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Metagenomic engineering of the mammalian gut microbiome in situ

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Engineering of microbial communities in open environments remains challenging. Here we describe a platform used to identify and modify genetically tractable mammalian microbiota by engineering community-wide horizontal gene transfer events in situ. With this approach, we demonstrate that diverse taxa in the mouse gut microbiome can be modified directly with a desired genetic payload. In situ microbiome engineering in living animals allows novel capabilities to be introduced into established communities in their native milieu.

In nature, microbes live in open, dynamic, and complex habitats that are difficult to recapitulate in a laboratory setting. Although recent advances in deep sequencing have shed light on the vast microbial diversity in nature, the ability to genetically alter these microbiomes remains limited, despite advances in culturomics and synthetic biology[1–4]. Genetic intractability is often attributed to host immunity, such as restriction methylation[5] or CRISPR–Cas processes[6], although myriad other factors (e.g., DNA transformation, growth state, fitness burden) can also influence gene transfer potential[7]. Here we devised an approach, metagenomic alteration of gut microbiome by in situ conjugation (MAGIC), to genetically modify gut microbiota in their native habitat by engineering the mobilome—the repertoire of mobile genetic elements in the gut microbiome.

We applied MAGIC to the mammalian gut because it harbors a diverse microbial community with key functional roles in host physiology[8]. We constructed an *Escherichia coli* donor strain that can deliver a genetic payload into target recipients by broad-host-range bacterial conjugation (Fig. 1). We integrated the IncPα-family RP4 conjugation system[9], which can efficiently conjugate into both Gram-positive and Gram-negative cells, into the EcGT1 donor genome, along with a constitutively expressing mCherry-specR cassette (Δ*galK::mCherry-specR*). To strengthen biocontainment of the donor and to facilitate in vitro selection of recipients, we generated an alternative strain, EcGT2 (Δ*asd::mCherry-specR*), to be auxotrophic for the essential cell-wall component diaminopimelic acid (DAP), thus requiring DAP supplementation in the growth media[10].

We developed a modular suite of mobile plasmids (pGT) that featured replicative origins with narrow to broad host ranges, an RP4 transfer origin, a selectable marker, and the desired genetic payload (Supplementary Tables 1–3, Supplementary Fig. 1). We also used a broad-host-range Himar transposon system for delivering integrative payloads. As a demonstration of the system, we used a dual-reporter payload harboring a green fluorescent protein (GFP) and an antibiotic-resistance gene (AbR). The use of fluorescence-activated cell sorting (FACS) combined with 16S metagenomic analysis enabled us to identify successfully modified recipients or transconjugants, which could then be readily isolated on antibiotic selective plates. This multi-pronged strategy can increase the diversity of genetically tractable microbiota that can be captured. We first validated and optimized MAGIC protocols in vitro by assessing the gating stringency of FACS with control spike-ins of GFP-tagged bacteria into a complex sample community (Supplementary Fig. 2). Subsequently, in vitro conjugations with defined recipient species (Supplementary Fig. 3) and live bacterial communities extracted from mouse feces (Supplementary Fig. 4) demonstrated the transfer of the payload from donors to recipients to yield GFP^{+} transconjugants that could be enriched by FACS (Supplementary Fig. 5), which we confirmed by fluorescence microscopy (Supplementary Fig. 6). 16S rRNA sequencing of FACS-enriched transconjugant populations revealed a diverse range of recipient bacteria (Supplementary Fig. 7).

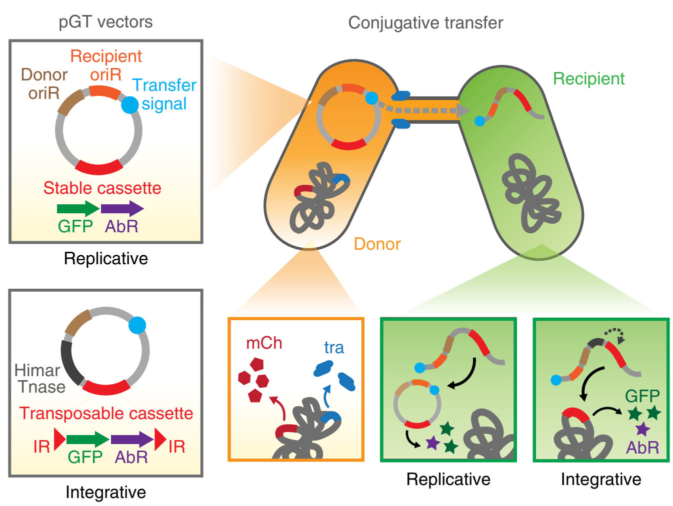

Fig. 1 | Overview of metagenomic alteration of gut microbiome by in situ conjugation (MAGIC). MAGIC implementation to transfer replicative or integrative pGT vectors from an engineered donor strain into amenable recipients in a complex microbiome. Replicative vectors feature a broad-host-range origin of replication (oriR), whereas integrative vectors contain a transposable Himar cassette and transposase (Tnase). The donor *E. coli* strain contains genomically integrated conjugative transfer genes (tra) and an mCherry gene (mCh). Transconjugant bacteria are detectable on the basis of expression of an engineered payload that includes GFP and an antibiotic-resistance gene (AbR).

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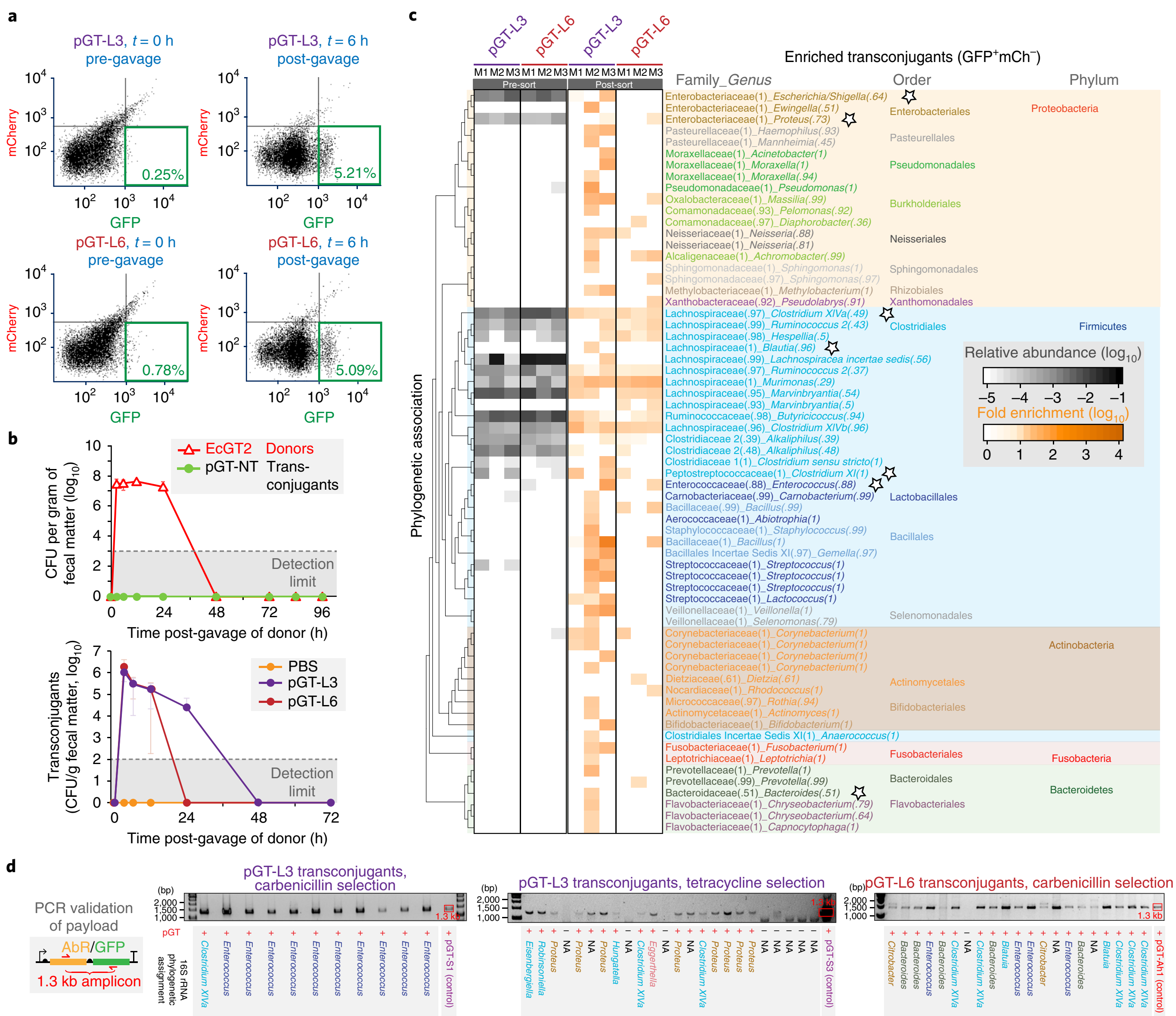

Fig. 2 | Identification and isolation of genetically tractable bacteria from the mouse gut with MAGIC. a, Fecal bacterial analysis by FACS, antibiotic selection, and sequencing after implementation of MAGIC in a mouse model. The dot plots represent FACS analysis of fecal bacteria from EcGT2 donors, pre- and post-gavage with pGT-L3 or pGT-L6 vector libraries. Green boxes define the sorted GFP$^+$mCherry$^-$ transconjugant populations. For each vector library, fecal samples from three cohoused mice were independently evaluated by flow cytometry, with similar results. **b**, Longitudinal analysis of fecal microbiome by flow cytometry for the presence of EcGT2 pGT-NT donor cells ($n=4$ mice) and of transconjugants of vector libraries pGT-L3 ($n=3$ mice), pGT-L6 ($n=3$ mice), pGT-NT control ($n=4$ mice), or PBS (no donor) control ($n=2$ mice). Donor cells and transconjugants were lost within 48 h. The dashed line indicates the detection limit. **c**, 16S taxonomic classification of transconjugants (GFP$^+$mCherry$^-$) enriched by FACS of pGT-L3 and pGT-L6 recipient groups at 6 h post-gavage. Each heat map column represents transconjugants from one mouse. The relative abundance of each operational taxonomic unit (OTU) in the total bacterial population is shown in the grayscale heat map, and each OTU's fold enrichment among transconjugants is shown in the orange heat map. In the table on the right, numbers in parentheses indicate the confidence of taxonomic assignment by RDP Classifier. Genera with successfully cultivated isolates are denoted by white stars. **d**, PCR confirmed the presence of the antibiotic resistance–GFP payload cassette from pGT-L3 and pGT-L6 vectors in diverse isolates that were engineered in the mouse gut and isolated by selective plating with carbenicillin or tetracycline. "NA" indicates 16S sequences that were not available.

Next, we explored the possibility of implementing MAGIC in vivo, directly in the native gut microbiome of an animal. We hypothesized that different groups of microbiota could be modified through the use of a library of pGT vectors with a range of gene expression levels and plasmid replication elements suitable for different gut bacteria. We generated libraries of pGT vectors (pGT-L1 to pGT-L6) by modularly permuting pGT parts, including regulatory sequences of varying activity, payload-selectable genes (*bla*, *catP*, *tetQ*), transposon elements (Himar), and plasmid origins (RSF1010, pBBR1, p15A) (Supplementary Tables 1 and 2). We carried out four separate in vivo studies in which EcGT2 donors containing pGT libraries were orally gavaged into conventionally raised C57BL/6J mice obtained from commercial vendors (Supplementary Fig. 8a). To assess the transfer capacity of individual pGT replicative or integrative designs (pBBR1, p15A-Himar, and RSF1010), we introduced the pGT libraries pGT-L1, pGT-L2, and pGT-L3

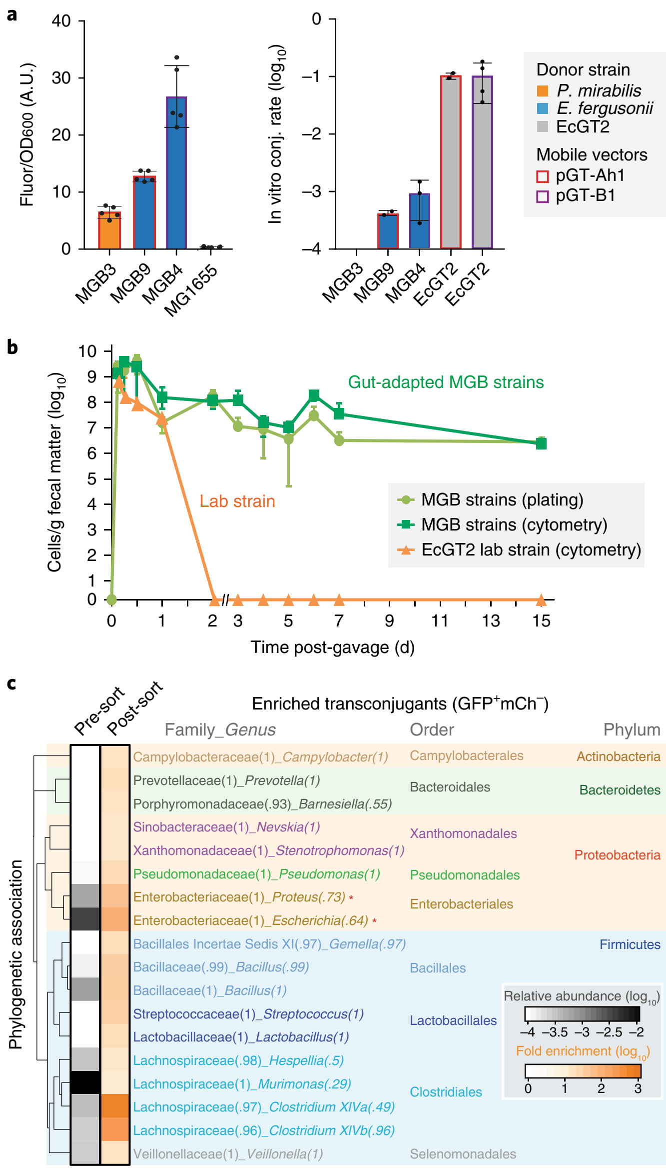

Fig. 3 | Transconjugant native gut bacteria recolonize the gut and mediate secondary transfer of engineered genetic payloads. a, Left, GFP expression profiles of three isolates (MGB3, MGB4, and MGB9; $n=5$ for each) versus the control strain (*E. coli* MG1655; $n=5$). MGB isolates were *P. mirabilis* (orange bar) and *E. fergusonii* (blue bars) containing either vector pGT-Ah1 (red border) or vector pGT-B1 (purple border). *E. fergusonii* strains were genetically identical, but received two different vectors. Right, efficiency of in vitro conjugation (conj.) of pGT vectors from MGB strains to *E. coli* MG1655 recipients. EcGT2 donors were used as positive controls (gray bars). Sample sizes: $n=2$–4. Data shown as mean ± s.d. **b**, Colonization of MGB strains and the EcGT2 lab strain in mice ($n=6$ and 4, respectively) over time, after initial oral gavage. Cell densities were determined by both plating (light green) and flow cytometry (dark green) of fecal bacteria, and by flow cytometry only for *E. coli* (orange). Data shown as mean ± s.d. **c**, FACS enrichment and 16S taxonomic classification of the top in vivo transconjugants at 6 h post-gavage with MGB strains. Fecal samples from 6 mice were combined for analysis. The relative abundance of each operational taxonomic unit (OTU) in the total bacterial population is shown in the grayscale heat map, and each OTU's fold enrichment among transconjugants is shown in the orange heat map. In the table on the right, numbers in parentheses indicate the confidence of taxonomic assignment by RDP classifier. Red asterisks denote OTUs that share the same genus as MGB donors.

separately into a cohort of mice from Taconic (Supplementary Fig. 8b–d). We tested larger combinatorial libraries (pGT-L3 to pGT-L6) in two independent mouse cohorts to assess variability across cohorts (Fig. 2, Supplementary Fig. 9). To compare in situ transfer in different gut communities, we tested the pGT-L6 library in mice from a different source (Charles River) (Supplementary Fig. 10).

We carried out FACS enrichment and 16S metagenomic analysis on fecal material from all mice studied, collected over time after oral gavage with pGT libraries. Across in situ studies, up to 5% of resulting bacteria seemed to be successful transconjugants (i.e., $GFP^+mCherry^-$) 6 h post-gavage, compared with those in samples from control groups (mice gavaged with PBS or EcGT2 carrying a nontransferable vector, pGT-NT) (Fig. 2a and Supplementary Figs. 8b, 9a, and 10a). These $GFP^+mCherry^-$ transconjugants persisted for up to 72 h post-gavage (Fig. 2b, Supplementary Fig. 9b). 16S metagenomic sequencing of these transconjugant populations revealed a wide phylogenetic breadth (Fig. 2c and Supplementary Figs. 8c, 9c, and 10b). We observed substantial reproducible enrichment of Proteobacteria and Firmicutes, especially Clostridiales and Bacillales, among successful transconjugants across multiple independent experiments. Use of the same pGT-L6 library in mice from different vendors, which harbored distinct microbiomes (Supplementary Fig. 10c), yielded shared and distinct transconjugants (Supplementary Fig. 10d). In parallel to FACS metagenomic studies, we isolated individual transconjugants from these fecal samples by selective plating for the payload AbR, and confirmed the presence of the GFP–AbR payload by PCR (Fig. 2d). Across all experiments, we isolated and validated more than 297 transconjugants belonging to 19 genera across 4 phyla (Supplementary Fig. 11, Supplementary Table 4), thus validating the capacity of MAGIC to broadly transfer genetic material in situ to diverse recipients in the mammalian gut. In contrast, we could isolate only seven genera from in vitro conjugation experiments using the same pGT vectors, despite a similar diversity of transconjugants detected by FACS metagenomics (Supplementary Fig. 7). This difference may be due to in vitro conditions that suboptimally support the growth of diverse species during conjugation reactions, which underscores the value of implementing MAGIC in situ in an established complex microbiome.

As transconjugants were no longer detected by 72 h in situ (Fig. 2b, Supplementary Fig. 9b), we speculated that the genetic payload on pGT vectors might be unstable or toxic, thus causing its negative selection in transconjugants. We tested this hypothesis in vitro by carrying out 20–30 serial passages of two transconjugant isolates of *Escherichia fergusonii* that contained the GFP–carbenicillin resistance (carbR) payload on either a pGT-B1 (replicative pBBR1 origin) or a pGT-Ah1 (integrative Himar transposon) plasmid (Supplementary Fig. 12). For the pGT-B1 population, we observed a considerable decrease in the fraction of GFP^+ cells (Supplementary Fig. 12a–c). PCR assay of the origin of replication indicated that the pGT-B1 plasmid was no longer present in the GFP^- cells (Supplementary Fig. 12d). In contrast, cells in the pGT-Ah1 population remained GFP^+ despite a detectable loss of the plasmid in parts of the population over time (Supplementary Fig. 12e–g), which suggests a more stable maintenance of the GFP–carbR payload as an integrative transposon within the host genome. Together, these results highlight the challenges of maintaining the long-term in vivo stability of engineered genetic constructs in complex microbial communities, and suggest design considerations for more precise tuning of payload life span and improved payload biocontainment.

Whole-genome sequencing of three transconjugant strains of *Proteus mirabilis* and *E. fergusonii* from our studies (designated as modifiable gut bacteria MGB3, MGB4, and MBG9) revealed the presence of putative endogenous DNA mobilization systems (Supplementary Fig. 13a–c). We wondered whether these native mobilization systems could interface with our engineered pGT vectors, and thus carried out in vitro conjugations of the MGB strains with laboratory *E. coli* recipients. We discovered that MGB4 and MGB9 (both *E. fergusonii*) were able to mobilize pGT vectors into recipients, although less efficiently than our engineered EcGT2 donor (Fig. 3a, Supplementary Fig. 13d). These results suggest that some native gut bacteria can promote secondary transfer of engineered payloads by using their endogenous conjugation machinery, which may improve payload transfer in situ.

