Insertion sequence driven evolution of the microbiota revealed by a novel metagenomics pipeline

Joshua M. Kirsch[1], Andrew J. Hryckowian[2, 3], and Breck A. Duerkop[1]*

[1]Department of Immunology and Microbiology, University of Colorado - Anschutz Medical Campus, School of Medicine, Aurora, Colorado, USA

[2]Department of Medicine, Division of Gastroenterology and Hepatology, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA

[3]Department of Medical Microbiology & Immunology, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA

*Correspondence: Breck A. Duerkop breck.duerkop@cuanschutz.edu

Abstract

Insertion sequence (IS) elements are mobile genetic elements in bacterial genomes that support adaptation. We developed a database of IS elements coupled to a computational pipeline that identifies IS element insertions in the microbiota. We discovered that diverse IS elements insert into the genomes of intestinal bacteria regardless of human host lifestyle. These insertions targeted bacterial accessory genes that aid in their adaptation to unique environmental conditions. Using IS expansion in *Bacteroides*, we show that IS activity leads to insertion "hot spots" in accessory genes. We show that IS insertions are stable and can be transferred between humans. Extreme environmental perturbations force IS elements to fall out of the microbiota and many fail to rebound following homeostasis. Our work shows that IS elements drive bacterial genome diversification within the microbiota and establishes a framework for understanding how strain level variation within the microbiota impacts human health.

Introduction

Bacteria can rapidly evolve their genomes through the mobilization of genetic elements, including bacteriophages (phages), plasmids, DNA inversions, and transposable elements (1-3). Genetic rearrangements have a strong impact on bacterial fitness, influencing diverse phenotypes including virulence, antibiotic resistance, interbacterial competition, and secondary metabolism. For example, pathogenicity islands can distribute virulence traits to previously non-pathogenic bacteria and DNA inversions can alter the expression of antibiotic resistance genes (4, 5). Additionally, novel genetic traits such as metabolic and virulence genes are acquired by bacteria through plasmid exchange, bacteriophage lysogeny, and conjugative transposition (6, 7). Thus, mobile elements actively drive bacterial adaptation and evolution.

Insertion sequence (IS) elements are small (~1 kB) simple transposons that are common to bacterial genomes (8). IS elements are capable of self-mobilization by activating an encoded transposase that recognizes sequence-specific inverted repeats at IS element termini.

IS transposases are diverse (9), and include enzymes with a DD(E/D) motif that support “copy-and-paste” and “cut-and-paste” mechanisms or those with HUH nuclease chemistry which transpose as ssDNA molecules at the replication fork (10-12). Gene expression and genome fidelity can be modulated by IS activity in a variety of ways, including IS insertions into protein coding regions inactivating genes, insertions into intergenic regions forming strong hybrid promoters increasing gene expression, or recombination with other IS elements resulting in large deletions (13-16). Additionally, IS elements can rapidly expand in bacterial genomes when commensal bacteria transition to become pathogens (15, 17, 18). Thus, IS elements have a profound impact on the evolution and physiological traits of bacteria (19).

Despite a deep knowledge of fundamental IS biology from diverse bacteria, we have a rather rudimentary understanding of how these elements function in polymicrobial communities. This knowledge is critical for deciphering how bacterial communities evolve in ecosystems such as the human microbiota. A barrier to studying IS elements in complex environments results from imperfect methodologies for measuring *in situ* IS dynamics. This stems from the poor recovery of multi-copy genes with repetitive sequences by short-read assemblers, leading to fragmented assemblies where IS elements are absent or become break-points between contigs (20). Therefore, assembly-level analyses of IS elements from metagenomic datasets are often underpowered. Despite these limitations, previous culture-based studies show that IS elements are active in the human intestine. Sampling of *Bacteroides fragilis* isolates revealed the gain and loss of IS transposases (21). Furthermore, IS elements were shown to rapidly expand in copy number in the genome of *Escherichia coli* during intestinal colonization of mice, driving increased virulence in a mouse model of Crohn’s disease (22, 23). Recently, longitudinal intestinal metagenomic samples from a single individual were sequenced using long DNA fragment partitioning, producing higher quality assemblies compared to short-read methodologies. This analysis revealed that *Bacteroides caccae* gained and lost numerous IS insertions over the course of sampling (20). Finally, we previously utilized a targeted IS

sequencing approach to identify novel insertions of the IS element IS256 in intestinal *Enterococcus faecium* populations isolated from an individual undergoing treatment with multiple antibiotics (19). Antibiotic exposure was associated with increased abundances of IS256 insertions in genes related to antibiotic resistance and virulence, suggesting that antibiotic treatment drives IS-mediated pathoadaptation in the human intestine.

In this work, we developed an open-source IS database that greatly expands the diversity of IS elements compared to current databases. We built a computational pipeline that utilizes this database to find IS insertions in public metagenomic datasets. Our analysis reveals widespread abundance and expansion of IS insertions in the human microbiota. We show that IS insertions are transferable between individuals and are stable for years. Distinct families of IS elements are favored for insertional activity within the microbiota and these preferentially insert into specific classes of genes. Such genes are specific to distinct microbial taxa with an overrepresentation within the *Bacteroidia* and *Clostridia*. Gene classes targeted by IS elements are primarily metabolic, cell surface, and mobile genetic element genes. Using an *in vitro* ISOSDB412 (IS4351) expansion model, we confirm that identical and related accessory genes are preferentially targeted. Finally, we show that the stability of IS insertions is lost following antibiotic perturbation and diet intervention. Following these alterations, new IS abundances and insertion site locations arise. Together, this work establishes a framework for studying IS elements within the microbiota and is the first step toward understanding how IS elements contribute to the function of the microbiota that impacts human health.

Results

ISOSDB: a comprehensive open-source IS database

To facilitate the study of IS elements in bacterial genomes and metagenomic datasets, we sought to build an updated and exhaustive IS database that can serve as an open-source tool for the scientific community. Prior to our development of this database, the main repository

for IS elements was the ISFinder database (24). While ISFinder has served as a gold-standard for the systematic naming of IS elements and their identification in single isolate genomes (25), it is underpowered for high-throughput genomics. ISFinder lacks integration into downstream tools that can facilitate in-depth analyses of IS elements, the entirety of the database cannot be downloaded for personal use, and it relies solely on manual curation and submission of new IS elements, decreasing the speed at which new IS elements can be reported. These are drawbacks in the current genomic age, where the identification of IS elements from complex datasets such as metagenomes would greatly improve our understanding of their function.

To address the need for an unrestricted open-access IS database to support genomic research, we built the Insertion Sequence Open-Source Database (ISOSDB), which consists of IS elements identified from 39,878 complete bacterial genomes and 4,497 metagenome assembled genomes (MAGs) (26).These IS elements were identified using OASIS, a rigorously tested IS identification tool that allows for the high-throughput analysis of multiple genomes (27). IS elements were considered valid and included in ISOSDB if: (1) there were at least two copies of the IS in a single genome, (2) it was flanked by terminal inverted repeats (IRs), and (3) has significant nucleotide homology to an IS in ISFinder or has a putative transposase. Redundant IS elements were deduplicated at 95% nucleotide identity. The resulting set of IS elements totaled 22,713 distinct IS elements, an almost five-fold excess to the ISFinder database (Fig. 1A). We identified transposase amino acid homologs, in addition to nucleotide homology over the whole sequence of an IS element, in the ISFinder database. 97.5% of the transposases included in ISOSDB had protein homologs in ISFinder, but only 37.9% had nucleotide homologs in ISFinder (Fig. 1B). The ISOSDB also has a wide range of transposases representing multiple IS families and contains distinct clusters of IS elements at the nucleic acid and protein level (Fig. 1C-D & Table S1). In summary, the ISOSDB is an expansive, freely available database that contains substantially improved IS diversity compared to current databases.

Development of an IS detection pipeline that uses ISOSDB

IS elements drive genomic diversity in almost all bacterial species (9, 28, 29). However, a systematic method to identify novel IS insertions in complex microbial communities has not been previously developed. This has precluded the study of IS biology in the microbiota at the population scale. To obtain a deeper understanding of IS biology in the microbiota, we developed the pseudoR pipeline that utilizes ISOSDB to identify IS insertions in previously assembled genomic sequences. pseudoR is named for its ability to find IS inactivated pseudogenes and is implemented in the R programming language. This pipeline is built for fragmented, incomplete assemblies such as metagenomes. Our approach was inspired by previous tools developed for the analysis of transposon insertions from eukaryotic (30) and bacterial genomes (31, 32). These tools either require *a priori* knowledge of the target site duplications which is often not consistent for IS insertions (33), use read pairing to infer IS insertions which limits the data available to verify IS insertions, or require a matched reference assembly.

The backbone of the pseudoR pipeline is a split read approach. First, reads are mapped against assembled contigs and unmapped reads are binned as separate read files. These unmapped reads are aligned against a database of IS terminal ends (150 bp on either end of an IS) compiled from ISOSDB (Fig. S1). If an unmapped read has an IS termini, the termini is trimmed and the remaining read is remapped against the assembled contigs. To ensure that insertions are genuine, a minimum depth of four reads is required and these reads must include at least one left and one right termini.

To compare between samples and multiple disparate assemblies, we built two modes (multi and single). Multi-mode takes each sample and maps its reads against its own assembly. These reads are then subsequently mapped against a deduplicated gene database built from all assemblies in the dataset. Single-mode was developed to analyze time series datasets, where a

single assembly can be used for multiple samples (such as one from the start of the time series).

IS insertions are widespread in the healthy human intestinal microbiota

We utilized the pseudoR pipeline to identify IS insertions in healthy human fecal metagenomes by analyzing data from three geographically disparate studies: a survey of healthy Italian adults ranging in age from 30-105 years old (ITA) (34), healthy individuals from a Japanese colorectal cancer study (JPN) (35), and healthy rural Madagascan adults (MDG) (36). Each study showed evidence of widespread IS insertion heterogeneity (Fig. 2A). Some individuals had few detectable IS insertions while others had over 100 unique IS insertions (Fig. 2B). We used relative IS depth (IS depth divided by the sum of the IS depth and the depth of reads that map to the insertion site without the insertion present) as a metric to report the abundance of IS alleles. Most insertions had a relative IS depth between 10% and 100%, demonstrating that the inserted allele exists in equilibrium with the wild type allele (Fig. 2A). We next determined the bacterial taxa that underwent extensive IS insertional activity (Fig. 2C, Fig. S2A). IS insertions were most abundant in the *Bacteroidia* and *Clostridia*. These two classes make up the majority of the healthy intestinal microbiota (37) and the genera *Bacteroides*, *Phocaeicola*, *Ruminococcus*, and *Blautia* accounted for the majority of IS element insertion diversity (Fig. S2A). While *Bacteroidia* IS insertions were present across all studies, the IS elements underlying these insertions varied between studies (Fig. 2D). Unique IS types for the *Bacteroidia* included ISOSDB412 (IS30 family) insertions for ITA individuals, ISOSDB18121 (IS1380 family) insertions for JPN individuals, and ISOSDB33 (IS1182 family) insertions for MDG individuals (Fig. 2D). Insertions in the *Bacteroidia* from JPN and ITA individuals were formed from shared IS elements, including ISOSDB4584 (ISL3 family), ISOSDB634 (IS982 family), and ISOSDB445 (IS66 family), whereas the IS diversity in the *Bacteroidia* from MDG individuals consisted of ISOSDB250 (IS66 family), ISOSDB426 (IS630 family), and ISOSDB45

(IS256 family) insertions. This suggests that regional differences contribute to IS element content or activity that drives insertion events.

IS insertion types among the *Clostridia* were found across all three geographic locations, with many more IS elements shared between JPN and ITA individuals (Fig. 2D). Interestingly, the *Clostridia* shared three IS family types (IS982, IS66, and IS30) with the *Bacteroidia*, yet the specific IS family members were different IS elements (Fig 2D). We conclude from this data that although the IS sequence space of these two classes of bacteria are unique, some IS families are shared and certain IS families may be more promiscuous.

MDG individuals, despite having similar numbers of IS insertions among their bacterial communities (Fig. 2B), had relatively low abundances of IS insertions in *Bacteroides*, *Phocaeicola, Blautia,* and *Bifidobacterium* and much higher abundance of IS elements associated with pathobionts including *Escherichia* and *Prevotella* (Fig. S2A) (38-41). This is supported by the presence of ISOSDB45 (IS256 family) insertions that are associated with pathogenic bacteria (Fig. 2D) (19, 42-44).

IS elements can insert into coding sequences or intergenic regions of the genome which can inactivate genes or influence the expression of adjacent genes (28). To measure IS insertions in intragenic versus intergenic sites, we analyzed the precise positions of all IS elements in ITA, JPN, and MDG individuals by comparing the number of intragenic insertions to intergenic insertions on a per individual basis. We found that specific IS elements had preference for intragenic or intergenic insertions (Fig. 2E). ISOSDB1159 (IS3 family), ISOSDB805 (IS3 family), ISOSDB178 (ISAS1 family), and ISOSDB237 (IS4 family) preferentially inserted into intergenic loci, while ISOSDB18121 (IS1380 family), ISOSDB445 (IS66 family), and ISOSDB412 (IS30 family) inserted more frequently into intragenic loci. These data indicate that some IS elements are suited to diversify genomes through mutation whereas others may preferentially insert adjacent to coding sequences to alter gene expression.

