Course title: **Advances in Genome Engineering Technologies**

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Objective

With the emergence of highly versatile genome-editing technologies investigators are able to rapidly and economically introduce sequence-specific modifications into the genomes of a broad spectrum of cell types and organisms. Presently the core technologies which are commonly used for genome editing are (1) clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9), (2) transcription activator-like effector nucleases (TALENs), (3) zinc-finger nucleases (ZFNs), and (4) *Piggy-Bac* and *Sleeping beauty* methodologies. The ease with which CRISPR/Cas9 technologies can induced genome editing has made it a forerunner and driven a revolution in genome editing thus accelerating scientific breakthroughs and discoveries in disciplines as diverse as synthetic biology, human gene therapy, disease modeling, drug discovery, neuroscience, and the agricultural sciences. The diverse array of genetic outcomes made possible by these technologies is because of their ability to efficiently induce targeted DNA double-strand breaks (DSBs) which then by activating DNA repair pathways such as nonhomologous end joining (NHEJ) and in presence of donor template, homology-directed repair (HDR) facilitate indel mutations or site-specific genomic modifications respectively.

Understanding the underlying chemical mechanisms of RNA-guided DNA and RNA cleavage provides a foundation for both conceptual advances and medical technology development. The course will focus on how bacterial CRISPR adaptive immune systems inspire creation of powerful genome engineering tools. Furthermore recent advances in CRISPR technology with engineered Cas9 are enabling advances in both fundamental biology and applications based medicine. This course will provide a glimpse into several CRISPR based application systems in different model organism which should expose the students to this recent emerging technique in the field of biology and medicine. This course will also discuss briefly about the ethical challenges of some of these applications that are evoking a huge controversy in present day biology.

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Content

1. Preface to genome engineering through transcriptional regulation (4 lectures)
   - ✓ The knockdown vs knockout paradigm
   - ✓ RNAi methodology, its applications and limitations
   - ✓ 10-23 DNAzyme an overshadowed technology
2. Introduction to basic concept of genome editing and genome editing tool. (2 lecture)
3. Zinc finger nucleases (ZFNs) (4 lectures)
   - ✓ Zinc finger DNA binding domains, versatility to specificity
   - ✓ Fok1 endonulcease, Engineering Methods and Specificity
   - ✓ Application of ZFN as a genome editing tool.
4. Transcription activator like effector nucleases (TALENs) (4 lectures)
   - ✓ TAL effectors and Repeat Variable Diresidues

- ✓ DNA cleavage domains and Engineering TALEN constructs
- ✓ Engineering Methodology of TALEN
- ✓ Enormous Specificity and overshadowing by simplicity of CRISPR

5. CRISPR –based gene editing (2 lectures)
   - ✓ The CRISPR system- journey from bacterial immunity to genome editing
   - ✓ Different types of CRISPR-Cas system (Type I, Type II, Type III),
   - ✓ Biogenesis

6. Mechanism and Designing (3 lectures/exercise)
   - ✓ Mode of action
   - ✓ Designing of guide RNA
   - ✓ Off-target effect
   - ✓ PAM sequence
   - ✓ Generation of Knock in
   - ✓ Generation of Knock out
   - ✓ Advantages over ZFNs/TALENs
   - ✓ Design your own CRISPR

7. Expression system and delivery methods (2 lectures)
   - ✓ Multiplex expression system
   - ✓ Multiplex plant genome editing
   - ✓ Mammalian and Adenoviral expression system
   - ✓ Single Injection method.

8. Application based designing of Model system (3 lectures)
   - ✓ Dead Cas9 and its application, Cas9 Nickase, Inducible Cas9.
   - ✓ Tetracycline repressor system
   - ✓ DiCre system
   - ✓ U1 snRNA
   - ✓ DD fusion system
   - ✓ Auxin-inducible degron (AID)

9. Applications in health and disease (4 lectures)
   - ✓ Genome wide CRISPR screen
   - ✓ Therapeutic application to edit genome in disease model.
   - ✓ CRISPR concerns beyond the lab bench
   - ✓ Practical application of CRISPR in human disease model
10. Transposon mediated genome editing concepts (3 lectures)
    - ✓ Revival of *Sleeping beauty* and *Piggy-Bac*
    - ✓ Engineering principles and methodology
    - ✓ Footprint free genome editing and other applications

11. RNA interference and CRISPR: A combined prospective in terms of application (1 lecture)

Text books:

1. A Crack in Creation: Gene Editing and the Unthinkable Power to Control Evolution (Jennifer A. Doudna and Samuel H. Sternberg, Boston: Houghton Mifflin Harcourt, 2017)
2. Genetic Engineering – Basics, New Applications and Responsibilities, (Hugo A. Barrera-Saldaña, 2011).

3. An Introduction to Genetic Engineering, Third Edition, Desmond S.T.Nicholl
4. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. Science. 2012; 337(6096):816-21.
5. Principles of Gene Manipulation and Genomics , Primrose, Edition 7E

Further reading and references

1. The Heroes of CRISPR. Eric S. Lander, Cell Volume 164, Issues 1-2, p18–28, 2016.
2. Genome engineering using the CRISPR-Cas9 system. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Nat Protoc. 2013; 8(11):2281-2308
3. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. Larson, M. H.; Gilbert, L. A.; Wang, X; Lim, W. A.; Weissman, J. S.; Qi, L. S. (2013) Nature Protocols. 8 (11): 2180–96
4. Correction of a pathogenic gene mutation in human embryos. Hong Ma, et al., Nature (2017) doi: 10 .1038/nature23305