DNA-TECHNOLOGY

GENETICS

MONOGENSCREEN

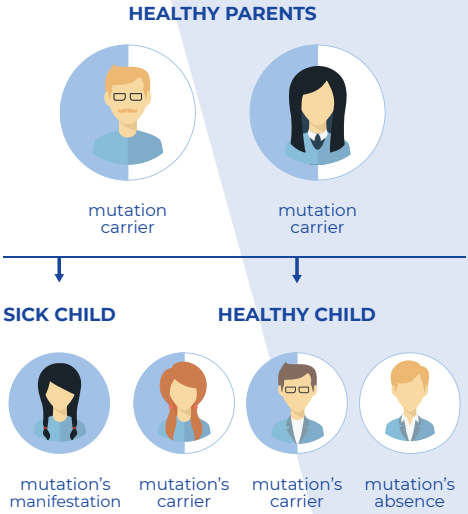
IDENTIFICATION OF MUTATIONS ASSOCIATED WITH CYSTIC FIBROSIS, PHENYLKETONURIA, GALACTOSEMIA AND NEUROSENSORY NON-SYNDROMIC HEARING LOSS BY REAL TIME PCR

CLINICAL SIGNIFICANCE

Monogenic diseases are hereditary diseases that develop as a result of mutations in one gene, leading to a change in the function of a protein. Among them, a group of autosomal recessive diseases stands out, which are inherited according to the laws of classical Mendelian genetics and manifest themselves in recessive homozygotes.

Two healthy parents who are heterozygotes for the same mutation have a 25% chance of having a child homozygous for a recessive trait.

In Russia, neonatal screening for monogenic diseases — cystic fibrosis, phenylketonuria, galactosemia, and sensorineural non-syndromic hearing loss — is mandatory for all newborns [1]. Currently, biochemical and audiological screenings are used, which capture the phenotypic manifestations of the disease and allow treatment to be prescribed to minimize symptoms.



Genetic screening is a different approach to the problem of monogenic diseases.

Carriers of recessive mutations do not have any manifestations of the disease and remain outside the field of medicine; only genetic testing can reveal them. So healthy heterozygous carriers planning a pregnancy will be warned about possible risks and will have the opportunity to use assisted reproductive technologies (ART) [2].

Genetic testing of future parents makes it possible to assess the risk of having a child with monogenic diseases and can become the basis for their primary prevention.

The MonogenScreen panel is designed to detect mutations in the genes that lead to cystic fibrosis, phenylketonuria, galactosemia, and sensorineural non-syndromic hearing loss.

Table 1. Genes included in the MonogenScreen test and related diseases.

Gene	Disease	Prevalence in Russia	Clinical manifestations
CFTR	cystic fibrosis	1:10 000	Elevated viscosity of the secretion of the glands of external secretion. Damage to the respiratory tract, gastrointestinal tract, liver, pancreas, biliary system, genitourinary system and sweat glands [3]. The progression of pulmonary and heart failure is the cause of death of patients in 95% of cases of cystic fibrosis [4-6]
GJB2	sensorineural non-syndromic hearing loss	1:1 000	Congenital impairment of auditory function [7]. It occurs in more than 50% of children with persistent bilateral hearing loss
GALT	Galactosemia	1:20 000	Deficiency of galactose-1-phosphate uridyltransferase – an enzyme involved in the conversion of galactose to glucose. The body accumulates an excess of galactose and its metabolites. Leads to cognitive disorders, cataracts, hepatic and renal dysfunctions [8-9]
PAH	Phenylketonuria	1:10 000	Disorder of the metabolism of phenylalanine due to deficiency of the enzyme phenylalanine hydroxylase. Phenylalanine and its toxic metabolites are accumulated in the body and lead to severe damage to the central nervous system and impaired mental development [10-11]

TEST IS RECOMMENDED FOR:

- pregnancy planning
- suspected carriage of mutant alleles of the CFTR, GJB2, GALT, PAH genes
- neonatal screening

BIOMATERIAL:

