Tn*6026* and Tn*6029* are found in complex resistance regions mobilized by diverse plasmids and chromosomal islands in multiple antibiotic resistant Enterobacteriaceae

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Abstract

Transposons flanked by direct copies of IS*26* are important contributors to the evolution of multiple antibiotic resistance. Tn*6029* and Tn*6026* are examples of composite transposons that have become widely disseminated on small and large plasmids with different incompatibility markers in pathogenic and commensal *Escherichia coli* and various serovars of *Salmonella enterica*. Some of the plasmids that harbour these transposons also carry combinations of virulence genes. Recently Tn*6029* and Tn*6026* and derivatives thereof have been found on chromosomal islands on both established and recently emerged pathogens. While Tn*6029* and Tn*6026* carry genes encoding resistance to older generation antibiotics, they also provide a scaffold for the introduction of genes encoding resistance to a wide variety of clinically relevant antibiotics that are mobilized by IS*26*. As a consequence, Tn*6029* and Tn*6026* or variants are likely to increasingly feature in complex resistance regions in multiple antibiotic resistant *Enterobacteriaceae* that threaten the health of humans and food production animals.

1.1 Introduction

IS*26* has garnered a great deal of interest for its role in the formation of multiple antibiotic resistance regions on plasmids and in the chromosomes of phylogenetically diverse Gram negative bacteria including *Escherichia coli* [1-5], various *Salmonella enterica* serovars[6, 7], *Pseudomonas aeruginosa* [8, 9], *Klebsiella pneumoniae*[10], *Actinobacter baumannii* [11, 12], *Proteus mirabilis*[13, 14], *Citrobacter freundii* [15], *Serratia marcescens*[16], *Enterobacter cloacae*, *Pantoea* spp[17], *Stenotrophomonas maltophilia* (GenBank entry KM649682.1), *Klebsiella oxytoca* (GenBank entry KJ541681.1), *Aeromonas salmonicida* [18]and *Vibrio cholerae* (GenBank entry KM083064.1). IS*26* is found flanking genes encoding resistance to a wide range of antibiotics including fosfomycin[19], kanamycin and neomycin[20], ampicillin, streptomycin and sulfonamides[6], chloramphenicol, florfenicol and clindamycin [21, 22], and antibiotic classes including extended spectrum β-lactamases (ESBLs, e.g. $bla_{\text{SHV-2}}$) [23, 24], aminoglycosides[24], and fluoroquinolones[24, 25]. These antibiotics are used widely in food animal production, aquaculture, the production of agriculturally important crop species and human medicine.

IS*26* is an 820 bp insertion element that comprises a transposase gene flanked by 14-bp terminal inverted repeats [26]. Two distinct models of movement have been described for IS*26* and this versatility underpins its importance in the formation of complex resistance loci (CRL). One mode involves co-integrate formation between an incoming molecule with an IS*26* and a recipient molecule that is devoid of IS*26* [27-29]. Resolution of the co-integrate generates directly orientated copies of IS*26* flanking the incoming DNA molecule at the boundaries along with an 8-bp sequence duplication of the insertion site [27-29]. Another mode involves the incorporation of a translocatable unit (TU) comprising one copy of IS*26* and passenger DNA (typically, but not restricted to, a resistance gene) next to an existing copy of IS*26* in a recipient molecule. Target site selection relies on IS*26* recognising another copy of IS*26*. Mobilisation of TUs is described as a conservative mechanism because it does not require duplication of IS*26*, nor does it generate another 8-bp duplication of the target site and occurs at a higher frequency compared to co-integrate formation described above [29]. The formation of a TU can explain the

appearance of multiple tandem copies of IS*26* interspersed with genes encoding resistance to different classes of antibiotics that are reported with increasing frequency in multiple antibiotic resistant Gram negative pathogens [1, 11, 24, 30]. As such IS*26* plays a major role in the formation of CRL [29].

The significance of IS*26* in the formation of a mobile complex resistance locus is well illustrated by the events that led to the formation of Tn*6029* and the related transposon, Tn*6026*. Tn*6029* is a composite transposon that carries a $bla_{\text{TEM-1}}$ gene (conferring resistance to β-lactams) derived from Tn*1*, *2* or *3* and a region of RSF1010 which accounts for the Δ*repA-repC-sul2-strA-strB* module encoding resistance to sulfonamides and streptomycin. Tn*6026* confers resistance to ampicillin, sulphonamides, and streptomycin through expression of $bla_{\text{TEM-1}}$, *sul2*, *strA* and *strB* (s*trAB*) genes respectively. Tn*4352* carries *aphA1*, which confers resistance to neomycin and kanamycin. As such, Tn*6026* consists of two composite transposon-like structures, described as Tn*6029* and Tn*4352*, which share a copy of IS*26*. These two composite transposon-like structures are flanked by direct copies of IS*26* and have been identified on plasmids of various incompatibility groups and in genomic islands in *Escherichia coli* and *Salmonella enterica* serovars from geographically diverse food animal, human and environmental sources [1, 2, 6, 25, 31-39].

The resistance genes found in Tn*6026* are effective against antibiotics used frequently in agriculture. The ability of IS*26* to target regions containing IS*26* coupled with the observation that direct copies of IS*26* are found flanking resistance genes encoding resistance to antibiotics used widely in human clinical medicine shows that widely disseminated transposons like Tn*6026* are likely to become components of larger CRL encoding resistance to multiple antibiotic classes. For example, Tn*6026* has been found in close proximity to class 1 integrons [1, 2, 32, 40]. In enteroaggregative haemorrhagic *E. coli* (EAHEC) O104:H4 outbreak strain 2011C, the insertion of IS*26* has led to the formation of novel CRL and the potential for a class 1 integron to be mobilized as a composite transposon-like structure flanked by direct copies of IS*26* [40].

In this mini-review we provide an overview of the plasmids and genomic islands that carry Tn*6029* and Tn*6026* and examine the precise location of these transposons in CRL. These analyses provide insight into the mechanisms that mobilise these transposons in geographically dispersed enterobacterial populations and highlight the emerging role they play in the evolution of CRL.

2.1 Structure of Tn*6029* and Tn*6026*

Regions within Tn*6029* and Tn*6026* bounded by IS*26* have separate evolutionary origins [6, 36]. The $bla_{\text{TEM-1}}$ gene segment originates from the closely related transposons Tn*1*, Tn*2* or Tn*3* [41]. The bla_{TEM} genes differ between each transposon with $bla_{\text{TEM-1a,}}$ $bla_{\text{TEM-1b}}$ and $bla_{\text{TEM-2}}$ found within Tn*3*, Tn*2* and Tn*1* respectively [42]. 1a and 1b produce the same protein despite 3 nucleotide differences whilst TEM-1 and TEM-2 differ by a single amino acid [43]. These transposons have been identified predominantly in *E. coli, K. pneumoniae, S. enterica* and *P. aeruginosa* [44-47]. In the majority of cases these transposons are plasmid borne and thus widely disseminated [42].