IS elements frequently insert into accessory genes important for bacterial adaptation

Having identified numerous IS insertions within predicted coding sequences, we analyzed open reading frames (ORFs) carrying IS insertions, termed iORFs, in the ITA, JPN, and MDG individuals. A deduplicated gene database of all predicted ORFs from the metagenomes of each study were assessed for iORF's. iORF's with insertions from a single individual were ~10-fold more abundant than iORFs with insertions from multiple individuals (Fig. 3A). This demonstrates that intestinal metagenomic IS insertions have a high degree of inter-individual variation. We next performed functional annotation of the iORF's and found five broad categories that were shared between individuals and encompassed 20.7% of iORFs (Fig. 3B). Many iORFs were annotated as *susC*, *susD*, or *tonB* receptor genes in ITA and JPN individuals. These genes encode high-affinity substrate-uptake receptors for carbohydrates and cofactors (45, 46). *susC-D* homologs are frequently involved in the uptake of polysaccharides used for metabolism, with *Bacteroidia* species containing many different *susC-D* homologs (45). Mobilome genes, including transposases, integrases, prophages, and mobile element defense genes, such as restriction modification systems, contained insertions in almost every individual across all studies. Exopolysaccharide biogenesis genes, such as LPS modifying enzymes and cell wall synthesis glycosyltransferases, were consistent IS element insertion targets. Cell wall and membrane modifications are crucial for *in vivo* survival by avoiding host immunity and during competition with other bacteria (47). Finally, antibiotic resistance genes frequently contained IS insertions, including the genes *tetQ* and *ermF* in JPN and ITA individuals. IS insertions were enriched in all functional categories except the EPS biogenesis genes, when comparing the abundance of iORFs with all predicted protein coding sequences (Fig. 3C, Fig. S2B). Together, these data show that genes involved in accessory metabolic functions, antibiotic resistance, and genomic plasticity are common IS insertion targets.

We next asked if certain iORFs were shared between individuals from the three studies. Although infrequent, a few iORFs were shared. 14% and 37% of ITA and JPN individuals,

respectively, had IS insertions in the gene *ermF*, a macrolide resistance gene, and 3.2% and 9.8% of ITA and JPN individuals, respectively, had IS insertions in *KAP*, a P-loop NTPase predicted to be involved in phage defense (Fig. 3D) (48, 49). 7.9% and 6.1% of ITA and MDG individuals, respectively, shared IS insertions in *shufflon*, a gene encoding an invertase that regulates pilin phase variation (Fig. 3D) (50). We found that iORF functional classes were conserved across diverse intestinal bacteria (Fig. 3E) yet the IS types responsible for these insertions differed among the various classes of bacteria (Fig. 3F). This demonstrates that although IS elements target similar genes within disparate bacteria, the types of IS elements that promote these diversification events are specific to certain classes of bacteria.

Multiple iORF's were classified as transposases (Fig. 3B) and examples of IS elements inserting in other transposases has been previously reported (51). In order to understand the dynamics of IS insertions in other IS elements, we classified both the transposase iORF and the IS forming the insertion by IS family (Fig. 3G). We found multiple examples of both self-targeting (an IS inserting into a closely-related IS) and orthologous-targeting (an IS element inserting into an unrelated IS). We found that IS families such as ISL3, IS4, IS110, and IS256 experienced promiscuous insertion events. These results suggest that IS self- and orthologous-targeting could be a mechanism to control IS mobilization of genetic traits.

IS* elements *provide mutational diversity to Bacteroides species

Genome wide mutagenesis of *Bacteroides thetaiotaomicron* (*Bt*) identified genes that support adaptation to environmental pressures, including antibiotics, bile acids, and carbon sources (52). We used this dataset to evaluate whether we could identify similar fitness determinants as iORFs within intestinal *Bacteroides*. We searched our iORF database for homologs to these fitness-associated ORFs from *Bt* and found multiple closely related iORFs that likely influence *Bacteroides* fitness both positively and negatively (Fig. S3A). Examples include iORFs that provide fitness advantages during growth in the presence of the antibiotics

doxycycline and vancomycin and during hyaluronic acid and glucosamine consumption (Fig. S3A). Interestingly, we found iORFs associated with bacterial fitness during exposure to the antipsychotic medication chlorpromazine (Fig S3A) which is associated with compositional changes and antibiotic resistance of the microbiota (53-56). Additionally, we found multiple IS insertions in homologs of the *Bt susC* gene BT1119 (Fig. S3B). Inactivation of BT1119 decreases fitness during growth on galacturonic acid (52).

Our *in silico* analysis shows that the intestinal *Bacteroidia* experience large-scale IS mobilization into discrete groups of genes involved in carbohydrate utilization, exopolysaccharide synthesis, and mobile genetic element interactions. To test if the activation of an IS element leads to insertion into these classes of genes, we focused on ISOSDB412 (IS30 family member IS4351), an IS element is that is native to strains of *Bt* and *Bacteroides fragilis* (*Bf*) (57). We transformed a low-copy number plasmid carrying an *ermF* cassette disrupted by ISOSDB412 into *Bt* VPI-5482 and *Bf* NCTC 9343. These strains lack native copies of ISOSDB412 which allowed us to study this IS element in the context of a genetic arrangement that we found from our analysis of the microbiota. Following growth, we isolated their genomic DNA and performed IS-Seq which enriches for ISOSDB412 amplicons to identify their insertion locations (19). Both *Bt* and *Bf* strains carrying the ISOSDB412 plasmid obtained numerous ISOSDB412 insertions throughout their genomes compared to controls that lacked the ISOSDB412 plasmid construct (Fig. 4A, Fig. 4D). As predicted, ISOSDB412 insertions were enriched in gene categories reflected from our findings from insertions from the human microbiota (Fig. 4B, Fig. 4E). In *Bt*, ISOSDB412 insertions were enriched in *susC-D/tonB* and EPS biogenesis genes, whereas *Bf* acquired an abundance of insertions in *susC-D/tonB* genes despite a significantly lower number of ISOSDB412 insertion events compared to *Bt* (Fig. S4A). Additionally, insertions were more frequently found in intragenic loci compared to intergenic loci, confirming our findings from the intestinal microbiota (Fig. 4C, Fig. 4F). These results demonstrate that IS elements can rapidly diversify *Bacteroidia* genomes.

Our discovery that IS insertions target accessory genes suggests that these IS insertions inactivate genes that are currently dispensable for intestinal survival. To explore this, we measured IS insertional dynamics in a nutrient-deplete environment with glucose as the sole carbon source. We hypothesized that a lack of fiber and complex carbon sources, high levels of glucose, and single sources of iron and nitrogen, would incentivize higher rates of IS insertions to inactivate costly metabolic machinery needed for competition in complex nutritional environments. We measured ISOSDB412 insertions from three *Bt* colonies after three sequential passages in either complex media (BHIS) or minimal media (MM) (Fig. 4G, Fig. 4H, Fig. 4I). We found that colonies passaged in MM had substantially more ISOSDB412 insertions compared to BHIS-passaged colonies. Numerous insertions were found in the open reading frame BT3642, a Na^+-dependent transporter (Fig. S4B) in MM colonies, but not in BHIS colonies. This gene has been shown to be detrimental for growth in glucose minimal media (52).

Next, we measured ISOSDB412 abundance in *Bt* cells chronically infected with the *Crassvirales* DAC15 or DAC17 (Fig. 4J, 4K, 4L). *Crassvirales* are bacteriophages that infect *Bacteroides* species and are abundant in the human intestine (58). *Bt* cells become chronically infected by these phages and shed them during growth (Fig. S4C). While DAC15 and DAC17 share 99.24% nucleotide identity over 98% of their genomes, DAC17 infection is associated with an increased IS insertions genome-wide (Fig. 4J, Fig. 4K) which inserted into genes involved in EPS biogenesis (Fig. 4L). Furthermore, almost all of the infected strains carried IS insertions in the CPS3 locus (Fig. S4D), which is crucial for productive phage infection and likely leads to phage resistance (59). Together, these results show that IS diversification of the *Bt* genome is more prevalent under nutrient-limited conditions and during infection with intestinal-resident phages. This indicates that IS activity supports *Bt* adaptation when faced with fitness constraints.

Temporal monitoring of IS activity shows that IS insertions are maintained over time.

IS insertions were found in specific genetic loci coexisting with the wild type allele lacking the IS insertion, indicating that both versions of the allele are maintained in equilibrium. Previous work has shown that many phyla of intestinal bacteria harbor variable numbers of IS insertions (60). To understand the temporal longevity of IS insertions in the microbiota, we analyzed longitudinal fecal metagenomic samples from healthy individuals (61, 62). Using a set of two individuals whose fecal samples were collected over the course of 76 and 91 weeks (61), we measured the IS landscape of their microbiotas using pseudoR (Fig. 5A & Fig. S5). We also analyzed longitudinal samples from an additional 10 individuals from a separate study (62) (Fig. S6). For each individual, we used the assemblies from the first timepoint (indicated as week 0 in Fig. 5A) as the reference for the pseudoR pipeline which was compared to all other time points to assess the maintenance of ancestral IS insertions and acquisition of new IS insertions. We found that many IS insertions are maintained for the entire time course and that some of these insertions frequently went in and out of detection (Fig. 5A, Fig. S5-6). New insertions not present at week 0 arose at almost every timepoint, with a variable number of insertions per timepoint. New insertions were maintained for extended periods of time while others were only detected at the initial timepoint. These results demonstrate that IS elements are stably carried in the microbiota and are actively forming new insertions, similar to the accumulation of mutations in laboratory-evolved strains (63-65).

Next, we analyzed whether the host's bacterial abundance accounts for the detection of new IS elements (Fig. S7A). Increasing levels of an host bacterial IS elements could generate higher sequencing read depth and more detection power for new IS insertions. For every insertion not present at week 0, we compared the insertion site's read depth (a marker of host abundance) to the maximum insertion site depth of all earlier timepoints (prior to the detection of the insertion). Insertions with depths equal to or less than 200% of the previous maximum insertion site depth were considered to arise from new insertional activity (Fig. S7B). We found

that the majority of new insertions could be accounted for as active IS insertions independent of bacterial abundance (Fig. S7C).

Insertions across the time series included diverse IS families and were frequently associated with the *Clostridia* and *Bacilli* (Fig. 5B, S5-6). Additionally, we observed that the rate of new insertions per week for each individual ranged on average from around 1 to 10 new insertions, assuming that. This value was calculated by dividing the number of new insertions per timepoint by the number of weeks between the current timepoint and the previous timepoint. We assumed a constant rate of transposition as has been used in previous studies (63, 64). Intra- and inter-personal variations in this rate could be due to both variations in selective pressures in the microbiota and technical variations, such as altered sequencing depth and library diversity. We also found that the maintenance, loss, and gain of IS elements tracked with common bacterial accessory genes that we established to be “hot spots” for IS insertions (Fig. 5C, Fig. 5D).

IS elements are efficiently transferred and maintained within new individuals

Having discovered that individual microbiotas have unique patterns of IS insertions, we wanted to understand how the human host influences the dynamics of IS insertions within the microbiota. To test this, we used pseudoR to profile the IS insertions during fecal microbiota transplantation (FMT) where the microbiotas of FMT donors and recipients were longitudinally sampled before and after fecal transplantation (61). We compared the IS insertional landscape in both the donors and recipients using the assembly of the donor’s transplanted microbiota as a reference. Donor-derived communities harbored IS insertions that were stably maintained over one year in the FMT recipients (Fig. 6A & Fig. S8). Insertions present prior to transplantation in recipients were lost, but a minority returned at later timepoints. Recipients maintained significantly less IS insertions that were present at week 0 compared to the donors (Fig. 6C), but both donor and recipients had similar rates of new insertions (Fig. 6D). To understand if IS

insertional activity was similar between host and recipient, we compared the pattern of new shared insertions in donors and recipients at all timepoints post transplantation (Fig. 6B & Fig. S9). We found multiple instances of the same newly detected insertion arising in both donor and recipients at the same timepoint. These results demonstrate that IS insertions can be stably maintained in new hosts for extended periods of time and that new IS mobilization occurs at similar rates and patterns in new hosts.

Antibiotics drive widespread loss of IS insertions within the microbiota

Considering IS insertions were found to persist for prolonged periods of time within the microbiota and were portable between individuals, we wanted to know what would happen to IS diversity if a stable community was disrupted. To test this, we analyzed the IS composition before, during, and after broad spectrum antibiotic treatment (66). The metagenome of the microbiota preceding antibiotic treatment was used as the reference for IS comparison using pseudoR. As expected, antibiotic treatment caused a substantial loss of IS diversity, which was not fully restored after treatment (Fig. 7A). The starkest example was sample ERAS10. Extensive IS diversity was present at week 0 in ERAS10 before antibiotic treatment and by at week 0.6 there were no detectable IS signatures nor did any of the original ISs return by week 26 post antibiotic therapy. To summarize these findings, we compared the rate of maintenance of pre-antibiotic treatment insertions in individuals treated with antibiotics and those who were not. Antibiotic treatment was associated with a significant decrease in the ability of the microbiota to maintain IS insertions (Fig. 7D).

IS insertion into susC-D/tonB loci is associated with diet intervention.

Our analysis had identified *susC-D/tonB* genes as major targets for IS insertions, suggesting that ISs can modulate carbohydrate metabolism in the *Bacteroidia* (Fig. 3), and we showed in *Bt* that nutrient stress promotes insertions into predictable metabolic loci (Fig. 4).

