

Supplementary Table 1. Primers used for molecular biology work.

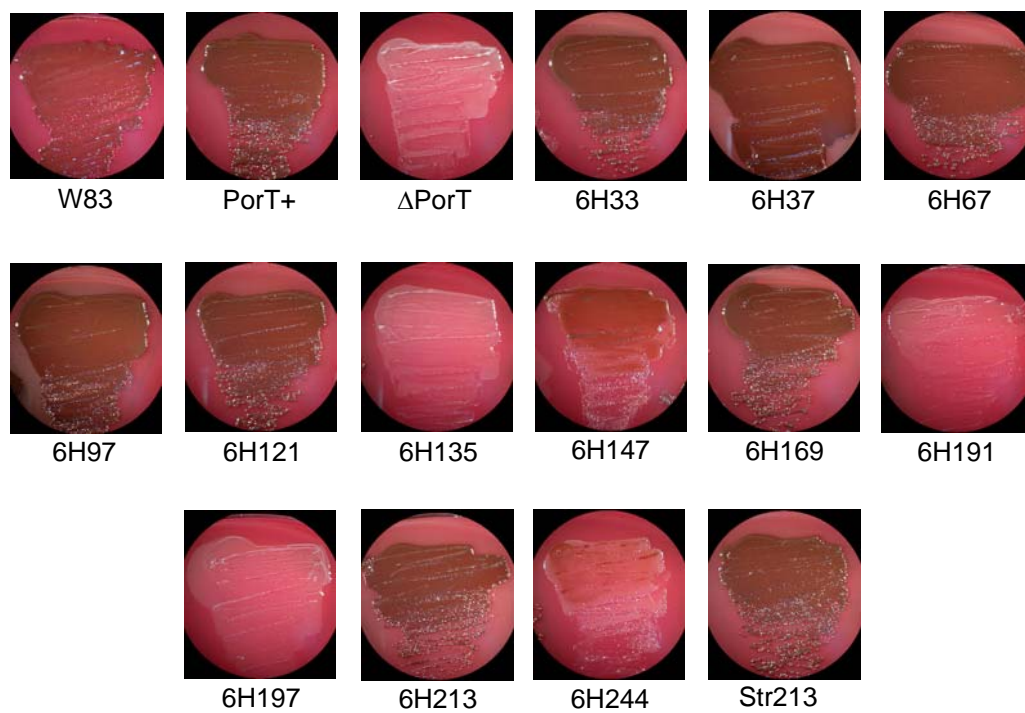
For creation of plasmids ^a :	Primer Name	Sequence ^b (5' – 3')
Cloning primers		
pPorTAtB-C (PorT+ mutant)	PorTFrBxbalF	ac TCTAGA AGTGGCGCATATCCTTGCA
	PorTFrBPstIR2	tata CTGCAG ACCTTCAGAACCGGAATCTGA
	tetQXmalF	tata CCCGGG ACAACGAATTATCTCCTAACGT
	tetQXbalR	cgc TCTAGA TTTTTATTGCCAAGTTCTAATGCT
	PorTSaclF	at GAGCTC CGTATATAGTCCACGGCTTGCT
	PorTSmaIR	aata CCCGGG CCACTGACCTGTGCGAAAAGAG
Mutagenesis primers		
pΔPorT-C (ΔPorT mutant)	DelPorTFA	TGAAGATTATTCTCTCAG TAGTTGTCACGCTCTTTTCGAC
	DelPorTFB	TAGTTGTCACGCTCTTTTCGACA
	DelPorTRA	CTGAGAGAATAATCTTCA ATTCTTTATTTGGTGTGT
	DelPorTRB	ATTCTTTATTTGGTGTGTAAGATCAACGC
p6HPorT-A (6H33 mutant)	6HPorTFA	CATCACCATCACCATCAC GTACAAAATCGCCCCTACGC
	6HPorTFB	GTACAAAATCGCCCCTACGC
	6HPorTRA	GTGATGGTGTATGGTGTATG TTTTTCCGTTTGCGCCGA
	6HPorTRB	TTTTTCCGTTTGCGCCGACA
pPorT6HPYA-A (6H37 mutant)	Por6HPYAFA	CATCACCATCACCATCAC CCCTACGCCGACTACAAAC
	Por6HPYAFB	CCCTACGCCGACTACAAAC
	Por6HPYARA	GTGATGGTGTATGGTGTATG GCGATTTTGTACTTTTTCCGTTTG
	Por6HPYARB	GCGATTTTGTACTTTTTCCGTTTG
pPorT6HEGS-A (6H67 mutant)	Por6HEGSFA	CATCACCATCACCATCAC GAAGGGAGTACGTGCGACTC
	Por6HEGSFB	GAAGGGAGTACGTGCGACTC
	Por6HEGSRA	GTGATGGTGTATGGTGTATG GGGTACAAACCCGTTGTTG
	Por6HEGSRB	GGGTACAAACCCGTTGTTG
pPorT6HPNL-A (6H97 mutant)	Por6HPNLFA	CATCACCATCACCATCAC CCCAATCTGAATTTACGTTTGCT
	Por6HPNLFB	CCCAATCTGAATTTACGTTTGCT
	Por6HPNLRA	GTGATGGTGTATGGTGTATG CAGAAGGAACATATCACCGATCA
	Por6HPNLRB	CAGAAGGAACATATCACCGATCA
