

The influence of processing platform on morphology, immunohistochemistry and DNA quality

Lone Bojesen¹, Dorthe Vestergaard Hansen¹, Linda Thyregod¹, Christina Grønhøj¹, Julie Ramstedt¹, Mette Eriksen¹, Peter Ingeholm¹, Gitte Wooler¹, Sofie Vetli Hjort¹ and Mai-Britt Naumann Pedersen¹

1. Department of Pathology, Herlev and Gentofte Hospital, University of Copenhagen, Denmark

INTRODUCTION:

In our laboratory we routinely process intestine biopsies and skin samples on the Tissue Tek® Xpress® for 1 to 2 hours, but in a previous project done on intestine biopsies, we found that especially the DNA analysis was negatively affected by this. We therefore wanted to test another rapid processing platform Pathos Delta and its program for 3 mm samples.

Aim: to test the influence of processing platforms on morphology, immunohistochemistry and DNA quality.

MATERIALS AND METHODS:

Samples: normal tissue from large intestine cut into small biopsies 2x2x4 mm, and skin samples cut into slices no more than 2 mm thick.

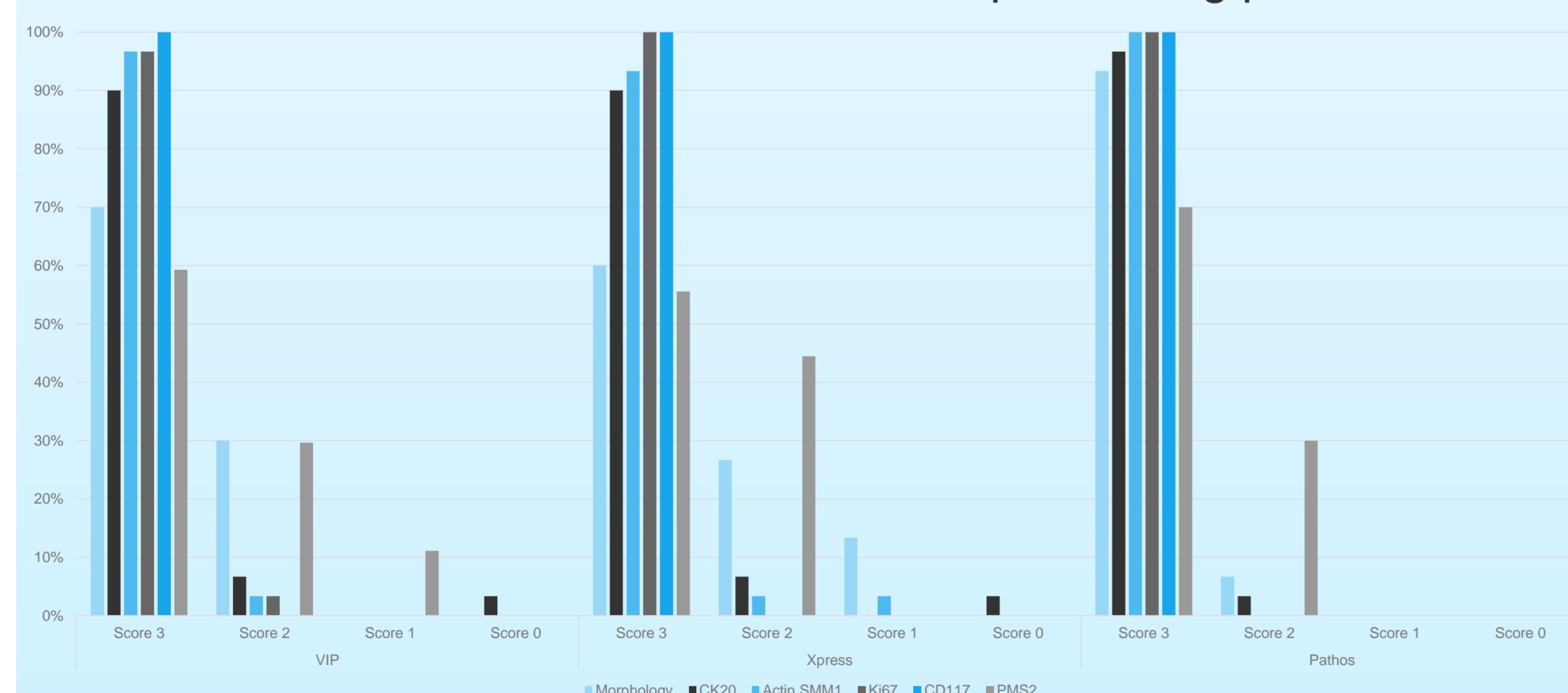
Fixation: 4% Neutrally buffered formaldehyde (NBF) for a minimum of 4 hours for Tissue Tek® VIP® 5 and Tissue Tek® Xpress® and 30-60 minutes for Pathos Delta.

