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UNL Announces Opening of Core for Applied Genomics and Ecology (CAGE).

The UNL Core for Applied Genomics and Ecology (CAGE) announces that it is now offering a new suite of services to UNL investigators and outside entities. Formerly known as the UNL Printed Microarray Core Facility, CAGE now offers Next Gen sequencing on its newly installed Roche-454 GS FLX. In support of the UNL Gut Function Initiative, CAGE specializes in application of the GS-FLX platform for analysis of complex microbial communities by massively parallel sequencing of 16S rRNA amplicons. CAGE also has expertise in shotgun sequencing applications on the instrument, including whole genome sequencing and analysis of BACs and metagenome libraries.

CAGE is directed by Dr. Andrew Benson, Professor of Food Microbiology in the Department of Food Science and Technology. "We have been training hard as teams of personnel to provide expert services for shotgun and amplicon sequencing protocols on the GS-FLX", said Benson. "The range of applications is growing rapidly -- this enabling technology is literally blowing the doors wide open to fields that have previously been experimentally intractable."

CAGE specialization in microbial community analysis is one example of just how enabling this technology is. Until now, high-resolution analysis of complex microbial communities such as those found in soil, water, or the GI tract has not been possible. Since most of the species in these environments are not culturable, researchers in the 1990's began applying non-culture based approaches, often using analytical methods such as Denaturing Gradient Gel Electrophoresis to analyze complex mixtures of amplicons from 16S rRNA PCR reactions. These methods provided only limited capabilities, capturing perhaps the top 10% of the entire population. The Next Gen sequencing strategy, however, now provides high resolution, sequenced-based analysis of hundreds of thousands of amplicons from these PCR reactions, corresponding to the top 100,000-1,000,000 members of a microbial community. "Until the Next Gen sequencing strategies became available, such studies could only be conducted at genome sequence centers with tens to hundreds of machines and an army of personnel. Now, a single GS-FLX machine and a small team of staff can accomplish the same thing in a week", said Benson.

Benson's teams are currently applying the technology to study the GI microflora in mouse, hamster, and human subjects. In addition, the team was recently funded by USDA to begin applying the technology to food testing. "The ability to examine the entire community of microorganisms in food samples completely changes how we think about food safety and quality testing", said Benson. Whereas most food microbiology testing has been done classically using indicator organisms, such as *E. coli*, Next Gen sequencing now allows the researchers to examine the entire community of organisms in a sample to determine if species that are unique to soil or feces are contaminating the sample. "Having a snapshot of the entire community should dramatically increase our

sensitivity because the classically used indicator organisms such as *E. coli* are only a minor fraction (0.1%) of the species found in the GI tract.”

The Next Gen sequencing platform has become widely popular among researchers. Published applications include whole genomes, BACs (and other large plasmid-based libraries), resequencing, cDNA analysis, SAGE, metagenomes, microbial communities, ultra-deep sequencing of PCR products, and virus populations.

CAGE will initially be offering services that include:

- Whole genome sequencing (Shotgun protocol)
- cDNA sequencing for transcriptome and EST analysis (Shotgun protocol)
- Metagenome libraries and BACs (Shotgun protocol)
- Community analysis (amplicon protocol)
- Variant analysis (amplicon protocol)

The GS-FLX platform provides up to 100Mb of sequence in a single run. Samples can be physically separated in different sequencing regions on the pico-titre plates with 2, 4, 8, or 16 different regions. Alternatively, samples can be pooled into a single reaction with bar-coded primers (amplicon sequencing) or using bar-coded adapters (shotgun sequencing).

Dr. Jaehyoung Kim, the CAGE manager, said that “We will provide researchers with complete service, from experimental design to execution, data storage, and primary data analysis.” CAGE provides an initial information gathering and pricing estimate service on its web page, allowing interested investigators to determine how the GS-FLX platform fits their application and an initial estimate of the cost. CAGE will also host a Next Gen GS-FLX workshop on September 15-16, and Dr. Benson will teach a course on Next Gen applications in the spring semester. “Interest in the platform is growing rapidly. The line of users is already around the corner. Our goal is to continue to grow Next Gen applications on campus. It is exciting to see the unique applications that creative researchers come up with. The next few years will indeed be a golden era for us”, said Benson.

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