Mentype® **Chimera®** CE IVD

Tailored to monitor chimerism status

Bio type® Diagnostic GmbH

Technical Report

Introduction

The EBMT transplant activity survey clearly illustrated the steadily increasing importance of hematopoietic stem cell transplantation as treatment for malignant and non-malignant disorders [1]. This implies a growing need for specifically designed diagnostics to investigate chimerism post-transplant. Mentype® **Chimera®** represents one of the first diagnostic assays specifically developed for chimerism analysis. The kit was designed as a mulitplex-PCR application targeting 12 highly polymorphic STR markers with very high overall degree of heterozygosity and balanced allelic distribution. Mentype® **Chimera®** was validated on 203 HLA matched related donor-recipient pairs and, ever since, is successfully applied in routine diagnostics. Following, the predominance of STR-markers applied in Mentype® **Chimera®** is presented in comparison to genetic loci deployed by STR-kits that were developed for non-medical human identification purposes.

Experimental work

Identification of informative markers

A comparative survey was set up in which Mentype® **Chimera®** was compared to a 16-STR multiplex PCR assay developed for forensic purposes. The probability to identify informative allelic constellations for unambiguous chimerism analysis was analysed. Therefore, several artificial DNA-mixtures were investigated for genetic loci that are separated by at least 2 alleles located outside the stutter area of donor or recipient-signals, respectively.

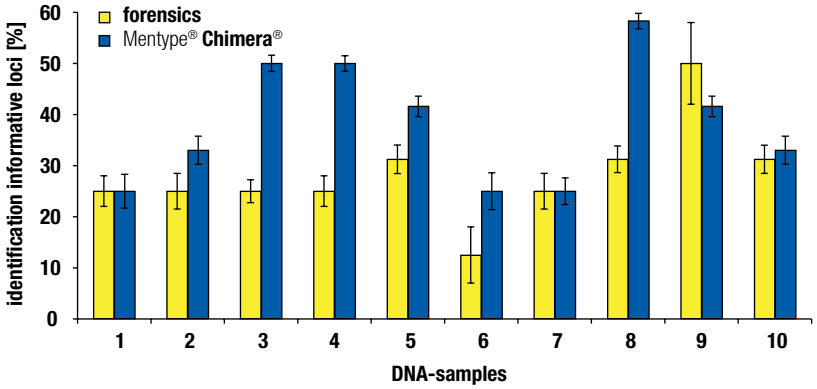

Figure 1: Probability to identify informative loci; to simulate chimerism, DNA-mixtures were prepared and screened. The number of informative loci in dependence to the number of STR-markers included in either kit is depicted in percent. Error-bars indicate the ration of ABCD/ABC-type loci; in total 10 different DNA-mixtures were investigated.

Results and Discussion

Mentype® **Chimera®** clearly shows the stronger capability to identify informative constellations (Fig.1). In proportion to the number of loci targeted by the respective kit, Mentype® **Chimera®** mediates a 10 % higher chance to detect informative constellations allowing unambiguous and reliable follow-up studies. These results are in accordance with a other comparative study that evaluated STR markers for their informativity in context of chimerism analysis [2]. Analyzing 203 HLA-matched donor/recipient pairs resulted in a clear ranking of markers towards their value in chimerism analysis (Table 1). Moreover, corroborate findings were published also recently (Table 1, highlighted loci) [3]. Shown results verify the importance of deliberate assortment of STR-loci for chimerism analysis and illustrate the efficiency of Mentype® **Chimera®** in medical diagnostics.

Forensic		Mentype® **Chimera®**	
inspeced loci	**chance for "ABCD" constellations [%]**	**inspeced loci**	**chance for "ABCD" constellations [%]**
D2S1338	32,2	D2S1360	22,1
D3S1358	13,6	D3S1744	20
D8S1179	17	D4S2366	20,6
D16S539	16,5	D5S2500	18,1
D19S433	8,7	D7S1517	24,9
D21S11	12,6	D8S1132	23,1
FGA	19,4	D10S2325	24,1
TH01	14,1		
vWA	17		
D12S391	25,4	D12S391	25,4
D18S51	27,7	D18S51	27,7
SE33	45,1	SE33	45,1

Table 1: Probability of different STR-markers to identify informative type ABCD constellations in related individuals; modified from Thiede et al., 2004 [2]. Only loci that were inspected in the presented study are depicted. Highlighted loci refer to Lion et al., 2012 [3] and were shown to mediate 99% combined probability to identify patient - and donor-specific alleles that are separated by at least two repeat lengths.

