UNC CRISPR Screening Facility

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UNC CRISPR Screening Facility

- Opened October 2021
- Located in state-of-the-art Genetic Medicine Building on UNC-Chapel Hill’s campus
- Whole human genome pooled and arrayed libraries
- Competitive pricing (contact us)
• **Types of CRISPR Screens:**
  - **Direct screens** for the desired phenotype in “normal” cells
  - **Genetic modifier screens:** “rescue” an existing phenotype in “disease” cells

• **Common Assay Methodologies:**
  - **Reporter assays** (fluorescence, cell membrane marker expression, etc.): desired phenotype translates to a way of sorting cells with causative mutations
  - **Selection** (e.g., antibiotic resistance): desired phenotype translates to survival of cells carrying the causative mutations
Pooled Screens: Fluorescence Reporter

1. Engineered cells of interest
2. Transduce cells with gRNAs + cas9
3. Specific CRISPR mutations activate gene
4. Sort
5. Sequence CRISPR hits
TYPES OF CRISPR SCREENS

Pooled Screens: Membrane Marker

1. Engineer cells of interest
2. Transduce cells with gRNAs + cas9
3. Specific CRISPR mutations activate gene
4. Membrane marker
5. Sort
6. Sequence CRISPR hits

- OFF
- ON
- anti-marker Ab
- membrane marker

strategic genomic location or promoter
Types of CRISPR Screens

Pooled Screens: Selection

1. Engineer cells of interest
2. Transduce cells with gRNAs + cas9
3. Add antibiotic resistance
4. Select resistant colonies
5. Sequence CRISPR hits

- Strategic genomic location or promoter
- Specific CRISPR mutations activate gene
- Antibiotic resistance
- Resistant colonies
Arrayed Screens

Choose specific set of genes to study → Transduce cells with gRNAs + cas9 → Cells of interest in multi-well plates → Selection, reporter, marker, etc. → CRISPR hits identified by well location.

- ONE CHOSEN GENE PER WELL
Availability of both Pooled and Arrayed Screens

**Arrayed Screens Include:**
1) Consultation on screen design, benchmarks, and objectives
2) High quality CRISPR sgRNA arrayed into 96-well or 384-well format
3) Automated assay with in-house robotics
4) Individual acquisition and analysis for each target gene
5) Deliverable: high quality data sets for individual genes

**Pooled Screens Include:**
1) Consultation on screen design, benchmarks, and objectives
2) High quality whole mammalian genome CRISPR sgRNA validated pools
3) Assay run by our experienced team, or PI can choose to run assay in their lab
4) Isolation of ‘hit’ genes, next generation sequencing and bioinformatics
5) Deliverable: rank list of genes identified in screen
Faculty and Staff

**Nate Hathaway, PhD**

*Facility Director*

Nate Hathaway, PhD is the Director of the UNC CRISPR Screening Facility. He is also an associate professor in Chemical Biology and Medicinal Chemistry, a member of the Center for Integrative Chemical Biology and Drug Discovery, and a member of the Lineberger Comprehensive Cancer Center. His lab studies how chromatin is dynamically regulated in the cell, allowing for varying levels of gene expression or gene silencing. Dr. Hathaway’s research group also actively develops new CRISPR gene regulation technology and has a drug discovery program that has identified small molecules that inhibit epigenetic pathways with potential therapeutic applications.

**Brian Golitz**

*Facility Manager*

Brian Golitz, who joined UNC in 2010, has a combined 20 years of industrial and academia experience, specializing in small molecule and cellular high-throughput screening. He led the successful genome-wide siRNA Screening Facility from launch and now works closely with a respected team in transitioning the facility to the new CRISPR Screening Facility. With a passion for miniaturization, automated liquid handling robotic platforms, data processing and sensitive detection systems, he is excited to use these tools to interrogate the functionality of the human genome. Brian has a degree in mechanical engineering with a focus on fluid dynamics from San Jose State University.

**Andy Snipes, PhD**

*Research Specialist*

Andy Snipes, PhD is driven by the application of cutting-edge technology to answer complex biological questions and has over a decade of experience in applied molecular biology and genetics approaches. His dissertation research involved dynamic imaging, inducible target expression with fluorescent reporters, and site directed mutagenesis to study the homeostatic regulation of stem cell populations in plant apical meristems. He then incorporated multi-target CRISPR/Cas9 gene editing to identify the function of a highly redundant gene family in guard cell dynamics. Andy is excited to be applying his molecular biology expertise in the high-throughput analyses of the human genome at the CRISPR Screening Facility.
Advisory Board

Samantha Pattenden, Ph.D.
Director of Applied Epigenetic Technologies, Center for Integrative Chemical Biology and Drug Discovery
Associate Professor, Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy

The Pattenden lab is focused on the development of innovative techniques in chromatin-based therapeutic target discovery and cancer diagnostics. Through the Center for Integrative Chemical Biology and Drug Discovery, we enable our collaborators to apply chemical biology methods to the discovery of novel molecular targets, pathways, and mechanisms.

J. Mauro Calabrese, Ph.D.
Department of Pharmacology and Lineberger Comprehensive Cancer Center, UNC School of Medicine.

The Calabrese lab studies how long noncoding RNAs regulate gene expression using approaches that include genomics, biochemistry, and molecular and computational biology.

Erin Heinzen, Pharm.D., Ph.D.
Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy
Department of Genetics, UNC School of Medicine

The Heinzen lab studies the genetic, genomic, and molecular bases of epilepsy and other neurodevelopmental disorders using high-throughput sequencing methodologies and human induced pluripotent stem cell models of genetic epilepsies.
Advisory Board

Hector L. Franco, Ph.D.
Assistant Professor, Department of Genetics, School of Medicine

The Franco lab uses genetic and genomic tools to study mechanisms of gene expression in breast and ovarian cancer. They leverage the CRISPR toolkit to perturb salient regulatory elements throughout the genome to study their functions.

Jesse Raab, Ph.D.
Assistant Professor, Department of Genetics, School of Medicine

The Raab Lab uses high-throughput genomics approaches to understand how disruption of chromatin affect gene regulation and contributes to liver disease and liver cancer. They use CRISPR screens to develop better mouse models of tumor formation and identify new therapeutic targets in liver cancer.

Alex Abuin, Ph.D.
Assistant Director for Translational Studies, Eshelman Institute for Innovation, Eshelman School of Pharmacy

Alex has a background in molecular and human genetics with over 20 years of biopharma experience in therapeutic target identification and validation in oncology, immunology and diabetes. His lab at Lexicon Genetics carried out large scale mutagenesis screens using gene-trapping retroviral vectors in mouse embryonic stem cells.
A special thank-you to those who have financially supported the launch of the UNC CRISPR Screening Facility:

Eshelman Institute for Innovation, NC Biotech, UNC School of Medicine, UNC Eshelman School of Pharmacy, Lineberger Comprehensive Cancer Center, UNC Research

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