Validation of a Pyrosequencing Assay for Severe Combined Immunodeficiency

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Background: Severe combined immunodeficiency, or SCID, is a category of inherited disorders that are defined by defective T and B lymphocyte-mediated immune response. One form of SCID is caused by a point mutation (646 G>A) in the adenosine deaminase (ADA) gene on chromosome 20. A molecular assay known as pyrosequencing can be used to determine the genotype and allele frequency of this mutation. This sequence-based detection technique utilizes the natural process of pyrophosphate (PPi) release as a nucleotide is incorporated into the complementary DNA sequence during replication.

Objective: This study aimed to create and validate a polymerase chain reaction (PCR)-based pyrosequencing assay to genotype SCID patients with the ADA gene mutation.

Methods: To achieve this, primers were designed, and PCR parameters were optimized for DNA amplification. PCR products were analyzed by gel electrophoresis, and the PyroMark Q24 (Qiagen) instrument was used determine the frequency of each allele present at the 646 G>A mutation locus. For validation, 14 DNA samples were tested.

Results: The assay produced valid allele frequencies for the locus of interest, and SCID control DNA was genotyped with 100% accuracy. For intra-run precision, the mean coefficient of variation was 2.79% (0.91-4.25%). For inter-run precision, the mean coefficient of variation was 3.89% (1.50-5.93%). The analytical sensitivity of the assay was as low as 1ng of DNA for PCR, with as little as 4µL of PCR product into pyrosequencing. The assay was challenged with heparin, an anticoagulant found in some blood collection tubes that can inhibit PCR, and it was determined that the assay could tolerate up to 5% heparin.

Conclusion: In conclusion, this pyrosequencing assay is a robust and reliable method for genotyping the 646 G>A mutation in ADA SCID. Future studies include addition of other relevant SCID mutations.