Development of Novel Method Using RNA Template for Detection of Mutation with Superior Sensitivity

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Mutation detection contributes great significance in the early diagnosis, prognosis and monitoring of cancer. Therefore, the emergence of a highly efficient assay for detecting mutation is greatly desired for improvements in the treatment outcome and survival rate of this lethal disease. Currently, mutation detection assays often utilize DNA as template and fail to detect somatic point mutations at a frequency below 1% due to limited selectivity. This hindrance would lead to misdiagnosis and misclassification, hence resulting in irrelevant treatment. In our study, we aim at developing a novel mutation-identifying method utilizing RNA as templates for enhanced sensitivity that enables demanding applications. To achieve the targets, we have substantially eliminated cross-priming in reverse transcription by using a recently-developed hot-start reverse transcriptase in combination with an extendable blocking probe. Additionally, we have designed the mutation-specific primer in the reverse transcription for improved specificity and better adaptation to clinical settings. Using in-vitro RNA transcripts of V600E mutation and that of wildtype BRAF alleles as model, the novel method that was established in this study has been proven to allow detection of the mutated allele with superior sensitivity of 0.5 per 10,000. Analysis of RNA samples isolated from FFPE tissue has confirmed the feasibility and superiority of the new method in comparison with the “gold-standard” DNA-based sequencing. Interestingly, this study has demonstrated the applicability of our method to an even more challenging sample type, peripheral blood, which would entitle circulating mutant RNA a promising biomarker for accurate non-invasive mutation analysis based on liquid biopsy.