Development of induced pluripotent stem cells from cryopreserved lung tissue of pygmy sperm whale (*kogia breviceps*).

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Concerns about marine mammal health and environmental threats have risen in the last decade due to increases in mortality, unusual mortality events, and previously unseen pathologies. Because opportunities for *in vitro* studies on pelagic organisms, such as marine mammals, are limited, it is necessary to establish systems whereby cultures of cells and reconstitution of functional tissues from these organisms can be used as surrogates for the animal in the wild. Isolated primary cells are, in fact, common models for the study of protein functions, cellular mechanisms, organ-specific functions, and responses to environmental parameters. Because there is a limited availability of marine mammalian samples and primary cell types we first developed a method to cryopreserve tissues and thereby generate a tissue bank (or biorepository). Starting from fragments of long-term cryopreserved tissues (several months after the stranding event), we were able to establish cultures of viable primary cultures of different lung cell types. To further circumvent the limited availability of samples, we then used one of these primary cultures (a primary culture of lung fibroblasts) to generate induced pluripotent stem (iPS) cells. iPS cells are a potential source for most primary cell types in large numbers, and therefore a useful approach to circumvent the limited availability of pygmy sperm whale samples. In a field with a lack of basic knowledge due to the limited availability of tools and samples and to the protected status of the animals, the development and establishment of primary cultures from 1) cryopreserved tissues and 2) reprogramming of pluripotent stem cells, has the potential to create the reagents necessary to revolutionize our understanding of the basic biology of marine mammals and understand the impact of stressors on marine and land animal health.

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