in the same reaction vial together with the template, amplified copy numbers can be quantified. We have constructed a standard for the PCR-detection of the insertion element IS 6110 of *Mycobacterium tuberculosis*. The PCR-MIMIC construction kit of Clontech was used. In this reaction the same primers amplify a template sequence of 123 bp and the standard of 360 bp. The standard is added in 3 different concentrations (6, 60, 600 molecules per sample), the probe is amplified and the products are separated, blotted and detected with enhanced chemiluminiscence.
The results show that the lower detection limit is not constant. In some experiments only 600 molecules of the standard were detectable. This varying efficiency may be caused by inhibitors within the individual sample. Especially in the case of negative results, one has to consider this phenomenon. The use of internal standards gives information concerning the lower detection limit and prevents false negative results.

Identification and characterization of medically important *Candida* species by using PCR-fingerprinting

M. Thanos, H. J. Tietz, C. Schweynoch, Y. Gräser, W. Presber and G. Schönian

Institut für Mikrobiologie und Hygiene und Hautklinik, Universitätsklinikum Charité, Humboldt Universität, Clara-Zetkin-Str. 96, D-10098 Berlin (Berlin)

A PCR fingerprinting assay was applied to identify and to characterize the genetic relatedness among medically important *Candida* species. By using different primers of arbitrary nucleotide sequence in the polymerase chain reaction, distinctive and reproducible sets of amplification products were observed for reference strains of all *Candida* species tested. Primers were chosen which generated only little if any intraspecies DNA polymorphisms. DNA was extracted from yeasts by a minipreparation method. PCR protocols used for different primers were optimized with regard to primer concentration, amount of template DNA, annealing temperature and cycling program. Amplified products were separated by electrophoresis in agarose gels and detected after straining with ethidium bromide. The PCR patterns were evaluated by a laser densitometer using an appropriate computer software.
Comparing the PCR profiles obtained from clinical isolates with those yielded from reference strains it was possible to identify different *Candida* species even if they were not typable by the conventional biochemotyping. Furthermore, this PCR technique allowed to establish the genetic relatedness of distinct *Candida* species and might therefore be, useful for phylogenetic and taxonomic studies within this genus.

Detection of DNA polymorphisms in *Leishmania* species by using single primers in the polymerase chain reaction

K. Zlateva, G. Schönian, C. Schweynoch and W. Presber

Institut für Mikrobiologie und Hygiene, Universitätsklinikum Charité, Humboldt Universität, Clara-Zetkin-Str. 96, D-10098 Berlin (Germany)

DNA polymorphisms were assessed in different *Leishmania* species by amplifying genomic DNA with single primers of arbitrary nucleotide sequence. The method is simple and fast. It requires only small amounts of DNA and can be applied to any species from which DNA can be prepared. No prior sequence information is required.
Primers used as hybridization probes in conventional DNA fingerprinting or reported by other authors were tested for their applicability in this PCR fingerprinting technique. Reaction conditions were optimized for each primer. The number, reproducibility and intensity of bands in a fingerprint should be a function of several parameters, including the concentration of salts, primer, annealing temperature, template concentration, primer length and primer sequence. PCR products that are not shared among all strains act as polymorphic markers. As each primer gave a different pattern of AP-PCR products, the data allow the differentiation of closely related strains even of the same species.
The amplification products were electrophoresed in an agarose gel and photographed after staining with ethidium bromide.
Several primers were found which enabled the detection of variable DNA sequences in different *Leishmania* species in this PCR approach. The PCR patterns could be used for species identification in *Leishmania* isolates. Using an appropriate computer software this method is well suited for the determination of genetic relatedness among *Leishmania* species and can be applied to phylogenetic and taxonomic studies.