Docket #: S21-375

Methylation-dependent enrichment of DNA by species of origin

Metagenomic sequencing offers a powerful approach for the comprehensive monitoring and detection of pathogenic bacteria in food, clinical samples, and the environment. However, many samples collected in clinical or food safety contexts can contain mixed prokaryotic and eukaryotic populations of genetic material, often with DNA from species of interest being far exceeded by other sources of DNA in the sample. This high background consumes vast amounts of wasted sequencing reads, increasing costs and decreasing coverage for the DNA of interest. Currently available enrichment techniques for prokaryotic DNA are expensive or time consuming.

Researchers in the Fire lab have leveraged the distinct DNA methylation patterns between prokaryotes and eukaryotes to develop a simple, cost-effective, and fast method for enriching a subset of prokaryotic DNAs from mixed prokaryotic and eukaryotic DNA populations for library construction and next-generation sequencing. Of note, the enrichable species include prominent species of enterobacteria including E. coli and Salmonella.

Applications

- Metagenomic detection and characterization of microbial populations
    - Food safety
    - Clinical samples
    - Environmental testing
- Selective prokaryotic DNA enrichment from mixed prokaryotic and eukaryotic DNA populations
    - Selectively enrich for DNA from *Enterobacteriaceae*

    - *E. coli*
    - *Salmonella*
    - *Shigella*
- Alternative embodiments can enrich for other cellular and viral DNA sources

Advantages

- Fast (90min)
- Simple (1-tube reaction)
- Cost-effective

Publications

- Syed Usman Enam, Joshua L. Cherry, Susan R. Leonard, Ivan N. Zheludev, David J. Lipman, Andrew Z. Fire. " Restriction Endonuclease-Based Modification-Dependent Enrichment (REMoDE) of DNA for Metagenomic Sequencing." *Applied and Environmental Microbiology* (ahead of print). 15 December 2022.

Patents

- Published Application: WO2023192492
- Published Application: 20250207206

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