

Polymerase Chain Reaction Amplification of Genomic Fragments of Bovine Herpesvirus-1

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Especial conditions were developed for the amplification of five DNA segments from US region of BHV-1 by polymerase chain reaction. In order to eliminate most nonspecific products it was found that addition of three cosolvents DMSO, glycerol and NP 40 was a simple method for increasing the specificity of amplification.

Key words: bovine herpesvirus-1 - cosolvents - polymerase chain reaction

Bovine herpesvirus-1 (BHV-1), commonly known as infectious bovine rhinotracheitis virus, is a prominent cause of disease in cattle (Gibbs et al. 1977). In Brazil, over 50 millions of animals may be infected (Lovato et al. 1995, Vidor et al. 1995). Virus isolation in tissue culture is the used method for detection of BHV-1 in clinical samples or semen but the principal drawback of the method is the length of time required to obtain results. Depending on the number of blind passages in tissue culture, the procedure may take one to three weeks to be completed. To overcome these difficulties attention has been directed toward the polymerase chain reaction (PCR) assays but major problems in the amplification of DNA from certain herpesvirus may arise due probably to their GC rich genomes. The BHV-1 genome is more than 70% GC rich (Leung-Tack et al. 1994).

We have developed PCR conditions allowing the efficient amplification of five DNA segments from US region of BHV-1 with primers designed on the published sequences of Leung-Tack et al. (1994) (Table). The nucleotide sequence data used in this paper are deposited under Genbank accession number Z23068. The optimization used the logical system of changes in pH, magnesium, potassium chloride and annealing temperatures (Payne et al. 1995). We found that specificity was

difficult to achieve under these conditions for all five fragments with high GC contents. The reaction conditions were 2.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-Cl pH 8.3, 0.2 mM dNTP, 2.5 U Taq polymerase in a final volume of 100 µl overlaid with 50 µl of silicone oil. All amplifications were performed in a MJ Research Minicycle. For amplifications the hot start protocol was featured when the samples were first heated at 95°C for 10 min to completely denature the template. After the denaturation, at 80°C, the enzyme was added to each reaction beneath the layer of oil. The denaturation, annealing and extension temperatures and times were 94°C (30 sec), 52°C (1 min), 72°C (1 min) respectively for 35 cycles. The amplification was followed by a final elongation step of 10 min. It was found that the parameters for denaturation, annealing and extension must be worked carefully. The reaction products were analyzed by agarose 2% (Seakem® Gold Agarose, FMC) gel electrophoresis after ethidium bromide staining.

In order to eliminate most nonspecific products and increase the efficiency of the amplification the effect of the addition of several cosolvents such as formamide, DMSO, glycerol, NP40, tetramethylammonium chloride (TMAC) and replacement of 70% of dGTP by de⁷GTP to the reaction was verified. It was found that the success rate of the amplification of the five GC-rich BHV-1 ORFs was significantly higher with the addition of 1% DMSO, 5% glycerol and 0.1% NP40 (Figure). The denaturant formamide, TMAC, replacement of dGTP by de⁷GTP did not give the desired specificity.

In conclusion, the addition of the three cosolvents DMSO, glycerol and NP40 was found to be a simple and successful method for increasing the specificity of selected fragments of the BHV-1 genome.

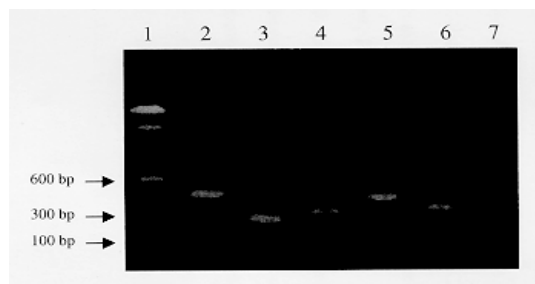
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Specificity of amplifications of the BH-83 Brazilian strain with the addition of cosolvents DMSO, glycerol and NP40. Lane 1: 100 bp DNA ladder. Lane 2: fragment of 456 bp (ORF 1). Lane 3: fragment of 267 bp (ORF 2). Lane 4: fragment of 303 bp (ORF 3). Lane 5: fragment of 411 bp (ORF 4). Lane 6: fragment of 352 bp (ORF 7). Lane 7: SHV-1 strain LA 031 (negative control).

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TABLE

Primers position, open reading frames and size of amplified product

Primers	Position	Orfs US	Product amplified
LVC 10/11	5' 430-417/885-868 3'	ORF 1	456 bp
LVC 12/13	5' 1159-1177/1444-1426 3'	ORF 2	267 bp
LCV 14/15	5' 2397-2405/2682-2700 3'	ORF 3	303 bp
LVC 16/17	5' 3272-3289/3682-3655 3'	ORF 4	411 bp
LVC 18/19	5' 7578-7596/7912-7930 3'	ORF 7	352 bp