The second segment shared by both Tn*6026* and Tn*6029* contains the *sul2-strAB* resistance gene cluster in addition to plasmid replication genes Δ*repA* and *repC.* The cluster is derived from a plasmid RSF1010 (GenBank: M28829) [36, 48] a broad host range, widely disseminated IncQ plasmid first isolated from the faeces of pigs in the early 1970s [48, 49]. The cluster present in RSF1010 likely arose via the transposition of Tn*5393* containing *strAB,* into a *sul2* gene associated with a mobile element known as CR2 [48]. The CR2 region was subsequently lost when IS*26* insertion elements captured the Δ*repA-repC-sul2-strAB* cluster [36]. RSF1010 and related plasmids are widely distributed geographically and in various bacterial species of human and animal origin [50-52].

An evolutionary pathway for the emergence of Tn*6029* has been proposed and involves the insertion of Tn*1*, Tn*2* or Tn*3* into RSF1010. Three independent insertion events involving IS*26* are critical to both delineating the boundaries and shaping the events that formed Tn*6029* [6]. The Δ*repA-repC-sul2-strA-strB* module is flanked by two inward facing IS*26* elements and enables an inversion of the region.

Notably, a CRL containing IS*26*-Δ*repA-repC-sul2-strA-strB*-IS*26* in the opposite orientation to how it appears in Tn*6029* was reported in a MDR *S. enterica* serovar Typhimurium from Italy [25, 36].

Variants of Tn*6029* including Tn*6029*B [42], Tn*6029*C and Tn*6029*D[40] have been reported. Tn*6029*B has an IS*26*-mediated deletion of 85-bp upstream of the bla_{TEM} gene and has been identified on the plasmid pHCM1 isolated from *S. enterica* Typhi implicated in a clinical case of typhoid fever in Vietnam [31] as well as a chromosomal region known as RD1 in *E. coli* O104:H4 strain 2009EL-2050 isolated from a patient with bloody diarrhea [35, 40]. Tn*6029*C was originally identified on a small plasmid in a MDR commensal isolate of *E. coli* in West Africa [37, 40]. The Tn*6029* variant is characterized by an additional 11-bp deletion in the *ΔtnpR-*bla_{TEM-1}ΔCR2 segment seen in Tn*6029*B and by the inverted orientation of the module compared to the structures seen in Tn*6029* or Tn*6029*B[40]. Tn*6029*D is identical to Tn*6029*C except that the bla_{TEM} module is missing due to an IS*26*-mediated event. The key features that distinguish Tn*6029,* Tn*6029*B, Tn*6029*C and Tn*6029*D are depicted in Figure 1.

Tn*4352* was initially identified on a plasmid NTP16 isolated from *S. enterica* Typhimurium and consists of two direct copies of IS*26* at opposite ends of an *aphA1* gene that confers resistance to kanamycin and neomycin [20]. The introduction of Tn*4352* into Tn*6029* involves the formation of a TU consisting of the *aphA1* gene and a single copy of IS*26.* The TU forms a co-integrate with the target IS*26* that abuts *strB* in Tn*6029* and inserts without creating a new copy of IS*26* or a target site duplication [29]. The 8-bp sequences that abut the three copies of IS*26* in Tn*6029* (four copies of IS*26* in Tn*6026*) can therefore be used to track the movement of the two elements when they move following typical features of composite transposons. BLAST analysis of direct repeats abutting IS*26* in CRL carrying Tn*6029* and Tn*6026* provides evidence of evolutionary events and will be discussed later.

3.1 Enterobacteria that carry Tn*6029* and Tn*6026*

To date Tn*6026,* Tn*6029* and its variants have been identified in *Salmonella enterica* serovars Typhi, Typhimurium and Paratyphi A as well as pathogenic and commensal *Escherichia coli* [1, 2, 6, 25, 31-39].

S. enterica Typhimurium is typically food or water-borne and causes disease in both humans and animals by colonisation of the intestinal epithelium [53]. *S. enterica* serovars Typhi and Paratyphi are aetiological agents of related systemic diseases typhoid and paratyphoid fever [33]. These enteric diseases are uncommon in developed countries, however it remains an issue in developing areas such as Africa and Southeast Asia [54, 55]. Antimicrobial therapy is essential when infections become septic. As such the acquisition of multiple antibiotic resistance on plasmids and in the chromosome of invasive *S. enterica* serovars poses a serious threat to human health [33].

Enterohaemorrhagic *E. coli* (EHEC) are important enteric pathogens that cause various diseases in humans and animals [56, 57]. EHEC cause haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) and carry a number of virulence genes implicated in the etiology of these afflictions including chromosomally-encoded *stx*$_1$ and *stx*$_2$ encoding shiga toxins 1 and 2, *eaeA* which encodes intimin, an adhesin that is found on the locus of enterocyte effacements (LEE), and plasmid encoded factors including *ehxA* (encodes enterohaemolysin), *espP* (encodes a serine protease implicated in pathogenesis) and *msbB* [1, 56]. Shigatoxigenic *E. coli* (STEC) are readily isolated from the faeces of healthy slaughter-age cattle and sheep and young calves with gastrointestinal disease [57-59] and are regarded as major reservoirs of EHEC that cause serious gastrointestinal and systemic diseases [56]. The association of antibiotic resistance genes with plasmid encoded virulence factors represents an added threat to human health [60].

Virulence plasmids carried by EHEC serotypes O26:H- and O111:H- isolated from patients suffering intestinal disease have been shown to carry Tn*6026* [1, 2, 34]. Two large co-resident MDR plasmids pO26-CRL$_{111}$ and pO26-CRL$_{125}$ carrying virulence genes have been sequenced from EHEC O26:H- strain O6877. The IncI1 plasmid pO26-CRL$_{111}$ carries a suite of virulence factors with roles in haemolysis, biofilm formation, cell adhesion and protection from oxidative stress [1]. In pO26-CRL$_{111}$, a derivate of Tn*21* is found inserted into the *traC* gene generating 5-bp direct repeats typical of Tn*3* family transposons. Tn*6026* abuts a 24-bp remnant of the 3´-CS of the class 1 integron found in the derivate

Tn*21* transposon. An IS*26*-mediated deletion presumably caused the loss of all but 24-bp of the 3´-CS and any evidence of the insertion of Tn*6026*. Strain O6877 also carries a 125-kb IncZ plasmid known as pO26-CRL$_{125}$ that carries a novel serine protease autotransporter of unknown function. Identical copies of the derivate Tn*21* are found in both plasmids with Tn*6026* abutting 24-bp of the 3´-CS [2]. However a more complex evolutionary path created the CRL in pO26-CRL$_{125}$ as it comprises the derivate Tn*21* and a truncated version of Tn*1721* that is separated by an IncP-1a oriV sequence. Both plasmids appear to be stably maintained in strain O6877.

