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Engineering the human gut commensal *Bacteroides thetaiotaomicron* with synthetic biology

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Abstract

The role of the microbiome in health and disease is attracting the attention of researchers seeking to engineer microorganisms for diagnostic and therapeutic applications. Recent progress in synthetic biology may enable the dissection of host–microbiota interactions. Sophisticated genetic circuits that can sense, compute, memorize, and respond to signals have been developed for the stable commensal bacterium *Bacteroides thetaiotaomicron*, dominant in the human gut. In this review, we highlight recent advances in expanding the genetic toolkit for *B. thetaiotaomicron* and foresee several applications of this species for microbiome engineering. We provide our perspective on the challenges and future opportunities for the engineering of human gut-associated bacteria as living therapeutic agents.

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Introduction

The gut microbiota ecosystem is intimately associated with numerous aspects of the human host, influencing human health and disease [1]. The diversity and composition of these microbial communities have a broad impact on human health, affecting metabolism, immunity, and behavior [2]. For instance, the gut microbiota converts ingested dietary fiber and host mucosal glycans to short-chain fatty acids (SCFAs), which are crucial for human intestinal health [3]. The gut microbiota also influences the responses of cancer patients to anti-PD-1 immunotherapy [4]. Fecal microbiota transplantation (FMT) has become a powerful strategy to treat recurrent *Clostridium difficile* infection and other disorders by restoring balance to disturbed microbiota [5]. Our understanding of the human gut microbiota has been dramatically shaped by -omics technologies, which provide a snapshot of the composition and metabolism of microbial communities in the human gut. However, targeted approaches to elucidate the mechanistic relationships between gut microbiota and host have lagged behind. Specifically, the molecular mechanisms of how the gut microbiota affects human health and disease are still poorly understood.

Synthetic biology provides attractive approaches to facilitate our understanding of host–microbiota interactions but also to manipulate the gut microbiota, ideally reconfiguring these microbial communities to promote health [6]. In this article, we review recently developed genetic tools for the obligate anaerobe *Bacteroides* spp., which is one of the most abundant and stable genera in the healthy human gut [7]. Of the species in the *Bacteroides* genus, *Bacteroides thetaiotaomicron* (*B. thetaiotaomicron*) was the first to be sequenced [8]. This prominent human gut commensal species has been reported to attenuate gut inflammation [9], enhance innate immunity against pathogen invasion [10], and process important dietary nutrients [11] and is well tolerated as a live biotherapeutic in patients with Crohn's disease [12]. *B. thetaiotaomicron* has emerged as a key organism for both understanding and manipulating the gut microbiota ecosystem [13]. This review focuses on recent advances in expanding the genetic toolbox (Table 1) for *B. thetaiotaomicron*, which can enable the construction of sophisticated genetic circuits to sense

Table 1

The synthetic biology toolbox for *Bacteroides* engineering.

