BIOTYPE

Mentype® **Chimera**®

Instructions for Use

The new standard for chimerism analysis

For in vitro diagnostic use

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Lot number

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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® **Chimera**® PCR amplification kit, please do not hesitate to get in touch or visit www.biotype.de

Product description

Mentype® **Chimera**® is a multiplex-PCR application specifically developed for chimerism monitoring after blood stem cell and bone marrow transplantation, respectively. The assay was validated by chimerism analysis of over 200 HLA-matched related donor-recipient-pairs and its suitability was confirmed in a comparative clinical evaluation study. The assay has been successfully in use ever since for routine diagnostics.
Genetic markers that are addressed by Mentype® **Chimera**® are distributed over 12 chromosomes and represent highly polymorphic short tandem repeats (STRs) with a very high rate of heterozygosity and a balanced allelic distribution. Together, this significantly increases the chance to identify informative loci for donor-recipient discrimination and provides reliability and robustness of chimerism analyses.

One PCR reaction simultaneously amplifies the autosomal loci **D2S1360**, **D3S1744**, **D4S2366**, **D5S2500**, **D6S474**, **D7S1517**, **D8S1132**, **D10S2325**, **D12S391**, **D18S51**, **D21S2055**, **SE33 (ACTBP2)**, and the gender-specific locus **Amelogenin**. One primer for each locus is fluorescence-labelled with **6-FAM**, **BTG**, or **BTY**.

The detection limit of the Mentype® **Chimera**® PCR amplification kit is **200 pg genomic DNA**. The optimal range under standard conditions is **0.2-1.0 ng DNA**.

The test kit has been validated using the GeneAmp® PCR System 9700 Aluminium, Eppendorf Mastercycler ep-S, Biometra T1, ABI PRISM® 310 Genetic Analyzer and ABI PRISM® 3130 Genetic Analyzer applying the POP-4® polymer.

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1. Description of Mentype® Chimera®

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

Locus	GenBank accession	Repeat motif of the reference allele	Reference allele	Allele range
Amelogenin X	M55418			
Amelogenin Y	M55419			
D2S1360	G08130	$[TATC]_9$ $[TGTC]_9$ $[TATC]_5$	23	19-32
D3S1744	G08246	$[TCTA]_2$ TA$[TCTA]_{12}$ TCA $[TCTA]_2$	16	13-22
D4S2366	G08339	$[ATAG]_9$ ATTG $[ATAG]_2$	12	9-15
D5S2500	G08468	$[ATAG]_{12}$	12	9-18
D6S474	G08540	$[TAGA]_5$ TGA $[TAGA]_{12}$	17	11-20
D7S1517	G18365	$[GAAA]_{11}$ CAAA $[GAAA]_2$ CAAA $[GAAA]_2$	17	14-31
D8S1132	G08685	$[TCTA]_9$ TCA $[TCTA]_9$ TCTGTCTA	20	12.1-27
D10S2325	G08790	$[TCTTA]_{12}$	12	6-23
D12S391	G08921	$[AGAT]_5$ GAT $[AGAT]_7$ $[AGAC]_6$ AGAT	19.3	13-28
D18S51	L18333	$[AGAA]_{13}$	13	5.3-42
D21S2055	G27274	$[CTAT]_2$ CTAA $[CTAT]_9$ CTA $[CTAT]_3$ TAT $[CTAT]_3$ TAT $[CTAT]_4$ CAT$[CTAT]_2$	24	16.1-39
SE33 (ACTBP2)	NG000840	$[AAAG]_9$ AA $[AAAG]_{16}$	25.2	3-50

Table 1 shows STR loci with respective repeat motifs and alleles that are concordant with the guidelines for the use of microsatellite markers of the International Society for Forensic Genetics (ISFG; Bär *et al.*, 1997). The nomenclature for STR loci D8S1132 and D12S391 is in accordance with Hering and Müller (2001), for loci D4S2366 and D6S474 with Becker *et al.* (2007), for locus D10S2325 with Wiegand *et al.* (1999) and the nomenclature for locus D7S1517 is in accordance with Wiegand and Klintschar (2002). Allele ranges include all known alleles of the National Institute of Standards and Technology (NIST as of 12/2008) and the current literature.

Table 2. Chromosomal mapping for Mentype® **Chimera**®

Locus	Chromosomal mapping
Amelogenin X	Xp22.1-22.3
Amelogenin Y	Yp11.2
D2S1360	2p24-p22
D3S1744	3p24
D4S2366	4p16-15.2
D5S2500	5q11.2
D6S474	6q21-22
D7S1517	7q31.33
D8S1132	8q23.1
D10S2325	10p12
D12S391	12p13.2
D18S51	18q21.3
D21S2055	21q22
SE33	6q14.2

Kit content

Mentype® **Chimera**® PCR Amplification Kit

Reagent	Volume per packaging size		
	25 reactions	100 reactions	400 reactions
Nuclease-Free Water	1,5 mL	2x 1,5 mL	6x 1,5 mL
Reaction Mix A	125 µL	500 µL	2x 1,0 mL
Mentype® **Chimera**® Primer Mix	63 µL	250 µL	4x 250 µL
Multi Taq 2 DNA Polymerase	10 µL	40 µL	160 µL
Mentype® **Chimera**® Control DNA XY5 (2 ng/µL)	10 µL	10 µL	10 µL
DNA Size Standard 550 (BTO)	13 µL	50 µL	200 µL
Mentype® **Chimera**® Allelic Ladder	25 µL	25 µL	4x 25 µL

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

Note: The packaging size 1000 reactions cannot be ordered anymore.

