



A straightforward technique to obtain genomic DNA from nasal swabs suitable for sheep SNP genotyping analysis

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Abstract

Isolation of high quality and quantity genomic DNA is essential for molecular studies. It is crucial to select a non-invasive and straightforward technique to ensure the efficient collection of DNA, particularly at the farm level. The aim of this study was to determine if nasal swabs are an appropriate biological matrix to obtain good quality genomic DNA suitable for SNP genotyping. In this study, two biological matrices (blood and nasal swabs) were evaluated and compared for the isolation of genomic DNA obtained from 15 female Texel sheep. DNA quality and quantity were assessed using spectrophotometry and gel electrophoreses. Genotype concordance rates were used for comparison. Results showed that the highest concentration mean was obtained from blood samples (159.14 ng/ μ l), while from nasal swab samples the concentration mean was lower (130.12 ng/ μ l), but the difference was non-significant. Regarding purity, DNA obtained from nasal swabs presented a higher A260/A280 ratio (1.96), while the one obtained from blood samples was 1.90. Total DNA yield obtained from blood samples (15.91 μ g) was significantly higher than the one obtained from nasal swabs (6.51). Blood and nasal swab genotyping concordance rates were high (mean = 0.984). In conclusion, our results indicate that nasal swabs can yield good quality DNA; however, the DNA extraction protocol should be optimized.

Keywords: nasal swabs, ovine, DNA extraction, SNP array, concordance analysis

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Técnica simple para obtener ADN genómico adecuado para análisis de genotipado en ovinos a partir de hisopados nasales

Resumen

El aislamiento de ADN genómico de alta calidad y cantidad es fundamental para los estudios moleculares. Es crucial seleccionar una técnica sencilla y no invasiva para garantizar la recolección eficiente de ADN, particularmente a nivel de los establecimientos. El objetivo de este estudio fue determinar si los hisopados nasales son una matriz biológica apropiada para obtener ADN genómico de buena calidad para el genotipado de SNP. En el estudio se evaluaron y compararon dos matrices biológicas (sangre e hisopados nasales) para el aislamiento de ADN genómico obtenido de 15 ovejas Texel. La determinación de la calidad y la cantidad de ADN se realizó mediante espectrofotometría y electroforesis en gel. Se utilizaron tasas de concordancia de genotipado para comparación. Los resultados mostraron que la concentra-





ción media más alta se obtuvo de las muestras de sangre (159,14 ng/μl), mientras que de las muestras de hisopados nasales la concentración media fue menor (130,12 ng/μl), pero la diferencia no fue significativa. En cuanto a la pureza, el ADN obtenido a partir de hisopados nasales presentó una relación A260/A280 mayor (1,96), mientras que el obtenido de muestras de sangre fue de 1,90. El rendimiento total del ADN obtenido a partir de muestras de sangre (15,91 μg) fue significativamente mayor que el obtenido a partir de hisopados nasales (6,51). Las tasas de concordancia de sangre e hisopados nasales fueron altas (media = 0,984). En conclusión, nuestros resultados indican que es posible obtener ADN de buena calidad a partir de hisopados nasales; sin embargo, se debe optimizar el protocolo de extracción de ADN.

Palabras clave: chip de SNP, extracción de ADN, hisopado nasal, ovinos, análisis de concordancia

Uma técnica simples para obter DNA genômico a partir de esfregaços nasais adequado para análise de genotipagem em ovinos

Resumo

O isolamento do DNA genômico com alta qualidade e quantidade é essencial para estudos moleculares. É crucial selecionar uma técnica não invasiva e simples para garantir a recolha eficiente de ADN, especialmente ao nível das fazendas. O objectivo deste estudo foi determinar se os esfregaços nasais são uma matriz biológica apropriada para obter ADN genômico de boa qualidade adequado para genotipagem de SNP. Neste estudo, duas matrizes biológicas (sangue e esfregaço nasal) foram avaliadas e comparadas para o isolamento de DNA genômico obtido de 15 ovelhas fêmeas Texel. A determinação da qualidade e quantidade do DNA foi realizada por espectrofotometria e eletroforese em gel. As taxas de concordância genotípica foram utilizadas para comparação. Os resultados mostraram que a média de concentração mais alta foi obtida nas amostras de sangue (159,14 ng/μl), enquanto nas amostras de esfregaço nasal a média de concentração foi menor (130,12 ng/μl), mas a diferença não foi significativa. Em relação à pureza, o DNA obtido em esfregaços nasais apresentou maior relação A260/A280 (1,96), enquanto o obtido em amostras de sangue foi de 1,90. O rendimento total de DNA obtido em amostras de sangue (15,91 μg) foi significativamente superior ao obtido em esfregaços nasais (6,51). As taxas de concordância de sangue e esfregaço nasal foram elevadas (média = 0,984). Concluindo, nossos resultados indicam que esfregaços nasais podem produzir DNA de boa qualidade; no entanto, o protocolo de extração de DNA deve ser otimizado.

