

## Supplementary Figure legends

**Figure S1:** Sequences of new split-inteins, grouped by the type of host protein.

**Figure S2:** Output of a CD-search (Marchler-Bauer and Bryant 2004) sequence-to-multiple alignments comparison, of the putative DNA-repair exonuclease synthetically joined products (excluding the VSR region) with the SbcD multiple sequence alignment (COG0420). The multiple alignment is represented by its consensus sequence. The joining point of the putative DNA-repair sequence parts includes an “x” and is marked by an arrow.

**Figure S3:** (A) Annotated multiple sequence alignment of group I introns that include Vsr-like ORFs. The *Bacillus thuringiensis* phage 0305 $\phi$ 8-36 (BP0305phi; NCBI accession NC\_009760.1) and *Bacillus cereus* AH1134 (bacce-AH1; NCBI accession NZ\_ABDA01000203.1). Group I introns of recA genes are aligned with the *Bacillus anthracis* Sterne recA group I intron (bacan-Ste; NCBI accession AF229167.1). The group I intron regions are indicated by a red line. Their predicted secondary structures (P1 to P10) and conserved sequence motifs (R and S) are annotated according to the *B. anthracis* intron (Ko et al. 2002). Protein coding regions have their amino acids sequence above them. The recA exons are highlighted in yellow, the Vsr-like ORFs are highlighted in green, the putative hairpins at the 5' of the Vsr-like ORFs are double underlined, the conserved Vsr-like motifs (Figure 3) are highlighted in grey, and group I introns conserved core regions are highlighted in cyan. (B) Predicted RNA secondary structure of the group I intron of recA gene from *Bacillus thuringiensis* phage 0305 $\phi$ 8-36 (BP0305phi; NCBI accession NC\_009760.1). Predicted secondary structures P1 to P10, conserved sequence elements R and S, the G site, and the ORF insertion point and its stop codon are shown according to (A).

**Figure S4:** Conserved nucleotide motifs in 5' untranslated regions of endonuclease ORFs. Motifs were searched using MEME. The position is off the distance of the sequence 3' ends from the translation initiation codons. The motifs are shown aligned with their logos (calculated using the WebLogo server at <http://weblogo.berkeley.edu/>) (A) and in the context of the whole region (B). In the later view, the sequences are aligned by their initiation codons, at their 3' termini, that are also boxed. “...” indicates unknown sequence data.

**Table S1**

Protein hosts and the GOS reads used as a source for their assembly.

