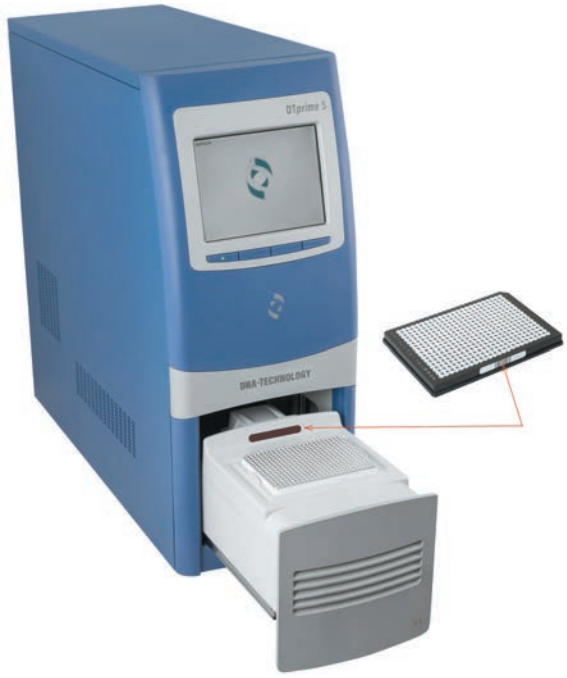


Fig. 18. DT*prime* in X1 version with built-in barcode scanner

- ***Durable light sources*** *(LED lamps)* lasting for up to 100,000 hours. They feature high stability as compared with halogen lamps.
- The optical system of the device (includes an LED light source, CCD, and a system of lens, mirrors and optical filters) provides high sensitivity, ***optimum signal/noise ratio and low level of interference between detection channels***
- ***Unique design of the optical system*** of DT devices allows simultaneous detection of a fluorescent signal in all the wells of the thermal unit. This increases detection uniformity and functioning speed of the device as a whole
- The ***highly selective filters*** of the optical system and the ***cross-current compensation feature***, supported by original software, increase fluorescence detection specificity and allow to effectively work with reagent kits in multiplex format
- The ***lightproof isolation system*** of the design completely isolates the optical path from external lighting.

- The ***built-in high efficiency radiator in the thermal unit*** increases the service life of Peltier elements and maintains uniform temperature throughout the analysis
- The thermal unit, case and power supply unit all have ***separate fans***. This ensures stable operation of the device during prolonged use, preventing it from overheating and ensuring low noise
- The built-in microcontrollers allow to provide devices with ***standalone (internal) software*** that supports self-diagnosis, including:
  - Diagnosis of current state of Peltier elements prevents the likelihood of obtaining incorrect PCR results while using the device. The diagnosis involves alerting the user about changes in the operation of Peltier elements before they affect the analysis results;
  - Thermal unit position control determines tube positioning accuracy
- ***Hot cap*** – designed to provide good thermal contact between the tubes and the template, and to maintain the temperature of tube caps at 105±1 °C. This is necessary to prevent formation of condensate on the tube caps, which can significantly distort luminous flux measurement.
- The heatblock design allows for ***temperature drop in the template***:
  - by creating *separated pseudo units* (3-section or 6-section blocks for DT*prime* devices) (Fig. 19)
  - introduction of a *bi-directional (horizontal or vertical) temperature gradient* in single-block DT*prime* devices.

Introduction of a temperature gradient opens ample opportunities in the development and/or adaptation of reagent kits for PCR analysis, because it allows choosing optimum operating conditions for the reagents and maximizing PCR efficiency. The maximum value of the gradient in the template is ±10 °C.

When using DT*prime* devices with 96-well template, horizontal gradient programming allows to create maximum number of temperature conditions (up to twelve) in eight replicates each (Fig. 19).

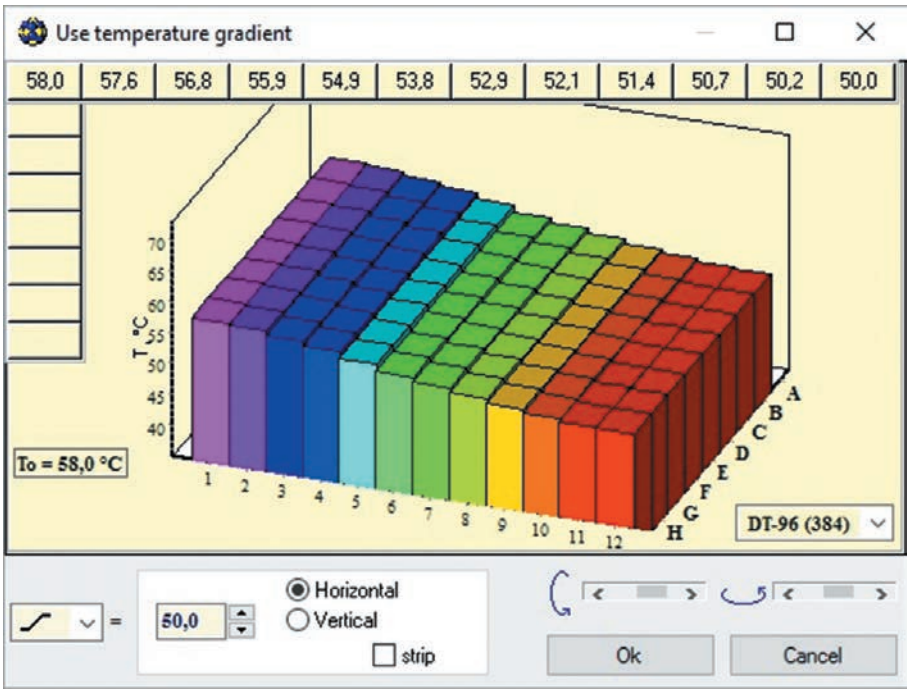


Fig. 19. Horizontal gradient for DT*prime* monoblock in M1 version

The vertical gradient provides eight temperature options in twelve replicates (for DT*prime* devices) (Fig. 20)

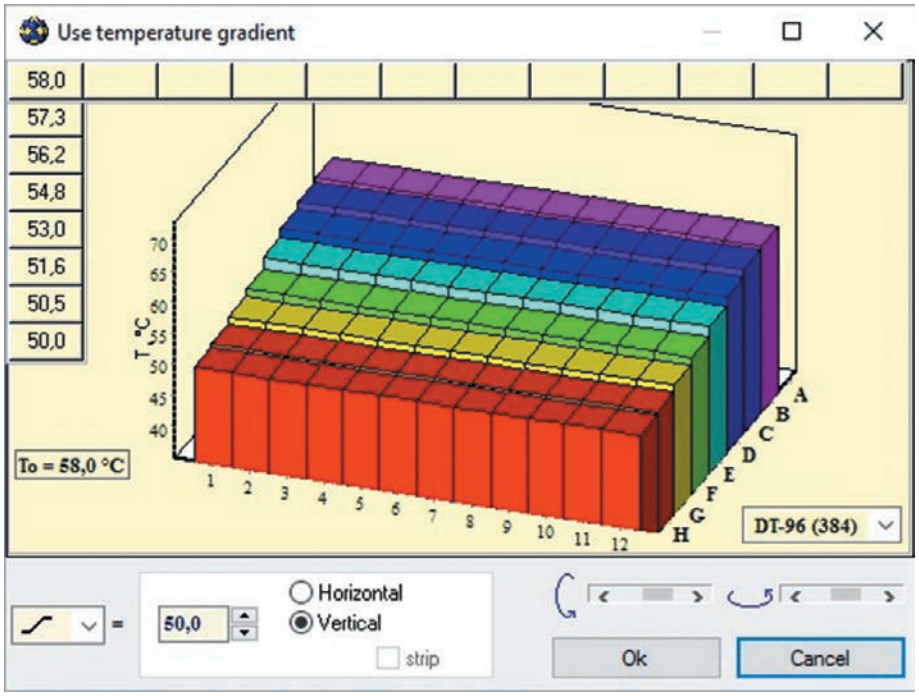
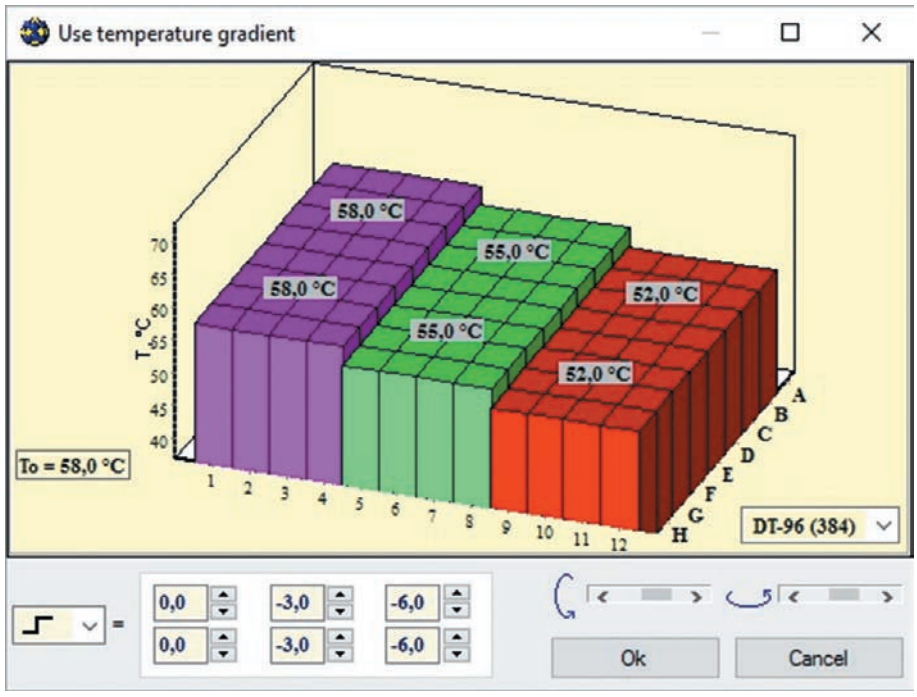


