

Plasmid name	Description
pET-RctB wt	pET28b <i>rctB</i> wt
pGZ1119EH	ColD ori
pBluescript II KS+	pUC ori
pBS-12x6	pBluescript II KS+ 12x6
pBS-inc 11	pBluescript II KS+ inc 11
pBS-inc 12	pBluescript II KS+ inc 12
pBS-11x6	pBluescript II KS+ 11x6
pBS-11+1x6	pBluescript II KS+ 11+1x6
pBS-Rev-12x6	pBluescript II KS+ Rev-12x6
pBS-12x2	pBluescript II KS+ 12x2
pBS-12x4	pBluescript II KS+ 12x4
pBS-12-1	pBluescript II KS+ 12-1
pBS-12-2	pBluescript II KS+ 12-2
pBS-12-3	pBluescript II KS+ 12-3
pBS-12-4	pBluescript II KS+ 12-4
pBS-12-5	pBluescript II KS+ 12-5
pBS-12-6	pBluescript II KS+ 12-6
pBS-12-135	pBluescript II KS+ 12-135
pBS-12-246	pBluescript II KS+ 12-246
pBS-12-12456	pBluescript II KS+ 12-12456
pCR2.1-pseudo- <i>oriCII</i> -wt	<i>oriCII</i> bps 520-887
pCR2.1-pseudo- <i>oriCII</i> -11x6	<i>oriCII</i> wt where 6 12-mers replaced with 11-mers
pCR2.1-pseudo- <i>oriCII</i> -11+1x6	<i>oriCII</i> wt where 6 12-mers replaced with 11-mers & last base of 12-mer
pCR2.1-pseudo- <i>oriCII</i> -Rev-12x6	<i>oriCII</i> wt where entire 6 12-mer section reversed
pCR2.1-pseudo- <i>oriCII</i> -Delta-DnaA	<i>oriCII</i> wt where DnaA box is deleted
pCR2.1-pseudo- <i>oriCII</i> -Rev-DnaA	<i>oriCII</i> wt where DnaA box is reversed
pCR2.1-pseudo- <i>oriCII</i> -12x2	<i>oriCII</i> wt where 1st, 2nd, 5th, 6th 12-mers deleted
pCR2.1-pseudo- <i>oriCII</i> -12x4	<i>oriCII</i> wt where 1st, 2nd 12-mers deleted
pCR2.1-pseudo- <i>oriCII</i> -12-1	<i>oriCII</i> wt where GATC of 1st 12-mer changed to GTAC
pCR2.1-pseudo- <i>oriCII</i> -12-2	<i>oriCII</i> wt where GATC of 2nd 12-mer changed to GTAC
pCR2.1-pseudo- <i>oriCII</i> -12-3	<i>oriCII</i> wt where GATC of 3rd 12-mer changed to GTAC
pCR2.1-pseudo- <i>oriCII</i> -12-4	<i>oriCII</i> wt where GATC of 4th 12-mer changed to GTAC
pCR2.1-pseudo- <i>oriCII</i> -12-5	<i>oriCII</i> wt where GATC of 5th 12-mer changed to GTAC
pCR2.1-pseudo- <i>oriCII</i> -12-6	<i>oriCII</i> wt where GATC of 6th 12-mer changed to GTAC

pCR2.1-pseudo- <i>oriCII</i> -12-135	<i>oriCII</i> wt where GATC of 1st, 3rd, 5th 12-mers changed to GTAC
pCR2.1-pseudo- <i>oriCII</i> -12-246	<i>oriCII</i> wt where GATC of 2nd, 4th, 6th 12-mers changed to GTAC
pCR2.1-pseudo- <i>oriCII</i> -12-12456	<i>oriCII</i> wt where GATC of 1st, 2nd, 4th, 5th, 6th 12-mers changed to GTAC
pCR2.1-pseudo- <i>oriCII</i> -1+5	<i>oriCII</i> wt where GTACT inserted between 1st and 2nd 12-mer
pCR2.1-pseudo- <i>oriCII</i> -3+5	<i>oriCII</i> wt where GTACT inserted between 3rd and 4th 12-mer
pCR2.1-pseudo- <i>oriCII</i> -6+5	<i>oriCII</i> wt where GTACT inserted between 6th 12-mer and AT-rich region
pCR2.1-pseudo- <i>oriCII</i> -1+10	<i>oriCII</i> wt where GTACTGTACT inserted between 1st and 2nd 12-mer
pCR2.1-pseudo- <i>oriCII</i> -3+10	<i>oriCII</i> wt where GTACTGTACT inserted between 3rd and 4th 12-mer
pCR2.1-pseudo- <i>oriCII</i> -6+10	<i>oriCII</i> wt where GTACTGTACT inserted between 6th 12-mer and AT-rich region
pCR2.1-pseudo- <i>oriCII</i> -1+21	<i>oriCII</i> wt where GTACTGTACTGGACCTCCGGG inserted between 1st and 2nd 12-mer
pCR2.1-pseudo- <i>oriCII</i> -3+21	<i>oriCII</i> wt where GTACTGTACTGGACCTCCGGG inserted between 3rd and 4th 12-mer
pCR2.1-pseudo- <i>oriCII</i> -DnaA+5	<i>oriCII</i> wt where GTACT inserted between DnaA box and 1st 12-mer
pCR2.1-pseudo- <i>oriCII</i> -DnaA+10	<i>oriCII</i> wt where GTACTGTACT inserted between DnaA box and 1st 12-mer
pCR2.1-pseudo- <i>oriCII</i> -DnaA+21	<i>oriCII</i> wt where GTACTGTACTGGACCTCCGGG inserted between DnaA box and 1st 12-mer
pCR2.1-pseudo- <i>oriCII</i> -Delta-AT	<i>oriCII</i> wt where AT-rich region deleted
pCR2.1-pseudo- <i>oriCII</i> - <i>oriCI</i> -AT	<i>oriCII</i> wt where AT-rich region replaced with <i>oriCI</i> AT-rich
pCR2.1-pseudo- <i>oriCII</i> -Delta-29-mer	<i>oriCII</i> wt where 29-mer and bases downstream up to RctB start codon deleted
pCR2.1-pseudo- <i>oriCII</i> -AT GTAC	<i>oriCII</i> wt where 3 GATC in AT-rich region changed to GTAC
pCR2.1-pseudo- <i>oriCII</i> -AT 1/2-1	
pCR2.1-pseudo- <i>oriCII</i> -AT 1/2-2	
pYB199-pseudo- <i>oriCII</i> -wt	
pYB199-pseudo- <i>oriCII</i> -11x6	
pYB199-pseudo- <i>oriCII</i> -11+1x6	
pYB199-pseudo- <i>oriCII</i> -Rev-12x6	
pYB199-pseudo- <i>oriCII</i> -Delta-DnaA	
pYB199-pseudo- <i>oriCII</i> -Rev-DnaA	
pYB199-pseudo- <i>oriCII</i> -12x2	
pYB199-pseudo- <i>oriCII</i> -12x4	
pYB199-pseudo- <i>oriCII</i> -12-1	
pYB199-pseudo- <i>oriCII</i> -12-2	
pYB199-pseudo- <i>oriCII</i> -12-3	
pYB199-pseudo- <i>oriCII</i> -12-4	
pYB199-pseudo- <i>oriCII</i> -12-5	
pYB199-pseudo- <i>oriCII</i> -12-6	
pYB199-pseudo- <i>oriCII</i> -12-135	

