

Supplementary information

Thiouridine residues in tRNAs are responsible for a synergistic effect of UVA and UVB light in photoinactivation of *Escherichia coli*

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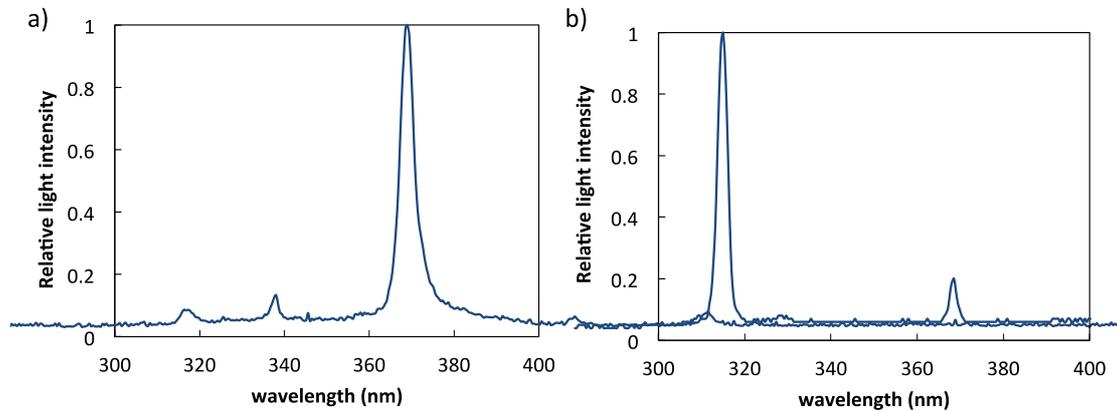
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SI Figure 1: Relative light intensity spectra of the UVA lamp (a) and the UVB lamp (b). The light intensity was normalized to 1 at 365 nm (UVA lamp) and 311 nm (UVB lamp) respectively.

SI Table 1: Strains used in this study

Strain	Genotype	Parental strain	Reference
wt	<i>E. coli</i> MG1655		(Blattner et al., 1997)
ancestor1	$\Delta lacZ::rfp$	<i>E. coli</i> MG1655	This study
ancestor2	$\Delta lacA::gfp$	<i>E. coli</i> MG1655	This study
SP40	$\Delta thil::kan$	<i>E. coli</i> MG1655	(Baba et al., 2006)
<i>Strains isolated from selection experiment (further mutations list in SI Table 2)</i>			
SP01	<i>thil</i> A41T	Ancestor1	This study
SP02	<i>thil</i> C815A	Ancestor2	This study
SP03	<i>thil</i> C815A	Ancestor1	This study
SP04	<i>thil</i> C554T	Ancestor2	This study
SP07	<i>thil</i> C578T	Ancestor1	This study
SP08	<i>thil</i> G862A	Ancestor2	This study
SP09	<i>thil</i> G862A	Ancestor1	This study

SP10	<i>thil</i> G1024A	Ancestor2	This study
SP11	<i>thil</i> G1024A	Ancestor1	This study
SP12	<i>thil</i> GA 28-29 AG	Ancestor2	This study
<i>Strains constructed by allelic replacement</i>			
SP21	<i>thil</i> A41T	<i>E. coli</i> MG1655	This study
SP22	<i>thil</i> C815A	Ancestor1	This study
SP23	<i>thil</i> C815A	<i>E. coli</i> MG1655	This study
SP24	<i>thil</i> C554T	Ancestor1	This study
SP27	<i>thil</i> C578T	<i>E. coli</i> MG1655	This study
SP28	<i>thil</i> G862A	Ancestor1	This study
SP29	<i>thil</i> G862A	<i>E. coli</i> MG1655	This study
SP30	<i>thil</i> G1024A	Ancestor1	This study
SP31	<i>thil</i> G1024A	<i>E. coli</i> MG1655	This study
SP32	<i>thil</i> GA 28-29 AG	Ancestor1	This study

SI Table 2: List of all mutations identified in the 10 evolved strains compared to the corresponding ancestor.

