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Optimization of the Real-Time Loop-Mediated Isothermal Amplification (LAMP) Technique for Detecting the Fat Mass and Obesity-Associated (FTO) Gene

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Abstract

The loop-mediated isothermal amplification (LAMP) technique enables rapid, highly sensitive, and specific nucleic acid amplification. In the field of genetic detection and amplification, polymerase chain reaction (PCR) has long been considered as a gold standard, though it has several limitations. This study aimed to optimize real-time LAMP (RT-LAMP) method for detecting the fat mass and obesity-associated (FTO) gene using blood samples. Six primers were used in the synthesis of the deoxyribonucleic acid (DNA) sequence by DNA polymerase, facilitating auto-cycling strand displacement. The primers included outer and inner primers, specifically F3 and B3, as well as forward inner primer (FIP that consists of F1c and F2) and backward inner primer (BIP that consists of B1c and B2). These primers were designed using Primer Explorer V5.0, and their sequences were checked for specificity using the BLAST program. A total of 2.5 mL of whole peripheral blood was extracted for DNA, which was then assessed for concentration, purity, and integrity. Twelve sets of primer concentrations were tested, and the most appropriate and optimal concentrations for amplifying and detecting the FTO gene were found to be 1.6 for FIP and BIP, and 0.8 for F3 and B3. The detection threshold value (Df) and threshold time (Tt) were 0.178 and 34:18 minutes, respectively, at a reaction temperature of 65°C. Validation of RT-LAMP optimization was performed using 2.0% agarose gel electrophoresis, which demonstrated as ladder-like pattern recognition, indicating the effectiveness of the RT-LAMP technique. The results showed that real-time LAMP can detect the FTO gene in facilitating rapid diagnosis. Therefore, real-time LAMP is a promising method that can be quantitatively studied to improve PCR performance in amplifying target DNA sequences.

Keywords: LAMP, FTO Gene, Primers

Introduction

Conventional polymerase chain reaction (PCR) has been used as a gold standard for gene detection. However, it has been predicted in several fields to provide simpler and more affordable methods for quantitative analysis. PCR has its own limitations, the necessity for agarose or polyacrylamide gel electrophoresis, which delays the detection and analysis, the potential for carry-over contamination, the inability to quantify amplification products in samples and the use of reagents like ethidium bromide, which poses health risks to the handler (Staggemeier et al., 2015). Therefore, this research will study the better way to detect gene of interest by optimizing real-time LAMP method.

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technique that enables rapid and high specificity amplification of large quantities of deoxyribonucleic acid (DNA). The reaction by product

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which are the pyrophosphate ions bind to magnesium ions as the LAMP reaction proceeds, forming a white precipitate of magnesium pyrophosphate (Mori et al., 2004). LAMP approach relies on a self-sustaining process of DNA synthesis known as auto-cycling strand displacement. This is carried out by an enzyme called DNA polymerase that is characterized with an exceptional strand shift capability, along with a specific set of two internal and two external primers. Initially, all four primers are utilized in the LAMP reaction, but as the cycling progresses, only the inner primers, called forward inner primer (FIP) and backward inner primer (BIP), are employed for strand displacement DNA synthesis. These inner primers each consist of two distinct sequences corresponding to the sense and antisense sequences of the target DNA. One sequence is utilized for priming in the initial stage, while the other serves for self-priming in subsequent stages (Notomi et al., 2000).

