Mentype® **DIPscreen**

Instructions for Use

The entry to a quantitative chimerism analysis

For in vitro diagnostic use

CE IVD

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Made in Germany

BIOTYPE GmbH develops, produces and markets their PCR-based rapid Mentype® Detection Kits. Our products provide customers with fast and reliable testing methods for professional medical diagnostics.

Our Mentype® Test Kits guarantee highest quality standards for clinical research and diagnostics.

For information and enquiries about the Mentype® **DIPscreen** please do not hesitate to get in touch or visit www.biotype.de

Product description

Mentype® **DIPscreen** is a multiplex-PCR application developed to identify DIP polymorphisms that individually occur in donor or recipient, and, constitute informative loci. In a single multiplex-PCR 33 DIP-loci are simultaneously screened together with the gender-specific locus amelogenin. Mentype® **DIPscreen** is a multiplex-PCR application that mediates monitoring of chimerism samples after stem cell transplantation. The flexible assay format allows individual diagnostics at any time required.

Analysis of molecular chimerism resulting from allogeneic stem cell transplantation has become a well-established method to control the course of transplant engraftment and to assess the risk of threatening relapse. Molecular chimerism analysis can be performed on diverse DNA-sequence motifs, of which biallelic short insertion/deletion polymorphisms (DIPs, INDELs) offer substantial benefits. Polymerase-mediated amplification of DIP-markers does not result in formation of stutter peaks that can hamper clear analysis. Moreover, these polymorphisms are best suited for allele-specific quantitative approaches. Mentype® **DIPscreen** is a DIP-based chimerism analysis and therefore accounts for an unambiguous donor/recipient differentiation and highly clear chimerism monitoring.

The 33 DIP loci addressed by Mentype® **DIPscreen** are distributed over 18 chromosomes, and are at least separated by 10 Mbp each (see Tab. 1). The detection limit of Mentype® **DIPscreen** is about **200 pg genomic DNA**. The optimal range under standard conditions is **1.0-2.0 ng DNA**. For fast and sensitive fragment length analysis primers are fluorescence-labelled with **6-FAM**, **BTG**, or **BTY**.

The test kit was validated and evaluated by using GeneAmp® 9700 Silver, Eppendorf Mastercycler ep-S, Biometra T1, ProFlex PCR System, and ABI PRISM® 3130 Genetic Analyzer as well as Applied Biosystems™ 3500 Genetic Analyzer running with 36 cm capillary array and POP4® polymer.

Content

1. Description of the Mentype® DIPscreen

Table 1. Locus-specific information of Mentype® **DIPscreen**

DIP Locus	Chromosomal position	Motive (-DIP / +DIP)
FAM Panel		
AM X	Xp22.1-22.3	
AM Y	Yp11.2	
HLD106	16q13	-/AATGCGT
HLD70	6q16.1	-/AGCA
HLD84	8q24.12	-/CTTTC
HLD103	12q23.1	-/GCTTATAA
HLD104	13q32.1	-/ACTC
HLD116	18p11.22	-/AGGTGTCGAACAACATGATAC
HLD112	17p12	-/TTGTA
HLD307	Xp11.23	-/TCAACCAA
HLD310	2p22.3	-/GTCTGGTT
HLD110	16q22.1	-/TCCCTG
HLD133	3p22.1	-/CAACCTGGATT
HLD79	7q31.2	-/AATCT
HLD105	14q24.3	-/ATAGACAA
HLD140	3q23	-/GGTAGTATGGGCCT
HLD163	12q24.31	-/AACTACGGCACGCCC
BTG Panel		
HLD91	11q14.1	-/GATA
HLD23	18p11.32	-/CTTTAA
HLD88	9q22.33	-/CCACAAAGA
HLD101	15q26.1	-/GTAG
HLD67	5q33.3	-/CTACTGAC
HLD301	17q21.32	-/CAGGGGCTC
HLD53	3q22.1	-/ATGT
HLD97	13q13.1	-/AGAGAAAGCTGAAG
HLD152	16p13.2	-/TGGTCAAAGGCA
HLD128	1q31.3	-/ATTAAATA
HLD134	5q11.2	-/ATGATGGTTCTTCAGA
HLD305	20q11.22	-/CAAGGTCCCACCACACTCGCGTGGGA
BTY Panel		
HLD48	2q11.2	-/GACTT
HLD114	17p13.2	-/TCCTATTCTACTCTGAAT
HLD304	9q34.3	-/GAGCTGCTCAAGAGAGAGG
HLD131	7q36.2	-/TTGGGCTTATT
HLD38	1q32.2	-/TAGTT
HLD82	7q21.3	-/ACCTCCTACTCCTTGGTCTATTCCTGGTCACATGTACT

Abbreviations: HLD = Human Locus DIP, -DIP = Deletion, +DIP = Insertion

Table 1 shows the chromosomal position, motif and respective reference allele of DIP-loci addressed by Mentype® **DIPscreen**.

Kit Content

Mentype® **DIPscreen**

Reagent	Volume per packaging size	
	25 reactions	100 reactions
Nuclease-Free Water	1,5 mL	2 x 1,5 mL
Reaction Mix A	125 µL	500 µL
Mentype® **DIPscreen** PrimerMix	125 µL	500 µL
Multi Taq 2 DNA Polymerase (hot start, 2,5 U/µL) **OR** Polymerase N*	15 µL	60 µL
Mentype® **DIPscreen** Control DNA XY82 (2 ng/µL)	10 µL	10 µL
DNA Size Standard 550 (BTO)	13 µL	50 µL
Mentype® **DIPscreen** Allelic Ladder	25 µL	25 µL

* From kit lot number LEUK01107 on, the kit contains the new Polymerase N.

