Biobanking DNA: Getting It Right from the Start

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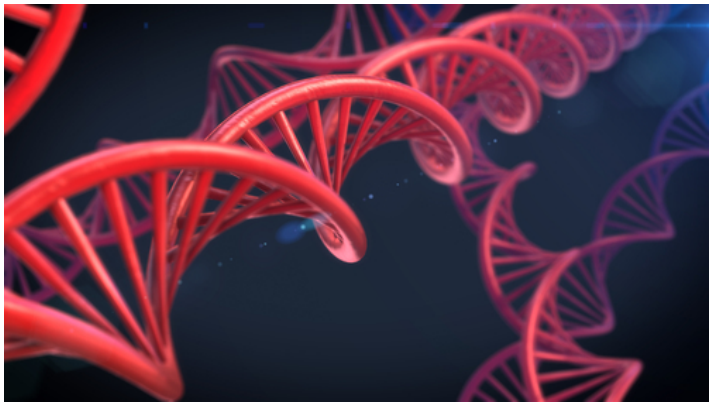

Preparing high-quality double-stranded DNA is essential for next-generation sequencing (NGS) and microarray techniques. Psifidi et al. (2015) compared eleven methods for preparing DNA from blood samples, scoring them to find the best extraction methods that give consistently high-quality material for biobanks.[1]

For best success and optimal data recovery, the DNA collected must be pure, free of contaminants that could inhibit genomic workflows and sufficiently concentrated to meet assay requirements. NGS assays are the current gold standard for genomic research, while microarrays offer a cheaper alternative, depending on the nature of the research. However, these workflows need more DNA than traditional methods such as polymerase chain reaction (PCR). Microarrays need approximately 100 times more DNA than a standard PCR reaction, and the starting material must be very pure. For large-scale genotyping studies, standardizing extraction workflows by using kits can maximize yields while maintaining consistency.

Psifidi et al. examined the quality and quantity of DNA extracted using three commercial silica-based kits. They also examined modifications of these kits, designed to maximize yields and quality. In addition to the commercial kits, they looked at two in-house methods (phenol-chloroform and a magnetic bead separation protocol) as well as an ion exchange–based commercial kit.

The researchers drew blood from 16 ewes in an experimental flock, collecting 11 samples from each animal by jugular venipuncture. They pooled the samples from each animal before subdividing aliquots to ensure a consistent number of leukocytes for each test extraction. The team used whole blood or buffy coat, depending on extraction requirements specified in the kit or protocol.

When examining how well the different protocols performed, the researchers looked at factors such as cost, time taken to process samples and labor requirements. They also considered

DNA quality by looking at purity, total yield, concentration, presence of inhibitors and integrity. In this way, the results reflected the value of each method as it pertained to DNA biobanking for onward NGS or microarray analysis.

The team used standard spectrophotometry to measure DNA purity and a Qubit 2.0 fluorometer to measure yield and concentration in the final preparations. They analyzed DNA purity by gel electrophoresis and then conducted real-time polymerase chain reactions (RT-PCR) for two different targets to check for replication inhibitors co-precipitated with the DNA. Using these and the physical assessments (time, cost and labor), the researchers assigned a final score for each method.

For the commercial kits examined, Psifidi et al. found that modifying the protocols in-house to maximize recovery and purity gave the best results, regardless of the intended starting materials for each kit. The modifications introduced included sample pretreatment, longer incubation with proteinase, and a chloroform extraction step, for example. Results for these modified kits gave the best returns in yield, purity and suitability for long-term DNA biobanking and NGS/microarray usage.

The researchers also found that one of the in-house protocols performed well. This protocol used a magnetic bead separation procedure following cell lysis during DNA extraction. Results were comparable to those obtained using the modified commercial kits described above, with yields, cost and purity being suitable for onward NGS/microarray assessment. The protocol also produced sample quality considered viable for long-term DNA biobanking.

After choosing the four highest-scoring DNA extraction techniques, the researchers looked at applying them for large-scale genotyping. Using blood obtained from 600 ewes of the same breed in the experimental flock, they used the four methods to extract DNA, then stored it at -20°C for three years. Following storage, the team examined the DNA, measuring quality, purity and integrity before carrying out microarray genotyping. Gel electrophoresis showed that the DNA extracted had not deteriorated during storage, while spectrophotometry indicated that the samples exceeded thresholds for purity. Moreover, genotyping results showed low variability between extraction procedures.

In summary, Psifidi et al. recommend the four protocols shown to perform best as suitable for extracting DNA destined for large-scale genotyping studies involving NGS or microarray assay. The researchers advise that optimizing sample preparation and extraction is essential for building high-quality DNA biobanks for the future.

Reference

1. Psifidi, A, et al. (2015) "Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using blood samples," PLoS ONE 10(1), doi:10.1371/journal.pone.0115960.