**Supplemental Table 1.** Primers used in this experiment.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Target** | **Sequence (5’→3’)** | **Tm**  **(°C)** | **Size**  **(bp)** | **Reference** |
| TiLV Segment9 | TiLV-S9-qF;  CTAGACAATGTTTTCGATCCAG  TiLV-S9-qR;  TTCTGTGTCAGTAATCTTGACAG | 54.9  56.2 | 137 | (Taengphu et al., 2022) |
| ISKNV - major capsid protein gene (*MCP*) | Meg-MCP160F;  TCAAAACAGACTGGCCATGC  Meg-MCP349R;  TAAATGACACCGACACCTCCTC | 59.0  59.7 | 190 | (Kawato et al., 2021) |
| *F. orientalis -*hypothetical protein (*HP*) gene | Francis-n-o-F1;  GGCGTAACTCCTTTTAGCTTCC  Francis-n-o-R1;  TTAGAGGAGCTTGGAAAAGCA | 59.3  57.2 | 203 | (Dong et al., 2016) |
| *S. agalactiae - groEL* gene | SagroEL2 F;  GCAAGTTTTAGGACAGTCTGCT  SagroEL2 R;  AGTTTCAGTGCCGCTACTTT | 58.9  57.7 | 351 | (Leigh et al., 2018) |

Tm, melting temperature, bp, base pair.

**References:**

Dong HT, Gangnonngiw W, Phiwsaiya K, Charoensapsri W, Nguyen VV, Nilsen P, Pradeep PJ, Withyachumnarnkul B, Senapin S, Rodkhum C. 2016. Duplex PCR assay and in situ hybridization for detection of Francisella spp. and Francisella noatunensis subsp. orientalis in red tilapia. *Diseases of Aquatic Organisms* 120:39–47. DOI: 10.3354/dao03021.

Kawato Y, Cummins DM, Valdeter S, Mohr PG, Ito T, Mizuno K, Kawakami H, Williams LM, Crane MSJ, Moody NJG. 2021. Development of New Real-time PCR Assays for Detecting *Megalocytivirus* Across Multiple Genotypes. *Fish Pathology* 56:177–186. DOI: 10.3147/jsfp.56.177.

Leigh WJ, Zadoks RN, Jaglarz A, Costa JZ, Foster G, Thompson KD. 2018. Evaluation of PCR primers targeting the groEL gene for the specific detection of Streptococcus agalactiae in the context of aquaculture. *Journal of Applied Microbiology* 125:666–674. DOI: 10.1111/jam.13925.

Taengphu S, Kayansamruaj P, Kawato Y, Delamare-Deboutteville J, Mohan CV, Dong HT, Senapin S. 2022. Concentration and quantification of Tilapia tilapinevirus from water using a simple iron flocculation coupled with probe-based RT-qPCR. *PeerJ* 10:e13157. DOI: 10.7717/peerj.13157.