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PCR Primer Design for 18s rRNA of Monogenean Parasite- *Bychowskyella*

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Abstract: Polymerase chain reaction (PCR) is a very important process in the field of molecular biology. Designing oligonucleotide primers is a critical first step in conducting successful molecular biology research involving polymerase chain reactions. The effectiveness and sensitivity of the enzymatic polymerase chain reaction are significantly affected by the primers' efficiency. The current work focuses on the *in silico* primer designing of 18s rRNA of *Bychowskyella* (Monogenea: Dactylogyridae) infecting siluriform fishes from India. In this study, 5 sets of Forward and Reverse primers of the 18S rRNA sequence have been designed. Designing of these specific primers for the species of *Bychowskyella* genus will contribute to meaningful insights into future research and management strategies for parasite biology.

Keywords: PCR, Monogenea, *Bychowskyella*, 18s rRNA

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Introduction

Monogeneans represent a diverse group of parasitic flatworms, many of which are of significant veterinary and ecological importance due to their pathogenic effects on fish and amphibians (Buchmann and Bresciani, 2006). *Bychowskyella* genus within the Monogenea class, includes several species known to parasitize aquatic hosts, making them subjects of interest in both clinical and ecological research (Illa *et al*., 2019). As the 18s region of the ribosomal RNA (rRNA) gene is conserved across taxa and contains variable sections that provide species-specific information, it is a commonly utilized molecular marker for phylogenetic and taxonomic investigations (Ranjithkumar *et al*., 2018). The 18s region of the rRNA gene is extremely useful for the parasitologists in gaining insights into the genetic diversity, population organization, and evolutionary relationships of parasitic species (Lymbery and Thompson, 2012). During the primer designing procedure, a stringent protocol is needed to ensure the accurate amplification of

