Appendix II: Primers and Oligonucleotides Used for Construction and Analysis of Recombination Libraries

Table AII-1. Primers used in the construction of RandE:APST and RandEPST libraries for the parent proteins: P, PSE-4; S, SED-1; A, AST-1; T, TEM-1. All primers are named for the parent P2F, the block P2F, and whether they are forward (coding strand) or reverse (noncoding strand) P2F. Underlined regions are 5' overhangs used for construction. Letters shown in bold are single base mutations from the native sequence to either make the overhangs match or remove restriction sites.

Primers that are annealed to form internal fragments all 5' to 3'

P2F	Cqcttcccgttaacaagtacttttaaaacaatagcttgcgctaaatta
P2R	**CAA**TAATTTAGCGCAAGCTATTGTTTTAAAAGTACTTGTTAACGGGAA
S2F	Cq**C**tttgcgatgtgcagcaccagtaaggtcatgaccgccgccgcggta
S2R	**C**AATACCGCGGCGGCGGTCATGACCTTACTGGTGCTGCACATCGCAAA
A2F	Cq**C**ttcccgatggcgtccacgttcaagggcctggcgtgcggggcgctg
A2R	**CAA**CAGCGCCCCGCACGCCAGGCCCTTGAACGTGGACGCCATCGGGAA
T2F	CG**c**TTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTA
T2R	**C**AATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAA
P3F	**TTG**tatgatgctgagcaaggaaaagttaatcccaatagtacagtcgagattaagaaagc
P3R	TC**A**GCTTTCTTAATCTCGACTGTACTATTGGGATTAACTTTTCCTTGCTCAGCATCATA
S3F	Tt**G**aaacagagtgaaacccatgacggtattttgcagcaaaaaatgaccattaaaaaagc
S3R	TC**A**GCTTTTTTAATGGTCATTTTTTGCTGCAAAATACCGTCATGGGTTTCACTCTGTTT
A3F	**TTG**cgcgagcatcccctgtcgacgGgctacttcgatcaggtgatccactactccgccgc
A3R	TC**A**GCGGCGGAGTAGTGGATCACCTGATCGAAGTAGCCCGTCGACAGGGGATGCTCGCG
T3F	TT**g**TCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA
T3R	TCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGA
P4F	**T**gatcttgtgacctattcccctgtaatagaaaagcaagtagggcaggcaatc
P4R	**C**GTGATTGCCTGCCCTACTTGCTTTTCTATTACAGGGGAATAGGTCACAAGA
S4F	**T**gatctgaccaactggaatcccgtaacagagaaatatgtgggtaatacgatg
S4R	CGTCATCGTATTACCCACATATTTCTCTGTTACGGGATTCCAGTTGGTCAGA
A4F	**T**gagctggtcgagtattcgccggtgaccgagacccgggtcgagaccggcatg
A4R	**C**GTCATGCCGGTCTCGACCCGGGTCTCGGTCACCGGCGAATACTCGACCAGC
T4F	TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATG
T4R	**C**GTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAG
P5F	Ac**g**ctcgatgatgcgtgcttcgcaactatgactacaagtgataatactgcggcaaatatcatc
P5R	TAGGATGATATTTGCCGCAGTATTATCACTTGTAGTCATAGTTGCGAAGCACGCATCATCGAG
S5F	Ac**g**ttagctgagctaagcgcagcgacgttacagtacagcgataataccgccatgaataaactg
S5R	**T**AGCAGTTTATTCATGGCGGTATTATCGCTGTACTGTAACGTCGCTGCGCTTAGCTCAGCTAA
A5F	Ac**g**gtccgggaactgtgcgacgccgcgatcacggtttccgacaacacggcgggcaatcagttg
A5R	**T**AGCAACTGATTGCCCGCCGTGTTGTCGGAAACCGTGATCGCGGCGTCGCACAGTTCCCGGAC
T5F	AC**g**GTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTT
T5R	**T**AGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTAC

P6F	Ctaagtgctgtaggtggccccaaaggcgttactgatttttaagacaaatt
P6R	CCCAATTTGTCTTAAAAAATCAGTAACGCCTTTGGGGCCACCTACAGCACT
S6F	Ct**A**gcgcatcttggcggccccggcaacgtcacggcgtttgcacgttccatt
S6R	**C**CCAATGGAACGTGCAAACGCCGTGACGTTGCCGGGGCCGCCAAGATGCGC
A6F	Ct**A**aaactgctcggtggaccggagggattcaccgcgtccctgcgttccctc
A6R	**C**CCGAGGGAACGCAGGGACGCGGTGAATCCCTCCGGTCCACCGAGCAGTTT
T6F	CT**a**ACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATG
T6R	CCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGT
P7F	Gqqgacaaagagactcgtctagaccgtattgagcctgatttaaatgaaggtaagctcggt
P7R	ATCACCGAGCTTACCTTCATTTAAATCAGGCTCAATACGGTCTAGACGAGTCTCTTTGTC
S7F	Gq**G**gacacgacgtttcgtctcgatcgcaaagagccggaattaaacaccgccattcccggc
S7R	ATCGCCGGGAATGGCGGTGTTTAATTCCGGCTCTTTGCGATCGAGACGAAACGTCGTGTC
A7F	Gq**G**gacgccacgtcgcggctggaccgctgggagaccgacctgaacaccgcgattcccggg
A7R	ATCCCCGGGAATCGCGGTGTTCAGGTCGGTCTCCCAGCGGTCCAGCCGCGACGTGGCGTC
T7F	GGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAAC
T7R	**A**TCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATC
P8F	Gatttgagggatacgacaactcctaaggcaatagccagtactttgaataaatttttattt
P8R	GCCAAATAAAAATTTATTCAAAGTACTGGCTATTGCCTTAGGAGTTGTCGTATCCCTCAA
S8F	Gatgagcgcgacacaacatcgccgctggc**g**atggccaaaagtctgcgtaaactcacgctg
S8R	GCCCAGCGTGAGTTTACGCAGACTTTTGGCCAT**c**GCCAGCGGCGATGTTGTGTCGCGCTC
A8F	Gatgagcgcgataccaccaccccggccgcgctcgccgccgactaccgcgcgctcgtcgtc
A8R	GCCGACGACGAGCGCGCGGTAGTCGGCGGCGAGCGCGGCCGGGGTGGTGGTATCGCGCTC
T8F	GA**t**GAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACT
T8R	GCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTC
P9F	Gg**c**tccgcgctatctgaaatgaaccagaaaaaattagagtct
P9R	CCAAGACTCTAATTTTTTCTGGTTCATTTCAGATAGCGCGGA
S9F	Ggcgacgcgctggcagggccccagcgcgcgcagcttgtcgac
S9R	CCAGTCGACAAGCTGCGCGCGCTGGGGCCCTGCCAGCGCGTC
A9F	Ggcgatgtcctcggcgcacccgaacgcgaccagct**t**aaggca
A9R	CCATGCCTT**a**AGCTGGTCGCGTTCGGGTGCGCCGAGGACATC
T9F	GGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGAC
T9R	CCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTC
	Primers to construct the plasmids containing the stop sequence
P1R	**GCATGC**TCAGCTACTTA**GCTCTTC**AGCGCTGATTGCCATTGTAATCCCAAT
S1R	**GCATGC**TCAGCTACTTA**GCTCTTC**AGCGCTCGTCTGCGCGGTACAG
A1R	**GCATGC**TCAGCTACTTA**GCTCTTC**AGCGTTCGTCGGCGCGGTGGGCGACG
T10R	**GCATGC**TCAGCTACTTA**GCTCTTC**AGCGTTCTTCGGGGCGAAAACTCTC
P10F	**Gaagagc**taagtagctga**gcatgcGCTCTTC**ttggatggtgaacaatcaagtcac
S10F	**Agc**taagtagctga**gcatgcGCTCTTC**c**Tgg**ctgaaaggcaacaccaccg
A10F	**c**taagtagctga**gcatgcGCTCTTC**a**tgg**ctcgtcgccaacaccaccgg
T10F	**gagc**taagtagctga**gcatgcGCTCTTC**CTGGATGGAGGCGGATAAAG

Table AII-2. Other primers involved with synthesis of RandE:APST and RandE:PST libraries. Including the primers necessary to construct the cassette between blocks 1 and 10 (first set of primers). The primers to PCR amplify from the 5' and 3' end to put blocks 1 and 10 into the plasmid. The primers to PCR amplify just blocks 2-9 with Sap1 sites on the ends so that overhangs can be regenerated.