Enteropathogenic (EPEC) *E. coli* are important enteric pathogens that cause various diseases in humans and animals [56]. EPEC do not possess Shiga toxin genes yet retain the ability to form attaching and effacing lesions on intestinal cell monolayers via the expression of the LEE-encoded intimin gene *eae*. pO111-CRL$_{115}$ is from atypical EPEC (aEPEC) strain D275 recovered from a cow with gastrointestinal disease [61]. pO26-CRL$_{125}$ and pO111-CRL$_{115}$ are essentially identical plasmids, with the exception of a 9.7 kb segment found only in pO26-CRL$_{125}$ and several small indels [2]. pO26-CRL$_{125}$ was isolated from human EHEC O26 strain O6877 from a case of haemorrhagic colitis in 1998. Thus, essentially identical plasmids can exist in serologically diverse *E. coli* that colonise and infect humans and food animals.

pO26-CRL$_{111}$, pO26-CRL$_{125}$ and pO111-CRL$_{115}$ carry identical copies of a derivate Tn*21* transposon with a complete copy of Tn*6026* that abuts 24 bp of the 3´-CS. The evolutionary events that formed this region created a molecular signature that can be exploited to track the plasmids that carry these and related CRL. We utilise a PCR with primers L1 in *intI1* and JL-D2 in IS*26* to track unusual deletion events caused by IS*26* that occur in close proximity to a class 1 integron [61]. From an epidemiological perspective, multiple antibiotic resistance is most frequently associated with the presence of class 1 integrons [62]. The presence of IS*26* within class 1 integrons provides a preferred site for subsequent insertion of laterally mobile scaffolds containing IS*26*. Thus, the formation of complex resistance regions minimises the creation of deleterious mutations occurring in genes that are functionally essential to the bacterial host. Using the L1/JL-D2 PCR, pO26-CRL$_{111}$, pO26-CRL$_{125}$ and pO111-CRL$_{115}$ share an

identical 848 bp signature [1, 2, 61]. Epidemiological screens suggests that aEPEC and STEC may be important carriers of Tn*6026* and Tn*6029*. Twenty seven isolates of aEPEC with serotypes O111:H11, O111:H-, O123:H11, Ont:H9, Ont:H32, O180:H- and O162:H9 from bovine faeces sourced from cattle located at nine NSW properties separated by up to 900 km displayed features indicating that they are carriers of Tn*6026*. These features included a characteristic 848 bp PCR product encompassing Δ*intI1*-*dfrA5*-Δ3´-CS-ΔIS*26* present in sequenced plasmids pO26-CRL_{111} and pO26-CRL_{125} that carry Tn*6026* and phenotypic resistance to ampicillin, streptomycin, sulfathiazoles, and kanamycin. Eighteen of the 27 isolates carried the Tn*21* transposition genes *tnpM, tnpA* and *tnpR* [61]. These data suggest that derivate Tn*21* transposons on plasmids play a key role in mobilizing Tn*6026* in Australian bovine populations. Further work is needed to examine the plasmids that carry these derivate Tn*21* transposons. Preliminary work suggests that both small and large plasmids with different incompatibility markers are critical in this regard (our unpublished data).

3.2 Enteroaggregative haemorrhagic *E. coli* (EAHEC) carry variants of Tn*6029*

In May 2011, a major foodborne outbreak caused by Shiga toxin (*stx2*) producing *E. coli* occurred in Germany followed by a smaller outbreak in France. Cumulatively these outbreaks resulted in 4320 cases of bloody diarrhoea (BD), 850 cases of haemolytic uremic syndrome (HUS) and 82 deaths [63]. Draft genome assemblies of representative isolates from the 2011 HUS outbreak in Germany provided insight into the hybrid nature of their genomes as well as evidence of the evolution of the *E. coli* O104:H4 lineage that led to the classification of the strains as enteroaggregative haemorrhagic *E. coli* (EAHEC) [64]. The outbreak isolates were resistant to multiple antibiotics including an extended spectrum β-lactams, ampicillin, streptomycin, sulfamethoxazole, tetracycline and trimethoprim, limiting treatment options. While the extended spectrum β-lactamase gene CTX-M-15 was identified on a large plasmid, all draft genome assemblies fell short of providing a complete picture of key evolutionary events that created the chromosomally-located CRL encoding resistance to the remaining antibiotics [65, 66]. Tn*6029* and variants of it created by IS*26*-mediated gene re-organisation events feature prominently in

chromosomally-located CRL in outbreak and progenitor strains of EAHEC O104:H4 (Table 1 and Figure 2).

We have recently created a model that depicts the possible sequence of events that shaped the CRL in Genomic Island 3 (GI3) in outbreak and progenitor strains of EAHEC O104:H4 [40]. We propose that one of the key steps in the evolution of the CRL arose by a homologous recombination between regions found in two In2 derivatives, one of which is identical to plasmid pASL01a (JQ480155.1) [37] and the other identical to that in *Salmonella enterica* plasmids like pYT2 (AB605179) or pSal8934b (JF274992) [67]. Different IS*26*-mediated events within the boundaries of Tn*6029*C shaped the CRL in EAHEC O104:H4 outbreak strains (See Figure 3). In EAHEC strain 2011C-3493, we propose that a copy of IS*26* inserted into the IR_L of an existing IS*26* that abuts the bla_{TEM-1} gene in Tn*6029*C in a progenitor (Figure 3). This event generated two direct copies of IS*26* that flank the bla_{TEM-1} module. Subsequent loss of the bla_{TEM-1} module is consistent with the formation of a TU and generates a new derivative of the Tn*6029*-family known as Tn*6029*D. The presence of a 12-nucleotide signature sequence (TTTGCCCCATAT) and evidence of 8-bp direct repeats provides evidence that IS*26* has driven the creation of the CRL in 2011C-3493 and that Tn*6029*D is related to Tn*6029*C (Figure 3).

IS*26* has also played an important role in the formation of the CRL in EAHEC O104:H4 strain 2009EL-2050 from the Republic of Georgia. Here we propose that a copy of IS*26* inserted in the *tnpM* gene of Tn*21* generating an 8 bp target site duplication. This insertion event created two inwardly facing copies of IS*26* that flank the class 1 integron in Tn*21,* which thereafter underwent an inversion giving rise to the structure depicted in Figure 3. These events created a novel transposon known as Tn*6222*, described in Roy Chowdhury *et al* [40] that comprises two modules one of which is composed of a functional class 1 integron containing a *dfrA7* gene cassette and another with the bla_{TEM-1} gene.

GI3 is located adjacent to the *selC* tRNA gene in EAHEC, a known hotspot for the acquisition of genomic islands in *E. coli* [68, 69]. GI3 has a role in carbohydrate metabolism and virulence in avian

pathogenic *E. coli*. GI3 is known to target 23 bp genomic signatures found in *E. coli* genomes and may be mobile. As such GI3 may become significant as a carrier of virulence and antibiotic resistance genes in future emerging *E. coli* pathogens.