Real-time LAMP is well known for its simplicity, sensitivity and rapid isothermal nucleic acid amplification (iNAAT) approach. Target regions can be amplified at a constant temperature, leading to the elimination of time-consuming steps (Gadkar et al., 2018). Real-time monitoring of LAMP amplification is predominantly achieved using fluorescence or turbidimetry, quantitative data (Papadakis et al., 2020). A prior study has developed a device that can simultaneously measure the turbidity of multiple samples, ensuring a consistent temperature to perform real-time assessments of the turbidity changes in LAMP reactions. The findings suggested that utilizing real-time turbidity measurements in LAMP reactions enables the precise quantitative analysis of minimal nucleic acid quantities in a sample across a broad range. The ability to measure template DNA or ribonucleic acid (RNA) quantitatively has been demonstrated through real-time monitoring of LAMP reactions, utilizing either a real-time thermal cycler or a real-time turbidimeter (Mori et al., 2004). As compared to conventional PCR that requires 3 to 4 hours, real-time LAMP only takes less than an hour. It is due to its higher amplification efficiency that is done without denaturation step. The method is extremely precise and increases the quantity of amplified DNA to as much as a billion copies within an hour, surpassing the million copies achieved through PCR. Moreover, isothermal amplification can be carried out without the need for advanced laboratory equipment, in which it only requires a dry block heater or a water bath. Another groundbreaking feature of LAMP is its high specificity facilitated by the use of multiple primers (ranging from four to six). This allows discrimination of up to eight specific locations on the DNA template, a notable advancement compared to the typical PCR, which only distinguishes two locations (Soroka et al., 2021).

In this study, fat mass and obesity-associated (FTO) gene was used for this optimization and development of the real-time LAMP method. *FTO*, or also known as alpha-ketoglutarate-dependent dioxygenase FTO gene is closely related to obesity. It is a gene located on chromosome 16q12.2 and composed of nine exons, exhibiting significant conservation throughout different vertebrate species, including fish and chickens. *FTO* primarily expresses itself in adipose tissues and skeletal muscle of human tissues (Lan et al., 2020). It has been discovered that *FTO* encodes a N^6-methyladenosine (m^6A) RNA demethylase and exerts a significant impact on numerous metabolic and biological processes. Single nucleotide polymorphisms (SNPs) in the first intron of the FTO gene have been successfully linked to obesity-related features including anthropometric traits, type 2 diabetes, early-onset obesity, and severe obesity (Huang et al., 2023). LAMP is particularly useful for rapid and reliable diagnostics in both clinical and research settings to overcome limitations arising from conventional PCR in gene detection. LAMP is a comparable, sensitive, specific, and rapid method that can be used to diagnosis a disease during fieldwork by less-equipped laboratories. This study will provide the details of the development and optimization of the methods that can be used in detection of FTO gene that contributes to the acceleration of obesity in Malaysia. The method is extremely precise and increases the quantity of amplified DNA to as much as a billion copies within an hour, surpassing the million copies achieved through PCR. Optimization guarantees that LAMP approach is both specific and efficient, eliminating false positives while also ensuring robust and stable amplification (Notomi et al., 2000). This is especially crucial for the FTO gene since it plays a role in a complex phenotype such as obesity, which requires precise measurements.

Methods

This experimental study was designed to optimize the real-time LAMP method for identifying FTO gene in blood samples. Ethical approval was obtained from the UniSZA Human Research Ethics Committee (UHREC) with reference number UniSZA/UHREC/2023/599. This study used 2.5 mL of whole peripheral blood from a volunteer who was a student at Universiti Sultan Zainal Abidin (UniSZA). A written consent form was given to the subject after human ethical approval was obtained from UHREC.

Primer Design

Sets of internal primers, FIP (consists of F1c and F2) and BIP (consists of B1c and B2), and external primers (F3 and B3) utilized in this assay, were designed based on the sequence of FTO gene using the Primer Explorer V5.0 software (http://primerexplorer.jp/elamp4.0.0/index.htmL). To ensure specificity, all designed primers undergone Basic Local Alignment Search Tool (BLAST) program searches using the National Center for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Validation of Designed Primers

The designed primers were also validated using PCR to ensure that the primers would amplify the specific target DNA sequence without producing non-specific bands. The annealing temperature was calculated based on the outer primers (F3 and B3). Samples containing positive and negative controls were also tested. The PCR master mix reaction was as follows: 10 µL Master Mix (BiotechRabbit, Berlin, Germany), 1 µl of 20 µM forward primer (F3), 1 µl of 20 µM reverse primer (B3), 6 µL deionized water and 2 µL DNA template.

The master mix was transferred into 0.2 mL PCR tube, and briefly centrifuged. Tubes were then placed in thermal cycler (Thermo Fisher Scientific Inc., Massachusetts, United States). For the initial denaturation phase, the temperature was set at 95°C for 2 minutes. This was followed by 35 cycles of denaturation phase at 95°C for 20 seconds, the annealing phase at 56°C for 20 seconds, and the elongation phase at 72°C for 20 seconds. After amplification, the PCR products were analyzed using 1.5% agarose gel electrophoresis.

