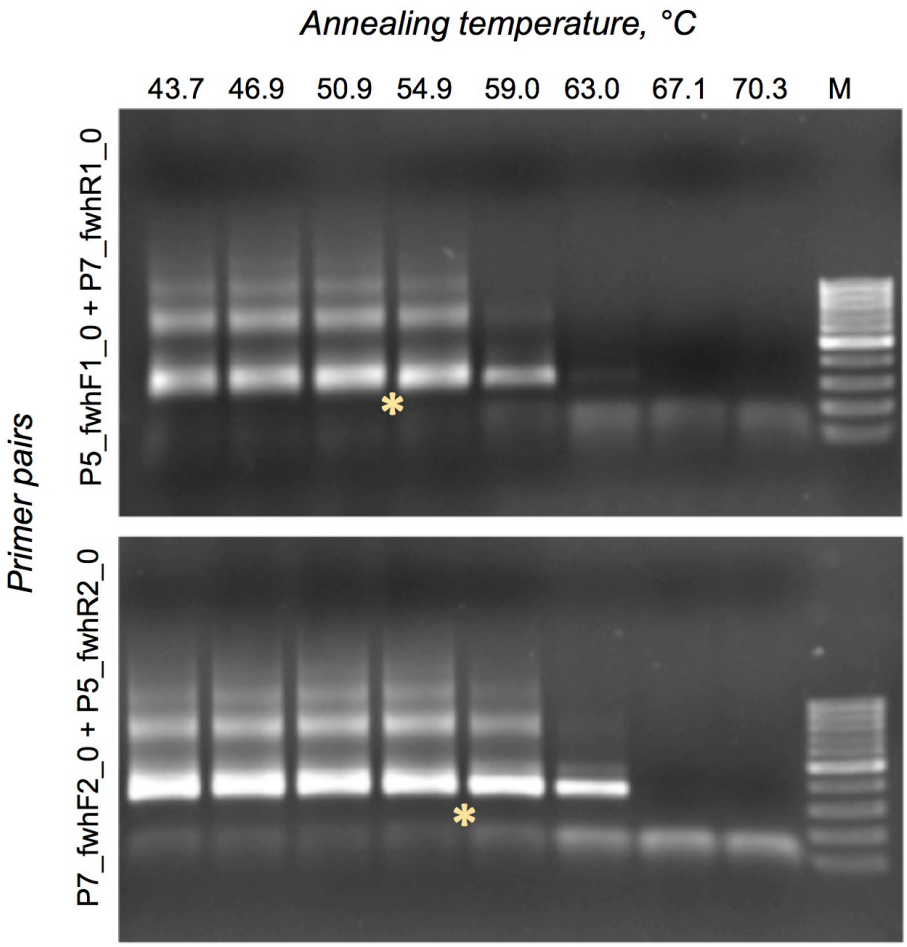

Figure S4: Gradient PCR optimization for the fwh1 and fwh2 primer sets using DNA template from the multi species mock samples (Elbrecht & Leese 2015). The primers amplified the expected fragment sizes that included the Illumina flow cell binding sequence (fwh1 - 294 bp and fwh2 - 327 bp). The upper non-specific bands are the effect of excessive cycling (34x). Yellow asterisks indicate the optimal annealing temperature we chose for further PCR reactions (52°C for fwhF1+fwhR1 and 58°C for fwhF2+fwhR2). M indicates the O'GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific, Darmstadt, Germany).