

# Supplementary Materials: Establishing a System for Testing Replication Inhibition of the *Vibrio cholerae* Secondary Chromosome in *Escherichia coli*

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Table S1. Strains used in this study.

Strain	Characteristics	Resistance	Reference
<i>E. coli</i>			
MG1555	wild type	-	[1]
AB330	<i>cf.</i> DY330, <i>lacZ+</i> , <i>gal+</i>	-	Alexander Böhm
XL1 Blue	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac[F' proAB lacIqZΔM15 Tn10 (TetR)]</i>	tetracycline, nalidixic acid	Stratagene
DH5αpir	F- Φ80 <i>lacZΔM15 Δ(lacZYA-argF)</i> U169 <i>recA1 endA1 hsdR17</i> (rK-, mK+) <i>phoA supE44 thi-1 gyrA96 relA1 λ+</i> Donor strain for conjugation: <i>thrB1004 pro thi rpsL</i>	nalidixic acid	[2]
WM3064	<i>hsdS lacZΔM15 RP4-1360 Δ(araBAD)567 ΔdapA1341::[erm pir(wt)]</i>	erythromycin	William Metcalf
#1	MG1655 <i>oriC</i> (wt), <i>tos1</i>	-	[3]
#15	MG1655 <i>oriC</i> (wt), <i>oriV2</i> , <i>tos1</i>	kanamycin	[3]
#16	MG1655 Δ <i>oriC</i> (wt), <i>oriV2</i> , <i>tos1</i>	kanamycin	[3]
SMS18	MG1655 + pMA100	ampicilin	[4]
SMS29	MG1655 + pMA106	ampicilin	[4]
FSK18	MG1655 <i>parB</i> (1070231-1070357) <i>kanR</i>	kanamycin	[4]
SMS62	MG1655 + pMA129	ampicilin	[4]
NZ90	<i>cf.</i> #16; flipped KanR	-	this study
SM112	<i>cf.</i> #1; <i>lacZ::TetO/LacO-array-FRT</i> + pMA289	chloramphenicol	[5]
SM113	<i>cf.</i> #16; <i>lacZ::TetO/LacO-array-FRT</i> + pMA289	chloramphenicol, kanamycin	this study
NZ127	AB330 with <i>crtS</i> on chromosome	kanamycin	this study
NZ133	MG1655 with <i>crtS</i> on chromosome	kanamycin	this study
NZ139	MG1655 with <i>crtS</i> on chromosome, flipped KanR cassette	-	this study
NZ134	MG1655 Δ <i>oriC::oriII</i>	chloramphenicol	this study
NZ135	AB330 Δ <i>oriC::oriII</i>	chloramphenicol	this study
NZ138	MG1655 Δ <i>oriC::oriIII</i> , flipped CmR cassette	-	this study
NZ140	NZ139 + pMA568	ampicilin	this study
NZ141	NZ139 + pMA899	ampicilin	this study
NZ72	MG1655 + pMA568	ampicilin	this study
NZ119	MG1655 + pMA899	ampicilin	this study
SMS169	WM3064 + pMA899	erythromycin, ampicilin	this study
NZ122	WM3064 + pMA206	erythromycin, ampicilin	this study
SM141	WM3064 + pMA308	erythromycin, ampicilin	this study
NZ115	WM3064 + pMA200	erythromycin, ampicilin	this study
<i>V. cholerae</i>			
El Tor N16961	wild type	streptomycin	[6]
MCH1	fused chromosomes lacking <i>oriII</i>	streptomycin	[7]
NSCV1	natural isolate with fused chromosomes	-	[8]

Table S2. Plasmids used in this study.