Optimization of Real-Time LAMP Reaction

The assay was performed in a total volume of 25 µL of reaction mixture using the New England BioLabs E1700 WarmStart LAMP Kit, according to the manufacturer's protocol. LAMP reaction was employed using WarmStart® LAMP Kit (DNA & RNA) and Real-time Turbidimeter LA-500 device (Eiken Chemical Co., Ltd., Tokyo, Japan). The optimized reaction temperature was 65°C based on the recommendation for the appropriate range between 60°C until 65°C (Khan et al., 2017). To optimize RT-LAMP procedure, various concentrations of FIP, BIP, F3, and B3 primers were tested, which were 1.4 µM, 1.6 µM and 1.8 µM concentration of FIP and BIP, and 0.2 µM, 0.4 µM, 0.6 µM and 0.8 µM concentration of F3 and B3 (refer Table 1).

Table 1. Real-time LAMP reaction mixture containing primers FIP and BIP with F3 and B3 primers in optimization

F3/B3 (µM)	**0.2**			**0.4**			**0.6**			**0.8**		
FIP/BIP (µM)	**1.4**	**1.6**	**1.8**	**1.4**	**1.6**	**1.8**	**1.4**	**1.6**	**1.8**	**1.4**	**1.6**	**1.8**
WS (µl)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
FIP (µl)	0.88	1	1.13	0.88	1	1.13	0.88	1	1.13	0.88	1	1.13
BIP (µl)	0.88	1	1.13	0.88	1	1.13	0.88	1	1.13	0.88	1	1.13
F3 (µl)	0.25	0.25	0.25	0.5	0.5	0.5	0.75	0.75	0.75	1	1	1
B3 (µl)	0.25	0.25	0.25	0.5	0.5	0.5	0.75	0.75	0.75	1	1	1
dH_2O (µl)	8.74	8.5	8.24	8.24	8	7.74	7.74	7.5	7.24	7.24	7	6.74
DNA (µl)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
TOTAL (µl)	25	25	25	25	25	25	25	25	25	25	25	25

Abbreviations: WS: WarmStart, FIP: Forward Inner Primer, BIP: Backward Inner Primer, dH_20: Distilled water

All reaction tubes were then briefly centrifuged to ensure the mixture was well-mixed prior to placing the tubes in LA-500 device for RT-LAMP reaction. LAMP reaction was done for 1 hour and inactivation was at 80°C. Cooling phase was activated until the temperature reached 65°C.

Results and Discussion

Primer Design of FTO Gene for Real-Time LAMP

A set of internal primers, FIP (F1c and B2) and BIP (B1c and B2) and external primers, (F3 and B3) were designed using the Primer Explorer V5.0 software (Table 2). These primers were designed based on the FTO gene, with the accession number NC_000016.10. They are located at chromosome 16q12.2, specifically at position 53,662,164 - 54,163,738. The primers' sequences, along with their melting temperature (T_m) and base pairs are tabulated in Table 3. Figure 1 shows the DNA sequences, highlighting the specific sites of the primers within the FTO gene.

Table 2. Details on primer sequences

Primers	5'pos	3'pos	len	Tm	5'dG	3'dG	GC rate	Sequence
F3	178	197	20	60.56	-5.35	-5.57	0.50	GCCTTTCTCACACTGCACAA
B3	353	371	19	59.01	-3.57	-4.72	0.47	ATTTCAGCCTCGGTGTGTT
FIP			42					GCGAGATACCGGAGTGAGCAGA-CATGGCTGCTTATTTCGGGA
BIP			40					ATTGGTAATCCAGGCTGCACCT-CTTTCACTGGCCAGGGGA
F2	199	218	20	59.99	-5.90	-6.39	0.50	CATGGCTGCTTATTTCGGGA
F1c	243	264	22	65.49	-6.28	-5.49	0.59	GCGAGATACCGGAGTGAGCAGA
B2	323	340	18	60.76	-4.02	-6.04	0.61	CTTTCACTGGCCAGGGGA
B1c	271	292	22	64.10	-4.55	-6.24	0.50	ATTGGTAATCCAGGCTGCACCT

