Optimization of DNA recovery: Effect of temperature, elution volume, and DNA fragment length on DNA release from AMPure XP

Genomics

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DNA binding to magnetic beads is an integral part of many DNA extraction and library preparation workflows for next generation sequencing (NGS). This process is utilized for nucleic acid purification, concentration, and size selection, and can be run multiple times in a single workflow. It is a key step in the workflow, and optimization of DNA recovery from the magnetic beads has a large impact on DNA recovery from the entire process. In this study, the effects of potential mechanisms to increase DNA recovery modifications are quantified using AMPure XP, a commonly used magnetic bead for library preparation.

The rate of DNA release from SPRI beads at room temperature and 60° C was measured, and a large DNA fragment (49 kb) was released from beads slightly faster at the higher temperature. At room temperature, samples reached equilibrium after 2 min of incubation with the beads, so the time savings from a heated elution is not significant. The elution buffer was also varied, and higher yields were seen in buffered solutions at pH 6-8 than in nuclease-free water. This effect was greatest with larger DNA fragments (49 kb) and decreased as DNA size decreased (to 1 kb). DNA elution does not appear to be affected by changing the volume of the eluant; the smallest elution volumes are limited by pipette capability rather than DNA elution kinetics. Elution volumes can thus be optimized based on workflow and labware capabilities rather than chemistry limitations.

The analysis of these variables in DNA release will lead to an understanding of which variables are critical to good DNA recovery and which can be modified for ease of use and optimization of recovery. This study establishes these parameters and will lead to more informed decision-making when optimizing library preparation workflows.