

**BAND-TAILED PIGEON** is genetically the closest related species to the Passenger pigeon still alive today.

[a] DNA from Band-tailed pigeon tissue was obtained from a blood sample from "Sally," a female Band-tailed pigeon raised by professional breeder Sal Alvarez. The DNA code was sequenced and the resulting data assembled computationally to produce the genome of the species.

[b] In order to determine which sections of the genetic code are used to regulate cell functions and to create traits, the RNA needs to be sequenced. To discover and map RNA elements multiple tissues need to be sequenced. RNA from the brain, heart, lungs, liver, kidney and ovary tissues of one of Sally's embryonic offspring was therefore sequenced to identify the protein-coding genes of the genome.

**THE GREAT PASSENGER PIGEON COMEBACK** began in 2012 with a central paradigm: de-extinction needed a model candidate.

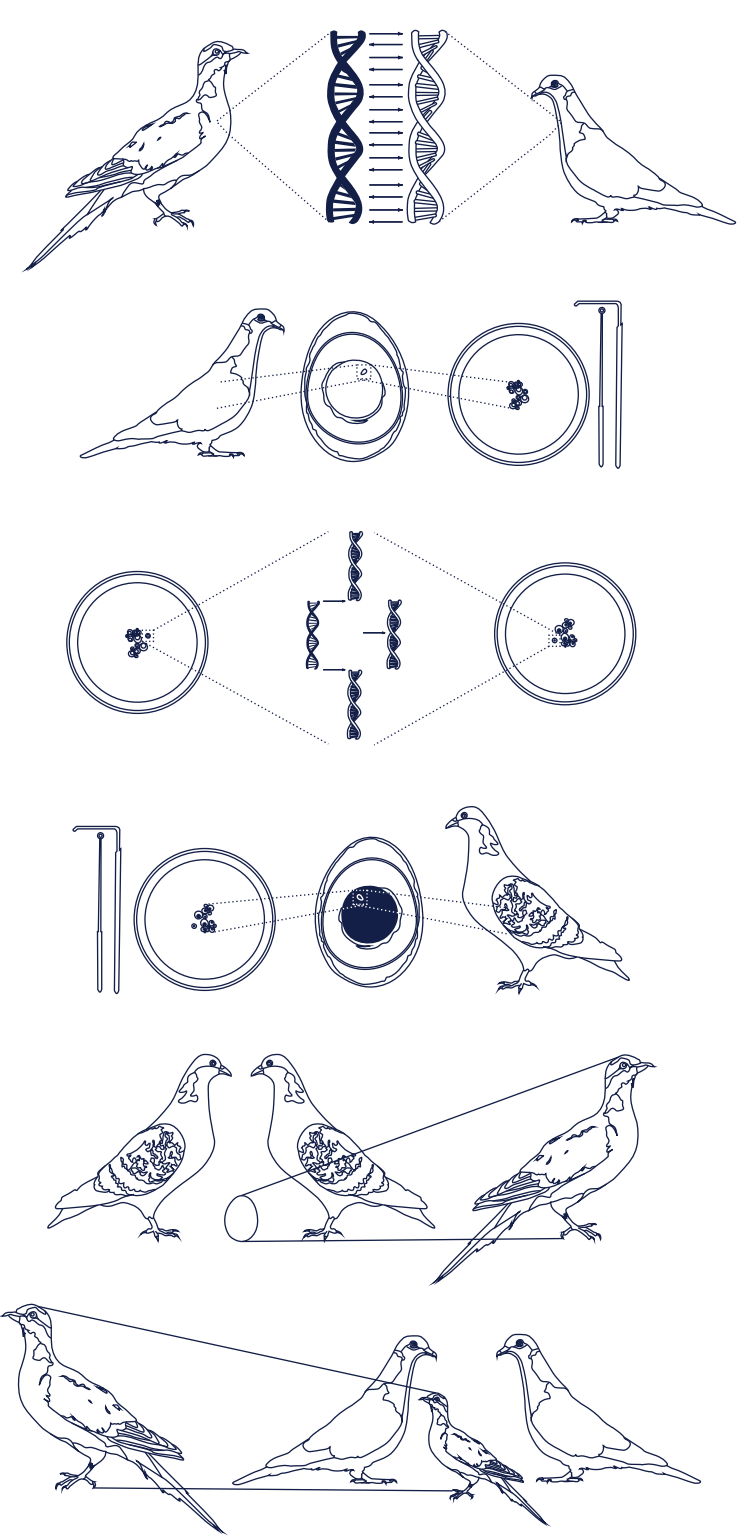
[a] The conservation movement itself formed in response to the extinction of the Passenger pigeon. When the last birds were shot in the wild, mere decades after their population numbered in the billions, their absence from the skies demonstrated that even the most abundant of natural resources could be exhausted by unchecked human consumption, beginning a new age of conservation regulation and game management.

