TnCentral: A Prokaryotic Transposable Element Database and Web Portal for Transposon Analysis

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Importance

The ability of bacteria to undergo rapid evolution and adapt to changing environmental circumstances drives the public health crisis of multiple antibiotic resistance as well as outbreaks of disease in economically important agricultural crops and animal husbandry. Prokaryotic transposable elements (TE) play a critical role in this. Many carry "passenger genes" (not required for the transposition process) conferring resistance to antibiotics or heavy metals or causing disease in plants and animals. Passenger genes are spread by the normal TE transposition activities, by insertion into plasmids which then spread via conjugation within and across bacterial populations. Thus, an understanding of TE composition and transposition mechanisms is key to developing strategies to combat bacterial pathogenesis. Toward this end, we have developed TnCentral, a bioinformatics resource dedicated to describing and exploring the structural and functional features of prokaryotic TE and whose use is intuitive and accessible to users with or without bioinformatics expertise.

Abstract

We describe here the structure and organization of TnCentral (https://tncentral.proteininformationresource.org/), a web resource for prokaryotic transposable elements (TE). TnCentral currently contains ~400 carefully annotated TE, including transposons from the Tn*3,* Tn*7,* Tn*402* and Tn*554* families, compound transposons, integrons and associated insertion sequences (IS). These TE carry passenger genes, including genes conferring resistance to over 25 classes of antibiotics and nine types of heavy metal as well as genes responsible for pathogenesis in plants, toxin/antitoxin gene pairs, transcription factors and genes involved in metabolism. Each TE has its own entry page providing details about its transposition genes, passenger genes, and other sequence features required for transposition as well as a graphical map of all features. TnCentral content can be browsed and queried through text and sequence-based searches with a graphic output. We describe three use cases, which illustrate how the search interface, results tables, and entry pages can be used to explore and compare TEs.

TnCentral also includes downloadable software to facilitate user-driven identification, with manual annotation, of certain types of TE in genomic sequences.

Through the TnCentral homepage, users can also access TnPedia which provides comprehensive reviews of the major TE families including an extensive general section, and specialised sections with descriptions of insertion sequence and transposon families. TnCentral and TnPedia are intuitive resources that can be used by clinicians and scientists to assess TE diversity in clinical, veterinary and environmental samples.

Introduction

Transposable elements (TE) are key facilitators of bacterial evolution and adaptation and central players in the emergence of antibiotic and heavy metal resistance and to the transmission of virulence and pathogenic traits. Some TE can capture "passenger genes" (genes not involved in the transposition process) encoding these traits and transmit them to plasmids, where they accumulate and are then transferred within and between bacterial populations by conjugation. TE also contribute significantly to the on-going reorganization of bacterial genomes giving rise to new strains that are more adept at proliferating in clinical and agricultural environments, as well as in natural ecosystems.

Understanding TE nature, distribution, and activity is therefore an indispensable part of the struggle to cope with the public health crisis of multiple antibiotic resistance (ABR) [1,2]. To understand the impact of TE on bacterial populations, it is essential to provide a detailed description and catalog of TE structures and diversity. The simplest TE, known as Insertion Sequences (IS), have a profound impact on genome organization and function (see [3–7]) but do not themselves generally carry integrated passenger genes. There are a large number of significantly more complex TE (Figure 1), arguably even more important in the global emergence of ABR and other virulence and pathogenicity traits. These are generically called transposons and may carry multiple passenger genes, including some of the most clinically important antibiotic resistance genes. Like IS, these TE are grouped into a number of distinct families with characteristic organizations [3]. Their transposition activities facilitate the rapid spread of groups of antibiotic resistance genes and promote their horizontal transfer. Yet another important aspect of their impact is their ability to assemble passenger genes into resistance clusters [8,9]. While there appears to be a wide-spread appreciation that mobile plasmids are responsible for the spread of antibiotic resistance, it

96 is less well-known that IS and transposons are the conduits that transfer this information
97 between chromosomes and plasmids.

98 There are a number of other bioinformatics resources that cover aspects of
99 prokaryotic TE biology. These include databases for TE passenger genes such as antibiotic
100 resistance (CARD [10], ARDB [11]) or toxin/antitoxin gene pairs (TADB [12], TASmania [13])
101 as well as the various classes of TE themselves such as insertion sequences (ISfinder [14]),
102 integrons (INTEGRALL [15]), integrative conjugative elements, ICE (ICEberg [16,17]),
103 plasmids (PlasmidFinder [18]) or more general databases which include a variety of these
104 genome components (ACLAME [19–21]). However, there is a need for a resource that
105 collects, compares and collates detailed information on the various different classes of TE
106 that are responsible for the transmission of medically and economically important
107 passenger genes in an intuitive and accessible way.

108 Here, we describe TnCentral (https://tncentral.proteininformationresource.org/), a
109 database of detailed structural and functional information on bacterial TE. Additionally,
110 TnCentral provides access to TnPedia (https://tnpedia.fcav.unesp.br/), a comprehensive
111 encyclopedia describing the current state of our knowledge of the biology of IS and
112 transposons. Together, TnCentral and TnPedia provide a detailed description of TE diversity
113 with easy-to-understand graphics outputs that are accessible to users without significant
114 bioinformatic knowledge. They allow users to rapidly analyse the landscape of TE in
115 genomes (chromosomes and plasmids) isolated from clinical, veterinary and environmental
116 samples.

117 Results

118 TnCentral Website Content

119 As of May 2021, TnCentral contains information on ~400 TE. About half of these TE are *Tn3*-
120 family transposons. The remainder are integrons, compound transposons, transposons from
121 the *Tn402*, *Tn554* and *Tn7* families, and IS that are associated with TE or are part of
122 compound transposons (Supplementary Table 1). They include TE with resistance to over 25
123 different classes of antibiotics and nine different heavy metals. The collection also contains
124 TE that carry a toxin/antitoxin system for bacterial plasmid maintenance [22–24] and TE
125 from xanthomonads carrying genes for plant pathogenicity.

126

127 **TnCental Web Portal**

128 The TnCentral home page is designed to give the user easy access to the contents of
129 TnCentral with a number of options (Figure 2A), including:

130 **TnCentral Search** *(search of the TnCentral database),*

131 **Sequence Search** *(BLAST-like search for sequence similarities in the database),*

132 **Browse Tn list** *(view all TE in TnCentral),*

133 **Tnfinder Software** *(access to downloadable scripts for identifying potential TE in*
134 *sequence databases),*

135 **Documentation** *(downloadable documentation for TnCentral),*

136 **For Curators** *(detailed curation guidelines),*

137 **TnPedia** *(TE Encyclopedia),*

138 **Related links***, and*

139 **Feedback***.*

140

141 **TnCentral Search.** The interface provides a variety of search functions divided into two
142 search types: ***Transposon search*** and ***Gene search*** (Figure 2B).

143

144 ***Transposon search****.* The transposon collection can be searched using the transposon
145 **name**, **synonyms** which may have been used in the literature, the **type** of mobile genetic
146 element (e.g., insertion sequence, transposon or integron), the **family** and **subgroup** to
147 which it belongs, the **host organism**, **country** of identification and **date** of identification. The
148 latter three search terms are intended for use in epidemiological tracking. These search
149 terms result in a table that can be sorted, customized and downloaded (See Use Case #1,
150 below).

151 ***Gene search****.* It is also possible to search for TE-associated genes by name, by class
152 (Transposase, Accessory Gene or Passenger Gene) or by function (Antibiotic Resistance,
153 Heavy Metal Resistance) and to retrieve information on the transposons in which they are
154 found (see Use Case #2, below).

155

156 ***Sequence Search.*** Sequence Search allows users to perform sequence similarity

searches using BLAST [26,27] (see Use Case #3, below). By default, the search database is the TnCentral database, but the page also provides links to BLAST against the ISfinder (https://isfinder.biotoul.fr/blast.php), NCBI https://blast.ncbi.nlm.nih.gov/Blast.cgi), Comprehensive Antibiotic Resistance Database (CARD; https://card.mcmaster.ca/analyze/blast) and the Toxin-Anitoxin (TADB; https://bioinfo-mml.sjtu.edu.cn/TADB2/) databases. The BLAST tool automatically distinguishes between DNA and protein query sequences.

