Supporting Information

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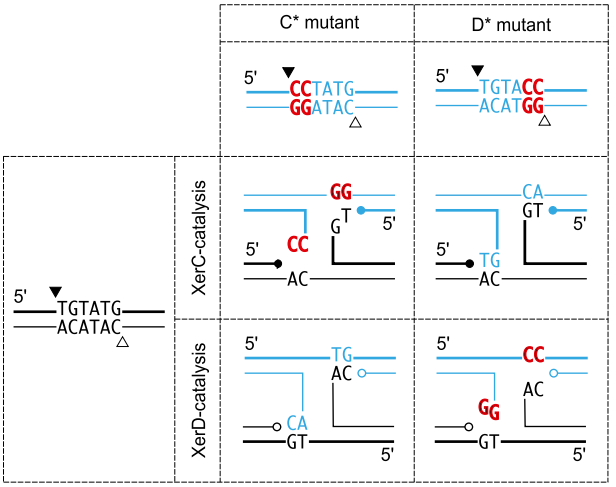

Fig. S1. C* and D* recombination sites that prevent XerC and XerD strand exchanges, respectively. WT recombination sites (black) and a mutated recombination site (blue) are indicated; mutations are indicated in bold and red. XerC (▼) and XerD (△) cleavage sites and XerC (●) and XerD (○) 3′-phosphotyrosyl links are indicated. A space between opposed bases indicates no stabilization by Watson–Crick or Wobble base-pairing interactions.

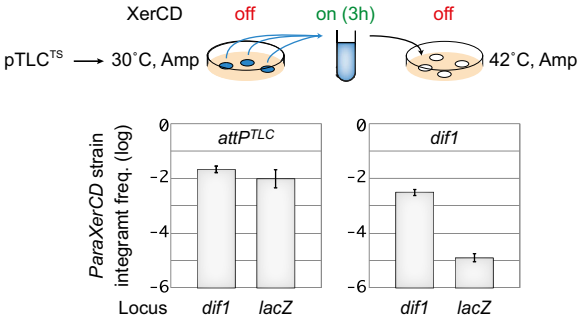

Fig. S2. TLCϕ integration is not spatially restricted. Frequency of integrants after 3 h of growth at the permissive temperature in production of recombinases from a *xerC-xerD* operon under the pAra promoter integrated at the *xerC* locus (p_{ara}*XerCD*) cells. The legend is as in Fig. 3*C*.