Functions	Genetic circuits	Promoter	RBS	Payload	Vector backbone	Genome integration	Additional information	Year	Refs
Sense and respond	YES and NOT gates	Constitutive promoters: P_{BT1311} variants; Rhamonse-, chondroitin sulfate-, arabinogalactan-, and IPTG-inducible promoters.	rpiL* and randomized rpiL* RBS libraries (142); weak RBS RC500	Serine integrases (memory); NanoLuc; dCas9; BT1854 (LpxF); BT1754	Integration vector pNBU2	attBT2	Controlled gene expression (>10^4 fold dynamic range); Genetic circuits validated in mouse model.	2015	[18]
Regulated gene expression	YES gate	The mannan-inducible promoter (P_{BT3784}).	RBSxyl120; SD8	*Lactobacillus* pepl; BtCepA	pGH		10^3-fold range of promoter activity	2016	[21]
Regulated gene expression		Constitutive phage promoter: P_{BfP1E6}	AT-rich, AG-rich, and random RBS libraries.	GFP and mCherry	pNBU2	attBT2	High-throughput cloning and genomic integration pipeline; unique fluorescent protein signature for *in vivo* imaging.	2017	[19]
Regulated gene expression	YES gate	aTc inducible P1 and P2 promoters	RBS panel [18]	Nanoluc; BT1854 (LpxF); BT1754; Ss-Bfe1 and Ss-Bte1; BT0455 (Sialidase)	pNBU2; pExchange_*tdk* (allelic exchange)	attBT2; between BT3743 and BT3744; between BT4719 and BT4720.	10^5-fold range of promoter activity; Tested in 11 different species	2017	[23]
Exclusive nutrient access		Native promoters of porphyran polysaccharide utilization loci (PULs)	Native RBSs	Porphyran utilization PULs	pWD011 and pEZ-BAC for yeast assembly	attBT2	Transfer of the 60-kb PUL into a native strain of *Bacteroides*	2018	[33]
Expression of metagenomic DNA		Native promoters of gut-derived DNA	Native RBSs of gut-derived DNA	Glycan utilization genes	pCC1FOS (fosmid vector)		Functional metagenomic screens	2018	[39]
Genetic maniputlation	YES gate	aTc- and Rhamnose-inducible promoters; P_{BT1311}		ss-Bfe1 toxic effector (counter-selection); inulin utilization cassette	pNBU2	attBT2	Genetic manipulation in antibiotic-resistant *Bacteroides* isolates	2019	[31]
Regulated gene expression	AND gate	Dextran and arabinogalactan-responsive promoters; P_{BT1311}	rpiL*RBS	NanoLuc; agarase (BuGH16C)	pINT; pNBU2; pEpisomalPromoter (pEP)	attBT2; PUL75	Dual-glycan expression system	2019	[22]

