INSTRUCTIONS FOR USE

Reagent kit for determining the mutation status of BRCA1 and BRCA2 genes by mass parallel sequencing in human genomic DNA sample “Quasar-BRCA1/2” according to

TS 21.20.23-038-97638376-2020

Version 8 dated 25.05.2023.

*Red-marked rules, standards etc. are local. They should be replaced by relevant ones applicable in a given country.

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Introduction

Hereditary forms of breast cancer and ovarian cancer are autosomal dominant inheritance disorders and are caused by mutations in the BRCA1 and BRCA2 genes. Breast cancer and ovarian cancer early diagnosis is an important modern oncogenetics problem due to the tumors detection significant frequency at late stages. Mutations in the BRCA1 and BRCA2 genes are extremely diverse, therefore, for targeted genetic testing, a complete sequencing of the coding regions of the BRCA1 and BRCA2 genes is an appropriate approach. [1] [2]

Quasar-BRCA1/2 reagent kit determines **target analytes** that are DNA sequences of the coding regions of the human BRCA1 and BRCA2 genes.

The test material is genomic DNA samples isolated from clinical material (whole blood, tissue fixed in 10% formalin solution and embedded in a paraffin block (FFPE block)).

The scientific validity of the target analyte lies in the presence of various mutations associated with hereditary forms of breast cancer and ovarian cancer.

The *BRCA1,2* genes belong to suppressor genes that encode proteins involved in double-strand DNA breaks repair. Mutations in these genes lead to the proteins function loss, as a result the main mechanism of double-strand DNA break repair gets disrupted.

A test for mutations detection in the *BRCA1* and *BRCA2* genes helps to determine hereditary cancer predisposition syndromes[3]. The disease development pathogenetic mechanism is mediated by a deoxyribonucleic acid (DNA) repair damage of a different penetrance degree in these genes. Most clinical cases of hereditary cancer are associated with mutations in these genes, which also protect human body from mutated cells that provoke tumor neoplasms appearance[4].

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[1] Clinical Guidelines "Breast cancer", Age group – patients aged over 18, approved by the Ministry of Health of the Russian Federation, 2018

[2] Clinical guidelines "Ovarian cancer/fallopian tube cancer/primary peritoneal cancer" Age group – patients aged over 18, approved by the Ministry of Health of the Russian Federation, 2018

Wild-type *BRCA1* and *BRCA2* genes are tumor suppressor genes, and encoded by them proteins play a leading role in double-stranded DNAs reparation by homologous recombination[5]. Functional activity loss due to congenital or acquired mutations in the *BRCA1* and *BRCA2* genes leads to cell cycle dysregulation, apoptosis and cell differentiation, increasing chromosomal instability that leads to increasing risk for breast cancer, prostate cancer, ovarian cancer, and breast cancer in men[1].

Assay for mutations detection in the *BRCA1* and *BRCA2* genes helps to determine the most effective treatment strategy with both targeted drugs (PARP inhibitors) and with various chemotherapy regimens, and also makes it possible to predict the course of breast cancer, ovarian cancer, pancreatic cancer, stomach cancer[6].

It was found that BRCA1-associated breast cancer has better response to drug therapy comparing to sporadic breast cancer, till the complete remission. It is determined that the survival rate in patients with hereditary gynecological cancer is significantly higher than in the group of patients without hereditary predisposition, regardless of the stage and treatment: five-year survival rate in patients with hereditary breast cancer is 58,9±6,3% while with sporadic cancer it is 39.7± 4.6%. Genetic test assessment importance is also determined by the fact that BRCA-status can be potentially used as a predictive marker for chemotherapy treatment. Defects in repair system imply that DNA-damaging agents such as ionizing radiation and drugs are highly effective. High efficacy of neoadjuvant therapy with anthracyclines and taxanes in carriers of mutations in the BRCA1 and BRCA2 genes is shown. Cells with impaired mechanisms of homologous recombination are characterized by high sensitivity to platinum derivatives.

[3] Boychuk SV1, Ramazanov BR DNA repair system defects - role in oncogenesis and cancer therapy // Kazan Medical Journal - 2014. – Vol. 95. - № 3. – P. 307-314.

[4] Imyanitov E.N. The Biology of Tumor Growth // Practical oncology. - 2017. – Vol. 18. - №4. – P. 307-315.

[5] Imyanitov E.N. The Biology of Breast Cancer // Practical oncology. - 2017. Vol. 18. - No 3. - P. 221-231.

[6] Imyanitov E.N General ideas about hereditary tumor syndromes // Practical oncology. - 2014. – Vol. 15. - № 3. – P. 101-106.

A number of studies showed that neoadjuvant therapy with Cisplatin is effective for patients with BRCA1-associated breast cancer, a significant reaction to the drug is associated with a triple-negative phenotype with mutation in the RCA1 gene[7].

It has been shown that BRCA1/2-deficient tumor cells selectively die if PARP inhibitors are used (PARP (poly (ADP-ribose) polymerase) — enzymes that catalyze poly-ADP-ribosylation; participate in DNA repair)[8].

According to the OVATAR study results conducted in collaboration with the Russian Society of Clinical Oncology (RUSSCO) and a pharmaceutical company AstraZeneca Russia, pathogenic mutations in the BRCA1/2 genes are detected in 35% (140/400) of female patients, according to the blood and tumor tissue parallel sequencing results in 400 patients with primary ovarian cancer in Russia. At the same time, there are 8 frequent mutations that are officially registered in Russia for a PCR panel, were detected only in 49% of cases (69/140). The other 51% (71/140) of cases were rare pathogenic mutations (occurrence less than 2%) in the BRCA1/2 genes by the full gene analysis methods (NGS and MLPA). 30.7% (43/140) of these were rare germline mutations, 15% (21/140) — somatic mutations, and major rearrangements — 5% (7/140).[9]

The scope of the reagent kit is clinical laboratory diagnostics, oncology.

Indications and contraindications for use

Sequence determination of the BRCA1 and BRCA2 genes coding regions by high-throughput sequencing is recommended for screening hereditary breast cancer, ovarian cancer in potentially healthy women and for testing patients diagnosed with breast cancer and ovarian cancer in order to determine an effective treatment strategy and to predict the treatment effectiveness.

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7 Gelmon K. et al. Targeting triple-negative breast cancer: optimizing therapeutic outcomes // Ann Oncol. — 2012. — Vol. 23. — № 9. — P. 2223–2234.

8 Oza A. M. et al. Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomized phase 2 trial // Lancet Oncol. — 2015. — Vol. 16. — № 1. — P. 87–89

9 Savets V.V. [and others] Final analysis of the OVATAR non-interventional study: diagnostic and therapeutic approaches to the treatment of ovarian cancer in Russia. Analysis of a group with BRCA mutations // Journal of Malignant Tumors. – 2019. – vol. 9. – No.3 S1. – pp. 90-91.

Contraindications for use: none were identified if used by well-trained personnel and taken into account the intended use.

Population and demographic aspects of the reagent kit usage:

According to the clinical guidelines " Breast cancer", Age group — patients older 18 years old, approved by the Ministry of Health of the Russian Federation, 2018:

"It is recommended to detect the most common germinal mutations in the BRCA1/2 genes by polymerase chain reaction in lymphocytes and to consult a geneticist for treatment tactics determination in the following cases:

- in women with confirmed breast cancer with a burdened family history (presence of breast cancer in close relatives of the ≤50 years old, ovarian or fallopian tube cancer, pancreatic cancer, male breast cancer, metastatic prostate cancer);
- in women with confirmed breast cancer aged <45 years old;
- in women < 60 years old with triple negative breast cancer phenotype;
- for premier multifocal breast cancer (including but not limited by established contralateral breast cancer, ovarian or fallopian tube cancer, pancreatic cancer);
- for breast cancer in men.

Comment: female patients having personal/hereditary history who do not have frequent hereditary mutations should be referred for an extended germline and/or somatic mutation testing using high-throughput sequencing (NGS)."

- According to the clinical guidelines "Ovarian cancer/fallopian tube cancer primary peritoneal cancer", Age group — patients over 18 years old, approved by the Ministry of Health of the Russian Federation, 2018:

"All female patients with high-grade serous and endometrial carcinomas are recommended to have molecular genetic testing of mutations in the BRCA1 and BRCA2 genes in blood or oral mucous membrane scraping and/or in biopsy (surgical) material as predictors of the disease outcome and the choice of the patient's treatment strategy.

Comment: mutation frequency in the BRCA1 and BRCA2 genes in the mentioned above types of tumor is 15%. Information about the BRCA gene mutations is useful for determination whether a tumor is more sensitive to therapy with alkylating drugs, platinum derivatives, and PARP inhibitors."

1. Intended use

Intended use: Quasar-BRCA1/2 reagent kit is designed for qualitative determination of the BRCA1 and BRCA2 genes mutation status associated with ovarian cancer and breast cancer risk factors by mass parallel sequencing in human genomic DNA sample isolated from clinical material (whole blood, tissue samples fixed in 10% formalin solution and embedded in a paraffin block (FFPE-block)) using Illumina platforms for breast cancer and ovarian cancer screening in potentially healthy women and in patients with diagnosed breast cancer and ovarian cancer in order to determine an effective treatment strategy and to predict the treatment effectiveness.

Functional use: results obtained by high-throughput DNA libraries sequencing prepared with Quasar-BRCA1/2 reagent kit after subsequent bioinformatic analysis can be used for screening of hereditary breast cancer types and ovarian cancer in potentially healthy women and in testing patients diagnosed with breast cancer and ovarian cancer in order to determine an effective treatment strategy and to predict the treatment effectiveness.

Reagent kit potential consumers: reagent kit for research use only.

2. Method principle

Method

Real-time multiplex polymerase chain reaction with subsequent indexing of amplicons and obtaining DNA libraries for high-throughput parallel sequencing using Illumina MiSeq.

Wrench time ofthe DNA libraries sample preparation protocol for sequencing is about 3 hours without operator's work time. Sequencing wrench time using Illumina MiSeq and Illumina MiSeq Reagent Kit v3 (600-cycles) MS-102-3003 in 2x150 bp read length is about 30 hours.

Test sample type

PCR test material is human genomic DNA samples isolated from clinical material (whole blood, formalin-fixed and paraffin-embedded tissue (FFPE-block)).

