Alignment of Colossal's Dire Wolf De-Extinction Project with IUCN SSC Guiding Principles

Introduction

The primary objective of our dire wolf de-extinction project is to develop new tools with the capacity to augment the conservation and careful stewardship of species, both extant and functionally extinct. The goal of Colossal's dire wolf proxy project is not merely symbolic restoration; it is the development and rigorous assessment of next-generation conservation technologies. In this document, we address how our dire wolf de-extinction project aligns with the 2016 IUCN SSC Guiding Principles [GP] on Creating Proxies of Extinct Species for Conservation Benefit.[1] Consistent with Section II of the IUCN Guidelines – which stresses that proxy creation must support, not replace, the preservation of extant biodiversity – we position the dire-wolf project as an additive conservation tool rather than a technological substitute for traditional protection measures.

[1] https://portals.iucn.org/library/efiles/documents/Rep-2016-009.pdf

Expectation of conservation benefit (GP 1)

Principle 1 (positive conservation benefit)

IUCN Principle: "There should be a priori positive justification for engaging in proxy species creation based on the expectation of a positive conservation benefit…"

Colossal Alignment: We acknowledge and endorse the IUCN SSC position that any "proxy species" effort must demonstrate a clear conservation benefit. Our dire wolf de-extinction project is fundamentally a conservation technology platform designed to advance techniques that can benefit extant endangered canids. The conservation benefit arises not from reintroducing our proxy dire wolves into already established gray wolf habitats, but to provide a foundation for evaluating genomic biotechnologies as tools for broad conservation application.This work aligns with the IUCN SSC's call for “demonstrable, explicit, additive, conservation benefits” by contributing directly to genetic rescue, biobanking, and veterinary innovations for threatened species. One of this project’s primary benefits is enabling evaluation of the safety and conservation benefits of future genome editing applications in conservation biology, as rigorous evaluation is only practical when there is significant scientific infrastructure to act as a control. We emphasize the following conservation benefits that this work provides:

- A testbed for developing precise multi-gene editing techniques applicable to genetic rescue of endangered canids. We position the dire wolf project as a pioneering technology development platform, focusing on genetic rescue and veterinary advancements for endangered canids globally.
- A pathway to improve veterinary care and reproductive technologies for threatened canid species. Work stemming from this project has already resulted in significant practical conservation outcomes, including the birth of four critically endangered red wolves[2] through our innovative non-invasive blood cloning technology, demonstrating direct and immediate applicability to living species conservation. The successful birth of three proxy dire wolves and four critically endangered red wolves demonstrates that the same technology stack immediately benefits living taxa as well as extinct phenotypes.
- Publicly available protocols on canid genomics,[3] canid deep sequencing data,[4] assisted reproduction, and husbandry[5] that benefit the broader conservation community.
- Biobanking innovations such as EPC cell line cloning from non-invasive blood draws.
- A public-facing demonstration that de-extinction is compatible with IUCN's "additive benefit" test, as the project drew significant funding from new private technology development capital that was not diverted from existing wolf recovery budgets.

Ecological and candidate selection criteria (GP 2-5)

Principle 2 (ecological role)

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[2] De-extinction startup Colossal Biosciences believes it can help North Carolina's dwindling red wolf population. https://www.axios.com/local/raleigh/2025/04/08/colossal-biosciences-clone-red-wolves-and-extinct-direwolves.

[3] See the methods section in our manuscript: Gedman, Gregory, et al. "On the ancestry and evolution of the extinct dire wolf." bioRxiv (2025) https://www.biorxiv.org/content/10.1101/2025.04.09.647074v1.

[4] All data generated for the dire wolf ancestry and evolution paper is available at NCBI BioProject PRJNA1222369.