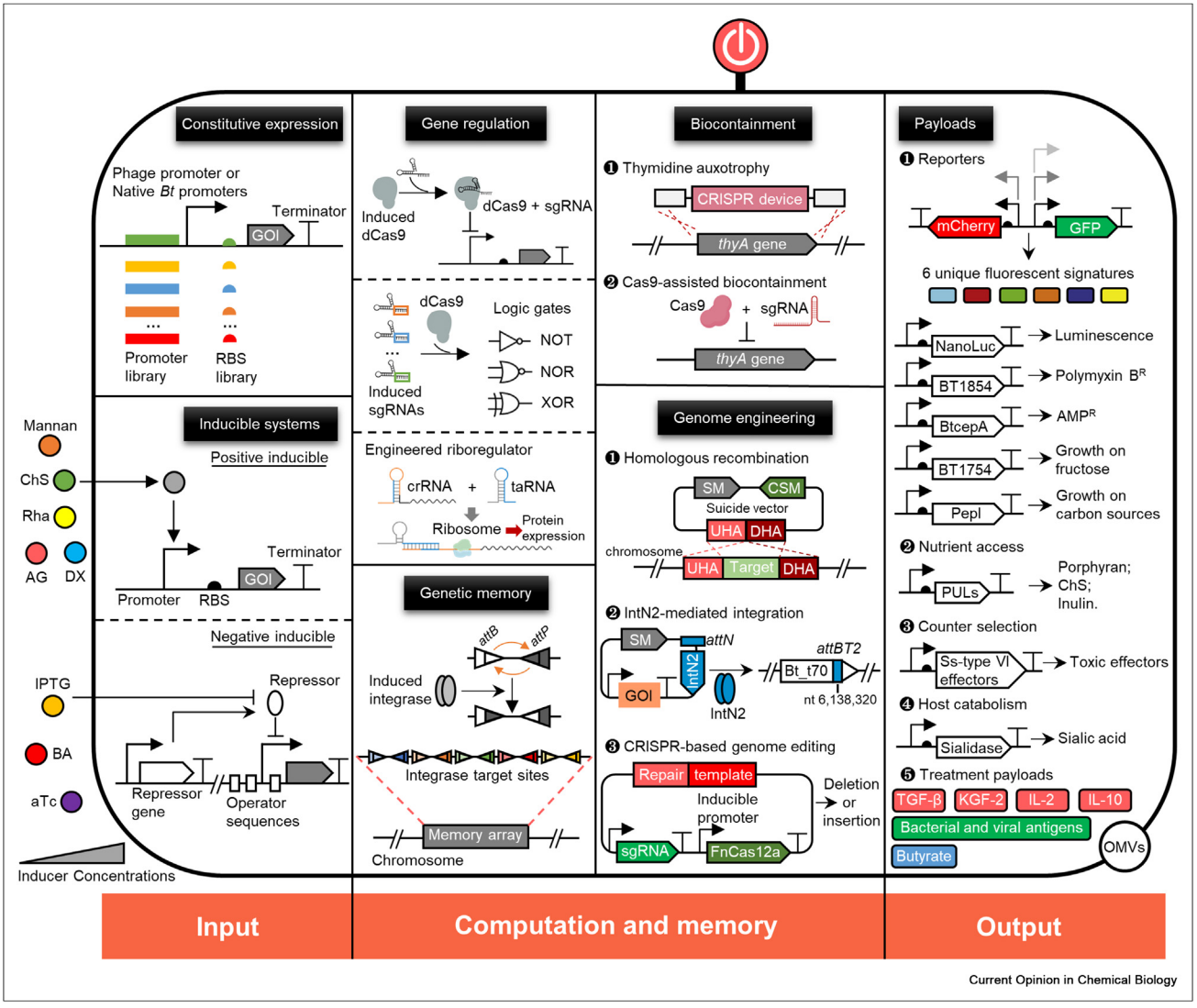
Sense and respond	NOT; NOR; XOR; the 2-input 3-output circuit	Constitutive promoters: P_{BT1311}, P_{PAM3}, P1, *etc*; aTc- and BA-inducible promoters	rpiL*RBS *etc*.	NanoLuc; dCas9; sgRNAs *etc*.	L3S2P21 *etc*.	attBT1-1; attBT2	Circuit design automation; Development of complex genetic logic gates in *Bacteroides*.	2020	[24]
Regulated gene expression		Constitutive promoters: P_{BT1311} and P_{BfP1E6}	RBS panel [18,19]	Butyrate biosynthetic pathway: *thil, hbd, crt, bcd, etfAB, ptb, buk*	pNBU2-based vectors (pMM710 and pLGB13); Replicon plasmid pFD340	BT4681-2; *pta* and *ldhD* gene loci.	GEM (*i*KS1119)-guided metabolic engineering design. The maximum butyrate titer in the Δ*pta*Δ*ldhD* mutant was 41 ± 1 mg/L in BHIS medium.	2021	[43]
Genetic maniputlation	YES gate	Constitutive promoters: P_{BT1311} and P_{cfxA}; aTc- and IPTG-inducible promoter		SpCas9; SpRY; FnCas12a; NanoLuc.	pNBU2; Replicon plasmids		Deletion of large genomic DNA up to 50-kb; Work in multiple *Bacteroides* species.	2022	[29]
Containment and regulated gene expression		Constitutive promoters: P_{cepA}, P_{cfiA}, P_{cfxA}, P_{BT1311}, and P_1.	rpiL*RBS	NanoLuc; SpCas9; sgRNAs; Engineered Riboregulators.	pNBU2	attBT2; *thyA* gene	Cas9-assisted biocontainment; engineered riboregulator for controlled gene expression.	2022	[14]

and respond to environmental signals (Figure 1). We also provide our vision for future research and discuss challenges facing gut commensal bacteria engineering.

