Supplementary data (Valens et al.)

Construction of strains

Strain FBG140 contains at the original $\lambda$ attB site an artificial operon carrying attB inserted in frame in lacZ upstream of the cat gene. The insertion of attB in frame in lacZ resulted in a reduction of two-fold of $\beta$-galactosidase activity but still allowed a blue coloration on a medium containing 5-Bromo 4-chloro 3-indolyl $\beta$-D-galactopyranoside (X-Gal) of the strain carrying the lacZ-attB cassette. This strain has been constructed in two steps: firstly, the linearized ApaI-NotI fragment from plasmid pG has been introduced by homologous recombination in MG1655–Δlac-bet-exo. This recipient strain (MG1655 Δlac ΔMluI Δ(recC ptr recB recD)::P$_{lac-}$ bet exo kan recJ) has been constructed by P1 transduction using the strain KM29 (Murphy, 1998) as donor. The region carrying aadA-P$_{GBM3}$-lacZ-cat has been transferred in MG1655 Δlac by P1 transduction.

Strain FBG146 contains at the original $\lambda$ attB site an artificial promoter-less operon carrying attR fused to the 3’ part of lacZ upstream of cat gene. This strain has been constructed in two steps. The attR-lacZ-cat cassette has been first integrated in MC1061 in place of attB by using pLN135-attR, a derivative of pLN135 (Cornet et al., 1996) carrying the attR-lacZ-cat cassette and the aadA gene between ybhC and ybhB. Strain FBG146 has been obtained after transferring in MG1655 ΔlacIZ the attR-lacZ-cat cassette linked to aadA by P1 transduction.

Strain FBG146$^{off}$ has been obtained by using pI-pKO3-attR$^{off}$, a pKO3 derivative (Link et al., 1997) carrying the aadA gene, attR fused to the 3’ part of lacZ, the cat gene between “ybhc” and “ybhb”. In this strain, orientation of attR is opposite to that of strain FBG146.
Strain FBG147 has been constructed by site specific integration of attR fused to the 3’ part of lacZ into the attB site of phage HK022 (Rossignol et al., 2002) using pHK10-Apra-attRlacZ.

Strain FBG150 (MG1655 ΔlacIZ ΔattB) has been obtained by using pI-pKO3, a pKO3 derivative (Link et al., 1997) carrying the aadA gene between “ybhC” and “ybhB”; in this strain, a 25-bp region corresponding to attB has been substituted by the aadA gene.

Strains FBG150-attL_{82}, FBG150-attL_{66}, FBG150-attL_{29}, FBG150-attL_{7}, FBG150-attL_{83}, FBG150-attL_{70} were obtained by conjugal transposition of pUT-Tn5-attL.

Strain FBG150-attL_{87} has been obtained by transposition of NCBOR-attL in strain FBG150.

Strain FBG150-attR_{53} has been obtained by transferring attR_{53} from collection FBG150-attL_{82} into FBG150 using P1 transduction.

