

# Horizontal transfer of antibiotic resistance genes in the human gut microbiome

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1 **Horizontal transfer of antibiotic resistance genes in the human gut microbiome**

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10 **Abstract**

11

12 Infections caused by antibiotic-resistant bacteria are a major threat to public health.  
13 The pathogens causing these infections can acquire antibiotic resistance genes in a  
14 process termed horizontal gene transfer (HGT). HGT is a common event in the  
15 human gut microbiome, i.e. the microbial ecosystem of the human intestinal tract.  
16 HGT in the gut microbiome can occur via different mechanisms of which transduction  
17 and conjugation have been best characterised. Novel bioinformatic tools and  
18 experimental approaches have been developed to determine the association of  
19 antibiotic resistance genes with their microbial hosts and to quantify the extent of  
20 HGT in the gut microbiome. Insights from studies into HGT in the gut microbiome  
21 may lead to the development of novel interventions to minimise the spread of  
22 antibiotic resistance genes among commensals and opportunistic pathogens.

23 **Highlights**

24

- 25 - The human gut microbiome harbours antibiotic resistance genes ('the  
26 resistome')
- 27 - Antibiotic resistance genes can spread in the gut microbiome through  
28 horizontal gene transfer (HGT)
- 29 - The best characterised mechanisms of HGT in the human gut microbiome are  
30 transduction and conjugation
- 31 - Novel bioinformatic and experimental approaches have been developed to  
32 study HGT in the gut

### 33 **The human gut microbiome and antimicrobial resistance**

34 The term 'human gut microbiome' describes the microorganisms, their genomes and  
35 the environmental conditions of the human intestinal tract [1]. Over the last decade,  
36 the implementation of high-throughput, low-cost sequencing methods has fuelled  
37 research into the gut microbiome, with the aim to uncover its composition, function,  
38 and role in health and disease [2]. The gut contains hundreds of bacterial species,  
39 collectively called the microbiota, with those belonging to the phyla Bacteroidetes  
40 and Firmicutes accounting for 90% of all species in the gut of healthy adults [3]. The  
41 phyla Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia are  
42 generally less abundant, but can contribute to important functions, such as providing  
43 protection against invading pathogens [4,5]. Most gut bacteria have a symbiotic or  
44 commensal relationship with the human host. However, the gut microbiota also  
45 contains opportunistic pathogens, including those belonging to the families  
46 Enterobacteriaceae, particularly *Escherichia coli* and *Klebsiella pneumoniae*, and  
47 Enterococcaceae, most notably *Enterococcus faecalis* and *Enterococcus faecium*.  
48 Intestinal carriage of opportunistic pathogens can predispose an individual to urinary  
49 tract infections and, in immunocompromised patients, more serious systemic  
50 infections [6–8]. Recent decades have seen a global rise of infections caused by  
51 antibiotic-resistant clones of *E. coli*, *K. pneumoniae* [9], and *E. faecium* [10].

52 Infections caused by antibiotic-resistant bacteria are a significant threat to global  
53 public health. The annual attributable mortality due to antibiotic-resistant infections in  
54 the EU alone is estimated to number over 30,000 deaths, with the highest cases  
55 occurring in Italy and Greece [11]. Morbidity and mortality due to multi-drug resistant

56 infections are even higher in low- and middle-income countries in Asia, Africa and  
57 South America [12–14].

58 The main mechanisms by which bacteria can become resistant to antibiotics are  
59 prevention of the antibiotic from reaching toxic levels inside the cell, modification of  
60 the antibiotic target, and modification or degradation of the antibiotic itself [15].  
61 These resistance mechanisms can arise through mutations of chromosomal genes  
62 and the acquisition of antibiotic resistance genes (ARGs) from other strains of the  
63 same, or different, species in a process termed horizontal gene transfer (HGT). The  
64 sharing of genes through HGT contributes importantly to the global dissemination of  
65 ARGs [16]. HGT can occur in any environment, particularly when bacterial loads are  
66 high, e.g. in soil [17], in wastewater treatment plants [18,19] and in the gut  
67 microbiome of humans and animals [20–22].

