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DEVELOPMENT AND OPTIMIZATION OF A TARGETED NGS PANEL FOR MODY DIAGNOSIS

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ABSTRACT

This study presents the development and optimization of a targeted next-generation sequencing (NGS) panel for the diagnosis of maturity-onset diabetes of the young (MODY). MODY is frequently misdiagnosed as type 1 or type 2 diabetes, highlighting the need for accurate genetic testing approaches.

This study aimed to design a primer panel targeting MODY-associated genes, optimize multiplex PCR conditions, and evaluate the performance of the developed workflow.

A total of 124 primer pairs were designed using PCRpanel software and tested. Specific amplification of target regions (300-400 bp) was confirmed by agarose gel electrophoresis. Multiplex PCR conditions were optimized. DNA libraries were prepared and successfully sequenced using the Illumina MiSeq platform. The minimum sequencing coverage achieved was 30×, with most regions exceeding 100×.

The developed panel demonstrated high specificity and may serve as an effective diagnostic tool for MODY.

Keywords: MODY, targeted panel, NGS, multiplex PCR, genetic diagnostics, WES

INTRODUCTION

Monogenic forms of diabetes mellitus are a heterogeneous group of inherited diseases caused by variants in genes regulating pancreatic β -cell function [1, 2]. One of the best-studied forms is MODY (Maturity-Onset Diabetes of the Young), which accounts for 2-5% of all cases of diabetes [3]. Despite this, this form of the disease often remains underdiagnosed and is frequently misclassified as type 1 or type 2 diabetes, leading to inappropriate treatment [4].

MODY is characterized by early onset, autosomal dominant inheritance, and impaired insulin secretion in the absence of pronounced insulin resistance. Currently, more than 14 genes have been described, with variants that are associated with various MODY subtypes that differ in their clinical course, prognosis, and treatment approaches [2].

Accurate diagnosis of MODY is crucial for determining patient management strategies, disease prognosis, and genetic counseling. In some cases, establishing the genetic cause of the disease allows for modification of therapy, including discontinuing insulin and switching to oral hypoglycemic agents. Therefore, molecular genetic testing is a key tool for confirming the diagnosis.

Modern next-generation sequencing (NGS) methods allow for the simultaneous analysis of multiple genes associated with the disease, making them an effective diagnostic tool for monogenic forms of diabetes [5]. However, the use of whole-exome or whole-genome sequencing is limited by high cost, excessive data volume, and the need for complex bioinformatics processing.

An alternative approach is targeted NGS-based panels, which allow for the targeted analysis of a preselected set of genes. Such panels are more cost-effective, provide high coverage, and are suitable for routine diagnostic use [6]. A commercial amplicon-based multiplex PCR kit for MODY diagnostics (MASTR/SureMASTR MODY, Agilent) is available, targeting seven MODY-associated genes (ABCC8, GCK,

HNF1A, HNF4A, HNF1B, INS, KCNJ11) across 118 amplicons distributed into five separate multiplex PCR reactions. This kit has been used in large-scale clinical cohorts, including a Hungarian study of 450 patients with suspected MODY, alongside Sanger sequencing and hybridization-capture-based approaches [7]. However, detailed descriptions of the underlying multiplex PCR primer design, pool optimization strategy, and thermal cycling parameters for such commercial kits are proprietary and not published in the peer-reviewed literature, limiting their reproducibility and adaptability for laboratories developing in-house diagnostic workflows. To our knowledge, no peer-reviewed study has reported the complete design and experimental optimization process of a custom multiplex PCR-based targeted NGS panel for MODY diagnostics, including primer pool composition and thermal cycling optimization data.

However, the development of targeted panels requires careful optimization, particularly at the multiplex polymerase chain reaction (PCR) stage. Simultaneous amplification of a large number of genetic targets can be associated with a number of technical challenges, including non-uniform amplification, competition between primers, and the formation of non-specific products.

Despite the widespread adoption of NGS in clinical practice, optimization of multiplex PCR protocols for targeted panels remains understudied, particularly in the context of MODY diagnostics. The development of stable and reproducible methodological approaches is essential for the creation of reliable diagnostic tools [8].