[5] https://colossal.com/wp-content/themes/colossal/Dire_Wolf_Husbandry_Manual.pdf

IUCN Principle: "Alternative ecological replacements must be considered because suitable ecological proxies might be found among extant species…"

Colossal Alignment: Colossal recognizes that the ecological roles previously occupied by the dire wolf are now effectively filled by extant species such as the gray wolf. Therefore, rather than serving as an ecological proxy, the proxy dire wolf primarily acts as a model species for pioneering conservation technologies and genomic editing techniques beneficial to modern species conservation. We have no plans to release dire wolf proxies into existing gray wolf habitats or to disrupt established canid communities. We are not pursuing ecological replacement as the primary goal, but rather technological advancement that will provide a critical "second aid" to critically endangered and demographically recovered but genetically depleted populations, complementary to traditional conservation approaches and habitat restoration.

Principle 3 (de-extinction selection criteria)

IUCN Principle: Selection should apply comprehensive criteria, including conservation benefits, invasive potential, generation time, human-wildlife conflict potential, and ecological roles.

Colossal Alignment: In selecting the dire wolf, we prioritized criteria including the need for minimal genetic editing due to substantial genetic overlap with the extant gray wolf. The project also emphasized strong animal welfare infrastructure and ethical veterinary protocols to mitigate risks to the animals throughout the project.

Our selection of the dire wolf as our initial de-extinction candidate was based on several factors:

- Minimal genetic edits required to achieve a predictable result, due to genomic and phenotypic similarity between extinct dire wolves and extant gray wolves. This positions the dire wolf de-extinction project as the best model system to develop the technology to benefit canids (and conservation more broadly) in a way that is consistent with IUCN goals.
- Genomic infrastructure from gray wolves and dogs that enable prioritization of animal welfare when choosing gene editing targets.
- Existing veterinary expertise and husbandry protocols for gray wolf (dog) care.
- The use of domestic dogs as gestational surrogates was explicitly chosen due to extensive veterinary knowledge, minimizing risks to surrogates and offspring.
- Established research infrastructure for ethical and welfare-centered development.

Target quantitative success metrics include:

- ≥ 2 conservation programs adopting genome editing or cloning methods developed here by 2027.
- ≥ 5 scientific publications citing dire wolf genome datasets within 3 years.
- **Zero** containment breaches from secure preserve.

Principle 4 (re-establishment)

IUCN Principle: Evaluate re-establishment of natural assemblages in suitable habitats where threats leading to extinction are controlled.

Colossal Alignment: Colossal recognizes that re-creating original Pleistocene ecosystems is neither feasible nor ecologically sound given contemporary environments. Thus, Colossal's approach emphasizes

highly controlled managed care environments (detail below) to mitigate ecological risks and effectively manage animal welfare and research outcomes.

Principle 5 (IUCN translocation guidelines)

IUCN Principle: Acknowledging IUCN Guidelines for reintroductions and translocations.

Colossal Alignment: Adhering strictly to this principle, Colossal maintains all proxy dire wolves within secured, controlled environments without any current or immediate future plans for environmental reintroduction. This controlled setting ensures comprehensive monitoring, risk assessment, and animal welfare management.

Managed release, containment, risk management (GP 6-21)

Principles 6-8 (release planning)

IUCN Principles: Principles 6-8 emphasize meticulous planning and phased evaluation for any potential translocation, highlighting the need for thorough assessments of predicted population performance, behavior, ecological roles, obtaining informed consent from local and indigenous communities, and matching habitat suitability to species needs.

Colossal Alignment: Colossal employs a rigorously phased and controlled approach, ensuring comprehensive health, genetic, behavioral, and ecological assessments are conducted exclusively in specialized managed-care settings. There are no immediate plans for environmental release. Future considerations for any potential release or translocation of other de-extinct species would only advance following extensive ecological suitability evaluations, detailed behavioral monitoring, and secured informed consent from local community and indigenous partners,[6] aligning fully with established IUCN guidelines and ethical standards. We believe that as a first practical application of de-extinction, dire wolf proxies can reveal vital information about CRISPR gene editing outcomes, mult-gene interactions, and canid evolutionary biology.

Principles 9-12 (phased approaches)

IUCN Principles: Use phased approaches including confinement and staged releases to evaluate performance and manage risks. Engage local communities, match habitat suitability to needs of focal species. Detailed risk assessment on proxy species welfare and ecosystem impacts.