| Protein host | GOS location    | JCVI reads  | JCVI assemblies                                   | Probable origin                |
|--------------|-----------------|---|---|--------------------------------|
| gp41-1       | Lake Gatun      | 1095368026018+1095366023924+1<br>095333010713+1093023041874+10<br>95333009945+1093023002130 | 1097207246556+<br>1097207259355+<br>1101669430723 | T4-like viruses                |
| gp41-2       | Lake Gatun      | 1101669426331   |   |                                |
| gp41-3       | Lake Gatun      | 1095351007293+1095356001149   | 1061005454744                                     |                                |
| gp41-4       | Lake Gatun      | 093022163129+1093022103376  | 1097207258370                                     |                                |
| gp41-5       | Lake Gatun      | 1091143037864   | 1097207240963                                     |                                |
| gp41-6       | Lake Gatun      | 1095337027236+1095306084054   | 1097207277771                                     |                                |
| gp41-7       | Off Nags Head   | 1093012253551+1092963600993   | 1097205056197                                     |                                |
| gp41-8       | Lake Gatun      | 1091143028835   | 1097207240798                                     |                                |
| gp41-9       | Punta Cormorant | 1097156422586   |   |                                |
| IMPDH-1      | Lake Gatun      | 1091143056039+1095333020518+1<br>091142153811+1095333021862                                 | 1101669394623                                     | Bacterial                      |
| IMPDH-2      | Lake Gatun      | 1095351023224+1093022056516+1<br>093022126404+1095349055864                                 | 1097207259656                                     |                                |
| IMPDH-3      | Delaware Bay    | 1095899230244+1095898151644   | 1101669181432                                     |                                |
| DnaE-1       | Rangirora Atoll | 1092963363647+1092963253032   | 1097263593299                                     | Bacterial                      |
| DnaE-2       | Lake Gatun      | 1095349061806+1095328030710   | 1101669426735                                     |                                |
| DnaE-3       | Gulf Of Mexico  | 1093022142172+1093022142942   | 1097205348472                                     |                                |
| NrdJ-1       | Lake Gatun      |   | 1101669428687                                     | Bacterial and<br>dsDNA viruses |
| NrdJ-2       | Lake Gatun      |   | 1101669429579                                     |                                |
| NrdA-1       | Lake Gatun      | 1095368021404+1095349056558   | 1101669410105                                     | Bacteria and<br>viruses        |
| NrdA-2       | Rangirora Atoll | 1092963091065+1093006402449   | 1097263589660                                     |                                |
| NrdA-3       | Lake Gatun      | 1091143060848   | 1101669394741                                     |                                |
| NrdA-4       | Lake Gatun      | 1091143047300+1091142207694   | 1101669393609                                     |                                |
| NrdA-5       | Punta Cormorant | 1095521346405   | 1101670305357                                     |                                |
| NrdA-6       | Lake Gatun      | 1091142141262+1091143128742   | 1097207263468                                     |                                |
| NrdA-7       | Lake Gatun      | 1097207240466   |   |                                |
| DNA ligase   | Lake Gatun      | 1095326019642+1095337013586   | 1097207283029                                     | viruses                        |
| Terminase    | Lake Gatun      | 1095306046644+1095328063912   | 1101669405733                                     | Myoviridae<br>viruses          |
| Unknown      | Off Key West    | 1097205249970   | 1091138266427                                     |                                |

**Table S2**

List of salt bridges across the intein halves as calculated from their models.

**gp41-1**

3 ASP A - 103 ARG B  
41 LYS A - 107 ASP B  
45 LYS A - 102 GLU B  
48 LYS A - 98 GLU B  
52 GLU A - 92 LYS B  
52 GLU A - 93 LYS B  
53 ASP A - 92 LYS B  
55 LYS A - 120 ASP B  
80 GLU A - 96 LYS B  
87 LYS A - 120 ASP B

**IMPDH-1**

18 GLU A - 120 LYS B  
35 GLU A - 103 LYS B  
72 ARG A - 113 GLU B

**NrdA-2**

32 ASP A - 101 ARG B  
65 HIS A - 104 ASP B

**NrdJ-1**

55 ARG A - 128 ASP B  
72 LYS A - 140 ASP B  
75 ARG A - 124 GLU B  
97 ASP A - 116 LYS B  
98 ASP A - 114 LYS B  
98 ASP A - 116 LYS B  
100 GLU A - 114 LYS B

**gp41-8**

18 ARG A - 114 GLU B  
18 ARG A - 115 GLU B  
43 LYS A - 115 GLU B  
52 LYS A - 104 GLU B  
60 ARG A - 114 GLU B  
91 LYS A - 97 GLU B  
91 LYS A - 99 GLU B

