Back-extraction of PNA affinity tags following HIC purification of DNA targets

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Conventional nucleic acid purification techniques rely on differences in size, shape, and charge density to separate target samples from heterogeneous mixtures. However, the non-specific nature of these processes does not allow for separation of nucleic acid strands of comparable size but different sequence. This is a critical concern for a variety of applications; for example, to ensure sequence homology in plasmids used for gene therapy, for PCR-based amplification of low copy-number targets, and for extraction of trace mRNAs for expression analysis. While sequence-specific affinity tags have been used for DNA purification, they are typically immobilized on solid supports, thereby inhibiting recovery yields by slow DNA binding kinetics and non-specific surface fouling. These difficulties may be alleviated with the use of soluble, self-assembling affinity tags.

Previous work in our group has shown that peptide nucleic acid amphiphiles (PNAAs) can bind target ssDNA and dsDNA, and using hydrophobic interaction chromatography (HIC), these complexes can be separated from non-target strands. A schematic of a PNAA molecule is featured in Figure 1. Like most existing nucleic acid purification techniques, separation of similarly-sized strands is impossible in traditional HIC. The affinity tag selectively alters the hydrophobicity, and hence the retention, of the resulting complex, providing a large shift in retention volume for target sequences compared to unlabeled DNA. As an extension of this work, we have demonstrated that target ssDNA and dsDNA can be separated from heterogeneous DNA mixtures. By careful manipulation of common HIC parameters, we have achieved high-resolution separation of a 60-base target ssDNA and a 40bp dsDNA oligomer from non-target DNA with single base mismatch sensitivity. However, PNAA binds dsDNA with high affinity, making PNAA removal under non-DNA denaturing conditions exceedingly difficult. The removal, or back-extraction, of the PNAA affinity tag is critical before performing PCR amplification of HIC products, or using HIC products for gene therapy.

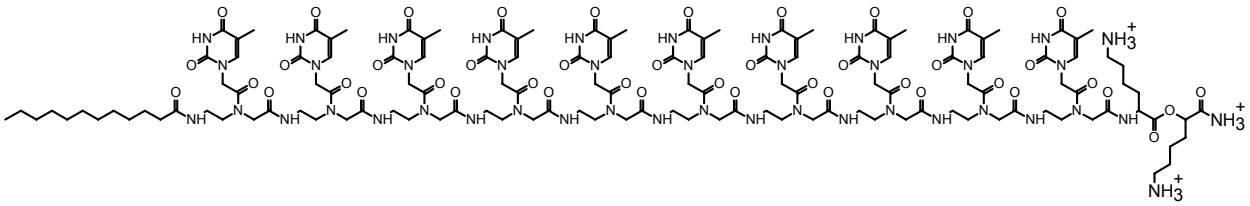

Figure 1: Structural diagram of C_{12}-t_{10}-$(Lys)_2$, one of the PNA amphiphiles used in this study.

Recently, we have discovered that the PNAA affinity tag can be removed from a sub-population of PNAA/dsDNA complexes using centrifugally-driven size exclusion, and that this enriched sample can undergo PCR amplification without complication. HIC fractions were collected and subjected to centrifugation at 12,000g in Microcon (Millipore, Billerica, MA) spin filters at room temperature. Subsequent PAGE analysis illustrated the reappearance of intact

target DNA, devoid of affinity tag, along with two distinct PNAA-DNA complex stoichiometries, one of which dynamically forms as a result of HIC processing. By adding non-target strands with a molecular weight distinct from target prior to HIC, we have also shown that DNA capture and subsequent removal is sequence-specific, as no non-target DNA, free or PNAA-bound, appears in the gel (Figure 2). Furthermore, it is possible to achieve complete target DNA recovery by treating PNAA-DNA HIC fractions with a chemical denaturant and removing the PNAA by size exclusion. This method has been successfully applied to the separation of sheared plasmid from DNA/protein mixtures and its subsequent identification via PCR.

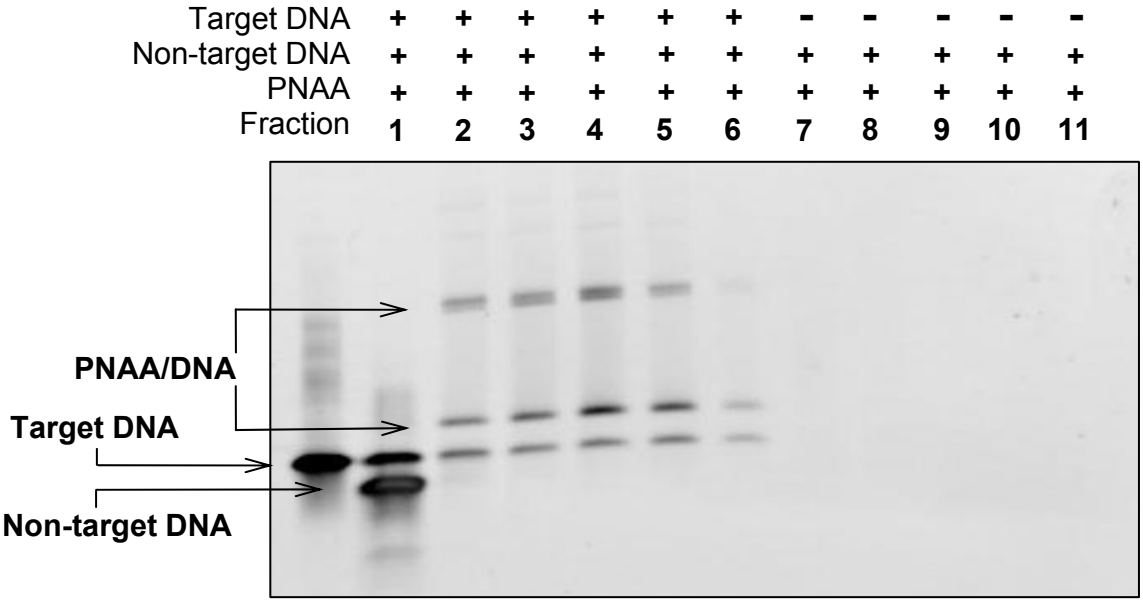

Figure 2: Capture of dsDNA by PNAA is sequence specific, even in the presence of non-target nucleic acids. Target DNA is 40bp and contains a PNAA binding site, while non-target dsDNA is 30bp. Each fraction corresponds to a 1 mL sample taken at the appearance of void volume peak (fraction 1), and the putative PNA-DNA peak (2-6). Fractions 7-11 represent a negative control in which non-target DNA and PNAA were incubated and introduced to the column. Gel: 4-20% TBE/polyacrylamide, 120 minutes at 100V.