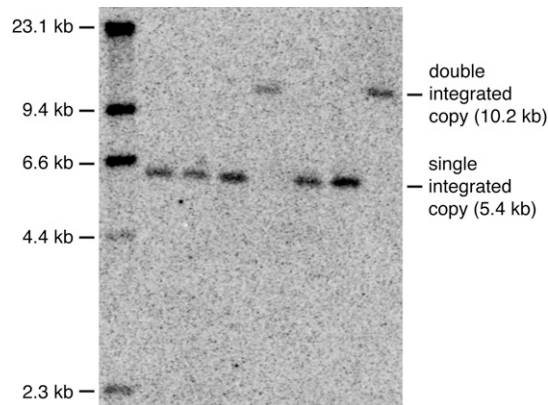


# Supporting Information

Das et al. 10.1073/pnas.0910212107



**Fig. S1.** Tandem integration of the classical variant of CTX $\phi$ . The genomic DNA from BS1 bacteria in which RS<sup>Cl</sup> had integrated successfully was subjected to Hpa1 restriction digest. Restriction fragments were separated by agarose gel electrophoresis and blotted on a PVDF membrane. The *dif1*-containing fragment was revealed by hybridization with a radioactive probe made from the Hpa1 *lacZ* fragment.

**Table S1. Strain list.**

Strains	Genotype/phenotypes	References/resources
N16961	O1 El Tor strain, St <sup>r</sup>	(1)
569B	O1 classical strain, St <sup>r</sup>	D. Mazel Lab., Pasteur
H12	CTX $\phi$ - O1 El Tor strain	RK Bhadra Lab., IICB
H25	CTX $\phi$ - O1 El Tor strain	RK Bhadra Lab., IICB
WO-5	CTX $\phi$ - O1 El Tor strain	RK Bhadra Lab., IICB
MV18	N16961 $\Delta lacZ \Delta(RS2 RS1 dif1)::aadA1$ ; Sp <sup>r</sup> St <sup>r</sup>	This study
MV78	MV18 <i>lacZ<sub>EC</sub>::dif1</i> ; St <sup>r</sup>	This study
BS1	MV78 $\Delta dif2::aadA1$ ; Sp <sup>r</sup> St <sup>r</sup>	This study
BS2	MV18 $\Delta dif2::arr2$ ; Sp <sup>r</sup> St <sup>r</sup> Rf <sup>r</sup>	This study
BS3	BS2 in which $\Delta dif2::arr2$ was replaced by <i>lacZ<sub>EC</sub>::dif2</i>	This study
BS10	BS1 $\Delta xerC::arr2$ ; Sp <sup>r</sup> St <sup>r</sup> Rf <sup>r</sup>	This study
BS11	BS1 $\Delta recA$ ; Sp <sup>r</sup> St <sup>r</sup>	This study
BS12	BS2 in which $\Delta dif2::arr2$ was replaced by <i>lacZ<sub>EC</sub>::dif1</i>	This study
BS13	BS2 in which $\Delta dif1::aadA1$ was replaced by <i>lacZ<sub>EC</sub>::dif2</i>	This study
BS14	BS2 in which $\Delta dif1::aadA1$ was replaced by <i>lacZ<sub>EC</sub>::difG</i>	This study
BS15	BS2 in which $\Delta dif2::arr2$ was replaced by <i>lacZ<sub>EC</sub>::difG</i>	This study