Palavras-chave: chip do SNP, esfregaço nasal, extração de DNA, ovinos, análise de concordância

1. Introduction

Molecular studies significantly depend on the extraction of high-quality DNA to ensure its suitability for subsequent downstream applications, such as DNA genotyping⁽¹⁾. Single nucleotide polymorphisms (SNPs) are sequence variations caused by a single nucleotide mutation at a specific locus in the DNA⁽²⁾. Currently, SNP markers are one of the preferred genotyping approaches due to their abundance in the genome, genetic stability, and compatibility with high-throughput automated analysis⁽³⁾. SNP genotyping technologies serve as powerful resources for investigating quantitative genetics, population genetics and molecular evolution⁽⁴⁾. Therefore, ensuring a robust DNA extraction method is essential for obtaining genomic DNA with both high quality and yield, crucial for accurate SNP genotyping.

Different biological samples, such as blood, ear tissue and semen, are suitable for sheep DNA analysis using conventional manual techniques. However, these materials vary in terms of ease of collection, storage requirements, and necessary shipment conditions. Furthermore, while numerous studies comparing DNA extraction from various sources have been published in cattle⁽⁵⁾⁽⁶⁾⁽⁷⁾⁽⁸⁾, fewer studies focus on sheep⁽⁹⁾⁽¹⁰⁾⁽¹¹⁾.

Although blood remains the primary source of genomic DNA in sheep due to its reliability in providing high-quality DNA, blood collection may induce stress in animals, and proficient technicians are essential to ensure



proper sample collection. Another drawback is the need to preserve blood at 4°C for shipment and storage. Semen provides an alternative method for DNA collection, but its applicability is primarily limited to high-genetic-value rams. Ear punching, in addition to being an invasive procedure, requires preservation, typically achieved using alcohol. In contrast, nasal swabbing, a technique predominantly used in humans⁽¹²⁾, offers the advantages of non-invasiveness, ease of sampling, and room temperature storage, making it an appealing option alongside traditional collection methods.

Identifying a non-invasive and straightforward technique for obtaining high quality DNA is critical to ensure successful sampling at the farm level. Thus, in this study, we assessed and compared two biological matrices—nasal swabs and blood—for the isolation of genomic DNA from sheep. Our goal was to determine the suitability of nasal swabs as a biological matrix to obtain high-quality DNA suitable for SNP genotyping in sheep.

2. Materials and methods

2.1 Sample collection

Blood samples were collected from 15 female Texel lambs raised at INIA's (National Agricultural Research Institute) Animal Production Unit, an experimental farm located at INIA Las Brujas (Canelones, Uruguay). Peripheral blood samples were obtained through jugular vein puncture using 6 ml K₂ EDTA blood collection tubes (BD, New Jersey, US). These samples were gently inverted to prevent clotting and immediately placed in insulated boxes with ice. Once in the laboratory, buffy coat was extracted from blood by spinning whole blood at 3500 RPM (1300 xg) for 10 min in an Eppendorf (5415R) centrifuge at 4°C. Buffy coat was removed, mixed with 1 ml of red blood cell lysis buffer (0.14 M NH₄Cl, 17 mM Tris base) and centrifuged at 8000 RPM (6797 xg) for 1 min at 4°C. The liquid was discarded, and the process was repeated until a clean buffy coat was obtained.

