

Comparison of 12 DNA extraction kits for vertebrate samples

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Abstract

Comparison of 12 DNA extraction kits for vertebrate samples. Obtaining high quality DNA extractions is a crucial step for molecular biology research projects. At present, numerous protocols are available for vertebrate tissue extractions. In the present study we compared eleven column-based protocols and one HotSHOT protocol using similar conditions (i.e., type of sample, weight of starting material). We evaluated time of extraction, quality and quantity of DNA yield, and price of extraction for a single sample. Based on our analysis, the most successful kits for producing DNA with the highest concentration and purity are the JetQuick® Genomic DNA Purification Kit (Genomed) and the NucleoSpin® Tissue (Macherey–Nagel). Nevertheless, it is highly recommended to test various extraction kits with specific samples to find the optimal kit in all aspects of time, quality and cost for a particular project.

Key words: DNA isolation, DNA concentration, DNA purity, Price of extraction kit

Resumen

Comparación de 12 kits de extracción de ADN de muestras de vertebrados. Obtener extracciones de ADN de buena calidad es un paso crucial para los proyectos de investigación del ámbito de la biología molecular. En la actualidad, existen numerosos protocolos para la extracción en tejidos de vertebrados. En el presente estudio comparamos 11 protocolos de extracción por columnas y un protocolo HotSHOT utilizando condiciones parecidas (como el tipo de muestra o el peso del material inicial). Evaluamos el tiempo de extracción, la calidad y la cantidad de ADN obtenido y el precio de la extracción de una única muestra. Según nuestro análisis, los kits que dieron mejores resultados para producir ADN con la mayor concentración y pureza son JetQuick® Genomic DNA Purification Kit (Genomed) y el NucleoSpin® Tissue (Macherey–Nagel). No obstante, se recomienda encarecidamente probar varios kits de extracción con muestras específicas para encontrar el que sea mejor en cuanto al tiempo, la calidad y el costo en relación con un proyecto concreto.

Palabras clave: Aislamiento de ADN, Concentración de ADN, Pureza del ADN, Precio del kit de extracción

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Introduction

Recent expansion of new approaches in molecular genetics allows researchers to obtain genetic information from a variety of sources: e.g., hair (Grisedale et al., 2018), faeces (De Barba et al., 2017), urine (Hausknecht et al., 2007), shed feathers (Valsecchi, 1998), eggshells (Lee and Prys-Jones, 2008), and owl pellets (Taberlet and Fumagalli, 1996). Additionally, improved protocols for non-invasive samples enable DNA isolation from environmental samples such as water and soil (Taberlet et al., 1999; Thomsen et al., 2012; Pilliod et al., 2013; Català et al., 2015; Jerde et al., 2019; Stat et al., 2019) and from museum samples (Payne and Sorenson, 2002; Wandeler et al., 2003; Flagstad et al., 2003; Wisely et al., 2004; Hedmark and Ellegren, 2005; Horváth et al., 2005; Harper et al., 2006; Stuart et al., 2006; Ciborowski et al., 2007; Wandeler et al., 2007).

Regardless of the source or eventual use of the DNA, the goals of all extraction methods are the same: 1) to release genetic material from its source (fluid, tissue or microbe); 2) to stabilize nucleic acids against degradation; 3) to remove amplification inhibitors; 4) to concentrate the nucleic acid material into an appropriate volume of an aqueous solution compatible with downstream application; and 5) to standardize the methods to support accurate, sensitive and reproducible laboratory assays (Attia et al., 1996; Fox et al., 2007; Hill, 2011; Boesenberg-Smith et al., 2012).

The few previous studies that compared DNA extraction methods include: bacterial and fungal communities (e.g. Queipo-Ortuño et al., 2008; Tomaso et al., 2010; Vesty et al., 2017; Rodrigues et al., 2018); mollusc (Der Sarkissian et al., 2017); invertebrate (Kranzfelder et al., 2016; Schiebelhut et al., 2017); and ancient samples and formalin-fixed tissue samples (Rohland and Hofreiter, 2007; Janecka et al., 2015; Gamba et al., 2016). Furthermore, these studies are not comparable, as each experiment employed a different source of genetic material (e.g. different tissue, age of material, storage conditions) and different extraction kits. The extraction of DNA from vertebrate samples is usually less challenging than in the previously mentioned groups. However, the comparison of extraction kits for vertebrate samples is still lacking. Advances in Next Generation Sequencing demand the use of high quality DNA. Therefore, we decided to compare 12 DNA extraction protocols under similar conditions, including: initial weight of isolated sample, equipment, laboratory technician, and measurements of quality and quantity. The goal of this study was to compare the concentration and purity of isolated DNA, and the time and price of single extraction from vertebrate tissue.

Material and methods

For the purpose of this experiment we used 12 different extraction kits from eight manufacturers. We tried to cover the diversity of extraction kits, including those kits commonly cited in the literature (based on Google Scholar Search, 25th of June 2019). The kits were: High Pure PCR Template Preparation Kit (Roche)

72,200 Google Scholar hits; Dneasy® Blood & Tissue Kit (Qiagen) 46,700 hits; NucleoSpin® Tissue (Macherey–Nagel) 26,800 hits followed by less-known and/or less cited in literature; E.Z.N.A.® MicroElute Genomic DNA Kit (Omega) 128 hits; Quick-gDNA™ MiniPrep (Zymo Research) 219 hits; Invisorb® Spin Tissue Mini Kit (Strattec) 381 hits. Beside the DNA concentration and purity, we also assessed the price of kits and hand-on time demanded for single extraction. The eleven extraction kits are based on silica membrane columns and the last extraction is HotSHOT (Truett et al., 2000). The list of used extraction kits: <1> E.Z.N.A.® Tissue DNA Kit (Omega); <2> High Pure PCR Template Preparation Kit (Roche); <3> E.Z.N.A.® MicroElute Genomic DNA Kit (Omega); <4> JetQuick® Genomic DNA Purification Kits (Genomed); <5> Dneasy® Blood & Tissue Kit (Qiagen); <6> E.Z.N.A.® Forensic DNA Kit (Omega); <7> NucleoSpin® Tissue (Macherey–Nagel); <8> Quick-gDNA™ MiniPrep (Zymo Research); <9> UltraClean® Tissue & Cells DNA Isolation Kit (MoBio); <10> UltraClean® Tissue & Cells DNA Isolation Kit (MoBio) with Proteinase K; <11> Invisorb® Spin Tissue Mini Kit (Strattec); and <12> HotSHOT (Truett et al., 2000).