Stains: Hematoxylin-Eosin (HE),
Immunohistochemistry: CD117, Ki67, Actin SMM-1, CK20 and PMS2 for intestine. Melan-A, Sox10, S100, CKA and EP4 for skin.

DNA quantity and quality: Concentration measured by Qubit and fragment analysis with GeneScan™ 400HD ROX: Sizing DNA fragments.

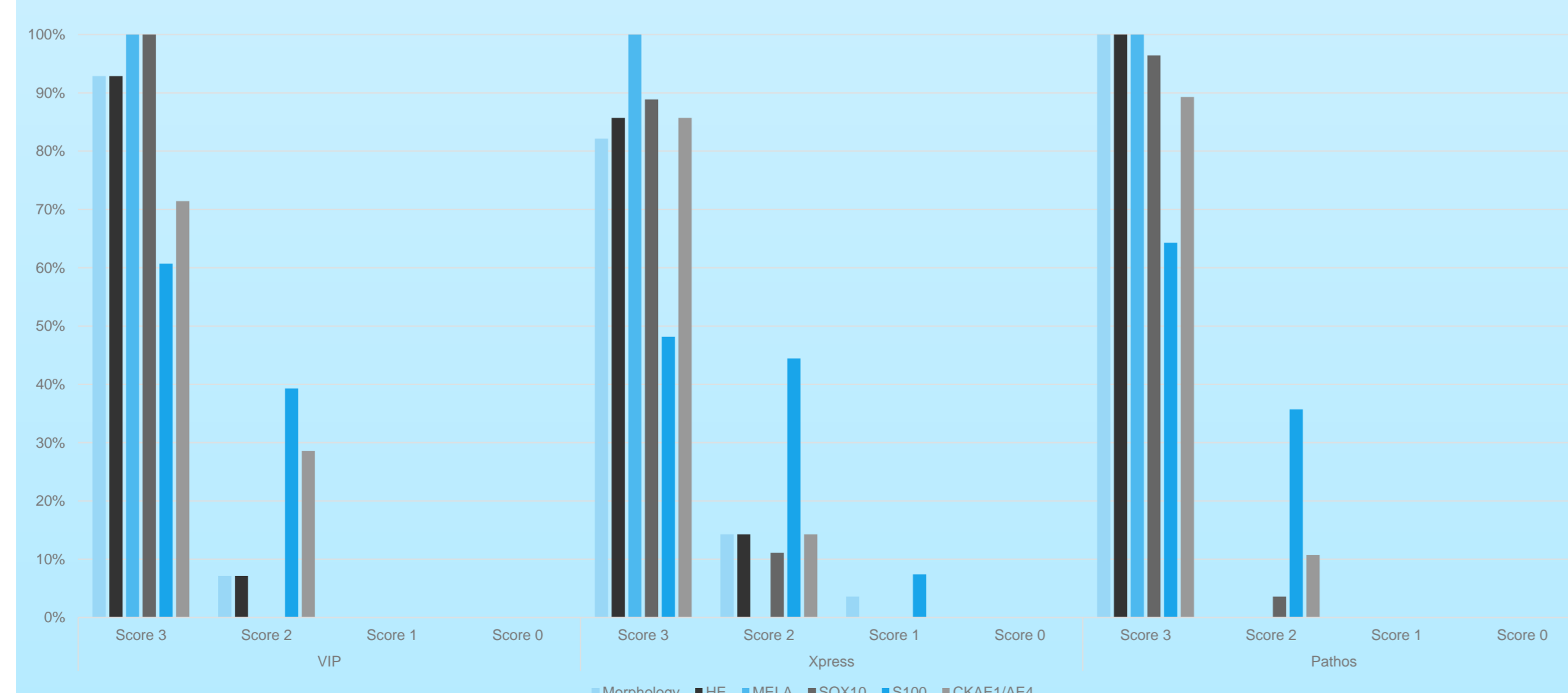
RESULTS:

For intestine biopsies morphology and PMS2 are marginally better on tissue processed on Pathos Delta. The other immunohistochemical stains are almost similar in score between all three processing platforms.



Figur 1: Results for morphology, HE and immunohistochemistry in intestine biopsies

For skin samples the results are similar to that of intestine biopsies. Here S100 performs inferior on all platforms, which indicates a need to optimize the staining.



