Greenstone Biosciences Gut New Approach Method (NAM) Portfolio: Modeling Human Gastrointestinal Disease *In Vitro*

Gut hiPSC Derived NAMs

Human-induced pluripotent stem cell (iPSC)-derived gut organoids and intestinal epithelial cells represent a groundbreaking platform for studying intestinal development, barrier biology, inflammation, and interactions between the host and microbes. These systems provide scalable, genetically defined, and patient-specific models that address significant limitations associated with traditional 2D cell lines and animal models. Recently, the FDA announced an initiative to reduce reliance on animals in drug discovery and development, aiming to shorten the time and costs of developing new gastrointestinal drugs. The FDA Modernization Act 2.0/3.0 has further facilitated the use of human-based in vitro assays, referred to as New Approach Methods (NAMs), in drug discovery.

Our hiPSC biobank includes a variety of healthy and disease-specific iPSC lines that can be utilized to develop human gut NAMs for drug screening, evaluating drug pharmacokinetics (ADME) and pharmacodynamics, and conducting preclinical safety assessments. These methods will expedite the discovery of new therapies for several chronic gastrointestinal diseases, including inflammatory bowel disease (IBD), colorectal cancer (CRC), celiac disease, and more.

Contents

iPSC-Derived Gut Organoids

iPSC-derived gut organoids are three-dimensional, self-organizing models of the intestine created from human induced pluripotent stem cells (iPSCs). They replicate key structural and functional aspects of the native intestinal epithelium. During directed differentiation, iPSCs progress through stages into definitive endoderm, then mid/hindgut progenitors, culminating in complex organoids that contain various intestinal cell types such as enterocytes, goblet cells, enteroendocrine cells, and Paneth cells. These organoids develop polarized epithelial layers with working barrier functions, mucus secretion, and relevant signaling pathways. This makes them highly useful for studying epithelial integrity, host–microbe interactions, and inflammation-related diseases like inflammatory bowel disease. Additionally, because they can be derived from patient-specific iPSCs, gut organoids support personalized disease modeling, drug testing, and mechanistic research in a controlled, human-relevant environment.

Product Specifications

Identity Markers:

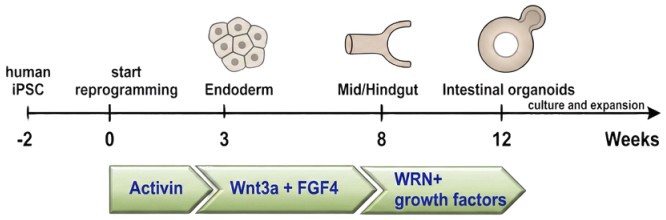
Sucrase isomaltase (enterocytes)
MUC2 (goblet cells), LGR5 (stem cells)
Lyz (Paneth cells)

Quantities:

≥ 500 organoids per vial

Shipping Conditions

Cryopreserved cells; Shipped on Dry Ice;
Store in liquid nitrogen upon arrival



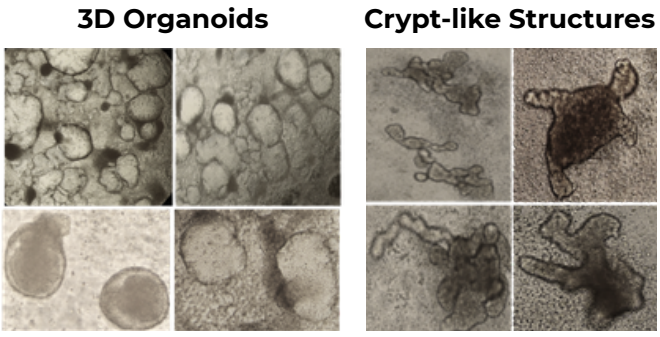


Figure 1. (Top)IPSCs are differentiated into intestinal organoids via two intermediate stages; endoderm and midgut. (Bottom) Representative images of different stages of iPSC-derived intestinal organoids: 3D spheroids, and crypt structure organoids.