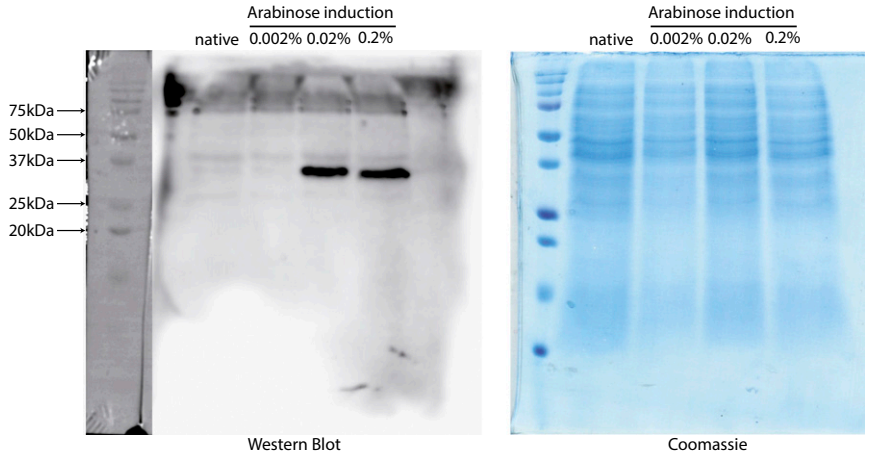

Fig. S3. Western blot analysis of strains carrying tagged XerD. Native, protein extract from cells producing 6His-XerD under the native promoter at the *xerD* locus; Arabinose induction, protein extract from cells producing XerC and 6His-XerD from an operon under the arabinose-inducible promoter inserted at the *xerC* locus. Cells were grown for 3 h in LB supplemented with 0.002%, 0.02%, or 0.2% arabinose. Cells were broken in 400 μL of boiling 10% SDS before Laemmli treatment. Proteins were separated on a 14% acrylamide/bisacrylamide (29:1) SDS/PAGE gel. Two gels with the same amount of samples were done in parallel for Coomassie coloration and for transfer (Western blot). Blocking of the membrane was in TBS-Tween 0.1% + 5% milk. Washing steps were in TBS-Tween 0.1%. The primary antibody was anti–Tetra-His of mouse, and the secondary antibody was goat X-mouse HRP (Biorad), and they were used at a ratio of 1:1,000 in blocking buffer. The signals were detected with the SuperSignal West Pico Chemioluminescent Substrate (Pierce) with a LAS3000 image reader (GE Healthcare). The molecular mass of 6His-XerD is 36.7 kDa. Bands were quantified using ImageJ (NIH). Arabinose (0.02% and 0.2%) led to 100-fold more XerD than native promoter.

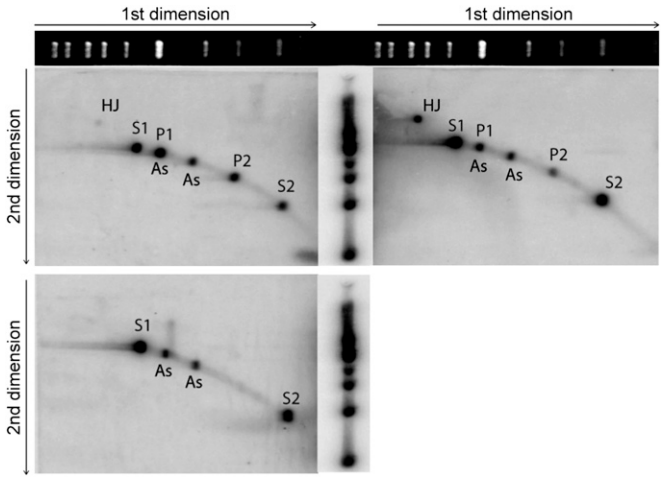

Fig. S4. HJ intermediate resolution by XerC catalysis. Plasmid intramolecular recombination between *dif1* and $attP^{TLC}$ was induced for 3 h using 0.2% arabinose. The total genomic DNA of the strains was extracted and separated in the first dimension on a 0.7% agarose gel and in the second dimension on a 1.4% agarose gel in the presence of 10 μg/mL ethidium bromide. (*Top Left*) Plasmid carrying a WT $attP^{TLC}$ site. (*Top Right*) Plasmid carrying a C*-modified $attP^{TLC}$ site. (*Bottom Left*) Plasmid carrying a D*-modified $attP^{TLC}$ site. The radiolabeled plasmid was used as a probe for the assay. Horizontal scale: 1-kb ladder. Vertical scale: 1-kb ladder partially revealed by the Southern blot. S1, S2, P1, and P2 are plasmid substrate and products digested by HpaI and AlwNI. As, nonspecific labeling (also observed in cells lacking the plasmid).

