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Rapid diagnosis of common deletional α-thalassemia in the Chinese population by qPCR based on identical primer homologous fragments

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ABSTRACT

Objective: In China, $-^{SEA}$, $-\alpha^{3.7}$ and $-\alpha^{4.2}$ are common deletional α-thalassemia alleles. Gap-PCR is the currently used detection method for these alleles, whose disadvantages include time-consuming procedure and increased potential for PCR product contamination. Therefore, this detection method needs to be improved. Based on identical-primer homologous fragments, a qPCR system was developed for deletional α-thalassemia genotyping, which was composed of a group of quantitatively-related primers and their corresponding probes plus two groups of qualitatively-related primers and their corresponding probes. In order to verify the accuracy of the qPCR system, known genotype samples and random samples are employed.
Result: The standard curve result demonstrated that designed primers and probes all yielded good amplification efficiency. In the tests of known genotype samples and random samples, sample detection results were consistent with verification results.
Conclusions: In detecting αα, $-^{SEA}$, $-\alpha^{3.7}$ and $-\alpha^{4.2}$ alleles, deletional α-thalassemia alleles are accurately detected by this method. In addition, this method is provided with a wider detection range, greater speed and reduced PCR product contamination risk when compared with current common gap-PCR detection reagents.
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1. Introduction

Thalassemia is a common monogenic recessive disease whose carriers primarily inhabit in the tropical and subtropical regions. Guangxi, Guangdong and Hainan are the provinces that the majority of thalassemia allele carriers in the Chinese population are concentrated. Composed of two normal α chains and two normal β chains, normal hemoglobin (Hb) is a tetramer (α2β2). According to the locations of their defect types, α-thalassemia and β-thalassemia are two of the most common thalassemia types. In the case of α-thalassemia, the molecular biological mechanism is a defect in the α-globin gene cluster (NG_000006.1) and the most severe form of α-thalassemia gives rise to Hb Bart's hydrops fetalis syndrome and HbH disease. Hb Bart's hydrops fetalis syndrome is commonly fatal to fetuses and newborns, which can also increase puerperal infection risk. Moreover, patients with severe HbH disease can suffer from anemia or hemolysis [1]. Since thalassemia is a recessive genetic disease, risk is high for birth of children with severe thalassemia or Hb Bart's hydrops fetalis syndrome on the condition that both of parents are thalassemia allele carriers of the same type. Researcher teams, Pan et al. plus Xu et al., demonstrated that carrying rates of α-thalassemia alleles are respectively 15.5% and 8.53% in Guangxi and Guangdong of China [2,3]; Therefore, occurrence rates for α-thalassemia in these regions are higher than those in China as a whole. As there are no clinical drugs to treat α-thalassemia currently, strengthened pre-conception genetic couples-screening for thalassemia is the most effective method to reduce severe thalassemia [4,5].

Principally, α-thalassemia is caused by deletion of a large fragment in the α-globin gene cluster DNA, that is the molecular biological mechanism of deletional α-thalassemia alleles. In China, $-^{SEA}$, rightward deletion ($-\alpha^{3.7}$) and leftward deletion ($-\alpha^{4.2}$) are common deletional α-thalassemia alleles, which are main detection objects for deletional α-thalassemia alleles in Chinese multilevel thalassemia detection centers at present [6]. Currently, key detection methods for $-^{SEA}$, $-\alpha^{3.7}$ and $-\alpha^{4.2}$ involve gap-PCR [7], quantitative fluorescent PCR (QF-PCR) [6], multiplex ligation-dependent probe amplification assay (MLPA) [8,9] and qPCR [10,11]. For Chinese multilevel thalassemia detection laboratories, gap-PCR is the most widely-adopted detection method, while MLPA is mainly used for novel genotype detection due to its operational complexity. Agarose gel electrophoresis is required for analysis in gap-PCR, therefore PCR product contamination risk is increased and test performance is time-consuming. For this reason, an experimental method with accurate typing, speed, ease of operation and low contamination risk is most needed for routine detection in thalassemia laboratories.

Copy number variant (CNV) detection is a potential technique of choice to determine the number of copies for a genomic gene [12]. As the molecular biological mechanism of deletional thalassemia is the deletion of large DNA sequence fragments, CNV-based qPCR is an appropriate method for α-thalassemia diagnosis. Currently, the majority of

