



SafeForever™: Validation Report

Validation Report

Validation of all the subcomponents of biospecimens namely DNA, RNA and Proteins isolation from biospecimen stored in SafeForever™ and its application in genotyping, Next Generations Sequencing, Metagenomics, ELISA and Hematoxylin and eosin (H&E) staining.

Sample type	Process	Application	Inference *	Reference Image No.
Blood	DNA extraction [1-2]	Polymerase Chain Reaction (PCR)	DNA is amplifiable quality	Fig. 1. The agarose gel electrophoresis of the PCR products obtained with CYP2D6 primers
		SNP based Genotyping	DNA is suitable to assess the SNP calling using RT-PCR assay accurately.	Fig 2. Real time PCR assay of HLA B27 from the DNA
		Next Generation Sequencing (NGS)	DNA isolated from samples stored in SafeForever™ is suitable for identification of genetic variants in NGS platform	Fig 3. Coverage analysis report and Variants called
	RNA [5]	Realtime PCR assay	Downstream gene expression profiling is possible with the RNA obtained	Fig 4. Amplification plot of RNase P gene
	Protein extraction [3]	Enzyme-Linked Immunosorbent assay (ELISA)	The results highlighted that the total protein isolated from the samples stored in SafeForever™ is as suitable for ELISA	Fig 5. Human Amylase Alpha 1, AMY1 ELISA results
Saliva	DNA extraction [4]	Polymerase Chain Reaction (PCR)	DNA is amplifiable quality	Fig. 6. The agarose gel electrophoresis of the PCR products obtained with CYP2D6 primers
		SNP based Genotyping	DNA is suitable to assess the SNP calling using RT-PCR assay accurately.	Fig 7. Real time PCR assay of HLA B27 from the DNA
		Next Generation Sequencing (NGS)	DNA isolated from samples stored in SafeForever™ is suitable for identification of genetic variants in NGS platform	Fig 8. Coverage analysis report and Variants called
	Protein extraction [3]	Enzyme-Linked Immunosorbent assay (ELISA)	The results highlighted that the total protein isolated from the samples stored in SafeForever™ is as suitable for ELISA	Fig 9. Human Amylase Alpha 1, AMY1 ELISA results

Sample type	Process	Application	Inference *	Reference Image No.
Tissue	DNA extraction	Next Generation Sequencing (NGS)	DNA isolated from tissue stored in SafeForever™ is suitable for identification of genetic variants in NGS platform	Fig 10. Library conc. QC of the reads obtained from Ion AmpliSeq panel and the final variants called
		16s rRNA metagenomics	Useful in understanding the microbial community in the samples and help clinician in better diagnosis based on microbiome report	Fig 11. 16SMicrobial QC report of endoscopic biopsy stored in SafeForever™
	RNA extraction [5]	---	The tissue sample stored in SafeForever™ for 24hrs can be used for RNA extraction and can be used similar to RNA storage buffer like RNAlater or similar products.	Fig 12. Agarose gel electrophoresis showing total RNA extracted from uterus tissue
	Tissue staining [6]	Hematoxylin and eosin (H&E) stain	The data indicate that sample stored in SafeForever™ has maintained tissue architecture and is suitable for H&E staining studies	Fig 13. H&E image of the tissue after 24hrs preservation in SafeForever™
Plant materials	DNA extraction [7]	---	The plant parts stored in SafeForever™ can be used for RNA extraction and downstream application.	Fig 14. Agarose gel electrophoresis showing total DNA extracted from plant material
	RNA extraction [5]	---		Fig 15. Agarose gel electrophoresis showing total RNA from plant
Soil	DNA extraction [8]	16s rRNA metagenomics	Useful in understanding the microbial community in the environmental samples.	Fig 16. 16s rRNA sequencing analysis data performed on Oxford Nanopore system

Usability Note:

- DNA isolation can be performed from different sources of samples like blood, saliva, tissue, plant materials and soil after storing the sample in SafeForever™ version 1. DNA isolation from cell cultures and rumen fluid etc. will be undertaken in the second phase of the study.
- As proof-of-concept RNA isolation from solid materials like tissue and plant parts has been established using SafeForever™ version 1, further modifications in the SafeForever™ version is under process to establish its utility in RNA isolation from liquid samples like blood, saliva and serum.
- Total Protein isolation from blood and saliva is validated using SafeForever™ version 1 of the product. Further validation required to test protein isolation or its application when storing serum/plasma/urine samples.
- Metabolomics analysis profiling of the sample stored in SafeForever™ has to be validated.