Based on this data, we suspected that a radical diet intervention in humans would lead to a shift in IS insertions in metabolic-associated genes such as *susC-D/tonB* loci. To test this hypothesis, we analyzed the IS insertions in the intestinal microbiota of obese individuals before, during, and after a year-long diet intervention (67). Participants in this study received a low-calorie formula-based diet for 12 weeks, followed by a 6-week period of phasing out the formula diet for solid food without changing total caloric intake, followed by a 7-week phase where caloric intake was increased while preventing weight gain (68). We found that ancestral *susC-D/tonB* insertions were frequently lost during the course of the diet study and new insertions in *susC-D/tonB* genes arose after the diet intervention ended (Fig. 7C). Additionally, insertions arose in the *susC-D/tonB* homolog BT1119 (Fig. 7D). Together, these results demonstrate that diet alteration is associated with perturbations in IS insertions in *susC-D/tonB* genes.

Discussion

IS elements are fundamental to the evolution of bacterial genomes. Despite this, IS diversity and function within polymicrobial communities is understudied. Here, we built a novel computational pipeline for identifying IS insertions within complex metagenomic DNA sequences. This pipeline, which we call pseudoR, relies on an open-source IS database (ISOSDB), which vastly improves nucleotide diversity compared to current state-of-the-art databases. Using pseudoR, we found that IS elements contribute to high levels of bacterial genomic diversification within the microbiota, are maintained at specific loci for extended periods of time, can be efficiently transferred between individuals, and experience fluctuations within bacterial populations following perturbation. Together, these results demonstrate that IS elements are important genetic elements that can dictate genotype diversity within the microbiota.

Using previously published metagenomic datasets, we show that the majority of open reading frames containing IS insertions (iORFs) are unique and not shared between individuals

(Fig. 3A). This is similar to the observation that polymorphism diversity in the human intestinal microbiota is more likely to be unique than shared (69). Our work expands on this idea and suggests that IS insertional diversity is a part of each person's "fingerprint" of microbial diversity.

iORFs were frequently annotated within five broad categories: *susC-D/tonB*, mobilome genes, EPS genes, antibiotic resistance genes, and MGE resistance genes. Genes belonging to these categories are considered accessory genes and are often non-essential. Interestingly, genes regulated by DNA inversions, including phase variation, fall into similar functional categories as those targeted by IS insertions (70, 71). This suggests that insertions and inversions control gene expression of the microbiota to position bacteria for optimal adaptation. Additionally, *susC-D/tonB* receptors are often targets of nucleotide evolution in the human intestine, possibly to help gain new abilities based on substrate availability (21). In support of this, a SusC epitope activates T cells to promote an anti-inflammatory IL-10 response in healthy individuals and induces a pro-inflammatory IL-17A response in people with Crohn's disease (72). This suggests that IS elements influence the immunomodulatory capacity of the microbiota.

The microbiome of urban individuals is less-well suited to degrade diverse polysaccharides compared to rural individuals (73). Here, we found that urban individuals (those in the ITA and JPN cohorts) have extensive modulation of the microbiota with high rates of IS insertions in *susC-D/tonB* genes, compared to individuals living in rural settings (MDG cohort). This could partly be explained by in ITA and JPN individuals as they have a dominance of IS insertions in *Bacteroides* and *Phocaeicola* species, while MDG individuals have limited *Bacteroidia* IS insertions. However, we expect that the varied diet of MDG individuals, which includes wild meat and plants (74), partially selects for a more diverse set of functional *susC-D/tonB* receptors compared to individuals consuming an industrialized diet. Further work to characterize IS insertions in individuals with rural lifestyles, such as the Hadza hunter gatherers (75), will better demonstrate how IS elements impact nutrient acquisition.

iORFs were common among MGE resistance and macrolide resistance (*ermF*) genes (76). Both of these classes of genes have been reported to be targets of IS inactivation (19, 33). Since these are heterogenous populations that co-exist between genes carrying or lacking IS insertions, we hypothesize that IS insertions in these genes helps maintain a low level of population-wide MGE and antibiotic resistance and helps reduce the burden of expressing the resistance gene. Such a “hedge betting” strategy would be advantageous upon antibiotic or phage exposure where the population can regain resistance thorough selection of variants.

Prior to this study, it was not known how stable IS elements were in bacterial genomes within host associated microbiotas. We found that specific IS insertions were detectable for just under two years, demonstrating that IS insertions within specific genetic loci are often not sanitized from the population. These findings are analogous to the discovery of nucleotide polymorphism conservation within the microbiota (77). Additionally, we also established that IS elements can be stably transferred between individuals. Donor-derived bacterial communities have been shown to persist in recipients following FMT (78-80). We conclude that IS insertions are stable in host associated microbiotas and may impart a minimal fitness cost to their bacterial host.

Bacterial diversity frequently rebounds after antibiotic treatment (66, 81), but how bacterial communities assemble at the strain level is more varied and includes widespread loss of mutations and enrichment of others (82). We found that IS insertions were readily lost after antibiotic treatment. Our results suggest that ISs can be viewed as strain-level features that are more variable than taxonomic-level diversity following perturbation.

In summary, the development of a contemporary and stringently curated IS database, combined with a streamlined computational approach to identify IS elements from metagenomic data, has revealed novel insights into how IS elements shape the genomes of the microbiota and sets the stage for exploring how specific IS variants in the microbiota influence human health. However, a few limitations to the tools and study exist. First, delineation of IS insertion

sites between closely related strains is imperfect, thus insertions between very closely related strains may group together and fail to resolve. Work from the intestinal microbiota has found that most species are represented by one dominant strain and that these strains are stable over long periods (83), suggesting that the insertion sites that we measured are from subpopulations originating from single strains. Second, we do not understand how IS insertions influence the fitness of the microbiota. We can begin to overcome this problem by assembling and assessing Tn-Seq libraries of diverse intestinal bacteria under conditions relevant to the intestine and in animal colonization experiments. Coupling such studies with pseudoR analyses of microbiota datasets would begin to help determine what IS insertion sites are beneficial or detrimental to commensal bacteria. Third, the pseudoR pipeline relies on assemblies containing some level of wild type allele being measured in comparison to an IS insertion at that same allele. This limits the detection of IS elements that assemble as part of the reference contig. Conceivably, an inverse method to find wild type alleles in IS-allele assemblies can be built and be implemented into a future release of pseudoR. Fourth, the single reference mode of pseudoR excludes assemblies from later timepoints to prevent over-assembly and focuses the analysis on the initial timepoint. This hinders our understanding of how IS elements are impacting the microbiota not present at the initial timepoints.

METHODS

EXPERIMENTAL STRAINS

Bacteroides thetaiotaomicron VPI-5482, *Bacteroides thetaiotaomicron* VPI-5482 CPS3, and *Bacteroides fragilis* NCTC 9343 were cultured as previously described (84, 85). Briefly, these strains were grown in brain-heart infusion (BHI) broth (Becton Dickinson, Franklin Lakes, NJ) supplemented with 1 g/L cysteine, 5% w/v $NaHCO_3$, and 5 mg/L hemin (BHIS), Varel-Bryant minimal media (86) supplemented with 28 mM glucose, or *Bacteroides* phage recovery medium

(BPRM) (87) at 37° C in a Coy Type A vinyl anaerobic chamber in an atmosphere of 5% hydrogen, 20% carbon dioxide, and balanced nitrogen (Coy Lab Products, Grass Lake, MI). For growth on solid-media we used BHI agar supplemented with 10% defibrinated calf blood (Colorado Serum Company, Denver, CO). *Escherichia coli* S17-1 was grown in Lennox L broth (Fisher Scientific, Hampton, NH) with aeration at 37° C. Tetracycline was used at 2 µg/mL, gentamicin was used at 200 µg/mL, and ampicillin was used at 100 µg/mL.

QUANTIFICATION AND STATISTICAL ANALYSIS

Details of statistical analyses for specific figures can be found in the figure legends. All statistical analyses were performed using Graphpad Prism, with the exception of Fisher's exact test which was performed using R.

METHOD DETAILS

Creation of the ISOSDB

All genomes classified as "complete" in NCBI Assembly were downloaded on 05/08/2023 using bit v1.8.57 (88). IS elements were identified using OASIS (27). This program uses annotated transposases as points for sequence extension and comparison to find complete IS elements. Identified IS elements were filtered using the following criteria: 1) multiple (minimum of 2) complete copies of the element must be present in at least one genome and 2) the elements must contain identified inverted repeat sequences. To supplement these genomes, we also identified IS elements in hybrid-assembled MAGs from a large human intestinal microbiota study (26). These metagenomes were annotated with Prokka v1.14.6 (89), processed as described above, and then combined with the IS elements identified from the

genomes from NCBI Assembly. Redundant IS elements in the final database were deduplicated using CD-HIT-EST (90) with a 95% sequence cutoff and a word length of 9. IS element homologs in ISFinder were found using either blastn (complete elements) with a minimum e-value of 0.000001 and a minimum length of alignment of 224 bp or blastp (transposases) with a minimum e-value of 0.000001 (91). To further confirm that IS elements in the ISOSDB are legitimate, the transposases of IS elements that lacked nucleotide homology to IS elements in ISFinder were profiled using InterProScan (92) and NCBI Conserved Domain Database (93) using default settings. Putative transposases that lacked domains associated with transposases (such as “DDE superfamily endonuclease”, “Integrase core domain”, and “Transposase”) were removed from the final ISOSDB. The ISOSDB and pseudoR pipeline is freely available at https://github.com/joshuakirsch/pseudoR. Each IS element in this database is given a unique numeric identifier. Clustering at the amino acid and nucleotide level was performed using MMSeqs2 v15.6f452 (94) with coverage mode 0 and a minimum coverage of 80%.

pseudoR pipeline

To characterize IS elements and their insertion sites in metagenomic DNA sequences we built the pseudoR pipeline. This tool relies on deeply-sequenced short read Illumina data and can be used for both metagenomic and single isolate genome data.

For this study, reads were downloaded from NCBI SRA (95) using fasterq-dump v2.11.0 from these studies (34-36, 61, 66, 67). Study accession numbers are provided in the Key Resources Table. Duplications, quality trimming, and read decontamination was performed using programs from the BBTools software suite (96). PCR duplicated reads were removed with clumpify.sh with the flag “subs=0”. Adapter and quality trimming was performed with bbduk.sh and human, mouse, and phi29 read contamination was removed using bbsplit.sh. Cleaned and

deduplicated reads were assembled into contigs using MEGAHIT v1.2.7 using the “meta-large” preset (-k-max 127 -k-min 27 -k-step 10) (97). All contigs used in downstream analyses were greater than 1 kB.

We built two modes for IS identification: single and multi mode. Single mode is primarily used to identify IS insertions within the microbiota from longitudinal samples from the same individual. In this mode, only the individual’s starting timepoint assembly is used to find novel IS insertions. This approach reduces the chance that meaningful information is lost during clustering of multiple assemblies from multiple timepoints. The single reference mode can be used for evolution studies where a single population is compared between different treatments. Multi mode is used for comparing the insertional patterns between multiple individuals. When using the multi reference mode, reads are first mapped against the sample’s own assembly and then remapped against a combined and deduplicated ORF database built from every sample’s assembly.

Before the pipeline was initially run, a database of IS termini was built by merging the first and last 150 bp of each IS in the ISOSDB together and deduplicating these merged sequences using CD-HIT-EST (90) with a minimum sequence identity of 90%. The merged sequences were split into 150 bp sequences and a blastn database (91) was built from this deduplicated dataset.

At the beginning of the pipeline, ORFs in assemblies are predicted using pprodigal.py (a parallelizable wrapper of prodigal (98)) and deduplicated using dedupe.sh. Reads are aligned first to the reference assembly using Bowtie2 (99). Any mapped read, including discordantly mapped reads, are considered positive hits. Unmapped reads are collected and aligned to the IS termini database using blastn and then filtered using a custom R script. This filtering script ensures that the IS termini is properly positioned on the read such that the remainder of the read is outside of the IS element. The IS termini are removed and the trimmed reads are then

re-mapped to the sample's assembly as unpaired reads using Bowtie2. Following this, the original mapped reads and IS-termini trimmed reads are re-mapped against the deduplicated gene database in nucleotide format.

Following read mapping completion in both single and multi mode, a custom R script is used to identify DNA insertion sites with read mapping from both the left and right terminus of the IS element at most 20 bp away from one another and this script compiles read abundances for these regions. Mosdepth v0.3.3 (100) is used to determine the sequencing depth of the uninserted allele for the loci described above. Seqkit v2.2.0 (101), samtools v1.6 (102), R, ggplot2, tidyr, and MetBrewer are used for various computational tasks. Scripts to reproduce all data analysis presented here are available in the GitHub repository.

