064 - Megarich: a pre-sequencing capture system for enriching and counting resistance genes within metagenomic samples

December 5, 2016, 3:30 - 3:45 PM | Salon E - 5th Floor

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Abstract

Use of shotgun metagenomic sequencing to study antimicrobial resistance has increased over the past several years. However, the resistome comprises a very small proportion (<0.1%) of all the DNA in a metagenomic sample such as feces, creating a major challenge. Here we describe development and validation of an efficient pre-sequencing capture-enrichment system with unique molecular identifiers (UMIs) to correct for amplification bias. This system was designed to capture all known antimicrobial, metal and biocide resistance (target) genes, as well as pathogen virulence factors. The system was validated using fecal samples collected from finishing beef cattle, swine, broilers, and biosolids from human waste water treatment plants. Sequencing results were compared for shotgun sequencing with and without use of UMIs, and capture sequencing with and without use of UMIs. Gene sequences included comprehensive databases of published resistance genes (ARG-ANNOT, CARD, Resfinder, BacMet and VFDB) were used to design the customized baits for the capture-enrichment process using Agilent SureSelect^{XT}. This system uses customized RNA 120-mer baits bound to magnetic beads to bind and capture target sequence, which are then PCR-amplified prior to sequencing. Overall, the capture system resulted in 2- to 4-fold increase in on-target sequencing, from a median of 0.14% (range 0.002 - 0.37%) for shotgun sequencing (n=32) to 15.8% (range 0.28 - 68.2%) for capture sequencing (n=32). While UMIs were critical for understanding PCR amplification bias, which was highly variable among samples, inclusion resulted in marked decrease in on-target sequencing efficiency. Variability in the proportion of singleton UMI families (a measure of PCR bias) was not associated with sample source, resistome abundance and diversity, or number of total reads sequenced, but was associated with amount of DNA present in samples after PCR amplification. This highly successful customized approach increased resistome sequencing efficiency, allowing detection of 100× to 10,000× more on-target reads, and identification of thousands of additional resistance genes that were undetected in non-enriched samples.