**Supplementary methods**

**Evaluation of assay performance in reference samples**

To confirm the accuracy of the NSG-based deep sequencing method, we constructed a mutant: a *TP53* mutant (NM\_000546.6: c.844C>A) in a pcDNA3.1 vector. Then, we mixed the mutant and wild type to at ratios of 100%, 10%, 1%, and 0.1% by using a 1: 10 serial dilution method. The above samples with different mutant concentrations were used to test whether the experimental method could detect these mutants.

First, we needed to distinguish the true existence of low-abundance mutants from background errors due to polymerase chain reaction (PCR) or deep sequencing. We calculated the mean and standard error (SD) of the background errors of each DNA region from raw FASTQ data with a quality score of 30. The mean background error per base was 9.2 × 10-4, and the SD was 5.09 × 10-5. The upper limit of the 99% confidence interval for background errors was 2.23 × 10-3 (Supplementary Figure S1A). The measured level of the 0.1% mutant was 3.17× 10-3,which is significantly higher than the range of background errors. This result suggests that our NGS testing procedures can accurately detect 0.1% mutants. Second, we observed that the mutation level could be measured in a linear manner. In reference samples, we achieved a mutation-level sensitivity of 99.50%, 11.76%, 1.35% and 0.32% for 100%, 10%, 1%, and 0.1% mutants, respectively, consistent with the decreasing number of mutant molecules (Supplementary Figure S1B).