Development of In Silico Allele Specific Polymerase Chain Reaction for Determining the Variant of PLOD1 rs7551175

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ABSTRACT

Abnormality in Procollagen lysine, 2-oxyglutrate, 5-dioxygenase enzyme can cause hereditary connective tissue disorder known as the kyphoscoliotic type of Ehlers-Danlos Syndrome (EDS). It might be caused by mutation in the human lysyl hydroxylase 1 gene (PLOD1). Insight is normal, life span may be normal, but individuals at risk of medium-sized artery rupture are directly impacted. Diagnosis is only considered very late in most cases. This study aims to develop a methodology used to calculate theoretical PCR outcomes or In-Silico PCR. The In-Silico PCR method is useful for analysing primers or probes rapidly against target sequences, determining the position of the primers, orientation, binding efficiency, and calculating their Tm's. The study mainly focuses on the determination of the rs7551175 variant of PLOD1, as preparative works before utilizing the actual or hands on of PCR in the molecular laboratory. In order to determine the variant of PLOD1, two pairs of forward and reverse primers were designed with the aid of Oligo Explorer 1.2 software by referring to the primer design guidelines. Several sizes of PCR products were produced theoretically on computer simulation to distinguish the genotype of sample whether it is homozygous wild-type, heterozygous, or homozygous variant.

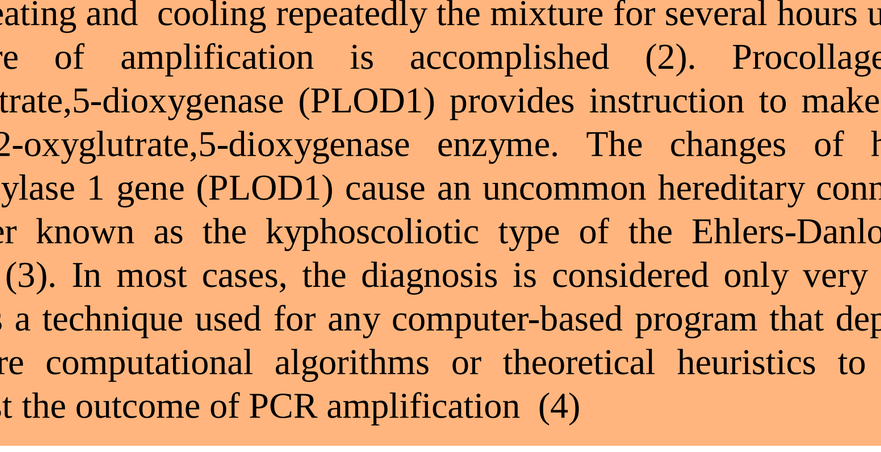
INTRODUCTION

Allele-specific polymerase chain reaction (AS-PCR) is a polymerase chain reaction based method which can be utilized to recognize the known single nucleotide polymorphism. A specific primer is designed to enable amplification by DNA polymerase if the nucleotide at '3 - end of the primer correspond to the base at wild-type sequences (1). PCR is an in vitro technique use to make genetic material DNA in several order of magnitude also used for nucleic acid amplification in molecular biology, it is among the most important laboratory techniques in genetics and molecular diagnostics, PCR totally includes combining a DNA sample with deoxynucleotides, oligonucleotide primers, triphosphates, and the Taq thermostable DNA polymerase in a preferable buffer, then heating and cooling repeatedly the mixture for several hours until the ideal measure of amplification is accomplished (2). Procollagen lysine,2-oxyglutrate,5-dioxygenase (PLOD1) provides instruction to make Procollagen lysine,2-oxyglutrate,5-dioxygenase enzyme. The changes of human lysyl hydroxylase 1 gene (PLOD1) cause an uncommon hereditary connective tissue disorder known as the kyphoscoliotic type of the Ehlers-Danlos Syndrome (EDS) (3). In most cases, the diagnosis is considered only very late.In silico PCR is a technique used for any computer-based program that depends on one or more computational algorithms or theoretical heuristics to calculate or forecast the outcome of PCR amplification (4)

OBJECTIVES

To develop In Silico allele specific polymerase chain reaction technique for the detection of PLOD1 rs7551175 variant, and to make a systematic review on the relationship between PLOD1 and Kyphoscoliotic Ehlers Danlos Syndrome.