This study aims to develop and optimize a targeted NGS panel for MODY diagnostics, including primer design, multiplex pool formation, and selection of optimal PCR conditions. Additionally, a pilot validation of the developed approach is conducted using NGS sequencing of clinical samples. To our knowledge, this is the first study in Kazakhstan focusing on the development and optimization of a targeted MODY panel with a multiplex PCR-based workflow.

METHODS AND MATERIALS

Patient recruitment

Peripheral blood samples (~2-3 mL each) were collected from 17 individuals with clinical suspicion of MODY.

Clinical evaluation included patient interviews, analysis of medical records, and laboratory data. All samples were collected after obtaining informed consent in accordance with ethical standards. Written informed consent was obtained from parents/legal guardians of all pediatric participants before sample collection, covering the study aims, expected outcomes, blood sample collection, and data protection in accordance with the legislation of the Republic of Kazakhstan. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the National Center for Biotechnology (No. 5, dated 14 May 2024, Astana, Kazakhstan).

The study cohort consisted of 17 individuals, predominantly pediatric and young adult probands (age at onset under 25 years, consistent with standard MODY diagnostic criteria) along with several affected family members recruited for segregation analysis, with clinical suspicion of MODY or early-onset diabetes atypical for classical type 1 or type 2 diabetes. Clinical presentations included elevated HbA1c (ranging from approximately 6.5% to 12%), variable C-peptide and fasting glucose levels, and in several cases a positive family history of diabetes across multiple generations. A subset of patients also presented with overweight/obesity, while others had normal body mass index, reflecting the phenotypic heterogeneity characteristic of MODY. Patients were recruited at the Center for Motherhood and Childhood, UMC (United Medical Center) (Astana, Kazakhstan), with the participation of pediatric endocrinologists and medical geneticists, based on clinical evaluation, family history, and laboratory findings. All 17 recruited patients subsequently underwent whole-exome sequencing (WES) as part of standard diagnostic workup. Samples P1, P9, and P17 were selected for pilot targeted NGS sequencing because WES had already identified confirmed pathogenic or likely pathogenic variants with established MODY subtypes in these three patients, allowing the developed targeted panel to be validated against samples of known, genetically confirmed status.

DNA extraction and quality assessment

Peripheral blood was collected in EDTA-containing tubes (AVATUBE, Kazakhstan). Genomic DNA was extracted from whole blood using GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) and QIAamp DNA Blood Kit (Qiagen, Hilden, Germany), according to the manufacturers' instructions.

DNA concentration was measured using a Qubit® 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) with the Qubit dsDNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). DNA purity was additionally assessed using a NanoDrop™ 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

DNA integrity was evaluated using the TapeStation 4150 system (Agilent Technologies, Santa Clara, CA, USA). Primer design and panel development

PCR amplification and gel electrophoresis

Primers were designed using PCRpanel, an in-house Ja-

va-based platform developed for ultra-high multiplex primer design, incorporating thermodynamic modeling, primer-dimer prediction, sequence complexity assessment, and repeat-aware specificity filtering into a unified automated workflow. The software is an internally developed platform and is not currently publicly available; all primer sequences, target genomic coordinates, and PCR reaction parameters generated using this platform are provided in full in this manuscript (Table 1 and Methods and Materials) to ensure experimental reproducibility. Individual primer pairs were tested by single-PCR in a total reaction volume of 20 µL, containing 5 µL MilliQ water, 1 µL human genomic DNA (10 ng/µL; final amount 10 ng per reaction), 10 µL 2× Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), and 2 µL each of forward and reverse primer (1 µM stock; final concentration 100 nM each). PCR amplification was performed using a VeritiPro™ Thermal Cycler (Applied Biosystems, Waltham, MA, USA) under the following conditions: initial denaturation at 98°C for 30 s, followed by 32 cycles of denaturation at 98°C for 5 s, annealing at 60°C for 60 s, and extension at 72°C for 60 s, with a final extension at 72°C for 1 min. Amplification products were analyzed by horizontal electrophoresis in a 1.5% agarose gel using a Bio-Rad electrophoresis system. DNA fragments were visualized under UV illumination to assess product size and specificity.

