Pseudoloma neurophilia

Diagnostic assay

This assay was designed for diagnosis of *Pseudoloma neurophilia* infection of zebrafish (*Danio rerio*) neural tissue. The brain and spinal cord are dissected post-mortem from fresh or frozen tissue and Proteinase K-digested for PCR. Specific primers were designed to amplify a segment of the small subunit rRNA of *P. neurophilia*. Control primers are utilized in each reaction to amplify a segment of zebrafish DNA.

Reagent concentrations in the PCR reaction:
dNTP concentration: 0.3 mM each
P. neurophilia–specific primer concentration (PNA_03 and PNA_04): 0.5 μM each
zebrafish control primer concentration (POA05 and POA06): 0.125 μM each

***Pseudoloma neurophilia*-specific primers:**
PNA_03: 5' TGA AAT GTG GTG ACC CGT TTA GG 3'
PNA_04: 5' TCC TTG ACC CAT CCT TCC TGT G 3'

Control primers:
POA05: 5' GCG TCT AGC TTT GCC CTT TGA TG 3'
POA06: 5' CCG TTT TTG AAG ACA TCT GGT CG 3'

PCR program (60_C_40):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **60**ºC for **40** sec
4. 72ºC for **40** sec
5. Go to step 2 (above) for 39 cycles
6. 72ºC for 5 min
7. 8.0ºC hold
8. END

Product size: 441 bp for PNA_03 and PNA_04
186 bp for POA05 and POA06

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