	Primers to construct the plasmids containing the stop sequence 3' end of block 1 and 5' end of block 10
P1R	**GCATGC**TCAGCTACTTA**GCTCTTC**AGCGCTGATTGCCATTGTAATCCCAAT
S1R	**GCATGC**TCAGCTACTTA**GCTCTTC**AGCGCTCGTCTGCGCGGTACAG
A1R	**GCATGC**TCAGCTACTTA**GCTCTTC**AGCGTTCGTCGGCGCGGTGGGCGACG
T10R	**GCATGC**TCAGCTACTTA**GCTCTTC**AGCGTTCTTCGGGGCGAAAACTCTC
P10F	**Gaagagc**taagtagctga**gcatgcGCTCTTC**ttggatggtgaacaatcaagtcac
S10F	**Agc**taagtagctga**gcatgcGCTCTTC**c**Tgg**ctgaaaggcaacaccaccg
A10F	**c**taagtagctga**gcatgcGCTCTTC**a**tgg**ctcgtcgccaacaccaccgg
T10F	**gagc**taagtagctga**gcatgcGCTCTTC**CTGGATGGAGGCGGATAAAG

	Primers for KpnF at 5' end of block 1
PkpnF	CAAGCTTGGTACCCatgcttttatataaaatgtgtgacaa
SkpnF	CAAGCTTGGTACCCatgcttaaggaacggtttcgccag
AkpnF	CAAGCTTGGTACCCgtgactttctccgctctccccttcc
TkpnF	CAAGCTTGGTACCCatgagtattcaacatttccgtgtc
	Primers for Pst1 Rev at 3' end of block 10
PSE4PstRMk	CAACCTGCAGCCATGGGtcagcgcgactgtgatgtataa
SED1PstRMk	CAACCTGCAGGAATTCGTTACTTTCCTTCCGTCACAATTTTCGC
AST1PstRMk	CAACCTGCAGactagtGCTATCCGAGCGCGTCGACCACC
TEMPstRmk	CAACCTGCAGCAGCTGGttaccaatgcttaatcagtgagg
	Primers to PCR amplify insert and add Sap1 site to 5'
PSE4PCRSAPF	CggcGacta**gctcttc**g**cgc**ttcccgttaacaagtactt
SED1PCRSAPF	cggcGacta**gctcttc**g**cgC**tttgcgatgTgcagcaccagt
TEM1PCRSAPF	cggcGacta**gctcttc**A**CGc**TTTCCAATGATG**AGC**ACTTTT
	Primers to PCR amplify insert and add Sap1 site to 3'
PSE4PCRSAPR	CggcGacta**gctcttc**TCCAAGACTCTAATTTTTTCTGGTTC
TEM1PCRSAPR	cggcGacta**gctcttc**TCCAGTCTATTAATTGTTGCCGGGAA
SED1PCRSAPR	**cggcGactagctcttcGCCAGTCGACAAGCTGCGCGCGC**

Table AII-3. Primers for construction of RASPP:PST using SISDC (Hiraga and Arnold 2003). First, the tag sequences for each recombination site are shown. The overhangs are in italics, the BsaX1 site is in bold and the Nde1 site is underlined. The primers used for all PCR reactions are shown below. They are named for the parent P64F, the recombination site amino acid P64F, and whether they are for the coding sequence (F), or noncoding sequence (R). For the primers, capital letters are part of the tags, lower-case letters match the gene.