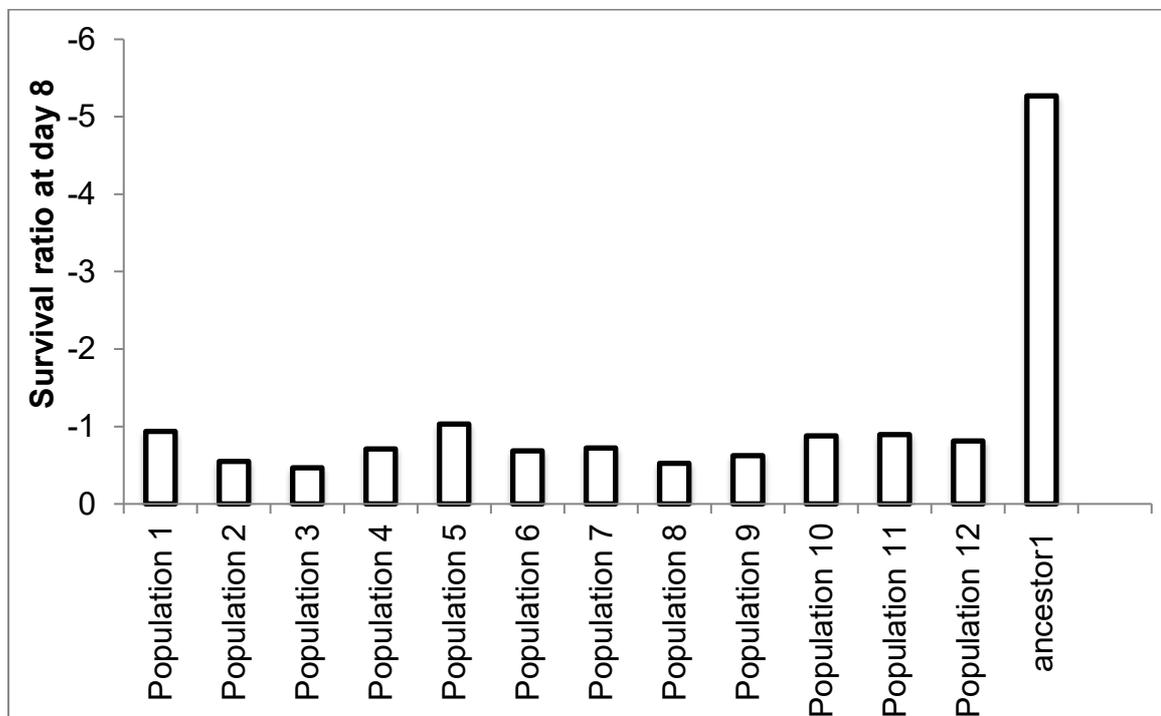
Strain	Position	Mutation	Annotation	Gene
SP01	119,896	Δ 3 bp	coding (616-618/855 nt)	<i>ampE</i> →
SP01	440,813	A→T	K14I (<u>A</u> AA→A <u>T</u> A)	<i>thil</i> →
SP01	831,883	Δ 3 bp	coding (289-291/483 nt)	<i>ybiA</i> ←
SP01	1,396,660	Δ 3 bp	intergenic (-14/+136)	<i>uspE</i> ← / ← <i>fnr</i>
SP01	1,484,505	Δ 4 bp	coding (3421-3424/3903 nt)	<i>hrpA</i> →
SP01	1,880,643	Δ 3 bp	coding (708-710/1086 nt)	<i>dmlA</i> →
SP01	2,423,266	Δ 3 bp	coding (671-673/687 nt)	<i>hisQ</i> ←
SP01	2,426,540	Δ 3 bp	coding (107-109/570 nt)	<i>ubiX</i> ←

