

**Introduction:** Before the dawn of the 22nd century, we face the huge risk of losing our genetic heritage accumulated during aeons of evolution. The losses include hundreds of vertebrates, hundreds of thousands of plants and over a million insect species. The gene pools of many human ethnic groups are also threatened. As we have observed, adequate conservation of habitat is unfeasible and active breeding programs cover only a handful of the many thousand species threatened.

Against such indispensable losses scientists are starting cryopreservation of germplasm by creation of gene banks. I propose to construct a cDNA library based gene bank for endangered species in the permanently shadowed polar lunar craters that would provide immunity from both natural disadvantages and humanitarian intrusions [4].

**Rationale:** In the pursuit of conservation of biodiversity, enormous money is spent all over the globe but they are unable to address the severity of the problem. Under such alarming circumstances, we turned to cryopreservation as an option but over thousands of years economic depression, sabotage, conflicts, warfare or even a brief disruption to the precise cryopreservation can hamper the storage of genetic samples. When we are considering conservation it is always preferable to go for a more secure and permanent solution. It was found out that the climatic and strategic location of the lunar polar craters are adequately hospitable, remote and free of maintenance and human observation as they provide naturally cryogenic temperature, reduced gravity and vacuum environment, non-reactive surface, safety from celestial intrusion and permanent shadow which doesn't allow the temperature to fluctuate thus providing most suitable storage facilities for the germplasm. PSRs provide steady temperature of 40-60K and immunity to earthquakes due to low seismic activity. At these sites, burial in one meter or more of the regolith will provide protection against the solar wind, solar and galactic cosmic rays and micrometeorite impact. It provides the minimum necessary barrier from human intervention and at the same time enables easy retrieval for future usage. Genetic samples of endangered species can enable restoration even after its extinction. Preserved tissues can secure the genetic heritage of species, and may allow future cloning to restore biodiversity. Furthermore, there would be no scientific extinction [4].

**Biological Processes:** For storage of a huge number of genetic samples, we need to follow the basic protocols of construction of a cDNA library based gene bank. cDNA library represents the genes that were being actively transcribed in that particular source under the physiological, developmental, or environmental conditions that existed when the mRNA was purified [2]. Total RNA can be extracted from plants by using LiCl method [5]. The message RNA can be isolated and purified double-strand cDNA can be synthesized using the cDNA Library Construction Kit in a PCR machine. Five microliters of PCR products can be labeled with  $\alpha^{32}\text{P}$ dATP fractionated by electrophoresis in 1.0% alkaline agarose gel to check the ds-cDNA quality along with the single

stranded cDNA. One microliter of the purified cDNA can be ligated into the predigested vector (1  $\mu\text{g}$ ) digested by EcoR I-Xho I following the protocol of overnight ligation at 16 °C in a sense orientation. The lambda library can be packaged in a high-efficiency system and plated on the E. coli cell line. After amplification of the library titre can be calculated as per the manufacturer's recommendation and it came to  $1 \times 10^7$  cfu. The library can be stored in 7% DMSO at -80°C until further screening of the gene of interest. The size of the insert fragments can be measured by PCR method using random selection of 10-15 clones from the SOLR infected positive clones (growing in LB ampicillin agar plates) [5]. Using 0.1 gram of genetic material from about 20 individuals per species can allow the future restoration of a species. A realistic payload of 2,000 kg can save one million species. The storage medium would contain liquid nitrogen [4].

**Location:** After a thorough search, it has been concluded that the gene bank containing container should be buried under the regolith of the PSR of the base of Shoemaker Crater located near the Lunar South Pole, centered at 88.1 S, 45E [1]. It provides diameter of 20-51km with an immense  $100\text{m}^2$  of PSR [6].

The physical properties of the floor material can be modeled. This floor is known to be flat, providing simple geometry for understanding impact dynamics and the Ejecta plume in case required. In addition, about half of the crater floor is invisible from earth but access from polar lunar orbiter is good because a spacecraft would pass overhead every two hours [6]. Hence, it enables easy storage, surveillance and prolonged retention of the proposed gene bank.

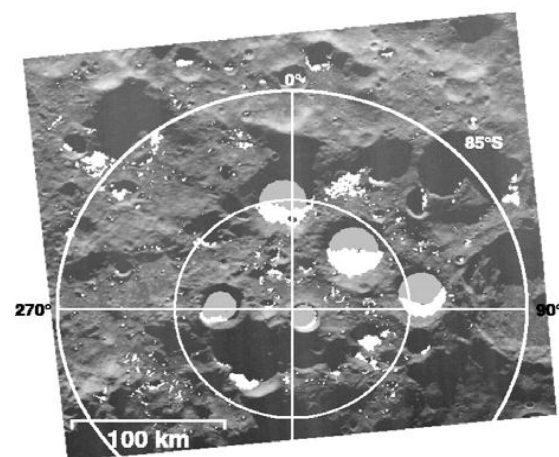


Figure 1. Radar DEM of south polar craters, Shoemaker crater indicated by arrow; white – permanent shadow (calculated), gray – no radar return; from [3]

**Conclusion:** Cryoconservation can add an important ethical component to the space programme and help raising public support. Conversely, the permanent safety of the genetic

and fundable. Many nations may wish to participate to secure the genetic heritage of their unique biota and ethnic groups[4]. It is highly advisable that developed countries associate with biologically rich countries and biotechnologists all over the world to collect the genetic samples as many as possible which they can include in their future lunar missions to secure the future of the living world. Until habitat losses are controlled, cryoconservation may provide the best chance to secure and eventually revive many endangered species. For this purpose, space-based depositories can provide precise conservation, the most cost-effective and secure means for permanent storage of irreplaceable genetic materials with a single one time expenditure for ages instead of the prevalent ineffective conservation programs.

[1] Allen, C. C., NASA Johnson Space Center, Mail Code KT, Houston, TX 77058 carlton.c.allen@nasa.gov. Shoemaker crater – going where we can “see” [2] Anonymous, [http://en.wikipedia.org/wiki/Library\\_\(biology\)](http://en.wikipedia.org/wiki/Library_(biology)) [3] Margot J. L. et al. (1999) *Science*, 284, 1658-1660 [4] Mautner, M.N. (1996) Space-based Genetic Cryoconservation of Endangered Species. *Journal of The British Interplanetary Society.*, Vol. 49, pp 319-320 [5] Sambrook J, Fritschi EF and Maniatis T (1989) *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratory Press, New York [6] Shevchenko, V.V. and E.A.Kozlova, Sternberg State Astronomical Institute, Moscow University, 13 Universitetsky pr., 119992 Moscow, Russia; e-mail: [shev@sai.msu.ru](mailto:shev@sai.msu.ru) Permanently shadowed areas at the lunar poles: nature and possible utilization.