

# **Nucleic acid separations utilizing immobilized metal affinity chromatography.**

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Immobilized metal affinity chromatography (IMAC) is widely used for protein purification, e.g., in the isolation of proteins bearing the well-known hexahistidine affinity tag. We report that IMAC matrixes can also adsorb single-stranded nucleic acids through metal ion interactions with aromatic base nitrogens and propose that metal affinity technologies may find widespread application in nucleic acid technology. Oligonucleotide duplexes, plasmid, and genomic DNA show low IMAC binding affinity, while RNA and single-stranded oligonucleotides bind strongly to matrixes such as Cu(II) iminodiacetic acid (IDA) agarose. The affinity of yeast RNA for IDA-chelated metal ions decreases in the following order: Cu(II), Ni(II), Zn(II), and Co(II). Adsorption isotherms for 20-mer oligonucleotide homopolymers show that purines are strongly favored over pyrimidines and that double-stranded duplexes are not bound. IMAC columns have been used to purify plasmid DNA from *E. coli* alkaline lysates, to purify a ribozyme, to remove primers and imperfect products from PCR reactions, and to separate 20-mer oligonucleotide duplexes containing centered single-base mismatches. Potential further applications include SNP scoring, hybridization assays, and the isolation of polyadenylated messenger RNA.

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