Tolerance intervals from probe-specific mixed-models to detect changes in copy number using multiplex ligation-dependent probe amplification (MLPA)

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Copy number variations play an important role in genes and other regulatory elements that may have phenotypical consequences. Several techniques and platforms have been developed for genome-wide analysis of DNA copy number, such as array-based comparative genomic hybridization (aCGH). However, the ability of aCGH to discern between different number of copies is very limited. MLPA is a recent method that aims to detect copy number alterations at the genomic level (gains or loses) in a test DNA with respect to a reference. In this work, we propose a method for the normalization procedure based on a non-linear mixed-model, as well as a new approach for determining the statistical significance of altered probes based on linear mixed-model. This method establishes a threshold by using different tolerance intervals that accommodates the specific random error variability observed in each test sample. Through simulation studies we have shown that our proposed method outperforms the existing ones based on threshold rule or iterative regression. We illustrate the method using a controlled MLPA assay in which probes interrogate regions that are variable in copy number in individuals suffering from different genomic disorders such as Prader-Willi, DiGeorge or Autism.