Constitutive promoter and ribosome binding site (RBS) design

In prokaryotic cells, gene expression is highly controlled at the transcriptional level. Given that the transcriptional control mechanisms in *Bacteroides* differ from those of well characterized organisms like *Escherichia coli* [15–17], strong constitutive promoters, such as native *Bacteroides* promoters [18] and phage promoters [19], were characterized and engineered to express heterologous and homologous proteins in *B. thetaiotaomicron*. Based on the constitutive promoter for the housekeeping sigma factor BT1311, Mimee et al. designed four promoter variants by introducing a 26-bp sequence in the regions surrounding and including the −33

Figure 1



Engineered genetic circuits in *B. thetaiotaomicron* with multiple functional modules. Genetically engineered gut commensal *B. thetaiotaomicron* sense inputs, process signals with bio-computation and memory, and generate output according to the "decision" made by the genetic circuits. Input sensors, constructed with constitutive and inducible promoters, detect environmental or disease biomarkers [Input]. Upon recognition of these biomarkers, modules involved in gene regulation and genetic memory can integrate the inputs [Computation and Memory] and, in response, drive specific and appropriate output functions [Output]. Genetic circuits can be integrated into the genome of *B. thetaiotaomicron*. Biocontainment modules can prevent horizontal gene transfer and unintended spread of engineered *B. thetaiotaomicron* [14]. RBS, ribosome binding site; GOI, gene of interest; ChS, chondroitin sulfate; Rha, rhamnose; AG, arabinogalactan; DX, dextran; IPTG, isopropyl β-D-1-thiogalactopyranoside; BA, bile acid; aTc, anhydrotetracycline; SM, selectable marker; CSM, counter selectable marker; UHA, upstream homologous arm; DHA, downstream homologous arm; AMP, ampicillin; PULs, the polysaccharide utilization loci; OMVs, outer membrane vesicles.

and −7 promoter structures, which are known to be important for *B. thetaiotaomicron* transcription [18]. Whitaker et al. identified the strong constitutive promoter P_{BfP1E6} in *Bacteroides fragilis* (*B. fragilis*) phage genomes and created eight synthetic promoters by introducing single or multiple mutations in this promoter spanning a 3×10^4-fold expression range [19]. To expand the range of constitutive gene expression, the RBS, which regulates protein production via translation, was characterized. When native *B. thetaiotaomicron* promoters and phage promoters were combined with RBS libraries, gene expression was programmable across ranges of 1×10^4-fold and $\sim 1 \times 10^6$-fold, respectively [18,19]. In addition, analysis of the RBS libraries revealed that the AT-rich RBSs strengthened protein expression more than other RBS designs [18,19]. Intriguingly, Townsend et al. showed that dietary fructose and glucose can silence a regulator of colonization (Roc) in *B. thetaiotaomicron* by controlling its protein translation [20]. Four nucleotides located between the putative RBS and the start codon of the *roc* gene are required for such control by fructose and glucose [20]. Thus, engineered *B. thetaiotaomicron* with another mRNA leader sequence from the BT3334 gene preceding the *roc* coding region can render the expression of this colonization factor active in mice fed glucose and sucrose [20]. The identification and characterization of constitutive promoters and RBSs will extend our genetic toolkit for the manipulation of *B. thetaiotaomicron*.