Method Principle

The assay includes the following stages:

1. Target multiplex DNA PCR amplification with possible real-time fluorescence detection of amplification products;
2. Target amplicons purification;
3. Amplicon indexing using PCR amplification with limited cycles;
4. Target DNA libraries mixing and purification;
5. Mass parallel sequencing using Illumina MiSeq.
6. Results interpretation.

Multiplex amplification reactions are performed with DNA samples to amplify gene regions using primers specific to these DNA regions in a reaction buffer.

The PCR-buffer contains all the main reagents including thermostable hot-start DNA polymerase, dNTP, intercalating dye and optimized buffer. The primer-mix contains specific primers for a multiplex PCR.

After purification with magnetic beads, specific amplification products undergo indexing with adding specific for sequencing adapters on the Illumina platform.

Method limitations

Contamination during the DNA isolation or during multiplex and index PCR reaction stages can be a possible reason for obtaining a non–specific result. A non-specific result can be detected using a positive control sample.

Damaged packaging during transportation.

Using an expired kit or kit storage conditions violation.

Storage conditions violation during samples transportation.

3. Reagent Kit Components

Configuration forms

Quasar-BRCA1/2 reagent kit is designed in three configuration forms:

- Quasar-BRCA1/2-96A;
- Quasar-BRCA1/2-96B;
- Quasar-BRCA1/2-48V.

Test samples number

The reagent kit is designed for a single use only.

Quasar-BRCA1/2-96A and Quasar-BRCA1/2-96B reagent kit configuration forms are designed for 96 reactions of each BRCA1/2 multiplex. It corresponds to detection of 94 test samples, a negative and a positive control samples during a simultaneous installation in a cycler and a single run of the Illumina MiSeq sequencer.

Quasar-BRCA1/2-48V reagent kit configuration form is designed for 48 reactions. It corresponds to detection of 46 test samples, a negative and a positive control samples during a single run of amplification reaction and a single run of the Illumina MiSeq sequencer.

Possibility of multiplexing samples for sequencing

Reagent kit configuration forms Quasar-BRCA1/2-96A, Quasar-BRCA1/2-96B, Quasar-BRCA1/2-48V differ by index plates A, B, V for double samples sequencing:

- Index plate A for 96 samples;
- Index plate B for 96 samples;
- Index plate V for 48 samples.

Indexes in the plates A and B are not repeated. It allows to conduct simultaneous sample preparation and sequencing of up to 192 samples during a single run of the Illumina MiSeq sequencer (including negative and positive control samples). The Index plate B is a half of the Index plate A. The layout of the unique index pairs in the index plates is shown in Annex 2.

The reagent kit components

Table 1 — Quasar-BRCA1/2-96A configuration form

No	Reagent	Description	Quantity, Volume
Packaging No. 1			
1	PCR-buffer-1	Transparent colorless liquid in a tube with a lilac lid	1 tube, 384 µl
2	PCR-buffer-2	Transparent colorless liquid in a tube with an orange lid	1 tube, 960 µl
3	Primer-mix-QB	Transparent liquid with a yellow shade in a tube with a colorless lid	1 tube, 384 µl
4	PC	Transparent colorless liquid in a tube with a red lid	1 tube, 24 µl
5	NC	Transparent colorless liquid in a tube with a blue lid	1 tube, 24 µl
6	Index plate A for 96 samples	Polymer 96-well plate with transparent colorless liquid in each well	1 plate, 30 µl in each well
Packaging No. 2			
1	Beads-1	A brown suspension in a colorless matte bottle with a lid	1 bottle, 3840 µl
2	Beads-2	A brown suspension in a tube with a brown lid	1 tube, 52 µl

3	Diluent-1	Transparent colorless liquid in a colorless matte bottle with a lid	1 bottle, 1920 µl
4	Diluent-2	Transparent colorless liquid in a tube with a yellow lid	1 tube, 40 µl

Table 2 — Quasar-BRCA1/2-96B configuration form

No	Reagent	Description	Quantity, Volume
Packaging No. 1			
1	PCR-buffer-1	Transparent colorless liquid in a tube with a lilac lid	1 tube, 384 µl
2	PCR-buffer-2	Transparent colorless liquid in a tube with an orange lid	1 tube, 960 µl
3	Primer-mix-QB	Transparent liquid with a yellow shade in a tube with a colorless lid	1 tube, 384 µl
4	PC	Transparent colorless liquid in a tube with a red lid	1 tube, 24 µl
5	NC	Transparent colorless liquid in a tube with a blue lid	1 tube, 24 µl
6	Index plate B for 96 samples	Polymer 96-well plate with transparent colorless liquid in each well	1 plate, 30 µl in each well

Packaging No. 2			
1	Beads-1	A brown suspension in a colorless matte bottle with a lid	1 bottle, 3840 µl
2	Beads-2	A brown suspension in a tube with a brown lid	1 tube, 52 µl
3	Diluent-1	Transparent colorless liquid in a colorless matte bottle with a lid	1 bottle, 1920 µl
4	Diluent-2	Transparent colorless liquid in a tube with a yellow lid	1 tube, 40 µl

Table 3 — Quasar-BRCA1/2-48V configuration form

No	Reagent	Description	Quantity, Volume
Packaging No. 1			
1	PCR-buffer-1	Transparent colorless liquid in a tube with a lilac lid	1 tube, 192 µl
2	PCR-buffer-2	Transparent colorless liquid in a tube with an orange lid	1 tube, 480 µl
3	Primer-mix-QB	Transparent colorless liquid with a yellow shade in a tube with a colorless lid	1 tube, 192 µl
4	PC	Transparent colorless liquid in a tube with a red lid	1 tube, 24 µl

5	NC	Transparent colorless liquid in a tube with a blue lid	1 tube, 24 µl
6	Index plate V for 48 samples	A polymer 96-well plate, 48 wells with a transparent colorless liquid, and the other 48 wells are empty	1 plate, 30 µl in each well
Packaging No. 2			
1	Beads-1	A brown suspension in a colorless matte bottle with a lid	1 bottle, 1920 µl
2	Beads-2	A brown suspension in a tube with a brown lid	1 tube, 52 µl
3	Diluent-1	Transparent colorless liquid in a colorless matte bottle with a lid	1 bottle, 960 µl
4	Diluent-2	Transparent colorless liquid in a tube with a yellow lid	1 tube, 40 µl

PCR buffer-1 and PCR buffer-2 are ready for use and contain all the main reagents, including thermostable DNA hot start polymerase deoxynucleotide triphosphates and an optimized buffer that allows to perform a PCR amplification. **PCR-buffer-1** is used in the PCR mixtures preparation area, **PCR-buffer-2** is used in the area of work with amplicons.

Primer-mix-QB is ready for use and contains a multiplex primer mixture for the BRCA1 and BRCA2 genes regions amplification.

Positive control sample (PC) is ready for use and is the Jurkat cell-line purified genomic DNA.

Negative control sample (NC) is ready for use and is DNase-free deionized water.

Index plates A, B, and V are ready for use and are oligonucleotides that contain short-sequences of 8 bp (i7/i5) used for unique labeling of each DNA fragment in NGS library. Combinatorial dual sequencing is used in the Index plates A, B, and V — the indexes repeat in rows and columns (see Figure 1, 2). The combination of indexes i7 and i5 is unique for each initial DNA sample. Implementation of these indexes allows simultaneous sequencing of multiple samples in one run of a device.

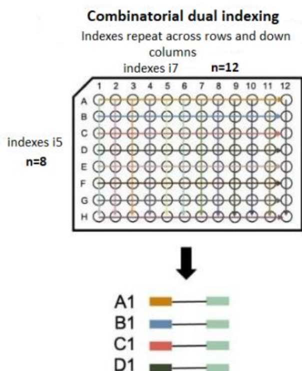


Figure 1 — combinatorial dual indexing used in Index plates A and B for 96 samples

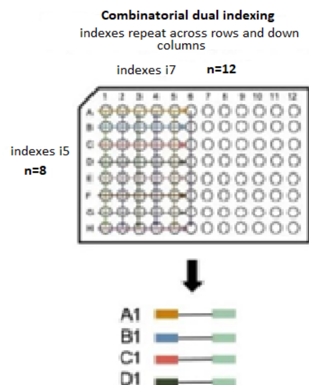


Figure 2 — combinatorial dual indexing used in the Index plate V for 48 samples

The index sequences i7 and i5 are shown in Annex 1. The layout of unique index pairs in index plates A, B and V is shown in Annex 2.

Beads-1 and Beads-2 are two-component suspensions of magnetic beads in a buffer and are designed to purify DNA from reaction mixtures.

ATTENTION: store the magnetic beads in a fridge at +4°C, **do not freeze!**

Diluent-1 and Diluent-2 are diluents for **Beads-1**, **Beads-2** and are deionized MilliQ water.

The kit contains no substances for medical use, substances of human or animal origin.

The Illumina MiSeq Reagent Kit v3 (600-cycles) MS-102-3003 (Illumina, USA) is required for sequencing.

