Updated Aug. 5, 2019

This report should be submitted within 2 weeks after you return to Japan. Please do not change the formatting

(Abroad / Domestic) Internship report form (Student) 2019/10/17 (Year/Month/Day)

Name	Kevin Christian Montecillo GULAY
Laboratory	Comparative Pathology
Year (Grade)	DC3
Internship institution	Yan Laboratory, Yale School of Medicine, Yale University
Internship period	Internship period: 07/02/2019 - 08/23/2019 (Departure Date from Sapporo: 07/02/2019, Arrival Date in Sapporo: 08/24/2019)
Purpose	1. To acquire knowledge and skills necessary to perform RNA-sequencing, ChIP-sequencing and ChIP-qPCR and other necessary experiments. 2. To learn and understand laboratory management practices in Yale University. 3. To establish a good professional relationship with the members of the Yan Laboratory and to familiarize myself with work practices that exists in an academic laboratory especially in Yale University. 4. To leave a good impression in the laboratory for future work opportunity.

- The reason why you chose this institute

There are four major reasons why I chose this Institute. The first reason is because the Principal Investigator in this lab is one of the leading experts in my field-of-interest, cancer epigenetics. The second reason is because the lab is performing very similar experiments to my own so I knew that I would be able to learn new techniques from them that would be helpful for my experiments. The third is because this institute, the Yale University, is one of the most prestigious universities in the US and in the world and I hoped to establish professional relationships with researchers in Yale for my future career path, and the last reason would be because my supervisor, Dr. Keisuke Aoshima, is a visiting researcher in this institute so I hoped to update him about my research.

- Result of the activity **(about 800 words、provide photos, tables and figures that clearly show the activities during the period)**

I was able to perform all of the goals I listed in my application form without any problems. During the first few weeks of my internship, I was able to take classes on Medical surveillance for animal handlers, regulatory training for animal care and use, biosafety, HIPAA privacy and security training, Patent policy acknowledgement agreement, shipping and export controls general awareness, and standards of business conduct. I was also able to prepare the necessary reagents and materials for my experiments (**Fig. 1**).

Fig. 1. Preparation of reagents and culturing of cell lines. **A**. Mr. Gulay culturing cell lines upon arrival in Yale University in a BSL-2 safety cabinet. **B**. Mr. Gulay preparing necessary reagents for his experiments in his own BSL-1 bench.

Prior to the internship, I sent my cell line samples and antibodies from Hokkaido University to Yale University following strict protocols and guidelines made by the USDA. I then used this cell lines to perform *in vivo* experiments, gene silencing experiments, and Chromatin Immunoprecipitation (ChIP).

To know the proper inoculation cell count *in vivo*, I first performed a preliminary experiment following the Yale IACUC protocols. I inoculated 1, 2, and 3 million cells subcutaneously in Balb/c nude mice, measured the tumor growth twice weekly, and then euthanized the mice when humane endpoints are met (**Fig. 2A and 2B**). From this experiment, I concluded that my cell line grows slowly *in vivo* and that 3 million cells per inoculation site is the best tumor inoculation volume when using my cell line (**Fig. 2C**).

Before coming to Yale University I was having a hard time generating a doxycycline inducible gene silencing vector for my target gene using the tet-pLKO.1 plasmid. Yan lab uses a different silencing vector, pINDUCER10, which is one of the best silencing vectors. I was able to construct a doxycycline-inducible

silencing vector for my target genes, KDM1A, KDM2A, and KDM2B (**Fig. 3A**) and was also able to measure the silencing efficiencies of the vector using real-time qPCR (**Fig 3B**) and western blot analysis (**Fig 3C**) and compare it to the scramble control. I was also able to confirm the resulting phenotype when my target gene was silenced using colony formation assay (**Fig. 3D**).

