Broad possibilities

- non-bacteriophage-related extra-chromosomal linear plasmid
- linearised chromosome
- linear phage

How bacteria replicate

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Linear plasmid

Useful facts

<u>Baker et al. 2007</u>
"Molecular analysis of H:z66þ S.Typhi revealed that the H:z66 flagellin structural gene (fljBz66) is encoded on a linear plasmid that we have named pBSSB1. The DNA sequence of pBSSB1 was determined to be just over 27kbp, and was predicted to encode 33 coding sequences"

"Analysis of the ... linear plasmid sequence confirmed that it encoded the fljBz66 gene and identified identical inverted repeat sequences present at both termini (terminal inverted repeats [tirs])"

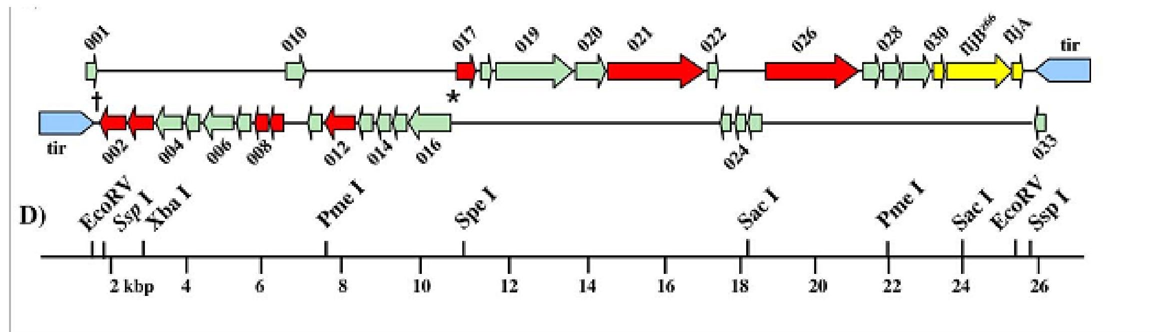
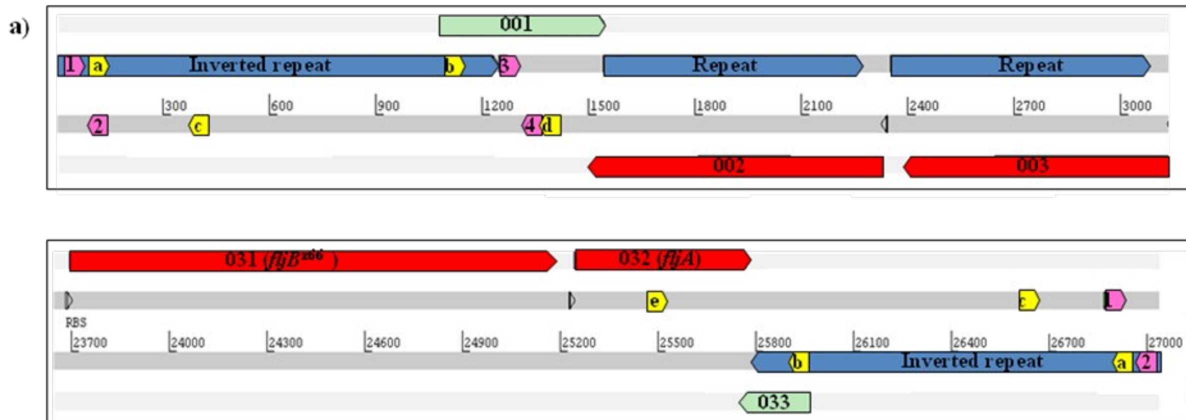


Figure 2. Map of the Complete Sequence of pBSSB1
(A) Plot of percentage GC content of pBSSB1, ranging from 24% to 49%, with an average of 36.6%.
(B) Plot of GC skew ((G–C)/(G+C)), ranging from –0.08 to 0.14 and averaging 0.03.
(C) CDS map of pBSSB1, consisting of the forward (upper) and the reverse (lower) DNA strand. The CDSs are identified with sequential numbers. The previously sequenced genes of Huang et al. [11] (030, *fljB*^{z66}, and *fljA*) are labelled yellow. The tirs are labelled in blue, CDSs with similarity to previously described sequences are coloured red, and CDSs with no similarity to previous sequences are coloured green. *, the change in coding bias associated with a possible origin of replication. †, the location of the kanamycin resistance gene insertion.
(D) Size scale in 2-kbp fragments of pBSSB1 (27,037 bp). Endonuclease cut sites are labelled for XbaI (2,708 bp), PmeI (7,704 and 21,995 bp), SacI (18,611 and 23,778 bp), and SpeI (11,137 bp); additionally, the cut sites located nearest the tirs are labelled for EcoRV (1,279 and 25,255 bp) and SspI (1,715 and 25,799 bp).



