

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）」 2020 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	Professor, IRCMS, Kumamoto University	Supervising avian sample collection, cell culture and pluripotency gene selection and analysis
Sofiane HAMIDI	Postdoctoral researcher, IRCMS, Kumamoto university	Support in pluripotency gene selection and analysis
Hiroki NAGAI	Research specialist, IRCMS, Kumamoto university	Collecting and culturing of embryonic fibroblasts from non-endangered species; Obtaining embryonic epiblast tissues for transcriptomics analysis
Galym ISMAGLOV	Master course student, Kumamoto University	Collection and culture of embryonic fibroblasts from non-endangered species and pluripotency gene selection and analysis

2. 日本側研究チームの研究目標及び計画概要

In FY2020, the Japan team will collect and expand fibroblast cells from non-endangered avian species (WP1); participate in collection and expansion of endangered green peafowls and purebred RJFs (WP1); participate in in silico analysis of pluripotency genes of different avian species (WP2); collect epiblast tissues from embryos of non-endangered species and prepare samples for omics analysis (WP2); and clone conserved pluripotency regulatory genes for generating expression constructs (WP2).

3. 日本側研究チームの実施概要

To facilitate the study of pluripotency network in avian species, our goal for FY2020 was to a) obtain total RNA samples from avian pluripotent epiblast tissues (seven species including chicken and blue peafowl) and from lineage-restricted primary embryonic fibroblast cells (six species including chicken and blue peafowl); b) complete RNA sequencing of epiblast and fibroblast samples; c) perform RNA-seq data analysis; d) prepare and maintain stocks of primary fibroblast cells for future reprogramming experiments; e) obtain adult fibroblast samples from green peafowl. Despite restrictions associated with the ongoing pandemic, we have accomplished most of our FY2020 aims as planned. Obtaining green peafowl samples met with some delay due to pandemic-related travel ban.

More specifically, the Japan team has collected primary embryonic fibroblast cells from six avian species (chicken, quail, emu, duck, blue peafowl, and turkey) and have cultured and established stocks of those primary fibroblast cells for future use. We have also collected RNA from those cultured cells for RNA-seq analysis and have completed CAGE-based RNA-seq analysis of those fibroblast cells (with 3-6 samples per species). Furthermore, we collected embryos from fresh eggs of seven avian species (the above six plus zebra finch) and have collected RNA from freshly-laid embryos (triplicates for each species), with sufficient amounts needed for RNA-seq (>5ug each sample). We have completed RNA-seq of those embryo samples. We have also helped establish Thai local network for obtaining green peafowl samples (genomic DNA and tissue specific RNA samples). We have performed preliminary bioinformatics analyses of RNA-seq data from cell and embryo samples and have done targeted bioinformatics analyses of pluripotency regulatory genes for potential conservation and diversification among avian species.