SP01	3,133,999	Δ6 bp	coding (390-395/1500 nt)	<i>pitB</i> ←
SP01	3,186,567	Δ3 bp	pseudogene (1319-1321/3805 nt)	<i>ypqIG</i> →
SP01	3,244,484	Δ12 bp	coding (1359-1370/1419 nt)	<i>exuT</i> →
SP01	3,371,541	G→A	Q20* (<u>C</u> AA→ <u>I</u> AA)	<i>nanA</i> ←
SP01	3,688,311	Δ3 bp	coding (2318-2320/2340 nt)	<i>bcsB</i> ←
SP01	3,956,672	Δ4 bp	coding (680-683/1476 nt)	<i>ilvC</i> →
SP01	4,160,722	Δ4 bp	coding (569-572/1101 nt)	<i>trmA</i> ←
SP02	441,587	C→A	A272E (<u>G</u> CA→ <u>G</u> AA)	<i>thil</i> →
SP02	965,156	G→A	L538L (<u>C</u> TG→ <u>C</u> TA)	<i>ycal</i> →
SP02	1,379,600	C→T	pseudogene (327/1082 nt)	<i>ycjV</i> →
SP02	1,666,422	T→C	E56G (<u>G</u> AA→ <u>G</u> GA)	<i>mIc</i> ←
SP02	2,990,422	G→A	G103R (<u>G</u> GG→ <u>A</u> GG)	<i>ygeH</i> →
SP03	441,326	C→T	S185F (<u>T</u> CC→ <u>T</u> TC)	<i>thil</i> →
SP03	1,195,443	C→T	H366H (<u>C</u> AC→ <u>C</u> AT)	<i>icd</i> →
SP03	1,195,455	C→T	T370T (<u>A</u> CC→ <u>A</u> CT)	<i>icd</i> →
SP03	1,195,468	T→C	L375L (<u>T</u> TA→ <u>C</u> TG)	<i>icd</i> →
SP03	1,195,470	A→G	L375L (<u>T</u> TA→ <u>C</u> TG)	<i>icd</i> →
SP03	1,195,500	C→T	N385N (<u>A</u> AC→ <u>A</u> AT)	<i>icd</i> →
SP03	1,195,503	G→C	A386A (<u>G</u> CG→ <u>G</u> CC)	<i>icd</i> →
SP03	1,195,506	A→G	K387K (<u>A</u> AA→ <u>A</u> AG)	<i>icd</i> →
SP03	1,299,502	C→G	T99T (<u>A</u> CC→ <u>A</u> CG)	<i>oppA</i> →
SP03	1,379,943	T→G	intergenic (-17/-28)	<i>ycjV</i> → / → <i>ompG</i>
SP03	1,603,237	G→A	E55K (<u>G</u> AG→ <u>A</u> AG)	<i>IsrB</i> →
SP03	2,783,653	T→A	intergenic (-620/+131)	<i>ypjC</i> ← / ← <i>ileY</i>
SP03	2,783,664	C→T	intergenic (-631/+120)	<i>ypjC</i> ← / ← <i>ileY</i>
SP03	2,875,564	G→T	G321V (<u>G</u> GA→ <u>G</u> TA)	<i>iap</i> →
SP04	441,326	C→T	S185F (<u>T</u> CC→ <u>T</u> TC)	<i>thil</i> →

