Frozen in time

a biological back-up of species

Kim Labuschagne explains how the SANBI wildlife biobank supports biodiversity research and conservation

A biobank is a large collection of biological samples and associated data, amassed for research and conservation purposes. These collections of samples can be from plants, animals, microorganisms or humans, but whatever material is stored, it needs to be kept at an optimal temperature to ensure its viability for further use.

The wildlife biobank at the South African National Biodiversity Institute (SANBI) has been dedicated to the long-term collection, curation and archiving of biodiversity samples for more than 20 years. Its samples include those from species assessed according to the IUCN Red List categories as extinct in the wild, critically endangered, endangered and vulnerable. The samples are collected from wild (*in situ*) and captive (*ex situ*) animals – both alive and dead – and may be blood, skin or tissue, hair and feathers, nails, scales, organs, bones, hooves, sperm or other materials.

A wide range of current and retrospective (or backward-looking) studies can be done using these collections. For example, if a disease ravages an animal population, researchers would be able to compare samples with those collected years ago to understand the progression of the disease, its impact on certain tissues, or any genetic changes that increased susceptibility.

The SANBI wildlife biobank serves as a science platform to both the national and international research and conservation community. It is able to support a variety of disciplines, including forensic sciences, comparative nutrition, epidemiological surveys, reproductive technologies, genetics and pathology diagnoses.

Currently, the biobank collection consists of more than 180 000 different samples from approximately 70 000 individual animals. Over 880 species from 204 families are represented, and the samples were collected from 24 countries. These samples are stored at a range of different temperatures – from room temperature (approximately 21°C) and normal fridge (4°C) and freezer (−20°C) temperatures, to ultralow (−80 °C) and cryogenic (−150°C to −196°C) temperatures.

The majority of the collection consists of blood samples, and these are stored in −80°C freezers in 1.8 ml tubes. Such low temperatures ensure that the sample is not exposed to freeze–thaw cycles that often occur in warmer freezers. Blood is one of the most important samples to store from an animal as it is able to answer a variety of questions. For example, has it been exposed to any poisons or toxins in the environment? What does the blood indicate about the animal's health? What species is it, and where is the individual from? These last two questions can be answered by extracting DNA from a tiny sample of blood and using it to complete a DNA profile that is unique for each individual. This genetic information could even help investigators determine whether confiscated animals or animal products were legitimately traded or illegally trafficked.

More than 20 000 samples in the collection are stored either in mechanical freezers at −50°C, or in tanks holding

Sperm samples stored in liquid nitrogen tanks.

liquid nitrogen at a very chilly −196°C (just below the boiling point of liquid nitrogen). We refer to this method of storage as cryopreservation. Along with a dose of biological antifreeze, this ensures that the cells remain in a somewhat live state. They become biologically inert, and can be preserved for years if kept at temperatures below −130°C. The tanks are maintained by adding liquid nitrogen on a weekly basis to ensure that the samples remain submerged. This topping up is necessary to counteract the liquid nitrogen lost through 'boil off' as heat leaks into the tank, although even the vapour inside the tanks can remain below −130°C.

The cryopreserved samples include sperm samples collected from pre-2000 to the current day, as well as an irreplaceable fibroblast (or cell culture) collection that dates back to the early 1980s. This includes important species such as the now extinct western black rhinoceros (*Diceros bicornis longipes*) and the critically endangered northern white rhinoceros (*Ceratotherium simum cottoni*) – of which only two surviving animals remain in the world, both females – as well as whale and dolphin species, various cat species, and even a hybrid between a black and white rhinoceros.

In 2010 the SANBI wildlife biobank was part of an international collaboration called Operation Frozen Dumbo. Sperm samples were collected from wild African elephants (*Loxodonta africana*) and stored in liquid nitrogen tanks at the biobank. Once all the health and export permits were obtained, these tanks were sent to France and the sperm samples were used to artificially inseminate a female elephant at one of the European zoos. The procedure was successful, and 22 months later a calf was born.

A cell culture is grown from a biopsy of skin or tissue, which is chopped into chunks the size of a pinhead and then placed in a petri dish with a nutritious media. The petri dish is left in an incubator set at the same body temperature of the animal it was collected from. The cells begin to divide (mitosis) and eventually cover the entire petri dish. When the cells' growth starts to slow down, it is time to freeze them. And this is where the antifreeze comes in – it is added to make sure that freezing them at such low temperatures won't kill them, but rather just inactivate them, so that they are 'frozen in time'.

As technologies advance, more uses for these cryopreserved samples are being discovered, the most recent being stem cell research. Stem cells are cells that can develop into many different cell types in the body. Scientists have even been able to reprogramme skin-derived stem cells into heart cells that beat in the petri dish.

Samples stored in biobanks around the world will continue to be used in ever more advanced ways to conserve our precious biodiversity, and in some cases, to rescue species on the brink of extinction.

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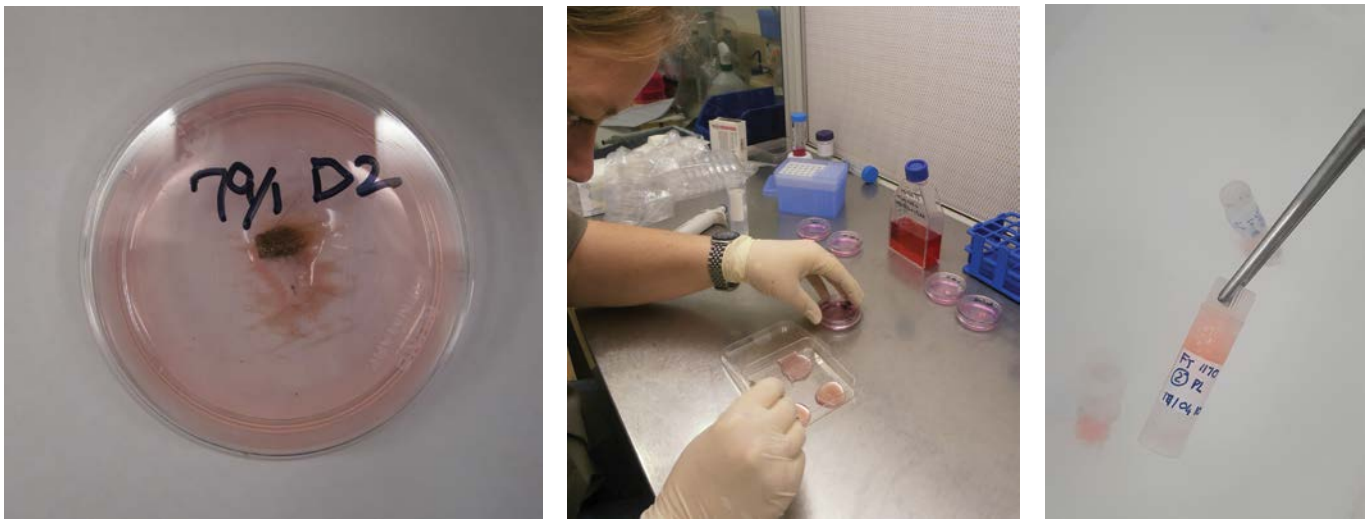

A lion biopsy is broken up into tiny chunks to be incubated in petri dishes filled with nutritious media. The resulting cell culture is then frozen in liquid nitrogen.