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State of the art

Evidence for a higher resolution of HLA genotyping by a new NGS-based approach

Optimisation du génotypage HLA de haute résolution par une nouvelle approche basée sur la technologie NGS

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Abstract

With more than 16,000 alleles identified, the human leucocyte antigen (HLA) system is one of the most polymorphic regions of the human genome. Regarding the crucial role of HLA compatibility in transplantation and especially in Hematopoietic Stem Cell Transplantation, identification of HLA polymorphisms at a high-resolution level is of major interest. Recently, NGS technology has been proposed which appears to be simpler and more informative than the classical molecular methods such as SSP, SSOr and SBT. In the present report, a new set of NGS reagents and the appropriate associated software for sequence analysis are described. Through different studies, the performances of the system are illustrated and demonstrate that the method herein described overcomes current limitations in performing high-resolution HLA typing in clinical laboratories.

Keywords: HLA; High-resolution genotyping; NGS; Transplantation

Résumé

Avec plus de 16 000 allèles décrits, le système HLA est l'un des systèmes génétiques les plus polymorphes du génome humain. Si l'on considère le rôle crucial de la compatibilité HLA dans l'acceptation et le rejet de greffe, et ceci particulièrement en ce qui concerne la greffe de cellules souches hématopoïétiques, la détermination du génotype HLA du donneur et du receveur au plus haut niveau de résolution présente un intérêt majeur. Récemment, la technologie « Nouvelle génération de séquençage » (NGS) a été proposée pour le génotypage HLA, qui s'avère être plus simple d'utilisation, plus performante que les approches moléculaires jusque-là classiquement utilisées telles que la PCR SSO, le PCR-SSP et le séquençage par la méthode de Sanger. Cet article a pour objectif de présenter les résultats obtenus avec des nouveaux réactifs NGS et le logiciel associé. À partir de plusieurs études, les performances du système proposé seront décrites permettant de démontrer l'intérêt de la méthode pour optimiser la qualité et le niveau de résolution des typages et ainsi permettre aux laboratoires HLA de résoudre les difficultés rencontrées pour accéder à un haut niveau de résolution avec le minimum d'ambiguïtés.

Mots clés : HLA ; Génotypage de haute résolution ; NGS ; Transplantation

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1. Introduction

The human leucocyte antigen (HLA) system is one of the most polymorphic regions of the human genome.

To date, more than 16,000 alleles have been identified for the HLA class I and class II loci, and this number is still fastly growing. Even if this system is one of the most extensively studied region, this level of polymorphism remains a challenge when it comes to type HLA genes. HLA typing is routinely performed in connection with many medical indications such as transplantation and association of HLA with a lot of different diseases [1,2]. Specially, in the case of Hematopoietic Stem Cell Transplantation, high-resolution typing is required to respect the best compatibility between donor and recipient, in order to decrease the risk of graft versus host disease and mortality [3]. During the last decades, the techniques commonly used in HLA laboratories were molecular methods based on polymerase chain reaction (PCR) using sequence-specific primers (SSP), reverse sequence-specific oligonucleotide hybridization (SSOr) or sequence-based typing (SBT). Until recently, SBT method based on Sanger sequencing was considered as the most advantageous method, in terms of higher resolution with minimal ambiguity typing as well as from an economical point of view. However, genotyping ambiguities due either to failure to interrogate all polymorphic positions, or when two or more different allele combinations produce identical sequences (cis/trans ambiguities) remain an issue in a lot of cases [4].

Recently, new HLA typing strategies using next-generation sequencing (NGS) have been proposed to solve the SBT approach limits [5–9]. By amplifying and sequencing the entire HLA loci, NGS-based techniques significantly reduce the phase related problems and take into account all possible polymorphisms.

Therefore, it was a great challenge for HLA laboratories to develop new NGS-based HLA typing strategies that are more informative as well as simpler, faster and cost-effective.

