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The effect of DNA quality on the sequencing success of cattle

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Abstract

The extraction of amplifiable DNA is a crucial step for genetic analysis of farm animals. The aim of present study was to determine the quality and the quantity of DNA extracted from blood, milk, hair and tissue in cattle. It has been shown that milk and hair samples can be used as a reliable source for obtaining good quality and quantity of DNA ready for the next generation sequencing (NGS). Commercially available kit, with several modifications, was included in the evaluation. Raw milk or milk somatic cell pellet storage under -80 °C or -20 °C has shown to be a reliable procedure for preservation when large number of milk samples were collected at once. The quality (purity) and the quantity of extracted DNA were measured using a nanophotometer. The adequacy of the DNA extract was assessed by estimating average read depth of all captured bases of whole NGS mitogenome sequences. Although DNA extracted from different types of samples was suitable for NGS analysis, hair samples showed the largest variation of DNA quantity, low purity and DNA integrity. Non-invasive sampling methods such as milk and hair collection can be used for dairy cattle while sampling from free-range requires handling, capturing and caution. Milk and hair can be used for obtaining optimal DNA concentrations for NGS analysis.

Keywords: cattle, DNA extraction, NGS, quality and quantity of DNA

Introduction

Optimal quality and quantity of DNA are parameters which influence the sequencing success. Since no specific guidance on the choice of protocol is given by the genotyping centers, it is important to choose the appropriate material and DNA extraction method. It is known that genomic selection drastically improves cattle breeding. There are several aspects that have to be accomplished while collecting samples for DNA extraction. First of all, sample has to be well preserved to obtain

optimal DNA quality. Secondly, sampling has to be simple and performed in accordance with animal welfare regulations (Directive 2010/63/EU, Legislation for the protection of animals used for scientific purposes). Milk sampling is routinely performed, less expensive, easy to accomplish and less stressful in aspects of capturing and handling than blood collection, which is fraught with technical difficulties and requires trained personnel (d'Angelo et al., 2007; Psifidi et al., 2010). Hair sampling from the tail is easy and can be performed during milk sampling while tissue sampling is usually performed during animal registration. Sampling from free-range animals can be challenging. It requires careful handling, capturing, caution and trained personnel. The purpose of this study was to determine and compare DNA quality and quantity obtained from milk, hair, blood and tissue samples using commercially available DNeasy Blood & Tissue Kit (Qiagen, Germany).

Materials and methods

Individual milk samples were collected from lactating Holstein cows (n=165). Two 50 ml tubes were filled equally from the each of the four quarters by hand milking after cleaning the udders. Samples were stored at -80 °C or 4 °C. Hair samples were collected from non-lactating Holstein cows (n=45) by plucking 3 tufts of hair from each animal's tail. Hair samples from Croatian autochthonous cattle breeds (Busa Cattle n=27; Istrian Cattle n=28; Slavonian-Syrmian Podolian n=4) were collected by plucking several tufts of hair from earlobes. Hair samples were transferred to individual envelopes and later stored at -20 °C. Blood samples were obtained from the jugular vein of Croatian autochthonous cattle breeds (Busa Cattle n=7; Slavonian-Syrmian Podolian n=13) during veterinary inspection. A volume of 3 ml of blood was collected in EDTA vacutainers stored at -80 °C. Tissue samples were collected from Croatian autochthonous cattle breeds during animal registration (Busa Cattle n=45; Istrian Cattle n=68; Slavonian-Syrmian Podolian n=56) using tissue sample applicator for ear biopsy and stored at -20 °C. Commercially available DNeasy Blood & Tissue Kit (Qiagen, Germany) was used for DNA extraction. DNA extraction was performed according to the manufacturer's protocol with several modifications depending on the sample. In all protocols, one extra centrifugation, prior to elution, was performed at 20,000 *g* at room temperature for 1 min to remove the remaining ethanol. In order to confirm the best method which gives sufficient amount of DNA, preliminary analysis was performed on four Holstein cows including different volumes of starting material (50 – 10 – 1.5 ml), temperatures of storage (-80 °C, -20 °C, 4 °C), storage time to the DNA extraction process after collecting the samples (within 4 and after 24 days) and cell pellets storage (-80 °C). DNA from 165 milk samples was extracted following pre-treatment phase as modification by Usman et al. (2014). A volume of 50 ml milk samples was centrifuged at 2,000 *g* at room temperature for 20 min. The fat layer was removed using a sterile spatula while the supernatant was discarded. The somatic cell pellets were resuspended in 20 ml phosphate-buffered saline (PBS, pH 7.4), vortexed and centrifuged at 4,000 *g* at room temperature for 10 min to dissolve milk casein. The somatic cell pellets were again resuspended in 200 µl PBS and transferred into a 1.5 ml tube by pipetting. A volume of 20 µl of proteinase K was added as extraction continued with the use of the DNeasy Blood & Tissue manual. DNA was extracted from hair using the following protocol 'Purification of DNA from nails, hair, or feathers' (Qiagen, Germany). As the

