IMSUT International Joint Usage/Research Center

International Project-completion Report (FY2022 ver.)

Date of submission: **05** / **11** / **2023**

Principal Investigator	Position, Institution: Professor, ETH Zurich Name: Jacob Corn
IMSUT Host Researcher	Division: Animal genetics, Professor Name: Tomoji Mashimo
Project Title	Development of a novel off-target detection tool for CRISPR-Cas3
Duration	From 04/01/2022 to 03/31/2023 *Please enter the entire research period.

Project Members *Please enter all of your project members, including IMSUT members.

Name	Position, Institution
Jacob Corn	Professor, ETH Zurich
Tomoji Mashimo	Professor, IMSUT
Kohei Takeshita	Researcher, RIKEN
Kazuto Yoshimi	Assistant Professor, IMSUT
Yuko Yamauchi	Technical staff, IMSUT
Hiromi Taniguchi	Technical staff, IMSUT

Project-completion Report on achievements/progress through the entire project period

In the field of life sciences, genome editing tools are currently one of the most anticipated areas of research due to their promising versatility and potential. The Mashimo Research Lab at the University of Tokyo has successfully identified a gene-editing tool called the CRISPR-Cas3 system that can be used to edit the eukaryotic cell genome of the bacterium Escherichia coli. As CRISPR-Cas3 technology differs significantly from CRISPR-Cas9 in terms of DNA cleavage and the size of the mutation introduced, there is potential for Cas3 to be used as a new genome editing tool in industry and medicine. The problem with CRISPR-Cas3 is that no analysis method has been established to evaluate when CRISPR-Cas3 cleaves an off-target sequence, so the problem lies in Cas3's safety and efficiency, which current methods cannot evaluate.

Previously, we have developed the DISCOVER-seq method to evaluate in vivo genome editing, which was used to detect the double-stranded cleavage repair factors such as MRE11 and Ku70. It is expected that by applying this strategy to the analysis of CRISPR-Cas3-mediated genome editing, we will be able to discover the mutation efficiency, deletion pattern and effect on off-target regions of CRISPR-Cas3.

In this collaborative project, we optimised the DISCOVER-seq for CRISPR-Cas3 system to develop a new method for off-target evaluation in human cells. Interestingly, Cas3 showed different peak patterns and time course changes compared to Cas9. This analysis tool could detect multiple on/off-target sites by CRISPR-Cas3, which can evaluate and quantify the effect of CRISPR-Cas3 on off-target cleavages throughout the genome for genome editing. In the future, this will be an extremely useful method that can be analysed in almost all cells and species, including patient-derived primary cells and organs in vivo.

Research Results from the Project during FY2022

<Publications>

Dynamic mechanisms of CRISPR interference by Escherichia coli CRISPR-Cas3.

Kazuto Yoshimi, Kohei Takeshita, Noriyuki Kodera, Satomi Shibumura, Yuko Yamauchi, Mine Omatsu, Kenichi Umeda, Yayoi Kunihiro, Masaki Yamamoto, Tomoji Mashimo

Nature communications, 13(1) 4917-4917, Aug 30, 2022

<Patent Applications>

Days of visits to IMSUT during FY2022

*Please include visits without travel allowances.

*If the project members could not visit IMSUT due to the pandemic of COVID-19 during FY2022, please present how many days in total your project has held online meetings, discussions via e-mail or communication tools such as Slack, etc. among your project members since April 1st, 2022.

*For the “Sex” and “Age” sections, the information shall be used only for statistical purposes.

*Please select the age range based on the age at the end of FY2022.

Name	Position, Institution	Sex	Age	Visits to IMSUT (Days)
Kohei Takeshita	Researcher, RIKEN	Male	**40 or older**	2
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	

		Pull-down▼	Pull-down▼	
Name	**Position, Institution**	**Sex**	**Age**	**Online Meetings (Days)**
Jacob Corn	Professor, ETH Zurich	Male	**40 or older**	1
Kazuto Yoshimi	Assistant Professor, IMSUT	Male	**36 to 39**	3
Kohei Takeshita	Researcher, RIKEN	Male	**40 or older**	2
		Pull-down▼	Pull-down▼	
Name	**Position, Institution**	**Sex**	**Age**	**Discussions via E-mail, Slack, etc. (Days)**
Jacob Corn	Professor, ETH Zurich	Male	**40 or older**	2
Kazuto Yoshimi	Assistant Professor, IMSUT	Male	**36 to 39**	10
Kohei Takeshita	Researcher, RIKEN	Male	**40 or older**	10
		Pull-down▼	Pull-down▼	

Usage of Facilities/Equipment during FY2022

*Please enter '0' or 'N/A' if you have not used any facilities.

*For this fiscal year only, if the project members could not visit IMSUT due to the pandemic of COVID-19, please include the uses by IMSUT faculty members to conduct this joint research project.

Name of Facility	Equipment	Number of Use (Times)	Usage time (Hours)
FACS Core Laboratory	e.g.) FACS Aria (BD)	1	3
Medical Proteomics Laboratory	e.g.) Orbitrap QSTAR Elite	N/A	
Imaging Core Laboratory	e.g.) Zeiss Multiphoton Microscopy（LSM710NLO)	N/A	
Gene Manipulated Mouse Section	Creation and cryopreservation embryo of Knockout mouse	N/A	
Human Genome Center	Supercomputer	N/A	
Amami Laboratory of Injurious Animals	Experimental lab	N/A	
Other		N/A	

Usage of Scientific Resources *Please enter'0' or 'N/A' if you have not used any.

Name of Scientific Resource	**Number of Samples/Lines**
Serum (BioBank Japan)	N/A
DNA (BioBank Japan)	N/A
Knockout mouse	N/A
Pathogenic bacteria	N/A
Other	N/A
Usage of Database *Please enter‘0' or ‘N/A’ if you have not used any.	
Name of Database	**Number of Use (Times)**