pKSMSufI amber mutant	Primer 1 (5'→ 3')	Primer 2 (5'→ 3')
F22Bpa	C CGT GGG CAA CCG CTG TAG ATG ACT GTA CAA CGT GC	GC ACG TTG TAC AGT CAT CTA CAG CGG TTG CCC ACG G
F167Bpa	ATC CAG GAT AAA CGG CTG GAT AAC TAG GGT ACG CCA GAA T	A TTC TGG CGT ACC CTA GTT ATC CAG CCG TTT ATC CTG GAT
L301Bpa	CTG CGC CCA ACC GGC TAG CTG CCG CTG GTC ACA	TGT GAC CAG CGG CAG CTA GCC GGT TGG GCG CAG
M311Bpa	C ACA GAC AGT CTT CCG TAG CGC TTG CTG CCA ACT	AGT TGG CAG CAA GCG CTA CGG AAG ACT GTC TGT G
L341Bpa	CCG GGT ATT AAT GGA CAG TAG TGG GAC GTC AAC CGT AT	AT ACG GTT GAC GTC CCA CTA CTG TCC ATT AAT ACC CGG
W414Bpa	TT TAT TTC GGT CAG CCT TCC TAG GCG CAC TTC C	G GAA GTG CGC CTA GGA AGG CTG ACC GAA ATA AA
W113pBpa	G AAC GCA GCT ACT CTG TAG TAT CAC GCC AAT ACT C	G AGT ATT GGC GTG ATA CTA CAG AGT AGC TGC GTT C
W134Bpa	C GGC CTT GCC GGA ATG TAG CTG GTG GA	TC CAC CAG CTA CAT TCC GGC AAG GCC G
L206Bpa	C TGG GTG CGC TTG CGA TAG CTG AAC GCG TCG	CGA CGC GTT CAG CTA TCG CAA GCG CAC CCA G
F234Bpa	G ATT TCT GGC GAT CAG GGA TAG CTG CCT GCT CC	GG AGC AGG CAG CTA TCC CTG ATC GCC AGA AAT C

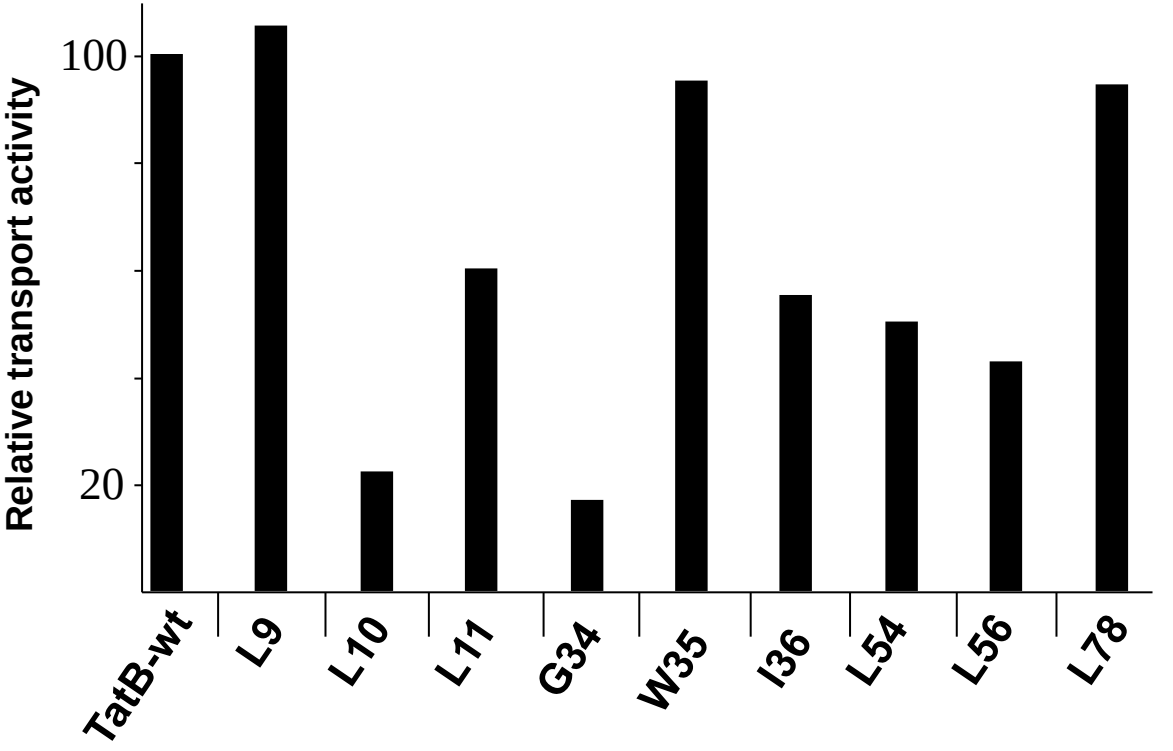
pET28aTorA-PhoA amber mutant	Primer 1 (5'→3')	Primer 2 (5'→ 3')
I124Bpa	GCG CTG GGC GTC GAT TAG CAC GAA AAA GAT CAC	GTG ATC TTT TTC GTG CTA ATC GAC GCC CAG CGC
W220Bpa	GCA ACC GCC GGT GAA TAG CAG GGA AAA ACG CTG	CAG CGT TTT TCC CTG CTA TTC ACC GGC GGT TGC
I279Bpa	ACG TAC CAC GGC AAT TAG GAC AAG CCC GCA GTT ACC	GGT AAC TGC GGG CTT GTC CTA ATT GCC GTG GTA CGT
L417Bpa	CAT ACC GGT AGT CAG TAG CGT ATT GCG GCG TAT	ATA CGC CGC AAT ACG CTA CTG ACT ACC GGT ATG
I99Bpa	CCT GCA AAA AAT ATT ATT TTG CTG TAG GGC GAT GGG ATG GGG GAT TCG G	C CGA ATC CCC CAT CCC ATC GCC CTA CAG CAA AAT AAT ATT TTT TGC AGG
V196	GGT CTG GCG ACC GGT AAC TAG TCT ACC GCA GAG TTG CAG	CTG CAA CTC TGC GGT AGA CTA GTT ACC GGT CGC CAG ACC