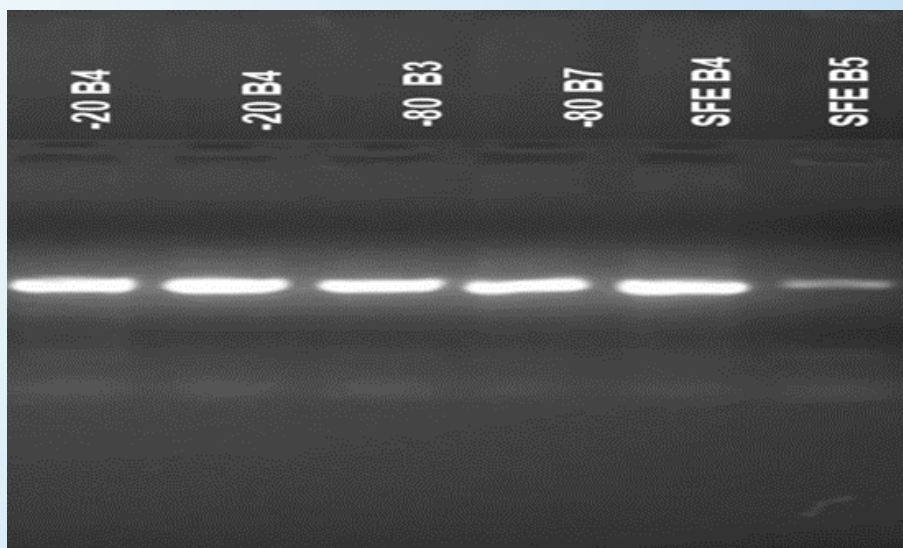


Figure 1. PCR product of CYP2D6 gene on 2% agarose gel. DNA isolated from blood stored at -20oC, -80oC, and SFE (SafeForever™) were used.

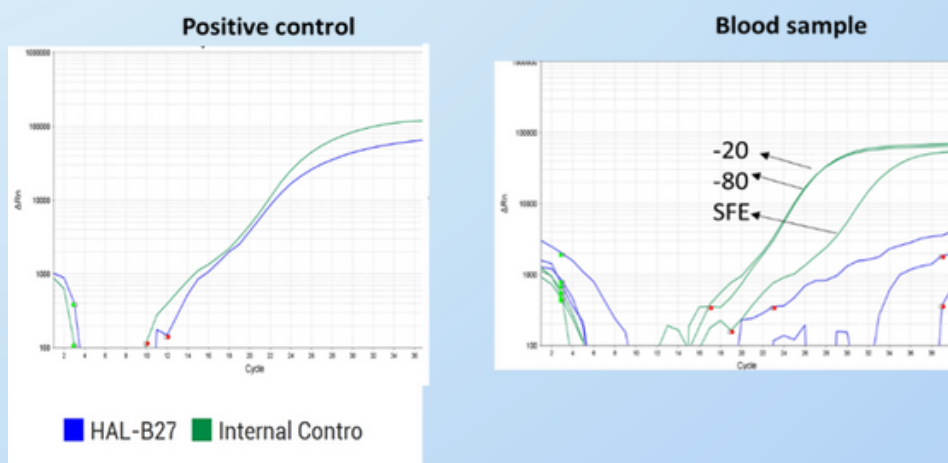


Figure 2. Real time PCR assay of HLA B27 from the DNA obtained from blood samples stored at different temperature and SFE (SafeForever™)