In general, non-gut-adapted bacteria (e.g., probiotics) do not colonize an established gut microbiome. Infiltration of foreign species usually requires drastic perturbations, such as the use of broad-spectrum antibiotics to suppress the natural flora. Even then, exogenous species do not persist after discontinuation of antibiotic suppression[11]. As our donor strains did not readily colonize the mouse gut and transconjugants were lost soon after (Fig. 2b, Supplementary Figs. 9b and 14a), we reasoned that using a colonizing donor strain might extend the persistence of payload constructs in situ. To explore this possibility, we tested whether a mixed population of MGB strains (MGB3, MGB4, and MGB9) could stably recolonize the native mouse gut after a single oral dose without any antibiotic coadministration (Supplementary Fig. 15a). In contrast to the rapid disappearance of a non-gut-adapted strain (EcGT1) within 48 h, MGB strains (especially MGB4) recolonized the mouse gut and stably persisted for at least 15 d (Fig. 3b, Supplementary Fig. 15b), populating the entire gastrointestinal tract (Supplementary Fig. 15c). FACS enrichment and 16S sequencing of GFP-expressing bacteria in feces from these mice revealed transconjugants resulting from in situ transfer of the pGT payload from MGB strains to the native microbiome 6 h (Fig. 3c) and 11 d post-gavage (Supplementary Fig. 15d). These transconjugant populations had similar phylogeny but less diversity than those from prior in situ experiments using the noncolonizing EcGT2 donor (Fig. 2c, Supplementary Fig. 9c). These results highlight the utility of MAGIC for the isolation of host-derived engineerable strains that can be modified and then used to stably recolonize the native community and mediate further transfer of engineered functions in situ.

In summary, MAGIC enables metagenomic infiltration of genetic payloads into a native microbiome, and isolation of genetically modifiable strains from diverse communities. These strains can be reintroduced into their original community to maintain engineered functions via sustained vertical and horizontal transmission in situ. Future improvements to the system, such as optimization of vector stability and donor-strain dosage (Supplementary Fig. 14b), could allow for better quantitative and temporal control of retention of genetic payloads in situ, which might be useful in applications requiring short-term or long-term actuation of engineered functions[12–14]. Designing genetic programs based on recipient-specific properties should enhance the targeted execution of desired functions in a defined subset of species in a community[15,16]. MAGIC and complementary strategies to engineer the horizontal gene pool can facilitate programmable execution of genetic circuits in other microbial communities[17–20]. The isolation of genetically tractable representatives from diverse microbiomes will expand the repertoire of new microbial chassis for emerging applications in synthetic biology and microbial ecology.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41592-018-0301-y.

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Author contributions
C.R., V.C., S.P.C., S.J.Y., and H.H.W. designed the study. C.R., S.P.C., and V.C. performed the experiments. C.R., S.P.C., V.C., and H.H.W. analyzed the data and wrote the manuscript, with input from all other authors.

Competing interests
A provisional patent application has been filed by the Trustees of Columbia University in the City of New York based on this work.

Additional information
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Methods

Media, chemicals, and reagents. *E. coli*, *Salmonella enterica*, *Vibrio cholerae*, and *Pseudomonas aeruginosa* strains were grown in rich LB-Lennox media (BD) buffered to pH 7.45 with NaOH in aerobic conditions at 37 °C. *Lactobacillus reuteri* was grown in MRS media (BD). *Bacteroides thetaiotaomicron* and *Enterococcus faecalis* were grown anaerobically at 37 °C in Gifu anaerobic modified medium (GAM) (Nissui Pharmaceutical) or BHI media (BD) supplemented with cysteine (1 g/liter), hemin (5 mg/liter), resazurin (1 mg/liter), and vitamin K (1 µl/liter). All gut bacteria used in the study were grown in LB-Lennox media or GAM. Antibiotics were used at the following concentrations to select for *E. coli*: chloramphenicol at 20 µg/ml, carbenicillin (carb) at 50 µg/ml, spectinomycin (spec) at 250 µg/ml, kanamycin at 50 µg/ml, tetracycline at 25 µg/ml, and erythromycin at 25 µg/ml. Antibiotics were used at the following ranges of concentrations to select for transconjugant gut bacteria: chloramphenicol at 5–20 µg/ml, carb at 10–50 µg/ml, tetracycline at 5–25 µg/ml. DAP was supplemented at 50 µM as needed.

Animal ethics statement. All animal experiments were performed in compliance with Columbia University Medical Center IACUC protocols AC-AAAU6464 and AC-AAAL2503.

Isolation of live mouse gut bacteria. Fresh fecal pellets were collected from mice, and live gut bacteria were isolated by mechanical homogenization. Briefly, 250 µl of PBS was added to previously weighed pellets in a microcentrifuge tube. Pellets were thoroughly mechanically disrupted with a motorized pellet pestle, and then 750 µl of PBS was added. The disrupted pellets in PBS were then subjected to four iterations of vortex mixing for 15 s at medium speed, centrifugation at 1,000 r.p.m. for 30 s at room temperature, recovery of 750 µl of supernatant in a new tube, and replacement of that volume of PBS before the next iteration. The resulting 3 ml of isolated cells were pelleted by centrifugation at 4,000*g* for 5 min at room temperature, the supernatant was discarded, and cells were resuspended in 0.5–1.0 ml of PBS. All gut bacteria isolations were performed in an anaerobic chamber (Coy Labs).

Donor strain construction. We derived donor strains EcGT1 and EcGT2 from the S17 λpir *E. coli* strain[21] by generating modifications Δ*galK::mCherry-specR* and Δ*asd::mCherry-specR*, respectively, with λ-red recombineering using the pKD46 system[22]. Synthetic cassettes containing constitutively active mCherry and spec-resistance genes were constructed with ~40 bp of homology on both ends to *galK* or *asd* flanking regions on the *E. coli* genome. 100 ng of mCherry-specR cassette DNA were electroporated into recombineering-competent S17-pKD46 cells. Cells were allowed to recover in 3 ml of LB media plus carb at 30 °C for 3 h before being plated on LB media plus spec. Spec-resistant colonies were genotyped by PCR for validation of mutations. The pKD46 recombineering plasmid was cured out of validated recombinants by growth at 37 °C in the absence of carb to yield the EcGT1 and EcGT2 strains used throughout the study. When generating the EcGT2 strain, we supplemented the growth media with DAP at all stages of the protocol.

Plasmid construction. pGT vectors were designed to have modular components (e.g., selectable markers, regulatory elements, replication origins) that are interchangeable by isothermal assembly (ITA) or Golden Gate assembly. Vector selection markers for *E. coli* were constitutively expressed, whereas the deliverable cargo and transposase cassettes were expressed using different regulatory elements to enable broad-host-range or narrow-host-range gene expression. The regulatory elements used in this study exhibit a range of activity (Supplementary Table 1). Vector libraries used in this study are detailed in Supplementary Table 2. Full vector component sequences are listed in Supplementary Table 3. The nontransferable vector pGT-NT used as a negative control was a minimal p15A cloning vector with no origin of transfer, containing a constitutively expressed sfGFP gene.

All plasmids were constructed by ITA with NEBuilder HiFi DNA assembly master mix (New England Biolabs). Component parts were made by high-fidelity PCR with Q5 (NEB) or KAPA Hifi (Kapa Biosystems) polymerase, using existing vectors or gBlocks (Integrated DNA Technologies) as PCR templates. PCR products were digested with DpnI (NEB) and purified with the QIAquick PCR purification kit (Qiagen) before ITA and transformation into *E. coli*. All assembled plasmids were Sanger-sequence verified.

In vitro MAGIC studies on synthetic recipient community. Donor strains harboring pGT vectors and representative recipients (*E. coli* MG1655, *S. enterica* ATCC 700931, *V. cholerae* C9503, *P. aeruginosa* PA01, *E. faecalis* ATCC 29200, *L. reuteri* ATCC 23272, *B. thetaiotaomicron* ATCC 29148) were grown overnight in appropriate media and cultivation conditions, and a 1:1,000 dilution culture was regrown for 14 h at 37 °C before conjugation studies. To prepare cells for in vitro conjugation, we washed donor and recipient populations twice in PBS and quantified cells by OD_{600} or flow cytometry using SYTO9 staining (Thermo Fisher). 10^8 donor cells and 10^8 recipient cells were mixed together, pelleted by centrifugation, and resuspended in 10 µl of PBS. Donor and recipient mixes were spotted on an agar plate and incubated for 5 h at 30 °C or 37 °C for conjugation. In vitro conjugations were performed on LB-Lennox (*E. coli*, *S. enterica*, *V. cholerae*, *P. aeruginosa*, *E. faecalis*), MRS media (*L. reuteri*), or supplemented BHI agar (*B. thetaiotaomicron*). After conjugation, cells were scraped from the plate into 1 ml of PBS, and 100 µl was plated on appropriate antibiotics and incubated overnight at 30 °C or 37 °C so we could determine the number of colony-forming units (CFUs) of transconjugants.

In vitro MAGIC studies on natural recipient community. Donor strains harboring pGT vectors were streaked onto LB-Lennox agar plates with appropriate antibiotics and supplements, grown at 37 °C overnight, and then grown from a single colony in 2 ml of liquid media for 10 h at 37 °C before conjugation. The recipient community was isolated anaerobically from fresh mouse feces as described above, immediately before conjugation. Donor cells were washed twice in PBS and quantified by OD_{600}, whereas recipient cells were quantified by flow cytometry using SYTO9 staining. 10^8 donor cells and 10^9 recipient cells were mixed, pelleted by centrifugation at 5,000*g*, and resuspended in 25 µl of PBS. The mixes were spotted on PBS + 1.5% agar plates and incubated at 37 °C either aerobically or anaerobically overnight (9–10 h). After conjugation, cells were scraped from the plate into 1 ml of PBS and subjected to antibiotic selection on GAM, FACS enrichment, and metagenomic 16S analysis (see below).

In vitro assessment of pGT vector horizontal gene transfer mediated by natural isolates. MGB natural isolates harboring pGT vectors (MGB3, MGB9, and MBG4) were conjugated with a recipient *E. coli* strain harboring a kanamycin-resistance plasmid compatible with pGT vectors. Prior to conjugations, all strains were streaked onto GAM agar plates with appropriate antibiotics, grown at 37 °C overnight, and then grown from a single colony in 5 ml of liquid GAM for 10 h at 37 °C before conjugation. MGB donor and recipient cells were washed twice in PBS and quantified by OD_{600}. 10^9 cells each of MGB and recipient strains were mixed, pelleted by centrifugation at 5,000*g*, and resuspended in 15 µl of PBS. The mixtures were spotted on GAM agar plates and incubated at 37 °C aerobically for 6 h. After conjugation, cells were scraped from the plate into 1 ml of PBS and plated on selective and nonselective GAM. Conjugation efficiency was calculated as t/n, where t is the number of *E. coli* transconjugant CFUs and n is the total number of *E. coli* CFUs.

Measurement of GFP expression in MGB strains. MGB isolates harboring pGT vectors (MGB3, MGB9, MBG4) were streaked onto GAM agar plates with appropriate antibiotics, grown at 37 °C overnight, and then diluted to OD_{600} 0.001 in liquid GAM into a 96-well plate. The plate was incubated in a Synergy H1 (BioTek) microplate reader for 24 h at 37 °C with orbital shaking. Measurements of OD_{600} and GFP expression (excitation, 488 nm; emission, 510 nm) were acquired with Gen5 software (BioTek) at the end of 24 h.

In vivo MAGIC studies in mice. Conventionally raised C57BL/6 female mice (Taconic Biosciences or Charles River Laboratories) were used throughout the study. Two control groups of four mice each were gavaged with PBS and EcGT2 containing a nontransferable GFP vector (pGT-NT). Three to four mice were used in each group gavaged with a pGT donor mix or with MGB strains. To equilibrate the mouse gut microbiome ahead of time, we mixed mice from multiple litters, cohoused them for at least 1 week before all experiments, and randomly allocated them into groups. Mice were gavaged with 10^9 donor cells (EcGT2 or MGB strains) in 300 µl of PBS at 8–10 weeks old. Control mice were gavaged with 300 µl of PBS. Fecal matter was collected immediately before gavage and periodically after gavage for analysis of the resulting microbiome populations by FACS, metagenomic 16S sequencing, and plating. Upon completion of the study, mice were euthanized, and small and large intestinal tissues were extracted. Luminal contents were washed from each tissue sample with PBS, and bacteria were extracted by homogenization of the luminal contents for plating and final CFU determination.

Flow cytometry and FACS measurements. Gut bacteria isolated from fresh fecal pellets were analyzed for evidence of successful conjugation on a flow cytometer (Guava easyCyte HT) using red (642 nm) and blue (488 nm) lasers with Red2 and Green photodiodes to detect mCherry (587/610 nm) and sfGFP (485/510 nm) fluorescence, respectively. Bacteria at 100× and 1,000× dilutions in PBS were used for optimal detection of donor material ($GFP^+mCherry^+$), gut microbes without a transferred vector ($GFP^-mCherry^-$), and transconjugants ($GFP^+mCherry^-$). Data were collected and analyzed with InCyte 3.1 software. For FACS enrichment studies, a BD FACSAria II cell sorter operated with BD FACSDiva software was used to gate for sfGFP (FITC filter 515/10 nm) and mCherry (mCherry filter 616/26 nm). Double-gating on GFP and mCherry channels was used to select for cells with $GFP^+mCherry^-$ fluorescence. In addition, we took background events into account by using the $GFP^+mCherry^-$ fluorescence detected in the fecal sample before gavage as the baseline signal. An increase over the baseline signified an enrichment of transconjugants. Population density (cells per gram of fecal matter) was calculated as the number of cells sorted over the mass of the sorted fecal sample. Additional plating and direct colony counting were used to validate flow cytometry measurements. FACS plots were formatted with FCS Express 6.

Fluorescence microscopy of fecal bacteria. We suspended bacteria in PBS and centrifuged them at 5,000*g* to concentrate them into a smaller volume, which varied depending on the concentration of bacteria. The bacteria were resuspended by pipetting, and a volume of 15 µl was dropped onto a Superfrost Plus microscope slide (Thermo Shandon) and covered with a glass coverslip. Slides were air-dried until the PBS receded from the edges of the coverslip and then were sealed with clear nail polish. Bacteria were imaged at 40× magnification on a Nikon Eclipse Ti2 microscope on bright-field, RFP, and GFP channels using NIS-Elements-AR software.

Validation of pGT vectors in transconjugants. Transconjugant validation was done by colony PCR of the GFP–antibiotic resistance payload and/or the pGT vector backbone. PCR products with the expected size were further verified by Sanger sequencing. Taxonomy assignment of isolated colonies was based on 16S rRNA PCR amplification and Sanger sequencing. All transconjugant strains validated in the study are listed in Supplementary Table 4.

In vitro evolution of transconjugant gut bacteria. *E. fergusonii* transconjugants MGB4 and MGB9 were serially passaged in LB media for 11–15 d. Starting from a single colony, the strains were inoculated into LB and grown at 37 °C with shaking. Every 12 h the liquid culture was diluted 1:1,000 into fresh LB media. At selected time points an aliquot of the saturated culture was plated on selective (50 µg/ml carb) and nonselective plates for quantification of the percentage of cells expressing the payload antibiotic-resistance and GFP genes. MGB9 cultures were also plated on selective plates with 20 µg/ml chloramphenicol to check for maintenance of the plasmid backbone.

Metagenomic 16S sequencing. Genomic DNA was extracted from isolated bacteria populations with the MasterPure Gram-positive DNA purification kit (Epicentre). PCR amplification of the 16S rRNA V4 region and multiplexed barcoding of samples were done in accordance with previous protocols[23]. The V4 region of the 16S rRNA gene was amplified with customized primers according to the method described by Kozich et al.[23], with the following modifications: (i) alteration of 16S primers to match updated EMP 505f and 806rB primers[24–26] and (ii) use of NexteraXT indices such that each index pair was separated by a Hamming distance of >2 and Illumina low-plex pooling guidelines could be used. Sequencing was done with the Illumina MiSeq system (500V2 kit).

Analysis of 16S next-generation sequencing data. Bacteria from fecal samples taken right before gavage (T0) and 6 h post-gavage (T6) were sorted by FACS to enrich for transconjugants. The compositions of the sorted transconjugant and total populations for each sample were determined from 16S sequencing data via the UPARSE pipeline[27] (USEARCH version 10.0.240) to generate operational taxonomic unit (OTU) tables and abundances and the RDP Classifier[28] to assign the taxonomy. Phylogenetic associations were analyzed at the genus level with at least 90% confidence for 16S assignment. In all MiSeq runs, two blank controls with sterile water as input material were included to check for contaminants in the reagents and to filter out contaminant OTUs if present. Reads mapping to nonbacterial DNA (e.g., mitochondria, plastids, or other eukaryotic DNA) were also excluded from analysis. Only OTUs with more than ten reads were considered in downstream analysis.

Relative abundances of OTUs in unsorted total fecal populations were calculated as the normalized number of reads in a sample. Relative abundances of OTUs in T0 FACS-enriched populations were used to measure false positive background fluorescence, which was subtracted from the fluorescence of T6 transconjugant populations. The corrected relative abundance of each OTU in a T6 FACS-enriched population is given by the following formula:

$$\mathrm{RA}_{6,i,\mathrm{sorted}} = \frac{A_{6,i} \times N_6 - A_{0,i} \times N_0}{\sum_i (A_{6,i} \times N_6 - A_{0,i} \times N_0)}$$

where $\mathrm{RA}_{t,i,\mathrm{sorted}}$ is the corrected relative abundance of OTU i at time t, $A_{t,i}$ is the normalized number of reads of OTU i at time t in the FACS-sorted sample, and N_t is the fraction of $\mathrm{GFP^+mCherry^-}$ FACS-sorted events at time t. OTUs for which $\mathrm{RA}_{6,i,\mathrm{sorted}}$ is negative are eliminated from subsequent analysis, and all remaining $\mathrm{RA}_{6,i,\mathrm{sorted}}$ values are renormalized.

The fold enrichment of each OTU in the FACS-sorted population is defined as its relative abundance in the FACS-sorted population divided by its relative abundance in the unsorted total population at T6. To overcome the problem of detection limits (i.e., OTU i appears in the sorted population but is present at levels below the detection limit in the total population), we added a pseudo-count of p to all relative abundances when calculating fold enrichments. p is given by

$$p = 10^{\lfloor -\log_{10} n \rfloor}$$

where n is the total number of reads in the FACS-sorted sample, and $\lfloor -\log_{10} n \rfloor$ is the floor function of (i.e., the greatest integer less than or equal to) $-\log_{10} n$. The fold enrichment of OTU i with the pseudo-count correction is calculated as

$$F_i = \frac{\mathrm{RA}_{6,i,\mathrm{sorted}} + p}{\mathrm{RA}_{6,i,\mathrm{unsorted}} + p}$$

If the relative abundance of OTU i in the unsorted population is below the detection limit, then the fold enrichment is calculable as $(\mathrm{RA}_{6,i,\mathrm{sorted}} + p)/p$, instead of $\mathrm{RA}_{6,i,\mathrm{sorted}}/0$.

The pseudo-count-corrected fold enrichment F_i overestimates the true fold enrichment ($\mathrm{RA}_{6,i,\mathrm{sorted}}/\mathrm{RA}_{6,i,\mathrm{unsorted}}$) by at most 10%, or possibly underestimates it. Because $0 < p \le 1/n$ and $\mathrm{RA}_{6,i,\mathrm{sorted}} \ge 10/n$,

$$F_i = \frac{\mathrm{RA}_{6,i,\mathrm{sorted}} + p}{\mathrm{RA}_{6,i,\mathrm{unsorted}} + p} \le \frac{\mathrm{RA}_{6,i,\mathrm{sorted}} + p}{\mathrm{RA}_{6,i,\mathrm{unsorted}}} \le \frac{1.1 \times \mathrm{RA}_{6,i,\mathrm{sorted}}}{\mathrm{RA}_{6,i,\mathrm{unsorted}}}$$

In all heat maps showing fold enrichment versus relative abundance, only OTUs with $F_i > 10$ are displayed, to show more stringent and high-confidence results. R code for this analysis is available from the corresponding author upon request.

Whole-genome sequencing of engineered mouse gut bacteria isolates. To sequence MGB isolates, we prepared a sequencing library using the Nextera kit (Illumina) and used the Illumina HiSeq 2500 platform for 100-bp single-end reads. The SPAdes single-cell assembler pipeline (version 3.9.1)[29] was used to generate whole-genome contigs. BLAST and PlasmidFinder (version 1.3)[30] were used to analyze the sequences and identify native mobilization systems. Geneious (version 7.1.5) was used to visualize contig alignments to genomes and plasmids.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Materials availability. All modular vector part sequences are listed in Supplementary Table 3. Full plasmid maps, vectors, and strains used in this study are available from the corresponding author upon request or will be available on Addgene.

Data availability

The raw data from this study are available from the corresponding author upon request.

