Loxodonta Localizer (LL): Frequently Asked Questions and Additional Information

Please send additional questions or comments to: roca@illinois.edu using the email subject "_Loxodonta_ Localizer".

Important note: Many haplotypes (sequences) show a limited geographic distribution among African elephants and will be informative for establishing the provenance of elephants or their ivory. However, some haplotypes are less informative due to the widespread distribution of elephants carrying them.

Summary of Frequently Asked Questions (FAQs)

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What happens to sequences used as input to the LL? Are the sequences saved or stored, and is the user recorded?

The sequences input into LL are neither saved nor stored, nor is the user recorded.

Are there sequences in GenBank or in published sources that are not listed in the* Loxodonta *Localizer?

Published sequences may not be present in the LL for the following reasons:
-The sequence may have been excluded if it contained ambiguous sites, i.e., character states other than A, C, G or T
-The sequence may not completely overlap the required 316 bp
-The sequence may have been published too recently to be included in the LL

However, should missing sequences or any other omissions or errors be discovered, please inform us by sending an email to roca@illinois.edu.

List of abbreviations

FR: Forest Reserve
GR: Game Reserve
NP: National Park
WS: Wildlife Sanctuary

What are the references for papers that previously published mitochondrial DNA sequences that are reported by the* Loxodonta *Localizer?

We thank our colleagues who have published mtDNA sequences and made them publicly available in Genbank. The sequenes used by the LL were obtained from GenBank entries deposited for the following papers:

1. Archie EA, Moss CJ, Alberts SC. The ties that bind: genetic relatedness predicts the fission and fusion of social groups in wild African elephants. Proceedings of the Royal Society B: Biological Sciences. 2006;273(1586):513-22. doi: 10.1098/rspb.2005.3361. PubMed PMID: PMC1560064.
2. Barriel V, Thuet E, Tassy P. Molecular phylogeny of Elephantidae. Extreme divergence of the extant forest African elephant. C R Acad Sci III. 1999;322(6):447-54.
3. Brandt AL, Hagos Y, Yacob Y, David VA, Georgiadis NJ, Shoshani J, Roca AL. The Elephants of Gash-Barka, Eritrea: Nuclear and Mitochondrial Genetic Patterns. Journal of Heredity. 2014;105(1):82-90. doi: 10.1093/jhered/est078.
4. Charif R, Ramey R, Jr W, B. Payne K, B. Martin R, M. Brown L. Spatial relationships and matrilineal kinship in African savanna elephant (*Loxodonta africana*) clans. Behavioral Ecology and Sociobiology. 2005;57(4):327-38. doi: 10.1007/s00265-004-0867-5.
5. Debruyne R. A case study of apparent conflict between molecular phylogenies: the interrelationships of African elephants. Cladistics. 2005;21:31-50.
6. Debruyne R, Van Holt A, Barriel V, Tassy P. Status of the so-called African pygmy elephant (*Loxodonta pumilio* [NOACK 1906]): phylogeny of cytochrome b and mitochondrial control region sequences. C R Biol. 2003;326(7):687-97. PubMed PMID: 14556388.
7. de Flamingh A, Roca AL, van Aarde RJ. Origin and phylogeography of African savannah elephants (*Loxodonta africana*) in Kruger and nearby parks in southern Africa. Conservation Genetics. 2018;19(1):155-67. doi: 10.1007/s10592-017-1005-z.
8. Eggert LS, Rasner CA, Woodruff DS. The evolution and phylogeography of the African elephant inferred from mitochondrial DNA sequence and nuclear microsatellite markers. Proc R Soc Lond B Biol Sci. 2002;269(1504):1993-2006. PubMed PMID: 12396498.
9. Eggert LS, Buiji R, Lee ME, Cambell P, Dallmeier F, Fleischer RC, Alonso A, Maldonado JE. Using genetic profiles of African forest elephants to infer population structure, movements, and habitat use in a conservation and development landscape in Gabon. Conservation Biology. 2014;28(1):107-18. doi: doi:10.1111/cobi.12161.
10. Finch TM, Zhao N, Korkin D, Frederick KH, Eggert LS. Evidence of positive selection in mitochondrial complexes I and V of the African elephant. PLoS ONE. 2014;9(4):e92587. doi: 10.1371/journal.pone.0092587. PubMed PMID: PMC3973626.
11. Ishida Y, Georgiadis NJ, Hondo T, Roca AL. Triangulating the provenance of African elephants using mitochondrial DNA. Evol Appl. 2013;6(2):253-65.
12. Johnson MB, Clifford SL, Goossens B, Nyakaana S, Curran B, White LJ, Wickings JE, Bruford MW. Complex phylogeographic history of central African forest elephants and its implications for taxonomy. BMC Evol Biol. 2007;7(1):244.