Plasmid	Characteristics	Resistance	Reference
pCP20	FLP <sup>+</sup> , $\lambda$ cI857 <sup>+</sup> , $\lambda$ p <sub>R</sub> Rep <sup>ts</sup>	ampicillin, chloramphenicol	[9]
pICH50927	Endlinker for MoClo reaction	ampicillin	[10]
pICH50900	Endlinker for MoClo reaction	ampicillin	[10]
pMA87	= synVicII-1.0	ampicillin	[4]
pMA90	= synVicII-0.2	ampicillin	[4]
pMA100	<i>oriII</i> -minichromosome (=synVicII-1.3)	ampicillin	[4]
pMA106	<i>oriC</i> -minichromosome(=synEsc-1.3)	ampicillin	[4]
pMA108	<i>oriII</i> minichromosome with scrambled DnaA box	ampicillin	this study
pMA109	<i>oriII</i> minichromosome with inverted DnaA box	ampicillin	this study
pMA110	<i>oriII</i> minichromosome with deleted DnaA box	ampicillin	this study
pMA111	<i>oriII</i> minichromosome with R3 DnaA box	ampicillin	this study
pMA112	<i>oriII</i> minichromosome with R2 DnaA box	ampicillin	this study
pMA113	<i>oriII</i> minichromosome with deletion left of DnaA box	ampicillin	this study
pMA114	<i>oriII</i> minichromosome with insertion right of DnaA box	ampicillin	this study
pMA115	<i>oriII</i> minichromosome with insertion left of DnaA box	ampicillin	this study
pMA116	<i>oriII</i> minichromosome with deletion right of DnaA box	ampicillin	this study
pMA129	replicon with oriF (=synF-plasmid)	ampicillin	[4]
pMA132	= synVicII-1.35	ampicillin	[11]
pMA135	=synVicII-1.351	ampicillin	[11]
pMA157	<i>crtS</i> for MoClo assembly	ampicillin	this study
pMA200	pMA308 with <i>crtS</i>	ampicillin	this study
pMA206	pMA899 with <i>crtS</i>	ampicillin	this study
pMA207	<i>crtS</i> insertion into <i>fucR-rlmM</i> region on <i>E. coli</i> chromosome	spectinomycin, kanamycin	this study
pMA208	<i>mmmG</i> flank for MoClo assembly	ampicillin	this study
pMA209	<i>mioC</i> flank for MoClo assembly	ampicillin	this study
pMA210	<i>oriII</i> insertion into <i>oriC</i> of <i>E. coli</i>	spectinomycin, chloramphenicol	this study
pMA289	pKG110 <i>tetR-mVenus</i>	chloramphenicol	[5]
pMA308	<i>oriC</i> -minichromosome with <i>oriT</i> for conjugation (longer region of <i>oriC</i> compared to pMA106)	ampicillin	this study
pMA327	MoClo backbone vector for pMA207	spectinomycin	[5]
pMA329	MoClo backbone vector for pMA210	spectinomycin	[5]
pMA349	MoClo backbone vector for <i>crtS</i>	ampicillin	[12]
pMA350	MoClo backbone vector for <i>mmmG</i> flank (for <i>oriC</i> to <i>oriII</i> exchange)	ampicillin	[12]
pMA351	MoClo backbone vector for <i>oriII</i> (for <i>oriC</i> to <i>oriII</i> exchange)	ampicillin	[12]
pMA352	MoClo backbone vector for CAT (for <i>oriC</i> to <i>oriII</i> exchange)	ampicillin	[12]
pMA353	MoClo backbone vector for <i>mioC</i> flank (for <i>oriC</i> to <i>oriII</i> exchange)	ampicillin	[12]
pMA431	kanamycin cassette for MoClo assembly	ampicillin, kanamycin	this study
pMA568	<i>oriII</i> -minichromosome (with NotI-flanked <i>lacZ</i> )	ampicillin	this study
pMA650	= synVicII-2.01	ampicillin	[11]
pMA709	<i>fucR-rlmM</i> 5' flank for MoClo assembly	ampicillin	[5]
pMA710	<i>fucR-rlmM</i> 3' flank for MoClo assembly	ampicillin	[5]
pMA734	chloramphenicol cassette for MoClo assembly	ampicillin, chloramphenicol	this study
pMA740	<i>oriII</i> for MoClo assembly	ampicillin	this study
pMA899	<i>oriF</i> -replicon with <i>oriT</i> for conjugation	ampicillin	this study

Table S3. Oligonucleotides used in this study.