4. Reagent kit characteristics

4.1 Technical and functional characteristics

Table 4

Indicator	Characteristics and standards	
1.1. Technical characteristics		
Reagent	**Appearance**	**Quantity, volume, µl (±5%)**
Quasar-BRCA1/2-96A configuration form		
Packaging No. 1		
PCR-buffer-1	Transparent colorless liquid in a tube with a lilac lid	1 tube, 384 µl
PCR-buffer-2	Transparent colorless liquid in a tube with an orange lid	1 tube, 960 µl
Primer-mix-QB	Transparent liquid with a yellow shade in a tube with a colorless lid	1 tube, 384 µl
PC	Transparent colorless liquid in a tube with a red lid	1 tube, 24 µl
NC	Transparent colorless liquid in a tube with a blue lid	1 tube, 24 µl
Index plate A for 96 samples	96-well plate with transparent colorless liquid in each well	1 plate, 30 µl in each well
Packaging No. 2		

Beads-1	A brown suspension in a colorless matte bottle with a lid	1 bottle, 3840 µl
Beads-2	A brown suspension in a tube with a brown lid	1 tube, 52 µl
Diluent-1	Transparent colorless liquid in a colorless matte bottle with a lid	1 bottle, 1920 µl
Diluent-2	Transparent colorless liquid in a tube with a yellow lid lid	1 tube, 40 µl
Quasar-BRCA1/2-96B reagent kit configuration form		
Packaging No. 1		
PCR-buffer-1	Transparent colorless liquid in a tube with a lilac lid	1 tube, 384 µl
PCR-buffer-2	Transparent colorless liquid in a tube with an orange lid	1 tube, 960 µl
Primer-mix-QB	Transparent liquid with a yellow shade in a tube with a colorless lid	1 tube, 384 µl
PC	Transparent colorless liquid in a tube with a red lid	1 tube, 24 µl
NC	Transparent colorless liquid in a tube with a blue lid	1 tube, 24 µl
Index plate B for 96 samples	Polymer 96-well plate with transparent colorless liquid in each well	1 plate, 30 µl in each well
Packaging No. 2		

Beads-1	A brown suspension in a colorless matte bottle with a lid	1 bottle, 3840 µl
Beads-2	A brown suspension in a tube with a brown lid	1 tube, 52 µl
Diluent-1	Transparent colorless liquid in a colorless matte bottle with a lid	1 bottle, 1920 µl
Diluent-2	Transparent colorless liquid in a tube with a yellow lid	1 tube, 40 µl
Quasar-BRCA1/2-48V reagent kit configuration form		
Packaging No. 1		
Indicator	**Characteristics and standards**	
PCR-buffer-1	Transparent colorless liquid in a tube with a lilac lid	1 tube, 192 µl
PCR-buffer-2	Transparent colorless liquid in a tube with an orange lid	1 tube, 480 µl
Primer-mix-QB	Transparent liquid with a yellow shade in a tube with a colorless lid	1 tube, 192 µl
PC	Transparent colorless liquid in a tube with a red lid	1 tube, 24 µl
NC	Transparent colorless liquid in a tube with a blue lid	1 tube, 24 µl

Index plate V for 48 samples	A polymer 96-well plate, 48 wells with a transparent colorless liquid, and the other 48 wells are empty	1 plate, 30 µl in each well
Packaging No. 2		
Beads-1	A brown suspension in a colorless matte bottle with a lid	1 bottle, 1920 µl
Beads-2	A brown suspension in a tube with a brown lid	1 tube, 52 µl
Diluent-1	Transparent colorless liquid in a colorless matte bottle with a lid	1 bottle, 960 µl
Diluent-2	Transparent colorless liquid in a tube with a yellow lid	1 tube, 40 µl
1.2. Completeness	Clause 1.4 TS	
1.3. Marking	Clause 4 TS	
1.4. Packaging	Clause 5 TS	
2. Functional characteristics		
Amplification control	In tubes 1-24 with PC: fluorescence increases, amplification does not exceed the exponential phase. In tubes 25-28 with reaction mixture "P-": there is no fluorescence signal. In the tube 29 with NC: fluorescence increases, amplification does not exceed exponential phase.	
Purification and indexing control	In tubes 1-192 with PC: fluorescence increases. Specific convex arc-shaped fluorescence accumulation curve. In tube 193 with NC: fluorescence increases. Specific convex arc-shaped fluorescence accumulation curve.	

DNA-library purification control	Purified DNA-libraries concentration is higher than 0.92 ng/µl and fluorescence increases in all 4 amplification samples.
DNA-libraries sequencing control	VCF file does not contain ALT genotypes

In case of the reagent kit failure, functional deviations that may affect the kit safety or the kit analytical characteristics — immediately stop using the kit and the manufacturer (see section 13 of the Instruction).

Metrological traceability of the control sample – PC

PC metrological traceability is confirmed by a spectrophotometric method by checking the Jurkat stock solution concentration (manufactured by Thermo Fisher Scientific, USA) which contains in the PC in 1 ng/µl concentration.

Subsequent targeted multiplex PCR conduction and DNA-libraries sequencing using Illumina MiSeq have confirmed that PC ensures stable work of the Quasar-BRCA1/2 Reagent Kit. PC is the Jurkat cell-line human genomic DNA in 1 ng/µl concentration in TE-buffer (10 mM Tris, 1 mM EDTA).

4.2 Analytical efficiency characteristics

4.2.1 Analytical specificity

Specificity for mutations in the BRCA1 (c.5266dupC, c.181T>G, c.5251C>T, c.4035delA, c.5161C>T, c.4675G>A, c.68_69del, c.3700_3704del, c.1961delA, c.4689C>G, c.3756_3759del), BRCA2BRCA2 (c.3749dupA, c.961_962insAA, c.2897_2898del, c.8754+1G>A, c.6174delT) genes has been proven using clinical samples with positive mutations in the *BRCA1, BRCA2* genes (tissue fixed in 10% formalin solution and embedded in a paraffin block (FFPE- block), whole blood), no nonspecific reactions were detected.

4.2.2 Analytical sensitivity

300 BRCA1, BRCA2 genes copies in 1 µl of DNA solution

4.2.3 The minimum tumor amount for analysis is 20% according to the results of tumor tissue morphological examination by a histotechnologist.

4.2.4 Average read depth for the test samples and PC is considered sufficient if it exceeds the 150x value for germline mutations and 500x value for somatic mutations.

4.2.4 Precision under reproducibility conditions

Precision evaluation under repeatability conditions was carried out using positive and negative clinical samples (tissue fixed in 10% formalin solution and embedded in a paraffin block (FFPE-block) and whole blood) depending on the presence of mutations in the BRCA1, BRCA2 genes by the Quasar-BRCA1/2 reagent kit 3 configuration forms.

To assess accuracy under repeatability conditions clinical samples were examined in 10 repetitions.

Repeatability data were obtained within one laboratory for specific equipment and within one reagent kit batch. False positive and false negative results were not obtained. Precision under repeatability conditions is 100%.

4.2.5 Precision under reproducibility conditions

Precision evaluation under reproducibility conditions was carried out using positive and negative clinical samples (tissue fixed in 10% formalin solution and embedded in a paraffin block (FFPE-block) and whole blood) depending on the presence of mutations in the BRCA1, BRCA2 genes by the Quasar-BRCA1/2 reagent kit in 3 configuration forms.

The test system reproducibility assessment was carried out similarly to the accuracy under repeatability conditions calculation. However, different batches of the reagent kit are used for testing and testings are carried out in different laboratories, by different operators, on different days, via different PCR cyclers (Reproducibility test Block 1, Reproducibility test Block 2, Reproducibility test Block 3, Reproducibility test Block 4).

When conducting precision testing under reproducibility conditions false positive and false negative results were not obtained. Precision under reproducibility conditions is 100%.

4.2.6 Interfering substances effect

Assay results on the interfering substances effects evaluation are described in Section 8.3 of the Instruction.

4.3. Clinical effectiveness

241 samples were selected during clinical trials: 140 whole blood samples and 101 samples of tissue fixed in 10% formalin solution and embedded in a paraffin block (FFPE-block) of potentially healthy women with personal/hereditary history and patients diagnosed with breast and ovarian cancer.

Cyclers recommended by the reagent kit manufacturer that were

used to perform target multiplex DNA PCR amplification:

- DTprime (DNA-Technology LLC, Russian Federation, registration certificate № FSR 2011/10229 dated 03.03.2011);
- CFX 96 (Bio-Rad, USA, registration certificate № FSZ 2008/03399 dated 21.06.2016);
- Rotor-Gene Q (Qiagen, Germany, registration certificate № FSZ 2010/07595 dated 10.08.2010);
- QuantStudio 5 (Thermo Fisher Scientific, USA, registration certificate № RZN 2019/8446 dated 06.06.2019).

Results reproducibility for all cyclers is 100%.

Every sample was tested in two rounds with each complectation form of the Quasar-BRCA1/2 reagent kit manufactured by TestGene LLC for inter-lot repeatability evaluation.

The reagent kit quality, safety and efficiency were examined using tissue samples fixed in 10% formalin solution and embedded in a paraffin block (FFPE-block) — in 202 tests, using whole blood — in 280 tests.

Complete intra-state, inter-stage and inter-series reproducibility was observed under reproducibility conditions.

Table 5. Diagnostic characteristics of the Quasar-BRCA1/2 reagent kit in relation to each tested clinical material

Test sample type	Positive samples observation Number	Negative samples observation number	Diagnostic sensitivity with 95% confidence probability	Diagnostic specificity with 95% confidence probability
Whole blood	206	74	100% (95% diagnostic interval: 98,23%-100%)	100% (95% diagnostic interval: 95,14%-100%)
10% formalin-fixed paraffin-embedded tissue (FFPE-block)	142	60	100% (95% diagnostic interval: 97,44%-100%)	100% (95% diagnostic interval:94.04%-100%)

Table 6. Quasar-BRCA1/2 reagent kit diagnostic characteristics in relation to each studied analyte

Test sample type	Studied analyte	Positive samples observation number	Negative samples observation number	Diagnostic sensitivity with 95% confidence probability	Diagnostic specificity with 95% confidence probability
10% formalin-fixed paraffin-embedded tissue (FFPE-block)	*BRCA1* c.5266dupC	30	172	100% (95% diagnostic interval: 88,43%-100%)	100% (95% diagnostic interval: 97,88%-100%)
	BRCA1 c.5251C>T	4	198	100% (95% diagnostic interval: 39,76%-100%)	100% (95% diagnostic interval: 98,15%-100%)
	BRCA1 c.4035delA	16	186	100% (95% diagnostic interval: 79,41%-100%)	100% (95% diagnostic interval: 98,04%-100%)
	BRCA2 c.3749dupA	4	198	100% (95% diagnostic interval: 39,76%-100%)	100% (95% diagnostic interval: 98,15%-100%)
	BRCA2 c.961_962insAA	2	200	100% (95% diagnostic interval: 15,81%-100%)	100% (95% diagnostic interval: 98,17%-100%)
	BRCA1 c.68_69del	10	192	100% (95% diagnostic interval: 69,15%-100%)	100% (95% diagnostic interval: 98,10%-100%)
	BRCA2 c.8754+1G>A	2	200	100% (95% diagnostic interval: 15,81%-100%)	100% (95% diagnostic interval: 98,17%-100%)
	BRCA1 c.3756_3759del	10	192	100% (95% diagnostic interval: 69,15%-100%)	100% (95% diagnostic interval: 98,10%-100%)
	BRCA1 c.181T>G	7	188	100% (95% diagnostic interval: 59,04%-100%)	100% (95% diagnostic interval: 98,06%-100%)