Be aware that the kit components of different kit lots must not be mixed. An overview of the lot numbers can be found on the label which is situated on the inside of the box flap. Aliquoting the kit components into other reaction vessels is not permitted.

Ordering information

Table 2. Ordering information for Mentype® **DIPscreen** kits

Product	Reactions	Order number
Mentype® **DIPscreen**	25 reactions	45-45410-0025
Mentype® **DIPscreen**	100 reactions	45-45410-0100

Storage

Store all components at -25 °C to -15 °C and avoid repeated thawing and freezing. The primer mix and the allelic ladder must be stored protected from light. The DNA samples and post-PCR reagents (allelic ladder and DNA size standard) should be stored separately from the PCR reagents. The expiry date is indicated on the kit cover.

Additionally required reagents

Additional reagents are required in order to use the BIOTYPE PCR Amplification Kit:

Table 3. Additional required reagents for Mentype® **DIPscreen** kits

Reagent	Supplier	Order Number
Hi-Di™ Formamide, 25 mL	Applied Biosystems	4311320
Matrix Standards BT5 multi-capillary instruments (25 µL)	BIOTYPE GmbH	00-10421-0025
Matrix Standards BT5 multi-capillary instruments (2 x 25 µL)	BIOTYPE GmbH	00-10421-0050

Warning and safety instructions

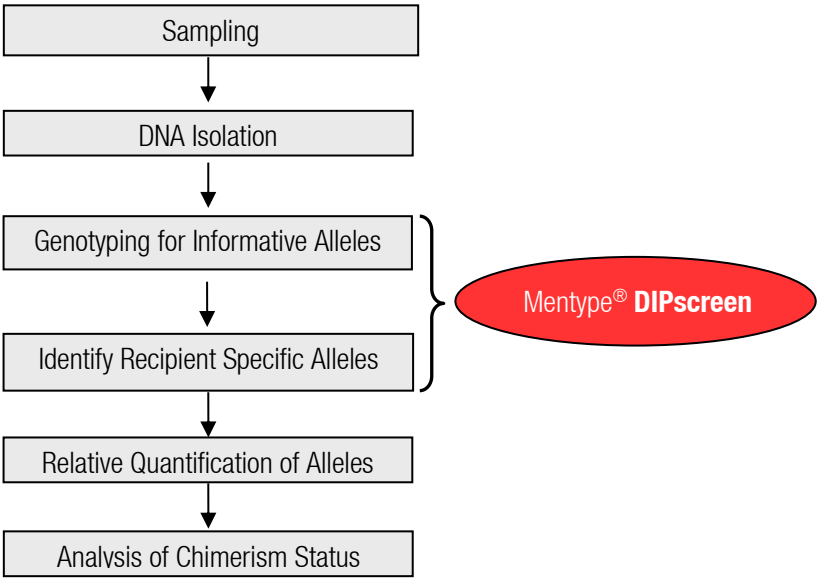
Observe the Safety Data Sheets (SDS) for all BIOTYPE products, which are available on request. Please contact the respective manufacturers for copies of the SDS for any additionally needed reagents.

Quality assurance

All kit components undergo an intensive quality assurance process at BIOTYPE GmbH. The quality of the test kits is permanently monitored to ensure unrestricted usability. Please contact us if you have any questions regarding quality assurance.

Trademarks and patents

Outline of working steps performed with Mentype® DIP-products



From sample to analysis – Monitoring chimerism with the Mentype® **DIPscreen**

Protocols for PCR amplification, electrophoresis and analysis

2. PCR amplification

2.1 Master mix preparation

The table below shows the volumes of all PCR reagents per 25 µL reaction volume, including a sample volume of 1.0 µL (template DNA). The number of reactions to be set up shall be determined taking into account positive and negative control reactions. Add one or two reactions to this number to compensate for the pipetting error.

Table 4. Master mix preparation for Mentype® **DIPscreen**

Component	Volume
Nuclease-Free Water	13.4 µL
Reaction Mix A*	5.0 µL
Mentype® **DIPscreen** PrimerMix	5.0 µL
Multi Taq 2 DNA Polymerase (hot start, 2,5 U/µL) **OR** Polymerase N	0.6 µL
Volume of master mix	**24.0 µL**
DNA template or control DNA	1.0 µL

* contains Mg^{2+}, dNTPs, BSA

All components should be mixed (vortex) and centrifuged for about 10 s before preparing the master mix. The DNA volume applied to the assay depends on its concentration. For reference samples 1 µL is mostly sufficient. For critical patient samples the amount of template can be increased appropriately. Fill up the final reaction volume to 25 µL with nuclease-free water.

Generally, DNA templates shall be stored in nuclease-free water or in diluted TE buffer (10 mM Tris HCl, pH 8.0 and 1 mM EDTA), e. g. 0.1 x TE buffer.

The primer mixes are adjusted for balanced peak heights at **28 PCR cycles** and **1 ng Control DNA XY82** in a reaction volume of 25 µL. If more DNA template is applied, higher peaks can be expected for small PCR fragments and relatively low peaks for large fragments. Reduce the amount of DNA template to correct this imbalance.

Positive control

For positive amplification control, dilute the Control DNA XY82 to 1 ng/µL.
Instead of the template DNA, pipette the diluted Control DNA into a reaction tube containing the PCR master mix.

Negative control

For negative amplification control, pipette nuclease-free water instead of template DNA into a reaction tube that contains the PCR master mix.

Template DNA

Sometimes, measured DNA concentration varies depending on the quantification method used. It might thus be necessary to adjust the optimal DNA amount.

2.2 PCR amplification parameter

Perform a “hot start” PCR in order to activate the Multi Taq 2 DNA Polymerase and to prevent formation of non-specific amplification products.

The number of cycles depends on the amount of DNA applied. 28 PCR cycles are recommended for all samples.

