

The seven loci of the *Tenacibaculum maritimum* MLST scheme and the PCR and sequencing protocols.

Locus	Forward primer (5'-3')	Reverse primer (5'-3')	Length ^a (bp)
atpA	CAGGAAACAGCTATGACC ATTGGWGAYCGTCAAACWGG	TGTAAAACGACGGCCAGT CCAAAYTTAGCRAAHGCTTC	567
dnaK	CAGGAAACAGCTATGACC GGWACYACNAAYTCDTGTGT	TGTAAAACGACGGCCAGT TCWATCTTMGCTTTYTCAGC	573
glyA	CAGGAAACAGCTATGACC CAYTTAACWCAYGGWTCDC	TGTAAAACGACGGCCAGT ACCATRTTTTTRTTTACHGT	558
gyrB	CAGGAAACAGCTATGACC AGTATYCARGCRCTRGAAGG	TGTAAAACGACGGCCAGT GTWCCTCCTTCRTGYGTRTT	597
infB	CAGGAAACAGCTATGACC ATGCCDCAAACWAAAGARGC	TGTAAAACGACGGCCAGT GTAATHGCTCCAACYCCTTT	564
rlmN	CAGGAAACAGCTATGACC GCKTGTGTDTCDAGYCARGT	TGTAAAACGACGGCCAGT CCRCADGCDGCATCWATRTC	549
tgt	CAGGAAACAGCTATGACC GAAACWCCWATWTTYATGCC	TGTAAAACGACGGCCAGT TAYAWYTCTTCNGCWGGTTC	486

^aLength of the target sequence.

forward sequencing primer: 5'-CAGGAAACAGCTATGACC-3'

reverse sequencing primer: 5'-TGTAAAACGACGGCCAGT-3'

PCR and sequencing protocols

Tenacibaculum strains were grown in marine 2216E broth (Difco) for 24-h at 28°C and 70 rpm and the genomic DNA was extracted from the pellet using the Wizard Genomic DNA purification kit (Promega). PCR amplification was performed in a 20-μL reaction volume using GoTaq polymerase (Promega) and the following touchdown protocol: 94°C for 5 min; 24 cycles at 94°C for 0.5 min, 55°C for 0.5 min (-0.4°C/ cycle), and 72°C for 1 min (+2 sec/ cycle); 12 cycles at 94°C for 0.5 min, 45°C for 0.5 min, and 72°C for 2 min (+3 sec/ cycle); and a final extension step at 72°C for 10 min. Five microliters of the PCR products was resolved on a 1% agarose/TBE gel to check amplification. For sequencing, one microliter of the PCR products was purified by using exonuclease I (Biolabs)-alkaline phosphatase (USB) for 1 h at 37°C, followed by enzyme inactivation for 5 min at 94°C. One-tenth of the purified PCR products was sequenced on both strands, using the sequencing primers, the BigDye Terminator version 3.1 sequencing kit (Applied Biosystems), and an Applied Biosystems 3730 automated sequencer.