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Use of milk samples from a milk evaluation program for the genotyping of cows

Abstract

Milk can be an attractive DNA-resource for genotyping milking cows, *e.g.* for paternity control or QTL analysis within a daughter design. The use of milk collected within the established milk evaluation programs enables the collection of large numbers of samples. Nevertheless, there are limitations when using the remedies of tested milk samples, *e.g.* permutations of samples or partially degraded DNA. A DNA preparation method suitable for samples from routine milk recording has been developed that combines an initial centrifugation step with direct lysis of cells and purification from comparatively high volumes by using silica membrane spin columns. The method yields high quality genomic DNA from fresh samples and PCR grade DNA from remedies of tested samples. In addition the potential use of milk samples within a daughter design was evaluated. We collected reference samples from 119 Simmental dairy cows from 6 half sib families. From 89 of these dairy cows remedies of milk samples were obtained from the routine milk laboratory. Paternity could be established by microsatellite analyses for all 119 reference cases. From the 89 milk laboratory samples 86 were successfully microsatellite typed. In 81 cases the genotypes from milk and reference sample were identical. In summary it could be shown that it is possible to genotype dairy cows from test laboratory milk samples, but results have to be used carefully taking into account inherent limitations. The use of milk as compared with tissue samples as a source for DNA within daughter designs is discussed.

Key Words: DNA preparation, milk samples, lactocorder, paternity control, Simmental breed

Zusammenfassung

Titel der Arbeit: **Eignung von Proben aus der Milchleistungsprüfung zur Genotypisierung von Milchrindern**

Milchproben sind eine interessante Alternative zur DNA-Gewinnung für die Genotypisierung von Milchrindern z. B. für Abstammungskontrollen oder QTL-Analysen. Werden Milchproben aus bestehenden Programmen zur Milchprüfung verwendet, können darüber große Probenzahlen einfach gewonnen werden. Mögliche Nachteile dieser Proben sind Probenverwechslungen oder partiell degradierte DNA. Es wurde eine Methode entwickelt, welche die Isolierung genomischer DNA aus Proben ermöglicht, die über den Milchprüfring am Zentrallabor in Wolnzach untersucht worden waren. Dabei wird nach Zentrifugation der Proben eine direkte Zelllyse durchgeführt und die DNA aus relativ großem Volumen über Silka-Säulen aufgereinigt. Die gewonnene DNA ist bei frischen Proben von hoher Qualität und bei älteren Proben für PCR-Anwendungen ausreichend. Desweiteren wurde die mögliche Anwendung von Milchproben innerhalb eines Töchter-Designs untersucht. Dazu wurden 119 Referenz-Proben von Fleckvieh-Kühen aus 6 Halbgeschwisterfamilien gesammelt. Von 89 dieser Kühe konnten Milchprobenreste aus der Milchprüfung gesammelt werden. Die Abstammung wurde für keine der 119 Referenztiere bestritten. 86 von 89 Milchproben konnten genotypisiert werden. Dabei waren 81 Genotypen mit dem Genotyp der entsprechenden Referenzprobe identisch. Zusammenfassend konnte gezeigt werden, dass die Verwendung von Milchproben aus den Milchprüflaboren möglich ist. Allerdings müssen methodisch bedingte Einschränkungen bei der Auswertung der Typisierungsergebnisse berücksichtigt werden. Die Verwendung von Milchproben im Vergleich zu Gewebeproben in Tocher-Designs wird diskutiert.

Schlüsselwörter: DNA Isolierung, Milchproben, Laktocorder, Abstammungskontrolle, Fleckvieh

Introduction

DNA for routine identity and paternity control or genetic testing in cattle is isolated from different tissue sources such as traditional blood samples, a drop of blood blotted