Greenstone Biosciences (GSB) iPSC-Derived Gut Organoids

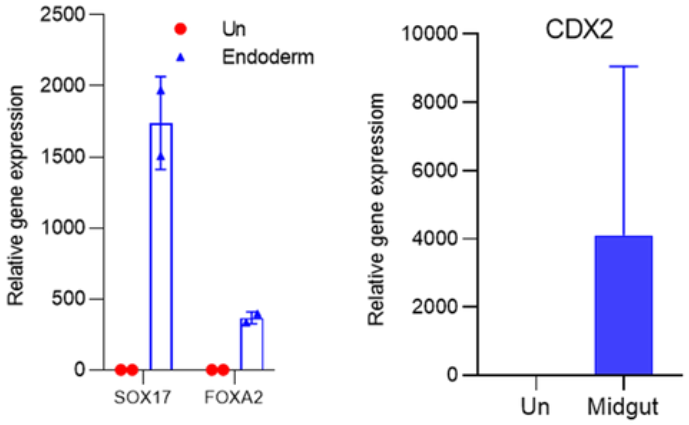


Figure 2. GSB endoderm is characterized by the expression of SOX17 and FOXA2. GSB midgut is characteized by the expression of CDX2.

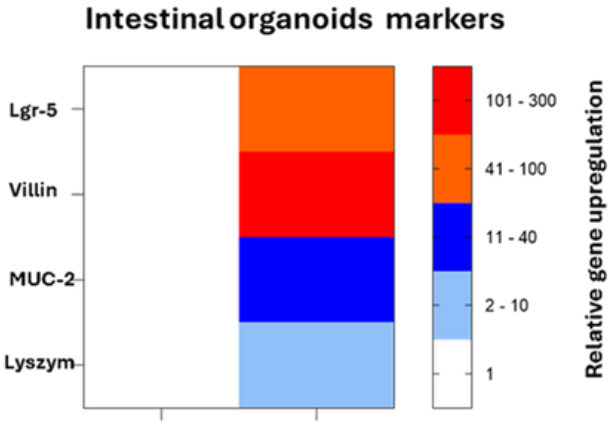
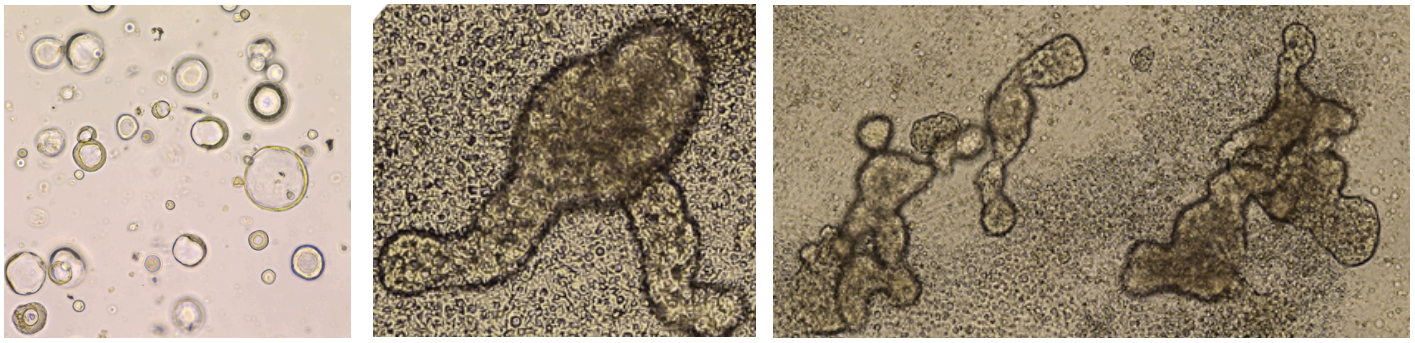


Figure 3. GSB intestinal organoids are characteized by the stemness marker of proliferating organoids (Lgr5), and other markers for differentiated epithelial cells , such as Sucrase-Isomaltase (SI) and villin for enterocytes, MUC 2 for Goblet cells, and Lyz for Paneth cells.