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

Storage

Store all components at -25 °C to -15 °C and avoid repeated thawing and freezing. Primer mix and 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 PCR reagents. The expiry date is indicated on the kit cover.

Additionally required reagents

Additional reagents required in order to use the Biotype® PCR amplification kit:

Table 3. Additional required reagents for Mentype® **Chimera**®

Reagent	Supplier	Order number
Hi-Di™ Formamide, 25 mL	Life Technologies Corporation	4311320
Matrix Standards BT5 single-capillary instruments (5 x 25 µL)	Biotype GmbH	00-10411-0025
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 instruction

The PCR amplification kit contains the following potentially hazardous chemicals:

Kit component	Chemical	Hazards
Reaction Mix A	Sodium azide NaN_3	toxic if swallowed, develops toxic gases when it gets in contact with acids

Read the material safety data sheets (MSDS) for all Biotype® products, which are available on request. Please contact the respective manufacturers for copies of the MSDS for any additionally needed reagents.

Quality assurance

All kit components undergo an intensive quality assurance process at Biotype GmbH. 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

Mentype® and **Chimera**® are registered trademarks of Biotype GmbH.
ABI PRISM®, GeneMapper®, GeneAmp® and Applied Biosystems® are registered trademarks of Applied Biosystems LLC.
Under the law of Europe POP-4® is a registered trademark of Applied Biosystems LLC.
POP-4® is registered as trademark of Life Technologies Corporation in the US.
The PCR is covered by patents. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche).

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. PCR master mix approach for Mentype® **Chimera**®

Component	Volume
Nuclease-Free Water	16.1 µL
Reaction Mix A*	5.0 µL
Mentype® **Chimera**® Primer Mix	2.5 µL
Multi Taq 2 DNA Polymerase (hot start, 2.5 U/µL)	0.4 µL
Volume of master mix	24.0 µL

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

All components should be mixed (vortex) and centrifuged for about 10 seconds before preparing the master mix. The volume of DNA 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), for example 0.1 x TE buffer.

The primer mixes are adjusted for balanced peak heights at **30 PCR cycles** and **0.5 ng control DNA XY5** 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 the positive amplification control, dilute control DNA XY5 to 0.5 ng/µL.
Instead of template DNA, pipette diluted control DNA into a reaction tube containing the PCR master mix.

Negative control

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

Template DNA

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

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 PCR cycles depends on the amount of DNA applied. 30 PCR cycles are recommended for all samples. In case of critical samples (< 100 pg DNA), the number of PCR cycles can be increased to 32.

Standard method

Recommended for all DNA samples

Table 5. Standard PCR amplification protocol for Mentype® **Chimera**®

Temperature	Time	
94 °C	4 min (hot start for activation of the Multi Taq2 DNA Polymerase)	
94 °C	30 s	
60 °C	**120 s**	**30 cycles**
72 °C	75 s	
68 °C	60 min	
10 °C	∞	hold

Optional

Recommended for small amounts of DNA

Table 6. Optional PCR amplification protocol for small DNA amounts

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**	**32 cycles**
72 °C	75 s	
68 °C	60 min	
10 °C	∞	hold

Note: If thermal cyclers with rapid heating and cooling steps (> 2 °C/s) are used, ramping should be adjusted to 2 °C/s in order to provide an optimal kit balance.

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, unspecific amplification products could appear.

3. Capillary gel electrophoresis

3.1 Preparation of PCR products

After the 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 7 consisting of Hi-Di™ Formamide and the DNA Size Standard 550 (BTO), add one or two reactions to the approach to compensate for pipetting variations.

Table 7. Approach of the denaturation mixture containing Hi-Di™ Formamide and DNA Size Standard 550 (BTO)

Component	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 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 Mentype® **Chimera**® Allelic Ladder into the 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 and then 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 (Biotype GmbH) has been successfully run, create a specific run module (ABI 310, ABI 3130) or instrument protocol (ABI 3500) with the following parameters:

Table 8. Specific parameters for the run module resp. instrument protocol of the capillary gel electrophoresis device

	ABI 310	ABI 3130	ABI 3500
Injections Voltage [kV]	15.0	3.0	3.0
Run Time	28 min	1560 s	1560 s
Injection Time [s]	5	10	10

Differing from the values given in Table 8, 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 supplied upon request via support@biotype.de by Biotype GmbH.

4. Analysis

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

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

Finding the exact lengths of amplified products depends on the device type, the conditions of electrophoresis, as well as the DNA size standard used. Due to the complexity of some STR loci, size-determination should be based on evenly distributed references. 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.

