Appendix S1 – Room temperature tissue preservative

The following protocol (originally provided by Ed Louis, Henry Doorly Zoo) was used to make the tissue preservative used in this study. Toe-clip tissue samples stored in this solution at room temperature for up to one (Ed's statement) or two (my results) years have consistently produced good DNA yields.

To make 500 ml of tissue preservative:

1. Use a clean bottle and put it on a stir plate, add stir bar.

2. Add 250 ml of 0.5 M EDTA

3. Add 100 ml DMSO

4. Start adding NaCl until saturated and slowly bring the volume up to 500 ml with double-deionized water, making sure the NaCl is still saturated. You do not want the NaCl to go into solution, nor do you want to exceed 500 ml or the concentration will be off.

The 0.5 M EDTA is already at the correct pH, so you will not need to adjust the pH when making the tissue preservative. The tissue preservative can be made at a pH of 7.5 to 8.0.

You do not need to autoclave this solution.