NTU-KU Joint Funding

Final Report

Section 1

NTU principle investigator	
Name (last name, first name)	**Hui-Wen Chen**
Position	**Professor**
Faculty/Department	**Department of Veterinary Medicine**

KU principle investigator	
Name (last name, first name)	**Keizo Tomonaga**
Position	**Professor**
Faculty/Department	**Department of Virus Research, Institute for Frontier Life and Medical Sciences**
Visiting ECR*	
Name (last name, first name)	**Jing-Yuan Chen/ Ai-Ai Chou**
Position	**Postdoctoral researcher/Master student**
Faculty/Department	**Department of Veterinary Medicine**

*Please complete this section if the KU principal investigators hosted ECRs from NTU.

Host researcher*	
Name (last name, first name)	**Takehiro Kanda**
Position	**Assistant professor**
Faculty/Department	**Department of Virus Research, Institute for Frontier Life and Medical Sciences**

*Please complete this section if the host researcher is different from the KU principal investigator.

Section 2

Project title
International collaboration on epidemiological surveillance and vaccine development of avian bornaviruses

Section 3

Period of project	
From dd/mm/yy to dd/mm/yy	13/02/23 – 23/02/23

Section 4

Summary of the project (approx. 100 words)
*KU PIs are required to submit a summary of the project in Japanese in addition to the English summary (approx. 200–300 characters).

(Please enter the summary of the project)

一本鎖RNAウイルスであるボルナウイルスは、哺乳類から鳥類まで幅広い宿主に重篤な神経疾患を引き起こすが、その詳細な発症機序は不明である。現在、ボルナウイルスに対して認可された治療薬やワクチンは存在していない。本プロジェクトでは、鳥ボルナウイルスのワクチン開発を目標に、組換えボルナウイルス作製技術であるリバースジェネティクスシステムの確立を目指した。目標達成のため。NTUから参加したECRに鳥ボルナウイルスの取り扱いと組換えボルナウイルスの作製のための材料作製等に関する共同研究を進めた。NTUとの共同研究より、ボルナウイルス感染の制御に向けた研究を加速させることができると考えられた。

Bornavirus causes severe neurological diseases in a wide range of mammalian and avian species, but the pathogenesis remains unclear. Currently, there is no authorized treatment or vaccine against bornavirus. Due to the slow replication rate of bornavirus, establishing a reverse genetic system is a quicker approach to generate recombinant viruses for investigating gene functions and advancing clinical applications. In this project, our goal is to establish a reverse genetic system for bornaviruses. Additionally, we aim to determine whether the ratio of RNA-dependent RNA polymerase helper plasmids transfected into cells impacts the recombinant virus titer. In conclusion, this project will enable us to generate recombinant viruses more efficiently and expedite bornavirus-related research.

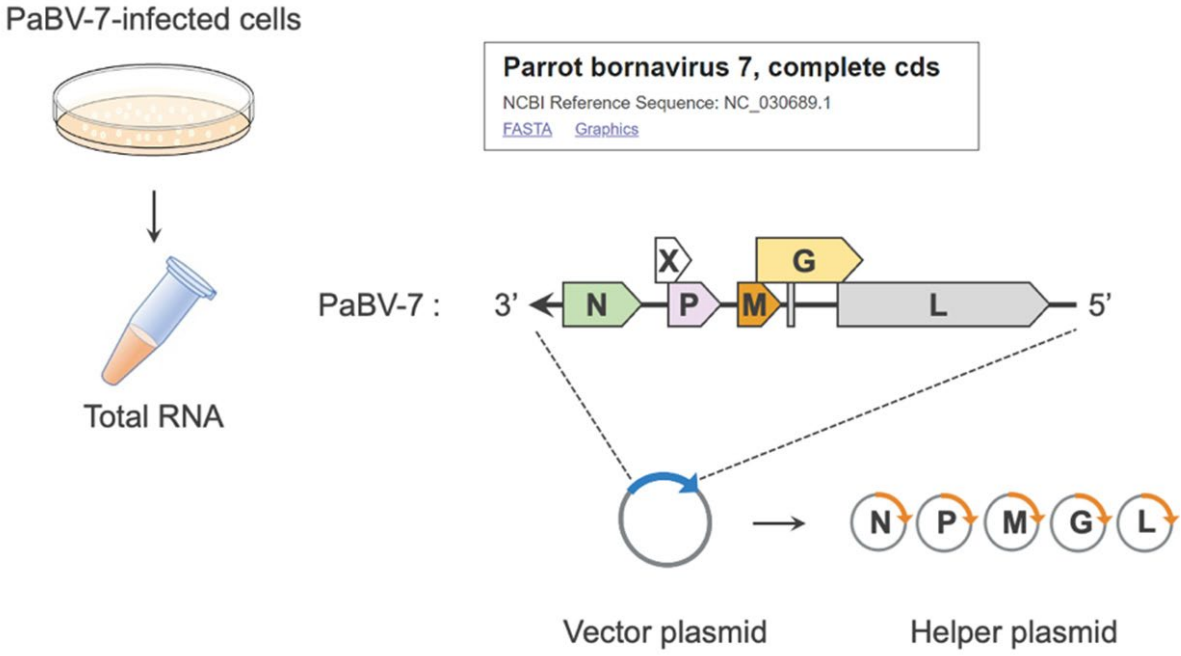
Section 5 (Please complete this section if ECRs from NTU participated in collaborative research at KU)