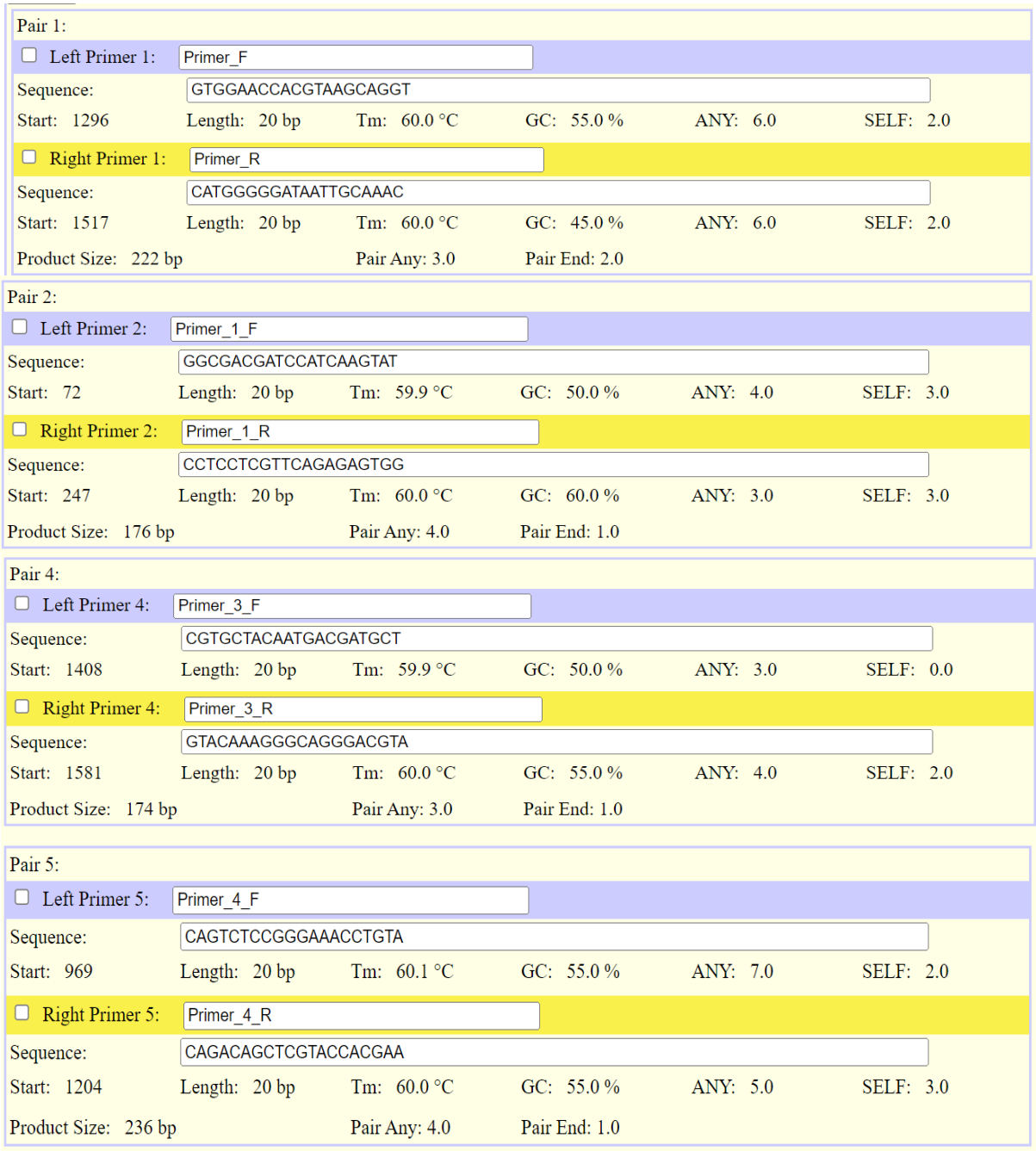

Fig. 1: Designed 5 pairs of primer.

the target sequence for the 18S rRNA gene of this parasite (Weiss, 1995). Their specific genetic structure requires specific primers to enable accurate identification (Pereira *et al*., 2008). To discover conserved regions, several sequence alignments of the 18S rRNA gene from different *Bychowskyella* species is usually the first step in primer designing (Mendoza-Palmero *et al*., 2015). These conserved areas are essential because they serve as the basis for the design of primers (Apte and Daniel, 2009). The length, GC content, melting temperature (Tm), and possible secondary structures of the primers are some of the elements that affect primer design (Abd-Elsalam, 2003). Furthermore, cross-reactivity with non-target sequences must be avoided at all costs as this

could produce unreliable results (Wang and Seed, 2003). While designing a primer, various aspects viz.,to predict primer binding sites, analyze primer secondary structures, and determine primer specificity using sequence similarity searches, bioinformatics techniques are essential (Kalendar *et al*., 2017). After potential primers are found, their specificity and efficiency are evaluated by *in silico* validation (Ficetola *et al*., 2010). This work aims to improve the molecular toolkit available to researchers studying the biology and genomics of these parasites by presenting the design and validation of PCR primers targeting the 18S rRNA gene of *Bychowskyella*. This designed primer sequence will help expand the use of molecular methods in the study of the parasite and advance our knowledge of the organism's evolutionary background, host-parasite relationships, and its effects on host health and ecosystem dynamics (Froeschke and von der Heyden, 2014).

Materials and Methods

Retrieval of the Sequence and Primer designing

The first stage in the primer design process was to retrieve the nucleotide sequence of the 18s rRNA of *Bychowskyella sp.*, from the NCBI (National Centre for Biotechnology Information) database (httpa:/www.ncbi.nlm.nih.gov) under accession number KT852455 (Verma *et al*., 2017). In the next step for the designing of primer pairs, Primer3Plus software was used. This retrieved sequence was input into this software with specific parameters, and specific and efficient primers were designed to amplify the 18s rRNA gene of *Bychowskyella*. In the final step, OligoCalc software was used to check the accuracy of the designed primers.

Results and Discussion

For the five pairs of designed sequences (Fig. 1) using Primer 3 Plus software, a rigorous optimization process was used to obtain the desired primer pair candidates. (Li *et al*., 2008). Only primer pairs having at least 35% GC content, GC lock at the 3 prime ends, having no chance of primer dimers or self-annealing with the pair, having no secondary priming sites, and having a melting temperature between 58 and 62 °C were chosen (Mackay, 2007). The results from Primer3 Plus offered comprehensive information to assist in selecting optimal primer pairs for PCR amplification of the target sequence, ensuring efficient and specific amplification in subsequent experiments (Ye *et al*., 2012). The outcome displayed a list of recommended primers along with their qualities, ranked from best to worst (Dolan, 2010). The stability of any base pairing of that primer to itself was displayed by the numbers under the heading "ANY" (Yakovchuk *et al*., 2006). The accuracy of the primers such as self-complementary and hairpin formation was also checked by OligoCalc software. The length of of the designed primer was 20bp that fulfilled almost all the criteria of a good primer.

Conclusion

The 5 pairs of primers so designed will allow for the targeted amplification of parasite DNA, which will make it easier to comprehend the parasite's molecular level information and will also be useful for monitoring medication resistance, host-parasite interactions, detection, and understanding phylogenomics.

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