METHODOLOGY



RESULT

Table 1 Upper Primer Self Annealing(FAS)

PRIMER SEQUENCE	Dg	Tm
5'-GAGGATCTGGTCATTCTCTTCG-3' |||| 3'-GCTTCTCTTACTGGTCTAGGAG-5'	-3.30 kcal/mol	12.00°C
5'-GAGGATCTGGTCATTCTCTTCG-3' ||| :: :: ::: 3'-GCTTCTCTTACTGGTCTAGGAG-5'	-1.38 kcal/mol	10.00°C

Table 2 Upper Primer Loop(FAS)

PRIMER SEQUENCE	dG	Tm
5'-GAGGATCTG ||| ::) 3'-GCTTCTCTTACTG	-0.96 kcal/mol	10.00°C
5'-GAGGATCTGGTCATTCTCTTCG-3' ||| :: :: ::: 3'-GCTTCTCTTACTGGTCTAGGAG-5'	-1.38 kcal/mol	10.00°C

Table 3 Lower Primer Self Annealing(FAS)

PRIMER SEQUENCE	dG	Tm
5'-GGACCTGTCTCTGTCTGC-3' ||| ::: 3'-CGTCTGTCTCTGTCCAGG-5'	-1.89 kcal/mol	10.00° C

Table 4. Lower Primer Loop(FAS)

PRIMER SEQUENCE	dG	Tm
5'-GAGGATCTGGTCATTCTCTTCG-3' |||| 3'-GCTTCTCTTACTGGTCTAGGAG-5'	-3.30 kcal/mol	12.00°C
5'-GAGGATCTGGTCATTCTCTTCG-3' ||| :: :: ::: 3'-GCTTCTCTTACTGGTCTAGGAG-5'	-1.38 kcal/mol	10.00°C

Table 6 Upper Primer Self Annealing(RAS

PRIMER SEQUENCE	dG	Tm
5'-GGTCAGAGTTGCTGGACC-3' :::: |||| 3'-CCAGGTCGTTGAGACTGG-5'	-5.24 kcal/mol	14.00 ºC
5'-GGTCAGAGTTGCTGGACC-3' : ||| ::: : 3'-CCAGGTCGTTGAGACTGG-5'	-0.88 kcal/mol	10.00 ºC

Table 7 Upper Primer Loop(RAS)

PRIMER SEQUENCE	dG	Tm
5'-GGTCAGAGT ||||) 3'-CCAGGTCGT	-4.83 kcal/mol	14.00 ºC
5'-GGTCAGAG : ||| T 3'-CCAGGTCGT	-0.46 kcal/mol	10.00 ºC

Primer Sequence for the detection of PLOD1 rs7551175

No	Primer Name	Primer Sequence (5' to 3')
1	FAS_rs7551175_PLOD1	GAGGATCTGGTCATTCTCTTCG
2	RC_rs7551175_PLOD1	GGACCTGTCTCTGTCTGC
3	RAS_rs7551175_PLOD1	CCCACCTACCTGTCTGT
4	FC_rs7551175_PLOD1	GGTCAGAGTTGCTGGACC

FAS x RC = 339 bp
RAS x FC = 534 bp
RC x FC = 835 bp

CONCLUSION

This study clarifies the use of in silico PCR in producing good designed primers with theoretical results, which may help the researchers to carry out the actual PCR genotyping test in the future. Hence, it will enable researchers to find out whether variants can cause the illness so monitoring task towards the patient can be carried out earlier.

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REFERENCES

1 Darawi, M. N., Ai-Vyrn, C., Ramasamy, K., Poi, P., Hua, J., Pin, T. M., … Majeed, A. (2013). *Allele-specific polymerase chain reaction for the detection of Alzheimer's disease-related single nucleotide polymorphisms*.

2 Ruslan Kalendar, Bekbolat Khessenove, Yerlan Ramankulov, Olga Samuilova, K. I. I. (2017). FastPCR: An in silico tool for fast primer and probe design and advanced sequence analysis. *Genomics*, *109*(3–4), 312–319. 4 Chukwuemeka, O., Umar, H. I., Folake Olukunle, O., Oretade, O. M., Olowosoke, C. B., Akinsola, E. O., … Oretade, O. J. (2020). In silico design and validation of a highly degenerate primer pair: a systematic approach. *Journal of Genetic Engineering and Biotechnology Research*, 1–17.

5 Elsalam, K. A. A.-. (2003). Bioinformatics tools and guideline for PCR primer design. *African Journal of Biotechnology*, *2*(5), 91–95