The variety of normal and diseased human tissues needed to perform comprehensive screening of drug candidates is a key resource for next-generation preclinical models, but obtaining these tissues is challenging. Gene editing enables Altis Biosystems to offer a diverse array of RepliGut® human gut models tailored for specific research programs.

ENHANCING HUMAN GUT TISSUE MODELS THROUGH GENE EDITING

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ALTIS BIOSYSTEMS

Many Challenges Around Accessing Tissues

Disease modeling provides the best results when actual human tissues are used. However, obtaining the full diversity of tissue types and capturing the variation of human cellular responses to pharmaceuticals in a dish is difficult. Accessing normal, healthy tissue to use as controls is one primary issue. Another, even more challenging issue is accessing tissues

from many different people that have varied genetic backgrounds, which many times influence disease penetrance or drug efficacies. As a result, tissue access represents a bottleneck and a substantial challenge for disease modeling using tissue culture. Ultimately, the lack of this precious resource negatively impacts preclinical studies trying to evaluate drug efficacy across the human population or disease cohort.

The Potential of Genetic Engineering

Genetic engineering provides a novel opportunity to model diseases. Gene-editing techniques, such as the CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9) technology and associated variants, can be applied to introduce disease-causing mutations into stem cells in a controlled manner. If tissues are available from individuals with appropriate genetic diversity, the consequences of specific mutations superimposed onto different genetic backgrounds can be observed, providing a better understanding of how drugs will perform across a population with a certain disease.

Gene-Editing Opportunities for RepliGut® Models

Altis Biosystems has developed RepliGut®, a next-generation commercial intestinal tissue model platform for in vitro testing during drug development and for basic science applications. The RepliGut® platform produces a polarized monolayer of human intestinal stem and differentiated cells — either of the large or small intestine — that can be used for modeling diseases or screening compounds for effects on the intestine.

We have developed efficient methods for introducing or knocking out genes in the cells of our RepliGut® system to create customized disease models for drug development and other applications. With our gene-edited models, it is possible to investigate how a specific genetic variation affects the performance of a drug, and the effects of a disease-causing mutation can be analyzed in donor cells across different genetic backgrounds.

The methodological leap is being able to target mutations to a specific gene in small intestinal or colonic stem cells. Once prepared, the genetically engineered, self-renewing stem cells can be frozen, providing an indefinite supply of cells with the causative or relevant mutation for that disease state. In essence, we see RepliGut® as a platform upon which gene editing of cells can serve as a unique resource to pharmaceutical companies and/or basic biologists interested in evaluating stem cell maintenance and differentiation in physiologic or disease states (such as barrier function and inflammation) or when evaluating on-/off-target drug effects.

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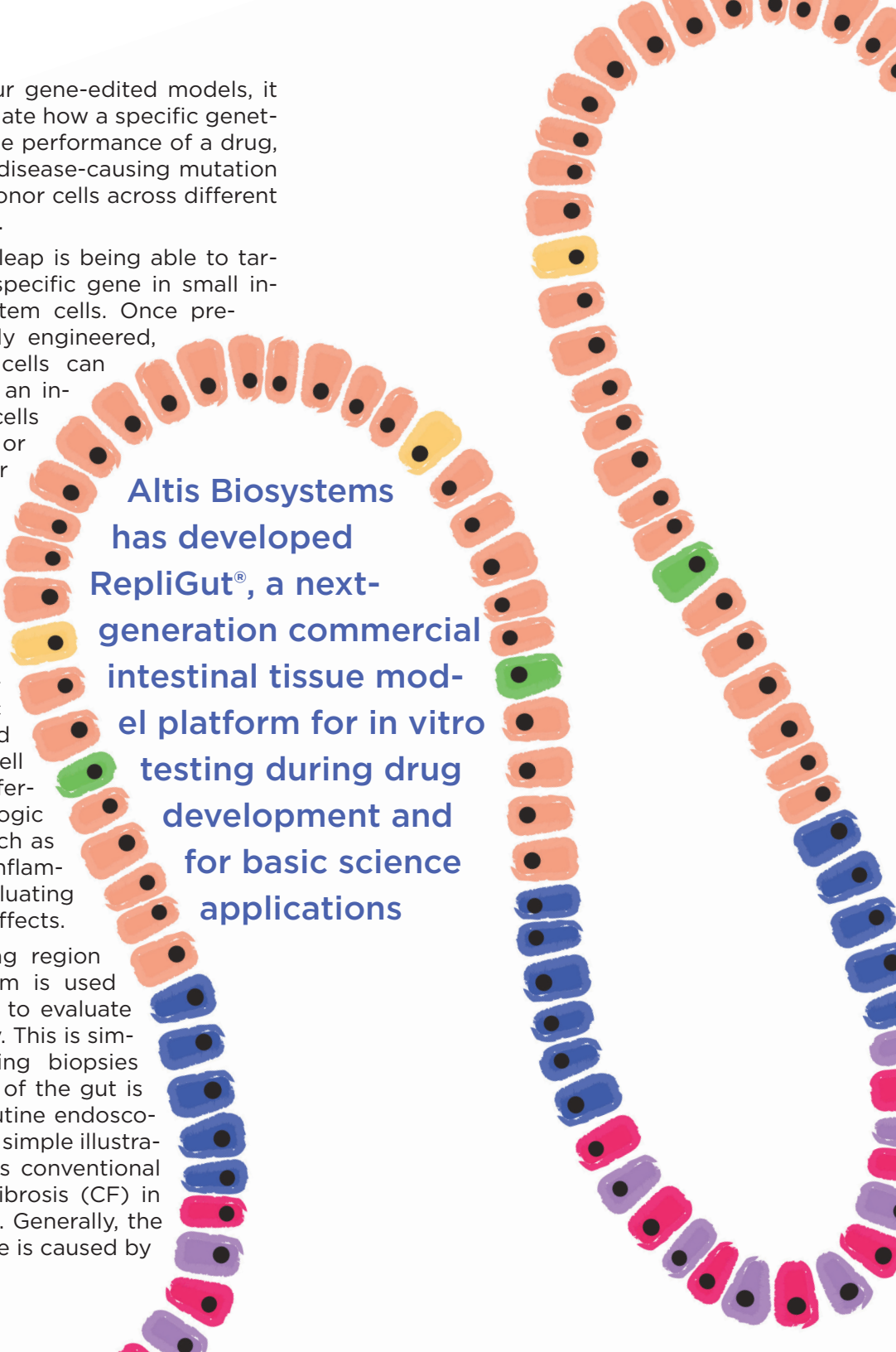
Too often, the wrong region of the gut epithelium is used to study diseases or to evaluate drug efficacy/toxicity. This is simply because obtaining biopsies from a large portion of the gut is not accessible by routine endoscopy or colonoscopy. A simple illustration of this is issue is conventional modeling of cystic fibrosis (CF) in 3D gut tissue culture. Generally, the GI form of the disease is caused by