Multiplex PCR

Primers were designed using PCRpanel based on the human reference genome (GRCh38/hg38), targeting coding exons and flanking intron-exon boundaries of the five MODY-associated genes. Gene-specific design was based on the following RefSeqGene reference sequences (NCBI): NG_009818 for HNF4A (MODY1), NG_011731 for GCK (MODY2), NG_008847 for HNF1A (MODY3), NG_013019 for HNF1B (MODY5), and NG_011820 for NEUROD1 (MODY6). A total of 124 primers were designed across the five genes and organized into gene-specific multiplex pools, as detailed in Table 1.

Following individual primer validation, primer pairs were combined into gene-specific multiplex pools (Table 1) for further optimization. Each 100 µL multiplex PCR reaction contained 71 µL MilliQ water, 5 µL human genomic DNA (10 ng/µL; 50 ng total input), 20 µL 5× Phusion Plus Buffer, 2 µL dNTPs (10 mM; final concentration 0.2 mM), 1 µL of the pooled primer panel (1 µM stock; final concentration 50 nM per primer), and 1 µL Phusion Plus II Hot Start DNA Polymerase (2 U/µL). PCR amplification was carried out under the following conditions: initial denaturation at 98°C for 90 s, followed by 32 cycles of denaturation at 98°C for 5 s, annealing at 60°C for 240 s, and extension at 72°C for 30 s, with a final extension at 72°C for 1 min.

Library preparation and next-generation sequencing

PCR products obtained from multiplex amplification were purified and used for library preparation. For pilot validation of the developed panel, three patient samples were selected for next-generation sequencing. Purified PCR amplicons (5 ng input DNA per reaction) were indexed by PCR using dual-index primers in a reaction containing 5× Phusion Plus Buffer, 10 mM dNTPs, and Phusion Plus DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA). Index PCR was performed under the following conditions: initial denaturation

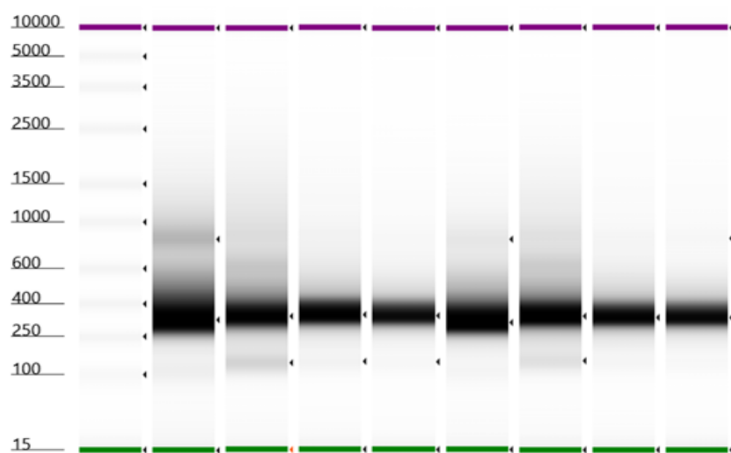


Figure 1. TapeStation electropherogram of prepared sequencing libraries.

Fragment size distribution of indexed libraries prepared for all patient samples designated for NGS sequencing, assessed using the TapeStation 4150 system (Agilent Technologies, Santa Clara, CA, USA). Lanes correspond to individual patient libraries; the upper (purple) and lower (green) bands indicate the internal size standards (10,000 bp and 15 bp, respectively). Library fragments predominantly fall within the range of approximately 250-400 bp, consistent with the expected amplicon size of the developed panel, confirming successful library preparation prior to sequencing.

