# Comparative analysis of different methods of DNA extraction from human breast tumor samples

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## RESUMO

**Introdução:** Uma grande parte das pesquisas realizadas em todo o mundo depende das amostras armazenadas, que em sua maioria, serão submetidas ao processo de extração do material genético para análises em biologia molecular. Essas são fontes extremamente valiosa para muitos estudos. Estes incluem a pesquisa de mutações em genes críticos para o câncer, bem como a detecção de patógenos. **Objetivo:** O objetivo desse estudo foi avaliar o impacto de diferentes métodos de extração de DNA de tecido parafinado, congelado e amostras de sangue. **Resultado:** Nós comparamos 13 métodos diferentes para a extração de DNA. Todas 63 amostras (20 tecidos parafinados, 23 tecidos congelados frozen e 20 amostras de sangue) foram obtidas de mulheres que realizavam tratamento de câncer de mama no Hospital de Câncer de Barretos. A pureza e rendimento do DNA extraído foi avaliado pelo NanoDrop® Spectophotometer. O fragmento de DNA amplificável foi avaliado por amplificação por PCR do gene APC e todas as extrações utilizadas independentemente da amostra foram consideradas adequadas para a amplificação de fragmentos de até 311 pb. **Conclusão:** Foi verificado que o QIAamp DNA FFPE Tissue kit apresentou melhor qualidade para as amostras de FFPE e também ótimo rendimento. Em relação as amostras de tecido fresco, foi visto ótimos valores de pureza e rendimento para o QIAamp DNA Mini and Blood Mini Kit. As amostras de buffy coat apresentaram melhores resultados com o QIAamp DNA Mini Blood and Mini Kit. Nós concluímos que o método de extração desempenha um papel importante em relação ao desempenho em aplicações moleculares.

**Palavras-chave**: extração de DNA, Buffy coat, FFPE, tecido congelado.

## ABSTRACT

**Introduction:** A large portion of researches conducted worldwide depend on stored samples that will most often be subjected to the process of extracting genetic material for molecular biology analysis. These samples archived are an extremely valuable source for retrospective genetic studies. These include mutation screening in cancer-critical genes as well as pathogen detection. **Aim:** The aimed of this study were to evaluate different methods of DNA extraction in frozen tissue, FFPE and blood. **Results:** We compared 13 different DNA extraction methods. All 63 samples (20 FFPE tissues, 23 frozen tissues and 20 blood) were obtained from women undergoing breast cancer treatment at Barretos Cancer Hospital. The purity and yield of the extracted DNA were evaluated by NanoDrop® Spectophotometer. All extractions independently sample used were found suitable for amplification of fragments up 311 bp amplification was marginally successful. **Conclusion:** QIAamp DNA FFPE Tissue kit was found to have better quality for FFPE samples and also excellent yield. For frozen tissue samples, excellent purity and yield values were seen for QIAamp DNA Mini and Blood Mini Kit. Buffy coat samples showed better results with QIAamp DNA Mini Blood and Mini Kit. We conclude that the extraction method plays an important role regarding performance in molecular applications.

**Keywords**: DNA extraction, Buffy coat, FFPE, frozen tissue.

## INTRODUCTION

Human cancer research is generally conducted on a large scale to find molecular aberrations at various levels, such as hybridization, DNA sequencing, transcriptomics and proteomics analyses[1]. The initial condition to development molecular analysis is quality control of acid nucleic. However, several factors including extraction and storage methods, type of sample as well as type of the extracted nucleic acid (DNA or RNA) have a direct impact on the success of the purification[2].

Sample storage routinely done biobanks with research purposes. Even even though extracted DNA is recognized for remaining stables under long storages at low temperatures, some studies have shown that DNA isolated from different samples can have issues concerning quality and integrity[3,4]. The initial step start research projects in molecular biology is the quality of biological material, and there are different types of methods that can be used to nucleic acids isolation including DNA2 and the extraction of biological material may affect posterior efficacy[5].

Currently, efficient methods for DNA extraction have been used and reported in the literature[6–8.] Technology advancement has become possible to extract DNA and RNA several commercial kits, it is necessary to know which method should be used for certain samples types. Dietrich et al., 2013 demonstrate that Formalin-fixed, Paraffin-embedded (FFPE) tissue is the most widely used for molecular testing[9]. However, the extraction of nucleic acid from FFPE may be a hindrance to some experiments performed later, because most these samples have high levels of degradation and represents a poor substrate for molecular biology methods[10,11].

