Overseas Practice on （Field Epidemiology・Collaborative Research）
report form（For Student）

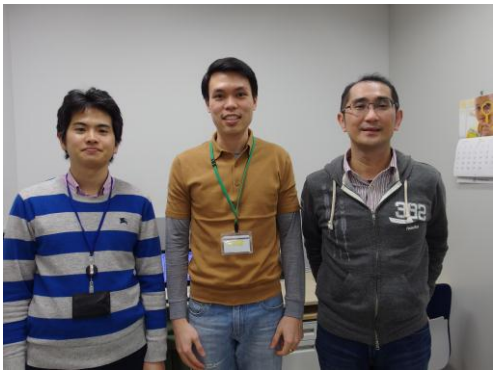
2019/02/15 (Year/Month/Day)

Name	Tussapon Boonyarattanasoonthorn
Laboratory	Laboratory Animal Science and Medicine
Year (Grade)	DC4
Place of practice	Genome Information Research Center, Research Institute for Microbial Diseases, Osaka University
Period of practice	February 1-16, 2019
Purpose	Training in genome editing of laboratory animals

Summary of activities（about 800 words, provide photos, tables and figures that clearly show the activities during the period）

In the past, animals harboring natural mutations have been used to elucidate the mechanisms underlying various diseases. Genetically modified animals play a key role in basic molecular biological investigations and act as models of human disease. Research institute for microbial diseases (RIMD), Osaka University mainly studies the mechanisms underlying mammalian reproductive systems and infectious diseases through genetic manipulation of animal models. As I am interested in the genetic analysis of infectious diseases in mouse, I decided to collaborate with this research institute to learn the method for generating knock-out mouse that lack a gene of interested to verify the function of the gene. Gaining of knowledge and experiences form the activity gave me an idea and skills for using in my current research and fostering collaborative projects with this research institute in the future. During the collaborative activities, I participated in a variety of the activities relevant to genome editing in mouse as describe below.

February 1st, 2019, arrived in RIMD and met with Prof. Masahito Ikawa, and Assistant prof. Shimada Keisuke, who was my supervisor during the activity. We discussed and modified the schedule of my working plan for fitting in to next 2 weeks of time.

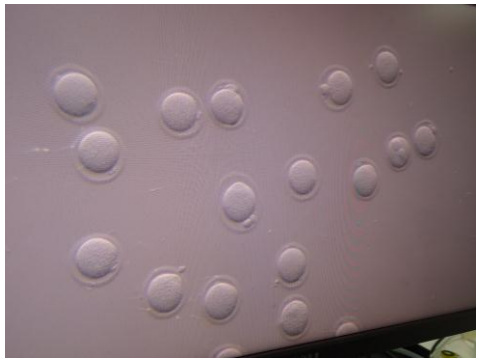


Prof. Ikawa (right) and Assistant Prof. Keisuke (left)

February 4th, 2019, at Genome information research center, basic ethics for performing animal experiment and rules for using animal facilities with orientation were the first lesson for new comers to make them familiar with the settings and procedures. After the orientation, I submitted the document for a permission to enter into the animal facilities.

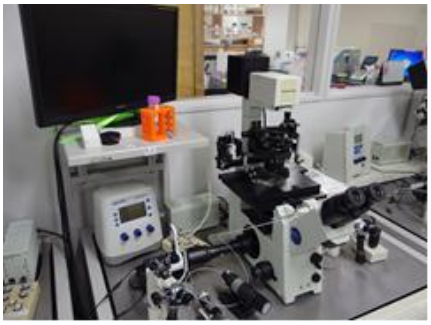
February 5th - 6th, 2019, went to the animal facility to collect oviduct from superovulated female mice and caudal epididymis from male mice, then brought the samples to laboratory for oocyte and sperm collection. After preparation of oocyte and sperm, I performed In Vitro Fertilization (IVF) and observed the development of fertilized oocytes. I also joined in lab seminar that present about a progression and result of each lab members.