DNA extraction and IS-Seq

Genomic DNA was extracted from 1.5 mL cultures of *Bacteroides* strains using the Zymobionics DNA Miniprep kit (Zymo Research, Irvine, CA) and used as input for IS-Seq as described previously (19). Briefly, NGS libraries were produced using the Illumina DNA Prep Kit (Illumina, San Diego, CA) and amplified using ISOSDB412 IS-Seq Step1 and p7 primers for 13 cycles using Q5 Master Mix (New England Biolabs). The products of this reaction were amplified with IS-Seq Step2 and p7 primers for 9 cycles using Q5 Master Mix and sequenced on a Novaseq 6000 at Novogene (Sacramento, CA). R1 reads were binned and trimmed of the ISOSDB412 terminus and adapters using cutadapt v1.1.18 (103). To remove PCR duplicates, R2 pairs of R1 reads that contained the ISOSDB412 terminus were binned and deduplicated using dedupe.sh from the BBTools suite with the flag minidentity=100. Deduplication of R2 reads ensures that each amplified read pair is a unique molecule, while maintaining read depth information of the R1 (IS-amplified) read. This set of deduplicated R2 reads was re-paired to

trimmed R1 reads with reformat.sh from the BBTools suite and these pairs were aligned to the host genome using Bowtie2. R1 read depth at each genomic position was calculated using bedtools v2.30.0 (104) IS-seq reads can be found at the European Nucleotide Archive under study accession number PRJEB66483.

Acknowledgements

We would like to thank the members of the Duerkop lab, Dr. A. Murat Eren, and Dr. Alejandro Reyes Muñoz for providing useful insights related to this project.

Funding

This work was supported by the National Institutes of Health grants R01AI141479 (B.A.D.), T32AI052066 (J.M.K), F31AI169976 (J.M.K.), and R35GM150996 (A.J.H). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Figure Legends

Figure 1. The ISOSDB: a database of diverse and complete ISs. (A) Number of unique IS sequences in ISFinder and the ISOSDB. (B) Comparison of transposase (Tnp amino acid) and nucleotide sequences of the full ISs from the ISOSDB to the ISs from ISFinder. (C) Numbers of clusters of ORFs and full nucleotide sequences in the ISOSDB for a range of minimum sequence identities. (D) Number of IS elements per IS family in the ISOSDB. Only families with 100 or more IS elements are shown.

Figure 2. IS insertions are abundant in intestinal bacteria. (A) Abundance of ISs from ITA, JPN, and MDG individuals. Each data point represents one new insertion not found in the

reference assembly. The y-axis is the relative IS depth percentage (IS depth/ (WT allele depth + IS depth)). (B) Number of insertions per sample shown in A. (C) IS insertions per bacterial class. The intensity of each bar is proportional to the number of IS insertions. (D) IS insertions in *Bacteroidia* and *Clostridia* bacteria organized by IS family. The intensity of each bar is proportional to the number of IS insertions. (E) Preferential IS insertion in either intergenic (left side, red) or intragenic (right side, blue) loci for highly abundant ISs (paired T test with FDR multiple testing correction, maximum adjusted *p* value = 0.05). The difference between intragenic and intergenic insertions individual IS elements was averaged between multiple individuals and is shown on the X axis.

Figure 3. IS insertions are commonly found in bacterial accessory genes. (A) Number of shared and unique iORFs within ITA, JPN, and MDG individuals. (B) Heatmap of IS insertions in different gene functional categories. (C) Statistically significant enrichment of ISs in the functional categories from B. White asterisks represent categories where the iORFs are over-abundant compared to all genes and blue asterisks represent categories where the iORFs are under-abundant compared to all genes (Fisher's exact test with FDR multiple comparison correction, ***$p < 10^{-25}$, **$p < 10^{-4}$, *$p < 0.05$). (D) Location and relative IS depth in shared iORFs. The location of the insertion (either above or below the gene representation) is representative of the study. (E-F) Number of IS insertions in iORFs in the functional categories in B from different bacterial classes (E) and IS families (F). (G) Rate of IS insertions in transposases. Rectangles in the red dashed line are self-targets and rectangles outside of the red dashed line are non-synonymous-targets.

Figure 4. ISOSDB412 insertion in *Bacteroides* species replicates findings from intestinal metagenomic data. (A and D) Representative IS-Seq deep sequencing of ISOSDB412 insertions in *Bt* (A) and *Bf* (D). (B and E) Functional enrichment of ISOSDB412 insertions in *Bt*

(B) and *Bf* (E) (Fisher's exact test, *$p < 0.06$, ***$p < 0.001$). (C and F) ISOSDB412 insertions were counted in intergenic and intragenic loci in *Bt* (C) and *Bf* (F) (paired T-test, *$p < 0.05$). (G) Representative IS-Seq of ISOSDB412 insertions of *Bt* passaged in either BHIS or MM. (H) Number of ISOSDB412 insertions in each condition in G (paired T test). (I) Number of ISOSDB412 insertions in EPS biogenesis genes in G (paired T test). (J) Representative IS-Seq of ISOSDB412 insertions of *Bt* chronically infected with DAC15 or DAC17. (K) Number of ISOSDB412 insertions in each condition in J (unpaired T test, *$p < 0.05$). (L) Number of ISOSDB412 insertions in EPS biogenesis genes in J (unpaired T test, *$p < 0.05$).

Figure 5. IS insertions are maintained in the intestinal microbiota for extended time periods. (A) IS insertions are depicted as rectangles, with the color intensity proportional to the relative IS depth percentage. Each insertion is unique based on insertion position and the same insertions are aligned vertically. Orange-colored IS insertions are present at the first timepoint (week 0), green IS insertions are new insertions not present at the initial timepoint, and blue IS insertions are new insertions at timepoints after their first appearance. (B) Number of IS insertions per timepoint for IS families (red) or taxonomic classes (blue). (C) Rate of new IS insertions per week. (D) Dynamics of IS insertions over time for gene functional category iORFs. Data from every individual was used for this figure. The legend for this panel is the same as in A.

Figure 6. FMT transfer of IS insertions between donors and recipients. (A) Blue rectangles are IS element insertions in donor samples and red rectangles are IS insertions in recipient samples. The same insertions in donor and recipients are aligned vertically. Week 0 is the transplanted fecal material. (B) Heatmap showing new IS insertions that were detected 2 weeks after transplantation and are detected in both donor and recipient samples. (C) Loss of IS

insertions detected in the transplanted fecal material (week 0) from recipients (paired T test, ***p* < 0.01). (D) Similar rates of new IS insertions are found in both donor and recipients (unpaired T test. Outliers removed prior to this test using the ROUT method where Q = 1%).

Figure 7. Antibiotic treatment and diet intervention alters the IS insertional landscape. (A) Heatmap showing IS insertion dynamics during antibiotic treatment. Only insertions in alleles with less than or equal to 200% of the week 0 read depth are shown in this panel. Horizontal black bars at the 0.6 Week timepoint are present in individuals who did not have an available sample for this timepoint. (B) Antibiotic treatment significantly decreases the number of IS insertions present following week 0 (unpaired T test, *** $p < 0.005$). (C) Dynamics of IS insertions in *susCD/tonB* genes during the diet timecourse. Data from every individual was used for this figure panel. (D) New *BT1119* IS insertions arise after the diet intervention has ended. Wild type allele depth is shown as a black line and IS insertion allele depth is shown as a red bar.

References

1. Shkoporov AN, Clooney AG, Sutton TDS, Ryan FJ, Daly KM, Nolan JA, McDonnell SA, Khokhlova EV, Draper LA, Forde A, Guerin E, Velayudhan V, Ross RP, Hill C. 2019. The human gut virome is highly diverse, stable, and individual specific. Cell Host Microbe 26:527-541.e5.

2. Forster SC, Liu J, Kumar N, Gulliver EL, Gould JA, Escobar-Zepeda A, Mkandawire T, Pike LJ, Shao Y, Stares MD, Browne HP, Neville BA, Lawley TD. 2022. Strain-level characterization of broad host range mobile genetic elements transferring antibiotic resistance from the human microbiome. Nat Commun 13:1445.

3. Jiang X, Hall AB, Xavier RJ, Alm EJ. 2019. Comprehensive analysis of chromosomal mobile genetic elements in the gut microbiome reveals phylum-level niche-adaptive gene pools. PLoS One 14:e0223680.

4. Jiang X, Hall AB, Arthur TD, Plichta DR, Covington CT, Poyet M, Crothers J, Moses PL, Tolonen AC, Vlamakis H, Alm EJ, Xavier RJ. 2019. Invertible promoters mediate bacterial phase variation, antibiotic resistance, and host adaptation in the gut. Science 363:181-187.

5. Desvaux M, Dalmasso G, Beyrouthy R, Barnich N, Delmas J, Bonnet R. 2020. Pathogenicity factors of genomic islands in intestinal and extraintestinal *Escherichia coli*. Front Microbiol 11:2065.

6. Groussin M, Poyet M, Sistiaga A, Kearney SM, Moniz K, Noel M, Hooker J, Gibbons SM, Segurel L, Froment A, Mohamed RS, Fezeu A, Juimo VA, Lafosse S, Tabe FE, Girard C, Iqaluk D, Nguyen LTT, Shapiro BJ, Lehtimäki J, Ruokolainen L, Kettunen PP, Vatanen T, Sigwazi S, Mabulla A, Domínguez-Rodrigo M, Nartey YA, Agyei-Nkansah A, Duah A, Awuku YA, Valles KA, Asibey SO, Afihene MY, Roberts LR, Plymoth A, Onyekwere CA, Summons RE, Xavier RJ, Alm EJ. 2021. Elevated rates of horizontal gene transfer in the industrialized human microbiome. Cell 184:2053-2067.e18.

7. Brito IL, Yilmaz S, Huang K, Xu L, Jupiter SD, Jenkins AP, Naisilisili W, Tamminen M, Smillie CS, Wortman JR, Birren BW, Xavier RJ, Blainey PC, Singh AK, Gevers D, Alm EJ. 2016. Mobile genes in the human microbiome are structured from global to individual scales. Nature 535:435-439.

8. Aziz RK, Breitbart M, Edwards RA. 2010. Transposases are the most abundant, most ubiquitous genes in nature. Nucleic Acids Res 38:4207-17.

9. Siguier P, Gourbeyre E, Chandler M. 2014. Bacterial insertion sequences: their genomic impact and diversity. FEMS Microbiol Rev 38:865-91.

10. He S, Corneloup A, Guynet C, Lavatine L, Caumont-Sarcos A, Siguier P, Marty B, Dyda F, Chandler M, Ton Hoang B. 2015. The IS200/IS605 family and "peel and paste" single-strand transposition mechanism. Microbiol Spectr 3.

11. Piegu B, Bire S, Arensburger P, Bigot Y. 2015. A survey of transposable element classification systems--a call for a fundamental update to meet the challenge of their diversity and complexity. Mol Phylogenet Evol 86:90-109.

12. Kosek D, Hickman AB, Ghirlando R, He S, Dyda F. 2021. Structures of ISCth4 transposososomes reveal the role of asymmetry in copy-out/paste-in DNA transposition. EMBO J 40:e105666.

13. Jellen-Ritter AS, Kern WV. 2001. Enhanced expression of the multidrug efflux pumps AcrAB and AcrEF associated with insertion element transposition in *Escherichia coli* mutants selected with a fluoroquinolone. Antimicrob Agents Chemother 45:1467-72.

14. Shankar N, Baghdayan AS, Gilmore MS. 2002. Modulation of virulence within a pathogenicity island in vancomycin-resistant *Enterococcus faecalis*. Nature 417:746-50.

15. Chain PS, Carniel E, Larimer FW, Lamerdin J, Stoutland PO, Regala WM, Georgescu AM, Vergez LM, Land ML, Motin VL, Brubaker RR, Fowler J, Hinnebusch J, Marceau M, Medigue C, Simonet M, Chenal-Francisque V, Souza B, Dacheux D, Elliott JM, Derbise A, Hauser LJ, Garcia E. 2004. Insights into the evolution of *Yersinia pestis* through whole-genome comparison with *Yersinia pseudotuberculosis*. Proc Natl Acad Sci U S A 101:13826-31.

16. Manson JM, Hancock LE, Gilmore MS. 2010. Mechanism of chromosomal transfer of *Enterococcus faecalis* pathogenicity island, capsule, antimicrobial resistance, and other traits. Proc Natl Acad Sci U S A 107:12269-74.

17. Hawkey J, Monk JM, Billman-Jacobe H, Palsson B, Holt KE. 2020. Impact of insertion sequences on convergent evolution of *Shigella* species. PLoS Genet 16:e1008931.

18. Leavis HL, Willems RJ, van Wamel WJ, Schuren FH, Caspers MP, Bonten MJ. 2007. Insertion sequence-driven diversification creates a globally dispersed emerging multiresistant subspecies of *E. faecium*. PLoS Pathog 3:e7.

19. Kirsch JM, Ely S, Stellfox ME, Hullahalli K, Luong P, Palmer KL, Van Tyne D, Duerkop BA. 2023. Targeted IS-element sequencing uncovers transposition dynamics during selective pressure in enterococci. PLoS Pathog 19:e1011424.

20. Zlitni S, Bishara A, Moss EL, Tkachenko E, Kang JB, Culver RN, Andermann TM, Weng Z, Wood C, Handy C, Ji HP, Batzoglou S, Bhatt AS. 2020. Strain-resolved microbiome sequencing reveals mobile elements that drive bacterial competition on a clinical timescale. Genome Med 12:50.

21. Zhao S, Lieberman TD, Poyet M, Kauffman KM, Gibbons SM, Groussin M, Xavier RJ, Alm EJ. 2019. Adaptive evolution within gut microbiomes of healthy people. Cell Host Microbe 25:656-667.e8.

22. Elhenawy W, Tsai CN, Coombes BK. 2019. Host-specific adaptive diversification of Crohn's disease-associated adherent-invasive *Escherichia coli*. Cell Host Microbe 25:301-312.e5.

