

## **Cloning and characterization of the 5S rRNA genes from *Eimeria* spp.**

(Pengklonan dan pencirian gen 5S rRNA daripada *Eimeria* spp.)

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Key words: avian *Eimeria*, ribosomal RNA, coccidiosis

### **Abstract**

Poultry coccidiosis is an economically important disease worldwide. It is caused by intracellular protozoan parasites of the genus *Eimeria* (phylum Apicomplexa). Traditional methods of relying on disease pathology or oocyst morphology have limitations particularly in detecting minor contaminating populations of *Eimeria* in chicken. Therefore, a DNA-based test using ribosomal RNA (rRNA) was chosen to identify a molecular marker to enable faster and more sensitive identification of a particular species.

The 5S rRNA gene was chosen because of its high degree of conservation, ubiquity and the relative ease with which it can be cloned. The 5S rRNA genes from *Eimeria* spp. were amplified by the polymerase chain reaction (PCR) using purified DNAs of the sporozoites. TA Cloning method was used to clone the PCR products (600–900 bp) into plasmid vector pCR 2.1 (3.9 kb) and transformed into *Escherichia coli* strain TOP10F'. Recombinant plasmids with size of 4.6–4.8 kb were found. Clones containing the inserts of the appropriate size were sequenced by automated sequencing whereby M13 forward and reverse primers were used. The 5S rRNA genes from the seven *Eimeria* species were successfully sequenced.

The sequences obtained were then sent to the Basic Local Alignment Search Tool (BLAST) program and results showed that all sequences were identical to the 5S rRNA gene from other organism. Sequences of 726, 738, 697, 673, 732 and 931 bp were each shown by *E. tenella*, *E. acervulina*, *E. praecox*, *E. maxima*, *E. brunetti* and *E. necatrix* whilst *E. mitis* has two sequences of 710 and 592 bp. For each species, at least 2 clones of PCR-generated fragments were sequenced. The results indicated that the presence of unique amplified DNA segments could be exploited as molecular markers to identify *Eimeria* species of the chicken.

### **Introduction**

Coccidiosis, caused by species of intracellular protozoan parasites belonging to the genus *Eimeria* (phylum

Apicomplexa), is one of the economically important diseases in modern poultry production (Schnitzler et al. 1998). Currently, seven species of *Eimeria* are

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known to infect chickens. These species differ considerably in their biology and pathogenicity (Mc Dougald and Reid 1997). Being able to accurately identify *Eimeria* species and strains has major implications for diagnosis and control as well as for studying their epidemiology and population biology. In the domestic fowl, seven species with different degrees of pathogenicity are recognized i.e. *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella*. Of these, three of them (*E. acervulina*, *E. maxima* and *E. tenella*) are the most prevalent in the broiler industry (Mc Dougald and Reid 1997).

The various species can be distinguished on the basis of the morphology of their oocysts and the sites of infection within the intestine. Quick, precise identification of *Eimeria* spp. is required for the selection of anticoccidial drugs, diagnosis and epidemiological surveys. Traditional methods for diagnosing coccidial infections are relatively insensitive and do not reliably differentiate between species. In mixed infections, only the major species present is likely to be identified. The oocysts of *E. tenella*, one of the most pathogenic of several species causing chicken coccidiosis, are difficult to distinguish microscopically from several other infective *Eimeria* species. Through molecular biology, other techniques are now available for the discrimination of *Eimeria* species from chickens (Ellis and Bumstead 1990; Procnier et al. 1993; Shirley 1994). Polymerase chain reaction (PCR) can amplify a specific nucleic acid sequence and exponentially increases the number of copies for detection.

One copy of a gene coding for 5S ribosomal RNA has been cloned from *E. tenella* and sequenced. A coding region of 120 nucleotides and an intergenic region of nucleotides together make up a 5S rRNA repeat unit, of which there are many copies tandemly repeated in the genome. The intergenic region is species-specific and sequences derived from it can be used for

species identification by PCR (Ellis et al. 1992).

Small subunit ribosomal RNA is a genetic unit of broad evolutionary interest, found in both prokaryotic and eukaryotic organisms (Sogin 1990; Escalante and Ayala 1995). The rRNA gene contains more variable regions useful in the discrimination of the species or strains of pathogenic microorganisms (Ellis et al. 1992; Jensen et al. 1993; Allsopp et al. 1994). The conserved of the rRNA gene sequence facilitated development of generic DNA probes for discrimination of parasites.

In chicken *Eimeria*, the rRNA gene has been used for the discrimination of species and strains (Ellis and Bumstead 1990). The srRNA gene-based diagnosis procedure, which enables rapid and precise identification of the parasite species from infected chickens, would be useful for disease control. However, no sequence information of the rRNA gene has been documented for constructing the species-specific primers to amplify the target gene except for *E. tenella* (Stucki et al. 1993). In this study, the 5S rRNA gene was chosen because of its high degree of conservation, ubiquity and the relative ease with which it can be cloned. The purpose of this project was to clone and characterise the 5S rRNA gene from the seven species of *Eimeria* that infected chickens.

## Materials and methods

### *Parasites*

Purified DNAs of all the seven *Eimeria* species: *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella*, Houghton isolate, were supplied by F. Tomley (Institute of Animal Health, Compton, UK).

### *Polymerase chain reaction (PCR)*

Sequences corresponding to the intergenic region between 5S rRNA genes were amplified using an antisense primer beginning 14 bases upstream of the 5S gene SSR (5' TACGCGGCAGGGGACTCCGCG)

and a sense primer beginning 2 bases downstream of the gene 5SL (5' CTAACCGGGCCTTGCGCC). PCR was performed in 20 µL total reaction volume with 40 pM of each primer, 0.2 mM dNTPs and 5 units *Taq* DNA polymerase (Promega, USA). Reaction cycles consisted of an initial denaturing step at 94 °C for 3 min followed by 30 cycles at 94 °C for 60 s, 60 °C for 60 s and 72 °C for 60 s with final extension cycle at 72 °C for 7 min using a Perkin-Elmer 480 (Norwalk, CT, USA). The amplified DNA fragments resulting from PCR were analysed directly on 1% agarose gels by ethidium bromide staining. The gels were run in 1 x TAE buffer [40 mM Tris (pH 8.0), 10 mM sodium acetate, 1 mM ethylenediaminetetraacetic acid (EDTA)] for 1 h at a constant 90 V, stained with ethidium bromide (10 mg/mL) and photographed. The negatives were analysed and the molecular sizes of the fragments were determined using 100 bp ladder (Promega, USA).