Colossal Alignment: Colossal employs a phased, methodical approach emphasizing extensive animal welfare, genetic stability monitoring, and health assessments. This phased strategy is essential for

[6] The effort to de-extinct the dire wolf, genetic rescue of the red wolf, and innovative gray wolf conservation programs are all made possible through Colossal's collaborative work with a broad spectrum of Indigenous communities, conservation organizations, and scientific experts. We extend our deepest gratitude to the MHA Nation, the Nez Perce Tribe, the Karankawa Tribe of Texas, INDIGENOUS LED, and the Wind River Tribal Buffalo Initiative, whose ancestral knowledge and insights have guided our efforts. We also work with many conservation organizations whose expertise and passion fuel these initiatives, including the American Wolf Foundation, Re:wild, Conservation Nation, Gulf Coast Canid Project, International Coexistence Network, Wolf Connection, Grizzly Systems and the Yellowstone Wolf Project.

responsibly managing risks and ensuring animal welfare while systematically documenting scientific outcomes. We affirm that the IUCN SSC's guiding principles on caution and risk assessment (GP 9 and 11) are integral to our phased methodology. Colossal implements comprehensive genetic and veterinary health monitoring to identify and mitigate risks proactively. This approach ensures ecological stability, disease prevention, and management of socio-economic impacts through rigorous, transparent methodologies.

Managed care is a strategic compromise and provides phased steps toward a science-driven conservation outcome. Following the guiding principles' emphasis on phased approaches (Principles 6-8), we elected to keep our proxy dire wolves in a carefully controlled environment. This strategy is not about creating "zoo animals"; rather, it is an essential and ethical step enabling:

- Longitudinal health monitoring: We will track cancer rates, immune and epi/genome function, aging patterns, and stress indicators over the lifespan. These data offer a rare longitudinal look at precise multi-gene edits in a large carnivore.
- CRISPR safety baseline: We aim to detect any unexpected "off-target" or secondary effects that may arise during development, gleaning lessons crucial for using gene editing in threatened canid populations.
- Epigenetic effects monitoring: Monitoring whether and how gene editing impacts gene expression or epigenetic regulation across tissue types. This provides knowledge directly relevant to genetic rescue scenarios for extant species.
- Physiological integration: Establishing how these edits influence the dire wolf proxy's complete organ systems helps refine gene targets and editing protocols, ensuring better welfare and reducing the likelihood of harmful ecosystem impacts in any future reintroduction project for other species.

By employing such systematic evaluation, we believe we meet the IUCN SSC's call for "in-depth risk assessment" (Principle 9) and "monitoring ... to retain the ability to terminate the trial" if unforeseen problems arise (Principle 13, below).

Principles 13-21 (staged release, risk management)

IUCN Principles: "A trial translocation, involving confinement facilities and staged releases, should be used to evaluate post-release performance, and to enable refinement for future translocations…" These principles call for risk assessment for disease, hybridization, close behavioral and genetic monitoring, socioeconomic impacts, exit strategy, and needs of released species.

Colossal Alignment: Colossal maintains rigorous health and genetic monitoring protocols that allow continuous assessment and immediate intervention, including potential project refinement or termination, based on clearly established thresholds to prevent negative impacts. From the outset, we have followed the guidelines urging a structured, stepwise approach, akin to a "trial translocation" under controlled conditions (Principle 13). These animals are maintained in specialized managed care, allowing rigorous health and welfare monitoring before any potential for broader introduction is ever considered. This phased strategy aligns with the IUCN SSC's emphasis on risk assessment. The proxy dire wolves reside within a state-of-the-art, highly secure 2,000-acre preserve certified by the American Humane Society. The facility features extensive perimeter security, real-time monitoring technologies, and protocols that far exceed standard containment measures, ensuring responsible population management. Colossal explicitly evaluates habitat suitability and recognizes contemporary ecosystems differ significantly from those existing during the dire wolf's era. Consequently, Colossal ensures the dire wolves are kept in environments meticulously designed to meet species-specific needs, thereby avoiding potential adverse ecological

impacts. Long-term management plans emphasize continual animal welfare assessments and controlled, scientific observations to gather data beneficial for future conservation applications.

