

## Developing a Set of Primers Targeting Chondroitin Ac Lyase Gene for Specific and Sensitive Detection of Flavobacterium Columnare, a Causative Agent of Freshwater Columnaris

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**Abstract :** Flavobacterium columnare is one of the devastating pathogen that causes noticeable economic losses in freshwater cultured fish. Like other filamentous bacteria, F. columnare tends to aggregate and fluctuate to all kind of media, thus revealing obstacles in recognition of its colonies. Since the molecular typing is the only fundamental tool for rapid and precise detection of this pathogen. The present study developed a species-specific PCR assay based on cslA unique gene of F. columnare. The cslA gene sequences of 13 F. columnare, strains retrieved from gene bank database, were aligned to identify a conserved homologous segment prior to primers design. The new primers yielded amplicons of 287 bp from F. columnare strains but not from relevant or other pathogens, unlike to other published set that showed no specificity and cross-reactivity with F. indicum. The primers were sensitive and detected as few as 7 CFUs of bacteria and 3 pg of gDNA template. The sensitivity was reduced ten times when using tissue samples. These primers precisely defined all field isolates in a double-blind study, proposing their applicable use for field detection.

**Keywords :** Columnaris infection, cslA gene, Flavobacterium columnare, PCR

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