

**Supplementary Figure 2.** Frequencies of predicted nucleotide base-pairing per position for N21 insert and 3' HD adapter (a) and 5' HD adapter, insert and 3' HD adapter (b). Vertical dotted line indicates ligation point. Blue line denotes data obtained with HD protocol and grey line randomly generated sets of 21nt sequences. Bars indicate minimum and maximum values in all replicates.