Success measurement and transparency (GP 22-27)

Principles 22-25 (success metrics)

IUCN Principles: Principles 22-25 require clearly articulated definitions of success, including specific goals detailing the intended conservation benefit. Tangible, measurable objectives must be explicitly associated with each goal, addressing various components of the project, such as alternative strategies, feasibility studies, planning, consultation, implementation, and evaluation phases.

Colossal Alignment: Colossal's dire wolf de-extinction initiative explicitly adheres to these guiding principles by establishing comprehensive, measurable, and transparent success criteria across all aspects of the project:

- Technical objectives and validation: Clear technical success goals were defined, including the precise introduction and validation of 20 genomic edits across 14 genes. Each genomic edit was rigorously confirmed via high quality whole-genome sequencing, karyotyping, and comprehensive bioinformatic analysis, setting a new benchmark in genomic editing precision for conservation applications.
- Animal welfare goals: Animal welfare success criteria are clearly established, monitored, and externally validated by the American Humane Society. Metrics for animal well-being include regular assessments of physiological health, stress indicators, enrichment effectiveness, and social behavior, ensuring the highest standards of ethical care.[7]
- Scientific transparency and knowledge dissemination: Colossal maintains complete transparency and accountability by openly sharing methodologies, protocols, and outcomes through peer-reviewed publications.[8] Key findings, such as the genomic characterization of dire wolves, are publicly documented, facilitating rigorous scientific scrutiny, replication, and application in broader conservation contexts.
- Public data availability: All genetic, genomic, and husbandry-related data generated by the dire wolf evolution and ancestry project are publicly available.[9] This accessibility ensures broad stakeholder engagement, supports global conservation collaboration, and enhances knowledge transfer to projects conserving extant endangered canids.
- Comprehensive project implementation and evaluation: Structured feasibility assessments and stakeholder consultations were systematically conducted before project initiation. Ongoing iterative evaluations ensure continuous alignment with conservation objectives, allowing proactive adjustment based on defined success metrics. This structured, data-driven approach ensures transparent decision-making and informed progression through each phase of the project, in alignment with international conservation standards and ethical guidelines.

Principles 26-27 (genetic and phenotypic variation)

[7] Dire wolf husbandry manual: https://colossal.com/wp-content/themes/colossal/Dire_Wolf_Husbandry_Manual.pdf
[8] Gedman, Gregory, et al. "On the ancestry and evolution of the extinct dire wolf." bioRxiv (2025) https://www.biorxiv.org/content/10.1101/2025.04.09.647074v1.
[9] All data generated for the dire wolf ancestry and evolution paper is available at NCBI BioProject PRJNA1222369.

IUCN Principles: Principles 26 and 27 highlight the possibility of genetic, phenotypic, and behavioral differences from the original species, highlighting issues such as genetic variation, limited genetic diversity, epigenetic effects, genetic stability, social and learning deficits, and potential alterations to ecosystem functions. They stress the importance of comprehensive genome-wide assessments and understanding genetic architecture to gauge likely project outcomes. They explicitly note the “possibility of significant differences from the original species due to genetic variability, epigenetic effects, genetic stability, social and learning deficits, and potential altered ecosystem functions.” and explore “genome-wide assessment and genetic architecture across taxonomic groups and their likely influence on success"

Colossal Alignment: Colossal explicitly acknowledges that significant genetic and phenotypic differences from the original dire wolf species are inevitable due to factors including genetic variability, epigenetic influences, and potential behavioral divergence. To understand these variables, Colossal employed comprehensive genome-wide assessments in the recent dire wolf evolution and ancestry manuscript.[10] Additionally, the dire wolf’s close genetic and phenotypic similarity with the gray wolf makes it a good first candidate for functional de-extinction. Genetic, epigenetic, immunological, and general physiological responses to novel editing can be studied reliably in a group for which reference data exist. Continuous analyses of genetic architecture and associated outcomes ensure proactive management of these differences. Colossal’s rigorous monitoring protocols provide essential data to refine techniques for future conservation initiatives, and underscore the importance of maintaining dire wolf proxies strictly within controlled environments without plans for ecological release to avoid unforeseen ecological disruptions.

