Term Paper Biology:
Cloning Technology

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Table of Contents

1. Preface

When you hear the word "cloning" the first things that may come to mind are science fiction movies, fantasy novels and a world very different from ours, but surely not a regular laboratory similar to the ones we have at our school. Well, it might come as a surprise, but it is not that far from the truth because cloning is nowadays being done all around the world and has already become reality more than 70 years ago with the cloning of northern leopard frogs by Robert Briggs and Thomas J King. Since then, cloning technology has seen big improvements, the biggest milestone reached being the successful cloning of the first mammal, Dolly the Sheep. So cloning is one of the most extravagant and intriguing topics in science, something that's hard to wrap your head around. We often use this term in everyday conversations but seldomly someone really knows how the process of cloning takes place. That is why we have chosen this topic. We want to find out how this supposed miracle is becoming reality, how it could change our lives and how we can benefit from it.

2. Introduction

Cloning technologies have been a topic of significant scientific and ethical interest over the past several decades. With the successful cloning of Dolly the sheep in 1996, the concept of cloning shifted from science fiction to a tangible reality. Today, cloning technologies are used in a variety of contexts, including agriculture, biotechnology, and medical research. In this term paper, we will explore the different types, current applications, and the ethical considerations all around these cloning technologies.

3. Cloning Technologies

There are four main types of cloning technologies which are currently being used in science with three of those using somatic cell nuclear transferring (SCNT).

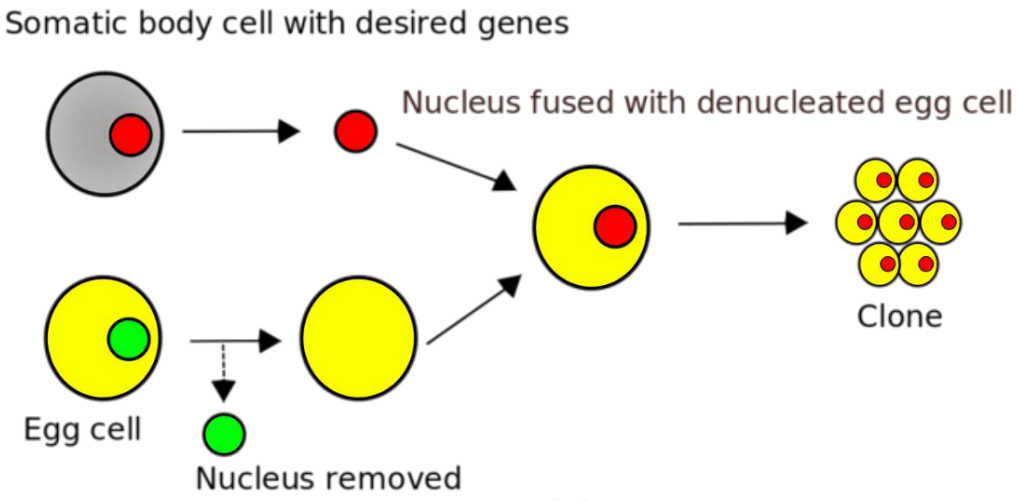

Figure 1: Process of somatic cell nuclear transfer

In the process of SCNT, first a somatic cell is taken (not a germ cell) e.g., a blood cell or a skin cell and its nucleus is extracted (Host 1). Then the nucleus is ingested into an unfertilized egg cell/oocyte (Host 2) whose nucleus has been removed, replacing it. It starts to multiply eventually becoming an embryo. This embryo either stays inside of the second host or is removed and inserted into a third host, both acting as a surrogate mother. Afterwards the cloned animal is born.

Types of cloning techniques:

- Somatic cell nuclear transfer
  - The nucleus of host 1 is inserted into the oocyte of host 2 (as explained above).

- Roslin technique (variation of SCNT)
  - The nucleus of host 1 and the unfertilized oocyte are put close together and shocked, so they fuse together (electrofusion).
  - The egg can now develop into a healthy embryo.

- Honolulu technique (variation of SCNT)
  - The fertilized oocyte is placed into a chemical solution which cultures it.
  - After a certain amount of time, it is implanted into a surrogate mother.

