WOOLLY MAMMOTH: FROM ICE TO LIFE BY 2027?

Bachelor's Degree in Genetics

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Bibliographic review project

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OBJECTIVES

1. To assess Colossal Biosciences' woolly mammoth de-extinction project.
2. To review the state of the art of the different techniques presented.
3. Based on current knowledge, to identify the main challenges associated with the project.
4. To consider the genetic viability of the created populations.

METHODOLOGY

1. Initial analysis of the Colossal Biosciences project, including identification of critical steps.
2. Primary searches on each project challenge using Scopus.
3. Complementation with information from other cited papers and patents.
4. Analysis of the collected information to draw conclusions about the current state of the project.

COLOSSAL BIOSCIENCES PROJECT

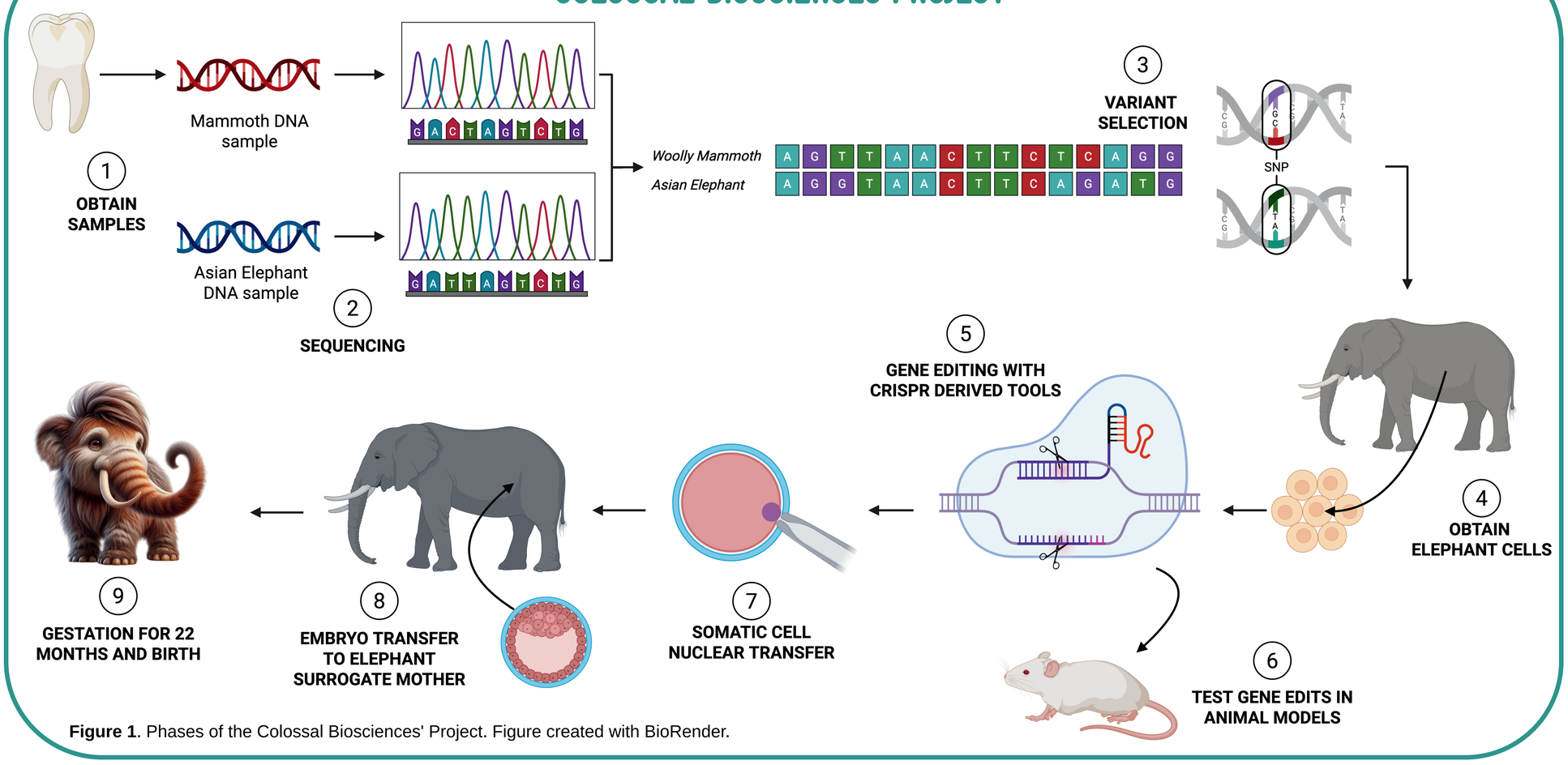


Figure 1. Phases of the Colossal Biosciences' Project. Figure created with BioRender.

MAIN CHALLENGES

1. SEQUENCING

- Due to the high fragmentation state of the mammoth DNA samples, assembly with a reference genome is currently necessary.
- Lin *et al.* (2022), in their experiment on Christmas Island rats, showed that this type of assembly can make important regions for adaptation unrecoverable, such as olfactory and immune system genes.
- Sandoval-Velasco *et al.* (2025) discovered that the three-dimensional genome architecture persisted in a mammoth DNA sample. This suggests a future use of a variant of the HI-C technique (PaleoHI-C) to achieve a *de novo* assembly.