Abbreviations: Len- length; Tm- Melting temperature; dG- free energy

Table 3. Set of primers sequence with the temperature and base pairs

Primer	Sequence (5'- 3')	T_m (°C)	bp
F3_FTO	GCCTTTCTCACACTGCACAA	60.1	20
B3_FTO	ATTTCAGCCTCGGTGTGTT	59.0	19
FIP_FTO	GCGAGATACCGGAGTGAGCAGACATGGCTGCTTATTTCGGGA	64.8	42
BIP_FTO	ATTGGTAATCCAGGCTGCACCTCTTTCACTGGCCAGGGGA	60.4	40

CCCCACAACTCCCCAAATGCTTACAAAGAAACACACCTTTGGAAATAATACAATTGTAGCTATATATCAACGTCTGTTTTTGCTTT

GGTTTTGTTTTAGTGGCAGCTGAAATATCCTAAACTAATTCTCCGAGAAGCCAGCAGTGTATCTGAGGAGCTCCATAAAGAGGT

TCAAGAAGCCTTTCTCACACTGCACAAGCATGGCTGCTTATTTCGGGACCTGGTTAGGATCCAAGGCAAAGATCTGCTCACTC
F3 F2 F1c

CGGTATCTCGCATCCTCATTGGTAATCCAGGCTGCACCTACAAGTACCTGAACACCAGGCTCTTTACGGTCCCCTGGCCAGTG
B1c B2

AAAGGGTCTAATATAAAACACACCGAGGCTGAAATAGCCGCTGCTTGTGAGACCTTCCTCAAGCTCAATGACTACCTGCAGAT
B3

AGAAACCATCCAGGCTTTGGAAGAACTTGCTGCCAAAGAGAAGGCTAATGAGGATGCTGTGCCATTGTGTATGTCTGCAGATT

TCCCCAGGGTTGGGATGGGTTCATCCTACAACGGACAAGATGAAGTGGACATTAAGAGCAGAGCAGCATACAACGTAACTTT

GCTGAATTTCATGGATCCTCAGAAAATGCCATACCTGAAAGAGGAACCTTATTTTGGCATGGGGAAAATGGCAGTGAGCTGGC

ATCATGATGAAAATCTGGTGGACAGGTCAGCGGTGGCAGTGTACAGTTATAGCTGTGAAGGTACAGTCTGCTCTTGGAAAAAG

CAGCCCTGTATGTAATAATATGACCCGAGTTGTTTAGGCTCTGGAGATACACACGCATATACATGAACATGTTTGCATGTGTGCT

TGCGTGTGTACATGCACATGCGTGTGTGTGTATCATGTGTCCTTT

Figure 1. Primers' location and binding site for FTO Gene retrieved from Primer Explorer V5.0 Software.

Validation of Designed Primer

The efficiency of the designed primer was confirmed by detecting PCR products using 1.5% agarose gel electrophoresis. A 100 base pair DNA marker was employed, and the gel showed the expected PCR product size of 170 base pairs (Figure 2).

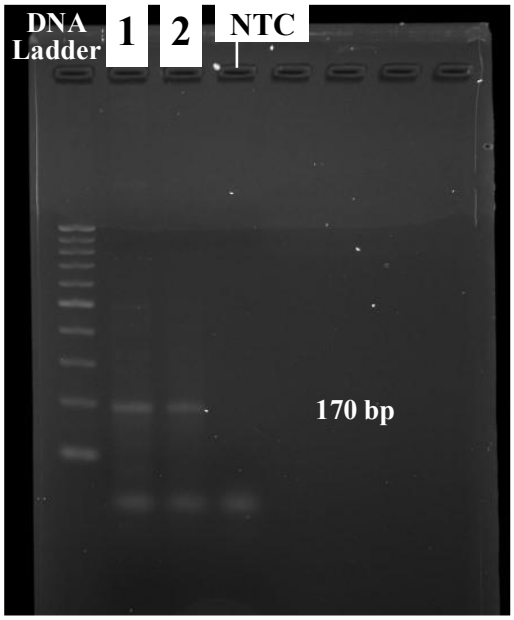

Figure 2. FTO gene detection on 1.5% agarose gel electrophoresis. 100bp DNA marker was used as DNA ladder. Lane 2 and Lane 3: Sample 1, Sample 2; Lane 4: Negative Template Control (NTC)

