Ultra-sensitive detection of rare mutation by next generation sequencing(NGS) error validation

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The advent of next generation sequencing has led to revolutionary impact on biological research and clinical field. Accordingly, with high throughput and low cost, it is possible to identify heterogeneous mutation and monitoring its change in cancer. However, NGS sequencing is hard to assess cancer which have rare mutation, <1% frequency, since its background error is too high. Until now, in order to remove its background noise, most people depend on deep sequencing with barcoding strategy, although barcoding method have limitation to capture ultra rare mutation and occur bias, and deep sequencing require high cost. To overcome this problem, we present novel approach that validate NGS sequencing error and can determine under 0.01% error rate. Since optical sensor that identify fluorescence signal of sequencing probe make base calling error, we approached to validation method that extract DNA molecule and determine whether it has real variant or sequencing error by amplifying the molecule and re-sequencing. In this experiment, by using DNA laser-based extraction, the nucleotide molecules that have 0.01% variant rate were physically separated from NGS plate, then verified by Illumina sequencing platform.

References

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