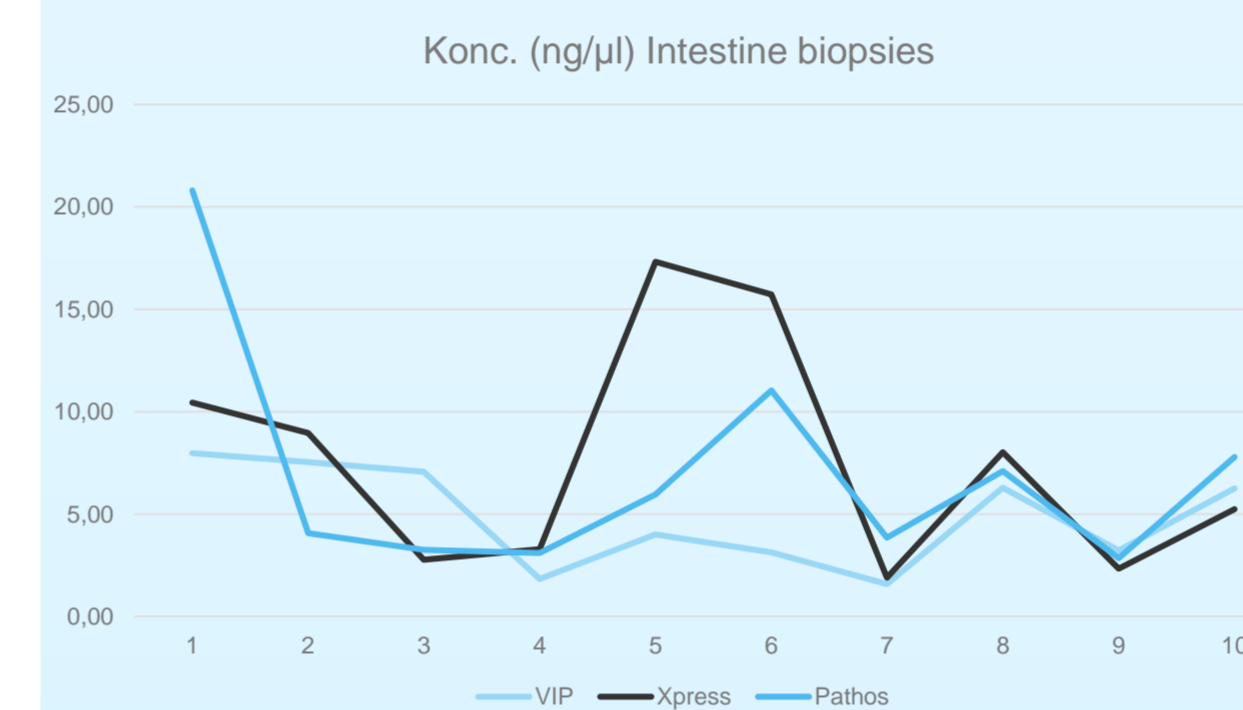
Figur 2: Results for morphology, HE and immunohistochemistry in skin samples

CONCLUSION:

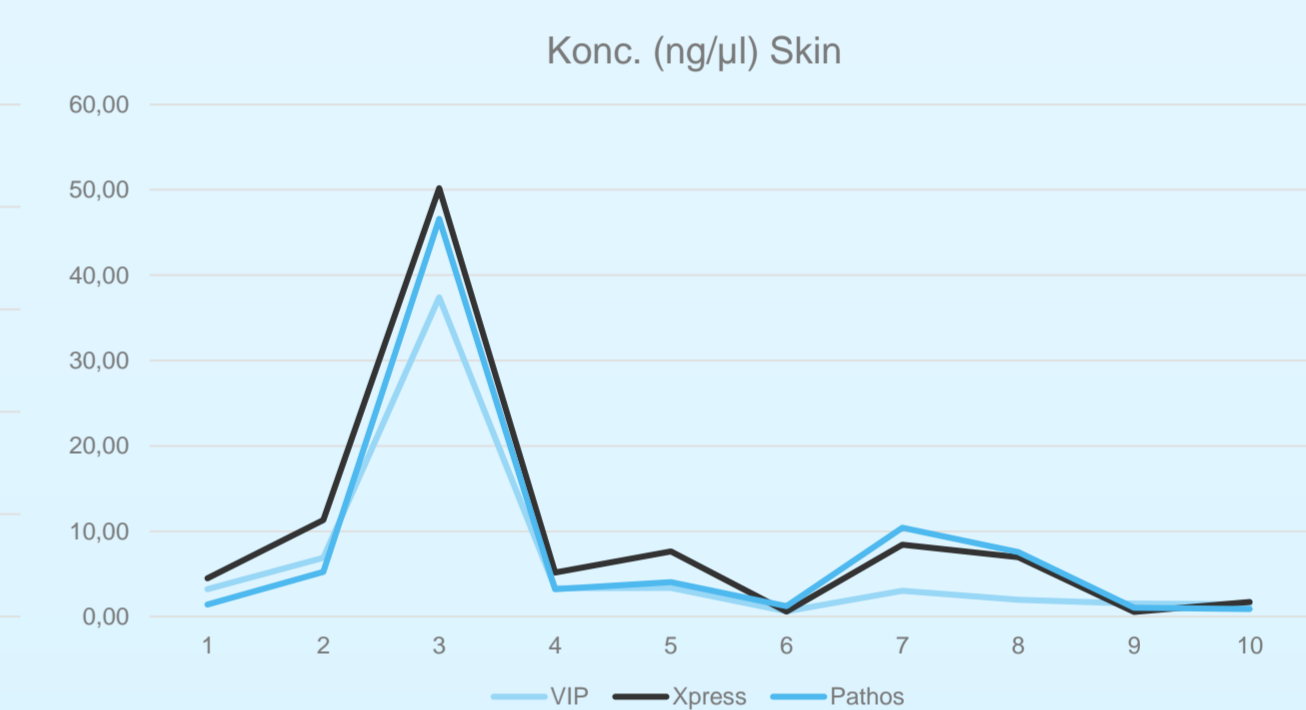
In this limited study we found that morphology, histochemistry and immunohistochemistry are almost similar in all three processing platforms, but DNA quality is negatively affected by processing on the Xpress.

