Validation of Devyser Chimerism NGS using the DNBSeq-G99 (MGI-Tech)

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INTRODUCTION

Accurate quantification of chimerism is essential for effective post-transplant monitoring, particularly in patients undergoing hematopoietic stem cell transplantation. The quantification of donor and recipient cell populations allows the assessment of engraftment and the early detection of clinically relevant events, such as graft failure and disease relapse. In this context, next-generation sequencing (NGS)-based assays have emerged as powerful tools, offering high sensitivity, reproducibility, and resolution when compared to conventional methods such as STR analysis. The Devyser Chimerism NGS assay uses targeted sequencing of insertion/deletion (indel) markers to quantify the proportion of donor-derived cells in post-transplant samples. This study aimed to evaluate the analytical performance of an NGS-based chimerism assay implemented on the MGI sequencing platform and using the Devyser Chimerism NGS kit.

MATERIALS & METHODS

Assay principle, Devyser Chimerism is a targeted NGS assay that quantifies chimerism from a panel of 24 genetic markers (short insertions/deletions, indels) distributed across the genome. It uses two sample types: screening samples (pre-transplant donor and recipient) to identify informative markers, and monitoring samples (post-transplant recipient) to quantify the donor fraction.

Samples, artificial chimeras were generated by mixing donor and recipient genomic DNA at proportions representing clinically relevant donor levels — 99%, 95%, 85% and 70% — with ten independent replicates per level. In total, 60 samples were processed (40 monitoring + 20 screening for informative-marker identification).

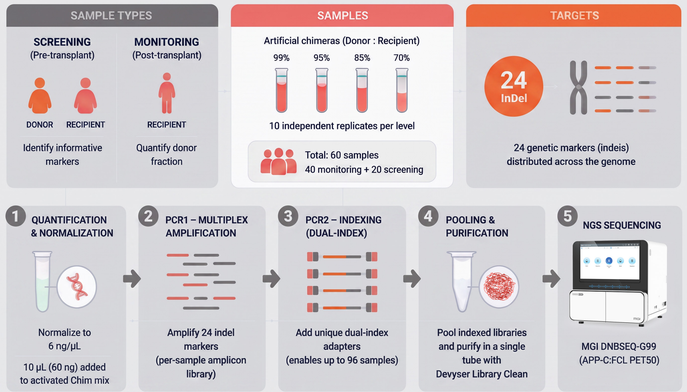
Protocol, after quantification, each sample was normalized to 6 ng/µL, and 10 µL (60 ng) was added to the activated Chim mix. A multiplex PCR1amplified the 24 markers into a per-sample amplicon library; PCR2 incorporated unique dual-index adapters. Indexed libraries were pooled and purified in a single tube with Devyser Library Clean and sequenced on the MGI DNBSEQ-G99 (APP-C FCL PE150).

Data analysis was performed using Devyser's chimerism software. For each informative marker (defined as opposite homozygous, or recipient-homozygous/donor-heterozygous), chimerism was calculated from allele frequencies. The final sample result was defined as the average across all informative markers (minimum of two). Assay performance was evaluated based on accuracy (bias vs. expected values), precision (SD and CV across replicates), and linearity.

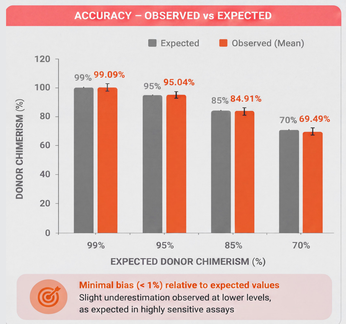
RESULTS

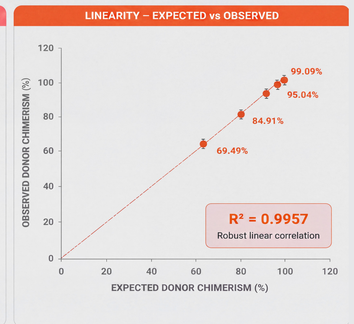
The assay demonstrated excellent accuracy across all evaluated concentrations, with a minimal bias of less than 1% relative to expected values. In the table below, we can observe the accuracy, precision, and linearity of the test.

DEVYSER CHIMERISM – ASSAY WORKFLOW



DEVYSER CHIMERISM – RESULTS





SUMMARY OF PERFORMANCE

EXPECTED DONOR CHIMERISM (%)	OBSERVED MEAN (%)	BIAS (%) (Observed − Expected)	COEFFICIENT OF VARIATION (CV)
99%	99.09%	+0.09%	1.26%
95%	95.04%	+0.04%	1.55%
85%	84.91%	−0.09%	1.59%
70%	69.49%	−0.51%	1.70%

DISCUSSION

The assay's precision substantially exceeds that of qPCR-based methods and matches traditional STR, supporting reliable quantification across the full spectrum of clinically relevant chimerism.

A slight trend toward underestimation was noted at lower chimerism levels, which is consistent with the expected behavior of highly sensitive quantitative assays. However, this deviation remained minimal and did not impact on the overall analytical performance or accuracy of the assay.

CONCLUSION

The evaluated NGS-based chimerism assay demonstrated high levels of accuracy, precision, and linearity when performed on the MGI sequencing platform. These results confirm the assay's analytical robustness across clinically relevant ranges, supporting its implementation as a reliable tool for routine chimerism monitoring in clinical laboratories and contributing to improved post-transplant patient management.

DEVYSER CHIMISM – RESULTS

ACCURACY	PRECISION	LINEARITY	PERFORMANCE
Bias < 1% across all levels	CV < 2% at all evaluated levels	R^2 = 0.9957 strong correlation	High accuracy, precision and linearity across clinically relevant ranges

BIOMETRIX DIAGNÓSTICA