"pBSSB2 was stably inherited by E.coli SGB33, even in the absence of antibiotic selection."

"Restriction endonucleases that were predicted to cut [pBSSB1] once (SpeI and XbaI)"

"pBSSB2 DNA was entirely degraded by 3'-5' exonuclease III ... but not by lambda exonuclease, which digests in a 5'-3' direction."

"the plasmid was not inserted into the chromosome at a detectable level."

"pBSSB1 contains tirs, and our data demonstrate that the ends are capped with covalently bound protein, as found in Streptomyces linear plasmids[28,29],and not closed hairpin loops".

Where does the protein come from – where is its gene located? To research still:

Qin,Z.,andCohen,S.N.(1998)Replicationatthetelomeres ofthe Streptomyces linearplasmidpSLA2. MolMicrobiol 28: 893–903.

Yang CC, Huang CH, Li CY, Tsay YG, Lee SC, et al. The terminal proteins of linear *Streptomyces* chromosomes and plasmids: A novel class of replication priming proteins. *Mol Microbiol.* 2002;43:297–305. [PubMed]

Lobry JR, Louarn JM. Polarisation of prokaryotic chromosomes. *Curr Opin Microbiol.* 2003;6:101–108. [PubMed]

Bao K, Cohen SN. Terminal proteins essential for the replication of linear plasmids and chromosomes in *Streptomyces*. *Genes Dev.* 2001;15:1518–1527. [PubMed]

9 Stewart, P. et al. (2004) Linear plasmids in bacteria: common origins, uncommon ends. In Plasmid biology (Phillips, G. and Funnell, B.E., eds), p. 613, ASM Press

"The tir, GC skew, and coding bias suggest that pBSSB1 replicates from a central internal origin, as do all small and large Streptomyces linear plasmids,such as pSLA2 from S.rochei [28,29]."

<u>Baker et al 2007b</u>

"To study the role of fljAz66, three mutant derivatives of S. Typhi 404ty-fliC(d) were constructed, individually replacing fljBz66 and fljAz66 with a kanamycin resistance determinant (DfljBz66, DfljAz66::kan respectively)as well as generating a double DfljBz66, DfljAz66::kan mutant. All three derivatives were stable and the deletions had no obvious effect on maintenance of pBSSB2."

“pBSSB2 was maintained over ~500 generations, representing 20 subcultures without antibiotic selection in both E.coli and S. Typhi.”