68 As *E. coli*, *K. pneumoniae*, *C. difficile*, enterococci and other opportunistic pathogens  
69 inhabit the human gut, there is a distinct possibility that they can acquire resistance  
70 determinants from other members of the gut microbiota. Indeed, previous work has  
71 shown that the gut harbours many and diverse antibiotic resistance determinants,  
72 which have collectively been termed the ‘gut resistome’ [23,24]. In this review, we  
73 will provide an overview of the different mechanisms of HGT in the human gut  
74 microbiome and of innovative methods to study HGT in this microbial ecosystem.

75

## 76 **Mechanisms of horizontal gene transfer**

77 ARGs can be horizontally transferred through a number of mechanisms, the most  
78 important of which are transformation, transduction, and conjugation. More recently,

79 the role of membrane vesicles (MVs) in HGT has also been recognised. In this  
80 section, we will shortly discuss the relevance of these HGT mechanisms in the  
81 context of the spread of ARGs in the gut microbiome (Figure 1).

82 In transformation, naked DNA from the extracellular environment is taken up by  
83 bacteria and inserted into their genomes. The process of transformation requires  
84 bacteria to be naturally transformable or competent. Over 80 bacterial species have  
85 been experimentally proven to be naturally competent, and the presence of genes  
86 involved in DNA uptake in a number of other species suggests that this trait is more  
87 widespread [25]. The stimuli that lead to a competent state in naturally transformable  
88 bacteria are only partially understood, but nutrient starvation and the presence of  
89 competence-inducing peptides have been identified as triggers [26]. Important  
90 pathogens, including *Neisseria gonorrhoeae*, *Vibrio cholerae*, and *Streptococcus*  
91 *pneumoniae* are naturally competent pathogens and have acquired antibiotic  
92 resistance through this process [27]. While DNase activity in the gut will degrade  
93 most DNA [28], intact plasmid DNA can be isolated from the gut contents of rats that  
94 are fed plasmids [29]. This finding indicates that there is potential for extracellular  
95 DNA to be taken up by naturally competent bacteria. Observations showing that *E.*  
96 *coli* can be transformed by plasmid DNA under natural conditions [30,31], suggest  
97 that *E. coli* could take up DNA in the gut, with transformation thus conceivably  
98 contributing to the spread of ARGs. The extent by which DNA can spread  
99 horizontally in the gut through the process of transformation is, however, currently  
100 unknown.

101 MVs Membrane vesicles (MVs) are 20–250 nm spherical structures that are  
102 predominantly produced by Gram-negative bacteria when the outer membrane

103 bulges away from the cell and is then released through constriction [32]. MVs fuse  
104 with their target cells, thus delivering their cargo. MVs that are produced *in vitro* by  
105 the gut commensal *Bacteroides* can contain  $\beta$ -lactamases and these vesicles protect  
106 target cells against  $\beta$ -lactam antibiotics [33]. In the context of HGT, it is relevant that  
107 MVs produced by gut bacteria can also contain cytoplasmic contents, including DNA  
108 [34]. DNA-containing MVs are thought to be formed by protrusion of both the outer  
109 and inner membrane, which leads to the inclusion of cytoplasmic components into  
110 the vesicles [35]. Indeed, membrane vesicles isolated from bacteria in the genus  
111 *Acinetobacter* can transfer antibiotic resistance plasmids *in vitro* [36]. Similarly,  
112 vesicle-mediated transfer of DNA has also been reported for *E. coli* [37]. While MVs  
113 are produced in the gut and can potentially influence host immune responses [38], it  
114 is as yet not clear whether they can also contribute to HGT in the gut microbiome.

115 Transduction describes the transfer of chromosomal and extrachromosomal DNA  
116 between bacteria via a viral intermediate known as a bacteriophage. The main  
117 mechanisms of transduction are generalised transduction, specialised transduction,  
118 and lateral transduction. Collectively these can cause the mobilisation of any  
119 fragment of a bacterial genome [39]. Generalised transduction occurs when  
120 bacteriophages in the lytic cycle incorporate sections of the DNA of the bacterial host  
121 during capsid synthesis. In specialised transduction, regions immediately flanking the  
122 integration site of a lysogenic phage are excised and packaged into the capsid [39].  
123 Lateral transduction, in contrast, occurs when prophages initiate DNA replication  
124 while they are still integrated into the host. This process generates multiple copies of  
125 DNA before excision from the host genome occurs. Once excised, the DNA – which  
126 may contain both phage and the adjacent genes up to hundreds of kilobases in  
127 length – are packaged into new phage particles and transferred to other bacterial