Construction of plasmids

Plasmid pG was constructed in seven steps. First the XbaI site of pOM5 has been destroyed by Klenow filling in treatment, giving pOM5ΔX. A SalI-HindIII 3.9 kb fragment of pOM5ΔX carrying the artificial promoter P_{GBM3}, lacZ and cat genes has been cloned in Bluescript pSK(-) digested by SalI-HindIII, giving plasmid pA. Plasmid pB has been obtained by cloning a double-stranded oligonucleotide corresponding to attB in the SalI site using complementary oligonucleotides 5’-TCGAGGAAGCCTGCTTTTTTATACTAACTTGAGCGAC-3’ and 5’-TCGAGTCGCTCAAGTTAGTATAAAAAAGCAGGCTTCC-3’. pC has been obtained by cloning in pB digested by XhoI a XhoI-SalI PCR fragment carrying aadA generated by PCR using oligonucleotides aadA-up 5’-GGCAGTCGACAGATCTCGCAGCGGTGGTAACGG-3’ and aadA-do 5’-AACGCGCTCGAGAAAAAGCCGCGCCGGAAGC-3’. Plasmid pD was constructed by cloning in pC digested by HindIII and XbaI a HindIII-XbaI PCR fragment containing...
ybhB, i.e. the region located downstream from \( \lambda \ attB \) in the *E. coli* MG1655 chromosome using oligonucleotides right-up 5′-CCGCGCAAGCTTAAAGGTTAAAAAGACAAAAAGTTG-3′ and right-do 5′-TTCCGTCTAGACTCGGACTCCGGTTCG-3′. Plasmid pE was constructed by cloning in pD digested by *XhoI* a *SalI*-*XhoI* PCR fragment containing *ybhC*, i.e. the region located upstream from \( \lambda \ attB \) (“*ybhC*”) in the *E. coli* MG1655 chromosome using oligonucleotides left-up 5′-AATTGGGCCTTGCGCATCTTATCCGAACCT-3′ and left-do 5′-GCGACTCGAGGGATTCATTTTTCTATTTCA-3′. A double-stranded oligonucleotide corresponding to *attB* has been inserted in frame, in the *ClaI* site of *lacZ* carried by pOM5 giving pOM5-*attB* using complementary oligonucleotides 5′-CGACAGCCTGCTTTTTTATACGCTGCT-3′ and 5′-CGAGCAAGTTAGTATAAAAAAGCAGGCTGT-3′. Plasmid pG has been obtained after replacing the *lacZ-cat* operon of pE by the *lacZ::attB-cat* operon of pOM5-*attB* by cloning a 3940-bp *SmaI*-*HindIII* fragment in pG digested by *BglII*, treated by Klenow fragment and digested by *HindIII*.

Plasmid pG-*attB-attP* was obtained by cloning a 416-bp *EagI*-*HindIII* PCR fragment carrying *attP* and the transcriptional terminator of pNKBOR-attP (using oligonucleotides 5′-AAAGGCGGCCGTCAGGTACGCGTGCTA-3′ and 5′-GGAAGGAAGCTTGTGACAAAAATCAAATAATGATTTT-3′) in pG in such a way that *attB* and *attP* are inversely orientated. The *attR* site fused to the 3′ part of *lacZ* and the *attL* site fused to the 5′ part of *lacZ* have been obtained by in vivo recombination of plasmid pG-*attP* (giving plasmid pG-*attL-attR*) in a strain expressing *int* (pTSA29-CITD).

Plasmid pI has been obtained by deleting in plasmid pG the *HindIII*-*StuI* fragment. It carries *aadA* between *ybhC* and *ybhB*.

Plasmid pI-pKO3 has been obtained by cloning in pKO3 digested by *XbaI* and *SmaI* the *EcoRV*-*XbaI* fragment of pI carrying *ybhC*, *aadA* and *ybhB*.
Plasmid pI-pKO3-attR and pI-pKO3-attR<sup>off</sup> have been obtained by cloning a SalI fragment of pG-attL-attR carrying attR fused to the 3’ part of lacZ.

Plasmid pLN135-pI has been obtained by cloning in pLN135 digested by BglIII, treated with T4 DNA polymerase and subsequently digested by Nsil a fragment of pI carrying ybhC, aadA and ybhB obtained by ApaI digestion followed by a T4 DNA polymerase treatment and subsequently digested by Nsil.

Plasmid pLN135-attR has been obtained by cloning in pLN135-pI digested by SalI a SalI fragment of pG-attL-attR carrying attR fused to the 3’ part of lacZ.

pNKBOR-attP has been obtained by cloning in pNKBOR digested by BamHI and PstI a 419-bp BglII-PstI PCR fragment carrying attP and the transcriptional terminator T1 of rrnB ribosomal operon obtained from plasmid pATTΔP (FB, unpublished results). In the resulting plasmid, attP is located close to the transcription terminator T1 of rrnB operon such that, after inversion with attB, transcription of the region corresponding to attR and the 3’ part of lacZ is prevented (Figure 1B). This mini-transposon is carried by a conditional replicon that will replicate only in strains containing the R6K replicase Pir.

