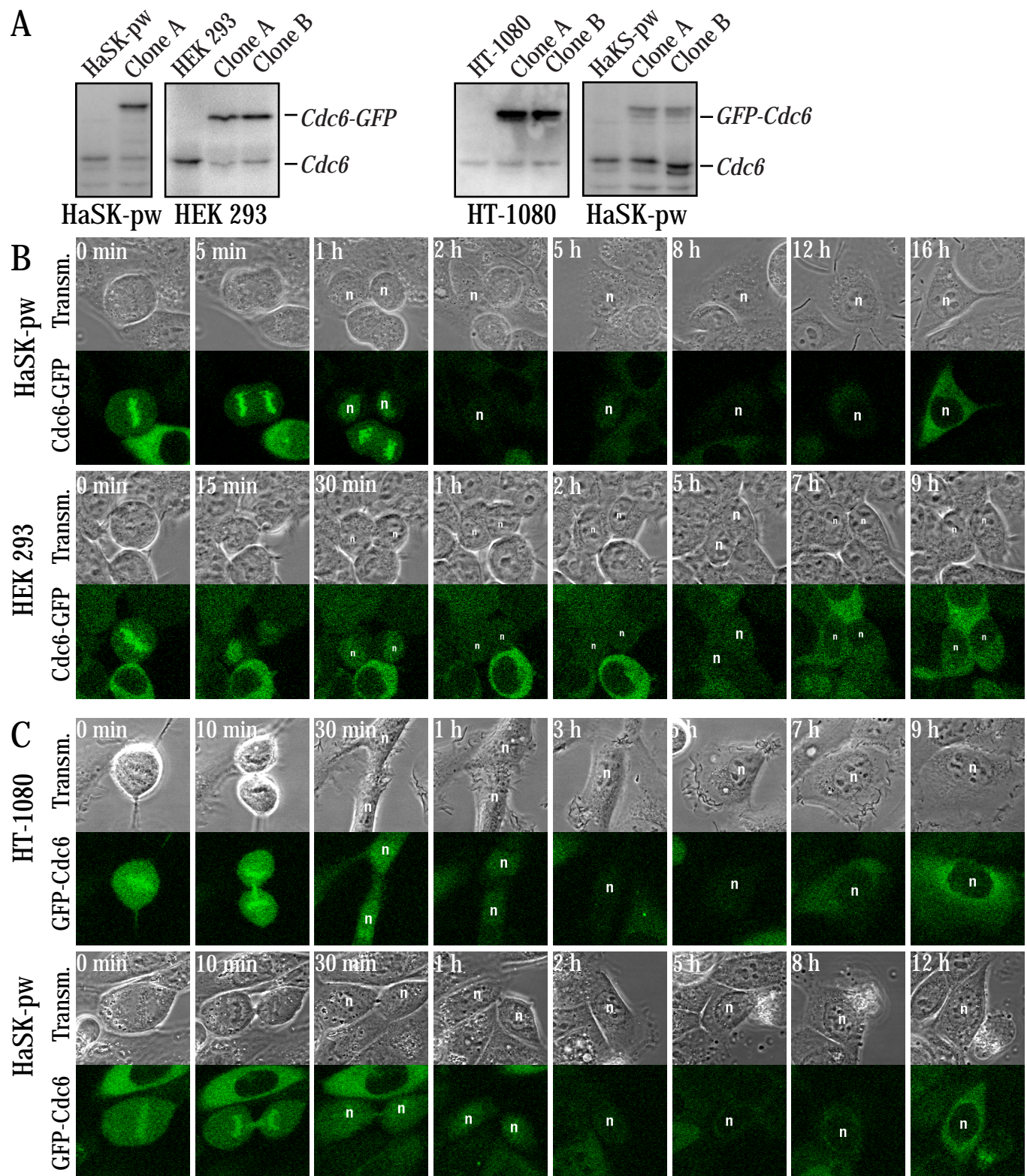