1. Heidelberg JF, et al. (2000) DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature* 406:477–483.

**Table S2. Plasmid list.**

Name	Description	References/resources
pSW23T	pSW23::oriTRP4; oriVR6Kγ; Cm <sup>r</sup>	(1)
pDS132	pCVD442 derivative carrying the <i>sacB</i> counter selectable marker; Cm <sup>r</sup>	(2)
pKAS32	pGP704 derivative carrying the <i>rpsL</i> counter selectable marker; Ap <sup>r</sup>	(3)
pBS22	RS <sub>Cl</sub> ; pSW23T harboring the replication and integration machinery of the CTXφ prophage of <i>V. cholerae</i> strain 569B; Cm <sup>r</sup>	This study
pBS39	pBS22 where <i>attP</i> <sup>Cl</sup> was modified into <i>attP</i> <sup>ET</sup> ; Cm <sup>r</sup>	This study
pMEV30	RS <sub>ET</sub> ; pSW23T harboring the replication and integration machinery of RS1, a CTXφ satellite phage of <i>V. cholerae</i> strain N16961; Cm <sup>r</sup>	This study
pBS4	pMEV30 in which <i>attP</i> <sup>ET</sup> was modified into <i>attP</i> <sup>Cl</sup> ; Cm <sup>r</sup>	This study
pBS15	pBS4 in which <i>attP</i> <sup>Cl</sup> was modified into <i>attP</i> <sup>mutCl</sup> ; Cm <sup>r</sup>	This study
pBS35	pMEV30 in which <i>attP</i> <sup>ET</sup> was modified into <i>attP</i> <sup>G</sup> ; Cm <sup>r</sup>	This study
pMEV136	pDS132 carrying an <i>aad1</i> cassette flanked by the upstream and downstream regions of <i>dif1</i> ; Cm <sup>r</sup> , Sp <sup>r</sup>	This study
pBS8	pKAS32 carrying an <i>aad1</i> cassette flanked by the upstream and downstream regions of <i>dif2</i> ; Ap <sup>r</sup> , Sp <sup>r</sup>	This study
pMEV235	pDS132 carrying an <i>arr2</i> cassette flanked by the upstream and downstream regions of <i>dif2</i> ; Cm <sup>r</sup> , Rf <sup>r</sup>	This study
pMEV78	pDS132 carrying the <i>lacZ::dif1</i> allele flanked by the upstream and downstream regions of <i>dif1</i> ; Cm <sup>r</sup>	This study
pBS3	pDS132 carrying the <i>lacZ::dif2</i> allele flanked by the upstream and downstream regions of <i>dif2</i> ; Cm <sup>r</sup>	This study
pBS24	pDS132 carrying the <i>lacZ::dif2</i> allele flanked by the upstream and downstream regions of <i>dif1</i> ; Cm <sup>r</sup>	This study
pMEV184	pDS132 carrying the <i>lacZ::dif1</i> allele flanked by the upstream and downstream regions of <i>dif2</i> ; Cm <sup>r</sup>	This study
pBS42	pDS132 carrying the <i>lacZ::difG</i> allele flanked by the upstream and downstream regions of <i>dif1</i> ; Cm <sup>r</sup>	This study
pBS38	pDS132 carrying the <i>lacZ::difG</i> allele flanked by the upstream and downstream regions of <i>dif2</i> ; Cm <sup>r</sup>	This study
pMEV245	pDS132 carrying an <i>arr2</i> cassette flanked by the upstream and downstream region of <i>V. cholerae xerC</i> ; Cm <sup>r</sup> , Rf <sup>r</sup>	This study
pMEV68	pDS132 carrying the upstream and downstream regions of <i>V. cholerae recA</i> ; Cm <sup>r</sup>	(4)

- Demarre G, et al. (2005) A new family of mobilizable suicide plasmids based on broad host range R388 plasmid (IncW) and RP4 plasmid (IncPalph) conjugative machineries and their cognate *Escherichia coli* host strains. *Res Microbiol* 156:245–255.
- Philippe N, Alcaraz JP, Coursange E, Geiselmann J, Schneider D (2004) Improvement of pCVD442, a suicide plasmid for gene allele exchange in bacteria. *Plasmid* 51:246–255.
- Skorupski K, Taylor RK (1996) Positive selection vectors for allelic exchange. *Gene* 169:47–52.
- Val M-E, et al. (2008) FtsK-dependent dimer resolution on multiple chromosomes in the pathogen *Vibrio cholerae*. *PLoS Genet* 4:e1000201.

**Table S3. *dif1* integration of CTXφ in *recA*<sup>-</sup> and *xerC*<sup>-</sup> strains**

Phage machinery	<i>attP</i> sequence	Host machinery	% Integration	Screened colonies
El Tor	El Tor	<i>recA</i> <sup>-</sup>	100.0	180
El Tor	El Tor	<i>xerC</i> <sup>-</sup>	<0.5	183
Classical	Classical	<i>recA</i> <sup>-</sup>	47.4	76
Classical	Classical	<i>xerC</i> <sup>-</sup>	<0.2	550

**Table S4. The category of the *attP* attachment region of various CTXφ variants**

Phage origin	<i>attP</i> category
VC44RS1J2E	El Tor
AB299799	El Tor
VC44RS1J1E	El Tor
N16961	El Tor
O139AY101180	El Tor
VCU83796	El Tor
AF510994	Classical
AF175708	Classical
AY349175	Classical
DQ012295	Classical
O395LRS2C	Classical
O395SRS2C	Classical
AF110029	Classical
AF238372	Classical
AF30279	G
AF416590	G