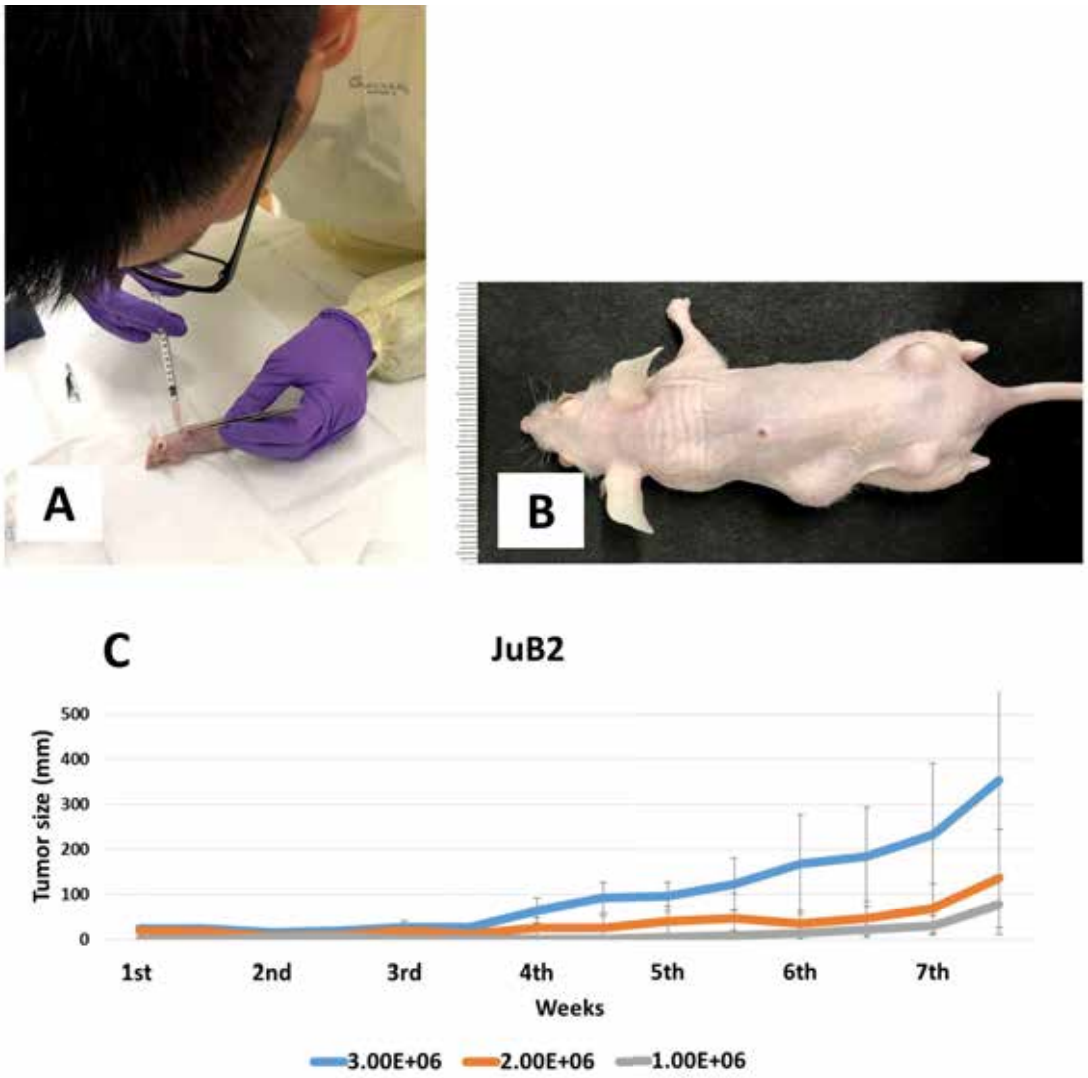

Fig. 2. *In vivo* experiments in Yale University. **A**. 1, 2, or 3 million JuB2 cells were inoculated per inoculation site in Balb/c nude mice. **B**. Balb/c nude mouse with the tumor xenografts that was euthanized when the humane endpoint criteria were met. **C**. Growth curve of JuB2 cells in Balb/c nude mice showing that 3 million cells per site is the best inoculation volume.