Figure 4. GSB intestinal organoids: (Left) typical proliferating colon oragnoids. (Middle and Right) Intestinal oragnoids show the crypt- and villus-like domains.



Gastrointestinal Disease Patient Selection: Clinical Trial in a Dish (CTiD)

The Greenstone Bioscience Biobank contains more than 2,000 unique patient hiPSC lines, many of which are derived from individuals diagnosed with gastrointestinal diseases, including Crohn's disease, ulcerative colitis, celiac disease, and colorectal cancer. Additional lines are available from patients with a range of oncology, neuromuscular, metabolic, and rare diseases.

iPSC-derived intestinal organoids faithfully preserve the inflammatory features and disease phenotypes of the original patient samples. For instance, organoids generated from IBD patients reproduce the human intestinal epithelium and recapitulate characteristic inflammatory traits, such as elevated expression of inflammatory cytokines MCP-1, IL-8/CXCL-8, and MIP-1. These IBD-derived organoids also display impaired barrier integrity: tight junction (TJ) proteins occludin and zonula occludens-1 (ZO-1) are disrupted, and the gut leakiness marker claudin-2 is upregulated.

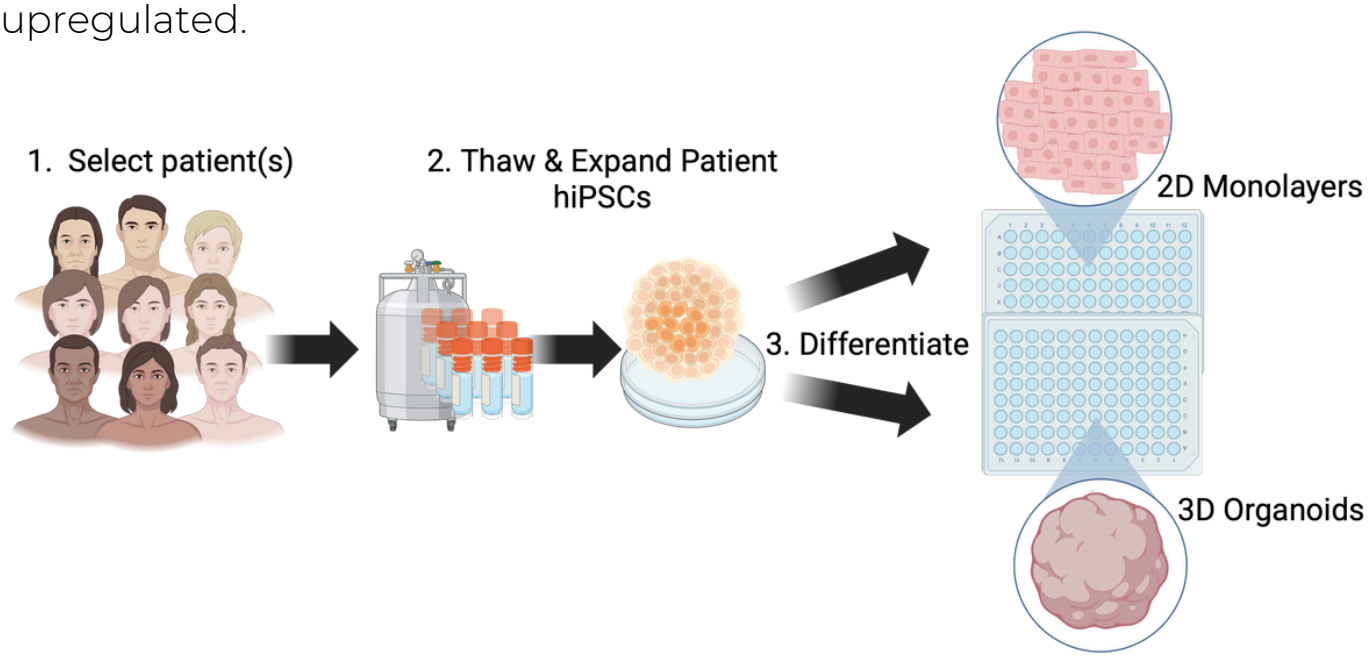


Figure 5. Greenstone Biosciences offers a complete beginning to end Clinical Trial in a Dish service. Step 1. choose patient(s) to include for in vitro CTiD. Step 2. GSB scientists retrieve, thaw and expand patient hiPSCs. Step 3. GSB scientists differentiate patient hiPSCs to 3D gut organoids and 2D differentiated intestinal epithelial cells.

Gut-In-A-Dish Platform

3D intestinal organoids (enteroids) are enriched in LGR+ stem cell progenitors, which drive self-renewal and proliferation. These organoids can be differentiated into polarized enteroid-derived monolayers (EDMs), where stem cell progenitors give rise to multiple epithelial lineages, including enterocytes, enteroendocrine cells, goblet cells, Paneth cells, and tuft cells. In EDMs, the proportion of stem cell progenitors is markedly reduced (Figure 7), resulting in a model that more closely resembles the native intestinal epithelium. In this gut-in-a-dish system, differentiated enterocytes exhibit apical–basolateral polarity, enabling quantitative assessment of drug pharmacokinetics through transport from the apical surface (facing the gut lumen in the model) to the basolateral surface (facing the blood circulation), as well as bidirectional movement.

