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

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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.k45cyy6>

Authors: Nafiul Huda, Md. Ismail Hosen, Pankaj Kumar Sarkar, A.K.M. Mahbub Hasan, A.H.M. Nurun Nabi

Summary: 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.

Associated Publications: Huda N, Hosen MI, Yasmin T, Sarkar PK, Hasan AKMM, Nabi AHMN (2018) Genetic variation of the transcription factor GATA3, not STAT4, is associated with the risk of type 2 diabetes in the Bangladeshi population. PLoS ONE 13(7): e0198507. doi: [10.1371/journal.pone.0198507](https://doi.org/10.1371/journal.pone.0198507)

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