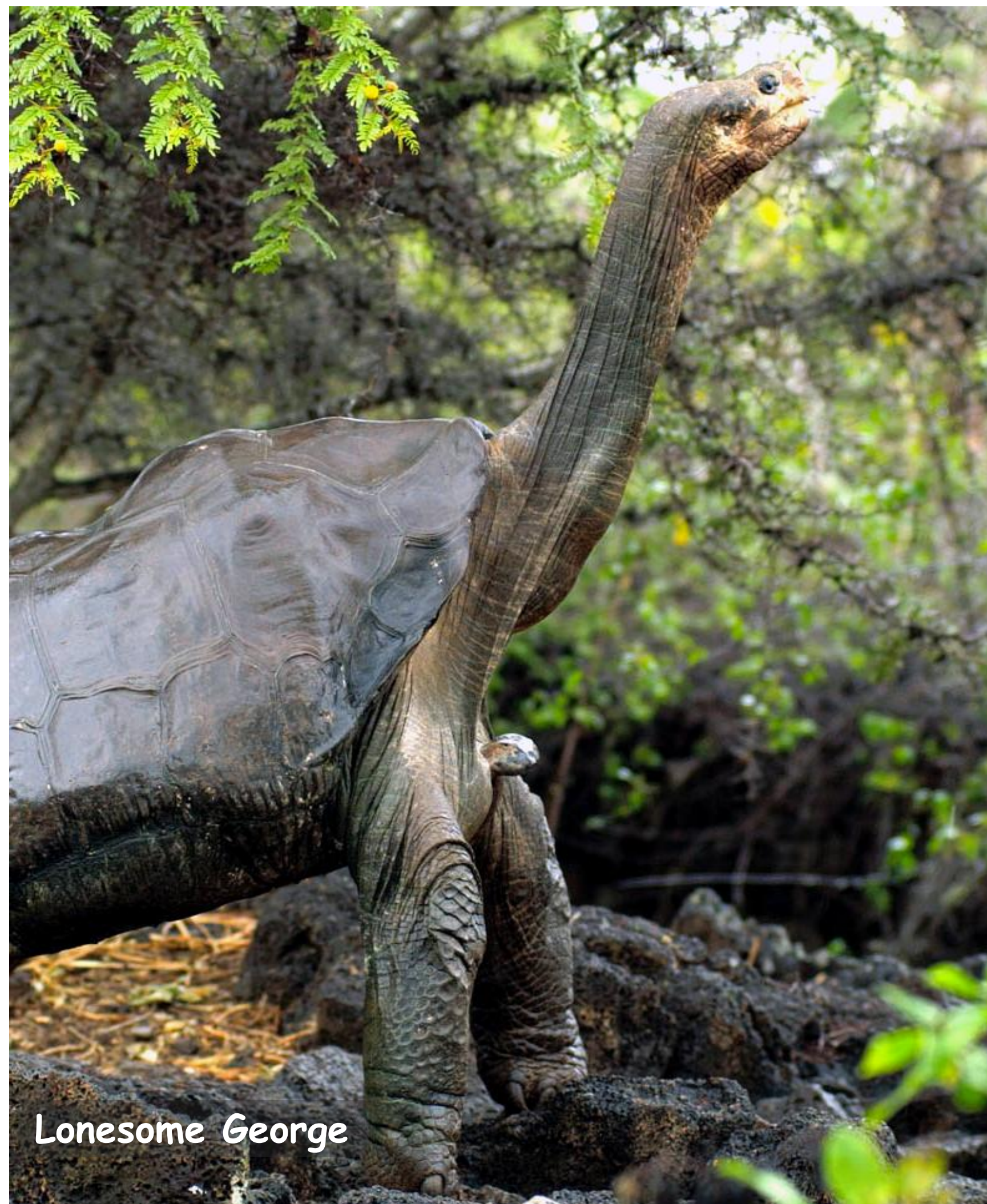


# Conservation biology meets genomics: resurrecting Galápagos tortoise species via high-resolution marker-assisted breeding



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Molecular genetic techniques offer conservation biologists critical information upon which to design effective management strategies. Galápagos tortoises are a flagship taxon in this respect, because captive breeding programs have been largely facilitated using genetic tools. We are embarking on an ambitious project aimed at developing and screening an information-rich panel of SNP loci capable of identifying extant hybrids carrying genes of the rarest species in the world (*Geochelone abingdoni*, represented by a single living individual, Lonesome George), as well those with genes from a recently extinct species (*G. elephantopus*, 150 years ago). Molecular identification of admixed individuals of high conservation value will be coupled with a marker-assisted captive breeding program aimed at resurrecting the evolutionary heritage of lineages that will otherwise be lost. A series of recent discoveries that have generated much excitement in the scientific community underscore the present project. We aim to develop a novel, general-purpose framework for SNP discovery in non-model organisms that can be applied in the absence of a reference genome. This is currently perceived as a major hurdle by molecular ecologists and researchers working in related fields, and so our work will contribute an important technical advance.



