

(ATI-gene) was developed to distinguish cowpox virus from other orthopoxvirus species. For this purpose, two primer pairs were designed, with one primer of each pair radioactively labelled. The discriminating bases are located at the 3'-end of the upper upstream primer (TT), at the 5'-end of the lower downstream primer (A), and at the 3'-end of the lower upstream primer (A), forming a one base overhang at the ligation site. Two mismatching bases (GG) were added at the 3'-ends of the upstream primers to prevent ligation at this end. Detection of the cowpox ligation product was achieved by autoradiography after denaturing polyacrylamide gel electrophoresis. To increase the sensitivity, the corresponding region of the ATI-gene was initially amplified by consensus primer detected polymerase chain reaction (PCR) prior to LCR.

All 9 cowpox viruses examined could be clearly discriminated from reference strains of camelpox virus, mousepox virus and monkeypox virus, as well as seven vaccinia virus isolates. The PCR-coupled LCR was shown to be a reliable tool for the screening of cowpox virus infections, which is of particular importance with respect to the increasing number of unusual hosts.

To our knowledge, this is the first report of a LCR that uses a double nucleotide deletion. This LCR does not require any fill-in reaction (substitution of bases) and is not limited to the detection of base pair changes from A-T/T-A to G-C/C-G or vice versa.

### Identification of Epstein-Barr virus transactivator BZLF1 mRNA in uncultured peripheral blood lymphocytes by RT-polymerase chain reaction

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The cascade of gene expression during the lytic replication of Epstein-Barr virus (EBV) is initiated by three immediate early regulatory proteins. Based on a nucleic acid detection method for one of these factors, the proof of active EBV replication is possible even before the onset of serological markers and will therefore be of great value for diagnosis and treatment. For early detection of lytic replication in the case of EBV associated diseases, we developed a reverse transcription polymerase chain reaction (RT-PCR), followed by a second

'nested' PCR with primers overlapping the first and second exon coding for BZLF1.

We report detection of mRNA for the immediate early transactivator BZLF1 in peripheral blood lymphocytes (PBL) of donors with reactivation of EBV and chronic infection (CAEBV). No transcripts were detected in PBL of normally latently infected individuals of EBV-negative BJAB cells.

The significance of this observation for pathobiology, diagnosis and treatment of clinical forms of EBV infections like infectious mononucleosis (IM), CAEBV, chronic fatigue syndrome (CFS) or reactivation of latent EBV and rejection of organ transplants needs further evaluation and will be discussed.

### Dependence on the taxon composition of clone libraries for PCR amplified, naturally occurring 16S rDNA, on the primer pair and the cloning system used

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Recent advances in molecular techniques have allowed the determination of the genetic variety of microbial inhabitants in the environment. Encouraging progress has been made in the elucidation of as yet undetectable biodiversity in different natural samples, such as marine environments, forest soil, acidothermal soil, hot spring environments and the environment of a continuous bioleach bioreactor. Despite the novelty of the results, certain problems associated with the methods applied to quantitatively describe the microbial populations have been recognized. Unsolved problems in the cloning strategy include: 1) the recovery of cells and/or nucleic acids from the environment, 2) the conditions under which PCR amplification of the target genes is performed, including selection and specificity of primers, the dependence of PCR amplification on the quality of DNA and the possibility of capture of *Taq* polymerase by abundantly occurring DNA, 3) the influence of the cloning strategy and cloning vectors, such as shotgun cloning (minute percentage of DNA are *rrn* operons), sticky end cloning (presence of restriction sites within the *rrn* operons may prevent cloning of the amplified product), blunt end cloning (low transformation efficiency), and clonability of heterologous rDNA, 4) the detection of clones by sequence analysis