

Table 2. Oligonucleotides for construction of chimeric and truncated ϵ -cyclases and site-directed mutants

Construct	Primer name and junction	Primer sequence*
Lse/Ste 262/108	(none) ELGG/PRVS	Used conserved <i>Ava</i> II sites
Lse/Ste 285/6	(none) YDPD/LGLQ	Used <i>Bgl</i> III sites
Lse/Ate 323/320	Lse323N KIFF/EETC	cgaagaatattttgttgagaca
	Ate324C	aggagacatgtttggcctcaa
Lse/Ate 443/435	Lse443N WPLE/RKRQ	ttcaagtggccacaatgtttc
	Ate444C	aggaaaagacagagagcattctt
Lse/Ate 461/453	Lse461N IVLM/DTEG	attagcacgatgtgatagtc
	Ate462C	ggataccgaaggcattagaag
Lse/Ate 498/490	Lse498N IIFA/LYMF	gcaaatattatcaaatccgttgaaga
	Ate499C	ttatacatgttcgctattcaccaaa
Ate/Lse 390/395	Ate394N ATGY/SVVR	tagcctgttgcgggatga
	Lse395C	ttcagttgttcgatctttgtcag
Ate/Lse 408/413	AteN412 VIAE/ILRQ	tctgcatgactgatgcatatt
	LseC314 (typo by vendor)	gattttaagacaagatcaatctaaagagatg
Ate/Lse 420/430	AteN429 QINS/NISK	ctgttgatctgtttgtagtctcttc
	LseC430	taacattcaaaaacaagcatgggaaa
Ate/Lse 440/450	AteN449 RQRA/FFLF	atgctctctgtcttttcttctg
	LseC450	tctttctattcggactatcacacac
Ate/Lse 455/465	AteN464 FDTE/GTRT	cttcggatcgaattgaactatgag
	LseC465	gaacacgtacattttccgactttc
Ate	(none)	Full length cDNA in pBluescript SK-; not a lacZ fusion
Ate Δ 11N	EPS- Δ 11 (NcoI)	caAcCatggcggttcaacatttc
Ate Δ 58N	EPS- Δ 59 (NcoI)	tgtgCCATggtgagagaagattcgtgac
Ate Δ 82N	EPS- Δ 82 (NcoI)	ttcCCatgGagcagaacaaagataggatga
Ate Δ 88N	EPS- Δ 88 (NcoI)	aagCCatggatgaacagtctaagcttgtg
Ate Δ 103N	(none)	Used MunI/mung bean + fused to NcoI/Klenow of pTrcHisA
Ate Δ 23C	Ate/K511tag (=taa)	gatgattgatgagaccttAtctcaattgttggg
Ate ALIVQF447-52/SHIVLM	Ate/456-61SHIVLM (DraIII)	gccttcgggtatcCaTtAgCacGatgTgtgAaagaccaagag
Ate L448H	AteL457H/FspI	ggtatcgaattgaactatgTgCgcaagaccaagagaaag
Ate L448D	AteL457D/EcoRV	ggtatcgaattgaacGatATCtcaagaccaagagaaag
Ate L448R	AteL457R/BssHII	ggtatcgaattgaactatgCgCgcaagaccaagagaaag
Ate A447D	AteA456D/BglII	cgaattgaactatgagATcTagaccaagagaaagaaatgc
Lse	(none)	Full length cDNA in pBluescript SK-; not a lacZ fusion
Lse Δ 92N	(none)	Used pre-existing NcoI; fusion in TrcHisA

Lse Δ107N	(none)	Used MunI/mung bean + fused to NcoI/Klenow of pTrcHisA
Lse Δ23C	Lse/M511tag	atgtctaaccagttcc <u>TA</u> tctcaagetgtgaggtgc
Lse H457L	Lse/H457L	ccctctagatccattagcacgatg A gtgatagtcggaatagaag
Lse H457D	LseH457D/EcoRV	cctctagatccattagcacgat AtC tgatagtcggaatagaag
Lse H457R	Lse/H457R/XhoI	ctagatccattagcacgat TCTC ga G agtccggaatagaagaagg
Lse L460Q	Lse/L460Q	gtccctctagatccatt T gcacgatgtgtgatag

* Bases altered or introduced by primers are in bold uppercase letters. Restriction enzyme sites introduced by primers are underlined.