- Artificial twinning
  - Unlike the above elucidated methods, artificial twinning does not require somatic cell nuclear transfer.
  - Here natural fertilization of the female gamete (egg) occurs, whose embryonic cells are later separated in early stages of development.
  - The individual cells can later be implanted into a surrogate with all the oocytes being genetically identical. [1]

3.1 Dolly the Sheep

Dolly the Sheep was the first mammal to be successfully cloned from an adult cell, and her birth marked a turning point in the field of biotechnology (Griffin, 1999).

Dolly was created by scientists at the Roslin Institute in Edinburgh, Scotland, using the somatic cell nuclear transfer process (Griffin, 1999). The process, as explained before, involves removing the nucleus of an egg cell and replacing it with the nucleus of a donor cell. The modified egg is then stimulated to develop into an embryo, which is implanted into a surrogate mother. In the case of Dolly, the donor cell was taken from an adult sheep's mammary gland, and the egg cell was taken from another sheep (Griffin, 1999). The scientists used the Roslin technique to bring the egg and donor cell together. The modified egg was then stimulated to develop into an embryo and was implanted into a surrogate mother.

The birth of Dolly was a scientific milestone because it showed that it was possible to create a genetically identical copy of an adult animal (Griffin, 1999). Prior to Dolly's birth, scientists had only been able to clone animals from embryonic cells. The cloning of Dolly from an adult cell showed that it was possible to clone an animal with all the characteristics and experiences of an adult. However, when Dolly was around one year old, analysis of her DNA showed that her telomeres were shorter than would be expected for a normal sheep of the same age (Shiels et al., 2004). Telomeres are sequences of DNA located at the end of chromosomes in eukaryotic cells. They serve as protective caps that prevent the loss of important genetic information during DNA replication (Aubert et al., 2008). It was speculated that this had something to do with premature aging since telomeres become progressively shorter, exposing the DNA to more damage as one ages. It's thought that Dolly had shorter telomeres because her DNA came from an adult sheep and the telomeres had not been fully renewed during her development (Xu et al., 2003).

However, she died due to a case of arthritis and a progressive lung disease which is unknown if it was caused by her being a clone or if it was just an unfortunate accident (Williams, 2003).

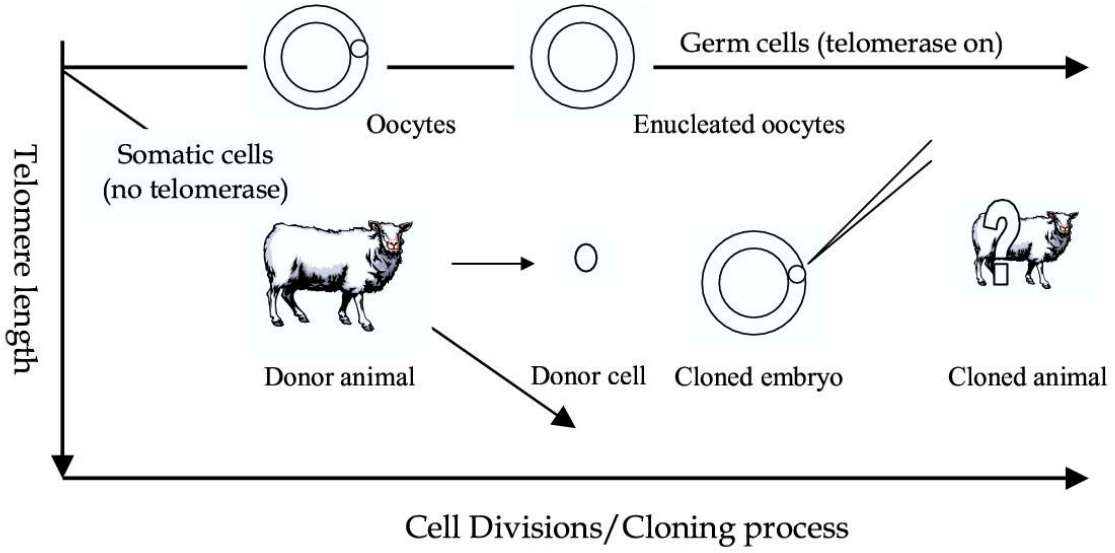

Figure 2: Graph showing length of telomeres with each cell division

In conclusion, Dolly the Sheep was a remarkable animal that changed the way we think about cloning. Her birth marked a turning point in the field and paved the way for new avenues of research of cloning technology.