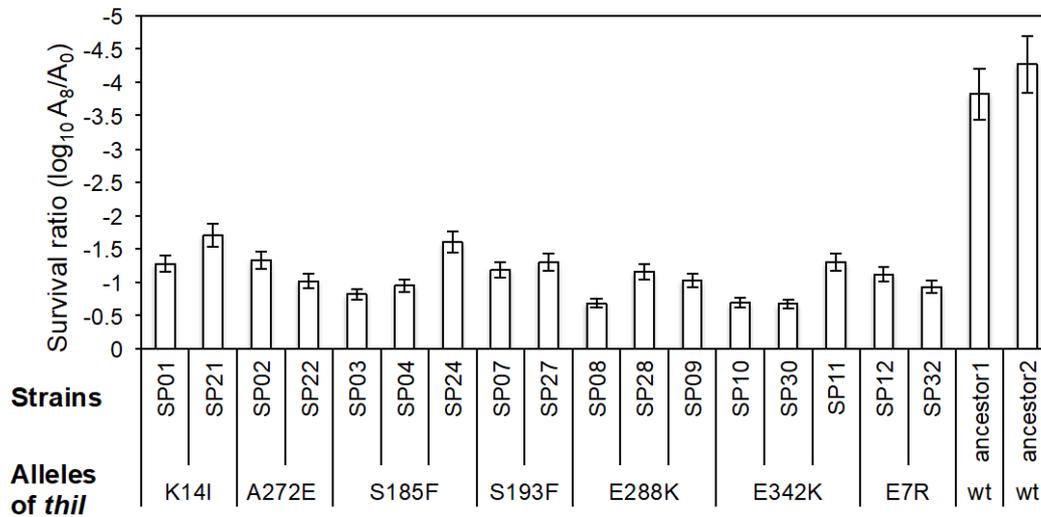
SP04	583,842	T→G	intergenic (+189/+61)	<i>appY</i> → / ← <i>ompT</i>
SP04	583,851	C→T	intergenic (+198/+52)	<i>appY</i> → / ← <i>ompT</i>
SP04	1,190,636	C→T	E192K (<u>G</u> AG→ <u>A</u> AG)	<i>purB</i> ←
SP04	1,195,443	C→T	H366H (CAC <u>C</u> →CAT <u>I</u>)	<i>icd</i> →
SP04	1,195,455	C→T	T370T (ACC <u>C</u> →ACT <u>I</u>)	<i>icd</i> →
SP04	1,195,468	T→C	L375L (<u>I</u> TA→ <u>C</u> TG)	<i>icd</i> →
SP04	1,195,470	A→G	L375L (TT <u>A</u> →CT <u>G</u>)	<i>icd</i> →
SP04	1,898,419	G→A	I397I (AT <u>C</u> →AT <u>I</u>)	<i>yoaE</i> ←
SP04	3,491,924	C→T	intergenic (+153/-109)	<i>tsgA</i> → / → <i>nirB</i>
SP07	441,350	C→T	S193F (T <u>C</u> C→T <u>I</u> C)	<i>thil</i> →
SP07	1,195,443	C→T	H366H (CAC <u>C</u> →CAT <u>I</u>)	<i>icd</i> →
SP07	1,195,455	C→T	T370T (ACC <u>C</u> →ACT <u>I</u>)	<i>icd</i> →
SP07	1,195,468	T→C	L375L (<u>I</u> TA→ <u>C</u> TG)	<i>icd</i> →
SP07	1,195,470	A→G	L375L (TT <u>A</u> →CT <u>G</u>)	<i>icd</i> →
SP07	1,195,500	C→T	N385N (AAC <u>C</u> →AA <u>I</u>)	<i>icd</i> →
SP07	1,195,503	G→C	A386A (G <u>C</u> G→G <u>C</u> C)	<i>icd</i> →
SP07	1,195,506	A→G	K387K (AAA <u>A</u> →AA <u>G</u>)	<i>icd</i> →
SP07	1,195,515	C→T	T390T (ACC <u>C</u> →ACT <u>I</u>)	<i>icd</i> →
SP07	1,351,487	C→T	M56M (<u>G</u> TG→ <u>A</u> TG)	<i>sapD</i> ←
SP07	1,410,536	C→T	D242N (<u>G</u> AT→ <u>A</u> AT)	<i>intR</i> ←
SP08	181,690	G→A	P269P (CC <u>G</u> →CC <u>A</u>)	<i>degP</i> →
SP08	366,933	Δ39 bp	coding (674-712/834 nt)	<i>mhpR</i> ←
SP08	441,634	G→A	E288K (<u>G</u> AA→ <u>A</u> AA)	<i>thil</i> →
SP08	754,164	C→A	intergenic (-473/-236)	<i>gltA</i> ← / → <i>sdhC</i>
SP08	877,433	T→C	intergenic (-175/-38)	<i>rimO</i> ← / → <i>bssR</i>
SP08	1,570,856	C→T	D791N (<u>G</u> AT→ <u>A</u> AT)	<i>pqqL</i> ←
SP08	1,886,015	A→G	M1M (<u>I</u> TG→ <u>C</u> TG)	<i>rnd</i> ←
SP08	2,333,620	C→T	G349G (GG <u>G</u> →GG <u>A</u>)	<i>yfaA</i> ←