	BRCA1 c.5161C>T	4	198	100% (95% diagnostic interval: 39,76%-100%)	100% (95% diagnostic interval: 98,15%-100%)
	BRCA1 c.1961delA	14	188	100% (95% diagnostic interval: 76,84%-100%)	100% (95% diagnostic interval: 98,06%-100%)
	BRCA1 c.4675G>A	4	198	100% (95% diagnostic interval: 39,76%-100%)	100% (95% diagnostic interval: 98,15%-100%)
Whole blood	*BRCA2* c.2897_2898 del	2	200	100% (95% diagnostic interval: 15,81%-100%)	100% (95% diagnostic interval: 98,17%-100%)
	BRCA1 c.3700_3704 del	12	190	100% (95% diagnostic interval: 73,54%-100%)	100% (95% diagnostic interval: 98,08%-100%)
	BRCA1 c.4689C>G	2	200	100% (95% diagnostic interval: 15,81%-100%)	100% (95% diagnostic interval: 98,17%-100%)
	BRCA2 c.6174delT	12	190	100% (95% diagnostic interval: 73,54%-100%)	100% (95% diagnostic interval: 98,08%-100%)
	BRCA1 c.5266dupC	46	234	100% (95% diagnostic interval: 92,29%-100%)	100% (95% diagnostic interval: 98,44%-100%)
	BRCA1 c.5251C>T	6	274	100% (95% diagnostic interval: 54,07%-100%)	100% (95% diagnostic interval: 98,66%-100%)
	BRCA1 c.4035delA	30	250	100% (95% diagnostic interval: 88,43%-100%)	100% (95% diagnostic interval: 98,54%-100%)
	BRCA2 c.3749dupA	4	276	100% (95% diagnostic interval: 39,76%-100%)	100% (95% diagnostic interval: 98,67%-100%)

	BRCA2 c.961_962ins AA	4	276	100% (95% diagnostic interval: 39,76%-100%)	100% (95% diagnostic interval: 98,67%-100%)
	BRCA1 c.68_69del	18	262	100% (95% diagnostic interval: 81,47%-100%)	100% (95% diagnostic interval: 98,60%-100%)
	BRCA2 c.8754+1G>A	4	276	100% (95% diagnostic interval: 39,76%-100%)	100% (95% diagnostic interval: 98,67%-100%)
	BRCA1 c.3756_3759 del	10	270	100% (95% diagnostic interval: 69,15%-100%)	100% (95% diagnostic interval: 98,64%-100%)
	BRCA1 c.181T>G	24	256	100% (95% diagnostic interval: 85,75%-100%)	100% (95% diagnostic interval: 98,57%-100%)
	BRCA1 c.5161C>T	6	274	100% (95% diagnostic interval: 54,07%-100%)	100% (95% diagnostic interval: 98,66%-100%)
	BRCA1 c.1961delA	14	266	100% (95% diagnostic interval: 76,84%-100%)	100% (95% diagnostic interval: 98,62%-100%)
	BRCA1 c.4675G>A	4	276	100% (95% diagnostic interval: 39,76%-100%)	100% (95% diagnostic interval: 98,67%-100%)
	BRCA2 c.2897_2898 del	6	274	100% (95% diagnostic interval: 54,07%-100%)	100% (95% diagnostic interval: 98,66%-100%)
	BRCA1 c.3700_3704 del	14	266	100% (95% diagnostic interval: 76,84%-100%)	100% (95% diagnostic interval: 98,62%-100%)
	BRCA1 c.4689C>G	4	276	100% (95% diagnostic interval: 39,76%-100%)	100% (95% diagnostic interval: 98,67%-100%)

	BRCA2 c.6174delT	12	268	100% (95% diagnostic interval: 73,54%-100%)	100% (95% diagnostic interval: 98,63%-100%)

5. Risks associated with the reagent kit usage

The risk zone includes the following hazards:

1.The kit reagents functional properties loss due to transportation, storage or usage under inappropriate conditions;

2. Reaction mixtures and test NDA samples contamination with contents from a PCR tube or with PCR products;

3. Testing with a poor-quality DNA sample (low concentration and/or poor purification);

4. Failure to comply with the requirements for sample preparation, analysis and disposal due to unqualified personnel work;

5. Usage of an unusable reagent kit (after the expiration date or in case of damaged packaging).

No risks have been identified in the risk zone area.

Total residual risk of using the Quasar-BRCA1/2 reagent kit for determining the mutation status of BRCA1 and BRCA2 genes by mass parallel sequencing in human genomic DNA sample according to TS 21.20.23-038-97638376-2020 is acceptable; the benefit of its usage exceeds the risk.

6. Safety precautions

Potential risk class — 2b — in accordance with Nomenclature Classification of Medical Devices approved by the Order of the Ministry of Health of the Russian Federation No.4n dated June 6, 2012.

All components and reagents included in the Quasar-BRCA1/2 reagent kit belong to hazard class 4 (low-hazard substances) in accordance with GOST 12.1.007-76 "OSSS. Harmful substances. Classification general safety requirements".

The reagents included in the Quasar-BRCA1/2 reagent kit have low vapor pressure and exclude the possibility of inhalation poisoning.

The reagents included in the Quasar-BRCA1/2 reagent kit are non-toxic, as they are prepared by mixing separate non-toxic components.

Infected or suspected of being infected material should be handled in accordance with SanPiN 3.3686-21 "Sanitary Epidemiological requirements for the Prevention of Infectious Diseases", MU "Work organization of laboratories using nucleic acid amplification methods

when working with material containing microorganisms of patches of groups I - IV" (MU 1.3.2569-09).

Personnel should ensure and comply with the biological safety rules and work requirements for the organization and conduct it in order to prevent contamination with nucleic acids and (or) amplicons of the tested samples, premises and equipment.

The work should be carried out in a laboratory performing molecular-biological (PCR) testing of clinical material in accordance with SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures". Follow the recommendations laid out in MU 287-113, MU 1.3.2569-09.

The following requirements should always be met when working:

1. remove unused reagents in accordance with SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures";

ATTENTION! When removing waste after amplification (tubes containing PCR products), it is not allowed to open the tubes and spill the contents, as this may lead to contamination of a laboratory area, equipment and reagents with PCR products.

1. use the kit strictly for its intended purpose, according to this instruction;

2. do not use the kit after the expiration date or if the packaging is violated;

3. only specially trained personnel are allowed to work with the kit (a specialist with higher medical education who has been trained in licensed qualification courses to conduct PCR diagnostics and who can work with the Illumina MiSeq sequencer (Illumina, USA) with installed GenerateFastq module (registration certificate No RZN 2014/1568 dated 29.04.2014);

4. avoid contact with skin, eyes and mucous membrane. In case of contact, immediately flush the affected area with water and seek medical assistance.

The necessary precautions regarding the influence of magnetic

fields, external electrical influences, electrostatic discharges, pressure or pressure changes, overload, sources of thermal ignition are not provided.

The kit contains no substances of human or animal origin with a potential infectious nature, therefore, precautions against any special, unusual risks during product use or sale are not provided.

7. Required equipment and materials

Work with the Quasar-BRCA1/2 reagent kit is carried out in 2 working areas.

Specific PCR is conducted in working area 3a. The next stages till "Libraries mixing" are conducted in working area 3b.

It is allowed to carry out the work in a single zone with at least two separate class II or III biosafety cabinets: the first one for conducting specific PCR and the second for the other sample preparation stages including "Libraries mixing".

Library sequencing is carried out in working areas 4-2 (to account the results (detection) of nucleic acid amplification products by sequencing) (MU 1.3.2569-09).

Equipment:

1. Class II and III biological safety PCR cabinet;
2. Vortex centrifuge, 6000 rpm;
3. A set of electronic or automatic variable volume dispensers;
4. Refrigerator for 2°C... 8°C with a freezer for less than -16 °C;
5. Cycler with real-time fluorescence detection via channels corresponding to the FAM/Green: CFX96 («Bio-Rad», USA, registration certificate № FSZ 2008/03399 dated 21.06.2016), Dtprime (DNA-Technology LLC, Russian Federation, registration certificate № FSR 2011/10229 dated 03.03.2011). Rotor-Gene Q (Qiagen, Germany, registration certificate № FSZ 2010/07595 dated 10.08.2010); QuantStudio 5 (Thermo Fisher Scientific, USA, registration certificate № RZN 2019/8446 dated 06.06.2019).
6. Illumina MiSeq system (Illumina, USA) with installed GenerateFastq software module (Registration certificate No. RZN 2014/1568 dated 29.04.2014).
7. To measure the concentration of the tested DNA samples right before the assay start — Spectrometer (manufactured by Thermo Fisher Scientific, USA) with 220-350 nm emission spectra.

Included into the National Register of Measuring Instruments, has an Approval Certificate for Measuring Instruments US.C.31.004.A №53590.

Materials and reagents not included in the kit:

ATTENTION! It is required to use only disposable sterile plastic consumables that have a special "DNase-free" label when working with DNA.

1. Disposable tips with an aerosol barrier up to 1,000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA);
2. 1.5ml disposable Eppendorf type sterile tubes;
3. Thin-walled disposable tubes with an optically transparent lid (if detecting through a lid) or with optically transparent walls (if detecting through a tube wall): 0.2 ml PCR tubes or 0.2ml PCR tubes in strips or PCR plates with an optically transparent film, compatible with the used cycler;
4. Lab coat and disposable talc-free gloves;
5. Container with disinfectant;
6. Test tube rack for 0.2ml tubes or for 0.2ml tube strips or PCR plates with 0.2 ml well volume;
7. Magnetic stand for 0.2ml tubes or magnetic stand for PCR plate with 96 wells;
8. Ethanol 96%.
9. Illumina MiSeq Reagent Kit v3 (600-cycles) MS-102-3003 (Illumina, USA), registration certificate No 2020/13097 dated 30.12.2020.
10. 2M NaOH solution
11. Deionized mQ water
12. Reagent kit for human DNA isolation from clinical material (see Section 8.2).

8. Test samples

Test sample type

PCR test material is human genomic DNA samples isolated from clinical material (whole blood, formalin-fixed paraffin-embedded tissue (FFPE-block)).

8.1 Clinical material collection procedure

ATTENTION! Before starting work, it is required to study the Methodological recommendations "Sampling, Transportation and Storage of Clinical Material for PCR Diagnostics", developed by the

FBIS Central Research Institute of Rospotrebnadzor, Moscow, 2012.

