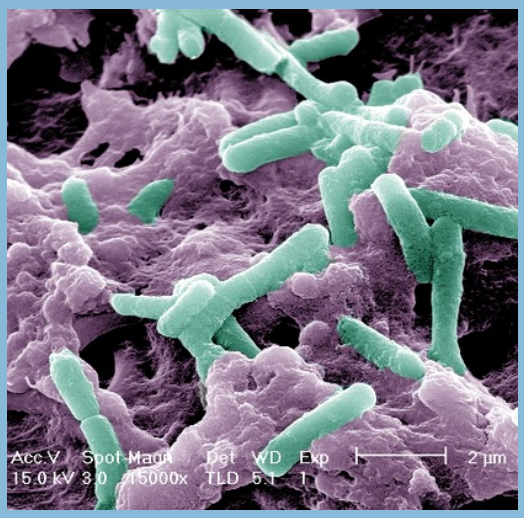
DDRC DIGEST

Digestive Disease Research Center
Medical University of South Carolina

Volume 1, Issue 4 **December 1, 2020**



Scanning Electron Micrograph of C. difficile bacteria on mucus generated by LS174T colonic cells. Courtesy of M. Engevik Lab (unpublished). See the article below about or most recent new full member investigators.

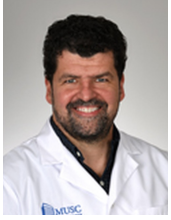
On the Inside

Welcome New DDRCC Members

As we pass mid-year, and in addition to the COBRE Junior Investigator and Pilot and Feasibility Awardees, we have accepted a number of new investigators into our **Full Member** ranks. Our new members span a broad range of clinical and basic research departments at MUSC. We welcome the addition of their diverse expertise, energy and talents to our community. A listing of all of the current DDRCC Full Membership may be found on the **Member Directory** page of the **MUSC DDRCC Website**.

Jessica H. Hartman, PhD Biochemistry and Molecular Biology

Metabolism and xenobiotic and mechanistic toxicology in liver disease.



John P. O'Bryan, PhD Cellular and Molecular Pharmacology

Therapeutics targeting RAS function in GI cancer.

Vanessa A. Diaz, MD Family Medicine

Telehealth and Health Information Technology to improve colorectal cancer screening and treatment.

Mindy A. Engevik, PhD Regenerative Medicine and Cell Biology

Microbial-host crosstalk and microbe-mucus interactions in the GI tract.

In addition to their participation in DDRCC community activities, conferences and Enrichment Series, Full Members will receive subsidized usage of our cores.

Look for the upcoming request for applications for the 2021 Pilot and Feasibility Award cycle early in 2021.