Browse Tn list. This option allows the user to browse the entire TnCentral database.

The Transposon entry page. All of the search and browse options provide links to entry pages for each TE (Figure 3), which provide detailed information about TE features and origins. The page includes: 1) host information: host species, strain, and plasmid/chromosome in which the transposon was found as well as the date and geographic location of the isolate; 2) a graphic representation of the annotated sequence with color-coded features; 3) Terminal Inverted Repeats (IR); 4) DNA sequence; 5) internal recombination sites (e.g. *res* sites) including their coordinates, length and DNA sequence; 6) ORF summary, which includes all protein coding genes in the order in which they appear, 5'-3', in the TE sequence, the element with which they are associated (important for nested TE in which one TE is inserted into another), their coordinates, their class (e.g., Transposase, Accessory Gene, Passenger Gene) and subclass (e.g., Antibiotic Resistance, Heavy Metal Resistance) and their relative orientation within the TE; 7) a detailed ORF description including the amino acid sequence; 8) if applicable, a table of Internal Transposable Elements (TE inserted in the main element) including the name, type location and length; 9) if applicable, a table of Internal Repeats (repeat elements, other than the terminal inverted repeats, that are found within the TE), including the associated TE, coordinates and DNA sequence; 10) Bibliographic references with direct links to PubMed [25]. Each section can be collapsed using a button on the right-hand side of the section heading. Sections can be viewed either by scrolling down on the page or by clicking on the section name in the menu located on the left side of the page. Sequence files in FASTA and GenBank format can be downloaded using the links on the left side of the page under the menu.

188

189

190 **Tnfinder Software.** This section provides three user-downloadable scripts written in-house
191 for identifying transposons. They provide users with local control over analyses and help
192 them screen datasets containing large numbers of genomic sequences using their own
193 servers for identifying potential candidates and which are then manually curated.

194

195 ***Tn3 Transposon Finder*** (Tn3_finder) performs the automatic prediction of
196 transposable elements of the Tn*3* family in bacteria and archaea. It compares user-provided
197 bacterial and archaeal genome sequences to custom Tn*3* transposase and resolvase
198 databases by BLAST alignments. The criteria for identifying potential transposon regions
199 according to similarity, coverage and distance values can be adjusted by the user. Additional
200 ORFs that might be related to passenger genes are also predicted, and flanking regions can
201 also be retrieved and analyzed. The automatic prediction results are written in report files
202 and pre-annotated GenBank files to help in subsequent manual curation. Tn*3*_finder allows
203 for the concurrent analysis of multiple genomes by multithreading.

204 ***Composite Transposon Finder*** (TnComp_finder) predicts the putative composite
205 transposons in bacterial and archaeal genomes based on insertion sequence replicas in a
206 relatively short span. It works by comparing nucleotide sequences from bacterial and
207 archaeal genomes to a custom transposon database and identifying duplicated transposons
208 in user-defined genomic regions from BLAST alignments. Similar to Tn3_finder,
209 multithreaded analyses of multiple genomes are available and parameters for similarity,
210 coverage, distance and flanking regions can be adjusted by the user. Results are written in
211 report files and pre-annotated GenBank files to help in subsequent manual curation.

212 ***Antibiotic Resistance Gene-associated IS Finder*** (ISAbR_finder) is an experimental
213 program for the automatic prediction of antibiotic resistance genes associated with known
214 IS elements derived from the ISfinder database and has yet to be tested extensively. It
215 works by comparing IS nucleotide sequences from bacterial and archaeal genomes to a
216 custom antibiotic resistance database based on the parsing of BLAST alignment results,
217 using a number of parameters that can be customized by the user for stricter or more
218 relaxed criteria and allowing multithreaded alignments of multiple genomes. ISAbR_finder

219 also produces report files and pre-annotated GenBank files on which the recommended
220 manual curation should be performed.
221
222 **Documentation.** This section, which can be downloaded as a pdf file, provides a short
223 background description of transposons and TnCentral together with a short description of
224 the **curation workflow** and of planned future developments.
225
226 **For Curators.** This section provides a detailed description of the curation workflow used in
227 generating the annotated TnCentral data.

228 **TnPedia.** TnCentral provides access from the homepage to TnPedia, an online knowledge
229 base which contains information concerning transposition in prokaryotes. TnPedia is
230 developed using MediaWiki (https://www.mediawiki.org) and can also be accessed directly
231 (https://tnpedia.fcav.unesp.br/). It is structured into three main sections: **General**
232 **Information, IS Families and Transposon Families** (Figure 4).
233 The **General Information** section provides a series of clickable sections with an
234 extensive bibliography and direct links to the articles in PubMed. It includes a historical
235 perspective, definitions and descriptions of a variety of prokaryotic TE, the basic
236 mechanisms involved in their movement and the enzymes involved in these processes. It
237 also contains information describing their impact on their host genomes and how their
238 activities are controlled.
239 The **IS Families** section consists of individual chapters describing each of the ~25 IS
240 families in detail and covers, where possible, the identification of the founding members,
241 their organisation, distribution, variability and phylogenetic relationships, regulation of their
242 transposition, impact on their host genomes, and their transposition mechanisms including
243 genetic, biochemical and structural studies.
244 The **Transposon Families** section describes each transposon family with similar
245 information to that included in the IS family descriptions but, in addition, including a
246 detailed description of their structures and the passenger genes which they may carry.
247
248 **Examples of TnCentral Use**

249 **Use Case #1: Comparing Protein Coding Genes in Tn*554* Family Members**

250 The Tn*554* family is a small family restricted to the Firmicutes. Members encode three
251 genes, *tnpA*, *tnpB* and *tnpC*, involved in transposition [28,29]
252 (https://tnpedia.fcav.unesp.br/index.php/Transposons_families/Tn554_family). TnpA and
253 TnpB both exhibit a C-terminal motif which shares all the important catalytic residues of a
254 typical tyrosine site-specific recombinase [28,29]. They insert in a sequence-specific way
255 into the DNA repair gene *radC* [30,31] and can also be found in a circular form [32–36]. To
256 compare the protein coding genes in Tn*554* family members side by side, we searched for
257 Tn*554* in the TE family field of the Transposon Search interface (Figure 5A). Fourteen Tn*554*
258 family members were found (of which only 10 are shown in Figure 5B). In order to perform a
259 side-by-side comparison of the protein-coding genes in these TE, we used the Customize
260 Display option on the search results page, to add the "All Gene Fields" columns, which
261 provide information about the protein coding genes, to the display and to remove several
262 columns (e.g., Host Organism, Country) (Figure 5B). Results for two of the Tn*554*
263 transposons (Tn*558.3* and Tn*559*) are shown in Figure 5C. Both transposons have the three-
264 part transposition module (*tnpA*, *tnpB*, *tnpC*) characteristic of the family. However, the two
265 transposons are quite diverse in their passenger genes. Tn*558.3* has gene called *fla*, which
266 contains a flavodoxin-like domain, and the ABR gene *fexA*, which confers resistance to
267 phenicol antibiotics. Tn*559* has just a single passenger gene, the ABR gene, *dfrK*, which
268 confers resistance to diaminopyrimidine antibiotics. As shown by this example, the flexible
269 search results page makes it easy to compare features across multiple transposons.