Tag 64:	--- TCT GGC AGA **AC** GGACT **CTCC** ATATGGC *CGC*
	GCG AGA CCG TCT **TG** CCTGA **GAGG** TATACCG ---
Tag 73:	*aaa* ACC CTT GAG **AC** GTTGC **CTCC** ATATGCT *AAA*
	TTT TGG GAA CTC **TG** CAACG **GAGG** TATACGA ---
Tag 148:	Acc GGC AAC CGT **AC** CGGTA **CTCC** ATATGAT *ACC*
	TGG CCG TTG GCA **TG** GCCAT **GAGG** TATACTA ---
Tag 176:	--- TCG TTA GCC **AC** AAGGC **CTCC** ATATGCG *GAT*
	CTA AGC AAT CGG **TG** TTCCG **GAGG** TATACGC ---
Tag 190:	--- CAA TGC GTG **AC** ATTCG **CTCC** ATATGTC *TTG*
	AAC GTT ACG CAC **TG** TAAGC **GAGG** TATACAG ---
Tag 218:	--- CGC CTT GAC **AC** TGCCA **CTCC** ATATGTA *GGC*
	CCG GCG GAA CTG **TG** ACGGT **GAGG** TATACAT ---

P1F	ccgCTCGAGGGTACCCatgcttttatataaaatgtgtgaca
T1F	ccgCTCGAGGGTACCCatgagtattcaacatttccgtgt
S1F	ccgCTCGAGGGTACCCatgcttaaggaacggtttcgcc
P64F	GGCAGAACGGACTCTCCATATGGCCGCttcccgttaacaagta
P64R	GGAGAGTCCGTTCTGCCAGAGCGctgattgccattgtaatccc
T64F	GGCAGAACGGACTCTCCATATGGCCGCtttccaatgatgagca
T64R	GGAGAGTCCGTTCTGCCAGAGCGttcttcggggcgaaaac
S64F	GGCAGAACGGACTCTCCATATGGCCGCtttgcgatgtgcagca
S64R	GGAGAGTCCGTTCTGCCAGAGCGctcgtctgcgcggtacagc
P73F	CTTGAGACGTTGCCTCCATATGCTAAAacaatagcttgcgctaaat
P73R	GGAGGCAACGTCTCAAGGGTTTTaaaagtacttgttaacgg
T73F	CTTGAGACGTTGCCTCCATATGCTAAAgttctgctatgtggcgcgg
T73R	GGAGGCAACGTCTCAAGGGTTTTaaaagtgctcatcattgg
S73F	CTTGAGACGTTGCCTCCATATGCTAAAgtcatgaccgccgccgcgg
S73R	GGAGGCAACGTCTCAAGGGTTTTactggtgctgcacatcgc
P149F	AACCGTACCGGTACTCCATATGATACCgatttttaagacaaattgggga
P149R	GGAGTACCGGTACGGTTGCCGGTaacgcctttggggccacct
T149F	AACCGTACCGGTACTCCATATGATACCgctttttttgcacaacatgggga
T149R	GGAGTACCGGTACGGTTGCCGGTtagctccttcggtcctccga
S149F	AACCGTACCGGTACTCCATATGATACCgcgtttgcacgttccattggcg
S149R	GGAGTACCGGTACGGTTGCCGGTgacgttgccggggccgc