Standard method

Recommended for all DNA samples

Table 5. PCR amplification protocol for Mentype® **DIPscreen**

Temperature	Time	
94 °C	4 min (hot start for activation of the Multi Taq 2 DNA polymerase)	
94 °C	30 s	
60 °C	**120 s**	**28 cycles**
72 °C	75 s	
68 °C	60 min	
10 °C	∞	hold

Note: To provide an optimal kit balance the ramping rate of the thermal cycler should be adjusted to 4-5 °C/s.

Very small amounts of DNA may result in statistical dropouts and imbalances of the peaks. Increasing numbers of PCR cycles raises the risk of cross-contamination caused by minimal amounts of impurities. Furthermore, nonspecific amplification products could appear.

3. Capillary Gel Electrophoresis

3.1 Preparation of PCR products

After completion of the PCR, remove the samples from the cycler and centrifuge briefly. Thaw the reagents Hi-Di™ Formamide (not included in the kit) and DNA Size Standard 550 (BTO), mix and centrifuge the tubes briefly. Prepare the approach described in Table 6 consisting of Hi-Di™ Formamide and the Size Standard 550 (BTO), add one or two reactions to the approach to compensate for pipetting variations.

Table 6. Approach of the denaturation mixture containing Hi-Di Formamide and DNA Size Standard 550 (BTO)

Componente	Volume per reaction
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (BTO)	0.5 µL

Pipette 12 µL of the denaturation mixture of Formamide and DNA Size Standard 550 (BTO) in the appropriate number of wells of a PCR plate (suitable for use in the Genetic Analyzer). Then add either 1 µL PCR product or 1 µL Allelic Ladder of the Mentype® **DIPscreen** per well. Seal the PCR plate with a suitable foil, vortex and centrifuge the plate briefly.

Note: The Allelic Ladder is used to correctly determine the fragments analyzed during data analysis. In each fragment length analysis run, the Allelic Ladder must be analyzed at least once to ensure successful data analysis.

Note: The capillaries of the gel electrophoresis device should never run dry. If the samples do not occupy all capillary positions, fill the additional wells of the plate with 12 µL Hi-Di™ Formamide according to the capillary number

Denature the prepared PCR products on a PCR cycler for 3 minutes at 95 °C, cool the samples to 4 °C in the cycler. Centrifuge the samples briefly before fragment length analysis.

3.2 Fragment Length Analysis

After the spectral calibration of the capillary gel electrophoresis device with the reagent Matrix Standard BT5 multi (BIOTYPE GmbH) has been successfully run, create a specific run module (ABI 3130) or instrument protocol (ABI 3500) with the following parameters:

Table 7. Specific parameters for the run module resp. instrument protocol of the capillary gel electrophoresis device

	ABI 3130	ABI 3500
Injections Voltage [kV]	3.0	3.0
Run Time	1500 s	1500 s
Injection Time [s]	10	10

Differing from the values given in Table 7, the run time can be adjusted to analyze all fragments (60-550 bp) of DNA Size Standard 550 (BTO).
Note: Follow the instructions for use of the manufacturer of the capillary gel electrophoresis device to set the specific running parameters.

Note: Also refer to the additional information leaflets available for calibration and application of the Mentype® products on the capillary gel electrophoresis instruments. These are available on request via support@biotype.de by BIOTYPE GmbH.

4. Analysis

For general instructions on automatic sample analysis, refer to the *GeneMapper® ID or GeneMapper®ID-X Software User's Manual.*

Note: Within the Mentype® **DIPscreen** the red panel should be faded out.

Finding the exact lengths of the amplified products depends on the device type, the conditions of electrophoresis, as well as the DNA size standard used. The DNA Size Standard 550 (BTO) shall thus be used with the following lengths of fragments: **60**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250, 260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550** bp.

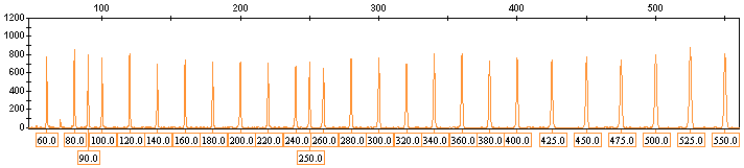


Fig. 1 Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

Note: The provided template files for the DNA Size Standard SST-BTO_60-450bp can be applied for the evaluation and analysis of the Mentype® **DIPscreen** using the GeneMapper® ID or ID-X Software.

4.1 BIOTYPE template files

Allele allocation should be carried out with suitable analysis software, e. g. the GeneMapper® ID/ID-X software in combination with the Mentype® **DIPscreen** template files from BIOTYPE. Template files are available on our homepage.

Recommended BIOTYPE templates for GeneMapper® ID/ID-X Software are:

Panels	DIPscreen_Panels_v1/v1X	or higher versions
BinSets	DIPscreen_Bins_v1/v1X	or higher versions
Size Standard	SST-BTO_60-450bp	
Analysis Method	Analysis_DIPscreen_3130_200rfu Analysis_DIPscreen_3130_1000rfu	
Plot Settings	PlotsBT5_4dyes	
Table Settings	Table for 2 alleles	

Panels and BinSets always have to be used whereas the other template files are optional.
The prepared BIOTYPE templates for GeneMapper® ID/ID-X Software were generated for POP4® runs. In case of using other polymer types, changes may be necessary on Panels and Bins or within the Analysis Method before analyzing the data.
For detailed instructions please refer to the instruction BIOTYPE Template Files for GeneMapper® which can be downloaded from our homepage (www.biotype.de).

Important note: Import and allele calling with provided template files is only guaranteed if the GeneMapper® ID/ID-X software is used. If GeneMapper® software is applied you may experience import problems with some template files. You may have to adjust Panels and Bins with one or more runs of the allelic ladder on your specific instrument setup. Contact us for support (support@biotype.de).