270

271 **Use Case #2: Type II Toxin/Antitoxin Systems in Tn*3* Transposons**

272 Toxin/Antitoxin (TA) systems are implicated in plasmid maintenance in bacterial populations
273 [37]. These systems are characterized by a stable toxin and an unstable antitoxin that binds
274 to the toxin and inhibits its lethal effect. Loss of a plasmid carrying a TA system will lead to
275 rapid depletion of the antitoxin, allowing the persistent toxin to kill the cell. Thus, only
276 members of a population that retain the plasmid will survive. Recently, a set of Tn*3*-family
277 transposons carrying TA systems were characterized and included in the TnCentral database
278 [22]. To explore these Tn, we used the TnCentral Gene Search function, selecting "Passenger
279 Gene" from the Gene Class pull-down menu and "Toxin" from the Gene Sub-Class pull-down

280 menu (Figure 6A, red box). The search results included eight different toxin genes (Gp49,
281 HEPN, PIN, PIN_3, *abiEii*, *higB*, *parE*, and zeta) found in 43 different transposons. Similarly,
282 transposons carrying antitoxin genes were identified using the Gene Search function with
283 the Gene Sub-Class menu set to "Antitoxin" (Figure 6B, red box). There were 44 transposons
284 carrying 11 different antitoxin genes. Combinations of toxin and antitoxin genes in individual
285 transposons were examined by going to the ORF Summary section of the entry pages for the
286 TA transposons. For example, Tn*Sku1* (Figure 6B, yellow box; Figure 6C) has a Gp49 toxin
287 gene and an antitoxin gene containing an HTH domain (referred to as HTH). Most
288 transposons have a single toxin/antitoxin gene pair except for Tn*Xca1*, which has two TA
289 pairs, and Tn*5501.5*, which has a *parD* antitoxin gene and no toxin gene. The majority of
290 Tn*5501* derivatives in TnCentral have a *parE* toxin gene as well as the *parD* antitoxin,
291 suggesting that Tn*5501.5* may have undergone a deletion in the region containing *parE*
292 (Supplementary Figure 1).
293
294 **Use Case #3: Tn*21* and its Relatives**
295 Tn*21* is the canonical member of a subfamily of Tn*3* transposons that confers a variety of
296 antibiotic resistances [38–40] and several analyses have proposed mechanisms to explain
297 how *Tn21* arose from simpler ancestor transposons (e.g., [40,41]). Tn*21* has a mercury
298 resistance operon at the 5'- (left) end, a *tnpA*/*tnpR* transposition module at the 3'-(right)
299 end, and a transposition-deficient integron (In2) carrying several ABR genes (a GCN5-related
300 N-acetyltransferase (GNAT_fam), *sul1*, *qacEdelta1*, and *aadA*) in the middle (Supplementary
301 Figure 2). These ABR genes confer resistance to aminoglycosides, sulfones, sulfonamides,
302 quaternary ammonium salts, and acridine dye. More recently, a transposon that lacks the
303 integron insertion but is otherwise identical to Tn*21* (the hypothetical Tn*21* backbone Tn21Δ
304 in [40]) was discovered [42]. This transposon, Tn*5060*, was proposed to be the ancestor of
305 Tn*21* [42]. Tn*21* also has numerous relatives that carry different combinations of antibiotic
306 resistance genes within and outside the integron. To explore the Tn*21* subfamily, we
307 performed a TnCentral Sequence Search (BLAST) using the putative ancestral Tn*5060*
308 sequence (Figure 7A). In addition to Tn*5060* itself, we identified ten transposons in the
309 database (Tn*20*, Tn*21*, Tn*21.1*, Tn*21.2,* Tn*5086,* Tn*2411,* Tn*2424,* Tn*4*, Tn*1935*, and Tn*As3*;
310 Supplementary Figure 2) that contain all (or nearly all) of the Tn*5060* sequence. With the

311 exception of Tn*20*, which is almost identical to Tn*5060* (99.5%), these transposons have two
312 or more discontinuous sub-regions that align to Tn*5060*. For example, Tn*21* has two sub-
313 regions, one of which is a close match to the left half of Tn*5060* and the other of which is a
314 close match to the right half of Tn*5060* (red bars in Figure 7B). This suggests that these
315 transposons arose from Tn*5060* via the insertion of other sequences.
316
317 We compared the antibiotic resistance profiles of the ten transposons by inspecting their
318 TnCentral entry pages. Tn*20*, like Tn*5060*, carries no ABR genes. The other nine transposons
319 carry ABR genes targeting aminoglycosides, sulfones, sulfonamides, and quaternary
320 ammonium salts (Figure 7C). Other resistances found in a subset of the six include acridine
321 dye (Tn*1935*, Tn*21*, Tn*2411*, Tn*4*, TnA*s3,* Tn*2424,* Tn*5086*), carbapenams (Tn*1935* and Tn*4*),
322 cephalosporins (Tn*1935* and Tn*4*), carbapenems (Tn*4*), monobactams (Tn*4*), phenicols
323 (Tn*As3*, Tn2*424*, Tn*21.1*, Tn*21.2*), diaminopyrimidines (Tn*5086*, Tn*21.1*, *Tn21.2*), and
324 tetracyclines (Tn*21.2*)**.** Interestingly, in some cases where the transposons have resistances
325 in common, they are conferred by different genes (Figure 7C). For example, phenicol
326 resistance is conferred by *CAT* in Tn*As3, catB2 in* Tn*2424*, and *cmlA6* in Tn*21.1* and Tn*21.2*.
327 Similarly, sulfonamide and sulfone resistance is conferred by *sul1* in all of the antibiotic-
328 resistant family members except for Tn*21.1* and Tn*21.2*, where those resistances are
329 conferred by *sul3*. Thus, even this closely related subfamily of transposons shows diversity in
330 its antibiotic resistance genes. This is partially due to the flexibility of the integron to
331 incorporate new antibiotic resistance gene cassettes but also to insertion of ABR-gene
332 containing elements outside of the integron region (e.g., Tn*3.1* in Tn*4*, Supplementary Figure
333 2).
334

335 **Discussion**

336 Here, we have described TnCentral, a user-friendly resource for exploration of prokaryotic
337 TE. TnCentral provides a flexible search interface, TE-specific entry pages with intuitive
338 graphics and detailed information about TE features, and a BLAST interface that allows users
339 to identify TE that carry features of interest or to identify TE that are present in sequences
340 of interest (e.g., plasmids). As shown in the use cases, the flexible search results page makes
341 it easy to compare features across multiple transposons, the detailed entry pages allow

342 exploration of TE passenger genes, such as ABR genes, and the Sequence Search enables
343 retrieval of TE with related sequences that could be used as a starting point for evolutionary
344 analyses. Moreover, TnCentral provides access to Tnfinder software for locating candidate
345 TE in sequence data and to TnPedia, a comprehensive review of the biology of selected TE
346 families.
347
348 As discussed in the Introduction, a variety of resources dedicated to aspects of prokaryotic
349 TE biology currently exist. TnCentral's unique contribution to this universe of resources lies
350 in its coverage of a variety of TE (e.g. different transposon families and compound
351 transposons with their associated IS and integrons) and its detailed focus on both core
352 transposition genes and passenger genes of clinical, environmental, and economic
353 importance. It has the additional feature of providing a clear graphic output for visualizing
354 the often complex structures of TE.

355 The next step beyond annotation of individual TE is to annotate and visualize the TE content
356 of prokaryotic chromosomes and plasmids. These studies are critical for understanding the
357 propagation of high impact passenger genes, such as those that confer antibiotic resistance.
358 Several tools that address this problem are available. For example, ISsaga [43], which is
359 integrated into ISfinder, annotates IS present in user-provided sequences. Other software
360 suites have been designed specifically to annotate IS in short read raw data (e.g. ISQuest
361 [44], Transposon Insertion Finder [45], ISMapper [46] and panISa [47]) using preassembled
362 libraries of TE and their components, while yet other approaches are based on *ab initio*
363 prediction (e.g., OASIS [48], ISseeker [49] ISEscan [50], or provide a comparative view of IS
364 mobilisation events (e.g. ISCompare [51]). These annotation tools are only as good as their
365 underlying TE databases. ISfinder, which includes nearly 6000 individual examples of IS
366 classified in distinct families and subfamilies according to their transposition mechanism and
367 structural organization, provides such a rigorous framework for IS and has been
368 incorporated into a number of annotation pipelines (e.g., ISsaga [43], MobileElementFinder
369 [52]). However, IS represent only a fraction of prokaryotic TE, and unlike transposons and
370 integrons, they rarely carry passenger genes. We hope that TnCentral will become a
371 benchmark for more complex TE as ISfinder is for IS.

