The *Streptococcus pyogenes* MLST scheme uses internal fragments of seven housekeeping genes amplified by PCR using the following primer pairs (described in Enright MC, Spratt BG, Kalia A, Cross JH and Bessen DE. 2001. Multilocus sequence typing of *Streptococcus pyogenes* and the relationships between *emm* type and clone. Infection and Immunity 69:2416-2427):

Genes and Function	Sequences (5' to 3')	Size (bp) of the amplicon used for assigning alleles
glucose kinase		
gki-up	GGCATTGGAATGGGATCACC	498
gki-dn	TCTCCTGCTGCTGACAC	
glutamine transporter protein		
gtr-up	GAGGTTGTGGTGATTATTGG	450
gtr-dn	GCAAAGCCCATTTCATGAGTC	
glutamate racemase		
murI-up	TGCTGACTCAAAATGTTAAAATGATTG	438
murI-dn	GATGATAATTCACCGTTAATGTCAAAATAG	
DNA mismatch repair protein		
mutS-up	GAAGAGTCATCTAGTTTAGAATACGAT	405
mutS-dn	AGAGAGTTGTCACTTGCGCGTTTGATTGCT	
transketolase		
recP-up	GCAAATTCTGGACACCCAGG	459
recP-dn	CTTTCACAAGGATATGTTGCC	
xanthine phosphoribosyl transferase		
xpt-up	TTACTTGAAGAACGCATCTTA	450
xpt-dn	ATGAGGTCACTTCAATGCCC	
acetyl-CoA acetyltransferase		
yqiL-up	TGCAACAGTATGGACTGACCAGAGAACAAGATGC	434
yqiL-dn	CAAGGTCTCGTGAAACCGCTAAAGCCTGAG	

PCR conditions

The PCR reactions are performed in volumes of 50 mL, with an initial denaturation at 95oC for five min, followed by 28 cycles of 95oC for 1 min, 55oC for 1 min and 72oC for 1 min. The amplified DNA fragments are purified either by precipitation with polyethylene glycol or using a commercial PCR purification kit. The sequence of each fragment is obtained on both strands using the same primers as those in the initial PCR amplifications.

As the same primers are used for amplification and sequencing, it is important that only a single DNA fragment is amplified in the initial PCR. This may involve some optimisation of the annealing temperature and other PCR conditions in individual laboratories.