General procedure for the analysis

1. Check the DNA size standard
2. Check the allelic ladder
3. Check the positive control
4. Check the negative control
5. Analyze and interpret the sample data

4.2 Controls

The Control DNA XY82 of the test kit and other commercially available DNA from standard cell lines represent the following alleles:

Table 8. Allele determinations of Mentype® **DIPscreen**, - = Deletion, + = Insertion

Locus	Control DNA XY82	Control- DNA XY13	ATCC K-562	CCR 9947A	CCR 9948	CCR 3657
AM	XY	XY	XX	XX	XY	XY
HLD106	+/+	+/+	-/-	+/+	+/+	+/+
HLD70	-/+	-/+	-/+	+/+	-/+	-/-
HLD84	+/+	-/+	+/+	-/-	-/+	-/-
HLD103	-/+	+/+	-/-	-/+	+/+	-/+
HLD104	-/+	-/+	-/-	-/+	+/+	-/-
HLD116	-/+	-/+	+/+	-/-	-/+	-/-
HLD112	-/+	-/+	+/+	-/+	-/+	-/+
HLD307	+/+	+/+	+/+	-/+	+/+	+/+
HLD310	-/+	+/+	-/+	-/+	-/-	-/+
HLD110	-/+	-/+	-/+	-/+	-/+	-/+
HLD133	-/+	-/+	-/-	+/+	+/+	-/+
HLD79	+/+	+/+	+/+	+/+	-/+	+/+
HLD105	-/-	-/+	-/-	-/+	-/+	-/+
HLD140	-/+	+/+	+/+	-/-	-/+	+/+
HLD163	-/+	+/+	-/+	-/+	+/+	-/+
HLD91	+/+	-/+	-/+	-/-	-/-	-/+
HLD23	-/+	-/+	+/+	-/-	-/+	-/+
HLD88	+/+	+/+	-/-	-/-	-/+	+/+
HLD101	-/+	-/+	-/+	-/+	-/+	-/+
HLD67	-/+	-/+	-/+	+/+	+/+	+/+
HLD301	-/+	-/+	-/+	-/+	-/+	-/-
HLD53	+/+	+/+	-/-	-/+	+/+	-/-
HLD97	-/+	-/-	-/-	-/+	-/+	+/+
HLD152	-/+	-/-	+/+	+/+	-/+	+/+
HLD128	-/+	-/+	-/+	-/+	-/-	-/+
HLD134	+/+	-/+	-/-	+/+	+/+	-/-
HLD305	+/+	-/+	-/+	-/+	+/+	-/+
HLD48	-/-	-/+	+/+	+/+	-/+	+/+
HLD114	-/-	+/+	-/-	-/-	+/+	-/+
HLD304	-/+	+/+	-/-	-/+	-/+	-/-
HLD131	+/+	+/+	-/+	-/-	-/+	+/+
HLD38	+/+	+/+	-/+	-/+	+/+	+/+
HLD82	+/+	+/+	+/+	+/+	-/+	+/+

The reference DNA K-562 is available from ATCC. DNA 9947A, 9948 and 3657 are available from Coriell Cell Repositories.

4.3 Lengths of fragments and alleles

Table 9 shows the fragment lengths of individual alleles that refer to the DNA Size Standard 550 (BTO). All analyses have been performed on an ABI PRISM® 3130 Genetic Analyzer with POP4® polymer. Different analysis instruments, DNA size standards or polymers may result in different fragment lengths. In addition, a visual alignment with the allelic ladder is recommended.

Scaling

Horizontal: 70-430 bp (see Figs. 2 and 3)
Vertical: depending on signal intensity

Figure 2

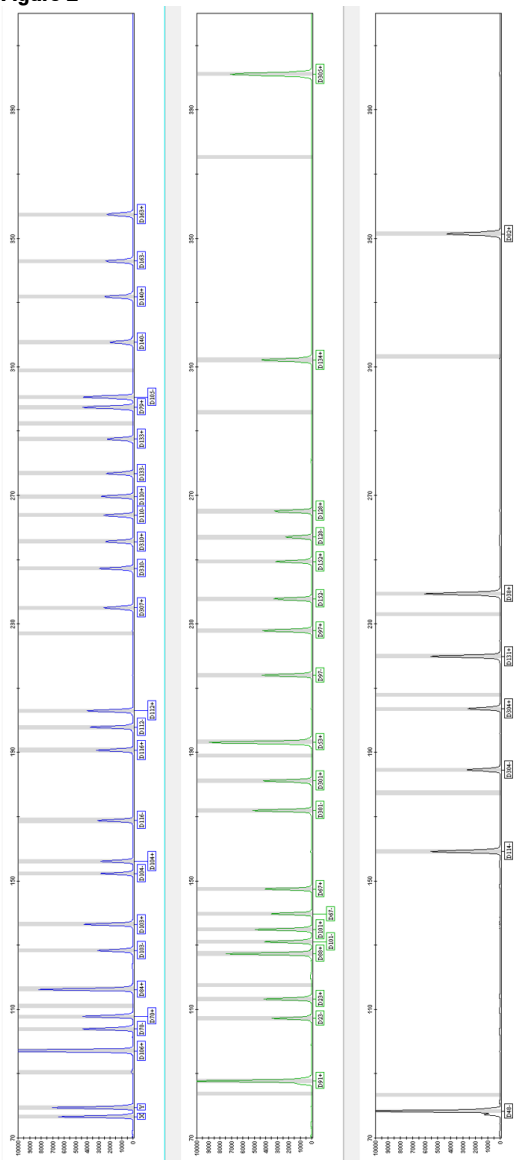


Fig. 2 Electropherogram of the Mentype® **DIPscreen** using 1 ng Control DNA XY82. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper® ID-X Software and the Mentype® **DIPscreen** template file.

