PATHONEXT

Application of the transposase based SureSelect ^{QXT} protocol for *BRCA1* and *BRCA2* FFPE sequencing

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Introduction

Next generation Sequencing (NGS) has become a key method for analyzing genetic variants in formalin fixed paraffin embedded (FFPE) tumor tissues. However, despite widespread application and significant technical progress in NGS formalin treated tissues represent still a challenging material for several reasons:

(1) Degradation of the DNA

(2) Erroneous base changes caused by deamination or cross linking

(3) PCR artifacts

Table 1: Testing approaches with different cleaning methods (NEB), various amounts of starting material, different degrees of degradation and diverse DNA extraction methods

	(ng/µl)	DIN	Isolation	Figure 1	Lane
GM14096	22,6	8,2	Control	(1)	H1
NA14094	23,0	9,2	Control	(1)	A2
1	27,6	4,7	GR	(1)	B1
1-NEB	30,6	3,8	GR	(1)	C1
2	22,6	5,4	GR	(1)	D1
2-NEB	20,8	2,8	GR	(1)	E1
3	31,2	3,2	GR	(1)	F1
3-NEB	16,5	3,2	GR	(1)	G1
4_GR	45,0	2,1	GR	(-)	Not shown
4_GR	26,6	2,1	GR	(-)	Not shown
4_XY	7,0	2,4	XY	(-)	Not shown
BRCA7	43,2	2,1	MW	(2)	E1
BRCA7	23,8	2,1	MW	(2)	E1
BRCA4	25,0	6,4	MW	(2)	B1
5_GR	27,2	3,8	GR	(2)	G1
5_XY	23,8	5,8	XY	(2)	H1

Methods

Seven samples with different treatments (table 1) where prepared with the SureSelect ^{QXT} protocol (Agilent, Santa Clara, CA).
DNA isolation followed three protocols: (XY) Xylol deparaffination/QIAamp DNA FFPE Tissue Kit (Qiagen); (GR) GeneRead DNA FFPE Kit (Qiagen), (MW) Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (Promega). Two commercial cell culture DNA's served as controls (Control). For evaluating the DNA (fig. 1) and library (fig. 2) quality we used the Tape Station (Agilent). Enzymatic DNA repair (NEBNext® FFPE DNA Repair Mix, NEB, Ipswich, MA) was carried out on three samples.
The library was sequenced on the MiSeq desktop sequencer (Illumina, San Diego, CA). Data analysis was performed using the CLCbio Biomedical Genomics Workbench (v2.5.1) (Qiagen, Hilden).