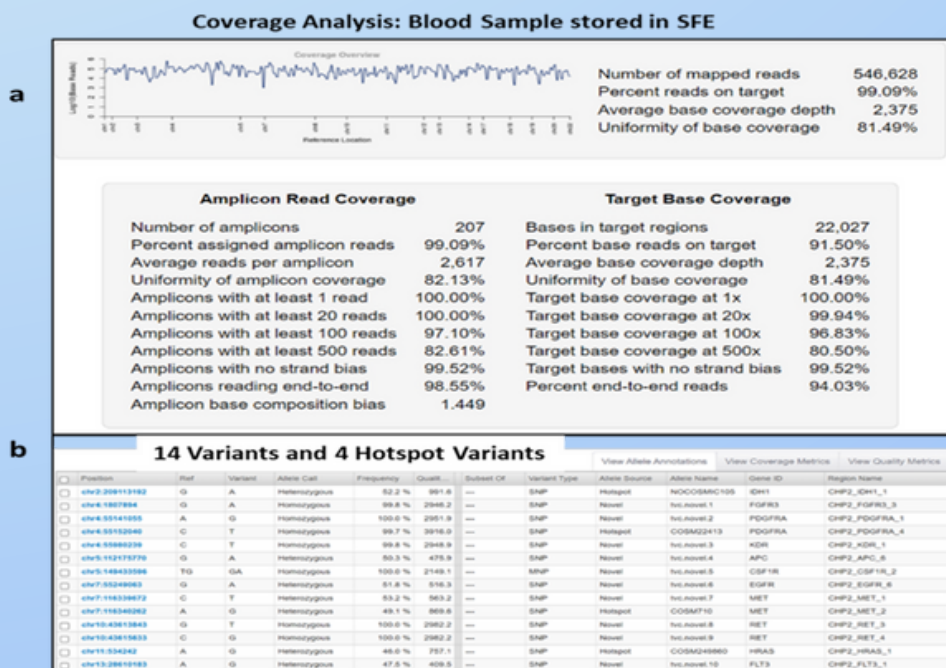


Figure 3. Coverage analysis and report and the variants called (a) Coverage overview from the alignment regions of Ion AmpliSeq Cancer Hotspot panel (b) Target regions containing variants detected in the Ion AmpliSeq panel

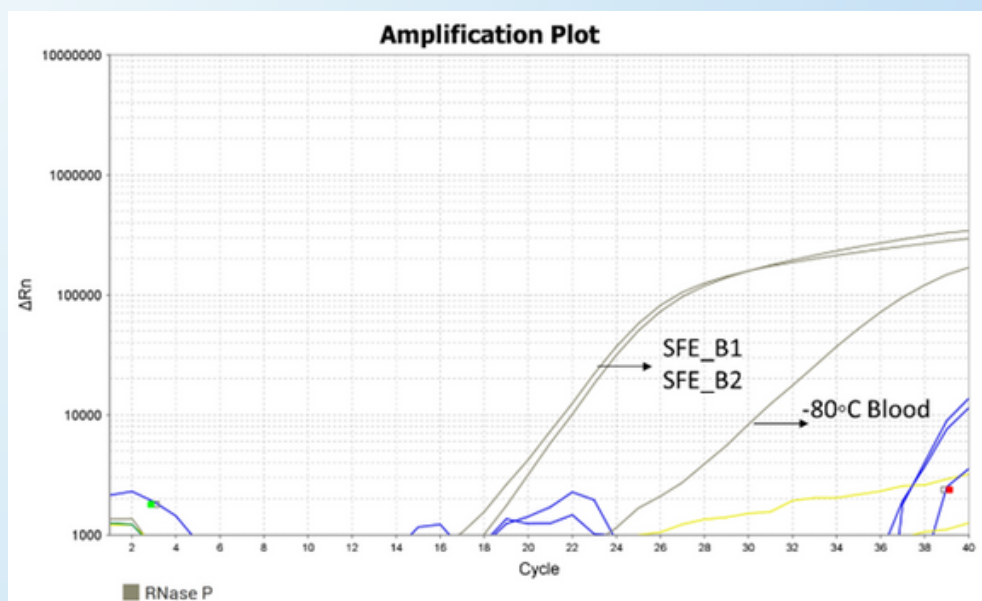


Figure 4. Amplification of RNase P with RNA isolated from rom -80°C blood and SFE (SafeForever™) stored

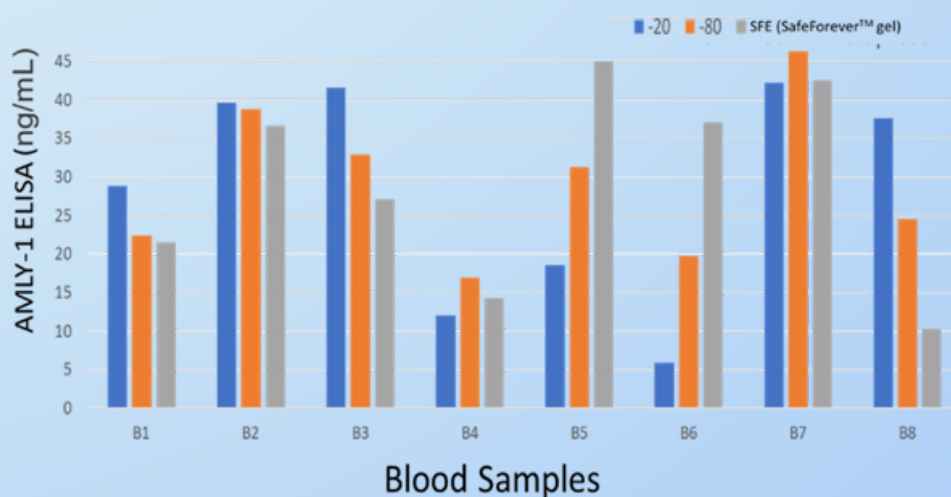


Figure 5. Human Amylase Alpha 1, AMY1 ELISA results analysed from the protein obtained from blood samples stored at different temperature and SFE (SafeForever™)