Nasal swabs were collected from the same 15 Texel lambs using standard commercial nasal swabs (Johnson & Johnson, New Jersey, US). The nasal swab sponge was gently rubbed inside each animal's nostril for 3 s, ensuring it did not extend beyond a depth of 1 cm. Subsequently, the swabs were air-dried for 5 min. Following this, the swabs were carefully placed in envelopes and stored at room temperature.

2.2 DNA extraction

We employed a single DNA extraction method for both matrices⁽¹³⁾: buffy coat from blood and nasal swab. For the buffy coat, leucocytes were suspended in 600 µl of digestion buffer [10 mM Tris-HCl (pH 8.0), 0.4 M NaCl, 2% SDS, 50 mM EDTA], and 5 µl of proteinase K (20 mg/ml, Meridian Bioscience, Cincinnati, OH) were added. The mixture was incubated overnight at 55°C. The following day, 5 µl of RNase A (10 mg/ml, Fermentas, Waltham, MA) were added to the lysate and incubated at 37°C for 30 min. Subsequently, 300 µl of potassium acetate 3 M were added and left at -20°C for 10 min. After centrifugation at 13000 RPM (17949 xg) for 10 min, 600 µl of the supernatant were transferred to a 1.5 ml tube, and 500 µl of isopropanol were added. After 20 min at -20°C, the mixture was centrifuged at 13000 RPM (17949 xg) for 10 min and the supernatant was discarded. The pellet was washed with 600 µl of 70% ethanol, and after centrifugation at 13000 RPM (17949 xg) for 5 min, the supernatant was discarded. The pellet was washed again with 100% ethanol. Following a final centrifugation, the DNA pellet was dried and re-suspended in 100 µl elution buffer (10 mM Tris-HCl, pH=8.0).

For the nasal swab, the sponge was cut inside a 1.5 ml tube, and the extraction procedure employed for the buffy coat was replicated, with the following adjustments in the quantities of certain reagents: 700 µl of digestion buffer, 200 µl of potassium acetate 3 M, and 50 µl of elution buffer.



2.3 Spectrophotometer measurements

Conducting spectrophotometric measurements of ultraviolet light absorbance at various wavelengths (260 nm and 280 nm) provides a quick and efficient method for the initial assessment of purity and concentration in nucleic acid samples⁽¹⁴⁾. In this study, both quantity and quality were assessed using a NanoDrop 8000 spectrophotometer (Thermo Scientific, Waltham, MA). DNA concentration was determined by utilizing the absorbance reading at 260 nm through the application of the Beer–Lambert law⁽¹⁵⁾. Additionally, the 260/280 nm absorbance ratio (A260/A280 ratio) was computed to assess protein contamination, with values falling within the 1.8–2.0 range considered optimal⁽¹⁴⁾. Total DNA yield was calculated by multiplying extracted DNA concentration by elution volume.

2.4 Gel electrophoresis

DNA integrity was evaluated through gel electrophoresis, where 1 µl of each DNA extract was analyzed in a 1% agarose gel containing 0.5X TBE buffer (Tris-Borate-EDTA). The electrophoresis was conducted for 25 min at 100V, and the results were visualized under UV illumination.

2.5 SNP genotyping

SNP genotyping was performed using the Applied Biosystems™ Axiom™ Ovine Genotyping Array 50K (Thermo Fisher Scientific, Waltham, MA, US) at Genexa Laboratory (Montevideo, Uruguay). Genomic data quality control was conducted using QCF90 software⁽¹⁶⁾, which involved the removal of SNPs located in sexual chromosomes, monomorphic and those with minor allele frequencies (MAF) <0.05, with call rate <90%, and significant deviation from HWE (Hardy–Weinberg Equilibrium) test at $p < 10^{-6}$. Additionally, animals with call rate <90% were removed. Following quality control, 30,302 SNPs remained for further analysis.

2.6 Animal Welfare

Animal handling and blood collection procedures were approved by the Animal Ethics Committee of INIA (approval number INIA_2018.2; unreferenced), in accordance with Uruguayan laws for the care and management of experimental animals (Law 18611)⁽¹⁷⁾.