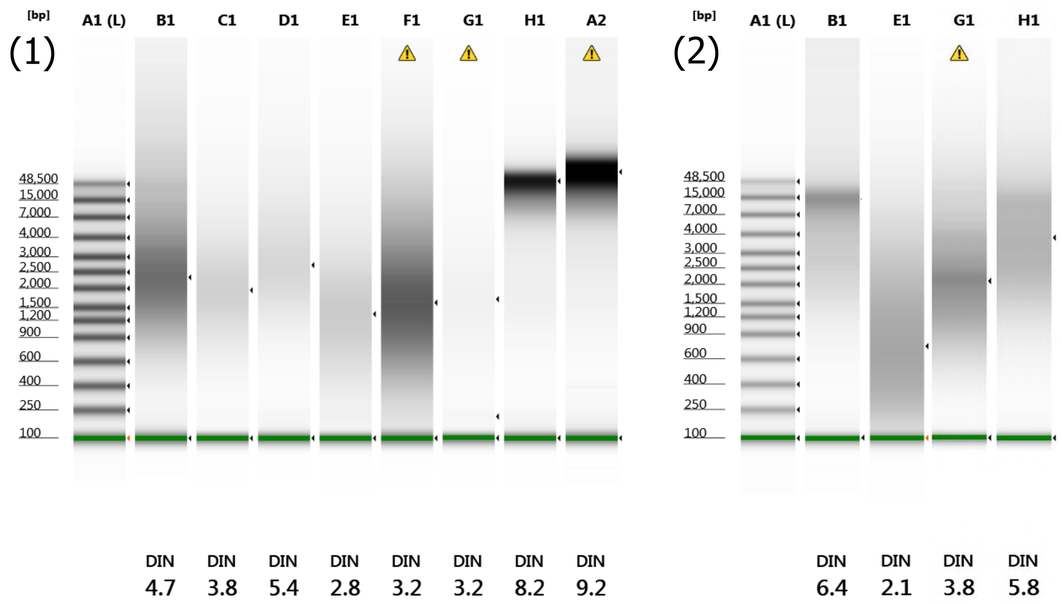
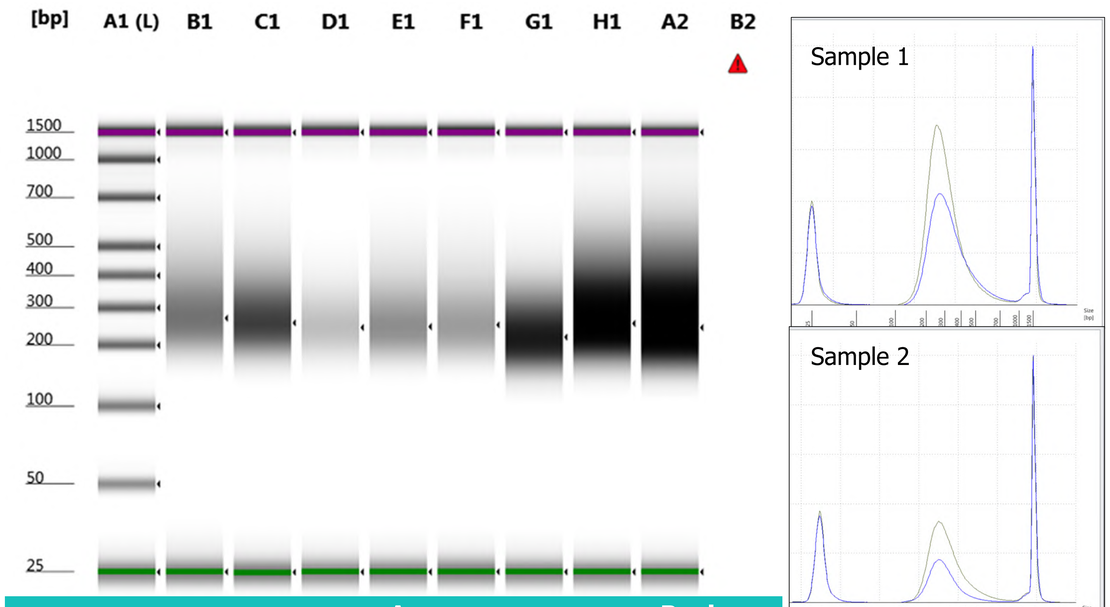


Figure 1: quality control of genomic DNA by using the DNA Integrity Number (DIN)



	Sample Description	From [bp]	To [bp]	Average Size [bp]	Conc. [ng/µl]	Region Molarity [nmol/l]
B1	1	157	710	330	23	118
C1	1-NEB	143	672	304	33.9	187
D1	2	153	518	277	7.99	47.4
E1	2-NEB	147	665	298	17.8	101
F1	3	152	646	302	14.3	80.3
G1	3-NEB	114	611	250	47.5	315
H1	GM14096	132	800	309	97.1	531
A2	NA14094	120	872	306	118	665

Figure 2: quality control of the adaptor tagged library, right side Comparison between NEB treated (green) and non-treated FFPE samples (blue)

Results

NEB treatment reduced DIN values in all three cases, but at the same time increased the amount of the adapter-tagged library as well as the DNA content of the final library. Additionally we observed that the NEB treatment resulted in a loss of 30-40% of the original input DNA. The on target rate was between 14-20%. This correlates with the level of degradation. The on target rate of the NEB treated probes was on average 2% higher than of the non-treated probes. Doubling the amount of input DNA did not increase the performance.