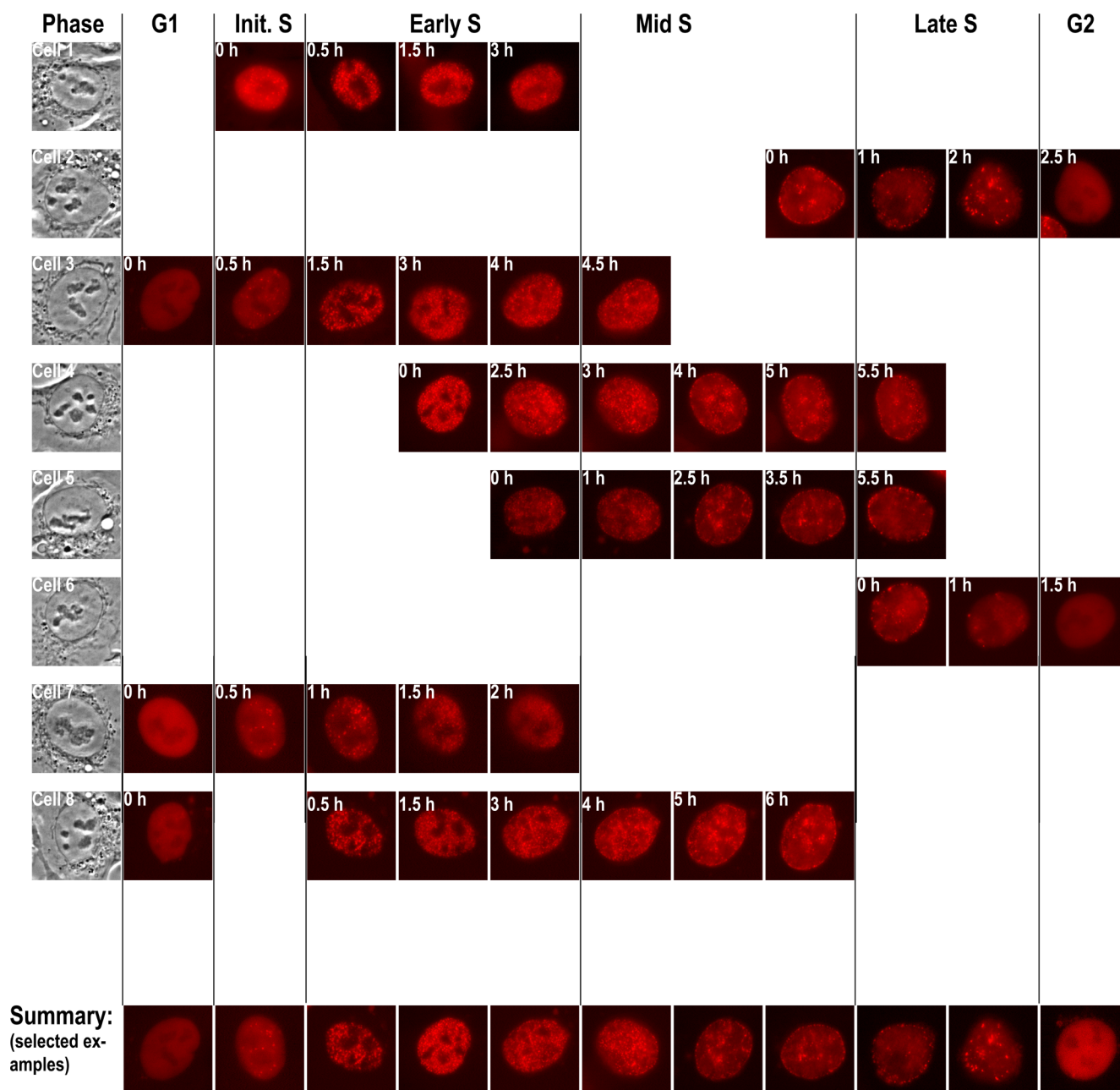


