1st FNIH Course on Gene Editing Technologies and Their Applications in the Life Sciences and Medicine in Africa

June 20 - 22, 2019

ICIPE, Nairobi, Kenya

"Established researchers and academics will extend their professional expertise by obtaining a rigorous understanding of how "gene editing technologies" work and their underlying mechanisms"

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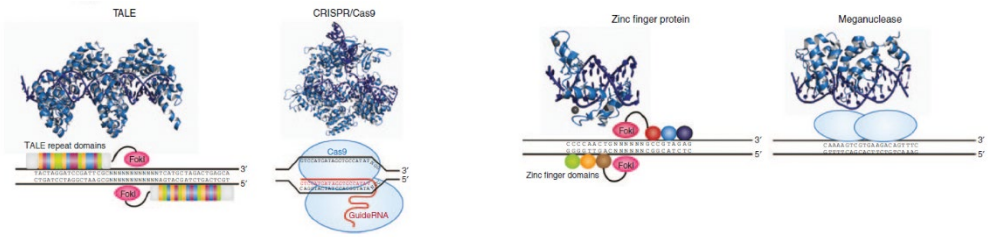
Why This Course?

The body of technologies collectively referred to as "gene editing technologies" have been transformative in the fields of life science and medicine. Intellectual access to the latest published cutting-edge research is highly dependent upon solid comprehension of these technologies in order to understand the methods, results, significance and implications of contemporary research, and to the ability to conceive of and develop applications of the technologies to one's research. This course will provide an opportunity for established researchers and academics to extend their professional expertise by obtaining a rigorous understanding of "gene editing technologies" and their underlying mechanisms. There are notable applications of these technologies with direct relevance to Africa such as their use to create "gene drive" systems as potentially powerful tools in the fight to eradicate malaria, crop improvement and gene therapy. These and other examples will be studied.



Learning Objectives and Content

The course will be comprised of short instructional lectures, active learning activities, formative assessments, reading scientific papers– all designed to promote learning. After completing this course participants will:

- Understand and be able to explain the biochemical and genetic mechanisms associated with the use of programmable DNA endonucleases such a CRISPR/Cas9.
- Understand and be able to explain the diversity of programmable DNA endonucleases and their key distinguishing features and uses.
- Understand and be able to explain how programmable DNA endonucleases in their various forms are being used as platforms for a variety of genomic technologies.
- Be able to describe and explain how specific applications of these technologies in the life sciences and medicine are being developed in Africa.
- Be competent in reading and understanding the material and methods sections of research publications making use of "genome editing technologies".
- Be able to deliver a lecture on the basics of gene editing to colleagues, trainees and students.

Instructors

David O'Brochta, Ph. D.,

Technical Lead for Gene Drive Research, Foundation for the National Institutes of Health, Bethesda, Maryland, USA

Professor Emeritus, Department of Entomology, University of Maryland, College Park, Maryland, USA

Dr. O'Brochta is the Technical Lead for FNIH's activities related to gene drive technology. Previously, as a Professor at the University of Maryland, he taught extensively in the areas of Genetics and Insect Biotechnologies. He is trained in entomology and molecular genetics and until recently had an active research program focused on the development of transgenic insect technologies and their applications to fundamental and applied problems in medical and agricultural entomology. His work in vector biology focused on the development of transgenic mosquito technologies and their applications to the study of mosquito/parasite interactions and he has used "gene editing technologies" in his research programs. He is a Fellow of the Royal Entomological Society, President of the Physiology, Biochemistry and Toxicology Section of the Entomological Society of America and past Editor of the Royal Entomological Society's journal, Insect Molecular Biology. He was the founding Director of the Insect Transformation Facility in the University of Maryland's Institute for Bioscience and Biotechnology Research, a facility providing genetic technical services, including gene editing, and training to insect scientists in support of their research

Daniel Maeda, Ph. D.,

Lecturer, Department of Molecular Biology and Biotechnology, University of Dar Es Salaam, Dar Es Salaam, Tanzania

Dr. Maeda is a lecturer and researcher in molecular and cellular biology and biotechnology. He is trained in stem cell biology and regenerative sciences, molecular and cellular biology and virology. Currently has research activities that intersect virology, molecular diagnostics, stem cell biology, chemical biology and pharmaceutical biotechnology. His work in stem cell biology and regenerative sciences focused on the development of hepatic cells upon differentiation from induced pluripotent stem cells and their applications as cellular models, suitability for gene editing/correction, and potential cell therapy use. As a lecturer he has taught extensively in the areas of molecular and cellular biology, molecular developmental biology, virology and pharmaceutical biotechnology covering the various applications of molecular biology tools including gene editing in basic and applied biomedical research. He is a professional member of the Cancer Epigenetics Society, Biotechnology Society of Tanzania, and one of the founding members of the Tanzania Society for Human Genetics. He serves as a member of the Technical Advisory Committee on Medical Devices and *In Vitro* Diagnostics of the Tanzania Food and Drugs Authority (TFDA), and a member of the National Biotechnology Advisory Committee (NBAC) of the Tanzania Commission for Science and Technology (COSTECH)