RESULTS:

The quantity of DNA is similar on all three platforms.

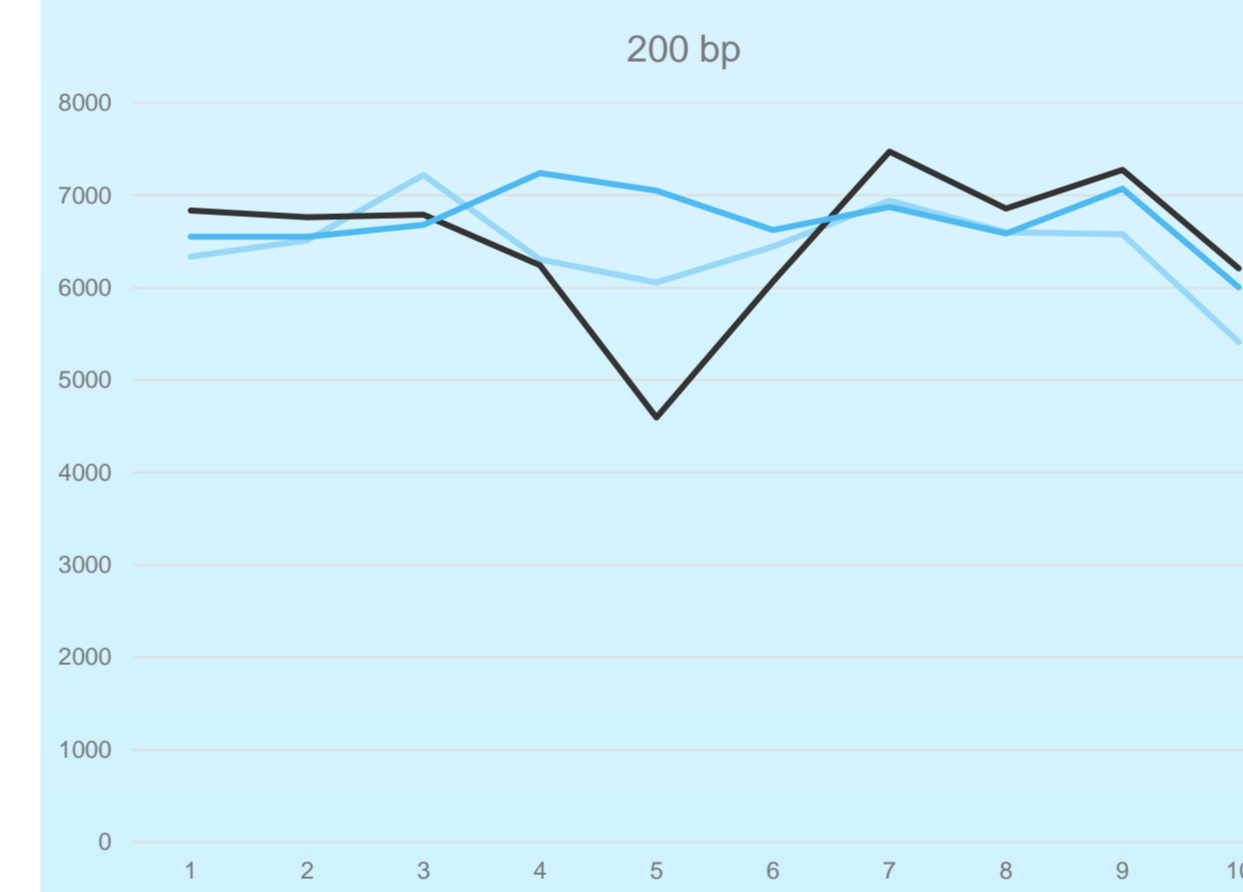


Figur 4: DNA concentration intestine biopsies