a single or compound mutations that cause improper mucous hydration and clinical sequela in the small intestine. However, these regions are typically not accessible for obtaining tissue samples from CF patients by routine endoscopy or colonoscopy; therefore, rectal biopsies are often used. At Altis, however, we can readily engineer CF mutations into the relevant region of the small intestine that is susceptible to the disease and provide those types of tissues to our customers.

In many cases unknown variants within a population can cause drugs to fail in preclinical screening, because they do not provide a similar pattern of behavior across the broad population. Gene editing of RepliGut® models across varied human genetic backgrounds enables us to develop better tools for use as preclinical models. The statistical power of these models will only increase as we expand our donor biobanks and add samples from more individuals representing a wider range of demographics.

ing in real time and in high-throughput where the proteins are being expressed and whether they are being disrupted or whether their morphology is altered when exposed to classes of drugs or synthetic diets.

One of the limitations of gene editing today is its efficiency. To introduce numerous edits requires multiple passages of the cell through the process, which not only takes time but often has unintended consequences. Ideally, we would like gene editing to reach a level of efficiency that will allow the simultaneous introduction of many multiple mutations known to cause a complex disease, something that very much appears to be on the horizon.

In the meantime, Altis has developed solutions to increase throughput on the current version of RepliGut®: a two-dimensional tissue construct that can be placed into higher-throughput plate-well formats for screening of different guide RNAs or donor plasmids.

implicated in roles in a broad range of diseases. For instance, engineering a mutation in a specific cell receptor could provide information about how certain types of microbes interact with host cells and transmit inflammatory signals, or whether mutations that impact mucous hydration levels regulate microbiota colonization or mucous infiltration.

Speedy Access to Customized Tissues

One of the greatest advantages of having gene-editing capability at Altis is that we are able to provide drug developers with specific RepliGut® models tailored to their research needs. We can create RepliGut® models that contain cells that will help drug developers understand specific behaviors and can do so quite rapidly.

If a drug developer is looking to understand how different candidate drugs will perform in patients with the same mutation but with varied underlying genetic landscapes, we can generate the relevant mutant RepliGut® lines in the same region of the small or large intestine across a number of different tissue donors. The customer can then screen the compounds across the cohort of engineered disease model, or Altis can provide that service for them. The key is creation of a custom gene population tailored to the research program, which is very exciting — I don't believe that anyone else offers this capability, particularly in a high-throughput format.

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Leveraging CRISPR-Cas9

Gene editing at Altis is largely performed using CRISPR-Cas9 and RNAs to target certain loci and create point mutations to destroy the function of a specific gene or to knock-in reporter genes that "report" specific physiological properties of cells or tissue monolayers.

For instance, we can influence stem cell differentiation to investigate the temporal dynamics of differentiation (e.g., rate, cell ratios) or stem cell state (e.g., level of proliferation/self-renewal) and how certain drugs affect these cellular states. Alternatively, we can link a fluorescent protein to the tight junction proteins responsible for gut barrier function, thereby monitor-

Exploring Impacts on the Microbiome

Gene editing of RepliGut® can also enable more detailed exploration of the impact of single-gene mutations on the microflora within the gut. This aspect of the technology is important, as the gut microbiome is increasingly

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In addition to his roles with Altis, Scott Magness is an Associate Professor in the Joint Department of Biomedical Engineering at the University of North Carolina at Chapel Hill (UNC) and North Carolina State University (NC State) and in the Departments of Medicine, and Cell Biology & Physiology at UNC. He obtained his Ph.D. in genetics and molecular biology from UNC Chapel Hill. Dr. Magness' expertise is in stem cell biology, intestinal physiology, single-cell genomics, and high-throughput platforms to study factors that influence GI stem cell maintenance & differentiation and the microbiome.