Starting material

We applied extraction protocols to four types of vertebrate samples: finger (phalange), spleen and tail from the domestic house mouse (*Mus musculus*) and blood samples obtained from grey partridge (*Perdix perdix*). Quality bias of samples (due origin, age, storage conditions, etc.) was eliminated by using only fresh samples. The tissue was weighed using Kern ABT analytical balances (Kern, ABT 120–5 DNM, resolution 0.1 mg). The average weight of finger was 1.78 mg (median 1.77 mg, 1.00–2.33 mg), spleen: 5.70 mg (median 5.95 mg, 2.08–7.70 mg), tail: 7.75 mg (median 8.00 mg, 2.54–10.30 mg). For the blood samples we used a comparable volume of clot (cca 5 ul). Details are available in table 1s in supplementary material.

To confirm the consistency of results obtained with the same protocol, three extraction kits were used: <4> JetQuick, <2> Roche, and <5> Qiagen to extract several samples of the same tissue type (N = 16, 12, 12 respectively, for samples of comparable weight for finger, spleen and tail).

Protocol

We followed the manufacturers' recommendations for lysis and purification of DNA (table 1). For three protocols <8, 9, 10> we used a homogenization step using the MagNA Lyser Instrument (Roche) and bead tubes (provided in Mobio kit). The tissue was mixed using a thermo-shaker until complete lysis. The extent of disruption (in %) was recorded after two hours (more details in table 2s in supplementary material). For protocols <1–11> we used the same amount of elution buffer, 100 µl for finger, and 200 µl for all other tissues. The elution step was repeated twice with the same amount of elution buffer (100 µl and 200 µl, respectively).

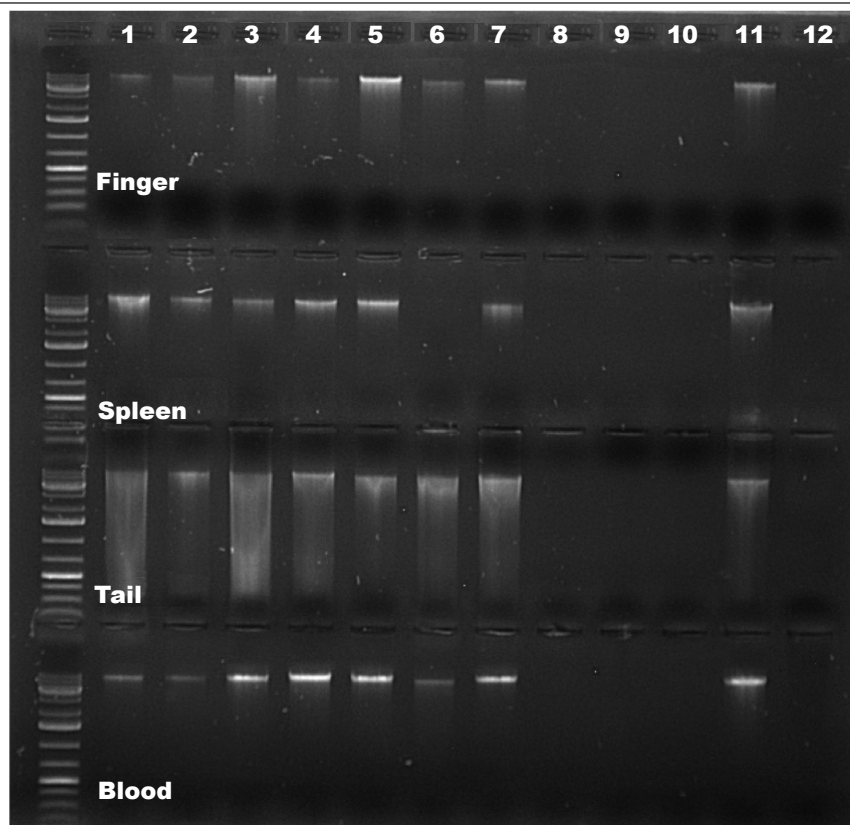


Fig. 1. DNA quality visualisation using 5 μ l of DNA on 1% agarose gel for 30 min with 1 kb ladder.

Fig. 1. Visualización de la calidad del ADN utilizando 5 μ l de ADN en gel de agarosa al 1% durante 30 min con 1 kb Ladder.

Extraction protocol <12> does not employ silica-based columns. Tissues were incubated in 75 μ l of extraction solution (25 mM NaOH, 0.2 mM EDTA, pH12) 95°C for 50 min and then the same amount of 40 mM Tris-HCl (pH 5) was added. The final solution was incubated for one hour at 4°C (Truett et al., 2000; Reichard et al., 2008).

Repeatability of DNA extraction

The effect of tissue weight on the final DNA concentration was tested by multiple extractions of samples of a comparable size (ranging from 0.72 mg to 3 mg in weight) using three kits: JetQuick® Genomic DNA Purification Kits (Genomed) <4> was used for extraction of 16 samples, <2> High Pure PCR Template Preparation Kit (Roche) and <5> Dneasy® Blood & Tissue Kit (Qiagen) for extraction of 12 samples each. We reported the relative concentration as the ratio of concentration per 1 mg of weight (c/W).

Measuring

Primary verification of DNA quality was tested using gel agarose electrophoresis under the following

conditions: 5 μ l of DNA in 1% agarose gel, running for 40 min. The gel was visualized by GenoPlex documentation system and GenoCapture software (fig. 1). The isolates were subsequently assessed for quantity and quality using Qubit® fluorometer and DS-11 Spectrophotometer. We measured the 1st and 2nd elution (5 μ l of DNA for each) using Qubit® fluorometer with Qubit dsDNA BR Assay kit. DNA purity was evaluated using A_{260}/A_{280} ratio via DS-11 Spectrophotometer (DeNovix). Following the manufacturers' recommendation, the blank (i.e., water or elution buffer) for each extraction kit was used together with 1 μ l of DNA. The A_{260}/A_{280} ratio measured with fluorometry should be ~1.8 (Santos et al., 2009). Values higher than 2.0 indicate basic contamination, while values lower than 1.7 relate to acidic contamination of phenol or proteins.