most of the DNA in hair is located in the root and the surrounding sheath cells (Higuchi et al., 1988), hairs were cut just above that part while being careful and patient. Approximately, 30 to 70 plucked hairs from each animal were put in the tube depending on the availability of samples. Blood (200 µl) and tissue (25 mg) samples were also extracted using DNeasy Blood & Tissue manual. The quality (purity) and the quantity of extracted DNA were measured using NanoPhotometer P330 Spectrophotometer (IMPLEN, Germany). The ratio of absorbance at 260 nm and 280 nm (A260/A280) reveals the purity of DNA. If the ratio is within the range 1.8-2, the purity of DNA is suitable. Where the ratio >2, indicates RNA contamination, while the ratio <1.8 indicates protein or phenol contamination (Eeles and Stamps, 1993). The quality (integrity) of extracted DNA was also assessed by gel electrophoresis. Descriptive statistics of the DNA concentration and quality measurements were calculated using SAS (SAS Inst. Inc., 2009). One of the quality control parameter for the next generation sequencing (NGS) of the mitochondrial genome, average read depth of all the captured bases, was estimated using Samtools 1.3.1 (Li et al., 2009).

Results and discussion

In preliminary analysis, DNA isolated from four individuals from a volume of 50 ml of raw milk, showed the highest concentrations within the range of 61.5-141 ng/µl. Also, high concentrations of DNA were obtained from 10 ml, whereas volume of 1.5 ml of raw milk showed very low DNA concentration (Figure 1. a-b-c). Considering effect of temperature storage, 50 ml of milk at -20 °C for 24 days and frozen somatic cell pellets obtained from 50 ml of milk stored at -80 °C for 24 days, showed high DNA concentrations (Figure 1. d-e). DNA purity, characterized by A_{260}/A_{280} ratio greater than 1.8, was optimal for all samples of 50 ml and 10 ml volume, no matter of storage temperature, except for volume of 1.5 ml whereas ratio was lower, within the range 1.4-1.6. DNA bands exhibited concomitant smearing in lane where the low intensity bands were observed in DNA extracted from a volume of 1.5 ml of milk. Descriptive statistics of extracted DNA from four different samples are summarized in Table 1. Higher mean values of DNA concentration were observed in the hair sampled from tails and earlobes, 414.69 and 127.32 ng/µl, and widest range, 16.5-844 ng/µl and 11.5-800 ng/µl, respectively. Despite the higher concentration obtained from fresh milk, frozen blood and tissue, higher and lower mean A260/A280 ratios of hair samples, indicate contamination by RNA and proteins. Lower DNA degradation was observed in the samples isolated from stored blood and tissue samples as well from milk samples (Figure 1) Extracted DNA from longer stored earlobes hair and fresh hair from tail was much more degraded, whereas DNA bands from stored earlobes were less noticeable. Complexity of DNA extraction from different material depends on the sample preparation. The most precise and time consuming was preparing a 0.5–1 cm pieces from the base of the hair especially if the hair was thin. Concentration and 260/280 ratio of extracted DNA samples, satisfied the recommended quantity of 1 µg of good quality DNA (concentration >= 20 ng/µl, OD 260/280 1.8-2) given by the protocol 'Illumina Library Construction Services - Sample Requirements' (UC Davis Genome Center, USA).