Epub 2007/12/21. doi: 1471-2148-7-244 [pii] 10.1186/1471-2148-7-244. PubMed PMID: 18093290.

13. Mondol S, Moltke I, Hart J, Keigwin M, Brown L, Stephens M, Wasser SK. New evidence for hybrid zones of forest and savanna elephants in Central and West Africa. Mol Ecol. 2015;24(24):6134-47. doi: 10.1111/mec.13472. PubMed PMID: 26577954.

14. Nyakaana S, Arctander P, Siegismund HR. Population structure of the African savannah elephant inferred from mitochondrial control region sequences and nuclear microsatellite loci. Heredity. 2002;89(2):90-8. PubMed PMID: 12136410.

How should sequences with heteroplasmy, indels or ambiguous sites be handled?

The *Loxodonta* Localizer is designed to handle mitochondrial DNA sequences with unambiguous character states (A, C, G or T).

A very small proportion of published sequences in Genbank include one or more nucleotide sites with "N" as the character state (unknown nucleotide), within the region examined by the *Loxodonta* Localizer. Because these can represent low quality or low coverage of reads, we excluded this small proportion of GenBank entries from the *Loxodonta* Localizer database, to minimize the use of sequence data that is sometimes not as reliable as published sequences with only unambiguous character states.

Heteroplasmy is defined as the presence, within a cell or individual, of more than one distinct sequence among organellar genomes. This leads to polymorphisms among DNA reads, producing ambiguous character states in sequence data that is otherwise of high quality.

Indels are insertion-deletion variants that show up as gaps or additional characters in the sequence when it is aligned to similar sequences

For those using the *Loxodonta* Localizer, we provide the following guidelines should indels or heteroplasmy be putatively detected at a site within the region of mtDNA used as input by the software:

-If there is a large batch of samples, and the sample with the indel or heteroplasmy is not critical, then consider removing the sample from further consideration.

-If the sample is critical or additional sequencing is being conducted, then verify that the indel or heteroplasmy is real and not an artifact due to a technical issue. For example, the DNA can be re-extracted if contamination after extraction is suspected. If Sanger sequencing is being conducted, it would be important to verify the indel or heteroplasmy by sequencing the mtDNA in both directions, and/or conducting the sequencing reactions several times, and also verifying that surrounding sequences can be identified unambiguously with high confidence.

-If the sample is critical, and the sequence is confirmed as containing a *single* heteroplasmic site, then both nucleotides present at the site can be coded and entered separately as input into the software. For example, if the sequence contains:

…ACTGA**R**GTCAA… where R indicates that some reads contain A and other reads contain G at a nucleotide position.

Then both variants of the sequence can be input twice into the *Loxodonta* Localizer:
…ACTGA**A**GTCAA…
…ACTGA**G**GTCAA…

And the geographic localities can be recorded for both sequences.

-For an indel, it may be worthwhile to find the most similar sequence in Genbank, and use this as input to the localizer. However, we strongly caution that Genbank can produce results that may be strongly subject to misinterpretation, and would suggest excluding the sequence with the indel.

-If the sequence contains more than one variable nucleotide, we strongly suggest excluding it, though potentially the variants could be cloned and sequenced separately.