

A Novel DNA Methylation Analysis Method using Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA)

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As late-stage cancer diagnosis results high death rate, it is desirable to develop methods for early diagnosis. As aberrant DNA methylation of CpG islands in promoter has been described in human cancer, it is suggested as a strong candidate for diagnostic biomarker. Though several studies have identified aberrant DNA methylation of many genes in cancer, critical gene target remain largely unknown. To find critical genes, validation through population study is needed.

Methylation sensitive (MS-) multiplex ligation-dependent probe amplification (MLPA) has great potential as a screening tool for critical genes target. But this method has limitations: designing a custom MLPA probe is difficult due to stuffer sequence, and only limited target site can be analyzed due to sequence specific restriction enzyme.

In this study, we developed a novel DNA methylation analysis method based on MS-MLPA, in which stuffer-free MLPA probes and three restriction enzymes are used for precise multiplex quantitative analysis. We tested our new method to analysis 28 cancer candidate genes related with hepatocellular carcinoma, which is common cancer type.