- whole peripheral blood
- dried blood spots

FEATURES OF THE PANEL

- A wide range of the **most common mutations associated with monogenic diseases** (70–87%) [12–14]
- **Multiplex format** — multiple targets are determined simultaneously in one tube, **which reduces the cost of testing by 18%** compared to running several individual tests
- **The Ability to use dried blood spots**
- **Relatively short time of the test (from 2.5 hours)**
- **Automatic generation of the results report** when using the recommended thermocyclers of the DT series and RealTime_PCR software
- **Availability of a file with test parameters** to automatically set the necessary parameters and calculate the results

PANEL SET

The composition of the PCR kit (package A):

- 16 Stream amplification mixtures;
- TechnoTaq MAX polymerase;
- Stream PCR buffer;
- DNA dilution buffer;
- positive control;
- strips of 8 test tubes;
- strip's caps.

The PCR kit is designed for 24 tests, including control samples. In one run, it is possible to examine 12 or 24 samples, including controls

Estimated time from sample to result (excluding sample preparation): from 2.5 hours

THE LIST OF INDICATORS DETERMINED BY THE PANEL, AND CHANNELS FOR THE DETECTION OF AMPLIFICATION PRODUCTS

Strip type	Tube №	Detection Channel				
		Mutation	Fam	Hex	Rox	Cy 5
«Strip №1»	1	CFTR: 3944delGT	N	m	—	—
		CFTR: F508del	—	—	N	m
	2	GALT: K285N	N	m	—	—
		CFTR: N1303K	—	—	N	m
	3	CFTR: 1677delTA	N	m	—	—
		CFTR: 3849+10kbC>T	—	—	N	m
	4	PAH: IVS10nt546	N	m	—	—
		CFTR: W1282X	—	—	N	m
	5	PAH: R261Q	N	m	—	—
		CFTR: E92K	—	—	N	m
	6	PAH: E280K	N	m	—	—
		PAH: R408W	—	—	N	m
	7	PAH: P281L	N	m	—	—
		CFTR:G542X	—	—	N	m
	8	PAH: IVS12+1G>A	N	m	—	—
		PAH: Y414C	—	—	N	m
«Strip №2»	1	PAH: IVS4+5G>T	N	m	—	—
		PAH: R158Q	—	—	N	m
	2	CFTR: 2143delT	N	m	—	—
		CFTR: R334W	—	—	N	m
	3	PAH: R252W	N	m	—	—
		CFTR: 394delTT	—	—	N	m
	4	CFTR: 2184insA	N	m	—	—
		CFTR: 3821delT	—	—	N	m
	5	CFTR: S466X	N	m	—	—
		GALT: N314D	—	—	N	m
	6	GJB2: 35delG	N	m	—	—
	7	CFTR: dele2,3 (21kb)	N	m	—	—
		PAH: D222X	—	—	N	m
	8	GALT: Q188R	N	m	—	—
		BK	—	—	—	+

Notation used: IC — internal control, N — norm, m — mutation

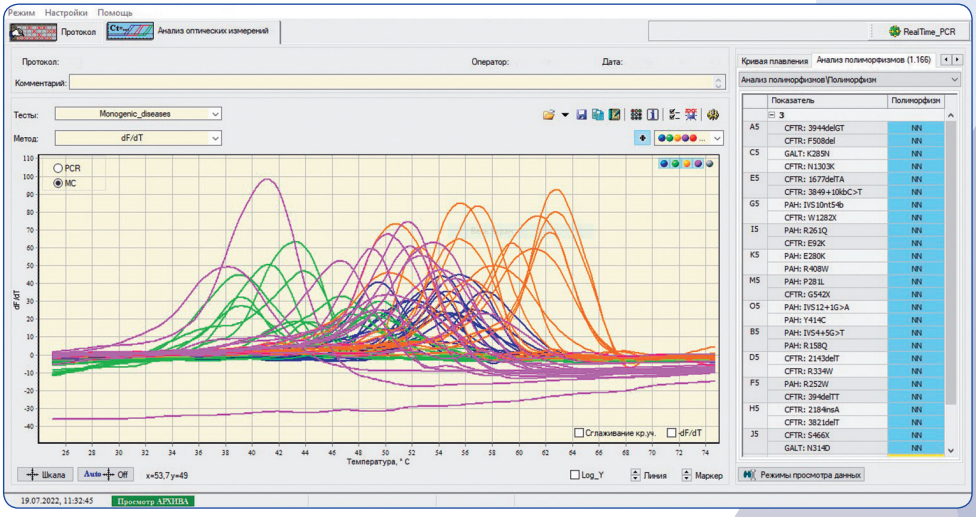
Analytical sensitivity	5.0 ng of human DNA per amplification tube