SP08	3,602,891	A→G	E159G (<u>G</u> AA→ <u>G</u> GA)	<i>rsmD</i> →
SP08	4,045,092	Δ33 bp	coding (104-136/2787 nt)	<i>polA</i> →
SP08	4,136,491	Δ3 bp	coding (566-568/1104 nt)	<i>gldA</i> ←
SP08	4,185,300	A→G	D643G (<u>G</u> AC→ <u>G</u> GC)	<i>rpoC</i> →
SP09	441,634	G→A	E288K (<u>G</u> AA→ <u>A</u> AA)	<i>thil</i> →
SP09	1,791,355	G→C	A76A (<u>G</u> CC→ <u>G</u> CG)	<i>btuD</i> ←
SP09	1,802,994	G→A	pseudogene (24/1900 nt)	<i>arpB</i> →
SP09	3,436,067	C→T	R8C (<u>C</u> GC→ <u>I</u> GC)	<i>mscL</i> →
SP09	3,906,461	C→A	intergenic (-72/+111)	<i>pstB</i> ← / ← <i>pstA</i>
SP10	261,229	T→C	L168S (<u>T</u> TA→ <u>T</u> CA)	<i>proA</i> →
SP10	438,083	A→T	L440Q (<u>C</u> TG→ <u>C</u> AG)	<i>dxs</i> ←
SP10	441,796	G→A	E342K (<u>G</u> AA→ <u>A</u> AA)	<i>thil</i> →
SP10	1,195,443	C→T	H366H (<u>C</u> AC→ <u>C</u> AT)	<i>icd</i> →
SP10	1,195,455	C→T	T370T (<u>A</u> CC→ <u>A</u> CT)	<i>icd</i> →
SP10	1,195,468	T→C	L375L (<u>T</u> TA→ <u>C</u> TG)	<i>icd</i> →
SP10	1,195,470	A→G	L375L (<u>T</u> TA→ <u>C</u> TG)	<i>icd</i> →
SP10	1,195,500	C→T	N385N (<u>A</u> AC→ <u>A</u> AT)	<i>icd</i> →
SP10	1,195,503	G→C	A386A (<u>G</u> CG→ <u>G</u> CC)	<i>icd</i> →
SP10	1,195,506	A→G	K387K (<u>A</u> AA→ <u>A</u> AG)	<i>icd</i> →
SP10	1,195,515	C→T	T390T (<u>A</u> CC→ <u>A</u> CT)	<i>icd</i> →
SP10	1,195,527	G→A	E394E (<u>G</u> AG→ <u>G</u> AA)	<i>icd</i> →
SP10	1,412,828	C→T	K861K (<u>A</u> AG→ <u>A</u> AA)	<i>recE</i> ←
SP10	1,500,251	+T	intergenic (+72/-230)	<i>tehB</i> → / → <i>ydcl</i>
SP10	1,682,194	T→A	F430Y (<u>T</u> IT→ <u>T</u> AT)	<i>rstB</i> →
SP10	1,859,197	C→T	E54K (<u>G</u> AG→ <u>A</u> AG)	<i>ydjL</i> ←
SP10	1,870,478	C→T	R138R (<u>C</u> GC→ <u>C</u> GT)	<i>yeaJ</i> →
SP10	1,871,630	T→A	H11Q (<u>C</u> AT→ <u>C</u> AA)	<i>yeaK</i> →

