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using an internal reference and a background buffer considerably improved the reproducibility of the melting point. for the hoogsteen based parallel triplex, the ph 5.5 buffer was found to be most suited. ph 5.5 had the advantage of stable triplex formation but not higher temperatures. we recommend the use of 5 mM sodium acetate ph 5.5 as background buffer for the lightcycler method. the buffer chosen for the duplexes should be adjusted to the ph of the triplexes and the background buffer should be adjusted to the ph of the triplexes. an extensive buffer screening is necessary to find the optimal conditions for each individual system. for the parallel triplexes, the optimum buffer composition depends on the stringency of the hybridisation. at low stringency conditions, for example in preamplification of the vic signals, the stability of the triplexes is the most important property. this means that the buffer used should not inhibit the hybridisation. at higher stringency conditions, which are needed to distinguish sequences with point mutations, the stability of the triplex is less important and the hybridisation buffer can be more diverse. there are many examples for buffer-dependent hybridisations, for example hybridisations to a high gc-content

target, for which low-stringency conditions are preferred. the results shown in the paper are in agreement with earlier studies and demonstrate the great power of the lightcycler technology for high-throughput analysis of dna-oligonucleotides. the analysis showed that lightcycler can be used to differentiate between parallel triplexes with different sequence mismatches. this is an important requirement for an efficient screening method for several mutations in the same amplicon. the method can be applied for the detection of mutations in the human genome.

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