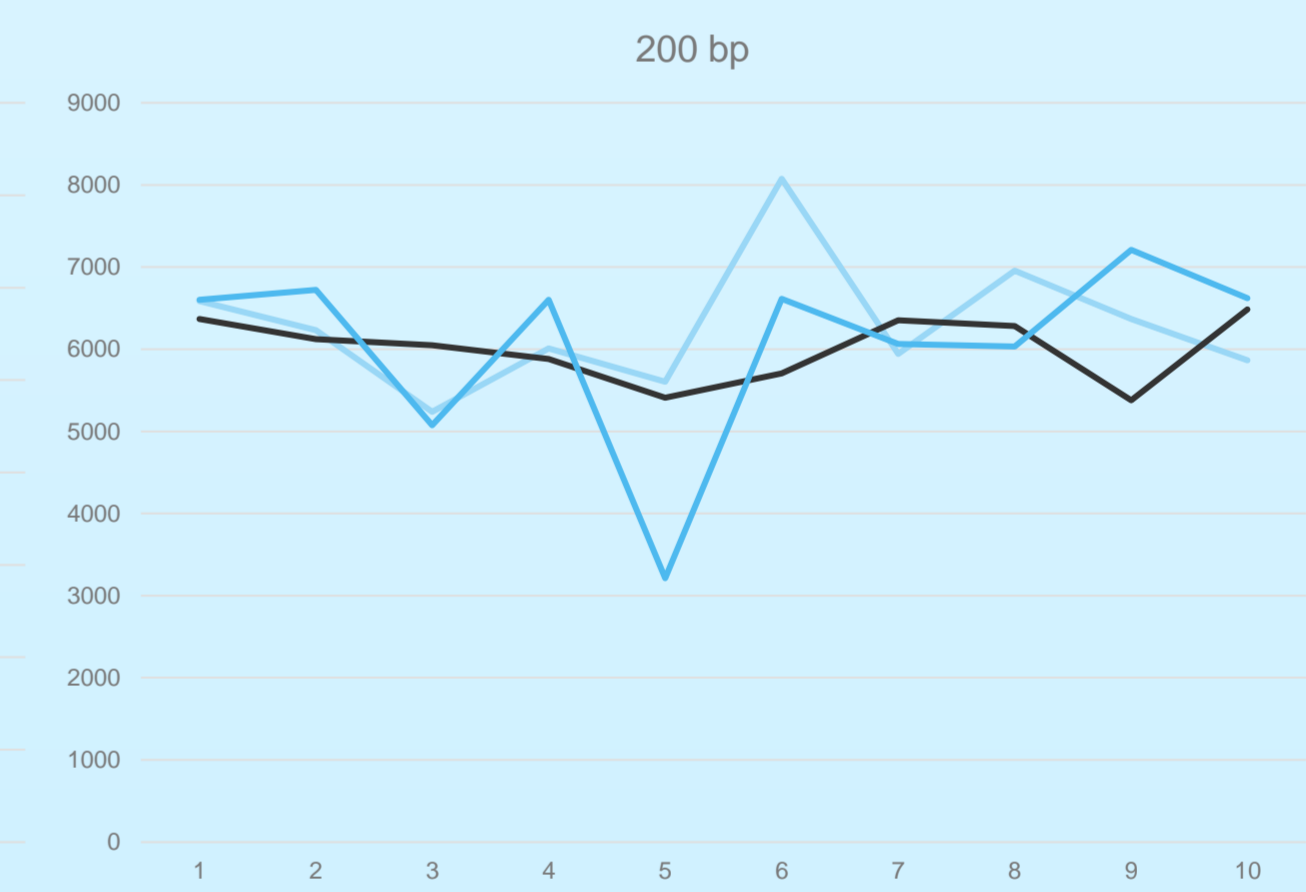


Figur 5: DNA concentration skin samples

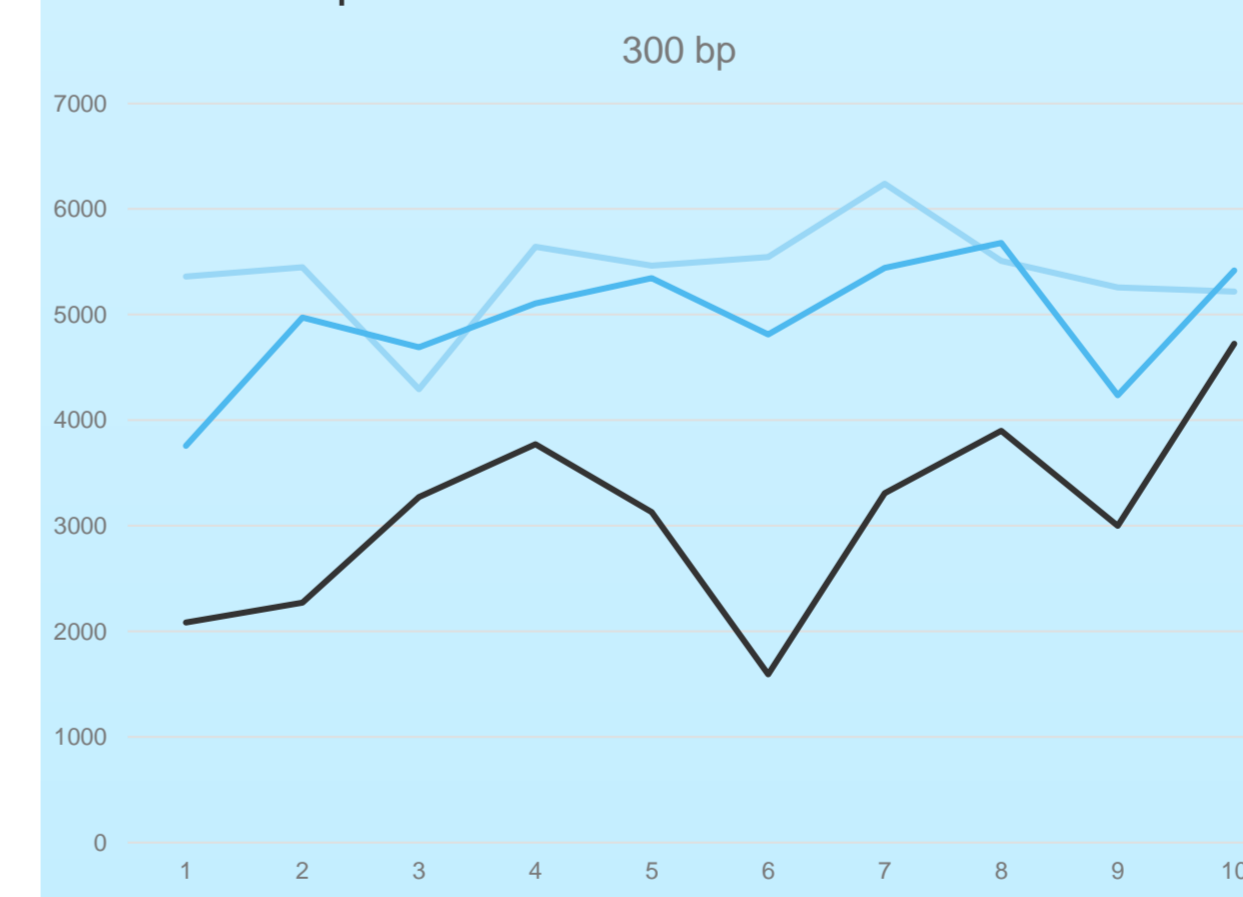
Xpress processed tissue gives