Supplemental figure S1



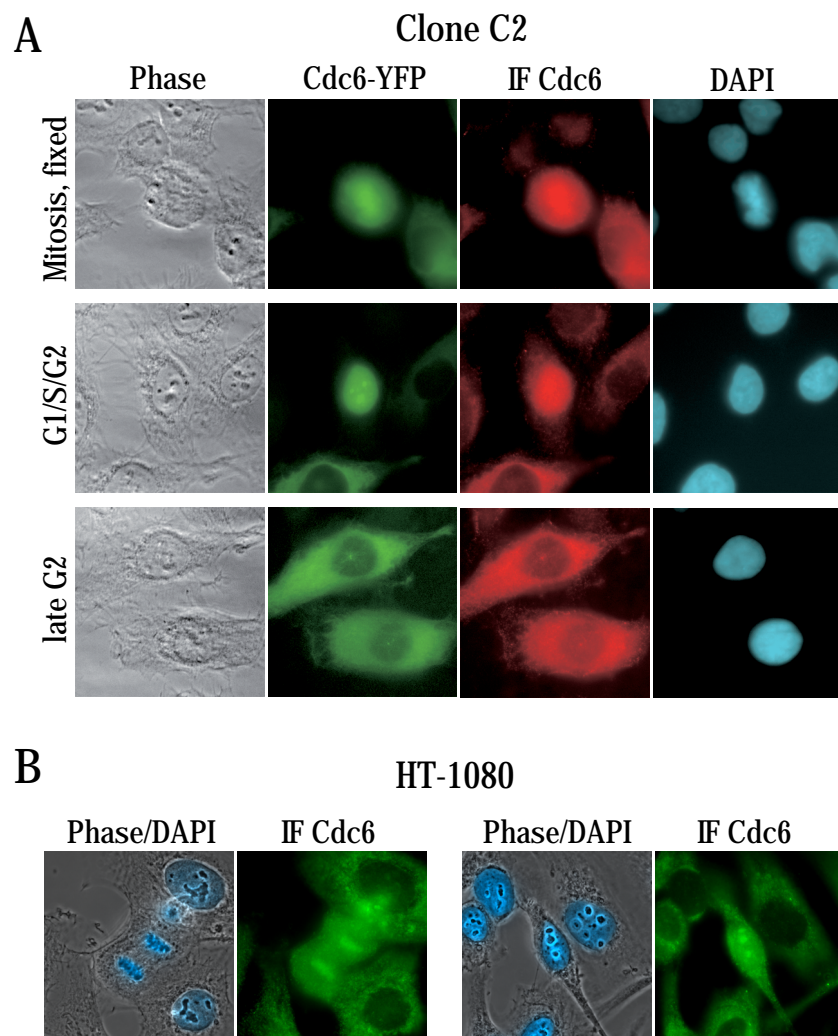
Similar time-dependent regulation of GFP-fused Cdc6 in different cell lines and constructs. (A) Western blots showing the expression level of C- (left) and N-terminal (right) GFP-fusions to Cdc6 of single clones selected from the indicated cell lines. (B, C) Representative confocal images were selected from a series of consecutive images taken every 5 - 30 min. In each case, the respective clone A is shown. C-terminal fusions to Cdc6 are shown in (B), N-terminal fusions in (C).

Supplemental figure S2



Typical changes in the distribution pattern of CFP-PCNA in the course of interphase. HT-1080 cells expressing CFP-PCNA (pseudocoloured in red) were cultivated under an epifluorescent microscope for several hours. A phase contrast image was taken in the beginning (*left*), and fluorescent images were then recorded every half hour. Eight examples of individual cells are shown. The lower panel represents a synopsis from these cells revealing typical distribution patterns. Note that these patterns match the distribution published in e.g. Refs 29 and 30.

