

**Table S1:**

Primers:

Primer name	Sequence <sup>a</sup>
Construction of pRL1 and 2:	
Xcm-Xho-Nco-Bgl-R2:	GTAGCAGATCT <b>CCATGGCTCGAGTGG</b> TTCCCGACTGGAA AGCGG
Xcm-PvuII-F1	TACGCCAGCTGT <b>TCGAATGGGGG</b> GATGTGCTGCAAGG
pRL-Cla-MNA-R1 <sup>b</sup>	AGTGAGCATCGAT <b>ACGCGTGC</b> CGGCGCGCCGAGCCGAA CGACC
Pspac <sup>C</sup> - <i>pyrFE</i> -cassette:	
BspyrF-fusPspa-F1	GGCATAATGTGTGGAATCGGAAGGAGCTGGAATCA
Pspc-fusBspyrF-R1	CTCCTTCCGATTCCACACATTATGCCACA
pyrF-rBgl-rH1-F1	TTTAGAC <b>CTAAA</b> ACTTCATGACATCCCGACTA
pyrF-rBgl-rH1-R1	GATGTCATGAAG <b>TTTTAG</b> GTCTAAAAATAGCTCGCAG
BspyrE-rH2-F1	GGTATCAGCAA <b>ACTT</b> GTAAAGAGCATTTTCC
BspyrE-rH2-R1	CTTTAACAAG <b>TTTG</b> CTGATACCTGAAGCAAC
BspyrE-rH3-F1	CTTGAAGC <b>CTGT</b> GCAGCTTTACAAGCGG
BspyrE-rH3-R1	AAGCTGCACAG <b>GGCT</b> TCAAGCACGCTGCCTC
BspyrFE-Nco-R1	CCAGT <b>CCC</b> ATGGATTAATAAAAAAACAGGCCTCAC
Pspac-Bgl-F2	CCAGTCAGATCTACACAGCCAGTCCAGAC
Pspac-rREPfus-F1	GGCATAATGT <b>AT</b> GGAATCGGAAGG
Pspac-rREPfus-R1	CCGATTCCA <b>TAC</b> ATTATGCCACACC
CAT194-cassette:	
CAT-Bam-F1	GTATCAGGATCCATGTATTCTCAAGATAAGAAAG
CAT-Bgl-R1	GAGCATAGATCTTCTTCAACTAACGGGGC
CAT-rNco-F2	TCTATTATTCC <b>TTGG</b> ACTTCATTTACTGGG
CAT-rNco-R2	GAAGTCCA <b>AGGA</b> ATAATAGAAAGAGAAAAAGC
<i>ermC</i> -cassette:	
ERY-Bam-F1	ACTATGGATCCTTTAAGAACTTTCTTTTTTTTAC
ERY-Bgl-R1	TATCAAGATCTCACAAAAATAGGCACACG
EryC-rSac-R1	GCAC <b>AAG</b> CTCTGATAAATATGAAC
EryC-rSac-F1	CATATTTATCAGAGCT <b>TGT</b> GCTATAATT
<i>tetK</i> -cassette:	
TetK-Bam-F1	CGACGGATCCAATATAAGAATTTGATAAAAAGAAAT
TetK-Bgl-R1	GCGATAGATCTTACGTGTGCTCTGCGAGGCT
pE194ts-origin-cassette:	
pMAD-ORI-Asc-F1	GGACGCGGCGCGCCACACGCAAAAAGGAAATTGGAAT
pMAD-ORI-Asc-R1	ATGTCAGGCGCGCCGTCGGCTTAAACCAGTTTTTCG
pT181ts-origin-cassette:	
pNL-ORI-Asc-F1	CATGCAGGCGCGCCGAGAAAACTTATTGGTTGG
pNL-ORI-Asc-R1	TAATCTGGCGCGCCTCTTTACTTGAGGTGACTAAAGT
OR <sub>181</sub> cassette:	

