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Targeted next-generation sequencing can replace Sanger sequencing in clinical diagnostics.

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Abstract: Mutation detection through exome sequencing allows simultaneous analysis of all coding sequences of genes. However, it cannot yet replace Sanger sequencing (SS) in diagnostics because of incomplete representation and coverage of exons leading to missing clinically relevant mutations. Targeted next-generation sequencing (NGS), in which a selected fraction of genes is sequenced, may circumvent these shortcomings. We aimed to determine whether the sensitivity and specificity of targeted NGS is equal to those of SS. We constructed a targeted enrichment kit that includes 48 genes associated with hereditary cardiomyopathies. In total, 84 individuals with cardiomyopathies were sequenced using 151 bp paired-end reads on an Illumina MiSeq sequencer. The reproducibility was tested by repeating the entire procedure for five patients. The coverage of ?30 reads per nucleotide, our major quality criterion, was 99% and in total ?21,000 variants were identified. Confirmation with SS was performed for 168 variants (155 substitutions, 13 indels). All were confirmed, including a deletion of 18 bp and an insertion of 6 bp. The reproducibility was nearly 100%. We demonstrate that targeted NGS of a disease-specific subset of genes is equal to the quality of SS and it can therefore be reliably implemented as a stand-alone diagnostic test.