Fig. 20. Vertical gradient for DT*prime* monoblock in M1 version

So, various temperature profiles may be implemented when the program is launched once. This allows to simultaneously conduct reactions with different temperature parameters.

In the case of using DT*prime* devices with separated pseudo units, a temperature drop over the template is secured, allowing simultaneous use of multiple reagent kits (up to six, respectively), whose amplification programs differ by temperature within ±10 °C, but have the same temperature shelf length (Fig. 21).

A.



B.

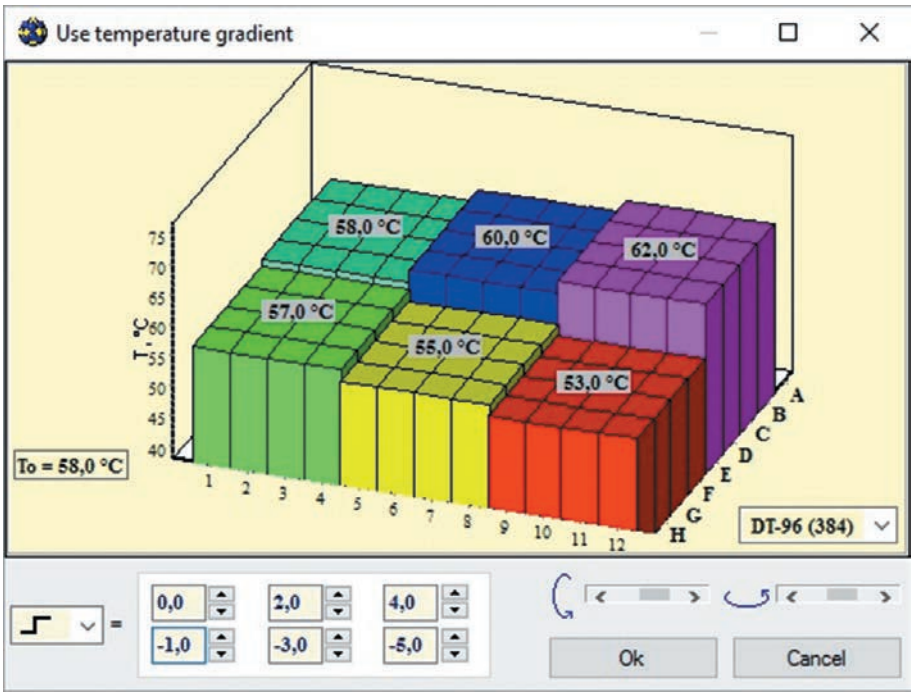


Fig. 21. Temperature drop over the template for DT*prime* device in M version
A. 3-section pseudo unit (M3 version)
B. 6-section pseudo unit (M6 version)

- ***Existence of a built-in memory*** provides essential advantages: saves analysis results in the event the control computer malfunctions (loss of communication with the computer) and stores information on the current status of the program in the device, which allows to resume the work of the program after momentary power failure.

All the data of the last amplification are recorded in own memory and can be read when there is a power failure (power outage) during amplification. Emergence of a network adapter will lead to continuation of work with full restoration of the state of the amplification program.

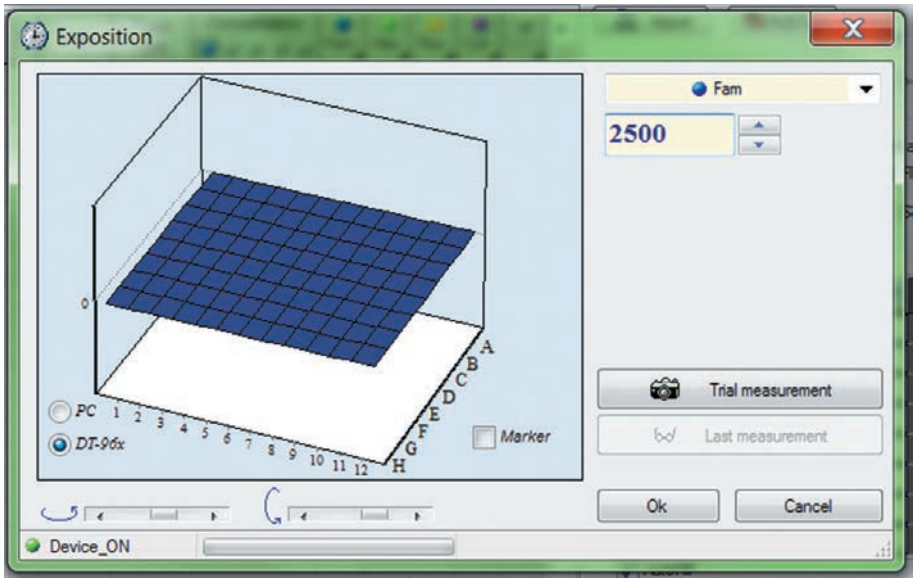
- The power supply unit of the device and fuses ensure stable and safe operation of the device during unstable power supply
- The device is calibrated at the manufacturer's factory and further calibration in the future is not required. This saves time and money for the device user

THE DT*master* SOFTWARE

- DT*master* is designed to work with DNA-Technology products and is ***unified for all DT devices***, regardless of their date of manufacture.
- ***Continuity of DTmaster versions "top-down"*** – all versions of the software are based on a single platform, thereby ensuring a familiar interface in the process of improving and expanding DTmaster options. This ensures correct operation of archival data (study protocols obtained using earlier versions of the software).
- When working with the reagent kits from DNA-Technology, the software supports ***loading of finished files (INI files) with test parameters***. This greatly simplifies the operator's routine work. The latest versions of ready files are available free of charge at the website of our company www.dna-technology.ru.
- ***Use of two expositions*** allows to work with reagent kits with different fluorescence levels at the same time. It prevents fluorescence values from passing the admissible range, provides consistently reproducible results and reduces the risk of inaccurate analysis results

- ***Device readiness for PCR is checked automatically each time the device is switched on.*** The temperature conditions of the thermal unit are checked – radiator temperature, thermostat temperature and cap temperature are checked whether they are within admissible values
- The software includes all the necessary ***routine diagnosis and device configuration options for the user:***
  - Checking of purity of wells;
  - Exposition configuration – a feature responsible for making the device compatible with third-party reagent kits (Fig. 22)

A.



