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IN-SILICO PRIMER DESIGNING USING 18S rRNA GENES OF MONOGENEAN PARASITE, *Thaparocleidus wallagonius*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Thaparocleidus wallagonius is a monogenean parasite and a fish-borne pathogen with a worldwide distribution. The present study focuses on in silico analysis of 18S rDNA of *Thaparocleidus wallagonius* collected from the fish *Wallago attu* from India. 18S rDNA serves as the source of taxonomic, standard reference sequences and systematic markers. The objective of genomics is the quantification, the collective characterization of the genes present in an organism, their influence, and interrelations on the organism. In this study, we designed 10 primers of the 18S rDNA sequence. 18S rRNA genes may be more useful in identifying the most efficient rRNA genes for genetic engineering and in understanding the relationship of the parasite with its host. The discovery of these primers will aid drug design studies, which could be a valuable tool for improving health of animal.

Keywords: *Thaparocleidus wallagonius;* primers; rDNA.

1. INTRODUCTION

Fish parasitic illnesses have been documented from many geographical places across the world. They exacerbate a major threat to fish farming, as fish are a key food source in developing nations [1]. Genus *Thaparocleidus* was first discovered by Jain (1952) for the species *Thaparocleidus wallagonius* from the gills of catfish, *Wallago attu* at Lucknow, India (Bloch & Schneider) [2].

To better identify drug targets and predict efficacy, new approaches are required. The use of computational modeling in biological science is exemplified by imaging in silico clinical trials. The simulation generates the source, objection, identification, and interpretation elements that are typically utilized in emerging technology assessments. Bioinformatics has provided new insights into parasite management, with the goal of providing optimal parasite control through parasite monitoring, genomic

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Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GAATGGCTTACGCGGGACTA	Plus	20	3	22	59.90	55.00	4.00	2.00
Reverse primer	AGCCATGAGCGAAGATACCG	Minus	20	619	600	59.97	55.00	4.00	2.00
Product length	617								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AATGGCTTACGCGGGACTAT	Plus	20	4	23	59.24	50.00	4.00	2.00
Reverse primer	CAACACCATCCAAATGCCCG	Minus	20	662	643	60.11	55.00	3.00	2.00
Product length	659								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCCGGTATCTTCGCTCATGG	Plus	20	598	617	60.95	60.00	4.00	2.00
Reverse primer	AAGACGAACACCCAACACCA	Minus	20	674	655	59.75	50.00	2.00	0.00
Product length	77								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGCTTAGGTATGCTTGGGTGG	Plus	21	577	597	60.06	52.38	4.00	0.00
Reverse primer	CCCAACACCATCCAAATGCC	Minus	20	664	645	59.75	55.00	3.00	1.00
Product length	88								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GAATGGCTTACGCGGGACTAT	Plus	21	3	23	60.27	52.38	4.00	2.00
Reverse primer	AACACCATCCAAATGCCCGA	Minus	20	661	642	60.25	50.00	3.00	0.00
Product length	659								

Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTGTCGGTCTCTCCTTTGCAT	Plus	21	557	577	59.65	47.62	4.00	2.00
Reverse primer	AGCAGATGGACACAGCTTCG	Minus	20	640	621	60.39	55.00	4.00	2.00
Product length	84								

Primer pair 7

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GGCCGGTATCTTCGCTCAT	Plus	19	597	615	59.63	57.89	4.00	2.00
Reverse primer	AGACGAACACCCAACACCAT	Minus	20	673	654	59.53	50.00	2.00	2.00
Product length	77								

Primer pair 8

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TCGGGCATTTGGATGGTGTT	Plus	20	642	661	60.25	50.00	3.00	0.00
Reverse primer	ATGATATTGAGCTGCCTCCGC	Minus	21	711	691	60.61	52.38	4.00	2.00
Product length	70								

Primer pair 9

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCATGCTTAGGTATGCTTGGG	Plus	21	574	594	59.39	52.38	8.00	2.00
Reverse primer	CACCATCCAAATGCCCGAGA	Minus	20	659	640	60.39	55.00	3.00	0.00
Product length	86								

Primer pair 10

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AATGGCTTACGCGGGACTA	Plus	19	4	22	58.80	52.63	4.00	2.00
Reverse primer	TCGAAGCCATGAGCGAAGAT	Minus	20	623	604	59.54	50.00	4.00	2.00
Product length	620								