less of the longer fragments, for both types of tissue.



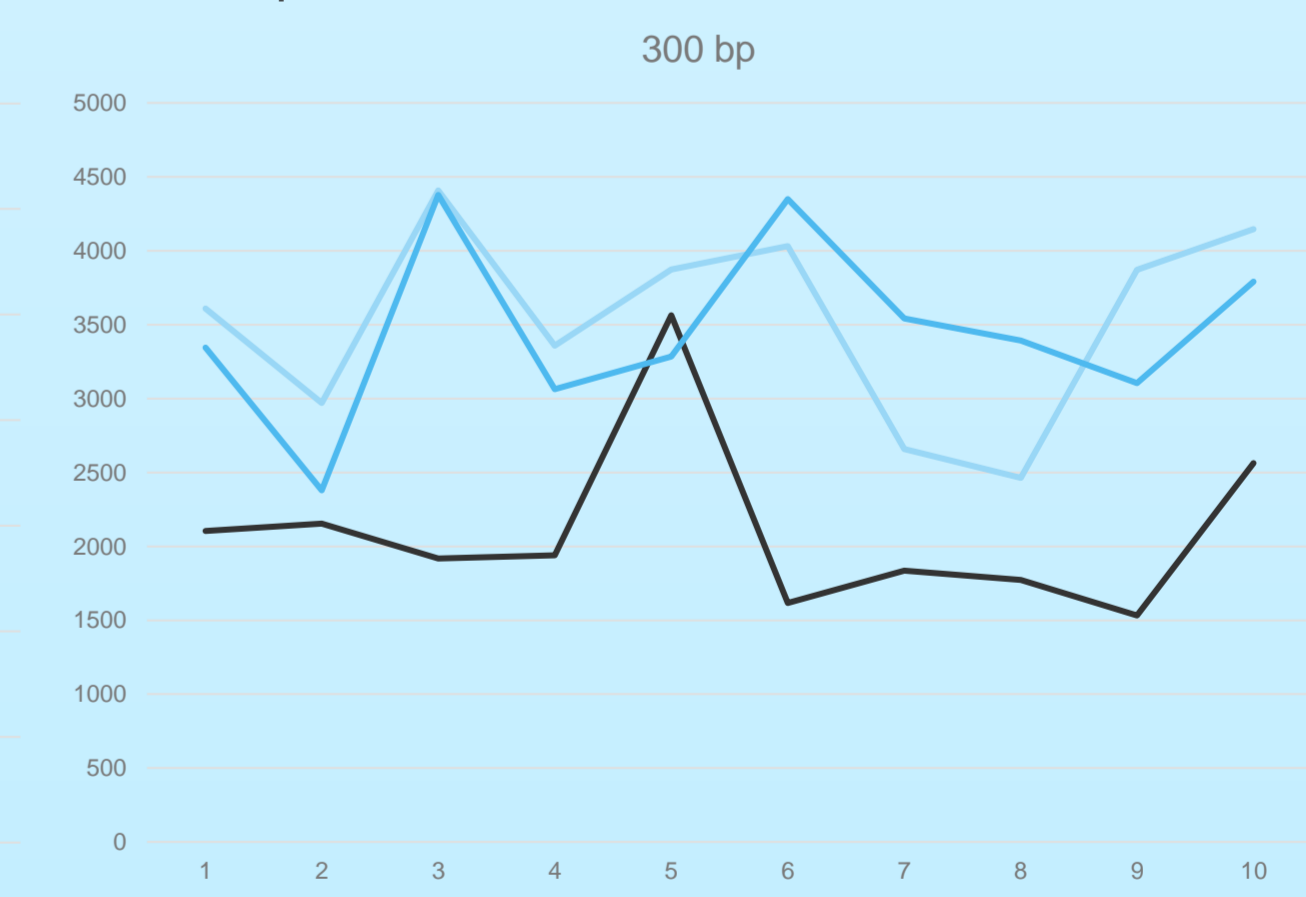
Figur 6: DNA fragments 200 basepair intestine biopsies