In both 2011C-3493 and 2009EL-2050 the 3´-CS comprises 1,210 nucleotides encoding *qacE*Δ*1* and a partial copy of the 3´ end of the *sul1* gene comprising only 79 nucleotides. Using the L1/JL-D2 PCR described above, a unique 2,082 bp amplicon is predicted and can be used to track the movement of EAHEC that carry this arrangement [70]. This 2,082 bp signature is unique to RD1 in 2011C-3493, 2009EL-2050 and to pASL01a indicating that the Tn*21* backbones in these mobile elements share evolutionary history. pASL01a contains a copy of Tn*6029*C in a derivate Tn*21* transposon (Tn*ASL01a*) located on a plasmid backbone comprising 5,168 bp that was unable to be typed using plasmid incompatibility typing methods [37]. PCR studies revealed that the transposon in pASL01a is found in commensal *E. coli* populations resistant to trimethoprim, sulfamethoxazole, and streptomycin from Nigeria and Ghana [37]. The unique 2,082 bp signature shared between commensal *E. coli* carrying pASL01a in West Africa and German EAHEC O104:H4 outbreak isolates is intriguing. Sprout seeds contaminated with multiply antibiotic resistant EAHEC O104:H4 sourced from Egypt and their subsequent trade to Germany and France is a key event that precipitated the 2011 outbreak [71]. Trimethoprim resistance occurs with high frequency in sub-Saharan Africa presumably in part because cotrimoxazole, a combination drug comprising trimethoprim and sulfamethoxazole is freely available without prescription and is used routinely to treat infections in the ~22 million HIV sufferers living in the region. Due to the limited availability and affordability of anti-retroviral treatments, HIV patients are reliant on the daily use of cotrimoxazole to control bacterial and parasitic infections [72]. In West Africa, resistance to trimethoprim among fecal commensal *E. coli* isolates has increased in frequency from about 40% in 1998 [73] to well over 60% from 2005 – 2009 [37]. Collectively these observations are consistent with the contention that MDR EAHEC O104:H4 originated in Africa.

4.1 Location of Tn*6029* and Tn*6026*

Tn*6026* and Tn*6029* variants are found on plasmids with different incompatibility groups including IncI1, IncZ, IncHI1, and IncHI2 several of which are widely disseminated. In addition to the identification of Tn*6029* and variants in GI3 [40], a Tn*6029* variant (Tn*6029*E) has been shown to reside within chromosomal islands in SGI11 and in strain 105/7/03 [25, 39]. Tn*6029* and Tn*6026* have been identified in serovars of *Salmonella enterica* of animal and human origin from Australia, Pakistan, Bangladesh, Vietnam and Italy [6, 25, 31, 33, 36, 39]. Studies in IncHI2 and IncHI1plasmids have shown that location of transposons can be used to delineate lineages [36, 74]. IncHI1 plasmids R27, pAKU1 and pHCM1 share highly similar backbone sequences comprising 164.4 kb but have showed divergence via the acquisition or loss of mobile elements [33]. Closely related IncHI1 plasmids pSRC27-H, pHCM1 and pAKU_1, carry Tn*6029*/Tn*6026* [33, 75]. Whilst the CRL in these three plasmids exhibit some differences they share common features like identical segments of DNA containing the same set of resistance determinants (Figure 2). This suggests that differences observed in the CRL have arisen by rearrangements of the segments subsequent to an insertion event involving a Tn*6026* or Tn*6029* family of transposon in an ancestral IncHI1 plasmid [36]. Tn*6026* has additionally been identified on two closely related IncF plasmids (pRSB107:AJ851089.1 and pRSB225:JX127248) isolated from the activated-sludge at a sewage treatment plant in Germany [32, 38]. IncF plasmids are known to carry antibiotic resistance genes as well as virulence genes in enteric bacteria and are commonly isolated from hospital patients [32, 76]

5.1 Mobilisation and evolution of CRL carrying Tn*6026* and Tn*6029*

Understanding how CRL evolve and move is critical to the future management of resistant infections. Genetic elements involved in the formation of CRL leave genetic signatures when transposition events occur [61]. Insertion elements and transposons generate duplications of specific length at the target site enabling evolutionary events to be deduced. The results of BLASTn searches using the sequence of Tn*6029* as a query and limiting our results to subjects with ≥99% nucleotide identity across the gene modules characteristic of Tn*6029* (i.e. the bla_{TEM} module and the RSF1010-derived Δ*repA-repC-sul2-*

strA-strB module), are depicted in Table 1. This search also identified copies of Tn*6026* of which Tn*6029* is a component. Sequence flanking the inverted repeats of each transposon-associated IS*26* element was also examined for 8-bp direct repeats.

First, it is apparent that Tn*6026*, typically arises as a result of insertion of Tn*4352* as a TU [29, 36]. When this occurs the direct repeat of the target IS*26* is shifted to the opposite side of the incoming TU. In support of this, six isolates containing a copy of Tn*6029* have the same duplication (CATCGGCG) at the terminal IS*26* as six isolates carrying a copy of Tn*6026*. In cases where this repeat is not present, it is most likely that homologous recombination between the terminal IS*26* element and a copy of IS*26* on another molecule removes evidence of the repeat. This is the case in pSRC27-H, an IncHI1 plasmid that has gained an additional segment of DNA containing bla_{TEM} and *aacC2* resistance genes. This has resulted in a different repeat at the terminal IS*26* and illustrates the rapid evolution that occurs within CRL due to insertion elements [77]. The gain and loss of Tn*4352* is likely to be driven by selective pressure of antibiotic use in environments where bacteria already possessing Tn*6029* exist [78]. Work in our lab, which found a predominance of Tn*6026* within MDR commensal *E. coli* exposed to extensive neomycin use, anecdotally supports this (unpublished results).

Tn*6026* and Tn*6029* appear to have been captured on larger mobile elements. These observations stem from the fact that there is little evidence of target site duplication at the boundaries of Tn*6026* and Tn*6029*. Both intact and truncated transposase modules and mercury resistance operons (derived in most part from Tn*21*) are present in all CRL where Tn*6069/6026* have been identified thus far. An important exception to this has been reported in *S. enterica* serovar Typhimurium [6]. Direct copies of an 8 bp duplication in the target sequence that arise when Tn*6026* is mobilized to a new location is found in multiple antibiotic resistant IncHI2 plasmid pSRC26 [6].

5.2 Chromosomal Islands that carry Tn*6029*

Two genomic islands, RR1/RR2 and SGI11 are found within monophasic *S. enterica* Typhimurium 105/7/03 and *S. enterica* Typhi respectively [25, 39]. These islands both contain the same variant of Tn*6029* in which the RSF1010 derived portion is inverted (Figure 2). In 105/7/03, the Tn*6029* variant is located within a glucose metabolism gene along with another region bounded by IS*26* elements containing a Tn*10* derivative and a Tn*21* mercury resistance module [25]. A transposase module is not present (Figure 2). PCR performed by Lucarelli indicates that these resistance regions are inserted at the same position in the chromosome of epidemiologically unrelated isolates of the same PFGE profile [25]. This is a concern for both human and animal health because resistance on the chromosome could easily disseminate amongst successful *S. enterica* clones causing enteric disease.

SGI11 in clinical *S. enterica* Typhi isolates from Bangladesh has a backbone similar to pAKU_1 [39]. Homologous regions include Tn*21* derivative transposition and mercury resistance modules and a Tn*9* backbone. Unlike pAKU_1, direct copies of IS*1* are present at each end of the resistance genes in SGI11 (Figure 2). Chiou reported that 9-bp direct repeats indicative of transposition flank each IS*1* [79]. The sequence of SGI11 in Genbank (KM023773.1) does not go beyond the IS*1* copies. If this is the case, it is an example of a larger scaffold mobilising Tn*6029* and Tn*21*.

