Point mutation detection by economic HRM protocol primer design

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ABSTRACT

Globally more than 100 million SNPs in populations. These variations approximately 4–5 million SNPs in a people genome, occur almost every 1000 nucleotides on average and present either unique or in many in individuals. They can act as genetic signs, associated with illness and respond to chemicals and drugs. SNPs occurrence within or near a gene play important role in disease throughout affecting gene task. Frequently many protocols have been used to study single nucleotide polymorphism (SNP) among human variants genome. Restriction fragment length polymorphism (RFLP), Amplification refractory mutation system PCR (ARMS-PCR), sequencing and SNaPshot assays considered familial methods. The potential risk of contamination after PCR is common due to further other steps. In this direction, a high resolution melting (HRM) real-time PCR method is an alternative, reducing the post-PCR transferring steps. uVariants is clarified as appropriate website for designing primers used for SNP recognition by easy and inexpensive protocol called HRM. The researchers can focus on the interest of reference SNP ID number, or “rs” ID to avoid loss time. In this article description how to uses uVariants website for primer design used in HRM technique.

Aims: To describe uVariants and uDesign software, application and usefulness of HRM technique primer design in the genotyping SNPs among people and public health.

Accessibility and requirements: uVariants and uDesign are freely accessible at: https://www.dna.utah.edu/variants/; https://www.dna.utah.edu/udesign/app.php respectively. The network server supports the browsers: Chrome, Firefox, Torch, CoolNovo, 360 Browser, Internet Explorer, Opera, and Safari.

1. Introduction

One of the most important output of human genome project is the use Single nucleotide polymorphism (SNP) genotyping [1]. It has been increased utilized in a diverse genetic disciplines especially in studying genetic determinants of diseases [2]. Variations and mutations as an example explained the difference of genetic responses to the environment in addition to variation effects of drug treatments. Several studies like molecular genetics and pharmacogenomics are associated with these variations [3]. Consequently, there are many genotyping technologies were suggested to detect and confirm these variations such as RFLP-PCR, and allele-specific (AS-PCR) assay [4,5]. Programs of primer design consider heart in optimizing the polymerase chain reaction (PCR). An unsuccessfully designed primer can give tiny or no product. For this mandatory matter, several web-based or secluded standard free program for designing primer in PCR are offered, but fluctuate in their quality and functionality [6,7]. Here, will focus on cost-effective and an easy protocol, namely HRM genotyping technique which designed there primer pairs by uVariants and uDesign.

High Resolution Melting (HRM) is a relatively new technique started from 2002 and increase their uses in the SNPs studies based on post-PCR analysis used for recognize genetic variation in nucleic acid sequences [8]. It is a highly effectiveness, robust and powerful protocol to identify DNA variants, particularly single nucleotide polymorphism (SNP) and forensic genotype [9]. HRM technology permits to determine large number of genotype genetic variants samples in short time and less expenditures with highly accuracy and simplicity [10]. HRM analysis depends mainly on the dismantling dsDNA throughout gradually raise temperature. In which amplicons are generated firstly by traditional amplification, after that increase temperature gradually from around 50 °C up to around 95 °C [5,11]. In typical procedure the temperature elevated in the presence of brighter dyes that bind to dsDNA at higher concentrations like SYBR green I, LC Green, SYTO Dye and EvaGreen in addition to an instrument have ability to collect fluorescence data in small temperature changes [12]. Thus, we recommended application of HRM analysis as a good means for SNP-genotyping for...
discriminate variants at target loci.

2. Designing primer pairs examples by uVariants and uDesign

Below at Table 1, sequences of two primer pairs designed for tumor necrosis factor alpha (TNFα) gene (6p21.33) (NC_000006.12) (dbSNP; rs180029 C > T) and TP53 gene (17p13.1) (NC_000017.11) (dbSNP; rs1800370 A > G) polymorphisms in the 5′ flanking region that can be used in the uVariants and uDesign respectively with default parameters settings (minimum primer length 10, maximum primer length 40, and primer Tm 60) to design primers for polymorphisms related to both SNPs.

| SNP       | Primer Sequences 5′-3′ | bp | Tm °C |
|-----------|------------------------|----|------|
| rs180029 C > T | FWD-CCCCACAAAGCTTCTTCTT REV-AAATCAGAATTTTTCGATTTT   | 20  | 60.1 |
|           |                        | 27  | 60.2 |
| rs1800370 A > G | FWD-GCATCAAATCATCCATTGCTGGG REV-ATCTACAGTCCCCCCTG   | 24  | 60.4 |
|           |                        | 18  | 60.0 |

3. Data input

At this time, uVariants (v1.9 b) and uDesign (v2.4.0) are able to deal with many sequences of genome. These programs are depend on the ENSEMBL database in their designing. The interested rs SNP ID can be defined principally by NCBI then pasting in the text box of programs as in (Fig. 1) and (Fig. 2) respectively.