SP11	441,796	G→A	E342K (<u>G</u> AA→ <u>A</u> AA)	<i>thil</i> →
SP12	440,800	2 bp→AG	coding (28-29/1449 nt)	<i>thil</i> →
SP12	2,147,053	G→A	intergenic (+3/+10)	<i>yegD</i> → / ← <i>yegI</i>
SP12	2,193,155	C→T	F278F (<u>TTC</u> → <u>TTT</u>)	<i>metG</i> →
SP12	2,953,473	C→T	E185K (<u>G</u> AA→ <u>A</u> AA)	<i>recB</i> ←
SP12	3,888,369	C→T	A37A (<u>GCC</u> → <u>GCT</u>)	<i>tnaB</i> →



SI Figure 2: The shown survival rates represent the sensitivity towards UV irradiation after eight hours of irradiation. The 12 populations were irradiated the eighth consecutive day while ancestor1 was not irradiated previously.



SI Figure 3: Comparison of the survival ratio for strains with different alleles in *thil*. The strains between SP01 and SP12 are evolved strains from the selection experiment. The strains between SP21 and SP32 are ancestor strains harboring a mutant version of *thil*. Error bars represent the standard errors of the means.

SI Table 3: Primer list of all primers used for the construction of strains SP21-SP32

Name	^a Sequence (5'→3')	Construction of strain	Used for
thil-mut1-f	gaagatctAGTTGCTGCGGAAAGTCCAT	SP21	Mutation construction
thil-mut1-r	gaagatctCAATACCTTCGTAGCGGCCT	SP21	Mutation construction
thil-seq1	CGTTCAGCGCCTCTTC	SP21	Fragment sequencing
thil-NLS1-f	ggCGGAAATCACCATCAT	SP21	Genotyping (TSP)

thil-NLS1-r	ggGGAATACGGGTCAGA	SP21	Genotyping (TSP)
thil-LS1-f	CTCTTCCAGCGGCAGGTCG	SP21	Genotyping (TSP)
thil-LS1-r	GATGGACAGTCACATCCGGATTG	SP21	Genotyping (TSP)
thil-mut2-f	<u>gaagatct</u> GATGTGGAACGTTACGTCGG	SP22, SP23	Mutation construction
thil-mut2-r	<u>gaagatct</u> TCTGGTCGAGATCGCCAAAT	SP22, SP23	Mutation construction
thil-seq2	AATCAGCATATTGAATCCGC	SP22, SP23	Fragment sequencing
thil-NLS2-f	ggTCAGCCACTTTAGATT	SP22, SP23	Genotyping (TSP)
thil-NLS2-r	gcGCATTATCTGTGGAAC	SP22, SP23	Genotyping (TSP)
thil-LS2-f	TTTCCACTTCCACCACTTCCTGC	SP22, SP23	Genotyping (TSP)
thil-LS2-r	GGCGGCGGTTTAAATCAGCATAT	SP22, SP23	Genotyping (TSP)
thil-mut4-f	<u>gaagatct</u> AAAGCCAATCTGTGCGCTTG	SP24	Mutation construction
thil-mut4-r	<u>gaagatct</u> CTTTCACCGTCGGGCTTTTG	SP24	Mutation construction
thil-seq4	TCCACCATATTCTCGAAGTC	SP24	Fragment sequencing
thil-NLS4-f	cgTCGAAACCACCGA	SP24	Genotyping (TSP)
thil-NLS4-r	ccCGGTTTAAATCAGCAT	SP24	Genotyping (TSP)
thil-LS4-f	CAGGATCAGCGTGTGGAGA	SP24	Genotyping (TSP)
thil-LS4-r	CCTTACCGGGAACATTCGTAACG	SP24	Genotyping (TSP)
thil-mut7-f	<u>gaagatct</u> AAAGCCAATCTGTGCGCTTG	SP27	Mutation construction
thil-mut7-r	<u>gaagatct</u> CTTTCACCGTCGGGCTTTTG	SP27	Mutation construction
thil-seq7	TATCAATCAGGCGCAGGT	SP27	Fragment sequencing
thil-NLS7-f	ggGCATCAACATATAACTGA	SP27	Genotyping (TSP)
thil-NLS7-r	ccCTCCTGCTGATTAAAG	SP27	Genotyping (TSP)
thil-LS7-f	GAAAGCGTTGGTTCAGTATCGCG	SP27	Genotyping (TSP)
thil-LS7-r	TCGATTTAACTGCTTTCACCGTCG	SP27	Genotyping (TSP)
thil-mut8-f	<u>gaagatct</u> GATGTGGAACGTTACGTCGG	SP28, SP29	Mutation construction
thil-mut8-r	<u>gaagatct</u> TCTGGTCGAGATCGCCAAAT	SP28, SP29	Mutation construction
thil-seq8	AATCAGCATATTGAATCCGC	SP28, SP29	Fragment sequencing
thil-NLS8-f	cgTGGTCACCGGCA	SP28, SP29	Genotyping (TSP)
thil-NLS8-r	cgCACACCACAATATTCC	SP28, SP29	Genotyping (TSP)