on a paper that is dried, ear tag systems that deposit a tissue sample in an enclosed container with bar code identification, hair follicles, or semen. In addition to routine applications, genotyping of dairy cows is of special interest within "daughter design" quantitative trait loci (QTL) detection, QTL analysis from "selected milk pools", and marker assisted selection (MAS). A daughter (or bottom-up) design enables the identification of segregating QTLs even in populations with small families. Furthermore, marker alleles favorably associated to QTL alleles can be early determined for young bulls prior to progeny testing. For this application it has been predicted that the bottom-up design is favorable to the top-down approach (MACKINNON and GEORGES, 1998). The main disadvantage is the large number of samples and typing reactions required (HEYEN et al., 1999; RON et al., 2001). Whereas typing costs are expected to decrease with new typing- and marker-technology, sampling costs are still high. To reduce sampling effort milk can be used as an alternative DNA resource. Nevertheless, there are drawbacks when using milk for DNA preparation. After collection these samples are subjected to varying, often unfavorable environmental conditions; the number of somatic cells varies in a broad range; a preservation-medium is used by the milk laboratory that is not optimized for molecular genetic applications; permutations of samples might occur at different stages prior to DNA-isolation. Therefore DNA often is of poor quality and low quantity (Amills and colleagues used for example 65 cycles to get sufficient amplification, AMILLS et al., 1997) or the sample identity is wrong. There are several studies that investigated methods for DNA isolation from milk, but most of them tested only a small number of samples that had been drawn especially for DNA preparation (AMILLS et al., 1997; DIETZ et al., 1997; ALATOSSAVA et al., 1998; WANNER et al., 1998, 1999). Only one QTL study in Israel used a larger number of milk samples within a selective pooling approach. Samples within this project were obtained under conditions well suited for subsequent DNA-preparation (only a few stations with large number of animals, milk sampled and directly shipped to the DNA lab) and effort was limited by pooling of milk samples prior to DNA preparation (LIPKIN et al., 1998). In many countries the structure of herds does not allow easy sampling of milk for DNA preparation. Instead dairy cows needed for a project are disseminated over a large number of dairy farms making it economically impossible to collect milk especially for DNA preparation. Here, remedies from milk laboratories after milk trait evaluations might be used as an alternative.
Within this study remedies of milk samples from milk content analysis from the central lab of the "Milchprüfring Bayern e. V." (ZL-MPR) were investigated as source for DNA genotyping. We developed a DNA preparation method suited for these samples and checked the identity of ZL-MPR milk samples by using tissue samples as reference.

Material and Methods

Samples

Tissue samples from 119 Simmental dairy cows were collected from February to April 2002 using TypiFix® container (Agrobiogen, Hilgertshausen, Germany). These cows were half sib progeny from six German Simmental bulls. The identification numbers of these animals were electronically transferred to the milk recording section of the "Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e. V."

(LKV). The corresponding tubes from these cows were marked and kept after routine laboratory evaluation of milk content by the central lab of the "Milchprüfring Bayern e. V." (ZL-MPR) in Wolnzach, Germany. Samples were identified and assigned by a bar code based system at the laboratory of the "Lehrstuhl für Tierzucht und Allgemeine Landwirtschaftslehre, Ludwig-Maximilians-Universität", Munich (Tz-LMU). They were frozen (-20°C) upon arrival in the laboratory. To establish and verify the DNA purification method bulk tank milk from the test station in Grub was sampled into tubes from the milk laboratory in Wolnzach. These tubes were prefilled with the routinely used preservation gel (Table 1).

Table 1
Preservation gel (Konservierungspuffer)

substance	amount
chlorampenicol	8.80 g (± 10 mg)
ethanol (96%)	~ 160 ml
NaN_3	210.0 g (± 100 mg)
citric acid	300.0 g (± 100 mg)
"Patentblau"	0.5 g
eosin	7.5 g
methyl cellulose	35.0 g
aqua bidest	ad 5.0 l
0.1 ml preservation gel at max. 40.0 ml milk	

DNA preparation
Different methods and modifications for DNA preparation were tested. Finally, a method was established that can be used for fresh or frozen samples and minimizes losses due to degraded material: 6 ml of the preserved milk were transferred to a 15-ml tube and centrifuged (2000 x *g* for 15 min). The lower 2 ml including the sediment were transferred to a fresh 15-ml tube. Lysis of cells and purification of DNA was done with NucleoSpin columns (# 740954.20, Machery&Nagel) as follows: after adding 2 ml lysis buffer and 150 µl proteinase K the suspension was vortexed for 10 sec and incubated 10 min at 70°C two times. After cooling down to room temperature 2 ml ethanol (96%) were added and after a short vortex step 2 ml of lysate were applied to the silica membrane spin column. The column was centrifuged (2000 x *g* for 5 min at 4°C), and the remaining lysate (2 ml) was loaded onto the column. Subsequent washing was done according to the protocol given by the manufacturer (Machery&Nagel). The DNA was eluted with 150 µl preheated (70°C) elution buffer. DNA concentrations were determined using a photometer and DNA quality was checked by agarose gel electrophoresis. DNA from tissue samples was extracted with the a 96-well tissue-kit (Machery&Nagel) by Agrobiogen.