pYB199-pseudo-oriCII-12-246	
pYB199-pseudo-oriCII-12-12456	
pYB199-pseudo-oriCII-1+5	
pYB199-pseudo-oriCII-3+5	
pYB199-pseudo-oriCII-6+5	
pYB199-pseudo-oriCII-1+10	
pYB199-pseudo-oriCII-3+10	
pYB199-pseudo-oriCII-6+10	
pYB199-pseudo-oriCII-1+21	
pYB199-pseudo-oriCII-3+21	
pYB199-pseudo-oriCII-DnaA+5	
pYB199-pseudo-oriCII-DnaA+10	
pYB199-pseudo-oriCII-DnaA+21	
pYB199-pseudo-oriCII-Delta-AT	
pYB199-pseudo-oriCII-oriCI-AT	
pYB199-pseudo-oriCII-Delta-29-mer	
pYB199-pseudo-oriCII-AT GTAC	
pYB199-pseudo-oriCII-AT 1/2-1	
pYB199-pseudo-oriCII-AT 1/2-2	
pYB190	pCRII
pYB199	pKD4 MCS*
pYB285	pGZ1119EH <i>rctB</i> wt
pYB289	<i>oriCII rctB</i>
pYB296	pGZ1119EH <i>rctB</i> 1-500
pYB355	pET28b <i>rctB</i> 1-500

Primer Name	Sequence (5'->3')
pseudo ori 5' Xma fuse	CTCTAGAACTAGTGGATCCCCTCGAGGTCATCCACTCAGGT
pseudo ori 3' Xho fuse	GCTGGGTACCGGGCCCCCCCCCGGGGGTTAGATCCGTATC
pseudo ori AT delta 3' Xho fuse	GCTGGGTACCGGGCCCCCCCCCGGGGCCATTTCAGAAGC
pseudo ori AT oriCI 3' Xho fuse	GCTGGGTACCGGGCCCCCCCCCGGGGAAATGTTTCGTGATAAAGC
pseudo ori DnaARev 5' Xma fuse	CTCTAGAACTAGTGGATCCCCTCGAGGTCATCCACTACAGT
pseudo ori PrctB dele 3' Xho fuse	GCTGGGTACCGGGCCCCCCCCCGGGGAGAATAAAGAAAAAGAGATC
oriCII FP 3'-1	CGATTTTGTAGTTCTAATTTATTGTAATTAAGAGATTATTTTAAGCC

oriCII FP 5'-2 Stu	TCGGAGGCCTGGCTTAAAATAATCTCTTTTAATTACAATAAATTAGAACTAAAAATCG
oriCII FP 3'-2 Nde	CTAGCATATGGATCAATCGTTTTTCTTCTGAGCTCATGG
inc-2 12mer 3' Nde	TAACCATATGCAATGAATACTTGTGACTTGTGCTTCC
inc-2 11mer 5' Stu	AAGGAGGCCTGGAAGCACAAGTCACAAGTATTCATTG
pseudo-ori mut 5' Xma fuse	CGGCCGCTCTAGAACTAGTGGATCCC
pseudo-ori mut 3' Xho fuse	GCTGGGTACCGGGCCCCCCC
kan 5' Xma fuse	GGGATCCACTAGTTCTAGAGCGGCCGTCTAGAGAATAGGAACTTCGGAATAGGAACTTCAAGATCC
kan 3' Xho fuse	GGGGGGGCCCGGTACCCAGC

Amp, Kan	This study
Amp, Kan	This study
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Amp, Kan	This study
Amp, Kan	This study
Amp, Kan	This study
Amp, Kan	This study
Amp, Kan	This study
Amp, Kan	This study
Amp, Kan	This study
Amp, Kan	12
Amp, Kan	14
Cam	39
Kan	39
Cam	This study
Kan	14

To construct	Method
pYB199-pseduo-oris, pBS-pseudo-oris	pcr, ita
pYB199-pseduo-oris	pcr, ita
pYB199-pseudo-oriCII-Delta-AT	pcr, ita
pYB199-pseudo-oriCII-oriCI-AT	pcr, ita
pYB199-pseduo-oriCII-Rev-DnaA	pcr, ita
pYB199-pseudooriCII-Delta-29-mer	pcr, ita
pBS-pseudo-oris	

pBS-inc-12	
pBS-inc-11	
pBS-inc-12	
pBS-inc-11	
<i>oriCII-min</i> -kan mutant library	pcr
<i>oriCII-min</i> -kan mutant library	pcr
<i>oriCII-min</i> -kan mutant library	pcr
<i>oriCII-min</i> -kan mutant library	pcr