pPorT6HKPV-B (6H121 mutant)	Por6HKPVFA	CATCACCATCACCATCAC AAACCCGTGGCCTCTTTC
	Por6HKPVFB	AAACCCGTGGCCTCTTTC
	Por6HKPVRA	GTGATGGTGTATGGTGTATG CTCCCCATCGGAAAAGACAA
	Por6HKPVRB	CTCCCCATCGGAAAAGACAA
pPorT6HFPL-B (6H135 mutant)	Por6HFPLFA	CATCACCATCACCATCAC TTTCCGCTATTATTGAAATATGGTTCTC
	Por6HFPLFB	TTTCCGCTATTATTGAAATATGGTTCTC
	Por6HFPLRA	GTGATGGTGTATGGTGTATG TTCCAAATAATTGGATCGAACGGA
	Por6HFPLRB	TTCCAAATAATTGGATCGAACGGA
pPorT6HNNM-B (6H147 mutant)	Por6HNNMFA	CATCACCATCACCATCAC AACAATATGCGTCCATACCTCAT
	Por6HNNMFB	AACAATATGCGTCCATACCTCAT
	Por6HNNMRA	GTGATGGTGTATGGTGTATG CAGGCGCCGAGAACCATA
	Por6HNNMRB	CAGGCGCCGAGAACCATA
pPorT6HLEI-A (6H169 mutant)	Por6HLEIFA	CATCACCATCACCATCAC TTGGAGATTTATACCAAAGCGAACGA
	Por6HLEIFB	TTGGAGATTTATACCAAAGCGAACGA
	Por6HLEIRA	GTGATGGTGTATGGTGTATG CCCCCTTCTTCGCCCCAG
	Por6HLEIRB	CCCCCTTCTTCGCCCCAG
pPorT6HPFF-B (6H191 mutant)	Por6HPFFFA	CATCACCATCACCATCAC CCCTTTTTTCAAATTGTGCCCCGAACCT
	Por6HPFFFB	CCTTTTTTCAAATTGTGCCCCGAACCT
	Por6HPFFRA	GTGATGGTGTATGGTGTATG CAGGTAATAATCGCATCCGAGACC
	Por6HPFFRB	CAGGTAATAATCGCATCCGAGACC
pPorT6HPEL-A (6H197 mutant)	Por6HPELFA	CATCACCATCACCATCAC CCCGAAGTGGCCTTTAGC
	Por6HPELFB	CCCGAAGTGGCCTTTAGC
	Por6HPELRA	GTGATGGTGTATGGTGTATG GCACAATTTGAAAAAAGGCAGGTAAAAATC
	Por6HPELRB	GCACAATTTGAAAAAAGGCAGGTAAAAATC
pPorT6HRPD-A (6H213 mutant)	Por6HRPDFA	CATCACCATCACCATCAC CGTCTGATCTTTGGATGATTATAAGT
	Por6HRPDFB	CGTCTGATCTTTGGATGATTATAAGT
	Por6HRPDRA	GTGATGGTGTATGGTGTATG CTCGTGTGTGATAACATCGGGAA
	Por6HRPDRB	CTCGTGTGTGATAACATCGGGAA