# Figure S1

## N-inteins:

>gp41-1  
CLDLKTQVQTPOGMKEISNIQVGDLVLSNTGYNEVLNVFPKSKKSYKITLEDGEIICSEEHLFPTQTGEMNISGGLKEGMC  
LYVKE

>gp41-2  
CLDLKTQVQTQQGLKDISNIQVGDLVL

>gp41-3  
CLDLKTQVQTPOGMKEISNIQVGDLVLSNTGYNEVLNVFPKSKKKS

>gp41-4  
CLDLKTQVQTPOGMKEISNIQVGDLVLSNTGYNEVLNVFPKSKKSYKIT  
LEDGEIICSEEHLFPTQTGEMNISGGLKEGMCLYVKE

>gp41-5  
CLDLKTQVQTPOGMKEISNIQVGDLVLSNTGYNEVLNVFPKSKKSYKIT  
LEDGEIICSEEHLFPTQTGEMNISGGLKEGMCLYVKE

>gp41-6  
SYKITLEDGEIICSEEHLFPTQNGEVNIKGLKEGMCLYVKE

>gp41-7  
CLDLKTQVQTPOGMKELSNIQVGDLVLSNTGYNQVLNVFPKSKKSYKIT  
LEDGEIICSEEHLFPTQNGEVNIKGLKEGMCLYVKE

>gp41-8  
CLSLDTMVTNGKAIEIRDVKVGDWLESECGPVQVTEVLPPIKQPVFEIV  
LKSGKKIRVSANHKPTKDGKLTINSGLKVGDFLRSRAK

>IMPDH-1  
CFVPGTLVNTENGLKKIEEIKVGDKVFSHTGKLQEVVDTLIFDRDEIIIS  
INGIDCTKNHEFYVIDKENANRVNEDNIHLFARWVHAEELDMKKHLLIELE

>DnaE-1  
CFTKDTNILTEHGFMDIEELDPKRDGVIYIDKEGKHRINHDYELHYMGRK  
EVFEIKTECGKTIKLTSDHEVMTQEGLYKVFELNENDVLIK

>DnaE-2  
ILTNNGEKFINEISCNDQIAVLNHSIEYNNDYEIIIFQGKKEIFEIILENTAELTED  
HEVMTQNGYKKVKELNDDDSLVNI

>NrdA-1  
CVAGDTKIKIKYPEVGQYGTWYWNVLEKEIQIEDLEDYIIMRECEIYDSAPQIEVLSYNIETGEQEWKPITAFQQTSPKA  
KVMKITDEESGKSIVVTPEHQVFTKNRGYVMAKDLIETDEPIIVNKDMNF

>NrdA-2  
CLTGDAKIDVLIDNIPISQISLEEVVNLNEGKEIYVLSYNIITKEVEYKEISDAGLISESAEVLEIIDETGQKIVCTPDHK  
VYTLNRGYVSAKDLKEDDELVFS

>NrdA-4  
CLAGDTTVTVLEGDIVFEMTLENLVSPLYKNVFSVSVLSFNPETQKQEFKPVTNAALMNPESKVLKITDSDTGKSIVCTPDHKV  
FTKNRGYVIASELNAEFILEIK

>NrdA-5  
HTETVRRVGTITAFQQTSPSKVMKITDEESGNSIVVTPEHKVFTKNRGYVMAKNLVETDELVIN

>NrdA-6  
YVCSRDDTTGFKLICTPDHMIYTKNRGYIMAKYLKEDDELLINEIHLPT

>NrdJ-1  
CLVGSSIEIITRNYGKTTIKEVVEIFDNDKNIQVLAFNTHTDNIEWAPIKAAQLTRPNAELVELEIDTLHGVKTIRCTPDHPVY  
TKNRGYVRADELTDDDELVVAI

>NrdJ-2  
CLVGSSIEIITRNYGKTTIKEVVEIFDNDKNIQVLAFNTHTDNIEWAPIKAAQLTRPNAELVELEINTLGVKTIRCTPDHPVY  
TKNRDYVRADELTDDDELVVAI

C-inteins:

>gp41-1  
MMLKKILKIEELDERELIDIEVSGNHLFYANDILTHNS  
>gp41-2  
MMLKKILKIEELDERELIDIEVSGNHLFYANAILTHNS  
>gp41-7  
MMLKKILKIEELDERELIDIEVSGNH

>gp41-8  
MCEIFENEIDWDEIASIEYVGVEETIDINVNDRLFFANGILTHNS  
>gp41-9  
MIMKNRERFITEKILNIEEIDDDLTVDIGMDNEDHYFVANDILTHNT

>IMPDH-1  
MKFKLKEITSIETKHYKGK-VHDLTVNQDHNSYNV-RGTVVHNS  
>IMPDH-2  
MKFTLEPITKIDSYEVTAEPVYDIEVENDHSFCVeNGFVVHNS  
>IMPDH-3  
MKFKLVEITSKETFNYSGQ-VHDLTVEDDHNSYSI-NNIVVHNS

