

## Genes

The *Yersinia* MLST scheme uses fragments of the following seven house-keeping genes:

*aarF* - putative ubiquinone biosynthesis protein UbiB

*dfp* - bifunctional phosphopantothenoilcysteine decarboxylase/phosphopantothenate synthase

*galR* - DNA-binding transcriptional regulator

*glnS* - glutaminyl-tRNA synthetase

*hemA* - glutamyl-tRNA reductase

*rfaE* - bifunctional heptose 7-phosphate kinase/heptose 1-phosphate adenylyltransferase

*speA* - arginine decarboxylase

## PCR amplification

The thermal cycler was set up as follows: stage 1 - initial denaturation 94°C for 5 minutes, stage 2 - denaturation 94°C for 30 seconds, stage 3 – annealing temperature dependent upon primer set for 30seconds, stage 4 – elongation 72°C for 30 seconds, stage 5 – 30 repeats of stages 2 to 4, stage 6 – final elongation 72°C for 5 minutes, and final hold at 4°C. The PCR reactions were carried out using the GoTaq® Flexi DNA polymerase kit (Promega, catalogue number:M8301), and dNTPs (Promega, catalogue number: U1330), in the quantities and concentrations as follows for one reaction: 5 µl 1.5 mM MgCl<sub>2</sub>, 5 µl PCR reaction buffer, 2 µl 10 µM dNTPs, 0.3 µl 5 U/µM Taq DNA polymerase, 40 µl sterilised distilled water, 0.5 µl 10 pmol of each forward and reverse primers, 1 µl ~10 ng/µl DNA. The amplification product was then purified using ExoSAP-IT (Affymetrix, catalogue number: 78201 1ML) and submitted for Sanger sequencing in duplicate to obtain independent forward and reverse reactions.

## MLST primers

The primers were used for both the amplification and sequencing reactions. The second set of primers for *rfaE* (labelled *rfaE\**) were designed for *Y. enterocolitica* serotype O:3 strains originating from Germany, due to low quality sequence produced with the original primers.

MLST gene	Forward Primer	Reverse Primer	PCR product length (bp)	MLST region length (bp)	Annealing Temperature (°C)
<i>aarF</i>	5'-ttccatgcagatatgcacc-3'	3'-ccactcactaatagttagc-5'	650	500	52
<i>dfp</i>	5'-gatccgggtacgctttatcag-3'	3'-cataacggctgacaatctcg-5'	547	455	59
<i>galR</i>	5'-attggtaacggttaccatg-3'	3'-gttgggctgaacatattggt-5'	648	500	59
<i>glnS</i>	5'-gaatcatgtatccgtgatg-3'	3'-gcacagaaataaccttcac-5'	557	442	56.5
<i>hemA</i>	5'-atgactctgctgcattagg-3'	3'-cggttgcaataatcatatg-5'	602	490	54
<i>speA</i>	5'-atgtctgatgataacttgatt-3'	3'-cagataaactttatggccc-5'	550	452	55.5
<i>rfaE</i>	5'-atgaaagtcactctgcctga-3'	3'-atcactgccttaggatc-5'	509	429	55.5
<b>Alternate <i>rfaE</i> primers specific for <i>Y. enterocolitica</i> serotype O:3</b>					
<i>rfaE*</i>	5'-atgaaagtcacgctgcctga-3'	3'-ggatagattgggtcagtagc-5'	550	429	57.5

Reference MLST regions from *Y. enterocolitica* 8081

>8081\_aarF

CACTAAATAAAGCAGATAAGCGCTATCTTGCTGAAAACCTTTATTGCTTTCTTTAATCGGGATTA  
TCGGCGGGTTGCAGAGCTGCATGTTGACTCTGGCTGGGTTCCCTTGTGATACCAATGTTGAAGAT  
TTTGAATTCGCTATCCGTACCGTCTGCGAACCAATTTTTGAAAAACCACTAGCCGAAATATCCT  
TTGGCCATGTGTTATTGAATCTCTTTAATACCGCACGCCGTTTTAATATGGAAGTACAACCACA  
GTTGGTTTTACTGCAAAAAACCTTGTGTATGTCGAAGGCTTAGGGCGTCAACTTTATCCTCAG  
CTTGACCTTTGGACGACTGCGAAGCCTTTCCTGGAAAGCTGGTTACGAGATCAAGTTGGGTTGC  
CTGCGGTTATTTCGTGCATTGAAAGAAAAAGCACCATTTTGGGCTGAAAAATTTCTGAGCTGCC  
AGAGCTTGTGTATGACAGTTTGCAACAGCATAAGCTATTACAGCAAAGTGTG

>8081\_dfp

TGCCAGAGGGGCAAAGTACTCTGGTTGCCGGGCCAGTGATGCTCCCTACTCCAGCGGGAGT  
GAATCGTATTGATGTTGTCAGTGCGCTAGAAATGCAGCAAACAGTTCAGAACTTAGCAGCCCA  
GCAGAATATTTTTATTTCTTGTGCTGCGGTAGCGGATTACCGCGCTGAGCAGGTTTCTGACGAG  
AAAATAAAAAAACAGGGCGATGAGATCACCTTAAGTTGGTGAAAAATCCAGACATTGTCGCT  
GGAGTCGCTTCGATGACTAAAAAACGTCCATTTGTTGTTGGATTTGCTGCCGAAACCCAGAATG  
TGGAAGAATACGCGCGACAAAAGCTAGCGCGGAAAAATCTGGATCTTATTTGCGCTAATGATG  
TATCGCTCGCAGAGCATGGTTTTAACAGTGATACTAATGCCTTGCACCTTTTTTGGCCGACTGG  
CGAGAAACGTT