23. Barreto HC, Frazão N, Sousa A, Konrad A, Gordo I. 2020. Mutation accumulation and horizontal gene transfer in *Escherichia coli* colonizing the gut of old mice. Commun Integr Biol 13:89-96.

24. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res 34:D32-6.

25. Kichenaradja P, Siguier P, Pérochon J, Chandler M. 2010. ISbrowser: an extension of ISfinder for visualizing insertion sequences in prokaryotic genomes. Nucleic Acids Res 38:D62-8.

26. Gounot JS, Chia M, Bertrand D, Saw WY, Ravikrishnan A, Low A, Ding Y, Ng AHQ, Tan LWL, Teo YY, Seedorf H, Nagarajan N. 2022. Genome-centric analysis of short and long read metagenomes reveals uncharacterized microbiome diversity in Southeast Asians. Nat Commun 13:6044.

27. Robinson DG, Lee MC, Marx CJ. 2012. OASIS: an automated program for global investigation of bacterial and archaeal insertion sequences. Nucleic Acids Res 40:e174.

28. Tempel S, Bedo J, Talla E. 2022. From a large-scale genomic analysis of insertion sequences to insights into their regulatory roles in prokaryotes. BMC Genomics 23:451.

29. Nzabarushimana E, Tang H. 2018. Insertion sequence elements-mediated structural variations in bacterial genomes. Mob DNA 9:29.

30. Quadrana L, Bortolini Silveira A, Mayhew GF, LeBlanc C, Martienssen RA, Jeddeloh JA, Colot V. 2016. The *Arabidopsis thaliana* mobilome and its impact at the species level. Elife 5:e15716.

31. Hawkey J, Hamidian M, Wick RR, Edwards DJ, Billman-Jacobe H, Hall RM, Holt KE. 2015. ISMapper: identifying transposase insertion sites in bacterial genomes from short read sequence data. BMC Genomics 16:667.

32. Durrant MG, Li MM, Siranosian BA, Montgomery SB, Bhatt AS. 2020. A bioinformatic analysis of integrative mobile genetic elements highlights their role in bacterial adaptation. Cell Host Microbe 27:140-153.e9.

33. Sheng Y, Wang H, Ou Y, Wu Y, Ding W, Tao M, Lin S, Deng Z, Bai L, Kang Q. 2023. Insertion sequence transposition inactivates CRISPR-Cas immunity. Nat Commun 14:4366.

34. Rampelli S, Soverini M, D'Amico F, Barone M, Tavella T, Monti D, Capri M, Astolfi A, Brigidi P, Biagi E, Franceschi C, Turroni S, Candela M. 2020. Shotgun metagenomics of gut microbiota in humans with up to extreme longevity and the increasing role of xenobiotic degradation. mSystems 5.

35. Yachida S, Mizutani S, Shiroma H, Shiba S, Nakajima T, Sakamoto T, Watanabe H, Masuda K, Nishimoto Y, Kubo M, Hosoda F, Rokutan H, Matsumoto M, Takamaru H, Yamada M, Matsuda T, Iwasaki M, Yamaji T, Yachida T, Soga T, Kurokawa K, Toyoda A, Ogura Y, Hayashi T, Hatakeyama M, Nakagama H, Saito Y, Fukuda S, Shibata T, Yamada T. 2019. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. Nat Med 25:968-976.

36. Pasolli E, Asnicar F, Manara S, Zolfo M, Karcher N, Armanini F, Beghini F, Manghi P, Tett A, Ghensi P, Collado MC, Rice BL, DuLong C, Morgan XC, Golden CD, Quince C, Huttenhower C, Segata N. 2019. Extensive unexplored human microbiome diversity

revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. Cell 176:649-662.e20.

37. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, Mele MC. 2019. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms 7.

38. Alpizar-Rodriguez D, Lesker TR, Gronow A, Gilbert B, Raemy E, Lamacchia C, Gabay C, Finckh A, Strowig T. 2019. *Prevotella copri* in individuals at risk for rheumatoid arthritis. Ann Rheum Dis 78:590-593.

39. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, Rostron T, Cerundolo V, Pamer EG, Abramson SB, Huttenhower C, Littman DR. 2013. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. Elife 2:e01202.

40. Hughes ER, Winter MG, Duerkop BA, Spiga L, Furtado de Carvalho T, Zhu W, Gillis CC, Buttner L, Smoot MP, Behrendt CL, Cherry S, Santos RL, Hooper LV, Winter SE. 2017. Microbial respiration and formate oxidation as metabolic signatures of inflammation-associated dysbiosis. Cell Host Microbe 21:208-219.

41. Conway T, Cohen PS. 2015. Commensal and pathogenic *Escherichia coli* metabolism in the gut. Microbiol Spectr 3.

42. Lebreton F, van Schaik W, McGuire AM, Godfrey P, Griggs A, Mazumdar V, Corander J, Cheng L, Saif S, Young S, Zeng Q, Wortman J, Birren B, Willems RJ, Earl AM, Gilmore MS. 2013. Emergence of epidemic multidrug-resistant *Enterococcus faecium* from animal and commensal strains. mBio 4.

43. McEvoy CR, Tsuji B, Gao W, Seemann T, Porter JL, Doig K, Ngo D, Howden BP, Stinear TP. 2013. Decreased vancomycin susceptibility in *Staphylococcus aureus* caused by IS256 tempering of WalKR expression. Antimicrob Agents Chemother 57:3240-9.

44. Kozitskaya S, Cho SH, Dietrich K, Marre R, Naber K, Ziebuhr W. 2004. The bacterial insertion sequence element IS256 occurs preferentially in nosocomial *Staphylococcus epidermidis* isolates: association with biofilm formation and resistance to aminoglycosides. Infect Immun 72:1210-5.

45. Martens EC, Koropatkin NM, Smith TJ, Gordon JI. 2009. Complex glycan catabolism by the human gut microbiota: the Bacteroidetes Sus-like paradigm. J Biol Chem 284:24673-7.

46. Noinaj N, Guillier M, Barnard TJ, Buchanan SK. 2010. TonB-dependent transporters: regulation, structure, and function. Annu Rev Microbiol 64:43-60.

47. Yadav AK, Espaillat A, Cava F. 2018. Bacterial Strategies to Preserve Cell Wall Integrity Against Environmental Threats. Front Microbiol 9:2064.

48. Vassallo CN, Doering CR, Littlehale ML, Teodoro GIC, Laub MT. 2022. A functional selection reveals previously undetected anti-phage defence systems in the *E. coli* pangenome. Nat Microbiol 7:1568-1579.

49. Aravind L, Iyer LM, Leipe DD, Koonin EV. 2004. A novel family of P-loop NTPases with an unusual phyletic distribution and transmembrane segments inserted within the NTPase domain. Genome Biol 5:R30.

50. Komano T. 1999. Shufflons: multiple inversion systems and integrons. Annu Rev Genet 33:171-91.

51. Blattner FR, Plunkett G, 3rd, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y. 1997. The complete genome sequence of *Escherichia coli* K-12. Science 277:1453-62.

52. Liu H, Shiver AL, Price MN, Carlson HK, Trotter VV, Chen Y, Escalante V, Ray J, Hern KE, Petzold CJ, Turnbaugh PJ, Huang KC, Arkin AP, Deutschbauer AM. 2021. Functional genetics of human gut commensal *Bacteroides thetaiotaomicron* reveals metabolic requirements for growth across environments. Cell Rep 34:108789.

53. Flowers SA, Evans SJ, Ward KM, McInnis MG, Ellingrod VL. 2017. Interaction between atypical antipsychotics and the gut microbiome in a bipolar disease cohort. Pharmacotherapy 37:261-267.

54. Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR, Fernandez KC, Dose H, Mori H, Patil KR, Bork P, Typas A. 2018. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature 555:623-628.

55. Ait Chait Y, Mottawea W, Tompkins TA, Hammami R. 2020. Unravelling the antimicrobial action of antidepressants on gut commensal microbes. Sci Rep 10:17878.

56. Kyono Y, Ellezian L, Hu Y, Eliadis K, Moy J, Hirsch EB, Federle MJ, Flowers SA. 2022. The atypical antipsychotic quetiapine promotes multiple antibiotic resistance in *Escherichia coli*. J Bacteriol 204:e0010222.

57. Tisza MJ, Smith DDN, Clark AE, Youn JH, Khil PP, Dekker JP. 2023. Roving methyltransferases generate a mosaic epigenetic landscape and influence evolution in Bacteroides fragilis group. Nat Commun 14:4082.

58. Dutilh BE, Cassman N, McNair K, Sanchez SE, Silva GG, Boling L, Barr JJ, Speth DR, Seguritan V, Aziz RK, Felts B, Dinsdale EA, Mokili JL, Edwards RA. 2014. A highly abundant bacteriophage discovered in the unknown sequences of human faecal metagenomes. Nat Commun 5:4498.

59. Hryckowian AJ, Merrill BD, Porter NT, Van Treuren W, Nelson EJ, Garlena RA, Russell DA, Martens EC, Sonnenburg JL. 2020. *Bacteroides thetaiotaomicron*-infecting bacteriophage isolates inform sequence-based host range predictions. Cell Host Microbe 28:371-379.e5.

60. Touchon M, Rocha EP. 2007. Causes of insertion sequences abundance in prokaryotic genomes. Mol Biol Evol 24:969-81.

61. Watson AR, Füssel J, Veseli I, DeLongchamp JZ, Silva M, Trigodet F, Lolans K, Shaiber A, Fogarty E, Runde JM, Quince C, Yu MK, Söylev A, Morrison HG, Lee STM, Kao D, Rubin DT, Jabri B, Louie T, Eren AM. 2023. Metabolic independence drives gut microbial colonization and resilience in health and disease. Genome Biol 24:78.

62. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, Andrews E, Ajami NJ, Bonham KS, Brislawn CJ, Casero D, Courtney H, Gonzalez A, Graeber TG, Hall AB, Lake K, Landers CJ, Mallick H, Plichta DR, Prasad M, Rahnavard G, Sauk J, Shungin D, Vázquez-Baeza Y, White RA, 3rd, Braun J, Denson LA, Jansson JK, Knight R, Kugathasan S, McGovern DPB, Petrosino JF, Stappenbeck TS, Winter

HS, Clish CB, Franzosa EA, Vlamakis H, Xavier RJ, Huttenhower C. 2019. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature 569:655-662.

63. Lee H, Doak TG, Popodi E, Foster PL, Tang H. 2016. Insertion sequence-caused large-scale rearrangements in the genome of Escherichia coli. Nucleic Acids Res 44:7109-19.

64. Sousa A, Bourgard C, Wahl LM, Gordo I. 2013. Rates of transposition in Escherichia coli. Biol Lett 9:20130838.

65. Consuegra J, Gaffe J, Lenski RE, Hindre T, Barrick JE, Tenaillon O, Schneider D. 2021. Insertion-sequence-mediated mutations both promote and constrain evolvability during a long-term experiment with bacteria. Nat Commun 12:980.

66. Palleja A, Mikkelsen KH, Forslund SK, Kashani A, Allin KH, Nielsen T, Hansen TH, Liang S, Feng Q, Zhang C, Pyl PT, Coelho LP, Yang H, Wang J, Typas A, Nielsen MF, Nielsen HB, Bork P, Wang J, Vilsbøll T, Hansen T, Knop FK, Arumugam M, Pedersen O. 2018. Recovery of gut microbiota of healthy adults following antibiotic exposure. Nat Microbiol 3:1255-1265.

67. Louis S, Tappu RM, Damms-Machado A, Huson DH, Bischoff SC. 2016. Characterization of the gut microbial community of obese patients following a weight-loss intervention using whole metagenome shotgun sequencing. PLoS One 11:e0149564.

68. Bischoff SC, Damms-Machado A, Betz C, Herpertz S, Legenbauer T, Löw T, Wechsler JG, Bischoff G, Austel A, Ellrott T. 2012. Multicenter evaluation of an interdisciplinary 52-week weight loss program for obesity with regard to body weight, comorbidities and quality of life--a prospective study. Int J Obes (Lond) 36:614-24.

69. Schloissnig S, Arumugam M, Sunagawa S, Mitreva M, Tap J, Zhu A, Waller A, Mende DR, Kultima JR, Martin J, Kota K, Sunyaev SR, Weinstock GM, Bork P. 2013. Genomic variation landscape of the human gut microbiome. Nature 493:45-50.

70. Chanin RB, West PT, Park RM, Wirbel J, Green GZM, Miklos AM, Gill MO, Hickey AS, Brooks EF, Bhatt AS. 2023. Intragenic DNA inversions expand bacterial coding capacity. bioRxiv doi:10.1101/2023.03.11.532203.

71. Milman O, Yelin I, Kishony R. 2023. Systematic identification of gene-altering programmed inversions across the bacterial domain. Nucleic Acids Res 51:553-573.

72. Pedersen TK, Brown EM, Plichta DR, Johansen J, Twardus SW, Delorey TM, Lau H, Vlamakis H, Moon JJ, Xavier RJ, Graham DB. 2022. The CD4(+) T cell response to a commensal-derived epitope transitions from a tolerant to an inflammatory state in Crohn's disease. Immunity 55:1909-1923.e6.

73. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 107:14691-6.

