

# Evaluation of Nucleic Acid performance and preservation in formalin-free PAXGene tissue fixative: a comparison to formalin

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## INTRODUCTION

Damage caused to nucleic acids in the fixation process by formalin limits application of molecular techniques. PAXGene tissue fixative is a methanol-based fixative aimed at preserving these nucleic acids. The aim of this work is to compare the quality of DNA and RNA from paired formalin- fixed paraffin embedded (FFPE) and PAXGene - fixed paraffin embedded (PFPE) samples.

## METHODS

- Paired tumour samples were fixed in Formalin and PAXgene, routinely processed overnight, and paraffin embedded
- DNA and RNA was extracted from 57 cases using QIAGEN kits and assessed for yield (Qubit), purity (Nanodrop) and fragmentation (Tapestation)
- To evaluate the functional application, Qualitative PCR was performed on the DNA and RT-qPCR and gene expression analysis of GUSB (housekeeping gene) was performed on the RNA

## DNA QUALITY

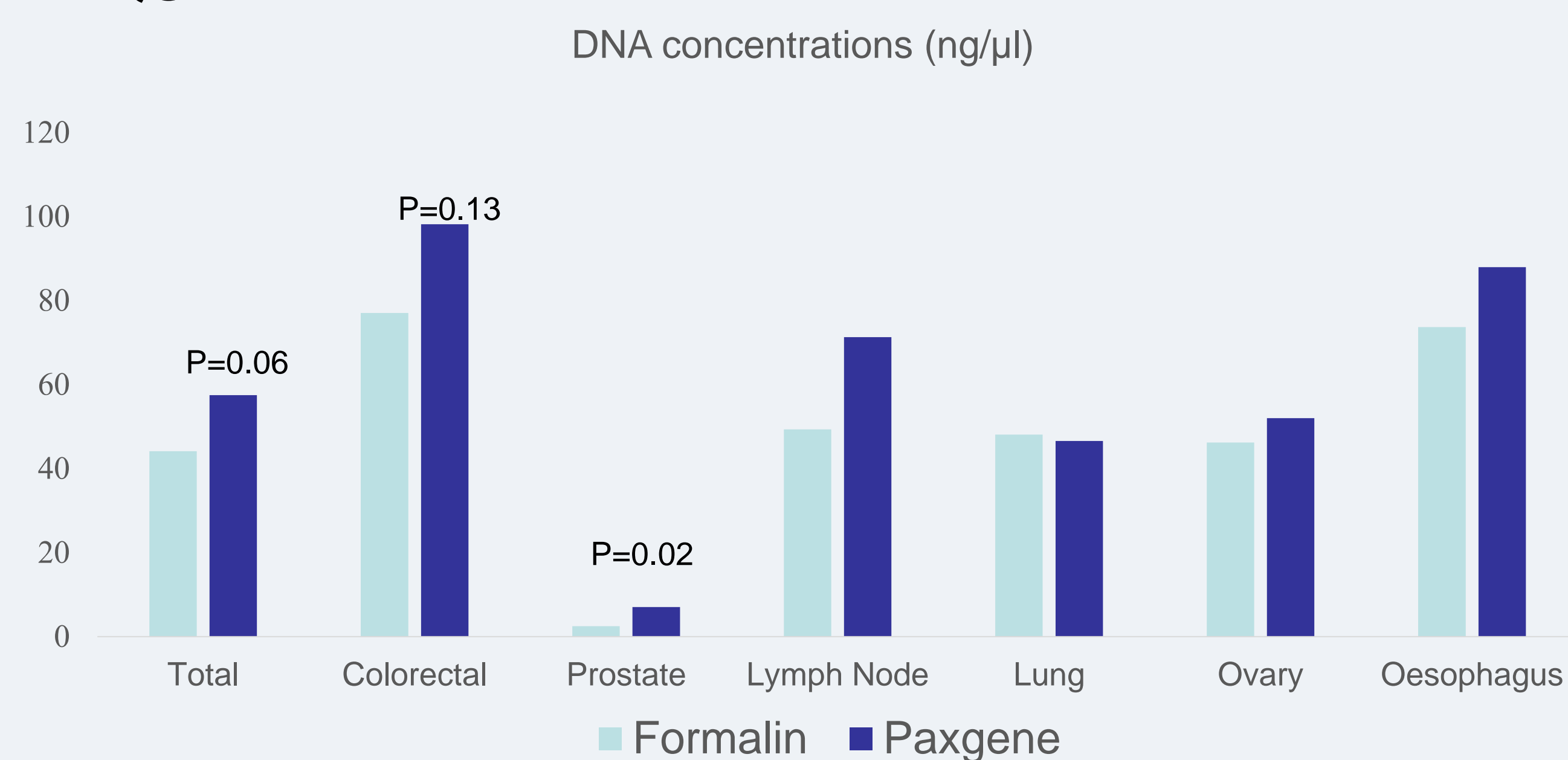
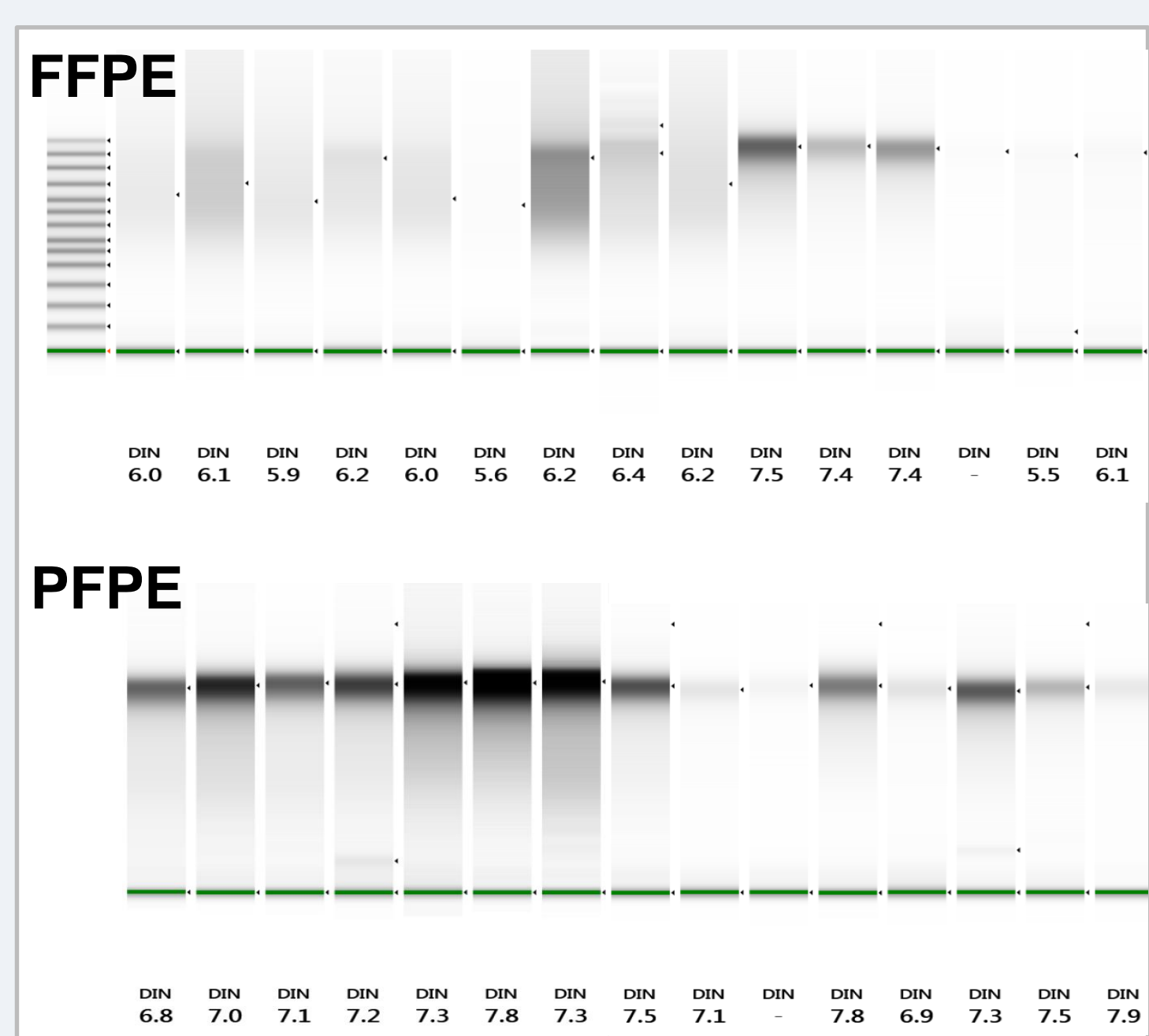


