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Method Article

Use of coagulation factor XIII (F13) gene as an internal control for normalization of genomic DNA's for HLA typing

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A B S T R A C T

Genomic DNA (gDNA) obtained from whole blood samples is a critical element for genomic research and clinical diagnosis. PCR efficiencies of the targeted genes like HLA-A, -B, -C, DPB1 and DRB1 using such isolated gDNAs were variable in spite of having similar amounts of gDNA taken for PCR. We addressed such PCR variabilities by normalizing the gDNA's using an internal control of human coagulation factor XIII that was found to be variable with all samples and did not correlate with the observed A_{260} nm readings. The PCR and Q-PCR methodologies for the human coagulation factor XIII have been optimized, and the advantages of normalizing gDNA preparations based on F13 copy numbers have been discussed. This method will serve as a suitable choice to be used in laboratories and research centres, particularly when dealing with a large number of samples for the next-generation sequencing purposes, and in forensic labs with limited sample availability.

A R T I C L E I N F O

Method name: Use of coagulation factor XIII (F13) gene as an internal control for normalization of genomic DNA's for HLA typing
Keywords: DNA, Electrophoresis, Extraction, Factor XIII, Human blood, PCR, Spectrophotometry, Spin-column
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Specifications table

Subject area	Biochemistry, Genetics and Molecular Biology
More specific subject area	Quantitation of genomic DNA
Method name	

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	Use of Coagulation Factor XIII (F13) Gene as an Internal Control for Normalization of Genomic DNA's for HLA Typing
Name and reference of original method	P. Khare, V. Raj, S. Chandra, S. Agarwal, Quantitative and qualitative assessment of DNA extracted from saliva for its use in forensic identification. J. Forensic Dent. Sci. 6 (2014) 81–85.
Resource availability	Not applicable

Background

Preparation of pure human genomic DNA (gDNA) from whole blood in appreciable quantities is critical for basic science research, genetics, metagenomics, and clinical diagnosis. Of all the methods available for isolation of human gDNA [1,2], spin column technology appears to be a relatively simple approach to extract nucleic acids from small amounts of biological samples [3,4]. The sensitivity of polymerase chain reaction (PCR) assays is decided by the purity of gDNA employed as templates, and hence, determining the purity of the gDNA preparations for any downstream applications assumes critical importance. It has been reported that there is a steep rise in the average primer-dimer rate and PCR cross-overs with increasing numbers of PCR cycles at DNA concentrations below 30 ng/μl, especially for applications of human leucocyte antigen (HLA) typing [5,6]. Hence, we realized that alternate methods to determine accurate concentrations of gDNAs would be immensely useful for metagenomics studies and other applications. We detailed out experiments carried out on the use of human coagulation factor XIII (F13) gene as an internal control (IC) for accurate quantification of gDNA and demonstration of the usefulness of this approach in HLA typing.

Method details

Method name

F13 gene as internal control for genomic DNA quantitation

Materials

- Isopropyl alcohol (Analytical Grade)
- Commercially available spin column kits (QiaAmp DNA Blood Mini Kit, Qiagen, GmbH).
- Oligonucleotides were synthesized from BioServe Technologies, Hyderabad, India.
- DMSO, BSA, Tween-20 were procured from Sigma Chemical Co., St. Louis, USA.
- Taq DNA polymerase and pTZ57R/T vector (Thermo Fisher Scientific, USA)
- LB-agar plates, LB broth and ampicillin (Hi-Media, Mumbai, India)
- 1X Thermo Scientific Maxima SYBR green master mix (Thermo Fisher Scientific, USA)

Procedure

Collection of blood samples

Written consent forms from all donors at the point of collection for the purposes of developing a Bone Marrow (BM) registry for organ and BM transplantation was obtained by BMCDT-Infosys Bone Marrow Registry, India. These consent forms are in accordance with the regulatory body guidelines. Specific approval from the local ethics committee was not sought as the purpose of the study was to assess the method of HLA typing in comparison with the existing HLA typing techniques. No new genetic information outside of the HLA genes that would affect the donors of the material used in this study has been gained.

Genomic DNA isolation

Since Qiagen columns have superior DNA properties with least PCR inhibitors [7], we used these columns for isolation of gDNA following the manufacturer's protocols. Briefly, 200 μl of whole blood