#### ***Cloning of the 5S gene from fragments into plasmid vector pCR 2.1***

PCR products were cloned into the *pCR 2.1* vector using Original TA Cloning (Invitrogen, USA) as described in the kit manufacturer's instructions. At least 10 transformed colonies were cultured for plasmid extraction. If the 5S rRNA cloned inserts were identical in size, at least two were sequenced and all inserts of different sizes were sequenced.

#### ***Analysis of recombinant plasmids***

White colonies were picked and cultured overnight at 37 °C in LB broth containing ampicillin (50 µg/mL). Plasmid minipreparations were carried out by the modified alkaline lysis method (*Wizard Plus SV Minipreps DNA Purification System*, Promega, USA). The recombinant plasmid was digested with *EcoRI* (10 unit/µL) (Promega, USA) and the inserts were analysed by 1% agarose gel electrophoresis separated and visualised by ethidium bromide staining.

#### ***DNA sequencing***

Clones with inserts were sequenced using the *ABI Prism BigDye Terminator cycle sequencing ready reaction kit* (Perkin-Elmer, CA, USA) using forward and reverse M13 primers, as described in the manufacturer's instructions. Sequences were submitted to the Centre for Gene Analysis and Technology (Universiti Kebangsaan Malaysia) for gel separation on *ABI 377 DNA sequencers* (Perkin-Elmer) and results were analysed and edited using *Sequence Navigator* (Applied Biosystems, Perkin-Elmer).

### **Results and discussion**

#### ***Polymerase chain reaction (PCR)***

On agarose gels (*Plate 1*), the sizes of the PCR products varied from about 600–900 bp. For 5S rRNA-PCR products, one band was detected for *E. acervulina*, *E. brunetti*, *E. maxima*, *E. praecox* and *E. tenella*, whereas two bands were resolved for the other two species (*E. mitis* and *E. necatrix*). The resolution of multiple 5S rRNA bands on agarose gels for some species indicated the existence of different sequence types within a PCR product. This was confirmed by sequencing (via cloning) of the 5S rRNA PCR products for selected samples representing each species.

#### ***DNA sequencing***

The TA Cloning method was used to clone the PCR products (600–900 bp) into the plasmid vector *pCR 2.1* (3.9 kb) and transformed into *E. coli* strain TOP10F'. Recombinant plasmids with sizes of 4.5–4.8 kb were observed using the Supercoil DNA markers (Promega, USA) (data not shown). Clones containing inserts of the appropriate size were sequenced by automated DNA sequencing whereby M13 forward and reverse primers were used. The 5S rRNA genes from seven *Eimeria* species were successfully sequenced. The sequences obtained were then sent to the Basic Local Alignment Search Tool (BLAST) program (Altschul et al. 1990) and results showed

