
THE PAST, PRESENT, AND FUTURE OF THE FROZEN ZOO®

Oliver A. Ryder, PhD,^{1*} Barbara S. Durrant, PhD,² and Marlys L. Houck, CLSp(CG)¹

¹Genetics Division, San Diego Zoo Institute for Conservation Research, San Diego Zoo Global, San Diego, CA 92112 USA; ²Reproductive Physiology Division, San Diego Zoo Institute for Conservation Research, San Diego Zoo Global, San Diego, CA 92112 USA

Abstract

The San Diego Zoo Institute for Conservation Research established 40 yr ago the first zoo-based repository for viable cell cultures, the Frozen Zoo®. Skin biopsies from mammals were collected aseptically, cultured, and viable early passage diploid fibroblast cells were frozen and maintained in liquid nitrogen storage. Early applications focused on chromosomal evaluation for species identification and potential chromosomally-based reproductive problems, resulting in many significant publications. In the intervening decades, the concept of the Frozen Zoo has been expanded, its taxonomic breadth enlarged, and the scope of its applications broadened in ways unimaginable at the time of its initiation. Now comprising more than 23,500 cryoprotected reproductive tissues and gamete samples, as well as frozen tissues for nucleic acid extraction, DNA samples, and frozen fibroblast cell cultures from approximately 10,000 individuals that encompass all classes of vertebrates. Altogether more than 1000 species are represented rendering the collections of the Frozen Zoo a unique resource. Viable individuals from two endangered species (*Bos gaurus* and *Bos javanicus*) have been cloned from cells in the Frozen Zoo using somatic cell nuclear transfer.² The Frozen Zoo is contributing scores of samples for whole genome sequencing projects of endangered species that pave the way for achieving a deeper understanding of aspects of the biology of numerous endangered species.³ Cells are now banked in the Frozen Zoo from one extinct species and multiple critically endangered species; these cell-based resources could be a source of genetic variation to rescue populations compromised by inbreeding and low population numbers. With our partners, genetic enrichment of the black-footed ferret, *Mustella nigripes*, utilizing frozen cell cultures banked from wild-caught individuals captured in the mid 1980s through somatic cell nuclear transfer (SCNT) cloning is being explored.⁵ The northern white rhinoceros, *Ceratotherium simum cottoni*, is at the brink of extinction with only three surviving individuals comprising an aged population of related animals. Previously, we have generated induced pluripotent stem cells (iPSC) from northern white rhinoceros¹ with the intention of using advanced genetic and reproductive technologies to rescue the species from extinction.⁴ Other species, such as Arabian oryx, *Oryx leucoryx*, and Somali wild ass, *Equus africanus somaliensis*, and others, may also benefit from initiatives that potentially increase the impact of the Frozen Zoo for maintaining sustainable populations. The need for establishing multiple centers into a global network of wildlife biobanks, especially including countries rich in biodiversity, in order to collect, establish, and maintain resources for global biodiversity conservation efforts will be discussed.

Key words: Cryopreservation, genetic disease, genetic rescue, genome sequencing, global wildlife Biobank

ACKNOWLEDGMENTS

Supported by IMLS, the Future MacDonal Foundation, Revive and Restore, the Caesar Kleberg Wildlife Research Foundation, and the John and Beverly Stauffer Foundation. The authors thank the numerous veterinarians and their institutions for contributing samples.

LITERATURE CITED

1. Ben-Nun IF, Montague SC, Houck ML, Tran HT, Garitaonandia I, Leonardo TR, Wang Y-C, Charter SJ, Laurent LC, Ryder OA, Loring JF. Induced pluripotent stem cells from highly endangered species. *Nature Methods* 10:829-831. 2011.
2. Friese C. *Cloning wild life: Zoos, captivity, and the future of endangered animals*. New York (NY): NYU Press; 2013.
3. Koepfli KP, Paten B, Genome 10K Community of Scientists, O'Brien SJ. The Genome 10K Project: a way forward. *Annu Rev Anim Biosci*.1: 261-282. 2015.
4. Ryder OA, Hermes R, Goeritz F, Durrant B, Stejskal J, Hrudý J, Vahala, J, Loring, JF, Hildebrandt T. The plight of the northern white rhinoceros: Is there still hope to prevent extinction. *Proc Int Conf Dis Zoo Wild Anim*; 2015. p. 63-64.
5. Wisely SM, Ryder OA, Santymire RM, Engelhardt JF, Novak BJ. A road map for 21st Century genetic restoration: gene pool enrichment of the black-footed ferret. *J Hered*. 5:581-92. 2015.