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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☒	☐	A description of all covariates tested
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☒	☐	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Flow cytometry was performed using InCyte 3.1 software on the Guava easyCyte HT flow cytometer. BD FACSDiva software was used for FACS on the BD FACSAria II cell sorter. NIS-Elements-AR software was used for fluorescence microscopy. Gen5 software was used to operate the plate reader for measurement of GFP expression in isolate strains.
Data analysis	All 16S data were processed using the UPARSE pipeline and the RDP classifier (USEARCH v.10.0.240) and subsequently analyzed in R, using the calculations stated in the Methods section. For whole genome sequencing assembly, we used SPAdes (v.3.9.1) software to generate contigs and then performed sequence analysis using BLAST, PlasmidFinder(v.1.3), and Geneious (v.7.1.5). FCS Express 6 was used for formatting FACS plots.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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- A list of figures that have associated raw data
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes of mice were chosen to ensure that the effect of treatment with the engineered bacteria was robust and replicable. At least 3 mice were used for each treated group, and at least 2 mice were used for control groups.
Data exclusions	No data were excluded from the analysis
Replication	All attempts at replication were successful. We ran multiple iterations of the study using different cohorts of mice, with multiple mice in each treatment group (see samples size above).
Randomization	Mice were randomly allocated to different treatments. We ensured that animals shipped to the animal facility in different cages were mixed appropriately in order to avoid microbiome cage bias.
Blinding	Blinding does not apply to this study because the investigators needed to identify the cages of mice for subsequent FACS sorting and analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study	
☐	☒	Unique biological materials
☒	☐	Antibodies
☒	☐	Eukaryotic cell lines
☒	☐	Palaeontology
☐	☒	Animals and other organisms
☒	☐	Human research participants

Methods

n/a	Involved in the study	
☒	☐	ChIP-seq
☐	☒	Flow cytometry
☒	☐	MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All strains used in the study are available upon request from the corresponding author.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	7-8 week old C57BL/6 female mice from Taconic and Charles River Laboratories were used.

Wild animals | No wild animals were used.

Field-collected samples | The study did not involve samples collected from the field.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Bacteria were extracted from murine feces as described in the Methods section by resuspension in PBS and filtration. The bacteria were run directly on the flow cytometer/cell sorter without additional treatment.
Instrument	BD FACSAria II, Guava easyCyte HT
Software	BD FACSAriaII was operated using BD FACSDiva. Guava easyCyte HT was operated using InCyte3.1. FCS Express 6 software was used to format FACS plots.
Cell population abundance	Representative population abundances pre- and post-sorting are shown in the manuscript. The purity of samples is addressed in the manuscript, as autofluorescent cells were filtered out of the post-sort population.
Gating strategy	FSC/SSC gates were determined by comparison of fecal bacterial samples and in vitro cultures of E. coli against the PBS background to gate in the signal for live bacteria and exclude noise. GFP and mCherry gates were set by comparing GFP+/mCherry+ E. coli, GFP+/mCherry- E. coli, GFP-/mCherry+ E. coli, and GFP-/mCherry- E. coli. To minimize sorting of autofluorescent fecal bacteria, we adjusted the fluorescence gates to stringently gate out the natural murine gut bacterial community.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

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SUPPLEMENTARY INFORMATION
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Metagenomic engineering of the mammalian gut microbiome in situ

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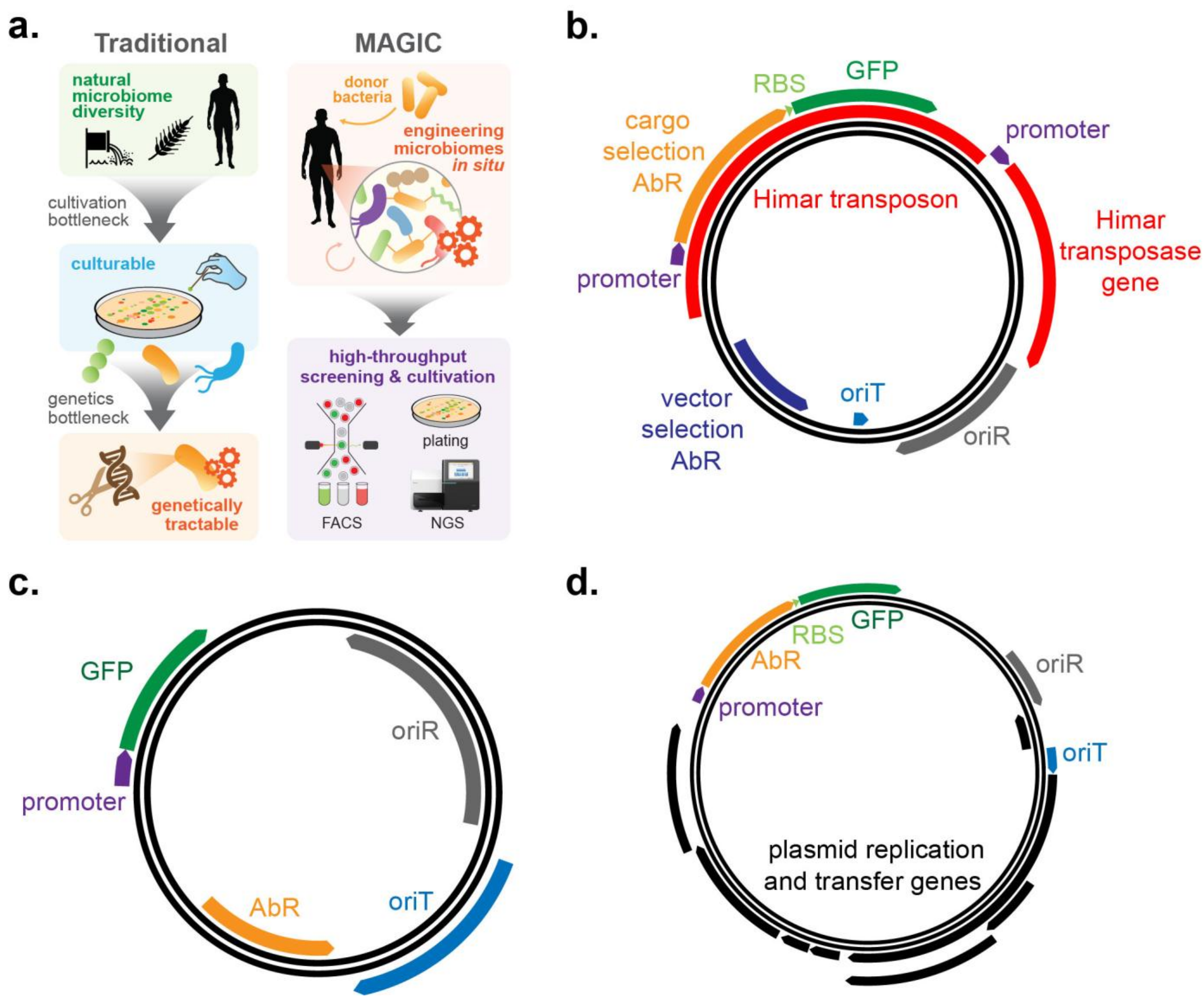
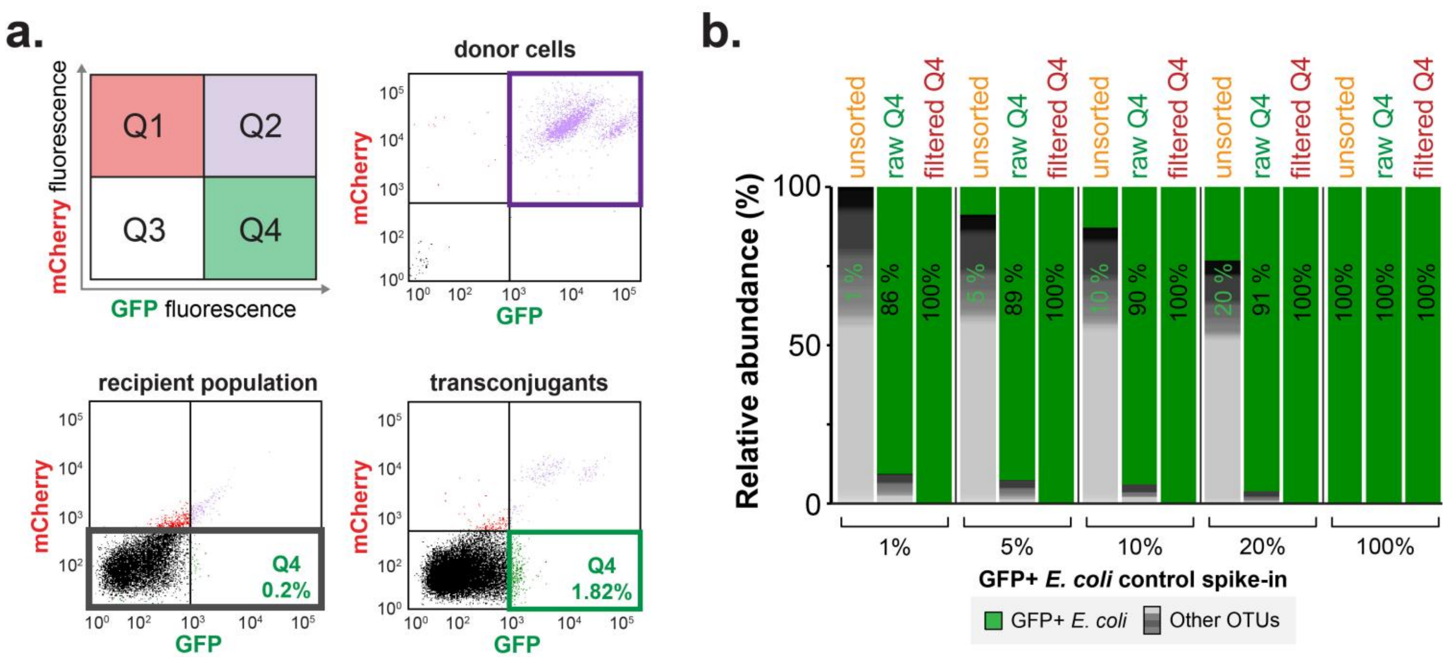


Supplementary Figure 1

Overview of metagenomic alteration of gut microbiome by in situ conjugation (MAGIC) and plasmid maps of MAGIC vectors.

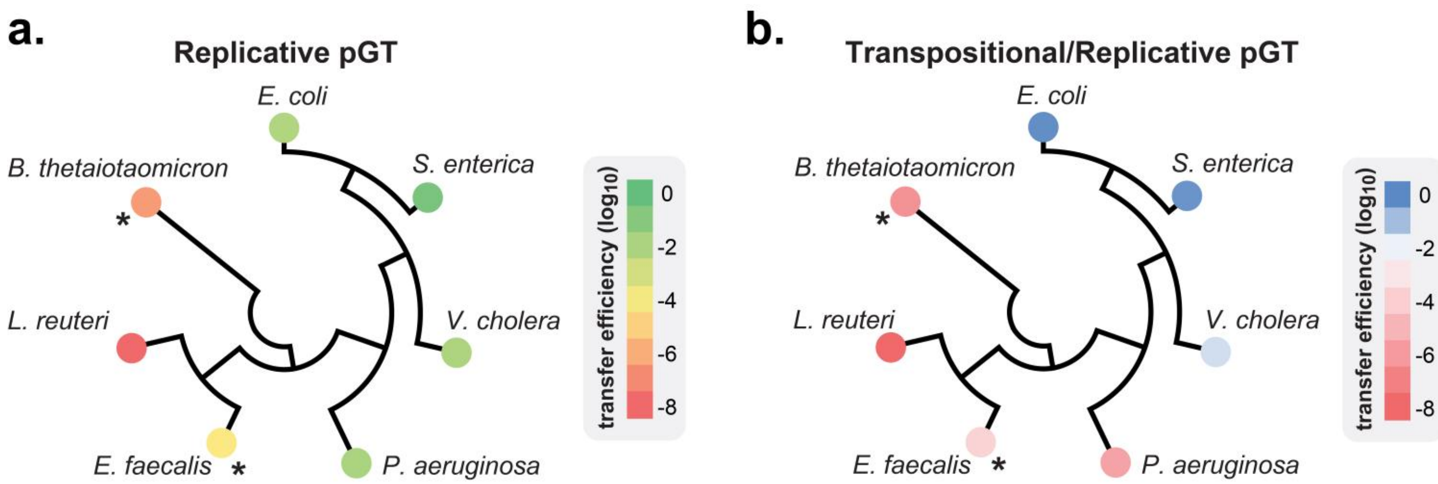
(a) In contrast to traditional approaches to cultivate microbes first and then test for genetic accessibility, MAGIC harnesses horizontal gene transfer in the native environment to genetically modify bacteria in situ. Transconjugant bacteria can be detected by FACS or antibiotic selection and further manipulated. **(b)** Map of Himar transposon integrative vectors (pGT-Ah and pGT-Kh variants found in libraries L2, L4, L5, L6, L7 and L8). **(c)** Map of replicative vectors with pBBR1 origin of replication (pGT-B variants found in libraries L1, L4, and L6). **(d)** Map of replicative vectors with RSF1010 origin of replication (pGT-S variants found in library L3). Although this vector backbone contains genes involved in conjugation (black), these vectors are not self-transmissible (*J. Bacteriol.* **117**, 619–630, 1974; *Gene* **75**, 271–288, 1989).



Supplementary Figure 2

FACS gating methodology for isolation of transconjugant bacteria.

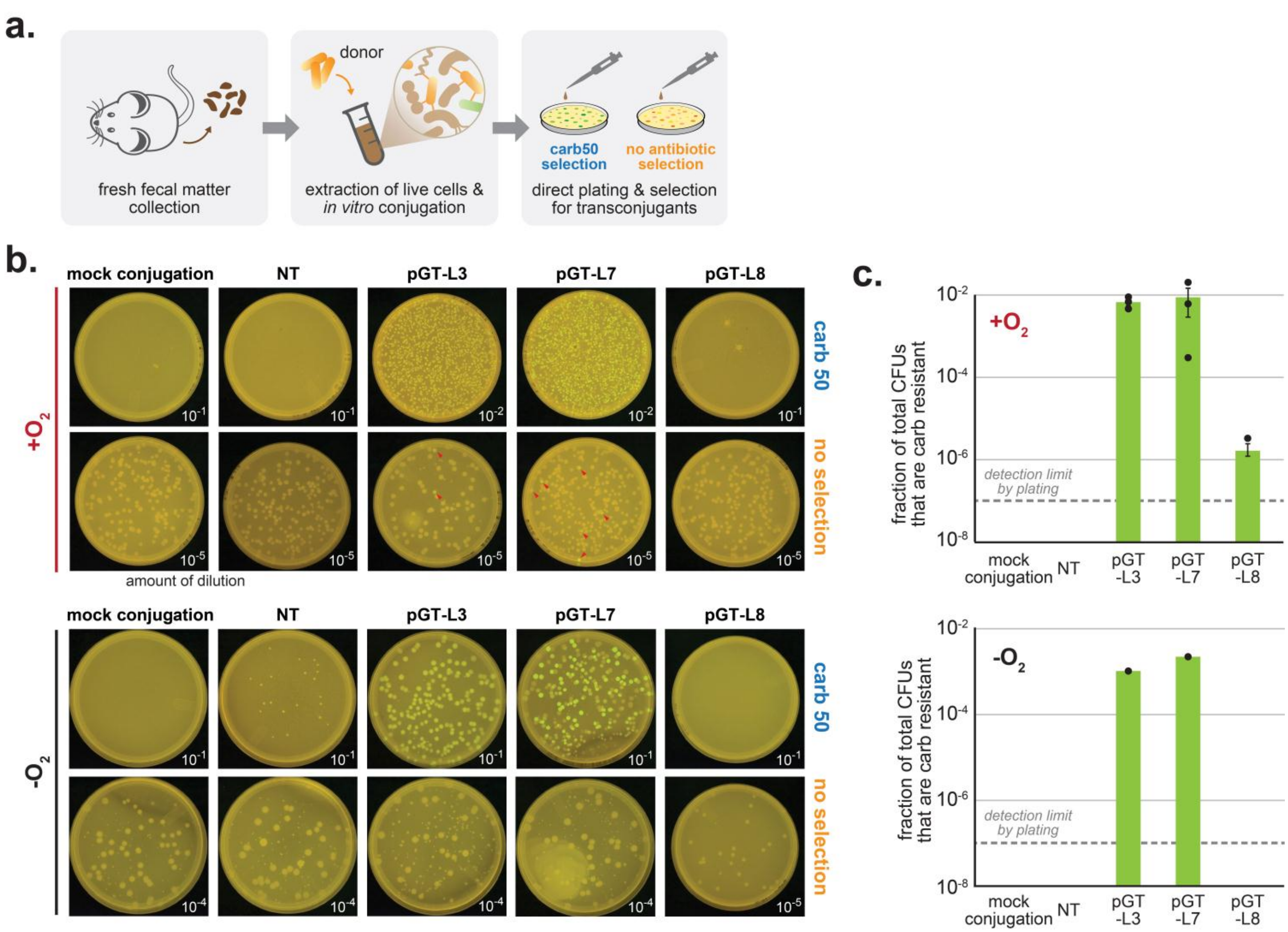
(a) Illustration of FACS enrichment method to isolate transconjugant cells from complex recipient populations. GFP and mCherry fluorescence are used to gate cell populations consisting of *E. coli* donors and diverse recipients. Quadrants Q1 and Q2 correspond to donor cells (mCh^{+}), and unmanipulated recipients are in quadrant Q3. Quadrant Q4 contains transconjugants that received the GFP gene cargo and are not naturally mCherry-fluorescent ($GFP^{+}mCh^{-}$). Q4 cells are isolated and further analyzed. This gating was used to analyze fecal samples from each individual mouse in each in situ experiment, as well as every in vitro conjugation in this study by flow cytometry. **(b)** To validate the FACS enrichment method, we mixed GFP^{+} *E. coli* with a natural mouse fecal bacterial community at given levels (1–100% of population) and retrieved by FACS. 16S sequencing of the samples showed that the fluorescent *E. coli* were efficiently and specifically enriched by FACS. Although the raw Q4 population contained some autofluorescent cells, the only remaining OTU in Q4 after application of an enrichment filter (see Methods) was *E. coli*.



Supplementary Figure 3

pGT vectors were transferred from *E. coli* donors to representative recipient species during in vitro conjugations.

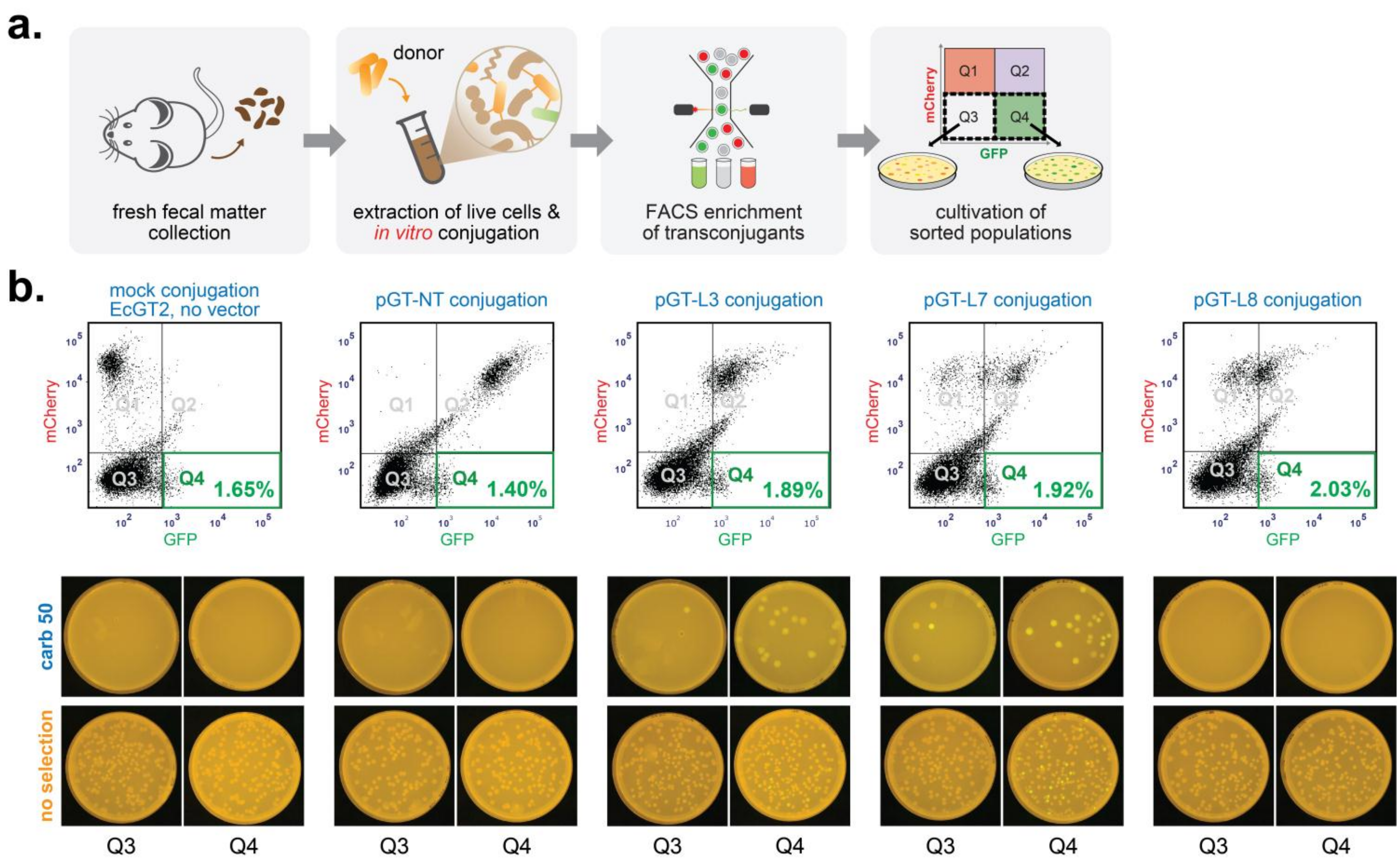
(a) In vitro conjugation efficiency of replicative vector pGT-B1 from *E. coli* donor to various recipients, which are plotted by phylogenetic relationships. **(b)** In vitro conjugation efficiency of vector pG-Ah1 between *E. coli* donor and various recipients. This vector is replicative only in Proteobacteria (*E. coli*, *S. enterica*, *V. cholerae*, *P. aeruginosa*) but delivered genetic cargo by transposition into a broader array of bacteria. Asterisks indicate cultures grown in anaerobic conditions; all other cultures were grown aerobically. Conjugation efficiencies were calculated from 2 independent conjugations.



