ASSESSMENT BY QPCR OF DNA BINDING TO PLASTIC MICROFUGE TUBES

Ram Kishore[1], Cristián Orrego[2]
[1]California Department of Justice, Jan Bashinski DNA Laboratory, Richmond, CA 94804, USA
[2]Human Rights Center, School of Law, University of California, Berkeley, CA 94705, USA

DNA tends to bind to plastic surfaces. Binding to microcentrifuge tubes can have a significant impact on recoveries of DNA, particularly from low copy DNA samples. It is therefore desirable that tubes used for processing and storing DNA be evaluated beforehand regarding their capacity for irreversible DNA binding. We find that qPCR can be a practical tool towards this end.

Serial dilutions of standard DNA were prepared in triplicates using Dynalab 1.5-mL, Axygen 1.7-mL and Eppendorf DNA LoBind 1.5-mL tubes. The Quadruplex (Jan Bashinski DNA Lab in-house qPCR assay) and Quantifiler® Duo protocols were performed after storing serial dilutions of the DNA standards at representative conditions of temperature and time. The slopes of the standard curves following qPCR (x axis = log [DNA], y axis = C_t) were evaluated. Slope shifts to more negative values would reflect proportionally larger amounts of DNA being removed from solution at increasingly lower dilutions due to capture by the plastic matrix.

The qPCR Quadruplex assay revealed that the slope of the nuSRY standard curve was -3.9 when dilutions were prepared in Axygen 1.7-mL tubes, compared to -3.5 for Dynalab 1.5-mL and Eppendorf DNA LoBind 1.5-mL tubes. Storage of serial dilutions at 4 °C for 24 hr yielded an average slope of -4.6 for nuSRY, nuTH01 and nuCSF standard curves with Axygen 1.7-mL tubes; slope values for tubes from the other two manufactures ranged from -3.5 to -3.8 for all three qPCR probes. Several DNA dilution panels displayed "dropout" (no C_t obtained by 40 cycles) at the highest dilutions when stored at -20 °C for 7 days in Axygen 1.7-mL tubes. The Quantifiler® Duo assay gave similar results.

DNA adhesion to tubes during DNA extraction was evaluated using an organic extraction procedure followed by final clean-up and concentration steps with a NucleoSpin® device. A single sample lysate was prepared by digesting a bloodstain for 2 hr in a 15-mL conical plastic tube followed by aliquoting equal portions in triplicates into sets 1 (Dynalab 1.5-mL, Axygen 1.7-mL and Eppendorf DNA LoBind 1.5-mL tubes) and 2 (Axygen 1.5-mL and 1.7-mL and Eppendorf DNA LoBind 1.5-mL tubes). Final fractions were quantified using the Quadruplex assay. Approximately 50% less DNA was recovered in Axygen 1.7-mL tubes compared with the 1.5-mL tubes from all three manufacturers. Further loss of DNA was observed when extracts were stored at -20 °C for longer times in Axygen 1.7-mL tubes, with DNA concentrations remaining unchanged in the other three types of tubes.

The values of the slopes of the standard curves in qPCR assays are diagnostic of DNA loss to tube surfaces. Routine assessment of microcentrifuge tube types (and different lots from each) for low DNA adhesion properties should be feasible using qPCR methods in forensic genetic laboratories. ⌘