at 95°C for 30 s, followed by 8 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 1 min. Indexed libraries were purified using AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) at a 1:1 bead-to-sample ratio, followed by two washes with freshly prepared 80% ethanol and elution in nuclease-free water. Library concentration was measured using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Library size distribution was assessed using the TapeStation 4150 system (Agilent Technologies, Santa Clara, CA, USA), confirming a predominant fragment size range of approximately 250-400 bp across all libraries, consistent with the expected amplicon size range of the developed panel (Figure 1). Libraries were normalized to 4 nM following the standard normalization (Illumina MiSeq Denature and Dilute Libraries Guide). Sequencing was performed on the Illumina MiSeq platform using the MiSeq Reagent Nano Kit v2 (Illumina, San Diego, CA, USA), enabling paired-end sequencing (2 × 250 bp).

Bioinformatics analysis

Raw sequencing data (FASTQ files) were subjected to quality control and filtering to remove low-quality reads and

adapter sequences. Reads were aligned to the human reference genome using the BWA mem algorithm. Subsequent analysis included evaluation of sequencing coverage, uniformity of read distribution across target regions, and overall performance of the developed panel.

RESULTS

As a result of the research, a targeted panel for analyzing genes associated with MODY was developed and optimized. The final panel included five key genes: HNF4A (MODY1), GCK (MODY2), HNF1A (MODY3), HNF1B (MODY5), and NEUROD1 (MODY6). A total of 124 primer pairs were developed, designed to amplify coding regions and adjacent exon-intron boundaries. The length of the amplicons ranged from 300 to 400 nucleotides, which corresponds to the optimal parameters for sequencing on the Illumina platform. The developed panel provided coverage of all exons of the selected genes, enabling a comprehensive analysis of genetic variants potentially associated with monogenic diabetes. The distribution of primers across genes and their main characteristics are presented in Table 1.

Table 1. Target Genes and Primer Coverage

Gene	Chr	Location	Exon count	Length	Primer pair ID	Reference sequence	Primers per pool
HNF4A (MODY1)	20	20q13.12	15	78,898 nt	53F-92R	NG_009818	42, 38 (two pools)
GCK (MODY2)	7	7p13	15	46,227 nt	25F-52R	NG_011731	30, 26 (two pools)
HNF1A (MODY3)	12	12q24.31	9	23,970 nt	1F-24R	NG_008847	28, 20 (two pools)
HNF1B (MODY5)	17	17q12	11	58,629 nt	93F-114R	NG_013019	22 (one pool)
NEUROD1 (MODY6)	2	2q31.3	4	12,223 nt	115F-124R	NG_011820	10 (one pool)

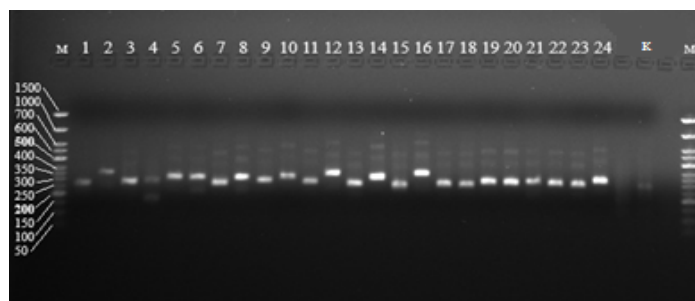


Figure 2. Electropherogram of individual PCR amplification.

Agarose gel electrophoresis image demonstrating the specificity of 24 individual primer pairs designed for the amplification of MODY3 (HNF1A) gene regions together with a negative control (K). Each lane (1-24) corresponds to a distinct primer pair, producing clear amplicons within the expected size range (~300-400). M - DNA marker.

Primer Efficiency Assessment

In the first stage of panel development, the efficacy of all 124 primer pairs targeting the amplification of MODY-associated genes was assessed individually using single-PCR. As a representative example, Figure 2 shows the electropherogram for 24 primer pairs targeting the HNF1A (MODY3) gene region (lanes 1-24), together with a negative control (K). Amplification products across all 124 tested primer pairs, including those targeting HNF4A, GCK, HNF1B, and NEUROD1, showed comparable specificity and expected fragment size (300-400 bp) by agarose gel electrophoresis, with high signal intensity and the absence of nonspecific products, including primer dimers. These results demonstrate the high specificity and efficacy of the developed primers, allowing us to proceed to the next stage, the formation of multiplex panels.