372 TnCentral is an ongoing project, and we will continue to expand and update the content. In
373 addition to the exporting annotated TE in GenBank format, we plan to make all files
374 available in a SnapGene file format which will allow users to use SnapGene
375 (https://www.snapgene.com/), a commercial software tool (with a free viewer version) for
376 visualizing and documenting nucleotide sequences and their features, to analyze and
377 explore them. We also intend to enhance the visualization of TnCentral Sequence Search
378 (i.e., BLAST) results to better support the analysis of plasmid sequences that may carry a
379 complex complement of TE although it should be noted that the Sequence Search tool can
380 already accommodate analysis of large plasmids. Ultimately, we envision that TnCentral
381 could be used to analyze the TE content of a collection of sequences, such as patient,
382 veterinary and environmental samples from an antibiotic resistance outbreak, to
383 understand TE-driven evolution of the prokaryotic mobilome.

384 Methods

385 Curation Workflow

386 The TnCentral curation workflow is depicted in Figure 8. Curation is performed by members
387 of the TnCentral development team as well as by graduate students in bioinformatics
388 courses at Georgetown University Medical Center. TnFinder scripts are run against RefSeq
389 and other sequence databases and GenBank files potentially containing TE are retrieved. TE
390 sequences are isolated and annotated using SnapGene (https://www.snapgene.com).
391 Features of interest (i.e., protein coding genes, TE, repeat elements, and recombination
392 sites) are annotated according to detailed curation guidelines (provided in the "For
393 Curators" of TnCentral). Fully annotated features are saved in a SnapGene Custom Library.
394 New transposon sequences can be searched against this library, enabling detection of
395 features previously identified in other TEs. All annotated TE files are checked by a second
396 curator. An enhanced GenBank file containing all annotations is exported from SnapGene
397 and checked for common curation formatting errors using a custom Perl script. Detected
398 errors are manually corrected in the SnapGene file, which is then exported as a revised
399 enhanced GenBank file. Information from this GenBank file is used to populate the
400 TnCentral database, which, in turn, serves as the backend for the TnCentral web portal. An

401 image file showing a color-coded map of TE features is also exported from SnapGene and
402 displayed on the TE entry page.
403
404 Although we have adhered to the standard nomenclature for transposons extracted from
405 the literature, for the many transposons newly identified during TnCentral database-
406 building, we have temporarily used names indicating their source. In all cases, the
407 Transposon Registry [53] accession number is provided as a synonym. There is some
408 ambiguity in the literature concerning class 1 integrons and members of the Tn*402*
409 transposon family. Class 1 integrons appear to be derivatives of this transposon family and
410 include members with a range of Tn*402* transposition genes with varying degrees of
411 completeness. We have therefore elected to include all Class 1 integrons as members of the
412 Tn*402* family (Supplementary Table S1). ISfinder classification is used for the individual IS
413 and in the case of compound transposons, the group to which they are belong is defined by
414 the flanking IS.
415
416 Properties of protein coding genes are annotated with cross-references to database or
417 ontology identifiers whenever possible. Antibiotic resistance gene properties, including gene
418 name, sequence family, antibiotic resistance mechanism, and target drug classes are
419 annotated according to the Antibiotic Resistance Ontology (ARO) as presented in
420 Comprehensive Antibiotic Resistance Database (CARD) [10]. The Pfam [54] and InterPro
421 resources [55] are used to define sequence family information.
422
423 **TnCentral Website implementation**
424 TE features and sequence information are extracted from the enhanced GenBank files. TE
425 feature information is used for the search and the entry pages, and the TE DNA and protein
426 sequence information are used for the Sequence Search and display. The extracted data is
427 loaded into the TnCentral database, implemented using MySQL. The website is built on a
428 Linux server with Apache, and the web application is built on Perl CGI. Apache Lucene is
429 used to index the data for flexible and fast search and retrieval. JavaScript is used for the
430 interactive web-interface and display. BLAST is used for similarity search.
431

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446 **Figure Legends**

447

448 **Figure 1.** Structural arrangement of prokaryotic Transposable Elements. The TE is indicated
449 by a pale-yellow horizontal bar at the top of each section. Open reading frames are shown
450 as horizontal arrows with the arrowheads indicating the direction of expression: purple,
451 transposition-associated genes; red, antibiotic resistance genes; green, other passenger
452 genes. The inverted terminal repeats found at the ends of the majority of TE are shown as
453 grey arrows and the direct target repeats generally produced by insertion are indicated by
454 small black arrows. **A.** Insertion Sequence (IS), a short DNA segment encoding only the
455 mobilization protein (Transposase, TnpA), flanked by two imperfect Inverted Repeats (IRs),
456 and generally containing a short flanking directly repeated duplication (DR) on the target of
457 insertion. **B.** tIS (transporter IS) are structurally similar to an IS, but contain passenger genes.
458 They are presently restricted to the IS*1595* and IS*66* families. **C.** Compound transposons are
459 formed by two IS in either direct or inverted orientation, flanking a variety of passenger
460 genes including those for antibiotic resistance. **D.** Transposons are more heterogeneous
461 structures and include different sets of transposition-related genes which are specific to
462 each Tn family and multiple antibiotic resistances, virulence and other passenger genes. This
463 is an example of a Tn*3* family transposon with transposon, *tnpA*, and resolvase genes, *tnpR*.

464

465 **Figure 2.** A) TnCentral homepage showing clickable links to various TnCentral sections in the
466 box on the left. B) TnCentral search interface showing search choices for TE on the left and
467 for transposition-related and passenger genes on the left.

468

469 **Figure 3.** TnCentral TE Entry Page. #1-10: Sections of the entry page (see text for details).

470

471 **Figure 4.** The main sections of TnPedia, a TnCentral-related wiki compiling information on
472 prokaryotic transposable elements. Only three of the four sections (General Information, IS
473 families and Transposon families) are illustrated. The fourth section is a Transposition
474 Glossary, which is under construction.

475

476 **Figure 5.** Comparing Protein Coding Genes in Tn*554* Family Members. A) TnCentral
477 Transposon Search interface, showing a search for Tn*554* in the TE family field. B) Interface
478 for customizing the columns in the search results display. Clicking on "Customize Display"
479 (red box) opens the interface. C) Partial Tn*554* family search results after customization to
480 show information on protein coding genes (All Gene Fields).
481
482 **Figure 6.** Exploring Toxin/Antitoxin Genes in TnCentral. A) Partial results of searching
483 TnCentral for toxin genes. The settings used to obtain these results are shown in the red
484 box. Links to entry pages for the TE carrying the indicated genes are provided in the MGE
485 Accession column (e.g., Tn*Sku1*-CP002358.1, yellow box). B) Partial search results for
486 antitoxin genes in TnCentral. Settings are shown in the red box. C) ORF Summary section of
487 the entry page for Tn*Sku1*-CP002358.1, showing the presence of a toxin/antitoxin gene pair
488 (Gp49 toxin/HTH antitoxin).
489
490 **Figure 7.** Analysis of ABR in Tn*21* Relatives. A) TnCentral Sequence Search using the
491 sequence of Tn*5060*, the proposed ancestor of Tn*21*, as a query. B) Sequence Search results.
492 The query sequence is represented by the width of the Alignment column. The red bars
493 represent regions of the matched transposons that are highly similar to regions of Tn*5060*.
494 C) ABR genes and targeted antibiotic classes in Tn*21* relatives. Red shading in the table cells
495 indicates that the transposon carries at least one gene targeting the antibiotic class; blue
496 shading indicates that it does not. The ABR genes found in each transposon are indicated in
497 the table cells.
498
499 **Figure 8.** TnCentral Curation Workflow (see text for description).
500
501

502 ## Supplementary Figures

503 **Supplementary Figure 1.** Maps of Tn*5501* and Tn*5501.5* showing the loss of *parE* toxin gene
504 in Tn*5501.5*. Feature color code: yellow--TE; purple--transposition genes; dark orange--
505 toxin/anti-toxin genes; light orange--other open reading frames; grey--repeat elements;
506 green--recombination sites. Maps were created with SnapGene.

507

508 **Supplementary Figure 2.** Maps of Tn*21* and its relatives. The feature color code is the same
509 as in Supplementary Figure 1. Maps were created with SnapGene. Note that the different
510 transposon derivatives are not to scale but their individual lengths are included.

511

512 **Supplementary Table S1.** The table displays the entire collection of TE at present in the
513 database (May 2021) with columns indicating their **TnCentral accession numbers**, their
514 **names**, **synonyms** from the literature and/or the Transposon Registry [53], TE **Type, Family**
515 **Group**), **Host Organism** and **Molecular Source** (e.g., plasmid or chromosome). If no
516 information is provided in the Molecular Source column, the source is chromosomal or
517 unknown.

