CRISPR/Cas9 technology in genome editing

General information:

Tutor	Dr. Weronika Sura (Department of Molecular Pathology, Institute of Human Genetics, Polish Academy of Science)
Number/types of classes	30 didactic classes (lectures and laboratories)
Semester	spring-summer 2020
Language	English
ECTS credits	2

Objective of the course

Exploring the applications of the CRISPR/Cas9 technology and its practical use to obtain *Arabidopsis thaliana* mutant line

Topics

1. Genome editing technologies (zinc-finger nucleases, TALENs)
2. Natural CRISPR systems
   a) occurrence
   b) components
   c) mode of action
3. Applicable CRISPR/Cas9 system
   a) necessary components
   b) stages of use (gRNA design, generation of genetic constructs, cloning methods, transformation of the target organism, assessing system efficiency, mutation identification, obtaining mutant lines – in practice)
   c) mode of action
   d) potential applications
   e) achievements
   f) prospects
   g) threats connected to the use of the technology
4. Transformation of plants
   a) methods
   b) use of *Agrobacterium tumefaciens*
   c) floral dip
   d) mechanism of BASTA selection

Effects of course (in terms of knowledge, skills)

PhD student:

- knows genome editing tools like zinc-finger nucleases and TALENs
- knows the origin, components and role of naturally occurring CRISPR systems
- understands the mechanism of natural CRISPR systems action in defense to infections
- knows potential research and therapeutic applications of CRISPR/Cas9 technology
- knows advantages and disadvantages of the technology (also in comparison to other genome editing tools)
- is aware of ethical threats resulting from the use of this technique
- can use the CRISPR/Cas9 system to generate mutations in genes of interest in *Arabidopsis thaliana*, in particular:
  - knows which components have to be introduced (in genetic constructs) to plants
  - is able to use online tools to find potential gRNA sequences and can choose optimal ones
  - is able to conduct all stages of generation of *Arabidopsis* mutant lines starting from obtaining binary vectors (cloning to binary vector, *E.coli* transformation and selection, plasmid amplification and isolation, *A. tumefaciens* transformation and selection, amplification of *A. tumefaciens,* cultivation and floral dip transformation of *Arabidopsis*, seed selection using herbicide BASTA, DNA isolation from plant material, PCR amplification of selected *loci* and observation of mutations after DNA electrophoresis)
- Knows techniques of plant transformation, in particular use of *Agrobacterium tumefaciens*
- Understands floral dip method
- Understands the mechanism of transformant selection with herbicide BASTA

Course content:

1. Introduction to the subject (lecture) and classes (2,5 h). Sowing of Col-0 seeds for transformation and T1 generation seeds for selection. Restriction digestions of plasmid carrying cassette for gRNA expression and binary vector. (2,5 h)
2. Dephosphorylation of the binary vector. Electrophoresis of digested DNA fragments, their purification and ligation. Transformation of *E. coli.* Preparation of plates with selection medium and plating bacteria. (5 h)
3. Colony PCR from selected colonies and DNA electrophoresis. Identification of colonies carrying proper product of ligation and medium inoculation. BASTA selection of T1 plants sowed on first classes. (5 h)
4. Plasmid miniprep. *A. tumefaciens* transformation. Preparation of plates with selection medium and plating bacteria. gRNA design (computers necessary). (5 h)
5. Colony PCR from *Agrobacterium* and electrophoresis, choosing colonies for amplification for floral dip (medium inoculation). Observation of BASTA selection outcomes. DNA isolation from plants after selection. (5 h)
6. Genotyping of BASTA-resistant plants (PCR, electrophoresis) and results interpretation.

Transformation of Col-0 plants using floral dip method. Exam. (5 h)

Teaching methods:

– lectures in English, using multimedia techniques
– laboratories

Evaluation of learning outcomes:

– oral exam