Supplemental figure S3

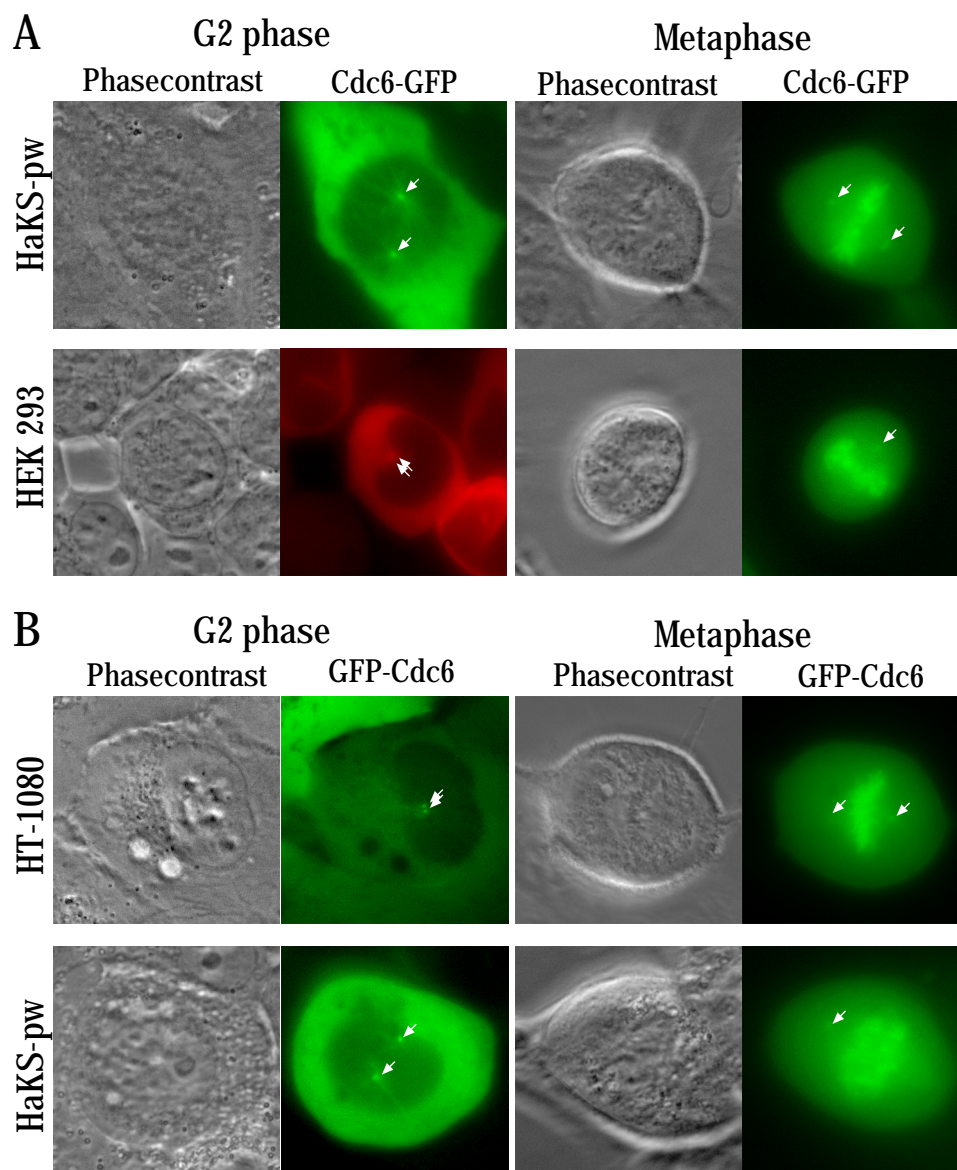


Immunofluorescent staining of endogenous and YFP-labelled Cdc6.

(A) Clone C2, expressing low levels of Cdc6-YFP was fixed first in Methanol and then Acetone at -20 °C, blocked, and incubated with an antibody to Cdc6 followed by a secondary, Cy3-labelled antibody. As opposed to alternative methods like PFA-fixation (not shown), this approach conserved to a large extent characteristic features of Cdc6-YFP distribution in living cells: Cdc6-YFP localized at least partly to mitotic chromosomes (upper panel), in interphase cells the protein was either localized in the nucleus, absent, or resided to a varying degree in the cytoplasm (middle panel). From our observations in living cells, this distribution suggests that these cells were in G1 phase, early S phase or late S/G2 phase. The lower panel shows a strong Cdc6-YFP-signal exclusively in the cytoplasm typical for G2 cells. The immunostaining with a monoclonal anti-Cdc6 antibody (IF Cdc6) reveals that Cdc6-YFP and potentially the endogenous protein, too, are readily recognized and detected by the antibody in these different cellular compartments.

(B) The same immunostaining protocol was applied to untransfected HT-1080 cells using the same primary monoclonal anti-Cdc6 antibody and a Cy2-labelled secondary antibody. The images shown are representative examples for the detection of endogenous Cdc6 on mitotic chromosomes (anaphase cell in the left image set), inside the nucleus with a weak concentration of Cdc6 in nucleoli (right), and in the cytoplasm of other cells.