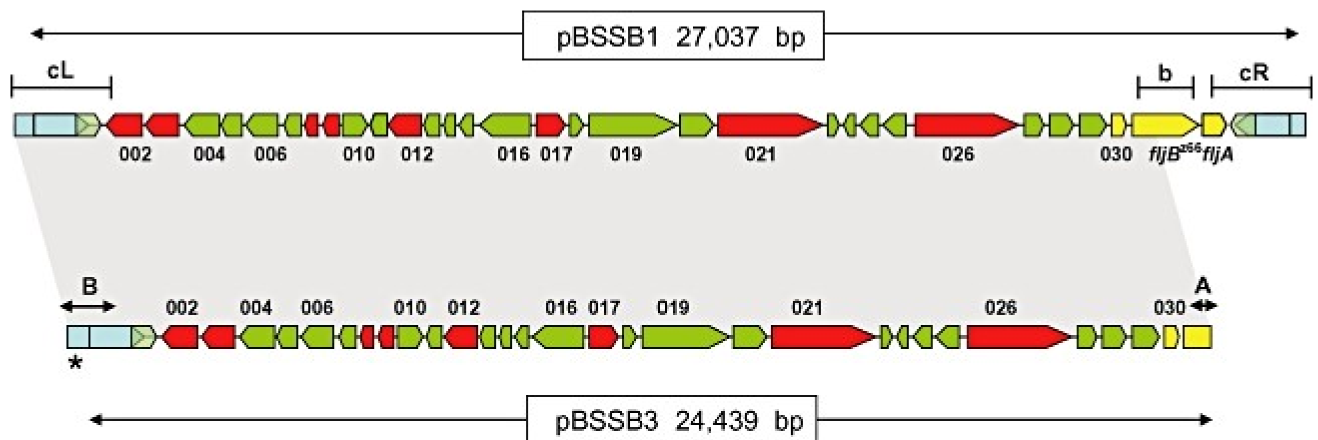


Fig. 5
Gene map alignment of pBSSB1 and pBSSB3. The map of pBSSB1(upper) is manipulated from Baker et al. (2007) and depicts the 33 open reading frames encoded on the element. ... The targeted locations for primers used in are demonstrated by; b, fljB^{z66} and the left and right tir, cL and cR respectively. The shaded region between pBSSB1 and pBSSB3 demonstrates identical sequence. The palindromic terminus sequence are labelled A and B. The asterisk distinguishes the location of the addition 120 bp at the left terminus.

“To precisely locate and characterize the nature of the deletion, plasmid DNA, named pBSSB3, was isolated and purified from 404tya and sequenced to completion... Deletion of the right tir and maintenance of the left tir was confirmed in all phase changed strains by Southern blotting (Fig.6A). Furthermore,the sequence demonstrated that the right end of pBSSB3 terminates at a 21bp imperfect palindrome;GGTGCTGTTCAGAATAGCACC (Fig.7A).The palindromic sequence in fljBz66 was confirmed to be the new terminus in 404tya and to be consistent in all phase-changed strains by Southern blotting.”

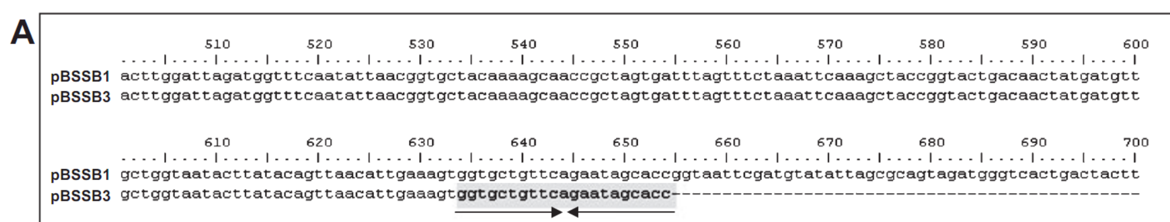


Fig.7. Palindromic sequences at the termini of pBSSB3. A.Sequence alignment of the fljBz66 gene from pBSSB1and the truncated fljBz66 gene from pBSSB3. Numbers correspond to the nucleotide position within the fljBz66 gene.The palindromic sequence at the termini is highlighted; arrows correspond to the direction of the repeated sequence.

Linear genome

Useful facts

Cui et al. 2007

“We linearized the Escherichia coli genome usingthe lysogenic k-like phage N15. Linear genome E.coli were viable and their genome structure was stable. There were no appreciable differences between cells with linear or circular genomes in growth rates, cell and nucleoid morphologies, genome-wide gene expression (with a few exceptions), and DNA gyrase-and topoisomeraseIV-dependent growth.

N15 is similar to the lambda phage in many aspects, such as genome size and in having cohesive ends (Rybchin&Svarchevsky,1999). However, unlike lambda, N15 is lysogenized as a linear plasmid with hair pin termini. On infection,the injected linear genome circularizes through cohesive ends, as with the l phage. For the linearization to lysogenize, two components—the tos site and protelomerase (TelN) protein—are required.