**Table S5. Oligonucleotides used in this study**

Name	Sequence
Dif1top	5'ATCAGTGCGCATTATGTATGTTATGTTAAATGGA
Dif1bot	5'CTGTCCATTTAACATAACATACATAATGCGCACTGAT
Dif2top	5'ATCAATGCGCATTACGTGCGTTATGTTAAATGGA
Dif2bot	5'CTGTCCATTTAACATAACGCACGTAATGCGCATTGAT
DifGtop	5'ATCAGTGCGCATTAGGTATATTATGTTAAATGGA
DifGbot	5'CTGTCCATTTAACATAATATACCTAATGCGCACTGAT
AttPdif1top	5'TACGCCCTTAGTGCGTATTATGTGGCGCGGCATTATGTTGAGGGTTCCG
AttPdif1bot	5'CTGCGGAACCCGTAACATAATGGCGTATAATACGCATTAAGGGCGTA
AttPmutClbot	5'CTGCGGAACCCGTAACATAATGGCGAGTAATACGCATTAAGGGCGTA
AttPmuCltop	5'TACGCCCTTAGTGCGTATTACGTGGCGCGGCATTATGTTGAGGGTTCCG
AttPGtop	5'TACGCCCTTAGTGCGTATTAGGTGGTGGCGGCATTATGTTGAGGGTTCCG
AttPGbot	5'CTGCGGAACCCGTAACATAATGCCCTAATACGCATTAAGGGCGTA
Dif2topextended	5'TAATCTAGATTATGCCTTAATTTAACATAACGCACGTAATGCGCATTAAAGTGTTCGATAGGTGCGACGAT
Dif2botextended	5'ATCGTCGACCTACGAACACTTAATGCGCATTACGTGCGTTATGTTAAATTAAGGCATAATCTAGATTA
DifGtopextended	5'CAATCTAGACCGCCGCTTAGTGCGCATTAGGTATATTATGTTAAATTAAGGCATAATGTCGACAA
DifGbotextended	5'TTGTCGACATTATGCCTTAATTTAACATAATATACCTAATGCGCACTAAGGGCGGCTAGATTG
Dif1topextended	5'CAATCTAGACCGCCGCTTAGTGCGCATTATGTATGTTATGTTAAATTAAGGCATAATGTCGACAA
Dif1botextended	5'TTGTCGACATTATGCCTTAATTTAACATAACATACATAATGCGCACTAAGGGCGGCTAGATTG

**Table S6. Sequence of *dif1* in different CTX $\phi$ -negative strains**

Strain	Serotype	<i>dif1</i> sequence	CTX	Sources
N16961	O1 (ET)	AGTGCGTATTA TGTATG TTATGTTAAAT	+	(1)
O395	O1 (CI)	AATGCGTATTA CGTGCG TTATGTTAAAT	+	CP000626.1
H25	O1 (ET)	AGTGCGTATTA GGTATA TTATGTTAAAT	-	This study
H12	O1 (ET)	AGTGCGTATTA GGTATA TTATGTTAAAT	-	This study
WO5	O1 (ET)	AGTGCGTATTA GGTATA TTATGTTAAAT	-	This study
Ku-40	O1 (ET)	AGTGCGTATTA GGTATA TTATGTTAAAT	-	AY368493.1
93333	O139	AGTGCGTATTA GGTATA TTATGTTAAAT	-	AY368494.1
AM19226	Non O1/ non O139	AGTGCGTATTA GGTATA TTATGTTAAAA	-	(2)
1587	Non O1/ non O139	AGTGCGTATTA GGTATA TTATGTTAAAT	-	(2)
RC385	Non O1/ non O139	AGTGCGTATTA GGTATA TTATGTTAAAA	-	(2)

1. Val M-E, et al. (2008) FtsK-dependent dimer resolution on multiple chromosomes in the pathogen *Vibrio cholerae*. *PLoS Genet* 4:e1000201.

2. Faruque SM, et al. (2007) Genomic analysis of the Mozambique strain of *Vibrio cholerae* O1 reveals the origin of El Tor strains carrying classical CTX prophage. *Proc Natl Acad Sci USA* 104:5151-5156.

**Table S7. *difG* integration of CTX $\phi$  on the second chromosome of *V. cholerae***

Phage machinery	<i>attP</i> sequence	<i>dif</i> sequence	% integration	Number of screened colonies
El Tor	El Tor	<i>difG</i>	<0.2	625
Classic	Classic	<i>difG</i>	<0.1	1526
El Tor	G	<i>difG</i>	2.2	1598