B.

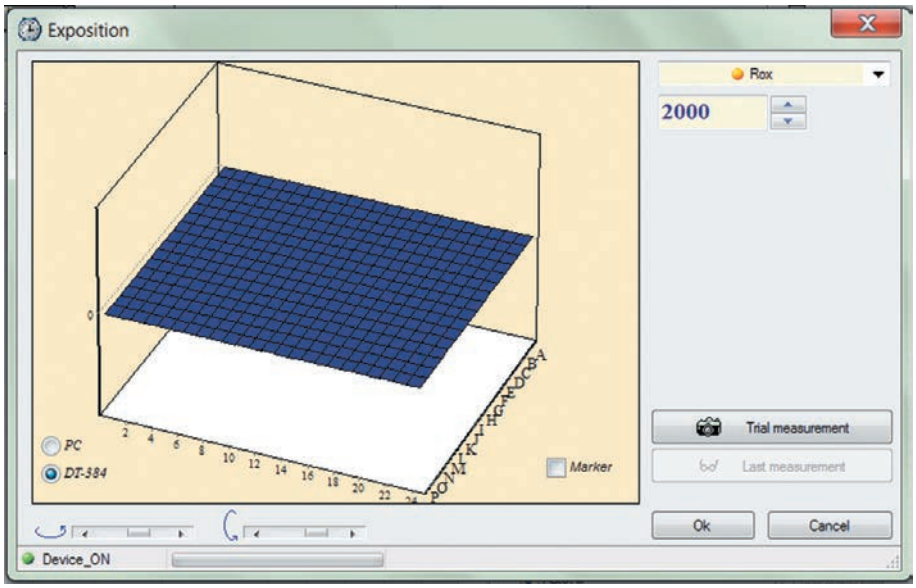


Fig. 22. Configuration of exposition measurements
A. Fam channel for DT*prime* device in M version
B. Rox channel for DT*prime* device in X1 version

- Checking procedure show the quality of the well mask settings (Fig. 23);

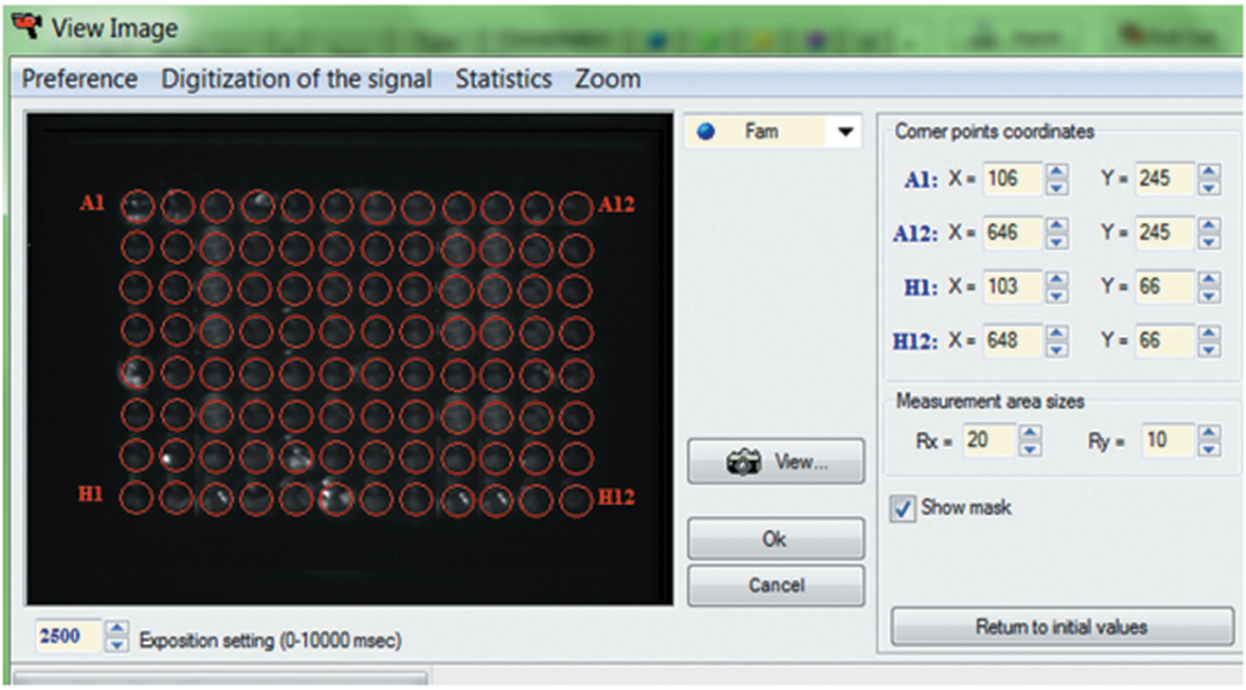


Fig. 23. Checking the well mask settings (for DT device in M version)

- Checking the health of mechanical drives and Peltier elements (under the manufacturer's supervision);
- Configuring the height of tubes – a unique solution that ensure that the device is «open» and provides high quality of analysis results when reagent kits with different types of plastic are used: low, medium and high profile plastic; tubes with convex or flat caps, strips; or if the quality of the hot cap clamp of tubes come into doubt.

- ***Easy and intuitive operation facilitates creation of analysis templates.*** This standardizes laboratory work in implementing a wide range of analyses, reduces the time required to launch a protocol, and reduces the errors committed by the operator (Fig. 24).

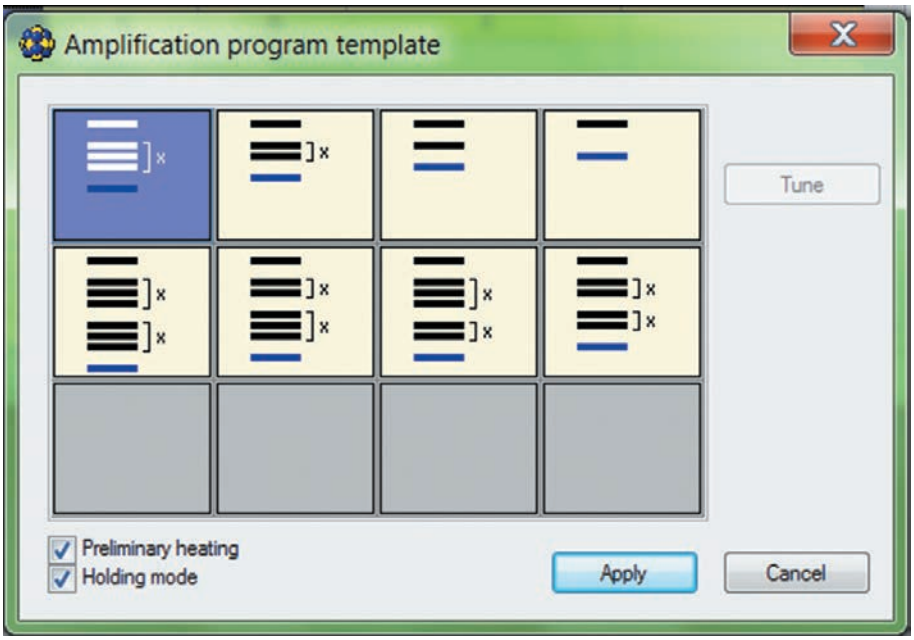


Fig. 24. Amplification program template

One-time configuration of the template will allow you to use it for any other similar analysis in the future (for example, when working with DNA-Technology reagent kit, you can create a common template for simultaneous amplification of samples with possibility of detecting, for example, twenty-seven species of microorganisms – sexually transmitted pathogens, herpes viruses, mycobacteria, respiratory tract infections, etc.) (Fig. 25)