Several studies[7,8] have compared different methods for genomic DNA extraction, through both manual and automated techniques. Nevertheless, the integrity of DNA extracted by each method has not been evaluated, which mostly been based on small sample sizes. Additionally, some methods used for DNA isolation may act as inhibitors for others technique, such as PCR real time and this factor may influence a reliable search result[5,9,12]. It became necessary to investigate witch methods may offer best yield and quality for the extracted biological material. The aimed of this study were to evaluate different methods of DNA extraction in frozen tissue, FFPE and blood.

## MATERIAL AND METHODS

### Study population and sample preparation

This study included a total of 63 samples (20 FFPE tissues, 23 frozen tissues and 20 blood) from breast cancer patients were provided from the Barretos Cancer Hospital between 2011 and 2012. The samples had been irreversibly anonymized and no clinical or patient-related information was available. Breast FFPE and frozen tissues samples were obtained from patients undergoing mastectomy. Five sections with 10 $\mu$m per sample per kit were cut from each patient block on a standard microtome (Reichert- Jung Hn40; Leica Instruments, Wetzlar, Germany) from FFPE samples, placed into individual 1.5-mL microcentrifuge tubes (Eppendorf, Hamburgo, Germany) and stored at 4 °C until using. Frozen tissue slides was prepare using a cryostat (CM 3050; Leica Instruments, Wetzlar, Germany) for tumor percentage verification. The cuts were performed with minimum weights of 20mg and maximum of 30mg. Venous blood samples collected in two collection tubes (4ml) with ethylenediamine tetraacetic acid (EDTA) from patients undergoing treatment at Barretos Cancer Hospital. Buffy coat fraction were isolated, processed and stored for -80 ° C. We do not evaluate the storage time of these materials.

### DNA extraction

We compared 13 different methods for extracting DNA, in which 3 from FFPE tissue, 6 from frozen tissue and 4 from buffy coat. For FFPE samples we used five section per sample and we extracted DNA whole tumor area each block. All samples were submitted to all analyzed methods. Three methods were developed at QIAamp DNA FFPE Tissue (Qiagen, Hilden, Germany), PureLink Genomic DNA Kit (Thermo Fisher Scientific, Waltham, MA, USA) and Illustra Nucleon Genomic DNA Extraction Kit (GE, Healthcare Life Sciences, Little Chalfont, United Kingdon). For frozen tissue sample we used five different methods for DNA extracting, DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), QIAamp DNA Mini and Blood Mini Kit (Qiagen, Hilden, Ger-

many), PureLink Genomic DNA Kit,Illustra Tissue and Cells genomicPrep Mini Flow Kit (Thermo Fisher Scientific, Waltham, MA, USA), AllPrep DNA/RNA Mini Kit, Illustra Triple Prep Kit (GE, Healthcare Life Sciences, Little Chalfont, United Kingdom) and AllPrep DNA/RNA Micro Kit (Qiagen, Hilden, Germany). For buffy coat DNA extraction were used four methods, QIAamp DNA Mini Blood and Mini Kit (Qiagen, Hilden, Germany), PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA), Illustra Blood GenomicPrep Mini Spin Kit (GE, Healthcare Life Sciences, Little Chalfont, United Kingdom) e DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). For all DNA extraction methods, we followed the manufacturer's instructions. The purity of total DNA was evaluated by NanoDrop® Spectophotometer v3.7 (Thermo Fisher Scientific, Waltham, MA, USA).

### Conventional PCR

We evaluated the presence of amplification inhibitors (compounds that impair the efficiency of the PCR reaction). This presence was assessed by amplifying the APC gene, amplifying 206 base pairs (bp) of exon 8 for samples extracted from FFPE, and 847 base pairs of exon 15B of samples extracted from frozen tissue and buffy coat. The amplification of genomic DNA for the APC promoter region was performed with the following primers: forward 5'-TTTTGTTTGTTGGGGATTG-3' and reverse 5'-CTCCAACACCTACCCCATTT-3'. PCR conditions consisted of an initial heating at 95°C for 15 minutes followed by 40 cycles at 95°C for 30 seconds, 60°C for 20 seconds and 72°C for 30 seconds. Subsequently, these PCR products were evaluated in a 2% agarose electrophoresis gel.

