

OLIGO	SEQUENCE (5'-3')	NT POSITION
Lyle01	GGA TCC AGA CTT GTC TAT GAT GTC GCG	-67 to -41
D41N	G CAT ACC AAT ■ AT CAT CAT GGG CAT TTT TGG	111 to 141
H43N	G CAT ACC AAT GAT CAT ■ AT GGG CAT TTT TGG	111 to 141
D84N	CTA CTT TCC GGT GGC ■ AC ATT AAC ACT GGC	235 to 264
H117N	GCG ATC GGT AAT ■ AT GAA TTT GAT AAT CCG	337 to 366
E118Q	GCG ATC GGT AAT CAT ■ AA TTT GAT AAT CCG	337 to 366
H217N	GCG GCG ACC ■ AT ATG GGG CAT TAC G	640 to 664
H252N	GCG ATG ATC GTC GGT GGT ■ AC TCG CAA GAT ACG	736 to 768

Table 4-4: Oligonucleotides used in the mutagenesis experiments and DNA sequencing discussed in Chapter 4

Nucleotide (NT) position indicates the location of the complementary sequence within the *E. coli ushA* gene and adjacent sequence (Burns and Beacham, 1986). Shaded bases indicate a variation from the *ushA* gene sequence used in mutagenesis.

4-2-3 OLIGONUCLEOTIDE DIRECTED MUTAGENESIS

Oligonucleotide directed mutagenesis was performed using the Muta-Gene phagemid *in vitro* mutagenesis kit, version 2, supplied by Bio-Rad. In brief, ssDNA enriched in uracil residues was used as a template to synthesise the complementary strand *in vitro*, with the mutagenic oligonucleotide acting as a primer. Subsequent transformation of the double-stranded DNA into a cell containing a proficient uracil N-glycosylase results in high efficiency inactivation of the uracil-containing strand, allowing the non-uracil containing mutagenic strand to replicate. This results in enrichment for the mutant (non-uracil containing) strand, and frequency of mutagenesis typically greater than 50%.

The *ushA* gene was isolated from purified pLM-2 by digestion with *Bam*HI and *Hind*III, followed by purification of the *ushA*-containing fragment by gel purification. The Muta-Gene host plasmid (pTZ19U) was digested with *Bam*HI and *Hind*III, and