128 strains [40]. The human gut contains an extensive community of bacteriophages  
129 [41,42] and significant numbers of ARG-carrying phages are present in the human  
130 gut and other environments [43]. The abundance of these ARG-carrying phages in  
131 the human gut increases upon antibiotic treatment [●44]. Experiments in mouse  
132 models have shown that transduction is a driving force behind genetic diversity in  
133 gut-colonising *E. coli* strains [●45] and can contribute to the emergence of drug  
134 resistance in gut bacteria [46]. However, the quantitative contribution of phages to  
135 the horizontal transfer of ARGs is still poorly understood. We foresee that novel  
136 methods to detect and quantify HGT in the gut (further outlined below) will be  
137 implemented to address this question.

138 In conjugation, mobile genetic elements such as plasmids and integrative and  
139 conjugative elements (ICEs) are transferred from one bacterium to another [47].  
140 Among conjugative elements, conjugative plasmids are arguably the most relevant  
141 for the spread of ARGs as they have the potential to carry multiple resistance genes,  
142 due to their substantial size (median of 90 kbp) and the common occurrence of one  
143 or more toxin-antitoxin modules which ensure that plasmids are retained within their  
144 microbial hosts [48]. In addition, conjugative plasmids frequently carry genes, other  
145 than ARGs, that contribute to microbial fitness, e.g. by coding for novel metabolic  
146 routes or tolerance to disinfectants or heavy metals. Antibiotic resistance  
147 determinants can thus be co-selected under a variety of conditions as they share a  
148 genetic platform with other fitness determinants [48]. Conjugation is a complex,  
149 multistage, and contact-dependent process where DNA is transported via a pilus  
150 between bacteria in close proximity to each other [49]. The gut, with its high density  
151 of bacterial cells and dense mucus layer, provides an environment conducive to  
152 conjugation. The spread of antibiotic resistance plasmids and ICEs has been

153 observed among commensals and opportunistic pathogens while colonising the  
154 human gut [50–52]. Notably, conjugative plasmids can provide the machinery  
155 allowing the mobilisation of DNA that is not self-transmissible, thus greatly increasing  
156 the potential for HGT of resistance determinants [53].

157

### 158 **Bioinformatic tools to study HGT in the gut**

159 The high-throughput sequencing of DNA isolated from microbial ecosystems, often  
160 referenced to as shotgun metagenomics, is widely used to study the microbial  
161 diversity of the human gut microbiome [54]. The Illumina DNA sequencing platform is  
162 most commonly used for shotgun metagenomic studies of the gut microbiome. The  
163 relatively short sequence reads generated by Illumina sequencers can be used to  
164 detect and quantify the abundance of ARGs in the human gut microbiome [55]. It is,  
165 however, difficult to resolve linkage of ARGs and MGEs as these elements are  
166 generally rich in DNA repeats that are difficult to resolve with short reads [56]. The  
167 advent of long-read technologies such as Oxford Nanopore and PacBio sequencing  
168 allows the reconstruction of genome and plasmid sequences from complex  
169 metagenomic samples [19]. Independent of the platform used for shotgun  
170 metagenomics, approaches based on DNA sequencing have long struggled to  
171 identify the microbial hosts of ARGs and to detect HGT events. Recently, advanced  
172 bioinformatic tools have been developed to address this issue (Table 1).

173 Different approaches have been developed to identify HGT events in metagenomic  
174 sequencing data [57]. These tools include MetaCHIP, which uses a combination of  
175 best-match and phylogenetic approaches to identify HGT candidates from genes that  
176 have a different taxonomic assignment to the contig or genome they have been

