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Application of representational difference analysis for cloning of DNA fragments with base sequences specific to a virulent strain of Marek's disease virus

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Representational difference analysis (RDA) [Lisitsyn, *et al.* 1993, Science 259 : 946–951] is a PCR-based method with improved efficiency of subtraction by combining the representation and kinetic enrichment. This method was employed to purify the restriction endonuclease-digested DNA fragments specific to Md5 (pathogenic strain of Marek's disease virus (MDV)) that were not common in two populations of DNA fragments obtained from Md5 (pathogenic) and CVI-988 (nonpathogenic) MDV strains. An excess amount of amplicon of the CVI-988 DNA digested with *Msp*I and subsequently amplified with PCR was added to the amplicon of Md5 to remove common DNA fragments existing in Md5 and CVI-988. After the subtraction, nineteen clones were obtained and restriction enzyme (*Bam*HI, *Eco*RI, *Hind*III and *Pst*I)-digested polymorphic patterns of DNAs from Md5 and CVI-988 were observed in seven clones. The fraction of the PCR products hybridized with the seven clones in all PCR products was estimated to be 50% by randomly selecting 100 PCR products after the subtraction and subsequently hybridizing them. The second subtraction using all PCR products of the first subtraction resulted in the selection and concentration of two clones.

Five of the seven clones were in accord with MDV sequences which had already been reported, the direct repeat sequence, a meq sequence having a partly unknown sequence and sequences having point mutations on the *Msp*I sites. The sixth sequence was in homology with that of the U3 promoter, a part of the long terminal repeat of avian erythroblastosis virus. The last one had a sequence which had not been identified yet but could hybridize with MDV DNA. These results suggested that three clones corresponded to the Md5-specific sequences. Thus, RDA was shown to be useful for cloning strain-specific DNA fragments of MDV.