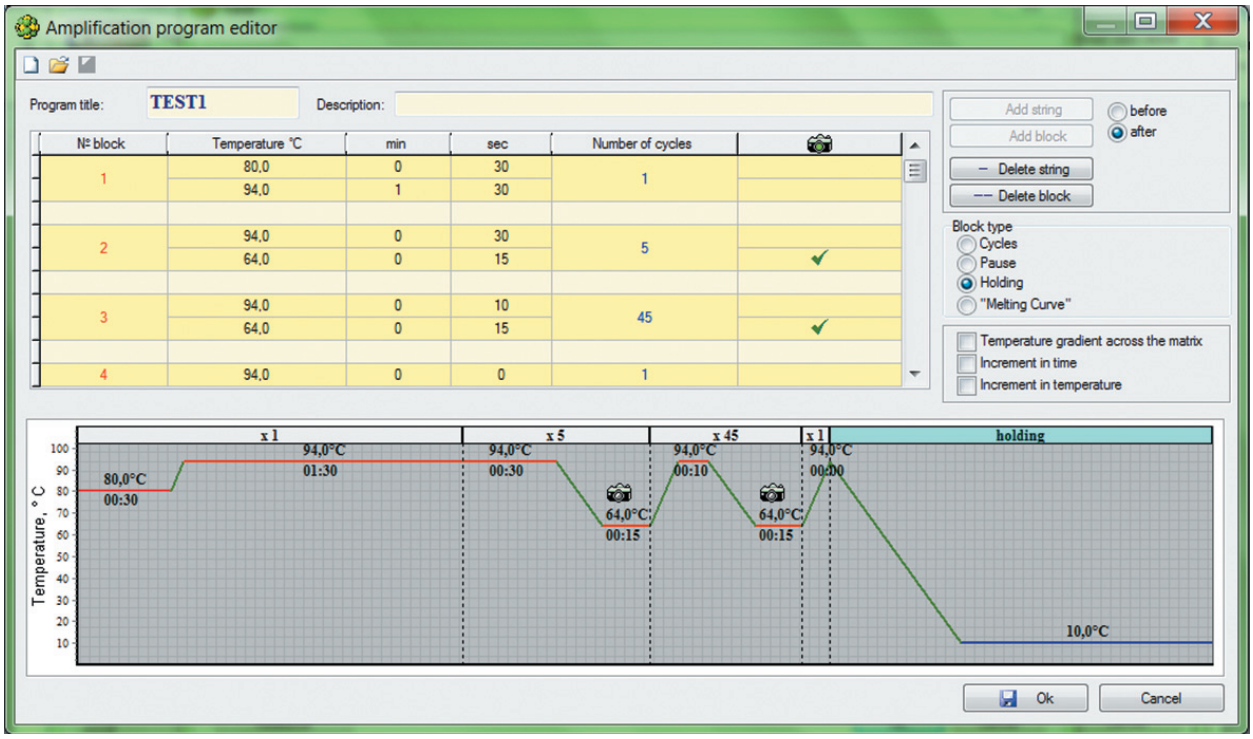


Fig. 25. Amplification program editor

- ***Multiple reuse of results of «standard curve» construction*** – the option is implemented for quantitative analysis using standards (reagent kits for HIV and viral hepatitis) and allows to load standard data from previous protocol to the current protocol, which significantly reduces duration of the pre-analytical phase, increases laboratory throughput, reduces cost of expendables *(the option can be used only when working with reagent kits of one series!)* (Fig. 26)

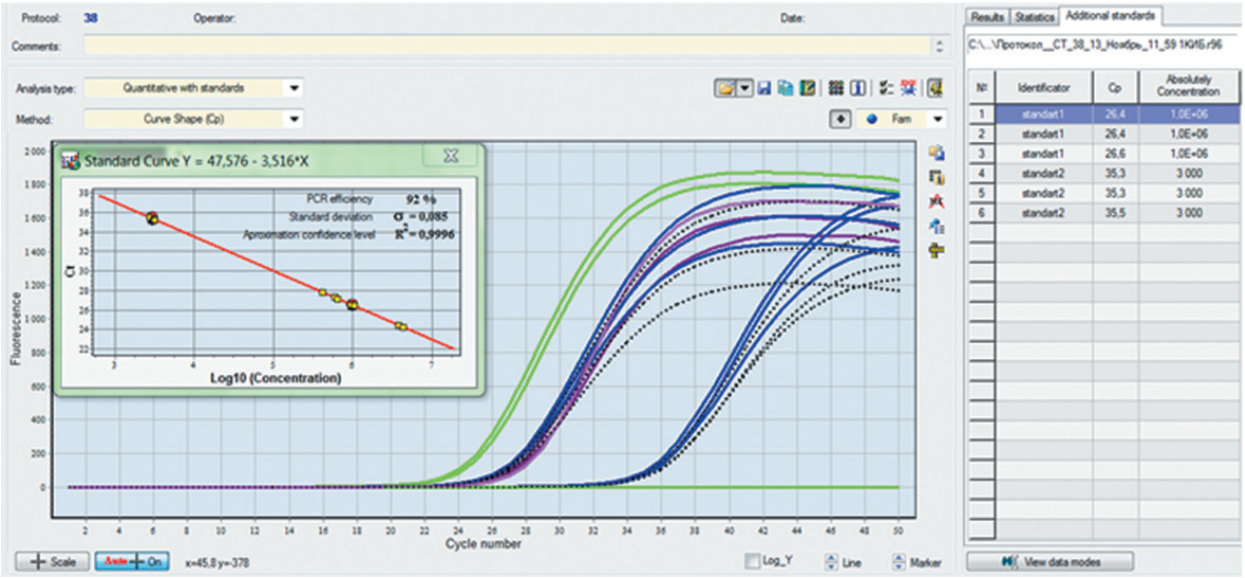


Fig. 26. Quantitative analysis protocol with additional standards (dotted curves)

- The software allows for a ***wide range of types of PCR studies***, including (Fig. 27):
  - Qualitative and quantitative (absolute and relative) PCR analysis;
  - Quantitative analysis using ready standards with multiple result-delivery formats (copies, picograms, international units);
  - Drawing of melting curves – carrying out genetic studies;
  - Multiplex analysis

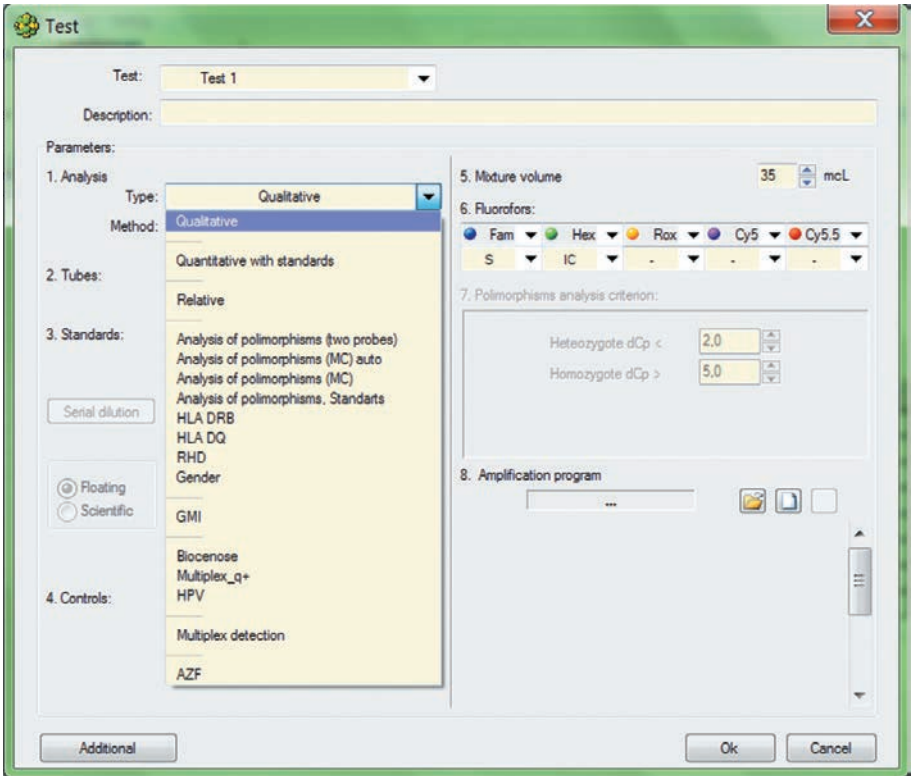


Fig. 27. Choosing PCR test options

- Unique characteristic of the software: ***multi-parameter analysis*** (assessment of condition of biocenoses, determination of predisposition to oncological diseases, HLA typing, etc).

