Rapid extraction of DNA from limited FFPE material derived from lung tumour of engineered mice

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Introduction

Biological material obtained from biopsies is highly valuable for clinical diagnostics, as well as pharmacological/clinical research. Millions of these tissues are preserved and archived in formalin-fixed paraffin-embedded (FFPE) blocks. These blocks are cut into slides and these slides are used for down-stream analysis such as immunohistochemistry, qPCR/PCR and sequencing. Several commercially available nucleic acid extraction kits require a minimum of one 10µm slide of starting material to extract enough genomic DNA (gDNA) for down-stream analysis. These blocks are extremely precious as they are obtained from human patients, Therefore, the kit used for extraction is crucial as it determines the size of the slide needed and therefore the number of analyses that can be carried out on each FFPE block. This limitation reduces the number of possible applications as some archived tissues are too small and cannot satisfy this criterion. In addition, DNA extraction with these kits can be time-consuming.

In this Application Note, *prep*GEM Universal kits for DNA extraction using a thermocycler (cat #PUN0100) and PDQeX *prep*GEM Universal kits for DNA extraction using the PDQeX nucleic acid extractor (cat #XPU0100 and XMA) were tested by extracting gDNA from FFPE slides of 2µm and 5µm collected from lung tumour of engineered mice. *prep*GEM Universal is a simple, rapid DNA extraction solution that leverages a temperature-driven and single-tube approach. The thermostable proteinase is activated at 75°C and inactivated at 95°C, and does not require toxic detergents such as SDS to work properly. In addition, the single-tube approach allows users to virtually recover 100% of the extracted material and preserve its integrity. The results showed that *prep*GEM Universal can extract gDNA sufficient for PCR-based applications from both 2µm and 5µm slides in 20 minutes, saving precious material and time. These findings were confirmed by Munkhbaatar et al. 2020 (Nat. Comm.), who used *prep*GEM Universal to extract gDNA from 5µm FFPE slides to evaluate Mcl-1 copy number in $Kras^{G12D/+}p53^{\Delta/\Delta}$ deletion lesions[1].

Materials and Methods

DNA Extraction

DNA was extracted from FFPE tissues derived from lung tumour induced in engineered mice. The size of the FFPE slides was 2µm and 5µm. To collect the tumour material, excess wax was trimmed away from the slide and the tissue was collected with a scalpel and transferred to a 0.2µl PCR tube or PDQeX cartridge. The extraction reaction mix was tested in 2 volumes: 25µl and 100µl. The *prep*GEM Universal components were mixed as follows:

Reagents	100 µl	25 µl
RED+ Buffer	10 µl	2.5 µl
Histosolv	10 µl	2.5 µl
*prep*GEM	1 µl	1 µl
Water	79 µl	19 µl

Table 1: Extraction mix volume of 100µl and 25µl for 1 sample.

The extraction mix of 100µl or 25µl was added to the PCR tube and PDQeX cartridge containing the collected FFPE tissue. The PCR tubes were incubated in a standard laboratory thermocycler, whereas the PDQeX cartridges were incubated in the PDQeX nucleic acid extractor (cat #XMA) as follows:

Degrees	Thermocycler	PDQeX
52 °C	5 min	5 min
75 °C	10 min	10 min
95 °C	5 min	5 min

Table 2: Incubation steps for thermocycler and PDQeX.

Fig 1: Workflow of the extraction with *prep*GEM Universal (cat #PUN0100) in a thermocycler. Sample and reagents are mixed in a 0.2µl PCR tube and placed in a standard thermocycler or heat-block. The extraction occurs at 52 °C and 75 °C. At the end of the extraction, the 95 °C step irreversibly inactivates the proteinase and denatures the DNA to become single-stranded, making the extract ready for PCR-based applications.

Fig 2: Workflow of the extraction with PDQeX *prep*GEM Universal (cat #XPU0100) in the PDQeX nucleic acid extractor (cat #XMA). Samples and reagents are loaded into a PDQeX cartridge and the cartridge is placed in the extractor along with collection tubes. The extraction occurs at 52 °C and 75 °C. At 95 °C, the thermo-responsive cartridge shrinks and pushes the lysate through a purification column that retains the proteinase and releases the lysate in a new collection tube. This allows the DNA to preserve the double strand configuration suitable for PCR-based applications and sequencing.

After extraction, the extracts were centrifuged at full speed for 1 min to pellet the paraffin, and the supernatants containing the nucleic acid were transferred to new tubes.

DNA Quantification

DNA was quantified using Nanodrop for both samples extracted using the PDQeX nucleic acid extractor and samples extracted using the thermocycler.

End-Point PCR

Extracted DNA was used to analyse Rosa26 genomic locus via End-Point PCR using Promega GoTaq Polymerase (cat #M7823). The reaction mix of 20µl was prepared as follows:

Reagents	1x
Nuclease-free water	7.6 µl
10 µM Forward	0.6 µl
10 µM Reverse	0.6 µl
GoTaq Polymerase	10 µl
gDNA	1.2 µl

The primer sequences used for Rosa26 were:

For Primer Rosa26 locus:
AGCACTTGCTCTCCCAAAGTC

Rev Primer Rosa26 locus:
CCGACAAAACCGAAAATCTGTGGG

The PCR reaction was carried out in a thermocycler using the following program:

Temperature	Time	Cycle
95°C	3 min	1
95°C	30 sec	
60°C	45 sec	35
72°C	45 sec	
72°C	5 min	1
4°C	∞	

The PCR product was then visualized on an agarose gel exposed to UV light following electrophoresis.