Cost

The cost per single extraction was calculated for the commercial kits by dividing the cost by the number of extractions. The prices are valid for 2019 in the Czech Republic.

Table 1. Overview of 12 extraction kits, information about lysis, purification of DNA from manufacturers' protocol, time extraction, concentration of DNA measured at Qubit® fluorometer, purity ration measured at spectrophotometer and costs in Czech Republic (2019). Lysis: SM, starting material (mg); LB, Lysis buffer (µl, nls, no lysis step); PK, proteinase K (µl); T, temperature (°C). Purification: BB, binding buffer (µl); I, incubation (°C for 10'); A, alcohol (µl; E, ethanol; I, isopropanol); EB, extra buffer (µl; HBC, HBC Buffer; IRB, Inhibitor Removal buffer); WB, wash buffer (µl); E, elution (µl); TEB (°C). Time: L, lysis (h); Ho, Hand-on (min). Concentration and Purity ratio (ng/µl: F, finger; S, spleen; T, tail; B, blood). C, cost for 1 reaction (€). ° in protocol was used Proteinase K for sample lysis; * Because of low concentration of DNA (< 10 ng/µl) spectrophotometer can't measure precise purity ratio; † price from 2016, after this date MoBio stop producing UltraClean® Tissue & Cells DNA Isolation Kit. (For abbreviations of extraction kits, see Material and methods and table 2).

	Lysis				Purification							
	SM	LB	PK	T	BB	I	A	EB	WB1	WB2	E	TEB
1	E.Z.N.A.® Tissue DNA Kit – Omega											
	30	200	25	55	220	70	220(E)	500HBC	700	700	100–200	70
2	High Pure PCR Template Preparation Kit – Roche											
	25–50	200	40	55	200	70	100(I)	500IRB	500	500	200	70
3	E.Z.N.A.® MicroElute GenomicDNA Kit – Omega											
	<10	200	20	55	220	70	220(E)	500HBC	700	700	10–50	70
4	JetQuick® Genomic DNA Purification Kit – Genomed											
	10–20	200	20	55	200	70	200(E)	no	500	500	25–200	65–70
5	Dneasy® Blood & Tissue Kit – Qiagen											
	<25	180	20	56	200	56	200(E)	no	500	500	200	no
6	E.Z.N.A.® Forensic DNA Kit – Omega											
	30	200	25	55–60	225	60	200(I)	500HBC	700	700	100	70
7	NucleoSpin® Tissue – Machery–Nagel											
	<25	180	25	56	100	70	210(E)	no	500	600	100	70
8	Quick-gDNA™ MiniPrep – Zymo Research											
	<25	500	nls		no	no	no	no	200	500	>50	no
9	UltraClean® Tissue & Cells DNA Isolation Kit – MoBio											
	1–25	700	nls		no	no	no	no	no	400	50	no
10	UltraClean® Tissue & Cells DNA Isolation Kit – MoBio [°]											
	1–25	700	20	56	no	no	no	no	no	400	50	no
11	Invisorb® Spin Tissue Mini Kit – Stratec											
	5–40	400	40	52	200	no	no	no	550	550	200	52
12	HotSHOT											
	NA	no	no	no	no	no	no	no	no	no	no	no

Visualisation

Results were visualized using packages *ggplot2* and *ggthemes* in the R statistical environment (R Team, 2015). The extraction kits are coded and numbered (1–12). A full list of names is available in table 1. The relative DNA concentration was calculated as the ratio of concentration per 1 mg of weight (c/W; table 1; fig. 2). For each extraction kit, four sample types were visualized (finger, spleen, tail and blood). First and second elution of each sample was shown. To provide a lucid comparison of concentrations

between various tissues and elution, a final list was created and provided in table 1s in supplementary material.

Results

Table 1 summarizes results. It contains information about lysis conditions and DNA purification, time of lysis and hands-on time, DNA yield, DNA purity, and the cost per sample obtained from the 12 extraction protocols.

Tabla 1. Resumen de los 12 kits de extracción, información sobre lisis, purificación del ADN del protocolo de los fabricantes, tiempo de extracción, concentración de ADN medida en el fluorímetro Qubit®, índice de pureza medido en un espectrofotómetro en costos en la República Checa (2019). (Para las abreviaturas, véase la cabecera de la tabla en inglés).

Time		Concentration				Purity ratio A_{260}/A_{280}				
L	Ho	F	S	T	B	F	S	T	B	C
3	50	5.26	21.10	86.40	11.90	2.16*	1.91	1.9	2.01	1.82
10	45	.44	11.50	49.60	2.63	2.33*	1.74	1.87	1.76*	3.05
10	50	5.23	8.16	93.50	13.30	2.07*	1.61*	1.87	1.68	3.10
10	55	3.68	12.00	43.00	10.80	2.07*	2.01	1.89	1.83	2.46
5	40	12.40	10.90	49.90	8.72	1.95	1.78	1.81	2.1	4.42
4	50	7.02	1.44	25.10	1.70	1.64	1.85*	1.74	1.33*	2.37
6	45	13.40	17.90	83.80	8.10	2.02	2.03	1.86	1.95	2.79
0	50	0.50	1.15	0.50	0.50	-0.99*	0.56*	0.36*	0.62*	2.19
0	50	0.50	0.50	0.50	0.50	1.04*	1.06*	0.97*	1.2*	11.46†
0.5	50	0.50	0.50	0.50	0.50	0.96*	1.01*	1.88*	1.38*	11.46†
6	35	25.00	28.70	87.00	16.40	2.09	2.12	1.87	1.96	3.37
0	120	1.19	4.08	1.41	1.46	1.7*	1.94*	1.66*	2.02*	<1.0

Time

The extraction protocols differ in time of lysis and hands-on time. Three of the protocols did not require a lysis step (8) Quick-gDNA™ MiniPrep (Zymo Research), (9) UltraClean® Tissue & Cells DNA Isolation Kit (MoBio) and (12) HotSHOT; therefore, the time of extraction was less than 2 hours. The lysis time was an interval from 3 hours (1) to overnight lysis (2, 3, 4). When we compared hands-on time after lysis, the time variability was minimal, ranging from 35 min (1) to 50 min (1, 6). More details are given in table 1.