Then using the antibodies and the cell line which I brought from Hokkaido University, I was able to successfully perform ChIP and concluded that only one of three antibodies that I have can properly work for ChIP experiments. Unfortunately, I was not able to perform RNA-sequencing (RNA-seq) using my cell

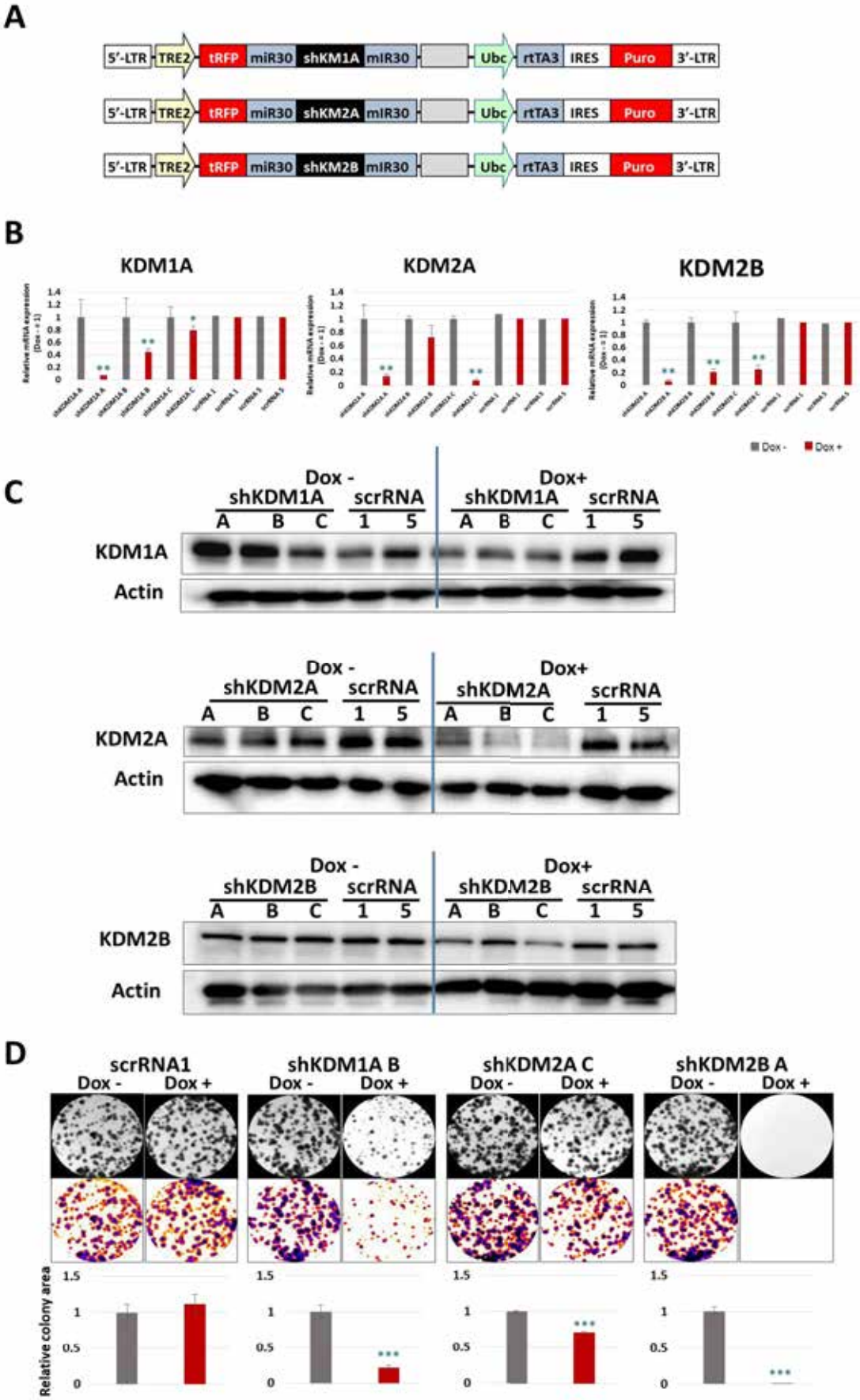

Fig. 3. Gene knockdown experiments. **A**. Construction of the doxycycline-inducible silencing vector for KDM1A, KDM2A, and KDM2B. **B**. Real time qPCR analysis for the KDM1A, KDM2A, and KDM2B mRNA expression in shKDM1A, shKDM2A, and shKDM2B stable cell lines maintained in Dox- or Dox+ medium. **C**. Western blot analyses for the KDM1A, KDM2A and KDM2B in shKDM1A, shKDM2A, and shKDM2B stable cell lines maintained in Dox- or Dox+ medium. **D**. Colony formation assay in shKDM1A, shKDM2A, and shKDM2B stable cell lines maintained in Dox- or Dox+ medium. (*; $p<0.05$, **; $p<0.01$, ***; $p<0.001$)

lines prior to the internship so I was not able to analyze my data but I was able to learn how to analyze RNA-seq results using RNA-seq data from Dr. Keisuke Aoshima. It would not be possible for me to learn this techniques in my laboratory in Hokkaido University since we do not have the necessary equipment for these experiments.

While I was learning new techniques and skills necessary for my own experiments, I was also able to establish connections with the members of the Yan lab. The Yan lab members, especially Dr. Sabine Lang, Dr. Shangmin Zhang were particularly helpful to me throughout my internship and gave me a lot of advice on how to pursue my career path and on how to properly perform my experiments.

On my last day in the Yan Lab, I was able to present my progress report in front of the yan lab members and Dr. Qin Yan. They were able to give me some constructive comments and advice on how I could improve my experiment (**Fig. 4**).

Fig. 4. Progress report presentation and discussion with Yan Lab members

Aside from the technical side of my internship, I was also able to have the chance to join the Yan lab members for their annual summer camp in the Yale camping site in Lyme, Connecticut. We were able to enjoy to grill and eat barbeque and other potluck food, kayak and paddleboard, and enjoy small talks about our private lives (**Fig. 5**). I was also able to take part in arranging some small lab celebrations and enjoy eating dinner with the lab members.

Overall, I think I left with a good impression not only for me but for all the researchers in Hokkaido University. Hopefully, this internship would open more