### Statistical analysis

Friedman test were used to compared quality of DNA extraction methods. Wilcoxon test was performed to find out the difference between each of the kits when compared. P-values of <0.05 were considered statistically significant. All analyses were performed with SPSS for Windows, v.21.0 (IBM Corporation).

### Ethics statement

This study was approved by the Research Ethics Committee of the Barretos Cancer Hospital under Protocol No. 131/2008. Each research participant provided written informed consent for the publication of any data. All information that could be used to identify the study participants was kept confidential and encrypted in a secure database to ensure full confidentiality of clinical information, laboratory findings and the anonymity of each participant.

## RESULTS

QIAamp DNA FFPE Tissue kits, PureLink Genomic DNA Kit and Illustra Nucleon Genomic DNA Extraction Kit that were used for DNA extraction from FFPE showed statistically significant differences (Table 1 and Figure 1A). The QIAamp DNA FFPE Tissue kit showed better quality over A260/280 absorbance, with an average of 2.1 and A260/230 absorbance with an average of 1.8. However, the Illustra Nucleon Genomic DNA Extraction Kit has shown a concentration that can be observed an average of 216 ng /$\mu$l. When compared A260/280 absorbance, we observed a significant differencel between Illustra Nucleon Genomic DNA Extraction Kit and the QIAamp DNA FFPE Tissue kits. For the A260/280 absorbance we verified PureLink Genomic DNA and QIAamp DNA FFPE Tissue Kits were statistically different with better quality scores compared to Purelink Genomic DNA.

All methods used for DNA extraction from frozen tissue were significantly different for all parameters analyzed (Table 2 and Figure 1B).

QIAamp DNA Mini Blood and Mini Kit, PureLink Genomic DNA Mini Kit, Illustra Blood GenomicPrep Mini Spin Kit and DNeasy Blood & Tissue Kit that were used for DNA extraction from buffy coat showed statistically significant differences for A260/230 and the concentration (ng /$\mu$l) (table 3 and figure 1c). The QlAmmp DNA Mini Blood Kit and Mini Kit demonstrated better quality for A260/280 (mean= 1.9, SD= 0.3) and A280/230 (mean= 1.8, SD= 0.2), well as the better concentration yield (mean= 274 ng/$\mu$l, SD= 205). However, we observed the comparison between Illustra Blood GenomicPrep Mini Spin and QlAamp Mini Blood and Mini Kit kits

**Table 1.** Comparative analysis between the different method used for DNA extraction from FFPE.

| | QIAamp DNA FFPE Tissue | PureLink Genomic DNA Kit | Illustra Necleon Genomic DNA Extration Kit | p value |
|---|---|---|---|---|
| **A260/280** | 2.1 (SD= 0.1)[a] | 2.3 (SD=1.3)[b] | 1.8 (SD= 0.3)[b] | 0.003* |
| **A260/230** | 1.7 (SD= 0.3)[b] | 1.1 (SD= 0.5)[a] | 1.4 (SD= 0.6)[b] | <0.001* |

Different letters (a and b) indicate statistical significance between the groups. *P< 0.05. The analysis was performed

**Table 2.** Comparative analysis between the different method used for DNA extraction from frozen tissue.

| | QIAamp DNA Mini and Blood Mini Kit | PureLink Genomic DNA Kit | Illustra Tissue and Cells genomicPrep | All Prep DNA/ RNA Mini Kit | Illustra Triple Prep Kit | All Prep DNA/RNA Micro Kit | p value |
|---|---|---|---|---|---|---|---|
| **A260/280** | 2.1 (SD= 0.1)[a] | 1.9 (SD= 0.1)[b] | 1.9 (SD= 0.1)[b] | 1.9 (SD= 0.1)[b] | 2.1 (SD= 0.1)[a] | 2.0 (SD= 0.1)[a] | <0.001* |
| **A260/230** | 1.9 (SD= 0.4)[b] | 2.2 (SD= 0.1)[b] | 2.8 (SD= 0.4)[a] | 1.3 (SD= 0.7)[d] | 1.7 (SD= 0.6)[b] | 1.0 (SD= 0.5)[b] | <0.001* |

Different letters (a, b, c and d) indicate statistical significance between the groups. *P< 0.05. The analysis was performed with the mean values.