Concentration and purity of DNA

The absolute and relative DNA concentration from the first and second elution for each tissue is shown in table 1 and figure 2. Overall, the second elution had similar or lower concentration than the first one. The only exception was blood sample isolation using extraction kits: (1, 2, 4, 5). The highest DNA yields were obtained using (11), which provided constantly high DNA concentration for all used tissues. Contrary to this, kits (8, 9) and (10) provided only low-concentration DNA in all cases.

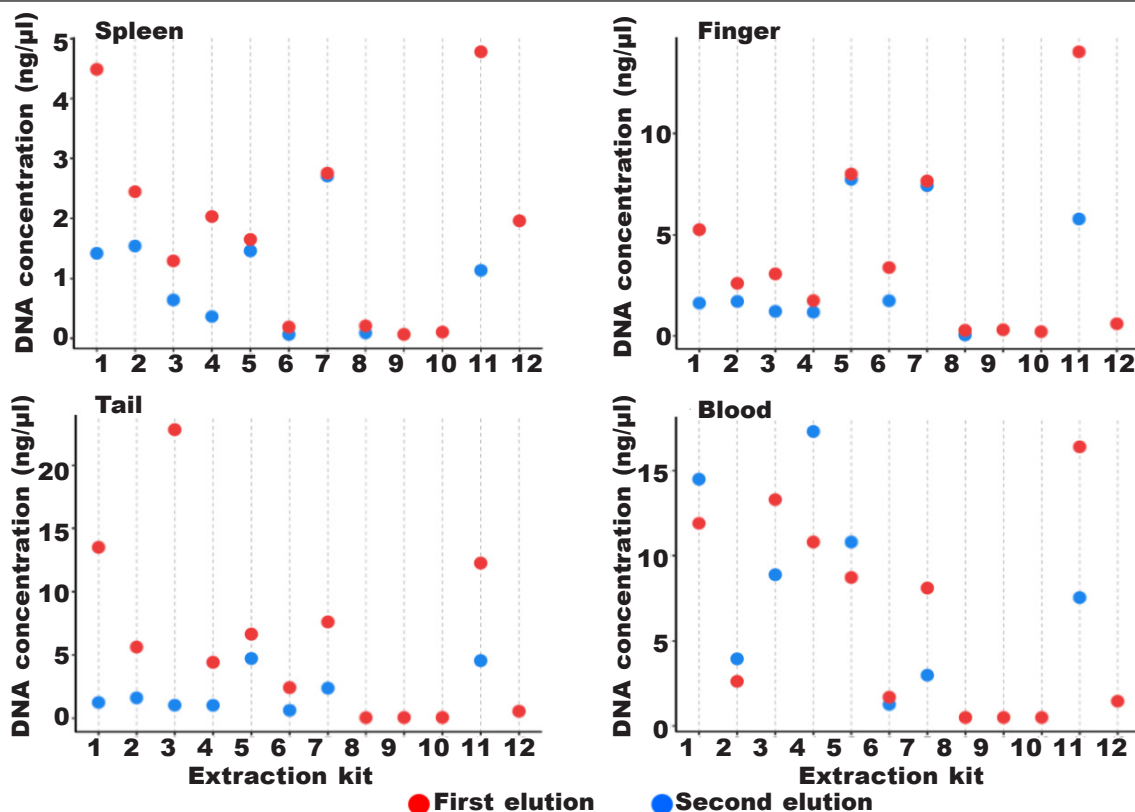


Fig. 2. Comparison of obtained DNA concentrations between 12 extraction kits. To visualize finger, spleen and tail the relative concentrations were used (c/w; absolute concentration available in table 1). The absolute concentration (ng/μl) was visualized for blood samples. In the case that only the first elution is visualised, the second elution was not provided (12), or the second elution was not measurable on fluorimeter (e.g. 8, 9, 10).

Fig. 2. Comparación entre las concentraciones del ADN obtenido con 12 kits de extracción. Para visualizar las muestras de dedo, bazo y cola se utilizaron las concentraciones relativas (c/w; concentración absoluta disponible en la tabla 1). La concentración absoluta (ng/μl) se visualizó para muestras de sangre. Cuando solo se visualizó la primera elución, la segunda (12) no se proporcionó o no fue mensurable en el fluorímetro (p. ej. 8, 9, 10).

The DNA quality was assessed using agarose gel electrophoresis. Several cases of very low DNA concentration (less than 0.5 ng/μl; 8, 9, 10, 12) produced no visible band. Additionally, the quality/rate of fragmentation in several of the extraction kits was dependent on tissue type. For example, the highest DNA concentration was obtained from tail and this DNA also displayed extensive degradation/fragmentation visible on gel.

The results of DNA extraction repeatability are shown in figure 3. 4 JetQuick® Genomic DNA Purification Kits (Genomed) and 2 High Pure PCR Template Preparation Kit (Roche) showed similar results (fig. 3) with concentrations ranging between 3.73 ng/μl to 3.92 ng/μl and 12.6 ng/μl and 12.7 ng/μl, respectively. Results from 5 Dneasy® Blood & Tissue Kit (Qiagen) displayed much higher variance. The minimal concentration of reference extraction (using the same amount of tissue) was 1.74 ng/μl, while the maximal concentration was 31.4 ng/μl.

The results of purity measurements are available in table 1. None of the tested kits showed A_{260}/A_{280} ratios within interval 1.8–2.0. Therefore, we defined pure DNA to be in interval 1.6–2.1. The best results according to these parameters were achieved using kits 4, 5 and 7. DNA concentration from other kits was lower than 10 ng/μl, which may have resulted in an inaccurate A_{260}/A_{280} ratio.