P161F	CCGCCTCGAGGCTCTTCCtcgtctagaccgtattgagcctga
P161R	AAAACTGCAGGCTCTTCAcgagtctctttgtccccaatttg
T161F	CCGCCTCGAGGCTCTTCCtcgccttgatcgttgggaaccgg
T161R	AAAACTGCAGGCTCTTCGcgagttacatgatcccccatgttg
S161F	CCGCCTCGAGGCTCTTCTtcgtctcgatcgcaaagagccgg
S161R	AAAACTGCAGGCTCTTCAcgaaacgtcgtgtcgccaatggaacg
P176F	TTAGCCACAAGGCCTCCATATGCGGATttgagggatacgacaacccc
P176R	GGAGGCCTTGTGGCTAACGAATCaccgagcttaccttcatttaa
T176F	TTAGCCACAAGGCCTCCATATGCGGATgagcgtgacaccacgatgcc
T176R	GGAGGCCTTGTGGCTAACGAATCgtttggtatggcttcattcag
S176F	TTAGCCACAAGGCCTCCATATGCGGATgagcgcgacacaacatcgcc
S176R	GGAGGCCTTGTGGCTAACGAATCgccgggaatggcggtgt
P190F	TGCGTGACATTCGCTCCATATGTCTTGaataaatttttatttggttccgc
P190R	GGAGCGAATGTCACGCATTGCAAagtactggctattgccttagg
T190F	TGCGTGACATTCGCTCCATATGTCTTGcgcaaactattaactggcgaacta
T190R	GGAGCGAATGTCACGCATTGCAAcgttgttgccattgctacag
S190F	TGCGTGACATTCGCTCCATATGTCTTGcgtaaactcacgctgggcgacgc
S190R	GGAGCGAATGTCACGCATTGCAAacttttggccatggccagcgg
P218F	CTTGACACTGCCACTCCATATGTAGGCaatttactacgttcagtattgcc
P218R	GGAGTGGCAGTGTCAAGGCGGCCagtgacttgattgttcaccatc
T218F	CTTGACACTGCCACTCCATATGTAGGCccacttctgcgctcggccc
T218R	GGAGTGGCAGTGTCAAGGCGGCCtgcaactttatccgcctccat
S218F	CTTGACACTGCCACTCCATATGTAGGCcagagcattcgtgccggcct
S218R	GGAGTGGCAGTGTCAAGGCGGCCtccggtggtgttgcctttc
PendR	AAAACTGCAGAAGCTTtcagcgcgactgtgatgtat
TendR	AAAACTGCAGAAGCTTttaccaatgcttaatcagtgagg
SendR	AAAACTGCAGAAGCTTttactttccttccgtcacaattttc

Table AII-4. PCR amplification of each half library of the small library during construction was done with the following primer sets. Primers sequences can be found on Table AII-3.

Front Half-Library	
P1F	P161R
P1F	S161R
P1F	T161R
S1F	P161R
S1F	S161R
S1F	T161R
T1F	P161R
T1F	S161R
T1F	T161R

Back Half-Library	
P161F	PendR
P161F	SendR
P161F	TendR
S161F	PendR
S161F	SendR
S161F	TendR
T161F	PendR
T161F	SendR
T161F	TendR

Table AII-5. Probes for DNA Hybridization to sequence chimeras in the smaller lactamase library. Stringency wash conditions: all washes contain 0.5% SDS and the indicated concentration of SSC.

	sequence	Stringency wash
PSEprobe1	GTTGAACAAGACGTTAAGGCAATTGAAG	2x
TEMprobe1	CGCTGGTGAAAGTAAAAGATGCTGAAG	1x
SEDprob1	GTTCAGAAAAAGCTGGCGGCG	0.5x
PSEprobe2	CGCTTCCCGTTAACAAGTACTTTT	2x
TEMprobe2	CGcTTTCCAATGATGAGCACTTTT	2x
SEDprobe2	CGCTTTGCGATGTGCAGCACCAGT	1x
PSEprobe3	GAAAAGTTAATCCCAATAGTACAGTCGAGATTAAG	2x
TEMprobe3	GCAACTCGGTCGCCGC	1x
SEDprobe3	GGTATTTTGCAGCAAAAAATGACCATTAAAAAAG	2x
PSEprobe4	GATTTTTTAAGACAAATTGGGGACAAAGAGAC	2x
TEMprobe4	CTTTTTTGCACAACATGGGGGATC	2x
SEDprobe4	GCACGTTCCATTGGCGACAC	2x
PSEprobe5	GCCTGATTTAAATGAAGGTAAGCTCGG	2x
TEMprobe5	CGGAGCTGAATGAAGCCATACC	1x
SEDprobe5	GGAATTAAACACCGCCATTCCCG	1x
PSEprobe6	CAACCCCTAAGGCAATAGCCAGTAC	2x
TEMprobe6	GCCTGTAGCAATGGCAACAacg	2x
SEDprobe6	CGCTGGCCATGGCCAAAAG	2x
PSEprobe7	GTTCCGCGCTATCTGAAATGAACC	2x
TEMprobe7	GAACTACTTACTCTAGCTTCCCGGC	2x
SEDprobe7	CTGAAAGGCAACACCACCGGA	0.5x
PSEprobe8	GGAGAGCATCAAGCCCCAATTATTG	2x
TEMprobe8	GGGGCCAGATGGTAAGCCC	2x
SEDprobe8	GATGCGAAATGGCGTAAAGATGTCC	0.5x