Supplemental figure S4



Binding of GFP-fused Cdc6 to centrosomes in different cell lines and constructs at G2- and metaphase. (A) Lightmicroscopic phasecontrast and green fluorescent images of representative HaKS-pw and HEK293 cells, respectively, expressing Cdc6 fused at its C-terminus to GFP. Centrosomes are marked with an arrow. (B) The same set of images as in (A) with HT-1080 and HaKS-pw cells expressing the N-terminal fusion of Cdc6 to GFP.

Supplemental material S5: Certificate and STR profiles (next page)

Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH



Leibniz-Institut DSMZ GmbH · Inhoffenstraße 7 B · 38124 Braunschweig

Prof. Petra Boukamp,
Deutsches Krebsforschungszentrum DKFZ
Abteilung A110
Genetik der Hautkarzinogenese
Im Neuenheimer Feld 280
69120 Heidelberg

Inhoffenstraße 7 B
38124 Braunschweig
GERMANY

Tel. +49(0)531 26 16-166
Fax: +49(0)531 26 16-150
E-Mail: wdi@dsmz.de
Internet: www.dsmz.de

Ihr Zeichen/Your ref.

Unser Zeichen/Our ref.wdi

+49(0)531-2616-166

Datum/Date 07.07.2014

Dear Prof. Boukamp,

Thank you very much for your order with regard to DNA fingerprinting of human cell lines. We have carried out DNA profiling using 8 different and highly polymorphic short tandem repeat (STR) loci. Furthermore, we have tested your samples for presence of mitochondrial DNA sequences from rodent cells as mouse, rat, chinese and syrian hamster. Results (table 1):

#	sample	parental/reference	comment/match
1	HaSK-pw (P1, 17.02.00)	unknown	no match in STR reference database, unique
2	HaSK-pw (P7, 21.01.03)	unknown	full-matching STR profile of HaSK-pw (P1), otherwise unique
3	HaSK-pw (P30, 20.04.04)	unknown	full-matching STR profile of HaSK-pw (P1), otherwise unique

At a detection limit of $1:10^5$ we could not detect any mitochondrial sequences from mouse, rat or chinese and syrian hamster cells in your samples. The samples are derived of pure human cell cultures.

Generated STR profile of your samples of HaSK-pw is not matching any other STR profile as indicated by a search of the reference STR database from cell banks ATCC (USA), HPACC (UK), JCRB (Japan), RIKEN (Japan), KCLB (Korea) and DSMZ. The sample has been taken from an unique cell line.

Your samples of HaSK-pw cell line reveal an unique STR profile not present in the STR database. To our knowledge the cell line HaSK-pw is not deposited in one of the major cell banks and no reference STR profile is available.

The exclusion rate of the applied STR system is indicating authenticity/uniqueness with a matching probability of the system ranging from 1 in 114,000,000 for Caucasians and Americans.

Please find enclosed the documentation (electropherograms) and the allelic list.

Best regards,
W. Dirks

Geschäftsführer/
Managing Director:
Prof. Dr. Jörg Overmann
Aufsichtsratsvorsitzender/Head of
Supervisory Board: MR Dr. Axel Kollatschny

Braunschweigische Landessparkasse
Kto.-Nr./Account: 2 039 220
BLZ/Bank Code: 250 500 00
IBAN DE22 2505 0000 0002 0392 20
SWIFT (BIC) NOLADE 2 H

Handelsregister/
Commercial Register:
Amtsgericht Braunschweig
HRB 2570
Steuer-Nr. 13/200/24030



	Cell line	Date	D5	D5'	D13	D13'	D7	D7'	D16	D16'	vWA	vWA'	TH01	TH01'	TPOX	TPOX'	CSF1	CSF1'	Amel	Amel'	Date Animal-PCR:	M	R	CH	SH
Boukamp	HaSK-pw p.1 (17.2.00)	03.07.14	11	11	11	11	10	12	12	13	14	17	6	7	8	11	10	11	X	X	03.07.14	-	-	-	-
Boukamp	HaSK-pw p.30 (20.04.04)	03.07.14	11	11	11	11	10	12	12	13	14	17	6	7	8	11	10	11	X	X	03.07.14	-	-	-	-
Boukamp	HaSK-pw p.7 (21.01.03)	03.07.14	11	11	11	11	10	12	12	13	14	17	6	7	8	11	10	11	X	X	03.07.14	-	-	-	-

STR-profile of reference

STR-Profil recently sample

STR-profile of analysed sample