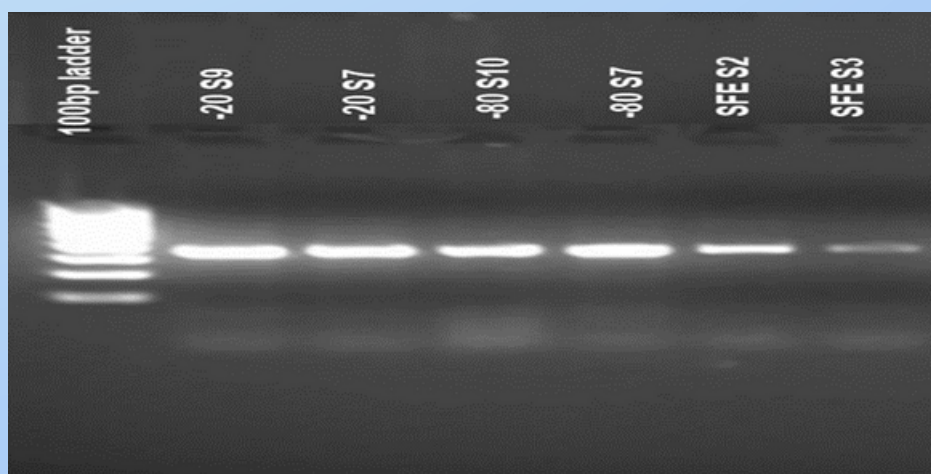


Figure 6. PCR product of CYP2D6 gene on 2% agarose gel. DNA isolated from Saliva stored at -20oC, -80oC, and SFE (SafeForever™) were used.

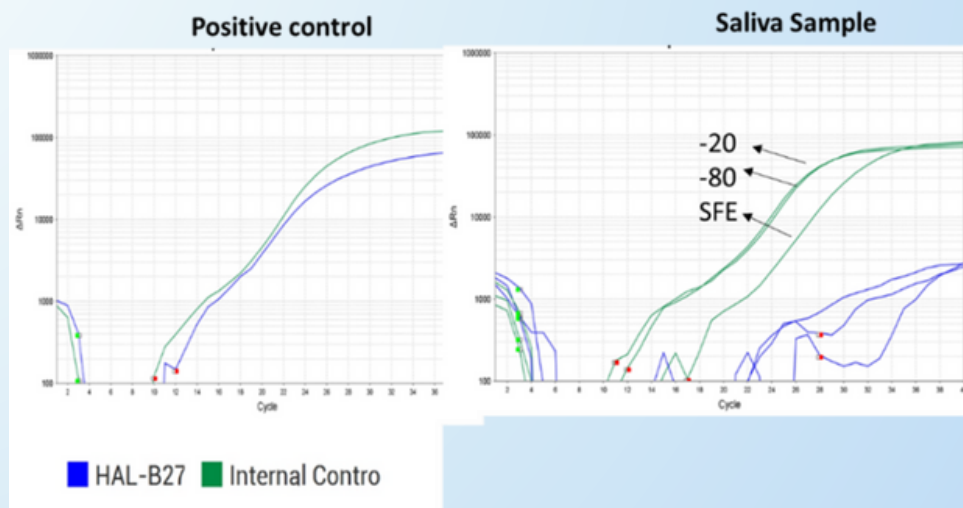


Figure 7. Real time PCR assay of HLA B27 from the DNA obtained from saliva samples stored at different temperature and SFE (SafeForever™)