p8737-TatB amber mutant	Primer 1 (5'→ 3')	Primer 2 (5'→ 3')
L9Bpa	ATC GGT TTT AGC GAA TAG CTA TTG GTG TTC ATC ATC GGC CTC	GAG GCC GAT GAT GAA CAC CAA TAG CTA TTC GCT AAA ACC GAT
L10Bpa	GAT ATC GGT TTT AGC GAA CTG TAG TTG GTG TTC ATC ATC GGC CTC	GAG GCC GAT GAT GAA CAC CAA CTA CAG TTC GCT AAA ACC GAT ATC
L11Bpa	C GGT TTT AGC GAA CTG CTA TAG GTG TTC ATC ATC GG	CC GAT GAT GAA CAC CTA TAG CAG TTC GCT AAA ACC G
G34Bpa	CG GTA AAA ACG GTA GCG TAG TGG ATT CGC GCG TTG CG	CG CAA CGC GCG AAT CCA CTA CGC TAC CGT TTT TAC CG
W35Bpa	ACG GTA GCG GGC TAG ATT CGC GCG TTG	CAA CGC GCG AAT CTA GCC CGC TAC CGT
I36Bpa	ACG GTA GCG GGC TGG TAG CGC GCG TTG CGT TCA	TGA ACG CAA CGC GCG CTA CCA GCC CGC TAC CGT
L54Bpa	AAC GAA CTG ACC CAG GAG TAG AAA CTC CAG GAG TTT CAG G	CTG AAA CTC CTG GAG TTT CTA CTC CTG GGT CAG TTC GTT

L56Bpa	GAA CTG ACC CAG GAG TTA AAA TAG CAG GAG TTT CAG GAC AGT CTG	CAG ACT GTC CTG AAA CTC CTG CTA TTT TAA CTC CTG GGT CAG TTC
L78Bpa	CT AAC CTG ACG CCC GAA TAG AAA GCG TCG ATG GAT G	C ATC CAT CGA CGC TTT CTA TTC GGG CGT CAG GTT AG

pKSMSufI ΔSP	**Primer 1 (5'→ 3')**	**Primer 2 (5'→ 3')**
SufI A27L	CCC CTG AAG GCC AGC **CTA** GCC GGG CAA CAG	CTG TTG CCC GGC **TAG** GCT GGC CTT CAG GGG
SufI S26L&A27L	CCC CTG AAG GCC **CTC** *CTA* GCC GGG CAA CAG	CTG TTG CCC GGC *TAG* **GAG** GGC CTT CAG GGG
SufI S26L&A27L&Q30L	CCC CTG AAG GCC **CTC** *CTA* GCC GGG *CTA* CAG	CTG *TAG* CCC GGC *TAG* **GAG** GGC CTT CAG GGG

Transport activity of *tatB* amber mutant vesicles



pSufI was synthesized in the presence of one of the indicated membrane vesicles. INVs were reisolated and then incubated in the presence of an ATP-regenerating system. The amount of PK-protected pSufI and SufI was determined, the translocation efficiency quantified and normalized with respect to that of wt-INVs (for further details, see Materials and Methods).