## 4. Identify hybrids

We will genotype ~1700 Galápagos tortoises sampled from V. Wolf, where admixed individuals occur. The ancestry of these individuals will be inferred by analyzing SNPs using standard multi-locus assignment tests<sup>4,5</sup> conditioned on genetically 'pure' reference individuals from targeted *Geochelone* species. Simulated datasets will be used to determine statistical power provided by the panel of SNPs for discriminating among hybrid individuals resulting from different levels of backcrossing<sup>6</sup>.

## 1. Background

Lonesome George is an iconic example of an 'evolutionary dead end'. Given the apparent absence of conspecific females, it seemed unlikely that he would produce any descendants and so loss of the species was considered inevitable. Russello et al.'s<sup>1</sup> exciting discovery of an extant hybrid male carrying *G. abingdoni* genes raises the possibility that there may be as-yet undiscovered female hybrids, and perhaps even 'pure' individuals. Molecular identification of otherwise indistinguishable hybrid (or pure) individuals for recruitment into the captive breeding program would play a leading role in efforts to rescue *G. abingdoni*.

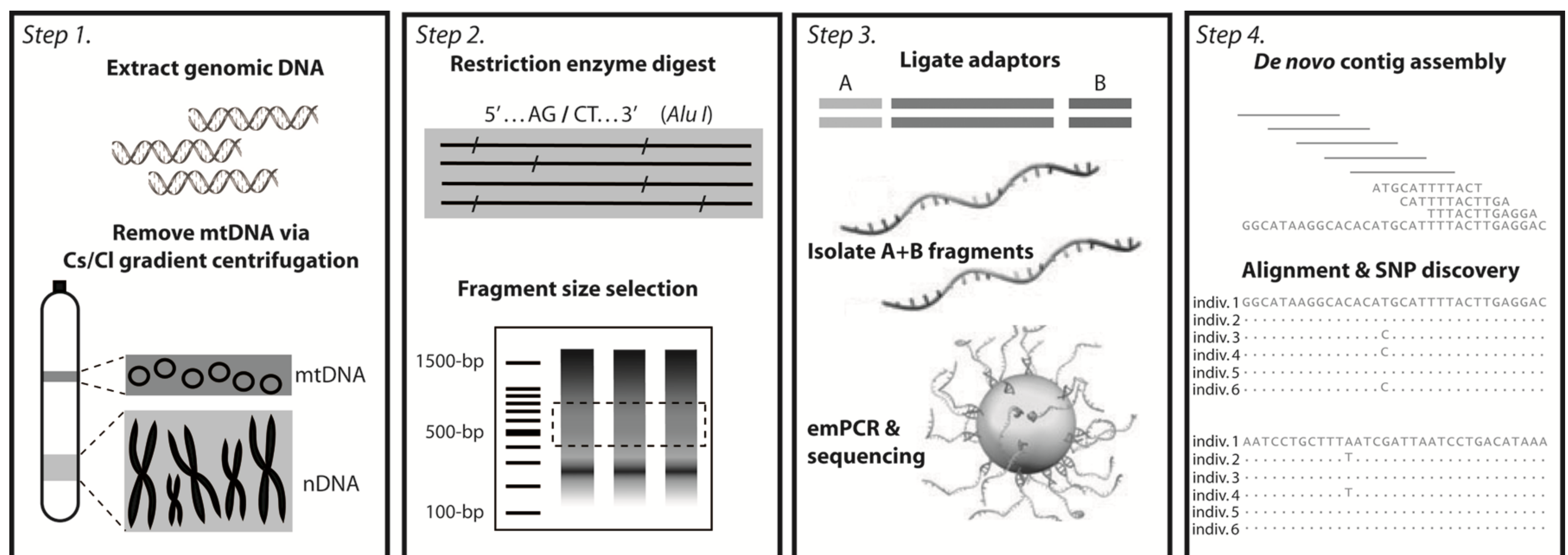
In a related development, Poulakakis et al.<sup>2</sup> showed that genes from an already extinct species (*G. elephantopus* from Floreana) live on in the genomes of extant admixed individuals. As with the *G. abingdoni* hybrid, these admixed individuals are from V. Wolf, on the island of Isabela. In this case, we will embark on an even more ambitious marker-assisted breeding program in which we attempt to resurrect *G. elephantopus*.