pPorT6H-A (6H244 mutant)	PorT6HFA	CATCACCATCACCATCACT AGTTGTCACGCTCTTTTCGACA
	DelPorTFB	TAGTTGTCACGCTCTTTTCGACA
	PorT6HRA	GTGATGGTGTGATGGTGTG CTCGAAATTGAACGTAAGCATAATCA
	PorT6HRB	CTCGAAATTGAACGTAAGCATAATCATT
pPorTStrRPD-A (Str213 mutant)	PorTStrRPDFA	TGGTCTCATCCTCAGTTTCGAAAAG CGTCCTGATCTTTTGGATGATTATAA
	Por6HRPDFB	CGTCCTGATCTTTTGGATGATTATAAGT
	PorTStrRPDRA	CTTTTCGAACTGAGGATGAGACCA CTCGTGTGTGATAACATCGG
	Por6HRPDRB	CTCGTGTGTGATAACATCGGGAA
Real-time PCR primers		
<i>rgpB</i> gene	RgpBTMF	CGATCGTAGCATTCTCCTCTCTGTTG
	RgpBTMR	CAGCGGAGAGCAGTCGTA
<i>gyrA</i> gene	GyrATMF2	GGGATCGGATACTCGCGAAGA
	GyrATMR	GGATTCATACACCTTCAGCCAATAGCA
	hGyrAProbeBFQ	ACTCGGCCTCCATGCACGCCACC
<i>porT</i> gene	PgPorTTMF	AGAGTCGCTCTCTTTGCCTGAT
	PgPorTTMR	TGGAATCCAAGATGATAGCGTTTGTAGTC
	fPgPorTProbeBFQ	CCTGGGCCGGACGTACGCTGTCG

