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PCR primer design for mitochondrial cox-1 gene from *Clinostomum complanatum* towards diagnosis

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Abstract:
Metacercariae of *Clinostomum* Leidy, 1856 are frequently encountered in freshwater fish. *Clinostomum complanatum* is a digenetic zoonotic parasite harbouring the intestine and body cavity of the fishes. 19 human incidences of *Clinostomum complanatum* infection have been

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reported to cause pharyngitis and lacramalitis from Japan, Thailand and Korea. Hence, adequate yet effective diagnosis is an issue. Designing primers used in the amplification of genes with adequate specificity and efficiency is of help in diagnosis. Hence, we describe primer design for cox-1 gene from the helminth parasite, *Clinostomum complanatum* parasitizing the intestine of fish *Channastriata* (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 complanatum*, zoonotic, primer, design, cox-1, gene, DNA**.**

Background:

Aquaculture has functioned as a pre-eminent key element in the economy of India. The increased incidences of parasitism and disease outbreaks have negative impacts not only on the fish production, but it also leads to economic losses **[1, 3]**. *Clinostomum complanatum*, is a digenetic trematode parasite of zoonotic importance that has been reported in various regions infecting the human population thus causing severe infections. Exploring and understanding the genome is the most prominent methodology for disease control. Till today many drugs and vaccines have been developed to minimize the parasitic infections caused by parasites thus preventing deaths caused by them. With the advent of high output technologies computer aided biology has played a significant role in understanding the genome, gene expression studies, physiochemical aspects of proteins, designing potent target drug molecules via docking and revealing the host–parasite interactions **[4]**. PCR based primers is helpful in this context. Hence, we describe primer design for cox-1 gene from the helminth parasite, *Clinostomum complanatum* parasitizing the intestine of fish *Channastriata* (Snakehead murrel).

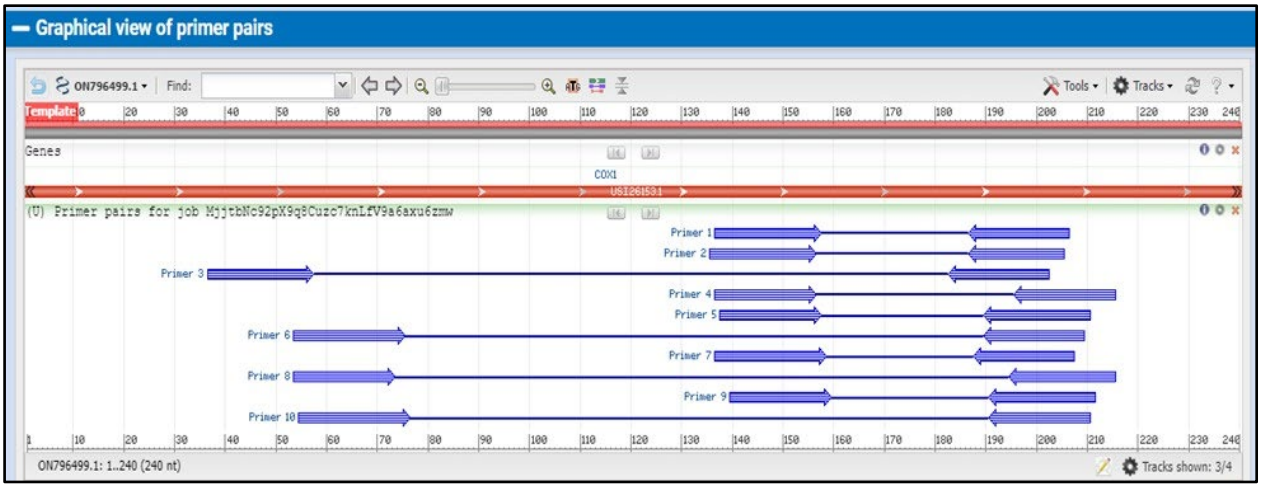

Figure 1: Depicting the ten primer sets.

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGATCGGTATACCCACAGGGA	Plus	21	137	157	59.78	52.38	8.00	1.00
Reverse primer	AACCGGACACGAGAACAACC	Minus	20	206	187	60.53	55.00	4.00	0.00
Product length	70								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTGATCGGTATACCCACAGGG	Plus	21	136	156	59.66	57.14	8.00	3.00
Reverse primer	ACCGGACACGAGAACAACC	Minus	19	205	187	59.93	57.89	4.00	0.00
Product length	70								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GGGGGTATCGTGTGTTTAGGT	Plus	21	37	57	59.44	52.38	2.00	0.00
Reverse primer	GGACACGAGAACAACCCAAC	Minus	20	202	183	59.06	55.00	2.00	0.00
Product length	166								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGATCGGTATACCCACAGGG	Plus	20	137	156	58.29	55.00	8.00	3.00
Reverse primer	GGATCTCAAAACCGGACACG	Minus	20	215	196	58.92	55.00	4.00	2.00
Product length	79								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GATCGGTATACCCACAGGGA	Plus	20	138	157	57.99	55.00	8.00	1.00
Reverse primer	TCAAAACCGGACACGAGAACA	Minus	21	210	190	60.13	47.62	4.00	0.00
Product length	73								

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Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGGTAGTGTTGTTTGGGCTCAT	Plus	22	54	75	59.89	45.45	4.00	2.00
Reverse primer	CAAAACCGGACACGAGAACA	Minus	20	209	190	58.71	50.00	4.00	0.00
Product length	156								

Primer pair 7

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGATCGGTATACCCACAGGGAT	Plus	22	137	158	60.16	50.00	8.00	3.00
Reverse primer	AAACCGGACACGAGAACAAC	Minus	20	207	188	58.71	50.00	4.00	0.00
Product length	71								

Primer pair 8

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGGTAGTGTTGTTTGGGCTC	Plus	20	54	73	57.73	50.00	2.00	2.00
Reverse primer	GGATCTCAAAACCGGACACGA	Minus	21	215	195	60.34	52.38	4.00	0.00
Product length	162								

Primer pair 9

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TCGGTATACCCACAGGGATT	Plus	20	140	159	57.52	50.00	8.00	1.00
Reverse primer	CTCAAAACCGGACACGAGAAC	Minus	21	211	191	59.47	52.38	4.00	0.00
Product length	72								

Primer pair 10

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GGTAGTGTTGTTTGGGCTCATC	Plus	22	55	76	59.51	50.00	4.00	1.00
Reverse primer	TCAAAACCGGACACGAGAAC	Minus	20	210	191	58.43	50.00	4.00	0.00
Product length	156								

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

Methodology:

Retrieval of the sequence:

We used the 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 ON796499.

Primer designing:

Primer designing was performed using the computational tools at NCBI.

Results and Discussion

Ten pair of primer sets was designed using computational tools (Figure 1 and 2). The average length of primer varies between 20-22 nucleotides. Usually, primer less than size 18 nucleotides is not considered an ideal primer as it cannot anneal with the genome of the target organism thus, unsuitable for use in wet labs. The GC content ranged from 47-55%. A high GC content requires a higher temperature to dissociate thus, the chances of amplification of gene is high **[2]**. The average temperature (Tm) was 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:

We illustrate the designing of 10 sets of primer pairs using the NCBI primer design tool. Thus, these designed primers set will be of use in the wet lab for amplification of concerned gene or DNA fragment.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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