6.1 Conclusions

Tn*6029* and Tn*6026* are disseminated worldwide and have been captured on plasmids with a range of different incompatibility groups including IncF, IncI1, IncHI1, IncHI2, and IncZ and on mobile chromosomal islands including variants of SGI1 and the potentially mobile GI3 in EAHEC (Figure 3). In most instances Tn*6029* and Tn*6026* are found in association with mercury resistance transposons, particularly Tn*21*. While the resistance genes present in Tn*6029/6026* confer resistance to older classes of antimicrobials these compounds are still utilised extensively in the developing countries where pathogens such as *S. enterica* remain a serious threat to human health [53]. Given the propensity for IS*26* to recognize copies of itself [29], variants of Tn*6029* and Tn*6026* are likely to increasingly feature in CRL

found in clinically relevant Gram negative bacteria. In support of this view, *aphA1*, *qnrB19*, and bla_{SHV-12}-like resistance genes flanked by direct copies of IS*26* have been reported in a multiple antibiotic resistant *S. enterica* serovar Typhimurium [30]. Further, direct copies of IS*26* flank a wide range of clinically relevant antibiotics including ESBLs, fluoroquinolones, fosfomycin and aminoglycosides [19, 21, 23, 24, 80]. Under antibiotic pressure, CRL carrying Tn*6029* or Tn*6026* are likely to acquire genes encoding resistance to third generation cephalosporins and ESBLs because they provide a suitable scaffold for the insertion of IS*26*-derived TUs carrying resistance genes.

References

1. Venturini, C., et al., *Multiple antibiotic resistance gene recruitment onto the enterohemorrhagic Escherichia coli virulence plasmid.* FASEB J, 2010. **24**(4): p. 1160-6.
2. Venturini, C., et al., *Sequences of two related multiple antibiotic resistance virulence plasmids sharing a unique IS26-related molecular signature isolated from different Escherichia coli pathotypes from different hosts.* PLoS One, 2013. **8**(11): p. e78862.
3. Hou, J., et al., *Detection of the plasmid-encoded fosfomycin resistance gene fosA3 in Escherichia coli of food-animal origin.* J Antimicrob Chemother, 2013. **68**(4): p. 766-70.
4. Deng, H., et al., *Identification of the multi-resistance gene cfr in Escherichia coli isolates of animal origin.* PLoS One, 2014. **9**(7): p. e102378.
5. He, L., et al., *Complete nucleotide sequence of pHN7A8, an F33:A-:B- type epidemic plasmid carrying blaCTX-M-65, fosA3 and rmtB from China.* J Antimicrob Chemother, 2013. **68**(1): p. 46-50.
6. Cain, A.K., et al., *Transposons related to Tn1696 in IncHI2 plasmids in multiply antibiotic resistant Salmonella enterica serovar Typhimurium from Australian animals.* Microb Drug Resist, 2010. **16**(3): p. 197-202.

7. Herrero-Fresno, A., et al., *Transposition and homologous recombination drive evolution of pUO-StVR2, a multidrug resistance derivative of pSLT, the virulence plasmid specific of Salmonella enterica serovar Typhimurium.* Infect Genet Evol, 2015. **29**: p. 99-102.
8. Martinez, E., et al., *Establishment and multi drug resistance evolution of ST235 Pseudomonas aeruginosa strains in the intensive care unit of a Colombian hospital.* Res Microbiol, 2014. **165**(10): p. 852-6.
9. Uemura, S., et al., *Acquisition of a transposon encoding extended-spectrum beta-lactamase SHV-12 by Pseudomonas aeruginosa isolates during the clinical course of a burn patient.* Antimicrob Agents Chemother, 2010. **54**(9): p. 3956-9.
10. Hudson, C.M., et al., *Resistance determinants and mobile genetic elements of an NDM-1-encoding Klebsiella pneumoniae strain.* PLoS One, 2014. **9**(6): p. e99209.
11. Nigro, S.J., et al., *A novel family of genomic resistance islands, AbGRI2, contributing to aminoglycoside resistance in Acinetobacter baumannii isolates belonging to global clone 2.* J Antimicrob Chemother, 2013. **68**(3): p. 554-7.
12. Post, V., P.A. White, and R.M. Hall, *Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant Acinetobacter baumannii.* J Antimicrob Chemother, 2010. **65**(6): p. 1162-70.
13. Siebor, E. and C. Neuwirth, *Proteus genomic island 1 (PGI1), a new resistance genomic island from two Proteus mirabilis French clinical isolates.* J Antimicrob Chemother, 2014. **69**(12): p. 3216-20.
14. Siebor, E. and C. Neuwirth, *Emergence of Salmonella genomic island 1 (SGI1) among Proteus mirabilis clinical isolates in Dijon, France.* J Antimicrob Chemother, 2013. **68**(8): p. 1750-6.
15. Du, X.X., et al., *Genetic characteristics of blaNDM-1-positive plasmid in Citrobacter freundii isolate separated from a clinical infectious patient.* J Med Microbiol, 2013. **62**(Pt 9): p. 1332-7.
16. Chu, P.Y. and C.F. Peng, *Genetic environments of the transferable plasmid-mediated blaCTX-M-3 gene in Serratia marcescens isolates.* Jpn J Infect Dis, 2014. **67**(1): p. 58-61.

17. Conlan, S., et al., *Single-molecule sequencing to track plasmid diversity of hospital-associated carbapenemase-producing Enterobacteriaceae.* Sci Transl Med, 2014. **6**(254): p. 254ra126.
18. Vincent, A.T., et al., *Detection of Variants of the pRAS3, pAB5S9, and pSN254 Plasmids in Aeromonas salmonicida subsp. salmonicida: Multidrug Resistance, Interspecies Exchanges, and Plasmid Reshaping.* Antimicrob Agents Chemother, 2014. **58**(12): p. 7367-74.
19. Jiang, Y., et al., *Dissemination of a clone carrying a fosA3-harbouring plasmid mediates high fosfomycin resistance rate of KPC-producing Klebsiella pneumoniae in China.* Int J Antimicrob Agents, 2014.
20. Wrighton, C.J. and P. Strike, *A pathway for the evolution of the plasmid NTP16 involving the novel kanamycin resistance transposon Tn4352.* Plasmid, 1987. **17**(1): p. 37-45.
21. Zhang, W.J., et al., *A novel conjugative plasmid from Escherichia coli of swine origin that co-harbors the multi-resistance gene cfr and the extended-spectrum beta-lactamase gene blaCTX-M-14b.* Antimicrob Agents Chemother, 2014.
22. Sun, J., et al., *Complete Nucleotide Sequence of cfr-Carrying IncX4 Plasmid pSD11 from Escherichia coli.* Antimicrob Agents Chemother, 2015. **59**(1): p. 738-41.
23. Billard-Pomares, T., et al., *Characterization of a P1-like bacteriophage carrying an SHV-2 extended-spectrum beta-lactamase from an Escherichia coli strain.* Antimicrob Agents Chemother, 2014. **58**(11): p. 6550-7.
24. Doi, Y., et al., *Whole-genome assembly of Klebsiella pneumoniae coproducing NDM-1 and OXA-232 carbapenemases using single-molecule, real-time sequencing.* Antimicrob Agents Chemother, 2014. **58**(10): p. 5947-53.
25. Lucarelli, C., et al., *Nucleotide sequence of the chromosomal region conferring multidrug resistance (R-type ASSuT) in Salmonella Typhimurium and monophasic Salmonella Typhimurium strains.* J Antimicrob Chemother, 2012. **67**(1): p. 111-4.
26. Mollet, B., et al., *Nucleotide sequence of IS26, a new prokaryotic mobile genetic element.* Nucleic Acids Res, 1983. **11**(18): p. 6319-30.

