

Publication

A selected pre-amplification strategy for genetic analysis using limited DNA targets

JournalArticle (Originalarbeit in einer wissenschaftlichen Zeitschrift)

ID 1196214

Author(s) Xia, Peng; Radpour, Ramin; Kohler, Corina; Dang, Cheng Xue; Fan, Alex Xiu Cheng; Holzgreve, Wolfgang; Zhong, Xiao Yan

Author(s) at UniBasel Zhong, Xiao Yan ;

Year 2009

Title A selected pre-amplification strategy for genetic analysis using limited DNA targets

Journal Clinical Chemistry and Laboratory Medicine

Volume 47

Number 3

Pages / Article-Number 288-293

Keywords genetic analysis, limited DNA resources, limited DNA target, pre-amplification, quantitative real-time PCR

BACKGROUND: Limited DNA resources or limited DNA targets in predominant backgrounds for genetic tests can lead to misdiagnosis. We developed a strategy to selectively increase the amount of minor targets through a specific pre-amplification procedure. METHODS: We used the model of circulating cell free (ccf) male fetal DNA as a minor target in the predominant maternal plasma DNA to evaluate the strategy. The sex determining region (SRY) locus on the Y chromosome was used to identify ccf fetal DNA, and the human glyceraldehydes-3-phosphate dehydrogenase (GAPDH) gene was used to identify ccf total DNA in maternal plasma. We selectively pre-amplified the minor target SRY locus using the Expand Long Template PCR system and assessed the efficiency of the pre-amplification by realtime PCR, for both SRY and GAPDH, to compare the quantities of pre-amplified fetal DNA with those of maternal total DNA without pre-amplification. RESULTS: The selected pre-amplification increased the amount of ccf fetal DNA dramatically (Wilcoxon test: p =0.000, the fold change = 11,596). After selected preamplification, a proportion of 2.19% of the ccf fetal minor part in the predominant maternal component was changed up to 25,334%. The increased amounts of ccf fetal DNA found with the pre-amplification are not correlated to the amounts found without the procedure (r=-0.017, p=0.949). CONCLUSIONS: This strategy may be useful in genetic analysis with limited DNA resources and limited DNA targets in predominant background molecules. However, this approach is not suitable for quantitative assessments, due to the fact that quantitative imbalanced amplification was observed as a result of the pre-amplification procedure.

Publisher De Gruyter

ISSN/ISBN 1434-6621; 1437-4331

edoc-URL http://edoc.unibas.ch/dok/A6006387

Full Text on edoc Available;

Digital Object Identifier DOI 10.1515/CCLM.2009.067

PubMed ID http://www.ncbi.nlm.nih.gov/pubmed/19676139

ISI-Number WOS:000265031000007

Document type (ISI) Article