Statistical result assessment is featured for research task execution.

- For user convenience, especially when the thermal unit die is loaded to the maximum (most important when using a 384-well plate format), the user can view the analysis protocol as a whole or by selecting individual probes and color coding them (Fig. 28)

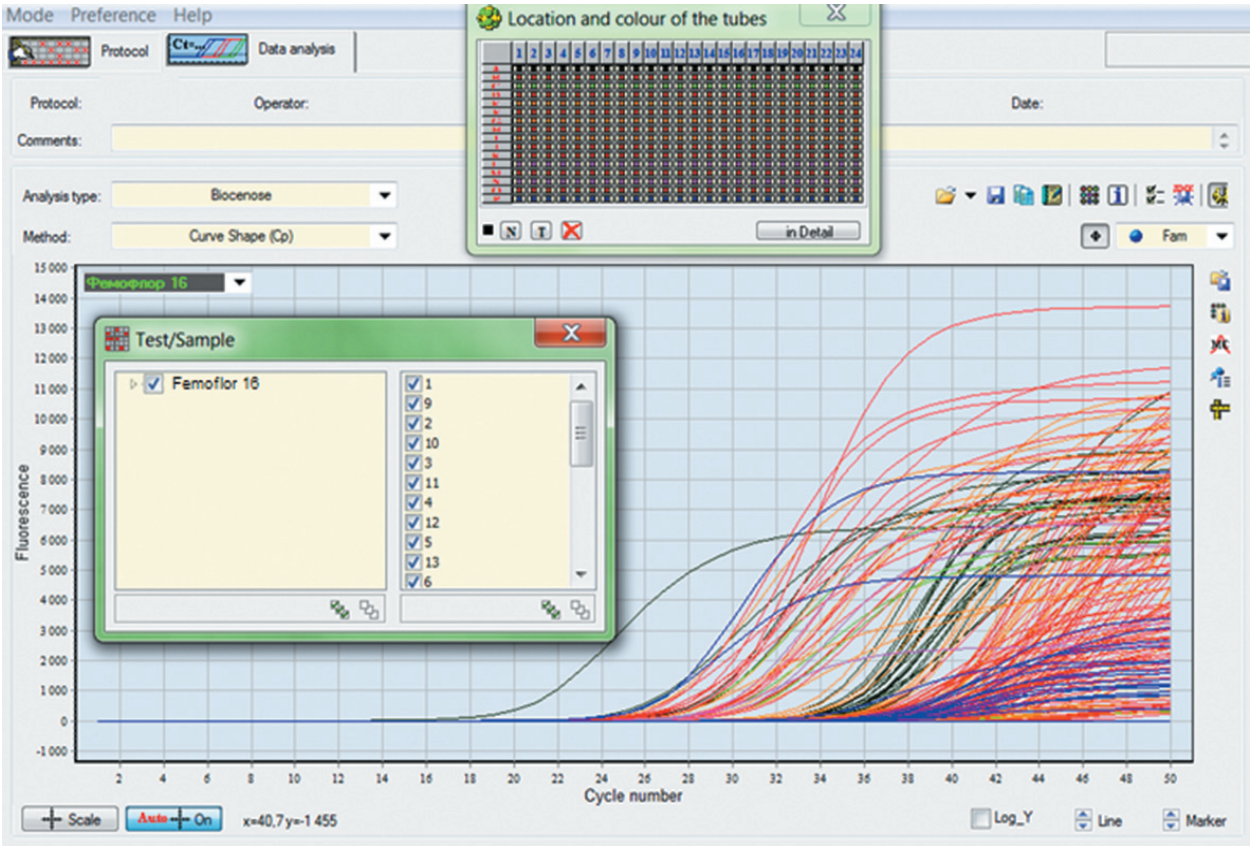


Fig. 28. Analysis protocol for fully loaded 384-well plate of DT*prime* thermocycler in X1 version

- ***Archiving of analysis protocols for an unlimited time:*** the user can store analysis results on his computer in a standard format, making them readable on any computer that has the software installed without connecting to the device. This feature also allows users to remotely consult with DNA-Technology specialists.
- Ability to work with analysis protocols on the host computer while PCR run is in progress
- ***Data can be exported in txt and xml formats to integrate the device into a laboratory information system (LIS). The user can also import analysis results***

3.1.1. DT*prime* detecting thermocycler

DT*prime* is the optimum choice for diagnostic laboratories requiring high throughput when performing routine analyses and for scientific institutions where optimization and fine-tuning adjustments are important.

The device is controlled by software *DTmaster* with a Russian/English user interface, common to all DT thermocyclers with real-time PCR detection.

Specifications

Parameter	Value
Thermal unit die format	• Monoblock (M1 version) – 96 0.2ml tubes (12x8 arrangement) • 3-section thermal unit (M3 version) – 96 0.2ml tubes (12x8 arrangement) • 6-section thermal unit (M6 version) – 96 0.2ml tubes (12x8 arrangement) • Monoblock (X1 version) – 384-well 0.045ml plate (24x16 arrangement)
Tube type	For M version: • PCR 0.2ml tubes (separate or in strips, 8 pieces each) • 96-well 0.2ml PCR plate For X1 version: • 384-well 0.045ml PCR plate
Thermal unit temperature range (°C)	From 0 to 100
Temperature shift (°C)	0.1
Minimum temperature maintenance accuracy (°C)	+/–0.2
Maximum heating rate of thermal unit (°C/sec)	• For M versions: 3.3 • For X versions: 2.1
Maximum cooling rate of thermal unit (°C/sec)	• For M versions: 2.1 • For X versions: 1.0
Thermal unit actuator	6 Peltier thermoelectric elements
Excitation source	LED
Detector	CCD
Number of fluorescence measurement channels	• 4 (versions 4M1, 4M3, 4M6) • 5* (versions 5M1, 5M3, 5M6 and 5X1)

Spectral range** (nm)	Detection channel: • Fam – 470/40 – 515/30 • Hex – 530/20 – 560/20 • Rox – 580/30 – 620/30 • Cy5 – 630/20 – 660/20 • Cy5.5 – 687/20 – 731/30
Color graphics display	29x97mm with 690x480pix resolution and increased service life (at least 30,000 hours)
Hot cap temperature (°C)	105
Supply voltage (V)	220
Power consumption (W)	Max 500
Utility frequency (Hz)	50
Dimensions (WxDxH) (mm)	210x540x540
Weight (kg)	27

* Base option for the X1 version
** Excitation/detection wavelengths can be changed

Benefits of using DT*prime*:

Fig. 29. DT*prime* in X1 version

- High performance and flexible: you can both apply separate tubes and strips, and standard 96-well plates as expendables, and 384-well microplates in X1 versions (Fig. 29).
- The software contains a lot of settings allowing advanced users to obtain more data and finely tune device for a given task.
- The user can work with robotized sample processing systems thanks to the special geometry of the body and the automatic pull-out thermal unit.
- Can be integrated into laboratory information systems to store data in standard graphic and text formats.
- Ability to control multiple devices from a single control computer increases lab productivity and allows you to simultaneously conduct a wide range of PCR studies using reagent kits and with different amplification programs
- The narrow case and compact design allows you to organize a complex of several devices in a small area.

3.1.2. DT*lite* detecting thermocycler

A device with minimum dimensions for this class of equipment – a 48-well thermal unit at high heating and cooling rate – optimal solution for small-throughput laboratories.

