Genome editing of breast cancer gene using isothermal amplification with CRISPR-Cas9 system

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We herein describe a novel strategy to detect breast cancer with Exponential amplification reaction (EXPAR) using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) -Cas9 system. Among of many gene editing technologies, the CRISPR-Cas9 system is the powerful tool. It causes DNA double-strand breaks(DSBs) at specific genomic loci. With this mechanism, we can target mutant gene and cut it out for gene therapy. Our research is combining CRISPR system with EXPAR, isothermal amplification assay. By NHEJ-based deletion of the insertion via the creation of two DSBs, it releases the deleterious mutated gene which triggers EXPAR. Finally, we can detect amplified dsDNA with the intercalating dye. As a result, we can distinguish the signal difference between a normal gene and mutant gene. Also, we can see the editing of gene mutation directly within 2 hours. Using this strategy, we save times and do less work comparing conventional methods such as gene sequencing, fluorescence - tagged plasmid and Cas9 labeled with fluorescent reporters. We can apply this mechanism to another mutant gene and screen Cas9 cleavage assay.