Achievements and Outcomes of ECRs' Stay (approx. 100–250 words)
*This section should be filled by each of the ECR(s) (one paragraph per ECR) based on his/her experience of staying in Japan.

(Please enter the achievements and outcomes for each of the ECR(s).)

Establishing the reverse genetic system of Parrot bornavirus type 7

1. Outline of the experiment design



2. Experiment schedule

Date	13 Mon	14 Tue	15 Wed	16 Thu	17 Fri	18 Sat	19 Sun
Experiment	Cell preparation	RNA extraction	RT-PCR Plasmid construction	Insert check (Colony PCR)	Submittion of sequence sample	Enjoy Kyoto	
Date	20 Mon	21 Tue	22 Wed	23 Thu	24 Fri	25 Sat	26 Sun
Experiment	Sequence analysis	Plasmid construction	Insert check (Colony PCR)	Submittion of sequence sample	Sequence analysis		

3. Experiment procedures

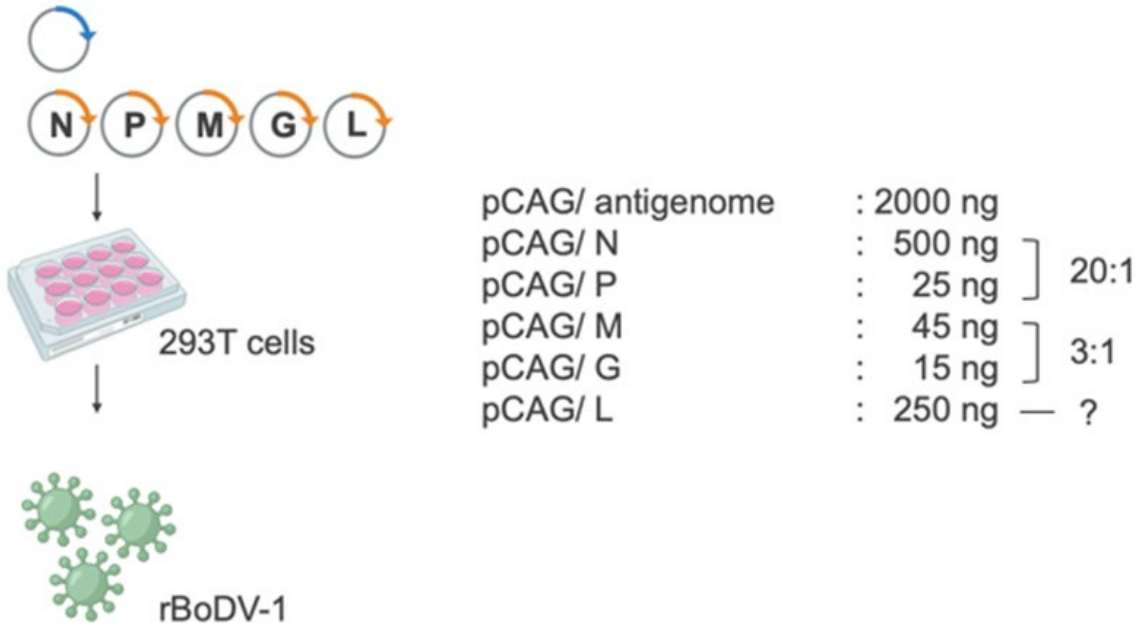
(1) PaBV-7-infected cell preparation and RNA extraction: We seeded PaBV-7-infected QT6 cells one day before extracting the viral RNA.

(2) RT-PCR & plasmid construction: After extracting PaBV-7 viral RNA, we used RTPCR to generate PaBV-7 antigenome fragments and two ribozymes (a hammerhead ribozyme and a hepatitis delta virus ribozyme). Then we assembled the fragments into a full-length antigenome flanked by ribozymes and insert it into the vector.