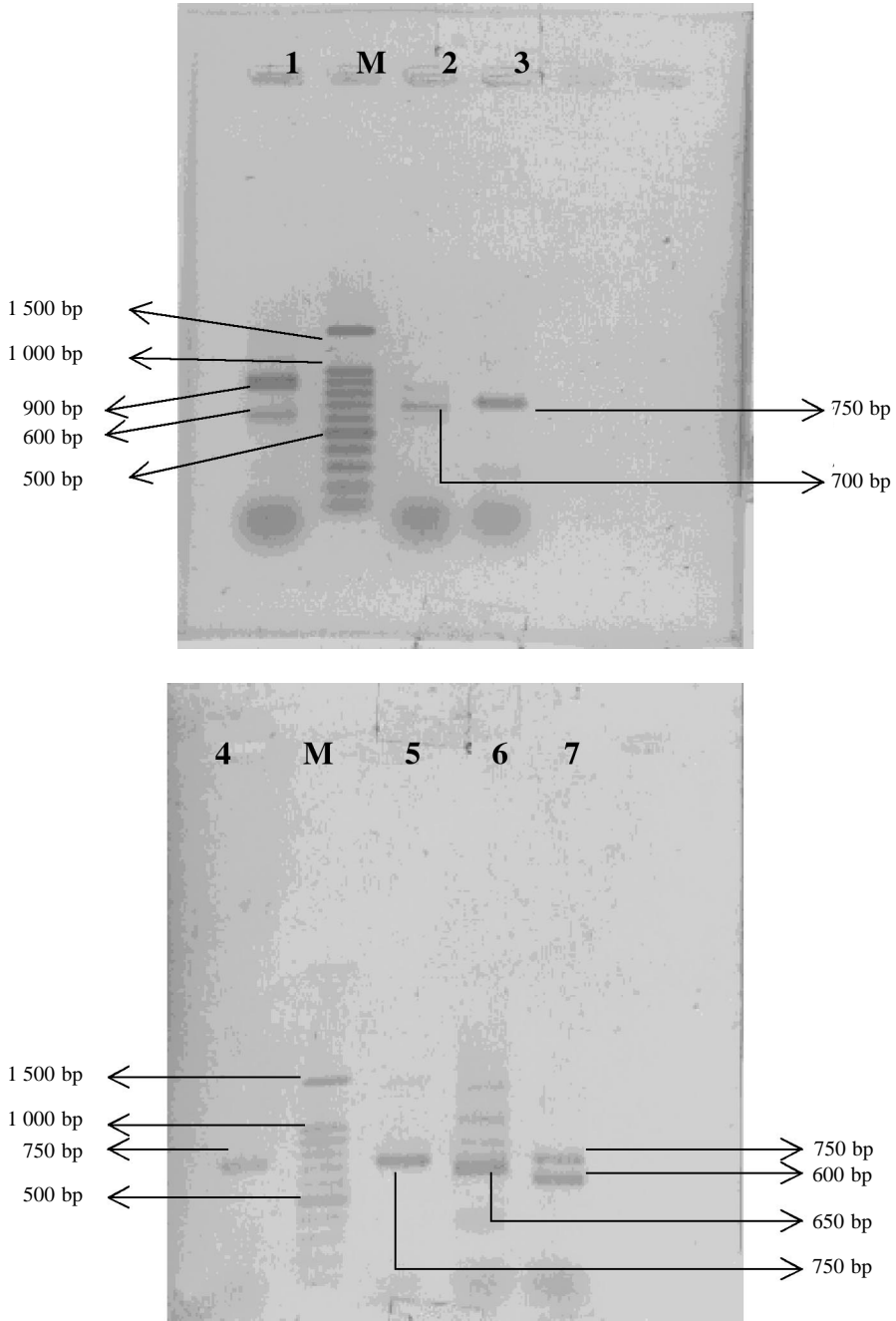


Plate 1. Ethidium bromide-stained agarose gel showing PCR-amplified segments of DNA from seven *Eimeria* spp. using 5SR and 5SL primers. Fragments were separated in 1% agarose gel and stained with ethidium bromide. Lane 1: *E. necatrix*, Lane 2: *E. praecox*, Lane 3: *E. tenella*, Lane 4: *E. acervulina*, Lane 5: *E. brunetti*, Lane 6: *E. maxima*, Lane 7: *E. mitis* and M: 100-bp DNA ladder (Promega, Madison, WI)

that all sequences were identical to the 5S rRNA gene from other organisms. This included two species i.e. *E. necatrix* and *E. mitis* which have two bands. Sequences of 726, 738, 697, 673 and 732 bp were each obtained for *E. tenella*, *E. acervulina*, *E. praecox*, *E. maxima* and *E. brunetti* respectively. *Eimeria necatrix* has two sequences of 930 bp and 609 bp while *E. mitis* has sequences of 710 bp and 592 bp (Figure 1). For each species, at least two clones of PCR-generated fragments were sequenced.

Using the BLAST program to send the information to a relevant database e.g. GenBank showed that all sequences were identical to the 5S rRNA gene, except for the 609 bp sequence from *E. necatrix*, which has a high percentage of similarity to the insertion sequence of *Pseudomonas aeruginosa*. This may be the result of contamination of the *E. necatrix* DNA. On the other hand, *E. mitis* showed the presence of polymorphism. As in other organisms, the 5S rRNA genes of *Eimeria* spp. are tandemly repeated in the genome (Stucki et al. 1993). The size of 5S rRNA gene is 120 nucleotides and the spacer is different in size and sequence for each species. It is not clear whether all the genes belong to a single cluster, but they appear to be localized on one chromosome (Shirley et al. 1990). Intergenic regions between repeated, conserved genes are usually very variable from species to species, since they are not subjected to selective pressure. They are therefore ideally suited as species-specific probes (Stucki et al. 1993).

The srRNA gene-based diagnosis procedure, which enables rapid and precise identification of the parasite species from infected chickens, would be useful for disease control. However, only 5S rRNA sequence from *E. tenella* has been reported so far (Stucki et al. 1993). The 5S rRNA sequences reported here is the first time reported for other species of *Eimeria*. The size of the PCR product of *E. tenella* from this study showed some similarity to that of

*E. tenella* Stucki's group (728 bp) (1993). The size of PCR product obtained in this study was 726 bp. It was suggested that this PCR product was 5S rRNA gene and had 97% homology to 5S rRNA sequence *E. tenella* of Stucki et al. (data not shown).

Ribosomal gene repeats have two advantages in DNA-based diagnosis. They are present in high copy numbers, which improve sensitivity. The spacer region between ribosomal genes are normally less conserved than the genes themselves, allowing discrimination between species.

### Conclusion

All the seven PCR products were cloned and the full sequences were successfully obtained from all species. The size of 5S rRNA gene is 120 nucleotides and the spacer is different in size and sequence for each species. The information about the 5S rRNA genes is useful as a molecular marker for future diagnostic work in parasite-based diseases.

The excellent sensitivity suggests that rRNA-based probes are capable of detecting individual parasites and can assay low levels of coccidial infections not detectable by other methods. The results of this study showed that it was possible to customize the specificity of rRNA-based probes for diagnostic, epidemiological or taxonomic purposes. Due to the economic impact of coccidiosis on the poultry industry, it is important that species of *Eimeria* are readily identifiable, such that rapid diagnosis of disease and early treatment can be carried out. Further sensitive and reliable identification of *Eimeria* is desirable for epidemiological study of the diseases and for controlling the purity of laboratory lines of *Eimeria*. Accordingly, there is a need to develop an assay for the rapid identification of species of *Eimeria* which does not have the limitations of previously described assays.

Cloning and characterization: 5S rRNA from *Eimeria* spp.