Fig 1. DNA concentrations (ng/ul) by tissue. Total n=57, CRC n=23, Prostate n=20, LN n=2, Ovary n=1, Oes n=1



- Yield was higher from PFPE (mean 5747ng) compared to FFPE (mean 4415ng), although not statistically significant (p=0.06)
- The mean fragment length was 12728bp for FFPE, compared to 20654 for PFPE (p<0.01)

Fig 2. Fragmentation of DNA measured by tapestation

- Mean dCT values were 4.07 (FFPE) and 0.67 (PFPE)(p<001)
- 20/53 FFPE samples had 260/280 ratios between 1.8 and 2.0 compared to 14/53 PFPE samples (outside of this range DNA is considered impure)

	FFPE	PFPE	P-value
Yield	4415	5747	0.06
Fragment Length	12728	20624	<0.01
dCT	4.01	0.67	<0.01
260/280 ratio	2.18	2.32	0.03

Table 1. Summary of QC and amplifiable quality of DNA

## RNA QUALITY

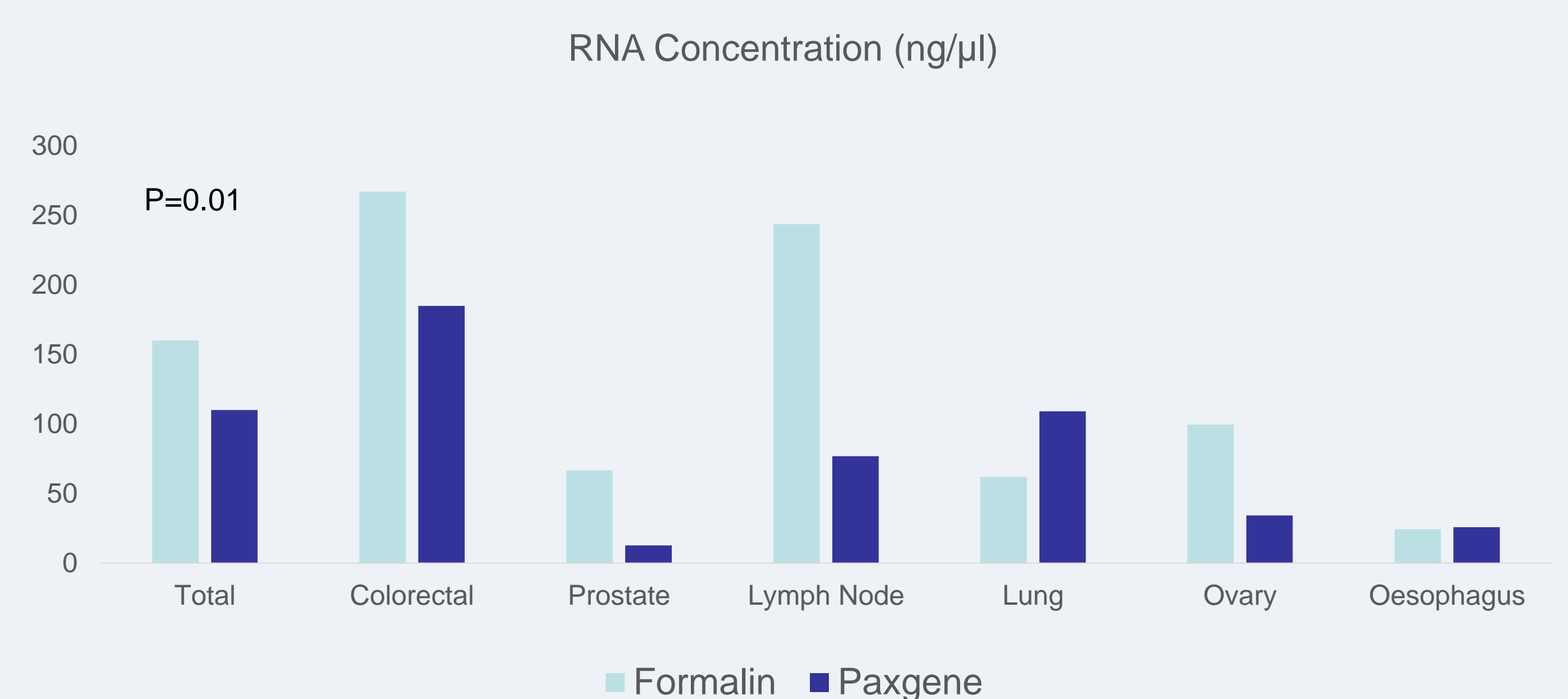


Fig 3. RNA concentrations (ng/ul) by tissue. Total n=42, CRC n=22, Prostate n=15, LN n=2, Ovary n=1, Oes n=1, Lung=1