"Conc.= concentration"; "sd= standard deviation".

**Table 3.** Comparative analysis between the different method used for DNA extraction from buffy coat.

| | QIAamp DNA Mini Blood and Mini Kit | PureLink Genomic DNA Mini Kit | Illustra Blood GenomicPrep Mini Spin Kit | DNeasy Blood & Tissues Kit | p value |
|---|---|---|---|---|---|
| **A260/280** | 1.9 (SD= 0.3) | 1.8 (SD= 0.3) | 1.8 (SD= 0.1) | 1.9 (SD= 0.1) | 0,08 |
| **A260/230** | 1.8 (SD= 0.2)[a] | 1.2 (SD= 0.4)[b] | 1.3 (SD= 0.5)[b] | 1.7 (SD= 0.4)[a] | <0,001* |

Different letters (a, b and c) indicate statistical significance between the groups. *P< 0.05. The analysis was performed with the mean values.

"Conc.= concentration"; "sd= standard deviation".

did not showed significant statistical differences for A260/280, A260/230 absorbance and yields in terms of concentration (ng/$\mu$l). We identify a significant difference when comparing the A260/230 absorbance, concentration between PureLink Genomic DNA Mini Kit and QlAamp DNA Mini Blood and mini Kit. This same result was found in the analysis between PureLink Genomic DNA Mini Kit and DNeasy Blood & Tissue Kit. Comparison between Illustra Blood GenomicPrep Mini Spin Kit and PureLink Genomic DNA Mini Kit we observed statistical difference only for concentration(ng/$\mu$l). We found that the desired amplification occurred for all DNA extracts regardless of DNA extraction procedure performed. Considering the PCR efficiency of each run, it was considered there are not PCR inhibitors in the DNA extracts regardless of the method used.

We performed a comparison between 11 DNA extraction kits. We estimated time to perform the procedure and the cost per sample of each kit (Table 4). Thus, we observe that the fastest method for extracting DNA from FFPE was QlAamp DNA FFPE Tissue. However, this was the most expensive kit compared to the others used, and the PureLink Genomic DNA being the most less expensive. Considering time analysis for DNA extraction from tissue, we observed that QIAamp DNA Mini and Blood Mini Kit, PureLink Genomic DNA, AllPrep DNA / RNA Micro Kit, and AllPrep DNA/RNA Mini Kit were the fastest kits. PureLink Genomic DNA and Illustra Tissue and Cells GenomicPrep Mini Flow Kit were the lowest cost kits, on the other hand, it was seen that AllPrep DNA/RNA Micro Kit and AllPrep DNA / RNA Mini Kit were the most expensive, priced approximately five times. larger than the cheapest kit for frozen tissue DNA extraction. The fastest kit for extracting DNA from blood was the PureLink Genomic DNA Mini Kit and Illustra Blood GenomicPrep Mini Spin Kit, which were also the least expensive.

## DISCUSSION

DNA extraction is the first step to develop molecular analysis from human samples and contributes to research in several fields such as treatment, diagnosis and prognosis of various diseases, including cancer. Given this, DNA extraction performed by kits is a very effective tool because they are often less toxic, have good quality, are faster, practical and reproducible. Different methods of DNA extraction from FFPE, buffy coat and frozen tissue samples have been reported in the literature demonstrating the low potential of FFPE extracted samples due to the high degradation rate of the genetic material that is conserved, as well as the PCR inhibitors that are present in all samples analyzed in this study[9]. For this reason, commercial kits can reduce the amount of PCR inhibitors of both the sample and the extraction method itself[13] as they use a relatively smaller amount of phenol-chloroform, which is already a huge advance in technology. We also need to know if the other characteristics such as yields (concentration) and qualities (A260/230 and A260/280 ratios) that are necessary for the realization of good molecular techniques are also present in these commercial kits.

Given these factors, we observed that the samples extracted from FFPE the QlAamp DNA FFPE Tissue kit showed A260/280, A260/230 ratios and good concentration when compared to the other analyzed kits more desirable compared to other kits. AllPrep DNA / RNA Micro Kit and Illustra Triple Prep Kit which showed good results on A260/280 ratios, but no good results were found in the other variables analyzed. The QlAamp DNA Mini Blood Kit and Mini Kit showed better results for samples extracted from blood, showing better ratios, concentrations when compared to the other kits used. For frozen tissue DNA extraction kits, we can also observe that QIAamp DNA Mini and Blood Mini Kit presented a good A260/230 ratio, however no good results were observed in the other variables.

