

Flanking Sequence Effects on Oligonucleotide Hybridization.

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Abstract

Microarray platforms measure both sequence content and prevalence of sequence by leveraging oligonucleotide hybridization. Computational probe design centers on short regions of high sequence similarity, most often between 25 and 50 base pairs in length. A variety of pattern-matching algorithms specific to the probe length are employed to determine the uniqueness of a target against its background, and a subset of these consider internal structure of the probe as a contra-indicator of successful hybridization. Since it is assumed that the target structure will mirror that of the probe, no consideration is given to the flanking sequences. In this study, we start to examine on the effects of total target sequence length in the context of common hybridization assay conditions. There are two considerations: due to the flexible nature of the single-stranded backbone, loops and bubbles can still yield stable hybridization between species with less than perfect sequence homology; the flanking sequences may loop back and occupy or obstruct the intended duplex region. Selecting a small subset of 33mer probes from the Affymetrix SNP6.0 Array we generated a pool of potentially cross-hybridizing sequence regions found by using the SeqNFind™ platform. Pools of length variant targets centered around the potentially cross hybridizing sequence were generated from human reference genome 36.3 and computationally examined using OMP™ to calculate the affinity constants. To demonstrate that the modeled properties affect measurements a small subset of these targets and probes were tested on microarrays.