DEVELOPMENT OF IN-SILICO ALLELE POLYMERASE CHAIN REACTION FOR THE DETECTION OF MAML3 RS58287721 VARIANTS

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ABSTRACT

MAML3 is the transcriptional coactivator in the NOTCH1 pathway. Both has strong correlation in development of an individual. Development of in-silico polymerase chain reaction (PCR) is a computational simulation that able to define the PCR product on wet laboratory. Previous study founded 11 bases of deletion on suspected Marfan syndrome sample on gene MAML3 rs58287721. Development of in-silico allele PCR of gene MAML3 rs58287721 variants is aimed. In-silico primer designing and in-silico primer validation are the methods. Primer designing using Oligo Explorer 1.2 aided in designing the primers according to the primer criteria while the primer validation using BLAST tool, analyse identical sequences measured by expected value less than 0.5. The outcome of the designed primers for validation recorded 0.003 through the BLAST tool for databases of Human G+T. The PCR product recorded 362 base pair. It would produce three genotypes with different gel bands reading. Further investigation on mutation of MAML3 rs58287721 through wet laboratory is recommended.

INTRODUCTION

- MAML3 gene is a coactivator transcriptional factor in NOTCH1 signalling pathway(1). It is acknowledged as widely express gene(2).
- The NOCTH1 receptor previously has been reported in patients with bicuspid aortic valves (BAV) and BAV aortopathy due to the presence of mutation(3).
- Previous pilot study has founded 11 bases of deletions removes DNA of GCTGCTGCTGC in a sample of suspected Marfan syndrome.
- National Center for Biotechnology Information (NCBI) is a common platform used for retrieving the gene data details(4).
- In-silico PCR is the most suitable technique for initial steps in studying the gene mutation(5).
- Developing a primer according to the criteria is the most crucial phase before proceeding to further phase of the lab(6).
- The primers were designed with the aid of Oligo Explorer software and the uniqueness of the primers were assessed using BLAST software(6).
- Formation of secondary structure while designing - hairpins, self-dimers or cross dimers(7).
- Efficacy of Gibbs free energy (ΔG) portrays the value to break the secondary form structure of DNA – the nearer to the 0, the better(8).

OBJECTIVE

To develop the in-silico allele polymerase chain reaction technique of gene MAML3 rs58287721 variants.

METHODOLOGY

IN-SILICO PRIMER DESIGNING

RETRIEVE NUCLEOTIDE DATA

- Collect the information of MAML3 rs58287721 on SNP database, Nucleotide database.

LOCATING SNP AND PRIMERS

- Guide in locating the primers in MAML3 sequence.

DESIGNING PRIMERS

- Design according to the primer criteria. (Tm, length, GC content)

IN-SILICO PRIMER VALIDATION

Table 1. The forward and reverse primer designed in Oligo Explorer 1.2 software

Primer Name	Primer Sequence (5' to 3')	T_m (°C)
forward_MAML3	GGCACTATAGGGTTTTGGTTGTTA	61.2
reverse_MAML3	GGAAATGGTTATCTCCTGAATCCG	60.7

SEARCH SIMILARITY

- Compares the range of sequence between biological sequences.
- Determine the significance of similarity and uniqueness

RESULTS

- 2 databases involved; Human G+T contains human RefSeq transcript and genomic sequence.
- Primers created is unique as the EV recorded 0.003.

Table 2. The designed primers uniqueness value through BLAST tool software

Database	Common name	Query cover	E value
TRANSCRIPTS			
Homo sapiens MAML3, mRNA	Homo sapiens	100%	0.003
GENOMIC SEQUENCES			
Homo sapiens chromosome 4, GRCh38.p12	Homo sapiens	100%	0.003

Table 3. Simplified result of secondary structure DNA from the designed primers

Primer Properties	ΔG	T_m (°C)	bp
Upper primer			
Self-annealing	-4.35	-16.00	6
Lower primer			
Self-annealing	-2.05	10.00	3
	-2.05	10.00	3
Primer loops	-2.43	10.00	3
	-2.43	10.00	3
Upper-lower primer annealing	-2.01	10.00	3
	0.77	6.00	3
	0.98	6.00	3

ΔG=Gibbs Free Energy, Tm=Melting Temperature, bp= Base Pair

- ΔG reported -4.35 kcal/mol of self anneal upper primer – slightly stable
- ΔG reported satisfactory value -2.05 till -2.43 kcal/mol for lower primer, primer loops and upper-lower primer – not stable
- Tm value is based on the bp value.

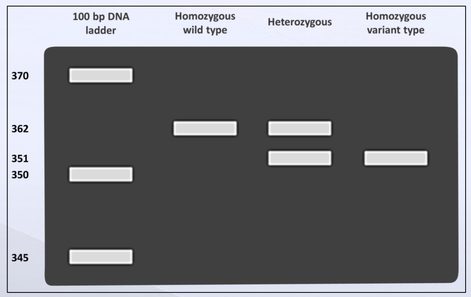

Figure 1. Expected variants of MAML3 rs58287721 in gel band electrophoresis based on the developed in-silico PCR

- Homozygous wild type – reading of normal alleles.
- Heterozygous – reading of one allele is mutated while the other allele is normal.
- Homozygous variant type – reading of mutated alleles

DISCUSSION

- Designing and planning the primers are crucial in PCR procedures as it will determine the optimization towards the targeted DNA.
- Set of primers were validated through the NCBI BLAST programme to examine the efficiency, specificity as well as sensitivity.
- The EV is a parameter that outlines the number of sequences that achieved to be expected.
- Best value that displayed from the EV is 0.5 and below shows that the primers are unique and specific towards the intended study gene.
- Outcome of the amplification PCR product depends on the designed primers and produce 3 genotypes that distinguished by the expression on allele.
- Secondary structure form of DNA could led to a poor or absent yield of PCR product in gel electrophoresis.

CONCLUSION

- MAML3 have a strong connection with the NOTCH1 signalling pathway acts as the transcriptional coactivator in the pathway.
- The development of in-silico PCR one of the most advantageous and competent approaches techniques.
- Aid in determining the possible discrepancy towards the primer.
- Further investigation on the mutation of MAML3 rs58287721 is recommended through the wet laboratory experiment.

ACKNOWLEDGEMENT

Gratitude is addressed to the supervisory of the research study, Dr Mohd Nazif bin Samat @ Darawi, the dean and senior lecturer of Faculty Health Sciences, UNISEL on his time, assistance, guidance and comments on this research study.

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