Figure 3

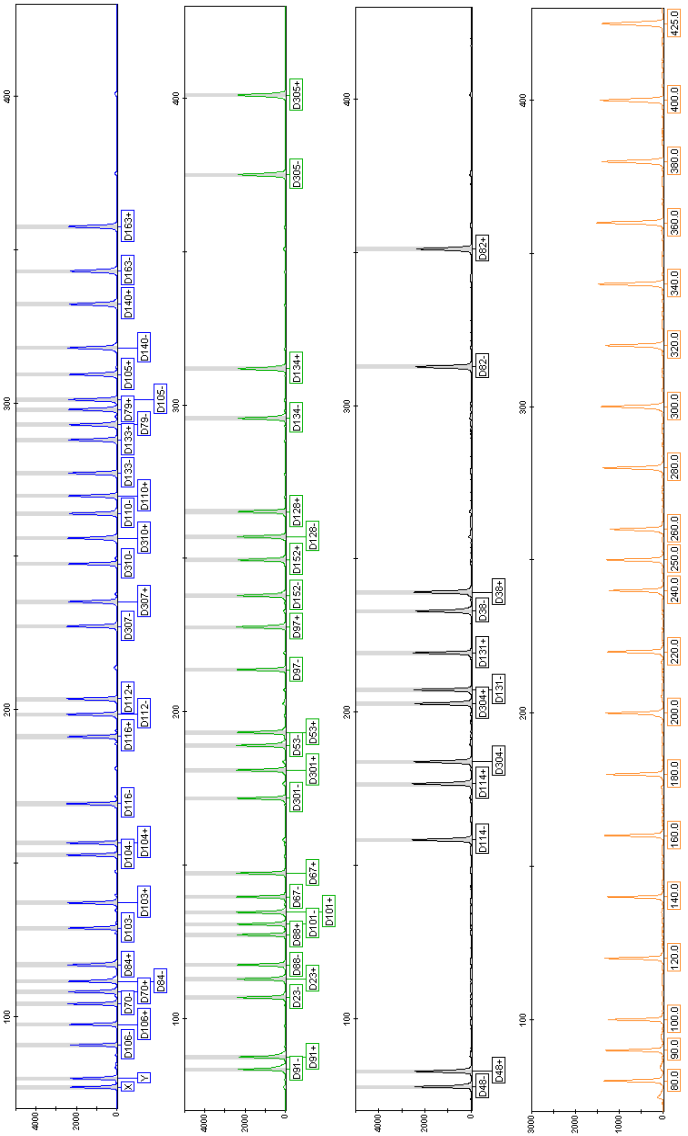


Fig. 3 Electropherogram of the allelic ladder Mentype® **DIPscreen**. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper® ID-X Software and the Mentype® **DIPscreen** template file.

Table 9. Fragment lengths of the Mentype® **DIPscreen** allelic ladder analyzed on an ABI PRISM® 3130 Genetic Analyzer with POP4® (FAM, BTG, BTY panel)

Marker/FAM	-DIP [bp]*	+DIP [bp]*	Marker/BTG	-DIP [bp]*	+DIP [bp]*
AM	77 (X)	80 (Y)	HLD91	84	88
HLD106	91	98	HLD23	107	113
HLD70	104	108	HLD88	118	128
HLD84	112	117	HLD101	131	135
HLD103	129	138	HLD67	140	148
HLD104	153	157	HLD301	172	182
HLD116	170	192	HLD53	190	194
HLD112	199	204	HLD97	214	228
HLD307	228	236	HLD152	239	250
HLD310	248	257	HLD128	258	266
HLD110	264	270	HLD134	296	312
HLD133	278	288	HLD305	375	401
HLD79	294	299			
HLD105	302	310	**Marker/BTY**	**-DIP [bp]***	**+DIP [bp]***
HLD140	318	333	HLD48	78	83
HLD163	344	358	HLD114	159	177
			HLD304	184	203
			HLD131	208	220
			HLD38	234	240
			HLD82	314	352

* rounded to integer

5. Interpretation of results

As mentioned above, post-PCR analysis and automatic allele allocation with suitable analysis software ensure a precise and reliable discrimination of alleles.

Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range (> 3 000 RFU), or if an incorrect matrix is applied. They appear at positions of specific peaks in other color channels, typically with lower signal intensities. Peak heights should not exceed 3 000 RFU in order to prevent pull-up peaks.

Template-independent addition of nucleotides

Because of its terminal transferase activity, the Multi Taq DNA Polymerase tends to add an adenosine radical at the 3'-end of the amplified DNA fragments. The artefact peak is one base shorter than expected (-1 bp peaks). All BIOTYPE primers are designed to minimize these artefacts. Artefact formation is further reduced by the final extension step of the PCR protocol at 68 °C for 60 min. Peak height of the artefact correlates with the amount of DNA. Laboratories should define their individual limits for analysis of the peaks.

Artefacts

Room temperature may influence the performance of PCR products on multi-capillary instruments; and shoulder peaks or split peaks occur. Furthermore, automated assignment could be influenced in some cases. If these effects occur, we recommend injecting the sample again at a higher room temperature and, maybe, using more than one allelic ladder sample per run.

Influence of polymers

Mentype® **DIPscreen** was validated and certified for the analysis on POP4® polymer. The use of other polymers (e. g. POP7™ or POP6™) might influence the run behavior of specific PCR products. Furthermore, background noise might increase through the different behavior of free fluorescent dyes.

6. References

Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C, Lamy T, Le Prise PY, Beauplet A, Bories D, Semana G, Quelvennec E. (2002) Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. *Blood 99, 4618-4625*.