27. Mollet, B., S. Iida, and W. Arber, *Gene organization and target specificity of the prokaryotic mobile genetic element IS26.* Mol Gen Genet, 1985. **201**(2): p. 198-203.
28. Iida, S., et al., *Functional characterization of the prokaryotic mobile genetic element IS26.* Mol Gen Genet, 1984. **198**(1): p. 84-9.
29. Harmer, C.J., R.A. Moran, and R.M. Hall, *Movement of IS26-associated antibiotic resistance genes occurs via a translocatable unit that includes a single IS26 and preferentially inserts adjacent to another IS26.* MBio, 2014. **5**(5): p. e01801-14.
30. Dionisi, A.M., et al., *Molecular characterization of multidrug-resistant strains of Salmonella enterica serotype Typhimurium and Monophasic variant (S. 4,[5],12:i:-) isolated from human infections in Italy.* Foodborne Pathog Dis, 2009. **6**(6): p. 711-7.
31. Parkhill, J., et al., *Complete genome sequence of a multiple drug resistant Salmonella enterica serovar Typhi CT18.* Nature, 2001. **413**(6858): p. 848-52.
32. Szczepanowski, R., et al., *The 120 592 bp IncF plasmid pRSB107 isolated from a sewage-treatment plant encodes nine different antibiotic-resistance determinants, two iron-acquisition systems and other putative virulence-associated functions.* Microbiology, 2005. **151**(Pt 4): p. 1095-111.
33. Holt, K.E., et al., *Multidrug-resistant Salmonella enterica serovar paratyphi A harbors IncHI1 plasmids similar to those found in serovar typhi.* J Bacteriol, 2007. **189**(11): p. 4257-64.
34. Ogura, Y., et al., *Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic Escherichia coli.* Proc Natl Acad Sci U S A, 2009. **106**(42): p. 17939-44.
35. Ahmed, S.A., et al., *Genomic comparison of Escherichia coli O104:H4 isolates from 2009 and 2011 reveals plasmid, and prophage heterogeneity, including shiga toxin encoding phage stx2.* PLoS One, 2012. **7**(11): p. e48228.
36. Cain, A.K. and R.M. Hall, *Evolution of a multiple antibiotic resistance region in IncHI1 plasmids: reshaping resistance regions in situ.* J Antimicrob Chemother, 2012. **67**(12): p. 2848-53.

37. Labar, A.S., et al., *Regional dissemination of a trimethoprim-resistance gene cassette via a successful transposable element.* PLoS One, 2012. **7**(5): p. e38142.
38. Wibberg, D., et al., *The IncF plasmid pRSB225 isolated from a municipal wastewater treatment plant's on-site preflooder combining antibiotic resistance and putative virulence functions is highly related to virulence plasmids identified in pathogenic E. coli isolates.* Plasmid, 2013. **69**(2): p. 127-37.
39. Chiou, C.S., et al., *Chromosome-mediated multidrug resistance in Salmonella enterica serovar Typhi.* Antimicrob Agents Chemother, 2014.
40. Roy Chowdhury, P., I.G. Charles, and S.P. Djordjevic, *A Role for Tn6029 in the Evolution of the Complex Antibiotic Resistance Gene Loci in Genomic Island 3 in Enteroaggregative Hemorrhagic Escherichia coli O104:H4.* PLoS One, 2015. **10**(2): p. e0115781.
41. Partridge, S.R. and R.M. Hall, *Evolution of transposons containing blaTEM genes.* Antimicrob Agents Chemother, 2005. **49**(3): p. 1267-8.
42. Bailey, J.K., et al., *Distribution of the blaTEM gene and blaTEM-containing transposons in commensal Escherichia coli.* J Antimicrob Chemother, 2011. **66**(4): p. 745-51.
43. Goussard, S. and P. Courvalin, *Sequence of the genes blaT-1B and blaT-2.* Gene, 1991. **102**(1): p. 71-3.
44. Pansegrau, W., et al., *Complete nucleotide sequence of Birmingham IncP alpha plasmids. Compilation and comparative analysis.* J Mol Biol, 1994. **239**(5): p. 623-63.
45. Partridge, S.R. and R.M. Hall, *In34, a complex In5 family class 1 integron containing orf513 and dfrA10.* Antimicrob Agents Chemother, 2003. **47**(1): p. 342-9.
46. Williams, L.E., et al., *Facile recovery of individual high-molecular-weight, low-copy-number natural plasmids for genomic sequencing.* Appl Environ Microbiol, 2006. **72**(7): p. 4899-906.
47. Cloeckaert, A., et al., *Dissemination of an extended-spectrum-beta-lactamase blaTEM-52 gene-carrying IncI1 plasmid in various Salmonella enterica serovars isolated from poultry and humans*

in Belgium and France between 2001 and 2005. Antimicrob Agents Chemother, 2007. **51**(5): p. 1872-5.

48. Yau, S., et al., *RSF1010-like plasmids in Australian Salmonella enterica serovar Typhimurium and origin of their sul2-strA-strB antibiotic resistance gene cluster.* Microb Drug Resist, 2010. **16**(4): p. 249-52.

49. Guerry, P., J. van Embden, and S. Falkow, *Molecular nature of two nonconjugative plasmids carrying drug resistance genes.* J Bacteriol, 1974. **117**(2): p. 619-30.

50. Radstrom, P., G. Swedberg, and O. Skold, *Genetic analyses of sulfonamide resistance and its dissemination in gram-negative bacteria illustrate new aspects of R plasmid evolution.* Antimicrob Agents Chemother, 1991. **35**(9): p. 1840-8.

51. Hochhut, B., et al., *Molecular analysis of antibiotic resistance gene clusters in vibrio cholerae O139 and O1 SXT constins.* Antimicrob Agents Chemother, 2001. **45**(11): p. 2991-3000.

52. Daly, M., et al., *Comparison of multidrug resistance gene regions between two geographically unrelated Salmonella serotypes.* J Antimicrob Chemother, 2005. **55**(4): p. 558-61.

53. Fabrega, A. and J. Vila, *Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation.* Clin Microbiol Rev, 2013. **26**(2): p. 308-41.

54. Kariuki, S., et al., *Characterization of multidrug-resistant typhoid outbreaks in Kenya.* J Clin Microbiol, 2004. **42**(4): p. 1477-82.

55. Tran, H.H., et al., *Risk factors associated with typhoid fever in Son La province, northern Vietnam.* Trans R Soc Trop Med Hyg, 2005. **99**(11): p. 819-26.