[1] J.R. Passweg, H. Baldomero, A. Gratwohl, et al.:The EBMT activity survey: 1990–2010; Bone Marrow Transplantation (2012), 47, 906-923;
[2] C. Thiede, M. Bornhäuser, G. Ehninger: Evaluation of STR inromativity for chimerism testing – comparative analysis of 27 STR systems in 203 matched related donor recipient pairs; Leukemia (2004) 18, 248-254;
[3] T. Lion, F. Watzinger, S. Preuner, et al.: The EuroChimerism concept for a standardized approach to chimerism analysis after allogeneic stem cell transplantation; Leukemia (2012) 1-8;

CHNTNv3en

Investigation of stutter ration

So called "stutter" are polymerase caused artifacts whose occurrence is dependent on the sequence of respective STR-loci as well as the number of STR-repeats. Deliberate primer-design and minute composition of reagents have good potential to diminish stutter-peak occurrence leading to accurate PCR-results. Here, the stutter appearance of several commercially available STR-kits is summarized in Table 2.

Results and Discussion

Mentype® **Chimera**® mediates a very low overall stutter peak occurrence (Table 2). This might result from deploying tetra- and penta - nucleotide STR-repeats as well as it might be a matter of primer-design and reagent composition. Together, polymerase-slippage appears reduced and accounts for clear results.

Accuracy in chimerism analysis

To illustrate the accuracy of Mentype® **Chimera**® distinct independent dilutions were set up to simulate chimerism. Samples were investigated by using two forensic STR kits in comparison. Sensitivity and accuracy, respectively, were analyzed and are depicted in Figure 2.

Table 2: Comparison of stutter peak occurrence mediated by distinct STR-Kits. Stutter category describes percentage of stutter-peak area on STR-peak area.

	Mentype® **Chimera**®	**Promega***	**ABI#**	**Qiagen^x^**
Average stutter [%]	9,8	11,6	12,1	10,3
Standard Deviation	3,2	4,0	3,1	2,7
Stutter Category				
0-10%	6	3	2	4
10-20%	6	11	13	12
>20%	0	1	0	0

(*, #, x, data taken from manufactures template files.)

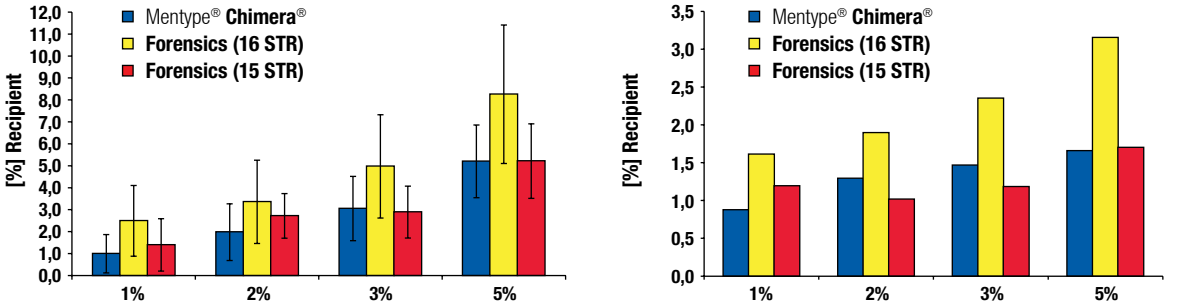

Figure 2: Comparative analysis for sensitivity and accuracy. To simulating chimerism three independent DNA mixtures of two DNAs were prepared (1 % to 5 %; total amount of DNA applied for PCR was 1 ng). Samples were investigated in four replicates; A) 1 to 5 % recipient DNA that corresponds to 10 – 50 pg artificial recipient DNA was applied and analysed by capillary-electrophoresis. Error-bars indicate standard deviation (n=12). B) Depicted are standard deviations of measurements shown in A).

Results and Discussion

Although all tested kits yielded evaluable results in simulated low percentage chimerism, quality of measurements greatly differed. As shown in Figure 2, highest accuracy of results was achieved by using Mentype® **Chimera**® as competitive products overestimated the amount of DNA. This analysis qualifies Mentype® **Chimera**® for accurate and reliable analysis, especially taking effect in low-percentage chimerism.

Conclusion

Deliberate assortment of STR loci is vital for chimerism analysis. The number of informative loci identified, rather depends on the characteristics of deployed loci, than the total number of targeted STR-markers. Moreover, specific validation on relevant patients samples allowing a delicate adjustment of reagents appears critical for performance of such applications. Fulfilling stated requirements Mentype® **Chimera**® has become a valuable standard in chimerism analysis.

For more information or if you have any questions please do not hesitate to contact us.

Ordering information

Mentype® **Chimera**®	**Order number**
25 reactions	45-13210-0025
100 reactions	45-13210-0100
400 reactions	45-13210-0400

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