Visualizing DNA for Long Read Sequencing by Moles, not Mass.

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Traditional measurements during NGS library preparation include the determination of the sample mass. This analysis is easily performed with the Agilent automated electrophoresis instruments, which provide visual results in the form of a digital gel and electropherogram. The electropherogram displays the fluorescent signal as a graphical representation, with the size on the X-axis and relative fluorescence units (RFUs) on the Y-axis. The height of the fluorescent signal is therefore directly proportional to the mass of sample at a given size. While this representation has been widely used for quality control of sheared gDNA and the final NGS library, examining the molarity of a sample may provide a better visual representation of the number of sequencing reads that can be produced by a sample, especially for long read sequencing. High molecular weight samples were analyzed using the Agilent Femto Pulse system and the accompanying ProSize data analysis software. ProSize allows the user to visualize the electropherogram image as a product of either mass or molarity by switching the Y-axis. By visualizing the data in moles and utilizing a smear analysis, the Femto Pulse can be used to determine the number of moles of sample found within different sizing brackets and providing a prediction of long read sequencing read length. This data can be used to make informed size selection experiments prior to sequencing, allowing customers to make informed long read sequencing decisions.