

**Melting Temperature Variance Study of Oligonucleotides for Sequence-Specific Oligonucleotide Probe Design****Lee, Inhan<sup>\*1</sup>, Dombkowski, Alan A.<sup>2</sup>, Athey, Brian D.<sup>1</sup>****<sup>1</sup>Department of Psychiatry and Michigan Center for Biological Information, University of Michigan, Ann Arbor, MI, USA; <sup>2</sup>Institute of Environmental Health Science, Wayne State University, Detroit, MI, USA**

Many modern biomedical techniques utilize sequence-specific oligonucleotide hybridization. Clear, interpretable results from PCR, DNA oligonucleotide arrays and RNA interference (RNAi) experiments greatly depend on the specificity of the interactions between probe and target sequences. Specificity to a target gene seems to be straightforward when distinctive genes are under consideration; however, it is highly difficult to distinguish genes with very similar sequences in DNA microarray experiments using conventional probes. Moreover, product quality diminishes considerably with an increase in the number of probe pairs in a multiplex PCR. Lack of specificity is of concern in RNAi experiments, given reports of untargeted gene regulation.

In order to obtain hybridization characteristics and extract the guidance to design target-specific probes without an off-target effect, we studied the melting temperature variance among perfectly matched and mismatched oligonucleotides as an initial stage. We used a nearest neighbor method for melting temperature calculations of oligonucleotides extracted from human mRNA sequences and analyzed the data. New insight into mismatch pairs could be obtained from these *in silico* experiments and we could generate guidelines for designing a DNA microarray probe that can discriminate between two similar nucleotide sequences. Applying the guidelines to find oligonucleotide microarray probes for P450 genes, we confirmed the ability of our method to differentiate the individual genes in terms of thermodynamic calculations of hybridization and sequence similarity. Surface effects should be modeled for substrate-bound oligonucleotides in the future.