Table S1. Plasmids

Name	Derived from	Properties	Source
pBS90	pSW23T	Ori R6K, Cm^R, carrying replicative form of TLC	This study
pBS126	pSW23T	Ori R6K, Cm^R, carrying suicide form of TLC (stop mutation in Cri = TLC*)	This study
pCM33	pSW23T	Ori R6K, Cm^R, with partial suicide TLC, efficient for integration	This study
pCM39	pSW23T	Ori R6K, Cm^R, partial suicide TLC with $attP^{TLC}$C*	This study
pCM40	pSW23T	Ori R6K, Cm^R, partial suicide TLC with $attP^{TLC}$D*	This study
pCM43	pSC101 $repA^{TS}$	TS vector, Amp^R, partial suicide TLC	This study
pCM41	pSC101 $repA^{TS}$	TS vector, Amp^R, partial suicide TLC with $attP^{TLC}$C*	This study
pCM42	pSC101 $repA^{TS}$	TS vector, Amp^R, partial suicide TLC with $attP^{TLC}$D*	This study
pCM13	pSC101 $repA^{TS}$	TS vector, Amp^R, with *dif1* site	This study
pCM18	pSC101 $repA^{TS}$	TS vector, Amp^R, with *dif1* C* site	This study
pCM19	pSC101 $repA^{TS}$	TS vector, Amp^R, with *dif1* D* site	This study
pBS79	pSW23T	Ori R6K, Cm^R, with *dif1* site	This study
pBS114	pSW23T	Ori R6K, Cm^R, with *dif1* C*	This study
pBS115	pSW23T	Ori R6K, Cm^R, with *dif1* D*	This study
pBS66	pSW23T	Ori R6K, Cm^R, CTXϕ deleted for morphogenic genes, efficient for integration	(2)
pCM56	pSW23T	Ori R6K, Cm^R, carrying sacB gene, with suicide TLC	This study
pEP68	pSW23T	Ori R6K, Cm^R, $attP^{CTX}$(+) + suicide TLCϕ*attP* + *sacB*	This study
pCM105	pSW23T	Ori R6K, Cm^R, $attP^{CTX}$(+) + suicide TLCϕ*attP* C*+ sacB	This study
pCM106	pSW23T	Ori R6K, Cm^R, $attP^{CTX}$(+) + suicide TLCϕ*attP* D* + *sacB*	This study
pCM103	pUC18	Vector, Amp^R, partial suicide TLC with $attP^{TLC}$ and *dif1* in direct orientation	This study
pCM116	pUC18	Vector, Amp^R, partial suicide TLC with $attP^{TLC}$C* and *dif1* in direct orientation	This study
pCM117	pUC18	Vector, Amp^R, partial suicide TLC with $attP^{TLC}$D* and *dif1* in direct orientation	This study
pMEV69	pDS132	Integration/excision vector with UP and DWN regions of *lacZ*, to make Δ_{vib}*lacZ* strains	Laboratory collection
pUXBF13 and pTn7 $hapR^+$		Complemented *hapR* + Gm^R	(3)
pCM011a	pUC18	Vector for natural transformation to have *dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb*	This study
pBJ31		pDS132-derived integration/excision vector to replace the *V. cholerae xerC* gene by an arabinose-inducible version (*araC-xerC-lacI-aadA1*)oriR6K, $spec^R$, cm^R	(4)
pCM54	pUC18	Vector of natural transformation carrying *SmR* ($spec^R$), *lacI, araC*, and $_{vib}$XerCD under ara promotor, flanking by UP and DWN regions of homologies around $_{vib}$*xerC* (1 kb). Replace $_{vib}$*xerC* by arabinose-inducible $_{vib}$*xerCD,* $Spec^R$, Amp^R	This study
pGD191	pSC101	Vector of natural transformation to delete the gamma domain of FtsK, Zeo^R, Amp^R	This study
pBS98	pUC18	Vector of natural transformation carrying *sh ble* gene (zeo^R) between UP and DWN region of homologies around $_{vib}$*xerD* (1 kb); serves to remove $_{vib}$*xerD*	This study
pBS104	pDS132	Integration/excision vector to replace $_{vib}$*xerD* by $_{vib}$*xerD*$_{YF}$	This study
pBS99	pKAS32	Integration/excision vector with UP and DWN regions of $_{vib}$*xerD* and *sh ble* gene between them, to delete $_{vib}$*xerD*	This study
pBS105	pDS132	Integration/excision vector to replace $_{vib}$*xerC* by *xerC*$_{YF}$	This study
pMEV245	pDS132	pDS132 carrying the *arr2* (Rif^R)cassette flanked by the upstream and downstream regions of $_{vib}$*xerC,* Cm^R	(1)
pKAS32		*rpsL (Strep*^{S}*)*, OriR6K, *bla* (Amp^R), oriT	(5)
pBJ37	pDS132	Integration/excision vector to replace $_{vib}$*lacZ::*$_{Ec}$*lacZa-dif1-lacZb*	(1)
pMEV70	pDS132	Integration/excision vector to replace *dif1-prophages::aaAd1*	
pCM144	pUC18	Vector of natural transformation to replace *XerD::FRT-sh ble-FRT* (Zeo^R)	This study
pCM145	pUC18	Vector of natural transformation to have Δ_{vib}*XerC::pAra* $_{vib}$*XerC-6His* $_{vib}$*XerD*	This study
pCM146	pUC18	Vector of natural transformation to have Δ_{vib}*XerD::6His* $_{vib}$*XerD* under native promotor	This study

AmpR, ampicillin resistance; CmR, chrloramphenicol resistance; KnR, kanamycin resistance; RifR, rifampicin resistance; SmR, spectinomycin resistance; SrepS, streptomycin sensitivity; zeoR, zeocin resistance.