Uniform software *DTmaster* with Russian/English user interface for all DT thermocyclers with real-time PCR detection

Specifications

Parameter	Value
Thermal unit die format	• Monoblock (S1 version) – 48 0.2ml tubes (8x6 arrangement) • 2-section thermal unit (S2 version) – 48 0.2ml tubes (8x6 arrangement) • Monoblock (L1 version) – 192-well 0.045ml plate (16X12 arrangement)
Tube type	For S versions: 0.2 ml PCR tubes (separate or in strips, 8 pieces each) For L1 versions: PCR 192-well 0.045ml plate
Thermal unit temperature range (°C)	From 0 to 100
Temperature shift (°C)	0.1
Temperature maintenance accuracy (°C)	+/– 0.2
Maximum heating rate of thermal unit (°C/sec)	• For S versions: 5.0 • For L versions: 3.5
Maximum cooling rate of thermal unit (°C/sec)	• For S versions: 2.5 • For L versions: 1.5
Thermal unit actuator	2 Peltier thermoelectric elements
Excitation source	LED
Detector	CCD
Number of fluorescence measurement channels	• 4 (versions 4S1, 4S2) • 5* (versions 5S1, 5S2 and 4L1)
Spectral range** (nm)	Detection channel: • Fam – 470/40 – 515/30 • Hex – 530/20 – 560/20 • Rox – 580/30 – 620/30 • Cy5 – 630/20 – 660/20 • Cy5.5 – 687/20 – 731/30

Hot cap temperature (°C)	105
Supply voltage (V)	220
Power consumption (W)	Max 500
Utility frequency (Hz)	50
Dimensions (WxDxH) (mm)	210x480x310
Weight (kg)	17

* Base option for the X1 version
** Excitation/detection Wavelengths can be changed

Benefits of using DT*lite*:

Fig. 30. DT*lite* in L1 version

- Ability to improve performance for die modification in 192-well format (Fig. 30)
- Compatible with automated sample processing systems
- Compatible with laboratory information systems (LIS)
- An open system that can work with third-party reagent kits
- Easy and flexible configuration
- Ability to manage multiple devices from a single control computer
- Ability to organize a complex of several devices in a small area.

3.2. Elf-4 power source

The Elf-4 power source is designed for electrophoresis of nucleic acids and proteins in agarose and acrylamide gels.

Specifications

Parameter	Value
Output voltage (V)	From 5 to 400
Output current (mA)	From 5 to 400
Output power (W)	From 0.5 to 80
Timer operating range	From 1 min to 16 hours
Dimensions (LxWxH), mm	180x120x60
Lock system	• Short circuit • Circuit breaking • Earth leakage • Sudden load change • Internal testing

Features of the Elf-4 power source

- It is preferable to be used for agarose gels
- Can work in voltage regulation, current regulation or power stabilization modes
- The built-in timer allows you to turn off electrophoresis after a specified period of time. This prevents loss of electrophoresis results caused by excessive distillation.

3.3. Elf-8 power source

The Elf-8 power source is designed for electrophoresis of nucleic acids and proteins in agarose and acrylamide gels.

Specifications

Parameter	Value
Output voltage (V)	From 10 to 800
Output current (mA)	From 3 to 200
Output power (W)	From 0.5 to 80
Timer operating range	From 1 min to 16 hours
Dimensions (LxWxH), mm	180x120x60
Lock system	• Short circuit • Circuit breaking • Earth leakage • Sudden load change • Internal testing

Features of the Elf-8 power source:

- It is preferable to be used for acrylamide gels
- Can work in voltage regulation, current regulation or power stabilization modes
- The built-in timer allows you to turn off electrophoresis after a specified period of time. This prevents loss of electrophoresis results caused by excessive distillation.

3.4. Termit thermostat

Termit is a solid-state thermostat used for research & clinical diagnostic studies. It is designed for use with 1.5ml and 0.5ml Eppendorf tubes.

Termit is the optimal choice in cases where there is no need to frequently change the incubation temperature during operation.

Specifications

Parameter	Value
Number of Eppendorf tubes 1.5 ml (pcs.)	40
Number of Eppendorf tubes 0.5 ml (pcs.)	28
Temperature range, °C	From room temperature to 99
Time taking	From 1 min to 99 hours
Temperature maintenance accuracy (°C)	± 1
Temperature shift (°C)	1
Power consumption 220 V (W) Initial heating (W) Setpoint temperature maintenance (W)	200 Max 200 Max 40
Dimensions (LxWxH) (mm)	250x120x80
Weight of device (kg)	1.5

Advantages of using Termit thermostat:

- Has a built-in timer
- Has an ergonomic design

3.5. Gnom thermostat (programmable)

Gnome is a programmable solid-state thermostat used for research & clinical diagnostic studies. It is designed for use with 1.5ml and 0.5ml Eppendorf tubes.

Gnom is especially convenient when applying methods consisting of several phases with different incubation temperatures, as well as for heating of tubes at high temperatures.

Specifications

Parameter	Value
Number of Eppendorf tubes 1.5 ml (pcs.)	40
Number of Eppendorf tubes 0.5 ml (pcs.)	28
Temperature range (°C)	From room temperature to 99
Time taking	From 1 min to 99 hours
Temperature maintenance accuracy (°C)	± 0.5
Temperature shift (°C)	1
Power consumption 220 V (W) Initial heating (W) Setpoint temperature maintenance (W)	200 Max 200 Max 40
Dimensions (LxWxH) (mm)	195x185x125
Weight of device (kg)	2

Advantages of using Gnom thermostat

- Programmability facilitates processes involving 1-3 consecutive time-temperature intervals
- The heat-insulating spring-loaded lid increases uniformity of temperature distribution in the block, prevents the tube lids from opening at high temperatures and reduces reaction mixture condensation on the tube lids
- The built-in fan significantly reduces the cooling time
- The LCD graphic screen makes operation of the device easy and convenient.

3.6. DT*pack* microplate sealing device

The device works with different types of microplates and films. The flexible configuration and compact design make DT*pack* an indispensable device during PCR studies in PCR plates.

The device is effective when working with plates of different heights and different execution materials. It can work with plastic of different configurations, including skirted PCR plates, semi-skirted PCR plates and non-skirted PCR plates.

The sealing parameters – optimum heating temperatures of the block and pressing force of the moveable carriage, as well as the sealing time – can be adjusted using special software and stored in the built-in memory of the device.

The LCD screen displays the device status, selected parameters, including the current temperature and sealing time.

Thanks to ability to select parameters optimized for combinations of specific plates and films, DT*pack* ensures reliable sealing of the samples, thereby preventing the risk of sample loss and laboratory contamination.

Specifications

Parameter	Value
Supply voltage (V)	220
Utility frequency (Hz)	50
Working surface temperature (°C)	100–200
Absolute temperature maintenance error (°C)	± 2
Sealing time (sec.)	0.1–9.9
Maximum pressing force (kg)	12
USB	2,0
Weight of device (kg)	4,5
Dimensions (WxHxD) (mm)	315x244x190

Advantages of using DT*pack*

- It has a simple 2-button control that allows you to seal up the microplate in four steps
- Compatible with a variety of plates and sealing films
- Replaceable blocks for 96-well and 384-well PCR plates (plate blocks, adapters and accessories of any shape can be custom-tailored)
- Optimized and controlled sealing process
- Electric drive with adjustable pressing force
- USB interface for programming of sealing parameters
- Adjustable temperature, sealing time and pressing force for formation of profiles of optimal operating parameters
- Built-in memory for storing up to four optimized profiles
- Coding on LCD screen using signs and symbols for information on the current status and selected sealing parameters
- Automatic standby mode for quick restart and energy saving
- Ergonomic design.