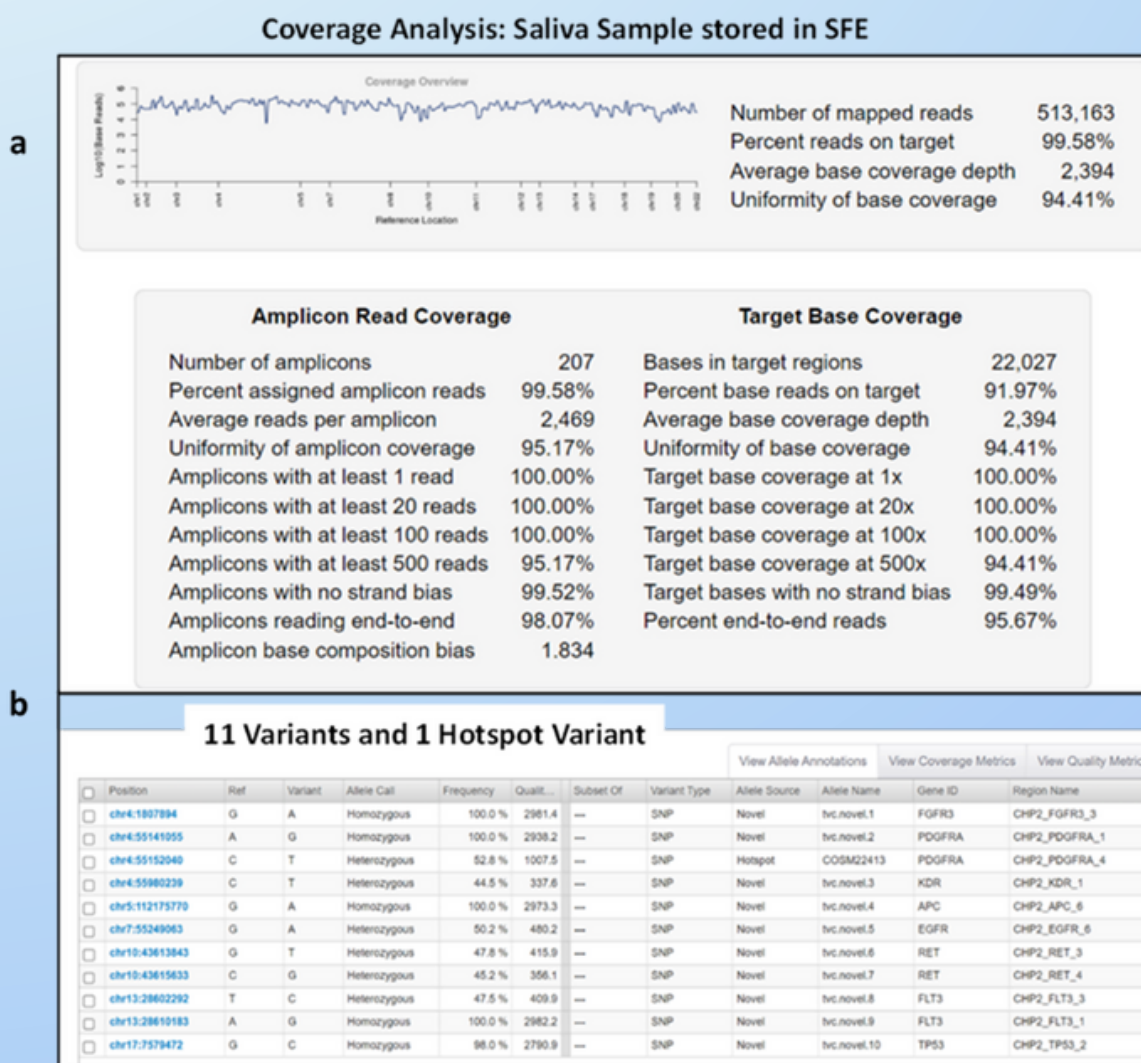


Figure 8. Coverage analysis and report and the variants called (a) Coverage overview from the alignment regions of Ion AmpliSeq Cancer Hotspot panel (b) Target regions containing variants detected in the Ion AmpliSeq panel

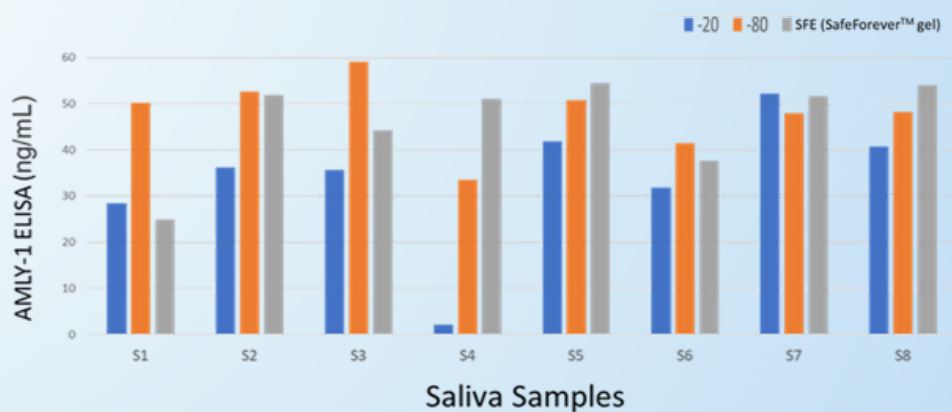


Figure 9. Human Amylase Alpha 1, AMY1 ELISA results analysed from the protein obtained from saliva samples stored at different temperature and SFE (SafeForever™)