518

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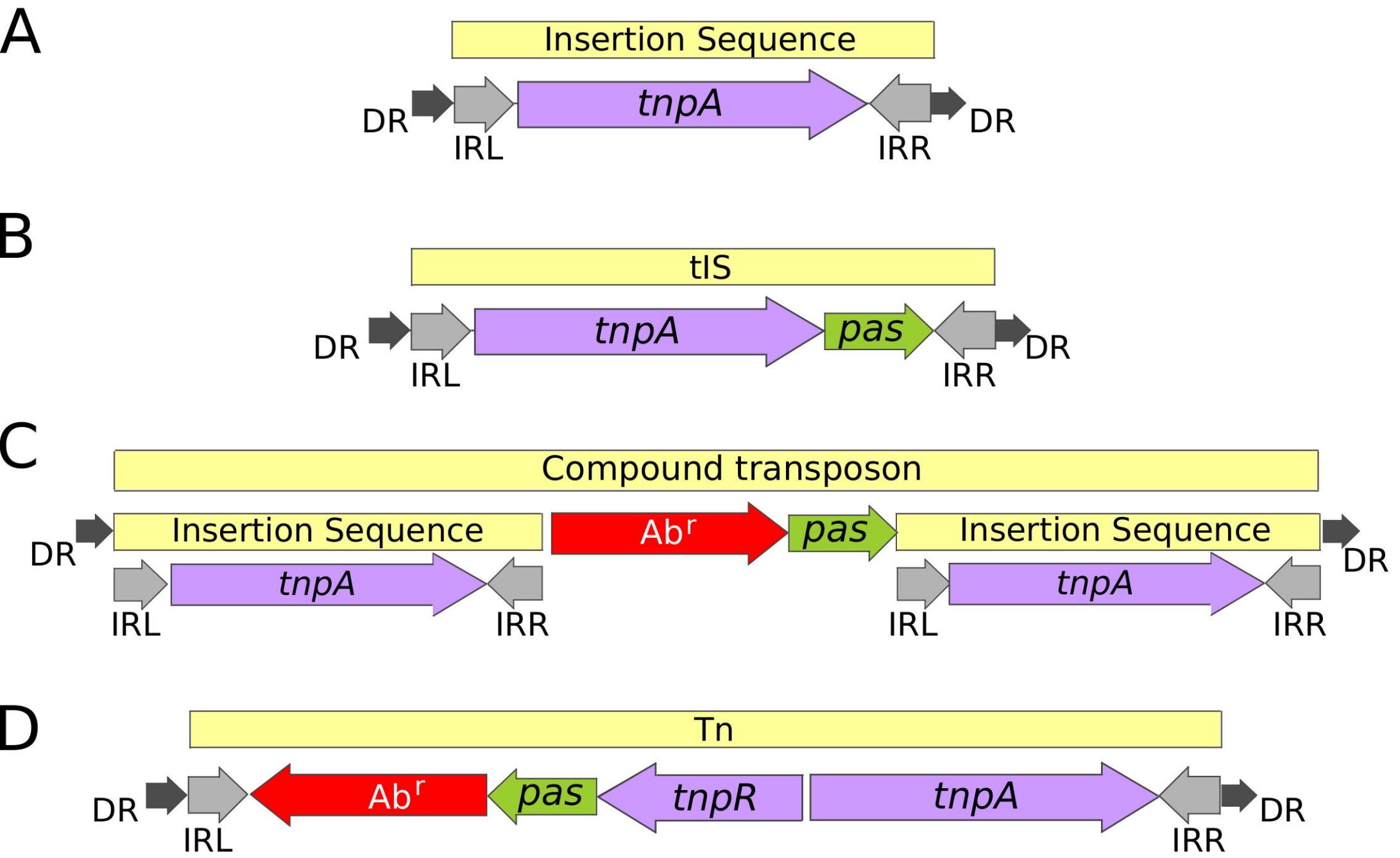
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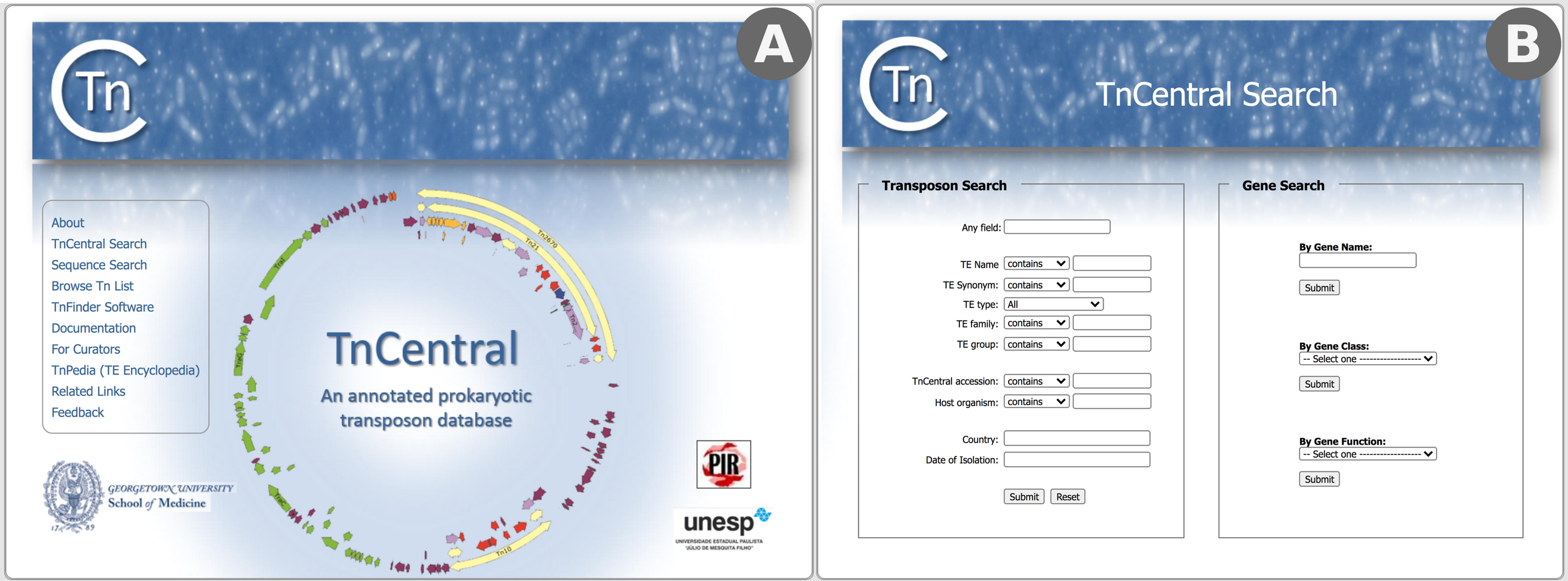
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An annotated prokaryotic transposon database
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TE Synonym: contains
TE type: All
TE family: contains
TE group: contains
TnCentral accession: contains
Host organism: contains
Country:
Date of Isolation:
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Reset
Gene Search
By Gene Name:
Submit
By Gene Class:
-- Select one ----------
Submit
By Gene Function:
-- Select one ----------
Submit

1

Transposon

Name: Tn5501

Family: Tn3 **Group:** Tn3000

Evidence of Transposition: Yes

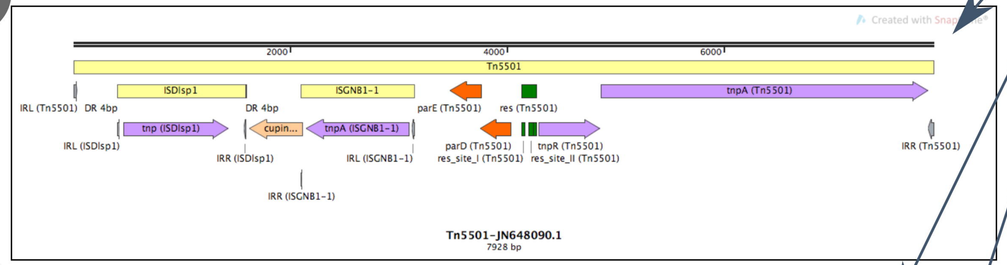
Host

Host Organism: Delftia sp. KV29

Molecular Source: plasmid pKV29
Date of Isolation: 1998

2

Map



3

Terminal Inverted Repeats (IR)