Paternity and identity control
A bovine microsatellite set was established for paternity and identity control. The set has been optimized for multiplex reactions and stable amplification from 10 ng of DNA (Table 2, Table 3). The microsatellites were amplified from 2 µl of DNA solution with standard buffer conditions, 3.0 mM $MgCl_2$, dNTP's (25 nM each), 0.15x Q-Solution, and 0.75 units of HotStar-taq polymerase (Qiagen, Hilden, Germany) in a final volume of 20 µl on a t-gradient 96-well thermocycler (Biometra, Göttingen, Germany). Primer concentrations were as given in Table 3. Cycling was for 15 min at

95°C, 35x[30 sec at 94°C, 1 min at 50°C or 60°C, respectively (see Table 2), 1 min 20 sec at 74°C] and 15 min at 74°C.
Fragment lengths of amplification products were analyzed on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). With the exception of microsatellite SPS115 the allele designations were done with category lists by the Genotyper® software (V. 2.5, Applied Biosystems). The data were transferred into a Microsoft® Access 2000 database. Preliminary allele frequencies were calculated from the maternal alleles within the half sib groups. Identity of tissue and milk samples was initially tested by an SQL-query. All conflicting genotypes were rechecked. The electropherograms of these genotypes were aligned and artifacts were identified manually. Genotypes were classified 1 to 4 according to quality. Class 1 and 2 genotypes could be transferred to the database without further editing whereas class 3 had to be rechecked and class 4 shows locus drop out (LDO) and allelic drop out (ADO) genotypes. PICs and the mean exclusion probabilities according to CHAKRAVARTI (1983) were calculated using the ALLELE FREQUENCIES option using Cervus 2.0 (MARSHALL et al., 1998). Parentage and identity were tested using the PARENTAGE and IDENTITY-CHECK options of Cervus.

Results

The usability of the remedies of ZL-MLP milk samples for genotyping purposes has been evaluated in two steps. Initially a DNA preparation method for samples used by the ZL-MPR has been developed. Based on this method, the paternity and identity of milk samples have been checked within dairy cow half sibs using DNA from tissue as reference.

DNA preparation from milk samples

A reliable and simple method for the preparation of DNA from fresh or degraded ZL-MPR samples has been developed based on silica-columns. Avoiding washing-steps prior to lysis minimizes loss of DNA from degraded nuclei. Intact cells are enriched and separated from the fat fraction by initial centrifugation. The method has been optimized with tank milk (homogeneous samples with about 80 somatic cells/µl) that has been collected into ZL-MPR tubes containing the routinely used conservation gel. To test the influence of different storage conditions (temperature and time) DNA prepared from fresh samples was compared to DNA prepared from frozen samples (-20°C) and samples kept in the fridge (4°C) or at 30°C for three and six days, respectively (Fig. 1). It turned out, that after six days at 30°C no high molecular weight DNA could be extracted, whereas three days old samples deliver DNA of similar quality as fresh samples. The quantity of DNA obtained from frozen samples was about half of that from fresh samples (Fig. 1). These results were obtained consistently from two independent experiments with all samples in duplicates.
ZL-MPR samples corresponding to the 119 reference cows were requested via the LKV. From 89 cows milk samples were obtained, some in duplicates. The volume of the left overs after the routine analyses (of percentage of milk fat, percentage of milk protein etc., and Fossomatic somatic cell count) differed from 20 ml to less than 3 ml. A distinct precipitate (coagulation-like) formed after thawing in 30 samples.
The isolated DNA from these samples varied widely with respect to quantity and quality (Fig. 2). The SCC varied from 10 to 2414 somatic cells/µl (mean 105) and the

DNA concentration from 0-150 ng/µl (mean 40 ng/µl corresponding to 6 µg DNA). Fragmentation varied from mainly high-molecular over partially (most of the samples) to highly degraded DNA (Fig. 2). DNA from some samples was not detectable on agarose gels.