Results

Tissues archived in FFPE blocks are highly valuable and precious for diagnostic purposes and clinical research, therefore, reducing the amount of starting material required for down-stream applications such as PCR/qPCR and sequencing is essential to be able to use these samples for multiple analyses. Nucleic acid extraction is crucial in determining the amount of starting material and a single-tube approach, such as the *prep*GEM Universal kit, is ideal to prevent loss of material.

In Figure 3, gDNA was extracted from 2µm and 5µm FFPE slides in 25µl of reagents. The process lasted in total 20 minutes and was carried out in a standard laboratory thermocycler, without the need of deparaffinization. The extracts were used to amplify the Rosa26 locus and analyse it via electrophoresis. The agarose gel showed a clear band at 346 bp size corresponding to the Rosa26 locus for both 2µm (lane 1) and 5µm (lane 2) slides. To control the process, a positive control was also inserted (lane 3). The intensity of the band was proportional to the amount of starting material.

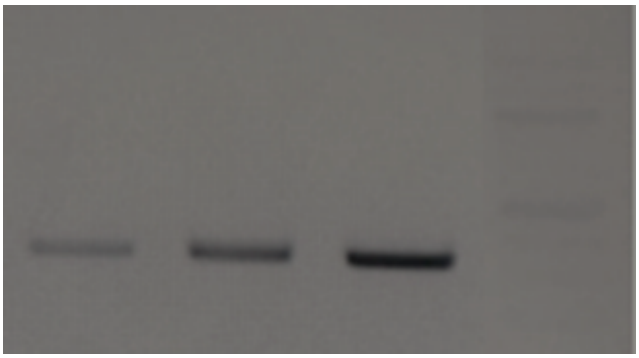

Fig 3: DNA extraction in 25µl volume from 2µm (lane 1) and 5µm (lane 2) FFPE slides. Lane 3 represents a positive control.

In the second experiment, gDNA was extracted from 2µm FFPE slides in a 100µl extraction using a thermocycler and as well as the PDQeX nucleic acid extractor. Table 1 shows DNA quantification of the two samples by Nano-drop. This quantification data shows that Nanodrop quantification is not suitable for use on *prep*GEM extracts because the two ratios A260/280 and A260/230 indicating the purity and quality of the sample and its suitability for down-stream applications are below the acceptable values ~1.8-1.9 and ~1.8-2.2 respectively.

Although, the two samples did not meet the standard criteria for quality control, Figure 4 shows that the down-stream PCR worked well for both samples indicating that these standards are not applicable to *prep*GEM extracts.

Sample	Nanodrop ng/µl	A260/280	A260/230
1	68.7	0.74	0.17
2	121.1	1.23	0.33

Table 3: DNA quantification with Nanodrop. Sample 1 represents the DNA obtained using a thermocycler and Sample 2 represents the extract obtained using the PDQeX extractor.

Moreover, Figure 4 shows that both extractions were successful and that it is possible to increase the extraction volume for 2µm slides to 100 µl, thus allowing more material available for down-stream analysis. Although there is reduced intensity of the band for the sample extracted with PDQeX, it is worth noting that the extraction from 2µm still led to sufficient material for the down-stream application. This indicates the possibility that with a higher amount of starting material, the PDQeX may produce dsDNA suitable for NGS sequencing.

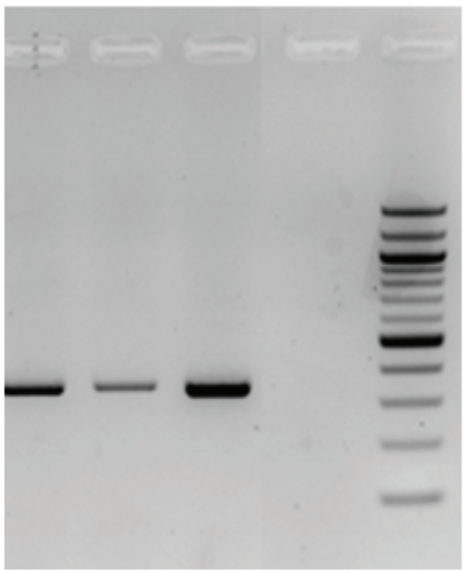

Fig 4: DNA extraction in 100µl volume from 1 single 2µm slide using a thermocycler (lane 1) and the PDQeX nucleic acid extractor (lane 2). Positive control in lane 3 and Negative control in lane 4.

These findings were confirmed by Munkhbaatar et al. 2020 (Nat. Comm.) who used *prep*GEM Universal (cat #PUN0100) to extract gDNA from 9 FFPE slides (5 vehicle group, 4 treated group) to evaluate Mcl-1 copy number in lesions from $Kras^{G12D/+}p53^{\Delta/\Delta}$ mouse lung tissue. In this study, the gDNA was extracted successfully from 5µm slides in an extraction volume of 100µl and Mcl-1 copy number variation was evaluated by Real-Time PCR (Taqman®)[1].

Taken together, the data demonstrates that *prep*GEM Universal kits are suitable for extracting gDNA from FFPE samples of a minimum size of 2µm and in a volume of up to 100µl, saving precious material. The process can be performed in a thermocycler, meaning that up to 96 samples can be analysed simultaneously in 20 minutes. Alternatively, the gDNA can be extracted in the PDQeX extractor to generate dsDNA. In conclusion, *prep*GEM Universal represents a rapid, simple and cost-effective solution to extract DNA from FFPE samples.

References

1. Munkhbaatar, E. *et al.* MCL-1 gains occur with high frequency in lung adenocarcinoma and can be targeted therapeutically. *Nat. Commun.* **11**, 4527 (2020).