IRL (Length: 38 bp) → GGGGTTCTAAGCCGGAACCGCCGAAAATTCCGTCAGCC
IRR (Length: 38 bp) → GGGGTTCTAAGCCAGAACCGCCGAAATTTCCGTCATCC

4

Sequence

DNA Sequence Length 7928

```
--------10 --------20 --------30 --------40 --------50 --------60 --------70 --------80 --------90 --------100
GGGGTTCTAA GCCGGAACCG CCGAAAATTC CGTCAGCCGA TCAACGTGGC TTGTCCCGCG CCCGGTCGAT GGGGTAGACC CAACAGTCGT GTCACTAGCC 100
GCCATTTCGA TCACGGCAAT GCCAGCCGGA CGTCACGTCC AGATTGTTCC GGTCTGGATG AGGCCGACTG ACGTCTCGGA TGACGGGTGG CATACAACTG 200
CTGTGAGTCC TGCAGGGGGG CAGCTGCCTG ACCGGACGGC GAGCATCAGC CCATCTCATG TATTAGTCAT GTCAGCTTTG ACACTGCGCA CGCGACGGCA 300
CCCGACCCGT TGCAGACCCC CAGACATATG GAAAGCTGAC GCTCAACGTG GAGTTAGCCG GCGCGGCGCG GCTTCATCGC GCAGCGTCCG TGTTGATGGA 400
```

Jump to:

Top

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- ORFs
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- Internal Transposable Elements
- Internal Repeats
- References

Download:

Source File (GenBank format)

Sequence File (FASTA format)

5

Recombination Sites

Name	Coordinates	Length	Sequence
res	4139-4269	131	GCCTGTCGGA AAACATTTGT TTTTCGACAG GCCTTCAACG GTCCTCTGCA CCAACCTCCG AGTGGCCGCA AAATTGTGCG GAAAACTCTG TCGCCAGACG CTACCATACG GAAACCTCGT CTTAATGGTT T

6

ORFs

ORF Summary

Gene Name	Associated TE	Coordinates	Class	Sub Class	Orientation
tnp	ISDlsp1	462-1436	Transposase		+
cupin2	Tn5501	1626-2111	Passenger Gene	Other	-
tnpA	ISGNB1-1	2150-3097	Transposase		-

7

ORF Details

Gene Name	Protein Name	Associated TE	Length	Coordinates	Strand
tnp	Tnp	ISDlsp1	975	462-1436	+

Class: Transposase
Transposase Chemistry: DDE
Protein Sequence:

```
MLTGMKQSSL ELNLSTRKTR KQELLAQMDR VVPWAALVEL IAPYYPEGKN GRPPFALEAM LRVHCMQQWF TLSDLAMEEA FFDTPIYREF AGLDAHGRMP
DESTILRFRH RLEKHRLAEQ ILATVNDLLA ARGLLLKAGT AVDATLIAAP SSTKNKDRKR DPEMHSSQKG NEWHFGMKAH IGVDADSGLV HTVIGTSGNV
ADVTEGNSLL HGEETDAFGD AGYQGAHKRP DARKDVTWHV AMRPGKRKEL DKENNPVDAL IDQVEKIKAS IRAKVEHPFR VIKRQFGYTK VRYRGLKKNT
LQLKTLFALS NLWMVRHQLL GAQG
```

8

Internal Transposable Elements (TE)

TnCentral Accession	TE Name	Type	Coordinates	Length
ISDlsp1-JN648090.1	ISDlsp1	Insertion Sequence	409-1592	1184
ISGNB1-1-EF628291	ISGNB1	Insertion Sequence	2097-3143	1047

9

Internal Repeat Elements

Name	Associated Mobile Element	Coordinates	Sequence (Top Strand)
IRL	ISDlsp1	409-428	GGAAATCCTG CAAAACCTCG
IRR	ISDlsp1	1574-1592	GCTCAACAAG TCCTGTAGG

10

References

Stolze Y, Eikmeyer F, Wibberg D, Brandis G, Karsten C, Krahn I, Schneiker-Bekel S, Viehover P, Barsch A, Keck M, Top EM, Niehaus K, Schluter A. *IncP-1beta plasmids of Comamonas sp. and Delftia sp. strains isolated from a wastewater treatment plant mediate resistance to and decolorization of the triphenylmethane dye crystal violet.* Microbiology. 2012 Aug;158(Pt 8):2060-2072. doi: 10.1099/mic.0.059220-0. Epub 2012 May 31. PubMed ID: 22653947

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Main Page

Welcome to TnPedia, the TnCentral Wiki

This Tnpedia has been written in an attempt to assemble a body of information (including many of the historical articles) generally dispersed in the literature as an aid to understanding how knowledge has been built up to our present view of the key role played by transposable elements (TE) in prokaryotes – both in influencing gene expression, in driving genome evolution and in facilitating horizontal gene transfer (HGT). It is divided into four sections:

General Information on Prokaryotic Elements

A section providing general information about Transposition and **T**ransposable **E**lements (TE) with emphasis on prokaryotic elements. It was originally written for ISfinder (https://www-is.biotoul.fr/index.php) with contributions from P. Siguier and E. Gourbeyre. It contains historical, mechanistic and genetic information.

Insertion Sequence (IS) Families

A second section describing each **I**nsertion **S**equence (IS) family in some detail. This section has been entirely updated compared to that which was included in a previous ISfinder version. It contains information from a number of reviews and from the primary literature together with analyses undertaken in the framework of ISfinder and TnCentral. P. Siguier and E. Gourbeyre provided a large proportion of these analyses.

Transposon families [In progress]

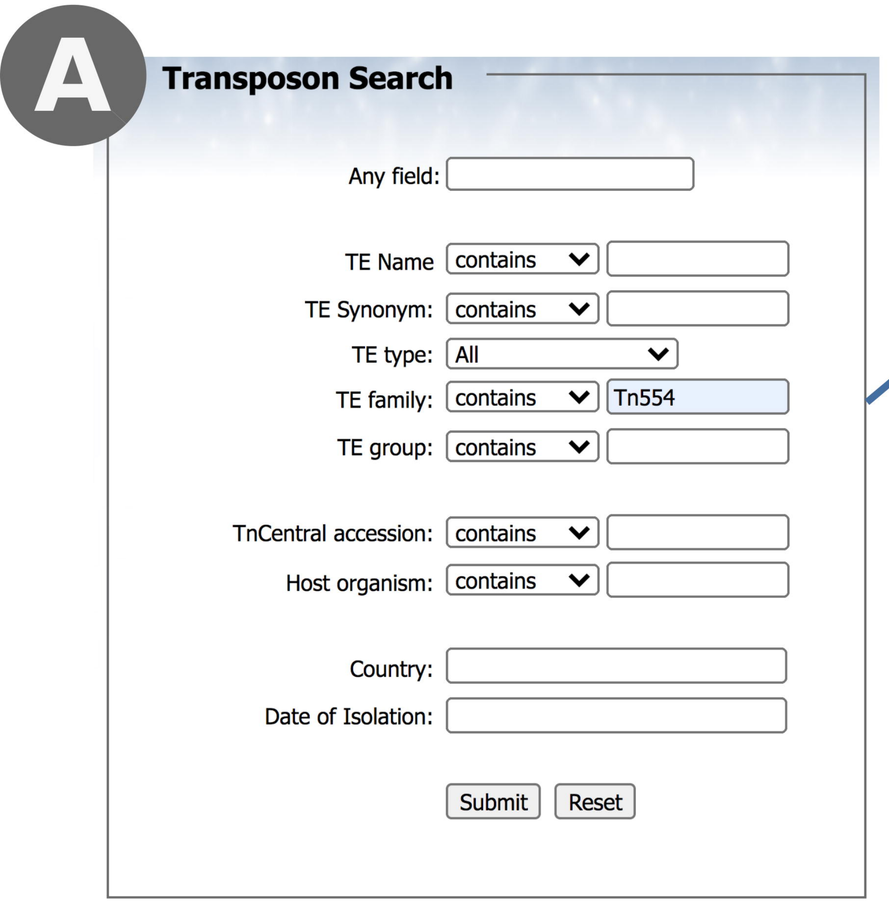
A third section presenting detailed descriptions of transposon and transposon families written in the framework of TnCentral.