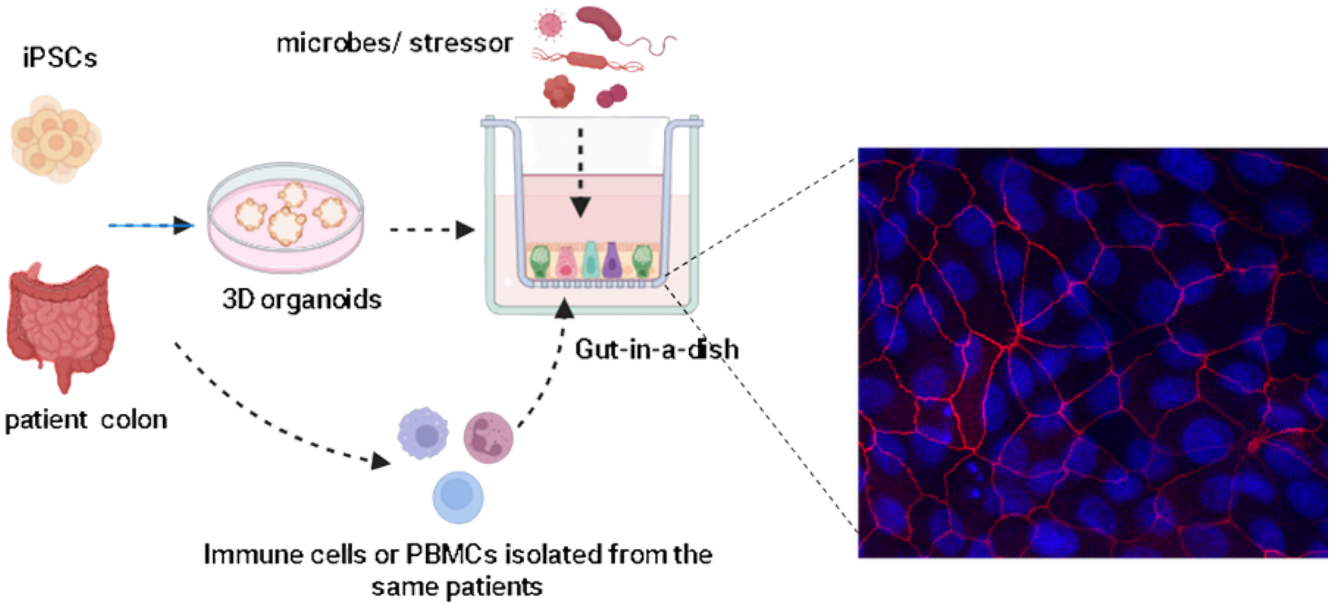


Figure 6. (Left) Development of Gut-in -a dish paltform. 3D organoids derived from iSPC or patient colon are differentiated into all types of intestinal epithelial cells on transwell, where all types of epithelial cells . The polarized epithelial cells have apical part where mircobes or stessorc can be added, and basolateral part where the immune cells, fibroblasts and other cell types are added. (Right) Immunofluorescence staining showing ZO-1 (red) in the polairzed epithelial cells.

Gut Permeability Assay

Gut permeability is assessed using transepithelial electrical resistance (TEER) and FITC-dextran flux assays to quantify epithelial barrier integrity and paracellular permeability. TEER measurements provide a real-time, noninvasive assessment of tight junction function by monitoring electrical resistance across polarized intestinal epithelial or organoid-derived monolayers, with decreased resistance indicating barrier disruption. In