Given these results we can see that the DNA extraction kits manufactured by Qiagen (Hilden, Germany) showed more satisfactory results as demonstrated by Clausen et al. (2007)[14], who evaluated DNA extraction by two distinct kits both from Qiagen (Hilden, Germany) and demonstrated good efficiency and quality. A study compared eleven methods for the extraction of genomic DNA from blood samples in which they found that the methods used by commercial kits and other in-house showed better results[15]. Huijsmans et al. 2010 found that molecular techniques for the extraction of paraffin DNA are important for the preparation of subsequent reactions, so it is necessary that this technique be performed better to reduce the obstacles to the methods that will be per-

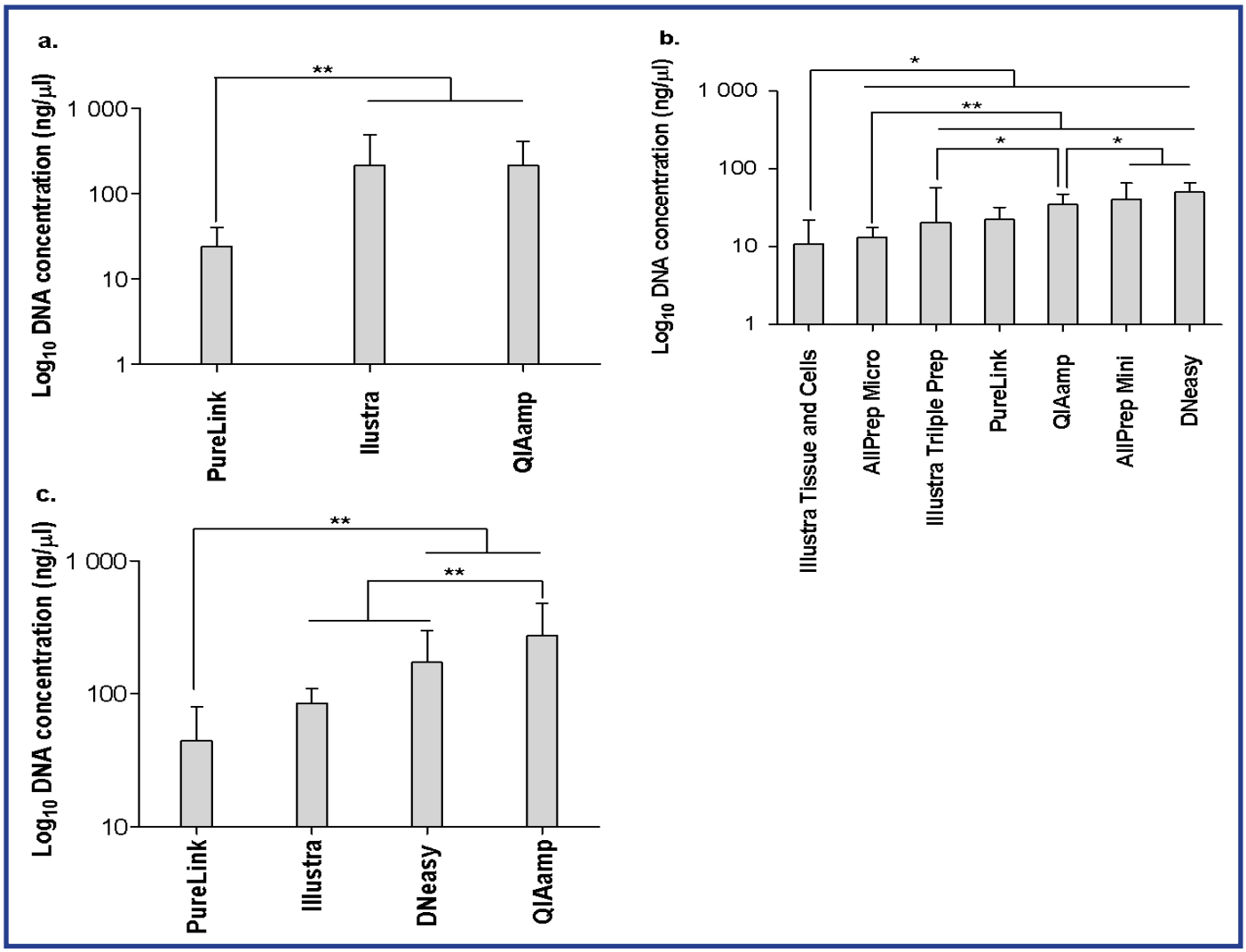


**Figure 1.** Bar graphs representing the DNA concentration between the different methods used for DNA extraction. (a) methods used for DNA extraction from FFPE tissue; (b) methods used for DNA extraction from frozen tissue; (c) methods used for buffy coat DNA extraction. *P ≤0.05.

formed later[16]. Some kits for FFPE extraction, and of the four methods tested, QIAamp DNA-blood-mini--kit extraction and EasyMAG NucliSens extraction showed better performance for SNP detection in real time PCR.

Illustra Triple Prep Kit and AllPrep DNA / RNA Micro Kit were tested in the work of Mathieson and Thomas (2013) and had poorer yields than Puregene Kit, and this may have been due to the fact that extractions were performed[2]. Simultaneous DNA and RNA in the same material, on the other hand, it was observed in this study that the kits tested in the mentioned work presented satisfactory yields. However, they were not the best when compared with the other kits used by the same samples. Another problem with the Illustra Triple Prep and AllPrep kits is that the RNA solution is centrifuged through a ligation column from previous DNA solutions, because of this there is a risk that RNA can bind to the DNA column and therefore the experiment becomes lost2. Therefore, greater care should be taken in conducting this experiment, as a lack of professional attention can lead to irreversible damage in the search for a good result.