[b] The research regarding the Passenger pigeon's ecology revealed that the species isn't simply a model candidate for de-extinction; it quite possibly is the most important species for the future of conserving the woodland biodiversity of the Eastern United States. As a result, the project is now not only a model for pioneering de-extinction methods, but it offers a new opportunity to achieve long-term conservation goals for woodland forests in the Eastern U.S.

**ROCK PIGEONS** are one of the most widely spread subspecies of the avian family. Crystal Bioscience has developed the protocols for isolating rock pigeon's PGC's.

[a] To produce genome-edited birds the most efficient method begins with editing genomes in cell cultures. For birds, the only cells that can be used are primordial germ cells (PGC's) – a kind of embryonic stem cell that becomes sperm and eggs in adult birds.

[b] Once isolated, these cells can be grown in a liquid medium of nutrients called the culture. Cultured cells can live and be grown in the lab, allowing scientists to make many gene changes without needing to hatch birds every time they make edits to the genome. The right culture conditions for different bird species requires experimentation to develop the correct formula. Developing PGC's cultures for pigeons is essential to accomplishing de-extinction.



1	IDENTIFYING DE-EXTINCTION MUTANTS
	Band-tailed pigeon genome is sequenced for the purpose of acting as a reference while assembling the genome of the Passenger pigeon.
	Passenger pigeon's fragmented DNA is extracted and sequenced from historic study skins. Those are mapped to the sequenced Band-tailed pigeon's genome to produce an assembly of the Passenger pigeon's genome.
	Differences from both genomes are analyzed to identify the mutations that changed trails between the species.
2	CULTURING PRIMORDIAL GERM CELLS
	Primordial germ cells (PGC's) are isolated from the germinal crescent of early stages Band-tailed pigeon's embryos and grown in vitro in culture media.
3	GENOME EDITING
	CRISPR/Cas9 - a genetic engineering tool cuts the Band-tailed pigeon's genome at specific target sites.
	Band-tailed pigeon's gene is removed as the natural DNA repair system of the body "wires" in the newly available gene - the assembled Passenger pigeon's gene.
	PGC's reengineered by the described process will therefore contain Passenger pigeon's genes.
4	HATCHING REVIVED PASSENGER PIGEONS
	Genome edited PGC's are injected into a developing Rock pigeon's embryo circulatory system where they migrate to the reproductive organs.
	A germ-line chimaera is produced - an animal that has all the tissues of the Rock pigeons but produces eggs/sperm from the genome edited PGC's.
5	INSERTING THE PGC's INTO THE HOST ORGANISM
	When the female and male germ-line chimaeras breed they will produce the first generation of the de-extinct Passenger pigeons.
6	RAISING REVIVED PASSENGER PIGEONS
	Germ-line chimaeras will be cycled to produce fertile eggs every 8-10 days through a controlled lighting regiment - a practice commonly used with poultry.
	First generation of Passenger pigeons will be raised by Band-tailed and Rock pigeon surrogate parents conditioned to breed in colonies like historic Passenger pigeons.
	Chimeras and surrogate flocks will be maintained long-term to propagate a sustainable captive population. Subsequent generations of Passenger pigeons will reis their own offsprings.

