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马立克氏病病毒强毒GA株基因组BamH Ⅰ-L片段的序列分析*

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关键词 马立克氏病病毒,致瘤毒株,核酸序列分析

马立克氏病病毒(Marek's disease virus, MDV)是一种能诱导鸡淋巴组织增生或淋巴肿瘤的细胞结合性疱疹病毒,由此而引起的马立克氏病(Marek's disease, MD)也是迄今为止唯一可以利用疫苗进行有效控制的肿瘤性疾病。鉴于此,作为研究动物病毒性肿瘤的理想模型,MDV在病毒学界、医学界和兽医界都得到了广泛重视。据其嗜淋巴细胞性特征,MDV隶属γ-疱疹病毒亚科,而病毒基因组在结构上更类似于单纯疱疹病毒(herpes simplex virus, HSV)——一种α-疱疹病毒[1]。最近我们的试验证明,由MDV强毒GA株BamHI基因文库中L片段制成的核酸探针能区分MDV致瘤株与疫苗火鸡疱疹病毒株(HVT)[2]。为了探索其本质,试验进一步对该片段进行了全序列分析,现将序列测定结果公布如下。

材料和方法

1 目的基因片段的回收与核酸探针的制备 按Sambrook等[3]方法,从MDV强毒GA株BamHI基因文库(美国农业部地区家禽肿瘤病研究所惠赠)中回收并纯化BamHI L片段。用Digoxigenin标记试剂盒(Boeheringe Mannhein产品)对此片段作随机引物标记,制备核酸探针[2]。

2 亚克隆的构建与DNA序列分析 参照文献[3],将经限制性内切酶消化后L片段各亚片段分别克隆进pUC_{118}或pUC_{119}中,重组转化子的鉴定采用酶切分析和Southern blot杂交。按Sanger等[4]介绍的双脱氧末端终止法,对各个亚克隆片段作碱基序列测定。应用Goldkey Version 2.0和DNASIS软件对所测序列进行分析。

结果与讨论

1 L片段的亚克隆及测序策略如图1所示。据所测序列重叠部分及相应的内切酶识别位点碱基组成拼出L片段的全序列,长2893 bp(图2)。

2 核酸序列分析结果表明,该片段内不含任何开读框(ORFs)。至于其中是否含某些调控因子序列或Northern blot对应序列,尚有待进一步研究。

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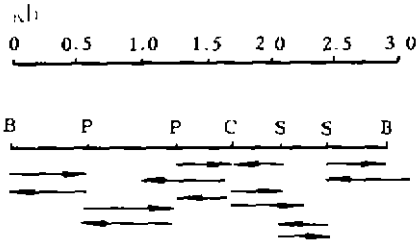

图 1　MDV BamHI L DNA 片段克隆及测序策略。选用 pUC_{118} 和 pUC_{119} 为克隆测序载体。箭头示出测序的方向，部分酶切位点为 BamHI (B)，PstI(P)，ClaI(C)和 SmaI(S)

Fig 1　Strategy and clones prepared for nucleotide sequence analysis of the BamHI－L fragment of MDV. All clones were prepared in either pUC_{118} or pUC_{119} as appropriate. Arrows indicate the direction and extent of DNA sequence analysis on a particular fragment. Partial restriction enzyme recognizing sites are BamHI (B), ClaI(C), PstI(P), and SmaI(S).