thil-LS8-f	AAAGGCCGCTACGAAGGTATTGG	SP28, SP29	Genotyping (TSP)
thil-LS8-r	AGAACGGCAGAGAAACCACATCA	SP28, SP29	Genotyping (TSP)
thil-mut10-f	<u>gaagatct</u> GCTACGAAGGTATTGGCGGT	SP30, SP31	Mutation construction
thil-mut10-r	<u>gaagatct</u> GCGTGAAAAAGCCCATGAAG T	SP30, SP31	Mutation construction
thil-seq10	TCCAGTTATATGTTGATGCG	SP30, SP31	Fragment sequencing
thil-NLS10-f	ccGATCACACCACAATATTT	SP30, SP31	Genotyping (TSP)
thil-NLS10-r	gcGCGCCTGATTGATAA	SP30, SP31	Genotyping (TSP)
thil-LS10-f	GGTGATGGCGGCAGGGATTATA	SP30, SP31	Genotyping (TSP)
thil-LS10-r	TATATGTTGATGCGTCGCGGCTG	SP30, SP31	Genotyping (TSP)
thil-mut12-f	<u>gaagatct</u> AGTTGCTGCGGAAAGTCCAT	SP32	Mutation construction
thil-mut12-r	<u>gaagatct</u> CAATACCTTCGTAGCGGCCT	SP32	Mutation construction
thil-seq12	TCGGCATAATGAATGTCTGA	SP32	Fragment sequencing
thil-NLS12-f	TTTATCATTAAATTGTTCC	SP32	Genotyping (TSP)
thil-NLS12-r	TCTCATCATAGTGCTT	SP32	Genotyping (TSP)
thil-LS12-f	CAGACAGCAGAATTTGTACGCGC	SP32	Genotyping (TSP)
thil-LS12-r	TCGTAGCGGCCTTTAATCAGCAG	SP32	Genotyping (TSP)
pKOV-unstuff-f	AGGGCAGGGTCGTTAAATAGC	All strains	Sequencing from vector
pKOV-unstuff-r	TTAATGCGCCGCTACAGGGCG	All strains	Sequencing from vector

^aCapital letters=pairs with target sequence, small letters=does not pair (*i.e.*, tail). BglIII restriction sites (AGATCT) underlined