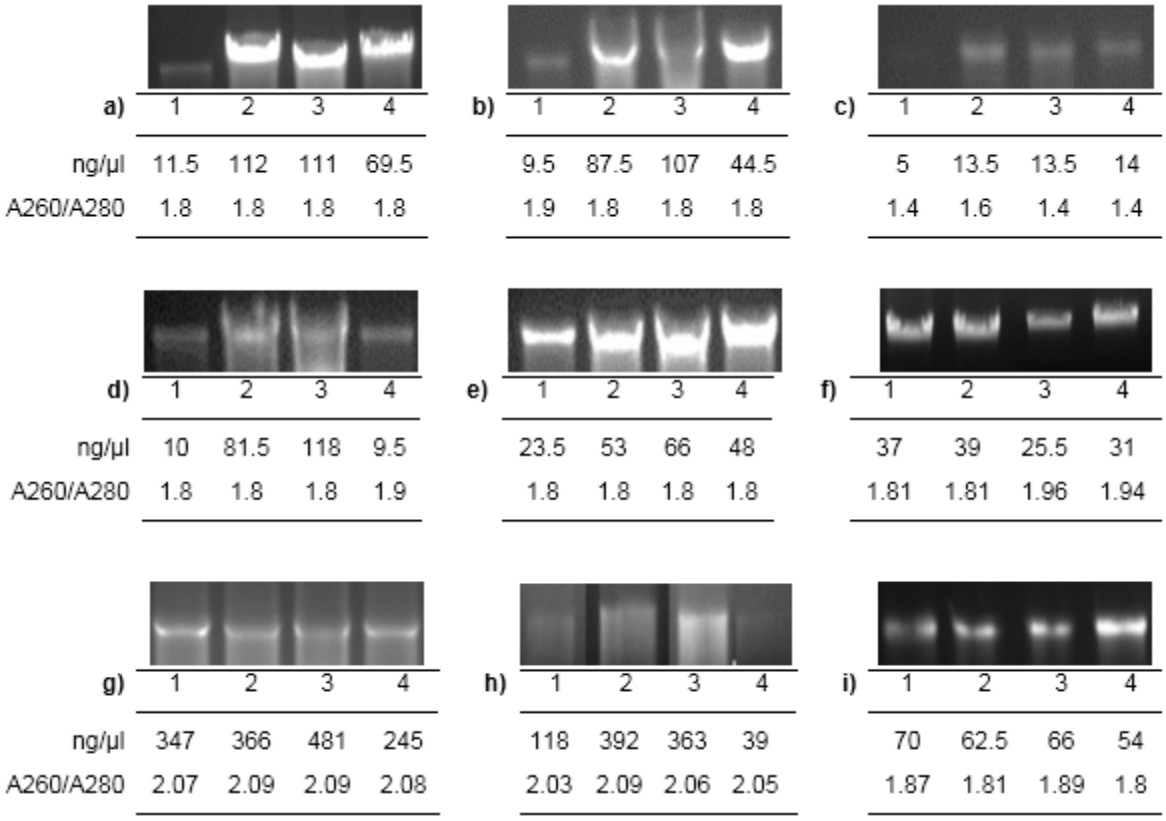

Figure 1. The analysis of total DNA from different cattle samples: a) 50 ml of fresh milk 4 °C/within 4 days; b) 10 ml of fresh milk 4 °C/within 4 days; c) 1.5 ml 4 °C/within 4 days; d) 50 ml of frozen milk -20 °C/24 days; e) frozen somatic cell pellets from 50 ml of milk -80 °C/24 days; f) frozen blood -80 °C/>6 months; g) fresh hair-tail; h) frozen hair-earlobe -20 °C/>1 year; i) frozen tissue -20 °C/>6 months.

If the total input of DNA material for library prep is below 100 ng, special library prep protocols need to be used. In total, 26 out of 458 extracted DNA were chosen for NGS mitochondrial genome analysis (108 milk, 44 hair-earlobe, 39 from tissue, 22 hair-tail, 13 blood) and all of 226 DNA samples were successfully sequenced. Average read depth of all the captured bases, estimated on 25 sequences, was 5051.

Table 1. Descriptive statistics of the extracted DNA from different material

		Concentration of DNA (ng/µl)			Purity of DNA 260/280 (nm)		
Material	No. of samples	Mean	Range	SE	Mean	Range	SE
Milk	165	68.72	3.5-273	4.46	1.86	1.03-2.8	0.01
Hair (tail)	45	414.69	16.5-844	30.33	2.07	1.83-2.11	0.01
Hair (earlobe)	59	127.32	11.5-800	18.03	1.72	1.25-2.15	0.04
Blood	20	29.4	10-59	2.59	1.84	1.53-2.04	0.03
Tissue	169	40.52	8.5-136	1.42	1.91	1.76-2.33	0.01

Conclusions

Different material could provide optimal quantity of DNA, but with different quality. Non-invasive sampling methods such as milk and hair collection can be used for dairy cattle while sampling from free-range requires handling, capturing and caution. Milk and hair can be used for obtaining optimal DNA concentrations for NGS analysis.

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