Price

Table 1 states the cost of DNA extraction. The price for 9 and 10 is from 2016; after this date MoBio Laboratory no longer produced the UltraClean® Tissue & Cells DNA Isolation Kit. We provide only an approximate price for 12 HotSHOT, because this approach uses basic chemicals, which are routinely available in molecular laboratories. The average price for a commercial kit per extraction is 4.12 € (median

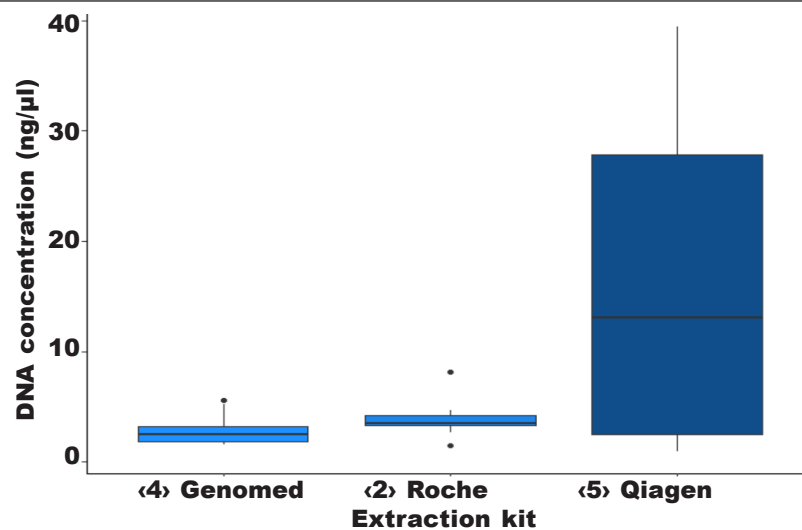


Fig. 3. Repeatability of DNA extraction using three reference kits: <4> JetQuick® Genomic DNA Purification Kits (Genomed), <2> High Pure PCR Template Preparation Kit (Roche), and <5> Dneasy® Blood & Tissue Kit (Qiagen). Boxplot represent relative DNA concentration accomplished by multiple isolation of DNA (16, 12, 12 samples respectively; more information in table 7s in supplementary material). Extraction kits <4> and <2> showed similar results, while <5> displayed much greater variance.

Fig. 3. Repetibilidad de la extracción de ADN utilizando tres kits de referencia: <4> JetQuick® Genomic DNA Purification Kits (Genomed), <2> High Pure PCR Template Preparation Kit (Roche) y <5> Dneasy® Blood & Tissue Kit (Qiagen). El diagrama de caja representa la concentración relativa de ADN alcanzada mediante el aislamiento múltiple de ADN (16, 12 y 12 muestras respectivamente; más información en la tabla 7s del material suplementario). Los kits de extracción <4> y <2> dieron resultados parecidos, mientras que con el <5> la varianza fue mucho mayor.

2.92 €). The price varied from less than 1€ <12> to 11.46 € <9>,10> per extraction (see table 1).

Discussion

The DNA quality, quantity and purity have crucial effect for downstream molecular analysis, therefore the methods of DNA extraction should be thoughtfully selected (Sagi et al., 2009). The main goal of this study was to compare DNA extraction (comprising yield, purity, cost and hands-on time) between twelve kits designed for DNA isolation. To make the comparison transparent, we defined three categories (best, average, worst) evaluating pros and cons of each kit in relation to hands-on time, DNA concentration, DNA purity and costs (table 2).

Time

One of the factors of DNA extraction is time (both the total extraction time and the time of lysis). Presented extraction kits differ in time of lysis, because of the different proteinase effectiveness. In general, longer lysis resulted in extracting higher amounts of DNA from the same amount of starting material (Janecka et al.,

2015). Nevertheless, there are extraction kits <1>, <3>, which provided 80–100% disrupted tissue 2 hours after lysis, while proteinases from other kits worked slower and took from 6 hours to overnight to provide results. To improve the yield of DNA and reduce lysis time, it is useful to disrupt tissue with pestles or beads and use extraction kits with effective Proteinase K (e.g. kits <1>, <3>).

There are extraction protocols without silica columns (e.g. <12>; Truett et al., 2000; Wang and Storm, 2006; Meeker et al., 2007; Montero-Pau, 2008; Jiang et al., 2014) that are not only fast (2 hours) and cheap but also allow extraction of 96 samples on a plate. Moreover, there are kits that provide super-fast DNA extraction (in less than 10'), such as QuickExtract™ DNA Extraction Solution (Lucigen). This protocol was used in recent studies: e.g., NGS barcoding (Kutty et al., 2018), gene expression (Fernández et al., 2012), and non-invasive seed extraction (Al-Amery et al., 2016). Al-Amery et al. (2016) compared this QuickExtract™ with the CTAB protocol and obtained similar results regarding quality and quantity of DNA.

Alternatively, there are extraction kits which use a bead homogenization instead of proteinase lysis (e.g. <8>, <9>; Guimaraes et al., 2011; Liakopoulos et al., 2014; Mavroidi et al., 2014; Delherbe, 2015; Minogue

Table 2. Comparison of the time, DNA concentrations, purity and costs between 12 extraction kits: *** short time, high concentration, good purity and good price; ** medium time, concentration, purity and price; * long time, low concentration of DNA, low purity and high price: \diamond in protocol was used Proteinase K for sample lysis. (For details see tables 3s–6s in supplementary material).

Tabla 2. Comparación del tiempo, las concentraciones de ADN, índice y los costos de los 12 kits de extracción. *** tiempo breve, concentración elevada, buena pureza y buen precio, ** tiempo, concentración, pureza y precio medios, * tiempo largo, concentración de ADN baja, baja pureza y precio elevado; \diamond en el protocolo se utilizó Proteinasa K para la lisis de la muestra). (Para obtener información más detallada, véanse las tablas 3s–6s del material suplementario).