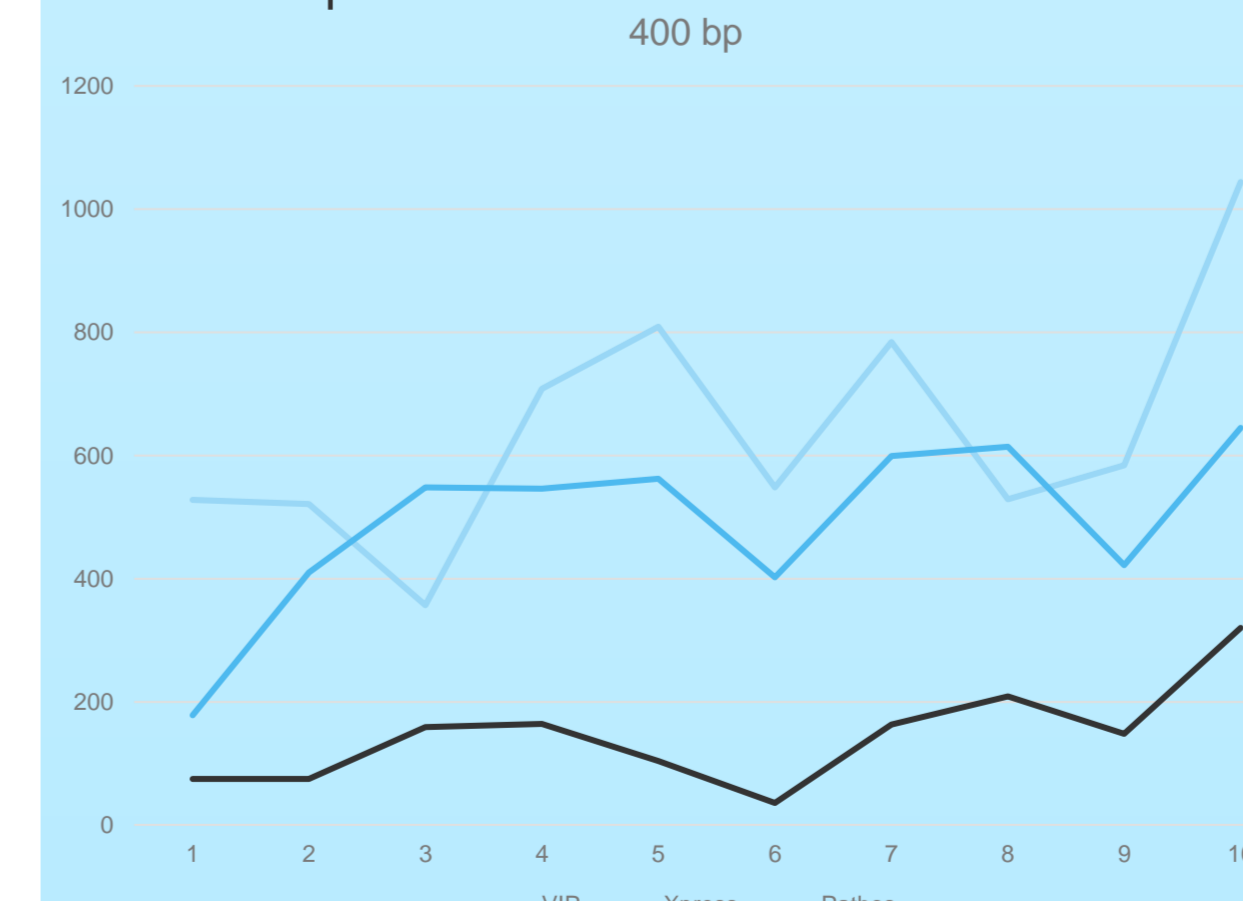
Figur 9: DNA fragments 200 basepair Skin samples