Inducible promoter-based biosensor

Although constitutive protein expression is suitable for many applications, inducible systems are often desirable to sense fluctuating environmental signals and precisely control output gene expression levels. Currently, there are three strategies to design and construct inducible promoters in *B. thetaiotaomicron*. By adapting genetic parts that control carbohydrate utilization, Mimee et al. developed a rhamnose-inducible promoter that is mediated by the transcriptional activator RhaR with an output dynamic range of 104-fold [18]. Promoters inducible by mannan (output range of 100-fold) and dextran (DX) (output range of 3- to 5-fold) have been derived by Horn et al. and Jones et al., respectively, from promoters of genes in polysaccharide utilization loci (PULs) [21,22]. Based on hybrid two-component systems that sense external stimuli and transduce signals in *Bacteroides*, Mimee et al. designed systems inducible by chondroitin sulfate (ChS) and arabinogalactan (AG) having output dynamic ranges of 60-fold and 29-fold, respectively [18]. The third strategy to design chemically inducible promoters is based on DNA operator-repressor systems. In the OFF state, repressor proteins bind to operator sequences in the promoter, preventing gene transcription. Once a chemical inducer binds to the repressor, the repressor releases the repression, allowing the gene to be transcribed. For instance, systems inducible by isopropyl β-D-1-thiogalactopyranoside (IPTG), anhydrotetracycline (aTc), and bile acid (BA) were developed in *B. thetaiotaomicron* by investigating the position effects of corresponding operator sequences in constitutive promoters [18,23,24]. As the orthogonality of genetic sensors is crucial for their combined use, Taketani et al. constructed a *B. thetaiotaomicron* strain with sensors inducible by IPTG, aTc, and BA; no cross-talk was recorded with this system [24]. Mimee et al. also tested systems inducible by rhamnose, ChS, AG, and IPTG with the full set of carbohydrate inducers [18]. Each inducible system was highly orthogonal to the others, and no cross-talk was observed [18]. Engineering genetic sensors, promoters, and RBSs enables scientists to manipulate outputs of genetically engineered *B. thetaiotaomicron* at different levels and in response to inputs from environmental signals.

Tunable and predictable gene regulation

CRISPRi-mediated gene knockdown can be used to modulate endogenous gene expression. Synthetic gene circuits encoding CRISPRi elements can be constructed and expressed in *B. thetaiotaomicron*. By connecting to chemically inducible systems, the regulated production of deactivated Cas9 (dCas9) or single guide RNAs (sgRNAs) can repress target promoters, resulting in the performance of complex response functions [18,24]. For instance, Mimee et al. implemented genetic one-input NOT gates in *B. thetaiotaomicron* that repressed distinct phenotypes in the presence of IPTG by regulating dCas9 expression; these phenotypes included the production of NanoLuc, resistance to antimicrobial peptides, and growth on fructose as the sole carbon source [18]. Taketani et al. designed more complex genetic circuits based on regulated sgRNAs with the help of Cello circuit design automation software [24]. In one of the systems designed by Taketani et al., the two-input NOR gates incorporate two separate copies of the same sgRNA, regulated by two inducers (aTc and BA) but targeting the same output promoter [24]. For another system, two-input XOR gates were designed by combining simpler NOR and OR gates based on different sgRNAs targeting different output promoters [24]. Additionally, a genetic circuit was implemented in *B. thetaiotaomicron* that senses the conditions of a fermenter (the sensor is induced by aTc) or the gut environment (the sensor is induced by BA) and controls three outputs of gene expression [24].

Recently, Hayashi and Lai et al. developed an engineered riboregulator (ER) for controlled gene expression in *B. thetaiotaomicron* [14]. The ER consists of two components: cis-repressed mRNA (crRNA) and trans-activating RNA (taRNA). The cis-repressive sequence in the 5′- untranslated region (UTR) of the crRNA blocks access of the ribosome to the RBS and represses downstream protein expression. In the presence of

taRNA, the trans-activating sequence hybridizes to the crRNA, exposing the RBS region to the ribosome and initiating protein translation. Unlike *E. coli* [25], the strongest RBSs of *Bacteroides* are AT-rich and more sensitive to secondary structure [18,19]. Based on the putative S1 protein binding site in the RBS, Hayashi and Lai et al. designed and optimized an ER system in *B. thetaiotaomicron*. The system could repress NanoLuc activity by 4258-fold in the ER-OFF state compared to the condition in the absence of the cis-repressive sequence, and induce NanoLuc activity, with a 69-fold increase from the OFF to the ON state [14]. Thus, tunable and predictable gene regulation systems for *B. thetaiotaomicron* have been developed that are likely to facilitate the design of genetic circuits.