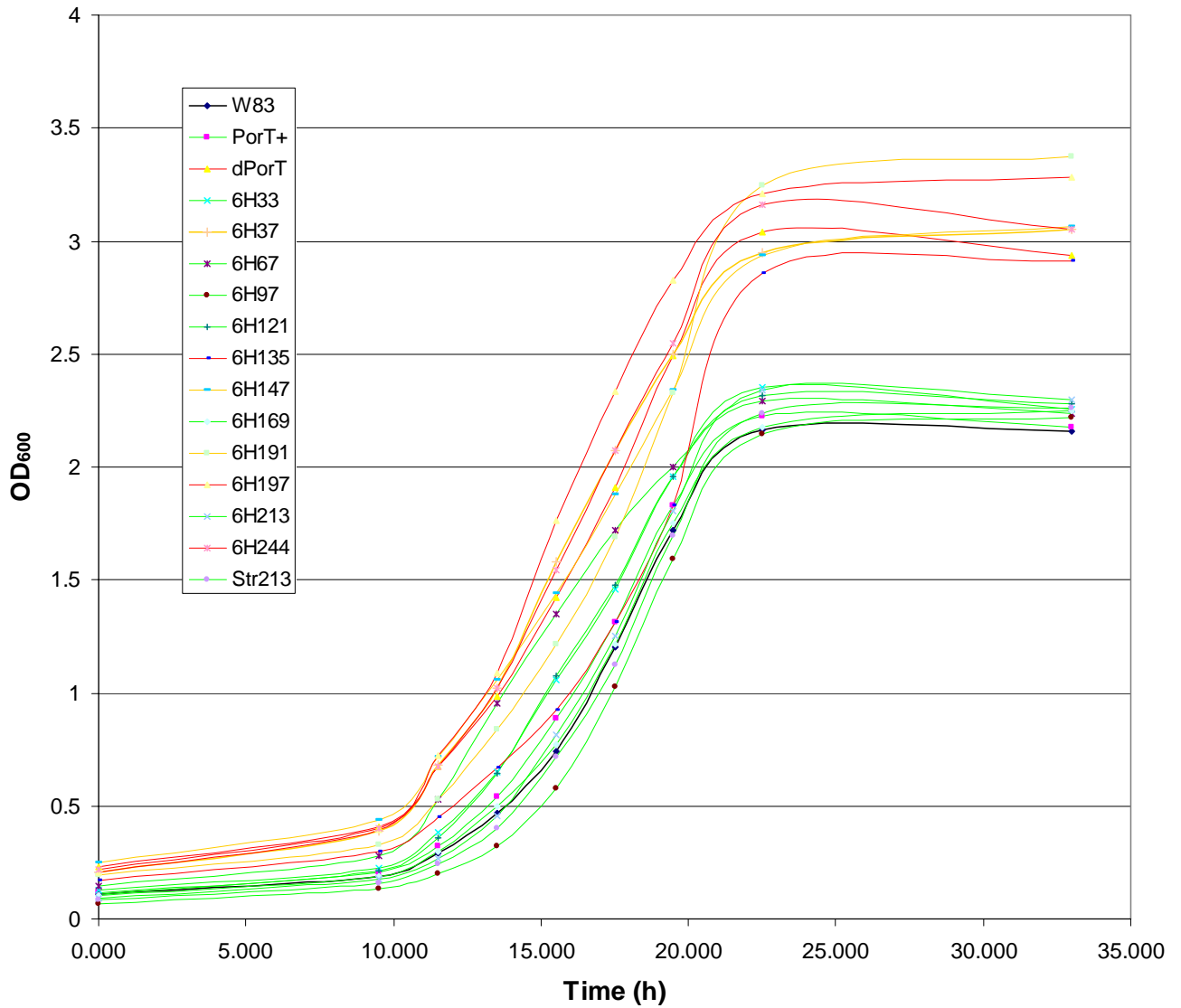
^a Named plasmids used for double crossover into *P. gingivalis* to create the desired mutant (in brackets).

^b Restriction sites are in bold and irrelevant bases for ease of restriction digest are in lowercase. Complementary overhangs for the SLIM mutagenesis technique are in bold italics.