56. Nataro, J.P. and J.B. Kaper, *Diarrheagenic Escherichia coli.* Clin Microbiol Rev, 1998. **11**(1): p. 142-201.

57. Hornitzky, M.A., et al., *Bovine feces from animals with gastrointestinal infections are a source of serologically diverse atypical enteropathogenic Escherichia coli and Shiga toxin-producing E. coli strains that commonly possess intimin.* Appl Environ Microbiol, 2005. **71**(7): p. 3405-12.

58. Djordjevic, S.P., et al., *Virulence properties and serotypes of Shiga toxin-producing Escherichia coli from healthy Australian slaughter-age sheep.* J Clin Microbiol, 2001. **39**(5): p. 2017-21.
59. Hornitzky, M.A., et al., *Virulence properties and serotypes of Shiga toxin-producing Escherichia coli from healthy Australian cattle.* Appl Environ Microbiol, 2002. **68**(12): p. 6439-45.
60. Martinez, J.L. and F. Baquero, *Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance.* Clin Microbiol Rev, 2002. **15**(4): p. 647-79.
61. Dawes, F.E., et al., *Distribution of class 1 integrons with IS26-mediated deletions in their 3'-conserved segments in Escherichia coli of human and animal origin.* PLoS One, 2010. **5**(9): p. e12754.
62. Leverstein-van Hall, M.A., et al., *Multidrug resistance among Enterobacteriaceae is strongly associated with the presence of integrons and is independent of species or isolate origin.* J Infect Dis, 2003. **187**(2): p. 251-9.
63. Mariani-Kurkdjian, P., et al., *Escherichia coli O104:H4 south-west France, June 2011.* Lancet Infect Dis, 2011. **11**(10): p. 732-3.
64. Brzuszkiewicz, E., et al., *Genome sequence analyses of two isolates from the recent Escherichia coli outbreak in Germany reveal the emergence of a new pathotype: Entero-Aggregative-Haemorrhagic Escherichia coli (EAHEC).* Arch Microbiol, 2011. **193**(12): p. 883-91.
65. Grad, Y.H., et al., *Comparative genomics of recent Shiga toxin-producing Escherichia coli O104:H4: short-term evolution of an emerging pathogen.* MBio, 2013. **4**(1): p. e00452-12.
66. Baquero, F. and R. Tobes, *Bloody coli: a gene cocktail in Escherichia coli O104:H4.* MBio, 2013. **4**(1): p. e00066-13.
67. Tamamura, Y., et al., *Complete nucleotide sequences of virulence-resistance plasmids carried by emerging multidrug-resistant Salmonella enterica Serovar Typhimurium isolated from cattle in Hokkaido, Japan.* PLoS One, 2013. **8**(10): p. e77644.

68. Schmidt, H., et al., *Identification and characterization of a novel genomic island integrated at selC in locus of enterocyte effacement-negative, Shiga toxin-producing Escherichia coli.* Infect Immun, 2001. **69**(11): p. 6863-73.

69. Chouikha, I., et al., *A selC-associated genomic island of the extraintestinal avian pathogenic Escherichia coli strain BEN2908 is involved in carbohydrate uptake and virulence.* J Bacteriol, 2006. **188**(3): p. 977-87.

70. Djordjevic, S.P., H.W. Stokes, and P. Roy Chowdhury, *Mobile elements, zoonotic pathogens and commensal bacteria: conduits for the delivery of resistance genes into humans, production animals and soil microbiota.* Front Microbiol, 2013. **4**: p. 86.

71. Buchholz, U., et al., *German outbreak of Escherichia coli O104:H4 associated with sprouts.* N Engl J Med, 2011. **365**(19): p. 1763-70.

72. Hamel, M.J., et al., *Does cotrimoxazole prophylaxis for the prevention of HIV-associated opportunistic infections select for resistant pathogens in Kenyan adults?* Am J Trop Med Hyg, 2008. **79**(3): p. 320-30.

73. Okeke, I.N., S.T. Fayinka, and A. Lamikanra, *Antibiotic resistance in Escherichia coli from Nigerian students, 1986 1998.* Emerg Infect Dis, 2000. **6**(4): p. 393-6.

74. Cain, A.K. and R.M. Hall, *Evolution of IncHI2 plasmids via acquisition of transposons carrying antibiotic resistance determinants.* J Antimicrob Chemother, 2012. **67**(5): p. 1121-7.

75. Wain, J., et al., *Molecular Analysis of incHI1 Antimicrobial Resistance Plasmids from Salmonella Serovar Typhi Strains Associated with Typhoid Fever.* Antimicrobial Agents and Chemotherapy, 2003. **47**(9): p. 2732-2739.

76. Burland, V., et al., *The complete DNA sequence and analysis of the large virulence plasmid of Escherichia coli O157:H7.* Nucleic Acids Res, 1998. **26**(18): p. 4196-204.

77. Golebiewski, M., et al., *Complete nucleotide sequence of the pCTX-M3 plasmid and its involvement in spread of the extended-spectrum beta-lactamase gene blaCTX-M-3.* Antimicrob Agents Chemother, 2007. **51**(11): p. 3789-95.

78. Lester, S.C., et al., *The carriage of Escherichia coli resistant to antimicrobial agents by healthy children in Boston, in Caracas, Venezuela, and in Qin Pu, China.* N Engl J Med, 1990. **323**(5): p. 285-9.

79. Shiga, Y., Y. Sekine, and E. Ohtsubo, *Transposition of IS1 circles.* Genes Cells, 1999. **4**(10): p. 551-61.

80. Dolejska, M., et al., *Complete sequences of IncHI1 plasmids carrying blaCTX-M-1 and qnrS1 in equine Escherichia coli provide new insights into plasmid evolution.* J Antimicrob Chemother, 2014. **69**(9): p. 2388-93.

Table Legend:

Table 1. Summary of reported instances of Tn*6026* and Tn*6029* within *Enterobacteriaceae*

Figure Legends:

Figure 1. Schematic representation of the composite transposon-like structures seen in Tn*6026*, *Tn6029* and derivatives. Figures not to scale. Open boxes represent genes; thick vertical bars represent inverted repeats of IS*26*; blue boxes represent transposition genes; red boxes represent mercury resistance genes; green boxes represent integron associated genes. Horizontal arrows represent direction of gene transcription. Initial and terminal inverted repeats of integrons are annotated IR*i* and IR*t* respectively.

Figure 2. Schematic representation of genetic backbones on which Tn*6026* and Tn*6029* derivatives have been observed. 8 bp direct repeats produced by the insertion of IS*26* insertion elements at the ends of each transposon are annotated.

Figure 3. Proposed evolutionary model for the formation of CRL within 2009EL-2050 and 2011C-3493 adapted from Roy Chowdhury et al[40].