Extraction kit	Time	Concentration of DNA	Purity of DNA	Cost
1 E.Z.N.A.® Tissue DNA Kit	**	***	**	***
2 High Pure PCR Template Preparation Kit	*	**	**	**
3 E.Z.N.A.® MicroElute Genomic DNA Kit	*	**	**	**
4 JetQuick® Genomic DNA Purification Kit	*	***	***	**
5 Dneasy® Blood & Tissue Kit	**	**	***	*
6 E.Z.N.A.® Forensic DNA Kit	**	*	**	***
7 NucleoSpin® Tissue	*	***	***	**
8 Quick-gDNA™ MiniPrep	***	*	*	***
9 UltraClean® Tissue & Cells DNA Isolation Kit	***	*	*	*
10 UltraClean® Tissue & Cells DNA Isolation Kit \diamond	***	*	*	*
11 Invisorb® Spin Tissue Mini Kit	**	***	**	*
12 HotSHOT	***	*	*	***

et al., 2015). These kits provide the possibility to extract DNA in less than one hour. Tomaso et al., (2010) compared five kits (QIAamp™ DNA Mini Kit (Qiagen), peqGold™ Tissue DNA Mini Kit (PeqLab), $\langle 9 \rangle$ UltraClean™ Tissue and Cells DNA Isolation Kit (MoBio), $\langle 2 \rangle$ DNA Isolation Kit for Cells and Tissues (Roche), and NucleoSpin™ Tissue (Macherey–Nagel) and with exception of $\langle 9 \rangle$ all of them yielded enough DNA for real-time PCR assay.

Measurement (Qubit, NanoDrop)

Determining DNA concentration and purity is an important step for downstream applications, such as polymerase chain reaction. Two types of measurements are preferable: fluorometry (Qubit) and spectrophotometry (e.g. NanoDrop, DeNovix). Qubit fluorometer calculates the total amount of DNA in one sample (O'Neill et al., 2011). The spectrophotometric instrument detects all particles that absorb light at 260 nm (DNA, RNA, single or double stranded, proteins, contaminants; O'Neill et al., 2011). Fluorometer and spectrophotometer results are not always correlated. Spectrophotometry measurements are usually higher than Qubit results, indicating that the DNA sample may contain a mixture of double- and single-stranded DNA, contaminants which scatter light, or UV-absorbing materials that are not nucleic acids (O'Neill et al., 2011). To take advantage we used both approaches for DNA quantification, Qubit values to determine concentration of double strand DNA and

spectrophotometer to obtain information about DNA purity. The difference in concentration measurements between Qubit and spectrophotometer could indicate the presence of single strand DNA, RNA, proteins and/or contaminants (in our case the biggest difference in extraction kit $\langle 12 \rangle$). The combination of both approaches provides the most complete and correct information about DNA sample quality (Simbolo et al., 2013) the quality of DNA can vary depending on the source or extraction method applied. Thus a standardized and cost-effective workflow for the qualification of DNA preparations is essential to guarantee interlaboratory reproducible results. The qualification process consists of the quantification of double strand DNA (dsDNA, Qubit measurements as the DNA quantity indication (DNA concentration), and spectrophotometry as the information about the DNA quality (purity ratio A_{260}/A_{280}).

Relationship of time, quality and price

When selecting an extraction method, many aspects are to be considered. The purity of the nucleic acid in obtained sample, the cost-effectiveness of the procedure, the duration of exposure to dangerous chemicals, the amount of hands-on time, and the related number of required steps must be taken into account (Boesenberg–Smith et al., 2012).

If the short-time extraction is needed, kits $\langle 8 \rangle$ – $\langle 10 \rangle$ or $\langle 12 \rangle$ should be used. If the high yield of DNA is the main concern, kits $\langle 1 \rangle$, $\langle 4 \rangle$, $\langle 7 \rangle$ or $\langle 11 \rangle$ are suitable. In case of

molecular methods requesting high quality pure DNA, kits <4>, <5> or <7> are preferable. Some laboratories have limited budgets, in which case we recommend kits <1>, <6>, <8>, <12>. In case of rare samples that demand good quality and quantity, it is advantageous to use <4> or <7>.

To sort this puzzle out, testing an extraction kit should belong to standard practice in any laboratory. The testing should include at least three different protocols. It is appropriate to optimize the selected extraction kit, e.g. amount of starting material, time of lysis and/or usefulness of performing 2nd elution. When the amount of starting material is larger than recommended, it is suggested in <11> protocol to double the amount of lysis buffer and proteinase. If extracting limited and rare samples, then the second elution is recommended because up to 42% of DNA is still bound to the membrane surface after the first elution (Janecka et al., 2015).

Conclusion and perspectives

Selection of the best DNA extraction kit depends on many factors: starting material, sample size, number of samples (single column extraction or 96-plate extraction), extraction time, expected concentration and purity (e.g., for microsatellites required DNA quality is lower compared to whole genome sequencing requirements) and price per one extraction. The kit choice can be adjusted according to special conditions of any samples (forensic, micro-samples, blood), price (big difference between kits), or extraction time. If many samples need to be processed, some extraction kits (Qiagen, Stratec, HotSHOT) provide the possibility to extract 96 samples in plates and thus significantly decrease the extraction time. Some kits, such as NucleoSpin (Macherey–Nagel), are forensic quality, treated to prevent DNA contamination. In conclusion, there is no best kit for any particular project, only the best solution. For this reason we strongly recommend trials be performed with 3–5 extraction kits in advance to check which kit provides the best results for specific samples.