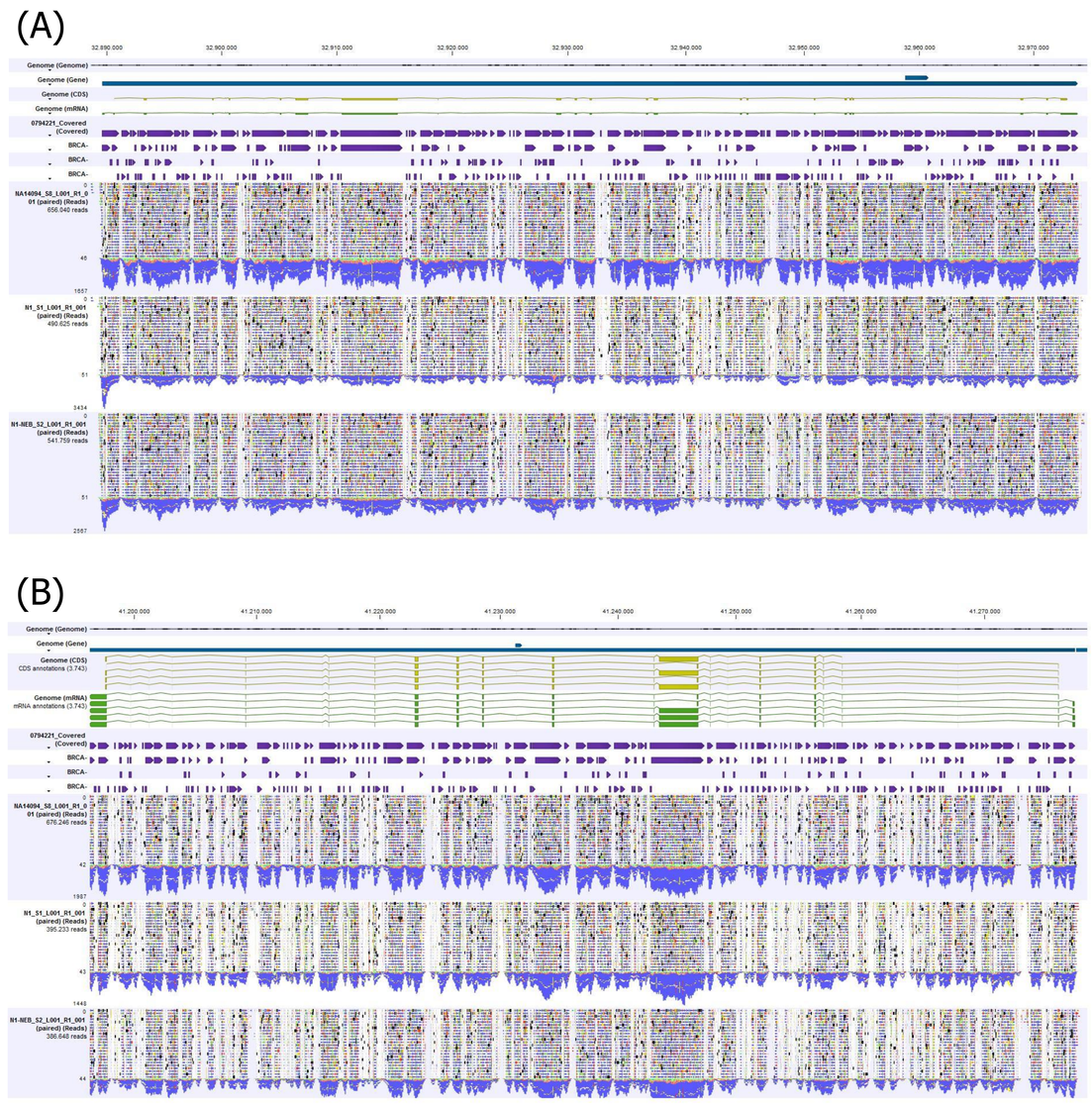


Figure 3: Mapping of (B) *BRCA1* and (A) *BRCA2* (comparison of control, FFPE, FFPE with NEB cleaning)

Conclusion

In this pilot study we could show that the SureSelect ^{QXT} target enrichment approach might be utilized for the analysis of FFPE DNA samples. The use of panels based on hybridization probes instead of PCR amplicons may at least partially overcome some of the problems with FFPE. Further experiments have to support these results.

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