Table 1

Transposon	Organism	Plasmid name	Plasmid type	Source	Location	Genbank	Reference
Tn*6026*	*S. enterica* Typhimurium	pSRC27-H	IncHI1	Equine	Australia	HQ840942.1	[36]
Tn*6026*	*S. enterica* Typhimurium	pSRC26	IncHI2	Bovine	Australia	GQ150541.1	[6]
Tn*6026*	*E. coli* O26:H- 'O6877' EHEC	pO26-CRL-111	IncI1	Human clinical	Australia	GQ259888.1	[1]
Tn*6026*	*E. coli* O26:H- 'O6877' EHEC	pO26-CRL-125	IncZ	Human clinical	Australia	KC340960.1	[2]
Tn*6026*	*E. coli* O111 aEPEC	pO111-CRL-115	IncZ	Bovine	Australia	KC340959.1	[2]
Tn*6026*	*E. coli* O111:H-	pO111_1	IncHI1	Human clinical	Japan	AP010961.1	[34]
Tn*6026*	Uncultured bacterium	pRSB107	IncF	Sewage effluent	Germany	AJ851089.1	[32]
Tn*6026*	Uncultured bacterium	pRSB225	IncF	Sewage effluent	Germany	JX127248.1	[38]
Tn*6029*	*S. enterica* Paratyphi A	pAKU_1	IncHI1	Human clinical	Pakistan	AM412236.1	[33]
Tn*6029B*	*S. enterica* Typhi	pHCM1	IncHI1	Human clinical	Vietnam	AL513383.1	[31]
Tn*6029B*	*E. coli* O104:H4 '2009EL-2050'	RD1	Chromosomal	Human clinical	Georgia	CP003297.1	[35]
Tn*6029C*	*E. coli* O104:H4 '2009EL-2071'	RD1	Chromosomal	Human clinical	Georgia	CP003301.1	[35]
Tn*6029C*	*E. coli*	pASL01a	No type	Healthy human	Nigeria	JQ480155.1	[37]
Tn*6029E*	*S. enterica* Typhi	SGI11	Chromosomal	Human clinical	Bangladesh	KM023773.1	[39]
Tn*6029E*	*S. enterica*	105/7/03 resistance region	Chromosomal	Human clinical	Italy	HQ331538.1	[25]

Figure 1. **2 column width**

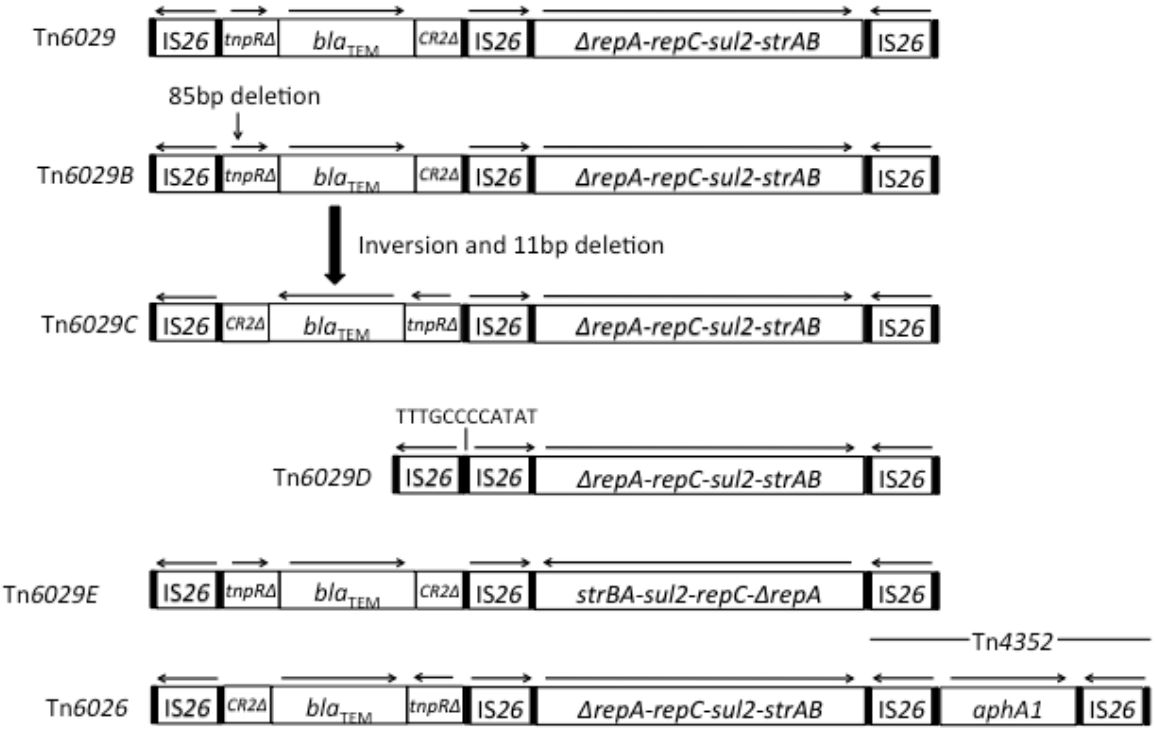

Figure 2. **1.5 column width**

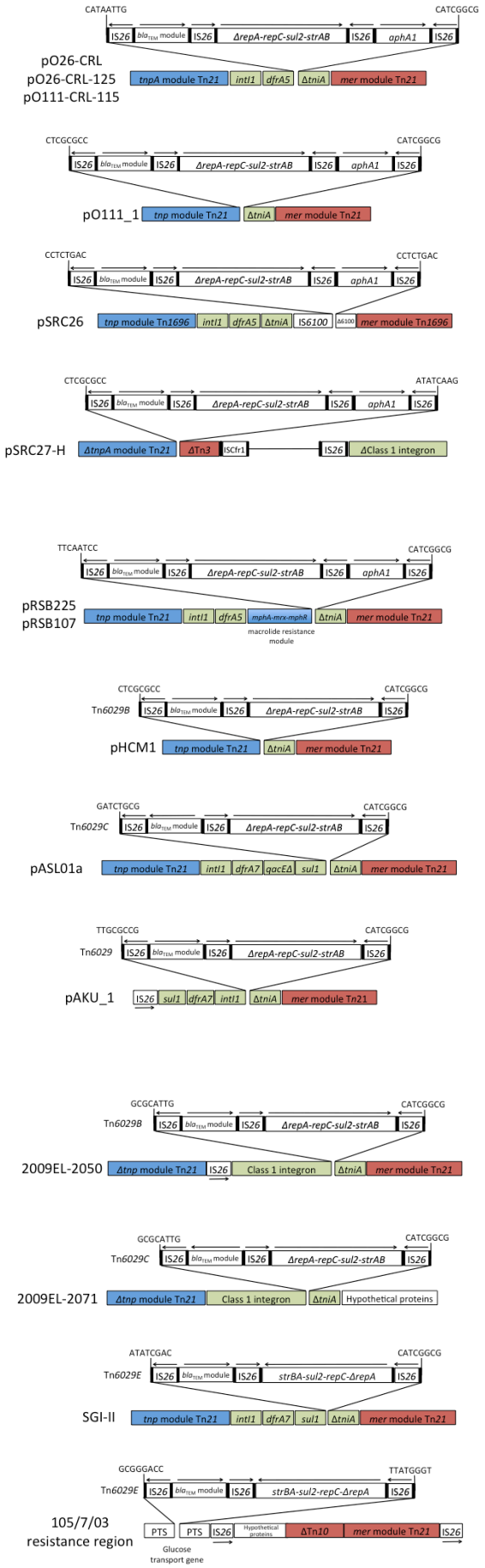

Figure 3. **2 column width**