# Advancements in microbiome engineering using CRISPR: unlocking the potential for better medicine

**Our gut microbiome is an ecosystem that science is only now starting to appreciate for its immense therapeutic opportunities. Here, Ziv Lifshitz, Vice President of R&D at Trobix Bio, describes some approaches using CRISPR technologies to engineer the microbiome for improved health.**

## The microbiome and human health

In recent years, the diverse and dynamic community of gut microorganisms, collectively termed microbiome, has emerged as a pivotal player in the realm of human health. From digestion to immunity, mental health to drug metabolism, this unseen world that resides within us all has been shown to exert dramatic influence over our health.[1]

Understanding the microbiome's crucial role in health and in various diseases has given rise to novel therapeutic strategies that aim to harness the microbiome to address both acute and chronic illnesses.

This article explores CRISPR-based technologies targeting the microbiome and their applications to the development of therapeutics, showcasing some of the innovative tactics employed in this swiftly evolving domain.

## Applying CRISPR to engineer the microbiome

Since its discovery, CRISPR genome editing technology stands as a ground-breaking tool that allows scientists to precisely edit targeted DNA to introduce changes that silence target genes, or alter or introduce new functionality. In short, CRISPR is a powerful tool for genetic research in medicine and biotechnology.[2]

It is worth noting, however, that although CRISPR has emerged as a game-changer in this field, it is not the sole technology that aims to restore the balance and functionality of the microbiome. Several other approaches are utilsed, notably the FDA-approved drug VOWST™ from Seres, a consortia of faecal microbiome spores that prevent reoccurrence of *Clostridium difficile* infection.

Within the context of microbiome engineering, the uses of CRISPR technologies can be roughly classified into two modalities, based on where the bacteria are genetically modified using CRISPR: outside the target organ (*in vitro*), or from within (*in situ*).

***In vitro* CRISPR** has been used as an indispensable tool for studying undomesticated microbiome bacteria, uncovering genes' functions, and unveiling complex interactions within microbial communities. CRISPR facilitates the development of live biotherapeutic products (LBPs) by equipping bacteria with novel functions, degrading harmful compounds, or enhancing immune responses to promote health or combat diseases.[3] Two examples in clinical development are Synlogic's *Escherichia coli* strain SYNB1934, engineered to degrade Phenylalanine in the gut to treat phenylketonuria (PKU) patients; and Advaxis' ADXS11-001, a *Listeria monocytogenes* strain engineered to secrete an antigen-adjuvant fusion protein to treat cancer.

Despite the promise of LBPs, this approach holds several challenges, mainly the ability to adjust to the microbiome structure of the individual and exist in it over varying time spans to consistently deliver the desired therapeutic impact. This means that LBPs often have a limited lifespan within the host's microbiome and their beneficial effects may be temporary.

One of the approaches of ***in situ* CRISPR** engineering utilises bacteriophages (phage) as delivery vehicles to introduce CRISPR-encoding DNA into the microbiome to modify them *in situ*. Phages are naturally highly selective, affording selectivity on one hand, but necessitating a 'cocktail' approach that uses various types of phages to target even closely related bacterial strains on the other hand.

The advantages of this *in situ*-phage approach is that it enables the modification of even unculturable bacteria with complex phenotypes and obviates the otherwise challenging need to colonise the targeted bacteria. The CRISPR modifies targeted cells by adding novel therapeutic function or inhibiting a specific detrimental function to drive therapeutic effect.

Companies such as Eligo Bioscience and SNIPR BIOME use modified phages to deliver their CRISPR/Cas9 systems into specific targeted gut bacteria. These CRISPR systems can kill bacterial cells by cutting their DNA to remove pathogenic gut bacteria, or to produce and secrete biotherapeutics in situ.

Locus bioscience delivers a cocktail of phages engineered with a CRISPR/Cas3 targeting *E. coli* to treat urinary tract infections.

With all of these approaches, however, the transient nature of the therapeutic effect, due to the rapid turnover of gut bacteria, remains a challenge.

At Trobix Bio we are working to address the transient nature of the gut microbiome. We have engineered phages to enable in situ microbiome engineering and also utilise CRISPR to favour survival and reproduction of targeted engineered bacteria. In other words, CRISPR is used as a protection system that favours *in vivo* selection of engineered bacteria carrying the desired trait. This enables therapeutic candidates to generate a robust and durable therapeutic effect, without disruption of the overall microbiome.

Our orally available therapeutic candidates use engineered phages to specifically target human *E. coli* in the