74. Golden CD, Anjaranirina EJG, Fernald LCH, Hartl DL, Kremen C, Milner DA, Jr., Ralalason DH, Ramihantaniarivo H, Randriamady H, Rice BL, Vaitla B, Volkman SK, Vonona MA, Myers SS. 2017. Cohort Profile: The Madagascar Health and Environmental Research (MAHERY) study in north-eastern Madagascar. Int J Epidemiol 46:1747-1748d.

75. Carter MM, Olm MR, Merrill BD, Dahan D, Tripathi S, Spencer SP, Yu FB, Jain S, Neff N, Jha AR, Sonnenburg ED, Sonnenburg JL. 2023. Ultra-deep sequencing of Hadza hunter-gatherers recovers vanishing gut microbes. Cell 186:3111-3124.e13.

76. Rasmussen JL, Odelson DA, Macrina FL. 1986. Complete nucleotide sequence and transcription of *ermF*, a macrolide-lincosamide-streptogramin B resistance determinant from *Bacteroides fragilis*. J Bacteriol 168:523-33.

77. Garud NR, Good BH, Hallatschek O, Pollard KS. 2019. Evolutionary dynamics of bacteria in the gut microbiome within and across hosts. PLoS Biol 17:e3000102.

78. Wilson BC, Vatanen T, Jayasinghe TN, Leong KSW, Derraik JGB, Albert BB, Chiavaroli V, Svirskis DM, Beck KL, Conlon CA, Jiang Y, Schierding W, Holland DJ, Cutfield WS, O'Sullivan JM. 2021. Strain engraftment competition and functional augmentation in a multi-donor fecal microbiota transplantation trial for obesity. Microbiome 9:107.

79. Li SS, Zhu A, Benes V, Costea PI, Hercog R, Hildebrand F, Huerta-Cepas J, Nieuwdorp M, Salojärvi J, Voigt AY, Zeller G, Sunagawa S, de Vos WM, Bork P. 2016. Durable coexistence of donor and recipient strains after fecal microbiota transplantation. Science 352:586-9.

80. Smillie CS, Sauk J, Gevers D, Friedman J, Sung J, Youngster I, Hohmann EL, Staley C, Khoruts A, Sadowsky MJ, Allegretti JR, Smith MB, Xavier RJ, Alm EJ. 2018. Strain tracking reveals the determinants of bacterial engraftment in the human gut following fecal microbiota transplantation. Cell Host Microbe 23:229-240.e5.

81. Ng KM, Aranda-Díaz A, Tropini C, Frankel MR, Van Treuren W, O'Loughlin CT, Merrill BD, Yu FB, Pruss KM, Oliveira RA, Higginbottom SK, Neff NF, Fischbach MA, Xavier KB, Sonnenburg JL, Huang KC. 2019. Recovery of the gut microbiota after antibiotics

depends on host diet, community context, and environmental reservoirs. Cell Host Microbe 26:650-665.e4.

82. Roodgar M, Good BH, Garud NR, Martis S, Avula M, Zhou W, Lancaster SM, Lee H, Babveyh A, Nesamoney S, Pollard KS, Snyder MP. 2021. Longitudinal linked-read sequencing reveals ecological and evolutionary responses of a human gut microbiome during antibiotic treatment. Genome Res 31:1433-1446.

83. Truong DT, Tett A, Pasolli E, Huttenhower C, Segata N. 2017. Microbial strain-level population structure and genetic diversity from metagenomes. Genome Res 27:626-638.

84. Bacic MK, Smith CJ. 2008. Laboratory maintenance and cultivation of *Bacteroides* species. Curr Protoc Microbiol Chapter 13:Unit 13C.1.

85. Hickey CA, Kuhn KA, Donermeyer DL, Porter NT, Jin C, Cameron EA, Jung H, Kaiko GE, Wegorzewska M, Malvin NP, Glowacki RW, Hansson GC, Allen PM, Martens EC, Stappenbeck TS. 2015. Colitogenic *Bacteroides thetaiotaomicron* antigens access host immune cells in a sulfatase-dependent manner via outer membrane vesicles. Cell Host Microbe 17:672-80.

86. Varel VH, Bryant MP. 1974. Nutritional features of *Bacteroides fragilis* subsp. *fragilis*. Appl Microbiol 28:251-7.

87. Tartera C, Araujo R, Michel T, Jofre J. 1992. Culture and decontamination methods affecting enumeration of phages infecting *Bacteroides fragilis* in sewage. Appl Environ Microbiol 58:2670-3.

88. Lee M. 2022. bit: a multipurpose collection of bioinformatics tools. F1000Research 11:122.

89. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068-9.

90. Li W, Godzik A. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658-9.

91. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403-10.

92. Zdobnov EM, Apweiler R. 2001. InterProScan--an integration platform for the signature-recognition methods in InterPro. Bioinformatics 17:847-8.

93. Marchler-Bauer A, Lu S, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C, Fong JH, Geer LY, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Jackson JD, Ke Z, Lanczycki CJ, Lu F, Marchler GH, Mullokandov M, Omelchenko MV, Robertson CL, Song JS, Thanki N, Yamashita RA, Zhang D, Zhang N, Zheng C, Bryant SH. 2011. CDD: a Conserved Domain Database for the functional annotation of proteins. Nucleic Acids Res 39:D225-9.

94. Steinegger M, Söding J. 2017. MMseqs2 enables sensitive protein sequence searching for the analysis of massive data sets. Nat Biotechnol 35:1026-1028.

95. Leinonen R, Sugawara H, Shumway M. 2011. The sequence read archive. Nucleic Acids Res 39:D19-21.

96. Bushnell B. BBTools, SourceForge. https://sourceforge.net/projects/bbmap/.

97. Li D, Liu CM, Luo R, Sadakane K, Lam TW. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31:1674-6.

98. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119.

99. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357-9.

100. Pedersen BS, Quinlan AR. 2018. Mosdepth: quick coverage calculation for genomes and exomes. Bioinformatics 34:867-868.

101. Shen W, Le S, Li Y, Hu F. 2016. SeqKit: A cross-platform and ultrafast toolkit for FASTA/Q file manipulation. PLoS One 11:e0163962.

102. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078-9.

103. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetjournal 17:3.

104. Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26:841-2.

105. Zheng L, Tan Y, Hu Y, Shen J, Qu Z, Chen X, Ho CL, Leung EL, Zhao W, Dai L. 2022. CRISPR/Cas-Based Genome Editing for Human Gut Commensal Bacteroides Species. ACS Synth Biol 11:464-472.

106. Willett JLE, Ji MM, Dunny GM. 2019. Exploiting biofilm phenotypes for functional characterization of hypothetical genes in Enterococcus faecalis. NPJ Biofilms Microbiomes 5:23.

107. Jones KR, Belvin BR, Macrina FL, Lewis JP. 2020. Sequence and characterization of shuttle vectors for molecular cloning in Porphyromonas, Bacteroides and related bacteria. Mol Oral Microbiol 35:181-191.

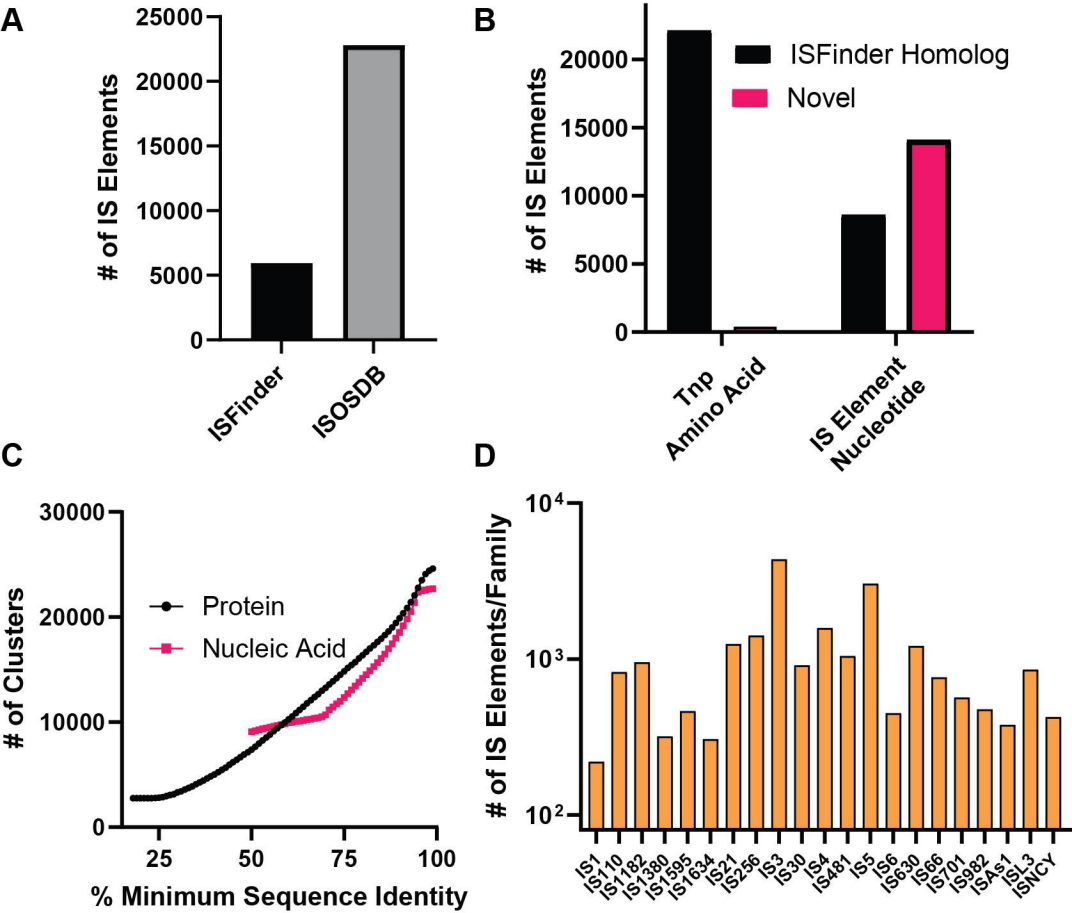
108. Cantalapiedra CP, Hernández-Plaza A, Letunic I, Bork P, Huerta-Cepas J. 2021. eggNOG-mapper v2: Functional annotation, orthology assignments, and domain prediction at the metagenomic scale. Mol Biol Evol 38:5825-5829.

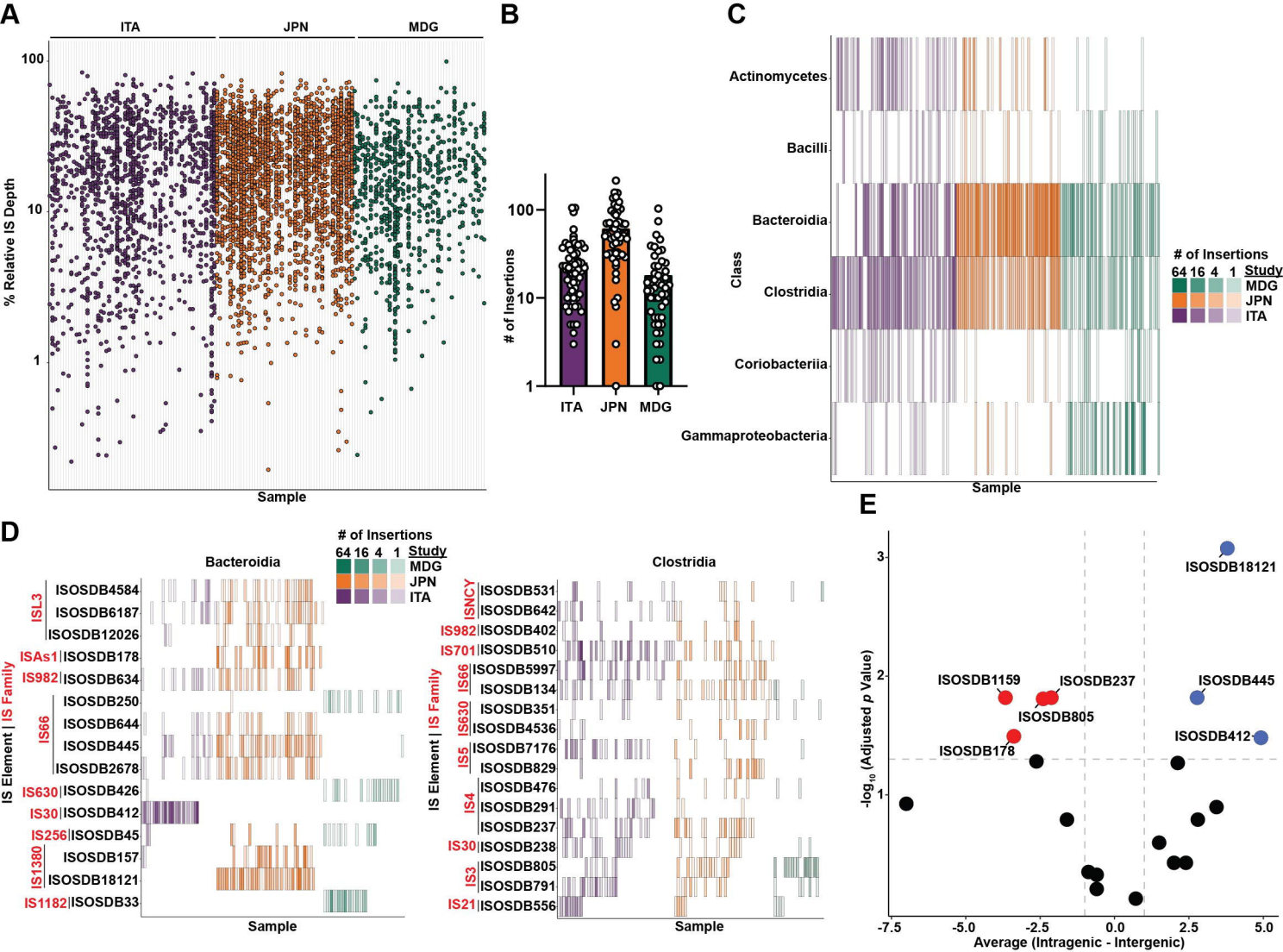
109. Tatusov RL, Galperin MY, Natale DA, Koonin EV. 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res 28:33-6.