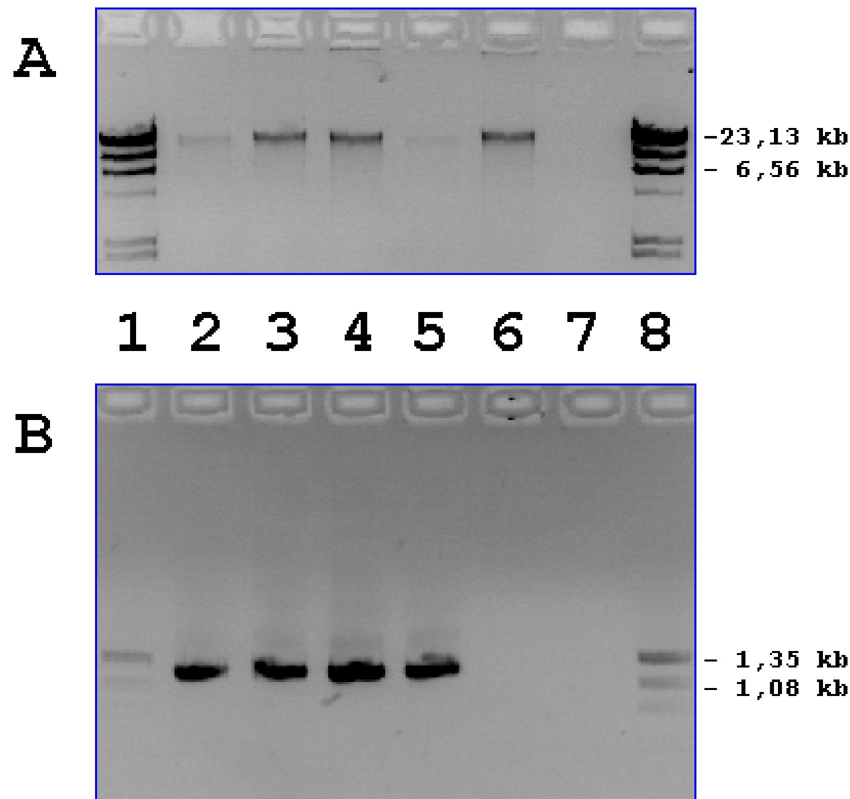

Fig. 1: Results from isolation of genomic DNA from tank milk samples stored under different conditions (Ergebnisse der DNA-Isolierung aus unter verschiedenen Bedingungen gelagerten Tankmilchproben)
A – genomic DNAs, 0.8% agarose gel; B – PCR products, 2.0% agarose gel
1 – length standard; 2 – samples stored at –20°C; 3 – freshly prepared; 4 – samples stored 3 days at 4°C; 5 – samples stored 6 days at 4°C; 6 – samples stored 3 days at 30°C; 7 – samples stored 6 days at 30°C; 8 – lenght standard

The influence of different parameters (SCC, storage time, day of milk sample investigation) onto DNA concentration was investigated statistically by regression analyses. Only the SCC was weakly correlated with DNA concentration (N = 91, correlation coefficient = 0.53, SD = 0.02).

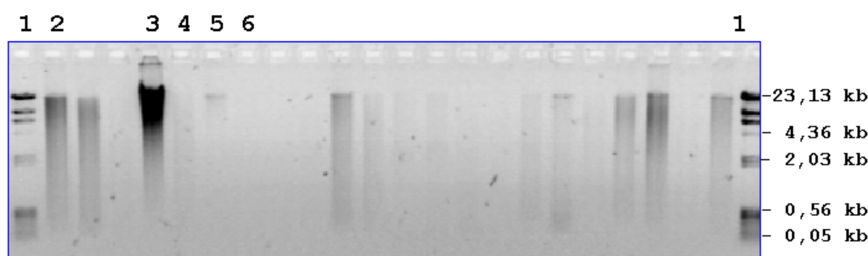

Fig. 2: Genomic DNA isolated from ZL-MLP samples (0.8% agarose gel) (Aus ZL-MLP-Proben isolierte genomische DNA (0,8%iges Agarosegel))
1 – length standard
Examples for different DNA quality: 2 – partially degraded DNA; 3 – mainly high molecular DNA, high concentration; 4 – degraded DNA, very low concentration; 5 - mainly high molecular DNA, low concentration
6 – DNA not visible

