

Procedure S1. Preparation of pools of colony PCR products from recombinant clones.

The preparation of pools of PCR products from recombinant clones is presented in a schematic diagram in Figure 1. Although Figure 1 is based on the processing of two clones (each for Tp1 and Tp2) selected from two samples from one sample group (for the sake of simplicity), clones from all TpAg genes for all samples in each sample group (n=12) were processed in this manner. Thus, for each sample group, the first pooling resulted in nine pools (except sample groups from Tanzania) where each pool represented each TpAg gene, and the second pooling resulted in one pool which included the nine TpAg gene products.

Up to 10 clones from each sample for each of the TpAg genes (Tp1, Tp2, Tp3, Tp4, Tp5, Tp6, Tp7, Tp8 and Tp10) were analyzed by colony PCR. For the first pooling, selected colony PCR products (hereinafter referred to as samples) were purified using QIAquick[®] PCR Purification Kit (Qiagen, Germany) following manufacturer's protocol except that the final elution was done using low salt Tris-EDTA (TE) buffer (10mM Tris, 0.1mM EDTA, pH 8.0) (Invitrogen, Carlsbad USA). In order to create equimolar pools (containing samples with equimolar concentrations) for each TpAg gene, the samples were quantified using Qubit 2.0 fluorometer (Invitrogen, Carlsbad USA). Appropriate volumes (determined based on the DNA concentrations) of the samples were pooled, resulting in nine pooled samples representing the nine TpAg genes for all groups, except the two Tanzania groups due to inadequate quantities of DNA. Hence, two pools were obtained from each of the two Tanzania groups. The pooled samples were resolved in 2% agarose gel electrophoresis using 1X TAE as the running buffer, and the DNA concentration was determined using Qubit 2.0 fluorometer (Invitrogen, Carlsbad USA). For the second pooling, equimolar concentrations of the pooled samples (from the first pooling) were further pooled together for each sample group, resulting in one final sample per sample group (Figure 1).

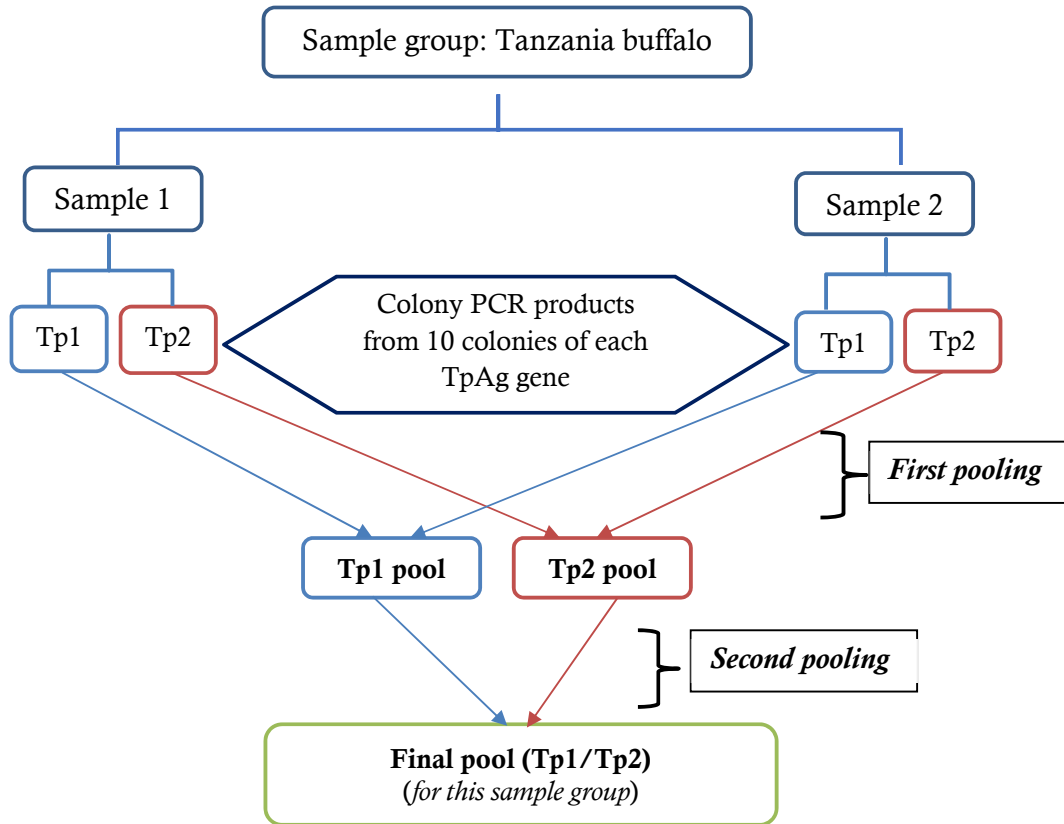


Figure 1. A schematic diagram showing how each of the 12 pools representing the different sample groups, each consisting of colony PCR products from recombinant clones of the nine TpAg genes were prepared.