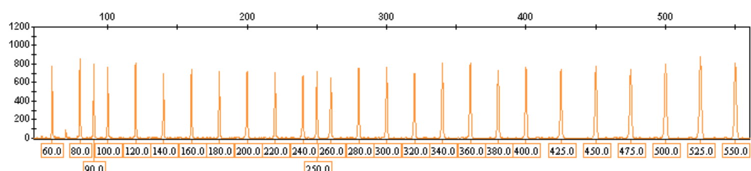

Fig. 1 Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

Note: The provided template files for the DNA size standard SST-BTO_60-500bp can be applied for the evaluation and analysis of the Mentype® **Chimera**® using the GeneMapper® ID or ID-X software.

4.1 Biotype template files

Allele allocation should be carried out with suitable analysis software, for example GeneMapper® ID/ID-X or Genotyper software in combination with the Mentype® **Chimera**® template files from Biotype GmbH. Biotype® template files are available on our homepage (www.biotype.de) for download or as CD-ROM on request.

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

Panels	Chimera_Panels_v1/v1X	or higher versions
BinSets	Chimera_Bins_v1/v1X	or higher versions
Size Standard	SST-BTO_60-500bp	
Analysis Method	Analysis_HID_310	
	Analysis_HID_3130	
	Analysis_HID_310_50rfu	
	Analysis_HID_3130_50rfu	
Plot Settings	PlotsBT5_4dyes	
Table Settings	Table for 2 Alleles	
	Table for 10 Alleles	

Panels and BinSets have to always be used, whereas the other template files are optional.

Additional Biotype® templates for GeneMapper® ID-X software:

Stutter* Chimera_Stutter_v1X or higher version

* When loading the above-mentioned panels, the stutter settings will not be accepted. Therfore, the stutter data has to be imported separately.

Recommended Biotype® template files for Genotyper Software are:

Mentype_Chimera_v1 or higher versions

Important Note: Import and allele calling with provided template files is only guarantied if the GeneMapper® ID/ID-X software is used. When the 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 XY5 which is part of the test kit and other commercially available DNA from standard cell lines represent the following alleles:

Table 9. Allele assignment of Mentype® **Chimera**®

Locus	Control DNA XY5	ATCC K-562	CCR 9947A	CCR 9948	CCR 3657
Amelogenin	X/Y	X/X	X/X	X/Y	X/Y
D2S1360	22/25	20/28	23/24	22/25	22/23
D3S1744	17/18	18/18	17/17	18/18	14/17
D4S2366	9/12	13/13	11/13	9/14	9/14
D5S2500	10/11	15/15	15/16	11/15	11/16
D6S474	15/16	14/17	13/17	16/16	15/16
D7S1517	22/27	21/24/25	19/25	20/22	24/25
D8S1132	18/20	20/24	19/21	20/24	17/18
D10S2325	13/14	7/13	9/10	8/14	9/14
D12S391	17/19	23/23	18/20	18/24	18/19
D18S51	13/15	15/16	15/19	15/18	12/20
D21S2055	25/27	28/35	19.1/26	19.1/26	19.1/25
SE33	15/21.2	26.2/28.2	19/29.2	23.2/26.2	22.2/27.2

For further confirmation, the table above displays alleles of reference DNA purchased from ATCC, as well as three assignments of reference DNA purchased from Coriell Cell Repositories standard of Szibor *et al.* (2003).

4.3 Lengths of fragments and alleles

Table 10-12 show the fragment lengths of individual alleles that refer to the DNA Size Standard 550 (BTO). All analyses have been performed on an
ABI PRISM® 310/3130 Genetic Analyzer with POP-4® 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: 70-480 bp
Vertical: Depending on signal intensity