## 2. Goals

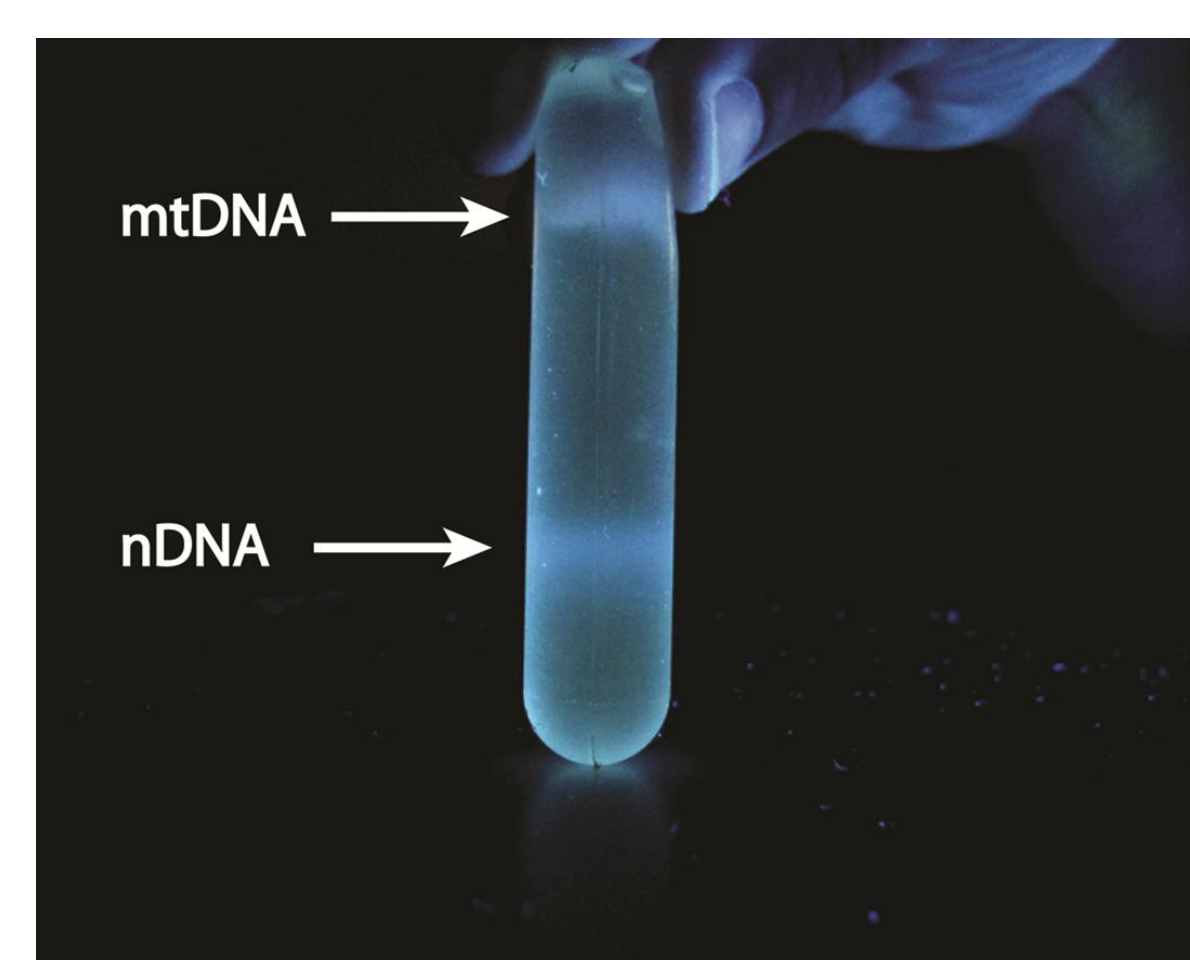
**Discovery of highly discriminating SNPs** using Roche 454 pyrosequencing, followed by high-throughput genotyping of ~1700 V. Wolf population samples (Sequenom assays).

**Marker-assisted selection of individuals** for captive breeding via identification of hybrids resulting from different levels of backcrossing.