DDRCC Core Profile: Advanced Cell Core

Leadership

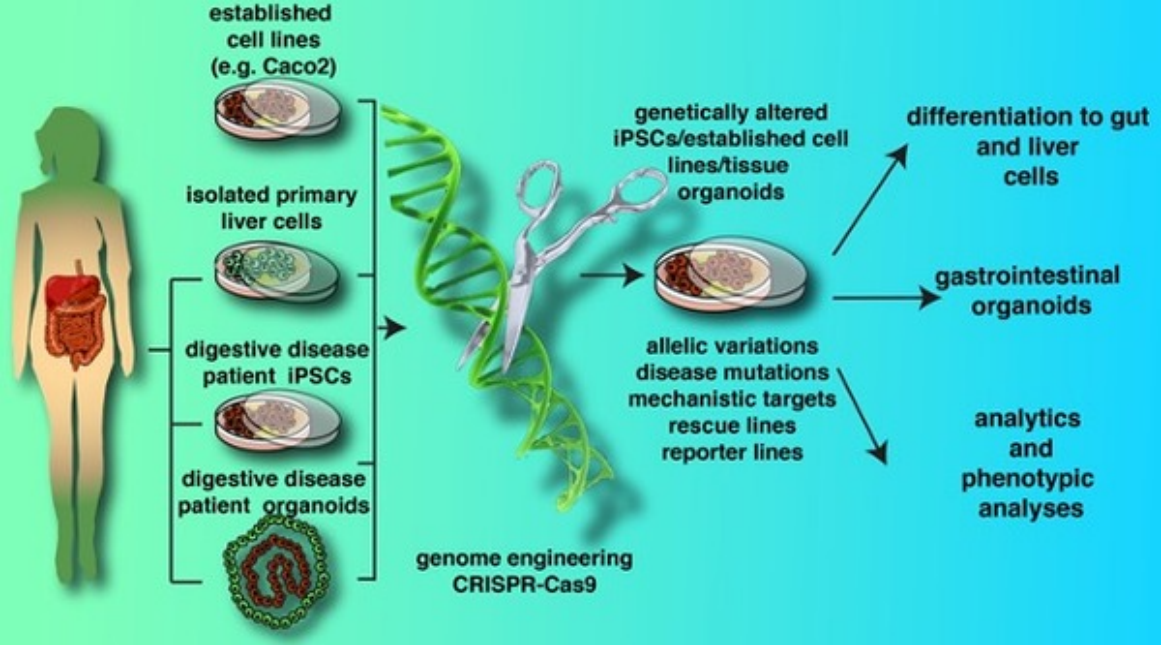
Stephen Duncan
Core Director

Jorge Munera
Associate Director

Christiana Kappler
Core Manager

Paige Lamprecht
Core Technician

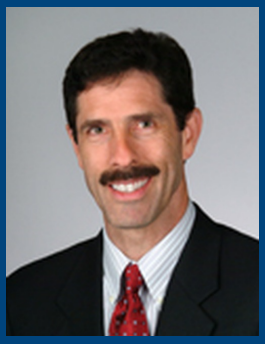
The Advanced Cell Core (ACC) offers production of state-of-the-art tissue culture models of digestive and liver disease, as well as training in the generation and use of these models to investigators within the CDLD and DDRCC. The core uses genome editing to generate human induced Pluripotent Stem Cells with genetic variations that can be used to recapitulate heritable diseases of the gut and liver. We offer investigators training in the differentiation of iPSCs to hepatocytes and gut organoids. The core also will isolate primary cells from the livers of mice.



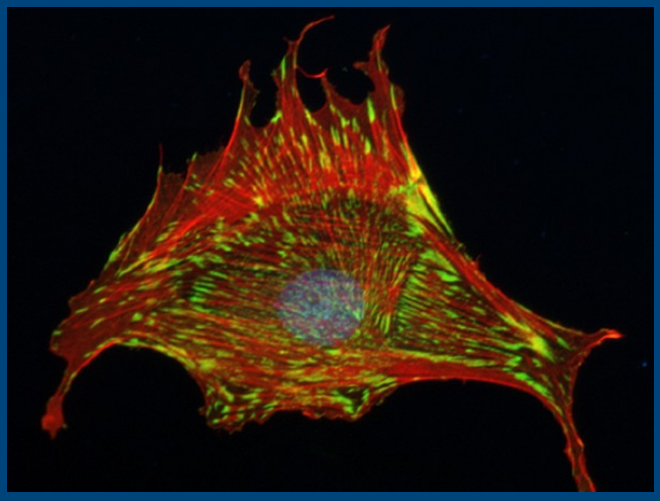
Overview of the Advanced Cell Core illustrating its function in producing and maintaining various cell models spanning the range from established immortalized human intestinal and liver cell lines to primary liver cells to genetically manipulable iPSC cells that are forward differentiated into GI and liver organoid cell model systems

The following services are available for users:

- **Generation of human iPSCs harboring indels in specific genes.**
- **Generation of specific variants in human iPSCs.**
- **Highly characterized wild type iPSCs and a subset of established cell lines.**
- **Provide validated reagents for the culture of iPSCs.**
- **Training in the handling and differentiation of iPSCs to hepatocytes and colonic and intestinal organoids.**
- **Isolation of primary hepatocytes and non-parenchymal cells from mice.**