Figure 2

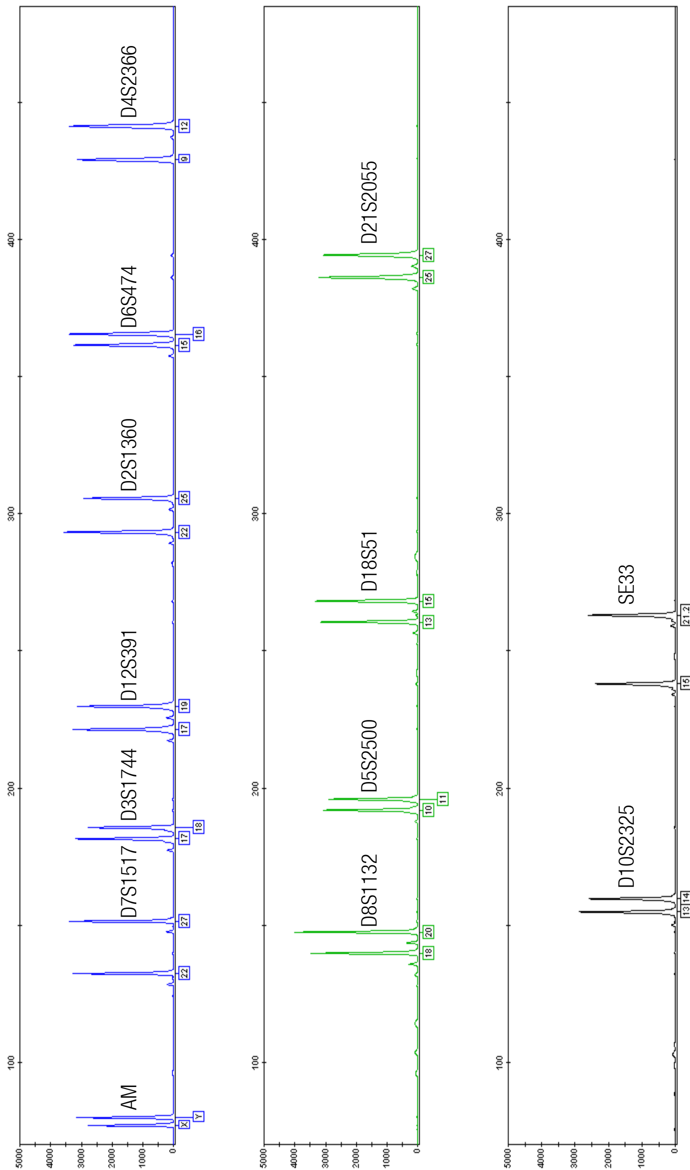

Fig. 2 Electropherogram of the Mentype® **Chimera**® using 500 pg control DNA XY5. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper® ID software and the Mentype® **Chimera®** template file.

Figure 3

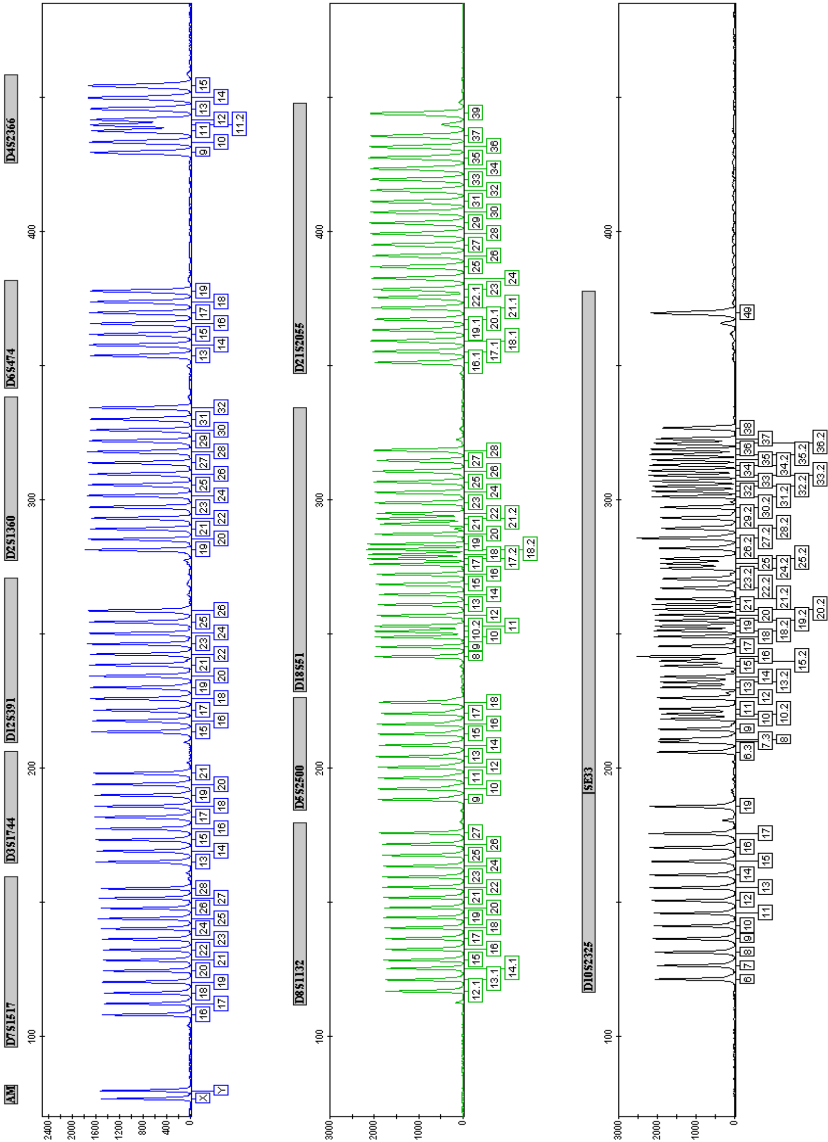

Fig. 3 Electropherogram of the allelic ladder Mentype® **Chimera®.** Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper® ID software and the Mentype® **Chimera®** template file.