EIMERIA ACERVULINA

738 bp

5SL

1	<b><u>CTAACCGGGC</u></b>	<b><u>CTTGCGCCGC</u></b>	<b><u>TAACTTCGG</u></b>	<b><u>AGTTCAGATG</u></b>	<b><u>GGATCCGGTG</u></b>	<b><u>CATTGACGCG</u></b>
61	<b><u>AGTATGAACG</u></b>	<b><u>ACGGCAAACC</u></b>	<b><u>AAACTCTCT</u></b>	<b><u>CAAAATACAC</u></b>	<b><u>ACAGCCATTA</u></b>	<b><u>CCATCTTCTC</u></b>
121	<b><u>CACATATGCT</u></b>	<b><u>ACAACCAAAA</u></b>	<b><u>ACAACCTCAGA</u></b>	<b><u>TCCAACCAAC</u></b>	<b><u>AAACCCCTTAT</u></b>	<b><u>TTTGCCCTCT</u></b>
181	<b><u>TCCAGGGCTA</u></b>	<b><u>CGATAGGGCT</u></b>	<b><u>GCGTGGACTC</u></b>	<b><u>GCGCTGGATA</u></b>	<b><u>CGGGTGGCGC</u></b>	<b><u>TAAACACTCC</u></b>
241	<b><u>ATTCTATGCT</u></b>	<b><u>CTCACATGCG</u></b>	<b><u>GATTTGCTCA</u></b>	<b><u>TGTCAAATCC</u></b>	<b><u>CTGCATCATG</u></b>	<b><u>GAAGCAAAAT</u></b>
301	<b><u>TAACGACGAT</u></b>	<b><u>GTTGGATTAG</u></b>	<b><u>AGCCACTTGG</u></b>	<b><u>TTGCATGTGA</u></b>	<b><u>GCGCCTGGAG</u></b>	<b><u>ACAGAACTGG</u></b>
361	<b><u>CAITTTGGAT</u></b>	<b><u>TTGAAGGGCT</u></b>	<b><u>CGGAGTGGGG</u></b>	<b><u>CAGCATGCCG</u></b>	<b><u>GTCGGACTGC</u></b>	<b><u>GCAAGTGTGA</u></b>
421	<b><u>AGGCCTATTG</u></b>	<b><u>AGGATAGAGG</u></b>	<b><u>AGGGGAATAA</u></b>	<b><u>TGATCATGGG</u></b>	<b><u>ACGTTACAGAA</u></b>	<b><u>GCCTGTGTGT</u></b>
481	<b><u>TGGAGAGTGG</u></b>	<b><u>ATTTTGTGTA</u></b>	<b><u>TGCGTGTGTG</u></b>	<b><u>TGTGTGTATA</u></b>	<b><u>TATGTGTGTG</u></b>	<b><u>TATGCGTATG</u></b>
541	<b><u>TGTATGTATG</u></b>	<b><u>TGCGTGTGCA</u></b>	<b><u>TTTGTCCGAG</u></b>	<b><u>CGACATGTGC</u></b>	<b><u>ATTTTGTATA</u></b>	<b><u>CATAAGCGTG</u></b>
601	<b><u>GGTAGCAGTG</u></b>	<b><u>GTGGCTATGA</u></b>	<b><u>ATAAAGAGTT</u></b>	<b><u>GAAATTGGTG</u></b>	<b><u>GAGAAATAAG</u></b>	<b><u>CGGAACTGTC</u></b>
661	<b><u>AAAAAATGTG</u></b>	<b><u>TGCACGGGAG</u></b>	<b><u>CTGTGTAAAT</u></b>	<b><u>TAAAAAGAAA</u></b>	<b><u>AGAGGCGTCA</u></b>	<b><u>TCACCCAAAG</u></b>
721	<b><u>GGATTTCGCC</u></b>	<b><u>GGAGTCCC</u></b>				

5SR

EIMERIA BRUNETTI

732 bp

5SL

1	<b><u>CTAACCGGGC</u></b>	<b><u>CTTGCGCCGC</u></b>	<b><u>TAACTTCGG</u></b>	<b><u>AGTTCAGATG</u></b>	<b><u>GGATCCGGTG</u></b>	<b><u>CATTGACGCG</u></b>
61	<b><u>AGTATGAACG</u></b>	<b><u>ACGGCAAATA</u></b>	<b><u>CAITACCCTT</u></b>	<b><u>CAAACAACAC</u></b>	<b><u>ACACCCTTAT</u></b>	<b><u>ACTTCTTATC</u></b>
121	<b><u>CACACTTCAT</u></b>	<b><u>GCCAACAATA</u></b>	<b><u>CACCTCAGC</u></b>	<b><u>CCACCACAAA</u></b>	<b><u>CGTCTTACCC</u></b>	<b><u>TCATCTCATA</u></b>
181	<b><u>CCCAACAGAA</u></b>	<b><u>CCCTAGCAAC</u></b>	<b><u>ACACCACTAT</u></b>	<b><u>ACCCGCCCTT</u></b>	<b><u>TTTCCCCCTT</u></b>	<b><u>TCTACACGCA</u></b>
241	<b><u>CACTTCGGCT</u></b>	<b><u>GCGCTTTCGA</u></b>	<b><u>TTTCTACGG</u></b>	<b><u>GCGTTGCTAA</u></b>	<b><u>CTGTGCGCCA</u></b>	<b><u>CTTGTGGTTC</u></b>
301	<b><u>TGGTCTACAC</u></b>	<b><u>AATCCCTAGC</u></b>	<b><u>ACACAGGCAG</u></b>	<b><u>GCGTAACGTT</u></b>	<b><u>GAAGGCACCTG</u></b>	<b><u>GGCACTGGTA</u></b>
361	<b><u>CTGGGTACTG</u></b>	<b><u>GCCAGGTCGC</u></b>	<b><u>GTCCCGAAG</u></b>	<b><u>ACAGCTGGTG</u></b>	<b><u>GTCGCTTTCG</u></b>	<b><u>AGGAGTTTGT</u></b>
421	<b><u>TGAGTTGAAT</u></b>	<b><u>CAAGGAATAT</u></b>	<b><u>AAAGGAATGT</u></b>	<b><u>TGAGGGCAGA</u></b>	<b><u>GGCTTGGAGT</u></b>	<b><u>ACTGTGTATG</u></b>
481	<b><u>AGACATTCAT</u></b>	<b><u>GCTTTTGCCT</u></b>	<b><u>TGGAGAGTGG</u></b>	<b><u>AGTGCGTTTG</u></b>	<b><u>CCGAAAGAGT</u></b>	<b><u>GCGGATGGCG</u></b>
541	<b><u>GAGGTGTGTG</u></b>	<b><u>GGCCTGTGTG</u></b>	<b><u>TGTAAGGATG</u></b>	<b><u>CCTACTTGAG</u></b>	<b><u>AGGGTGTGTC</u></b>	<b><u>GTTAGGAGAT</u></b>
601	<b><u>GAAAGGAGTT</u></b>	<b><u>TGAGAGTGAG</u></b>	<b><u>TAGAGCATGA</u></b>	<b><u>AAGCAGTGTG</u></b>	<b><u>CTCATGAGCG</u></b>	<b><u>TAGTGTGTAT</u></b>
661	<b><u>TTATCTGTAT</u></b>	<b><u>ATATATATGT</u></b>	<b><u>AATGTAATAA</u></b>	<b><u>GGCCGTCATC</u></b>	<b><u>ACCCAAAGGG</u></b>	<b><u>ATTCCCGGGA</u></b>
721	<b><u>GTCCCTACGC</u></b>	<b><u>AG</u></b>				

