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In Silico PCR Primer Designing using Mitochondrial (cox-1) Gene of *Clinostomum marginatum*

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Abstract: *Clinostomum marginatum* Rudolphi, 1819 is a digenetic fish trematode parasite. It is commonly known as "yellow grub" as it causes pinhead sized lumps in fishes that are white to yellow or black. Molecular studies employs usage of primer for gene amplification. In silico method helps in designing primers used in amplification of genes which can be further validated using various tools and software to check their specificity and efficiency. The present communication deals with the in silico primer designing of cox-1 gene of helminth trematode parasite, *Clinostomum marginatum* parasitizing the intestine of fish *Channa striata* (Snakehead murrel). Thus, these designed primers set will be of further use in the wet lab for amplification of concerned gene or DNA fragment.

Keywords: *Clinostomum marginatum*, In silico, Implification, Primer, Cox-1, Gene, DNA

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Introduction

Aquaculture has served as a prominent key factor in the economy of India. The global aquaculture production has now exceeded more than 91.4 million tons production and generates an average income of more than US$ 145.4 billion (Aohagi *et al*.,1992; Sosa *et al*., 2016). Increased parasitism and disease outbreaks have not only negative impacts on the fish production but also leads to economic losses. The metacercariae of *Clinostomum* species are known to have similar morphology even though if there are some distinct features, it is not enough to discriminate up to the species level.

The advent of high output in silico technologies significantly helps in understanding the genome, gene expression studies, physiochemical aspects of proteins, designing potent target drug molecules via docking and revealing the host –parasite interactions (Tyagi *et al*., 2021). To explore the genome usage of PCR

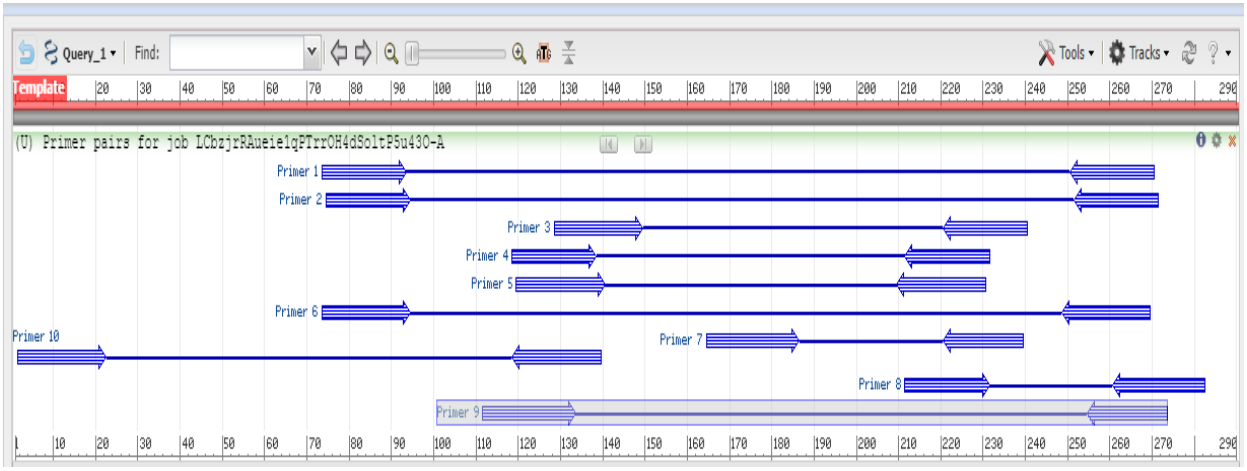

Fig. 1: Figure depicting the ten primer sets.