177 assembled into [58]. An additional method, MetaCherchant, has been developed  
178 which uses local de Bruijn graph assembly to uncover the wider context of ARGs.  
179 When metagenomes from a time series are compared, a change in gene context  
180 suggests that an HGT event has occurred [59]. A recently developed binning  
181 technique which utilises the methylation patterns of DNA, as determined by Oxford  
182 Nanopore and PacBio sequencing, has proven useful in linking MGEs to their host.  
183 Methylation motifs are given scores and DNA sequences from MGE and from  
184 bacterial genomes can be matched based on their methylation scores thus  
185 identifying the host of the MGE [60]. If the same MGE is identified in multiple hosts  
186 then an HGT event may have occurred. However, purely metagenomic methods are  
187 still limited in their ability to accurately identify all HGT events, because the  
188 conjugative transfer of ARG-carrying plasmids between different microbial hosts  
189 cannot be resolved by DNA sequencing alone. For this reason, novel experimental  
190 approaches have been developed to characterise HGT in the gut microbiome (Table  
191 1; Figure 2).

192

### 193 Novel experimental approaches to study HGT in the gut

194 HGT has been studied in the human gut since the 1960s [61]. In these human  
195 challenge studies, bacteria capable of transferring DNA ('donors') and recipient  
196 strains were administered to the host and transconjugants were then isolated by  
197 selective culture from the host faeces. However, these studies are fraught with  
198 ethical challenges as there is a risk that multidrug resistant donor strains or  
199 transconjugants colonize the gut of the volunteers, and thus animal models are more  
200 frequently used to study HGT in the gut (Figure 2A). More recently, culturing of

201 human faeces on selective media has identified novel hosts of ARGs, such as the  
202 *vanB* vancomycin resistance transposon in the gut commensals *Eggerthella lenta*  
203 and *Clostridium innocuum* [62], which can be transferred to the opportunistic  
204 pathogen *E. faecium* in the mouse gut [63]. These findings support the hypothesis  
205 that gut commensals act as a reservoir for ARGs. A drawback of these approaches  
206 is that they require the recipient cells to be cultured and while important advances  
207 have been made in the isolation of gut commensals [64], it remains technically  
208 challenging to systematically identify strains that acquire ARGs and uncover the  
209 hidden network of HGT in the gut.

210 In recent years, novel techniques have been developed which allow the high-  
211 throughput characterisation of HGT events in microbial communities. The inclusion  
212 of fluorescent markers on plasmids has enabled the use of technologies such as  
213 confocal fluorescence microscopy and Fluorescence Activated Cell Sorting (FACS)  
214 to uncover the host range of plasmids (Figure 2B). In one study, HGT events were  
215 identified by the use of plasmids that carry a green fluorescent protein gene (*gfp*) of  
216 which expression was controlled by a chromosomally encoded repressor. Upon  
217 conjugation of the plasmid to a new host, repression of *gfp* was lifted and  
218 transconjugants could be identified by their green fluorescence [65].  
219 Transconjugants were then isolated using FACS and subsequently subjected to 16S  
220 rRNA gene sequencing to identify which hosts the plasmids had spread to. A  
221 drawback of this approach is that typically only one plasmid is examined at a time,  
222 which limits our understanding of the wider picture of HGT events within a  
223 microbiome. In addition, there is a distinct lack of genetic tools to perform these  
224 studies in microbial hosts other than model strains like *E. coli* MG1655.

225 Several techniques have been developed that physically link ARGs to their microbial  
226 hosts in microbiomes. These data can be used to elucidate the patterns of ARG  
227 dissemination in microbiomes (Figure 2C). In Emulsion, Paired Isolation, and  
228 Concatenation PCR (epicPCR), single microbial cells are encapsulated in  
229 polyacrylamide beads followed by a fusion PCR step within individual beads. This  
230 results in a concatemer of the targeted gene (e.g. an ARG) and the 16S rRNA gene,  
231 which serves as a phylogenetic marker [66]. The resulting fusion PCR amplicon can  
232 be sequenced using Illumina technology to identify the host(s) of the gene targeted  
233 in epicPCR. Recently, epicPCR has been used to determine the diversity of ARG  
234 hosts in wastewater [67].

