

AGBT 2017 Poster Presentation

Accurate quantitation and normalisation of genomic DNA for high-throughput DNA library construction

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Abstract:

The Wellcome Trust Sanger Institute receives up to 20,000 DNA samples each month, the majority of which are destined to be processed through its high-throughput sequencing pipelines. In order to allow the slick passage of samples through these workflows, it is vitally important that the samples are accurately quantified and robustly normalised to target concentration and volume. Achieving this goal at scale is a difficult challenge intensified by the broad concentration range of incoming DNA samples, the viscoelastic properties of high molecular weight genomic DNA and because many of the samples are limited and/or irreplaceable.

We sought to develop a novel DNA quantitation and normalisation workflow that is accurate, scalable, inexpensive and minimizes the material lost to the measurement process. Central to this new process is the Mosquito liquid handler (TTP LabTech) which we use to remove small volumes of stock DNA from standard 96-well plates or low profile FluidX tubes. DNA (200 nl) is dispensed in to triplicate wells of a 384-well assay plate (together with DNA standards), combined with dye reagent and transferred to a fluorescence plate reader. The success of each assay is indicated by the quality of the standard curve and a set of internal controls. Values recorded are used to populate 'cherry-picking' robot files that are in turn used to generate plates of genomic DNA samples that are normalised for volume and concentration.

Validation tests indicated a dynamic range (stock genomic DNA) of 0.03-200 ng/ul for the assay. Combined with newly developed cherry-picking protocols, we were able to generate tightly normalised plates with a target of 250 ng per well (mean = 263 ng; CV= 4.1%). The production of high quality cherry-pick plates will allow us to develop highly streamlined DNA library construction workflows, improve first time success and ultimately drive down the cost of sample processing.