SETTING UP A PCR LABORATORY. PCR LABORATORY COMPONENTS

III. SETTING UP A PCR LABORATORY. PCR LABORATORY COMPONENTS

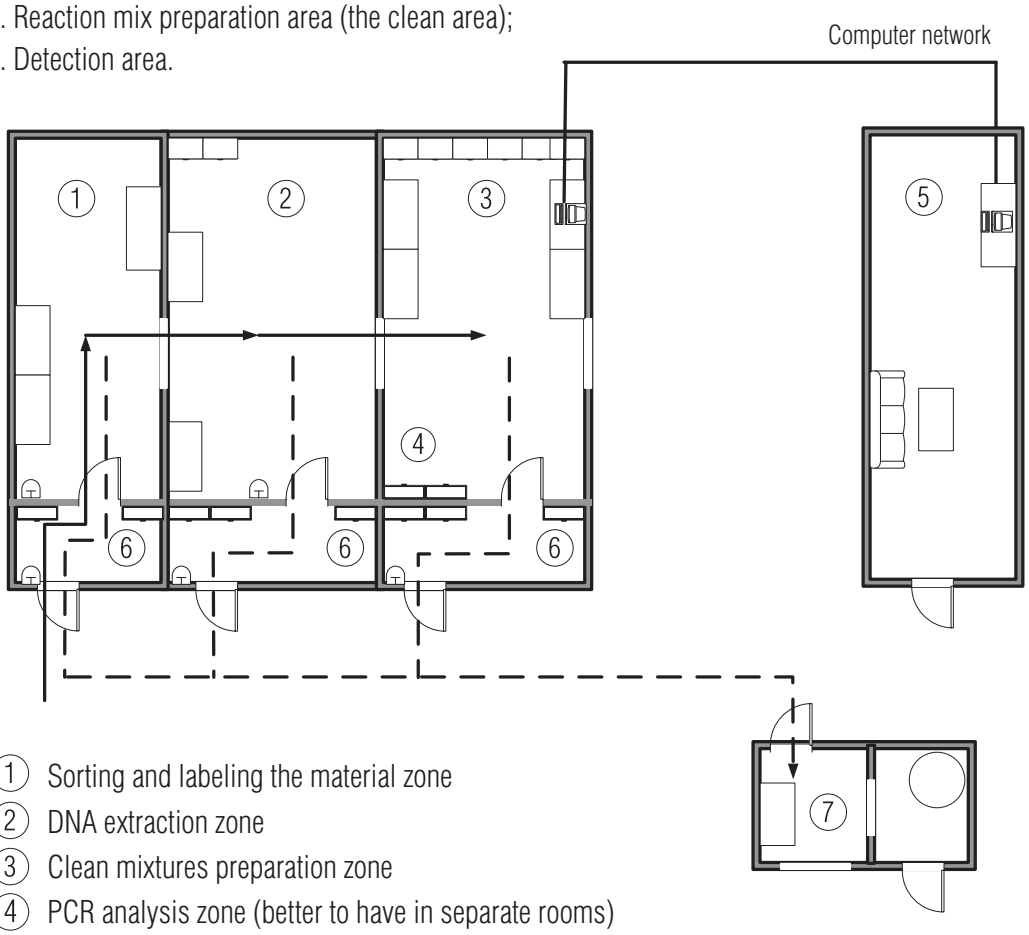
4.1. Setting up a PCR laboratory

PCR laboratory set up is currently regulated by E1873-06 *Standard Guide for Detection of Nucleic Acid Sequences by the Polymerase Chain Reaction Technique* (2006) and recommended by WHO's *Establishment of PCR Laboratory in Developing Countries* (2011).

A PCR laboratory should contain two functional work areas: a ***pre-amplification*** area and a ***post-amplification*** area (Fig.31). These two areas should ideally be in separate rooms, or (when there are space constraints) in separate work stations/biosafety cabinets in a single room. Supplies and equipment should be dedicated to each work area and should not be interchanged between areas.

A PCR-laboratory must be divided into three areas:

1. Sample preparation area;
2. Reaction mix preparation area (the clean area);
3. Detection area.



1. Sorting and labeling the material zone
2. DNA extraction zone
3. Clean mixtures preparation zone
4. PCR analysis zone (better to have in separate rooms)
5. Room for results analysis
6. Entry chambers
7. Utilization facility or autoclaving (optional)

Fig. 31. PCR laboratory organisation

The main source of contamination is the feedback of amplicons generated during the previous PCR reactions. Therefore by separating the area of activities with PCR reactions (post-PCR) from the previous activities (pre-PCR), the potential for contamination is significantly reduced. The most appropriate condition if there are separate rooms where these activities occur. Miffin (2007) outlines a possible separation of the pre-PCR and post-PCR laboratories in a way that the "forward flow" concept is implemented. Moreover, different air pressures inside the two laboratories decrease the risk of cross contamination. In the sample preparation room the pressure should be higher, while in the post PCR room it should be slightly reduced. It is ideal if each separated area has its own air supplier. For further reduction of the risk of contamination, it is recommended to maintain the doors closed in all rooms of the PCR laboratory. Besides these requirements, it is important to supply the separated PCR laboratory areas with equipments, devices and reagents, which are used only in the allotted room.

For prevention or reduction of potential contamination during PCR detection of special nucleic acid sequences, the unidirectional workflow must be applied in the molecular laboratory. It means that during the different work phases, analysis steps have to pass from the clean (pre-PCR) to the dirty (post-PCR) areas (referred to as forward flow).

The closed-tube system represent an additional safe mode against cross-contamination. In this case, the reaction tubes are not opened after the PCR processing, thus reducing the risk of contamination of the molecular laboratory by the amplicons; moreover elimination of laborious post-PCR sample Real- Time PCR representing a closed tube system, therefore it is less sensitive for the cross-contamination than the conventional PCR.

The *ISO 22174:2005* summarizes the requirements for PCR-based molecular techniques used for detection of microorganisms in food samples. For organisation of a PCR laboratory and for sample handling, the standard recommends the "forward flow" principle and systematic containment of the methodological steps involved in production of results. By keeping these measures it is ensured that the DNA from the test sample and the amplified PCR product remain physically separated during detection procedure. For this purpose, it is recommended that *minimum four distinct areas with their own working facilities* should be separated:

- The first area should be a laboratory for *nucleic acid preparation* from the test material.
- The second area should be the work area for *master mix preparation*, where all the reaction components necessary for the PCR amplification (except nucleic acid) are mixed together.
- The third area serves for the *addition of the separated nucleic acid* to the reaction mixture
- The fourth area is for the *detection and confirmation of PCR products*.

The PCR thermocycler can be placed in the third or in the forth work area, and so the amplification step has been separated from the nucleic acid extraction and from the master mix preparation.

The ideal situation is if all the four work areas are separated physically as distinct rooms and the pre-PCR and post-PCR areas have slightly increased and decreased air pressures, respectively.

4.1.1. Sample preparation

The analysis of a food matrix starts with sample preparation. The *ISO 20837:2006* document provides criteria for producing samples which are compatible with PCR and for separation of nucleic acid suitable for PCR analysis. This area has to have its own devices, which should not leave the room. In the sample preparation area, positive-displacement pipettes or pipettors with aerosol-resistant tips, disposable, powder-free gloves and laboratory coats assigned to that room are suggested to be used use. Fresh gloves and laboratory coats should be worn at all times to control contamination from this room to any other location.

This facility should be used for aliquoting of sample and preparation of positive and negative controls. As per the protocol used for extraction of nucleic acid, the required quantity of clinical sample should be added to lysis buffer and then transferred to RNA /nucleic acid extraction area. The processed samples and controls are then added to tubes containing PCR master mix in this room. PCR tubes should be capped as soon as the sample is added.

This room should be kept under negative pressure to prevent escape of infectious agent outside of the room. Biosafety cabinet (for pathogens), refrigerator, freezer and dry heat block/water bath are needed to be placed in this area. Reagents and solid items destined for the sample preparation room have to be autoclaved separately from the other reagents and materials.

4.1.2. Preparation of the reaction mixture

For preparation of reaction mixture which contains all the necessary reagents for the nucleic acid amplification filter tips, micropipettes, positive–displacement pipettes or pipettors, amplification reagent, appropriate supplies, PCR-cabinet, glows and dedicated laboratory coats are needed. The reagents have to be kept in fridge or in freezer, thus these apparatuses are also important equipment for this area. By applying a PCR-cabinet (PCR workstation) equipped with UV irradiation, it is possible to have a safe, nucleic acid-free environment which minimises the potential for PCR reaction contamination, since the UV light destroys the contaminating DNA inside the cabinet.

To prevent cross-contamination and to avoid repeated freezing and thawing, reagent-stock solutions should be aliquoted into smaller volumes. To deter contamination, the room should be under positive pressure.

