

Note S1: Temperature-dependent differences in off-target discrimination potential.

We have observed striking differences in the effective probe densities discovered when accounting for the thermodynamics of hybridization. Specifically, we observed a large drop in the hg38 'coverage' set probe density when using LDM that is not observed with UM, and we further observed the filtering of a sizeable number of UM and LDM probes when using kmerFilter with a mer-length of 16 (Fig. 2F and G). In both cases, we anticipate the effect is driven by the inability of the simulated hybridization temperature (37°C) to effectively destabilize off-target interactions: in the case of the 37°C LDM mode result, many off-target duplexes are predicted to have a probability of duplexing >0.2 , and many of these may have been missed in UM due to their alignments not being detected and/or reported using the less sensitive alignment parameters used for UM compared to LDM; in the case of kmerFilter, the low hybridization temperature necessitates the use of a 16 base mer-length compared to the 18 base length used for hybridization temperatures $\geq 42^\circ\text{C}$ (Fig. S4), and the choice of a common occurrence threshold '-k' of 5 (Methods) for both mer-lengths makes it more likely for a given probe to be filtered as 16mers tend to occur more frequently in a genome than 18mers due to their shorter lengths.