5SR

EIMERIA MAXIMA

673 bp

5/SL

1	<b><u>CTAACCGGGC</u></b>	<b><u>CTTGCGCCGC</u></b>	<b><u>TAACTTCGG</u></b>	<b><u>AGTTCAGATG</u></b>	<b><u>GGATCCGGTG</u></b>	<b><u>CATTAGACGCG</u></b>
61	<b><u>AGTATGAACG</u></b>	<b><u>ACGGCAAACA</u></b>	<b><u>CAACTCTCTC</u></b>	<b><u>CAAACAACAC</u></b>	<b><u>TCTACCATAC</u></b>	<b><u>ACACCTTGCC</u></b>
121	<b><u>CACTATTTCAT</u></b>	<b><u>ACCAACAATC</u></b>	<b><u>AACTCGCCTA</u></b>	<b><u>CCCTCGAACC</u></b>	<b><u>AAACTCCCTC</u></b>	<b><u>TCACCTACTT</u></b>
181	<b><u>CCACAGCAAA</u></b>	<b><u>GCCAAGGCAA</u></b>	<b><u>CGCCCTCTT</u></b>	<b><u>TGCCTTCTTC</u></b>	<b><u>CTACGCGCGC</u></b>	<b><u>CCTCAACTGA</u></b>
241	<b><u>TCACCCATTG</u></b>	<b><u>TACCAATACT</u></b>	<b><u>TGCTGTCTCC</u></b>	<b><u>CCCAACTCGA</u></b>	<b><u>ACAAGTGTCTG</u></b>	<b><u>TTGCATGAAA</u></b>
301	<b><u>GAGTGTACAA</u></b>	<b><u>TAACATTGAT</u></b>	<b><u>TCTGTGGCT</u></b>	<b><u>GCAGCACGGG</u></b>	<b><u>CTAGGTGTG</u></b>	<b><u>TATTTTTGTT</u></b>
361	<b><u>TGTCGAGGCG</u></b>	<b><u>ATCCGCGAGT</u></b>	<b><u>GTGTGGATAT</u></b>	<b><u>AGAGGAGTAA</u></b>	<b><u>TGAGGGTGGGA</u></b>	<b><u>TGGGAATTAG</u></b>
421	<b><u>GTAGCTCAGG</u></b>	<b><u>AGATATTGGA</u></b>	<b><u>AAAGTGGGTT</u></b>	<b><u>TGTGTTTGGC</u></b>	<b><u>TGTGTATAGA</u></b>	<b><u>AGTAGTTGTA</u></b>
481	<b><u>GTGTGAGTTG</u></b>	<b><u>TAGATGTGTG</u></b>	<b><u>TAAGCGTAGT</u></b>	<b><u>ATGTGTGTAA</u></b>	<b><u>GGTGGCTAG</u></b>	<b><u>GGGGCCATCT</u></b>
541	<b><u>GTATGCTAGG</u></b>	<b><u>ATGTGGATTG</u></b>	<b><u>AGTGTATAAA</u></b>	<b><u>TAAGTGAAGG</u></b>	<b><u>GAGGATAGTA</u></b>	<b><u>TAAGTGTAGG</u></b>
601	<b><u>GGTGTGGTGT</u></b>	<b><u>AAATGAGCAT</u></b>	<b><u>AAAAAGAGGC</u></b>	<b><u>CGTCATCACC</u></b>	<b><u>CAAAGGGATT</u></b>	<b><u>ACCGGGAGTCC</u></b>
661	<b><u>CCCTACCGCA</u></b>	<b><u>GTA</u></b>				

5SR

EIMERIA

MITIS (Jalur 1)

710 bp

5SL

1	<b><u>CTAACCGGGC</u></b>	<b><u>CTTGCGCCGC</u></b>	<b><u>TAACTTCGG</u></b>	<b><u>AGTTCAGATG</u></b>	<b><u>GGATCCGGTG</u></b>	<b><u>CATTAGACGCG</u></b>
61	<b><u>AGTATGAACG</u></b>	<b><u>ACGGCAAATC</u></b>	<b><u>AACAACCTCT</u></b>	<b><u>CAAATCACAC</u></b>	<b><u>CCAACCCGAC</u></b>	<b><u>ACTGCGCCTA</u></b>
121	<b><u>CACACTCGAC</u></b>	<b><u>ACCAACGAAC</u></b>	<b><u>GATTCTGAACC</u></b>	<b><u>AAACAACAAC</u></b>	<b><u>CACCCCCAGC</u></b>	<b><u>TCAAGTCTCA</u></b>
181	<b><u>GTCGAAAATC</u></b>	<b><u>TAAGGCTGCG</u></b>	<b><u>CTCTGCTCTT</u></b>	<b><u>TGTGAGGGCA</u></b>	<b><u>GCCACACTAA</u></b>	<b><u>GGCCTTCTGC</u></b>
241	<b><u>GTCCCTTCCC</u></b>	<b><u>GCGTCTACG</u></b>	<b><u>ACTGGGCTTT</u></b>	<b><u>TCTCTCGCTG</u></b>	<b><u>TGATGGCCCT</u></b>	<b><u>CAAGCGCCAT</u></b>
301	<b><u>TTATCCGTGG</u></b>	<b><u>ATCGTGTAT</u></b>	<b><u>GGCTTAGTGC</u></b>	<b><u>CACATGCAGG</u></b>	<b><u>CAGCGCAAGT</u></b>	<b><u>AGTGGCAATG</u></b>
361	<b><u>GTGTATGACA</u></b>	<b><u>GCTTCCTTGG</u></b>	<b><u>TGTTTGCAA</u></b>	<b><u>CGTCCCAGAG</u></b>	<b><u>GCGAGCGGCT</u></b>	<b><u>CGGTTGCGCA</u></b>
421	<b><u>GTGGCGAGGC</u></b>	<b><u>TGTACAGCGC</u></b>	<b><u>GTTGCCTCAC</u></b>	<b><u>ATCCGTGAGG</u></b>	<b><u>CTAGCCGTGT</u></b>	<b><u>TCTGAAAGCA</u></b>
481	<b><u>GAGGTGTGGA</u></b>	<b><u>ATGTTGCGCA</u></b>	<b><u>TGGAAAGCGC</u></b>	<b><u>TAGAGTTTGC</u></b>	<b><u>GTTGCGACGT</u></b>	<b><u>GGAAACTGTA</u></b>
541	<b><u>AGTGTAGGAT</u></b>	<b><u>GTGTGGCTTG</u></b>	<b><u>TGCGTGGCAA</u></b>	<b><u>GTGTGGACGG</u></b>	<b><u>GAACGGAAGA</u></b>	<b><u>GATGTGTGCG</u></b>
601	<b><u>TTGACGGAGT</u></b>	<b><u>GCTAGCGTGC</u></b>	<b><u>ATAATTGAAG</u></b>	<b><u>CGCAAACGTC</u></b>	<b><u>CGACTGCATG</u></b>	<b><u>AAGGGTGTGT</u></b>
661	<b><u>TGGATAAACA</u></b>	<b><u>AAAAAGGCCG</u></b>	<b><u>TCATCACCCA</u></b>	<b><u>AAGGGATTCC</u></b>	<b><u>CGCGGAGTCC</u></b>	