Genotyping, allelic and locus drop-out

119 reference samples and remedies from 100 (including the duplicates) ZL-MPR milk samples were genotyped at 10 microsatellite loci (Table 2, Table 3). ZL-MPR samples from 86 dairy cows could be genotyped (Table 4). From some milk DNAs only partial genotypes could be obtained despite of repeated typing, *i.e.* some loci did not amplify (locus drop out, LDO). Especially prone to LDOs were ETH3 and ETH10 (>10% of samples) whereas markers BM1824, BMS2113, ETH225 and TGLA227 showed low (<5%) LDO-rates.

Table 2
Microsatellites: primer sequences, concentrations and annealing temperature (Mikrosatelliten: Primersequenzen, Konzentrationen und Annealingtemperatur)

multiplex system	marker name	dye- and sequence (up and down-primer, 5'-3')	vol. added to a 20 µl PCR reaction (µl*)	annealing temperature
1	TGLA227	CGAATTCCAAATCTGTTAATTTGCT FAM-ACAGACAGAAACTCAATGAAAGCA	0.15	50°C
	BM2113	GCTGCCTTCTACCAAATACCC FAM-CTTCCTGAGAGAAGCAACACC	0.20	
	TGLA126	CTAATTTAGAATGAGAGAGGCTTCT TET-TTGGTCTCTATTCTCTGAATATTCC	0.35	
	INRA023	TET-GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACTC	0.30	
	BM1824	HEX-GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	0.08	
2	ETH10	GTTCAGGACTGGCCCTGCTAACA FAM-CCTCCAGCCCACTTTCTCTTCTC	0.08	60°C
	SPS115	FAM-AAAGTGACACAACAGCTTCTCCAG AACGAGTGTCCTAGTTTGGCTGTG	0.35	
	TGLA122	TET-CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	0.08	
	ETH3	HEX-GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	0.15	
	ETH225	HEX-GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	0.08	

*of a 10 pmol/µl solution

Following an initial analysis of identity and paternity some pairs of genotypes differed at one or more markers. These were checked for potential typing artifacts by inspecting the respective electropherograms. Two types of artifacts were observed: allelic drop out (ADO) and contamination. ADO was observed in 3 and contamination in 4 (from 86) milk genotypes. The genotypes of contaminated samples were characterized by several markers that showed one or two additional fragments when

compared to the reference genotype (Fig. 3). The SCCs for these samples were low (14, 20, 25, 28/µl). Similarly SCC values were correlated to genotype-quality classes. Class 3 and 4 milk genotypes showed low average SCCs compared to that of class 1 and 2 (Table 5).

Allele frequencies based on the maternal genotypes and exclusion probabilities have been calculated (Table 6). The mean exclusion probability when the genotypes from

Table 3
Microsatellites: number of alleles and range of fragment lengths (Mikrosatelliten: Anzahl der Allele und Fragmentlängenbereiche)

multiplex system	marker name	alleles Simmental (alleles MARC 99*)	
		N	range bp
1	TGLA227	10 (13)	71-93 (76-102)
	BM2113	7 (11)	126-140 (123-143)
	TGLA126	8 (3)	106-120 (116-122)
	INRA023	7 (-)	197-213 (-)
	BM1824	5 (7)	178-190 (178-192)
2	ETH10	6 (7)	216-226 (212-224)
	SPS115	8 (-)	244-258 (-)
	TGLA122	7 (18)	138-158 (137-181)
	ETH3	4 (9)	114-124 (105-125)
	ETH225	7 (10)	134-146 (141-159)

*cattle genome map or Meat Animal Research Center (http://sol.marc.usda.gov)

Table 4
Success of genotyping of ZL-MPR samples (Erfolg der Genotypisierungen der ZL-MPR Proben)

step	N	percent of samples requested	percent of samples received	percent of individuals typed
samples requested	119	100%		
samples received	89	74,8%	100%	
individuals typed successfully	86	72,3%	96,6%	100%
individuals typed with single pass*	59	49,6%	66,3%	68,6%
samples with correct identity	79	66,4%	88,8%	91,9%†
individuals typed for complete markerset#	71	59,7%	79,8%	82,6%

*less than 3 MDO; #including second pass typing; †about 8% samples with wrong identity

Table 5
Genotype quality and mean of somatic cell count (Qualität der Genotypen und mittlere Anzahl somatischer Zellen)

genotype quality	N	mean SCC [cells/µl]
1-2	72	126,60
3-4	20	28,30
Total	92	105,23

one or both parents are available were >97% and >99%, respectively. Markers ETH225 and TGLA127 provided the highest, marker ETH10 the lowest exclusion probabilities.