(3) Transformation and insert sequence confirmation: We transformed the antigenome plasmid into *E. coli*, then colony PCR and sequence analysis were conducted to confirm that the insert sequence is correct.

Rescuing rBoDV-1 by reverse genetic

1. Outline of the experiment design



To find out the best ratio of transfecting RNA-dependent RNA polymerase (L) helper plasmid, we transfected 293T cells with different amounts of L helper plasmid, then we determined the recombinant BoDV-1 virus titer by $TCID_{50}$ assay.

2. Experiment schedule:

Date	13	14	15	16	17	18	19
	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Experiment				Cell preparation	Plasmid transfection	Enjoy Kyoto	
Date	20	21	22	23	24	25	26
	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Experiment	Virus rescue & infection			Titration assay			

3. Experiment procedures:

1. Cell preparation and plasmid transfection: We seeded the 293T cells one day before transfection. Then we transfected the cells with 2000 ng antigenome, 500 ng of nucleoprotein helper plasmid, 25 ng of phosphoprotein plasmid, 45 ng of matrix protein, 15 ng of glycoprotein and different amounts of polymerase plasmid (0, 100, 250, 500, 750, 1000 ng).

2. Virus rescue & infection: After three days of incubation, we collected the recombinant virus and then infected Vero cells with ten-fold serial dilution of the virus in the 96 well plate. We examined the GFP fluorescent signal by microscope three days post-infection to determine the virus titer.

Research results

1. Establishing reverse genetic system of bornaviruses

We are still working on generating the right sequence of parrot bornavirus type 7.

2. Rescuing rBoDV-1 by reverse genetic

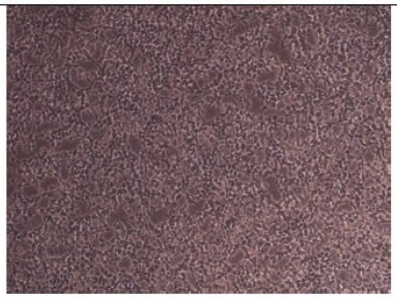
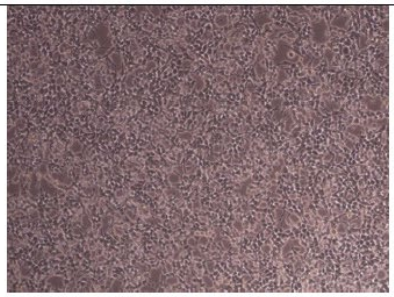
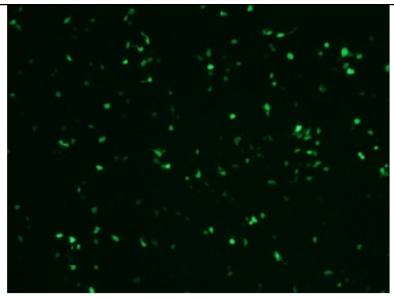
L plasmid (ng)	0	250
bright field		
GFP		

Figure 1. The representative photos showed that when the cells were transfected with L helper plasmid, the GFP signals can be observed under the microscope, indicating the recombinant viruses were successfully generated.

L plasmid (ng)	0	100	250	500	750	1000
sample 1 GFP+ cells in 100x dilution	-	25	21	29	12	23
sample 2	-	22	32	20	18	20
sample 3	-	24	35	23	20	18
ave GFP+ cell number	-	23.67	29.33	24.00	16.67	20.33
titer (x20 x 10^2), IFU/ml	-	4.73E+04	5.87E+04	4.80E+04	3.33E+04	4.07E+04

Figure 2. $TCID_{50}$ assay was conducted to determine the recombinant virus titer.