5SR

EIMERIA	MITIS (Jalur 2)	592 bp						
		5SL						
		1	<b><u>CTAACCGGGC</u></b>	<b><u>CTTGCGCCGT</u></b>	<b><u>TAACTTCGG</u></b>	<b><u>AGTTCAGATG</u></b>	<b><u>GGATCCGGTG</u></b>	<b><u>CATTAGACGC</u></b>
		61	<b><u>AGTATGAACG</u></b>	<b><u>ACGGCAAATC</u></b>	<b><u>AAACACCCCT</u></b>	<b><u>CAAATCACAC</u></b>	<b><u>CCACTCTTCA</u></b>	<b><u>CCTGCCACTC</u></b>
		121	<b><u>CTCAATTGCT</u></b>	<b><u>TCCAACACAC</u></b>	<b><u>CACTGGCAAC</u></b>	<b><u>TAACAACCTC</u></b>	<b><u>ATGCAGCCCC</u></b>	<b><u>ATCTCTCCCG</u></b>
		181	<b><u>TATACAACAG</u></b>	<b><u>CGCGACGCCG</u></b>	<b><u>AACCTCCCCA</u></b>	<b><u>TCCCAGTGGT</u></b>	<b><u>TACAAAAGTG</u></b>	<b><u>CTTAGCCCTT</u></b>
		241	<b><u>CCCACCTAC</u></b>	<b><u>AAGGTGGCGT</u></b>	<b><u>TCGCAGGTTG</u></b>	<b><u>TTGGGTGGAA</u></b>	<b><u>GTACCACAAG</u></b>	<b><u>TAATAATGGG</u></b>
		301	<b><u>TTTGGCTGTT</u></b>	<b><u>AGGGAGTGTG</u></b>	<b><u>GTAATTGTGA</u></b>	<b><u>GCGGACGCAG</u></b>	<b><u>AGATATGTGG</u></b>	<b><u>TATTTCTGTC</u></b>
		361	<b><u>GTGAAATGGA</u></b>	<b><u>GGATGTTGGG</u></b>	<b><u>ATGTTGTGCG</u></b>	<b><u>TGTTTGTGTG</u></b>	<b><u>CGTGTTTATT</u></b>	<b><u>GTATTTGGTT</u></b>
		421	<b><u>GTAGCAAATC</u></b>	<b><u>ACGCGCATA</u></b>	<b><u>GAGCGACGTT</u></b>	<b><u>GAGCGCTGAG</u></b>	<b><u>CATATGCTGT</u></b>	<b><u>CTCATGAATT</u></b>
		481	<b><u>TCGTGCGTGA</u></b>	<b><u>GTACGTGAGG</u></b>	<b><u>AATGCAAAAG</u></b>	<b><u>GTGTGTTGAG</u></b>	<b><u>CAATGCGTAG</u></b>	<b><u>ACGAAGAATA</u></b>
		541	<b><u>TGAAAAAGGC</u></b>	<b><u>CGTCAATCACC</u></b>	<b><u>CAAAGGGATT</u></b>	<b><u>CCCGGGAGTC</u></b>	<b><u>CCTACCGCAG</u></b>	<b><u>TA</u></b>
							5SR	
EIMERIA	NECATRIX	931 bp						
		5SL						
		1	<b><u>AATTCGGCTT</u></b>	<b><u>CTAACCGGGC</u></b>	<b><u>CTTGCGCCGC</u></b>	<b><u>TAACTTCGG</u></b>	<b><u>AGTTCAGATG</u></b>	<b><u>GGATCCGGTG</u></b>
		61	<b><u>CATTAGACGC</u></b>	<b><u>AGTATGAACG</u></b>	<b><u>ACGGCAAACC</u></b>	<b><u>TCACACTCCC</u></b>	<b><u>TCAATACACC</u></b>	<b><u>TCTACTTCAC</u></b>
		121	<b><u>ACACCTTCAA</u></b>	<b><u>CCAACAACCT</u></b>	<b><u>CAGACAACAA</u></b>	<b><u>CTTTCCACCC</u></b>	<b><u>AGCACCCTCT</u></b>	<b><u>CTCCAACACC</u></b>
		181	<b><u>TCACAGCGCA</u></b>	<b><u>TGTGATGCCC</u></b>	<b><u>ATCAATAACA</u></b>	<b><u>ACCCACTTAC</u></b>	<b><u>ACGCACACAC</u></b>	<b><u>AGCCCTTCGA</u></b>
		241	<b><u>CGACGCCCCC</u></b>	<b><u>CAACACCTTG</u></b>	<b><u>CGACGGCTTA</u></b>	<b><u>TCAACGTTCT</u></b>	<b><u>CCACAGTGCC</u></b>	<b><u>TCCTGATAAC</u></b>
		301	<b><u>CAACCTCAAC</u></b>	<b><u>ACAAAGTCTA</u></b>	<b><u>AAACATGTTG</u></b>	<b><u>CTGCCGCCGA</u></b>	<b><u>TATCTACTAC</u></b>	<b><u>TTTGCAGCTC</u></b>
		361	<b><u>CGCGCGAATG</u></b>	<b><u>CCCATACATA</u></b>	<b><u>GCTGGAACGA</u></b>	<b><u>ATGGGAGAGC</u></b>	<b><u>AGTGGAAAATC</u></b>	<b><u>AACAGTGGAC</u></b>
		421	<b><u>TGGTGTGTGAG</u></b>	<b><u>GCAACGCGCT</u></b>	<b><u>GGATTGATGT</u></b>	<b><u>GGAGGTTGAG</u></b>	<b><u>GGGAGAAAAA</u></b>	<b><u>ATGTAAGCAG</u></b>
		481	<b><u>AGCGAAATGG</u></b>	<b><u>TGTGGAGGAG</u></b>	<b><u>AGGGGGAAAA</u></b>	<b><u>ATGTAAGCAG</u></b>	<b><u>AGCGAAATGG</u></b>	<b><u>TGTGGAGGAG</u></b>
		541	<b><u>AAAAAAAGCT</u></b>	<b><u>ATGGCAAGGA</u></b>	<b><u>TGGATGTGAG</u></b>	<b><u>AGAGCGCGTG</u></b>	<b><u>TTGGTTGCGT</u></b>	<b><u>GTGTTGTGGG</u></b>
		601	<b><u>TTAGTGTCTGA</u></b>	<b><u>GGGTACTTTT</u></b>	<b><u>TGCATTTCCG</u></b>	<b><u>GTGTTGACCT</u></b>	<b><u>GTGGCAGTGG</u></b>	<b><u>TTAGGGACAG</u></b>
		661	<b><u>AAGCGCGGAA</u></b>	<b><u>GTGGCGTTAT</u></b>	<b><u>GGAGGATGGC</u></b>	<b><u>AGGGGTGTCT</u></b>	<b><u>AGTTGGGGGT</u></b>	<b><u>TTGTGGTGCC</u></b>
		721	<b><u>TGTGGGTTGA</u></b>	<b><u>GCATGTATAA</u></b>	<b><u>GGTTGAGGCG</u></b>	<b><u>GGAGGTGCGA</u></b>	<b><u>TTGAGAGATG</u></b>	<b><u>GGACCGAAGC</u></b>
		781	<b><u>AAATTGGGGT</u></b>	<b><u>GTTTGTCTGT</u></b>	<b><u>TGTTGAGTCG</u></b>	<b><u>AAGGCGAGCG</u></b>	<b><u>AGGTGTTAGA</u></b>	<b><u>GTTGAATAGA</u></b>
		841	<b><u>AGAGGGTTGA</u></b>	<b><u>GAGGGGAGCG</u></b>	<b><u>AACAAAACATT</u></b>	<b><u>GGGAAGCAAA</u></b>	<b><u>CAAAAAGGCC</u></b>	<b><u>GCCATCACCC</u></b>
