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Single-primer PCR correction: a strategy for false-positive exclusion

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ABSTRACT. Polymerase chain reaction (PCR) technology plays an important role in molecular biology research, but false-positive and nonspecific PCR amplification have plagued many researchers. Currently, research on the optimization of the PCR system focuses on double-primer-based PCR products. This research has shown that PCR amplification based on single-primer binding to the DNA template is an important contributing factor to obtaining false-positive results, fragment impurity, and nonspecific fragment amplification, when the PCR conditions are highly restricted during PCR-based target gene cloning, detection of transgenic plants, simple-sequence repeat marker-assisted selection, and mRNA differential display. Here, we compared single- and double-primer amplification and proposed "single-primer PCR correction"; improvements in PCR that eliminate interference caused by single-primer-based nonspecific PCR amplification were demonstrated and the precision and success rates of experiments were increased. Although for some kinds of experiments, the improvement effect of single-primer PCR correction was variable, the precision and success rate could be elevated at 12-50% in our experiment by this way.

Key words: PCR; False-positive exclusion; Single-primer PCR correction