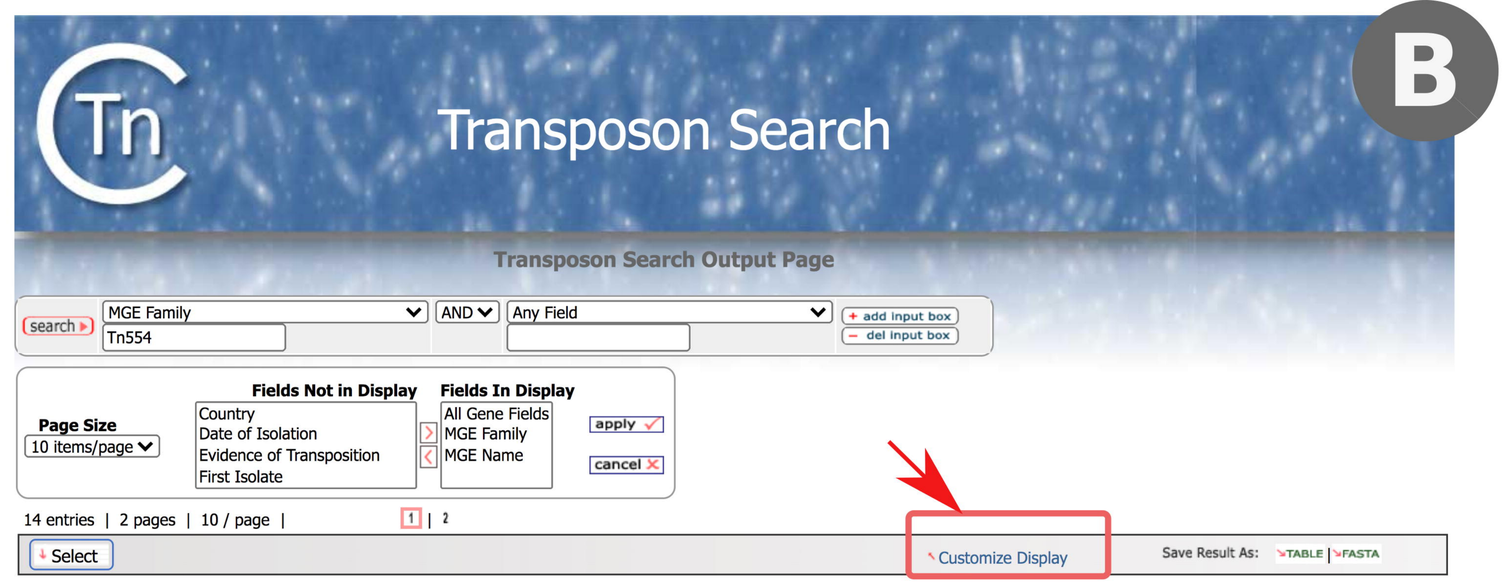
IS Families

Prokaryotic Insertion Sequences (IS)
1. IS*1* family
2. IS*1595* family
3. IS*3* family
4. IS*481* family
5. IS*4* and related families
5a. IS*701* family
5b. IS*H3* family
5c. IS*1634* family
6. IS*5* and related IS*1182* families
7. IS*6* family
8. IS*21* family
9. IS*30* family
10. IS*66* family
11. IS*110* and IS*1111* families
12. IS*256* family
13. IS*630* family
14. IS*982* family
15. IS*1380* family
16. IS*As1* family
17. IS*L3* family
18. IS*Azo13* family
19. IS*607* family [In progress]
20. IS*91* and related IS*CR* families
21. IS*200*/IS*605* family

Transposon families

Prokaryotic Transposon Families
1. Composite or compound transposons
2. Tn*3* family transposons
3. Tn*7* family transposons
4. Tn*402* family transposons
5. Tn*554* family transposons





TnCentral Accession	MGE Name	MGE Synonyms	MGE Type	MGE Family	MGE Group	Host Organism	Country	Date of Isolation
Tn5406-AF186237	Tn5406		transposon	Tn554		Staphylococcus aureus MRSA	Spain	January 2011
Tn554-X03216.1.1	Tn554		transposon	Tn554		Staphylococcus aureus	USA	1979
Tn554Cad-AB037671.1	Tn554Cad		transposon	Tn554		Staphylococcus aureus 85_2082	New Zealand	2001
Tn558-AJ715531.1	Tn558		transposon	Tn554		Staphylococcus lentus	USA	2004
Tn558.1-AM408573.1	Tn558.1		transposon	Tn554		Staphylococcus warneri	Denmark	2006
Tn558.2-MH018573	Tn558.2		transposon	Tn554		Enterococcus Enterococcus avium strain C674	China	2018
Tn558.3-CP025122	Tn558.3		transposon	Tn554		Bacillus sp. HBCD-sjtu	China	2017
Tn559-FN677369	Tn559		transposon	Tn554		Staphylococcus aureus ST398	Germany	2010
Tn6133-FR772051.1	Tn6133		transposon	Tn554		Staphylococcus aureus subsp. aureus ST398	Switzerland	2011
Tn6188-HF565366	Tn6188		transposon	Tn554		Listeria monocytogenes 6179	Austria	2013

C

TnCentral Accession	MGE Name	MGE Family	All Gene Fields							
			Gene Name	Gene Class	Gene Function	ORF Target	Gene Length (bp)	Protein Length (aa)	Protein Molecular Function	Sequence Family
Tn558.3-CP025122	Tn558.3	Tn554	tnpA	Transposase			1086	361		
			tnpB	Transposase			1920	639		
			tnpC	Accessory Gene	Helper		366	121		Tn554_family
			fla	Passenger Gene	Other		417	138		flavodoxin
			fexA (ARO:3002704)	Passenger Gene	Antibiotic Resistance	phenicol antibiotic (ARO:3000387)	1428	475	antibiotic efflux (ARO:0010000)	major facilitator superfamily (MFS) antibiotic efflux pump (ARO:0010002)
Tn559-FN677369	Tn559	Tn554	tnpA	Transposase			1086	361		
			tnpB	Transposase			1893	630		
			tnpC	Accessory Gene	Helper		378	125		Tn554_family
			dfrK (ARO:3002869)	Passenger Gene	Antibiotic Resistance	diaminopyrimidine antibiotic (ARO:3000171)	492	163	antibiotic target replacement (ARO:0001002)	trimethoprim resistant dihydrofolate reductase dfr (ARO:3001218)

A

Gene Search

Gene Search Output Page

Gene Class: Passenger Gene | Gene Sub Class: Toxin | Gene Target: -- Select one ----------

8 items | 1 page | 10 / page |

Select | Customize Display | Save As TABLE

Gene Name	Gene Class	Gene Function	ORF Target	MGE Accession	MGE Name	Host Organism	Country
Gp49	Passenger Gene	Toxin	ribosome associated mRNA	TnpPGH1-Y09450.1	TnpPGH1	Pseudomonas putida	
				TnSku1-CP002358.1	TnSku1	Sulfuricurvum kujiense DSM 16994	Japan
				Tn4662a.1-AY831462.1	Tn4662a.1	Pseudomonas putida GJ31	
				Tn4662a-NC_014124.1	Tn4662a	Pseudomonas putida HS	U.S.A
				Tn5501.12-CP017294.1	Tn5501.12	Pseudomonas aeruginosa PA83	Germany
HEPN	Passenger Gene	Toxin	RNA	TnSod9-NC_004349	TnSod9	Shewanella oneidensis MR-1	
PIN	Passenger Gene	Toxin	single stranded RNA	TnXca1-NC_007507	TnXca1	Xanthomonas campestris pv. vesicatoria	
				TnPsy42-KX009060.1	TnPsy42	Pseudomonas syringae pv. actinidiae RT594	Japan
				TnXax1.1-NC_016053	TnXax1.1	Xanthomonas arboricola pv. pruni CFBP 55306	