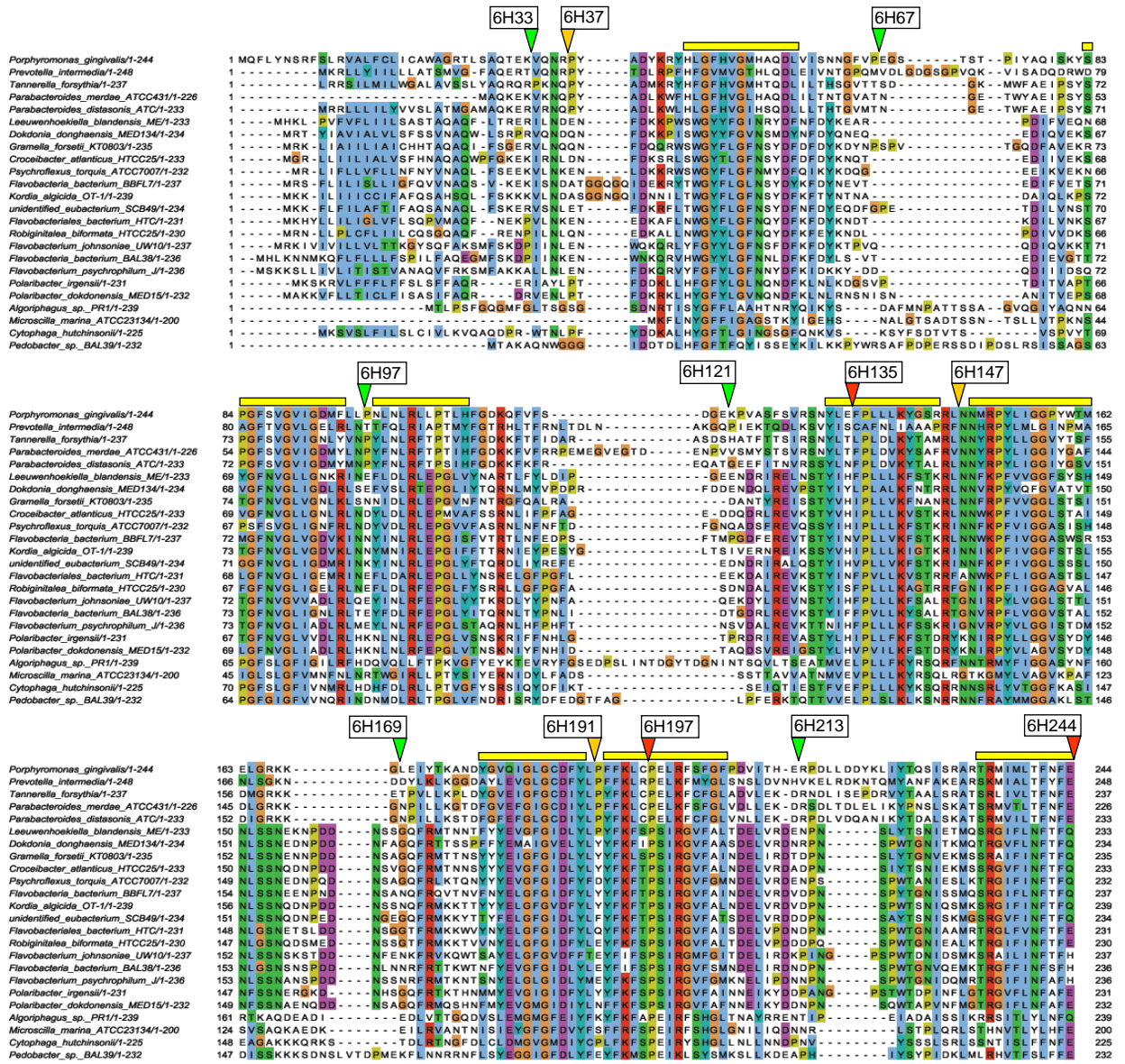
Supplementary Figure 1. Pigmentation of PorT mutants after 3 days growth on blood agar.



Supplementary Figure 2. Growth curve of PorT mutants in eTSB media. Pre-warmed 5 mL media in Teflon-capped tubes was inoculated with a 1:50 inoculum of an overnight starter culture that has been adjusted to OD₆₀₀ 1.0 using fresh media. Absorbance at 600 nm was measured at pre-determined time points and data shown were averaged from quadruplicate cultures with the SEM falling between 1-10% of the observed OD value. Mutants with fully functional PorT are represented with green lines, those with partially defective PorT with orange lines and non-functional PorT with red lines. Two independent runs gave similar results.



Supplemental Figure 3. Multiple alignment of PorT homologues in sequenced genomes using the ClustalW program at the European Bioinformatics Institute (www.ebi.ac.uk/clustalw/) and displayed using the JalView program (1). Highly conserved regions of predicted transmembrane β -sheets are indicated by yellow bars above the alignment. Arrowheads indicate the 6 \times His insertion sites in PorT of *P. gingivalis* along with the mutant names (boxed). Mutants with fully functional PorT are represented in green, those with partially defective PorT in orange and non-functional PorT in red.



Reference:

1. Clamp, M., J. Cuff, S. M. Searle, and G. J. Barton. 2004. The Jalview Java alignment editor. *Bioinformatics* 20:426-7.