parallel, permeability to fluorescently labeled FITC-dextran is evaluated by measuring its translocation across the epithelial layer, providing a sensitive readout of paracellular leak. Together, these assays enable quantitative evaluation of inflammation-induced barrier dysfunction and the capacity of therapeutic candidates to restore epithelial integrity in disease-relevant in vitro models of inflammatory bowel disease.

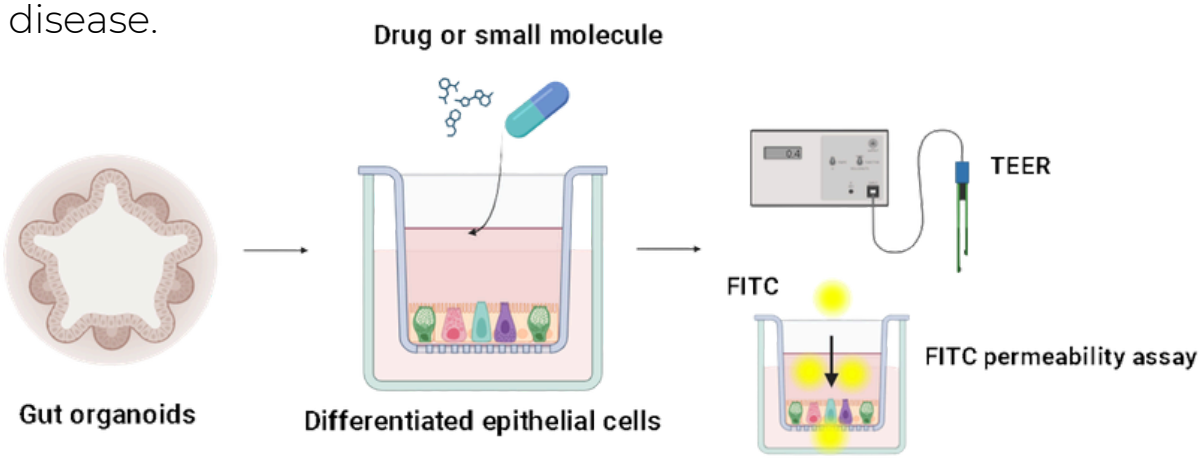
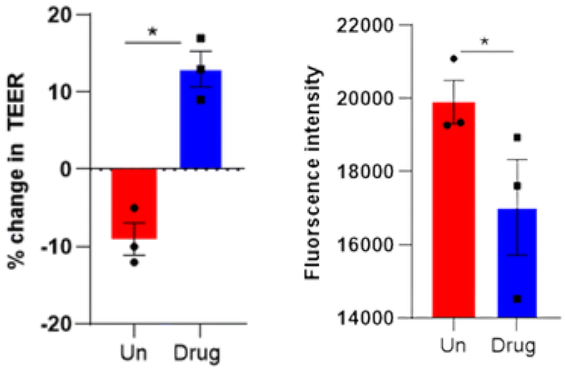


Figure 7. Gut organoids can be differentiated into polarized epithelial cells. The effect of drugs or small molecules on gut permeability by two methods: a) Measurement of transepithelial electrical resistance (TEER)., b) Perform FITC permeability assay

Figure 8. (Left) TEER measurement: A drug increases intestinal barrier function and prevents TEER reduction. (Right) A drug increases the intestinal barrier, reduces leakiness, and reduces the permeability of FITC.