Sample Type	Sample ID	Qubit DNA conc. (ng/ul)	Library Conc (ng/ml)
Live tissue at -80°C	LP-T2	22.7	2100
Liver tissue in SafeForever™ gel	SFE_LP-T2	22	1812

Sample	Bases	Q20	Reads	Mean Read Length	Read Length Histogram
Live tissue at -80°C	4,207,491	4,010,600	36,375	115 bp	
Liver tissue in SafeForever™ gel	4,408,493	4,200,249	38,381	114 bp	

Figure 10a. Library conc. and the QC of reads obtained from Ion AmpliSeq Library of liver tissue sample stored in -80°C and SafeForever™

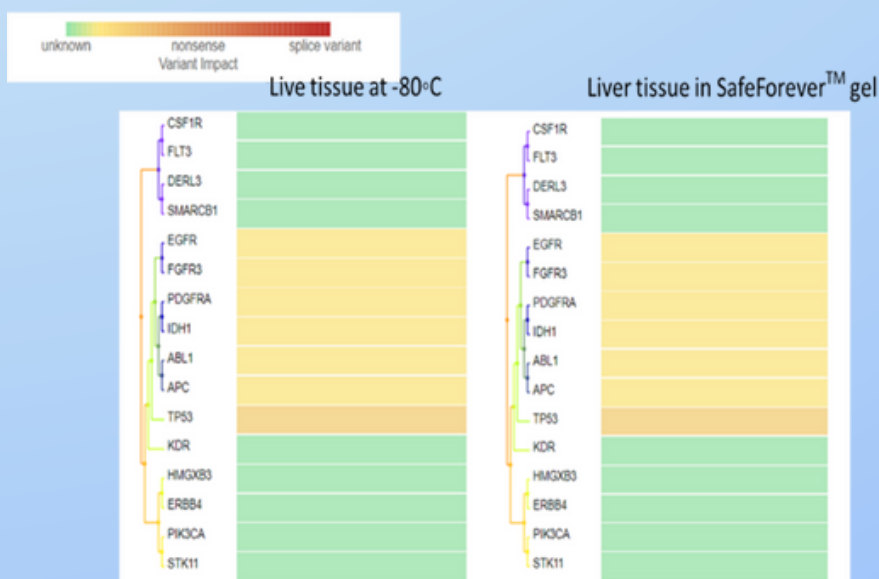


Figure 10b. Variant Visualization in the genes that was picked up by the Ion Reporter Software

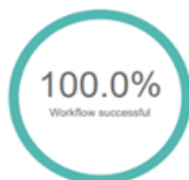
Fastq 16S ID: 406871

Human sample

QC and Barcoding

Reads per Exit Status

Showing the status of all the reads analysed



Workflow successful

27,896 Reads

Key Figures

READS ANALYSED

27,896

TOTAL YIELD

43.6 Mbases

AVG QUALITY SCORE

10.59

AVG SEQUENCE LENGTH

1,562

Quality Score

EXPORT PNG



Reads per Barcode ID

ID Read Count

barcode01 10,028

barcode02 11,393

barcode03 3,903

Figure 11. 16SMicrobial QC report of endoscopic biopsy samples stored in SafeForever™

Ladder RNA

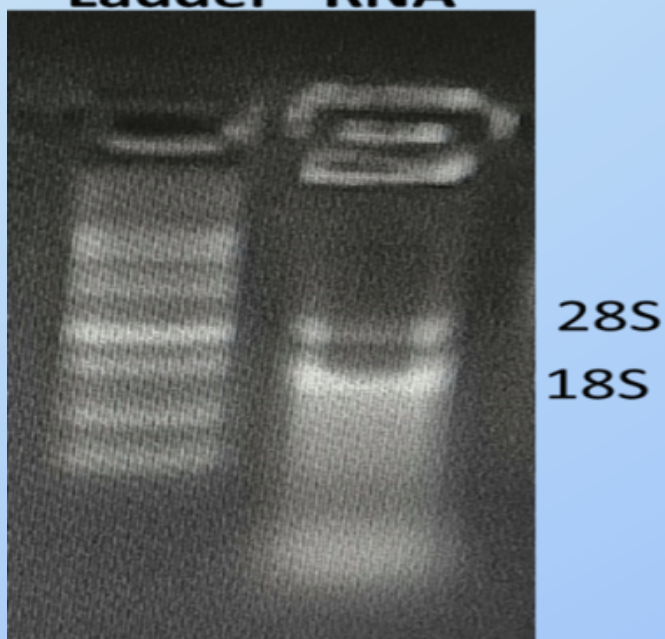


Figure 12. Agarose gel electrophoresis showing total RNA extracted from uterus tissue

