PCR and Sequencing

Internal fragments of six loci are amplified by PCR, using the primers listed below, with a chromosomal DNA template. PCR thermal cycling conditions include an initial denaturation of 95°C for 2 min; 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and a final extension of 72°C for 2 min. Sequencing is done with the same primers (at a lower concentration) as used for PCR amplification.

Locus and Primer	Primer sequence (5'-3')	Amplicon length (bp)	Trimmed allele length (bp)
arcC-F	TCGCGTTGTGCCCTCTCC	441	357 or 360
arcC-R	ATTTTACCTTCTAGCGCATCATTT		
glpK-F	GGCAATCTCGTCAAACACAACAT	490	435
glpK-R	CCATACGTATTTTTCACATCACCA		
gtr-F	GTTGTCACATTAATTGGTCGTTCC	438	393
gtr-R	ATTTACAAGTTCAGGGTCAAGTGC		
pta-F	GTCCGTCCTGCCTTACAAA	484	444
pta-R	CAATCGCTTCAAATCCACCTA		
tpiA-F	TGGTGCATATACAGGAGAAACTT	499	450
tpiA-R	TGATGCGCCACCAACTAA		
tuf-F	GCCAGTTGAGGACGTATTCT	412	371
tuf-R	CCATTTCAGTACCTTCTGGTAA		