Avoidance of opportunity costs (GP 28-30)

Principles 28-29 (not diverting resources)

IUCN Principles: Principles 28 and 29 stress that de-extinction efforts should neither directly nor indirectly endanger extant species, particularly by diverting resources (opportunity costs) away from their conservation. Additionally, while early-stage costs might be covered by non-traditional funding sources, transparency and stakeholder understanding of potential resource allocations are essential.

Colossal Alignment: Colossal has explicitly drawn in unique funding sources dedicated to conservation innovation, ensuring traditional conservation funding streams remain unaffected. Our outreach materials highlight our partnerships with conventional conservation NGOs, demonstrate their importance and work, and highlight that species conservation does not succeed without protected habitat. The dire wolf project is funded by alternative sources specifically dedicated to conservation technology innovations and de-extinction research, thus avoiding any competition for resources that would otherwise support extant species conservation. Colossal has not received nor has Colossal applied for traditional sources of conservation funding from research-focused governmental organizations like the National Science Foundation (NSF), Environmental Protection Agency (EPA), etc. Colossal transparently communicates all funding sources ensuring informed rational decision-making among stakeholders and avoiding hidden opportunity costs to existing conservation efforts. Rather than diverting funds away from conservation, the newly developed technologies funded by non-traditional tech investments directly enhance conservation strategies for current endangered species.

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[10] Gedman, Gregory, et al. "On the ancestry and evolution of the extinct dire wolf." bioRxiv (2025) https://www.biorxiv.org/content/10.1101/2025.04.09.647074v1.

Principle 30 (complementary to traditional conservation)

IUCN Principle: "Evaluation of the costs and benefits of proxy species creation compared to alternative management strategies for achieving similar conservation goals should be performed early in the assessment of project feasibility…"

Colossal Alignment: The dire wolf de-extinction project is not an alternative to traditional management strategies – it is a complement to traditional conservation. Benefits including the advancements of genetic rescue technologies and assisted reproductive technologies justified the investment, particularly given unique funding sources and the strategic importance of developing next-generation conservation tools applicable broadly to biodiversity preservation.

Ethical management of proxy species (GP 31-38)

Principle 31 (animal welfare)

IUCN Principle: "All efforts should be made to minimise the suffering of individuals, of focal species, of gestational surrogates, and of other affected species, at every stage of the 'de-extinction' process."

Colossal Alignment: Colossal prioritizes animal welfare by employing specialized animal care and health professionals with extensive expertise in canid health and welfare. Colossal employs an independent Institutional Animal Care and Use Committee (IACUC) to ensure that all managed care and research procedures are reviewed, approved and comply with USDA guidelines established under the Animal Welfare Act for domestic and wild canids under managed care. Our care facility is externally certified by the American Humane Society,[11] ensuring best-practice care standards. Domestic dogs are chosen as gestational surrogates specifically due to well-documented veterinary care protocols and minimized risk of adverse outcomes. Continuous monitoring protocols track health metrics, stress indicators, and overall welfare to promptly address any welfare concerns.

Principles 32-33 (captive management and humane care)

IUCN Principles: Principle 32 emphasizes captive management should not inherently be perpetual, although it recognizes some individuals may remain in captivity. Principle 33 mandates adherence to internationally acceptable standards for humane management.

Colossal Alignment: Our proxy dire wolves are maintained in facilities designed explicitly for large canids that meet international welfare standards and are certified by the American Humane Society. Comprehensive behavioral enrichment and welfare programs ensure robust psychological and social well-being. Wolves are housed in socially appropriate groupings informed by research on wolf behavior and husbandry. Complete transparency in husbandry practices is maintained through publicly available animal care protocols[12] and oversight by our external animal ethics committee (IACUC).