Personnel should complete tasks in this room before working in the sample processing or amplification/detection rooms and should not return from these rooms to the reagent preparation room.

4.1.3. Addition of nucleic acid to the reaction mixture

For this step a clean area is recommended. If there is no place in the molecular laboratory for separating a dedicated room for nucleic acid addition, this procedure can be made in the area of sample preparation. It is essential that handling of post-PCR materials is not allowed in this part of the room. Amplification is preferably carried out in this room, or if it is not possible (no available area), it can be done in the area used for detection and confirmation of PCR amplified nucleic acid.

4.1.4. Detection and confirmation of PCR amplified nucleic acid

The PCR thermocycler has to be placed in an area where only PCR products are going to be handled. The *ISO 20838:2006* document defines the general requirements for the specific amplification of target nucleic acid sequences and describes the way of detecting and confirming the amplified nucleic acid sequence. This ISO standard helps the food analytical laboratories for getting comparable and reproducible results. This standard concerns not only detection of pathogenic microorganisms from food and feed origin, but also of pathogens from environmental samples or detection of other investigated microorganisms.

Gloves and laboratory coats should be worn at all times and removed before leaving the room to control amplicon contamination of other locations. All equipment used for amplification and product detection should be dedicated to this room, including adjustable pipettes with plugged, aerosol-barrier tips. This room should be kept under negative pressure.

4.2. Equipping the laboratory

PCR laboratory components must meet modern requirements for PCR studies with chosen result detection format (according to WHO's Establishment of PCR laboratory in developing countries (2011) and ISO/TS 20836:2005).

To ensure that pre-PCR and post-PCR events remain separated, each room must have its own separate set of equipment, reagents, pipette tips and racks, etc. used in that location only.

The following are the most important devices and equipment for molecular biological work in a food analyzing laboratory:

- **Sample processing**
  - Stomacher for homogenisation
  - Vortex
  - Refrigerator
  - Thermostats
  - Gloves
  - Laboratory coat

- **Nucleic acid extraction**
  - Positive displacement pipettes or pipettors with aerosol-resistant tips
  - Refrigerator
  - Freezer
  - Water bath / dry heat block
  - Laminar flow biosafety cabinet

- Micro-centrifuge
- Vortex
- Equipment for determination of nucleic acid concentration
- equipment for preparation of Milli-Q water
- gloves
- laboratory coat

- **Preparation of reaction mixture**
  - Positive displacement pipettes or pipettors with aerosol-resistant tips dedicated to this area
  - Micropipettes dedicated to this area
  - Pcr cabinet (with uv sterilisation)
  - Freezer
  - Gloves
  - Laboratory coats dedicated to this area

- **Addition of nucleic acid and PCR amplification**
  - Positive displacement pipettes or pipettors with aerosol-resistant tips dedicated to this area
  - Dead air box for addition of nucleic acid
  - Micro-centrifuge
  - Freezer
  - Thermal cycler(s) (normal, gradient)
  - Real-time pcr instrument
  - Gloves
  - Laboratory coats

- **Detection and confirmation of PCR products**
  - Gel electrophoresis equipment
  - Gel imaging system
  - Pc with network connection
  - Hybridisation oven
  - Incubator
  - Refrigerator
  - Freezer
  - Gloves
  - Laboratory coats

Thermocyclers are essential to all PCR methods, and great care should be taken to ensure that they are well-maintained and reliable. The block temperature of thermocycler should be tested at least twice a year by the laboratory or under a maintenance agreement to ensure uniform heating throughout the block. Block temperature should be tested with an external probe that has been calibrated against a temperature standard. For testing, the probe is placed in several of the wells in the periphery and centre of the instrument. All temperatures should be within the manufacturers' specifications. The amplification programme used in each run should be printed to further verify the conditions of the PCR.

Real-time PCR instruments are equipped to perform fluorescence excitation and detection to monitor amplification throughout the PCR cycles. The design is usually different from the standard thermocycler, and calibration may be specific to the instrument design. Temperature, laser performance, alignment, and safety devices should be checked and optical systems calibrated.

The machine should be serviced annually. Real-time machines should be used with uninterrupted power supply (UPS) as these equipment are very delicate, sensitive and also to protect the laser from damage.

Separate centrifuges, including microfuges, are required for pre- and post-PCR procedures. The manufacturers' instructions for calibration should be followed. The centrifuge should be balanced before use to increase bearing life and minimize vibrations.

The vortex is an important equipment required for reagent preparation in the PCR clean room and for nucleic acid extraction.

Automatic, fixed-volume, adjustable, positive-displacement pipettes, and/or micropipettes are used in the PCR laboratory. These should be calibrated quarterly by the manufacturer or a technician. Each pipette should be sterilized according to manufacturers' recommendation on a regular basis or whenever contamination is suspected.

Laboratory users should pay careful attention to the specifications of the hood or cabinet to ensure that it is appropriate for its designated use by the laboratory. Class I cabinets have inward air flow and HEPA-filtered exhaust that provides personal and environmental protection, but no product protection.

Class II and III BSCs filter both air intake and exhaust, and prevent contaminants from entering and leaving the hood (reducing the likelihood of sample and work area contamination). Before use, hoods should be decontaminated using UV light for at least half an hour and cleaned with bleach or other effective nucleic acid inactivating agent. The airflow and HEPA filtration in all hoods should be monitored and certified as per manufacturers' recommendations at least annually.

Separate *refrigerators* for temporary storage of sample, extracted RNA/nucleic acid and final amplification products should be maintained in the respective laboratory. Usually long term storage is not recommended but if needed, separate deep freezers (-80 °C) can be maintained.

PCR clean reagents, enzymes, buffer, dNTPS and primers are required to be stored at -20 °C. The primers, dNTPS and water should be stored in small aliquots to avoid freezing and thawing effect and also to rule out contamination issues. To verify that equipment is functioning properly, the laboratory should have a schedule for maintaining equipment. The schedule should include the set-up, calibration, repair, record-keeping, and normal operation of all equipment used in sample analysis. The results of all tests should be documented in an equipment logbook and/or electronic database. The Quality Control (QC) personnel or the laboratory supervisor should check the logbook or database monthly, and any problems and corrective actions should be managed. Equipment should be dedicated to a specific laboratory room, and there should be instrument manuals from the manufacturer.

Special *tips for PCR analysis* include barrier tips and aerosol-resistant tips, both of which minimize cross-contamination of samples during pipetting. These tips can be purchased pre-sterilized and pre-loaded in hinged racks to provide tip protection and easy access. Pipette tips for PCR analyses should be RNase-free, DNase-free, and pyrogen-free.

Polypropylene tubes that are certified DNase-, RNase-, and pyrogen-free are best recommended for PCR laboratories. The size and style of PCR tubes or reaction plates should be compatible with the block and lid height of the thermocycler/real-time machine. Thin-walled tubes provide the best heat transfer, ensuring that the reaction volume reaches its specified temperature in the shortest amount of time, thereby improving specificity and reproducibility. Tubes containing stored samples and reagents should be centrifuged briefly before opening to ensure that all liquids are at the bottom of the tubes.

4.3. Regulations

To properly organize a PCR laboratory, obtain authorization documents and produce adequate analysis results, it is necessary to be guided by the following standard regulations:

ISO/IEC 17025:1999 General requirements for the competence of testing and calibration laboratories

ISO 22174:2005 Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – General requirements and definitions

ISO 20837:2006 Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – Requirements for sample preparation for qualitative detection

ISO 20838:2006 Microbiology of food and animal feeding stuffs – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – Requirements for amplification and detection for qualitative methods

Validation can be performed by following the relevant ISO, CEN (European Committee for Standardisation), AOACI (Association of Analytical Communities International) or AFNOR (Association Francaise de Normalisation) standard procedures.

There is an internationally recognised protocol to which proficiency testing schemes should comply. This is the IUPAC/AOAC/ISO Harmonised Protocol.

Address:
Varshavskoe shosse (high-way), 125Zh, Bld. 6, fl. 5, Moscow, 117587, Russia
Phone: +7 (495) 640-17-71

Web: www.dna-technology.ru
General sales: sales@dna-technology.ru