Fig. 1. 10 primer pairs designed

analysis, designing potent target drug molecules using docking, metagenomics, and other methods that allow for integrated analyses of the host-parasite relationship [3]. The main goal of these clinical studies is to assess imaging technology's scientific usefulness in contrast to present therapeutic choices. The evaluation of imaging techniques is divided into three categories: detection, diagnosis, and disease treatment guidance/monitoring [4]. Taxonomic, standard reference sequences and systematic markers are all derived from 18S rDNA. The goal of genomics is to quantify and characterize all of the genes in an organism, as well as their influence and interrelationships on the organism. 18S rDNA has the potential to preserve all animals' genetic information, which is why it is so important in taxonomic and molecular biodiversity research. The utilization of in silico approaches for the genomics has been successfully used in several helminthes like malarial parasite *Plasmodium vivax* and *P. falciparum, Leishmania donovani, Toxoplasma gondii, Explanatum explanatum* [5]. We designed different primers in this study. So, using computational tools, this study looked at the various primer designed in the 18S rDNA sequence of *Thaparocleidus wallagonius* from *Wallago attu*.

2. MATERIALS AND METHODS

Retrieval of the Sequence: The DNA sequence 18S rDNA of *Thaparocleidus wallagonius* was retrieved from NCBI (National Centre for Biotechnology Information) database (httpa:/www.ncbi.nlm.nih.gov) under accession number KX364086.1 for further computational analysis.

Primer designing: Primers for the retrieved sequence of 18S rDNA were designed using primer designer tool of NCBI where all the parameters were kept at default.

3. RESULTS AND DISCUSSION

The primers were created using the FASTA method. The benefit of using the NCBI-BLAST tool in primer design is that the program will alert you to any additional primer binding in the genome. For the design of primers, certain requirements were standardized. A total of 152880 BLAST hits were analyzed. 10 primer pairs varying in length were designed using the primer designer tool of NCBI (Fig. 1). The primers were 15 to 25 nucleotides long on average. The primer's ideal size was 19-21 nucleotides. A primer with less than 19 nucleotides cannot anneal with the genome, and primers with more than 21 nucleotides cause a self-complementary condition. The genome's total GC content ranged from 47 to 60 percent [6]. The binding affinity in the genome is governed by the GC content. A high GC content necessitates a higher temperature to dissociate. The average temperature (Tm) was 57-63 degrees; the small temperature difference was used to reconnect the forward primer and reverse primer to the RNA. The ideal temperature was 60°, and the primer should be at the same temperature. To avoid amplification, large introns were employed to separate genomic DNA primers. Of the ten primers, primer 4 produced the best results.. The forward primer was 21 nucleotides long, whereas the reverse primer was 20 nucleotides long. The forward primer had a Tm of 60.06 and the reverse primer had a tm of 59.75. Forward primer and reverse primer had a GC content of 52.38 and 55.00 percent, respectively. Both teams earned a 4 and a 3 on the self-complementary scale. The complementarity score for self 3’ is lower, which indicates a strong primer [7]. In the 10 primer pairs designed, the 3’ region of primer is almost from selected conserved sequence to avoid the formation of secondary structure i.e., hairpin like structure. Establishment of the sequencing reliability of genome is important for the phylogenetic studies and it is related to the reliability of primers directly. Outlook to these primer designing is having ability to amplify the reported sequence of genome [8]. We can identify primers and probes with reasonable sensitivity and specificity by testing the diagnostic capacity of the engineered primers and probes on several samples. In addition, the assay's reaction conditions, such as primer and probe concentrations, Tm, and annealing temperature, must be optimized for optimum accuracy of the performance.

4. CONCLUSION

In this study, 10 primer pairs were designed using computational tools. The primer data obtained is suitable for amplification of the genomic sequence. This analysis can effectively classify both known and unknown infections, revealing the approach's potential to significantly improve microbiota, pharmaceutical, environmental ecology, and food hygiene studies. The designed primer amplifies the DNA and shall also give insights on parasite ability to infect human beings. These primers will boost phylogenomics research on *Thaparocleidus* and expand the phylogenetic resolution of the parasite.

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AVAILABILITY OF DATA

The 18S rDNA sequence is available on NCBI under accession number KX364086.1.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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