

Table S1: Spontaneous mutations that suppress the *hfiA* overexpression phenotype.

<i>Group 1: Plasmid mutations in pMT805-hfiA identified by targeted sequencing of the plasmid</i>					
Type of change	Nucleotide change ¹		Coding change ²	Notes ³	
Nonsense SNP	C16T		R6stop		
Nonsense; 1 bp insert	TT(23-24)TTT		D9stop	Partially active	
Nonsense SNP	C28T		Q10stop	Partially active	
Nonsense SNP	C31T		R11stop		
2 non-synonymous SNPs	T68A, T71A		I23N, L24H		
Nonsense SNP	C73T		Q25stop		
Non-synonymous SNP	T101G		L34R		
Non-synonymous SNP	T104A		V35E		
In-frame 15 bp duplication	2X C109-G123		2X(L37-A42)	Dominant	
Frameshift; 1 bp insert	CG(117-118)CAG		Adds 68 codons after P39		
Frameshift; 82 bp deletion	ΔG123-C214		Adds 30 codons after A40		
Nonsense SNP	T155A		L52stop		
Frameshift; 1 bp insert	C ₆ (204-209)C ₇		Adds 41 codons after P66		
<i>Group 2: Chromosomal mutations</i>					
Mutant	Locus	Function	Type of change	Nucleotide change ^{4,5}	Coding change ⁵
<i>Group 2a: Mutations mapped by whole genome sequencing</i>					
256-39	CC_0095	UDP-glycosyl transferase	SNP	T778C	C260R
	CC_2868	NeuB, N-acetylneuraminatase synthase	SNP	T3118669C	5' non-coding
261-15	CC_0095	UDP-glycosyl transferase	2 SNPs	T778C, T790C	C260R, W264R
	CC_2864	ArsR family transcription regulator	SNP	A194G	H65R
<i>Group 2b: Mutations identified by targeted sequencing of only CC_0095 locus</i>					
256-112	CC_0095	UDP-glycosyl transferase	SNP	T743G	L248R
256-177	CC_0095	UDP-glycosyl transferase	Deletion: 68 bp	ΔT800-G867	Frame shift after L266
256-185	CC_0095	UDP-glycosyl transferase	Duplication: 30 bp	2X T736-G765	2X F246-L255

¹Nucleotide position is indicated with reference to the reannotated translation start site corresponding to position 938825 in the NA1000 reference genome (Genbank accession CP001340); see also Figure S3.

²Amino acid residues are indicated with reference to the reannotated HfiA primary sequence; see also Figure S3.

³*Partially active*: Surface binding, holdfast development and rosette formation not fully restored to WT / pMT805 (EV) levels in these mutants. *Dominant*: This mutation leads to enhanced surface binding, holdfast development and rosette formation compared to WT / pMT805 (EV) suggesting it may also affect endogenously expressed HfiA.

⁴Coding changes are in gene coordinates; coordinate of non-coding change corresponds to position in the NA1000 reference genome.

⁵CC_0095 coordinates correspond to the start codon annotated in the NA1000 reference genome (CCNA_0094)