>DnaE-2  
MKNFWRKLLKLLKIKSIKKSRIDNVYDIHHRINYKVFDEHPNLIAEKIVISNC  
>DnaE-3  
MNLLGKQOQTYPLEVAAHDHQYYLNNGILQSN

>NrdA-2  
MGLKIIKRESKEPVFDITVKDNSNFFANNILVHNC  
>NrdA-3  
MLKIEYLEEEIPVYDITVEETHNFFANDILIHNC  
>NrdA-5  
MLKIEYLEEEIPVYDITVEGTHNLAYSL  
>NrdA-6  
MGIKIRKLEQNRVYDIKVEKIIIFCNNILVHNC  
>NrdA-7  
MLKIEYLEEEIPVYDITVEKTNNFFANDILVHNC

>NrdJ-1  
MEAKTYIGKLKSRKIVSNEDTYDIQTSTHNFFANDILVHNS

>DNA ligase  
MQIVRVKKIAKVESRDKFDLEVKKNNNFFANGVLVHNC

>Terminase  
MLSDQVERKFTETVEVTDWQVDTDTGWQEVNASNQTIPYAVHELELDNGM  
FLSCADTHIVFDQYLNEIFVQDLVPGQQIQTSGVSAVKSLTATQDQQQMYDLSVNSKDQRYYTGGILSHNS

>unknown host  
MDDLYMLDEDEIVSIELIGEEDTIDITVDDTHMFFANDIYTHNS

# Figure S2

| Description   |   | Pssmid | Multi-dom | E-value |
|---|---|--------|-----------|---------|
| COG0420, SbcD, DNA repair exonuclease [DNA replication, recombination, and repair]                      |   | 30769  | yes       | 6e-06   |
| DNA repair exonuclease [DNA replication, recombination, and repair]                                     |   |        |           |         |
|   | CD Length: 390 Bit Score: 47.10 E-value: 6e-06  |        |           |         |
|   | 10 20 30 40 50 60 70 80   |        |           |         |
| SbcD-like 11 TEFLKNLADIA-PTFIIAGNHDLNLSNMNRLLDSLTPLVNSINHPNLUYLIKHS---IYTIVADTDFAVYSILDE 79             | ....*.... ....*.... ....*.... ....*.... ....*.... ....*.... ....*.... ....*.... ....*.... |        |           |         |
| <u>COG0420</u> 65 LEALRRLKDAGiPVVVIAGNHD-SPSRLSEASPLLLLNNLGLHGVVGRVLHEIrpppeivaapwLIPGPDPDVVFGLNG 143   |   |        |           |         |
|   | 90 100 110 120 130 140 150 160  |        |           |         |
| SbcD-like 80 KELWP-----SHKDCNAKKKVALYHGPVHGAETDAR--YVITMRHVNVSMFDGFDMVLLGDIHKYQVLQERseey 147            | ....*.... ....*.... ....*.... ....*.... ....*.... ....*.... ....*.... ....*....           |        |           |         |
| <u>COG0420</u> 144 LEKEQfelllhkgllSALDPDDPSILVLRHQSIDALTSGAErldALGTVDLSLLPKGGFDYVALGHIIHKRQVIPKE--- 219 |   |        |           |         |
|   | 170 180 190 200 210 220 230 240   |        |           |         |
| SbcD-like 148 ievdakevdsvylkkgwtidvxmrkvklkrvKPIIVYPSSTIQQNHGETVKGHGWLWDM-KNFTHIIFKEVPNNFGYYTLE 226     | ....*.... ....*.... ....*.... ....*.... ....*.... ....*.... ....*.... ....*....           |        |           |         |
| <u>COG0420</u> 220 -----DPPIVYPGSPERYSFGEGERKCVVLFVEFsGGKLWRFEELFVPLFERLEV 269                          | ↑   |        |           |         |
|   | 250 260 270 280 290 300 310 320   |        |           |         |
| SbcD-like 227 VKNGKVPPVLADWPKNVRMRiftGTLDTTGVKKLVSVLRKQHNIIIELSINKSRVNNTQEIKIKSGMELVDVQNVNVQNTLI 306    | ....*.... ....*.... ....*.... ....*.... ....*.... ....*.... ....*.... ....*....           |        |           |         |
| <u>COG0420</u> 270 DVLNGEDLEDELLKLDREE---DAKDEPVVRLTIEGETDILDERLKERILALVERIERLELLLEERATDLAEEPESSELE 346 |   |        |           |         |
|   | 330 340   |        |           |         |
| SbcD-like 307 QDWLERTYDNTISPNLMKKILD 328  | ....*.... ....*.... ...   |        |           |         |
| <u>COG0420</u> 347 ELLSELDVVEAFEEELLADEVLL 368  |   |        |           |         |

