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Chimerism analysis using next generation sequencing

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ABSTRACT

Hematopoietic stem cell transplantation (HSCT) is the predominant curative treatment for many malignant and non-malignant haematological diseases. Early detection of graft rejection and disease relapse following HSCT improves patient outcomes by allowing treatment to be initiated as quickly as possible. In order to evaluate the level of donor engraftment, mixed chimerism levels must be carefully monitored after transplantation. Short-tandem repeat (STR) genotyping is widely used to determine the proportions of donor and recipient cells after HSCT.

In this study, Devyser Chimerism NGS kit in combination with a MiSeq System was introduced in our laboratory for monitoring HSCT. This system is a complete workflow solution for labs, combining a reliable testing process with a designed for-purpose analytical software. Up to 24 informative markers in a recipient/donor pair distributed through the human genome have strong discriminative power with low bias from ethnic parameters. These IND/DEL genetic markers with population independent discriminative power are distributed across 17 chromosomes and were further selected to allow sensitive detection combined with accurate and precise quantification of mixed chimerism.

Streamlined, simple and robust NGS workflow uses just one multiplex PCR reaction per patient sample. Minimal hands-on time reduces assay complexity and risk of sample contamination and mix-up. User-friendly, designed-for-purpose software perfectly complements testing kit with an automatic detection of informative markers.

Insertion/deletion (indel) polymorphisms have been used in the fields of forensic investigations owing to the advantages of their low mutation rates, widespread distributions in the human genome and small amplicon sizes. Thus, forensic efficiency evaluation of this system for forensic individual identification will be also tested.

1. Introduction

In the workflow of our laboratory short-tandem repeat (STR) genotyping is used to determine the proportions of donor and recipient cells after hematopoietic stem-cell transplantation. This technique allow the early detection of relapse and in consequence, the early adjustment of the treatment to enhance donor-origin hematopoiesis in transplant recipients [1–5]. However, Next Generation Techniques (NGS) are widely used in our diagnostic routine and therefore the introduction of a commercial system for the analysis of chimerisms has been favorably viewed. The application of NGS to study chimerism is a novelty as far as we know. Thus, it was necessary to verify its effectiveness with sensitivity studies and comparing the results obtained by the STR method, on real cases. Moreover, this NGS system could also have a forensic interest, to increase the capacity of our forensic genetics validated internal method for the identification of individual variability [3,4]. This commercially available kit "Devyser Chimerism for NGS "(Devyser, AB Sweden [5]) uses 24 indel markers with a short amplicon size and low mutation rates and require simple analytical procedures. Application of the method requires a preliminary study of the donor/recipient couple to verify the indel structure of these subject, for identification of discriminative markers to be used for monitoring. Monitoring of chimerism status through the quantitative determination of mixed chimerism following transplantation can be performed with this innovative NGS system. Finally, a final fundamental step is to verify if this so sensitive method is able to ascertain early a relapse in the transplant.

2. Material and methods

Dilution series with recipient donor DNA were used to verify the sensibility of this technique. Subsequently, six donor/recipient pairs previously analyzed with the STR method were re-evaluated using this technique. DNA was extracted from bone marrow and/or blood samples

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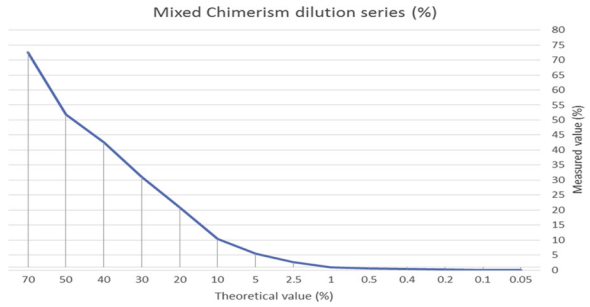

Fig. 1. Mixed Chimerism dilution evaluation.

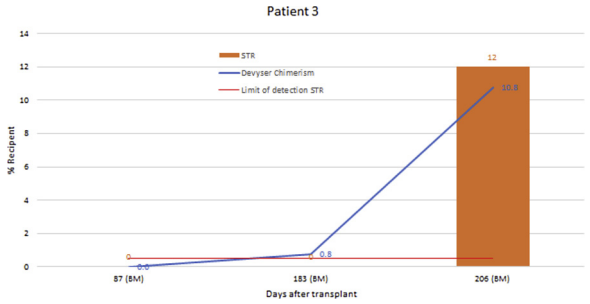

Fig. 2. STR and NGS comparison in a patient.

with QIAsymphony DSP DNA Kits with QIAsymphony instrument (Qiagen, Germany) and quantification was performed with Qubit dsDNA Broad Range Assay kit (Thermo Fischer, USA). NGS was performed in a single tube using MiSeq (Illumina, USA). Data analysis is performed using a program provided by the manufacturer.

3. Results and discussion

The aim of this study was to verify the sensitivity and accuracy of this indel NGS chimerism system recently introduced in the workflow of our laboratory to monitor hematopoietic stem cell transplantation. Dilution series of artificial mixed recipient/donor show that it was possible to identify up to 0.5% of the minor fractions in a mixture of two DNA (Fig. 1). Comparative results with STR showed that this NGS system was able to detect recurrence early after a transplant (for example Fig. 2)

4. Conclusion

The method is fast, allowing analysis to be carried out in a single day. Theoretically up to 96 samples could be analyzed in a single flow cell. We conclude that this technique can be a powerful tool for the determination of a genetic profile of clinical chimerism analysis when traditional techniques are not sensitive enough. Moreover, the amplification of a single indel by NGS was sensitive enough to detect a minor DNA contributor comprising down to 0.5% of the sample. Our results corroborate the high overall informative of this NGS system arguing for the applicability of this NGS system in routine for donor/recipient monitoring. Furthermore, our results provide evidence for higher sensitivity for the quantitative assessment of post-transplantation chimerism in samples with low DNA contents compared to STR-PCR. This gain in sensitivity can result in an earlier indication of disease relapse or graft failure and therefore provide better treatment options for the patient.

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Declaration of Competing Interest

None.

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