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGCCTGTTTTAATTGGCGGG	Plus	20	74	93	59.39	50.00	4.00	0.00
Reverse primer	CGAGTAACCAAACCCGGACA	Minus	20	270	251	59.97	55.00	4.00	0.00
Product length	197								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCCTGTTTTAATTGGCGGGT	Plus	20	75	94	59.39	50.00	4.00	0.00
Reverse primer	CCGAGTAACCAAACCCGGAC	Minus	20	271	252	60.67	60.00	4.00	1.00
Product length	197								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GGCGGATTTGAATCTTCCTCG	Plus	21	129	149	59.40	52.38	6.00	2.00
Reverse primer	AAAGGTCCATCCAACACCCG	Minus	20	240	221	60.25	55.00	3.00	2.00
Product length	112								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGTTAGGGTTGGCGGATTTG	Plus	20	119	138	58.46	50.00	2.00	0.00
Reverse primer	TCCAACACCCGCTCCATAAA	Minus	20	231	212	59.31	50.00	2.00	0.00
Product length	113								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTTAGGGTTGGCGGATTTGAA	Plus	21	120	140	58.84	47.62	3.00	3.00
Reverse primer	CCAACACCCGCTCCATAAAAC	Minus	21	230	210	59.80	52.38	2.00	0.00
Product length	111								

Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGCCTGTTTTAATTGGCGGGT	Plus	21	74	94	61.10	47.62	4.00	0.00
Reverse primer	GAGTAACCAAACCCGGACAGA	Minus	21	269	249	59.65	52.38	4.00	0.00
Product length	196								

Primer pair 7

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGCTTGATTATTGTTGCCGTCG	Plus	22	165	186	60.16	45.45	3.00	3.00
Reverse primer	AAGGTCCATCCAACACCCG	Minus	19	239	221	59.62	57.89	3.00	2.00
Product length	75								

Primer pair 8

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTTATGGAGCGGGTGTTGGA	Plus	20	212	231	59.31	50.00	2.00	0.00
Reverse primer	AGTACCAATTCCCGAGTAACCA	Minus	22	282	261	58.82	45.45	4.00	0.00
Product length	71								

Primer pair 9

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCTTTATTGTTAGGGTTGGCGG	Plus	22	112	133	59.57	50.00	3.00	1.00
Reverse primer	TCCCGAGTAACCAAACCCG	Minus	19	273	255	59.33	57.89	3.00	2.00
Product length	162								

Primer pair 10

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATCTGATCTCAACGGAGGTCT	Plus	21	2	22	57.97	47.62	5.00	2.00
Reverse primer	TCAAATCCGCCAACCCTAACA	Minus	21	139	119	59.93	47.62	2.00	0.00
Product length	138								

Fig. 2: Figure depicting the primer set designed showing its length, Tm, GC content and self compatibility.

based primers is of utmost usage. The present communication deals with the designed PCR primer sets to be used for amplifying the mitochondrial cox-1 genes of digenetic helminth, *Clinostomum marginatum*.

Materials and Methods

Retrieval of the sequence:

In the present work, we used mt gene (cox-1) of *C. complanatum* genome. The sequence was retrieved from NCBI (National Centre of Biotechnology Information) database (http://www.ncbi.nlm.nih.gov) under accession number ON803638.

Primer Designing:

Primer designing was performed using computational tools of NCBI.

Results and Discussion

Ten pair of primer sets were designed using in silico computational tools (Figs. 1, 2). The average length of primer varies between 20-22 nucleotides. Usually, primer less than size of 18 nucleotides is usually not considered a good primer choice as it cannot anneal with the genome of the target organism thus, comparatively unsuitable for use in wet labs. The GC content ranged from 45-60%. A higher GC content in the gene requires a more higher temperature to dissociate thus, the better its chances of amplification of gene (Sosa *et al*., 2016). The average temperature (Tm) was also analyzed for obtained primer set and it was found that it ranged between 57 -60 degrees which is usually considered as ideal temperature. Thus, the designed primer sets fulfills the criteria of ideal primers for amplification of genes.

Conclusion

In the present study, 10 set of primer pairs were designed using NCBI. Thus, these designed primers set will be of further use in the wet lab for amplification of concerned gene or DNA fragment.

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