In this context, the "Établissement français du sang" (EFS) decided in 2013 to promote an R&D project dedicated to the development of a HLA genotyping test-based on NGS technology. Today this project has been finalized with the availability of a set of reagents NG-Mix™ with CE marking pending. In the same time, specific software NG-View™ has been developed for sequence analysis. The aim of this report is to describe this NGS-based HLA method and to present its performances through a multisite clinical study.

2. Methods

Briefly, a long range PCR approach using My Fi™ DNA polymerase from Bioline Campany® is used. HLA class Ia (HLA-A, HLA-B, HLA-C), HLA class Ib (HLA-E, HLA-F, HLA-G, HLA–H, HLA-J, HLA-K) and HLA class II (HLA-DRB1, HLA-DRB3, HLA-DRB5, DQA1, DQB1, DPB1) genes are explored. HLA class I genes (Ia + Ib) are amplified from 5′ to 3′ UTR region in only two separate reactions. HLA-DRB1, DRB3 and DRB5 genes are simultaneously explored in two

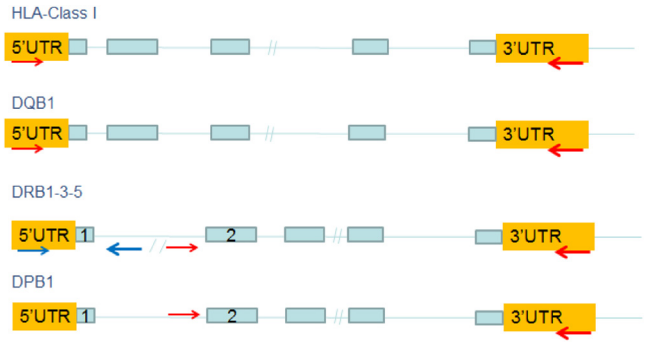

Fig. 1. PCR primer location.

different PCR, one from the 5′ untranslated region (UTR) to intron 1 and the second from Intron 1 to 3′ UTR. HLA-DQA1 and HLA-DQB1 are explored separately from 5′ to 3′ UTR, while HLA-DPB1 is explored from intron 1 to 3′ UTR (Fig. 1). All PCR primers are patented under the number 15705299.4. All loci are amplified in the same experimental PCR conditions which are as follows:

- denaturation at 96 °C for 20 s;
- annealing at 60 °C for 30 s;
- elongation at 72 °C for 5 min.

The PCR reaction is performed with 80 ng DNA in a total volume of 20 μl, containing, primers, PCR enzyme and 1X corresponding reaction buffer.

Gendx® library workflow has been selected, including the amplicon fragmentation and end repair in one step. After ligation to adaptor and purification, the libraries are normalized and tagged for indexation before a last clean-up step. All the fragments issued from the libraries are pooled and a size selection was applied using the Pippin-prep™ reagent (Sage Science®) in order to select the fragments with the optimal sizes (600–1300 bp). The selected fragments are quantified using Kapa™ library quantification kit (Kapa Biosystems®) before loading a total volume of 600 μl of a 9pM solution into the Miseq™ system (Illumina®) using a 2 × 250 bp-read-length kit. A total of 24 up to 96 samples are loaded at the same time on a Miseq™ reagent cartridge (MS-102-2003). All the workflow is presented in Fig. 2.

NG-View™, specific software dedicated to the sequence analysis, has been developed by the EFS IT system department. A Blast-n algorithm is integrated in the software and the sequence analysis follows two different algorithms. The first step of analysis consists in merging both R1 and R2 reads from Illumina®, based on the quality value of each nucleotide. The best 300,000 sequences are then selected by considering their sizes. The second step consists of phasing each sequence by comparison to IMGT data base using the Blast software and to find the best phase for each sequence. The third step consists in the merging of all phased sequences together. These constructions go through a second algorithm to find out the best assignment after comparison with HLA references issued from the IMGT data base. Results are presented in a user friendly