235 Another technique that can be used to identify the bacterial hosts of ARGs is  
236 chromosome conformation capture (3C). During 3C, cells are incubated with  
237 formaldehyde to cross-link the DNA, followed by restriction digestion, ligation of the  
238 cross-linked DNA and finally reversal of the cross-linking [68]. Sequencing the  
239 resulting 3C library will then reveal cross-links between MGEs carrying ARGs and  
240 chromosomal DNA, thus allowing the host of the MGEs to be identified.  
241 Metagenomic 3C (meta3C) was performed on mouse gut microbiome samples to link  
242 phage sequences to their bacterial hosts [69]. Recently, a derivative of 3C called  
243 Hi-C, which includes an additional step to enrich for ligated fragments of DNA, has  
244 been used to link ARGs to their bacterial hosts in a variety of complex samples. By  
245 performing Hi-C on a wastewater sample, the microbial hosts of ARGs and *in situ*  
246 host ranges of plasmids were assessed, where the authors found that the phylum  
247 Bacteroidetes was one of the most common reservoirs of ARGs in the sample [70].  
248 Also using Hi-C data, in combination with long-read assembly, the microbial hosts of  
249 viruses and ARGs were linked in a cow rumen microbiome [71]. Hi-C has similarly

250 been performed on human gut microbiome samples. In a pioneering study, an  
251 approximately 600-kbp megaplasmid was linked to several species in the order  
252 Clostridiales [72]. More recently, the transfer of accessory elements was tracked in  
253 two human stool samples collected 10 years apart using 3C, but the authors did not  
254 discuss linkage of ARGs to their microbial hosts [73]. While there has, thus far, been  
255 no extensive study using 3C or its derivatives to link ARGs to their host in a human  
256 gut microbiome sample, these studies have provided a foundation for the use of  
257 these novel techniques to study HGT in the gut.

258 A recent study [●●74] described an elegant recorder for HGT events based on the  
259 ability of the CRISPR-Cas9 system to integrate fragments of invading DNA into the  
260 bacterial genome (Figure 2D). CRISPR-Cas9 is an adaptive immune system that  
261 bacteria use to defend against invasion by foreign DNA [75]. Invading DNA is  
262 incorporated into the bacterial genome at the CRISPR locus by the Cas1-Cas2  
263 complex in a process known as spacer acquisition. These sequences are  
264 subsequently transcribed and used to target the Cas9 nuclease to invading DNA  
265 which matches the spacer sequence [76]. To record HGT events, Munck and co-  
266 workers engineered an *E. coli* strain containing a plasmid with the *cas1-cas2* operon  
267 under the control of an inducible promoter to capture DNA sequences which have  
268 entered the cell. Sequencing of the CRISPR spacer regions was then used to not  
269 only identify HGT events but also the order in which the events occurred. When this  
270 system was applied to clinical faecal samples, it showed that HGT is frequent in the  
271 gut and that IncX-type plasmids transferred most actively [●●74]. This technique  
272 promises to uncover HGT events at an unprecedented resolution, but to unveil the  
273 full extent of the gut mobilome it will need to be adapted to function in bacteria which  
274 fall outside of the Enterobacteriaceae family.

275

## 276 **Conclusions and future perspectives**

277 The human gut microbiome contains a wide variety of ARGs. Modern sequencing-  
278 based and experimental technologies are uncovering the extent by which resistant  
279 genes spread between strains and species that inhabit the gut. Genes that confer  
280 resistance against clinically relevant antibiotics and are carried on mobile genetic  
281 elements that replicate in pathogens are considered to be an immediate threat to the  
282 successful treatment of clinical infections [77]. While bacteriophages can potentially  
283 mobilise any chromosomal gene or mutation, the conjugative spread of plasmids is  
284 still widely considered to be the most important way resistance genes can be  
285 transferred among bacteria.

286 The observation that ARGs from opportunistic pathogens can also be found in the  
287 genomes of Gram-positive commensal bacteria [62,78] suggests that HGT of ARGs  
288 in the gut is pervasive, particularly among the Firmicutes. Carbapenemases and  
289 extended-spectrum beta-lactamases that are increasingly prevalent in the  
290 opportunistic pathogens *E. coli* and *K. pneumoniae* can readily spread among  
291 Proteobacteria in the gut [79,80] but HGT to other phyla have, to our knowledge, not  
292 been reported. The scarcity of HGT of resistance genes across phyla can be  
293 explained by the specific physiological contexts in which proteins conferring antibiotic  
294 resistance need to operate, which makes it unlikely that resistance genes can be  
295 functional, at a low biological cost, in phylogenetically disparate bacteria [●●81].  
296 However, soil microcosm experiments have provided evidence that plasmids can  
297 spread, albeit infrequently, among diverse phyla [65]. As HGT is a common event in  
298 the gut microbiome [●●74], it is not unlikely that broad-host range MGEs carrying

299 resistance genes will start to disseminate across phyla, although intra-phylum HGT  
300 remains quantitatively more important. There is still a lack of knowledge on the  
301 conditions in the gut microbiome that favour HGT events, but inflammation of the  
302 host and the production of membrane-destabilizing agents by both the microbiota  
303 and the host have been proposed to promote HGT in the gut [82].

