# Multiplex genotyping of BRCA gene mutations utilizing ligase reaction and nicking amplification by mass spectrometry

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A novel multiplex mutation genotyping method was developed by utilizing ligase reaction and nicking amplification based on mass spectrometry. The primary ligation probe is designed to contain a complementary base of that of mutation site at its 5′ end and a primer annealing site for nicking amplification at the 3’ end. The secondary ligation probe is designed to have a nicking enzyme recognition site and a mass marker sequences at its 5’ end. Therefore, the ligation probes were linked only in the case of mutant sample. The ligation product was then utilized as a template for nicking amplification reaction. As a result of the nicking amplification reaction by the universal primer, the cleaved short DNA segments were generated. Since, they are used as a mass marker, they were subjected to MALDI-TOF MS and the mass peak was detected only in the case of mutant samples. This strategy is very suitable for multiplex genotyping of target gene because the weight of the mass marker is variable. Using this strategy, we were successfully genotyping of 10 mutation sites in BRCA 1 gene in a single reaction tube.