		901	<b><u>AAAGGGATTC</u></b>	<b><u>CCGCGAGTCC</u></b>	<b><u>CTACCGCAGT</u></b>	<b><u>A</u></b>		
							5SR	
EIMERIA	PRAECOX	697 bp						
		5SL						
		1	<b><u>CTAACCGGGC</u></b>	<b><u>CTTGCGCCGC</u></b>	<b><u>TAACTTCGG</u></b>	<b><u>AGTTCAGATG</u></b>	<b><u>GGATCCGGTG</u></b>	<b><u>CATTGACGC</u></b>
		61	<b><u>AGTATGAACG</u></b>	<b><u>ACGGCAAACG</u></b>	<b><u>ACAAAACCCT</u></b>	<b><u>CTTTTCACAC</u></b>	<b><u>ATAACACAAT</u></b>	<b><u>ACTTCGCAGC</u></b>
		121	<b><u>CTCGCATAAG</u></b>	<b><u>CCCAAATACA</u></b>	<b><u>ACCAAACCAA</u></b>	<b><u>AACATATCAA</u></b>	<b><u>TAACACCCAC</u></b>	<b><u>ACATCTCTAT</u></b>
		181	<b><u>ACCCTCTCCC</u></b>	<b><u>CGCAGACTCT</u></b>	<b><u>ACAGAGCTGC</u></b>	<b><u>TGCTTTCGAA</u></b>	<b><u>CGCTTAAAGC</u></b>	<b><u>AAGACGCTAA</u></b>
		241	<b><u>CTGTCAAGTT</u></b>	<b><u>TCCCCTGGTG</u></b>	<b><u>AACTCGCCGG</u></b>	<b><u>TTACAAAACAC</u></b>	<b><u>TTGTACAGAA</u></b>	<b><u>GTAACACAAG</u></b>
		301	<b><u>TCGGGAGGAA</u></b>	<b><u>GCTCGCAACT</u></b>	<b><u>AGCCATCTTG</u></b>	<b><u>ATGTTTATGA</u></b>	<b><u>ACTCATGATG</u></b>	<b><u>AAGACGGAGG</u></b>
		361	<b><u>TAGTAATTTG</u></b>	<b><u>GTTGTGGGAA</u></b>	<b><u>GGGGGAGGAT</u></b>	<b><u>GTGGAGTTTG</u></b>	<b><u>GGATTTTCGCA</u></b>	<b><u>TGTTTCGAGTG</u></b>
		421	<b><u>GGGGGGTATG</u></b>	<b><u>TTGAGCAGAA</u></b>	<b><u>GCATATTATG</u></b>	<b><u>GAGGGCATGG</u></b>	<b><u>TGCATATGTG</u></b>	<b><u>ACGTTTCGAGG</u></b>
		481	<b><u>ATTTGCAAGG</u></b>	<b><u>GGCATTGGAG</u></b>	<b><u>TGTGTGAGCG</u></b>	<b><u>TTTGGAGTAG</u></b>	<b><u>GCAGTGGTGT</u></b>	<b><u>CTCTGCATGC</u></b>
		541	<b><u>GTTGGTGGAT</u></b>	<b><u>AGTTATGGGA</u></b>	<b><u>GGCGTGTGTG</u></b>	<b><u>AGGCAGTGGG</u></b>	<b><u>TTGTACGGGT</u></b>	<b><u>GCAGAAGTGA</u></b>
		601	<b><u>AGTGTGGAAA</u></b>	<b><u>GTCTGAGTGC</u></b>	<b><u>ATGAGAGGAG</u></b>	<b><u>TGTGTGAAGT</u></b>	<b><u>GGGAGCAAAA</u></b>	<b><u>AAGGCCGTCA</u></b>
		661	<b><u>TCACCCAAAAG</u></b>	<b><u>GGATTCCCGG</u></b>	<b><u>GAGTCCCTTA</u></b>	<b><u>CCGATTA</u></b>		
							5SR	
EIMERIA	TENELLA	726 bp						
		5SL						
		1	<b><u>CTAACCGGGC</u></b>	<b><u>CTTGCGCCGC</u></b>	<b><u>TAAATTTCGG</u></b>	<b><u>AGTTCAGATG</u></b>	<b><u>GGATCCGGTG</u></b>	<b><u>CATTAGACGC</u></b>
		61	<b><u>AGTATGAACG</u></b>	<b><u>ACGGCAAATC</u></b>	<b><u>ATACGCTCCC</u></b>	<b><u>TCAATACACC</u></b>	<b><u>TCTACTTCAC</u></b>	<b><u>ACACCTTCAA</u></b>
		121	<b><u>CCAACAACCT</u></b>	<b><u>CCCACGCAAC</u></b>	<b><u>ATCCCTCTC</u></b>	<b><u>CAACACCACT</u></b>	<b><u>CAACGCCCTT</u></b>	<b><u>CACGCCACA</u></b>
		181	<b><u>AAAAACAACC</u></b>	<b><u>CACGTCCACC</u></b>	<b><u>CACACACAGC</u></b>	<b><u>CTTTCGACCA</u></b>	<b><u>CGCGCCCCCA</u></b>	<b><u>CGCCTTGCGC</u></b>
		241	<b><u>TAACTTATGG</u></b>	<b><u>ACGCTCTCTC</u></b>	<b><u>CACAGCGCGT</u></b>	<b><u>CCTCTTACC</u></b>	<b><u>AACCTCAACA</u></b>	<b><u>CAAAGCCTAT</u></b>
		301	<b><u>AACATGCTGC</u></b>	<b><u>TGCTGCAGCT</u></b>	<b><u>AGCTCCCAAT</u></b>	<b><u>TTCCAGCTGC</u></b>	<b><u>GCGCGGTGTC</u></b>	<b><u>TCGCACCTAG</u></b>
		361	<b><u>CTGCAACGAA</u></b>	<b><u>TGCGAGGGCG</u></b>	<b><u>GTGGAGAGAG</u></b>	<b><u>AGCGTGTGG</u></b>	<b><u>TGTGCGTGTG</u></b>	<b><u>TGTGGGTTTG</u></b>
		421	<b><u>TGTTGAAGGT</u></b>	<b><u>ATTTAGTGA</u></b>	<b><u>TGTTGAGTGT</u></b>	<b><u>TACTCTGTGG</u></b>	<b><u>CTGTGGATAG</u></b>	<b><u>GGAGAGAAGC</u></b>
		481	<b><u>GCGGAAGTGG</u></b>	<b><u>CGTTATGGAG</u></b>	<b><u>GGATGCGAGT</u></b>	<b><u>GGTGCGCAAT</u></b>	<b><u>TGGGGTTGAG</u></b>	<b><u>CATATAGAAG</u></b>
		541	<b><u>GTTGAGGGTG</u></b>	<b><u>GCGGTTTGAT</u></b>	<b><u>GAAGAGGATG</u></b>	<b><u>GGAAGGACGT</u></b>	<b><u>AAATTGGGGT</u></b>	<b><u>GTTTGCTCCT</u></b>
		601	<b><u>TGCTGAGTCG</u></b>	<b><u>AAGGTGAGCT</u></b>	<b><u>GTTAGAGGTT</u></b>	<b><u>AATAGAGGAG</u></b>	<b><u>GGTTGAGAGG</u></b>	<b><u>CGTAGGCGTT</u></b>
		661	<b><u>GTTAGGGAAG</u></b>	<b><u>CCAACAAAAA</u></b>	<b><u>GGCCGTTCATC</u></b>	<b><u>ACCCAAAAGG</u></b>	<b><u>ATTCCCGCGG</u></b>	<b><u>AGTCCCTAAC</u></b>
		721	<b><u>GCGAGTA</u></b>					5SR