Genetic memory

Gut commensal *B. thetaiotaomicron* interacts with host cells and other microbial cells during transit through the human digestive tract, making this species attractive as a living system for potentially recording environmental inputs inside the body. By connecting biosensors to recombinase-based switches, *B. thetaiotaomicron* can be engineered to store long-term memories in its genomic DNA in response to exposure to environmental signals. Mimee et al. characterized four serine integrases in a DNA "memory array" in *B. thetaiotaomicron*, Int7, Int8, Int9, and Int12, which catalyze the unidirectional inversion of the DNA sequences between two respective recognition sequences [18]. To demonstrate the capability of chemical signal recording, the rhamnose-inducible recombinase Int12 circuit was constructed; this circuit responded within 2 h to increasing concentrations of rhamnose [18]. To test its function *in vivo*, mice were colonized with the engineered *B. thetaiotaomicron* strain and supplemented with exogenous rhamnose, and recombination was quantified by sequencing the DNA "memory array" of *B. thetaiotaomicron* in stool. The recombination frequency achieved >90% flipping of the targeted DNA sequences within one day after mice received rhamnose-supplemented water [18]. The recording of recombinase-mediated events demonstrates the potential of *B. thetaiotaomicron* for long-term living memory storage in a gut microorganism.

Genome engineering

To stabilize their functions in *B. thetaiotaomicron*, DNA genetic circuits can be integrated into the bacterial chromosome. Currently, there are three strategies for genome engineering in *B. thetaiotaomicron*: homologous recombination [26—28], tyrosine integrase-mediated integration [18], and CRISPR-based genome editing [29]. Homologous recombination, a commonly used method to engineer the genome of *Bacteroides* spp., allows DNA sequences to be deleted or inserted without considering the presence or location of restriction sites. However, this method requires the construction of a mutant for counterselection, such as the deletion of thymidylate synthase (*thyA*) or thymidine kinase (*tdk*), or the mutation of a subunit of phenylalanine tRNA synthetase (*pheS**).

Interestingly, Garcia-Bayona et al. and Bencivenga-Barry et al. have developed aTc-inducible counterselection cassettes using toxins from the type VI secretion system, which do not require the construction of *Bacteroides* mutants across diverse species [30,31]. In *B. thetaiotaomicron*, tyrosine integrase IntN1, encoded in pNBU1, mediates the sequence-specific recombination of the *attN* site of pNBU1 and the *attBT1-1* site located in the 3′ ends of the tRNA-Leu gene on the chromosome [24,32]. Similarly, tyrosine integrase IntN2 mediates recombination between the *attN* site of pNBU2 and one of two *attBT* sites (*attBT2-1* and *attBT2-2*) located in the 3' ends of two tRNA-Ser genes [18,32]. Shepherd et al. demonstrated that the 60-kb porphyran utilization locus, which includes 34 genes, can be integrated into the *Bacteroides* genome by a tyrosine integrase-mediated method [33]. Recently, Zheng et al. developed the aTc-inducible CRISPR/FnCas12a system, which deleted a 50-kb metabolic gene cluster in *B. thetaiotaomicron* and a target gene in four *Bacteroides* species: *B. fragilis*, *Bacteroides ovatus*, *Bacteroides vulgatus*, and *Bacteroides uniformis* [29]. Tajkarimi et al. have identified three types of native CRISPR-Cas systems (i.e. type IB, type IIIB, and type IIC) in the genome of a wide range of *B. fragilis* strains [34]. As more options for genome engineering of *B. thetaiotaomicron* become available, we envision that they may be applied to study linkages between microbiome states and important diseases.

Production and delivery of payloads

B. thetaiotaomicron can be engineered to produce and deliver payloads for specific tasks based on decisions calculated by genetic circuits. The payloads can be genes encoding reporter proteins to enable monitoring of colonized locations and functions of engineered gut commensal bacteria in *in vivo* environments. As a first step, Whitaker et al. characterized a set of constitutive strong promoters to drive distinct expression of GFP and mCherry, which encoded six unique fluorescent protein signatures and allowed differentiation of *Bacteroides* species within the gut [19]. Researchers have also tested the performance of artificial genetic circuits in *in vivo* models by measuring luminescence expressed by NanoLuc [14,18,22,23,29].

