PCR assay detects mutant genes with 50 to 100-fold increase in sensitivity

Published date: Sept. 22, 2012

Technology description

Summary

Increasing the sensitivity for detection of mutations in cancer cells through modified PCR
Polymerase chain reaction (PCR) involves separation of double stranded DNA and sequential amplification of target genes in order to detect gene sequences. This technology encompasses two modifications to increase the sensitivity of PCR, via preferential replication of the target of interest. One method involves a primer which allows for amplified alleles to denature at lower temperatures while creating a thermostable endonuclease site in wild-type alleles that lack a native restriction site. The endonuclease destroys the amplified wild-type allele through repeated PCR cycles, leaving behind the mutant alleles for further isolation and characterization. An alternate method requires a forward primer that halts replication of the wild-type allele by generating a product that can no longer be used as a template for PCR. A second forward primer, which is higher in concentration and specific to the mutant allele, in combination with a reverse primer are then used to amplify the mutant allele. These technologies could be used to enhance PCR techniques currently employed to detect markers of breast cancer and other solid organ cancers.
Modified PCR strategy allows for preferential replication of mutant alleles
Traditionally, PCR is used to amplify and detect mutant genetic sequences, but also amplifies normal (wild-type) genes. Current PCR technology can only detect a mutant gene when it represents 5-10% of the specimen; if a mutation is present in a tumor in only a small amount, normal PCR may be unable to detect the mutation. This new technology predicts an increase in sensitivity of PCR by 50-100 fold, consisting of a change in the PCR process that allows for selectively decreasing the amount of wild-type allele produced, thereby increasing the relative amount of mutant allele.
These processes have both been tested on single nucleotide base differences between rat (mutant) and mouse (wild-type) alleles. Experiments have demonstrated detection of the rat allele at concentrations as low as 0.1% of mouse allele.

Application area

• Amplify small quantities of known mutations in the presence of large amounts of the wild-type allele.
• Highly sensitive detection of mutations in breast cancer, and potentially other solid tumors such as colon and pancreatic.
• Diagnostic test for personalized cancer therapy.
• Detecting micro-metastases.
• Forensic applications (identifying small amounts of a specific DNA sequence).

Advantages

• Requires relatively simple modifications to current PCR processes.
• Detects mutant alleles present in very low concentrations.
• Sensitivity increase of 50-100 fold.

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