Blood collection procedure

The site of blood collection was disinfected with alcohol pads. The plastic cap from the needle tip was lifted and the needle was injected into the vein. The plunger was softly pulled back and let the blood flow into the tube until the syringe was filled. The animal was not anesthetized and blood collection was carried out according to the 'Regulations for Animal Experiments in Chittagong Veterinary and Animal Sciences University'

DNA Isolation methodology

At first 200 µl of whole blood sample of BBG (healthy and disease free) was transferred into the microcentrifuge tube with 20 µl of each Proteinase K and RNase A enzyme. Then the Binding solution (200 µl) was added to the lysate and mixed well by pulse-vortexing for 15 sec. the Lysate was then incubated at 56°C for 10 min and further 200 µl of absolute ethanol was added. Thereafter, pulse vortexing for 15 sec, the lysate was carefully transferred into the spin column and centrifuged at 13,000 rpm for 1 min. Subsequently, the flow through was discarded and 500 µl of Washing buffer 1 was added in the spin column and centrifuged (13,000 rpm for 1 min). This step was also repeated for Washing solution 2. Again the flow-through was discarded and the spin column was dried by centrifugation (13,000 rpm, 1 min). Then, the spin column was transferred into a microcentrifuge tube and 100 µl elution buffer was added and let it stand for 1 min. Finally, to elute the genomic DNA centrifugation was done at 13,000 rpm for 1 min. The collected genomic DNA was then stored at -20°C before going to further downstream processing.

Library preparation

At first, 1μg genomic DNA was randomly fragmented by Covaris. The fragmented DNA material was examined by Gel-Electrophotometric and then purified by AxyPrep Mag PCR clean up Kit. The fragmented DNA material was combined with End Repair Mix, incubated at 20°C for 30 min. The purified end-repaired DNA was combined with A-Tailing Mix and incubated at 37°C for 30 min. Then, Illumina adaptors were ligated to the Adenylate 3'Ends DNA and incubated at 16°C for 16h. The purified adapter-ligated DNA fragments were selected as based on the insert size. Several rounds of PCR amplification with PCR Primer Cocktail and PCR Master Mix were performed to enrich the Adapter-ligated DNA fragments. Subsequently, the PCR products were purified and the libraries were qualified by the Agilent Technologies 2100 bioanalyzer and ABI StepOnePlus Real-Time PCR System. Finally, the qualified libraries were sequenced pair-end using Hiseq 2500 System (Illumina).