Supplementary Figure 4

pGT vectors were transferred from *E. coli* donors to mouse fecal bacteria during in vitro conjugations.

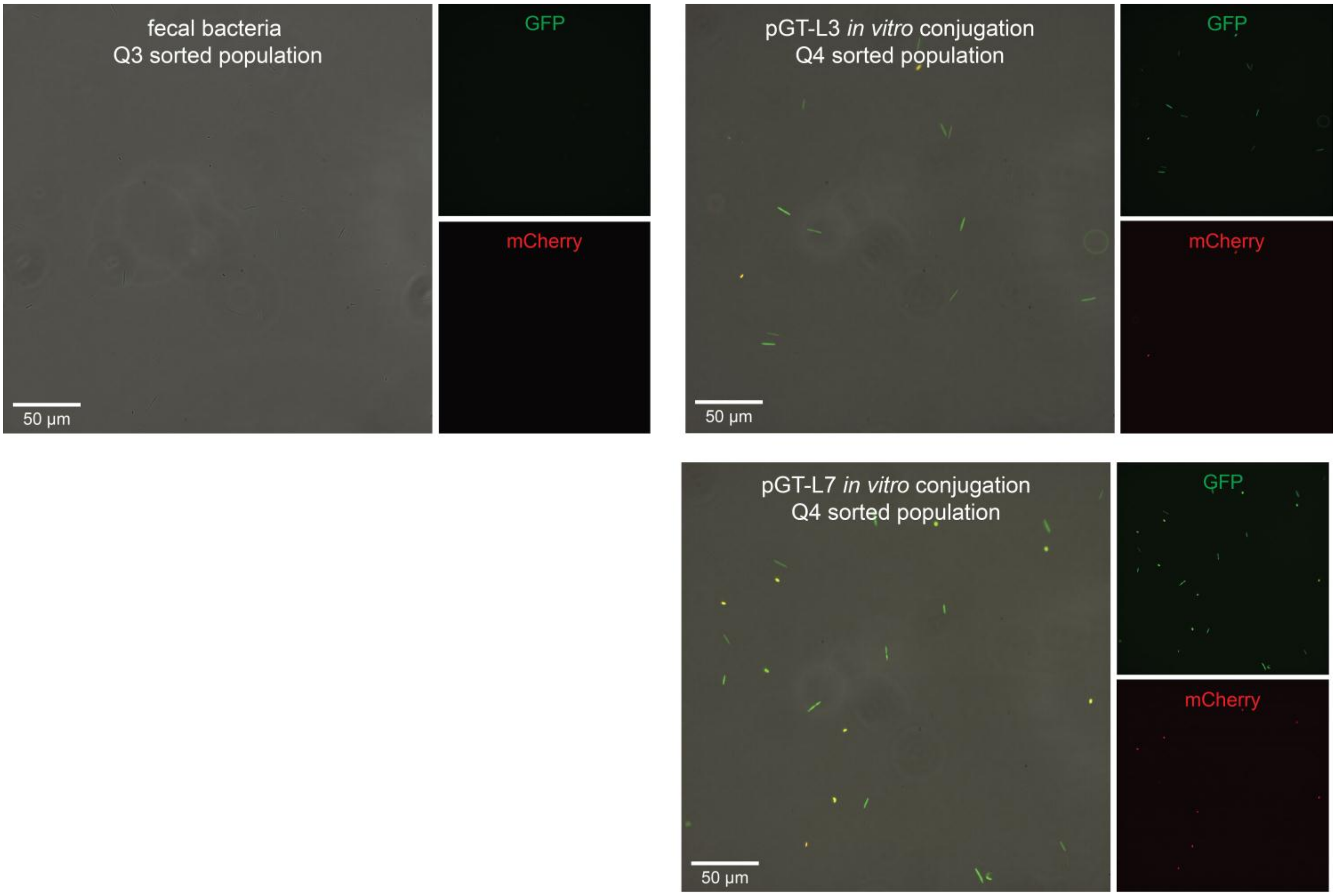
(a) In vitro conjugation of pGT vectors from EcGT2 donor strain into fecal bacteria extracted from mouse feces. **(b)** Aerobic (top) and anaerobic (bottom) conjugations were carried out with EcGT2 strains containing no vector (mock conjugation), a nontransferable vector (pGT-NT), pGT-L3, pGT-L7, and pGT-L8. Aerobic conjugations were plated on selective and nonselective media and grown aerobically at 37 °C for 24 h. Anaerobic conjugations were plated on selective and nonselective media, grown anaerobically at 37 °C for 48 h, and exposed to oxygen at room temperature for 48 h. Red arrows indicate GFP^{+} CFUs on nonselective plates. **(c)** Efficiencies of aerobic (top) and anaerobic (bottom) conjugations. Aerobic conjugation efficiencies were calculated from 3 independent conjugations; anaerobic conjugation efficiencies were calculated from 1 conjugation.



Supplementary Figure 5

FACS enriches for GFP$^+$, antibiotic-resistant transconjugant gut bacteria arising from in vitro conjugations.

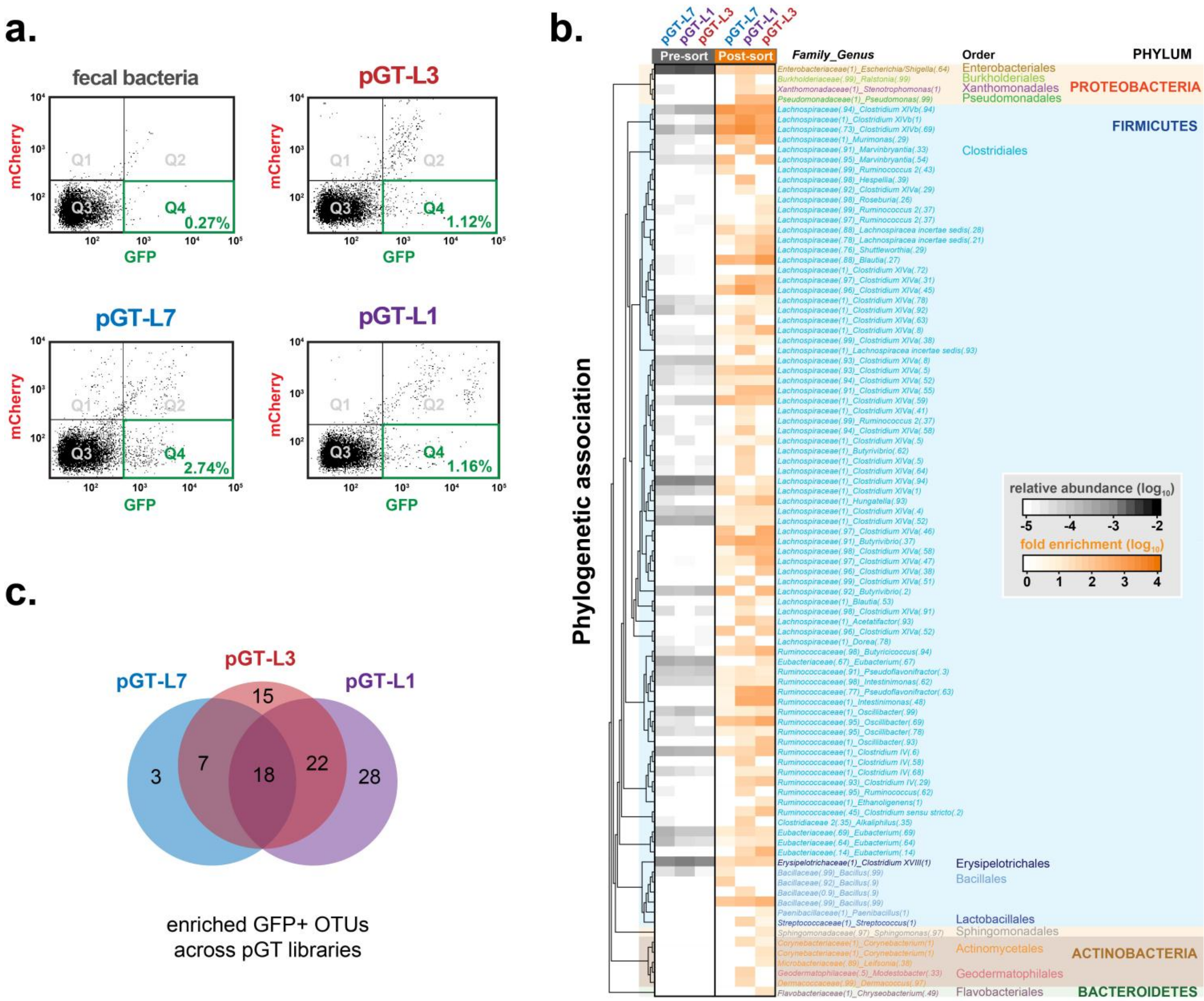
(a) Implementation of FACS enrichment of in vitro conjugations. **(b)** Conjugations between EcGT2 harboring vector libraries pGT-L3, pGT-L7, and pGT-L8 and mouse fecal bacteria were performed aerobically overnight. A mock conjugation using EcGT2 with no vector and a negative control conjugation using the pGT-NT nontransferable vector were also performed. 20,000 FACS-sorted events from Q3 (mCherry$^-$GFP$^-$) and Q4 (mCherry$^-$GFP$^+$) populations were plated on selective and nonselective media and grown aerobically to select for transconjugants. Cultivable aerobic transconjugants of pGT-L3 and pGT-L7 vectors were successfully enriched by FACS, although GFP$^+$ CFUs may appear dim against the autofluorescent media. This experiment was performed independently twice, with similar results.



Supplementary Figure 6

Fluorescence microscopy of FACS-sorted in vitro conjugations.

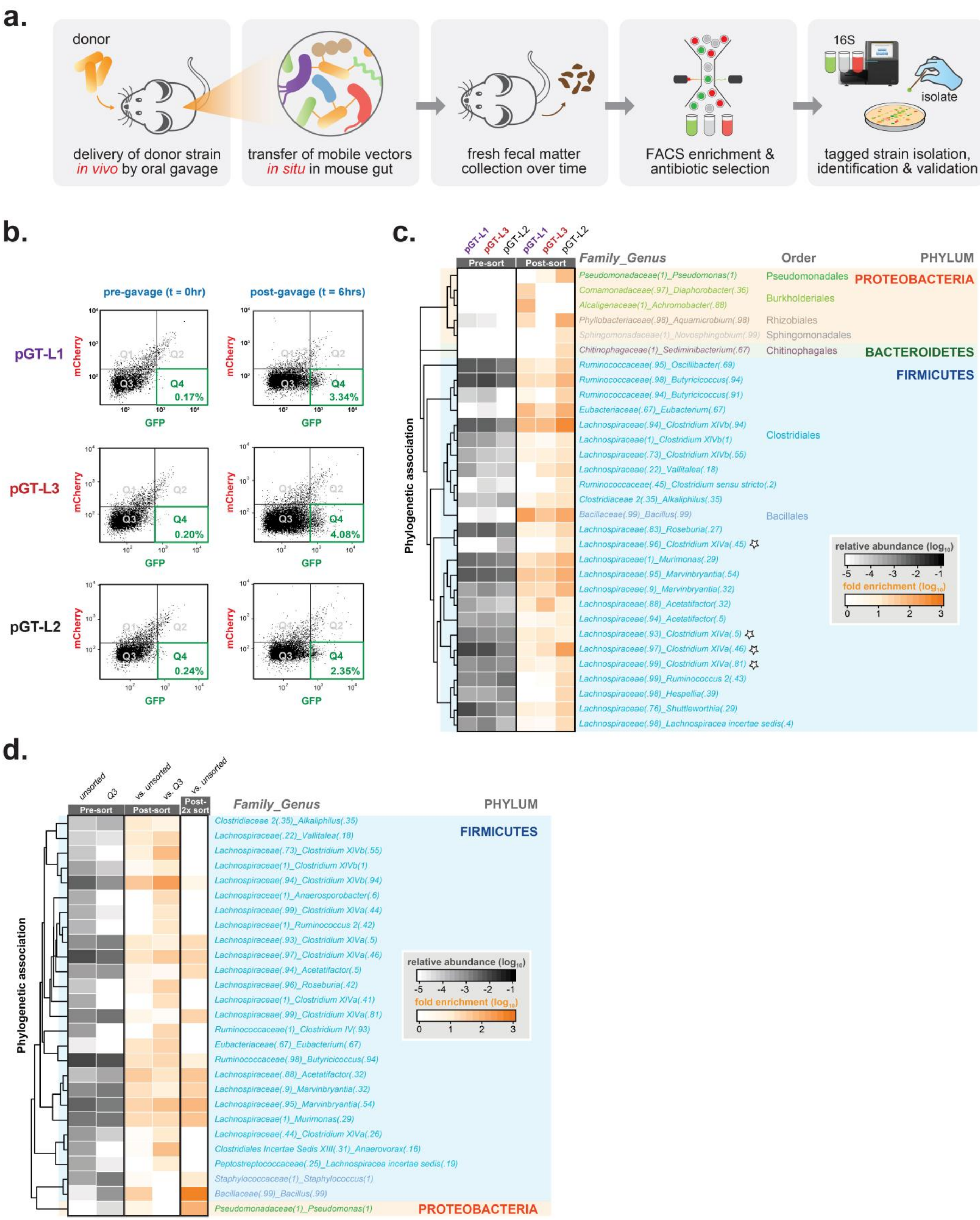
Overlays of bright-field, GFP, and mCherry channels are shown alongside GFP and mCherry channels. Q3 populations from unmodified fecal bacteria are negative for both GFP and mCherry, whereas Q4 populations from aerobic overnight in vitro conjugations of vector libraries pGT-L3 and pGT-L7 show enrichment of GFP-expressing cells, as well as some donor cells (mCherry^{+}GFP^{-}), which were eliminated in downstream sequencing analyses. This experiment was performed independently three times, with similar results.



Supplementary Figure 7

Identification of FACS-enriched in vitro transconjugants by 16S sequencing.

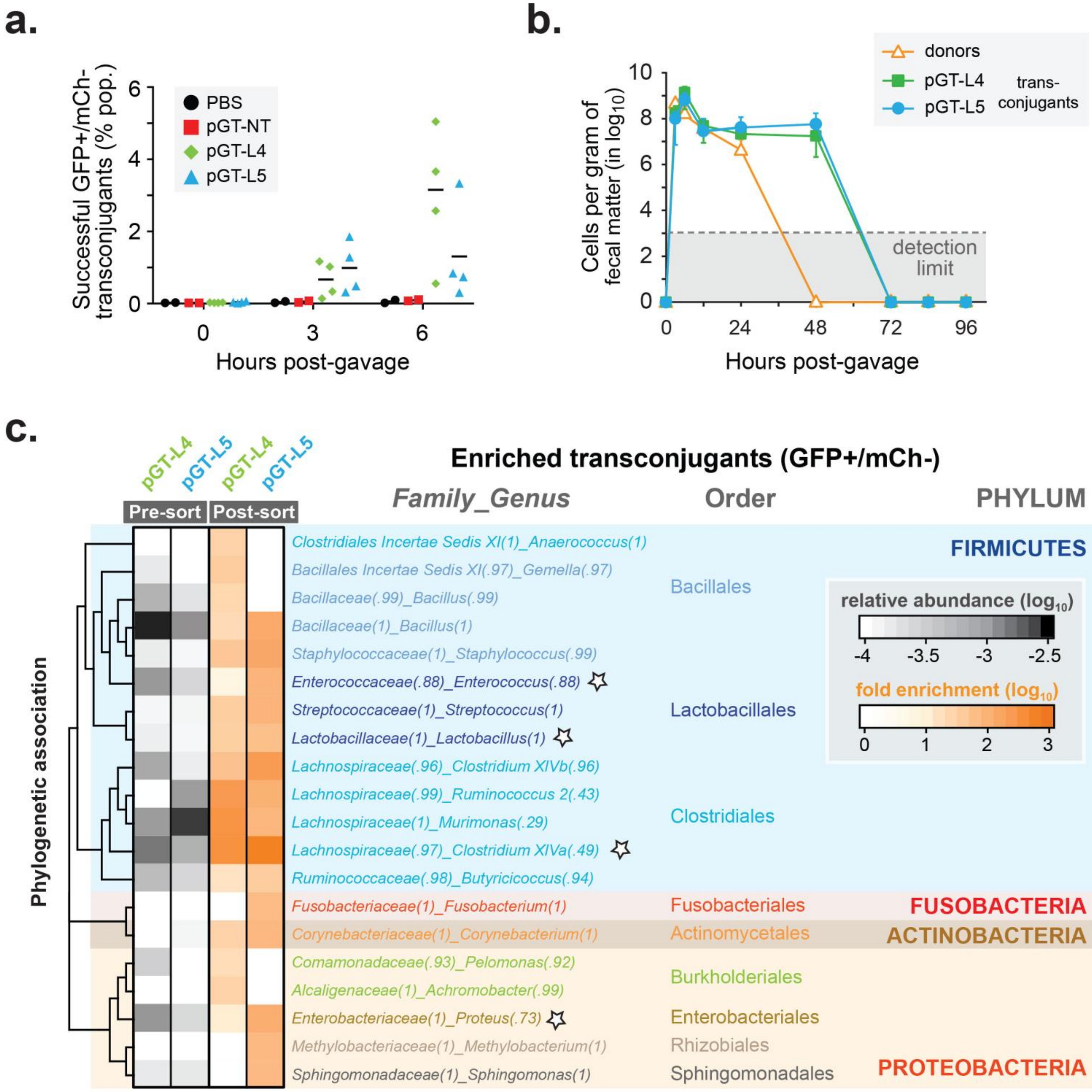
(a) FACS dot plots of in vitro conjugations of mouse gut bacteria and EcGT2 donors with vector libraries pGT-L1, L3, and L7. This experiment was performed 3 times, with similar results. Green boxes define the sorted $GFP^+mCherry^-$ transconjugant populations. **(b)** 16S taxonomic classification of in vitro $GFP^+mCherry^-$ transconjugants of pGT-L1, L3, and L7 enriched by FACS. Relative abundance of each OTU in the unsorted population is shown in the grayscale heat map, and fold enrichment for transconjugants of each OTU is shown in the orange heat map, with annotated taxonomic identities. Bracketed values indicate confidence of taxonomic assignment by RDP Classifier. Genera with successfully cultivated isolates are denoted by stars. Each column represents FACS-enriched transconjugants from one conjugation. **(c)** Comparison of OTUs shared between transconjugants arising from each vector library during in vitro conjugations. 18 OTUs were shared between all 3 libraries, with a total of 47 OTUs being shared between at least 2 libraries.


