***Loxodonta* Localizer: Guidelines for trimming the control region sequence**

Mitochondrial control region sequences will need to be in the 5' to 3' direction, and trimmed down to the appropriate length of 316 bp. Ambiguous character states (such as N or R) are not allowed in the sequence, which must only include the letters A, C, G and T. The sequences correspond to the same region of mtDNA as reported by Johnson et al. 2007; *BMC Evol Biol.* 7:244.

If you are using the primers CR-F1 and CR-R2, which are included in the *Loxodonta* Localizer protocol for amplifying and sequencing DNA, then follow these guidelines: the sequence to be entered as input will begin 94 bp downstream of the primer CR-F1 (or 93 bp if the sequence includes the deletion shown below) and end 112 bp upstream of the primer CR-R2. Below are shown only the 30 bp of sequences that immediately flank the appropriate 316 bp control sequence; these will be trimmed (along with the regions further upstream or downstream) in order to generate a sequence of the correct length. The numbers are positions in the elephant mitogenome reference sequence in GenBank with accession number AB443879 (Murata et al., 2009; *Gene* 441:176). Note that AB443879 has a deletion at position 15425 relative to many other elephant sequences, and this should be taken into account.

<u>Sequence immediately upstream and downstream of the sequences to be input:</u>

5' side

```
15396                                                           15425
A                T                                   A           A
G   T T C T      C T T A A A C T A T T C C C T G C A G C A A     Δ
                 C                                   G
                                                            (Deletion)
```

3' side

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15739                                                           15770
        C
T A     T C T T A A C T A C C T A C C T C C G A G A A A C C A T
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If using other primers or methods to generate sequences, the regions upstream and downstream of the mtDNA sequence must be trimmed, up to and including the sequences shown above.

Alternatively, one can copy sequences of the appropriate 316 bp length, which are available from the *Loxodonta* Localizer under the option to "select an existing sequence" in the input page. These existing sequences can then be aligned to newly generated African elephant mitochondrial sequences, and used to guide trimming of the novel sequences to the appropriate 316 bp region that can be used as input to the *Loxodonta* Localizer.