>8081\_galR

AAAATTATCTGATGAAGAATTGACGTCATTAATGCAGCAAATCCCTGGCATGGTGTGATTAAC  
CGCACCTCCCCGGTTTTTGAAACCCGTTGTGTGGCGTTGGATGACCGTTATGGTGCCTGGCTGG  
GAACCCGCCATCTGATTCAGCATGGGCATAAACGAATAGCGATTATTTGCTCCAATCATCAAAT  
TTCTGATGCCACCGATCGTCTGCAAGGTTATCTGGATGCTCTGGAAGAGTTTGGTATTGCGGTT  
GATGACCGTTTAATTTCTACGGTACGCCGGATGAAATCGGCGGTGAACAAGCCATGACTGAC  
TACTTGGCCGAGGCCGAAACTTCACTGCGGTCACTTGTATAACGACTCAATGGCAGCGGGA  
GCATTGTCCGTACTCAGCGATAACAGTATCGAAGTGCCACAAGAGATCTCACTGATCGGTTTTG  
ACGATGTGTTGATCTCCCGTTATCTGCGCCCGCGGCTGACCACCATTTCGCTATC

>8081\_glnS

GAGAATCGTGCGGCAGGGGAAGAGTGGCTGACCATGCCAAATCATCCGAATAATCCGGATATG  
GGCACCCGTCAGGTACCTTTCGACAGTGAGATTTATATCGATCGCGCTGATTTTCGTGAAGAAG  
CCAATAAGCAATAACAAGCGTCTGGTGCTGGGTAAAGAAGTGCGCCTGCGTAATGCTTATGTCA  
TCAAAGCCGAGCGTGTGAGAAAGACGCCGAAGGTCATGTCACGACACTTTATTGCAGCTATG  
ACGCAGAACTTTAAATAAAGACCCTGCCGACGGCCGTAAAGTGAAAGGGGTTATCCATTGGG  
TTTCTGTTAAGCATGCATTGCCTGCTGAAATCCGTTTATATGACCGTTTGTGTTAGTGTACCAAAC  
CCAGCGGCGGCGGAAGACTTCTTATCTACCATCAACCCAGAGTCTTTGATTATCCGTCAGG

>8081\_hemA

CACCTGAATCGATCGATCAGGCACTTGCTAGCTTGCTCCAACAGCCGTTGGTGCAGGGCGGTGT  
CGTGTGTGCTACGTGTAACCGAACTGAGTTATATCTCAGCGTAGAACAGCAAGAAAAACCTGCA  
CGAACAACCTGATTGCCTGGCTGTGTAACCTACCATAAACTCAGCCCGGATGAAGTAAAAAAAAG  
CCTCTATTGGCATCATGGCAATGACGCAGTTAGCCATCTGATGCGCGTTGCCAGCGGGCTGGAC  
TCGCTGGTGTGTTGGGCGAGCCACAAATTCTGGGACAGGTGAAAAAAGCCTTTGCAGAGTCGCAA  
CGCGAGCAATCGCTCTCTGGCGAATTGGAGCGGTTATCCAAAAAACATTTTCGGTCCGCAAA  
CGGGTTCGCACAGAACTGAAATTGGTGCTAGTGCTGTTTTCGGTGGCTTTCGCTGCCTGTACCT  
TGGCACGGCAAATCTTTGAGTCACTGTCAGAGCTGAATGTATTGTT

>8081\_rfaE

TGTTAGACCGTTATTGGTATGGCCCAACCAGCCGCATTTACCAGAAGCACCGGTGCCGGTTGT  
CAAAGTCGATACCATCGAAGAACGCCCGGTGGTGCCGCGAACGTCGCGATGAACATCGCCTC  
CCTTGGCGCAATCTCCCGGTTAGTCGGGTTGACGGGTATTGATGATGCCGCACGCGCACTGACC  
AGCAAGCTAAACGAAGTGCAGGTGCGTTGTGATTTTGTCTCGGTGCCGACTCACCCAACCATC  
ACCAAGTTGCGGGTACTTTCTCGTAACCAGCAGCTCATCCGTCTGGATTTTGAAGAAGGTTTTG  
ATGGTGTGACCCTCAACCTATCTTTGAGCGCATTCAACAAGCGCTGCCACAGATTGGTGCTCT  
GGTGCTGTCCGACTATGCCAAAGGTGCATTGAACAGTGTGCAGACGA

>8081\_speA

TGAATGATCGTAATGCCAGCAAGATGCTGAGCACGTATAACGTCGCCTACTGGGGGGGTA  
ACTATTATGACGTCAATGAACTCGGTCATATCAGTGTTTGTCTGATCCGGATGTTTCGTGAGGCACG  
TGTCGATCTAGCTGAATTGGTCAAAAAGATGCAGCTTGAGCAGGGGCAACGCCTGCCTGCGTT  
ATTCTGTTTCCCGCAAATTTTACAGCATCGCCTGCGCTCAATTAATGGCGCATTAAACGTGCA  
CGTGAGTCATTTGGCTATGAAGGCGATTACTTCTGGTTTACCCAATTAAGTCAATCAGCATC  
GCCGGGTTATTGAGTCACTGGTTAATTCCGGTGAGCCATTAGGGCTGGAAGCCGGTTCTAAAG  
CCGAAATGATGGCGGTTTTGGCACATGCCGGTATGACTCGCTCGGTCATCGTTTGTAATGGCTA  
TAAAGAC