Assessment of Drug-Induced Gut Injury or Toxicity

Human gut organoids provide a physiologically relevant platform to assess drug-induced intestinal toxicity by recapitulating key structural and functional features of the human intestinal epithelium, including polarized epithelial architecture, tight junction integrity, mucus production, and stem cell–driven regeneration. These three-dimensional systems, derived from primary tissue or pluripotent stem cells, enable evaluation of compound effects on epithelial viability, barrier function, cytokine release, and differentiation across multiple intestinal cell types.

Drug responses can be assessed using multiple complementary assays, including: (a) barrier function–related assays (TEER, FITC-dextran permeability, tight junction protein analysis); (b) membrane integrity/cytotoxicity assays (LDH release, propidium iodide [PI] uptake); (c) apoptosis-specific assays (TUNEL and Annexin assays); and (d) inflammatory signaling assays. Together, these human-relevant gut organoid strategies enhance prediction of gastrointestinal toxicity and support rational dose optimization.

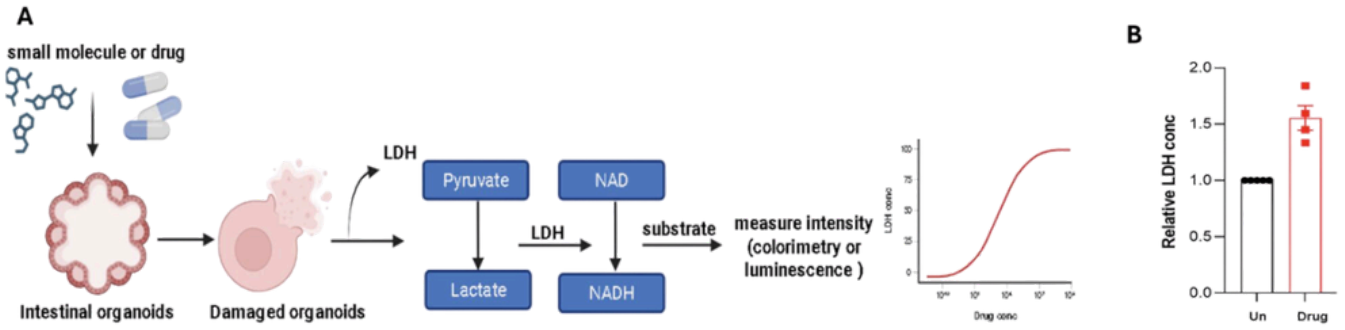


Figure 9. Assess GI toxicity using intestinal organoids and LDH assay. **(A)** Principle of the LDH assay. If the drug causes cell leakiness or damage, it increases the extracellular level of lactate dehydrogenase (LDH). LDH converts pyruvate into lactate, leading to the formation of NADH, which causes color changes or light emission when substrate is added. The signal intensity is proportional to LDH concentration, which reflects the degree of cell toxicity at different drug concentrations. **(B)** Measurement of relative LDH using intestinal organoids treated (drug) or not (untreated) with the drug. The level of LDH is higher in the supernatant of treated organoids than in the untreated ones.

Gut Organoids to Assess ADME

Human gut organoids provide a physiologically relevant platform to evaluate key pharmacokinetic (PK) properties, including drug absorption, metabolism, and epithelial transport. In this system, gut organoids and differentiated epithelial cells express clinically relevant drug transporters (e.g., P-gp, BCRP, MDR1, PEPT1) and metabolizing enzymes, including CYP3A4, CYP2C9, CYP2C19, and UDP-glucuronosyltransferases UGT1A1 and UGT1A3.