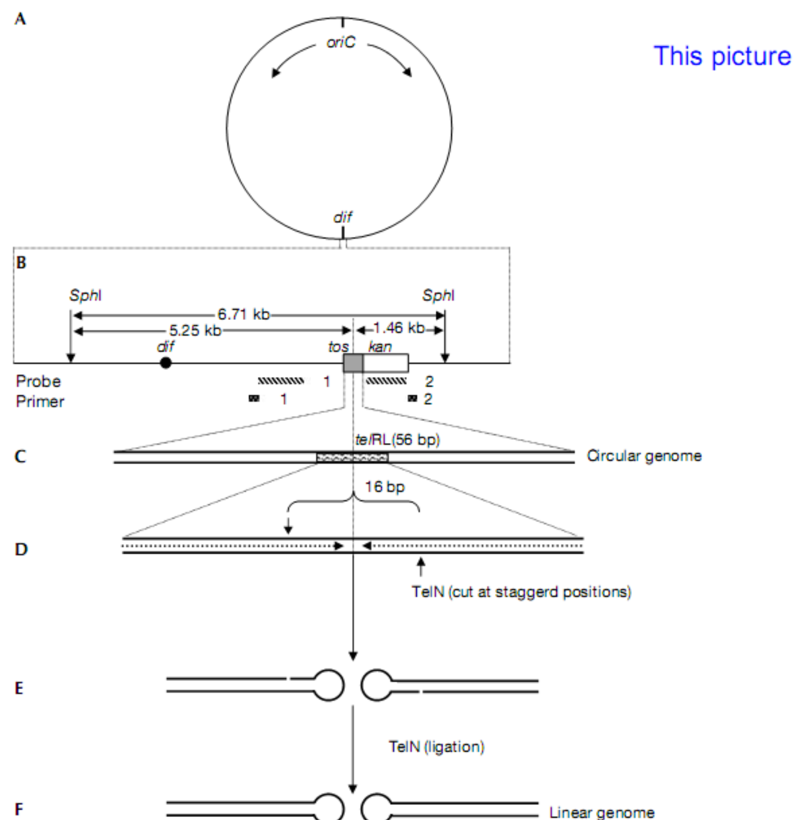


Fig 1 | Linearization process of a circular *Escherichia coli* chromosome mediated by the TelN protein. (A) The circle indicates the *E. coli* circular genome, in which *oriC* (replication origin) and the *dif* site are shown. The two arrows indicate bidirectional replication. (B) Expansion of the *dif* site from (A). The positions of the *dif*, *tos* and *kan* genes are shown as closed circles, grey and white boxes, respectively. The locations of the two probes used in hybridization experiments and the pair of primers used for the genome linearity PCR assay are shown beneath. The *tos* site (392 bp) shown in (B) is expanded in (C), and a part of *tos* (*telRL*: 56 bp) is expanded in (D). The almost perfect palindromic sequences are shown as a pair of dotted arrows in (D). TelN protein binds to the *telRL* site and cuts at the staggered positions indicated by vertical arrows in (D), producing two separate ends with long single-stranded regions, each of which can be self-annealed (E), and TelN seals the nicks, producing two ends with hairpin structure (F).

RJC: Suggest chopping off one end so that dna doesn’t circularise

“The tos sequence and a kan gene [selection marker] ... were inserted into a site(3kb from the dif site) in the replication termination region of the E.coli chromosome by using the linear transformation method (seeMethods;Fig1A,B).”

“Next, the tos–kan site was transduced into two wild-type strains— MG1655 and W3110—using the P1 vir phage to establish two tos–kan strains.The telN gene was then separately inserted in-frame downstream of the pBAD24 arabinose promoter.”

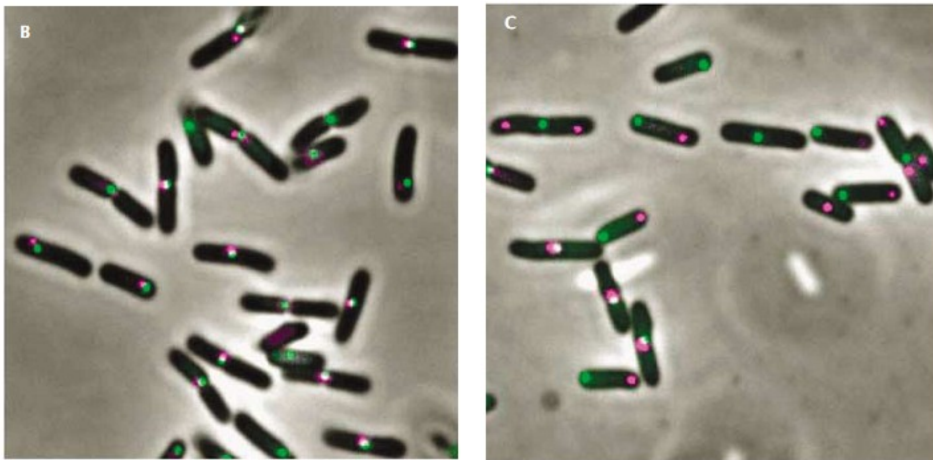
Grew strains in presence of arabinose and confirmed presence of TelN activity.

