

日本—ロシア・タイ 国際共同研究「農業（アジアの動物遺伝資源の保存、改良と活用）」 2021 年度 年次報告書	
研究課題名（和文）	人工多能性幹細胞(iPS 細胞)技術を用いた鳥類絶滅危惧種の 遺伝資源保全
研究課題名（英文）	Preservation of endangered avian genetic resources through induced pluripotent stem cell (iPSC) derivation
日本側研究代表者氏名	Guojun SHENG
所属・役職	International Research Center for Medical Sciences, Kumamoto University; Professor
研究期間	2020 年 4 月 1 日 ~ 2023 年 3 月 31 日

## 1. 日本側の研究実施体制

氏名	所属機関・部局・役職	役割
Guojun SHENG	熊本大学, IRCMS, 特別招聘教授	Supervising and coordinating the reprogramming of primary fibroblast cells and the test of differentiation potentials of reprogrammed cells
永井 宏樹	熊本大学, IRCMS, リサーチスペシャリスト, IRCMS	Generation of induced pluripotent cells from primary fibroblast cells Testing the differentiation potentials of generated reprogrammed cells
Galym ISMAGULOV	熊本大学, 医学教育部, 修士 2 年	Generation of induced pluripotent cells from primary fibroblast cells Testing the differentiation potentials of generated reprogrammed cells
Wei WENG	熊本大学, IRCMS, 特定事業研究員	Generation of induced pluripotent cells from primary fibroblast cells Testing the differentiation potentials of generated reprogrammed cells

## 2. 日本側研究チームの研究目標及び計画概要

As planned, we have reached most of our goals for FY2020, with a small delay in obtaining green peafowl samples due to pandemic-related travel restrictions. For FY2021, we aim to achieve our goals outlined in our original project application. These include:

- 1) Complete pluripotency network analysis using RNA-seq data obtained in FY2020;
- 2) Clone galliformes-specific pluripotency regulators as expression constructs for iPSC generation;
- 3) Test reprogramming will be done on fibroblast cells we have already obtained and stored, including chicken, quail, duck, turkey, emu and blue peafowl. The blue peafowl cells will be used as a test case for green peafowl fibroblast cells (which we aim to obtain within the first half of this fiscal year);
- 4) Red jungle fowl cell collection will be put on hold. We will aim to collect green peafowl samples as our first priority when pandemic-related travel restrictions ease;
- 5) We will use both feeder-based, and feederless culture methods for reprogramming.
- 6) We will optimize avian ESC culture method (currently published, not commercially available) for comparison;
- 7) We will generate fluorescent-marker positive iPSC;
- 8) Derived iPSCs will be tested for three-germ layer differentiation through chimera assays. Sub-germinal cavity injection method will be used for chimera generation.
- 9) Contributions of iPSCs to three germ layers will be analyzed through histology and lineage-specific marker analysis.

## 3. 日本側研究チームの実施概要 :

In FY2021, despite pandemic related restrictions in acquisition of experimental materials and exchange of research personnel, we have continued our research and collaboration in order to achieve the original goals. 1) We have carried out bio-informatics analysis of avian embryonic fibroblast cells (the source cells for reprogramming) and of avian embryonic epiblast cells (the in vivo equivalent of pluripotent cells). 2) We are in the process of summarizing these data for manuscript preparation, and the first manuscript is planned to come out within 2022 fiscal year. 3) We have cloned reprogramming genes (based on cross-species comparison) for gene expression analysis as validation of transcriptomics analysis. 4) We have started to test these constructs in chicken fibroblasts and systematic test will continue in FY2022. 5) We have obtained green peafowl embryonic fibroblast cells. In FY2022, we aim to 1) Publish our regulatory network analysis of RNA-seq data for avian embryonic fibroblast cells and embryonic epiblast cells. 2) Complete green peafowl sample fibroblast sample preparation, RNA-seq and analysis of RNA-seq data. Compare green peafowl data with those from other avian species. 3) Test reprogramming capacity of conserved pluripotency genes uncovered through comparative analysis and cloned into expression constructs. 4) Seek new collaborative and funding opportunities to achieve the final goal of using bio-technology to protect endangered avian species and conserve avian biodiversity. 5) We will organize another international symposium in FY2022 (the first one was held in the middle of FY2021, despite obstacles posed by the pandemic). We will strengthen future collaboration of team members of this project and seek new international collaborations.