2.7 Statistical Analysis

DNA concentration, DNA yield, and A260/A280 ratio obtained from both blood and nasal swabs were evaluated for normal distribution using the Shapiro-Wilk test, while equality of variances was assessed with the Levene's test. DNA concentration and DNA yield showed a normal distribution and homogeneity of the variances; therefore, a paired Student t test implemented in R⁽¹²⁾ was applied to compare the means for these variables. The A260/A280 ratio also showed a normal distribution, but the variances were not equal between the groups; therefore, we applied a Wilcoxon test to assess significant differences, also using R⁽¹⁸⁾.

Genotype quality was assessed by the call rate per animal. Call rate is defined as the ratio of genotypes passing the calling threshold to the total number of genotypes that require inference⁽¹⁹⁾ with values ranging from 0 to 100%. Higher values are preferred.

Concordance rates between genotypes obtained from blood and nasal swab matrices were assessed using SeekParentF90 software⁽²⁰⁾. Out of the initial 15 samples, four nasal swab samples failed genotyping, leading to their exclusion from the concordance analysis. Despite exhibiting concentrations in the lower range (69.4 to 110.7 ng/µl and a total yield between 3.5 and 5.5 µg), these concentrations surpassed the minimum requirement for SNP genotyping at Genexa Laboratory.



3. Results

3.1 DNA concentration, spectrophotometer measurements and total DNA yield

The two biological matrices yielded genomic DNA of varying quality and quantity (**Table 1**). For DNA concentration, the difference between mean values for blood (159.14 ng/ μ l) and nasal swab (130.12 ng/ μ l) matrices was non-significant ($p>0.05$).

On the other hand, significant differences were detected for A260/A280 ratio and total DNA yield ($p<0.05$), with blood matrix showing a slightly lower ratio (1.90) and a higher yield (15.91 μ g). Mean A260/A280 ratios for both samples were within the 1.8-2.0 range, which indicates the presence of pure DNA⁽⁹⁾.

Table 1. Comparison of mean DNA concentration (Conc), A260/A280 ratio and total DNA yield obtained from two different biological matrices, with standard errors in parentheses

Matrix	Conc. (ng/ μ l)	A260/A280 ratio	Total yield (μ g)
Blood	159.14(10.89) ^a	1.90(0.00) ^a	15.91(1.09) ^a
Nasal swab	130.12(15.32) ^a	1.96(0.01) ^b	6.51(0.77) ^b

^{a,b} Comparison of values within each column; values with the same superscript are not statistically different ($p>0.05$) from each other but they differ significantly ($p<0.05$) from values with different superscript.

3.2 Gel electrophoresis

Integrity of extracted genomic DNA was assessed by agarose gel electrophoresis (**Figure 1**). Gel electrophoresis analysis revealed that DNA extracted from blood samples exhibited a higher degree of integrity, evident through well-defined and concentrated bands. In contrast, DNA obtained from nasal swabs displayed some degree of smearing, indicative of DNA fragmentation.

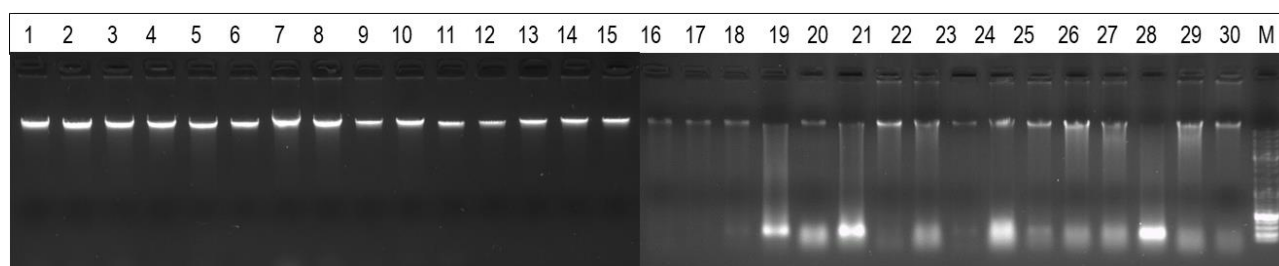


Figure 1. DNA quality assessed by gel electrophoresis. Blood samples (lines 1-15), nasal swab samples (lines 16-30), M molecular weight marker (GeneRuler 1kb Plus, Thermo Scientific)

3.3 Call rates and concordance analysis

In terms of call rates, both blood and nasal swabs yielded similar results. The mean call rate per individual obtained using blood (0.995) was slightly higher than the one obtained using nasal swab (0.994), but the difference was non-significant ($p>0.05$).