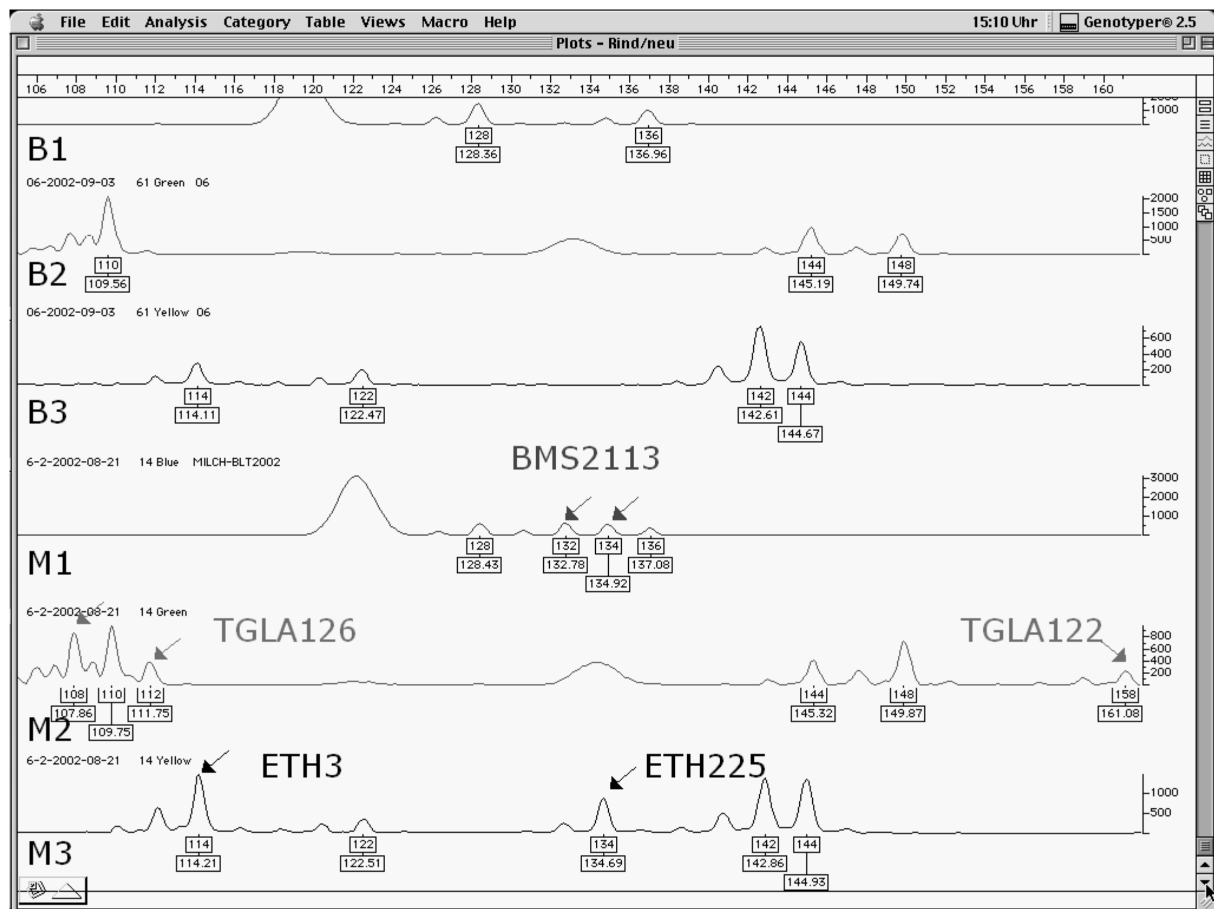

Fig. 3: Section of electropherograms derived from microsatellite typing tissue and milk sample from the same animal (Ausschnitte von Elektrogrammen der Mikrosatellitentypisierung von Gewebe- und Milchproben des gleichen Tieres)
Lanes B1-B3: electropherograms from DNA isolated from blood; M1-M3 from milk. Additional alleles (or threefold peak-heights at ETH3, respectively) within the genotype derived from milk sample are indicated with arrows

