Solid-phase PCR for rapid multiplex detection of Salmonella spp. at the subspecies level, with amplification efficiency comparable to conventional PCR

Solid-phase PCR (SP-PCR) has attracted considerable interest in different research fields since it allows parallel DNA amplification on the surface of a solid substrate. However, the applications of SP-PCR have been hampered by the low efficiency of the solid-phase amplification. In order to increase the yield of the solid-phase amplification, we studied various parameters including the length, the density, as well as the annealing position of the solid support primer. A dramatic increase in the signal-to-noise (S/N) ratio was observed when increasing the length of solid support primers from 45 to 80 bp. The density of the primer on the surface was found to be important for the S/N ratio of the SP-PCR, and the optimal S/N was obtained with a density of 1.49 × 10^{11} molecules/mm². In addition, the use of solid support primers with a short overhang at the 5’ end would help improve the S/N ratio of the SP-PCR. With optimized conditions, SP-PCR can achieve amplification efficiency comparable to conventional PCR, with a limit of detection of 1.5 copies/μl (37.5 copies/reaction). These improvements will pave the way for wider applications of SP-PCR in various fields such as clinical diagnosis, high-throughput DNA sequencing, and single-nucleotide polymorphism analysis. Graphical abstract Schematic representation of solid-phase PCR.