As shown in Figure 2, Sample 1 and Sample 2 were successfully amplified at the expected amplicon size, demonstrated the functionality of the primers. The negative control (Lane NTC) showed absence of amplicon. However, there was presence of primer dimer at the bottom of all of the samples. Primers can self-dimerize when a single primer contains complementary regions, leaving a free 3' end on the DNA template. As a result, primers bind to each other instead of the target sequence (Garafutdinov et al., 2020). Therefore, redesigning the primers, adjusting the annealing temperature and primer concentration, and increasing the number of amplification cycles are potential approaches to reduce primer dimer formation.

Optimization of Real-Time LAMP Reaction

As aforementioned, different concentrations of primers were used in RT-LAMP reaction which ranged from 1.4 μM to 1.8 μM for concentration of FIP and BIP, whilst a range of 0.2 μM to 0.8 μM was used for concentration of F3 and B3. Several optimization processes were conducted to produce a reliable result. A series of optimization was conducted for the various of concentrations of FIP with BIP and B3 with F3. However, the most reliable result in optimization was shown in a mixture of 1.6 μM concentration FIP and BIP with 0.8 μM concentration B3 and F3 (Table 4). Other mixtures of concentrations were considered fail as the readings were captured for Detection of Threshold Value (Df) and Threshold Time (Tt) values for negative control (NTC) indicating a presence of contamination. Df is the value where amplification of target sequence stops while Tt value as indication of time taken for amplification to occur. Figure 3 demonstrates the FTO gene detection using RT-LAMP. The figure shows the amplification curve interpreted the relationship between detection of threshold value (Df) values and threshold time (Tt) at different concentration of FIP and BIP as well as F3 and B3 primers. This is shown by the sharp curve with upward slope once the target DNA was amplified. The curse reaches a plateau phase as the reaction nears completion.

Table 4. Optimized concentration of FIP-BIP and F3-B3 in RT- LAMP

FIP	**F3**	**S**		**NTC**	
BIP	**B3**	**Df Value**	**Tt Value (min)**	**Df Value**	**Tt Value (min)**
1.6 μM	**0.8 μM**	0.178	34:18	0.011	-

Abbreviations: Df: Detection of Threshold Value (Df) and Threshold Time, Tt: Threshold Time, S: Sample, NTC: Negative Template Control

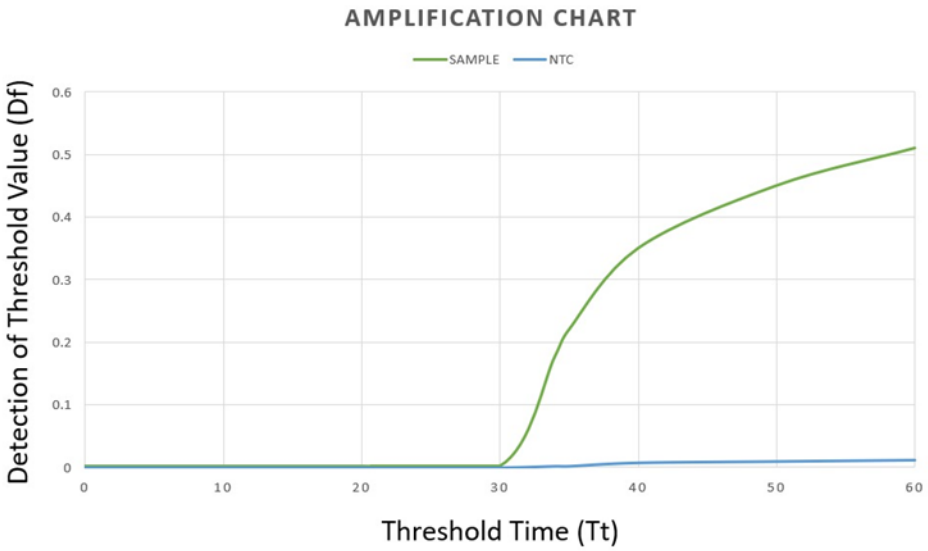

Figure 3. Amplification of FTO gene for the optimized 1.6 concentration, FIP and BIP and 0.8 concentration of F3 and B3 using real-time LAMP assay.