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Supplementary material

Table 1s. Details about the DNA extraction. For each extraction kit we note: W, weight of sample (in g); c1, concentration of the first elution (ng/ul); c2, concentration of the second elution (ng/ul); c/W, relative concentration. ([◊] in protocol was used Proteinase K for sample lysis)

Extraction kit	Finger				
	W	c1	c2	c1/W	c2/W
1 E.Z.N.A.® Tissue DNA Kit	1.00	5.26	1.63	5.26	1.63
2 High Pure PCR Template Preparation Kit	1.70	4.44	2.91	2.61	1.71
3 E.Z.N.A.® MicroElute Genomic DNA Kit	1.70	5.23	2.08	3.08	1.22
4 JetQuick® Genomic DNA Purification Kit	2.10	3.68	2.48	1.75	1.18
5 Dneasy® Blood & Tissue Kit	1.55	12.40	12.00	8.00	7.74
6 E.Z.N.A.® Forensic DNA Kit	2.07	7.02	3.61	3.39	1.74
7 NucleoSpin® Tissue	1.75	13.40	13.00	7.66	7.43
8 Quick-gDNA™ MiniPrep	1.78	0.50	0.10	0.28	0.06
9 UltraClean® Tissue & Cells DNA Isolation Kit	1.63	0.50	NA	0.31	NA
10 UltraClean® Tissue & Cells DNA Isolation Kit [◊]	2.33	0.50	NA	0.21	NA
11 Invisorb® Spin Tissue Mini Kit	1.78	25.00	10.30	14.04	5.79
12 HotSHOT	1.97	1.19	NA	0.60	NA

Table 2s. Information about the lysis progress after 2 hours. Percentage represent ratio of lysis tissue (100%. fully dissolved tissue). ([◊] in protocol was used Proteinase K for sample lysis)

Tabla 2s. Información sobre el progreso de la lisis a las 2 horas. El porcentaje representa el índice de lisis del tejido (100 % . tejido totalmente disuelto). ([◊] en el protocolo se utilizó Proteinasa K para la lisis de la muestra).

Extraction kit	Lysis after 2 hours			
	Finger	Spleen	Tail	Blood
1 E.Z.N.A.® Tissue DNA Kit	100 %	95 %	100 %	100 %
2 High Pure PCR Template Preparation Kit	85 %	85 %	85 %	85 %
3 E.Z.N.A.® MicroElute Genomic DNA Kit	100 %	50 %	30 %	100 %
4 JetQuick® Genomic DNA Purification Kit	90 %	60 %	60 %	100 %
5 Dneasy® Blood & Tissue Kit	100 %	70 %	100 %	100 %
6 E.Z.N.A.® Forensic DNA Kit	100 %	80 %	90 %	100 %
7 NucleoSpin® Tissue	80 %	60 %	60 %	80 %
8 Quick-gDNA™ MiniPrep	no lysis step			
9 UltraClean® Tissue & Cells DNA Isolation Kit	no lysis step			
10 UltraClean® Tissue & Cells DNA Isolation Kit [◊]	short lysis step			
11 Invisorb® Spin Tissue Mini Kit	100 %	100 %	40 %	100 %
12 HotSHOT	no lysis step			

Tabla 1s. Información detallada sobre la extracción de ADN. Para cada kit de extracción tomamos nota de: W, peso de la muestra (en g); c1, concentración de la primera elución; c2, concentración de la segunda elución; c/W, concentración relativa. (° en el protocolo se utilizó *Proteinasa K* para la lisis de la muestra).

Spleen					Tail				Blood			
W	c1	c2	c1/W	c2/W	W (g)	L (mm)	c1	c2	c1/W	c2/W	c1	c2
4.70	21.10	6.68	4.49	1.42	6.40	9.00	86.40	8.04	13.50	1.26	11.90	14.50
4.70	11.50	7.25	2.45	1.54	8.80	7.00	49.60	14.30	5.64	1.63	2.63	3.95
6.30	8.16	4.04	1.30	0.64	4.10	7.00	93.50	4.27	22.80	1.04	13.30	8.88
5.90	12.00	2.15	2.03	0.36	9.70	7.00	43.00	9.98	4.43	1.03	10.80	17.30
6.60	10.90	9.65	1.65	1.46	7.50	7.00	49.90	35.50	6.65	4.73	8.72	10.80
7.70	1.44	0.50	0.19	0.06	10.30	7.00	25.10	6.51	2.44	0.63	1.70	1.27
6.50	17.90	17.60	2.75	2.71	11.00	7.00	83.80	26.30	7.62	2.39	8.10	2.99
5.55	1.15	0.50	0.21	0.09	9.60	7.00	0.50	NA	0.05	NA	0.50	NA
7.58	0.50	NA	0.07	NA	8.50	3.00	0.50	NA	0.06	NA	0.50	NA
4.82	0.50	NA	0.10	NA	7.42	7.00	0.50	NA	0.07	NA	0.50	NA
6.00	28.70	6.82	4.78	1.14	7.10	7.00	87.00	32.40	12.25	4.56	16.40	7.54
2.08	4.08	NA	1.96	NA	2.54	7.00	1.41	NA	0.56	NA	1.46	NA

Table 3s. Order of extraction kits based on sum of relative concentration, including the first and the second elution for all four kind of tissue (finger, spleen, tail, blood). (° in protocol was used *Proteinase K* for sample lysis).

Tabla 3s. Kits de extracción ordenados en función de la suma de la concentración relativa, incluidas las eluciones primera y segunda para los cuatro tipos de tejido (dedo, bazo, cola y sangre). (° en el protocolo se utilizó *Proteinasa K* para la lisis de la muestra).

Extraction kit	Sum of reative concentration (1st + 2nd for finger, spleen, tail and blood)	
11 Invisorb® Spin Tissue Mini Kit	66.50836208	Good
1 E.Z.N.A.® Tissue DNA Kit	52.7006383	Good
7 NucleoSpin® Tissue	41.64634366	Good
4 JetQuick® Genomic DNA Purification Kit	38.89349409	Good
3 E.Z.N.A.® MicroElute Genomic DNA Kit	28.41650794	Medium
5 Dneasy® Blood & Tissue Kit	28.1037449	Medium
2 High Pure PCR Template Preparation Kit	20.52925475	Medium
6 E.Z.N.A.® Forensic DNA Kit	11.42614579	Medium
12 HotSHOT	4.580717485	Low
8 Quick-gDNA™ MiniPrep	1.134375949	Low
9 UltraClean® Tissue & Cells DNA Isolation Kit	0.872711527	Low
10 UltraClean® Tissue & Cells DNA Isolation Kit °	0.818326715	Low

Table 4s. Order of extraction kits based on sum of the time: L, lysis (h); HT, hand-on time (min.); TT, total time (min.). (° in protocol was used Proteinase K for sample lysis).