304 Our improved understanding of HGT in the gut opens up avenues for novel  
305 interventions to minimise transfer of ARGs, e.g. by the conjugative delivery of  
306 CRISPR-Cas to selectively deplete strains carrying resistance genes [83]. We  
307 envisage that the novel techniques reviewed here will aid in further elucidating the  
308 exchange networks of ARGs in the human gut. The information gleaned from these  
309 studies can then support the development of targeted approaches to control or  
310 reduce the number of antibiotic-resistant bacteria in the human gut, particularly in  
311 individuals that are at high risk of developing infections, such as neonates, the  
312 immunocompromised, and the elderly.

313



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315

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321

	Technique	Description	Limitations	Ref	
Bioinformatics	MetaCHIP	Identifies differences in taxonomic assignments between genes and the contig or genome they have been assembled into	Detection of recent HGT events is challenging	[58]	
	MetaCherchant	Uses local De Bruijn graph assembly to uncover wider context of ARGs	HGT of high copy-number MGEs may be difficult to detect due to complexity of the assembly graph	[59]	
	Binning by DNA methylation	DNA sequences from MGEs and bacterial genomes are matched based on their methylation	High numbers of genomes in sample limit uniqueness of methylation signal	[●60]	
Experimental	Culturing approaches	<i>in vivo</i> studies	Strains with MGEs are administered to a host and transconjugants are then isolated from faecal samples by culture	Ethical challenges; low throughput	[50]
		culturomics	High-throughput culture-based methods to identify bacterial hosts of antibiotic resistance genes	Laborious and technically challenging, particularly for bacteria that are present at low abundance	[64]
	Fluorescent reporter systems	Fluorescently-tagged plasmids are used to track the movement of plasmids in complex microbiomes	Needs to be optimised for each plasmid-host combination	[65]	
	epicPCR	Single microbial cells are captured in polyacrylamide beads, followed by fusion PCR on a target and the 16S rRNA gene, and sequencing of the amplicon	Targets only one gene per experiment; uses 16S rRNA gene as a low-resolution phylogenetic marker; requires multiple rounds of PCR introducing biases	[66]	
	Chromosome conformation capture	meta3C	By physically cross-linking DNA inside microbial cells, and subsequent sequencing of the cross-linked DNA, the hosts of genes of interest are identified	Requires high sequencing depth; can be difficult to accurately resolve hosts to strain- or species-level	[●69,73]
		Hi-C	Similar to meta3C but with an additional step to enrich for cross-linked fragments of DNA before sequencing.		[70–72]
	CRISPR recorder		A CRISPR-based system that utilises the spacer acquisition process to record HGT events. Uniquely able to track transient HGT events.	The system has presently only been developed for <i>E. coli</i>	[●●74]

323

324 **Table 1. Summary of current approaches to study horizontal gene transfer in**325 **the gut microbiome.** Bioinformatic approaches to identify HGT have recently been

326 reviewed in [●57] and the tools highlighted here are a selection of those that have

327 recently been developed. All bioinformatic tools listed in this table for detecting HGT

328 in metagenomes are reliant on accurate and contiguous metagenomic assembly.

329 Ref: references.

330

331 **Figure legends**

332

333 **Figure 1. Mechanisms of horizontal gene transfer (HGT).**

334 **Transformation:** physiologically competent bacteria can take up naked DNA from  
335 the environment. **Membrane vesicle fusion:** 20-250 nm spherical, lipid bilayer-  
336 enclosed vesicles can transport cargo between bacteria, including DNA.  
337 **Transduction:** genetic material can be transferred between donor and recipient  
338 bacteria via a bacteriophage intermediate. **Conjugation:** mobile genetic elements,  
339 such as plasmids, can transfer via a pilus formed between donor and recipient cells.  
340 The mechanisms of HGT illustrated in this figure can mediate the transfer of both  
341 chromosomal and extra-chromosomal DNA.