Validation of RT-LAMP

The validation of the RT-LAMP product was performed using gel electrophoresis to assess the characteristic the ladder-like pattern of LAMP products. Figure 4 exhibited a ladder-like pattern, demonstrating a successful amplification using RT-LAMP for sample with respective concentration of FIP, BIP, F3 and B3. No targeted sequence was amplified in Lane B for NTC.

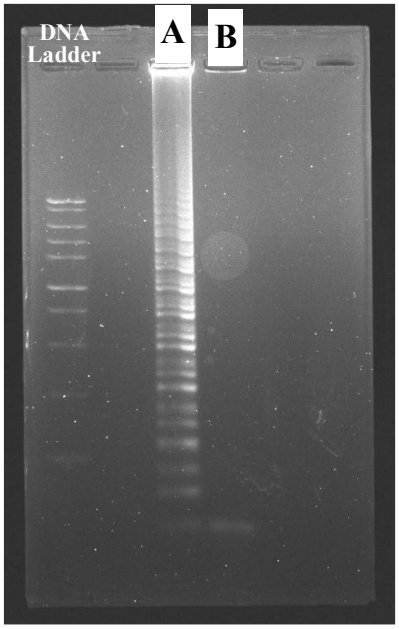

Figure 4. Validation of FTO detection on 2.0% agarose gel electrophoresis. 100bp DNA marker was used as DNA ladder. Lane A: concentration 1.6 for FIP and BIP, as well as concentration 0.8 of F3 and B3. Lane B: negative template control (NTC).

Discussion

Loop-mediated isothermal amplification (LAMP) is a technique for DNA and RNA amplification in a more rapid, specific and sensitive detection than conventional PCR. In this study, FTO gene was utilized to optimize RT-LAMP technique by detecting the presence of FTO gene and amplification process. LAMP method requires four to six primers to discriminate six to eight target sites on the target DNA. Primers include inner primers, outer primers and optionally, loop primers. Generally, this amplification assay can be employed under isothermal conditions, which is at optimum temperature, 65°C. Moreover, LAMP techniques have three steps which are initial, cyclic amplification and elongation step. Initially, DNA polymerase begins strand displacement synthesis by binding outer primers which are F3 and B3 to the target region. Meanwhile, inner primers which are FIP and BIP, start complementary strand synthesis by attaching to displaced single-stranded DNA, resulting in formation of loop structure (Panno et al., 2020).

Subsequently, the process continues with amplification step. Inner primers create loop shape that serves as templates for ongoing amplification and produce a new strand. Stretching out from the loop areas, DNA polymerase keeps creating new DNA strands during amplification process with several copies in a variety of looping and stem configurations forming a mixture of several loop cauliflower-like structures (Notomi et al., 2000). This permits DNA polymerase to initiate amplification by adding nucleotides and annealing of primers on the targeted sequence.

Development of Appropriate Primer Design

In the field of molecular genetics, the design of specific primers is essential, especially in establishing LAMP method due to few key factors and criteria that need to be followed. Both external primers worked as the starter of LAMP response while internal primers functioned to elongate the sequence (Aoi et al., 2006). All primers must comply with LAMP primer design criteria, which include melting temperature (T_m), stability at the end of primers, free energy of ends of primers, distance between designed primers as well as percentage of GC content (Aulia et al., 2023). All requirements are crucial to ensure that a primer is functioning well.

A set of primer that fit the most criteria was chosen to be utilized throughout the experiment. Melting temperature was generated when double stranded DNA product dissociates into single strands of DNA (Randhawa et al., 2013). T_m of all primers assigned as F3, B3, F2, B2, F1c and B1c were in range (59.01°C until

65.49°C) as tabulated in Table 2. Yet, the percentage of GC content of the primers was also in range between 47% to 61%. It was acceptable even though it was not considered as good primers (Aulia et al., 2023).