Genotyping data was compared to assess concordance rate between blood and nasal swab matrices (**Table 2**). High concordance rates were found, ranging from 0.898 to 0.999, with a mean of 0.984 and a standard error of 0.10. These results provide evidence that genotyping results using DNA isolated from both blood and nasal swabs show a substantial level of similarity.

**Table 2.** Genotype concordance rates (ratio) comparing blood matrix (Id_1) and nasal swab matrix (Id_2), call rates obtained with blood matrix (callrate_1), and call rates obtained with nasal swab matrix (callrate_2)

Animal Id	Id_1	Id_2	ratio	callrate_1	callrate_2
139020200029	20210409069	20220810071	0.998	0.999	0.998
139020200171	20210409075	20220810072	0.995	0.995	0.999
139020200215	20210409076	20220810073	0.997	0.998	0.999
139020200276	20210409078	20220810074	0.948	0.999	0.951
139020200277	20210409079	20220810075	0.998	0.999	0.999
139020200294	20210824013	20220810076	0.997	0.998	0.999
139020200324	20210409082	20220810077	0.898	0.964	0.999
139020200363	20210409086	20220810078	0.997	0.999	0.997
139020200369	20210824015	20220810079	0.999	0.999	0.999
139020200391	20210824018	20220810080	0.997	0.996	0.999
139020200401	20210824023	20220810081	0.998	0.998	0.999

4. Discussion

DNA concentration obtained from blood samples in our study exceeded the average amount reported by Nuñez and others⁽¹¹⁾, who used 50 µl of blood and a proteinase K protocol, yielding a concentration of 42.45 ng/µl. Our findings closely resembled the yield reported by Psifidi and others⁽¹⁰⁾, who used 10 ml of blood and a commercial kit (162 ng/µl), and Foley and others⁽⁷⁾, who used 450 µl of blood with a proteinase K protocol (158.2 ng/µl). However, in the case of nasal swabs, a previous study⁽⁷⁾ reported higher DNA concentrations (150.9 ng/µl) compared to our study. It's noteworthy that these authors used commercial nasal swabs (Performagene) containing a solution that acts as a preservative and cell lysis agent, whereas we employed regular nasal swabs. Consequently, our study reported lower total DNA yield values compared to Foley and others⁽⁷⁾ for nasal swabs (15.09 µg).

Concerning mean A260/A280 ratios, our results exhibited superior ratios compared to those reported by Neary and others⁽⁸⁾ for both blood (1.6) and nasal swabs (1.75), as well as by Foley and others⁽⁷⁾ for blood (1.7).

In comparison to a previous study⁽⁷⁾, our study showed a similar high degree of DNA integrity, characterized by well-defined bands in blood samples. However, this was not observed in nasal swab samples. Once again, this discrepancy could be attributed to the preservation solution used in the commercial swabs, which may have caused DNA fragmentation.

Even though DNA extracted from blood matrix exhibited superior performance, DNA derived from nasal swabs demonstrated satisfactory results, yielding high quantity (130.12 ng/µl), optimal A260/A280 ratio (1.96), and sufficient total yield (6.51 µg) suitable for SNP genotyping. Moreover, nasal swabs provide additional benefits, including ease of use (requiring minimal expertise and equipment for sample collection), accessibility (commercial swabs commonly found in stores), and the convenience of storing samples at room temperature. This confers a notable advantage over the use of blood samples, particularly for sampling in commercial farms. However, given the relatively high failure rate of 27% in nasal swab samples during genotyping, there is a need to revise and enhance the DNA extraction protocol from nasal swabs.



5. Conclusions

In conclusion, we observed a high concordance rate between genotypes obtained from blood and nasal swabs in this study. These results suggest that nasal swabs are a viable alternative to obtain high quality sheep DNA suitable for SNP genotyping at large scale. However, further studies are needed to improve the DNA extraction protocol from nasal swabs.

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Transparency of data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

BC: Conceptualization; Investigation; Formal analysis; Methodology; Software; Visualization; Writing – original draft

PP: Writing – review & editing

GC: Resources; Writing – review & editing

EAN: Supervision; Writing – review & editing

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