Table 10. Fragment lengths of the Mentype® **Chimera**® allelic ladder analyzed on an ABI PRISM® 3130 Genetic Analyzer with POP-4® polymer. (blue panel)

Marker/ allele	Size [bp]*	Further alleles**	Marker/ allele	Size [bp]*	Further alleles**	Marker/ allele	Size [bp]*	Further alleles**
Amelogenin	**6-FAM**		**D12S391**	**6-FAM**		**D6S474**	**6-FAM**	
X	77		15	213		13	354	11, 12
Y	80		16	217	16.3	14	358	
			17	221	17.3	15	362	
D7S1517	**6-FAM**		18	226	18.3	16	366	
16	108	14, 15	19	230	19.1, 19.3	17	370	
17	112		20	234	20.3	18	374	
18	116		21	238		19	378	
19	120		22	242				
20	124		23	246		**D4S2366**	**6-FAM**	
21	128		24	250		9	429	9.2
22	132		25	254		10	433	10.2
23	136		26	258	27	11	437	
24	140					11.2	440	
25	144		**D2S1360**	**6-FAM**		12	441	
26	148		19	281		13	445	
27	152		20	285		14	449	
28	155	29	21	289		15	454	
			22	293				
D3S1744	**6-FAM**		23	297				
13	165		24	302				
14	169		25	306				
15	173		26	310				
16	177		27	314				
17	182		28	318				
18	186		29	322				
19	190		30	326				
20	194		31	330				
21	198	22	32	334				

Table 11. Fragment lengths of the Mentype® **Chimera**® allelic ladder analyzed on an ABI PRISM® 3130 Genetic Analyzer with POP-4® polymer (green panel)

Marker/ allele	Size [bp]*	Further alleles**	Marker/ allele	Size [bp]*	Further alleles**	Marker/ allele	Size [bp]*	Further alleles**
D8S1132	**BTG**		**D18S51**	**BTG**		**D21S2055**	**BTG**	
12.1	117	12, 13	8	241	7	16.1	351	
13.1	121		9	245	9.2	17.1	355	
14.1	125	14.3	10	249		18.1	359	
15	128		10.2	251		19.1	363	
16	132		11	253	11.2	20.1	367	
17	136		12	257	12.2	21.1	371	
18	140		13	261	13.2	22.1	375	22
19	144		14	264	14.2	23	378	23.1
20	148		15	268		24	382	
21	151		16	272	16.2	25	386	
22	155		17	276		26	390	
23	159		17.2	278	17.3	27	395	
24	163		18	279		28	399	
25	167		18.2	281		29	403	
26	171		19	283	19.2	30	406	
27	175		20	287		31	411	
			21	291		32	415	
D5S2500	**BTG**		21.2	293		33	419	
9	188		22	295		34	423	
10	192		23	299	23.1	35	427	
11	196		24	302		36	431	
12	200		25	306		37	435	38
13	204		26	310		39	443	
14	208		27	314				
15	212		28	318	29			
16	216							
17	220							
18	224							

Table 12. Fragment lengths of the Mentype® **Chimera**® allelic ladder analyzed on an ABI PRISM® 3130 Genetic Analyzer with POP-4® polymer (yellow panel)

Marker/ allele	Size [bp]*	Further alleles**	Marker/ allele	Size [bp]*	Further alleles**	Marker/ allele	Size [bp]*	Further alleles**
D10S2325	**BTY**		**SE33**	**BTY**		**SE33**	**BTY**	
6	121		6.3	205	4.2, 5.3	25.2	278	
7	126		7.3	209	7	26.2	282	26
8	131		8	210	8.2	**27.2‡**	**285**	27
9	136		9	214	9.2	28.2	289	28, 28.3
10	141		10	218		29.2	293	29
11	145		10.2	220		30.2	297	30
12	150		11	222	11.2	31.2	301	31
13	155		12	226	12.2	32	303	
14	160		13	230		32.2	305	
15	165		13.2	232	13.3	33	307	
16	170		14	234	14.2, 14.3	33.2	309	
17	175	18	15	238		34	311	
19	185		15.2	240		34.2	313	
			16‡	**241**	16.2, 16.3	35	315	
			17	245	17.2, 17.3	35.2	317	
			18	249		36	318	
			18.2	251	18.3	36.2	321	
			19	253		37	322	37.2
			19.2	255		**38**	**326**	**39,42**
			20	257	20.1	49	369	50
			20.2	259				
			21	261				
			21.2	263	22			
			22.2	267				
			23.2	270	23			
			24.2	274	24			
			25	276				

* rounded to integer

** The “off-ladder” alleles of Biotype’s DNA pool are allocated with the actual Biotype® template files for GeneMapper® ID or Genotyper software. For further alleles see amongst others http://www.cstl.nist.gov/biotech/strbase/str_fact.htm

‡ For better orientation, these alleles are heightened within the allelic ladder.

5. Interpretation of results

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

An automated calculation of the donor/recipient DNA ratio, as well as standard deviations and detection limits can be obtained directly from the raw data of a fragment size analysis.

If results that are obtained with Mentype® **Chimera**® should be harmonized with results from cytological analyses, make sure that cytological analyses were performed with at least 500 leucocytes.

Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range, or if an incorrect matrix was applied. They appear at positions of specific peaks in other color channels, typically with lower signal intensities.

Stutter peaks

The occurrence of stutter peaks depends on the sequence of the repeat structure and the number of alleles. n-4 peaks are caused by a loss of a repeat unit during amplification of tetranucleotide STR motives, caused by slippage effects of the Taq DNA polymerase. Interpretation of those peaks should be done in accordance with the template files of the Genotyper and GeneMapper® ID/ID-X software.

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

The Mentype® **Chimera**® kit was validated and certified for the analysis on POP-4® polymer. The use of other polymers (e.g. POP-7™ or POP-6™) might influence the run behavior of specific PCR products. Furthermore, background noise might increase through the different behavior of free fluorescent dyes.