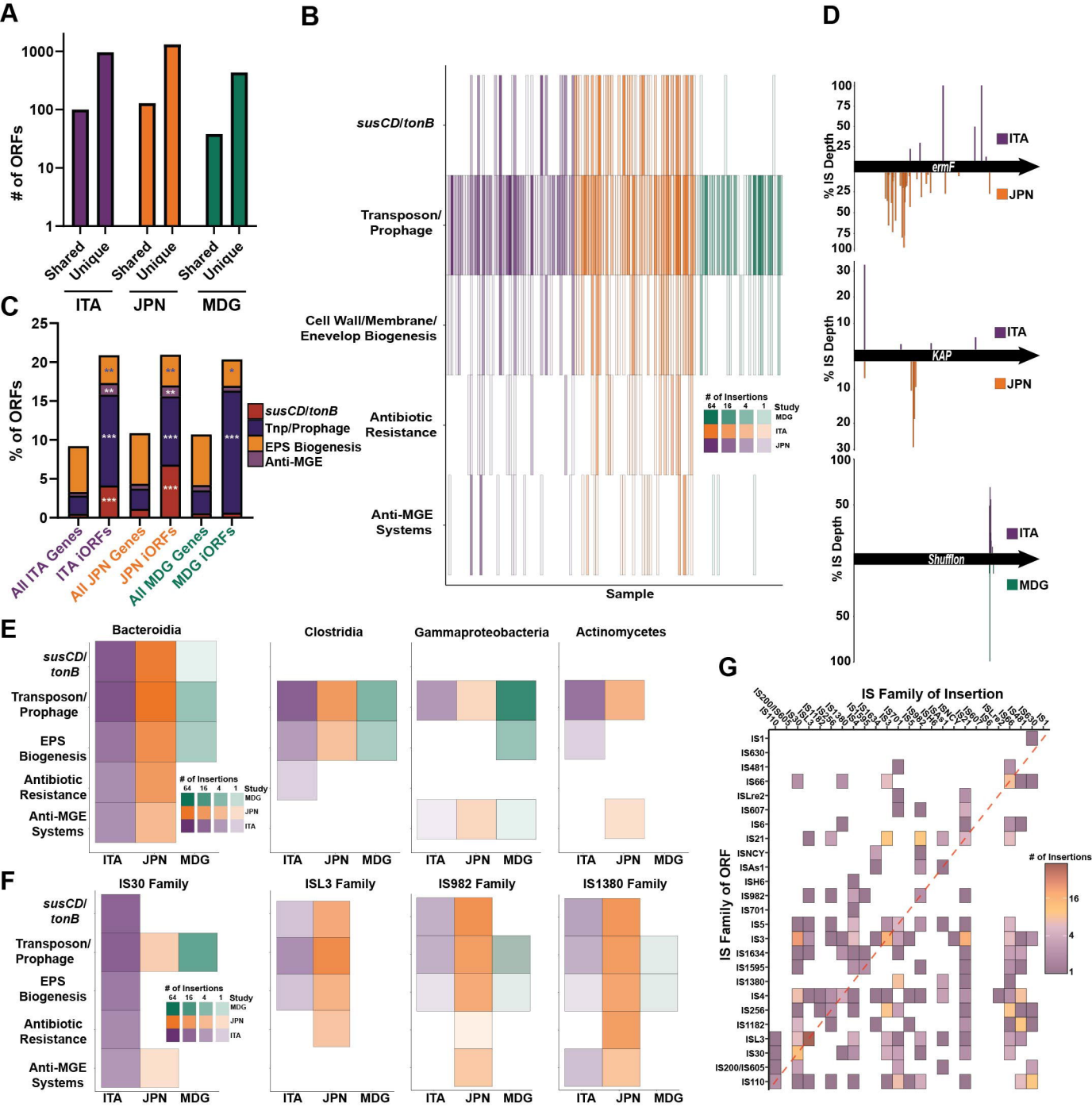
110. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, Huynh W, Nguyen AV, Cheng AA, Liu S, Min SY, Miroshnichenko A, Tran HK, Werfalli RE, Nasir JA, Oloni M, Speicher DJ, Florescu A, Singh B, Faltyn M, Hernandez-Koutoucheva A, Sharma AN, Bordeleau E, Pawlowski AC, Zubyk HL, Dooley D, Griffiths E, Maguire F, Winsor GL, Beiko RG, Brinkman FSL, Hsiao WWL, Domselaar GV, McArthur AG. 2020. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517-d525.

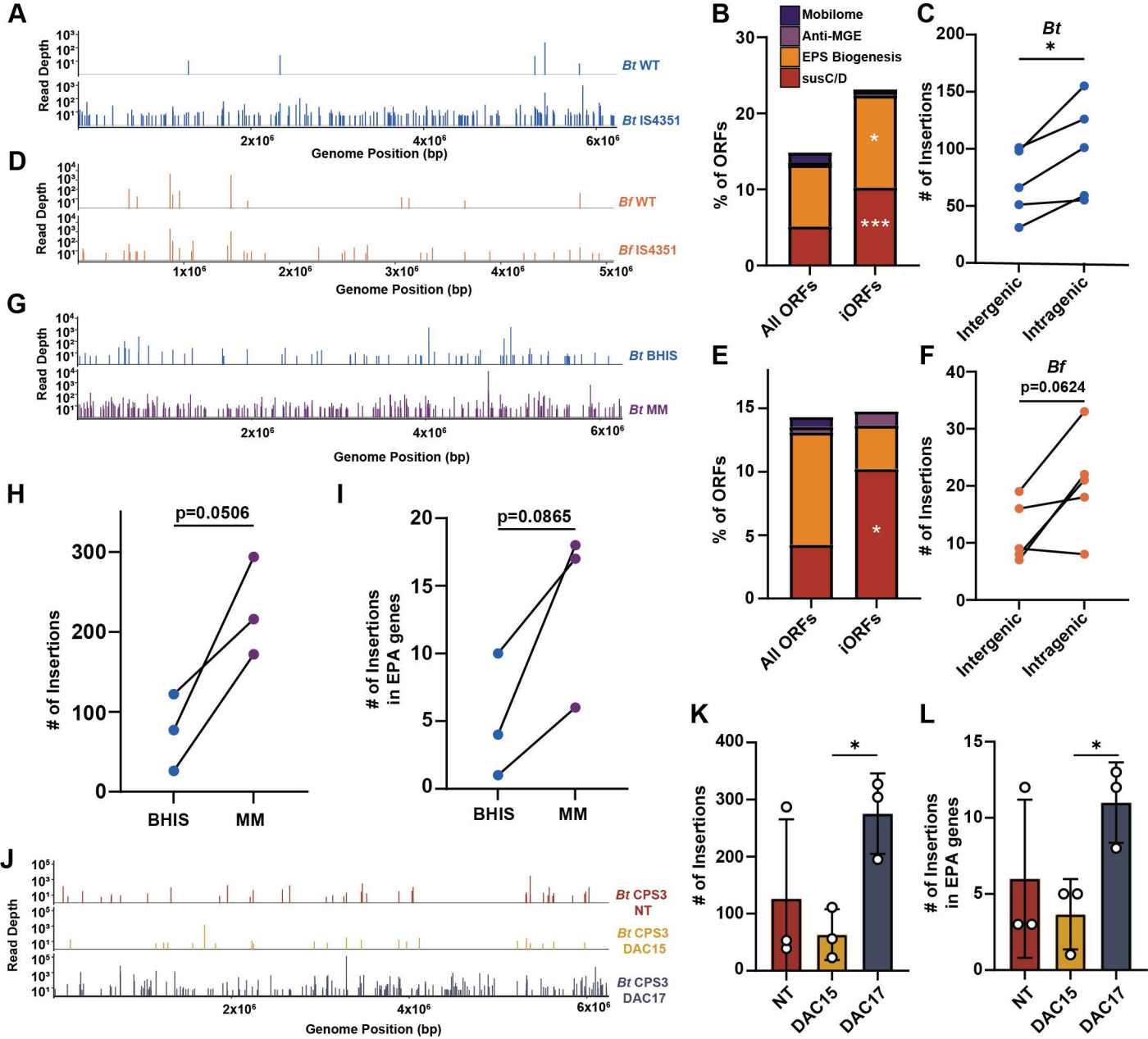
111. Wood DE, Lu J, Langmead B. 2019. Improved metagenomic analysis with Kraken 2. Genome Biol 20:257.

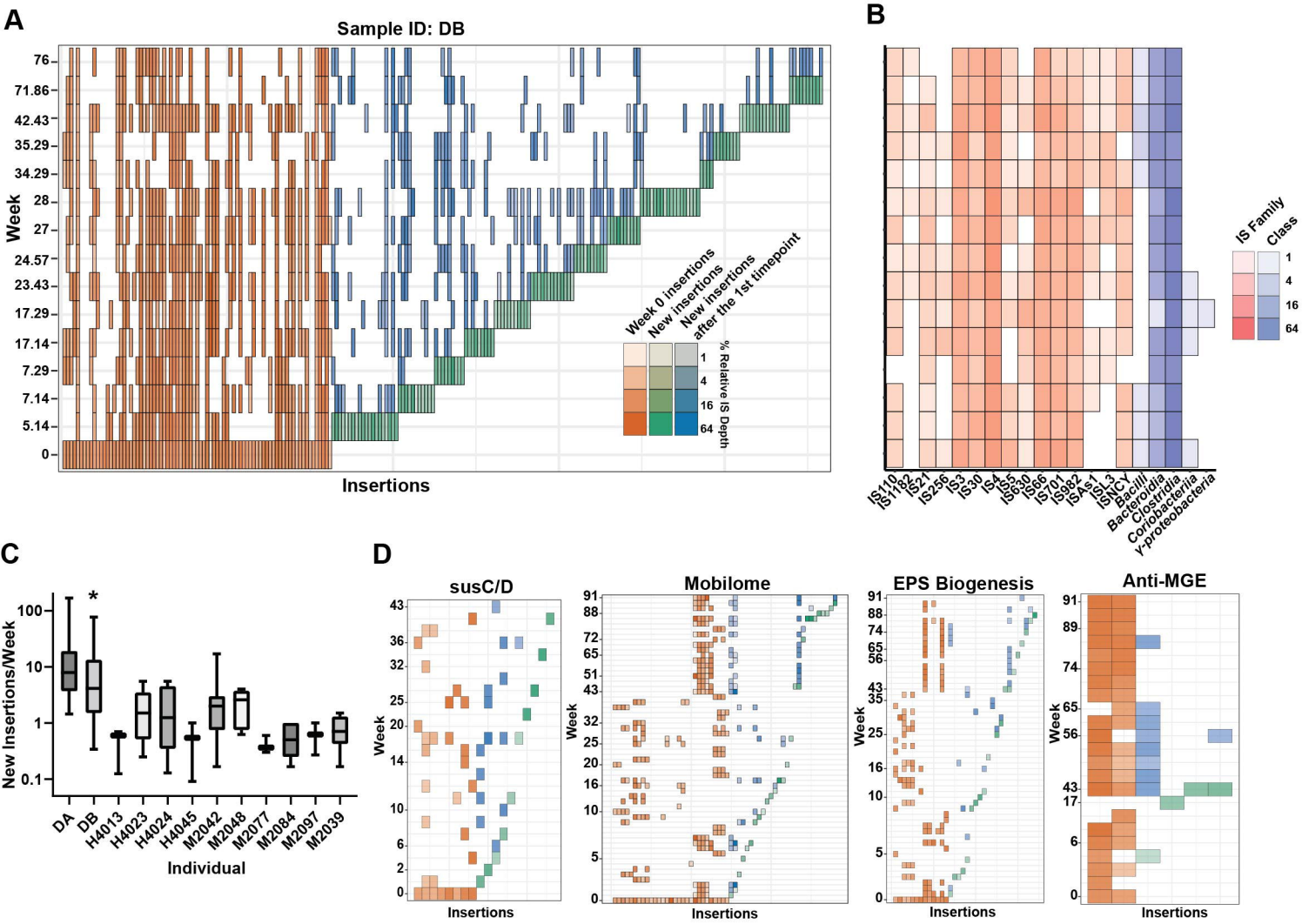
112. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772-80.

