Cryopreservation roadmap for EEP coordinators -- Version 1.0 – 16 August 2021

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What is cryopreservation? With cryopreservation, we refer to long-term storage of living reproductive material, cell-lines and their products. For most EEPs, cryopreserved sperm has the highest potential, since for many species sufficient cryo-protocols exist, and Artificial Insemination (AI) is technically the simplest future application. Samples can be collected during life and directly after death. Nevertheless, different tissues and techniques have different advantages and possibilities. Note that this is very different than the biobanking of materials (e.g. blood and tissue) for research purposes, for which it is much easier to take, store and use samples.

1. Is cryopreservation useful for your programme?

If you are reading this document, you might be interested in setting up a cryopreservation project for your EEP. And there are good reasons to be interested; In combination with other biotechnology applications, it can be a powerful tool to maintain a population's genetic diversity for the long-term. Especially when increasing the population size or the addition of unrelated individuals is not realistic, it may be crucial for your programme. Cryopreservation is in particular relevant for EEPs with an Ark or Insurance role because maintaining a high level of genetic diversity for a long-time is then usually important. Especially, when population size is limited, no new founders are coming in, genetically valuable animals are not breeding or if this allows the import of genetic material from other regions, then cryopreservation can make the difference between success of failure. That being said, it may also be unnecessary, unrealistic or a low priority for your EEP, because it can be time-consuming and expensive. So before investing time in it, determine if it is worth the effort. **The EAZA PMC can help you with identifying whether this is a priority and help evaluate alternatives.**

2. How feasible is cryopreservation?

If you determine that cryopreservation is important for your EEP, you will need to find out if this is already technically possible. The required techniques are complex and often differ per species. Generally, cryopreservation is likely more feasible for species that are closely related to domestics (e.g. hoof stock, felids), model species (e.g. rodents, xenopus) or humans (i.e. primates), where established protocols exists. It is usually (but not always) more challenging for birds, fish, amphibians and reptiles, due to lack of protocols and experience. **The EAZA PMC can share the contact details of experts who can assess the feasibility of cryopreservation for the species you manage.**

If the techniques are not developed, but cryopreservation is crucial, then working with relevant laboratories or universities to get the necessary research funded can still be a next step. Without you pushing for this, possibly no one will. You may also want to consider working on this with the TAG and other regions (e.g. AZA), as more programmes may have a cryopreservation need and may be dependent on similar techniques, or expertise is already available.

3. Identify a specialised storage facility and develop protocols

Next, identify and approach a specialised lab by contacting the EAZA Biobank coordinator, who keeps track of EAZA's Cryopreservation Service Provider Network. You will need this specialised lab for storage, but also to plan the technical aspects of the project. Note that only a few (at most) institutions will be relevant for your species. to ensure that you can quickly store samples when needed, and to avoid some legislative issues with transfers of samples, it will often make sense to work with multiple institutions in different countries.

Once you have found this expert institution, you will need to work through the following technical elements:

- First of all, determine what materials you will cryopreserve: The best material is again species and situation dependent. The most commonly preserved live-cell samples are sperm, but you may consider oocytes (and immediately create embryos), gonadal tissue from deceased individuals or tissue (like ear skin) to be transformed into a cell line. **The specialised lab and EEPs veterinary advisor can help with developing a clear and detailed strategy for this.**
- Second, together with the experts, develop a protocol to guide the process of sampling and storage. Samples often need to be shipped in a special manner, and soon after the sample has been taken (e.g. 24 hours), need to be received at the storage facility (that has been made aware that the sample is coming before shipment).
- Before sending samples to any institution, it is highly encouraged to consider and put in place the relevant documents and/or contracts that can be found on **the EAZA Biobank page**, including a "Material Transfer Agreement" outlining ownership and use of the samples and agreement that the samples are transferred somewhere else if the institution ceases to exist.

4. Planning to sample

It is important that veterinarians and specialists are informed so they take the right sample at the right time and are aware of the right storage and sending protocols. Distributing protocols to curators is often not sufficient; you will need to contact the relevant veterinarian directly. This also need active planning, especially when samples of specific individuals need to be taken, e.g. because these are genetically valuable or because there is an opportunity to take samples (castration, soon to be deceased, etc.). Also note that animal training protocols may need to be available in some cases, e.g. for taking sperm samples. **Contact the EAZA Reproductive Management Group (RMG) for support with sample collection.**

More than that, it will require follow-up and reminders. It can take years before a decent number of samples has been collected. Therefore, you may need to search for an advisor or Species Committee members that is willing to lead on this project.

5. Assisted Reproduction Techniques: Collecting sufficient samples and storing them is typically by far the most urgent step. Eventually however, you do need to ensure that these can also truly be used to revive lost genetic lines in the future. Most of the time, this step can be figured out years later and should not stop you from starting with cryopreservation. Nevertheless, the sooner relevant techniques, such as AI or reviving cell lines, have been trialled and are well-established, the safer your EEP will be from future disappointments. Again, the EAZA (RMG) can support this by connecting you to the relevant experts.