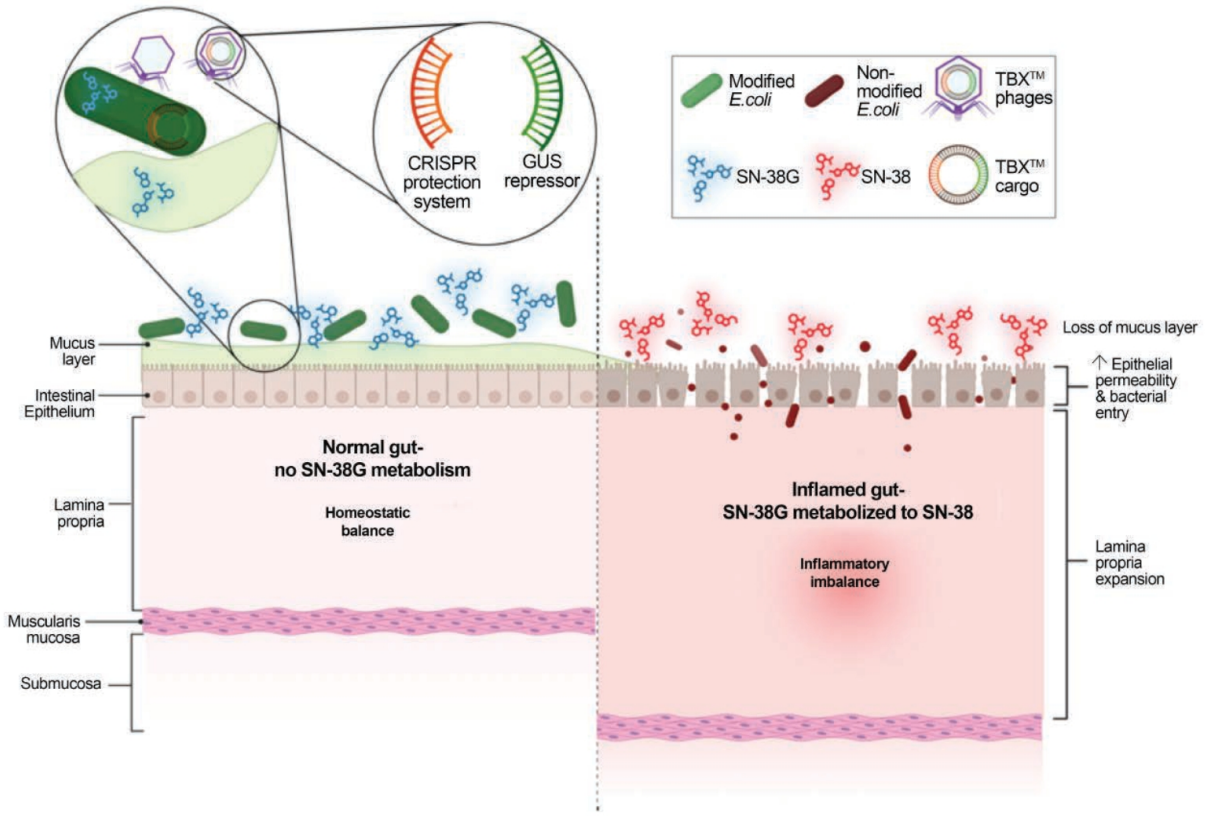


**Figure 1:** Illustration of Trobix Bio's TBX201 mechanism of action. TBX201 is a cocktail of phages that precisely delivers DNA cargo to target *E.coli* bacteria in the gut. The DNA cargo inhibits the bacterial GUS enzyme in the target bacteria, preventing the metabolism of Irinotecan's metabolite SN-38G to its toxic form SN-38, thus alleviating the toxicity of the cancer drug. The CRISPR/Cas3 protection system drives the *in vivo* selection of the desired, modified *E.coli*-GUS-inhibited bacteria.

microbiome. DNA cargos are packed within the phage's capsid, hold the genetic information to promote the production of therapeutic proteins in target bacteria or to control specific bacterial function, as well as a CRISPR/Cas3 protection system. To maintain this effect over time, a second type of phage, termed selective phages, are orally administered to kill any targeted bacteria not carrying the DNA cargo. Thus, during the period of treatment the modified therapeutic bacteria that hold the DNA cargos are provided positive reinforcement required to dominate. Preclinical proof of concept has been achieved with the first product candidate, an anti-infective agent for antimicrobial-resistant bacteria.

The enrichment of the desired modified gut bacterial population, driven by the protection of the CRISPR and the use of selection pressure in the form of engineered phages, differentiates the Trobix Bio approach in the field of microbiome-based biotherapeutics.

## Therapeutic candidates for cancer drug side effects

Two therapeutic candidates, TBX201 and TBX301, are currently being developed to treat life-threatening gastrointestinal side effects of leading cancer drugs, Irinotecan and immune checkpoint inhibitors (ICI), respectively. TBX201 was designed to reduce hospitalisations and improve quality of life for patients that may suffer from severe Irinotecan-induced diarrhoea. It specifically inhibits a specific bacterial enzymatic activity of *E. coli*, which is the underlying cause of Irinotecan-induced diarrhoea. TBX301 is designed to reprogramme the microbiome to secrete the anti-inflammatory cytokine IL-10 locally in the gut, enabling local treatment of colitis, a common ICI side effect. This local treatment aims to replace use of the immunosuppression effect of systemically administered anti-inflammatory drugs currently used to treat ICI-induced colitis, which can obviate the effects of ICI, thus improving treatment outcomes and patients' quality of life.

Despite impressive promise of microbiome therapeutics, there are still significant challenges in bringing microbiome-based biotherapeutics to patients: the regulatory pathway for microbiome therapeutics has yet to be paved; the underlying mechanisms of the microbiome-host relationship require further study; and industrial large-scale GMP manufacturing of microbiome-based therapeutics, while possible, remains challenging and expensive.

Nevertheless, microbiome engineering using CRISPR-based technologies holds the potential to reshape the future of healthcare with targeted treatments that optimise our microbiomes to support health and wellbeing.


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Ziv serves as Vice President of R&D at Trobix Bio. He holds a PhD in molecular microbiology and, together with Trobix's scientific team, leads the development of the company's platform technology and its products.



References

1. Aggarwal N, *et al.* Microbiome and Human Health: Current Understanding, Engineering, and Enabling Technologies. 1112313172nd ed.: *Chem Rev.*; 2023.
2. Nidhi S, *et al.* Novel CRISPR-Cas Systems: An Updated Review of the Current Achievements, Applications, and Future Research Perspectives. 242273327th ed.: Int J Mol Sci.; 2021.
3. Peng N, *et al.* CRISPR-Cas-Based Engineering of Probiotics. 520230017th ed.: *BioDesign Res*; 2023.