Parentage and sample identity

The control of sample identity and paternity was done based on the revised (ADO and LDO artifacts were skipped) data set. The paternal genotypes were derived from the half sib groups using the reference genotypes. No conflict was observed, *i.e.* the paternity was not disputed for any of the 119 dairy cows. Milk genotypes were available from 86 of the reference individuals. In 7 cases milk and reference genotypes were not identical (Table 4) and the identity had to be disputed. In addition all non-identical milk genotypes had conflicts to the paternal genotypes.

Table 6
Characteristics of microsatellites (Charakteristika der Mikrosatelliten)

name	expected heterozygosity*	PIC	mean exclusion probability (genotype of one parent available)*	mean exclusion probability (genotype of both parents available)*
BM1824	0.74	0.68	0.311	0.485
BMS2113	0.77	0.73	0.375	0.553
ETH10	0.58	0.53	0.184	0.353
ETH225	0.82	0.78	0.453	0.629
ETH3	0.72	0.66	0.288	0.457
INRA023	0.73	0.68	0.312	0.485
TGLA126	0.68 (0,71)	0.65	0.263 (0,29)	0.427 (0,45)
TGLA122	0.70 (0,70)	0.64	0.271 (0,30)	0.436 (0,48)
TGLA227	0.81 (0,61)	0.78	0.452 (0,20)	0.627 (0,37)
Mean	0,726	0,679		
cumulated			0,972	0,998

*in parentheses data given for US-Simmental (HEYEN et al., 1997)

Discussion

We established a DNA-preparation method that allows routine DNA isolation from milk samples. With this method, DNA yields well above half of the theoretically possible value can be obtained, no matter if fresh or frozen samples are used. The correlation of SCC and DNA amount is usually high when good quality milk samples are used (see also LIPKIN et al., 1998). Accordingly the yield from fresh tank milk samples (with standardized SCCs) was highly reproducible (*e.g.* numbers 3, 4, 6 fig. 1) in our study. In contrast to fresh milk, samples of the ZL-MPR are subject to variable storage conditions and mixed with a preservation gel. From our storage experiment it could be shown that fresh milk can be stored in completely filled ZL-MPR tubes at 4°C for 3 days without gross limitations. Longer storage or higher temperatures lead to progressing degradation of cells and DNA. The period from sampling to milk testing in the ZL-MPR ranges from 2-8 days in our data set. Additionally, the samples were exposed to different ambient temperatures from below 0°C (some samples may have been subjected to freeze-thaw cycles) to over 20°C before they reached the ZL-MPR laboratory. About one third of the samples showed a precipitate after storage at –20°C. For these samples, the DNA yield and quality varied broadly (0-22.5 µg, mean 6 µg) and the correlation of SCC and DNA yield was much lower than with fresh milk. This may distort milk pooling experiments for QTL analyses. Even DNA pooling using well defined concentration values is not straightforward since the DNA shows various degrees of degradation.

DNA prepared from ZL-MPR samples according to the developed protocol is suitable for standard genotyping when inherent properties are considered. First, the dairy cows will not completely be sampled, a few samples will be missing. Probably the missing dairy cows were not sampled due to failure of lactation for various reasons (culling, end of lactation). Second, there will be a higher rate of genotype failures (complete failure, LDO or ADO) compared to blood or tissue samples. In our experiment microsatellite amplification completely failed with 3 (from 89) samples that contained less than 0.2 ng DNA/µl (the standard protocol used allows some microsatellites to be analyzed from about 0.1 ng DNA/PCR reaction, data not shown). For 24 ZL-MPR

