

S3 Table. Individual, contig, and site-level quality filters used for genotyping.

1. Filtering at individual level

- (a) Remove individuals having extremely low or high coverage ($<1/5$ or >5 x the average coverage across all individuals).
 - (b) Remove individuals with excessively high sequencing error rates measured as the percentage of mismatched bases out of the total number of aligned bases in the mitochondrial genome. ($>3X$ increase in error rates relative to the per individual average – note, no individuals in the current study exceeded this filter threshold)
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2. Filtering at contig level

- (a) Remove contigs having extremely low (<1 st percentile) or high (>99 th percentile) coverage based on the empirical coverage distribution across contigs.
 - (b) Remove contigs with at least one SNP having allele frequencies highly deviating from Hardy–Weinberg equilibrium expectations ($p < 0.0001$) based on a two-tailed exact test.
 - (c) Retain only the contigs that pass all filters for both historic and contemporary samples
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3. Filtering at site level

- (a) Remove sites with excessively low (<1 st percentile) or high (>99 th percentile) coverage based on the empirical coverage distribution.
 - (b) Remove sites with biases associated with reference and alternative allele Phred quality ($1e-100$), mapping quality (0), and distance of alleles from the ends of reads (0.0001). Also remove sites that show a bias towards sequencing reads coming from the forward or reverse strand (0.0001).
 - (c) Remove sites for which there are not at least 80% of the individuals sequenced at a minimum of 3x coverage each.
 - (d) Remove sites with a Phred scaled root mean square mapping quality for SNPs below 10.
 - (e) Due to the high base misincorporation rate present in the historic samples, we remove sites from all individuals for which C to T and G to A SNPs are identified.
 - (f) Retain only the sites that pass all filters for both historic and contemporary samples.
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