Don Rockey
Associate Core Director
Cell Isolation Core



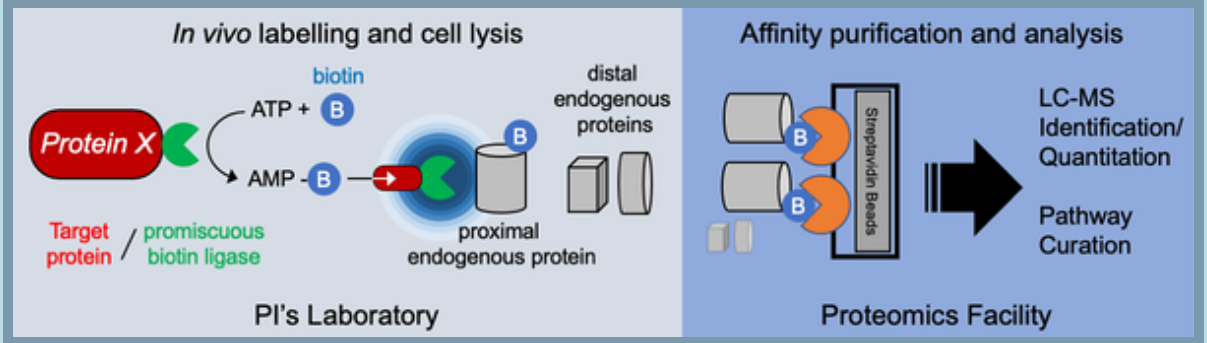
Hepatic Stellate Cell immunostained for Vinculin (*D. Rockey Lab*)

Initiating a project with the Advanced Cell Core:

The DDRCC and CDLD both fully subsidize the use of the ACC by its members. For initial consultation regarding starting an iPSC project with the core, please contact the Core Director, **Dr. Steve Duncan**. For primary cell isolation, please contact the Cell Isolation Core Associated Director, **Dr. Don Rockey**.

Core Spotlight: Proximity Labeling with BioID

Using *in vivo* proximity labeling and mass spectrometry-based proteomic analysis to detect novel protein-protein interactions and define biological network functions



Schematic overview of the BioID proximity labeling, affinity capture and LC-MS analysis of candidate in-vivo interacting proteins with a target protein of interest. *Adapted from Branon et al, Nat. Biotechnol. (2018) 36(9):880-887.*

A common challenge for determining the role of a particular macromolecular component or signaling element in cell function and phenotype is the delineation of its specific pathway interactions in space and time. Over the last several years, **proximity labeling** (**PL**) has gained traction over alternatives like cellular fractionation, co-localization and co-immunoprecipitation assays for this purpose. Conceptually, PL involves the in vivo expression of genetically-engineered fusion proteins combining a protein of interest with a promiscuous labeling enzyme, commonly **biotin ligase**, in a cell type of interest. Upon provision of a cell-permeable substrate, the ligase covalently attaches biotin moieties on proteins in the immediate neighborhood of their fused protein of interest. Following the biotinylation step, tagged proteins are then efficiently and cleanly purified from cell lysates using streptavidin beads and high stringency washing conditions facilitated by the extremely high affinity biotin-streptavidin interactions. LC-MS/MS analysis of the retrieved proteins then produces a “guilt-by-association” roadmap of the next-neighbor interactions between the tagged protein of interest and other cellular constituents in a particular cell type under specific sets of conditions (summarized in the accompanying figure).