pNKBOR-attL has been obtained by cloning in pNKBOR digested by BamHI a 999-bp BglII PCR fragment obtained from pG-attL-attR, using oligonucleotides 5’-AAAAGGAGATCTGGGTTGCATTCTGCAGCT-3’ and 5’-AAAAGGAGATCTAAATCAAATAATGATTTT-3’ containing the transcriptional promoter P<sub>GBM3</sub> and the attL site fused to the 5’ part of lacZ.

pNKBOR-attR has been obtained by cloning in pNKBOR digested by SalI a 3370-bp fragment from pG-attL-attR containing the transcriptional terminator T1 of rrnB ribosomal operon, the 3’ part of lacZ fused to attR. pNKBOR-attR-Δcat has been obtained by deleting the BamHI fragment carrying cat.
Plasmid pNCBOR has been obtained by replacing the fragment BssHII-Acc65I carrying the Kanamycin resistance gene of pNKBOR by a PCR BssHII-Acc65I fragment carrying cat using oligonucleotides 5’-AAAGGGGTACCTGAAATAAGATCACTACCG-3’ and 5’-AAAGGGCGGCGCGGAATTCTGCATTATC-3’.

Plasmid pNCBOR-attL has been obtained by cloning in pNCBOR digested by BamHI a 999-bp BglII PCR fragment obtained from pG-attL-attR, using oligonucleotides 5’-AAAAGGAGATCTGGGTTGCATTCTGCAGCT-3’ and 5’-AAAAGGAGATCTAAATCAAATAATGATTTT-3’ containing the transcriptional promoter P\text\textsubscript{GBM3} and the att\text{L} site fused to the 5’ part of lac\text{Z}.

Plasmid pUT-Tn5-attL was obtained in two steps. First, pWED-attL was constructed by cloning a 999-bp PCR fragment digested with BglII carrying the att\text{L} site fused to the 5’ part of lac\text{Z} in the BamHI site of pWED11. Plasmid pUT-Tn5-attL was obtained by cloning the NotI fragment of pWED-attL carrying the att\text{L} site fused to the 5’ part of lac\text{Z} in pUT-Tn5 (Herrero et al., 1990) digested by NotI.

For site-specific integration of att\text{R}-lac\text{Z} into the HK022 att\text{B} site, the SalI fragment of PG-attL-attR carrying att\text{R} fused to the 3’ part of lac\text{Z} was cloned in pHK10-Apra (Rossignol et al., 2002) digested by SalI.

To promote recombination, int gene or int and xis were PCR amplified and cloned downstream of P\text{r} and cI\text{857} in thermosensitive pSC101 derivatives pTSA29, pTSC29 and pTSK-29 (Phillips, 1999). These plasmids are present at 2 copies per cell and can be lost at 42°C. In plasmids pTSA29-CXI-AK, only int is expressed whereas both int and xis are expressed in plasmids pTSA29-CXI and pTSC29-CXI. At 30°C, expression of int or int and xis is repressed whereas a transient incubation at 42°C or at 36°C allows synthesis of Int or Int and Xis, respectively.

Nucleotide sequence of all plasmids is available upon request.
Construction of strains that support recombination

Strain FBG146 was transformed by pNKBOR-attL and KanR transformants were individually transformed by pTSA29-CXI. Clones that supported recombination were isolated; attL insertion points were determined (see Material and Methods). Duplication, deletion or inversion events were confirmed by a diagnostic PCR reactions assaying the presence of \( \text{attB} \), \( \text{attP} \), \( \text{attL} \) or \( \text{attR} \). Oligonucleotides used for these PCR reactions were: 5’-TTACGCCGGGAGAAAAACCG-3’, and 5’-TCAACCACCGACGATAGAG-3’ (\( \text{attB} \)), 5’-ATTGTAGTCACACAGGAAC-3’ and 5’-AAAAGGAGATCTAAATCAAATAATGATT-3’ (\( \text{attP} \)), 5’-AAATGGGGATCTGTGAGAATCGTACGACAGGAAC-3’ and 5’-TCAACCACCGACGATAGAG-3’ (\( \text{attR} \)), and 5’-AAAAGGAGATCTGGTGTTGCATTCTGAGTG-3’ and 5’-AAAAGGAGATCTCTAATCAATAATGATT-3’ (\( \text{attL} \)).