Proteins that mediate antibiotic resistance, such as polymyxin B and ampicillin resistance, or carbon metabolism, such as fructose utilization, were also expressed in *B. thetaiotaomicron* [18,21,23]. In addition, these payloads can include gene clusters (i.e., PULs) to metabolize plant- and host-derived polysaccharides. In the *B. thetaiotaomicron* genome, more than 80 PULs have

been identified, which contain gene clusters involved in the regulation, transport, and catabolism of complex polysaccharides [35]. In the sequencing era, large-scale functional genetics methods have been applied to *B. thetaiotaomicron*, such as transposon insertion sequencing (INSeq) [36,37] and a barcoded variant of TnSeq (RB-TnSeq) [38]; these studies have provided insights into the colonization of *in vivo* niches and nutrient acquisition strategies in gut ecosystems. By functional metagenomic screening, Lam et al. identified a fructan utilization PUL and transferred it into *B. thetaiotaomicron*, enabling the bacterium to utilize ChS as a carbon source [39]. To control the engraftment and abundance of the exogenous bacterial strain in the mouse gut, Shepherd et al. transferred a 60-kb PUL to *B. thetaiotaomicron* that provided access to porphyran, a marine polysaccharide, as a privileged nutrient [33]. To develop an alternative genetic tool for integrant selection in antibiotic-resistant *Bacteroides* species, Garcia-Bayona et al. constructed a three-gene inulin-utilizing PUL in *B. thetaiotaomicron* and selected integrants on inulin selection agar plates [31]. The expression of type VI toxic effectors (Ss-Bfe1 and Ss-Bte1) in *B. thetaiotaomicron* can be used for counterselection in genome engineering [30,31]. Lim et al. engineered an inducible genetic circuit in *B. thetaiotaomicron* to control the expression of sialidase in the mouse gut, revealing a non-linear relationship between commensal enzyme activity and host sialic acid levels [23].

A major strategy to improve human health with engineered gut commensal bacteria is to deliver and produce treatment payloads *in situ*, especially for chronic gastrointestinal disorders that require continuous treatment. *B. ovatus* was engineered for the xylan-regulated *in situ* delivery of TGF-β [40], KGF-2 [41], and murine IL-2 [42] to treat inflammatory gut diseases. Kim et al. heterologously expressed a biosynthetic pathway in *B. thetaiotaomicron* to produce non-native butyrate, a SCFA that maintains intestinal homeostasis [43]. Importantly, they maximized butyrate production in the *pta-ldhD* double knockout *B. thetaiotaomicron*, which was guided by an expanded genome-scale metabolic model (GEM) with the OptKnock algorithm [43].

Moreover, outer membrane vesicles (OMVs), naturally produced and secreted by *B. thetaiotaomicron*, are promising for development as drug delivery systems that can prevent dilution and proteolytic degradation during treatment. For example, Lu et al. characterized key components of OMVs to deliver IL-10 for the treatment of inflammatory bowel disease (IBD). Lu et al. engineered *Bacteroides* spp. to secrete therapeutic proteins via OMVs and tested secretion in animal models [44]. Carvalho et al. demonstrated that engineered *B. thetaiotaomicron* OMVs can be used to deliver antigens of *Salmonella enterica* ser. Typhimurium (OmpA and SseB) and H5N1 virus (the H-stalk protein H5) as a vaccine, as well as to deliver a human therapeutic protein (KGF-2) to the gastrointestinal and respiratory tracts [45]. In addition to using *Bacteroides* as a convenient chassis for synthetic gene circuits, researchers could potentially use OMVs as delivery systems for proteins and genes [44,45].

Biocontainment

Stringent regulations limit the use of the genetically modified bacteria to prevent them from being unintentionally released into the environment. There is also a potential risk that transgenes in the bacteria would be disseminated in an uncontrolled manner by horizontal gene transfer (HGT). Thus, appropriate containment of genetically modified microorganisms and their introduced genetic elements is a prerequisite for practical use.

