Good paper!

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The Magic of Magnetism

Separation of biomolecules has long been a challenge for scientists, but with recent developments and progresses in bioengineering fields, it has become a relevant issue for engineers as well. Scientists often must take a solution containing different proteins, nucleic acids, lipids, and/or other cellular constituents and purify out one specific molecule or family of molecules. One technique that has proven helpful in this endeavor is the use of streptavidin magnetic beads that can be easily precipitated with an external magnetic field.

Streptavidin is a small particle which, although not intrinsically magnetic, can be easily magnetized with an external magnetic field. This property allows scientists and engineers to localize magnetized streptavidin molecules with the application of a magnetic field. Conveniently, the endogenous metabolic catalyst Biotin strongly binds to streptavidin particles, and can also be used to label specific nucleic acids. The net result of these interactions resembles a co-immunoprecipitation or a chromatin-immunoprecipitation: biotin is used to label nucleic acids, then bound to streptavidin and precipitated with its binding partners (proteins or complementary strands) using an external magnetic field.

Good! bringing the point forward.

This technique has proven very useful for many different applications, but it is still subject to certain limitations due to the intrinsic error of DNA binding interactions. Biotin-streptavidin interactions are very good for separating molecules in situations where an error rate is unimportant or can be corrected; however, scientific or engineering applications that require high specificity and low error rates call for a more precise technique.

An example of a useful application of this technique is Complementary Strand Analysis (CSA), a method for allelic separation reported by Arguello et al. Biotinylated 3′ primers of an experimental heterozygous sample of DNA are used in a PCR reaction to amplify the anti-sense strand while a separate PCR reaction includes 5′ primers of the same locus in a homozygous control population to amplify the sense strand. Streptavidin is added to the mixture, and used to precipitate the biotinylated molecules, and complementary strands not attached to the biotin molecule are eluted with NaOH. The purified sense and anti-sense strands are now able to be hybridized to form a chimeric DNA duplex which can be separated in a polyacrylamide gel based on mismatches between the two strands that change the duplex's mobility.

CSA is a useful technique for following the inheritance of alleles in a family or for separation of alleles in a complex polymorphic genetic system. It is a simple procedure that does not require highly specialized equipment or expensive reagents; however, CSA has many limitations. It would probably only be effective for applications that differentiate between two or three alleles rather than a very large number, and could be difficult to use for delicate processes that could be affected by the reasonably small false-positive or -negative rates inherent in molecular binding interactions.

In 1994, Leonard Adleman published a paper entitled "Molecular Computation of Solutions to Combinatorial Problems" in which he claimed to have successfully used DNA binding interactions to compute a Hamiltonian solution to a simple directed graph. In step four of his algorithmic procedure, Adleman selected for DNA molecules encoding paths that enter each vertex once by attaching a biotinylated single-stranded-DNA probe to a streptavidin bead, selecting molecules containing sequences for each vertex and amplifying between each precipitation using PCR.

Adleman was using this technique as a proof-of-concept and not trying to optimize his protocols for selecting molecules of interest and *only* molecules of interest. In this setting, biotin-streptavidin precipitations are very useful; however, the procedure that Adleman followed was far from optimal for solving more complex problems or for situations which require a very pure product. It is impossible to eliminate unwanted background DNA interactions because of the inherent rate of nonspecific interactions in molecular binding. Adleman actually made this source of error more prominent by PCR amplification between each step and by not repeating his biotin-streptavidin precipitations for each vertex to minimize the rate of false-positives. Likewise, it is inevitable that candidate molecules that fit the criteria for extraction will remain in solution unbound to the biotin-labeled probes and will not be represented in the final products. While not a problem in Adleman's initial experiments due to the simple nature of the graph and the high concentrations of oligonucleotides, the fact that some "paths" will not be present in the final set would present a problem should this technique be applied to a more complicated problem requiring better specificity.

It is necessary to weigh the required level of specificity against the inherent error rate when deciding whether or not magnetic separation are a useful technique for a given application. For engineering applications which require high specificity and low error rates (such as a more complicated version of Adleman's "molecular computer" where biomolecules are being used to carry out computation), one must either improve and optimize this technique or find a new one all together. In his experiment, Adleman could have reduced his error rate by repeat applications, but biotin-streptavidin beads could be very useful, for engineering applications which simply attempt to purify a product where the erroneous possibilities could be easily eliminated.

how well does this scale to larger problems? loss of material in separations?

The same conditions hold for scientific application of this process. Experiments such those described in the CSA paper make very adept use of biotinylated primers to isolate certain molecules in an attempt to genotype an experimental population at a genetic locus known to have polymorphisms. The same error rate exists in these experiments, but erroneous DNA run on the final gels is in small enough quantities that it will not cloud the overall results. On the other hand, scientific experiments trying to examine the specific binding interactions between, for example, a stretch of DNA and its bound proteins may need to take error rates into account when examining final results.

Regardless of these limitations, this technique has many other potentially useful applications. It is easy to apply to the isolation/precipitation of certain biomolecules or their binding partners, similar to a co-IP, and could be used for purification/isolation of a specific molecule or screening for molecular binding interactions. Beyond these applications, however, one could even imagine targeting drugs or nucleic acids to a specific location in the body for therapeutic purposes with an external magnetic field, possibly eliminating some of the toxic effects of current methods.