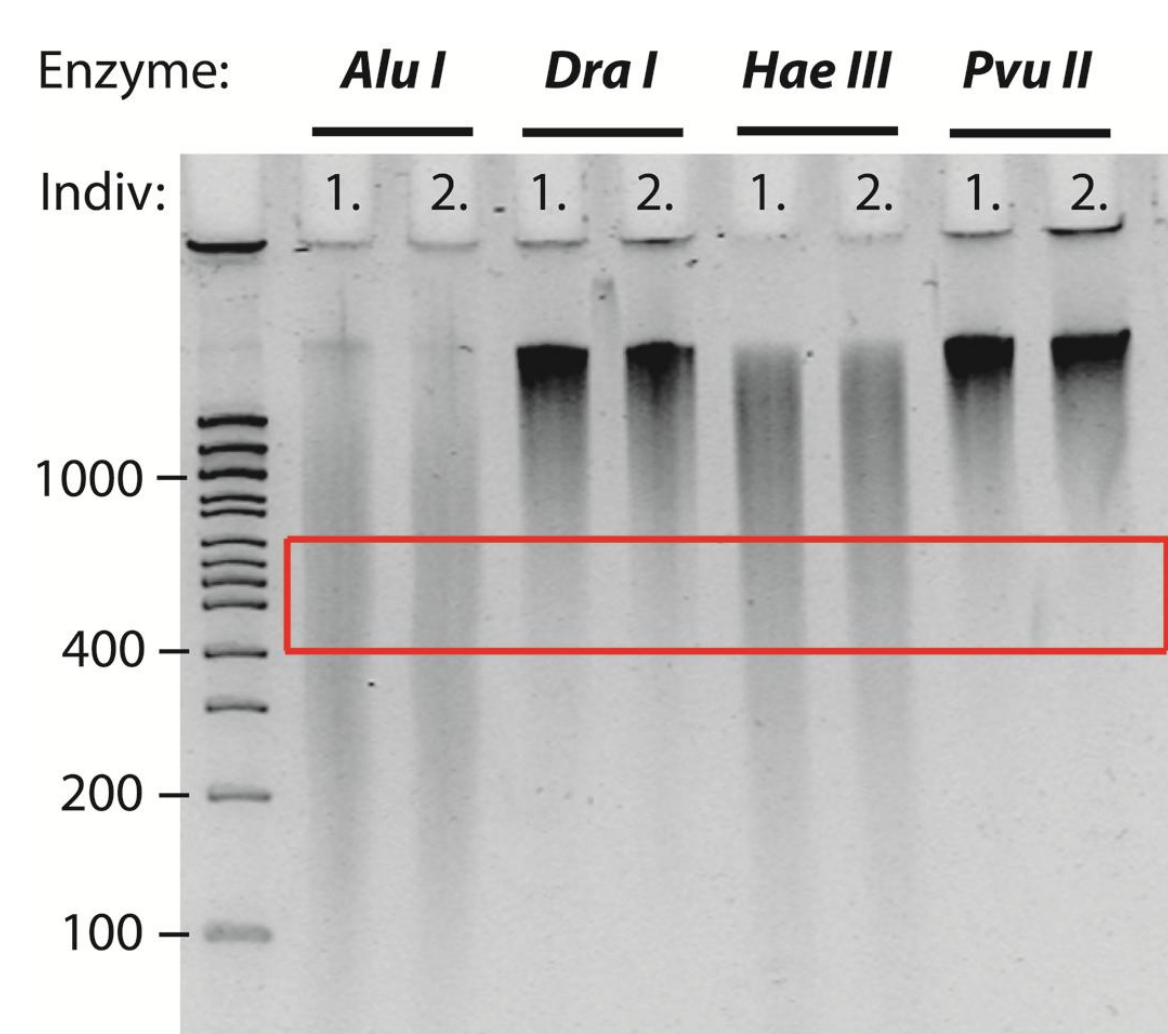
## 3. SNP development approach



**SNP development pipeline.** Step 1: isolate nDNA. Step 2: reduced representation library construction (digestion and gel size-selection of 400-800bp fragments<sup>3</sup>. Step 3: sstDNA library construction (ligation of MID barcodes, isolation of target ssDNA, emPCR and Roche/454 sequencing). Step 4: assembly of 250bp reads into contigs, homologue alignment, quality filtering, and SNP discovery.



**Cs/Cl spins.** mtDNA removal should improve sequencing coverage of nuclear SNPs.



**nDNA digests.** Testing several restriction enzymes may help reduce repetitive DNA content.

## Acknowledgements & References

We thank Chris Lane & Ian Misner (U. Rhode Island) for assistance with mtDNA removal, and Ken Nelson (Yale U.) for help with Agilent Bioanalyzer RRL library quantification. Matt Morgan (U. Texas at Austin) and Ben Evans (Yale U.) contributed valuable ideas.

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