Chen DP, Tseng CP, Wang WT, Wang MC, Tsai SH, Sun CF (2011) Real-time biallelic polymorphism-polymerase chain reaction for chimerism monitoring of hematopoietic stem cell transplantation relapsed patients. *Clin Chim. Acta 412, 625-630*.

Harries LW, Wickham CL, Evans JC, Rule SA, Joyner MV, Ellard S (2005) Analysis of haematopoietic chimaerism by quantitative real-time polymerase chain reaction. *Bone Marrow Transplant. 35, 283-290*.

Masmas TN, Madsen HO, Petersen SL, Ryder LP, Svejgaard A, Alizadeh M, Vindelov LL (2005) Evaluation and automation of hematopoietic chimerism analysis based on real-time quantitative polymerase chain reaction. *Biol Blood Marrow Transplant. 11, 558-566*.

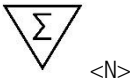
Mills RE, Luttig CT, Larkins CE, Beauchamp A, Tsui C, Pittard WS, Devine SE (2006) An initial map of insertion and deletion (INDEL) variation in the human genome. *Genome Res 16 (9):1182-1190, 2006*.

Qin XY, Li GX, Qin YZ, Wang Y, Wang FR, Liu DH, Xu LP, Chen H, Han W, Wang JZ, Zhang XH, Li JL, Li LD, Liu KY, Huang XJ (2011) Quantitative assessment of hematopoietic chimerism by quantitative real-time polymerase chain reaction of sequence polymorphism systems after hematopoietic stem cell transplantation. *Chin Med J (Engl.) 124, 2301-2308*.

Weber JL, David D, Heil J, Fan Y, Zhao C, Marth G (2002) Human diallelic insertion/deletion polymorphisms. *Am J Hum Genet 71(4):854-862*.

Wilhelm J, Reuter H, Tews B, Pingoud A, Hahn M (2002) Detection and quantification of insertion/deletion variations by allele-specific real-time PCR: application for genotyping and chimerism analysis. *Biol Chem 383, 1423-1433*.

7. Explanation of Symbols

	**Manufacturer**
	**Batch code**
	**Contains sufficient for <N> tests**
	**Consult electronic instructions for use (eIFU)**
	**Use-by date**
	**Temperature limit**
	**Catalogue number**
	**In vitro diagnostic medical device**
	**Keep away from sunlight**
	**Keep dry**

Specification Mentype® DIPscreen PCR-Amplification kit

A Analytical Validation

A a) Determination of the Standard Reaction and batch-specific Tolerance

Objective: The standard reaction and the batch-specific tolerances with respect to the absolute signal heights (RFU), the balance of the signal heights of the multiplex PCR and the baseline were determined.

Methodology: The test kit contains the Control DNA XY13 of a healthy donor, which is heterozygous in 17 DIP systems and amelogenin (AM). The standard reaction (28 PCR cycles) was carried out with this Control DNA in the nominal concentration of 1 ng in quadruple determination. Four blank values (no template control, NTC) without DNA were also carried out.

Results: For the batch-specific mixing of the PCR primers, the following specifications were established: Signal heights of 1 000-5 000 RFU were obtained using an ABI PRISM® 3130 Genetic Analyzer. The fluctuations for signal heights of heterozygous systems allowed a maximum of 50 % of the guide value. In the scaling range, no nonspecific signals 200 RFU were determined (baseline) for the blank values.

A b) Genotyping Accuracy

Objective: The accuracy of the allele assignment should be statistically secured under standard conditions. The testing examined the automatic allele calling with the allelic leader. Further the concordance of the allele assignment compared to the pre-typing of test DNAs by other methods (other PCR kits, direct sequencing, etc.) using the GeneMapper ID software. Based on the results, the test-specific device settings for genotyping by means of capillary electrophoresis (bins and panels) for the analysis templates of the DNA sequencer are defined.

Methodology: 100 pre-typed human DNAs from donors (whole blood, cheek swabs) were investigated in single determination. In addition, a blank was carried without DNA. Acceptance criterion was defined as full profiles with peak heights ≥ 200 RFU (manual evaluation) [3; 4].

Results: After determination of the test-specific device settings the correct genotype was assigned to all DNA samples for all HLD systems and the amelogenin marker.

A c) Analytical Specificity

Objective: The investigations served to exclude false-positive results due to cross-reactivity with selected non-human DNA samples. In clinical practice, however, non-human DNA can be largely excluded due to sterile sampling.

Method: 2.5 ng genomic DNA from *Bos Taurus* (cattle), *Sus scrofa domestica* (pig), *Canis lupus familiaris* (dog), *Felis catus* (cat) and *Oryctolagus cuniculus* (domestic rabbit) were tested. The DNA from animals was derived from blood samples, which were provided as a residual material of veterinary studies.

Results: No cross-reactivity was detected in the allele area (< 200 RFU).

A d) Analytical Sensitivity

Objective: The investigations were used to determine the analytical detection limit (sensitivity).

Method: A dilution series with 1 ng to 65 pg of reference DNA was tested in quadruplicate. As an acceptance criterion, complete DNA profiles with ≥ 100 RFU were defined.

Results: A detection limit of 200 pg of genomic DNA was determined.

A e) Assays Performance with Different PCR-Thermocyclers

Objective: PCR thermal cyclers of different manufacturers differ in their specifications. In particular, different heating and cooling rates as well as different temperature control techniques (hysteretic versus symmetrical settling of the actual values by the setpoint) can be observed.

Method: Testing of the standard reaction with Control DNA in the nominal concentration of 1 ng was carried out with all thermocyclers (described below) in quadruplicate determinations with the same master mix. In addition, two blank samples without DNA were examined.

