

Advanced Mitochondrial DNA Assay for Metabolic Syndrome

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To the Editor:

Dear Sir,

Recently, Kim et al. reported that reduced leukocyte mitochondrial DNA (mtDNA) copy number and increased mtDNA deletion ratios are independent predictors of new-onset metabolic syndrome (MetS) in a population-based longitudinal study [1]. Authors suggest that the risk of MetS development could be estimated by measuring mtDNA copy number and deletion ratio, baseline of which was measured with a quantitative polymerase chain reaction (qPCR) in leukocytes. The qPCR targets of the mitochondrial minor arc (mtMinArc), the mitochondrial major arc (mtMajArc), and the nuclear gene were in the D-loop, the NADH dehydrogenase subunit 4 (ND4), and the $\beta 2M$, respectively. The mtDNA copy number per cell was calculated by $\text{mtMinArc} / \beta 2M$. The deletion ratio of mtDNA was represented by $(\text{mtMinArc} - \text{mtMajArc}) / \text{mtMinArc}$.

The relative amount of mtDNA was estimated by a probe-based multiplex qPCR [2], or by a singleplex qPCR [3]. The disadvantages of the both methods are not accurate and they do not separate the amount of mtDNA and nuclear DNA.

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Here, I suggest modified assay to obtain a plasmid construct for standard curve generation. Standard plasmid vector for triplex qPCR assay simultaneously amplifies the D-loop gene in the mtMinArc, the ND4 gene in the mtMajArc, and the $\beta 2M$ gene in the nuclear DNA (Fig. 1). Multiplexing the three targets not only offers higher throughput analyses but also minimizes extraneous variability.

Suggested assay underline that triplex qPCR assay using a new standard plasmid vector may provide a vital tool in both research and diagnostic settings for identifying and quantifying the mtDNA changes in disease condition such as MetS and aging.

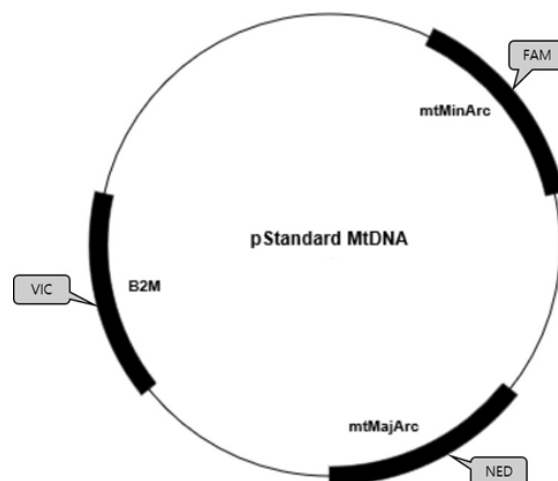


Fig. 1. A circular map of the new standard plasmid vector. The three targets: mtMinArc, mtMajArc, and the $\beta 2M$ gene in the nuclear DNA. The locations of TaqMan probes are depicted: FAM (recognizing D-loop), NED (recognizing ND4) and VIC (recognizing $\beta 2M$).

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