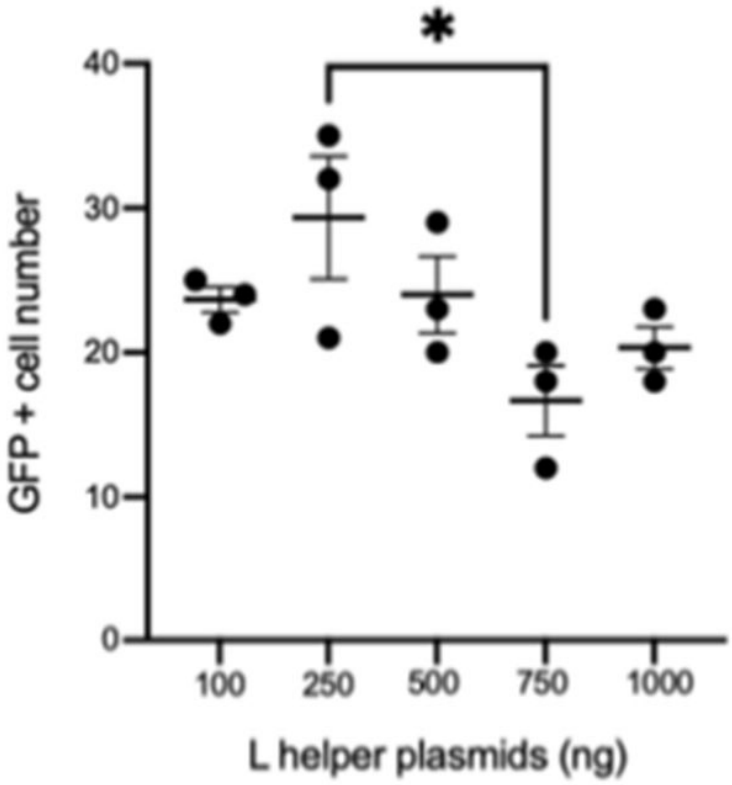
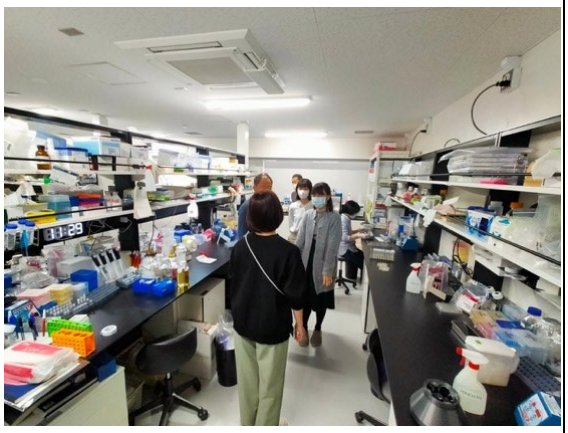
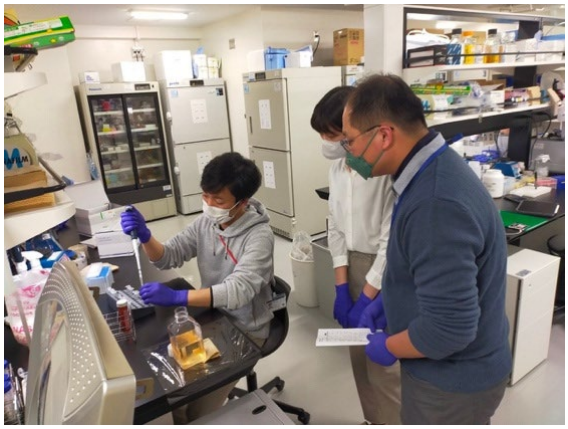


Figure 3. $TCID_{50}$ assay showed that the virus titer of transfecting 250 ng of L helper plasmid is the highest among all groups.

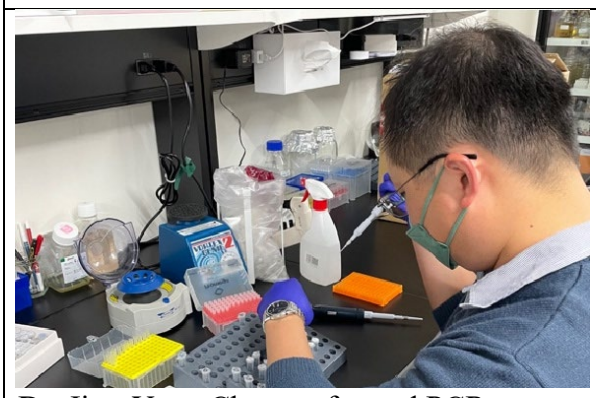
Section 6

Photographs with captions	
 Dr. Tomonaga and Dr. Kanda introduced the project.	 Visiting ECRs toured Dr. Tomonaga's lab.



Dr. Kanda demonstrated the experiment.

Dr. Hui-Wen Chen gave a seminar in KU.



Dr. Jing-Yuan Chen performed PCR experiment.

Master student, Ai-Ai Chou, conducted the RNA extraction.

*Please submit digital files (such as JPEG or GIF files) of the photographs used in your report as attachments. The size of each image should be at least 4MB, so that it can be used for printed materials. Please ensure that none of the photographs submitted will cause any issues relating to portrait rights.

URL at which project outcomes can be viewed (Optional)

*E.g. workshop notifications/programs/reports, evidence of academic papers published or otherwise made available, etc.

URL:

https://www.infront.kyoto-u.ac.jp/event/event-3781/