The quality checking of the extracted DNA samples was performed by amplifying the APC gene and after performing the PCR product run on an electrophoresis gel. Thus, it can be seen that this gene remained intact in all extracted samples. For all kits, this result was similar to that found by Russ et al. (2016) who obtained a reproducible PCR product from DNA extraction by Kit12.

**Table 4.** Cost-per-sample evaluation and estimated duration of DNA extraction from FFPE, frozen tissue and buffy coat.

| Method | Estimated process duration (24 samples) | Cost per sample (€) |
|---|---|---|
| **FFPE** | | |
| Illustra Nucleon Genomic DNA Extraction Kit | 2 days | 5.48 |
| PureLink Genomic DNA Kit | 2 days | 2.64 |
| QIAamp DNA FFPE Tissue | 3.5 hour | 7.42 |
| **Frozen tissue** | | |
| AllPrep DNA/RNA Micro Kit | 35 minutes | 14.68 |
| AllPrep DNA/RNA Mini Kit | 35 minutes | 10.40 |
| DNeasy Blood & Tissue Kit | 1 hour | 5.30 |
| Illustra Tissue and Cells GenomicPrep Mini Flow Kit | 3 hour | 3.18 |
| Illustra Triple Prep Kit | 2 hour | 9.38 |
| PureLink Genomic DNA Kit | 2 days | 2.64 |
| QIAamp DNA Mini Blood and Mini Kit | 40 minutes | 5.58 |
| **Buffy coat** | | |
| DNeasy Blood & Tissue Kit | 40 minutes | 5.30 |
| Illustra Blood GenomicPrep Mini Spin Kit | 30 minutes | 2.13 |
| PureLink Genomic DNA Mini Kit | 30 minutes | 2.64 |
| QIAamp DNA Mini Blood and Mini Kit | 40 minutes | 5.58 |

## CONCLUSION

We conclude that FFPE Tissue kit was found to have better quality for FFPE samples and also excellent yield. For frozen tissue samples, excellent purity and yield values were seen for QIAamp DNA Mini and Blood Mini Kit. Buffy coat samples showed better results with QIAamp DNA Mini Blood and Mini Kit. All extractions independently sample used were found suitable for amplification of fragments up 206bp amplification was marginally successful. Thus, the method used for DNA isolation those samples should improve next steps in molecular biology research.

## ABBREVIATIONS

DNA – Deoxyribonucleic acid
PCR – polymerase chain reaction quantitative real time
FFPE – Formalin-fixed, Paraffin-embedded bp – base pair

## ACKNOWLEDGMENTS

The authors would like Biobank of Barretos Cancer Hospital, 1331 Rua Antenor Duarte Villela, Barretos, São Paulo, 14784-400, Brazil for managing and performing DNA extraction.

