The Agilent 2100 Bioanalyzer as quality control for next generation sequencing

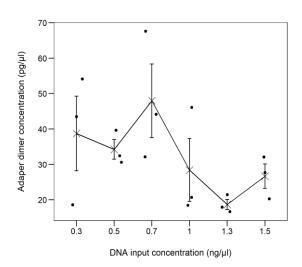


Figure 1: Adapter dimer concentrations of DNA samples with input concentrations ranging from 0.3 to 1.5 $\text{ng/}\mu\text{L}$. Displayed are the data points, SD, and the mean (x).

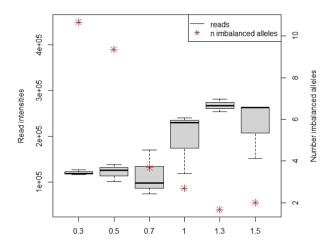


Figure 2: Read intensities and number of imbalanced alleles of samples with DNA input concentrations ranging from 0.3 to 1.5 ng/ μ L. Boxplots represent interquartile ranges and median of read intensities.

Next-generation sequencing (NGS) has advanced forensic DNA analysis, but challenges remain with degraded samples from deceased humans. This study examines the Agilent 2100 Bioanalyzer for quality control in NGS. We assessed DNA concentrations, fluorescence unit (FU) values, and adapter dimer concentrations. Lower DNA input led to reduced FU values and more adapter dimers, but the Bioanalyzer effectively detected adapter dimers in degraded remains, demonstrating its value in forensic NGS.

Next generation sequencing (NGS) technologies have expanded the spectrum of forensic DNA analysis by facilitating precise genotyping¹ for identification purposes of unknown human deceased. Yet, challenges persist regarding complex sample processing and assurance of equal molar concentrations across pooled samples. Since optimal cluster density is crucial for sequencing performance, the determination of both quantity and quality is indispensable².

We investigated the application of the Agilent 2100 Bioanalyzer for library quality control. Our analysis included assessing total DNA concentrations, FU values, and adapter dimer concentrations. The sensitivity study revealed a decrease in FU values and an increase in adapter dimers with declining DNA input concentrations. Deviations in total DNA concentrations and FU values between the runs indicated a lack of repeatability. Yet, the analysis of degraded samples from decomposed human remains has shown the ability to detect adapter dimer concentrations, important for the sequencing success. Therefore, the Agilent 2100 Bioanalyzer proves to be a valuable tool for NGS quality control.

Group Leader:

Dr. Iris Schulz Iris.schulz@unibas.ch

References:

(1) England R, Harbison S. A review of the method and validation of the MiSeq FGx™ Forensic Genomics Solution. WIREs Forensic Sci.2020;2(1):e1351. https:// doi.org/10.1002/wfs2.1351

(2) Head SR, Komori HK, LaMere SA, Whisenant T, Van Nieuwerburgh F, Salomon DR, et al. Library construction for next-generation sequencing: overviews and challenges. Biotechniques. 2014;56(2):61–77. https://doi.org/10.2144/000114133