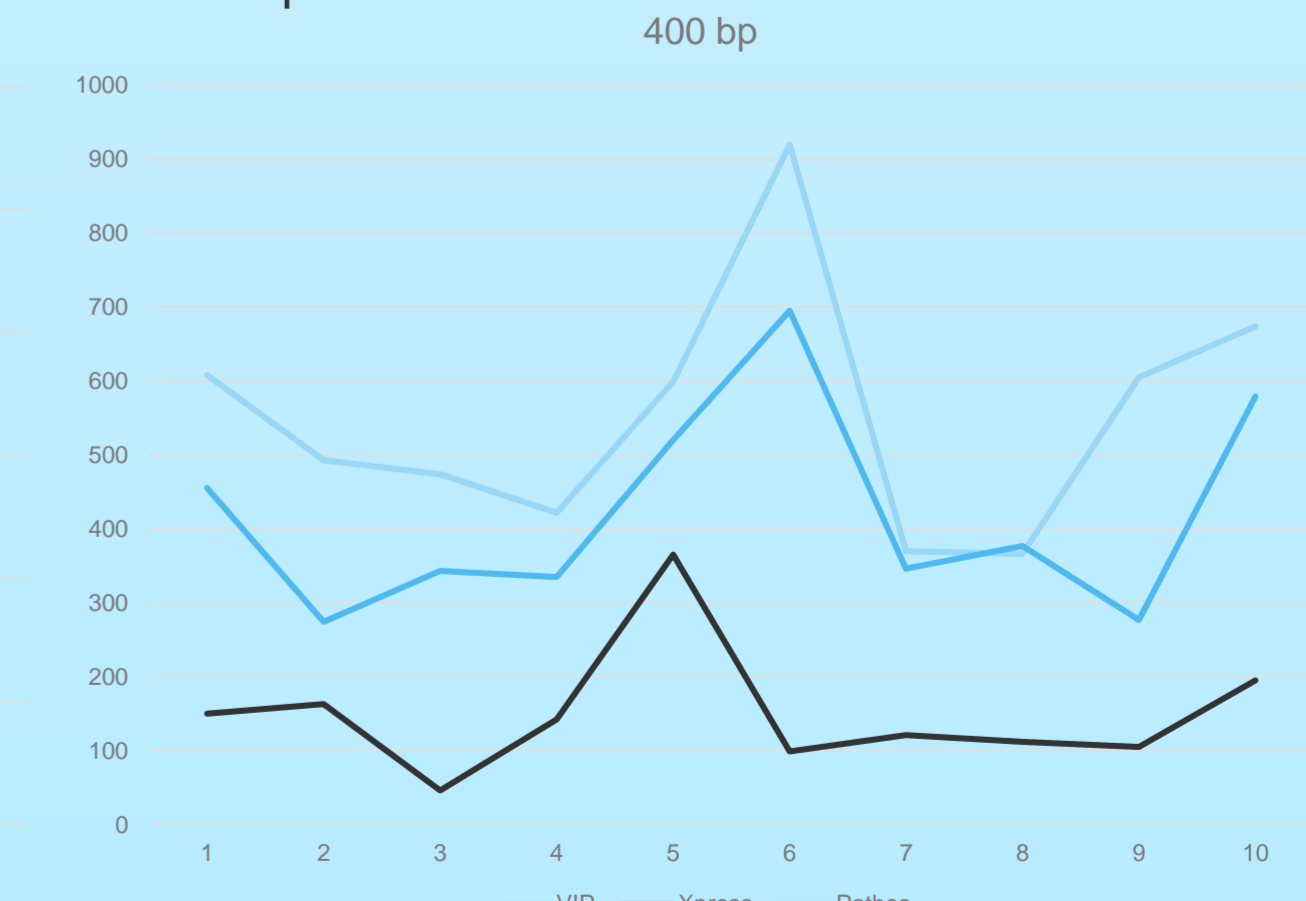
Figur 7: DNA fragments 300 basepair intestine biopsies



Figur 10: DNA fragments 300 basepair Skin samples



Figur 8: DNA fragments 400 basepair intestine biopsies



Figur 11: DNA fragments 400 basepair Skin samples

The poor DNA quality using Xpress may be explained by different solutions for dehydration and clearing.

REFERENCES: