Nested polymerase chain reaction (PCR)

Nested polymerase chain reaction (PCR) is used in situations in which it is necessary to increase the sensitivity and/or specificity of PCR, for example, when amplifying a particular member of a polymorphic gene family or when amplifying a cDNA copy of an mRNA present at very low abundance in a clinical specimen containing a heterogeneous population of cell types. Nested PCR usually involves two sequential amplification reactions, each of which uses a different pair of primers. The product of the first amplification reaction is used as the template for the second PCR, which is primed by oligonucleotides that are placed internal to the first primer pair. The use of two pairs of oligonucleotides allows a higher number of cycles to be performed, thereby increasing the sensitivity of the PCR. The improved specificity of the reaction derives from the binding of two separate sets of primers to the same target template. Nested PCR is an efficient method to amplify segments of long templates but requires knowledge of the sequence of the target.

nested PCR, two (rather than just a single) pairs of primers target a single locus. The first pair amplifies the target fragment in a conventional PCR reaction. The second pair anneals to sites within the first amplicon, and amplifies an internal (shorter) sequence . Clearly, the sequence of the full amplicon must be known to design appropriate primers.

The purpose of nested PCR is to increase assay sensitivity by re-amplifying the target from a template previously enriched by the first PCR. Non-target sequences amplified non-specifically in the first PCR are not re-amplified in the second reaction as they would be unlikely to possess the internal priming sites targeted by the second PCR. However, an increased risk of contamination is a major disadvantage of this method, due to possible carry-over contamination of PCR products, so great care must be exercised when performing it. Nested PCR has been used to detect the presence of verotoxinogenic *E. coli* in ground beef by

targeting the genes *vt1* and *vt2* . The sensitivity achieved was such that 110 cfu could be detected in a 10 g sample. Similarly, when nested PCR was used to detect *Shigella flexneri* in lettuce samples spiked with the pathogen, the level of sensitivity was higher than that achievable with single PCR. In order to reach the same level of sensitivity, a prior phase of pathogen enrichment by culture was necessary .