GeneAmp 9700 with Silverblock (Applied Biosystems®, Life Technology GmbH, Darmstadt), GeneAmp 9700 with Alu block (Life Technology GmbH, Darmstadt) and Eppendorf Mastercycler ep-S (Eppendorf AG, Hamburg)

Results: No unspecific by-products ≥ 200 RFU were detected in the allele region. The deviation of the mean peak heights compared to the standard reaction was a maximum of 20 % at a defined ramp of ≥ 2 °C./sec.

A f) Mixed DNA Samples

Objective: The aim of chimerism analysis after allogeneic blood stem cell transplantation is the separate detection and the relative quantification of donor and recipient DNA. In order to detect the minimal residual disease, the smallest possible amounts of recipient DNA should be detected in the mixture. Therefore, different mixtures of two defined DNAs with different genotypes were produced in analytical validation.

Method: 10 independent mixtures of each two unrelated DNAs were prepared using the deficient DNA as 0 %, 1 %, 5 %, 10 %, 30 %, 50 % and 70 %. Between two DNAs in the mixtures, an average of 13 DIP-Loci (12.8 ± 2.22) with informative alleles were added for evaluation. In each case 2 ng of the DNA mixtures was tested in the standard reaction. Signal heights of at least 50 RFU were evaluated.

Results: A detection limit of 1 % could be achieved for the deficient DNA. This corresponds to the values 1–5 %, which were achieved with forensic STR kits in the chimerism analysis [8-11].

A g) PCR Annealing Temperatures

Objective: To determine the robustness of the PCRs, temperature fluctuations for the primer attachment step (annealing) of the multiplex PCR were simulated. This temperature step is critical for the sensitivity and specificity of the PCR.

Method: The kit-specific annealing temperature of 60°C was varied by ± 1 °C and ± 2 °C in the standard reaction setting with Control DNA and a nominal concentration of 1 ng. A triple determination with the same master mix was performed.

Results: No nonspecific by-products ≥ 200 RFU were detected for ± 1 °C. The average peak heights deviated from the standard reaction at ± 1 °C to a maximum ± 30 %. At + 2 °C some systems (HLD 84, 103, 116, 112, 133, 105, 40, 67, 48), showed a reduction in performance. One system showed a complete profile loss (HLD 91) at that temperature.

A h) Fluctuation of PCR buffer Batches

Objective: The concentration ratios of the contents of the PCR buffer Reaction Mix A (dNTPs, ion concentrations, in particular Mg^{2+}) are critical for sensitivity, specificity and the balance of the signals in multiplex PCRs. Therefore, the robustness of the test is tested against batch fluctuations of the supplied PCR buffer.

Methods: 4 independent Reaction mix A batches were tested for performance in the standard reaction with Control DNA of the nominal concentration of 1 ng.

Results: No nonspecific by-products ≥ 200 RFU were detected. The deviation of averaged peak heights compared to the standard reaction was a maximum of 20 %.

A i) In-use Stability

Objective: The stability of the reagents of the PCR kit was tested after repeated freezing and thawing.

Methodology: The kit reagents were subjected to a 20-fold freezing and thawing cycle. The freezing was carried out for at least 1 h at -20 °C. The mixture was thawed at room temperature and the reagents were homogenized by shaking before use. Subsequently, a standard reaction with control DNA of the nominal concentration of 1 ng and additional blank values without DNA was performed in triplicate determinations. The evaluation was carried out in comparison to a standard reaction without freezing and thawing.

Results: The deviation of averaged peak heights compared to the standard reaction was a maximum of 20 % (in particular signal loss). Additional peaks > 200 RFU were observed in the blank samples; however, no peak occurred in the allelic range of the kit (free fluorescence dyes in the BTG Panel).

B Clinical Performance Data

B a) Study Design, ethics and regulatory aspects

A clinical performance study was conducted according to §§ 20-24 of German Medizinproduktegesetz. The protocol was approved by the National Competent Authority BfArM according to § 7 German Verordnung über klinische Prüfungen von Medizinprodukten and by the institutional ethics committee. All participants gave written informed consent.

B b) Reference Methods

The performance of the kit was compared to the Short-Tandem-Repeats (STRs) based CE-IVD Mentype® **Chimera**® PCR-Amplification kit (BIOTYPE GmbH, Dresden, DE) [12]. Further, a cytogenetic differentiation of donor and recipient leukocytes by means of fluorescence in situ hybridization (FISH) was performed using the sex chromosome-specific CE-IVD CEP® X SpectrumOrange ™ / Y SpectrumGreen ™ Direct Labeled Fluorescent DNA Probe Kit (Abbott GmbH & Co KG, Wiesbaden, DE; usage in according to the manufacturer's data) [11].

B c) DNA-Extraction and Purification

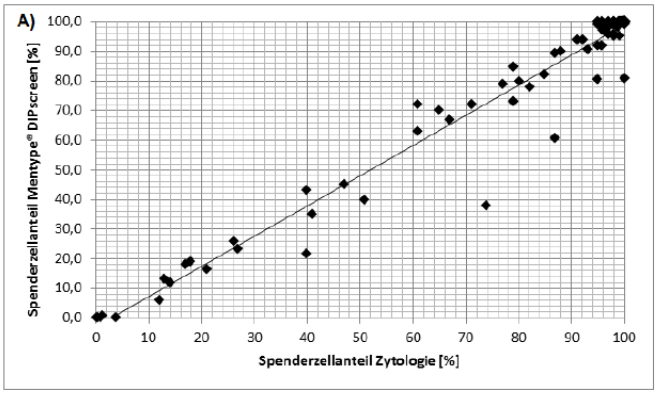
DNA extraction from heparinized whole blood samples was performed with the QIAamp® DNA Blood Mini Kit (Qiagen GmbH, Hilden, DE), according to the manufacturer.