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International Centre for Insect Physiology and Ecology
Mzinga Meeting Room
Nairobi, Kenya

June 20, 2019 - Basics

8:00 Introduction to Course
8:30 Icebreaker/Introductions
8:45 **What is gene editing?** Discussion to assess perspectives and align concepts and expectations
9:15 **Broken DNA- The engine that drive 'editing'** - lecture
9:45 Mastering DNA DSB repair pathways – active learning
10:30 Break
11:00 **How to Break DNA – DNA Endonucleases** - lecture
11:30 Mastering DNA Endonucleases – active learning
12:45 Lunch
1:45 **CRISPR/Cas9 – Basic structure & function** - lecture
2:15 Mastering Cas9/sgRNA structure & function – active learning
3:30 Break
4:00 **CRISPR – A bacterial anti-virus immune system** - lecture
4:30 Recap of Day 1 material
5:00 End of Day 1

June 21, 2019 - Going Deeper

8:00 Review Day 1 material, formative assessments, clarify and remediate misconceptions.
9:30 **Jennifer Doudna Lecture** (video)
10:00 Break
10:30 **Cas9 -Knock-outs – Basics** - lecture
11:00 Mastering Cas9 Knock-outs – active learning
12:30 Group Photo/Lunch
1:30 **sgRNA Design Practical** (on-line training using 2 sgRNA design/analysis tools; reinforce concepts)
3:30 Break
4:00 **Cas9 – Knock-in – Basics** - lecture
4:30 **Cas9 – Beyond Knock-out and Knock-ins** - lecture
5:00 Review Day 2

Assignment for Day 3 – Read Papers relevant to presentations

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June 22, 2019 - Africa-Relevant Applications & Ethics

8:00 Formative Assessments – Day 1 and 2 material – review and clarify
9:00 **Gene Drive and Malaria Eradication** – Dr. Jonathan Kayondo, UVRI - lecture/discussion
10:00 Break
10:30 **Plant Genome Editing – Crop improvement** – Dr. Valentine Ntui, IITA, lecture/discussion
12:00 Lunch
1:00 **Livestock Improvement** – Dr. Steven Kemp, ILRI -lecture/discussion
2:00 **Gene Therapies** – Dr. Daniel Maeda, Dar es Salaam University, -lecture/discussion Break
3:00 Break
3:30 **Ethical Considerations** -Group Discussion
4:30 Final Assessments
5:00 End of Course.

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Master Reading and Reference List.

References

Addgene. 2017**. CRISPR 101: A Desktop Resource**, pp. 195. Addgene, Watertown, Massachusetts, eBook, free download at https://info.addgene.org/download-addgenes-ebook-crispr-101-2nd-edition?_ga=2.81154327.1290750171.1559581143-1085826080.1553024087

This is a compilation of short online posts on the Addgene site. Although not highly detailed, the text covers a very wide range of topics and provides a good starting point for exploring further. A pdf of this eBook is available in the course shared folder.

abmgood.com. 2018. **dCas9 as a Tool for Transcriptional Modulation**. abmgood.com., https://www.abmgood.com/marketing/knowledge_base/CRISPR-Cas9-dCas9-Gene-Regulation.php

This is from abmgood.com's online knowledgebase. It is a concise, well-illustrated explanation on some of the ways dead Cas9 (dCas9) is being used as a platform to construct various technologies for regulating gene expression.

Adli M. 2018. **The CRISPR tool kit for genome editing and beyond**. Nature Communications 9: 1911, doi 10.1038/s41467-018-04252-2

This is a very good short but dense overview including excellent historical information that puts CRISPR in context. All of the topics covered in this course are covered in this paper and more. There is a robust discussion of CRISPR-mediated epigenome editing which is interesting but is a topic we will on briefly mention.

Arbuthnot P, Maepa MB, Ely A, Pepper MS. 2017. **The state of gene therapy research in Africa, its significance and implications for the future**. Gene Therapy 24: 581-589, doi 10.1038/gt.2017.57

This paper looks at the potential of gene editing technologies to impact Africa an identifies challenges that will need to be met before benefits can be realized.

Chen H, Choi J, Bailey S. 2014**. Cut site selection by the two nuclease domains of the Cas9 RNA-guided endonuclease**. The Journal of Biological Chemistry 289: 13284-13294, doi: 10.1074/jbc.M113.539726.

This paper will be the focus of an in-class paper-analysis exercise. We will read this paper and then answer questions. The paper introduces another Cas system and illustrates features that are distinct from SpCas9.

Dunn DA, Pinkert CA. 2014. **Chapter 8 - Gene Editing. in Transgenic Animal Technology** (Third Edition) (ed. CA Pinkert), pp. 229-248. Elsevier, London, doi 10.1016/B978-0-12-410490-7.00008-6.