4. Interview with Ben J. Novak, Lead Scientist, Program Manager, Biotechnology for Bird Conservation

We held an interview with Ben J. Novak from the Revive and Restore[2] organization conducted by a conversation through e-mail. Revive and Restore is the leading wildlife conservation organization promoting the incorporation of biotechnologies into standard conservation practice. They have several projects such as the pursuit of Woolly Mammoth de-extinction that has helped to identify immediate potential benefits to Asian elephant conservation. We had the following conversation with Ben J. Novak:

What cloning technique have you used to bring back the extinct species and why?

Cloning is a limited technology - only mammals, amphibians, fish, and one insect have ever been cloned. It's likely more type of invertebrates can be cloned, but birds and reptiles cannot be cloned. For birds, reptiles, and many other animals and plants scientists must use other technologies to turn single cells into embryos. However, cloning is not a viable technology to bring back extinct species currently. The only way to clone an organism is from living cells. Cloning can be used to reproduce an extinct species only if living cells were preserved (through cryopreservation) before the species went extinct. There are a few extinct species for which scientists saved some cells before extinction - such as the Bucardo ibex, Po'ouli honeyeater, some snails, and frogs - but sadly for most of these cells of only a single individual were saved, and therefore it is unlikely those species can be brought back without a lot of gene-editing and hybridization with living relatives. These species are also not entirely extinct, as living cells still exist for them - replicating genomes faithfully from one cell generation to another. If cells are saved for males and females of a species, with enough genetic diversity, then with reproductive technologies like cloning, it is possible to reproduce and save them. This is why biobanking is so

important - otherwise, de-extinction via gene-editing is required to revive an extinct species, meaning that the extinct species can never be truly recovered, only replaced ecologically with a carefully gene-edited living relative. This process requires ancient DNA sequencing, DNA synthesis, cell culture, gene-editing in cultured cells - and then a reproductive technology to turn those gene-edited cells into embryos, and in the case of many species, a surrogate mother to gestate and give birth (for birds the gene-edited cells are implanted into surrogate mothers and surrogate fathers, which then breed to produce the embryos and lay eggs (and for birds, also a surrogate father and gene-editing of a living relative - however this technology has only been achieved so far in chicken, but Revive & Restore is working to create reproductive technologies for more birds).

What differences are there in cloning an extinct animal compared to cloning a living one?

In Revive & Restore's collaborative conservation cloning efforts for the living endangered Przewalski's horse and black-footed ferret, we use a process called interspecies, or cross-species, cloning. We use the egg cells of a common, non-endangered relative of the endangered species to create the cloned embryos of these endangered species. We then use the same non-endangered relative to be the surrogate mother. A similar process would be used to clone an extinct species. However, cloning fish and amphibians doesn't not require surrogate mothers. The world's only effort to clone an extinct (or evolutionarily torpid species) is the Lazarus project, working to revive the gastric brooding frog of Australia.

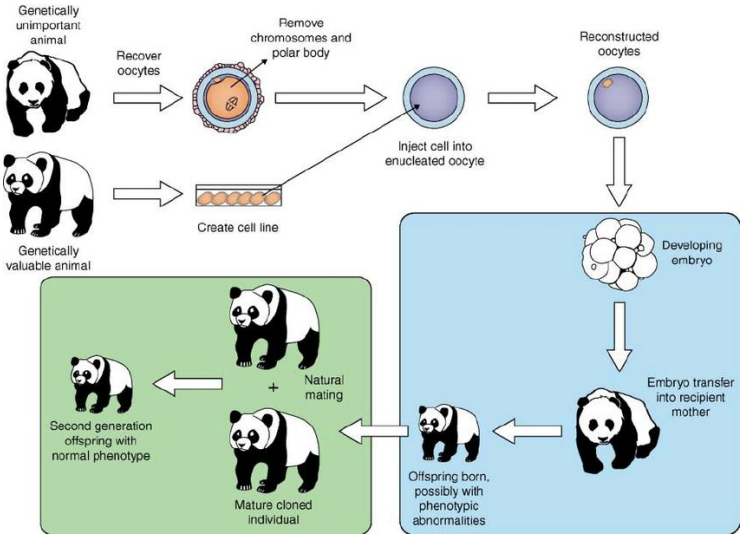

Figure 3: Cloning process of endangered species

What are the benefits of bringing back extinct creatures?