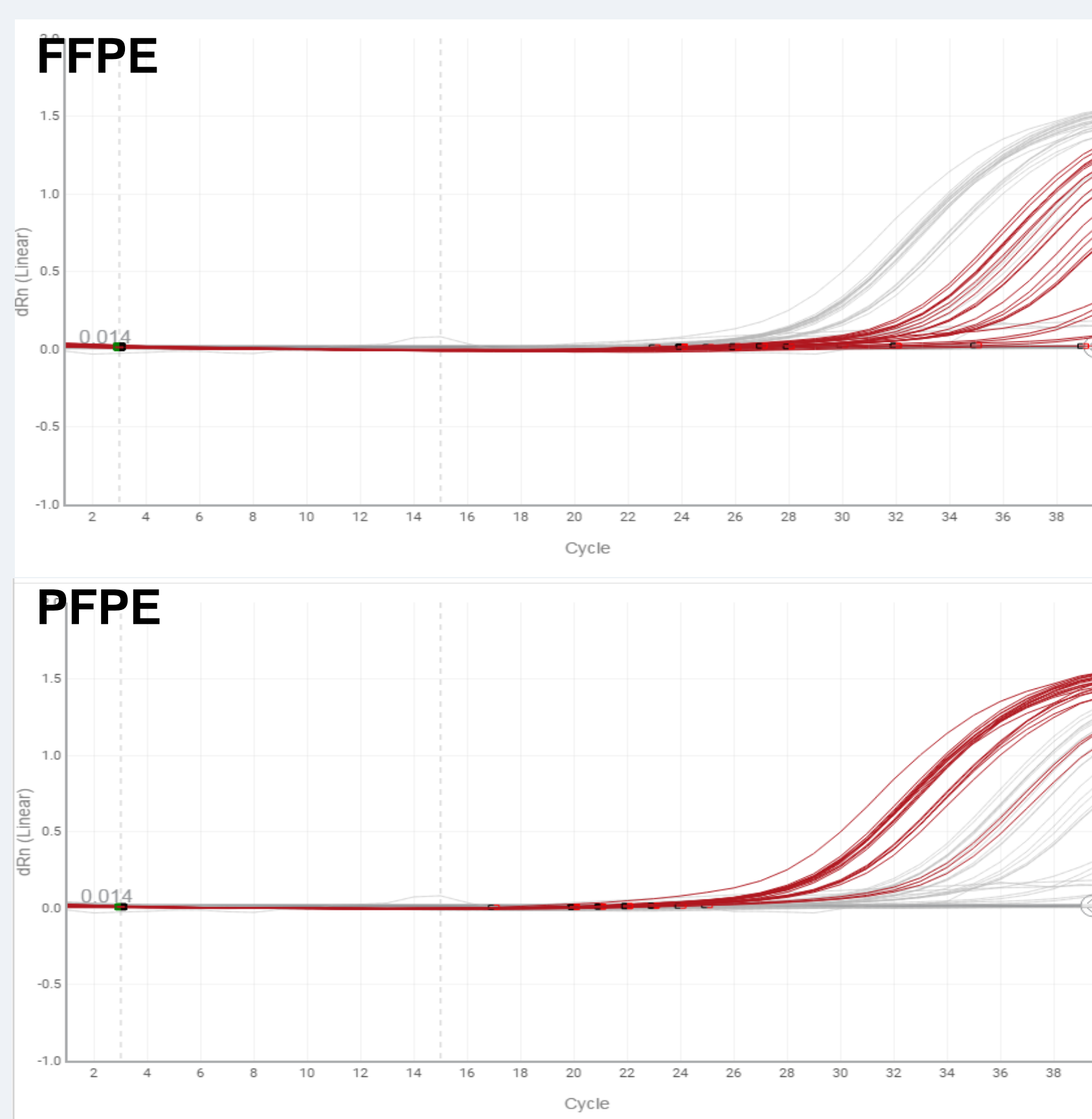
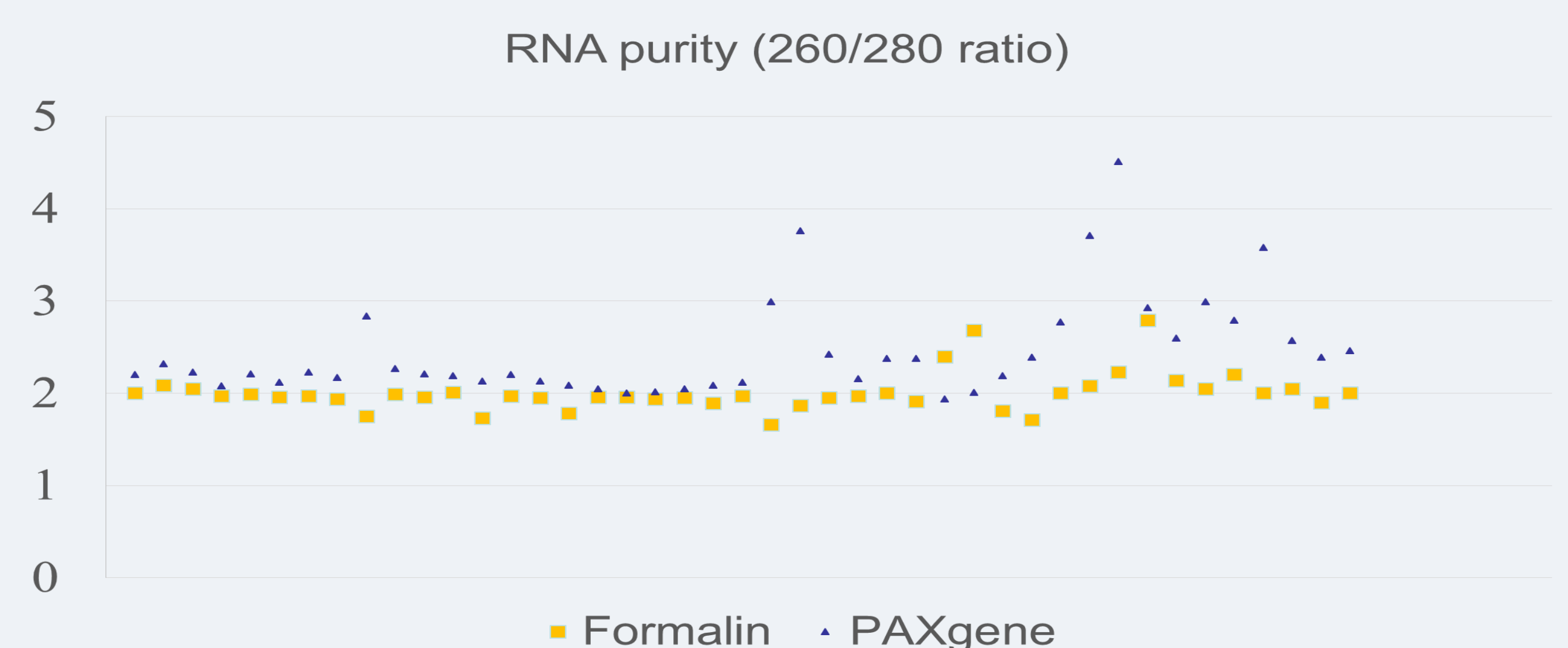


Fig 4. Amplification of GUSB

- Concentrations from FFPE (mean 160ng/μl) were higher compared to PFPE (mean 110ng/μl), p<0.01
- RNA Integrity numbers were higher in PFPE (mean 3.5) compared with FFPE (mean 2.9), p<0.01
- GUSB expression was undetectable in 42% FFPE samples. Ct means were 32.3 (FFPE) vs 27.5 (PFPE), p<0.01
- 260/280 ratios were consistently lower in FFPE (mean 2) compared with PFPE (2.46)(P<0.01) samples



## CONCLUSIONS

DNA yield from FFPE and PFPE were comparable, however the DNA was of greater quality. DNA fragment length was significantly longer in PFPE and was also of a higher amplifiable quality. The purity of PFPE DNA and RNA was however lower than FFPE.

RNA yield from PFPE was significantly lower than FFPE, however RIN scores were significantly higher and superior quality was demonstrated following RT-qPCR and quantification of gene expression.