1. Das B, Bischerour J, Barre F-X (2011) VGJphi integration and excision mechanisms contribute to the genetic diversity of Vibrio cholerae epidemic strains. *Proc Natl Acad Sci USA* 108(6): 2516–2521.
2. Bao Y, Lies DP, Fu H, Roberts GP (1991) An improved Tn7-based system for the single-copy insertion of cloned genes into chromosomes of gram-negative bacteria. *Gene* 109(1):167–168.
3. Demarre G, et al. (2014) Differential management of the replication terminus regions of the two Vibrio cholerae chromosomes during cell division. *PLoS Genet* 10(9):e1004557.
4. Das B, Bischerour J, Val M-E, Barre F-X (2010) Molecular keys of the tropism of integration of the cholera toxin phage. *Proc Natl Acad Sci USA* 107(9):4377–4382.
5. Skorupski K, Taylor RK (1996) Positive selection vectors for allelic exchange. *Gene* 169(1):47–52.

Table S2. Xer recombination promotes a conservative recombination event that leads to the joint excision of TLCϕ and CTXϕ

	Blue colonies		White colonies				
Xer status	Frequency	Cm^S, Kn^S	Frequency	Cm^S, Kn^S	Kn^S	Cm^S	Cm^R, Kn^R
Xer^+	2.8×10^{-4} (in 2.7×10^7 cells)	100% (246/246)	2.0×10^{-5} (in 2.9×10^6 cells)	66% (71/108)	7.4% (8/108)	15% (16/108)	11.6% (13/108)
Xer^-	$<10^{-6}$ (in 1.6×10^6 cells)	—	2.2×10^{-5} (in of 1.6×10^6 cells)				

Cm^S, chloramphenicol sensitive; Kn^S, ksnamycin sensitive.

Table S3. *Vibrio cholerae* strains

Name	Genotype	Source
BS47	*CVC301* (*N16961 StrR Pcp18 araE*); Δ*lacZ* Δ*dif1*-prophages::$_{Ec}$*lacZa-difA-lacZb*	This study
BS1	*CVC301* (*N16961 StrR Pcp18 araE*); Δ*lacZ* Δ*dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb;* Δ*dif2::aad1*	(1)
BS3	*CVC301* (*N16961 StrR Pcp18 araE*); Δ*lacZ,* Δ*dif1*-prophages*::aad1;* Δ*dif2::*$_{Ec}$*lacZa-dif2-lacZb*	(1)
BS10	*CVC301* (*N16961 StrR Pcp18 araE*); Δ*lacZ* Δ*dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb;* Δ*dif2::aad1;* Δ*xerC::rif*	(1)
BS49	*CVC301* (*N16961 StrR Pcp18 araE*); Δ*lacZ* Δ*dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb;* Δ*dif2::aad1;* Δ*xerD::zeo*	This study
EPV366	*N16961*Δ*lacZ; ChapR* Δ*dif1*-prophages*::aad1*; *LacZ::*$_{Ec}$*lacZa-dif1-lacZb* Δ*xerC::*p_{ara}*XerCD-Spec*^{R}	This study
CMV26	*N16961; ChapR;* Δ*dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb-FRT-sh ble-FRT* (*Zeo*^{R}); Δ*xerC::*p_{ara}*XerCD-Spec*^{R}	This study
BS50	*CVC301* (*N16961 StrR Pcp18 araE*); Δ*dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb;;* Δ*dif2::aad1; XerD::XerDyF* catalytic mutant	This study
BS51	*CVC301* (*N16961 StrR Pcp18 araE*); Δ*lacZ, dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb;;* Δ*dif2::aad1; XerC::XerCyF* catalytic mutant	This study
CMV01	*N16961;* Δ*lacZ; ChapR; dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb-FRT-sh ble-FRT* (*Zeo*^{R})	This study
CMV13	*N16961;* Δ*lacZ; ChapR; dif1-prophages::*$_{Ec}$*lacZa-dif1-lacZb, XerC::*p_{ara}*XerC-Spec*^{R}	This study
CMV14	*N16961;* Δ*lacZ; ChapR; dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb, XerC::*p_{ara}*XerC-Spec*^{R}*; ftsk*Δγ*::sh ble* (*Zeo*^{R})	This study
CMV30	*N16961;* Δ*lacZ; ChapR; dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb, XerC::*p_{ara}*XerCD-Spec*^{R}*;* Δ*XerD::sh ble* (*Zeo*^{R})	This study
CMV20	*N16961;* Δ*lacZ; ChapR; dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb, XerC::*p_{ara}*XerC-Spec*^{R}*;* with suicide *TLC-sacB* integrated at *dif1*	This study
CMV21	*N16961;* Δ*lacZ; ChapR; dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb, XerC::*p_{ara}*XerC-Spec*^{R}*; ftsk*Δγ*::sh ble* (*Zeo*^{R})*;* with suicide *TLC-sacB* integrated at *dif1*	This study
CMV36	*N16961;* Δ*lacZ; ChapR; dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb, XerC::*p_{ara}*XerCD-Spec*^{R}*;* Δ*XerD::sh ble* (*Zeo*^{R})*;* with suicide *TLC-sacB* integrated at *dif1*	This study
EPV158	*N16961;* Δ*lacZ; ChapR; dif1* and prophages::$_{Ec}$*lacZa-dif1-lacZb-FRT-sh ble-FRT* (*Zeo*^{R})*;* with integrated *pEP68 at dif1* (integration by *attP*^{CTX})	This study
CMV34	*N16961;* Δ*lacZ; ChapR; dif1*-prophages::$_{Ec}$*lacZa-dif1-lacZb-FRT-sh ble-FRT* (*Zeo*^{R})*;* with integrated *pCM105* at *dif1* (integration by *attP*^{CTX})	This study
CMV35	*N16961;* Δ*lacZ; ChapR; dif1* and prophages::$_{Ec}$*lacZa-dif1-lacZb-FRT-sh ble-FRT* (*Zeo*^{R})*;* with integrated pCM106 at *dif1* (integration by *attP*^{CTX})	This study
EPV361	*N16961;* Δ*lacZ; ChapR;* Δ*xerC::*p_{ara}*XerC-6HisXerD-Spec*^{R}*;* Δ*xerD::FRT-sh ble-FRT* (*Zeo*^{R})	This study
EPV363	*N16961;* Δ*lacZ; ChapR;* Δ*xerD::6HisXerD*	This study