People have been restoring locally extinct species and populations for the last 200 years for many reasons - sometimes to restore biodiversity and other times to restore ecological functions. Sometimes restoration is motivated by ecotourism. Bringing back species that are completely extinct is being done for the same reasons, but chiefly the extinct species that people are working on now (the passenger pigeon, woolly mammoth, thylacine) are species that can help prevent other living species from going extinct. These extinct species played vital roles in the good chain and habitat engineering that will help living ecosystems. In eastern North American forests, many species are struggling to thrive due to the loss of forest disturbances, which passenger pigeons once perpetuated.

What is your opinion regarding the ethics of cloning?

The cloning company we work with, ViaGen pets and Equine, are an incredible company that provides great welfare and care to the animals involved in the cloning process. When we cloned Elizabeth Ann the black-footed ferret, the domestic ferrets used were rehomed as pets and some went to a museum as animal ambassadors to help kids learn about cloning and conservation.

There is always a way to use biotechnology responsibly, and we strive to do the best for the species we work with toward the goal of saving biodiversity and ecosystems. Cloning is a good technology for conservation and could be used more to help other endangered species.

5. Ethical Views on Cloning

In what follows, I would like to refer to the two papers on the ethics of cloning and de-extinction that were linked to us by Ben J. Novak, one being his own book chapter.

5.1 Ethical Analysis of Cloning

Ethical analysis of cloning for genetic rescue is based on a framework, a table, focusing on three related assessments, these being (Sandler et al., 2021):

- Goal Assessment: Is the goal of the project well justified?
- Means Assessment: Can the goal of the project be accomplished responsibly?
- Desirability Assessment: Is the project overall desirable?

These assessments are again subdivided and defined. If a project passes all evaluations, it should be viewed as ethically justifiable. A controversial topic like this can lead to difficult discussions about right and wrong. In our opinion, it is beneficial to have a framework to draw upon. Therefore, taking stance on the framework, if a project passes all the assessments and can be shown to achieve the goal responsibly, we think it is defensible, even if there is room for debate.

Level of assessment	Areas of analysis	Relevant question
Means assessment Can the goal of the project be accomplished responsibly?	Opportunity cost	Would the resources used in support of the project be more efficiently or effectively used elsewhere?
	Comparable effectiveness	Are there alternative approaches to accomplishing the goal that would be more effective, immediate, less costly or more likely to succeed?
	Animal welfare	What are the direct and indirect effects on the non-human animals involved in or impacted by the project?
	Ethical conduct of research	Is the research design sensitive to the social and cultural values involved in the project, and does it avoid bias and conflicts of interest?
	Oversight	What are the oversight and accountability mechanisms for the project?
Desirability assessment Is the project overall desirable?	Public support	Is the project acceptable to the communities that are engaged with the population, species, and system involved?
	Conservation context/issues	How does the use of conservation cloning express, challenge or otherwise intersect with different conservation philosophies or paradigms?
	Project structure/ support	Do other participants in the broader project and the groups and organizations involved support the strategy, and how will it impact related project components?

Figure 4: Framework on ethical analysis of cloning

5.3 The Woolly Mammoth Welfare

The Woolly Mammoth Revival is a project focusing on bringing back the extinct species so that herds can repopulate tundra and boreal forest in Eurasia and North America. This is being achieved by using a close relative of the Mammoth that is still very much alive - the Asian elephant.[3] Even though the goal and cause of the project is very remarkable scientist have expressed their concerns on how justifiable the means are (Novak, 2019). Living elephants are threatened in the wild but providing an adequate ex situ habitat, a conservation in which the animal has been moved from its original home is difficult (Novak, 2019). Even though de-extinction projects can lead to a good outcome and the goal is plausible, the welfare of the animals is still questionable which might raise questions in the region of ethical rights and wrongs and the overall relevance of the project (more on this in Building De-Extinction Programs – Ben J. Novak)

5.3 Pros and Cons of Cloning

Cloning is being harshly debated, this is because of ethical, cultural, and religious reasons. Most of the themes mentioned here are only ideas and need many more years of thorough research.