Multiplex PCR Optimization

After confirming the performance of individual primers, they were combined into two multiplex pools, Pool 1 (primer pairs 1-14) and Pool 2 (primer pairs 15-24), targeting the HNF1A (MODY3) gene region, as a representative example of the multiplex pooling strategy applied across the full panel (Figure 3). When performing multiplex PCR on agarose gel, a characteristic broad band in the 350-400 bp range was observed, consistent with the total amplification of target regions of similar length. Optimization of reaction conditions, including primer concentration and temperature, ensured stable and reproducible amplification in all samples studied. Optimization of reaction conditions, including primer concentration and temperature, ensured stable and reproducible amplification in all samples studied. In particular, the annealing time was empirically extended from 60 s (used for single-primer

PCR) to 240 s during multiplex PCR optimization, which improved amplification uniformity across the large number of co-amplified targets within each pool and reduced competition-driven bias between primer pairs; this adjustment, together with primer concentration titration, was the key determinant of the final reaction conditions.

Agarose gel electrophoresis showing multiplex amplification of the HNF1A (MODY3) target region using two pooled primer sets: Pool 1 (P1; primer pairs 1-14) and Pool 2 (P2; primer pairs 15-24). Both lanes show a characteristic broad band in the 350-400 bp range, corresponding to the combined amplification of all target exon regions within each pool, consistent with successful multiplex PCR. DNA ladder (M) is shown on the left. *Four additional primer pools, P1(SA)-P4(SA), correspond to a separate targeted panel developed for Alport syndrome (COL4A genes) and are not related to the MODY panel described in this study. To evaluate the efficiency of the generated multiplex primer pools, amplification was performed, followed by analysis of the products by agarose gel electrophoresis (Figure 3). The electropherogram shows the formation of distinct bands in the range of approximately 300-400 bp in all analyzed lanes corresponding to samples (P1 and P2). The resulting amplicons are characterized by sufficient intensity and the absence of pronounced non-specific amplification products. The absence of additional bands and smearing indicates the high specificity of the multiplex PCR and the correct operation of the combined primer pools. Comparison with a molecular weight marker (ladder) confirms that the sizes of the amplified fragments correspond to the expected values. Thus, the obtained results demonstrate successful optimization of the multiplex

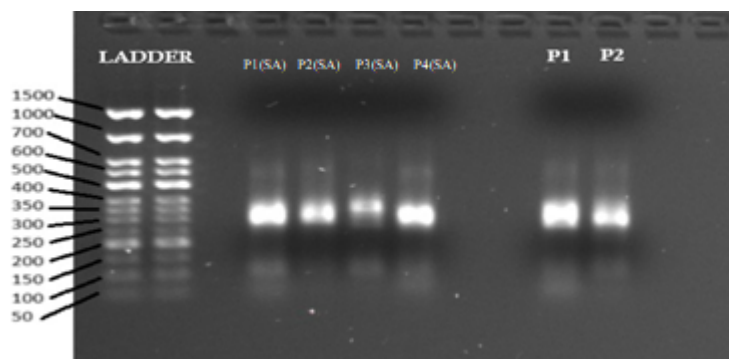


Figure 3. Electropherogram of multiplex PCR products.