a.
donor
delivery of donor strain in vivo by oral gavage
transfer of mobile vectors in situ in mouse gut
fresh fecal matter collection over time
FACS enrichment & antibiotic selection
16S
isolate
tagged strain isolation, identification & validation
b.
pre-gavage (t = 0hr)
post-gavage (t = 6hrs)
pGT-L1
pGT-L3
pGT-L2
mCherry
GFP
Q1
Q2
Q3
Q4
0.17%
3.34%
0.20%
4.08%
0.24%
2.35%
c.
pGT-L1
pGT-L3
pGT-L2
Pre-sort
Post-sort
Family_Genus
Order
PHYLUM
Phylogenetic association
Pseudomonadaceae(1)_Pseudomonas(1)
Comamonadaceae(.97)_Diaphorobacter(.36)
Alcaligenaceae(1)_Achromobacter(.88)
Phyllobacteriaceae(.98)_Aquamicrobium(.98)
Sphingomonadaceae(1)_Novosphingobium(.99)
Chitinophagaceae(1)_Sediminibacterium(.67)
Ruminococcaceae(.95)_Oscillibacter(.69)
Ruminococcaceae(.98)_Butyricicoccus(.94)
Ruminococcaceae(.94)_Butyricicoccus(.91)
Eubacteriaceae(.67)_Eubacterium(.67)
Lachnospiraceae(.94)_Clostridium XlVb(.94)
Lachnospiraceae(1)_Clostridium XlVb(1)
Lachnospiraceae(.73)_Clostridium XlVb(.55)
Lachnospiraceae(.22)_Vallitalea(.18)
Ruminococcaceae(.45)_Clostridium sensu stricto(.2)
Clostridiaceae 2(.35)_Alkaliphilus(.35)
Bacillaceae(.99)_Bacillus(.99)
Lachnospiraceae(.83)_Roseburia(.27)
Lachnospiraceae(.96)_Clostridium XlVa(.45)
Lachnospiraceae(1)_Murimonas(.29)
Lachnospiraceae(.95)_Marvinbryantia(.54)
Lachnospiraceae(.9)_Marvinbryantia(.32)
Lachnospiraceae(.88)_Acetatifactor(.32)
Lachnospiraceae(.94)_Acetatifactor(.5)
Lachnospiraceae(.93)_Clostridium XlVa(.5)
Lachnospiraceae(.97)_Clostridium XlVa(.46)
Lachnospiraceae(.99)_Clostridium XlVa(.81)
Lachnospiraceae(.99)_Ruminococcus 2(.43)
Lachnospiraceae(.98)_Hespellia(.39)
Lachnospiraceae(.76)_Shuttleworthia(.29)
Lachnospiraceae(.98)_Lachnospiracea incertae sedis(.4)
Pseudomonadales
Burkholderiales
Rhizobiales
Sphingomonadales
Chitinophagales
Clostridiales
Bacillales
PROTEOBACTERIA
BACTEROIDETES
FIRMICUTES
relative abundance (log10)
-5 -4 -3 -2 -1
fold enrichment (log10)
0 1 2 3
d.
unsorted
Q3
vs. unsorted
vs. Q3
vs. unsorted
Pre-sort
Post-sort
Post-2x sort
Family_Genus
PHYLUM
Phylogenetic association
Clostridiaceae 2(.35)_Alkaliphilus(.35)
Lachnospiraceae(.22)_Vallitalea(.18)
Lachnospiraceae(.73)_Clostridium XlVb(.55)
Lachnospiraceae(1)_Clostridium XlVb(1)
Lachnospiraceae(.94)_Clostridium XlVb(.94)
Lachnospiraceae(1)_Anaerosporobacter(.6)
Lachnospiraceae(.99)_Clostridium XlVa(.44)
Lachnospiraceae(1)_Ruminococcus 2(.42)
Lachnospiraceae(.93)_Clostridium XlVa(.5)
Lachnospiraceae(.97)_Clostridium XlVa(.46)
Lachnospiraceae(.94)_Acetatifactor(.5)
Lachnospiraceae(.96)_Roseburia(.42)
Lachnospiraceae(1)_Clostridium XlVa(.41)
Lachnospiraceae(.99)_Clostridium XlVa(.81)
Ruminococcaceae(1)_Clostridium IV(.93)
Eubacteriaceae(.67)_Eubacterium(.67)
Ruminococcaceae(.98)_Butyricicoccus(.94)
Lachnospiraceae(.88)_Acetatifactor(.32)
Lachnospiraceae(.9)_Marvinbryantia(.32)
Lachnospiraceae(.95)_Marvinbryantia(.54)
Lachnospiraceae(1)_Murimonas(.29)
Lachnospiraceae(.44)_Clostridium XlVa(.26)
Clostridiales Incertae Sedis XIII(.31)_Anaerovorax(.16)
Peptostreptococcaceae(.25)_Lachnospiracea incertae sedis(.19)
Staphylococcaceae(1)_Staphylococcus(1)
Bacillaceae(.99)_Bacillus(.99)
Pseudomonadaceae(1)_Pseudomonas(1)
FIRMICUTES
PROTEOBACTERIA
relative abundance (log10)
-5 -4 -3 -2 -1
fold enrichment (log10)
0 1 2 3

Supplementary Figure 8

Identification of FACS-enriched in situ transconjugants by 16S sequencing.

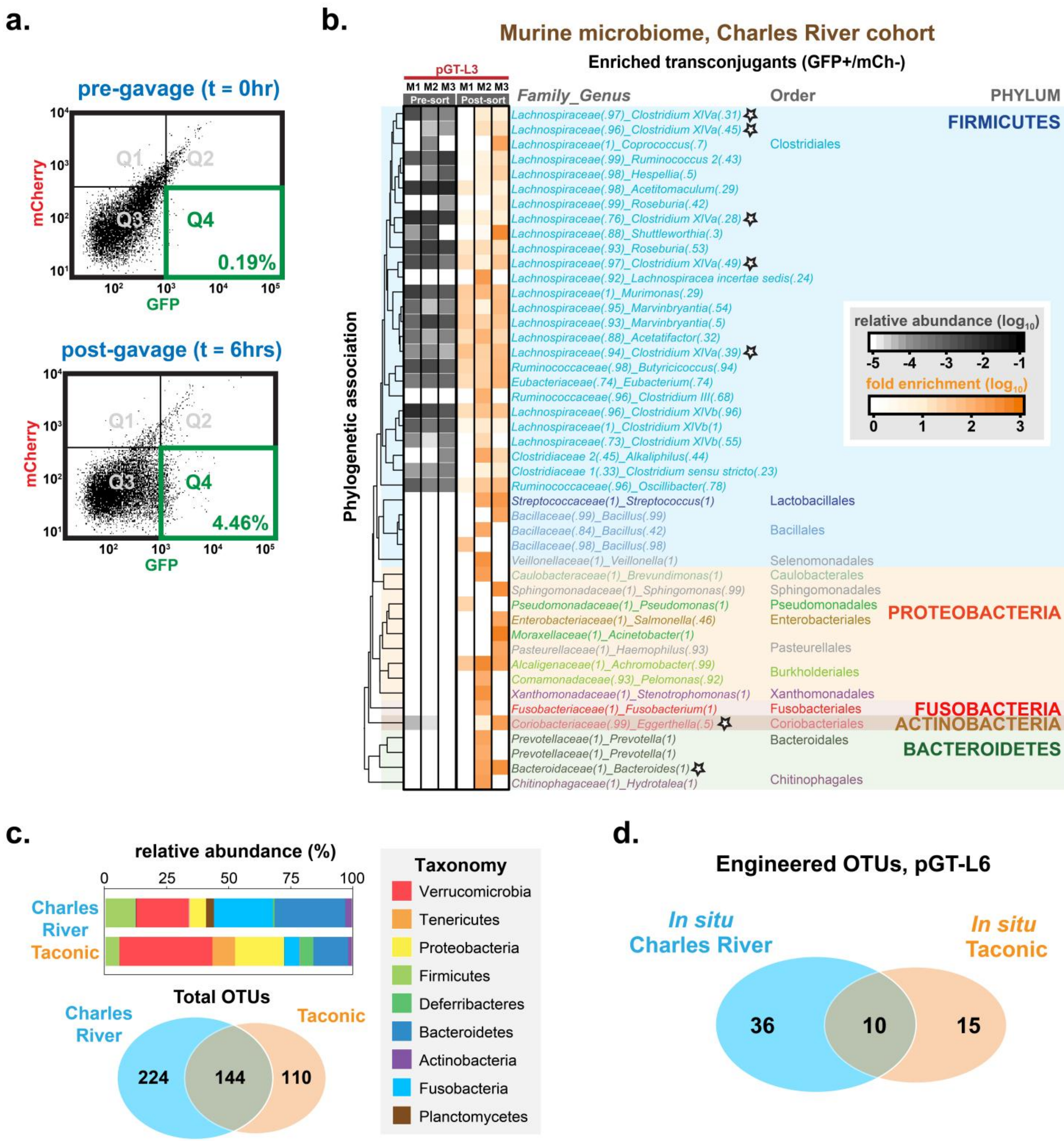
(a) Implementation of MAGIC in a mouse model with fecal bacterial analysis by FACS, antibiotic selection, and sequencing. **(b)** FACS dot plots of in situ conjugations using EcGT2 donors with vector libraries pGT-L1, L2, and L3. Green boxes define the sorted $GFP^{+}mCherry^{-}$ transconjugant populations. Each plot shows fluorescence expression of bacteria from the combined fecal samples of 3 cohoused mice. The experiment was run 3 independent times, with similar results. **(c)** 16S taxonomic classification of FACS-enriched transconjugants from in situ mouse experiments using vector libraries pGT-L1, L2, and L3. Relative abundance of each OTU in the unsorted population is shown in the grayscale heat map, and fold enrichment for transconjugants of each OTU is shown in the orange heat map, with annotated taxonomic identities. Bracketed values indicate confidence of taxonomic assignment by RDP Classifier. Each column represents data from a separately housed cohort of 3 mice whose fecal samples were combined for analysis. Genera with successfully cultivated isolates are denoted by stars. **(d)** The pGT-L3 transconjugant population from (b) was further analyzed by comparison of Q4 enriched OTUs against Q3 OTUs, which represent a sample of the GFP^{-} native bacteria population, and by enrichment analysis of Q4 samples that were sorted again for Q4. Enriched GFP^{+} transconjugants were robust whether compared against the total fecal population or against Q3. 7 out of 11 OTUs enriched in Q4 were present in the double-sorted Q4 population, indicating that Q4 sorting is robust. The OTUs lost upon double-sorting were obligate anaerobes and likely sensitive to prolonged aerobic conditions during double-sorting.



Supplementary Figure 9

Identification of FACS-enriched in situ transconjugants of multi-vector libraries.

(a) Flow cytometric quantification of in situ transconjugants in the total bacterial population after gavage of EcGT2 donors containing pGT-L4 (green; *n* = 4 mice) or pGT-L5 (blue; *n* = 4 mice) vector libraries. Control groups gavaged with PBS (black; *n* = 2 mice) or donors containing a nontransferable pGT-NT vector (red; *n* = 2 mice) produced no detectable transconjugants. Black bars indicate means. **(b)** Longitudinal analysis of mouse fecal microbiome by flow cytometry for presence of transconjugants after gavage of EcGT2 donors containing pGT-L4 (green; *n* = 6 mice) or pGT-L5 (blue; *n* = 6 mice). Donor cells of these libraries (orange; *n* = 12 mice) were lost within 48 h, whereas transconjugants were observed up to 72 h post-gavage. The dotted line indicates the detection limit of flow cytometry. Error bars indicate s.d. **(c)** 16S taxonomic classification of transconjugants (GFP^+mCh^-) enriched by FACS of pGT-L4 and pGT-L5 recipient groups. Relative abundance of each OTU in the unsorted population is shown in the grayscale heat map on the left, and fold enrichment for transconjugants of each OTU is shown in the orange heat map on the right, with annotated taxonomic identities. Bracketed values indicate confidence of taxonomic assignment by RDP Classifier. Each column represents data from 6 mice from 2 independent cohorts whose fecal samples were combined for analysis. Genera with successfully cultivated isolates are denoted by stars.

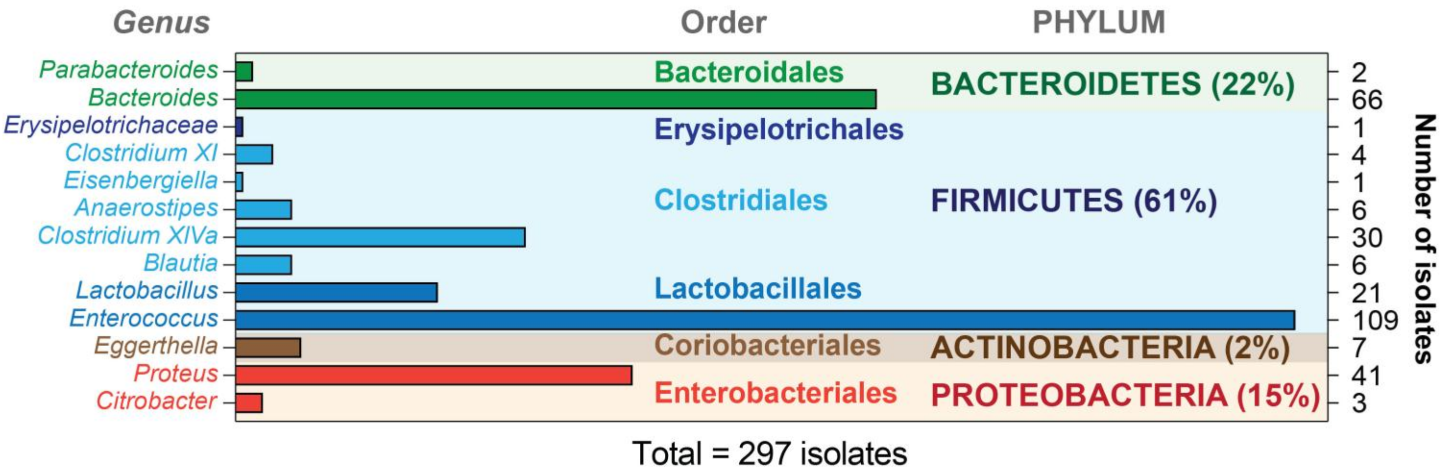


Supplementary Figure 10

Identification of FACS-enriched in situ transconjugants in mice from a different commercial vendor.

(a) FACS dot plots of in situ conjugations using EcGT2 pGT-L3 donors in a cohort of mice from a different vendor (Charles River Laboratories). Green boxes define the sorted $GFP^{+}mCherry^{-}$ transconjugant populations. Flow cytometry was performed 3 times, on fecal samples from individual cohoused mice, with similar results. **(b)** 16S taxonomic classification of FACS-enriched $GFP^{+}mCherry^{-}$

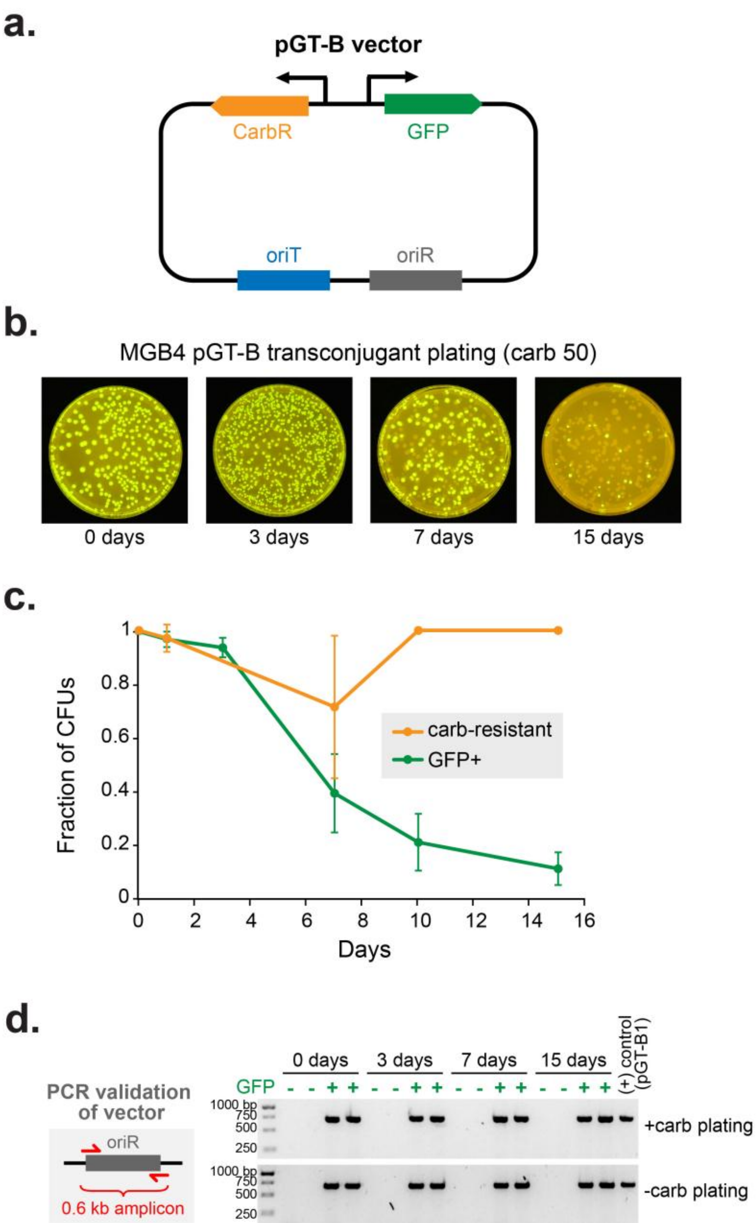
transconjugants of pGT-L3. Relative abundance of each OTU in the unsorted population is shown in the grayscale heat map, and fold enrichment for transconjugants of each OTU is shown in the orange heat map, with annotated taxonomic identities. Bracketed values indicate confidence of taxonomic assignment by RDP Classifier. Each column represents bacteria from one mouse. Genera with successfully cultivated isolates are denoted by stars. **(c)** Metagenomic 16S rRNA sequencing of mouse fecal samples shows that mice from different vendors have divergent gut microbiomes, with some shared OTUs. **(d)** In in situ experiments using the same vector library (pGT-L6) in cohorts of 3 mice each from different vendors, 10 transconjugant OTUs were shared between cohorts.



Supplementary Figure 11

PCR-validated transconjugant isolates from in situ mouse experiments.

297 PCR-validated isolates from in situ experiments using vector libraries pGT-L3 and pGT-L6 were identified by 16S Sanger sequencing and assigned to a genus using RDP Classifier with assignment confidence > 0.89.



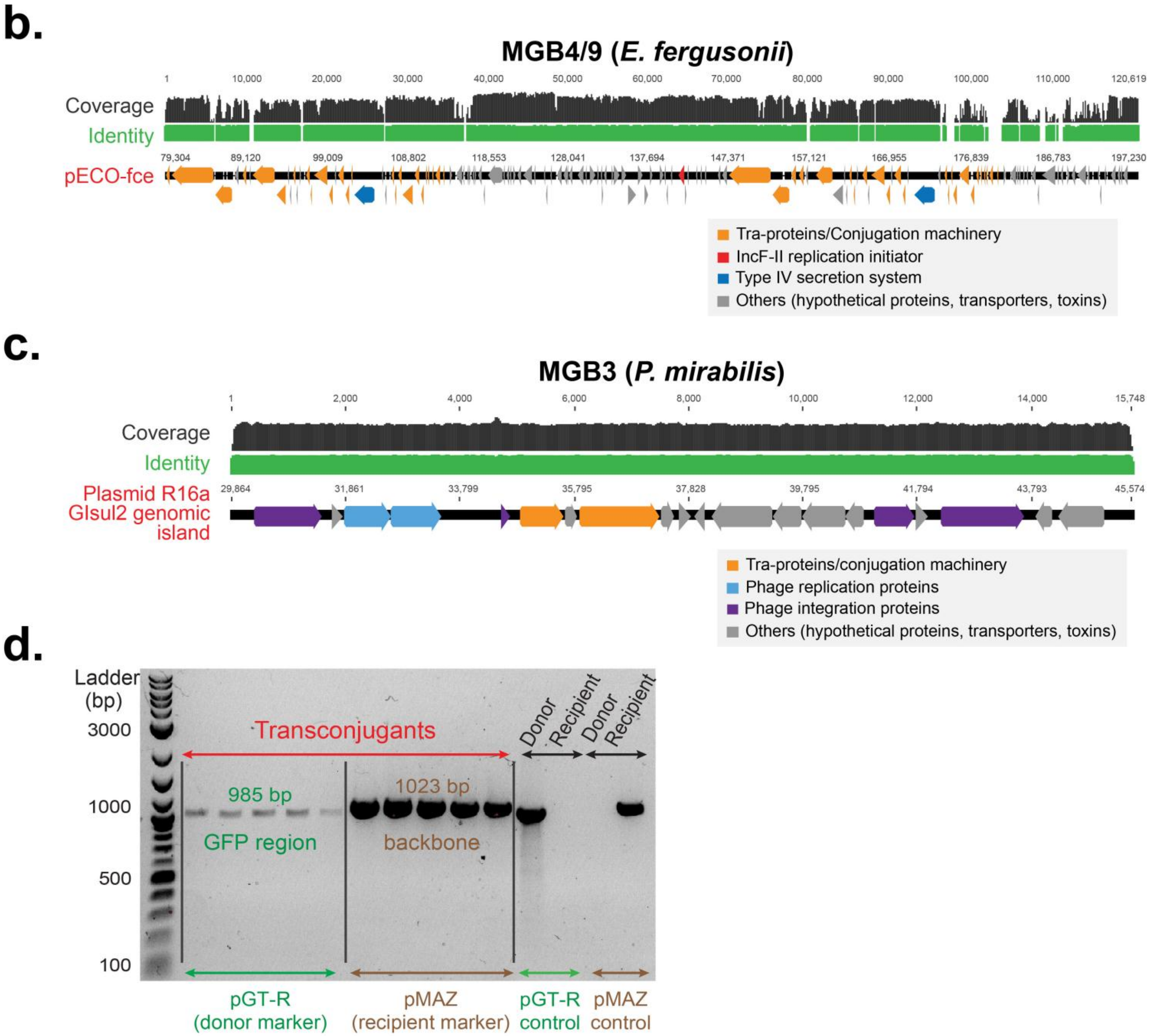
Supplementary Figure 12

Comparison of vector and payload stability in two transconjugant isolates.