Fig. 5. Establishing connections with Yan lab members during a summer lab trip.

doors for other hopeful students who wish to have an internship in Yale University in the future. This internship also helped me to gain a perspective on what career path I should follow after graduating from Hokkaido University and also helped me improve skills which would help me become a leading expert in my field.

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- What do you think the positive impact of the activity will have on your further career path?

This internship have three major positive impacts in my career path. Firstly, I was able to learn new techniques which could give me an advantage over other researchers in my field. This would be really helpful whether I would pursue a postdoc degree after my PhD and even if I would deviate from it and apply for a researcher position in a private company. Secondly, I also have established strong professional relationships with researchers in the Yan lab which would be very useful for my career path. And lastly, Dr. Qin Yan expressed that he would be very happy to accept me as a postdoc student after graduating from Hokkaido University so this internship activity helped me establish my career path as a postdoc student.

- Advice for your junior fellows

My advice would be to choose an institute which would have the most number of positive impacts in your career path. To have the internship sooner so If, in any case, the internship would not turn out well, there would still be plenty of time before graduation to change and think about your own career path. I would also

advise them to apply for the internship at least six months prior to the intended date of the internship so they would have ample time to prepare for the VISA, to prepare themselves mentally, and to plan their internship well.

Approval of supervisor	Institution・Official title・Name Qin Yan, PhD, Associate Professor, Yale University 

※1 Send the electronic file to the Leading School section, International Affairs Office
※2 Attach a copy certificate of the content of internship activity that is prepared by the counterpart at the internship institution (any form with a signature of the counterpart).
※3 The Steering Committee of the Leading Program will first confirm the content of this report and report will be forwarded to the Educational Affairs Committee for credits evaluation.

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