

# PRELIMINARY SCHEDULE

## CRISPR/Cas9 workshop at SNU, May 24-28 2018

Theory = 9:00 to 12:30

Practical = 14:00 to 18:00

### Day 1: May 24

**Theory:** Introduction to Genome Editing; CRISPR/Cas9-based Editing – NHEJ and HDR; Complete Workflow for Genome Editing

**Practical:**

- > Basic outline of the practical training planned for the 5 days (cloning, transfection & overview of CRISPR strategy);
- > Start gDNA cloning – annealing of gDNA oligos, ligation into pre-cut pDC2-Cas9 vector, transformation of ligation mix into bacteria.
- >> *P. falciparum* ring-stage transfection (preexisting construct)

### Day 2: May 25

**Theory:** Applications of CRISPR/Cas9 Technologies – Beyond Genome Editing

**Practical:**

- > Start screening of gDNA clones (inoculate for plasmid prep)
- >> Transfection of preexisting gDNA/Cas9 construct (OFP-expressing and cloned using ThermoFisher's GeneArt CRISPR Nuclease Vector with OFP Reporter Kit) into HEK293 cells using nucleofection
- >> If time permits: Transfection of gRNA-Cas9 ribonucleoprotein complex (pre-assembled using ThermoFisher's GeneArt™ Precision gRNA and Cas9 Platinum nuclease) with lipofectamine

### Day 3: May 26

**Theory:** Live projects of gDNA design, discussion of downstream steps, project-specific discussions (pre-defined for workshop participants), etc.

**Practical:**

- > Plasmid prep and PCR-based detection of gDNA positive clones
- > Cloning of Homology boxes 1 and 2 using GIBSON assembly, transformation of GIBSON assembly mix into bacteria
- >> GIEMSA staining of transfected *P. falciparum* parasites
- >> Observe transfected cells for OFP signal

#### Day 4: May 27

**Theory:** CRISPR/Cas9 advances in pathogenic parasites; selected 15 min talks from 4 workshop participants

**Practical:**

- > Start screening of homology box clones (inoculate for plasmid prep)
- >> Observe transfected cells for GFP signal; Harvest the transfected HEK293 cells, genomic DNA preparation, confirmation of deletion (using ThermoFisher's GeneArt GenomicCleavage Detect Kit)
- >> If time permits: Harvest cells transfected with gRNA-Cas9 ribonucleoprotein complex and analyze as described above.

#### Day 5: May 28

**Theory:** ThermoFisher CRISPR/Cas9 genome-editing resources

**Practical:**

- > Confirm homology box clones using restriction digestion of plasmids, Results analysis...
- >> GIEMSA staining of transfected *P. falciparum* parasites
- >> Results analysis and discussion...