ApplicationForum

Purification of High-quality DNA from Saliva Samples with DANAGENE Saliva System applied to TargetSeq-NGS protocols

Alberto Acedo, AC-Gen Reading Life Inc, Valladolid, SPAIN acedo@acgen.es
David Navarro, DanaGen-BioTed S.L, Barcelona , SPAIN david@danagen.es

Introduction

DANAGEN-BIOTED S.L has developed a method for the collection, stabilization, transportation and purification of DNA from saliva samples using the DANASALIVA Sample Collection Kit, a cost-effective collection and transportation device that effectively stabilizes buccal cells and white blood cells found in saliva over 1 year at room temperature. Then, saliva DNA is isolated from the preserved saliva samples via DANAGENE Saliva Kit. Here, it is demonstrated the efficiency genomic DNA samples extracted with DANAGENE Saliva System to prepare a custom library (HC-Gen Test) designed by AC-Gen Reading Life Inc. for Hereditary Cancer Diagnostic (http://www.ac-gen.com/hereditary-cancer).

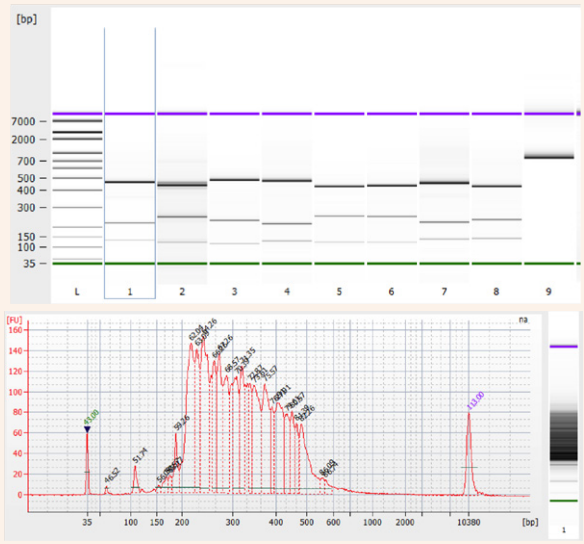
Materials and Methods

Genomic Isolation, DNA quality and quantification

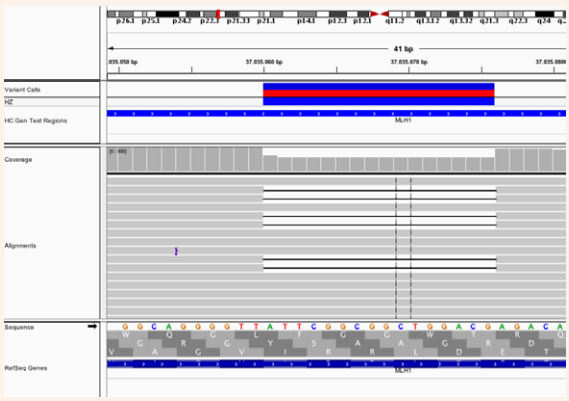
2ml saliva sample was collected using the DANASALIVA Sample Collection Kit from a hereditary colon cancer patient and gDNA was isolated from 600ul of saliva sample following DANAGENE Saliva Kit protocol. A fluorometry-based DNA method was used to accurately quantify DNA starting material. Size distribution of each DNA preparation was verified by gel electrophoresis and DNA purity was measured with a UV-Vis Spectrophotometer.

Library Target-Seq Preparation

gDNA sample was diluted to a final concentration of 5 ng/uL. 225ng of gDNA were digested in eight different restriction reactions, each containing two restriction enzymes. Restriction digestion reaction was validated by electrophoretic analysis of each sample. Then, all eight-digestion reactions corresponding to each DNA sample was transferred into appropriate tube. Next, biotinylated probes and barecode primers cassette were added and ligated. All interest target regions, corresponding with exonic regions of 37 genes related with Hereditary Cancer were captured using streptavidin-coated magnetic beads. Library was amplified and quantified for equimolar dilution. A pool of libraries were performed before proceeding to DNA sequencing with Ion PGM 200pb sequencing protocol using a 316V2 chip.



High sensitivity electrophoretic analysis showed a perfect restriction reaction for the eight combinations of restriction enzymes (Figure 1) and a correct library profile (Figure 2).



Variant calling of DNA sequences obtained in PGM system perfectly detected the frameshift mutation c.22_37del (p.I8Rdf*4) in MLH1 gene (Figure 3). This mutation had been previously analysed through Sanger sequencing for the same sample and it´s related with Lynch syndrome.

Results

DNA yield and quality from saliva sample purified using DANAGENE Saliva kit are shown in Table 1.

Gel electrophoresis performed to check gDNA integrity, didn´t show smearing below 2,5kb, indicating no sample degradation

Conclusion

As when building a house, any good NGS experiment is founded in a proper starting material. In this paper it has been demonstrated the high quality of DANAGENE Saliva System kit for the isolation of gDNA applied to mutation screening of clinically important DNA variants with NGS technologies. DNA isolation from saliva samples it´s a cost-effective method because samples can be collected directly for doctors without intervention of specially trained nurses and transported without special conservation requirements.

Table 1. DNA yield and purity from saliva sample

Saliva Sample	DNA Yield	Purity by absorbance 260/280	Purity by absorbance 260/230
600ul	20ug	1,85	1,92