Table 2. Variant analysis of MODY-associated genes in three patients

Sample	Gene	Variant type	Impact	AA change	Interpretation
P1	HNF1A	frameshift	HIGH	G/X	protein truncating
P1	HNF1A	missense	MODERATE	R/P	probably damaging
P1	HNF1A	missense	MODERATE	Y/N	probably damaging
P1	HNF1A	missense	MODERATE	Y/F	possibly damaging
P1	HNF1A	missense	MODERATE	W/R	probably damaging
P1	HNF1A	frameshift	HIGH	truncated	protein truncating
P1	HNF1A	missense	MODERATE	P/H	possibly damaging
P9	GCK	missense	MODERATE	H/L	probably damaging
P9	GCK	missense	MODERATE	I/N	probably damaging
P9	HNF1A	frameshift	HIGH	truncated	protein truncating
P9	HNF1A	inframe deletion	MODERATE	QF/H	protein altering
P9	HNF1A	missense	MODERATE	G/V	probably damaging
P9	HNF1A	missense	MODERATE	Y/N	probably damaging
P9	HNF4A	missense	MODERATE	G/W	probably damaging
P9	HNF4A	frameshift	HIGH	truncated	protein truncating
P9	HNF4A	inframe insertion	MODERATE	-/PD	protein altering
P9	HNF1B	frameshift	HIGH	truncated	protein truncating
P17	GCK	missense	MODERATE	M/K	probably damaging
P17	GCK	frameshift	HIGH	truncated	protein truncating
P17	HNF1A	missense	MODERATE	D/E	probably damaging
P17	HNF1A	frameshift	HIGH	truncated	protein truncating
P17	HNF4A	missense	MODERATE	D/N	probably damaging
P17	HNF4A	frameshift	HIGH	truncated	protein truncating

reaction and the suitability of the generated primer pools for further use in targeted sequencing.

During the initial stages of panel development, variability in amplification efficiency in multiplex reactions was observed, manifested by varying band intensities, indicating uneven representation of individual targets. To address these limitations, primer concentrations and PCR parameters, including the amplification temperature profile, were optimized. This resulted in uniform amplification of all target regions and high reproducibility of the results.

As part of the pilot validation of the developed panel, targeted sequencing was performed on three clinical samples (P1, P9, and P17), selected based on prior WES-confirmed pathogenic or likely pathogenic MODY variants, using the Illumina MiSeq platform. The resulting data demonstrated successful coverage of the target regions of genes associated with MODY. Variant analysis identified genetic alterations in key MODY-associated genes, including HNF1A, HNF4A, GCK, and HNF1B, which are illustrated in Table 2 [9].

The detected variants included both high-impact (frameshifts) and moderate-impact variants (missenses and inframe insertions/deletions). Characteristics of the identified variants with varying functional impacts on the protein were identified

in the analyzed samples. The most significant are frameshift variants (HIGH impact), potentially leading to protein truncation, and missense variants (MODERATE impact), some of which were predicted to be likely damaging. In patient P1, variants were detected exclusively in the HNF1A gene, including frameshift and several missense variants, consistent with the profile characteristic of MODY3. In patient P9, variants were identified in several genes, including GCK, HNF1A, HNF4A, and HNF1B. These included frameshift variants in the HNF1A, HNF4A, and HNF1B genes, as well as missense and inframe variants. This distribution of variants does not allow a definitive determination of a specific MODY subtype based on targeted sequencing data alone. Patient P17 also had variants in several genes, including GCK, HNF1A, and HNF4A, including frameshift and missense variants. Similar to P9, the presence of variants in multiple genes complicates precise MODY subtype classification.

DISCUSSION

The present study demonstrates the successful development and optimization of a targeted NGS panel for the molecular diagnosis of monogenic diabetes. A key focus of this work was the methodological optimization of multiplex PCR,

which represents one of the most challenging stages in targeted panel development [10, 11]. Importantly, all genetic variants identified using the developed targeted NGS panel was also detected by whole-exome sequencing (WES). This concordance confirms the reliability and accuracy of the designed primer panel, demonstrating that it effectively captures clinically relevant variants associated with MODY and can serve as a robust diagnostic tool.