8.1.1 Material collection for testing

Whole blood.

Sample blood on an empty stomach or after three hours after eating from the basilic vein with a disposable needle (0.8-1.1 mm in diameter) into a special vacuum system (lilac lids — 6% EDTA-K2) or by a disposable syringe into plastic tubes with sodium citrate (3,8% sodium citrate solution in a 1:9 ratio). Close the test tube with a lid and carefully turn it upside down several times to thoroughly mix blood with anticoagulant (otherwise the blood will clot and it will be impossible to isolate a DNA).

Using heparin as a coagulant is prohibited!

The initial biological material transportation, storage and disposal conditions:

Whole blood samples

- at +2°C...+8°C — up to 24 hours.
- at -18°C...-22°C — up to 6 months.

It is allowed to freeze material once if it cannot be delivered to a laboratory.

ATTENTION! Avoid repeated freezing and thawing of samples.

8.1.2 Biopsy and/or surgical specimen.

The material is sampled from a pathological lesion: from its central part and from the part bordering with unchanged tissues. The sampled material is placed in a container with 10% neutral formalin solution. Laboratory processing of biological material is performed after fixation, and includes the following procedures — impregnation (dehydration and impregnation with paraffin); embedding in paraffin and paraffin blocks (FFPE-blocks) preparation; microtomy (paraffin sectioning).

Histological preparations suitability criteria for DNA isolation for tumor cells subsequent molecular genetic analysis:

1. According to the morphological analysis results tumor zones should occupy at least 20% of the tissue in a FFPE slide;
2. According to the morphological analysis results hemorrhage and necrosis areas should occupy not more than 15% of the tissue in a FFPE slide;

If the sample does not meet at least one of the listed criteria, it is

recommended to use another sample.

When preparing paraffin slides, it is necessary to minimize the samples cross-contamination risk. For that:

- work in disposable talc-free gloves;
- perform the procedure in a PCR cabinet or in a laminar flow cabinet
- use disposable microtome blades and sterile tweezers;
- dispose the first two slides of each block, use slides starting from the third one for molecular research;
- do not place the slices in a water bath.

The initial biological material transportation, storage and disposal conditions:

- at room temperature — during 6 hours;
- at 2°C...8°C — for 3 days;
- at -20°C — for 1 week;
- at -70°C — for a long time.

ATTENTION! Avoid repeated freezing and thawing of samples.

FFPE-blocks transportation and storage conditions:

at 15°C... 25°C — not more than 3 years.

Disposal of clinical material (Class B) is carried out in accordance with SanPiN 2.1.3684-21.

8.2 Human DNA extraction from biological material (whole blood, 10% formalin-fixed and paraffin-embedded tissue (FFPE-block))

To isolate human genomic DNA from tissue samples fixed in 10% neutral formalin solution and embedded in a paraffin block — FFPE-block, it is recommended to use the following reagent kits:

- Reagent kit for human genomic DNA isolation from formalin-fixed and paraffin-embedded tissues (DNA-Tissue-M) according to TS 21.20.23-012-97638376-2019, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2021/14273 dated 06.05.2021);
- Reagent kit for human genomic DNA isolation from formalin-fixed and paraffin-embedded tissues (DNA-Tissue-F) according to TS 21.20.23-009-97638376-2016, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2018/7772 dated 10.30.2018).

To isolate human genomic DNA it is recommended to use the following reagent kits:

- Reagent kit for DNA/RNA isolation from clinical material "NA-

Extra" according to TS 21.20.23-013-97638376-2019, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2021/15428 dated 24.09.2021);

It is necessary to strictly follow the protocol and the instructions of the used reagent kit during the DNA isolation procedure.

Conditions for DNA test samples possible storage

- at 2°C... 8°C — up to 24 hours,
- at -18°C... -22°C — up to 1 month,
- at -80°C — for a long time.

8.3 Interfering substances and restrictions on the test material use

The potentially interfering substances effect on the Quasar-BRCA1/2 reagent kit performance has been examined for potentially interfering substances that may originate from the following external and internal sources:

1) substances used in patient treatment (e.g., medicines);

2) substances found in specific sample types — in this case clinical sample contamination with hemoglobin can inhibit a PCR if not sufficiently purified during the DNA isolation;

3) substances added during sample preparation — i.e. anticoagulants or paraffin, which is used for a FFPE block preparation.

Interfering substances concentrations that are expected to be found during the Quasar-BRCA1/2a reagent kit normal use are shown in Table 7.

Table 7.

Interfering substances	Maximum concentration
Endogenous interfering substances	
Hemoglobin	260 µl/ml
Exogenous interfering substances	
Substances added during sample preparation	
Heparin (anticoagulant)	0.15 IU/ml
Sodium citrate (anticoagulant)	0.1 mM/ml
EDTA-K2 (anticoagulant)	0.5 mM/ml
Paraffin	$1*10^{-4}$ µl/µl
Cancer treatment drugs	

Ropivacaine (painkiller)	0.02 mg/ml
Bevacizumab (used for ovarian cancer and breast cancer treatment)	0.02 mg/ml
Paclitaxel (ovarian cancer and breast cancer prevention and treatment)	0.006 mg/ml
Capecitabine (breast cancer treatment drug)	0.03 mg/ml

Based on the study results heparin (anticoagulant) in 0.15 IU/ml concentration was classified as a PCR inhibitor. It is not allowed to use heparin as anticoagulant while sampling peripheral blood.

To reduce the PCR inhibitors number, it is necessary to follow the rules for collection clinical material.

Limitations on test material usage:

4.2.3 Minimal tumor amount for analysis is 20% according to the results of tumor tissue morphological examination by a histotechnologist.

- it is not allowed to use test material under storage and transportation conditions violation (temperature, duration);
- it is not allowed to use samples contaminated with extraneous biological material.
- heparin is not allowed to be used as anticoagulant when sampling blood.

9. Kit components preparation for testing

Installation, adjustment, calibration of the kit is not required for commissioning.

ATTENTION! When working with a DNA, it is necessary to use only disposable sterile plastic consumables with a "DNase-free" label. It is mandatory to use a separate pipette tip with an aerosol barrier for each reaction component.

PCR cabinet, equipment and materials contained in it should be wet cleaned using disinfectants suitable for use in PCR laboratories, and exposed to UV-radiation for 20-30 minutes before the reactions preparation.

1. Mix thoroughly the tube contents with the isolated DNA, PCR-buffer-1, PCR-buffer-2, Primer-mix-QB and PC, turn over each tube 10 times or mix using vortex at low speed for 3-5 seconds, then remove the drops from the test tube lids by short centrifugation.

2. Take the Beads-1, Beads-2, Diluent-1, and Diluent-2 out of a refrigerator before the assay conduction. Mix Beads-1 and Beads-2 into a homogenous mixture. Incubate bottles for about 30 minutes at room temperature. Thoroughly mix the Beads every time before using.

3. Measure the DNA test samples concentration by spectrophotometric method right before the start of the assay. It is recommended to conduct DNA concentration determination with NanoDrop 2000c spectrophotometer (manufactured by Thermo Fisher Scientific, USA) with 220-350 nm emission spectra. Nanodrop 2000c spectrophotometer (manufactured by Thermo Fisher Scientific, USA) is included in the State Register of Measuring Instruments US.C.31.004.A №53590. Dilute DNA with pure deionized water mQ to 1 ng /µl concentration in 12 µl volume (12 ng in total). Accurate concentration measurement sample dilution are important for the assay efficiency. It is recommended to analyze DNA samples isolated using the same method.

4. Prepare 35 ml of 70% ethanol fresh solution. For that, mix 25.6 ml of 96% ethanol and 9.5 ml of deionized mQ water.

ATTENTION! It is forbidden to change the reactions volume. If the volume is changed, the method sensitivity decreases dramatically!

Reagents preparation for sequencing

It is required to use the Illumina MiSeq Reagent Kit v3 (600-cycles) MS-102-3003 (Illumina, USA) for sequencing with 2x150 bp paired-end sequencing protocol.

1. Take out a package with cartridge, reagents and a HT1 buffer from a freezer.

2. Place the reagent cartridge in a water bath filled with a room temperature water in amount sufficient to immerse the reagent cartridge base till the line indicated on its packaging.

3. Defrost the tube with HT1 buffer at room temperature.

4. Leave the reagent cartridge to defrost in a room temperature water bath until completely thawed (about 60 minutes).

5. Remove the cartridge from the water bath and gently tap it on the table surface to remove the water from the cartridge base. Wipe the cartridge base dry.

6. Turn the reagent cartridge over ten times to mix the melted reagents, then carefully inspect and check that all items have thawed out.

7. Check reagents in positions 1, 2, and 4 and make sure that they are evenly mixed and do not contain sediments.

8. Place the defrosted reagent cartridge and the tube with the

defrosted HT1 buffer in the refrigerator at +4°C temperature till the sample load.

9. Make 0.2M NaOH fresh dilution from a 2M NaOH stock solution. For that, mix 5 µl of 2M NaOH and 45 µl of deionized mQ water in a clean 0.5-1.5 ml tube. Centrifuge the tube for 1-3 seconds on a vortex centrifuge to discharge droplets from the walls.

10. Testing procedure

PCR testing includes the following steps:

1. Target multiplex PCR DNA amplification with possible fluorescence detection of amplification products in real time;
2. Target amplicons purification;
3. Amplicon indexing with DNA libraries creation using limited-cycle RE-PCR;
4. Target DNA libraries mixing and purification;
5. Mass parallel sequencing using Illumina MiSeq.
6. Results interpretation

10.1 Target multiplex real time PCR DNA amplification with possible fluorescence detection of amplification products

Total reaction volume — 20 µl.

The work is carried out with DNA samples diluted to 1 ng/µl concentration (Section 9)

1. Label 0.2 ml tubes or a 0.2 ml PCR plate according to the calculation: test samples number + 1 PC + 1 NC.
2. Prepare a reaction mixture in a separate disposable sterile 1.5 or 2.0 ml Eppendorf type tube: (n+3)x4 µl of PCR-buffer-1 and (n+3) x 4 µl of Primer-mix-QB, where n is the number of test samples, including PC and NC. Mix the reaction mixture thoroughly for 3-5 seconds on a vortex centrifuge.
3. Add 8 µl of the reaction mixture into each tube or well of the plate.
4. Add 12 µl of diluted DNA into the corresponding test tubes or wells of the plate. Do not add DNA into the tubes for PC and NC.
5. Add 12 µl of PC into the corresponding tube or well of the plate.
6. Add 12 µl of NC into the corresponding tube or well of the plate.
7. Close the tube lids or tape the plate with a film.
8. Centrifugate the test tubes or the plate for 1-3 seconds to remove drops from the walls. Use a microcentrifuge-vortex.
9. Install the test tubes or the plate into a reaction module of a real-time

PCR device. It is recommended to install the tubes in the center of the thermoblock to ensure that the tubes are pressed evenly by the heating lid. It is possible to use the PCR device without signal detection in real time mode.