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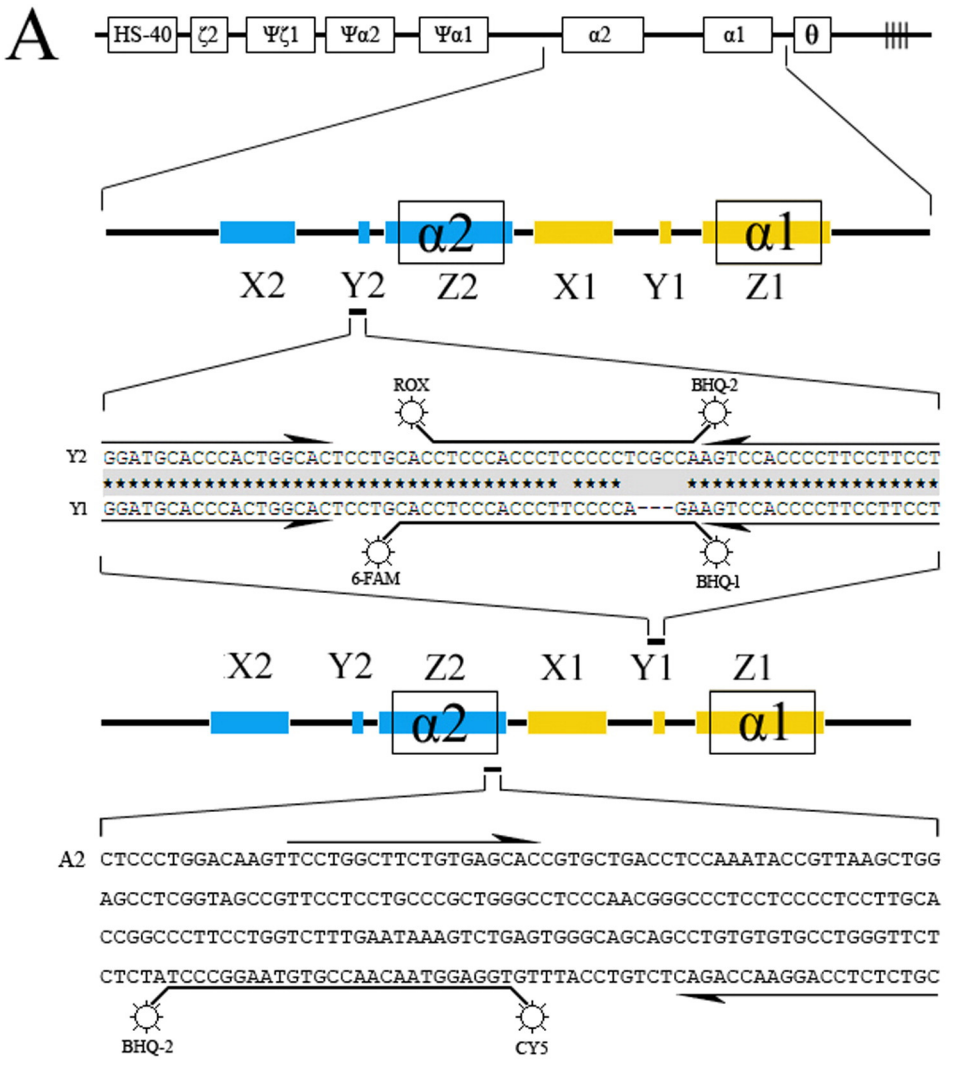
A
HS-40
ζ2
Ψζ1
Ψα2
Ψα1
α2
α1
θ
X2 Y2 Z2 X1 Y1 Z1
ROX
BHQ-2
Y2 GGATGCACCCACTGGCACTCCTGCACCTCCCACCCTCCCCCTCGCCAAGTCCACCCCTTCCTTCCT
Y1 GGATGCACCCACTGGCACTCCTGCACCTCCCACCCTTCCCCA---GAAGTCCACCCCTTCCTTCCT
6-FAM
BHQ-1
X2 Y2 Z2 X1 Y1 Z1
A2 CTCCCTGGACAAGTTCCTGGCTTCTGTGAGCACCGTGCTGACCTCCAAATACCGTTAAGCTGG
AGCCTCGGTAGCCGTTCCTCCTGCCCGCTGGGCCTCCCAACGGGCCCTCCTCCCCTCCTTGCA
CCGGCCCTTCCTGGTCTTTGAATAAAGTCTGAGTGGGCAGCAGCCTGTGTGTGCCTGGGTTCT
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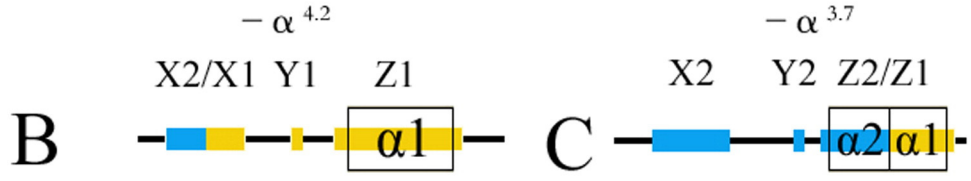
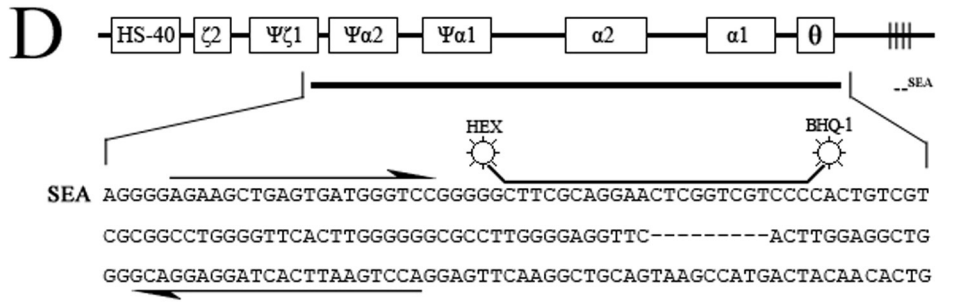
BHQ-2
CY5


B
− α 4.2
X2/X1 Y1 Z1
α1
C
− α 3.7
X2 Y2 Z2/Z1
α2 α1


D
HS-40
ζ2
Ψζ1
Ψα2
Ψα1
α2
α1
θ
--SEA
HEX
BHQ-1
SEA AGGGGAGAAGCTGAGTGATGGGTCCGGGGGCTTCGCAGGAACTCGGTCGTCCCCACTGTCGT
CGCGGCCTGGGGTTCACTTGGGGGGCGCCTTGGGGAGGTTC----------ACTTGGAGGCTG
GGGCAGGAGGATCACTTAAGTCCAGGAGTTCAAGGCTGCAGTAAGCCATGACTACAACACTG

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