

PCR confirmation of mitochondrial minicircles

We used polymerase chain reactions (PCR) to confirm the size and circularity of mitochondrial minicircles in *Columbicola passerinae* 2. First, we designed two pairs of outward-directed primers based on the assembled *rrnS* and *cob* genes (Table S2). Each 25 μ L PCR reaction contained 12.5 μ L of Promega GoTaq Green Master Mix (Promega Corporation, Madison, WI, USA), 0.5 μ L of each primer, 10.5 μ L of deionized water, and 1 μ L of genomic DNA. We set up reactions for each primer pair, including pairs of primers from different genes to confirm *rrnS* and *cob* are not on the same chromosome. We ran PCR with the following thermal cycler protocol: an initial denaturation step at 95°C for 2 minutes, 34 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 3 minutes, followed by a final extension at 72°C for 5 minutes. We then ran the PCR products on a 1% agarose gel with GelGreen Nucleic Acid Gel Stain (Biotium, Fremont, CA, USA) and the GeneRule 1kb Plus DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA).