Genotyping of rs3824662 polymorphism in GATA3 gene by allele-specific PCR

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Abstract

To amplify the target regions of GATA3 for studying rs3824662 polymorphism, a web-based primer designing tool available at http://bioinfo.ut.ee/primer3-0.4.0 facilitates crafting the set of outer primers [forward outer primer: 5'-TTGCAAATGGAAGAGGGTCT-3' and reverse outer primer: 5'-ACCCTGCAAATGAGAGGAAA-3'] and inner primers [G specific primer: 5'-TGAGATTAAACACAAACACGtTG-3' and T specific primer: 5'-CTGAGATTAAACACAAACACGaTT-3'] to perform allele-specific PCR that specifically amplify the GATA3 gene. G/T allele-specific primer is very sensitive and binds only with the DNA fragments containing G/T nucleotide template DNA. Forward outer and reverse outer primers will amplify a specific 691 base pair region of GATA3. This 691 base pair region contains the desired polymorphic site of interest. Allele-specific primers produce 506 bp products upon the presence of either a G allele or T allele. PCR reaction in a total volume of 15 µL with an initial denaturing step of 5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 45 s at 55°C, and 1 min at 72°C, and a final extension step of 5 min at 72°C will produce better reproducible result. The reaction mixture contains both forward outer and reverse outer primers and contains either the G allele-specific forward inner primer or the T allele-specific forward inner primer. Primer concentration used was 200 nM each. When the G allele is present in a specific DNA sequence, only the 506 base pair band will be found upon gel electrophoresis of PCR amplicons with the G allele-specific primer. In this case, no such 506 base pair band will be obtained upon gel electrophoresis with the T allele-specific primer. On the other hand, for mutant TT genotypes, the opposite phenomenon will occur when the T allele is present, which will be reflected by the presence of the 506 base pair DNA band. In the case of the heterozygous genotype (GT), both inner primers for G and T alleles will bind with specific DNA sequences to produce 506 base pair bands.

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Before start

Take precaution as required for a general PCR reaction.

Materials

GoTaq(R) G2 Hot Start Green Master Mix, 1,000 reactions M7423 by Promega

Agarose, LE, Analytical Grade, 500gm V3125 by Promega

Ethidium bromide [EB, EtBr] EB0195.SIZE.25g
by Bio Basic Inc.

primers by Contributed by users

Protocol

Primer design

Step 1.

The set of outer primers [forward outer primer: 5’-TTGCAAATGGAAGAGGGTCT-3’ and reverse outer primer: 5’-ACCCTGCAAATGAGAGGAAA-3’] and inner primers [G specific primer: 5’-TGAGATTAAACACAAACACGtTG-3’ and T specific primer: 5’-CTGAGATTAAACACAAACACGaTT-3’] were designed.

PCR condition

Step 2.