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[11] Statement from Robin Ganzert, Ph.D., CEO of American Humane Society on the dire wolf de-extinction: https://www.businesswire.com/news/home/20250407444322/en/Colossal-Announces-Worlds-First-De-Extinction-Birth-of-Dire-Wolves#:~:text=%E2%80%9CColossal%20has%20achieved%20American,of%20American%20Humane%20Society

[12] Dire wolf husbandry manual: https://colossal.com/wp-content/themes/colossal/Dire_Wolf_Husbandry_Manual.pdf

Principles 34-37 (translocation and release management)

IUCN Principles: Principles 34-37 outline necessary steps for responsible translocation, emphasizing best practices, thorough risk assessment, minimizing stress during translocation, and ongoing post-release monitoring.

Colossal Alignment: Although no immediate plans for environmental translocation exist, Colossal has committed to following IUCN best practices rigorously if future release or movement scenarios arise. All potential translocation activities would incorporate comprehensive risk assessments, clear communication of potential risks to stakeholders, and robust monitoring protocols, as outlined in our animal care protocol. Although we have no plans for environmental release of the dire wolf proxy, we nonetheless established detailed contingency plans for future welfare management should release ever be considered, aligning strictly with IUCN best practices. We are currently partnering with an external team of experts to build a conservation assessment and conservation evaluation report that will highlight the benefits and potential risks of de-extinction and rewilding. We will use this strategy document to identify areas where approaches to manage risk should be built in advance and to identify appropriate community members to help build these strategies.

Principle 38 (euthanasia)

IUCN Principle: "Euthanasia might be an appropriate action for individuals unsuitable for release or captive management that contributes to the establishment of wild populations."

Colossal Alignment: Colossal has clearly outlined humane criteria and conditions under which euthanasia may be considered, strictly prioritizing animal welfare and ethical best practices, only to be used as a last resort for critical welfare considerations.[13] Our independent IACUC reviewed and approved the criteria and methodology for humane euthanasia of all research animals following AVMA Guidelines for the Euthanasia of Animals: 2020 Edition[14] and in compliance with USDA reporting requirements. Decisions related to euthanasia are governed by comprehensive ethical guidelines, transparent communication, and strict oversight from veterinary and ethics committees. Commitment to ethical management ensures euthanasia is a rare, carefully considered option only when critical welfare considerations necessitate such intervention.

Conclusion

Colossal's dire wolf de-extinction initiative firmly aligns with the spirit and guidelines of the IUCN SSC by responsibly pioneering technologies critical for contemporary conservation needs. Our transparent and phased approach provides crucial insights into genetic editing, animal welfare, and ecological management, setting a robust ethical and scientific benchmark. We proactively acknowledge the complexities and uncertainties inherent in de-extinction and have implemented comprehensive measures to mitigate risks, enhance welfare, and support the broader conservation community. Through extensive collaboration, openness, and rigorous adherence to the IUCN SSC guidelines, we demonstrate how carefully conducted de-extinction research can complement and strengthen global biodiversity conservation efforts, rather than detract from them.

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[13] Dire wolf husbandry manual, pages 63 and 141. Available online at https://colossal.com/wp-content/themes/colossal/Dire_Wolf_Husbandry_Manual.pdf.
[14] https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf

Colossal at its core is a *species preservation company*. Our company's broader mission centers on species preservation and advancing conservation science. The dire wolf de-extinction initiative fits squarely within that mandate. By operating under the lens of the IUCN SSC Guiding Principles, we ensure that this project poses no risk to extant species, pursues tangible conservation benefits, and adopts a transparent, collaborative stance.

Ultimately, we recognize that no project can perfectly reconstitute an extinct species or replicate past ecosystems. Instead, we interpret "de-extinction" as a practical gateway to develop next-generation conservation tools: validating multi-gene editing approaches, refining animal welfare protocols, and gaining fresh insights into the complexities of large canid biology. Our goal is that the gene editing tools developed and demonstrated by the dire wolf project are a useful addition to our conservation toolbox. Far from undermining the urgency of efforts to conserve existing species, this project highlights the extraordinary effort needed to reverse such an extinction, underscoring the urgency to conserve existing species through habitat protection, population protection, and, if necessary, using modern genetic engineering tools like those developed through projects like this one.