To contain an engineered bacterial strain in unmonitored environments, such as clinical applications, auxotrophy is often used [46–48]. This approach was applied to *Bacteroides* spp. by deletion of the thymidylate synthase gene *thyA*, which leads to thymidine auxotropy [49]. However, the *thyA*-deficient *B. ovatus* acquired a *thyA* gene from surrounding bacterial cells and escaped from the containment. Hayashi and Lai et al. have devised a Cas9-assisted biocontainment system to prevent engineered *B. thetaiotaomicron* from acquiring *thyA* through HGT, by targeting the *thyA* gene [14]. Hayashi and Lai et al. designed an artificial gene cassette bearing a CRISPR Device, composed of a Cas9-encoding gene and single guide RNA (sgRNA), that could specifically recognize and destroy the *thyA* gene by introducing a double-strand break. In this system, when the gene cassette was introduced by genetic exchange with the *thyA* gene, the conjugation efficiency of plasmids having *thyA* was significantly decreased, at least 156-fold, and the CRISPR Device reliably functioned as a safeguard to avoid disrupting the thymidine-auxotrophic biocontainment of the genetically modified *B. thetaiotaomicron*. Additionally, the containment system had the capacity to prevent the dissemination of the gene cassette into wild-type *B. thetaiotaomicron*. The CRISPR Device transferred to the wild-type strain could destroy the *thyA* gene on the genome and have a bactericidal effect on the cells that acquired the gene cassette. The number of transconjugants that acquired plasmids with the CRISPR Device was markedly lowered by at least 18 times, compared to the number of transconjugants that acquired control plasmids. This Cas9-assisted auxotrophic biocontainment system simultaneously prevents the escape of the genetically modified microorganisms and the genetic elements *in vitro*. Experiments are in progress to determine the function and stability of the CRISPR Device in animal models.

Conclusion

The use of probiotic supplementation and FMT to treat human digestive tract disorders has sparked increasing interest in engineering gut commensal bacteria for living diagnostic and therapeutic applications [5,50]. With continuing advances in genome editing, DNA synthesis and sequencing, and automation of genetic circuit design, we are able to rapidly and efficiently construct artificial genetic circuits that enable gut commensal organisms to sense and respond to *in vivo* environmental signals. However, challenges remain — these are associated primarily with poor understanding of the mechanisms underlying the dynamics and functions of the microbiota and its interactions with the human host, especially for digestive tract disorders, such as IBD.

The stable and abundant colonization of the human gut by commensal *Bacteroides* species and the development of genetic circuits for this genus demonstrate the potential of these organisms as chassis for long-term sensors and medicines in the microbiota. This paradigm can be extended to other gut microorganisms as well. Multi-omics analysis, machine learning, and automation are accelerating the design-build-test-learn (DBTL) cycle of synthetic biology. This cycle could be applied to "domesticate" important gut microbes other than *Bacteroides*, which would provide new tools to study the structure–function relationships of the microbiota with the host and to develop novel therapies for human diseases. For example, bioengineered bacterial sensors could be put into disease models and humans to detect which biomarkers are relevant *in situ* in response to important clinical disease states. Libraries of consortia with various payloads could be created to determine which organisms and which payloads could change the microbiota to alleviate inflammation or treat disease. Human gastrointestinal organoid models could be designed to accelerate the study of interactions between the microbiota and human cells. So far, we have just scratched the surface of gut microbiome engineering, and the future is promising as synthetic biology and microbiome science continue to intersect.

Declaration of competing interest

T.K.L. is a co-founder of Senti Biosciences, Synlogic, Engine Biosciences, Tango Therapeutics, Corvium, BiomX, Eligo Biosciences, Bota.Bio, and Avendesora. T.K.L. also holds financial interests in nest.bio, Ampliphi, IndieBio, MedicusTek, Quark Biosciences, Personal Genomics, Thryve, Lexent Bio, MitoLab, Vulcan, Serotiny, and Avendesora. N.H. is an employee of JSR Corporation. Y.L. declares no competing interests.

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** of outstanding interest

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