

CRM 12688-2024 OF THE COMPOSITION OF HUMAN GENOMIC DNA E701 – AS A MEANS OF METROLOGICAL SUPPORT FOR DECODING THE HUMAN GENOME

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The latest scientific research development by Russian scientists – the certified reference material of the human full-genomic DNA composition E701 – CRM 12688-2024 – was able to give people confidence in their health and the opportunity to live at a new qualitative level. The CRM, developed by specialists from the Research Center for Applied Metrology - Rostest (formerly VNIIMS) – the national metrology institute of the Russian Federation, and the Russian Research Medical University named after N.I. Pirogov of the Ministry of Health of the Russian Federation, is a means of metrological support for measurements performed in the study of hereditary genetic diseases, establishing kinship, identifying mutations in the human genome, and determining oncological diseases.



The reference material is intended for:
- control of the accuracy of measurement results and certification of reference and routine methods for measuring the mass fraction of nucleotides, the mass concentration of human genomic DNA;
- ensuring metrological traceability of the results of measurements of mass fraction and mass concentration; verification and calibration.
Areas of the economy and spheres of activity where the use of the reference material: healthcare, scientific research, forensic medical and judicial examination, sanitary and epidemiological supervision, laboratory diagnostics, implementation of state control (supervision) measures.
The reference material's substance is a preparation of human genomic DNA (male Caucasian race with karyotype 46XY without hereditary pathologies in the anamnesis) in a buffer solution [1]. The composition of the solution is 10 mmol/dm³ Tris (hydroxymethyl) aminomethane, 0.1 mmol/dm³ ethylenediaminetetraacetic acid (EDTA), at pH 8.0. The material is packaged in 50 mm³ test tubes with a screw cap, freestanding, with a label, with a capacity of 2 cm³. The test tube is placed in a cardboard case, the design of which protects the RM from sharp impacts and contamination.
The following main measuring instruments (their registration numbers in the Federal Information Fund for Ensuring the Uniformity of Measurements (FIF OEI) [2] are listed below), equipment and materials were used to produce the reference material:
- spectrophotometer JASCO V-730, FIF OEI No. 67389-17; NanoPhotometer N60 (Implen GmbH), FIF OEI No. 85586-22;
- CFX96 as part of thermal cyclers for nucleic acid amplification C1000 Touch, Bio - Rad , FIF OEI No. 73436-18;
- sequencers DNBSEQ-G400, MGI Tech; NovaSeq , Illumina;
- droplet digital PCR QX200, Bio-Rad;
- pipette dispenser with variable dosing volume (0.5 - 10.0) mm³ , (2 - 20) mm³ , (20 - 200) mm³ , (100 - 1000) mm³ , standard deviation ± (2.5 - 8)%, FIF OEI No. 55543-13...

METHODS FOR ESTABLISHING CERTIFIED VALUES OF A REFERENCE MATERIAL AND JUSTIFICATION OF TRACEABILITY

Determination of the certified value of a reference material mass concentration
The certified value was determined in accordance with the “Methodology for measuring certified values of mass concentration and mass fraction of nucleotides of a reference material of human genomic DNA composition”, approved by the VNIIMS, No. ФП.1.31.2024.49632[6]. Using a spectrophotometer JASCO V-730 spectrophotometer for each analyzed sample, at least 3 measurements are performed. Based on the Bouguer-Lambert- Beer law – formula (1), equation (2) is obtained for determining the concentration.

where: A – absorption; ε – the molar extinction coefficient of DNA obtained from standard reference Data on physical constants and properties of substances and materials of the State Service of Standard Reference Data (SSRD) No. 452-2024 [3]; l – optical length; c – concentration CRM.

The obtained concentration values are used to further construct a calibration graph of the dependence of the number of threshold PCR cycles on the logarithm of the initial number of DNA copies (proportional to the mass concentration of DNA). Using a spectrophotometer NanoPhotometer N60 we selected “dsDNA”. The DNA concentration is determined by measuring the optical density values of a liquid biological sample and subsequent recalculation using built-in software. The mass concentration of DNA is measured by the RT PCR method. The possibility of using this method to obtain traceable measurement results was demonstrated earlier [4]. The procedure is described in detail in the measurement procedure [6].

