# Single Nucleotide Recognition and Mutation Site Sequencing based on a Barcode Assay and Rolling Circle Amplification

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Table S1. DNA sequences used in this experiment

| DNA | Sequence (5'-3') |
|---|---|
| Target A | TCCACGGGGTCTTTCCGTCTTGCCGC |
| Template A | AAGACCCCGTGGAACAGAGCAAGTCCGTCTTGCCCTC AGCATGCATGCGGCAAGACGGA |
| Target B | TCCACGGGGTCTCTCCGTCTTGCCGC |
| Target C | TCCACGGGGTCTTCCCGTCTTGCCGC |
| Occupy | GCCCTCAGCATGCAT |
| Hairpin | NH2-GGAACAGAGCAAGTCCGTCTTAAGCTCTGTTCC-BHQ2 |
| Forward primer | TTCAACGCTTCATCTTGCGACTGACGCATT |
| Reverse primer | ATACCACCCTTGATGTTTCTGTTAGCTAAC |

Table S2. Main apparatus used in this experiment

| Apparatus | Specification/Model | Manufacturer |
|---|---|---|
| Pure water/ultrapure water integrated system | Milli-Q Integarl | Millipore |
| Constant temperature metal bath | ThermoStat plus | Eppendorf |
| Transmission electron microscopy | HT7700 | Hitachi |
| Gel Imaging InstrμMent | ChemiDoc ™ Touch | Bio-Rad |
| Refrigerated high-speed centrifuge | H1850R | CENCE |
| Fluorescence spectrometer | Cary Eclipse | Agilent Technologies |
| Nano Particle Size Potentiometer | Zetasizer Nano ZS | Malvern Panalytical |
| Ultraviolet-visible spectrometer | UH4150 | Hitachi |
| Multi-function enzyme labeler | Infinite M200 PRO | TECAN |
| Confocal laser scanning microscopy | FluoviewSIM-A1 | Nikon |
| Constant temperature heating magnetic stirrer | DF-101D | YUHUA |

| | | |
|---|---|---|
| Real-time fluorescence quantitative PCR instrμMent | Millicell ERS-2 | Merck Millipore |
| Dark box ultraviolet analyzer | ZF-20D | YUHUA |
| Freeze dryer | FD-1A-50 | BIOCOOL |

Table S3. PCR reaction system

| Composition | Volume (μL) |
|---|---|
| 2× superReal PreMix Plus | 10 |
| Forward primer (10 μM) | 0.6 |
| Reverse primer (10 μM) | 0.6 |
| cDNA | 5 |
| 50×ROX Reference Dye | 2 |
| RNase-free ddH2O | 1.8 |

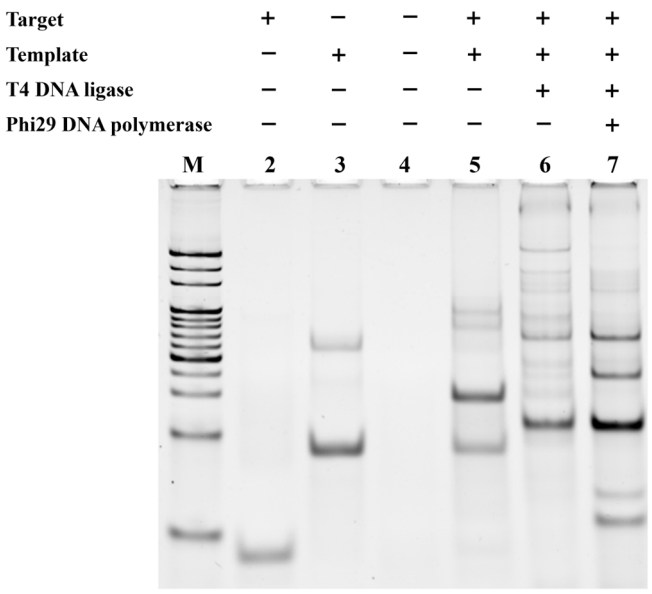



Figure S1. Gel electrophoretic verification of digestion of RCA amplification products

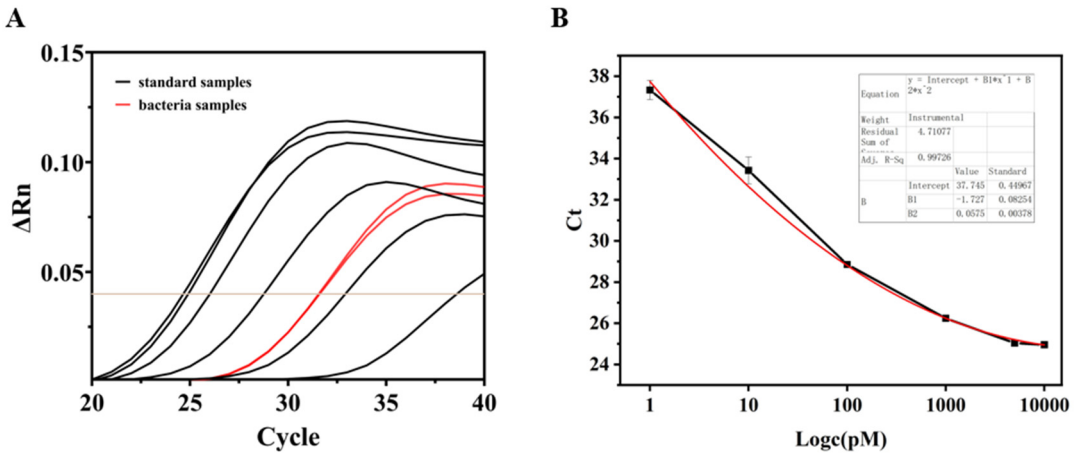



Figure S2. Validation of assays by standard PCR methods. (A) PCR amplification curves of standard samples with different concentrations. (B) Linear curves of standard samples with different concentrations and amplification times. Where the real sample to be measured is in the range of PCR amplification curve, the theoretical concentration value can be obtained by substituting linear curve. (*n*=3)