Existing approaches to MODY molecular diagnostics range from serial Sanger sequencing and commercial multiplex amplicon kits to hybridization capture-based NGS panels covering broader gene sets, such as a 17-gene custom capture panel used in a Hungarian cohort of 450 patients [7] and a 27-gene capture-based panel (KAPA HyperCapture, Illumina NovaSeq) used in a Russian pediatric HNF4A-MODY cohort [12]. In contrast to these capture-based strategies, the present study employed an amplicon-based multiplex PCR approach targeting five core MODY genes (HNF4A, GCK, HNF1A, HNF1B, NEUROD1). While covering fewer genes than broader capture-based panels, this amplicon-based design offers a simpler, more cost-effective workflow that does not require hybridization capture reagents or extended capture protocols, making it particularly suitable for laboratories with limited NGS infrastructure. However, amplicon-based multiplex PCR is inherently more sensitive to primer-primer interactions and coverage non-uniformity than capture-based approaches, which necessitated the extensive optimization described in this study. Notably, unlike the commercial and internationally developed panels described above, the panel presented here was designed and optimized entirely in-house, using domestically developed primers and software, representing the first locally developed targeted NGS panel for MODY diagnostics in Kazakhstan and reducing reliance on imported diagnostic kits.

At the initial stage, individual primer pairs showed high specificity, producing clear amplicons of the expected size (300-400 bp), as confirmed by agarose gel electrophoresis. However, when combined into multiplex pools, variability in amplification efficiency was observed, likely due to competition between primers and differences in amplification kinetics. Such challenges are commonly reported in multiplex PCR-based approaches and represent a critical limitation in panel design [13].

Through iterative optimization of primer concentrations and thermal cycling conditions, uniform amplification across all target regions was achieved. The appearance of consistent bands in the expected size range and the absence of nonspecific products or primer-dimers confirmed the robustness of the optimized protocol [14]. These results demonstrate the importance of careful experimental optimization for the development of multiplex-based targeted panels.

It should be noted that in one sample (P1), variants were identified exclusively in the *HNF1A* gene, consistent with a classical MODY3 genetic profile. In contrast, samples P9 and P17 exhibited variants across multiple MODY-associated genes, including *HNF1A*, *HNF4A*, *GCK*, and *HNF1B*. This multi-gene variant pattern complicates interpretation and highlights an important limitation of targeted sequencing approaches: the presence of multiple variants does not necessarily indicate causality. Importantly, all these patients

were additionally analyzed using whole-exome sequencing (WES), and the same genes were confirmed to harbor pathogenic or likely pathogenic variants, supporting the reliability of the results obtained with the developed targeted panel.

The panel was designed to target the five most clinically significant MODY-associated genes (*HNF4A*, *GCK*, *HNF1A*, *HNF1B*, *NEUROD1*), which together account for the majority of genetically confirmed MODY cases reported in the literature, with *GCK* and *HNF1A* representing the most prevalent subtypes. Rarer MODY subtypes were not included in the current panel version to keep the amplicon panel size manageable for cost-effective routine use; these may be incorporated in future panel iterations. The selected PCR parameters, including a two-step protocol with short denaturation and extension times and an extended annealing step for multiplex reactions, were empirically optimized to accommodate the shared amplicon size range (300-400 bp) across all target regions, balancing amplification efficiency against the risk of non-specific product formation inherent to high-multiplicity primer pools [15].

These findings emphasize the need for careful interpretation of NGS results in the clinical context, including consideration of variant pathogenicity, inheritance patterns, and phenotypic data. In particular, the presence of multiple variants may reflect benign polymorphisms, variants of uncertain significance, or potential oligogenic effects [8, 16].

This study highlights that the success of targeted NGS diagnostics depends not only on sequencing technology but also on careful design and optimization of upstream molecular workflows. However, the main limitation of this study is the small sample size, which covers only three patients for NGS validation, which does not allow full assessment of diagnostic performance.

CONCLUSION

In this study, a targeted NGS panel for the diagnosis of MODY was successfully developed and optimized. A total of 124 primer pairs were designed, and stable multiplex PCR conditions were established, ensuring efficient and specific amplification of target regions.

The developed workflow demonstrated high specificity, reproducibility, and suitability for downstream sequencing. Pilot NGS validation confirmed adequate coverage of target regions and the ability to detect clinically relevant genetic variants, including both high-impact and moderate-impact variants.