Name	Sequence (5' → 3')
14	CAATCTCAATTCGATCGGCCTGCACT
16	GGTAGATCCGTATCACACTTACCGT
421	GTACGAGAGGAAGGAGATACAACGACGCCTATCTTGATGTCATCCACTCAGGTGTAAAGT TGACTGTGTGAGCACCTTG
422	GTACGAGAGGAAGGAGATACAACGACGCCTATCTTGATGTCATCCACTCAGGTAATAGGT TGACTGTGTGAGCACCTTG
423	GTACGAGAGGAAGGAGATACAACGACGCCTATCTTGATGTCATCCACTCAGGTCTGTGTG AGCACCTTGATC
444	GTACGAGAGGAAGGAGATACAACGACGCCTATCTTGATGTCATCCACTCAGGTTTTGGAT AAACTGTGTGAGCACCTTGATCATGC
474	GTACGAGAGGAAGGAGATACAACGACGCCTATCTTGATGTCATCCGGTTGTGGATAAACT G
475	GTACGAGAGGAAGGAGATACAACGACGCCTATCTTGATGTCATCCGACTTACTCAGGTTG TGG
476	GTACGAGAGGAAGGAGATACAACGACGCCTATCTTGATGTCATCCACTCAGGTTGTGGAT AAATGAGCACCTTGATC
477	GTACGAGAGGAAGGAGATACAACGACGCCTATCTTGATGTCATCCACTCAGGTTGTGGAT AAAGACTTCTGTGTGAGCACCTTGATC
478	GTACGAGAGGAAGGAGATACAACGACGCCTATCTTGATGTCATCCACTCAGGTTGTGTAT AAACTGTGTGAGCACCTTGATCATGC
498	GTACGAGAGGAAGGAGATACAACGACGCCTATCTTGATGTCATCCACTCAGGTTNNNNAT AAACTGTGTGAGCACCTTG
703	AAGGTCTCGGGAGGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTT CATTAAATGG
704	TGGTCTCCAGCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGGCGCGCCTACCTGT GACCG
1002	TGGTGAGAATCCAAGTAGGGATAATTGCGGCCGAGCTGGCAGCAGGTTTGCCG
1004	AACTTCGACAGCTATATTACCTGATGCGGCCGCGACTATGCGGCATCAGAGC
1204	AAGGTCTCGGGAGCTAAAACGCACAAAGCCCG
1205	TGGTCTCCAGCGGTAGGCCAAAAAAGAGCGAGC
1349	GAACTTCTCGAGCATCTCCGACGGAAAGGTACCCGGTCCTTTGCTTGCCTTAGTATC GAAAAATAAAACAAATAGGGTTCGCGGGAAAGAATTCTAATCCATACTTTCCACAGG TAG
1439	AAGGTCTCGGGAGTGAGTGATCTTTTCACTATATTC
1440	TGGTCTCCAGCGCTCTTTTAAGCAGGATCCTAAC
1455	AAGGTCTCGGGAGGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTT CAAGATCCCC
1456	TGGTCTCCAGCGAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCAG AGCGCTTTTG
1487	GAGCATCTCCGACGGCGCGCCCATGGAGCGGCGTAACCGTC
1488	GGCCAGAGCTGCAGGCGCGCCCGTCGACAGCGACACACTTG
1593	TGGTGAGAATCCAAGTAGGGATAATGAGTGATCTTTTCACTATATTCTGTCTTAAGTCT
1613	AACTTCGACAGCTATATTACCTGTTATCTCTTTTAAGCAGGATCCTAACCGGA
1661	AAGGTCTCGGGAGGACCGCCGAGTGATCCACTTC
1662	TGGTCTCCAGCGCTTTTAAATACCCAGGATCC
1663	AAGGTCTCGGGAGATACTTATTTGAGTAAATTAAC
1664	TGGTCTCCAGCGCTGGCTGGTTATCAGCTCCAC
3921366fw	GAGAATATGGCGTACCAGCA
3921366rv	AAGACGCAGGTATTTTCGCTT
3921366p	CAACCTGACTTCGGTCCGCG 5'Fam-3'Tamra
ori2fw	CCTTGAGCTTGAGATTGCTG
ori2rv	GCCGCCCTACTATCGTTAAA
ori2probe	TGCTCACGCTGAGCCTCATTCA 5'Fam-3'Tamra

**Table S4.** Oligonucleotides used for construction of mutated *oriII*-minichromosomes.

Plasmid Name	Oligonucleotide Names
pMA108	421, 424
pMA109	422, 424
pMA110	423, 424
pMA111	444, 424
pMA112	478, 424
pMA113	474, 424
pMA114	477, 424
pMA115	475, 424
pMA116	476, 424

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