(a) Vector map of pGT-B1. GFP and β-lactamase genes are expressed from separate promoters on a replicative pBBR1 origin plasmid. **(b)** MGB4, an *Escherichia fergusonii* isolate containing pGT-B1, lost GFP expression over time when serially passaged without selection for 15 d. Plating was performed for 3 independent serial passages. **(c)** Quantification of carb-resistant and GFP^+ CFUs of MGB4 over time; all CFUs remained carb-resistant as the population lost GFP expression. Center values are the means of 3 serial passages; error bars represent s.d. **(d)** Colony PCR for the pGT-B1 backbone showed that the plasmid was absent in GFP^- CFUs at all time points surveyed. Each lane shows the PCR product for one colony. This PCR was performed once. **(e)** Vector map of pGT-Ah1, which contains GFP and β-lactamase genes on a transposable cassette. The plasmid backbone contains a chloramphenicol resistance gene for selection. **(f)** MGB9, an *Escherichia fergusonii* isolate containing pGT-Ah1, remained 100% GFP^+ during serial passaging without selection over 11 d. Plating was performed for 3 independent serial passages. **(g)** Over time the proportion of MGB9 CFUs expressing the genes on the transposable cassette (GFP^+ and carb-resistant) remained at 100%, whereas the chloramphenicol resistance conferred by the pGT-Ah1 backbone was lost in some of the population. Center values are the means of 3 serial passages; error bars represent s.d.

a.

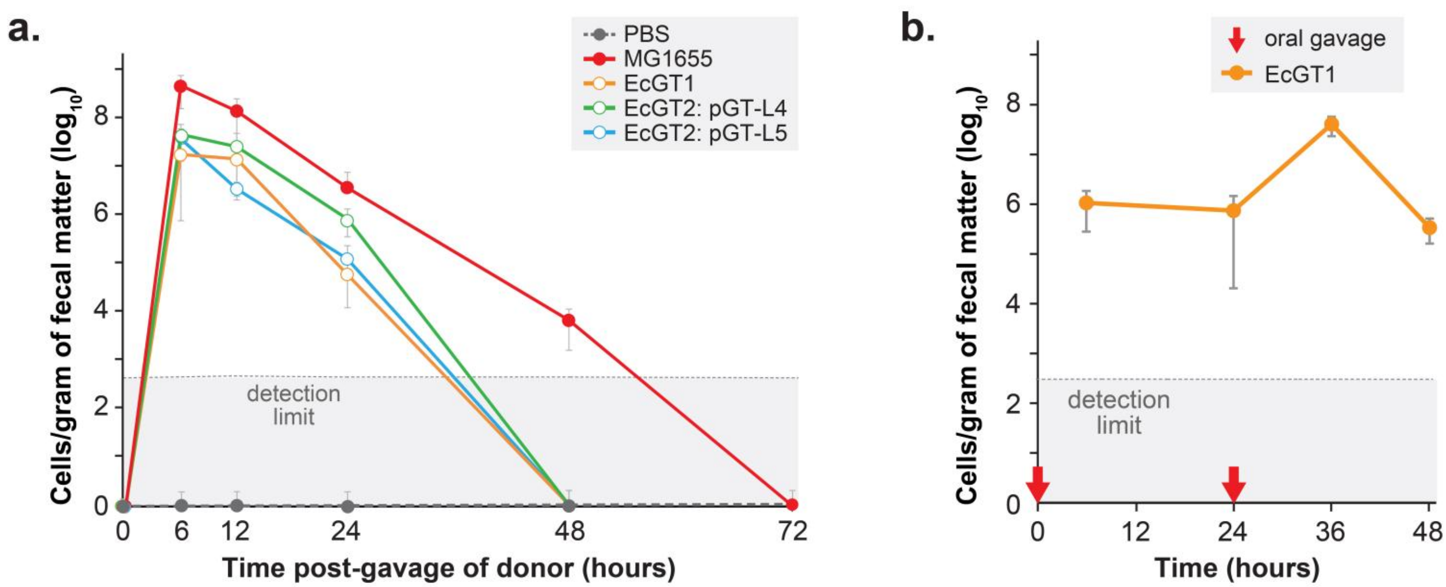
Isolate	Strain	Vector	N50 (bp)	# of contigs	Total length (bp)
MGB3	*P. mirabilis*	pGT-Ah1	153,603	67	3,843,607
MGB9	*E. fergusonii*	pGT-Ah1	92,709	153	4,938,839
MGB4	*E. fergusonii*	pGT-B1	103,260	148	4,917,568



Supplementary Figure 13

Characterization of 3 modifiable gut bacteria (MGB) strains by whole-genome sequencing and in vitro conjugation.

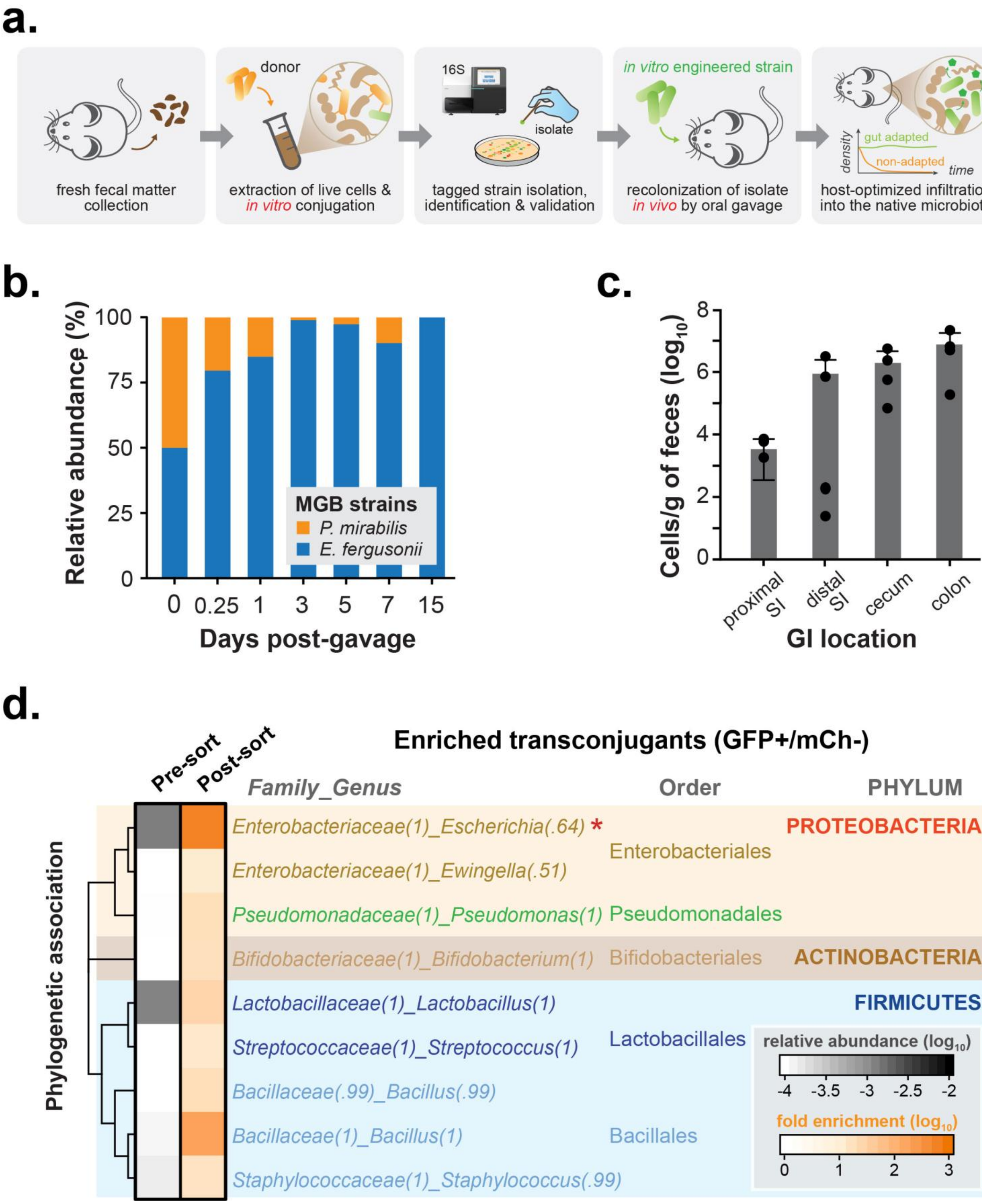
(a) Three distinct MGB strains, isolated from in vitro conjugations between *E. coli* pGT donors and mouse fecal bacteria, were analyzed by whole-genome sequencing. MGB4 and MGB9 appear to be the same strain isolated from separate experiments with different pGT vectors transferred. Sequencing of **(b)** MGB4/9 and **(c)** MGB3 revealed the presence of genes involved in conjugation and genetic transfer. However, only MGB4/9 strains that shared homology with the pECO-fce plasmid were observed to transfer their pGT vectors to *E. coli* during in vitro conjugations. **(d)** PCR confirmation of pGT vector transfer from MGB4 to an *E. coli* recipient following in vitro conjugation. The conjugation was performed 3 times, with similar results; 5 individual transconjugants were assessed by colony PCR.



Supplementary Figure 14

Longevity of donor *E. coli* strains in the mouse gut after oral gavage.

(a) In vivo gut colonization profiles of MAGIC donors EcGT1 (S17, *galK::mCherry),* EcGT2 (S17, *asd::mCherry*), and control *E. coli* MG1655 in C57BL/6 mice measured by flow cytometry of fecal bacteria after a single gavage of 10^9 cells. Mean values were calculated using feces from 2 gavaged mice; error bars indicate s.d. **(b)** Two orally gavaged doses of 10^9 EcGT1 cells resulted in a longer persistence of this donor in the gut. Mean values were calculated using feces from 2 gavaged mice; error bars indicate s.e.m.


a.
donor
16S
isolate
in vitro engineered strain
gut adapted
non-adapted
density
time
fresh fecal matter collection
extraction of live cells & in vitro conjugation
tagged strain isolation, identification & validation
recolonization of isolate in vivo by oral gavage
host-optimized infiltration into the native microbiota
b.
Relative abundance (%)
100
75
50
25
0
0
0.25
1
3
5
7
15
Days post-gavage
MGB strains
P. mirabilis
E. fergusonii
c.
Cells/g of feces (log10)
8
6
4
2
0
proximal SI
distal SI
cecum
colon
GI location
d.
Enriched transconjugants (GFP+/mCh-)
Pre-sort
Post-sort
Family_Genus
Order
PHYLUM
Phylogenetic association
Enterobacteriaceae(1)_Escherichia(.64) *
Enterobacteriaceae(1)_Ewingella(.51)
Enterobacteriales
PROTEOBACTERIA
Pseudomonadaceae(1)_Pseudomonas(1)
Pseudomonadales
Bifidobacteriaceae(1)_Bifidobacterium(1)
Bifidobacteriales
ACTINOBACTERIA
Lactobacillaceae(1)_Lactobacillus(1)
FIRMICUTES
Streptococcaceae(1)_Streptococcus(1)
Lactobacillales
Bacillaceae(.99)_Bacillus(.99)
Bacillaceae(1)_Bacillus(1)
Bacillales
Staphylococcaceae(1)_Staphylococcus(.99)
relative abundance (log10)
-4
-3.5
-3
-2.5
-2
fold enrichment (log10)
0
1
2
3

Supplementary Figure 15

Characterization of MGB recolonization of the mouse gut.

(a) Schematic diagram of experiment: genetically tractable gut microbiota were isolated from the mouse microbiome in vitro and then orally gavaged to recolonize the gut. **(b)** MGB3, MGB4, and MGB9 strains orally gavaged into mice (n = 4) as a mixture recolonized the GI tract without any antibiotic treatment. MGBs were detectable in fecal samples for at least 15 d post-gavage. **(c)** MGB strains (namely, MGB4) were present in all sampled locations along the GI tract when the mice (n = 4) were euthanized 15 d post-gavage. Error bars represent s.d. **(d)** Phylogenetic tree of FACS-sorted $GFP^{+}mCherry^{-}$ transconjugants in fecal samples from mice after 11 d post-gavage of MGB strains. Fecal samples from 4 mice were combined for analysis. Relative abundance of each OTU in the unsorted population is shown in the grayscale heat map, and fold enrichment for transconjugants of each OTU is shown in the orange heat map. Bracketed values indicate confidence of taxonomic assignment by RDP Classifier. The red asterisk denotes the *Escherichia/Shigella* OTU that shares a genus with the MGB4/9 donors.

Supplementary Table 1. List of vectors and vector components.

Origins of replication (oriR):

Origin	Copy #	Host range	Code
R6K[1]	10-20	Narrow (Proteobacteria)	**K**
p15A[2]	14-16	Narrow (Enterobacteria)	**A**
oriV[3]	4-7	Broad (Gram- and Gram+)	**V**
pBBR1[4]	15-40	Broad (preferably Gram-)	**B**
RSF1010[5]	12	Broad (Gram- and Gram+)	**S**
RCR[6]	250-350	Broad (Eubacteria)	**W**

Integrative elements:

Transposase	Transposon inverted repeat sequence	Host range	Code
none	-	-	-
Himar[7]	ACAGGTTGGATGATAAGTCCCCGGTCT	Broad	**h**
Tn5	CTGTCTCTTATACACATCT	Broad	**t**

Regulation sequences:

Promoter/UTR	Expression in E. coli	Origin of sequence	Code
GATTGCATTAGGTTTTAGTTTCTTGTATAATGCTTAA TGTTGGTCACTGACAGGCTACGATACGGAAGGTTGCT CACGCCCGGCCCCTTTGCCATGGCTAGTGTGTGGAAA TTTCCGAGGAGCAAGTCTATTTCCAAAAATGGGCGAA AAAGGAGGTAATACA	+++	*Bacillus cellulosilyticus*	**1**
GGGAGAGCTTCAACGGCGCTTCTACCCATTTGCTTGG AAAGGATGAGGAGCAGGAAGAAATTCCGTCCCCAATG CGACGGCCCTTTACATCCATGTTGTTTGATAGTATAA TGGATACGGATTGACCAAATTGTTCATTTAGTCAGTT TGAAGGATGAGGAGT	++	*Geobacillus sp.*	**2**
GTGAAGGATACGGCTGCGGCACTTCGACATCGCCCCA TGTGGCGGCTTTGAACTGGGCTTATGAAACGCGTTCA CAACCTTTTTTGACCATCGGCGCGAACGTGGTATCAT GCGTTCAGCTTTTGCCCATACATACTACGTGCTCAAT CTAGGAGGATTTCATAC	+++	*Eggerthella lenta*	**3**
CTCTAGAGTAGTAGATTATTTTAGGAATTTAGATGTT TTGTATGAAATAGATGCTTCGTATGGAATTAATGAAA TTTTTAGTCAGGTAAAAAAGGTAATAGGAGAATATT	+++	*Segmented filamentous bacteria*	**4**

GTTTTAAATGATGAAAAGAAATATTTAGGGAAGATTG TTTCGACGCGAATTGTTGATCTGGAAAATGATCACCT TATCGGACAAGCTTTAAAATAGGAGGATATAAAAAT	++	*Segmented filamentous bacteria*	**5**
ATAAGGATTCTTTAAAGAGAGATATAGTTATGTCAAA GACTGTAGAATTTTTAGTAAATCAAAATAAAAAAAGA GGTATTAAATAGAGTGTATTTTAAAGGAGGAGACTT	+++	*Segmented filamentous bacteria*	**6**
AAACACCAATAAAATTAGAATATTTAGGAGCGACTTT AAAAAAGTTTAATAAGAATTGTTTATGAGATATTTTT ATTATATTTAAACTCAATTTAAAGTAGGGAGAATAG	+	*Segmented filamentous bacteria*	**7**
GCAAGTGTTCAAGAAGTTATTAAGTCGGGAGTGCAGT CGAAGTGGGCAAGTTGAAAAATTCACAAAAATGTGGT ATAATATCTTTGTTCATTAGAGCGATAAACTTGAATT TGAGAGGGAACTTAG	+	*Clostridium perfringens*	**8**

Vector selection genes:

Resistance gene	Antibiotic selection	[Ab] in *E. coli*
Beta-lactamase	Carbenicillin	50 µg/ml
Chlor	Chloramphenicol	20 µg/ml
Tet	Tet	25 µg/ml
Spec	Spec	250 µg/ml
Kan	Kan	50 µg/ml

Cargo selection cassettes:

Resistance cassette	Antibiotic selection	[Ab] in *E. coli*
GFP-Beta-lactamase	Carb	50 µg/ml
GFP-CatP	Chlor	20 µg/ml
GFP-Tet	Tet	25 µg/ml
GFP-Spec	Spec	250 µg/ml
GFP-Kan	Kan	50 µg/ml
GFP-ErmG	Erm	-

Vector name	Cargo selection	Cargo promoter	Vector selection	Transposase promoter
pGT-Ah1	GFP-Beta-lactamase	4	Chlor	4
pGT-Ah2	GFP-Beta-lactamase	5	Chlor	5
pGT-Ah3	GFP-Beta-lactamase	6	Chlor	6
pGT-Ah4	GFP-Beta-lactamase	7	Chlor	7
pGT-Ah5	GFP-CatP	8	Kan	4
pGT-Ah6	GFP-CatP	8	Kan	5
pGT-Ah7	GFP-CatP	8	Kan	6
pGT-Ah8	GFP-CatP	8	Kan	7
pGT-Ah9	GFP-Tet	4	Chlor	4
pGT-Ah10	GFP-Tet	4	Chlor	5
pGT-Ah11	GFP-Tet	4	Chlor	6
pGT-B1	GFP	1	Beta-lactamase	-
pGT-B2	GFP	2	Beta-lactamase	-
pGT-B3	GFP	3	Beta-lactamase	-
pGT-S1	GFP-Beta-lactamase	4	Beta-lactamase	-
pGT-S2	GFP-Beta-lactamase	5	Beta-lactamase	-
pGT-S3	GFP-Tet	4	Tet	-
pGT-S4	GFP-Tet	5	Tet	-
pGT-Kh1	GFP-Beta-lactamase	4	Chlor	4
pGT-Kh2	GFP-Beta-lactamase	5	Chlor	5
pGT-Kh3	GFP-Beta-lactamase	7	Chlor	7

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Supplementary Table 2. Vector libraries used in this study.