The most commonly used biotinylating enzyme is **BioID/BioID-2**, an engineered mutant of E. coli biotin ligase. The BioID approach has been favored due to its robust function, versatility, and simplicity of use, requiring only the introduction of biotin to initiate efficient tagging. Its major limitation of relatively slow kinetics — requiring 18-24 hour incubation with biotin substrate and hence limiting its ability to detect spatially and temporally distinct interactions — has recently been overcome by further genetic engineering and the development of fast kinetic variants. The resulting **TurboID** enzyme allows for efficient labeling of MS-detectable levels of interacting proteins in as little as 1 hour. A further truncated variant, **mini-TurboID**, combines rapid biotinylating kinetics with a lower probability of steric hindrance due to the presence of the enzyme tag. Even further refinement of this technology enables biotin labeling to be restricted to specific cell compartments or interfaces using a **split-TurboID** variant.

The DDRCC Proteomics Core has a steadily accruing experience with BioID, and has guided several investigators through the initial considerations of fusion protein engineering, cellular expression level, experimental replicate and control design, sample preparation and downstream MS analysis. Recently, our Pilot and Feasibility awardee and DDRCC member **Wenjian Gan** has successfully used BioID-2 and PL in his quest to define the interaction network of the arginine methylase PRMT5 in collaboration with Lauren Ball and the DDRCC Proteomics Core or Mass Spectrometry Facility. In work currently under review for publication, he was able to validate the method through biotin-streptavidin retrieval of known PRMT5 target proteins, and identify several other novel upstream and downstream candidates, of both known and currently unknown function. A brief discussion of his experience and findings are included in the video below:

A discussion with Wenjian Gan and Lauren Ball about Wenjian's collaboration with the DDRCC Proteomics Core to map PRMT5 interacting proteins via BioID-2 proximity ligation (a transcript is available **here**.)

Initiating a project with the Proteomics Core

For a prospective consultation about initiating a collaboration with the Proteomics Core for approaches available to facilitate the study of protein interactions, email **Dr. Lauren Ball** and/or visit the Mass Spectrometry Facility **webpage**.

Enrichment Series Seminars

DDRCC / CDLD / GI and Hepatology Grand Rounds: Wednesday, 7am EST (Zoom)

December 2

Jay Pasricha, MD Johns Hopkins Medical Institutions, Baltimore, MD

From POEM to Duodenoscope contamination: lessons in biodesign thinking.

December 9

GI and Hepatology Fellows MUSC

Clinical case presentations.

Decemeber 16

Vikesh Singh Johns Hopkins Medical Institutions, Baltimore, MD

Genetic testing for pancreatitis: Does it impact clinical management?

December 23

No Seminar

Holiday

January 6

Douglas K. Rex, MD Indiana University School of Medicine

Advances in polyp detection with colonoscopy

January 13

Gregory G. Ginsberg, MD Hospital of the University of Pennsylvania

Management of GIST tumors

January 20

GI & Hepatology Fellows, MUSC

Case Conference: GI & Hepatology Service

January 27

Vivek Kumbhari,, MBBCh, PhD Johns Hopkins Medical Institutes

Metabolic endoscopy: potential and proof

DDRCC/CDLD/ RMCB Virtual Seminar Series: Wednesday, 11 am EST (Zoom)

December 2

Fiona Watt, FRS, FMedSci King's College, London, England

Exploring Skin Cell Heterogeneity

December 9

Jiandie Lin, PhD University of Michigan Medical School

Hormonal signaling in metabolic health and disease

December 16

Ge Tao, PhD Regenerative Medicine and Cell Biology, MUSC (Work in Progress)

Programmed death of cardiomyocytes after myocardial infarction

December 23

No Seminar

Holiday

January 6

Joseph Wu, MD, PhD Stanford University School of Medicine

TBA

January 13

Elaine Fuchs, PhD HHMI and Rockefeller University

TBA

January 20

Henry Sucov, PhD MUSC Regenerative Medicine and Cell Biology (WIP)

TBA

To receive notifications for our Enrichment series seminars, please contact the DDRCC Center Manager.

Notes from the DDRCC

A Note from the Center Manager: Citing Publications

Center Manager
Kyu-Ho Lee, MD-PhD

One of the bigger ongoing challenges for our center, particularly for our progress reports and applications for renewal, is the proper documentation of DDRC-supported papers. As Center Manager, I will be tracking publications by our Full and Associate Members. However, I need your help in ensuring that these papers cite our P30 and P20 awards.