■■■.

1. Das B, Bischerour J, Val M-E, Barre F-X (2010) Molecular keys of the tropism of integration of the cholera toxin phage. *Proc Natl Acad Sci USA* 107(9):4377–4382.

Table S4. Oligonucleotides

Oligo	Used for	Sequence
1266	Check CTXϕ-Kn integration	CACGACGATTCACTCAACCTTCC
1398	Check CTXϕ-Kn integration	TTCGACGTTCAGACGTAGTG
1940	*dif1* C* mutation	CGATAGTGCGCATTACCTATGTTATGTTAAATTAAT
1941	*dif1* C* mutation	CGATTAATTTAACATAACATAGGTAATGCGCACTAT
1942	*dif1* D* mutation	CGATAGTGCGCATTATGTACCTTATGTTAAATTAAT
1943	*dif1* D* mutation	CGATTAATTTAACATAAGGTACATAATGCGCACTAT
1944	$attP^{TLC}$ C* mutation	GGTAATGCGCACTAGGATC
1945	$attP^{TLC}$ C* mutation	TATGTAGAGAAAGTGAAGAC
1946	$attP^{TLC}$ D* mutation	GGTACATAATGCGCACTAGG
1947	$attP^{TLC}$ D* mutation	TAGAGAAAGTGAAGACTACG
1878	TLC amplification on *N16961*	CCGTCTAGAGCTAGGAACATTTTGTCTCTAGG
1879	TLC amplification on *N16961*	CCGGATCCTAGTGCGCATTATGTATGTAGAG
1899	Check $attP^{CTX}$ integrants	GTCTCGTTGCTGCATAAACC
2479	Check $attP^{CTX}$ integrants	CCCGAGCTCTCAAAAGCTCAGCCTCCTAC
477	Check *TLC*ϕ–*CTX*ϕ excision	CGCAGGCTTCTGCTTCAATC
1565	Check *TLC*ϕ–*CTX*ϕ excision	cggcgtttcatctgtggtgc
1297	*dif1* gel shift	ATCAGTGCGCATTATGTATGTTATGTTAAATGGA
1298	*dif1* gel shift	CTGTCCATTTAACATAACATACATAATGCGCACTGAT
1958	$attP^{TLC}$ gel shift	ACGTCTAGTGCGCATTATGTATGTAGAGAAAGTGGACGT
1959	$attP^{TLC}$gel shift	ACGTCCACTTTCTCTACATACATAATGCGCACTAGACG