Library	Vectors
pGT-L1	B1, B2, B3
pGT-L2	Ah5, Ah6, Ah7, Ah8
pGT-L3	S1, S2, S3, S4
pGT-L4	Ah1, Ah3, B1, B2, B3
pGT-L5	Ah5, Ah6, Ah7, Ah8, Ah9, Ah10, Ah11
pGT-L6	Ah1, Ah3, Ah5, Ah6, Ah7, Ah8, Ah9, Ah10, Ah11, B1, B2, B3
pGT-L7	Ah1, Ah2, Ah3, Ah4
pGT-L8	Kh1, Kh2, Kh3

Supplementary Table 3: Full sequences of pGT vector parts

Cargo selection genes	Sequence	Notes
Beta-lactamase (carbenicillin/ampicillin resistance)	ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTG GGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGC GCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGC ATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAAC ATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAA CTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTT ATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCA ACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAA	
CatP (chloramphenicol resistance)	ATGGTATTTGAAAAAATTGATAAAAATAGTTGGAACAGAAAAGAGTATTTTGACCACTACTTTGCAAGTGTACCTTGTACATACAGCATGACCGTTAAAGTGGATATCACACAA ATAAAGGAAAAGGGAATGAAACTATATCCTGCAATGCTTTATTATATTGCAATGATTGTAAACCGCCATTCAGAGTTTAGGACGGCAATCAATCAAGATGGTGAATTGGGGATA TATGATGAGATGATACCAAGCTATACAATATTTCACAATGATACTGAAACATTTTCCAGCCTTTGGACTGAGTGTAAGTCTGACTTTAAATCATTTTTAGCAGATTATGAAAGT GATACGCAACGGTATGGAAACAATCATAGAATGGAAGGAAAGCCAAATGCTCCGGAAAACATTTTTAATGTATCTATGATACCGTGGTCAACCTTCGATGGCTTTAATCTGAAT TTGCAGAAAGGATATGATTATTTGATTCCTATTTTTACTATGGGGAAATATTATAAAGAAGATAACAAAATTATACTTCCTTTGGCAATTCAAGTTCATCACGCAGTATGTGAC GGATTTCACATTTGCCGTTTTGTAAACGAATTGCAGGAATTGATAAATAGTTAA	Derived from pJIR750 plasmid from *Clostridium perfringens*
Tet (tetracycline resistance)	ATGAATATTATAAATTTAGGAATTCTTGCTCACATTGATGCAGGAAAAACTTCCGTAACCGAGAATCTGCTGTTTGCCAGTGGAGCAACGGAAAAGTGCGGCTGTGTGGATAAT GGTGACACCATAACGGACTCTATGGATATAGAGAAACGTAGAGGAATTACTGTTCGGGCTTCTACGACATCTATTATCTGGAATGGTGTGAAATGCAATATCATTGACACTCCG GGACACATGGATTTTATTGCGGAAGTGGAGCGGACATTCAAAATGCTTGATGGAGCAGTCCTCATCTTATCCGCAAAGGAAGGCATACAAGCGCAGACAAAGTTGCTGTTCAAT ACTTTACAGAAGCTGCAAATCCCGACAATTATATTTATCAATAAGATTGACCGAGCCGGTGTGAATTTGGAGCGTTTGTATCTGGATATAAAAGCAAATCTGTCTCAAGATGTC CTGTTTATGCAAAATGTTGTCGATGGATCGGTTTATCCGGTTTGCTCCCAAACATATATAAAGGAAGAATACAAAGAATTTGTATGCAACCATGACGACAATATATTAGAACGA TATTTGGCGGATAGCGAAATTTCACCGGCTGATTATTGGAATACGATAATCGCTCTTGTGGCAAAAGCCAAAGTCTATCCGGTGCTACATGGATCAGCAATGTTCAATATCGGT ATCAATGAGTTGTTGGACGCCATCACTTCTTTTATACTTCCTCCGGCATCGGTCTCAAACAGACTTTCATCTTATCTTTATAAGATAGAGCATGACCCCAAAGGACATAAAAGA AGTTTTCTAAAAATAATTGACGGAAGTCTGAGACTTCGAGACGTTGTAAGAATCAACGATTCGGAAAAATTCATCAAGATTAAAAATCTAAAAACTATCAATCAGGGCAGAGAG ATAAATGTTGATGAAGTGGGCGCCAATGATATCGCGATTGTAGAGGATATGGATGATTTTCGAATCGGAAATTATTTAGGTGCTGAACCTTGTTTGATTCAAGGATTATCGCAT CAGCATCCCGCTCTCAAATCCTCCGTCCGGCCAGACAGGCCCGAAGAGAGAAGCAAGGTGATATCCGCTCTGAATACATTGTGGATTGAAGACCCGTCTTTGTCCTTTTCCATA AACTCATATAGTGATGAATTGGAAATCTCGTTATATGGTTTAACCCAAAAGGAAATCATACAGACATTGCTGGAAGAACGATTTTCCGTAAAGGTCCATTTTGATGAGATCAAG ACTATATACAAAGAACGACCTGTAAAAAAGGTCAATAAGATTATTCAGATCGAAGTGCCGCCCAACCCTTATTGGGCCACAATAGGGCTGACTCTTGAACCCTTACCGTTAGGG ACAGGGTTGCAAATCGAAAGTGACATCTCCTATGGTTATCTGAACCATTCTTTTCAAAATGCCGTTTTTGAAGGGATTCGTATGTCTTGCCAATCCGGGTTACATGGATGGGAA GTGACTGATCTGAAAGTAACTTTTACTCAAGCCGAGTATTATAGCCCGGTAAGTACACCTGCTGATTTCAGACAGCTGACCCCTTATGTCTTCAGGCTGGCCTTGCAACAGTCA GGTGTGGACATTCTCGAACCGATGCTCTATTTTGAGTTGCAGATACCCCAAGCGGCAAGTTCCAAAGCTATTACAGATTTGCAAAAAATGATGTCTGAGATTGAAGACATCAGT TGCAATAATGAGTGGTGTCATATTAAAGGGAAAGTTCCATTAAATACAAGTAAAGACTATGCATCAGAAGTAAGTTCATACACTAAGGGCTTAGGCATTTTTATGGTTAAGCCA TGCGGGTATCAAATAACAAAAGGCGGTTATTCTGATAATATCCGCATGAACGAAAAAGATAAACTTTTATTCATGTTCCAAAAATCAATGTCATCAAAATAA	
Spec (spectinomycin resistance)	ATGCGCTCACGCAACTGGTCCAGAACCTTGACCGAACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTCATGGCTTGTTATGACTGTTTTTTTGGGGTACAGTCTATGCCTCGG GCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTCGATGTTTGATGTTATGGAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAAACAAAGTTAAACATCATGAGGGAA GCGGTGATCGCCGAAGTATCGACTCAACTATCAGAGGTAGTTGGCGTCATCGAGCGCCATCTCGAACCGACGTTGCTGGCCGTACATTTGTACGGCTCCGCAGTGGATGGCGGC CTGAAGCCACACAGTGATATTGATTTGCTGGTTACGGTGACCGTAAGGCTTGATGAAACAACGCGGCGAGCTTTGATCAACGACCTTTTGGAAACTTCGGCTTCCCCTGGAGAG AGCGAGATTCTCCGCGCTGTAGAAGTCACCATTGTTGTGCACGACGACATCATTCCGTGGCGTTATCCAGCTAAGCGCGAACTGCAATTTGGAGAATGGCAGCGCAATGACATT CTTGCAGGTATCTTCGAGCCAGCCACGATCGACATTGATCTGGCTATCTTGCTGACAAAAGCAAGAGAACATAGCGTTGCCTTGGTAGGTCCAGCGGCGGAGGAACTCTTTGAT CCGGTTCCTGAACAGGATCTATTTGAGGCGCTAAATGAAACCTTAACGCTATGGAACTCGCCGCCCGACTGGGCTGGCGATGAGCGAAATGTAGTGCTTACGTTGTCCCGCATT TGGTACAGCGCAGTAACCGGCAAAATCGCGCCGAAGGATGTCGCTGCCGACTGGGCAATGGAGCGCCTGCCGGCCCAGTATCAGCCCGTCATACTTGAAGCTAGACAGGCTTAT CTTGGACAAGAAGAAGATCGCTTGGCCTCGCGCGCAGATCAGTTGGAAGAATTTGTCCACTACGTGAAAGGCGAGATCACCAAGGTAGTCGGCAAATAA	
Kan (kanamycin resistance)	ATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATC TATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTT ATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCA GGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCA CGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTCA GTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCC ATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGAT GAGTTTTTCTAA	

ermG (erythromycin resistance)	ATGAACAAAGTAAATATAAAAGATAGTCAAAATTTTATTACTTCAAAATATCACATAGAAAAAATAATGAATTGCATAAGTTTAGATGAAAAAGATAACATCTTTGAAATAGGT GCAGGGAAAGGTCATTTTACTGCTGGATTGGTAAAGAGATGTAATTTTGTAACGGCGATAGAAATTGATTCTAAATTATGTGAGGTAACTCGTAATAAGCTCTTAAATTATCCT AACTATCAAATAGTAAATGATGATATACTGAAATTTACATTTCCTAGCCACAATCCATATAAAATATTTGGCAGCATACCTTACAACATAAGCACAAATATAATTCGAAAAATT GTTTTTGAAAGTTCAGCCACAATAAGTTATTTAATAGTGGAATATGGTTTTGCTAAAATGTTATTAGATACAAACAGATCACTAGCATTGCTGTTAATGGCAGAGGTAGATATT TCTATATTAGCAAAAATTCCTAGGTATTATTTCCATCCAAAACCTAAAGTGGATAGCACATTAATTGTATTAAAAAGAAAGCCAGCAAAAATGGCATTTAAAGAGAGAAAAAAA TATGAAACTTTTGTAATGAAATGGGTTAACAAAGAGTACGAAAAACTGTTTACAAAAAATCAATTTAATAAAGCTTTAAAACATGCGAGAATATATGATATAAACAATATTAGT TTCGAACAATTTGTATCGCTATTTAATAGTTATAAAATATTTAACGGCTAA	
sfGFP	ATGCGTAAAGGCGAAGAGCTGTTCACTGGTTTCGTCACTATTCTGGTGGAACTGGATGGTGATGTCAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGGTGACGCAACT AATGGTAAACTGACGCTGAAGTTCATCTGTACTACTGGTAAACTGCCGGTACCTTGGCCGACTCTGGTAACGACGCTGACTTATGGTGTTCAGTGCTTTGCTCGTTATCCGGAC CACATGAAGCAGCATGACTTCTTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCACGATTTCCTTTAAGGATGACGGCACGTACAAAACGCGTGCGGAAGTGAAATTT GAAGGCGATACCCTGGTAAACCGCATTGAGCTGAAAGGCATTGACTTTAAAGAAGACGGCAATATCCTGGGCCATAAGCTGGAATACAATTTTAACAGCCACAATGTTTACATC ACCGCCGATAAACAAAAAAATGGCATTAAAGCGAATTTTAAAATTCGCCACAACGTGGAGGATGGCAGCGTGCAGCTGGCTGATCACTACCAGCAAAACACTCCAATCGGTGAT GGTCCTGTTCTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGATCCGAACGAGAAACGCGATCACATGGTTCTGCTGGAGTTCGTAACCGCAGCGGGC ATCACGCATGGTATGGATGAACTGTACAAATAA	

<u>Vector selection genes</u>	**Sequence**	**Notes**
Chlor (chloramphenicol resistance)	ATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAG CTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTACGTATGGCA ATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGG CAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGT TTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCAG GTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAA	All vector selection markers not listed here are the same as the ones in the "Cargo selection genes" section.

<u>Origins of</u>	**Sequence**	**Notes**
R6K origin of replication	ATCCCTGGCTTGTTGTCCACAACCGTTAAACCTTAAAAGCTTTAAAAGCCTTATATATTCTTTTTTTTCTTATAAAACTTAAAACCTTAGAGGCTATTTAAGTTGCTGATTTAT ATTAATTTTATTGTTCAAACATGAGAGCTTAGTACGTGAAACATGAGAGCTTAGTACGTTAGCCATGAGAGCTTAGTACGTTAGCCATGAGGGTTTAGTTCGTTAAACATGAGA GCTTAGTACGTTAAACATGAGAGCTTAGTACGTGAAACATGAGAGCTTAGTACGTACTATCAACAGGTTGAACTGCTGATCTTC	Requires additional pir gene for replication
p15A origin of replication	AACAACTTATATCGTATGGGGCTGACTTCAGGTGCTACATTTGAAGAGATAAATTGCACTGAAATCTAGAAATATTTTATCTGATTAATAAGATGATCTTCTTGAGATCGTTTT GGTCTGCGCGTAATCTCTTGCTCTGAAAACGAAAAAACCGCCTTGCAGGGCGGTTTTTCGAAGGTTCTCTGAGCTACCAACTCTTTGAACCGAGGTAACTGGCTTGGAGGAGCG CAGTCACCAAAACTTGTCCTTTCAGTTTAGCCTTAACCGGCGCATGACTTCAAGACTAACTCCTCTAAATCAATTACCAGTGGCTGCTGCCAGTGGTGCTTTTGCATGTCTTTC CGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGACTGAACGGGGGGTTCGTGCATACAGTCCAGCTTGGAGCGAACTGCCTACCCGGAACTGAGTGTCAG GCGTGGAATGAGACAAACGCGGCCATAACAGCGGAATGACACCGGTAAACCGAAAGGCAGGAACAGGAGAGCGCACGAGGGAGCCGCCAGGGGGAAACGCCTGGTATCTTTATA GTCCTGTCGGGTTTCGCCACCACTGATTTGAGCGTCAGATTTCGTGATGCTTGTCAGGGGGGCGGAGCCTATGGAAAAACGGCTTTGCCGCGGCCCTCTCACTTCCCTGTTAAG TATCTTCCTGGCATCTTCCAGGAAATCTCCGCCCCGTTCGTAAGCCATTTCCGCTCGCCGCAGTCGAACGACCGAGCGTAGCGAGTCAGTGAGCGAGGAAGCGGAATATATCC	
oriV	AGCGGGCCGGGAGGGTTCGAGAAGGGGGGGCACCCCCCTTCGGCGTGCGCGGTCACGCGCCAGGGCGCAGCCCTGGTTAAAAACAAGGTTTATAAATATTGGTTTAAAAGCAGG TTAAAAGACAGGTTAGCGGTGGCCGAAAAACGGGCGGAAACCCTTGCAAATGCTGGATTTTCTGCCTGTGGACAGCCCCTCAAATGTCAATAGGTGCGCCCCTCATCTGTCATC ACTCTGCCCCTCAAGTGTCAAGGATCGCGCCCCTCATCTGTCAGTAGTCGCGCCCCTCAAGTGTCAATACCGCAGGGCACTTATCCCCAGGCTTGTCCACATCATCTGTGGGAA ACTCGCGTAAAATCAGGCGTTTTCGCCGATTTGCGAGGCTGGCCAGCTCCACGTCGCCGGCCGAAATCGAGCCTGCCCCTCATCTGTCAACGCCGCGCCGGGTGAGTCGGCCCC TCAAGTGTCAACGTCCGCCCCTCATCTGTCAGTGAGGGCCAAGTTTTCCGCGTGGTATCCACAACGCCGGCGGCCGCGGTGTCTCGCACACGGCTTCGACGGCGTTTCTGGCGC GTTTGCAGGGCCATAGACGGCCGCCAGCCCAGCGGCGAGGGCAACCAGCCCGGTGAGCGTCGGAAAGGCGCTGGAAGCCCCGTAGCGACGCGGAGAGGGGCGAGACAAGCCAAG GGCGCAGGCTCGATGCGCAGCACGACATAGCCGGTTCTCGCAAGGACGAGAATTTCCCTGCGGTGCCCCTCAAGTGTCAATGAAAGTTTCCAACGCGAGCCATTCGCGAGAGCC TTGAGTCCACGCTAGATCTATCTCA	Requires trfA protein for replication