Moreover, the stability at the end of primers was assessed based on their free energy (dG), which should be -4kcal/mol or lower. The more negative dG value, the more stable the structure. In previous study, values for target end stabilities of primers were considered acceptable as stability for primers were less than -4kcal/mol (Davidson, 2021). Primers were validated through BLAST from NCBI to confirm matched sequence of primer from the database.

The amplicons observed for samples 1 and 2 confirmed the functionality of the primers. In contrast, the negative control (Lane NTC) showed no amplification, verifying that no unintended products were generated.

Optimization of Real-Time LAMP Technique in Amplification of FTO Gene

After several optimization processes, reliable results were obtained with no Df and Tt values observed for NTC in a mixture of concentration 1.6 μM of FIP and BIP that mixed with concentration of 0.8 μM of F3 and B3. To verify the results, RT-LAMP products were tested using 2.0% agarose gel electrophoresis. A ladder-like pattern was displayed as the confirmation of specificity of RT-LAMP products with the expected size of 102 to 115 base pairs indicating the product was specifically amplified (Ali et al., 2022; Notomi et al., 2000). The ladder-like pattern observed on the gel resulted from the synthesis of stem–loop DNA with variable stem lengths, as well as cauliflower-like structures containing multiple loops formed by progressively inverted repeats of the target sequence (Panno et al., 2020).

Function and Significance of Melting Temperature (T_m) and Betaine

Melting temperature (T_m) is the most crucial key factor to ensure optimal amplification of target sequence. Although the recommended range for LAMP primers is between 60°C and 65°C, the specific T_m may vary depending on the target sequence (Soroka et al., 2021).

Betaine concentration is another important factor for producing highly specific DNA amplification. Betaine, that was generally mixed into WarmStart master mix, enhances the amplification of FTO gene by reducing the formation of secondary structure that is caused by GC- rich regions (Foo et al., 2020). The optimum betaine concentration may vary depending on the specific DNA sequence.

Troubleshooting of RT-LAMP Optimization

Optimizing RT-LAMP for high specificity and sensitivity is challenging, with the risks of non-specific amplification and false positives. Achieving optimal conditions for temperature, primers, and reactions is time-consuming and complex. Due to time-constraint, the optimization of RT-LAMP using different temperature could not be done. The range of optimum temperature for primers are between 60°C until 65°C (Khan et al., 2017). The optimum temperature depends on the concentration of primers during the amplification process. As RT-LAMP technique is extremely sensitive, any possible contamination might occur. Apart from cross-contamination, Contamination may occur as a result of carryover during master mix preparation. All equipments must be autoclaved before running the assay and bench is required to be cleaned from any possible contaminants. Moreover, NTC manifested value might be due to non-specific amplification. Amplification can arise from the secondary structure and terminal transferase-like activity of the DNA polymerases employed in RT-LAMP because of the high concentration of the primers utilized in the RT-LAMP process (Jainonthee et al., 2022). During the experiment, the sample and reagents were required to be kept in iced box to avoid degradation (Oosting et al., 2020). Another pitfall in this study was the primer dimer formation. Precautionary measures should be taken to mitigate self-dimerization such as redesigning the primer to ensure the primer are designed with low complementarity to themselves particularly in the 3′ end regions, to avoid heterodimerization. In addition, reducing primer concentration in the PCR reaction can help minimize primer dimer formation as to reduce non-specific binding. Moreover, the use of a high-fidelity polymerase can also improve the specificity of the amplification and reduce the chances of primer dimer formation by enhancing the accuracy of DNA synthesis (Garafutdinov et al.,2020). Validation of the results via sequencing could not be done due to time constraints.

Conclusion

This study had successfully optimized the RT-LAMP technique for FTO gene detection using one primer set designed based on GC content and melting temperature. Even though the optimization of temperatures was not conducted due to time constraint, the temperature selection on 65°C was successful. However, this study can be improved by eliminating self-dimerization of primers. Real-time LAMP proved to be more sensitive and faster than the conventional PCR where it can complete amplification in 30 minutes. This demonstrates its potential for rapid FTO gene screening. Further development of this method can facilitate the widespread application of quantitative genetic diagnostics in various fields.

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Conflict Of Interest

No conflict of interest.

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