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                BamH I
   1 5'---GGATCCATTACGTAAAATTTAGATCTTTTGTAGAAATTCAATTTCCTTCCCTTTTACTT
  61       TGTTTGATGTGTGCCACTGTTGTACCGATAGTGCCGCGCGTGAAAGAGTGAACGGGAAGG
 121       GCTTACGTAAAGAACTGTCGGTGCCGGTACGAGGGGAAGCTGTAACGCATTTCGAGTCTT
 181       GATAAAGCAGAGCATGAAAATTAAATCGTAGCTCCTAAAAACTAACGGTTCGTGGTTTTT
 241       TTTTTTTTTTTTTACCTTAGTGCGTTTGTTGACGGAAATGTCCGCCATTATTTGAACGTC
 301       TGTGCGCATGCGCGATGAGTGCGCAGGGCGTATATAACGCAAGCGCGCAGGGCTGGTTCG
 361       GGTAAGGCGTTCACGCATGCGCCAGTTATGCATGACGCGCATGCGTACGGTGGAGTGTAT
 421       CTGCCATGCGCGTGTGTCGAAAACCAGGCATGCGCAGATAACCATACTTACGGCGCATGC
 481       TTGTTTTGGGTAATTGGTCTGCGCATGCGTTTTGTGTGTAGAGGGCGCATGCGTACTGCC
 541       GTAAGCGGTCTGCGCATGCGTTTTGGACTGCGCATGCGTTTTGTGTGTGGAGGGCGCATG
                Pst I
 601       CGTACTGCAGGGGTCCGTGGGAAAATTTCCTCATATACATTTAAGGGTCCCTCATAGTCT
 661       TGTGTTCCCAGTATTATCACCTTTTGATGCAAACTCCTGTTCATTTTAGTGTTTCGATGC
 721       TATAGTTTATGCCCCATCGAATGGAAATTTTCAATAAACAAAATGCATCTCACGGTCGTG
 781       GAATTTTAAGTTGGGGGTCTATCGCCCGAGGCATTACGGGTACGGTTCCATGGATATATA
 841       ATGCAGGGGGTGTGGGTTTGATGAGCAGTTGGGGCGGCAAAAATGCAGGCGTTGTTGCTA
 901       GTATTGGTTCTATTCATAGTACAGATCTATTTGTTGCCTGGAAATGGTAAGTAGGGGAAG
                Xba I                                   Sca I
 961       AGAACTCTAGACTGGAGGTTGTGGAATACGCTATACTGATGTAACCAGTACTCGTAAAAG
1021       CTGAGGGGTTTCGTCTCTGGCATGTGCAGCAATGGTGGTATAAGGAAAGGTAGCACGGGG
1081       GATGGTGTTGTTCTGATAAATGCATGCTCTTAATAATGTAGGCATATCACTGGAGAGTCT
                Sal I
1141       CGCTGTCGACAAGAGGTGCAAGTGCGTGAAAGTCACTAATCGGCCTACTGGCTTGGGGCC
                                        Pst I
1201       TATTATCGCTGTTGACGTGATACCACCGGGTATACACTGCAGGAGGACTGAAATTATGTA
                                                Nhe I
1261       AGTAAGCTCTATTACTTGGCCAAAATATGGGGTTGTCATCTTAGGTGTAGTGTCTGGCTA
1321       GCTTTGCTCTCAAGAAGAACAGGAAGGTATGTGTGGACCCTGAGGCGCCTTGGGTACAGC
1381       AGTTTATTAAAAAACTAGAACGACAGCATCGCACAAGGAAGGAAAATCTGATGGTTGGAG
1441       AAGATGGTGGCAAATCGACCGTGGGACCGGTAAAAAACACAATTGAGCCCACACCTCCTA
1501       CTATTGGTTCCCATATCTGTCTTTGATTAATTGCTACCTCCTGTAATTAAAAGAAGGCAC
                                              Cla I
1561       TTTTTTTTTCCGATTTTGACTCCTCGTACATATATCGATAATGTAGCTATTGGATGGAGC
1621       AGGGATACGGTCTCAGAACTGGATGTAGTAATGCAGGCAGGTGTACAACTGTCTGTCGGC
1681       CGCGAGAGGTTTTAGGGCCCCGGGGTTTATTCAGTGCTGCGCCGATTCTACCCCGAGAC
1741       TCGCACATTTCTGAGGGTATCGGACTTCTCTATCCCCACATATTCTAGCAACGGTTCTCC
1801       ATCGCGCAATTAAAGCACGATTAAGTACCCCAAGTTCACTGACTCTTCGTGCAAAAGGGG
1861       GAAAAAAGAAAGCCGAGACCACAAGATGGCACTGACCCCGTGCACGGGGATCTGCGGGAC
                Sma I
1921       GGGTGATGACCCGGGGGCATCGCACTGCCCTCAGCCCAAGCCCCAGGGCCACGTTTTTTT
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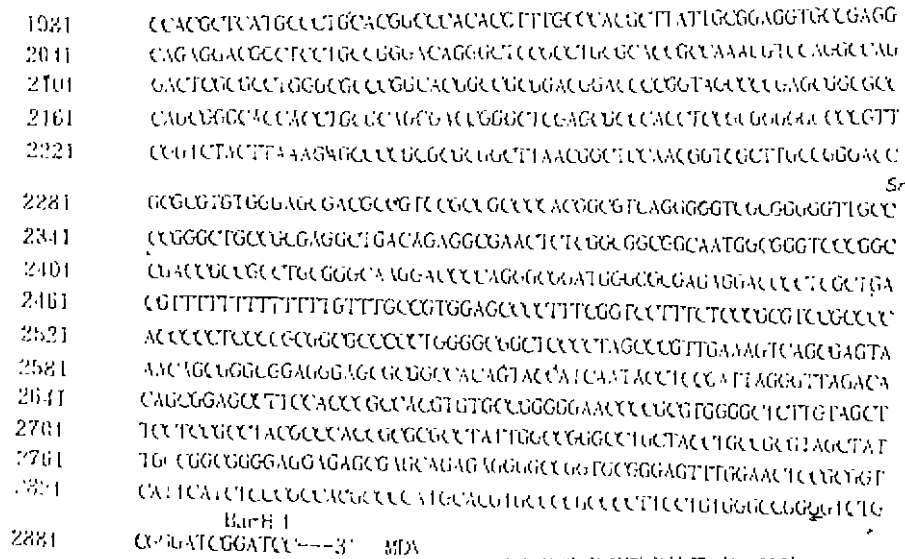

图2 MDV GA 株 BamHI L DNA 片段核酸序列测定结果，长 2893bp，常见的限制性内切酶识别位点及碱基数码也分别示出

Fig. 2 Nucleotide sequence of the 2893bp BamHI－L fragment within the region of IR_L in MDV genome (strain GA) Conventional restriction enzyme recognizing sites are given and nucleotides are numbered on the left of the sequences

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Sequence Determination of the Specific DNA Fragment from the Genome of Strong Virulent GA Strain of the Marek's Disease Virus

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Abstract Differentiation of strong virulent and vaccinal Marek's disease virus (MDV) was carried out by using DNA molecular hybridization. DNA probe was made of BamHI-L fragment from MDV gene library (strain GA) by labelled with digoxigenin using random priming. And the complete nucleotide sequence of BamHI-L fragment was further determined from subcloned DNAs by using double-stranded sequencing method. The results of computer analysis showed that, (1) the entire DNA fragment is 2 893 bp long and has a base composition of 55.91% G+C; (2) it has no any open reading frames (ORFs); (3) this DNA fragment is possibly involved in transcription of *meq* gene of MDV.

Key words Marek's disease virus, Strong virulent strain, Sequencing