pBBR1 origin of replication	CTACGGGCTTGCTCTCCGGGCTTCGCCCTGCGCGGTCGCTGCGCTCCCTTGCCAGCCCGTGGATATGTGGACGATGGCCGCGAGCGGCCACCGGCTGGCTCGCTTCGCTCGGCC CGTGGACAACCCTGCTGGACAAGCTGATGGACAGGCTGCGCCTGCCCACGAGCTTGACCACAGGGATTGCCCACCGGCTACCCAGCCTTCGACCACATACCCACCGGCTCCAAC TGCGCGGCCTGCGGCCTTGCCCCATCAATTTTTTTAATTTTCTCTGGGGAAAAGCCTCCGGCCTGCGGCCTGCGCGCTTCGCTTGCCGGTTGGACACCAAGTGGAAGGCGGGTC AAGGCTCGCGCAGCGACCGCGCAGCGGCTTGGCCTTGACGCGCCTGGAACGACCCAAGCCTATGCGAGTGGGGGCAGTCGAAGGCGAAGCCCGCCCGCCTGCCCCCCGAGCCTC ACGGCGGCGAGTGCGGGGGTTCCAAGGGGGCAGCGCCACCTTGGGCAAGGCCGAAGGCCGCGCAGTCGATCAACAAGCCCCGGAGGGGCCACTTTTTGCCGGAGGGGGAGCCGC GCCGAAGGCGTGGGGGAACCCCGCAGGGGTGCCCTTCTTTGGGCACCAAAGAACTAGATATAGGGCGAAATGCGAAAGACTTAAAAATCAACAACTTAAAAAAGGGGGGTACGC AACAGCTCATTGCGGCACCCCCCGCAATAGCTCATTGCGTAGGTTAAAGAAAATCTGTAATTGACTGCCACTTTTACGCAACGCATAATTGTTGTCGCGCTGCCGAAAAGTTGC AGCTGATTGCGCATGGTGCCGCAACCGTGCGGCACCCTACCGCATGGAGATAAGCATGGCCACGCAGTCCAGAGAAATCGGCATTCAAGCCAAGAACAAGCCCGGTCACTGGGT GCAAACGGAACGCAAAGCGCATGAGGCGTGGGCCGGGCTTATTGCGAGGAAACCCACGGCGGCAATGCTGCTGCATCACCTCGTGGCGCAGATGGGCCACCAGAACGCCGTGGT GGTCAGCCAGAAGACACTTTCCAAGCTCATCGGACGTTCTTTGCGGACGGTCCAATACGCAGTCAAGGACTTGGTGGCCGAGCGCTGGATCTCCGTCGTGAAGCTCAACGGCCC CGGCACCGTGTCGGCCTACGTGGTCAATGACCGCGTGGCGTGGGGCCAGCCCCGCGACCAGTTGCGCCTGTCGGTGTTCAGTGCCGCCGTGGTGGTTGATCACGACGACCAGGA CGAATCGCTGTTGGGGCATGGCGACCTGCGCCGCATCCCGACCCTGTATCCGGGCGAGCAGCAACTACCGACCGGCCCCGGCGAGGAGCCGCCCAGCCAGCCCGGCATTCCGGG CATGGAACCAGACCTGCCAGCCTTGACCGAAACGGAGGAATGGGAACGGCGCGGGCAGCAGCGCCTGCCGATGCCCGATGAGCCGTGTTTTCTGGACGATGGCGAGCCGTTGGA GCCGCCGACACGGGTCACGCTGCCGCGCCGGTAG	Includes coding sequence of required replication protein
RSF1010 plasmid backbone	GCTCGACCAGGCGTACGCTTATGGGTGCCTTTCCGCAGCTTGGAACGCGGATGGAGAAGAGGAGCAACGCGATCTAGCTATCGCGGCCGCGATCAAGCAGGTGCGACAGACGTC ATACTAGATATCAAGCGACTTCTCCTATCCCCTGGGAACACATCAATCTCACCGGAGAATATCGCTGGCCAAAGCCTTAGCGTAGGATTCCGCCCCTTCCCGCAAACGACCCCA AACAGGAAACGCAGCTGAAACGGAAGCTCAACACCCACTGACGCATGGGTTGTTCAGGCAGTACTTCATCAACCAGCAAGGCGGCACTTTCGGCCATCCGCCGCGCCCCACAG CTCGGGCAGAAACCGCGACGCTTACAGCTGAAAGCGACCAGGTGCTCGGCGTGGCAAGACTCGCAGCGAACCCGTAGAAAGCCATGCTCCAGCCGCCCGCATTGGAGAAATTCT TCAAATTCCCGTTGCACATAGCCCGGCAATTCCTTTCCCTGCTCTGCCATAAGCGCAGCGAATGCCGGGTAATACTCGTCAACGATCTGATAGAGAAGGGTTTGCTCGGGTCGG TGGCTCTGGTAACGACCAGTATCCCGATCCCGGCTGGCCGTCCTGGCCGCCACATGAGGCATGTTCCGCGTCCTTGCAATACTGTGTTTACATACAGTCTATCGCTTAGCGGAA AGTTCTTTTACCCTCAGCCGAAATGCCTGCCGTTGCTAGACATTGCCAGCCAGTGCCCGTCACTCCCGTACTAACTGTCACGAACCCCTGCAATAACTGTCACGCCCCCCTGCA ATAACTGTCACGAACCCCTGCAATAACTGTCACGCCCCCAAACCTGCAAACCCAGCAGGGGCGGGGGCTGGCGGGGTGTTGGAAAAATCCATCCATGATTATCTAAGAATAATC CACTAGGCGCGGTTATCAGCGCCCTTGTGGGGCGCTGCTGCCCTTGCCCAATATGCCCGGCCAGAGGCCGGATAGCTGGTCTATTCGCTGCGCTAGGCTACACACCGCCCCACC GCTGCGCGGCAGGGGGAAAGGCGGGCAAAGCCCGCTAAACCCCACACCAAACCCCGCAGAAATACGCTGGAGCGCTTTTAGCCGCTTTAGCGGCCTTTCCCCCTACCCGAAGGG TGGGGGCGCGTGTGCAGCCCCGCAGGGCCTGTCTCGGTCGATCATTCAGCCCGGCTCATCCTTCTGGCGTGGCGGCAGACCGAACAAGGCGCGGTCGTGGTCGCGTTCAAGGTA CGCATCCATTGCCGCCATGAGCCGATCCTCCGGCCACTCGCTGCTGTTCACCTTGGCCAAAATCATGGCCCCCACCAGCACCTTGCGCCTTGTTTCGTTCTTGCGCTCTTGCTG CTGTTCCCTTGCCCGCACCCGCTGAATTTCGGCATTGATTCGCGCTCGTTGTTCTTCGAGCTTGGCCAGCCGATCCGCCGCCTTGTTGCTCCCCTTAACCATCTTGACACCCCA TTGTTAATGTGCTGTCTCGTAGGCTATCATGGAGGCACAGCGGCGGCAATCCCGACCCTACTTTGTAGGGGAGGGCGCACTTACCGGTTTCTCTTCGAGAAACTGGCCTAACGG CCACCCTTCGGGCGGTGCGCTCTCCGAGGGCCATTGCATGGAGCCGAAAAGCAAAAGCAACAGCGAGGCAGCATGGCGATTTATCACCTTACGGCGAAAACCGGCAGCAGGTCG GGCGGCCAATCGGCCAGGGCCAAGGCCGACTACATCCAGCGCGAAGGCAAGTATGCCCGCGACATGGATGAAGTCTTGCACGCCGAATCCGGGCACATGCCGGAGTTCGTCGAG CGGCCCGCCGACTACTGGGATGCTGCCGACCTGTATGAACGCGCCAATGGGCGGCTGTTCAAGGAGGTCGAATTTGCCCTGCCGGTCGAGCTGACCCTCGACCAGCAGAAGGCG CTGGCGTCCGAGTTCGCCCAGCACCTGACCGGTGCCGAGCGCCTGCCGTATACGCTGGCCATCCATGCCGGTGGCGGCGAGAACCCGCACTGCCACCTGATGATCTCCGAGCGG ATCAATGACGGCATCGAGCGGCCCGCCGCTCAGTGGTTCAAGCGGTACAACGGCAAGACCCCGGAGAAGGGCGGGGCACAGAAGACCGAAGCGCTCAAGCCCAAGGCATGGCTT GAGCAGACCCGCGAGGCATGGGCCGACCATGCCAACCGGGCATTAGAGCGGGCTGGCCACGACGCCCGCATTGACCACAGAACACTTGAGGCGCAGGGCATCGAGCGCCTGCCC GGTGTTCACCTGGGGCCGAACGTGGTGGAGATGGAAGGCCGGGGCATCCGCACCGACCGGGCAGACGTGGCCCTGAACATCGACACCGCCAACGCCCAGATCATCGACTTACAG GAATACCGGGAGGCAATAGACCATGAACGCAATCGACAGAGTGAAGAAATCCAGAGGCATCAACGAGTTAGCGGAGCAGATCGAACCGCTGGCCCAGAGCATGGCGACACTGGC CGACGAAGCCCGGCAGGTCATGAGCCAGACCCAGCAGGCCAGCGAGGCGCAGGCGGCGGAGTGGCTGAAAGCCCAGCGCCAGACAGGGGCGGCATGGGTGGAGCTGGCCAAAGA GTTGCGGGAGGTAGCCGCCGAGGTGAGCAGCGCCGCGCAGAGCGCCCGGAGCGCGTCGCGGGGGTGGCACTGGAAGCTATGGCTAACCGTGATGCTGGCTTCCATGATGCCTAC GGTGGTGCTGCTGATCGCATCGTTGCTCTTGCTCGACCTGACGCCACTGACAACCGAGGACGGCTCGATCTGGCTGCGCTTGGTGGCCCGATGAAGAACGACAGGACTTTGCAG GCCATAGGCCGACAGCTCAAGGCCATGGGCTGTGAGCGCTTCGATATCGGCGTCAGGGACGCCACCACCGGCCAGATGATGAACCGGGAATGGTCAGCCGCCGAAGTGCTCCAG AACACGCCATGGCTCAAGCGGATGAATGCCCAGGGCAATGACGTGTATATCAGGCCCGCCGAGCAGGAGCGGCATGGTCTGGTGCTGGTGGACGACCTCAGCGAGTTTGACCTG GATGACATGAAAGCCGAGGGCCGGGAGCCTGCCCTGGTAGTGGAAACCAGCCCGAAGAACTATCAGGCATGGGTCAAGGTGGCCGACGCCGCAGGCGGTGAACTTCGGGGGCAG ATTGCCCGGACGCTGGCCAGCGAGTACGACGCCGACCCGGCCAGCGCCGACAGCCGCCACTATGGCCGCTTGGCGGGCTTCACCAACCGCAAGGACAAGCACACCACCCGCGCC GGTTATCAGCCGTGGGTGCTGCTGCGTGAATCCAAGGGCAAGACCGCCACCGCTGGCCCGGCGCTGGTGCAGCAGGCTGGCCAGCAGATCGAGCAGGCCCAGCGGCAGCAGGAG AAGGCCCGCAGGCTGGCCAGCCTCGAACTGCCCGAGCGGCAGCTTAGCCGCCACCGGCGCACGGCGCTGGACGAGTACCGCAGCGAGATGGCCGGGCTGGTCAAGCGCTTCGGT GATGACCTCAGCAAGTGCGACTTTATCGCCGCGCAGAAGCTGGCCAGCCGGGGCCGCAGTGCCGAGGAAATCGGCAAGGCCATGGCCGAGGCCAGCCCAGCGCTGGCAGAGCGC AAGCCCGGCCACGAAGCGGATTACATCGAGCGCACCGTCAGCAAGGTCATGGGTCTGCCCAGCGTCCAGCTTGCGCGGGCCGAGCTGGCACGGGCACCGGCACCCCGCCAGCGA GGCATGGACAGGGGCGGGCCAGATTTCAGCATGTAGTGCTTGCGTTGGTACTCACGCCTGTTATACTATGAGTACTCACGCACAGAAGGGGGTTTTATGGAATACGAAAAAAGC GCTTCAGGGTCGGTCTACCTGATCAAAAGTGACAAGGGCTATTGGTTGCCCGGTGGCTTTGGTTATACGTCAAACAAGGCCGAGGCTGGCCGCTTTTCAGTCGCTGATATGGCC AGCCTTAACCTTGACGGCTGCACCTTGTCCTTGTTCCGCGAAGACAAGCCTTTCGGCCCCGGCAAGTTTCTCGGTGACTGATATGAAAGACCAAAAGGACAAGCAGACCGGCGA CCTGCTGGCCAGCCCTGACGCTGTACGCCAAGCGCGATATGCCGAGCGCATGAAGGCCAAAGGGATGCGTCAGCGCAAGTTCTGGCTGACCGACGACGAATACGAGGCGCTGCG CGAGTGCCTGGAAGAACTCAGAGCGGCGCAGGGCGGGGGTAGTGACCCCGCCAGCGCCTAACCACCAACTGCCTGCAAAGGAGGCAATCAATGGCTACCCATAAGCCTATCAAT ATTCTGGAGGCGTTCGCAGCAGCGCCGCCACCGCTGGACTACGTTTTGCCCAACATGGTGGCCGGTACGGTCGGGGCGCTGGTGTCGCCCGGTGGTGCCGGTAAATCCATGCTG GCCCTGCAACTGGCCGCACAGATTGCAGGCGGGCCGGATCTGCTGGAGGTGGGCGAACTGCCCACCGGCCCGGTGATCTACCTGCCCGCCGAAGACCCGCCCACCGCCATTCAT CACCGCCTGCACGCCCTTGGGGCGCACCTCAGCGCCGAGGAACGGCAAGCCGTGGCTGACGGCCTGCTGATCCAGCCGCTGATCGGCAGCCTGCCCAACATCATGGCCCCGGAG TGGTTCGACGGCCTCAAGCGCGCCGCCGAGGGCCGCCGCCTGATGGTGCTGGACACGCTGCGCCGGTTCCACATCGAGGAAGAAAACGCCAGCGGCCCCATGGCCCAGGTCATC	Includes genes for mobilization proteins A, B, C and replication proteins A, B, C

RCR	TCCGCCGCCCTAGACCTAGTGTCATTTTATTTCCCCCGTTTCAGCATCAAGAACCTTTGCATAACTTGCTCTATATCCACACTGATAATTGCCCTCAAACCATAATCTAAAGGC GCTAGAGTTTGTTGAAACAATATCTTTTACATCATTCGTATTTAAAATTCCAAACTCCGCTCCCCTAAGGCGAATAAAAGCCATTAAATCTTTTGTATTTACCAAATTATAGTC ATCCACTATATCTAAGAGTAAATTCTTCAATTCTCTTTTTTGGCTTTCATCAAGTGTTATATAGCGGTCAATATCAAAATCATTAATGTTCAAAATATCTTTTTTGTCGTATAT ATGTTTATTCTTAGCAATAGCGTCCTTTGATTCATGAGTCAAATATTCATATGAACCTTTGATATAATCAAGTATCTCAACATGAGCAACTGAACTATTCCCCAATTTTCGCTT AATCTTGTTCCTAACGCTTTCTATTGTTACAGGATTTCGTGCAATATATATAACGTGATAGTGTGGTTTTTTATAGTGCTTTCCATTTCGTATAACATCACTACTATTCCATGT ATCTTTATCTTTTTTTTCGTCCATATCGTGTAAAGGACTGACAGCCATAGATACGCCCAAACTCTCTAATTTTTCCTTCCAATCATTAGGAATTGAGTCAGGATATAATAAAAA TCCAAAATTTCTAGCTTTAGTATTTTTAATAGCCATGATATAATTACCTTATCAAAAACAAGTAGCGAAAACTCGTATCCTTCTAAAAACGCGAGCTTTCGCTTATTTTTTTTG TTCTGATTCCTTTCTTGCATATTCTTCTATAGCTAACGCCGCAACCGCAGATTTTGAAAAACCTTTTTGTTTCGCCATATCTGTTAATTTTTTATCTTGCTCTTTTGTCAGAGA AATCATAACTCTTTTTTTCGATTCTGAAATCACCATTTAAAAAACTCCAATCAAATAATTTTATAAAGTTAGTGTATCACTTTGTAATCATAAAAACAACAATAAAGCTACTTA AATATAGATTTATAAAAAACGTTGGCGAAAACGTTGGCGATTCGTTGGCGATTGAAAAACCCCTTAAACCCTTGAGCCAGTTGGGATAGAGCGTTTTTGGCACAAAAATTGGCA CTCGGCACTTAATGGGGGGTCGTAGTACGGAAGCAAAATTCGCTTCCTTTCCCCCCATTTTTTTCCAAATTCCAAATTTTTTTCAAAAATTTTCCAGCGCTACCGCTCGGCAAA ATTGCAAGCAATTTTTAAAATCAAACCCATGAGGGAATTTCATTCCCTCATACTCCCTTGAGCCTCCTCCAACCGAAATAGAAGGGCGCTGCGCTTATTATTTCATTCAGTCAT CGGCTTTCATAATCTAACAGACAACATCTTCGCTGCAAAGCCACGCTACGCTCAAGGGCTTTTACGCTACGATAACGCCTGTTTTAACGATTATGCCGATAACTAAACGAAATA AACGCTAAAACGTCTCAGAAACGATTTTGAGACGTTTTAATAAAAAAATCGCCTAGTGC	

<u>Transposon inverted repeat sequences</u>	**Sequence**	**Notes**
Himar	ACAGGTTGGATGATAAGTCCCCGGTCT	
Tn5	CTGTCTCTTATACACATCT	

<u>Regulatory sequences (5' UTRs, incl. promoter and RBS)</u>	**Sequence**	**Notes**
1	GATTGCATTAGGTTTTAGTTTCTTGTATAATGCTTAATGTTGGTCACTGACAGGCTACGATACGGAAGGTTGCTCACGCCCGGCCCCTTTGCCATGGCTAGTGTGTGGAAATTT CCGAGGAGCAAGTCTATTTCCAAAAATGGGCGAAAAAGGAGGTAATACA	From *Bacillus cellulosilyticus*
2	GGGAGAGCTTCAACGGCGCTTCTACCCATTTGCTTGGAAAGGATGAGGAGCAGGAAGAAATTCCGTCCCCAATGCGACGGCCCTTTACATCCATGTTGTTTGATAGTATAATGG ATACGGATTGACCAAATTGTTCATTTAGTCAGTTTGAAGGATGAGGAGT	From *Geobacillus sp.*
3	GTGAAGGATACGGCTGCGGCACTTCGACATCGCCCCATGTGGCGGCTTTGAACTGGGCTTATGAAACGCGTTCACAACCTTTTTTGACCATCGGCGCGAACGTGGTATCATGCG TTCAGCTTTTGCCCATACATACTACGTGCTCAATCTAGGAGGATTTCATAC	From *Eggerthella lenta*
4	CTCTAGAGTAGTAGATTATTTTAGGAATTTAGATGTTTTGTATGAAATAGATGCTTCGTATGGAATTAATGAAATTTTTAGTCAGGTAAAAAAGGTAATAGGAGAATATT	From Segmented Filamentous Bacteria (SFB)
5	GTTTTAAATGATGAAAAGAAATATTTAGGGAAGATTGTTTCGACGCGAATTGTTGATCTGGAAAATGATCACCTTATCGGACAAGCTTTAAAATAGGAGGATATAAAAAT	From Segmented Filamentous Bacteria (SFB)
6	ATAAGGATTCTTTAAAGAGAGATATAGTTATGTCAAAGACTGTAGAATTTTTAGTAAATCAAAATAAAAAAAGAGGTATTAAATAGAGTGTATTTTAAAGGAGGAGACTT	From Segmented Filamentous Bacteria (SFB)
7	AAACACCAATAAAATTAGAATATTTAGGAGCGACTTTAAAAAAGTTTAATAAGAATTGTTTATGAGATATTTTTATTATATTTAAACTCAATTTAAAGTAGGGAGAATAG	From Segmented Filamentous Bacteria (SFB)
8	GCAAGTGTTCAAGAAGTTATTAAGTCGGGAGTGCAGTCGAAGTGGGCAAGTTGAAAAATTCACAAAAATGTGGTATAATATCTTTGTTCATTAGAGCGATAAACTTGAATTTGA GAGGGAACTTAG	From *Clostridium perfringens*

<u>Primers for PCR validation of transconjugants</u>

16S forward	AGAGTTTGATCATGGCTCAG
16S reverse	CGGTTACCTTGTTACGACTT
GFP validation primer forward	ATGCGTAAAGGCGAAGAGC
GFP validation primer reverse	TTATTTGTACAGTTCATCCATACCATG
Beta-lactamase validation primer forward	ATGAGTATTCAACATTTCCGTGTC
Beta-lactamase validation primer reverse	TTACCAATGCTTAATCAGTGAGGC

pGT-B backbone validation primer forward	CTGCGCAACCCAAGTGCTAC
pGT-B backbone validation primer reverse	CAGTCCAGAGAAATCGGCATTCA
pGT-Ah backbone validation primer forward	ATGGAAAAAAAGGAATTTCGTGTTTTG
pGT-Ah backbone validation primer reverse	TTATTCAACATAGTTCCCTTCAAGAGC
CarbR internal forward primer	CCGAAGAACGTTTTCCAATGATGAG
GFP internal reverse primer	TGATTGTCTGGCAGCAGAAC
catP (chlor resistance) validation primer forward	GCAAGTGTTCAAGAAGTTATTAAGTC
catP (chlor resistance) validation primer reverse	TTAACTATTTATCAATTCCTGCAATTCG
tetQ (tet resistance) internal forward primer	TGGAAGAACGATTTTCCGTAAAGGT

Supplementary Table 4. List of isolated transconjugant strains

Strains are grouped by the mouse cohort they were isolated from and the vector library used in the study.
All family-level assignments were made using the RDP classifier with confidence >0.89.

Taconic mice *in situ* conjugations

Vector library	Family	Genus	Genus-level assignment confidence	Vector received	Antibiotic resistance
pGT-L6	Erysipelotrichaceae (Clostridium XVIII)	*Erysipelotrichaceae incertae sedis*	1	pGT-Ah	carb
	Bacteroidaceae	*Bacteroides*	1	pGT-Ah	carb
	Enterobacteriaceae	*Proteus*	1	pGT-Ah	carb
	Enterobacteriaceae	*Citrobacter*	1	pGT-Ah	carb
	Enterococcaceae	*Enterococcus*	1	pGT-Ah	carb
	Lachnospiraceae	*Hungatella*	0.72	pGT-Ah	carb
	Lachnospiraceae	*Clostridium XIVa*	1	pGT-Ah	carb
	Lachnospiraceae	*Anaerostipes*	1	pGT-Ah	carb
	Lachnospiraceae	*Moryella*	0.19	pGT-Ah	carb
	Lachnospiraceae	*Blautia*	1	pGT-Ah	carb
	Lactobacillaceae	*Lactobacillus*	1	pGT-Ah	carb
	Peptostreptococcaceae	*Clostridium XI*	1	pGT-Ah	carb
pGT-L3	Coriobacteriaceae	*Eggerthella*	1	pGT-S	tet
	Enterobacteriaceae	*Cosenzaea*	0.73	pGT-S	tet
	Enterobacteriaceae	*Proteus*	1	pGT-S	tet
	Enterococcaceae	*Enterococcus*	1	pGT-S	carb
	Lachnospiraceae	*Lactonifactor*	0.7	pGT-S	tet
	Lachnospiraceae	*Clostridium XIVa*	1	pGT-S	carb
	Lachnospiraceae	*Hungatella*	0.71	pGT-S	tet
	Lachnospiraceae	*Clostridium XIVa*	1	pGT-S	tet
	Lachnospiraceae	*Blautia*	1	pGT-S	tet
	Lachnospiraceae	*Robinsoniella*	0.42	pGT-S	tet
	Lachnospiraceae	*Eisenbergiella*	0.99	pGT-S	tet
	Lactobacillaceae	*Lactobacillus*	0.89	pGT-S	tet

Charles River mice*in situ* conjugations

Vector library	Family	Genus	Genus-level assignment confidence	Vector received	Antibiotic resistance
pGT-L6	Bacteroidaceae	*Bacteroides*	1	pGT-Ah	carb
	Enterococcaceae	*Enterococcus*	1	pGT-Ah	carb
	Lactobacillaceae	*Lactobacillus*	1	pGT-Ah	carb
	Porphyromonadaceae	*Parabacteroides*	1	pGT-Ah	carb

***In vitro* conjugations**

Vector library	Family	Genus	Genus-level assignment confidence	Vector received	Antibiotic resistance
pGT-L7	Enterobacteriaceae	*Proteus*	1	pGT-Ah	carb
	Enterococcaceae	*Enterococcus*	1	pGT-Ah	carb
	Enterobacteriaceae	*Escherichia*	1	pGT-Ah	carb
	Lactobacillaceae	*Lactobacillus*	1	pGT-Ah	carb
	Bacillaceae	*Bacillus*	1	pGT-Ah	carb
pGT-L3	Enterobacteriaceae	*Escherichia*	1	pGT-S	carb
	Enterococcaceae	*Enterococcus*	1	pGT-S	carb
	Enterobacteriaceae	*Proteus*	1	pGT-S	carb
pGT-L5	Enterobacteriaceae	*Cosenzae*	0.89	pGT-Ah	chl
	Enterobacteriaceae	*Proteus*	1	pGT-Ah	chl
	Burkholderiaceae	*Cupriavidus*	1	pGT-Ah	chl
pGT-L4	Enterobacteriaceae	*Escherichia*	1	pGT-Ah	carb
	Enterobacteriaceae	*Proteus*	1	pGT-Ah	carb
	Enterobacteriaceae	*Escherichia*	1	pGT-B	carb