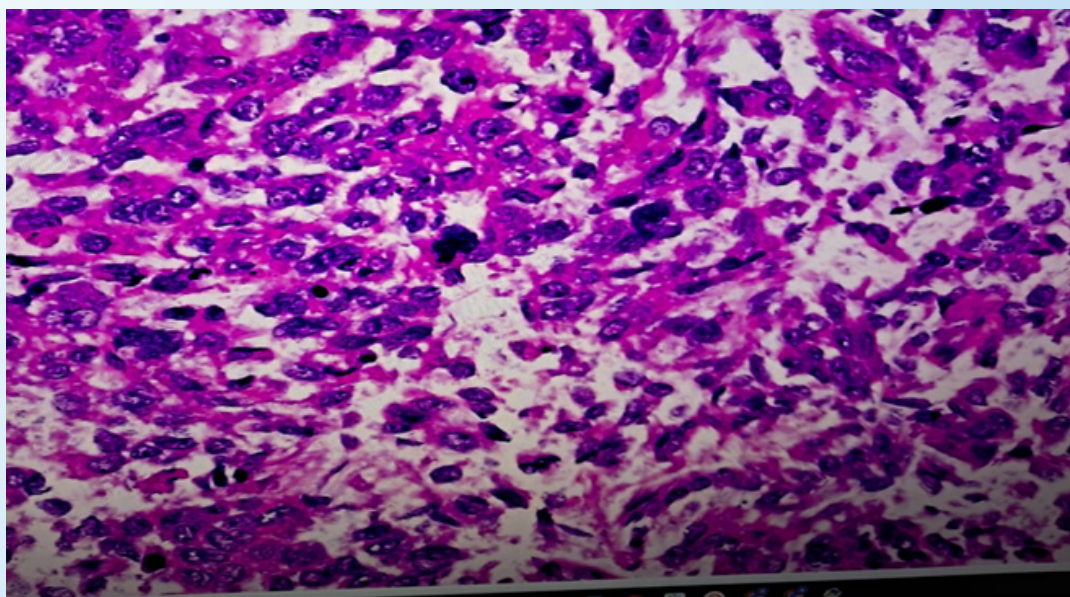
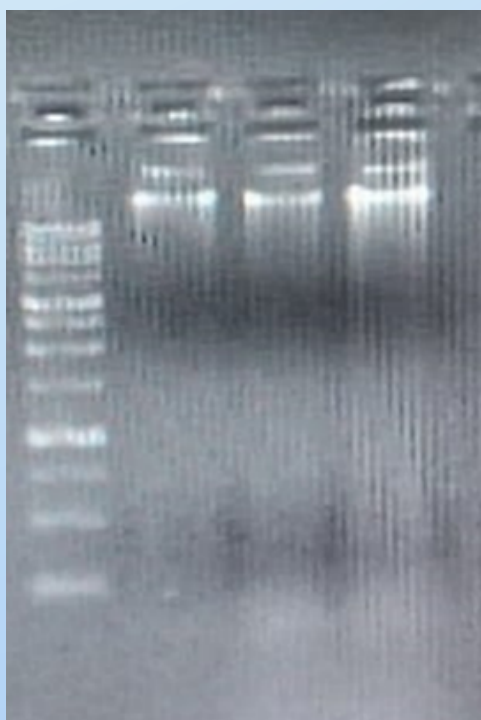


Figure 13. H&E image of the tissue after 24hrs preservation in SafeForever™



1. 1kb Ladder
2. SafeForever™ stored Leaf
3. SafeForever™ stored stem
4. SafeForever™ stored root

Figure 14. Agarose gel electrophoresis showing total DNA extracted from plant material