This is most readily done **during the initial manuscript submission**. Whether or not you are the corresponding author, please make sure that the proper NIH grant numbers are entered (**P30 DK123704** or **P20 GM120475**).

Under some circumstances, grant support can be added retroactively using **MyBibliography** in NCBI. For details please see the brief article below.

Retroactively Acknowledging the P30 / P20 on NCBI

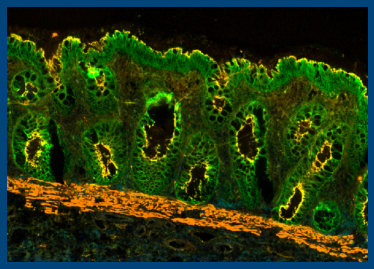
You can now affiliate or add acknowledgement of grant support for a paper after publication using the NIH ERA Commons system and MyBibliography in NCBI (National Center for Biomedical Information). FAQ's and step-by-step instructions for managing your MyBibliography account include details for checking compliance status for any of your publications, and associating funding from any NIH or HRA (Health Research Alliance) grant to individual publications, whether or not you are the corresponding author.

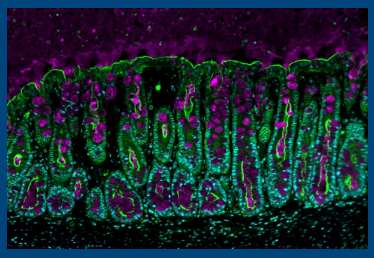
The process is straightforward, and compliance will be essential not only for DDRCC and CDLD progress reports and renewals, but also for your own grant applications.

For more individual assistance and consultation about handling compliance and associating P30 and P20 funding to your publications, please contact **Kyu-Ho Lee** or **Ms. Teri-Lynn Herbert** at the Colbert Education Center Library.

DDRCC Website Renovations

We are currently updating our DDRCC webpages, and would like to solicit contributions from our members. In addition to news items and open position notifications, we would like to include photos of labs or lab activities, journal cover images you may have had accepted, or interesting data images, published or unpublished. Please submit such items to the **Center Manager** for consideration.





Selected GI Publications by our Members

October - November, 2020

Clift CL, **Drake RR**, Mehta A, **Angel PM**. Multiplexed imaging mass spectrometry of the extracellular matrix using serial enzyme digests from formalin-fixed paraffin-embedded tissue sections. Anal Bioanal Chem. 2020 Nov 18. PubMed PMID: 33206215.

Blanding DP, Moran WP, Bian J, Zhang J, Marsden J, **Mauldin PD**, **Rockey DC**, **Schreiner AD**. Linkage to specialty care in the hepatitis C care cascade. J Investig Med. 2020 Nov 17. PubMed PMID: 33203787.

Desjardins M, Halstead L, Simpson A, Flume P, **Bonilha HS**. Voice and Respiratory Characteristics of Men and Women Seeking Treatment for Presbyphonia. J Voice. 2020 Nov 7. PubMed PMID: 33172730.

Engevik MA, Danhof HA, Shrestha R, Chang-Graham AL, Hyser JM, Haag AM, Mohammad MA, Britton RA, Versalovic J, Sorg JA, Spinler JK. Reuterin disrupts Clostridioides difficile metabolism and pathogenicity through reactive oxygen species generation. Gut Microbes. 2020 Nov 9;12(1):1788898. PubMed PMID: 32804011; PubMed Central PMCID: PMC7524292.

Hara Y, Yanatori I, Tanaka A, Kishi F, **Lemasters JJ**, Nishina S, Sasaki K, Hino K. Iron loss triggers mitophagy through induction of mitochondrial ferritin. EMBO Rep. 2020 Nov 5;21(11):e50202. PubMed PMID: 32975364; PubMed Central PMCID: PMC7645172.

