Validation table 1. List of Cell lines used

Cell line	Provider	RRID
MDA-MB-231	Institut Hiscia, Arlesheim, Switzerland	CVCL_0062
MCF-7	Institut Hiscia, Arlesheim, Switzerland	CVCL_0031
MRC5	RD Biotech, BESANCON, FRANCE	CVCL_0440
HMEC	Life Technologies,Carlsbad, CA, USA	Cat # A10565

Validation table 2. List of antibodies used

Target	RRID
EZH2	AB_2561022
SUZ12	AB_2614929
Myc	AB_310165
pp65	AB_631358
HCMV late Ag	Cat #11-005, BioMérieux, Craponne, France
Oct4	AB_445175
SSEA-4	AB_778073
Nanog	AB_446437
Tra-1-60	AB_778563
SOX2	AB_2341193
Ki67 Ag	AB_396302
Akt	AB_1118808
pAkt	AB_560378
STAT3	AB_628293
pSTAT3	AB_397015
EpCAM	AB_400261
CD49f	AB_396078
vimentin	AB_628437
E-cadherin	AB_2076666
CD24	AB_395822
CD44	AB_395870
Anti-mice FITC	AB_394862
Anti-rabbit FITC	AB_955238
Mice IgG PE	AB_10050483
H3K27me3	AB_2616029
H3K4me3	AB_306649
Total anti-H3	AB_302613

Validation table 3. List of Viral strains used

Strain name	Species	Host species	Initial description	Genebank reference
DB	Cytomegalovirus	Human	DOI: 10.4049/jimmunol.0803800	KT959235
BL	Cytomegalovirus	Human	DOI: 10.1038/s41388-021-01715-7	MW980585
B544	Cytomegalovirus	Human	DOI: 10.3389/fimmu.2021.772160	NA
B693	Cytomegalovirus	Human	DOI: 10.3389/fimmu.2021.772160	NA
TB40E	Cytomegalovirus	Human	DOI : 10.1099/0022-1317-80-11-2867	KF297339

Results:

DNA-System	DNA-criteria HuMEC-2013 CL181107_001
D3S1358	17, 17
vWA	15, 17
D16S539	12, 13
CSF1PO	11, 13
TPOX	8, 9
AM	X, X
D8S1179	13, 14
D21S11	28, 31
D18S51	14, 15
D2S441	13, 14
D19S433	13, 15
TH01	9, 9.3
FGA	22, 23
D22S1045	15, 15
D5S818	8, 10
D13S317	9, 12
D7S820	9, 11
SE33	18, 21
D10S1248	12, 15
D1S1656	13, 14
D12S391	21, 23
D2S1338	19, 25

Summary:
The following table shows the result of the comparison with the online database of the DSMZ (http://www.dsmz.de/de/service/services-human-and-animal-cell) and the Cellosaurus database (https://web.expasy.org/cellosaurus/):

Our sample number	Client sample name	Database name
CL181107_001	HuMEC-2013	Profile not found in databases

The samples HuMEC-2017, CTH-P50 and HuMEC-SP did not contain sufficient human DNA for a STR profile.

Dr. Burkhard Rolf
Director Forensic Services

Dr. Michaela Bosch
Project Manager DNA-Forensics

Cell_line-certificate_eng_V05_21032017

Vorlage_Verwandtschaftsanalyse_EUROFINS_v02_121127

Results:

DNA-System	DNA-criteria MDA-MB-231 CL181018_003	DNA-criteria MCF-7 CL181018_004
AM	X, X	X, X
D3S1358	16, 16	16, 16
D1S1656	15, 17	11, 15.3
D6S1043	18, 18	12, 18
D13S317	13, 13	11, 11
Penta E	11, 11	7, 12
D16S539	12, 12	11, 12
D18S51	11, 16	14, 14
D2S1338	20, 21	21, 23
CSF1PO	12, 13	10, 10
Penta D	11, 14	12, 12
TH01	7, 9.3	6, 6
vWA	15, 18	14, 15
D21S11	30, 33.2	30, 30
D7S820	8, 9	8, 9
D5S818	12, 12	11, 12
TPOX	8, 9	9, 12
D8S1179	13, 13	10, 14
D12S391	17, 18	18, 20
D19S433	11, 14	13, 14
FGA	22, 23	23, 25

