

ABSTRACT

A method for preparing a high-throughput sequencing library based on nested multiplex PCR and a kit thereof are provided. The method for preparing a high-throughput sequencing library based on nested multiplex PCR includes: amplifying a targeted region by using a forward primer and a reverse primer, where the forward primer is a forward specific sequence, and the reverse primer includes a reverse specific sequence at a 3'-end and a first universal sequencing sequence at a 5'-end; purifying a product of the first round of PCR amplification; and amplifying the purified product by using a nested primer, a first tag primer, and a second universal primer, where the nested primer is located downstream of the forward primer, and includes a second universal sequencing sequence at a 5'-end and a specific sequence at a 3'-end, a 3'-end sequence of the first tag primer is partially or completely the same as the first universal sequencing sequence of the reverse primer at the 5'-end, the first tag primer further includes a first tag sequence, and a 3'-end sequence of the second universal primer is partially or completely the same as the second universal sequencing sequence at the 5'-end of the nested primer. The method for preparing a high-throughput sequencing library based on nested multiplex PCR can effectively avoid dimers generated in the multiplex PCR amplification process, and greatly increase the specificity of PCR amplification by adopting a nested amplification method.

(Fig.2)