RECOMMENDED MATERIALS AND EQUIPMENT

Kits for nucleic acids isolation	PCR setup robot	Microplate sealers	Real-time PCR instruments
▪ PREP-GS Genetics ▪ PREP-CITO DBS ▪ PREP-MB MAX	▪ DTstream M4	▪ DTpack	▪ Dtprime X (384) modification
produced by «DNA-Technology TS» LLC	produced by «DNA-Technology R&D» LLC		

REALTIME_PCR SOFTWARE

Analysis and interpretation of the reaction results are carried out automatically for devices of the DT series manufactured by «DNA-Technology R&D», LLC using the RealTime_PCR software.



An example of the result of a PCR test using a detecting thermocycler of the DT series and related software: analysis of optical measurements

AN EXAMPLE OF THE RESULT FORM

MonogenScreen test result: detection of mutations in CFTR, GALT, GJB2, PAH genes by real-time PCR

Date:
Tube number:
Patient Full Name:
Sex:
Organization:
Doctor:
Notes:
Sample identifier number:

logo

Lab name and contacts

№	TEST SPECIFICATION	Genotype	Result
1	CFTR: 1677delTA	N/N	Norm
2	CFTR: 2143delT	N/N	Norm
3	CFTR: 2184insA	N/N	Norm
4	CFTR: 3821delT	N/N	Norm
5	CFTR: 3849+10kb C>T	N/N	Norm
6	CFTR: 3944delGT	N/N	Norm
7	CFTR: 394delTT	N/N	Norm
8	CFTR: dele2.3 (21kb)	N/N	Norm
9	CFTR: E92K	N/N	Norm
10	CFTR: F508del	N/N	Norm
11	CFTR: G542X	N/N	Norm
12	CFTR: N1303K	N/m	Heterozygote
13	CFTR: R334W	N/N	Norm
14	CFTR: S466X	N/N	Norm
15	CFTR: W1282X	N/N	Norm
16	GALT: K285N	N/N	Norm
17	GALT: N314D	N/N	Norm
18	GALT: Q188R	N/N	Norm
19	GJB2: 35delG	N/N	Norm
20	PAH: D222X	N/N	Norm
21	PAH: E280K	N/N	Norm
22	PAH: IVS10nt54b	N/N	Норма
23	PAH: IVS12+1G>A	N/N	Norm
24	PAH: IVS4+5G>T	N/N	Norm
25	PAH: P281L	N/N	Norm
26	PAH: R158Q	N/N	Norm
27	PAH: R252W	N/N	Norm
28	PAH: R261Q	N/N	Norm
29	PAH: R408W	N/N	Norm
30	PAH: Y414C	N/N	Norm

Conclusion:
Mutations in the following genes are detected: CFTR (N1303K).

Mutations in genes: CFTR (1677delTA, 2143delT, 2184insA, 3821delT,3849+10kbC>T, 3944delGT, 394delTT, dele2.3 (21kb), E92K, F508del, G542X, R334W, S466X, W1282X), GALT (K285N, N314D, Q188R), GJB2 (35delG), PAH (D222X, E280K, IVS 10nt54b, IVS12+1G>A, IVS4+5G>T, P281L, R158Q, R252W, R261Q, R408W, Y414C) are not detected.

Results Form of PCR analysis was obtained using a detecting thermocycler of the DT series and related software.