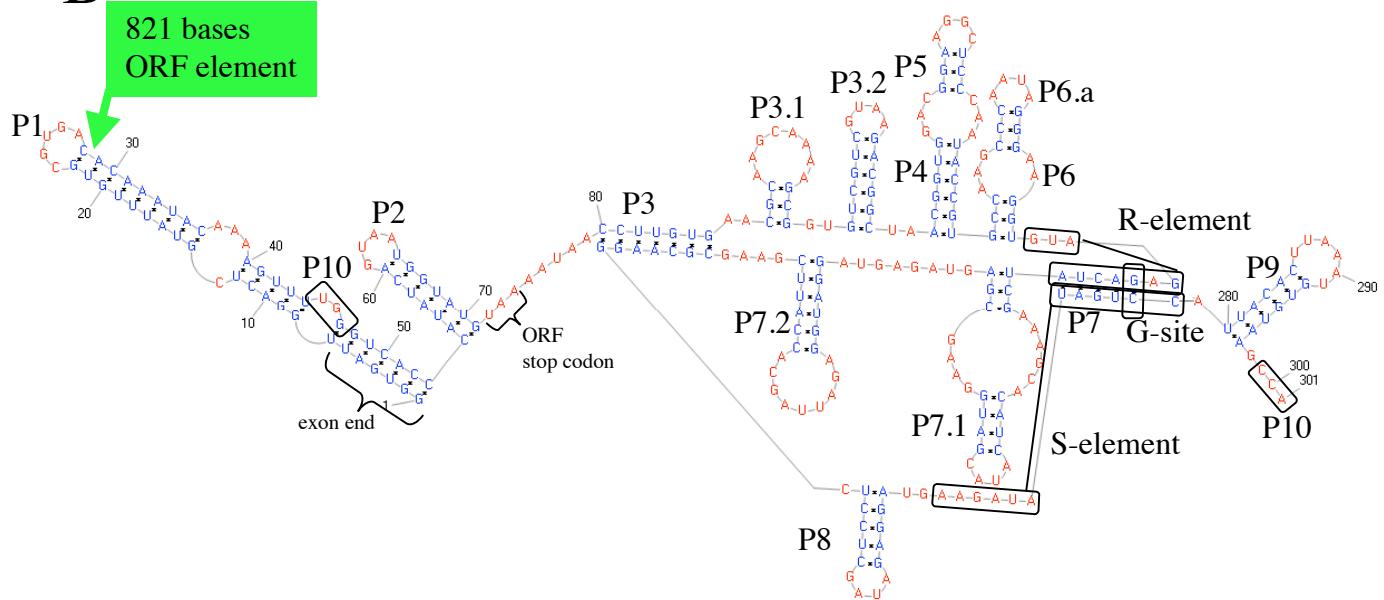
# Figure S3

A



# Figure S3

B



# Figure S4

A

**Motif 1** E-value = 1.7e-010

| Loci    | Position | P-Value  | Sequence             |
|---------|----------|----------|----------------------|
| gp41-1  | 22       | 1.30e-08 | AGTTACACTTCTTATAAAAT |
| NrdA-1  | 0        | 3.49e-08 | GATTACACTTCCTATTATG  |
| NrdA-4  | 0        | 3.49e-08 | GAATACACATCTTACATTT  |
| NrdA-5  | 0        | 1.99e-09 | GATTACACTTCCTATAATG  |
| DnaE-1  | 26       | 2.01e-07 | ATTTACACCGCTTGCAAT   |
| DnaE-2  | 24       | 9.84e-07 | TAATATACCTCCTGCAACT  |
| NrdJ-1  | 26       | 1.44e-05 | GAATACAAGTTCATCTAT   |
| NrdJ-2  | 38       | 1.64e-06 | GTTCACACATTCGAAAAG   |
| IMPDH-1 | 1        | 3.64e-06 | GGAGGCATATCCTGTGAAT  |