Tabla 4s. Kits de extracción ordenados en función de la suma del tiempo: L, lisis (h); HT, tiempo de manipulación (min.); TT, tiempo total (min.). (° en el protocolo se utilizó Proteinasa K para la lisis de la muestra).

Extraction kit	L	HT	TT	
8 Quick-gDNA™ MiniPrep	0	50	50	Short
9 UltraClean® Tissue & Cells DNA Isolation Kit	0	50	50	Short
10 UltraClean® Tissue & Cells DNA Isolation Kit °	0,5	50	80	Short
12 HotSHOT	0	120	120	Short
1 E.Z.N.A.® Tissue DNA Kit	3	50	230	Medium
6 E.Z.N.A.® Forensic DNA Kit	4	50	290	Medium
5 Dneasy® Blood & Tissue Kit	5	40	340	Medium
11 Invisorb® Spin Tissue Mini Kit	6	35	395	Medium
7 NucleoSpin® Tissue	6	45	405	Long
2 High Pure PCR Template Preparation Kit	10	45	645	Long
3 E.Z.N.A.® MicroElute Genomic DNA Kit	10	50	650	Long
4 JetQuick® Genomic DNA Purification Kit	10	55	655	Long

Table 5s. List of extraction kits with result of purity measurements obtained by spectrophotometer. Purity ratio A_{260}/A_{280} for finger, spleen, tail blood. * mean out of recommended values. (° in protocol was used Proteinase K for sample lysis).

*Tabla 5s. Lista de kits de extracción con los resultados de las mediciones de la pureza obtenidas en el espectrofotómetro: Relación de pureza A_{260}/A_{280} para el dedo, el bazo y sangre de la cola: * media fuera de los valores recomendados, (° en el protocolo se utilizó Proteinasa K para la lisis de la muestra).*

Extraction kit	Purity ration				
1 E.Z.N.A.® Tissue DNA Kit	2,16*	1,91	1,9	2,01	Medium
2 High Pure PCR Template Preparation Kit	2,33*	1,74	1,87	1,76*	Medium
3 E.Z.N.A.® MicroElute Genomic DNA Kit	2,07*	1,61*	1,87	1,68	Medium
4 JetQuick® Genomic DNA Purification Kit	2,07*	2,01	1,89	1,83	Good
5 Dneasy® Blood & Tissue Kit	1,95	1,78	1,81	2,1	Good
6 E.Z.N.A.® Forensic DNA Kit	1,64	1,85*	1,74	1,33*	Medium
7 NucleoSpin® Tissue	2,02	2,03	1,86	1,95	Good
8 Quick-gDNA™ MiniPrep	-0,99*	0,56*	0,36*	0,62*	Low
9 UltraClean® Tissue & Cells DNA Isolation Kit	1,04*	1,06*	0,97*	1,2*	Low
10 UltraClean® Tissue & Cells DNA Isolation Kit °	0,96*	1,01*	1,88*	1,38*	Low
11 Invisorb® Spin Tissue Mini Kit	2,09	2,12	1,87	1,96	Medium
12 HotSHOT	1,7*	1,94*	1,66*	2,02*	Low

Table 6s. Order of extraction kits based on cost per reaction (in €). ([◊] in protocol was used Proteinase K for sample lysis).

Tabla 6s. Kits de extracción ordenados en función del costo por reacción (euros). ([◊] en el protocolo se utilizó Proteinasa K para la lisis de la muestra.)

Extraction kit	Cost for 1 reaction
12 HotSHOT	< 1 Low
1 E.Z.N.A.® Tissue DNA Kit	1.82 Low
8 Quick-gDNA™ MiniPrep	2.19 Low
6 E.Z.N.A.® Forensic DNA Kit	2.37 Low
4 JetQuick® Genomic DNA Purification Kit	2.46 Medium
7 NucleoSpin® Tissue	2.79 Medium
2 High Pure PCR Template Preparation Kit	3.05 Medium
3 E.Z.N.A.® MicroElute Genomic DNA Kit	3.10 Medium
11 Invisorb® Spin Tissue Mini Kit	3.37 High
5 Dneasy® Blood & Tissue Kit	4.42 High
9 UltraClean® Tissue & Cells DNA Isolation Kit	11.46 High
10 UltraClean® Tissue & Cells DNA Isolation Kit [◊]	11.46 High

Table 7s. List of samples used for repeatability analysis: W, weight (mg); C, concentration (ng/ul).

Tabla 7s. Lista de las muestras utilizadas para los análisis de repetibilidad: W, peso (mg); C, concentración (ng/ul).

ID	Sample	W	C	ID	Sample	W	C
1	JetQuick	1.98	3.73	5	Roche	1.58	4.31
2	JetQuick	3.18	5	6	Roche	2.12	7.1
3	JetQuick	2.09	11.8	7	Roche	1.69	5.4
4	JetQuick	2.84	12.6	8	Roche	1.63	7.61
5	JetQuick	2.17	4.23	9	Roche	1.5	6.59
6	JetQuick	2.44	5.53	10	Roche	2.57	3.92
7	JetQuick	2.01	3.82	11	Roche	1.9	6.28
8	JetQuick	1.97	5.32	12	Roche	1.56	12.7
9	JetQuick	1.74	6.65	1	Qiagen	1.9	3.01
10	JetQuick	2.42	7.47	2	Qiagen	2.22	5.79
11	JetQuick	2.07	10.9	3	Qiagen	1.53	11.1
12	JetQuick	3	7.34	4	Qiagen	1.92	1.74
13	JetQuick	2.47	6.53	5	Qiagen	1.74	7.61
14	JetQuick	2.5	4.49	6	Qiagen	0.79	14.9
15	JetQuick	2.48	7.23	7	Qiagen	0.9	24.1
16	JetQuick	2.69	4.2	8	Qiagen	0.97	25.9
1	Roche	2.02	7.29	9	Qiagen	0.97	30.3
2	Roche	1.95	8.13	10	Qiagen	0.98	31.4
3	Roche	2.09	7.36	11	Qiagen	0.72	28.4
4	Roche	2.11	7.56	12	Qiagen	1.32	2.78