pNL-ORI-Asc-F2	CTCTGGGCGCGCCTATTTGGGTGAGCGATTCC
pNL-ORI-Asc-R2	CTCTGGGCGCGCCATCGTCATTGCATCAAACT
<i>pyrFE</i> region for pRLSAYx9:	
SA-pyrFE-A-Xba-F1	GGATGGTCTAGATTGAAGTTGATGCGATTTGTGA
SA-pyrFE-B-Bgl-R1	GGTTGAAGATCTAGCAAACCTCTCTGTATGGTGG
Construction of pRLBE2:	
pC194-REP-BsrGI-F2	CTGTCTGTACATTTAGTTGAAGAATAAAAGACCAC
pC194-BstBI-R1	TAAGTCTTCGAATGCCCGTTAGTTGAAGAAGGTT
EryC-BsrGI-F1	ACTATTGTACATTTAAGAAGCTTTCTTTTTTTTAC
EryC-BstBI-R1	TATCATTCGAAACACAAAAAATAGGCACACG
Construction of pRLBER1:	
pRLB-Mlu-Nco-R1	TTGAGCCATGGACGCGTAGTTATTGGGAGGTTAGC
SA-pyrFE-Nco-F1	ATGAGCCATGGTACAGTTGGTAAGGCGATGG
SA-pyrFE-BsrGI-R1	ATGAGTGTACATCGCGGATTGCGCCTCG
Cassettes for pRLBxR8 and 9 plasmids:	
CAT-BsrGI-F1	GGGACTGTACATGTATTCTCAAGATAAGAAAG
CAT-BstBI-R1	GGGAGTTCGAATCTTCAACTAACGGGGCAGG
pNL-ORI-Nco-F1	TTGAGCCATGGGAGAAAACCTTATTGGTTGG
pNL-ORI-Mlu-R1	GGTGAACGCGTCTCTTTACTTGAGGTGACTAAAGT
pMAD-ORI-Nco-F1	GAGTACCATGGACACGCAAAAAGGAAATTGGAAT
pMAD-ORI-Mlu-R1	ATGTCACGCGTCGGCTTAAACCAGTTTTTCG
RNase J1 deletion (PR01-01):	
RnaseJ1-L-Xho-F1:	CTGATCTCGAGTATCTTAACAACCTTTTTACTG
RNaseJ1-L-Xba-R1	CAGAATCTAGACCTCCAAGTGCATATACACC
RnaseJ1-R-Eco-F1	CAGATGAATTCATCATTGAAACATTACAACC
RnaseJ1-R-Nar-R1	CAGAAGGCGCCAGAAATGGGCTGGACATTAGTG
RNase J2 deletion (PR01-04)	
RnaseJ2-Xho-F1	ACGTGCTCGAGGTCGTCGTGAAATTGGACA
RnaseJ2-Bam-R1	TCTTGGATCCTAAATTTGTTACCCGTCC
RnaseJ2-Eco-F1	AACTAGAATTCGAAAGTACAAAACGTCGTC
RnaseJ2-Nar-R1	TTCGAGGCGCCACCAGCTTCTTCAATAGTGTT
RNase Y deletion (PR01-02):	
RnaseY-Xho-F1	GACGCTCGAGGCGGGGTGGCAGCATTTA
RnaseY-L-Xba-R1	TGGACTCTAGACCTTTTCTAGGGTTTGTCTTTAT
RnaseY-R-Eco-F1	GACAGGAATTCGAGAGACTAGAGCAGTAG
RnaseY-R-Nar-R1	CCTAAGGCGCCGTCATAAAAACCTGTCATACC
Removal of RNase Y N-terminal anchor (PR01-03):	
RnaseY-rTailfus-F1	GTGTTTGTGTGCGAAATTTGTTGCTTCAAAGCAATC
RnaseY-rTailfus-R1	CAACAAATTTGCGACACAAACACCTCCTTTTCTAGGG
RnaseY-Eco-F1	TAGACGGAATTCGCGGGGTGGCAGCATTTA
RnaseYrT-Nar-R1	AACGGTGGCGCCCGTTTCGCTAATGTCTCATCT
Enolase substitution (PR01-05):	

Eno-L-Xho-F1	ACTCGGCTCGAGTTATTATGCAATGGACCGTGAC
Eno-L-Bam-R1	CTCAAGGATCCATAATTGGCATGTTTATAATCTCC
Eno-R-Bam-F1	GACAAGGATCCGTACTGCCATAATTTTAGTTGAGG
Eno-R-Nar-R1	TCTGAAGGCGCCCGACATTTACATCGCCG
EC-Eno-Bgl-F1	CCTCAAGATCTGTCCAAAATCGTAAAAATCATC
EC-Eno-Bam-R1	CCTATGGATCCTAAAGTCAGTCTTATGCCTGG
<i>cshA</i> replacement with CAT194-cassette (PR01-06):	
SA1885-LL-Xho-F2	CATCATCTCGAGCAATGCCTGGATTTACGGCT
SA1885-L-Bam-R1	CCTCTGGATCCTAGCATTAGATAAGATGTAAGCG
SA1885-RS-Bam-F2	CAAAAGGATCCTAAAGTAAGAAGAAAAGTACAA
SA1885-RS-Nar-R2	TCACAGGGCGCCTTTAGTAGCTTCTTCTATAACTT
SA1386 and <i>cshB</i> deletions (PR01-08, PR01-09, and PR01-10):	
SA1387-INT-Xba-F1	GAAACTCTAGAACCCACAAGCAATCGTAGT
SA1387-Bam-R1	ACATGGGATCCTAATAACATAAAAACACAC
SA1386-DW-Eco-F1	GGTTAGAATTCAATAAAAATGATAATGCTCTCTTC
SA1386-DW-Nar-R1	ATTAGAGGCGCCATAAAACCTATCAAGATACCAC
SA1388-Xba-F1	ACATTTCTAGATCATCATGTGCCATTTAGT
SA1386-Nar-R1	TTTACC GGCGCCATTTGGTCCAACAATCGC
SA1386fusP1387-F1	GATGAACATATAAGGTTAGGTGTGTTTTATGTTA
P1387fusSA1386-R1	CACACCTAACCTTATATGTTTCATCTTTACTATTAT
SA1388-Bam-R1	TTTACGGATCCCGAGATTTTAGTTTAAATAT
<i>spa</i> deletion (PR07):	
SPA-R-Acc-R1	GCTAACGGTACCCCTGTATGTATTTGTAA
SPA-R-Nar-F1	GCGGATGGCGCCTAGCCTGAAGTCGATATGACTA
SPA-L-Xho-R1	GCTACCCTCGAGTGATTGGAAGCGTCTGAACAT
SPA-L-Bam-F1	GCTACGGATCCAAACAATACACAACGATAGAT
Primers for sequencing inserts in the MCS of pRLY-vectors:	
pRL-MCS-Seq-F1	GCGGCATCAGAGCAGATTG
pRLY-MCS-Seq-R1	GGAAACGAAATCCCGAGTC
Primers for sequencing inserts in the MCS of pRLB-vectors:	
pRLB-MCS-Seq-F1	CTAATACGACTCACTATAGGGC
pRLB-MCS-Seq-R1	TGCCCCGTTAGTTGAAGAAGGTT

a) Restriction sites used for cloning PCR products are underlined. Single bases that modify amplified sequence are indicated in bold-face. All primers except pRL-Cla-MNA-R1 were synthesised by Microsynth AG, Balgach, Switzerland.

b) Synthesised by Eurofins MWG GmbH, Anzinger Strasse 7a, D-855560 Ebersberg, Germany.