samples single markers could not be typed using standard PCR conditions (LDO) or showed ADO. Even repeated DNA isolation and amplification did not lead to complete genotypes and it can be assumed that the DNA yield from these samples with low SCCs dropped (by degradation) below a critical value. Only first-lactating cows had been investigated in our study and when investigating older cows a lower drop-out rate can be expected due to higher SCCs. The same is true for other breeds, which usually have higher SCC than German Simmental. Third, there will be a risk of contamination. Relevant degrees of contamination have been observed in our project. The peak heights of additional alleles within 4 samples are similar to that of the intrinsic origin, *i.e.* the amount of contaminating DNA is as high as that of the intrinsic DNA. This can be expected especially for samples with very low SCC values. If 1 ml of a sample with SCC of 25 is contaminated with 10 µl of a sample with SCC of 2500 the number of cells are equal. There are mainly two reasons for contamination of ZL-MPR samples: during flask sampling of test milk, *e.g.* with the lactocorder, there is the possibility that substantial traces of milk from the previously milked cow are present in the milk line and during testing in the laboratory (fat, protein percent and somatic cells) two needles are used without regular cleaning between individual flasks. Therefore some drops are transferred from the progenitor to the next flask. Similar observations have been described with milk samples collected via a milk robot (HÜTTL, 2001). Forth, the identity of samples may be confused in some cases.
We used tissue samples as references to confirm the identity of the milk samples. A microsatellite set was established for paternity and identity control in Simmental. With the exception of TGLA227, estimated PIC values and exclusion probabilities were in a similar range as found by other groups (Table 6). The paternity has not been disputed for any of the reference samples. This reflects the reliability of sampling by the personnel which does linear conformation scoring of test bull progeny. When investigating the collected ZL-MPR samples, identity could be confirmed for 81 (from 86) samples. Overall, this is a good result considering the complex conditions (several persons were involved, the identification of samples was not fully automatized, *e.g.* the animal identification number had to be typed in at the lactocorder) and the pilot character of the project.
Regarding these constraints ZL-MPR samples are potentially useful for a number of applications: spot-check paternity control or routine genotyping, *e. g.* for diagnosis of monogenic diseases or kappa-casein. Using current technology, mistyping is an intrinsic problem of low-cost, single-pass genotyping in domestic animals. Genotype mistyping probabilities have to be estimated. The rate of “mistyping” by distorted sample identity was comparatively high in our study. Nevertheless, when milk samples are used it is comparatively easy to request a second sample for control, *e. g.* to confirm mispaternity. It might be attractive and effective for breeding organizations when a high-throughput paternity control system shall be implemented. Furthermore, the rate of false identity will tend to decrease by further development of sample assignment and genotyping methods.
The use of a large number of milk samples within a daughter design is possible with the developed DNA preparation method. Nevertheless, the effort for DNA preparation is higher as compared to tissue or blood samples. Due to the high start-volume necessary to get useful DNA even from low-cell samples and initial centrifugation steps, the method is not easily automatable and requires a midi format, suited for 2 ml

starting material. Therefore the columns are more expensive than the mini-format used for tissue or blood samples. Alternative „quick methods" have the disadvantage of several washing steps prior to cell lysis with high hands-on time and are not optimal for degraded samples since DNA may be lost when discarding the supernatant.
One goal of the investigation was to develop a method for the collection of samples for modified daughter designs (GÖTZ and BECK, 2000). In this design samples from all daughters have to be collected. About 6 months later 10% of the collected families will be analyzed. The usability of the method depends on the number of correct genotypes since these determine the discrimination power for the detection of heterozygous sires. Basically, both alternatives used in our study are practical under field conditions: the collection of tissue samples using special containers in the course of linear scoring or the use of milk samples. About 40 (30-50) tissue samples per bull can be collected in the course of linear scoring. From the ZL-MPR samples requested about 75% arrived in the laboratory. From about 50% of the originally requested samples complete genotypes could be generated with a single pass investigation using standard methods and 8% of the samples did not stem from the expected animal. Based on these numbers it can be calculated that in the case of a test bull with 110 daughters in the testing scheme about 55 genotypes can be obtained using single-pass standard typing or 80 genotypes using optimized typing techniques and 8% of the samples may be of false identity that can be skipped from the analysis by checking for inconsistencies with the paternal genotype. In statistical terms a 25% smaller difference between daughter groups is sufficient to detect a bull to be heterozygous as compared to the use of tissue samples.
Nevertheless, when comparing the costs, one DNA sample from tissue is in our conditions delivered for 5 €, whereas the price for the used kit is about 4 € and with the costs for man-power and further lab usage the DNA from milk sample adds to about 7-8 €. As explained, the method is not fully optimized yet, but we consider it quite difficult to obtain further automatization.

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