



S4 Figure. Matching Tms improve production of a mismatch amplicon. Non-degenerate primer pairs were evaluated for their ability to co-amplify the mismatch V3-V4 template (MM) in the presence of an equal amount of the match template (M). The non-degenerate pair ND_F1 and ND_F2_Tm54 and ND_R1 had matching Tms of ~54 °C (left set, lanes 2-4); the non-degenerate pair ND_F1 and ND_R2_Tm57 had matching Tms of ~57 °C (right set, lanes 5-7). PCRs used Platinum II Taq polymerase and the EMP cycling protocol. Purified PCR products were digested with *Spe I* to detect the mismatch amplicon. The mismatch amplicon was detected in the mixed template reactions (lanes 4 and 6).