



RESEARCH PAPER

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Effect of single nucleotide mismatch at 3'-end of the primers in selected candidate micro RNA breast cancer genes

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Abstract

Polymerase chain reaction (PCR) is an important biological tool for qualitative and quantitative detection and amplification of desired sequence of DNA (deoxyribonucleic acid) in living organisms. The success of PCR reaction is based on the specificity of the primers used. The potential of these primers can be influenced by primer-template DNA, primer-primer complementarity, annealing, extension temperatures and nature of the mismatch. But, often the powers of thermal cycler are restricted due to primer-template mismatches which can lead to inaccuracies/false results. In the present study, the effect of single nucleotide mismatches (No change, T/A and C/G) at the 3'-end of primers on PCR reaction of breast cancer candidate microRNA genes i.e; hsa-mir-196a-2 (1), hsa-mir-196a-2 (2) and hsa-mir-146a respectively was evaluated in the human genome using conventional PCR. Here we conclude that no effect was found on the PCR product of these studied genes with introduced single mismatches at the 3'-end of primers under standard PCR conditions.

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Introduction

PCR is a powerful tool by which specific DNA sequences can be amplified more than a million times using thermal cycler (Kalle, *et al.*, 2014). Primer-template specificity is the key to successful PCR amplification (Simsek and Adnan, 2000). The degree of primer-template mismatch is directly proportional to success/failure of the PCR reaction. The 3'-end primer sequence is crucial for the sensitivity and specificity of PCR reaction. Mismatches at these sites significantly decrease the efficacy of the PCR reaction. In the present study selected candidate micro RNA breast cancer genes. To briefly explain, micro RNA are highly conserved, small (19–24 nt) non-coding RNA molecules that regulates the expression of protein coding genes (Kung, *et al.*, 2013). Variations in the DNA sequences of micro RNA genes leads to abnormal function of protein coding genes.

Such changes ultimately leads to the development of vast variety of several diseases and different types of cancers (Iorio, *et al.*, 2005; Farazi, *et al.*, 2013). HsamiR-146a is candidate micro RNA for breast cancer. It is already been investigated that hsa-mi R-146a is over expressed in different type of cancers including breast carcinomas, endocrine, pancreatic tumors and prostate cancer (Garcia, *et al.*, 2011; Volinia, *et al.*, 2006). Hsa-mi R-196a-2 polymorphism was related to lung cancer, colorectal cancer, and breast cancer (Hu, *et al.*, 2009; Jedlinski, *et al.*, 2011). Similarly it was already investigated that polymorphism in hsa-mi R-499 gene is connected with a potentially increased cancer risk (Qiu, *et al.*, 2012). The present study is designed to investigate the consequences of single mismatch at 3'-end of the primers in selected candidate micro RNA breast cancer genes during PCR reaction.

Materials and methods

Blood sample collection and DNA isolation

About 5 ml Blood samples were collected from five healthy female individuals. The current work will follow the guidelines of Helsinki declaration (Rickham, 1964). DNA was isolated from these blood samples using phenol chloroform protocol. The quantity and quality of isolated DNA was analyzed using spectrophotometer.

Primers designing

Primers for the studied micro RNA genes were designed using online tools. A single mismatch was introduced at the 3'-end (No change, T/A, C/G and C/T) in each primer as shown in Table 1.

PCR conditions

Two selected breast cancer candidate micro RNA genes were included in the present study. A small region (178bp, 149bp and 146bp) of hsa-mir-196a-2 (1), hsa-mir-196a-2 (2) and hsa-mir-146a (1) were amplified using pair of selected primers with mismatched insertion at 3'-end of primers as indicated in table. 1. 25 ul reaction volumes with 20 pmoles of each primer were used for amplified Isolated DNA. Dream Taq Green (Thermo Scientific #K1081) were used for amplification of the desired gene sequence. Each cycle of amplification contained the following steps: initial denaturation at 94°C for 5 minutes; 30 cycles for 1 minutes at 94°C, at 53°C, 60°C and 61°C for 1 minute, at 72°C for 1 minute and final elongation 8 at 72°C for hsa-mir-196a-2 (1), hsa-mir-196a-2 (2) and hsa-mir-146a (1) using thermal cycler (Kyratec, Model; SC300, Australia). The PCR products were run on 4 % agarose gel. 50bp marker (Thermo Scientific gene Ruler 50bp DNA Lader # SMO373) was used as standard. The PCR products were visualized using VILBER LOURMAT.

Results and discussion

In the present work the consequences of primer-template single mismatch at the 3'-end of primers was investigated. For this purpose two selected candidate micro RNA genes were chosen due to their smaller sequence size. These genes included hsa-mir-196a-2 (1), hsa-mir-196a-2 (2) and hsa-mir-146a (1). It was noticed that the PCR reactions gave perfect results despite mismatched introduced at the 3'-end of each primer (see the table 1). One gene hsa-mir-196a-2 was used as control with no alteration at the 3'-end of the primer pair designed for this gene. The PCR product obtained with all three mismatches were exactly the same as expected by the primers with no mismatch as shown in Fig. 2 at calculated annealing temperatures.

This resulted in specific PCR products (178bp, 149bp and 146bp) for each gene as shown in Table 1. Fig. 1, 2, 3 are the presentative PCR amplification results by using primers with alteration at the 3'-end. Amplification of the segment of DNA is based on the design of perfectly matching primers remains an unsolved problem.

The investigation of PCR parameters mainly primer-template perfect matching is crucial for successful PCR reaction. Latest modern online tools are required for designing of domain-specific primers that could reduce the chances of false positive/false negative results of the PCR reaction.

Table 1. List of primers with their relevant Primer Sequences (mismatch in each is primer sequence is highlighted with bold and underline letters).

Primer Name	Primer Sequence (mismatch in is indicated in bold and underline letters)	Mismatch	Expected PCR product size
hsa-mir-196a-2 (1)	FP:-5'-GGGCTGAATTTCTTCCTTCC-3' RP:-5'-CTCGACGAAAACCGACTGAT-3'	No change	178bp
hsa-mir-196a-2 (2)	FP:-5'-CCCCTTCCCTTCTCCTCCAGATA-3' RP:-5'-CGAAAACCGACTGATGTA <u>ACTCAG</u> -3'	T/A	149bp
hsa-mir-146a (1)	FP:-5'-CATGGGTTGTGTCAGTGT <u>CAGAG</u> CT-3' RP:-5'-TGCCCTC TGTCTCCAGTCTTCCAA-3'	C/G	146bp

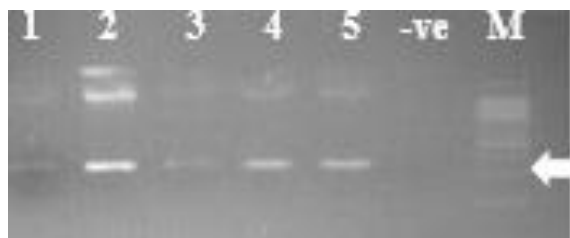


Fig. 1. Representative PCR product of hsa-miR-196-2 (1) with primer pairs (with no change at 3'-end) on 4% agarose gel.

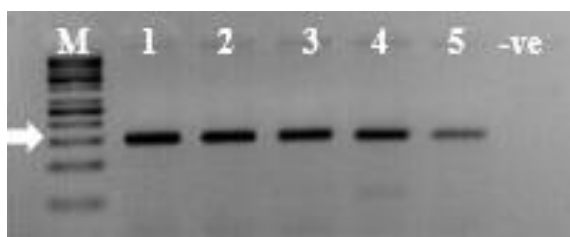


Fig. 2. Representative PCR product of hsa-miR-196-2 (2) with primer pairs (with change of T/A at 3'-end) on 4% agarose gel.

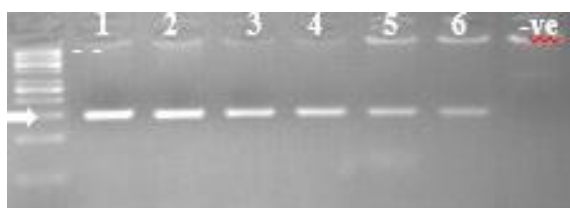


Fig. 3. Representative PCR product of hsa-miR-146a-1 with primer pairs (with change of C/G at 3'-end) on 4% agarose gel.

The effect of primer-template mismatch in the primer sequences were widely studied by introducing base alterations in different living organisms (Kwok *et al.*, 1990; Huang *et al.*, 1992; Christopherson *et al.*, 1997; Smith *et al.*, 2002; Bru *et al.*, 2008). The present study showed that there is no effect on PCR product using primers with introduced single discrepancy at the 3'-end which was in accordance with previous observation (Simsek and Adnan, 2000). Simsek *et al.*, further investigated the effect of three different mismatches at the 3'-end of a primer to amplify a human globin gene using different annealing temperatures. Our work is mainly focused on the effect of primer-template mismatched on conventional PCR in contrast to study performed by C. Christopherson *et al.* who focused on the effects of various modified bases in the primers on RT-PCR using samples that showed various HIV-1 subtypes. They also studied the effect of changing in annealing temperature in RT-PCR while annealing temperature was constant in our case (Christopherson *et al.*, 1997). Our findings were in contrast to previous studies by R. Stadhouders *et al.* who investigated that mismatches placed in the 3'-end region of the primers have greater effects on priming efficacy than more 5 located mismatches using 5 Nuclease Assay (Stadhouders *et al.*, 2010). D. Bru *et al.* also investigated the effect of single mismatch on efficiency of PCR amplification during real-Time PCR reaction.

It was concluded that the efficacy of PCR reaction is dependent on the mismatch position and the primer used (Bru *et al.*, 2008). In conclusion the no effect was detected on the PCR product of these studied genes with introduced single mismatches at the 3'-end of primers under standard PCR conditions. Further study is required to explore the consequences of primer-template mismatch on different locations at 3'-end of primer pairs in other microRNA candidate genes in breast cancer.

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Declaration of interests

No conflict of interest exist.

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