**Motif 2** E-value = 9.9e-003

| Loci    | Position | P-Value  | Sequence              |
|---------|----------|----------|-----------------------|
| gp41-1  | 0        | 1.06e-07 | TAGTATAACCTTACTGATATG |
| gp41-8  | 11       | 9.97e-07 | TGGCAACAGCTACTCAGAGG  |
| NrdA-1  | 34       | 1.52e-07 | TAGGAAGTGTAAATTCTATA  |
| NrdA-5  | 34       | 1.52e-07 | TAGGAAGGGTAATTTCTATA  |
| DnaE-1  | 0        | 7.03e-07 | CAGGAGGTGTAAATTAATATG |
| DnaE-2  | 0        | 6.08e-06 | TTGCAGGAGGTATATATTAA  |
| NrdJ-2  | 58       | 5.11e-06 | TGGTAGAAAGGATTCTTATA  |
| IMPDH-1 | 27       | 3.99e-07 | ATGTATGAGTGCCTCCTATA  |



# Figure S4

B

|         |  |                           |
|---------|--|---------------------------|
| gp41-1  | [1] 1.30e-08   | [2] 1.06e-07              |
|         | ATAGTGTGTGTA <b>AGTTACACTTCTTATAAAATAATAGTAGTATAACTTACTGATATG</b>  |                           |
| gp41-8  | [1] 9.97e-07   |                           |
|         | TAAATAATATT <b>TGGCAACAGCTACTCAGAGGATTTATG</b>   |                           |
| NrdA-1  | [2] 1.52e-07   | [1] 3.49e-08              |
|         | AATATTAT <b>AGGAAGTGTAAATTCTATA</b> TTTCATAAAATAGTTATGA <b>GATTACACTTCCTATTATG</b>   |                           |
| NrdA-4  | TAAAAAGGTGTATTCCATAATGTATGTAAAATATATATCATTATG <b>GAATACACATCTTA</b><br><b>CATT</b> TATGCATATTAGATCCTAGAAAACCTGGGAATTCAAATATGGAAATTATATTTC<br>GAATTTGAGCCTTTTATATTGGTAAAGGCAAACCACAACGTGTTACAATCGTATGTATA<br>GACATTAGAATTGTAAAATCGCTCCAGCTGC... | [1] 3.49e-08              |
| NrdA-5  | [2] 1.52e-07   | [1] 1.99e-09              |
|         | ATATTAT <b>AGGAAGGGTAATTCTATA</b> TTTTATAAAATAGTTATGA <b>GATTACACTTCCTATAATG</b>   |                           |
| DnaE-1  | [1] 2.01e-07   | [2] 7.03e-07              |
|         | AC <b>ATTACACCGCTTGCATATA</b> ATTAAATTG <b>CAGGAGGTGTAAATTATG</b>  |                           |
| DnaE-2  | [1] 9.84e-07   | [2] 6.08e-06              |
|         | TAAATTAGTA <b>TAATACCTCCTGCAACT</b> ATTG <b>TTGCAGGAGGTATATATTATG</b>  |                           |
| NrdJ-1  | GAAGTCAAGATATTAAACAAGGATAACAGA <b>GAATACAAGTTCATCTATTCAAGGAAGAA</b><br>TATAAATTGAATTGTATG  | [1] 1.44e-05              |
| NrdJ-2  | AATCGAGATATTATAAATAGTATAAGGGT <b>TGGTAGAAAGGATTCTTATATGTTACACATT</b><br><b>TCGAAAAG</b> CGACAACATAAGAGTGGTACCAATATAAAACAACAAAG...  | [2] 5.11e-06 [1] 1.64e-06 |
| IMPDH-1 | [2] 3.99e-07   | [1] 3.64e-06              |
|         | GGTACGAA <b>ATGTATGAGTGCCTCCTATATACATTA</b> GGAGGCATATCCTGTGAATATG   |                           |