10. Program the device to perform the corresponding amplification and fluorescence signal detection program according to the instructions for the used device. The PCR protocol is shown in Table 8
11. Specify the samples numbers and identifiers. In case of using PCR tubes mark the tubes location on the thermoblock matrix in accordance with their installation.
12. In case of using a cycler with real-time signal detection make sure that the FAM/Green detection channel is applied to the optical measurement parameter and automatic baseline mode are **disabled**.
13. Start amplification.
14. Make sure that the reaction is carried out according to the fluorescence level increase in the channel of test samples, PC and NC according to Table 9. Normal fluorescence increase graph is shown in Figure 2.

Table 8 – PCR protocol

Stage	Temperature, °C	Time, min.:sec.	Detection channels	Total cycles amoun
1	95	05:00	-	1
2	95	01:00	-	5
	61	04:00		
	63	02:00		
	66	01:00		
3	95	00:30	-	25
	72	03:00	FAM/Green	
4	8	constantly	-	1

Table 9 — PC and NC results

Added material	Fluorophore: FAM/Green
PC	Fluorescence increases. Amplification does not go beyond the exponential phase.
NC	Fluorescence increases. Amplification does not go beyond the exponential phase.

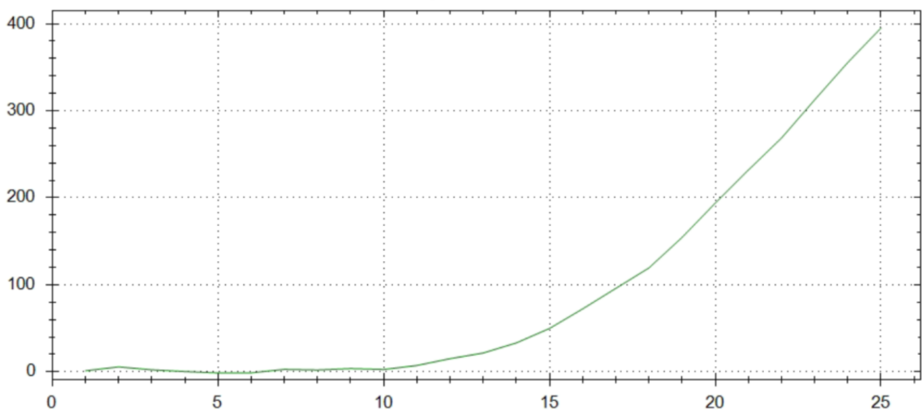


Figure 2 – normal fluorescence rise graph. General curve shape does not differ for the tested samples, PC and NC. Concave arc-shaped fluorescence curve without accumulation. Amplification does not exceed the exponential phase.

15. Proceed to the next stage at the end of the program.

ATTENTION! If necessary, the obtained amplicons can be stored for 12 hours at -20°C.

10.2 Target amplicons purification;

This section describes work with amplicons (PCR products from Section 10.1) to obtain purified amplicons.

1. Prepare a magnetic beads suspension in disposable 15 ml tubes according to the calculation: (n+1)x20 µl of Diluent-1 and (n+1)x40 µl of resuspended Beads-1 of a room temperature where n is number of tested samples, including PC and NC. Diluted magnetic beads precipitate rather quickly. If necessary, resuspend them more just before use. Prepared magnetic beads suspension **cannot be stored** and must be used immediately after preparation!
2. Add 60 µl of the prepared suspension from Section 1 into each tube or well of the plate with amplicons from Section 10.1, mix by pipetting.
3. Incubate the tubes or the plate for 2 minutes at room temperature in a test tube rack.
4. To precipitate the magnetic beads place the tubes or the plate on a magnetic rack and incubate for 2 minutes at room temperature or until the mixture in the tubes or wells of the plate becomes transparent.
5. Carefully remove the supernatant without touching magnetic beads

with a disposable tip and pour it into a container with a disinfectant solution. If the beads have been touched and got caught into the dispenser tip, remove the content into the test tube or well of the plate and repeat steps 4-5.

6. Add 180 µl of 70% ethanol into the test tubes or wells of the plate.

7. Place the test tubes or the plate with a reaction mixture on a magnetic rack. Carefully remove the supernatant without touching magnetic beads with a disposable tip and pour it into a container with a disinfectant solution. If the beads have been touched and got caught into the dispenser tip, remove the content into the test tube or well of the plate and repeat this step.

8. Repeat steps 6-7 one more time. Thus, target amplicons get washed with 70% ethanol twice.

9. Remove the remaining ethanol with a thin tip.

10. Dry the magnetic beads at room temperature for 2 minutes without removing the tube or the plate from the magnetic rack to remove residual alcohol.

11. Add 100 µl of deionized mQ water to the sediment of magnetic beads for elution, mix thoroughly on a vortex until the suspension gets homogenized. Remove the drops by short centrifugation.

12. Incubate the tubes or the plate for 2 minutes at room temperature in a test tube rack.

13. To precipitate the magnetic beads place the tubes or the plate on a magnetic rack and incubate for 2 minutes at room temperature or until the mixture in the tubes or wells of the plate becomes transparent. The supernatant contains purified amplicons. Immediately start the next stage.

10.3. Amplicon indexing with DNA libraries creation using limited-cycle RE-PCR

Total reaction volume — 50 µl.

This Section describes how to work with purified amplicons from Section 10.2 to obtain indexed DNA libraries.

1. Label 0.2 ml PCR tubes or a 0.2 ml PCR plate according to the calculation: test samples number + 1 PC + 1 NC
2. Add 10 µl of PCR-buffer-2 to each tube or well of the plate.
3. The unique combination of the QR7 and QR5 indexes is added to the index plates A, B and V in advance. Add 30 µl of the selected index mixture from the wells of the plate into each tube or well of the PCR plate. One index mixture is added to one sample.

ATTENTION! It is necessary to use a separate tip with an aerosol barrier for each selected index mixture from the wells of the plate.

The unique index pairs layout in index plates A, B and V is shown in Annex 2.

4. Add 10 µl of purified amplicons from Section 10.2 into the appropriate tubes or wells of the plate. The remained amplicon solution with magnetic beads can be stored at -20 °C for 30 days. **After freezing, the magnetic beads lose their properties and cannot be used for DNA precipitation.**

5. Close the tube lids or tape the plate with a film.

6. To discharge droplets from the walls centrifuge the tubes or the plate for 1-3 seconds on a vortex centrifuge.

7. Install the tubes or the plate into the reaction module of the real time PCR cycler. It is recommended to install the tubes in the center of the thermoblock to ensure that the tubes are pressed evenly by the heating lid. It is possible to use the PCR device without the signal detection in real time mode.

8. Program the device to perform a corresponding amplification and florescent signal detection program according to the instructions for the used device. The PCR protocol is shown in Table 10.

9. Specify the samples numbers and identifiers. In case of using PCR tubes mark the tubes location on the thermoblock matrix in accordance with their installation.

Table 10 – QB-IND Index PCR protocol

Stage	**Temperature, °C**	**Time, min.:sec.**	**Detection channels**	**Total cycles amoun**
1	95	03:00	-	1
2	95	00:30	-	3
	66	00:30		
	72	02:00		
3	95	00:30	-	6
	72	02:00	FAM/Green	
4	72	05:00	-	1
5	8	constantly	-	1

10. In case of using a cycler with real-time signal detection make sure that the FAM/Green detection channel is applied for the optical measurement parameter and automatic baseline mode is **disabled**.

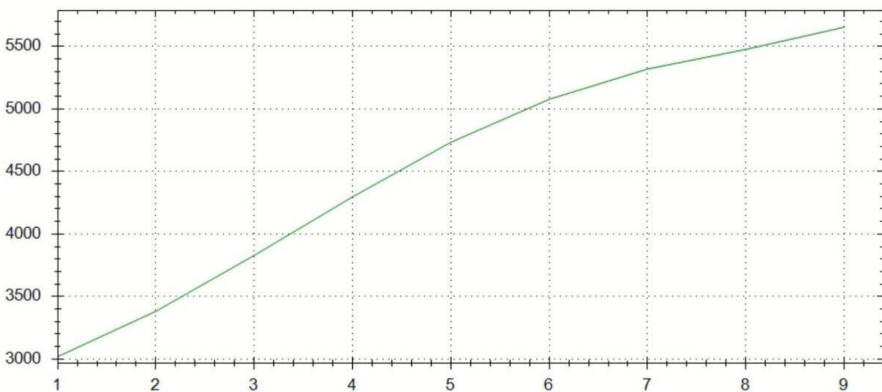
11. Start amplification.

12. Make sure that the reaction is carried out with increasing fluorescence level in the PC and NC channel according to Table 11. A normal fluorescence rise graph for test samples and PC is shown in Figure 2. DNA libraries are contained in test tubes or wells of the plate after the amplification completion.

Table 11 — PC and NC results

Added material	Fluorophore: FAM/Green
PC	Fluorescence increases. Specific convex arc-shaped fluorescence accumulation curve.
NC	Fluorescence increases. Specific convex arc-shaped fluorescence accumulation curve.

Figure 3 — normal fluorescence rise graph. The curve shape does not differ for the tested samples, PC and NC. Convex arc-shaped fluorescence accumulation curve.