TRANSPORT AND STORAGE

Transportation of a kit is carried out in thermoboxes with ice packs at a temperature inside the thermobox corresponding to the storage conditions of the components included in the kit.

It is allowed to transport TechnoTaq MAX polymerase at 2 °C to 8 °C for not more than 5 days.

All components of the kit, with the exception of the TechnoTaq MAX polymerase, should be stored in a refrigerator or cold room at 2 °C to 8 °C for the entire shelf life of the kit. Strips with amplification mixture should be stored in a place protected from light.

TechnoTaq MAX polymerase should be stored in a freezer at -18 °C to -22 °C for the entire shelf life of the kit.

REFERENCES

1. Order of the Ministry of Health of the Russian Federation dated April 21, 2022 No. 274n «On Approval of the Procedure for Providing Medical Care to Patients with Congenital and (or) Hereditary Diseases».

2. Handyside, A.H., Lesko, J.G., Tarín, J.J. Birth of a Normal Girl after in Vitro Fertilization and Preimplantation Diagnostic Testing for Cystic Fibrosis // New England Journal of Medicine. — 1992. — №.327. — C. 905–909.

3. Turcios N. Cystic Fibrosis Lung Disease: An Overview // Respiratory care. — 2020. — T.65. — № 2.

4. Cystic fibrosis. Edited by N.I. Kapranov, N.Yu. Kashirskaya. MEDPRAKTIKA-M.: 2014. 672 p.

5. National consensus «Cystic fibrosis: definition, diagnostic criteria, therapy» Edited by E.I. Kondratieva, N.Yu. Kashirskaya, N.I. Kapranov. Moscow, LLC «BORGES Company «2016, 205 p. https://mukoviscidoz.org/doc/konsensus/CF_consensus_2017.pd

6. P.J. Mogayzel, E.T. Naureckas, K.A. Robinson Cystic Fibrosis Pulmonary Guidelines. Am. J. Respir. Crit. Care Med. 2013; 187: 680–689.

7. Wilcox, S.A., Saunders, K., Dahl, H.H. M., High frequency hearing loss correlated with mutations in the GJB2 gene // Human Genetics. —2000. — №106. — C. 399–405.

8. Yablonskaya M.I., Novikov P.V., Borovik T.E. Federal clinical guidelines for the diagnosis and treatment of galactosemia // Moscow, 2013.

9. Clinical guidelines «Disorders of galactose metabolism (Galactosemia)» (2021)

10. Baranov A.A. Federal clinical guidelines for the provision of medical care to children with phenylketonuria and disorders of tetrahydrobiopterin metabolism. Moscow, 2015.

11. Clinical guidelines «Classic phenylketonuria and other types of hyperphenylalaninemia» (2020).

12. Nikiforova A. I., Abramov D. D., Kadochnikova V. V., Zobkova G. Yu., Ogurtsova K. A., Bryukhanova N. O., Shestopalova E. A., Kochetkova T. O., Shubina E. S.,Donnikov A. E., Trofimov D. Yu. Determination of the frequency of occurrence of mutations in the PAH gene using a combination of real-time PCR technologies and high-throughput sequencing in patients with phenylketonuria in the Moscow region. Bulletin of RSMU. — 2017. — №4. — sS. 42–49.

13. Abramov, D. D., Belousova, M. V., Kadochnikova, V. V., Ragimov, A. A., Trofimov, D. Yu. Carrier frequency in the Russian population of mutations in the GJB2 and GALT genes associated with the development of neurosensory hearing loss and galactosemia // Bulletin of RSMU. — 2016. — №6. — sS. 21–24.

14. Nikiforova A. I., Abramov D. D., Zobkova G. Yu., Goryainova A. V., Semykin S. Yu., Shubina E.S., Donnikov A.E., D. Trofimov D.Yu. Determination of CFTR gene mutations in children with cystic fibrosis // Bulletin of RSMU. — 2018 — №3. — sS. 35–41.

e030-1 2022.09.28

DNA-TECHNOLOGY

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:
Phone/Fax: +7 (495) 640-17-71
hotline@dna-technology.ru