“Utilization of pBAD-telN as a TelN protein supplier, however has two disadvantages: (i)controlling the arabinose concentration in the cell, and(ii) the presence of a mixture of replicons, both linear(chromosome)and circular(plasmid),in a single cell.”

“To avoid these problems, we used the N15 prophage as a TelN supplier.”

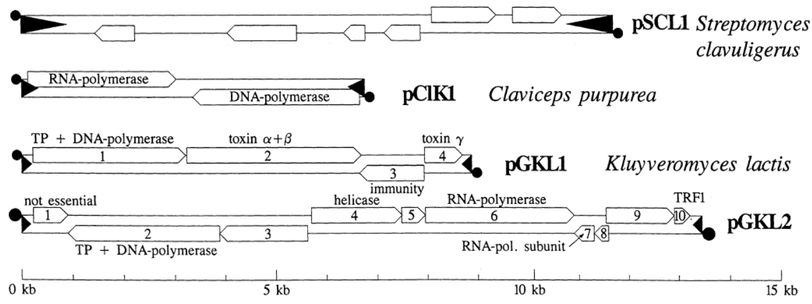
Linearity and whole genomic structure remained unchanged for at least 170 generations. “expression of ... 4,300 genes was compared and only three genes were found to have significantly different expression.

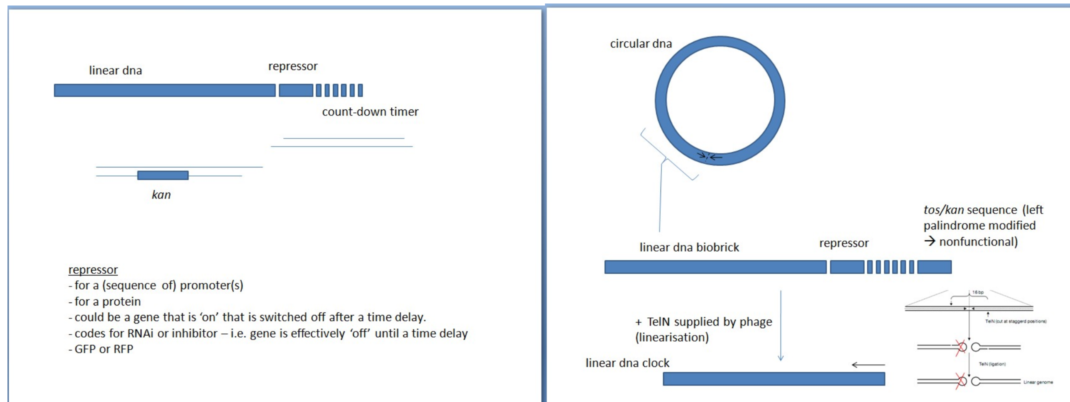
Important linearise as close to the ternini as possible. Termini in linear and circular genomes:



Procedure

1) Replicate work of Baker et al. 2007 by transforming *E.coli* with pBSSB2 (linear plasmid with kanamycin cassette using lambda red recombinase). See experimental details, stains, and supporting information in paper. Do same for Cui et al.
2) Perform same experiment but in *Bacillus subtilis*.


pSCL1 Streptomyces clavuligerus
RNA-polymerase
DNA-polymerase
pClK1 Claviceps purpurea
TP + DNA-polymerase
toxin α+β
toxin γ
immunity
pGKL1 Kluyveromyces lactis
not essential
helicase
RNA-polymerase
TRF1
TP + DNA-polymerase
RNA-pol. subunit
pGKL2
0 kb
5 kb
10 kb
15 kb


linear dna
repressor
count-down timer
kan
repressor
- for a (sequence of) promoter(s)
- for a protein
- could be a gene that is 'on' that is switched off after a time delay.
- codes for RNAi or inhibitor – i.e. gene is effectively 'off' until a time delay
- GFP or RFP
circular dna
linear dna biobrick
repressor
tos/kan sequence (left palindrome modified → nonfunctional)
+ TelN supplied by phage (linearisation)
linear dna clock