10.4 Mixing and purification of target DNA libraries

This Section describes how to work with indexed DNA libraries from Section 10.3

1. Mix 10 µl of each indexed DNA library from Section 10.3 (including PC and NC) in a new tube. Mix well and centrifugate briefly.
2. Add 20 µl aliquot of DNA libraries mixture into a new 0.2 ml tube.
3. Add 20 µl of Diluent-2 and 26 µl of resuspended Beads-2 of a room temperature, mix by pipetting.
4. Incubate the tubes or the plate for 2 minutes at room temperature in a test tube rack.
5. To precipitate the magnetic beads place the tubes or the plate on a magnetic rack and incubate for 2 minutes at room temperature or until the mixture in the tubes or wells of the plate becomes transparent.
6. Carefully remove the supernatant without touching magnetic beads with a disposable tip and pour it into a container with a disinfectant solution. If the beads have been touched and got caught into the dispenser tip, remove the content to the test tube or the well of the plate and repeat steps 5-6.
7. Add 180 µl of 70% ethanol into the test tubes or wells of the plate.
8. Place the test tubes or the plate with a reaction mixture on a magnetic rack and incubate for 1 minute at room temperature. Carefully remove the supernatant without touching magnetic beads with a disposable tip and pour it into a container with a disinfectant solution. If the beads have been touched and got caught into the dispenser tip, remove the content to the test tube or the well of the plate and repeat this step.
9. Repeat steps 7-8 one more time. Thus, target amplicons are washed with 70% ethanol twice.
10. Remove the remaining ethanol with a thin tip.
11. Dry the magnetic beads at room temperature for 2 minutes without removing the tube or the plate from the magnetic rack to remove residual alcohol.
12. Add 22 µl of deionized mQ water µl to the magnetic beads sediment for elution, mix thoroughly using vortex until the suspension gets homogenized. Remove the drops by short centrifugation.
13. Incubate the tubes or the plate for 2 minutes at room temperature in a test tube rack.
14. To precipitate the magnetic beads place the test tube on a magnetic rack and incubate for 2 minutes at room temperature, or until the

mixture in the test tube becomes transparent.

15. Take 20 µl of the supernatant into a new 0.2 ml tube.

16. Add 16 µl of resuspended Beads-2 of room temperature, mix by pipetting.

17. Incubate the tubes or the plate for 2 minutes at room temperature in a test tube rack.

18. To precipitate the magnetic beads place the tubes or the plate on a magnetic rack and incubate for 2 minutes at room temperature or until the mixture in the tubes or wells of the plate becomes transparent.

19. Carefully remove the supernatant without touching magnetic beads with a disposable tip and pour it into a container with a disinfectant solution. If the beads have been touched and got caught into the dispenser tip, remove the content into the test tube or the well of the plate and repeat steps 18-19.

20. Add 180 µl of 70% ethanol into the test tubes or wells of the plate.

21. Place test tubes or a plate with a reaction mixture on a magnetic rack and incubate for 1 minute at room temperature. Carefully remove the supernatant without touching magnetic beads with a disposable tip and pour it into a container with a disinfectant solution. If the beads have been touched and got caught into the dispenser tip, remove the content to the test tube or the well of the plate and repeat this step.

22. Repeat steps 20-21 one more time. Thus, target amplicons are washed with 70% ethanol twice.

23. Remove the remaining ethanol with a thin tip.

24. Dry the magnetic beads at room temperature for 2 minutes without removing the tube or the plate from the magnetic rack to remove residual alcohol.

25. Add 20 µl of deionized mQ water to the magnetic beads sediment for elution, mix thoroughly using vortex until the suspension is homogenized. Remove the drops by short centrifugation.

26. Incubate the tubes or the plate for 2 minutes at room temperature in a test tube rack.

27. To precipitate the magnetic beads place the test tube on a magnetic rack and incubate for 2 minutes at room temperature or until the mixture in the test tube becomes transparent.

28. Take 18 µl of the supernatant into a new 0.2 ml tube. The supernatant contains a single purified DNA library for sequencing (contains all previously mixed DNA libraries of test samples).

29. Measure the DNA library concentration in test tubes by spectrophotometric method using a NanoDrop 2000c spectrophotometer (manufactured by Thermo Fisher Scientific, USA) with emission spectra of 220-350 nm. according to instructions of the manufacturer.

NOTE: it is recommended to use 2 µl of the sample for measurement.

30. Select a 5 µl aliquot of the DNA library from Section 16 and add mQ water till 4 nM concentration in total. Calculate the amount of added mQ water according to the calculation:

$$V_{mQ} = 5.4 \cdot C_{\text{library}} - 5$$

V_{mQ} — amount of added deionized mQ water
C_{library} — library concentration (ng/µl)
0.92 ng/µl concentration corresponds to 4 nM molarity.

10.5 Massive parallel sequencing using Illumina MiSeq

This Section describes work with purified DNA library in 4 nM concentration from Section 10.4.

ATTENTION! Make sure that Generate Fastq module is installed on Illumina MiSeq sequencer. If necessary, download and install it from the Illumina website.

1. Mix 5 µl of DNA library in 4 nM concentration and 0,2M of NaOH in a 1.5 ml tube.
2. Centrifugate the test tubes for 1-3 seconds to remove the drops from the walls. Use a microcentrifuge-vortex.
3. Incubate the mixture for 5 minutes at room temperature.
4. Add into the test tube 1100 µl of cooled to +4 °C HT1 buffer which is included in the Illumina MiSeq Reagent Kit v3 (600-cycles) MS-102-3003 manufactured by Illumina, USA (registration certificate No RZN 2020/13097 dated 30.12.2020). The final DNA library sequencing solution concentration is 18 pM.
5. Then follow the manufacturer's instruction for Illumina MiSeq sequencer (Illumina, USA) with installed Generate Fastq module (Registration Certificate No. RZN 2014/1568 dated 29.04.2014) to load the cartridge and to start sequencing.
6. The analysis requires 2x150 bp sequencing with two 8-bp index reads (i7/i5)
7. QR7 and QR5 index sequences to be included in the Sample

Sheet are shown in Annex 1.

8. An example of filling out the Sample Sheet is shown in Annex 3.

10.6 Results Interpretation

Check the read depth using the Local Run Manager software and the Illumina MiSeq sequencer (Illumina, USA). The average read depth for test samples and PC is considered sufficient if it exceeds 150x value for germline mutations and 500x for somatic mutations. The average read depth for NC should not exceed 10x value.

Mutations in the BRCA1 and BRCA2 genes (BRCA1 c.5266dupC, c.181T>G, c.5251C>T, c.4035delA, c.5161C>T, c.4675G>A, c.68_69del, c.3700_3704del, c.1961delA, c.4689C>G, c.3756_3759del, BRCA2 c.3749dupA, c.961_962insAA, c.2897_2898del, c.8754+1G>A, c.6174delT) are considered detected if the VCF file (see the instructions of Illumina MiSeq system manufacturer) contains at least 1 line of the following type:

Table 12 — entries in the VCF file.

CHROM	POS	ID	REF	ALT	Interpretation
chr17	41209079	.	T	TG	BRCA1 c.5266dupC
chr17	41243512	.	CT	C	BRCA1 c.4035delA
chr17	41258504	.	A	C	BRCA1 c.181T>G
chr17	41245586	.	CT	C	BRCA1 c.1961delA
chr17	41215382	.	G	A	BRCA1 c.5161C>T
chr17	41209095	.	C	T	BRCA1 c.5251C>T
chr17	41226348	.	C	T	BRCA1 c.4675G>A
chr17	41276044	.	ACT	A	BRCA1 c.68_69del
chr17	41243843	.	GTTTAC	G	BRCA1 c.3700_3704del
chr17	41223242	.	G	C	BRCA1 c.4689C>G
chr17	41243788	.	TAGAC	T	BRCA1 c.3756_3759del

chr13	32914437	.	GT	G	BRCA2 c.6174delT
chr13	32912240	.	G	GA	BRCA2 c.3749dupA
chr13	32906576	.	C	CAA	BRCA2 c.961_962insAA
chr13	32911388	.	ACT	A	BRCA2 c.2897_2898del
chr13	32950929	.	G	A	BRCA2 c.8754+1G>A

The ID column value may differ from the one shown in the table.

If there are no lines from Table 12, then there are no mutations in the BRCA1 and BRCA2 genes (BRCA1 c.5266dupC, c.181T>G, c.5251C>T, c.4035delA, c.5161C>T, c.4675G>A, c.68_69del, c.3700_3704del, c.1961delA, c.4689C>G, c.3756_3759del, BRCA2 c.3749dupA, c.961_962insAA, c.2897_2898del, c.8754+1G>A, c.6174delT).

Diagnostic value of the obtained assay results:

Data obtained from high-throughput sequencing of DNA libraries prepared with the Quasar-BRCA1/2 reagent kit after the subsequent bioinformatic analysis can be used by a qualified specialist (oncologist), taking into account the clinical picture data and other research methods to determine an effective treatment strategy and to predict the treatment effectiveness as well as for genetic testing for hereditary forms of breast cancer, ovarian cancer in potentially healthy women according to the clinical guidelines "Breast cancer" (Age group – patients over 18 years old approved by the Ministry of Health of the Russian Federation, 2018) and by clinical guidelines "Ovarian cancer/fallopian tube cancer/primary peritoneal cancer" (Age group – patients over 18 years old, approved by the Ministry of Health Of the Russian Federation, 2018).

11. Storage, transportation and usage conditions

Storage

The Quasar-BRCA1/2 reagent kit manufacturer's packaging contains two packages.

Packaging No. 1 contents should be stored at -15°C... -25°C during the entire shelf-life period. It is allowed to freeze/thaw packaging No. 1 not more than 5 times.

Packaging No. 2 contents should be stored at +2°C... +8°C during the

entire shelf-life period. It is not allowed to store and transport Packaging No. 2 at temperatures below +2°C.

The reagentkit stored under the regulated conditions violation cannot be used.

Transportation

Quasar-BRCA1/2 reagent kit can be transported by all types of covered vehicles in accordance with the transportation rules applicable for the vehicle type.

Transport the the packaging No. 1 contents at -15°C… -25°C during the entire shelf-life period or at 2°C... 8°C — up to 5 days.

Transport the packaging No. 2 contents at 2°C... 8°C during the entire shelf-life period or at 2°C... 30°C — up to 5 days.

Atmospheric pressure is not subject to control as it does not affect the reagent kit quality.

To ensure compliance with transportation conditions throughout the entire transportation period, the reagent kit should be placed in a reusable polyurethane foam thermal container filled with ice packs for temporary storage and transportation. Ice packs type, volume and their number in a thermal container and the thermal container size varies according to the transportation duration and conditions.

Reagent kits transported under the temperature conditions violation cannot be used.