The results indicate that the proposed targeted panel represents a cost-effective and reliable approach for molecular diagnostics of MODY. Furthermore, this study emphasizes the critical role of multiplex PCR optimization in ensuring the performance of targeted sequencing assays.

Future work should focus on expanding the number of analyzed samples, validating detected variants using orthogonal methods, and integrating clinical data to improve diagnostic accuracy.

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ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the National Center for Biotechnology (No. 5, dated 14 May 2024, Astana, Kazakhstan).

AUTHOR CONTRIBUTIONS

Conceptualization, T.A.; methodology, Z.E., T.A., R.A., K.R.; statistical analysis, R.A., K.R.; study design, T.A., R.A.; data interpretation, T.A., Z.E.; preparation of the initial version of the manuscript, T.A.; review and editing of the text, T.A.; scientific supervision, Z.E. All authors have read and agreed to the published version of the manuscript.

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РАЗРАБОТКА И ОПТИМИЗАЦИЯ ТАРГЕТНОЙ NGS-ПАНЕЛИ ДЛЯ ДИАГНОСТИКИ MODY

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АННОТАЦИЯ

В данном исследовании представлена разработка и оптимизация таргетной панели на основе технологии секвенирования нового поколения (NGS) для диагностики MODY (диабета зрелого типа у молодых). MODY часто ошибочно диагностируется как сахарный диабет 1-го или 2-го типа, что подчеркивает необходимость точных методов генетической диагностики.

Целью исследования являлась разработка панели праймеров, нацеленной на гены, ассоциированные с MODY, оптимизация условий мультиплексной ПЦР и оценка эффективности разработанного подхода.

Было разработано и протестировано 124 пары праймеров с использованием программного обеспечения PCRpanel.. Специфическая амплификация целевых участков (300-400 п.н.) подтверждена методом агарозного гель-электрофореза. Условия мультиплексной ПЦР были оптимизированы. ДНК-библиотеки были успешно подготовлены и секвенированы с использованием платформы Illumina MiSeq. Минимальная глубина секвенирования составила 30×, при этом большинство регионов имели покрытие более 100×.

Разработанная панель продемонстрировала высокую специфичность и может служить эффективным инструментом для диагностики MODY.

Ключевые слова: MODY, таргетная панель, NGS, мультиплексная ПЦР, генетическая диагностика, WES

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MODY ДИАГНОСТИКАЛАУҒА АРНАЛҒАН ТАРГЕТТІК NGS-ПАНЕЛІН ӘЗІРЛЕУ ЖӘНЕ ОҢТАЙЛАНДЫРУ

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АННОТАЦИЯ

Бұл зерттеуде жасөспірімдердегі ересек типті қант диабетін (MODY) диагностикалауға арналған жаңа буындағы секвенирлеу (NGS) технологиясына негізделген таргеттік панельді әзірлеу және оңтайландыру сипатталады. MODY жиі 1 немесе 2 типті қант диабеті ретінде қате диагностикаланады, бұл дәл генетикалық диагностиканың маңыздылығын көрсетеді.

Зерттеудің мақсаты MODY-мен байланысты гендерге бағытталған праймерлік панельді әзірлеу, мультиплексті ПТР жағдайларын оңтайландыру және ұсынылған әдістің тиімділігін бағалау болды.

Жалпы саны 124 праймер жұбы PCRpanel бағдарламалық жасақтамасын пайдаланып әзірленіп, сынақтан өткізілді. Мақсатты аймақтардың (300-400 жұп негіз) спецификалық амплификациясы агарозды гель-электрофорез арқылы расталды. Мультиплексті ПТР жағдайлары оңтайландырылды. ДНҚ кітапханалары дайындалып, Illumina MiSeq платформасында сәтті секвенирленді. Секвенирлеудің ең төменгі тереңдігі 30× құрады, ал көптеген аймақтарда бұл көрсеткіш 100×-тен асты.

Әзірленген панель жоғары спецификалықты көрсетті және MODY диагностикасында тиімді құрал бола алады.

Түйінді сөздер: MODY, таргеттік панель, NGS, мультиплексті ПТР, генетикалық диагностика, WES