



Development of an SNP application: Based on real-time high resolution melting analysis of surface immobilized nucleic acids

Michael Martin Pieler

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The development of a real-time microarray-based single-nucleotide polymorphism (SNP) application is discussed in this book. The SNP discrimination is based on the real-time analysis, hybridization kinetics and melting analysis, of DNA attached to the microarray and DNA in solution. One of the major challenges of this real-time approach is the management of the huge amount of data generated by the measurements: the measurement data of approx. 1,100 spots is loaded into a self-designed MATLAB® program which fits the data, automatically generates diagrams of the kinetics and the melting process and views the fitting parameters in edited form, thus rendering the examination of the results easier and quicker. For the probe design a MATLAB® program was developed which automatically designs SNP microarray probes based on the sequenced gene sections from the biological material. The length of the used probes for the SNP detection was 60 nt. State-of-the-art methods use shorter oligos with endpoint detection for SNP discrimination. Long oligos are known to have higher reproducibility and higher sensitivity, but the specificity is not good enough for SNP detection. Within this work it is shown that it is possible to do SNP discrimination with long oligos using real-time analysis methods.

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