Resource Summary Report

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Ultrasensitive hybridization capture of short tuberculosis cell-free DNA from urine

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Protocol Information

URL: https://dx.doi.org/10.17504/protocols.io.bep4jdqw

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Summary: Overview: This protocol describes a method for highly sensitive sequence-specific purification of short tuberculosis (TB) urine cell-free DNA (cfDNA) from large-volume (10 mL) urine samples. Biotinylated oligonucleotide capture probes complementary to the target of interest are immobilized on streptavidin-coated magnetic beads and used to capture, concentrate, and purify target DNA via hybridization. This protocol improves upon the analytical performance of both existing silica-based extraction methods for urine cfDNA and previous hybridization capture protocols, and meets several previously unmet design criteria for urine cfDNA: (1) high recovery of short fragments, (2) large sample input volume, and (3) . There are two key innovations that contribute to the robustness and unprecedented sensitivity of this method: dual biotinylated capture probes, which increase recovery compared to single biotinylated probes by moderating probe density on the bead surface (a key variable affecting efficiency of surface-based hybridization), improving thermostability of the bead-probe linkage, and eliminating interference by endogenous biotin in urine, and a two-probe system for each target region, which enables recovery of both strands of double-stranded DNA. We designed this hybridization method for capture of short, dilute TB cfDNA fragments from urine, but anticipate that it will be versatile and may be useful for other applications and sample types requiring sensitive and efficient purification of DNA from large sample volumes. Design of primer and probe sequences for new targets is straightforward (see "Materials" tab).Expected performance:Near 100% recovery (95% CI: 82.6 - 117.6%) of synthetic ssDNA or dsDNA spiked into 10 mL urine samples, verified across concentrations of at least 1 – 10,000 copies/mL and fragment lengths of at least 25 – 150 bpLimit of detection of ?5 copies of dsDNA in 10 mL urine (0.5 copies/mL)Enables amplification of DNA from 10 mL urine in a single PCR well (500X concentration factor)Tolerant to variations in sample composition, including pH (5 – 8), salt (0 – 500 mM), and non-target DNA (0 – 10

µg)Graphic abstract: (A) Schematic of capture probe design. (B) Overview of workflow.

Associated Publications: Oreskovic A, Lutz BR (2021) Ultrasensitive hybridization capture: Reliable detection of 1 copy/mL short cell-free DNA from large-volume urine samples. PLoS ONE 16(2): e0247851. doi: 10.1371/journal.pone.0247851

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