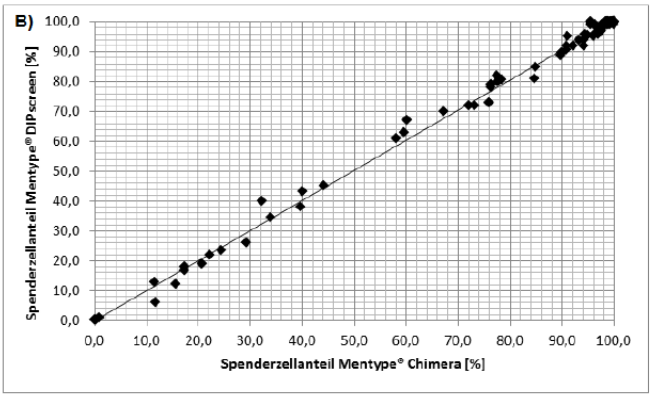
B d) Results

A total of 98 data sets of adult patients were collected on various days after allogeneic blood stem cell or bone marrow transplantation. The donor-recipient pairs differed in the genetic gender and were thus suitable for the sex chromosome-specific FISH [10]. At least 1.5 ng genomic DNA was used per PCR. Firstly, all informative STR or DIP systems of the donor-recipient pairs were determined and sex was confirmed by genotyping the amelogenin marker, which is part of the multiplex PCR. For the PCR results mean values of the signal heights of all informative STR- or DIP systems were used. The results of the concordance analysis are summarized in Fig.1.

Compared to cytogenetics, 11 samples, which had been analyzed with Mentype® **DIPscreen,** showed a deviation of the donor portion of more than 5 % (absolute) (see Fig. 4A). In 5 of these samples, cell numbers significantly less than 200 were counted for cytogenetics. However, according to the recommendations of the manufacturer of the FISH kit, at least 200 cells should be counted. According to practical recommendations, higher absolute cell numbers (500-1 000) yield better cytogenetic results [11, 13]. In contrast to cytogenetics, the differences from Mentype® **DIPscreen** to the STRs-based multiplex PCR kit Mentype® **Chimera**® were 7.9 % (see Fig. 4B). Only 3 of the 98 measurement data sets showed a deviation of more than 5 %.

Fig. 4 Concordance analysis of multiplex PCR Mentype® **DIPscreen** in comparison to cytology (A) and multiplex PCR Mentype® **Chimera®** (B)





B e) References

1) **Wenz H, Robertson JM, Menchen S, Oaks F, Demorest DM, Scheibler D, Rosenblum BB, Wike C, Gilbert DA, Efcavitch JW.** High-precision genotyping by denaturing capillary electrophoresis. *Genome Res 1998; 8: 69-80.*

2) **Sgueglia JB, Geiger S, Davis J.** Precision studies using the ABI prism 3100 genetic analyzer for forensic DNA analysis. *Anal Bioanal Chem 2003; 376: 1247-54.*

3) **Gilder JR, Doom TE, Inman K, Krane DE.** Run-specific limits of detection and quantitation for STR-based DNA testing. *J Forensic Sci 2007; 52: 97-101.*

4) **Schneider PM, Fimmers R, Keil W, Molsberger G, Patzelt D, Pflug W, Rothämel T, Schmitter H, Schneider H, Brinkmann B.** The German Stain Commission: recommendations for the interpretation of mixed stains. *Int J Legal Med. 2009; 123: 1-5.*

5) **Wheeler DL, Barrett T, Benson DA, Bryant SH, Canese K, Chetvernin V, Church DM, DiCuccio M, Edgar R, Federhen S, Geer LY, Kapustin Y, Khovayko O, Landsman D, Lipman DJ, Madden TL, Maglott DR, Ostell J, Miller V, Pruitt KD, Schuler GD, Sequeira E, Sherry ST, Sirotkin K, Souvorov A, Starchenko G, Tatusov RL,Tatusova TA, Wagner L, Yaschenko E.** Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res 2007; 35 (Database issue): D5-12.*

6) **Haas-Rochholz H, Weiler G.** Additional primer sets for an amelogenin gene PCR-based DNA-sex test. *Int J Legal Med 1997; 110: 312-5.*

7) **Peakall R and Smouse PE.** GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics 2012; 28: 2537-2539.*

8) **Thiede C, Lion T.** Quantitative analysis of chimerism after allogeneic stem cell transplantation using multiplex PCR amplification of short tandem repeat markers and fluorescence detection. Appendix: Method in focus. *Leukemia 2001; 15: 303–6.*

9) **Thiede C.** Diagnostic chimerism analysis after allogeneic stem cell transplantation: new methods and markers. *Am J Pharmacogenomics 2004; 4: 177-87.*

10) **Thiede C, Lion T.** Quantitative analysis of chimerism after allogeneic stem cell transplantation using multiplex PCR amplification of short tandem repeat markers and fluorescence detection. Appendix: Method in focus. *Leukemia 2001; 15: 303–6.*

11) **Buño I, Nava P, Simón A, González-Rivera M, Jiménez JL, Balsalobre P, Serrano D, Carrión R, Gómez-Pineda A, Díez-Martín JL.** A comparison of fluorescent in situ hybridization and multiplex short tandem repeat polymerase chain reaction for quantifying chimerism after stem cell transplantation. *Haematologica 2005; 90: 1373-9.*

12) **Henke L, Muche M, Blaauw A, Van Eede PH, Martin W, Helmken C, Budowle B, Henke J.** Validation of a "new" short tandem repeat (STR) fluorescent multiplex system and report of population genetic data. *Clin Lab 2007; 53:477-82.*

13) **Mohr B, Koch R, Thiede C, Kroschinsky F, Ehninger G, Bornhäuser M.** CD34+ cell dose, conditioning regimen and prior chemotherapy: factors with significant impact on the early kinetics of donor chimerism after allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant 2004; 34: 949-54.*

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