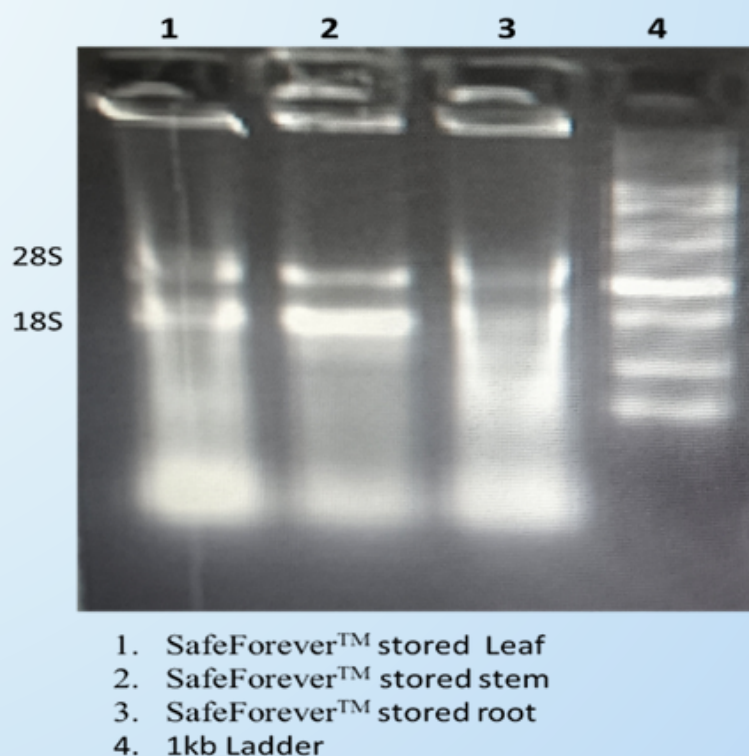


Figure 15. Agarose gel electrophoresis showing total RNA extracted from plant

a

Sample type	Storage	DNA conc. (ng/ul)	260/280	16srRNA read count
SFE1_Soil	SafeForever™	2.12	1.79	1,35,596

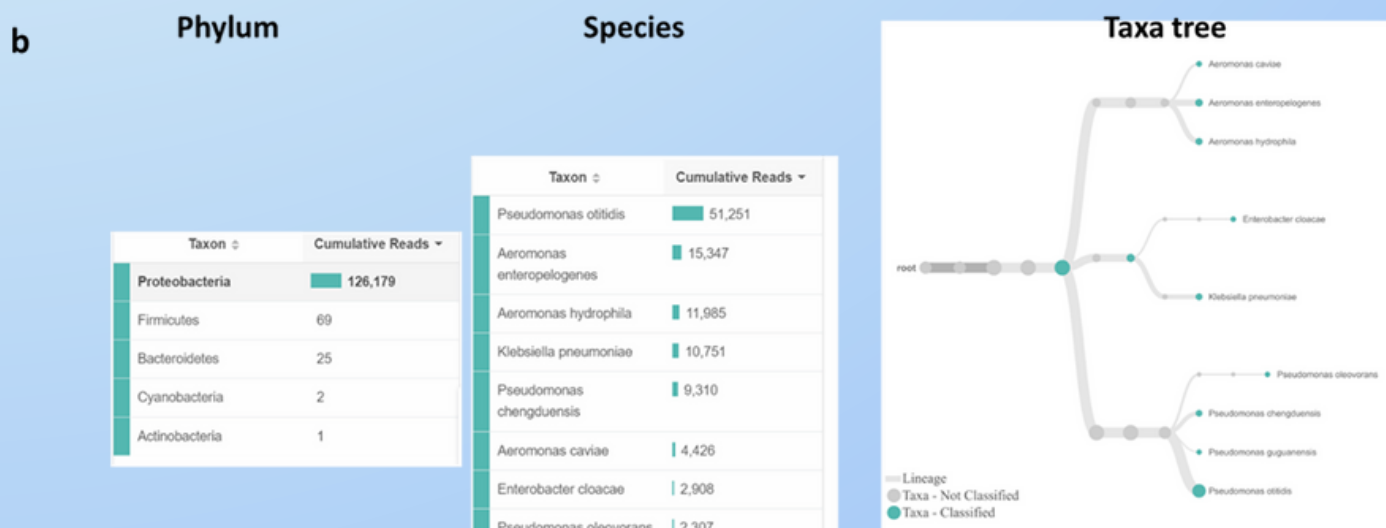


Figure 16. 16s rRNA sequencing performed on Oxford Nanopore system. (a) DNA conc of soil sample stored in SafeForever™ and the reads obtained (b) Phyla, species and the Taxa tree obtained from EPI2ME analysis of SafeForever™ stored soil sample



Bibliography

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