6. Population-genetic data

Most important population-genetic data of the STR markers are listed in table 13-16. The formula to calculate **Polymorphism Information Content** (PIC) was published by Botstein *et al.* (1980), **Expected Heterozygosity** (HET) by Nei and Roychoudhury et al. (1974), and, **Power of Discrimination** (PD) refers to Jones et al. (1972). All formulas are suitable for autosomal markers.

$$\mathrm{PIC} = 1 - \sum_{i=1}^{n} f_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} f_i^2 f_j^2$$

$$\mathrm{HET} = \frac{n}{n-1}\left(1 - \sum_{j=1}^{K} f^2\right)$$

$$\mathrm{PD} = 1 - \boldsymbol{\Sigma_i}\, \mathbf{f_i^2}$$

Table 13. Population-genetic data

Marker D2S1360		Marker D3S1744		Marker D4S2366	
Allele	**Allele frequency**	**Allele**	**Allele frequency**	**Allele**	**Allele frequency**
19	0.007	13	0.007	9	0.347
20	0.126	14	0.104	10	0.179
21	0.060	15	0.053	11	0.074
22	0.309	16	0.100	12	0.147
23	0.142	17	0.319	13	0.168
24	0.098	18	0.197	14	0.074
25	0.086	19	0.130	15	0.011
26	0.093	20	0.067		
27	0.035	21	0.023		
28	0.023				
29	0.012	PIC	0.790	PIC	0.760
30	0.002	PD	0.943	PD	0.919
31	0.005	HET	0.792	HET	0.795
32	0.002				
PIC	0.820				
PD	0.955				
HET	0.856				

Table 14. Population-genetic data

Marker D5S2500		Marker D6S474		Marker D7S1517	
Allele	Allele frequency	Allele	Allele frequency	Allele	Allele frequency
9	0.007	13	0.246	16	0.007
10	0.084	14	0.212	17	0.007
11	0.313	15	0.154	18	0.049
12	0.161	16	0.285	19	0.120
13	0.061	17	0.097	20	0.101
14	0.042	18	0.005	21	0.099
15	0.213			22	0.082
16	0.103	PIC	0.740	23	0.077
17	0.009	PD	0.918	24	0.155
18	0.007	HET	0.733	25	0.230
				26	0.054
PIC	0.780			27	0.014
PD	0.938			28	0.005
HET	0.804				
				PIC	0.860
				PD	0.967
				HET	0.826

Table 15. Population-genetic data

Marker D8S1132		Marker D10S2325		Marker D12S391	
Allele	Allele frequency	Allele	Allele frequency	Allele	Allele frequency
16	0.007	6	0.002	15	0.035
17	0.095	7	0.102	16	0.019
18	0.221	8	0.056	17	0.107
19	0.153	9	0.121	17.3	0.019
20	0.128	10	0.142	18	0.215
21	0.119	11	0.144	18.3	0.007
22	0.133	12	0.193	19	0.121
23	0.077	13	0.133	19.3	0.016
24	0.056	14	0.065	20	0.117
25	0.005	15	0.037	21	0.093
26	0.005	16	0.005	22	0.114
27	0.002			23	0.072
		PIC	0.860	24	0.040
PIC	0.850	PD	0.967	25	0.021
PD	0.964	HET	0.851	26	0.002
HET	0.828				
				PIC	0.870
				PD	0.971
				HET	0.893

Table 16. Population-genetic data

Marker D18S51		Marker D21S2055		Marker SE33 (ACTBP2)	
Allele	**Allele frequency**	**Allele**	**Allele frequency**	**Allele**	**Allele frequency**
10	0.005	16.1	0.056	11	0.002
12	0.103	17.1	0.021	12	0.014
13	0.110	18.1	0.023	13	0.002
14	0.157	19.1	0.274	13.2	0.002
15	0.199	20.1	0.040	14	0.026
16	0.161	21.1	0.019	15	0.049
17	0.112	22.1	0.005	16	0.047
18	0.072	23	0.007	17	0.070
19	0.028	25	0.112	17.3	0.002
20	0.030	26	0.116	18	0.044
21	0.021	27	0.016	18.3	0.002
24	0.002	28	0.007	19	0.082
		29	0.030	19.2	0.009
PIC	0.850	30	0.021	20	0.044
PD	0.964	31	0.023	20.2	0.009
HET	0.902	32	0.026	21	0.035
		33	0.067	21.2	0.019
		34	0.074	22	0.007
		35	0.053	22.2	0.035
		36	0.007	23.2	0.023
		37	0.002	24	0.002
				24.2	0.035
		PIC	0.870	25.2	0.044
		PD	0.971	26.2	0.040
		HET	0.856	27.2	0.084
				28.2	0.084
				29.2	0.051
				30	0.002
				30.2	0.061
				31.2	0.028
				32.2	0.023
				33	0.009
				33.2	0.005
				34	0.002
				36	0.002
				PIC	0.950
				PD	0.990
				HET	0.949

All population-genetic data is based on an analysis of ca. 210 unlinked Europeans performed by Biotype GmbH.