This a chapter from a book and is an easy read. One of the excellent features of this chapter is the authors' comparison of the various gene editing systems and their advantages and disadvantages.

Gakpo JO. 2018**. CRISPR poised to transform livestock breeding in Africa**. Alliance for Science, Cornell, New York, https://allianceforscience.cornell.edu/blog/2018/03/crispr-poised-transform-livestock-breeding-africa/

This is a short synopsis of an interview with an African scientist speaking about livestock improvement in Africa

Hille F, Richter H, Wong SP, Bratovič M, Ressel S, Charpentier E. 2018. **The Biology of CRISPR-Cas: Backward and Forward**. Cell 172: 1239-1259, doi 10.1016/j.cell.2017.11.032.

This is a comprehensive and detailed overview of CRISPR-Cas biology by one of the leaders in the field (Charpentier). We are only interested in the Introduction which provide us with enough information on the biology of CRISPR-Cas systems for our purposes

Maeder ML, Gersbach CA. 2016. **Genome-editing Technologies for Gene and Cell Therapy**. Molecular Therapy 24: 430-446.doi 10.1038/mt.2016.10

This paper reviews gene editing in the context of gene therapy has excellent brief descriptions of Meganucleases, ZFNs, TALENs and CRISPR system. There is also a nice overview of the possible applications of gene editing technologies to the field of gene therapy.

Nolan T, Crisanti A. 2017. **Driving out malaria**. Scientist 31: 24-31. <Go to ISI>://WOS:000391164200009

This is written in a simple way, consistent with the magazine in which it was published. It is a good synopsis of the efforts to develop gene drive mosquitoes and the infographic accompanying the pieces illustrates the concepts effectively.

Pandey M, Raghavan S. 2017. **DNA double-strand break repair in mammals**. Journal of Radiation and Cancer Research 8: 93-97, doi 10.4103/jrcr.jrcr_18_17.

This is a short paper that summarizes 3 DNA repair pathways. We will on concern ourselves with 2 (NHEJ and HDR). The authors provide an inventory of proteins involved in these processes and while the detail provided is beyond this course, we will learn 3 major proteins involved in each process.

Tripathi JN, Ntui VO, Ron M, Muiruri SK, Britt A, Tripathi L. 2019. **CRISPR/Cas9 editing of endogenous banana streak virus in the B genome of Musa spp. overcomes a major challenge in banana breeding**. Communications Biology 2: 46, doi 10.1038/s42003-019-0288-7

This paper will provide background for the presentation on Day 3 by the second author, D. Ntui

Specific Reading for Each Lesson

Day 1

Broken DNA – The Engine that Drives "Editing"

CRISPR 101/Addgene: pp 48, 51-52

Pandy and Ragavan, 2017 (*we will not concern ourselves with microhomology mediated end joining, MMEJ*).

How to Break DNA – DNA Endonucleases

CRISPR 101/Addgene: pp 7-10.

Maeder and Gersbach. 2016, pp 430-434

Dunn and Pinkert, 2014.pp 229-239

CRISPR- A Bacterial Anti-Virus Immune System

CRISPR-101/Addgene, pp 12-14

Hille, et al. 2018, p 1240

SpCRISPR/Cas9

CRISPR-101/Addgene, pp 19-26
Dunn and Pinkert, 2014.pp 237-239

Assignment Day 1 – read paper for analysis on Day 2

Chen et al 2014
Homework questions for Chen et al 2014.

Day 2

Paper Analysis

Chen et al 2014
Homework questions for Chen et al 2014

SpCas9 Knock-Outs

CRISPR-101/Addgene, pp 111-114; 130-132; 157-159

sgRNA Design Practical

CRISPR-101/Addgene, pp 111-114; 130-132; 157-159

SpCas9 Knock-Ins

CRISPR-101/Addgene, pp 51-55

Beyond Knock-Out & Knock-Ins

CRISPR-101/Addgene, pp 73-78; 82-84; 93-95
abmgood.com 2018

Pre-reads for Day 3 Guest Presentations

Plant Genome Editing – Crop Improvement
Tripathi JN, Ntui VO, Ron M, Muiruri SK, Britt A, Tripathi L. 2019. CRISPR/Cas9 editing of endogenous banana streak virus in the B genome of Musa spp. overcomes a major challenge in banana breeding. Communications Biology 2: 46, doi 10.1038/s42003-019-0288-7

Day 3

Gene Drive and Malaria Eradication.

Nolan T, Crisanti A. 2017

Plant Genome Editing – Crop Improvement.

Tripathi et al. 2019

Gene Editing Technologies and Livestock Improvement

Gakpo JO. 2018.

Gene Editing Technologies for Gene Therapies

Arbuthnot et al. 2017

Social and Ethical Considerations