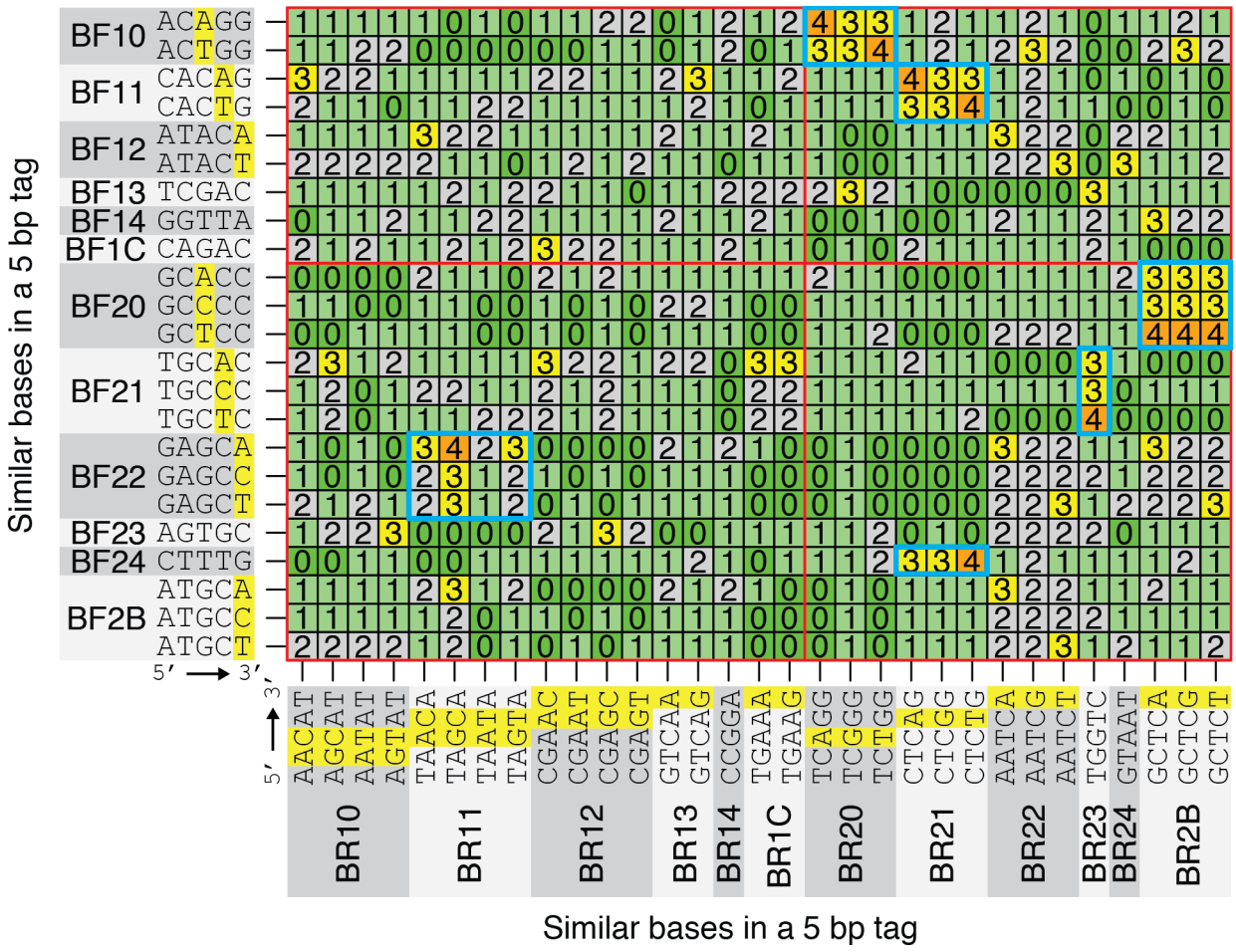

Figure S4: Matrix of similarities between all possible primer combinations using 5 bp inline tags. For some primers, several tagging-sequences are shown, due to nucleotide degeneracy in the tag sequences. Primer combinations which are similar at 4 sites (orange background) should be avoided for tagging as a single read error could lead to mistagging (blue squares). As we are using a parallel sequencing approach, also combinations like BF22+BR11 should be avoided, as both forward and reverse reads could occur together in sequencing read 1 or 2, possibly leading to mistagging.
With the presented primer sets a total of 276 samples can be securely tagged (excluding the problematic primer sequences in blue squares). Number of good tagging combinations for each primer set (tagging possiblities are doubled when using parallel sequencing, see Elbrecht & Leese 2015):
BF1+BR1 = 36 * 2 = 72
BF1+BR2 = 34 * 2 = 68
BF2+BR1 = 35 * 2 = 70
BF2+BR2 = 33 * 2 = 66
Total = 138 * 2 = **276**