

Fluorescent-based genetic analysis for a microfluidics device

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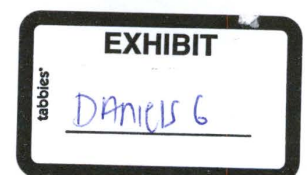
Abstract

This work concentrated on transferring polymerase chain reaction (PCR) assays to a microfluidics format with fluorescent detection, with the ultimate goal of creating a rugged microchip for performing PCR in the field. Several parameters were explored with regards to transferring the assay to a microchip, including substrate suitability; effect of additives; and decreased volume on product formation and reproducibility. The phosphoglucose isomerase (PGI) gene from *Erwinia herbicola* was successfully cloned and characterized as the template for all subsequent PCR reactions. Additives such as DMSO and formamide appeared to be useful to reduce the denaturation and annealing temperatures of real time PCR assays. BSA interfered with SYBR Green I fluorescent detection in real time but was required for PCR success. Complete PCR master mixes including enzyme and template were found to be stable for up to 24 hours at room temperature and dried PCR reagents stored at room temperature retained their activity for 14 days; The plastic polydimethyl siloxane (PMDS) was found to be a suitable reaction medium for PCR and fluorescent detection using SYBR Green I dye. The PCR reaction volume limited the amount of product obtained through PCR; reaction volumes of less than 10 μ L required special handling to ensure product was reproducibly obtained. The variability of results in small volume PCR was attributed to local reagent concentration effects due to evaporation and condensation of water fr

Keywords

PCR; polymerase chain reaction; microfluidics; tethered-dye gene probe; fluorescent detection; SYBR Green I; Genetic analysis

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