Using this model, we can assess:

- Drug permeability coefficients
- Drug half-life (t1/2)
- Metabolites present in both apical (gut-facing) and basolateral (blood-facing) compartments
- Drug–drug interactions

By quantifying compound permeability, transepithelial transport, metabolic stability, and first-pass metabolism in organoid systems, we can better predict oral bioavailability and intestinal clearance.

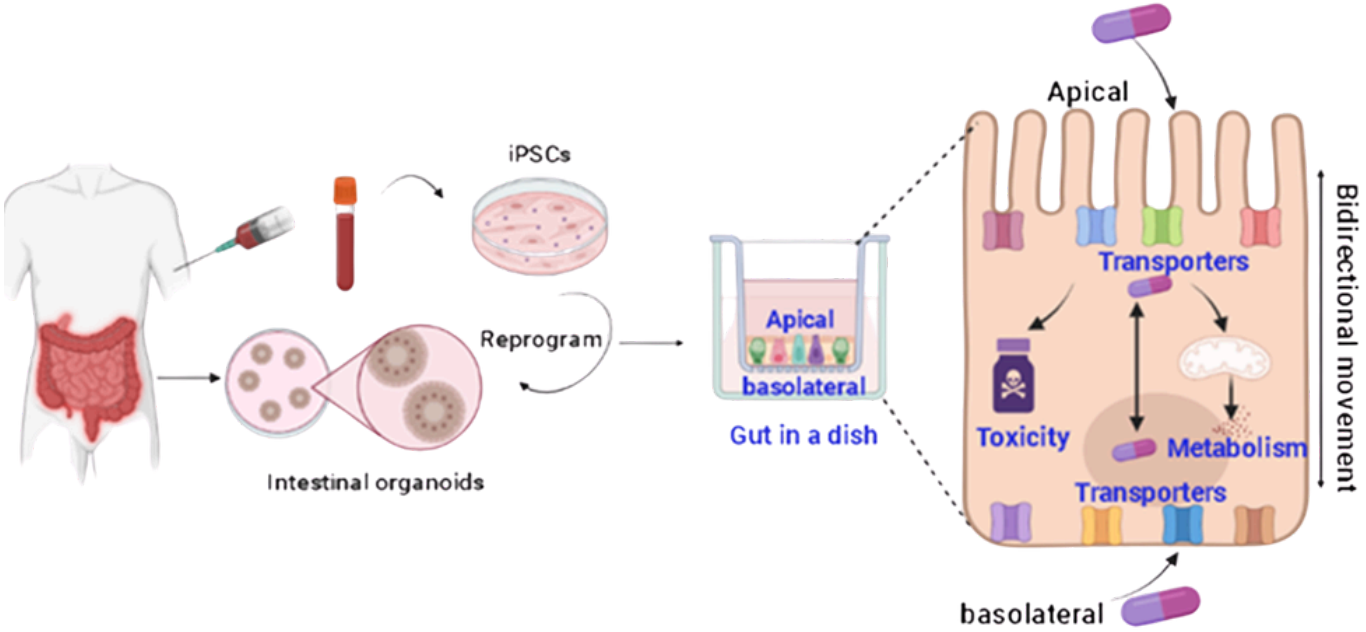


Figure 10. Schematic diagram for the use of intestinal organoids in drug pharmacokinetics and toxicology.

GSB Product Deliverables

GSB's products include intestinal organoids derived from healthy and diseased patients, generated from iPSCs and primary human colon epithelial cells. These 3D intestinal organoids will be used to develop two platforms: the gut-in-a-dish and gut-on-a-chip. We can also enhance the organoids further by introducing mutations or modifying gene expression—either overexpressing or knocking out genes—using CRISPR-Cas9 or other methods.