Treem WR, Palmer M, Lonjon-Domanec I, Seekins D, Dimick-Santos L, Avigan MI, Marcinak JF, Dash A, Regev A, Maller E, Patwardhan M, Lewis JH, **Rockey DC**, Di Bisceglie AM, Freston JW, Andrade RJ, Chalasani N. Consensus Guidelines: Best Practices for Detection, Assessment and Management of Suspected Acute Drug-Induced Liver Injury During Clinical Trials in Adults with Chronic Viral Hepatitis and Adults with Cirrhosis Secondary to Hepatitis B, C and Nonalcoholic Steatohepatitis. Drug Saf. 2020 Nov 3. PubMed PMID: 33141341.

Herro R, Miki H, Sethi GS, Mills D, Mehta AK, Nguyen XX, **Feghali-Bostwick C**, Miller M, Broide DH, Soloff R, Croft M. TL1A Promotes Lung Tissue Fibrosis and Airway Remodeling. J Immunol. 2020 Nov 1;205(9):2414-2422. PubMed PMID: 32958689; PubMed Central PMCID: PMC7577982.

Howell AV, Gebregziabher M, Thiers BH, Paulos CM, Wrangle JM, Hunt KJ, **Wallace K**. Immune checkpoint inhibitors retain effectiveness in older patients with cutaneous metastatic melanoma. J Geriatr Oncol. 2020 Oct 29. PubMed PMID: 33132048.

Dunbar E, Greer PJ, Melhem N, Alkaade S, Amann ST, Brand R, **Coté GA**, Forsmark CE, Gardner TB, Gelrud A, Guda NM, LaRusch J, Lewis MD, Machicado JD, Muniraj T, Papachristou GI, Romagnuolo J, Sandhu BS, Sherman S, Wilcox CM, Singh VK, Yadav D, Whitcomb DC. Constant-severe pain in chronic pancreatitis is associated with genetic loci for major depression in the NAPS2 cohort. J Gastroenterol. 2020 Oct;55(10):1000-1009. PubMed PMID: 32681239.

Pournasr B, **Duncan SA**. Generation of isogenic Propionyl-CoA carboxylase beta subunit (PCCB) deficient induced pluripotent stem cell lines. Stem Cell Res. 2020 Oct;48:101953. PubMed PMID: 32822967; PubMed Central PMCID: PMC7640943.

Blaschke CRK, Black AP, Mehta AS, **Angel PM**, **Drake RR**. Rapid N-glycan profiling of serum and plasma by a novel slide-based imaging mass spectrometry workflow. J Am Soc Mass Spectrom. 2020 Aug 18. PubMed PMID: 32809822.

Branch LL, **Elmunzer BJ**, **Forster E**, Hoffman B, Moran RA, **Coté GA**. High Frequency of Multiple Imaging Studies During the Diagnostic Workup for Pancreatic Ductal Adenocarcinoma. Pancreas. 2020 Oct;49(9):e79-e80. PubMed PMID: 33003088.

A complete listing of DDRCC publications may be found on NCBI.

CITE OUR GRANTS

FOR THE DDRCC:
P30 DK123704

This project was supported in part by NIH P30 DK123704 (*core facility*) at the MUSC Digestive Disease Research Core Center.

FOR THE COBRE CDLD:
P20 GM120457

This project was supported in part by NIH P20 GM120475 (core facility) at the MUSC Digestive Disease Research Core Center.

For queries regarding DDRCC news, membership and cores, please contact the Center Manager:

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Email Dr. Lee

For queries regarding the COBRE in Digestive and Liver Disease, please contact the COBRE PI:

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Email Dr. Duncan

Visit the DDRCC Website:
https://medicine.musc.edu/departments/dom/divisions/gastroenterology/research/labs-and-centers/ddrcc

Visit the CDLD Website:
https://medicine.musc.edu/departments/regenerative-medicine/cobre-digestive-liver-disease