7. References

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Hering S, Müller E (2001) New allele and mutational events in D12S391 and D8S1132: sequence data from an eastern German population. *Forensic Sci Int 124:187-191.*

Jones DA (1972) Blood samples: Probability of Discrimination. *J Forensic Sci Soc 12:355-359.*

Nei M, Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. *Genetics 76:379–390.*

Szibor R, Edelmann J, Hering S, Plate I, Wittig H, Roewer L, Wiegand P, Calì F, Romano V, Michael M (2003) Cell line DNA typing in forensic genetics – the necessity of reliable standards. *Forensic Sci Int 138: 37-43.*

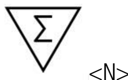
Wiegand P, Lareu M. V., Schürenkamp M (1999) D18S535, D1S1656 and D10S2325: three efficient short tandem repeats for forensic genetics. *Int J Legal Med 112:360-363.*

Wiegand P, Klintschar M (2002) Population genetic data, comparison of the repeat structure and mutation events of two short STRs. *Int J Legal Med 116:258-261.*

8. Explanation of Symbols

Manufacturer

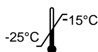
Batch code



Contains sufficient reagents for <N> tests

Reference to eIFU

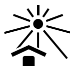
Expiry date



Storage temperature limitation

Catalogue number

In-Vitro-Diagnostics



Protect from light

Keep dry

Specification: Mentype® Chimera® PCR amplification kit

A Analytical validation

A a) Determination of the standard reaction and batch-specific tolerance

Objective: Determination of the standard reaction and batch-specific tolerances of the multiplex PCR and the baseline in the sense of relative signal heights (RFU) and balance of signal heights.

Methodology: The test kit contains control DNA, which is heterozygous in most STR systems. The standard reaction was carried out in quadruple determinations with the control DNA in the nominal concentration of 500 pg. Four blank values (no template control, NTC) without DNA were additionally applied.

Results: For batch-specific mixing of PCR primers, the following specifications were established: Using an ABI PRISM® 310 Genetic Analyzer signal heights of 1 000-4 000 RFU and using an ABI PRISM® 3130 Genetic Analyzer, signal heights of 1 000-5 000 RFU. The fluctuations for signal heights of heterozygous systems shall not exceed 30 % of the guideline value. In the scaling range, no unspecific signals ≥ 50 RFU were observed (baseline) for the blank values.

A b) Genotyping accuracy

Objective: The accuracy of allele allocation is statistically proven under standard conditions. The test checks the automatic allele calling with the allelic ladder and the concordance of the allele assignment compared to the pre-typing of the test DNAs by means of other methods (other PCR kits, direct sequencing and the like) using the GeneMapper ID software. Based on the results, the test-specific device settings for genotyping by means of capillary gel electrophoresis (bins and panels) and the proportion of stutter peaks for the analysis templates of the DNA sequencer are defined.

Methods: 80 pre-typed human DNAs from different sources (whole blood, cheek smears) were tested in single determinations. In addition, a blank (no DNA) was carried. Acceptance criteria were defined as full profiles with peak heights ≥ 50 RFU (manual evaluation).

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

A c) Analytical specificity

Objective: The purpose of the study is to exclude false-positive results as a consequence of 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) was tested. This animal DNA derived from blood samples, which were provided as residual material of veterinary studies.

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

A d) Analytical sensitivity

Objective: Determination of the analytical detection limit (sensitivity).

Method: A dilution series with 500 pg to 31.5 pg of reference DNA was tested in quadruplicates. As an acceptance criterion, complete DNA profiles with ≥ 200 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 thermocyclers from different manufacturers differ in their specifications. In particular, different heating and cooling rates as well as different temperature control techniques can be observed.

Method: Testing of the standard reactions with control DNA in the nominal concentration of 500 pg was performed with the following thermocyclers in 4-fold determinations with the same master mix and 2 blank samples (no DNA): Thermocycler *GeneAmp 9700* with Alu-block (Life Technologies, Division Applied Biosystems Deutschland GmbH, Darmstadt), *GeneAmp 9700* with Silver-block (Applied Biosystems Deutschland GmbH, Darmstadt), DNA Engine (PTC-200) Peltier Thermal Cycler (Bio-Rad Laboratories GmbH, München), *Biometra T1* (Biometra GmbH, Göttingen), *Techne® TC-512 Thermal Cycler* (biostep GmbH, Jahnsdorf) and *Eppendorf Mastercycler ep-S* (Eppendorf AG, Hamburg).

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

A f) Mixed DNA samples

Objective: The aim of the chimerism analysis after allogeneic blood stem cell transplantation is the detection and the relative quantification of the donor and recipient DNA fractions. In order to detect the minimal residual disease, the smallest possible amounts of donor or recipient DNA are to be detected in a mixed sample.

Method: Three independent mixtures of two DNAs were prepared using the deficient DNA as 0 %, 1 %, 2 %, 3 %, 5 %, 10 %, 30 % and 50 %. DNAs in the mixtures

showed at least three STR loci with four informative alleles. In each case, 1 ng of the DNA mixtures in four parallel mixtures was tested in the standard reaction manner. Signal heights of at least 50 RFU were evaluated.

