A PCR-Free and Label-Free Mutation Screening Method Based on Graphene Oxide-Assisted Isothermal Amplification and Mass Spectrometry Analysis

박정훈, 박현규†
KAIST
(hgpark@kaist.ac.kr†)

We developed a novel method for PCR-free and label-free mutation screening by utilizing graphene oxide (GO)-assisted exponential isothermal amplification reaction (EXPAR) and mass spectrometry analysis. In this method, allele-specific ligation is directly performed with human genomic DNA to identify mutations by utilizing a pair of ligation probes that flanked each mutation site. During ligation, the ligation probe pairs are ligated only in the presence of perfectly matched allele. The ligated products are then utilized as a template for GO-assisted EXPAR. In the presence of the ligated products, the primer is extended by polymerase and short oligonucleotide fragments are generated by nicking enzyme. Since the short oligonucleotide fragments could be utilized for trigger of GO-assisted EXPAR, the oligonucleotides are exponentially amplified only in the presence of the ligated products. The oligonucleotides are subjected to mass spectrometry acting as a mass marker and we successfully obtained the result mass peak, detected only in the presence of perfectly matched alleles.