## COMPETING INTEREST

The authors have no conflicts of interest to disclose.

## REFERENCES


1. Grzendowski M, Riemenschneider MJ, Hawranke E, Stefanski A, Meyer HE, Reifenberger G et al. Simultaneous extraction of nucleic acids and proteins from tissue specimens by ultracentrifugation: A protocol using the high-salt protein fraction for quantitative proteome analysis. Proteomics. 2009; 9: 4985–4990.
2. Mathieson W, Thomas GA. Simultaneously extracting DNA, RNA, and protein using kits: is sample quantity or quality prejudiced? Anal Biochem. 2013; 433: 10–18.
3. Mychaleckyj JC, Farber EA, Chmielewski J, Artale J, Light LS, Bowden DW et al. Buffy coat specimens remain viable as a DNA source for highly multiplexed genome-wide genetic tests after long term storage. J Transl Med. 2011; 9: 91.
4. Rahila C, Narayanan MBA, Kirubaharan JJ, Kumar SGR, Selvamary AL, Sujatha A. Influence of Storage Methods of Whole Blood Samples on DNA Integrity in Epidemiological Studies. J Pierre Fauchard Acad India Sect. 2018; 31: 89–92.
5. Hassani A, Khan G. A simple procedure for the extraction of DNA from long-term formalin-preserved brain tissues for the detection of EBV by PCR. Exp Mol Pathol. 2015; 99: 558–563.
6. Spalletti-Cernia D, Barbato S, Sorrentino R, Vallefuoco L, Rocco C, Di Costanzo P et al. Evaluation of the Automated QIAsymphony SP/AS Workflow for Cytomegalovirus DNA Extraction and Amplification from Dried Blood Spots. Intervirology. 2017; 59: 211–216.
7. Pérez-Barrios C, Nieto-Alcolado I, Torrente M, Jiménez--Sánchez C, Calvo V, Gutierrez-Sanz L et al. Comparison of methods for circulating cell-free DNA isolation using blood from cancer patients: impact on biomarker testing. Transl Lung Cancer Res. 2016; 5: 665–672.
8. Kalousová M, Levová K, Kuběna AA, Jáchymová M, Franková V, Zima T. Comparison of DNA Isolation Using Salting-Out Procedure and Automated Isolation (MagNA System). Prep Biochem Biotechnol. 2017. doi:10.1080/10826068.2017.1303613.
9. Dietrich D, Uhl B, Sailer V, Holmes EE, Jung M, Meller S et al. Improved PCR performance using template DNA from formalin-fixed and paraffin-embedded tissues by overcoming PCR inhibition. PloS One. 2013; 8: e77771.
10. Blow N. Tissue preparation: Tissue issues. Nature. 2007; 448: 959–963.
11. Bereczki L, Kis G, Bagdi E, Krenacs L. Optimization of PCR amplification for B- and T-cell clonality analysis on formalin-fixed and paraffin-embedded samples. Pathol Oncol Res POR. 2007; 13: 209–214.
12. Russ PK, Karhade AV, Bitting AL, Doyle A, Solinas F, Wright DW et al. A Prototype Biomarker Detector Combining Biomarker Extraction and Fixed Temperature PCR. J Lab Autom. 2016; 21: 590–598.
13. Huggett JF, Foy CA, Benes V, Emslie K, Garson JA, Haynes R et al. The digital MIQE guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments. Clin Chem. 2013; 59: 892–902.
14. Clausen FB, Krog GR, Rieneck K, Dziegiel MH. Improvement in fetal DNA extraction from maternal plasma. Evaluation of the NucliSens Magnetic Extraction system and the QIAamp DSP Virus Kit in comparison with the QIAamp DNA Blood Mini Kit. Prenat Diagn. 2007; 27: 6–10.
15. Psifidi A, Dovas CI, Bramis G, Lazou T, Russel CL, Arsenos G et al. Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using blood samples. PloS One. 2015; 10: e0115960.
16. Huijsmans CJ, Damen J, van der Linden JC, Savelkoul PH, Hermans MH. Comparative analysis of four methods to extract DNA from paraffin-embedded tissues: effect on downstream molecular applications. BMC Res Notes. 2010; 3: 239.



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