Determination of the mass fraction of each nucleotide
To measure the mass fraction of each nucleotide is obtained [5], the reference material is sequenced by the next-generation sequencing (NGS) method using DNBSEQ-G400 sequencers, by the Illumina high-throughput sequencing method using the NovaSeq sequencer in accordance with the operating manual for the devices and the measurement procedure [6]. The nucleotide sequence obtained is given in the passport for the reference material with a relative uncertainty in reading the nucleotide sequence of no more than 0.5%. From a given sequence, the mass fractions of each nucleotide in the sequence are calculated. If it is necessary to calculate the relative proportion of each nucleotide in the DNA nucleotide sequence – D_i, formula (3) is used:

where D_i– the relative proportion of each nucleotide in the DNA nucleotide sequence, %; K_i– the number of each nucleotide in the sequence, pcs.; i – is the designation of a nucleotide, which is a “ tetrit ” – a value that takes on 4 values A, G, C, T; N – is the size of the sequence, equal to the number of nucleotides of the DNA sequence, pcs.

In the case of calculating the mass fraction of each nucleotide in the DNA nucleotide sequence – W_i, taking into account the molecular mass of each nucleotide, formula (4) is used:

where W_i is the mass fraction of each nucleotide, %; M_i – is the molecular weight of each nucleotide, a.m.u.; m – is the mass of a DNA nucleotide sequence consisting of N nucleotides, a.m.u.

The nucleotide sequence of human genomic DNA E701 is presented in the database of the National Center for Biotechnology Information (NCBI) [7], the standard reference data - the nucleotide sequence of gene fragments in human genomic DNA E701 - GSSSD No. 453-2024 [8].
The traceability of the certified value of “mass concentration of human genomic DNA” to the unit of quantity “mass concentration of component”, reproduced by the State Primary Standard of units of spectral coefficients of directional transmittance, diffuse and specular reflections in the wavelength range of 0.2–20 μm [9], is ensured by carrying out measurements according to a certified measurement method that provides for the use of a reference material with established traceability – CRM 11368-2019 [10].
Traceability of certified values of the “mass fraction of adenine, guanine, cytosine, thymine” of the reference material to the unit of quantity “mass fraction of the component” within the framework of the interlaboratory experiment is ensured using verified measuring instruments and reference materials of approved types by competent testing laboratories, including those accredited for compliance with ISO/IEC 17025 [22].
The nucleotide sequence of the human DNA genome is consistent with the DNA sequence presented in the National Center for Biotechnology Information (NCBI) database [7].

CERTIFIED METROLOGICAL CHARACTERISTICS

Name certified characteristics	CRM Acceptable Certified Value Range	Borders permissible values of relative uncertaintys certified CRM values (P=0.95), ± 6, %
Mass fraction of adenine (A)*, %	29.0 – 29.5	1.5
Mass fraction of guanine (G)*, %	20.0 – 20.5	1.5
Mass fraction of cytosine (C)*, %	20.0 – 20.5	1.5
Mass fraction of thymine (T)*, %	29.0 – 29.5	1.5
* in relation to the nucleotide sequence of the genomic DNA of the reference material		
Mass concentration of human genomic DNA, ng/mm ³	from 9 to 10	8

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CONCLUSION

The conducted studies have shown the comparability of the results of measurements of the CRM metrological characteristics, obtained by various methods and on sequencing platforms, under conditions of the greatest spread of readings under reproducibility conditions.
The certified characteristics of the CRM are metrologically traceable to the state primary standards of the Russian Federation. Practical application of CRM in 18 organizations – laboratories of genetic research, research institutes demonstrated the comparability of results at the level of 8% of the total uncertainty in measuring the mass concentration, 1.5% of the total uncertainty in determining the nucleotide sequence of the CRM. This confirms the successful solution of the set task of metrological support of measurements performed in studies of hereditary genetic diseases of humans, on the vast territory of the Russian Federation European part.

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