Collecting samples from mice in animal facility



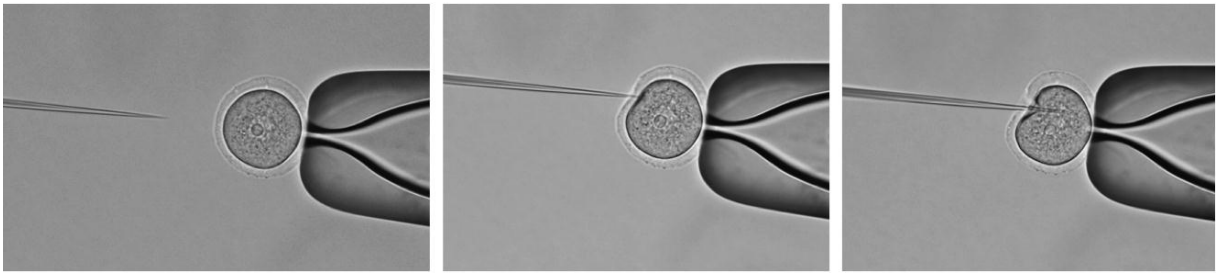
Preparation of oocyte and sperm for IVF

February 7th - 8th, 2019, observed preparation of compounds (gRNA/CAS9 protein) that were used for microinjection and electroporation. The preparation was performed by the staffs and Ph.D. students of RIMD. After the preparation, I observed how to perform electroporation and practiced for microinjection by using microinjector and microscope. The compounds were injected to pronuclear stage embryo. After incubation of injected embryos, the embryos that develop to 2-cell embryos were transfer into the oviduct of pseudopregnant mouse. Pups developed from injected embryos will be born about 19 days after the embryo transfer procedure. The offspring will be weaned when they are 18–21 days old. At weaning, ear-tags are applied and the tail tips are clipped for identification and genotyping.



Microinjector systems

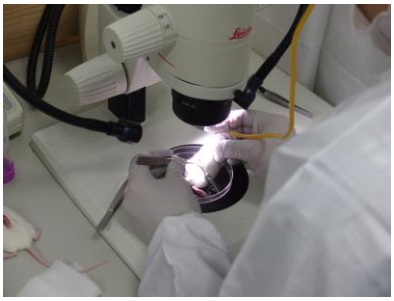
Embryo transfer



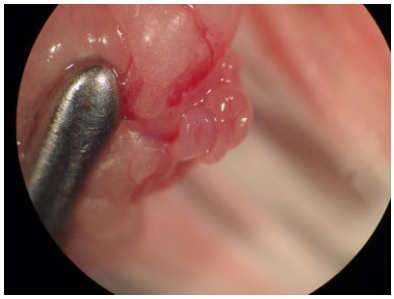
Microinjection in pronuclear stage embryo

February 12th - 13th, 2019, participated in the structure analysis of testicular tissue and sperm by transmission electron microscopy (TEM). In this laboratory, they have studied about reproductive system especially in male mouse. They generated many kinds of knock-out mice by targeting male fertility genes. From the samples, we observed the abnormal sperm development that affirmed the function of the interested genes.

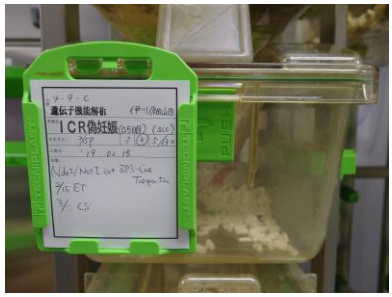
February 14th - 15th, 2019, participated in electroporation and performed embryos transfer to pseudopregnant mouse. I had opportunity to present about my current research to the lab members of RIMD in their weekly journal/data club and weekly laboratory meeting. I introduced my research and got many comments and suggestions from them that will benefit to my future career path. This opportunity of getting communicate and share the ideas with experts of this field was an immense experience for me.



Embryo transfer



Area of oviduct for transfer embryos



Cage for keeping my mouse

My current research at laboratory of laboratory animal science and medicine, Hokkaido University is focusing on genetic analysis of responsible gene for Mycoplasma infection in mouse. In order to detect the gene, knock-out mouse is necessary for proving and confirming a hypothesis. The connection we made with RIMD will use to make collaboration to exchange further technical knowledge and protocols in future.

Finally, I am grateful to Prof. Masahito Ikawa, for his acceptance for this activity and assistant prof. Shimada Keisuke, for his kind support during the activity. Furthermore, I would like to thank leading program coordinator Prof. Motohiro Horiuchi and committee for approving and financial supporting. Also, I would like to thank leading staff for their kind help throughout this activity.