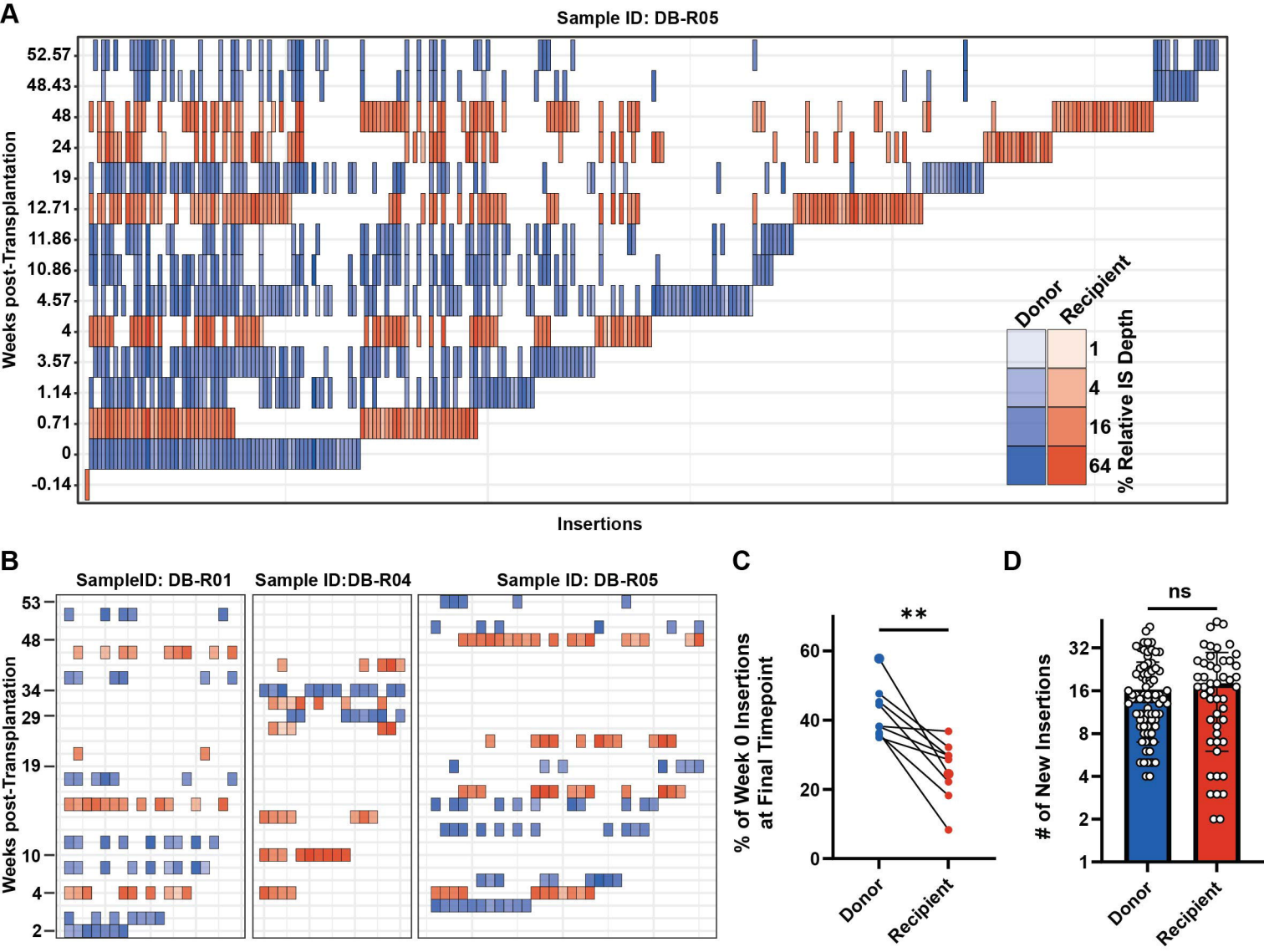

A
25000
20000
15000
10000
5000
0
# of IS Elements
ISFinder
ISOSDB
B
ISFinder Homolog
Novel
20000
15000
10000
5000
0
# of IS Elements
Tnp
Amino Acid
IS Element
Nucleotide
C
30000
20000
10000
0
# of Clusters
Protein
Nucleic Acid
25
50
75
100
% Minimum Sequence Identity
D
10^4
10^3
10^2
# of IS Elements/Family
IS1
IS110
IS1182
IS1380
IS1595
IS1634
IS21
IS256
IS3
IS30
IS4
IS481
IS5
IS6
IS630
IS66
IS701
IS982
ISAs1
ISL3
ISNCY

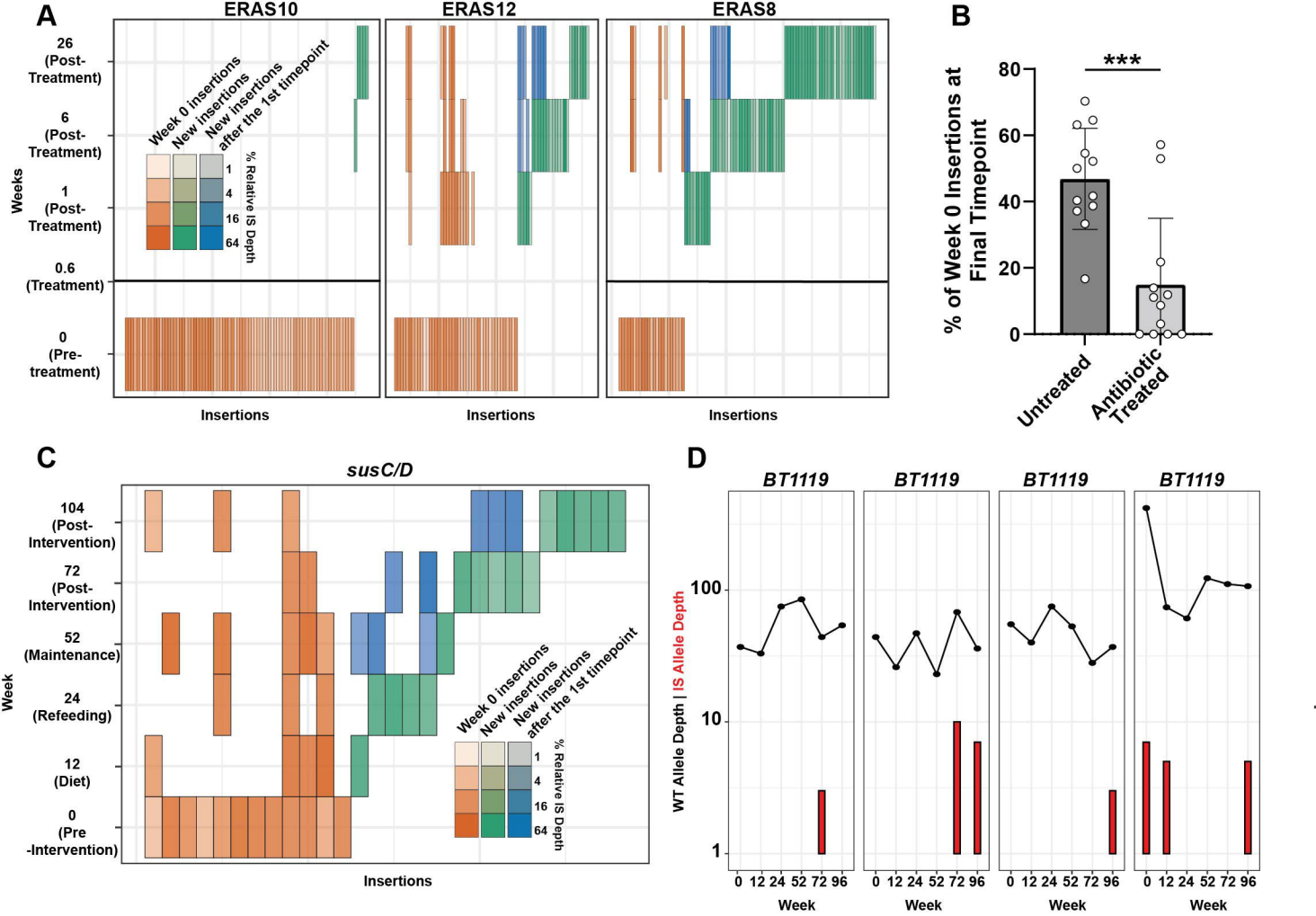

A
ITA
JPN
MDG
% Relative IS Depth
100
10
1
Sample
B
# of Insertions
100
10
1
ITA
JPN
MDG
C
Actinomycetes
Bacilli
Bacteroidia
Clostridia
Coriobacteriia
Gammaproteobacteria
Class
Sample
# of Insertions
64 16 4 1
Study
MDG
JPN
ITA
D
Bacteroidia
Clostridia
# of Insertions
64 16 4 1
Study
MDG
JPN
ITA
IS Element | IS Family
ISL3
ISOSDB4584
ISOSDB6187
ISOSDB12026
ISAs1
ISOSDB178
IS982
ISOSDB634
IS66
ISOSDB250
ISOSDB644
ISOSDB445
ISOSDB2678
IS630
ISOSDB426
IS30
ISOSDB412
IS256
ISOSDB45
IS1380
ISOSDB157
ISOSDB18121
IS1182
ISOSDB33
Sample
ISNCY
ISOSDB531
ISOSDB642
IS982
ISOSDB402
IS701
ISOSDB510
IS66
ISOSDB5997
ISOSDB134
IS630
ISOSDB351
ISOSDB4536
IS5
ISOSDB7176
ISOSDB829
IS4
ISOSDB476
ISOSDB291
ISOSDB237
IS30
ISOSDB238
IS3
ISOSDB805
ISOSDB791
IS21
ISOSDB556
Sample
E
ISOSDB18121
ISOSDB1159
ISOSDB237
ISOSDB445
ISOSDB805
ISOSDB412
ISOSDB178
-log10 (Adjusted p Value)
3
2
1
-7.5
-5.0
-2.5
0.0
2.5
5.0
Average (Intragenic - Intergenic)


A
# of ORFs
1000
100
10
1
Shared
Unique
ITA
JPN
MDG
B
susCD/tonB
Transposon/ Prophage
Cell Wall/Membrane/ Enevelop Biogenesis
Antibiotic Resistance
Anti-MGE Systems
Sample
# of Insertions
64 16 4 1
Study
MDG
ITA
JPN
C
% of ORFs
susCD/tonB
Tnp/Prophage
EPS Biogenesis
Anti-MGE
All ITA Genes
ITA iORFs
All JPN Genes
JPN iORFs
All MDG Genes
MDG iORFs
D
% IS Depth
ermF
KAP
Shufflon
ITA
JPN
MDG
E
Bacteroidia
Clostridia
Gammaproteobacteria
Actinomycetes
susCD/ tonB
Transposon/ Prophage
EPS Biogenesis
Antibiotic Resistance
Anti-MGE Systems
ITA
JPN
MDG
F
IS30 Family
ISL3 Family
IS982 Family
IS1380 Family
G
IS Family of Insertion
IS Family of ORF
# of Insertions
16
4
1


A
Read Depth
Bt WT
Bt IS4351
Genome Position (bp)
B
Mobilome
Anti-MGE
EPS Biogenesis
susC/D
% of ORFs
All ORFs
iORFs
C
Bt
# of Insertions
Intergenic
Intragenic
D
Bf WT
Bf IS4351
E
F
Bf
p=0.0624
G
Bt BHIS
Bt MM
H
p=0.0506
BHIS
MM
I
p=0.0865
# of Insertions in EPA genes
J
Bt CPS3 NT
Bt CPS3 DAC15
Bt CPS3 DAC17
K
NT
DAC15
DAC17
L


A
Sample ID: DB
Week
Insertions
Week 0 insertions
New insertions
New insertions after the 1st timepoint
% Relative IS Depth
B
IS Family
Class
IS110
IS1182
IS21
IS256
IS3
IS30
IS4
IS5
IS630
IS66
IS701
IS982
ISAs1
ISL3
ISNCY
Bacilli
Bacteroidia
Clostridia
Coriobacteria
γ-proteobacteria
C
New Insertions/Week
Individual
DA
DB
H4013
H4023
H4024
H4045
M2042
M2048
M2077
M2084
M2097
M2039
D
susC/D
Mobilome
EPS Biogenesis
Anti-MGE
Week
Insertions


A
Sample ID: DB-R05
Weeks post-Transplantation
52.57
48.43
48
24
19
12.71
11.86
10.86
4.57
4
3.57
1.14
0.71
0
-0.14
Insertions
Donor
Recipient
% Relative IS Depth
1
4
16
64
B
SampleID: DB-R01
Sample ID:DB-R04
Sample ID: DB-R05
Weeks post-Transplantation
53
48
34
29
19
10
4
2
C
% of Week 0 Insertions at Final Timepoint
**
60
40
20
0
Donor
Recipient
D
# of New Insertions
ns
32
16
8
4
2
1
Donor
Recipient


A
ERAS10
ERAS12
ERAS8
26 (Post-Treatment)
6 (Post-Treatment)
1 (Post-Treatment)
0.6 (Treatment)
0 (Pre-treatment)
Weeks
Week 0 insertions
New insertions
New insertions after the 1st timepoint
% Relative IS Depth
1
4
16
64
Insertions
B
% of Week 0 Insertions at Final Timepoint
***
80
60
40
20
0
Untreated
Antibiotic Treated
C
susC/D
104 (Post-Intervention)
72 (Post-Intervention)
52 (Maintenance)
24 (Refeeding)
12 (Diet)
0 (Pre-Intervention)
Week
Week 0 insertions
New insertions
New insertions after the 1st timepoint
% Relative IS Depth
1
4
16
64
Insertions
D
BT1119
BT1119
BT1119
BT1119
WT Allele Depth | IS Allele Depth
100
10
1
0 12 24 52 72 96
Week