The gut-on-a-chip is an advanced platform designed for drug screening and development. It enables co-culture of epithelial cells, microbiota, and other gut cell types within a microfluidic system. The platform simulates the gut environment through controlled fluid flow, oxygen levels, and peristalsis-like motions. When co-culturing intestinal organoids or derived epithelial cells with immune cells and microbiota in the chip, it closely mimics human gut physiology and provides a powerful tool for disease modeling.

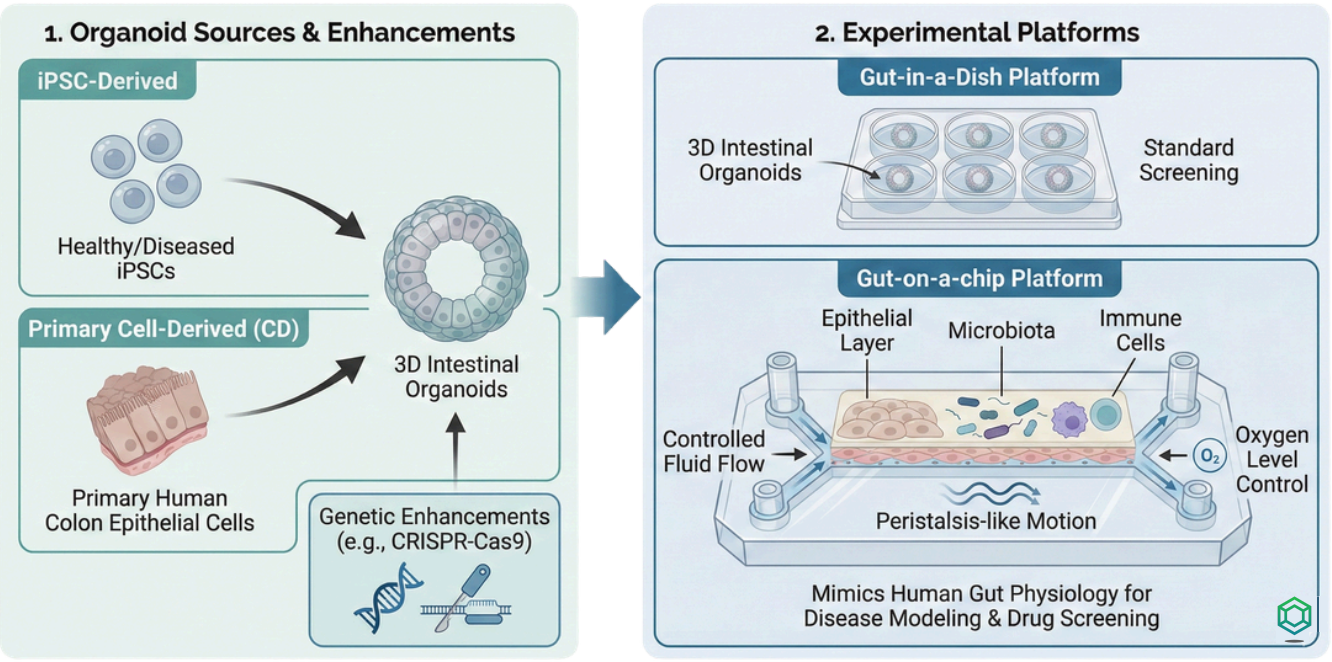
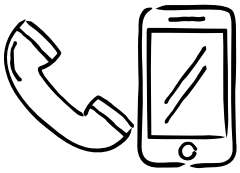


Figure 11. GSB products include 3D iPSCs-derived intestinal organoids, colon-derived organoids, gut-in-a-dish platform, and gut-on-a-chip system. The 3D organoids can be differentiated into polarized 2D epithelial cells for high-throughput screening or used in a chip system.

Contact Us



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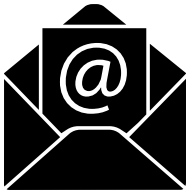

info@greenstonebio.com

https://greenstonebio.com

Alexandria Center for Life Sciences
At Stanford Research Park
3160 Porter Drive, Suite 140
Palo Alto, CA 94304

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BIOSCIENCES