4. uVariants and uDesign software workflow

uVariants and uDesign both are created to support the development of web application involving web services and resources. The submission is integrated into the SNP database running database server. Normally this database powered by ensembl and collects public SNP data and related reference statistical data from public SNP databases.
Fig. 3. Graphical uMelt preview of rs180029 (TNFα) gene designed by uVariants.

Fig. 4. Graphical uMelt preview of rs1800370 (TP53) gene designed by uVariants.
Fig. 5. Graphical uMelt preview of rs180029 (TNFα) gene designed by uDesign.
5. HRM primer design protocol overview

Since the intercalating dyes that used in HRM method have ability to bind generically to any double stranded DNA product (non-specific), this lead to design primer with avoiding template secondary structures and robust performance and must be specific to the intended region. Briefly, Design (identify target sequence), checking specificity (BLAST primer sequences) on the NCBI site, run PCR and HRM in appropriate real-time PCR instrument, and analyze results (real-time & HRM). The general specifications tips for HRM primer design were explained bellow.

1. Primer length should be ∼20 nt.
2. Primer Tm range between 58 62 °C, optimal 60 °C.
3. Primer Tm difference within < 2 °C for Forward and reverse.
4. Amplicons length preferred < 250 bp to ensure sensitive detection of variants.
5. GC contents 40–100%.
6. GC clamp Maximum of 2 G or C nucleotides in the last 5 nt at 3′ end.

6. Discussion

Currently, a new model of web calculation and interactivity used to web page using HTML5 and JavaScript. Scientific applications supported by this arrangement because the quick progress in the performance of JavaScript engines and HTML5-compatible browsers [13]. Primer design for PCR in eukaryotic genes to detect of variants is a complex and boring job. The reason of this work was to give an idea about designing primer that used in High Resolution Melting (HRM) technology by several disciplines for SNPs analysis [14]. This paper adopted a simplified scientific explanation method for all steps of the program used in this technique without deepening the mechanism of it and/or the complexities that accompany HRM technique to offer a clear and easy guide for anyone who wants to start using this program accurately, scientifically and simply. In conditions of this analysis process, the determination between at least two genotypes to be a significant distinguishable and carried out simply in a single closed tube with minimum expenditure and reagents consumption. However, HRM analysis of three genotypes based on dissociation curve behavior of dsDNA and melting peaks plots with high stability and accuracy during temperature rise. So, in typical method the amplicon is first produced by traditional amplification and then subjected to raise in temperature gradually in the presence intercalating dye like SYBR green I [15,16].

The principle of this technique depend mainly on the contents of the amplicon, and the region rich with GC pairs dissociate later compared with region rich with AT. From the most familiar application of HRM are genotyping of SNPs. SNP is a replacement one nucleotide instead another in genome that appear at greater than 1% in a given population. They classified into 4 types (type I, C/T and G/A and account 64%, temperature shift is high; type II, C/A and G/T and account 20%; type III, C/G and account 9%; type IV, A/T represent 7%, temperature shift is low) in human depending on the type of base exchange [17].

Fig. 6. Graphical uMelt preview of rs1800370 (TP53) gene designed by uDesign.
7. Conclusion

The Protocol of high resolution melting had been effective in analysis of SNPs variations. The beneficial of this technique was marked in fast, easy, and successful, but need optimization for their protocol.

Conflicts of interest

I would like to declare there is no confliction in this work.

Abbreviations

SNP Single nucleotide polymorphism
RFLP-PCR Restriction fragment length polymorphism polymerase chain reaction
ARMS-PCR Amplification refractory mutation system polymorphism polymerase chain reaction
BLAST Basic local alignment search tool

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2019.100628.

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