342

343 **Figure 2. Experimental approaches to study horizontal gene transfer (HGT) in**  
344 **the gut.**

345 **Panel A:** the use of culture-based animal models to study HGT. Laboratory animals  
346 are fed bacteria containing an antibiotic resistance gene (ARG)-plasmid and stool  
347 samples can then be cultured on antibiotic-containing media to isolate  
348 transconjugants. **Panel B:** fluorescent reporter systems to identify HGT events. A  
349 donor cell carries a green-fluorescent protein (GFP)-tagged plasmid and a repressor  
350 gene on the chromosome. GFP will only be expressed when the plasmid is  
351 transferred to another cell that does not contain the repressor and GFP-producing  
352 transconjugants can thus be isolated using fluorescence-activated cell sorting  
353 (FACS). **Panel C:** novel techniques for physically linking ARGs to their microbial

354 host. Emulsion, Paired Isolation, and Concatenation PCR (epicPCR) involves the  
355 encapsulation of single bacterial cells in polyacrylamide beads. After cell lysis, a  
356 PCR is performed in each bead individually. The PCR targets an ARG with custom  
357 primers where the reverse primer contains an overhang homologous to the 16S  
358 rRNA gene, of which another conserved region is targeted by a third primer. The  
359 resulting fusion PCR amplicon contains the ARG fused with the 16S rRNA gene of  
360 its host(s). Chromosome conformation capture approaches use formaldehyde to  
361 cross-link DNA inside cells. Cells are then lysed, followed by enzymatic digestion of  
362 the DNA, ligation of the cross-linked fragments, and finally removal of the cross-links,  
363 ultimately leaving linked DNA fragments that were in close physical proximity to each  
364 other. **Panel D:** CRISPR spacer acquisition as a tool for HGT detection. Using an  
365 engineered bacterial strain containing a plasmid with the *cas1-cas2* operon, the  
366 sequences of DNA entering the cell are captured and incorporated into the CRISPR  
367 array. By sequencing the CRISPR array, HGT events can be observed, including the  
368 order in which they occurred.