Shelf life

Quasar-BRCA1/2 reagent kit shelf life is 12 months from the acceptance date by the manufacturer’s Quality Control Department (QCD) under all the transportation, storage and usage conditions. A reagent kit with an expired shelf life cannot be used.

Opened kit components shelf life

The reagent kit is designed for single use. In this regard, stability assessment was not carried out during the use of a reagent kit.

Ready for usage kit components shelf life

One hour under conditions that prevent drying of the components as well as contamination by extraneous biological material.

12. Disposal

Reagent kits that have become unusable including the ones with expired shelf life, are subject to disposal in accordance with SanPiN 2.1.3684-21 requirements "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water

bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures".

According to medical waste classification the kits belong to Class A (epidemiologically safe waste, which is similar in composition to solid household waste). Unused reagents are collected in a single-use labeled packaging of any color (except yellow and red) in accordance with Clause 170 SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures".

Used test tubes and materials are disposed in accordance with MU 287-113 (Methodology Guidelines for Disinfection, Pre-Sterilization Cleaning and Sterilization of Medical Devices).

Liquid components (reagents, chemical agents) are disposed by draining into a sewer with a reagent preliminary dilution with tap water 1:100 and removing the packages remains as industrial or household garbage.

Consumer packaging is subject to mechanical destruction with the residues removal as industrial or household garbage.

Personnel carrying out the reagent kit destruction must comply with the safety rules for carrying out one or another destruction method.

13. Warranty, contacts

The manufacturer guarantees the reagent kit quality and safety during the shelf-life period in compliance with the product transportation and storage requirements, as well as in compliance with the usage rules.

In case of complaints about the reagent kit quality, undesirable events or incidents, submit information to:

Limited Liability Company TestGene (TestGene, LLC),

9, 44 Inzhenerny Proezd, office 13, Ulyanovsk, 432072, Russian Federation

www.testgene.com

Technical Support Service:

Phone number: +7 927 981 58 81

E-mail: help@testgen.ru

Instruction for use corresponds to requirements of the Order of the Ministry of Health of the Russian Federation dated 19.01.2017 No. 11n, GOST 51088-2013.

Annex 1

Index sequences i7 and i5 to be included in the Sample Sheet for MiSeq (as well as NovaSeq, HiSeq 2000/2500, NextSeq 2000 Sample Sheet v2).

i7 name	**i7 sequence**	**i5 name**	**i5 sequence**
QR701	AACTGAGC	QR501	GTGGAGCG
QR702	GGTCAGAT	QR502	ACAAGATA
QR703	GTCTCATA	QR503	TAGTAGCT
QR704	TTCCATAA	QR504	CCTGGTGG
QR705	ACGAGATT	QR505	ATTATCCT
QR706	ACATCGCG	QR506	CCACTTGT
QR707	TAGTGCTC	QR507	GAACAGTA
QR708	ATCAAGGC	QR508	TTGTTAAT
QR709	ACTGAGTA	QR509	TGATAGTG
QR710	GTCAGACG	QR510	CAGCGACA
QR711	CGTATGTT	QR511	TACACTGT
QR712	AGTCATAG	QR512	GTGGCGCT
		QR513	CACGAAGG
		QR514	GCTCTACT
		QR515	ATGCACGA
		QR516	GACTATAG

Annex 2

The layout of unique index pairs in index plates A, B and V.

Table 1 — layout of unique index pairs in the plate A

	1	2	3	4	5	6	7	8	9	10	11	12
A	QR701 QR501	QR702 QR501	QR703 QR501	QR704 QR501	QR705 QR501	QR706 QR501	QR707 QR501	QR708 QR501	QR709 QR501	QR710 QR501	QR711 QR501	QR712 QR501
B	QR701 QR502	QR702 QR502	QR703 QR502	QR704 QR502	QR705 QR502	QR706 QR502	QR707 QR502	QR708 QR502	QR709 QR502	QR710 QR502	QR711 QR502	QR712 QR502
C	QR701 QR503	QR702 QR503	QR703 QR503	QR704 QR503	QR705 QR503	QR706 QR503	QR707 QR503	QR708 QR503	QR709 QR503	QR710 QR503	QR711 QR503	QR712 QR503
D	QR701 QR504	QR702 QR504	QR703 QR504	QR704 QR504	QR705 QR504	QR706 QR504	QR707 QR504	QR708 QR504	QR709 QR504	QR710 QR504	QR711 QR504	QR712 QR504
E	QR701 QR505	QR702 QR505	QR703 QR505	QR704 QR505	QR705 QR505	QR706 QR505	QR707 QR505	QR708 QR505	QR709 QR505	QR710 QR505	QR711 QR505	QR712 QR505
F	QR701 QR506	QR702 QR506	QR703 QR506	QR704 QR506	QR705 QR506	QR706 QR506	QR707 QR506	QR708 QR506	QR709 QR506	QR710 QR506	QR711 QR506	QR712 QR506
G	QR701 QR507	QR702 QR507	QR703 QR507	QR704 QR507	QR705 QR507	QR706 QR507	QR707 QR507	QR708 QR507	QR709 QR507	QR710 QR507	QR711 QR507	QR712 QR507
H	QR701 QR508	QR702 QR508	QR703 QR508	QR704 QR508	QR705 QR508	QR706 QR508	QR707 QR508	QR708 QR508	QR709 QR508	QR710 QR508	QR711 QR508	QR712 QR508

Table 2 — layout of unique index pairs in the plate B

	1	2	3	4	5	6	7	8	9	10	11	12
A	QR701 QR509	QR702 QR509	QR703 QR509	QR704 QR509	QR705 QR509	QR706 QR509	QR707 QR509	QR708 QR509	QR709 QR509	QR710 QR509	QR711 QR509	QR712 QR509
B	QR701 QR510	QR702 QR510	QR703 QR510	QR704 QR510	QR705 QR510	QR706 QR510	QR707 QR510	QR708 QR510	QR709 QR510	QR710 QR510	QR711 QR510	QR712 QR510
C	QR701 QR511	QR702 QR511	QR703 QR511	QR704 QR511	QR705 QR511	QR706 QR511	QR707 QR511	QR708 QR511	QR709 QR511	QR710 QR511	QR711 QR511	QR712 QR511
D	QR701 QR512	QR702 QR512	QR703 QR512	QR704 QR512	QR705 QR512	QR706 QR512	QR707 QR512	QR708 QR512	QR709 QR512	QR710 QR512	QR711 QR512	QR712 QR512
E	QR701 QR513	QR702 QR513	QR703 QR513	QR704 QR513	QR705 QR513	QR706 QR513	QR707 QR513	QR708 QR513	QR709 QR513	QR710 QR513	QR711 QR513	QR712 QR513
F	QR701 QR514	QR702 QR514	QR703 QR514	QR704 QR514	QR705 QR514	QR706 QR514	QR707 QR514	QR708 QR514	QR709 QR514	QR710 QR514	QR711 QR514	QR712 QR514
G	QR701 QR515	QR702 QR515	QR703 QR515	QR704 QR515	QR705 QR515	QR706 QR515	QR707 QR515	QR708 QR515	QR709 QR515	QR710 QR515	QR711 QR515	QR712 QR515
H	QR701 QR516	QR702 QR516	QR703 QR516	QR704 QR516	QR705 QR516	QR706 QR516	QR707 QR516	QR708 QR516	QR709 QR516	QR710 QR516	QR711 QR516	QR712 QR516

Table 3 — layout of unique index pairs in the plate V

	1	2	3	4	5	6	7	8	9	10	11	12
A	QR701 QR501	QR702 QR501	QR703 QR501	QR704 QR501	QR705 QR501	QR706 QR501						
B	QR701 QR502	QR702 QR502	QR703 QR502	QR704 QR502	QR705 QR502	QR706 QR502						
C	QR701 QR503	QR702 QR503	QR703 QR503	QR704 QR503	QR705 QR503	QR706 QR503						
D	QR701 QR504	QR702 QR504	QR703 QR504	QR704 QR504	QR705 QR504	QR706 QR504						
E	QR701 QR505	QR702 QR505	QR703 QR505	QR704 QR505	QR705 QR505	QR706 QR505						
F	QR701 QR506	QR702 QR506	QR703 QR506	QR704 QR506	QR705 QR506	QR706 QR506						
G	QR701 QR507	QR702 QR507	QR703 QR507	QR704 QR507	QR705 QR507	QR706 QR507						
H	QR701 QR508	QR702 QR508	QR703 QR508	QR704 QR508	QR705 QR508	QR706 QR508						

Annex 3

Example of filling out Sample Sheet.

```
[Header]
IEMFileVersion,4
Experiment Name,QB1
Date,10/21/2021
Workflow,GenerateFASTQ
Application,FASTQ Only
Assay,TruSeq HT
Description,
Chemistry,Amplicon

[Reads]
151
151

[Settings]
ReverseComplement,0

[Data]
sample_id,Sample_Name,Sample_Plate,Sample_Well,I7_Index_ID,Index,I5_Index_ID,Index2
T1,,,,QR703,GTCTCATA,QR513,CACGAAGG
T2,,,,QR704,TTCCATAA,QR516,GACTATAG
```

Annex 4

Designation	Document name
GOST R ISO 23640-2015	Medical devices for in vitro diagnostics. Evaluation of stability of in vitro diagnostic reagents
GOST R 51088-2013	Medical devices for in vitro diagnostics. Reagents, reagent kits, test systems, control materials, nutritional mediums. Product requirements and accompanying documents.
GOST R ISO 15223-1-2020	Medical devices. Symbols used for labeling medical devices, labels and accompanying documents. Part 1. Essential requirements
GOST R ISO 18113-1-2024	Medical devices for in vitro diagnostics. Information provided by the manufacturer (labeling). Part 1. Terms, definitions and general requirements.
GOST R ISO 18113-2-2024	Medical devices for in vitro diagnostics. Information provided by the manufacturer (labeling). Part 2. In vitro diagnostics reagents for professional use only
GOST ISO 14971-2011	Medical devices. Application of risk management to medical devices
GOST ISO 13485-2017	Medical devices. Quality management systems. Requirements for regulatory purposes.
GOST R 15.309-98	System of product development and launching into manufacture. Test and acceptance of produced goods. Principal positions.
GOST R 51352-2013	Medical devices for in vitro diagnostics. Test methods
GOST R EN 13612-2010	Performance evaluation of in vitro diagnostic medical devices