Summary:
The following table shows the result of the comparison with the online database of the DSMZ (http://www.dsmz.de/de/service/services-human-and-animal-cell):

Our sample number	Client sample name	DSMZ name
CL181018_003	MDA-MB-231	MDA-MB-231
CL181018_004	MCF-7	MCF7

Dr. Burkhard Rolf
Director Forensic Services

Dr. Michaela Bosch
Project Manager DNA-Forensics

Analysis Report for Cell Line Typing

1. Sponsor

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2. Analysis Report

Report ID: 00999_005727
Report Version: 01
Issue Date: 24.06.2020
Report Approved: Joy Beyer

3. Descriptions

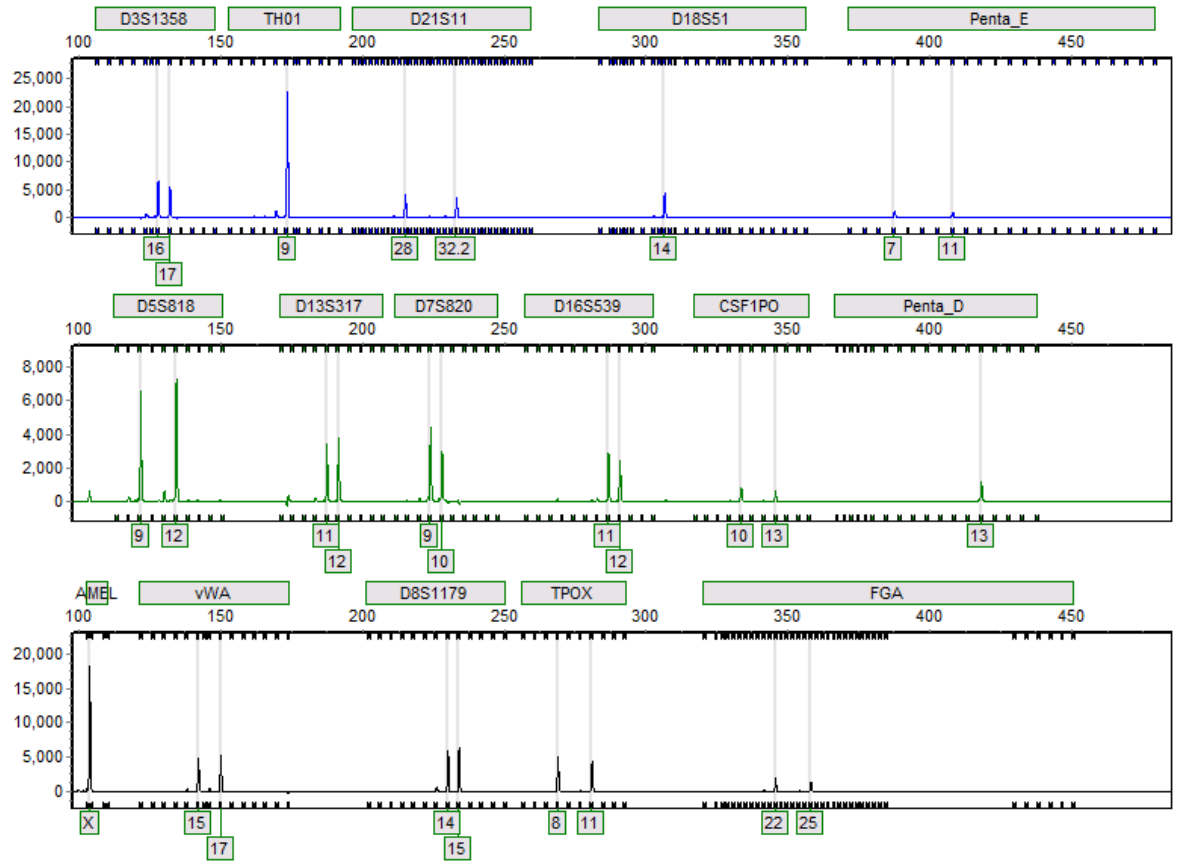
Customer Test Item ID: CTH-DB-2020-2
Analysis Method: Profiling of the human cell lines was done using highly polymorphic short tandem repeat loci (STRs). STR loci were amplified using the PowerPlex® 16 HS System (Promega). Fragment analysis was done on an ABI3730xl (Life Technologies) and the resulting data were analyzed with GeneMarker HID software (Softgenetics).