369

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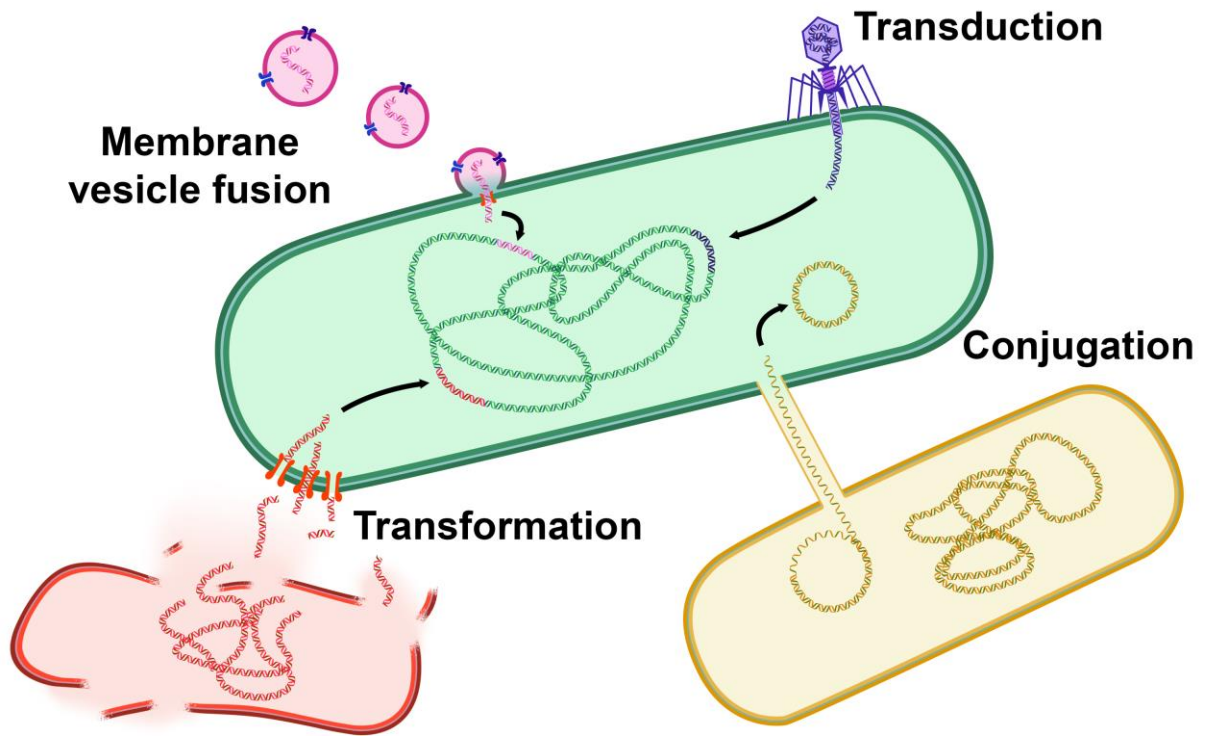
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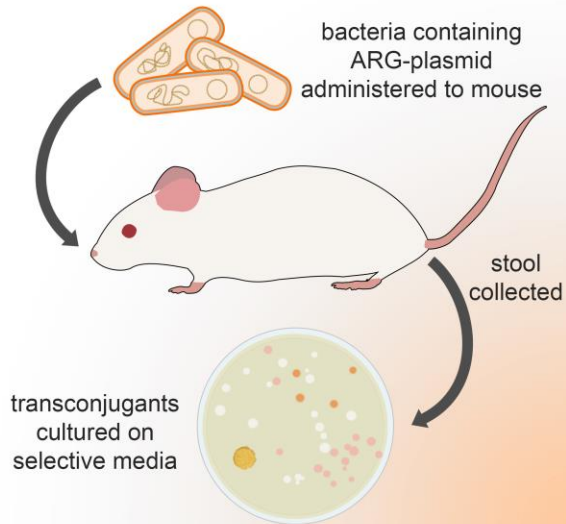
674 **Figure 1**



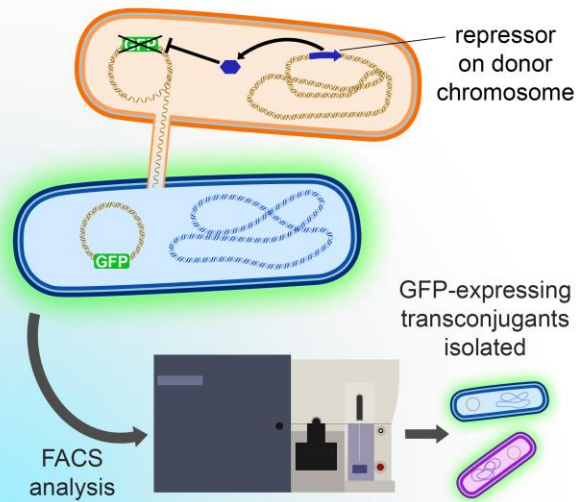
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**A. Culture-based techniques**

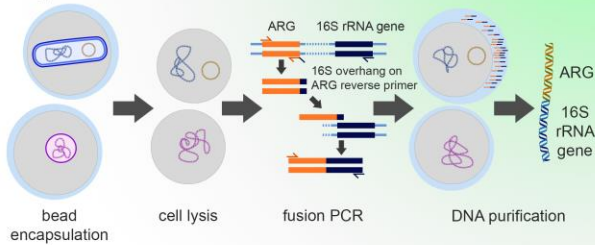


**B. Fluorescent reporter systems**

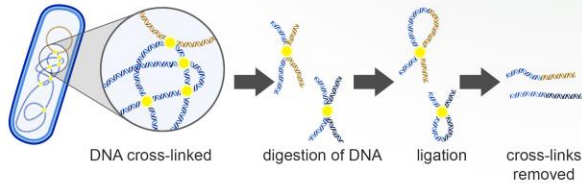


**C. Linkage-based techniques**

**epicPCR**



**Chromosome conformation capture**



**D. CRISPR-based recorders**