Figure 1. Nucleotide sequence of the 5S rRNA repeat unit of *Eimeria* spp. The 5S rRNA gene is marked by bold and the pair of primers 5SL and 5SR is marked by underline

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### **Abstrak**

Koksidiosis pada ayam merupakan penyakit yang penting kerana ia mengakibatkan kerugian yang besar kepada dunia. Penyakit ini disebabkan oleh parasit protozoa intrasel daripada genus *Eimeria* (filum Apicomplexa). Teknik tradisi yang bergantung kepada patologi penyakit dan morfologi oosista ini mempunyai kelemahan dari segi mengesan populasi kecil *Eimeria* yang menjangkiti ayam. Oleh itu ujian yang berasaskan DNA khususnya menggunakan gen RNA ribosom (rRNA) telah dipilih untuk mengenal pasti penanda molekul bagi membolehkan pengenalpastian spesies dilakukan dengan lebih cepat dan tepat.

Jujukan gen 5S rRNA dipilih kerana ia mempunyai bilangan salinan yang tinggi dalam genom sel-sel dan jujukannya juga amat terpelihara. Gen 5S rRNA daripada genom DNA peringkat sporozoit *Eimeria* sp. telah diamplifikasi dengan menggunakan teknik tindak balas berantai polimerase (PCR). Produk PCR yang bersaiz antara 600–900 pasangan bes (bp) telah diperolehi dan diklonkan dengan kaedah pengklonan TA menggunakan vektor *pCR2.1* (3.9 kb). Hasil ligasi kemudian telah ditransformasikan ke dalam strain *Escherichia coli* TOP10F'. Plasmid rekombinan yang bersaiz 4.6–4.8 kb telah didapati. Klon rekombinan kemudian diujukkan menggunakan kaedah penjujukan berautomasi menggunakan primer M13 ke hadapan (Forward) dan M13 mengundur (Reverse) yang terdapat pada vektor. Gen dan penjarak 5S rRNA daripada ketujuh-tujuh *Eimeria* spesies telah berjaya diujukkan.

Jujukan telah dianalisis menggunakan perisian 'Basic Local Alignment Search Tool' (BLAST) dan didapati semua jujukan berpadanan dengan gen 5S rRNA daripada organisma lain. Jujukan sebanyak 726, 738, 697, 673, 732 dan 931 bp masing-masing ditunjukkan oleh *E. tenella*, *E. acervulina*, *E. praecox*, *E. maxima*, *E. brunetti* dan *E. necatrix*. Manakala jujukan 710 bp dan 592 bp merupakan jujukan untuk *E. mitis*. Hasil kajian ini mendapati jujukan gen dan penjarak 5S rRNA daripada ketujuh-tujuh *Eimeria* spesies boleh dieksploitasi sebagai penanda molekul untuk mengenal pasti *Eimeria* spesies di dalam ayam.