4. Analysis Results

4.1. Summary Table of the STR Profile

Locus	*Chromosomal Location*	*Core STR Marker*	*Customer Sample Typed Alleles*	*Database Alleles*	*Comments*
D3S1358	Chr03		16/17	N/A	
TH01	Chr11	Yes	9	N/A	
D21S11	Chr21		28/32.2	N/A	
D18S51	Chr18		14	N/A	
Penta_E	Chr15		7/11	N/A	
D5S818	Chr05	Yes	9/12	N/A	
D13S317	Chr13	Yes	11/12	N/A	
D7S820	Chr07	Yes	9/10	N/A	
D16S539	Chr16	Yes	11/12	N/A	
CSF1PO	Chr05	Yes	10/13	N/A	
Penta_D	Chr21		13	N/A	
AMEL	X/Y	Yes	X	N/A	
vWA	Chr12	Yes	15/17	N/A	
D8S1179	Chr08		14/15	N/A	
TPOX	Chr2	Yes	8/11	N/A	
FGA	Chr04		22/25	N/A	

4.2. Electropherogram



5. Conclusion

According to our analysis of the submitted sample there is no detectable contamination with human origin.

A search with the analyzed data of the submitted sample in the Microsynth, Cellosaurus, ATCC and DSMZ databases didn't give any useful match with a reference DNA profile.

6. Customer Comment

Samples can be pooled

7. Glossary

Short Tandem Repeats (STRs)

Short tandem repeats (STRs) consists of a DNA motif of 2-13 bases that are repeated up to several hundred times. The number of repeats in a STR is highly variable among individuals, resulting in fragment length differences if amplified using PCR. These differences in fragment lengths at different loci are used for profiling the cell lines.

Stutter Peaks

Stutter peaks are small peaks which occur immediately before or after the true peak. Stutter peaks are commonly caused by a slippage of the polymerase during the PCR amplification.

Detection of Cell Line Mixtures

Contamination of one cell line by one or several other cell lines can be detected down to a frequency of the contaminating cell line of 3%. Typically, cell line mixtures will result in STR profiles including three or more peaks for single or multiple loci. If Microsynth notices a possible contamination of a cell line, we will comment the finding in the conclusion part of the analysis.

Peak height ratio

Peak height ratio < 25 % (to the highest peak within a STR) is mentioned in the summary table (comments). Peak height ratios < 25% need not necessarily have an effect on the behaviour or characteristics of the cell line. A small peak height may be due to reduced amplification efficiency, for example resulting from a mutation in the primer site. The reason for the difference in peak heights observed, however, would need some in depth analysis of the test item.

8. General Comment

The results refer only to the portion of the sample Microsynth has analyzed. The analysis results might not be assigned unconditionally to the whole sample. Microsynth shall not in any event be liable for incidental, consequential or special damages in relation to carried out analyses and corresponding results.

9. Compliance and Quality Assurance Statement

All aspects of this study were in accordance with ISO 9001:2015 standards. All the applied equipment is qualified and calibrated. The applied methods are validated.