Results: Results are shown in Figure 4. For the deficient DNA, a detection limit of 1 % could be achieved. This corresponds to the values 1-5 %, which are achieved with conventional forensic STR kits when used in chimerism analyses.

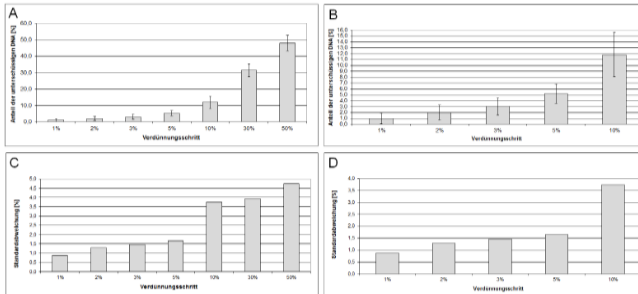


Fig. 4: Test of DNA mixtures. (A, B) Mean values and standard deviation of the fractions of the deficient DNA calculated from the signal heights of capillary electrophoresis (C, D) standard deviation to A and B

A g) PCR annealing temperatures

Objective: To determine the robustness of the PCR, temperature fluctuations are simulated for the primer annealing within the multiplex PCR. 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 500 pg. A triple determination with the same master mix was performed.

Results: No unspecific by-products ≥ 200 RFU were detected for ± 1 °C. The average peak heights deviated from the standard reaction at ± 1 °C to a maximum of ± 30 %. No allelic signal failure < 200 RFU was detected for ± 2 °C.

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 determined against batch fluctuations of the supplied PCR buffer.

Methods: Three independent Reaction mix A batches were tested for performance in the standard reaction with control DNA of the nominal concentration of 500 pg.

Results: No unspecific by-products ≥ 50 RFU were detected. The deviation of averaged peak heights compared to the standard reaction was a maximum of 20 %.

A i) PCR inhibitors

Objective: Hematin from hemoglobin is a potent inhibitor of Taq DNA polymerase if not completely removed during the DNA purification from stabilized whole blood.

Method: The effect of *hematin porcine* (Sigma-Aldrich, Freiburg) was tested in a final concentration of 0-250 µM in a standard reaction with control DNA of the nominal concentration of 500 pg.

Results: Complete profiles (≥ 50 RFU) were achieved up to an inhibitor end concentration of 100 µM *hematin porcine*. For a final concentration of 150 µM, complete profiles could no longer be achieved (partial profiles only).

A j) 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 500 pg and additional blank values without DNA was performed in triplicate determinations. The evaluation was carried out in comparison to a standard reaction that was performed without going through the freezing and thawing cycle.

Results: The deviation of averaged peak heights compared to the standard reaction was a maximum of 20 % (in particular signal loss). No additional peaks > 50 RFU were found within the scaling range for the blank values.

B Clinical performance data

B a) Study design, ethics and regulatory aspects

A clinical performance study was conducted according to §§ 20-24 of the 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 cytogenetic differentiation of donor and recipient leukocytes by means of fluorescence in situ hybridization (FISH) served as a test for comparison. The sex chromosome-specific CE-IVD CEP® X SpectrumOrange™ / Y SpectrumGreen™ Direct Labeled Fluorescent DNA Probe Kit (Abbott GmbH & Co KG, Wiesbaden) was used according to the manufacturer's instructions.

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), according to the manufacturer.

B d) Results

A total of 103 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. At least 1.5 ng genomic DNA was used per PCR. Firstly, all informative STR 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 were used (at least 2). The results of the concordance analysis are summarized in Fig.5.

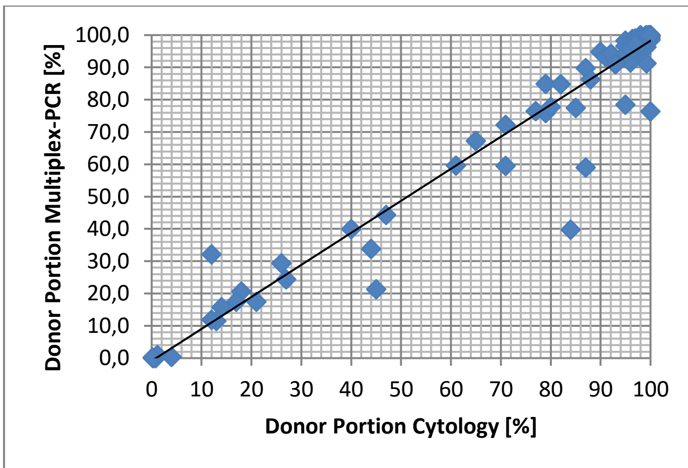


Fig. 5: Concordance analysis of multiplex PCR in comparison to cytology.

In 92 of the 103 data sets (90.3 %) the deviation of the results from multiplex PCR to cytogenetics was less than 5 %. Larger deviations were observed only in cytogenetic findings, in which the total number of cell counts was 500 or less. According to the recommendations of the manufacturer of the FISH-kit, at least 200 cells should be counted. According to practice recommendations, however, higher absolute cell numbers (500-1 000) yield better cytogenetic results [1, 2].

B e) References

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

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