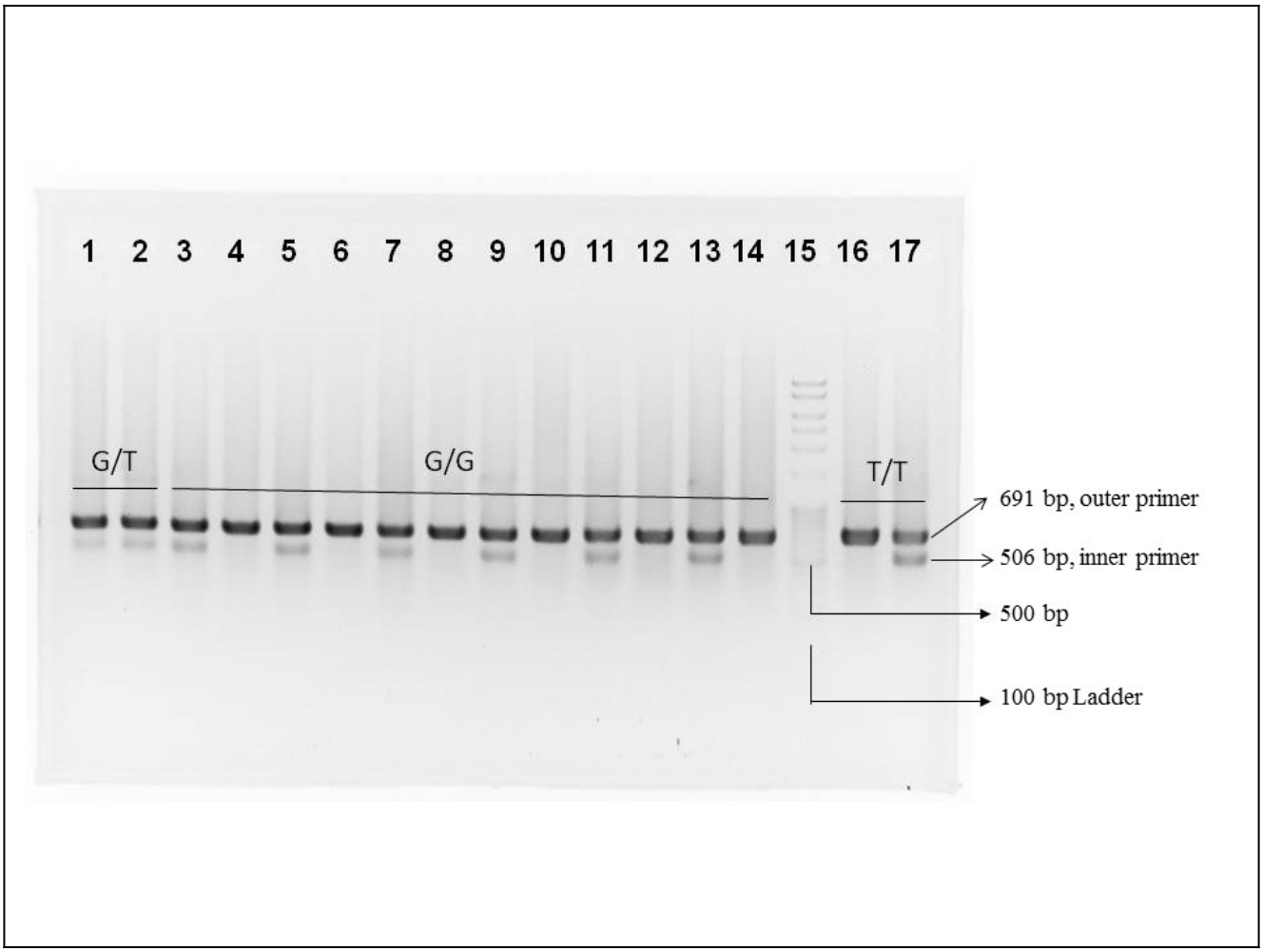
G/T allele-specific primer is very sensitive and binds only with the DNA fragments containing G/T nucleotide template DNA. Forward outer and reverse outer primers will amplify a specific 691 base pair region of GATA3. This 691 base pair region contains the desired polymorphic site of interest. Allele-specific primers produce 506 bp products upon the presence of either a G allele or T allele.
PCR reaction in a total volume of 15 µL with an initial denaturing step of 5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 45 s at 55°C, and 1 min at 72°C, and a final extension step of 5 min at 72°C will produce better reproducible result. The reaction mixture contains both forward outer and reverse outer primers and contains either the G allele-specific forward inner primer or the T allele-specific forward inner primer. Primer concentration used was 200 nM each.

Output

Step 3.

When the G allele is present in a specific DNA sequence, only the 506 base pair band will be found upon gel electrophoresis of PCR amplicons with the G allele-specific primer. In this case, no such 506 base pair band will be obtained upon gel electrophoresis with the T allele-specific primer. On the other hand, for mutant TT genotypes, the opposite phenomenon will occur when the T allele is present, which will be reflected by the presence of the 506 base pair DNA band. In the case of the heterozygous genotype (GT), both inner primers for G and T alleles will bind with specific DNA sequences to produce 506 base pair bands.

EXPECTED RESULTS



Warnings

1. As Tm value is critical so to run the PCR reaction, one should set its own Tm value considering +/- 2 degree centrigrade temperature.

2. Please use gloves when someone is using Ethidium bromide as it is a carcinogenic compound. Also, disposal of this compound should be taken care of.