Some arguments for the use of cloning technologies:

- Cloning could be used by scientists to **clone extinct animals**, even raising genetic diversity in animals on the brink of extinction.[4] This could save thousands of populations and species. For example, the northern white Rhinoceros, of which there are only two females left,[5] could be saved by using this technology and cloning a male.
- Cloning is one of, if not **the oldest form of reproduction**. Through asexual reproduction living organisms have been reproducing for eons.[4] Furthermore, all cells in our bodies are cloning themselves constantly through cell division.
- Another advantage of cloning is that **it helps with food production**. It is used in making more efficient, more nutritious and more disease resistant crops, produce, and livestock. By changing their biological processes and making them more desirable and getting rid of the genetic "lottery", these man-made organisms use less land, less water and need less fertilizer and pesticides[4]. This could also be used for genetic modification and cloning of livestock which emits less carbon dioxide and is less harmful for the environment and the climate.
- In the future the technology could be used **for infertile or same-sex couples** who want to have children. This would open opportunities for people who want to have children who biologically belong to them and therefore do not want to adopt. With cloning, we could genetically manipulate the children to have traits from both parents. Of course, assuming this could be done safely and without harm.[4]
- Using **cloning in health care** could be another advantage, for example cloning organs or whole body parts for people in need, rather than relying on organ donors, would moreover be possible. This would certainly make the chance higher for the body to accept the new organ. This could furthermore help people with prosthetics or amputations by, for example, making a new leg by cloning it and growing it in the lab.

Arguments against the use of cloning technologies:

- The process is **neither accurate nor safe**, it is not yet fully developed. If for example a baby is cloned, then it won't be exactly the same in behavior and physical appearance because of **epigenetics**. They will grow up in different habitats and will have different nutrition, which will lead to different characteristics[4,6]. Also another point is that most clones produced have health problems. This might influence their lifespans. For example, Dolly the sheep had abnormalities in her DNA, in essence shortened telomeres. They had not been fully renewed during her development, leaving her with shorter telomeres than would be expected for a normal sheep of the same age.[4]
- The strongest argument against cloning is that **it could be abused and is generally unethical**, for this reason the topic raises lots of moral, religious and societal questions. In the case of Dolly, the sheep, 277 attempts were made before a usable clone was made. This suggests that if humans were ever cloned, researchers would need to make numerous "failed" attempts[4]. There would certainly be concerns that the technology could be used to enhance people in sports and for military applications. For example, the creation of genetically superior individuals or the manipulation of human characteristics.

In conclusion the technology is still in its early stages, and it will take years until it will be usable on humans. The research for cloning is also not allowed in many countries, because of religious and cultural reasons.[4] If we're cloning animals to promote the conservation of species, then society should first acknowledge the main forces driving the extinction of animal populations: the destruction of habitats and hunting. The ethics of cloning will likely continue to be a subject of ongoing debate as technology develops and its potential uses are further explored.

6. Summary

There is still much to learn about cloning technologies, ethics, and applications but regarding where we were just a few decades ago, the progress we made is undeniable. Nowadays we have multiple ways of cloning different, fully functional organisms. From the leopard frogs to the birth of Dolly in 1996, to trying to bring back the woolly mammoth. Many may see this as a waste of time, money and skills, an unethical procedure with no benefits for either us humans or for mother nature. But on the other hand, there are many, that think of cloning as a profitable technology, saying it helps preserve biodiversity by supporting endangered species and even bringing back extinct ones, or that it promotes and makes agriculture more efficient directly raising society's life standards. Either way it can most definitely lead to very interesting topics and discussions with experts like Ben J. Novak, who spent years of his life studying the art of biotechnology and are more than capable of teaching us about less talked about section of science.

Sources

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[2]Revive & Restore | Genetic Rescue to Enhance Biodiversity (reviverestore.org)

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[4]Top 7 Pros and Cons of Cloning (www.bioexplorer.net)

[5] The white rhino (www.worldwildlife.org)

[6]Epigenetics (en.wikipedia.org)

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Figure, Dolly the sheep dolly-the-sheep.jpg (2048×1536) (independent.co.uk)

Figure 1, Process of somatic cell nuclear transfer R.a5c41d71fd2cfdcabbfcc2b74cf3f36f (1300×591) (bing.com)

Figure 2, Graph showing telomeres shortening in somatic cells with each cell division https://media.springernature.com/full/springer-static/image/art%3A10.1186%2F1477-7827-1-105/MediaObjects/12958_2003_Article_105_Fig1_HTML.jpg?as=webp

Figure 3, Cloning process of endangered species https://www.researchgate.net/profile/Amanda-Pickard/publication/224876350/figure/fig1/AS:302816196349967@1449208415959/Schematic-diagram-showing-the-steps-by-which-a-genetically-valuable-animal-nucleus.png

Figure 4, Framework on ethical analysis of cloning Sandler_Moses_Wisely_2021.pdf