Derivatives of FBG147 were constructed by transferring attL insertions from the FBG146 derivatives using bacteriophage P1 transduction.

Derivatives of FBG150-\( \text{attL}_{82} \) and FBG150-\( \text{attL}_{29} \) were obtained by transformation of pNKBOR-attR-\( \Delta \text{cat} \). Isolation and analyses of strains supporting recombination were as described for FBG146 derivatives.

Derivatives of FBG150-\( \text{attL}_{66} \), -\( \text{attL}_{7} \), -\( \text{attL}_{83} \), -\( \text{attL}_{70} \), and -\( \text{attL}_{87} \) were obtained by transferring attR insertions from FBG150-\( \text{attL}_{82} \) or FBG150-\( \text{attL}_{29} \) derivatives using bacteriophage P1 transduction.

FBG150-\( \text{attR}_{53} \) derivatives were obtained by transformation of pNCBORGattL and CamR colonies were individually transformed by pTSA29-CXI.

FBG140 derivatives were obtained by transformation of pNKBOR-attP and KanR colonies obtained were individually transformed by pTSA29-CXI-AK. Clones that supported recombination were isolated; attP insertion points were determined and deletion or inversion
events were confirmed by diagnostic PCR reactions assaying the presence of \textit{attL} and \textit{attR} as described above.

\textbf{Duplication by excisive recombination}

The assay of programmed genetic transactions allowed selecting for strains that can support deletions, duplications or inversions. As predicted, we could select strains promoting inversion of very long fragments. Concerning deletions, most of the strains supporting such events deleted small fragments; as reported before (Henson and Kuempel, 1985), only in the Ter domain were observed events that deleted fragments as long as 227-kb (data not shown).

Interestingly, a number of strains that gave rise to blue recombinants contained \textit{attL} in the same orientation of \textit{attR}, recombinant clones contained both \textit{attB}, \textit{attL} and \textit{attR}, and in the parent strain \textit{attL} was always found between \textit{attR} and the first replication arrest site located on the opposite replichore; in this configuration, the only way to reconstitute a \textit{lacZ} gene is by interchromatid recombination between \textit{attR} and \textit{attL} (Figure 1C, lane 2). These results indicated that blue recombinants originated from a duplication of the region located between \textit{attR} and \textit{attL} produced by interchromatid recombination. Insertions of \textit{attL} that gave rise to \textit{lacZ} recombinants were only found between \textit{attR} and the first replication arrest site found on the opposite replichore for two reasons. First if \textit{attL} was located on the other side of \textit{attR}, recombination would generate an \textit{attP} site at the junction of the duplication with no functional \textit{lacZ} and second, if the duplicated region contained an inversely oriented replication arrest site, it would generate a unviable chromosome with two replication fork traps (i.e. with two termination sites) that is predicted to be non replicable.

Intermolecular recombination events (Table 1) were very rare in strains containing \textit{attL}_{83} and \textit{attL}_{66} albeit a short distance between \textit{att} sites. The low percentage of recombinants did result from the impossibility to collide these loci intermolecularly since intramolecular
events were detected or predicted. In strain LC5-R33, the 22.7-kb deletion provoked by intramolecular recombination was not deleterious and recombination between att sites reconstituted attP at high frequency. In strains LC5-R30 and LC2-R82, the number of clones after recombination was reduced to a level in agreement with the presence of non viable recombinants that supported deletion of the intervening fragment. Deletion of the intervening fragment between att sites (70.5-kb and 10.7-kb, respectively) was likely detrimental to the cell because genes in the deleted region are analogues of essential genes in B. subtilis (Kobayashi et al., 2003) and thus presumably essential in E. coli (data not shown).

References