2. VARIANT SELECTION

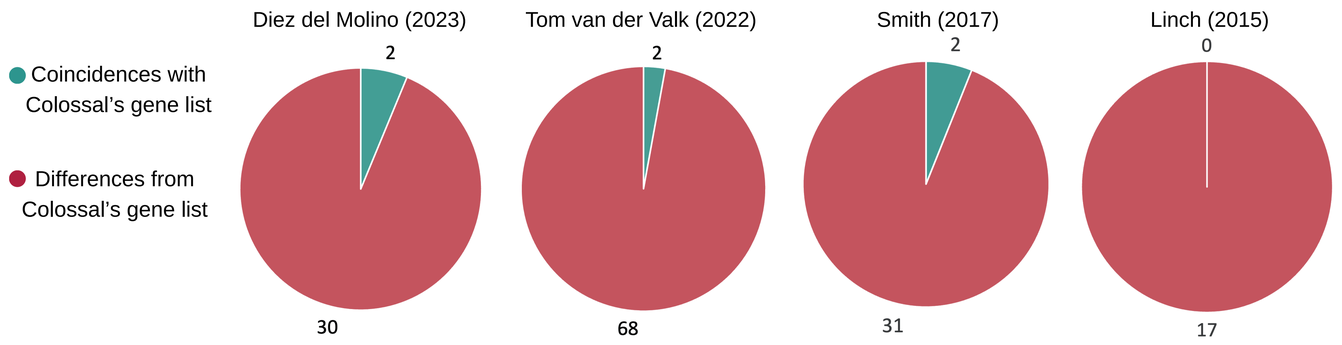
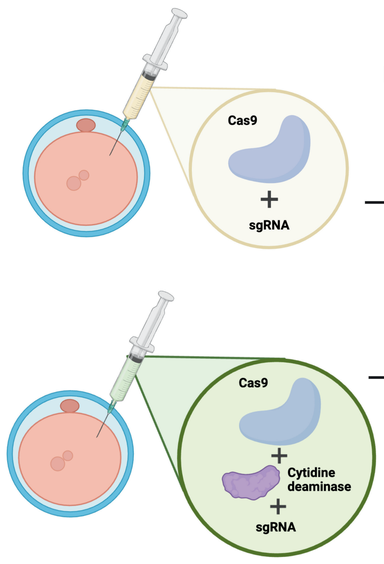


Figure 2. Gene selection comparison. Each graphic shows the number of genes in each study that are also in the Colossal Biosciences' patent. The results highlight significant discrepancies and the difficulty of the process. Figure created with Excel.

3. GENOME EDITING

Table 1. Genome editing tecniques

	CRISPR Cas9	Base editing	Prime editing
Mutations	Gene knockout	Transitions and transversions	Transitions, transversions, insertions and deletions
Advantages	Easy for multiplexing	Fewer double-strand breaks	Specificity and flexibility for target selection
Challenges	Target base selection, apoptosis, specificity and efficiency	Target base selection, bystander edits and off-target effects	Low efficiency, higher indels



COLOSSAL BIOSCIENCES' WOOLLY MOUSE EXPERIMENTS

Experiment	Genes	Blastocyst formation	Pups born	Mice fully edited	Overall efficiency of the process	Off-target modifications in 1 individual	Missense modifications?
A	*Fgf5 Mc1r Fzd6 Fam83g Fabp2*	47% (63 out of 134)	18% (11 out of 60)	9% (**1** out of 11)	0.78%	11	No
C	*Fgf5 Mc1r Fam83g Fzd6 Tgm3 Astn2 Fabp2*	70% (78 out of 111)	11% (5 out of 47)	0% (**0** out of 8) **2 mice fully KO for 6 genes*	0%	56	No

Figure 3. Colossal Biosciences' woolly mouse experiments illustrate the present inefficiency of multiplexing genome editing tools. Figure created with BioRender.

4. MITOCHONDRIAL DNA

- 13 mitochondrial genes encode important units in the oxidative phosphorylation pathway and metabolism.
- Positive selection of mitochondrial gene variants aids adaptation to cold environments in several species.
- Ngatia *et al.* (2019) found mutations in 5 woolly mammoth mitochondrial genes that could impact cold adaptation.
- Colossal Biosciences' project, however, does not involve mitogenome modification.

5. REPRODUCTIVE BIOLOGY

- Currently, no successful protocol exists for ovarian stimulation and oocyte recovery on living elephants.
- Somatic cell nuclear transfer (SCNT) is an inefficient process with potential risks to the resulting organisms.
- The endangered status of the Asian elephant precludes its use for gestating large numbers of de-extinct woolly mammoths. Colossal Biosciences plans to use artificial wombs instead, but this technique is not yet developed.

6. FUTURE GENETIC DIVERSITY

- The last woolly mammoths survived until 4,000 years ago on Wrangel Island and experienced genetic bottlenecks. Including their samples in variant selection could reduce the adaptive potential of the resulting organisms.
- The minimum viable population size for short-term survival is 100 genetically distinct individuals, whereas 1,000 are required for long-term survival. All project steps must be highly optimized to generate this number.

CONCLUSIONS

- Colossal Biosciences' woolly mammoth will never fully resemble a real one. Genetically, it will be an elephant with some engineered woolly mammoth traits.
- Advancements in technologies such as PaleoHi-C for genome assembly and studies to improve genome editing techniques could help solve some of the project's constraints in the future.
- Certain aspects of the process, however, such as assisted reproduction techniques and variant selection, seem challenging to address in the short term.
- Due to the technical limitations of the project, it is unlikely that Colossal Biosciences will be able to generate this mammoth-like elephant by 2027.

BIBLIOGRAPHY

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