Gene Class: Passenger Gene | Gene Sub Class: Antitoxin | Gene Target: -- Final --

11 items | 2 pages | 10 / page | 1 | 2

Select | Customize Display | Save As TABLE

B

Gene Name	Gene Class	Gene Function	ORF Target	MGE Accession	MGE Name	Host Organism	Country
HTH	Passenger Gene	Antitoxin		TnSku1-CP002358.1	TnSku1	Sulfuricurvum kujiense DSM 16994	Japan
HTH_37	Passenger Gene	Antitoxin		Tn4662a.1-AY831462.1	Tn4662a.1	Pseudomonas putida GJ31	
				Tn4662a-NC_014124.1	Tn4662a	Pseudomonas putida HS	U.S.A
				Tn5501.12-CP017294.1	Tn5501.12	Pseudomonas aeruginosa PA83	Germany
				TnpPGH1-Y09450.1	TnpPGH1	Pseudomonas putida	
PIN_12	Passenger Gene	Antitoxin		TnBth3-CP003766	TnBth3	Bacillus thuringiensis HD-789	

ORF Summary

C

Gene Name	Associated TE	Coordinates	Class	Sub Class	Orientation
HTH	TnSku1	126-416	Passenger Gene	Antitoxin	+
Gp49	TnSku1	419-712	Passenger Gene	Toxin	+
tnpR	TnSku1	713-1336	Accessory Gene	Resolvase	-
tnpA	TnSku1	1492-4410	Transposase		+

A

Sequence Search (BLAST)

Job Name:

Database

TnCentral

Other Sequence Search Services:

iSfinder: ○ CARD: ○ Toxin/Antitoxin: ○ NCBI: ○

Query Sequence

Enter a sequence here:

```
>Tn5060
GGGGGCACCTCAGAAAACGGAAAATAAAGCACGCTAAGGCATAGCTGACCTTGCCAGGCCTGCTTCGCCCTGTAGTGACGCGATCAACGGGCAG
GAAACATTCCCCTTTCGTGCATGGCAGGCGCACACGAGTTCAGACAGCACGGTTTCCATGCGCGCCAAGTCGGCCATCTTCTCGCGCACGTCCT
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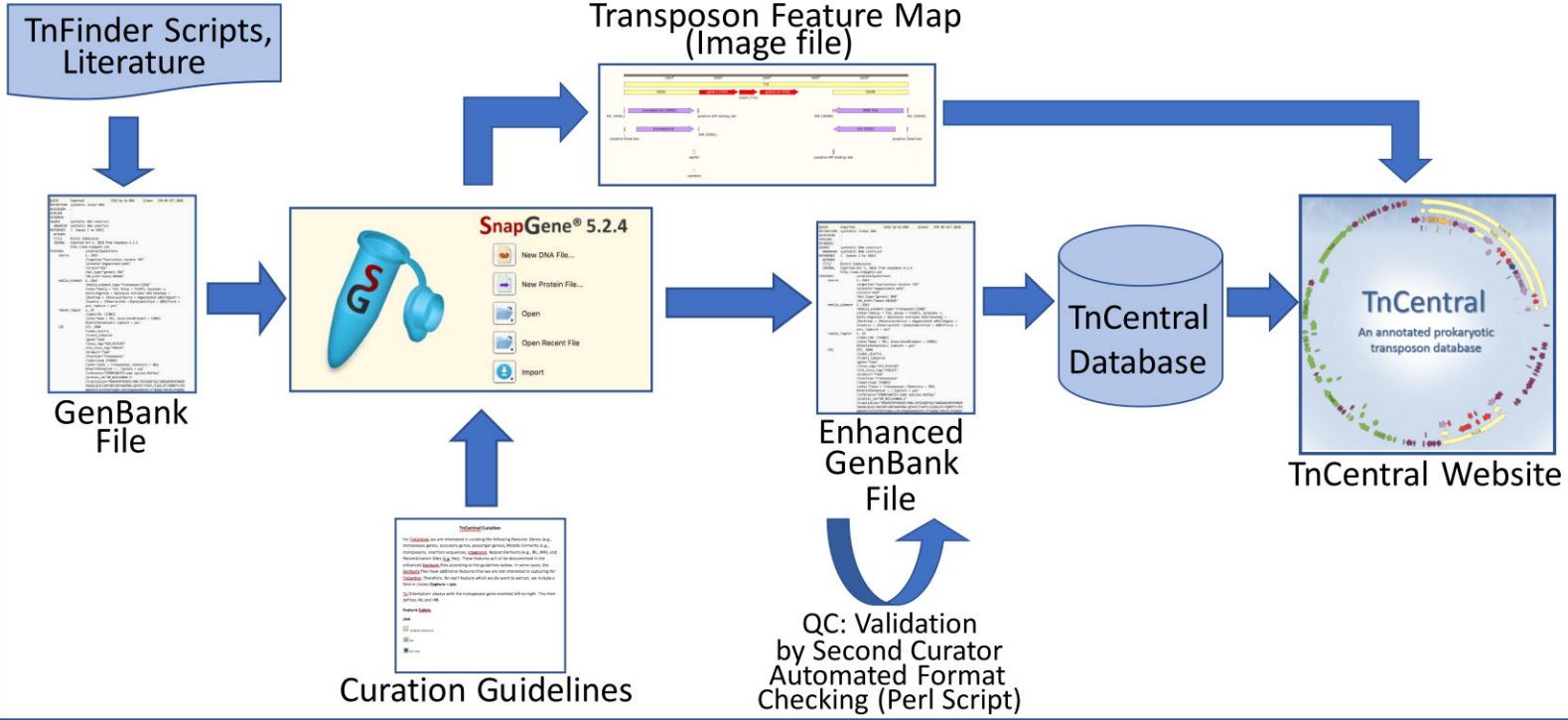
Or, upload file (Fasta format) : Choose File No file chosen

B

TnCentral Accession	TE Name	TE Length	Host Organism	TE Family	Alignment (by score)
Tn5060-AJ551280.1	Tn5060	8667	Pseudomonas sp. A19-1	Tn3	
Tn20-AF457211.1	Tn20	8644	Escherichia coli	Tn3	
Tn21-AF071413	Tn21	19672	Shigella flexneri	Tn3	
TnAs3-CP000645.1	TnAs3	18735		Tn3	
Tn21.2-MH626558	Tn21.2	35400	Salmonella enterica subsp. enterica serovar Typhimurium	Tn3	
Tn2424-UGCJ01000005	Tn2424	26008	Escherichia coli NCTC11186	Tn3	
Tn5086-CP054343	Tn5086	15341	Escherichia coli SCU-164	Tn3	
Tn1935-MK797990	Tn1935	23364	Salmonella enterica subsp. enterica serovar Wien ZM3	Tn3	
Tn21.1-MH257753	Tn21.1	21668	Salmonella enterica subsp. enterica serovar Typhimurium	Tn3	
Tn2411-FN554766	Tn2411	18055	Escherichia coli 042	Tn3	
Tn4-KY749247.1	Tn4	23009	Salmonella enterica subsp. enterica serovar Paratyphi B	Tn3	

C

	Tn1935	Tn21	Tn2411	Tn4	TnAs3	Tn2424	Tn5086	Tn21.1	Tn21.2
aminoglycosides	GNAT_fam, aadA, aph3'-Ia	GNAT_fam, aadA				GNAT_fam, AAC(6')-Ia, aadA3	GNAT_fam	aadA, aadA2	
sulfonamides	sul1							sul3	
sulfones									
quaternary ammonium salts	qacEdelta1							qacL	
acridine dye									
phenicols					CAT	catB2		cmlA6	
diaminopyrimidines							dfrA7	dfrA12	
tetracyclines									tetR, tet(B), tetC_p
penams	bla-OXA-1			bla-TEM-1					
cephalosporins									
penems									
monobactams									


TnFinder Scripts, Literature
Transposon Feature Map (Image file)
GenBank File
SnapGene® 5.2.4
New DNA File...
New Protein File...
Open
Open Recent File
Import
Enhanced GenBank File
TnCentral Database
TnCentral
An annotated prokaryotic transposon database
TnCentral Website
Curation Guidelines
QC: Validation by Second Curator Automated Format Checking (Perl Script)