Panel: State of the art for autonomous detection systems using nucleic acid signatures

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Nucleic Acid Signatures

Question:
How will the technology evolve signatures as increasing knowledge of targets and neighbor strains reveals both false positives and negative situations?
Nucleic Acid Signatures: Genetic Approach

1. Toxin...
   16S ...

2. Find a region unique to the target and common among strains

3. Compare

Pick a gene
Nucleic Acid Signatures: Genetic Approach

• Deductive Process
• Relies on deep knowledge of pathogen (pathogenicity, phylogenetic relationships, etc)
• Good for targeting key pathogenic determinants (e.g., toxin genes)
• Ignores most of the genome as signature candidates
Nucleic Acid Signatures: Genomic Approach

1. Begin with target organism full genome sequence data

2. Compare with full genome sequence data of all other organisms (background)

3. Find sequences unique to the target and not found in the background genomes
Nucleic Acid Signatures: Genomic Approach

• Utilizes entire genome of target and non-target organism
• Does not require deep knowledge of pathogen
• Enables discovery of multiple targets for signatures
• Relies on coherence between the virtual world (i.e., genome database) and the real world.
Genomics: Rapid growth of an enabling technology

- In 2005 there were ~200 bacterial genome sequences available
- In 2009 there were ~2,000
  - Doubling time of 1.3 y
- In 2013, many thousands of genomes
  - Still a small fraction of the real world

Simply...
as these data grow,
signature designs get better
(lowering probability of false results)
Nucleic Acid Signatures: Genomic Approach

Find sequences unique to the target and not found in the background genomes
The target is in a continuous genetic background
Pan-genomes

“Core” Genome

Species

Hu et al.,
Briefings in Functional Genomics
2011
Technology to evolve signatures

2016
• Multiplex capability: More targets interrogated
• Adaptive target sets: flexible assays to incorporate new information.

2020
• Direct sequencing signatures:
  – Probability-based analysis of total genomic content of samples
Assay technology for automated systems

• PCR-based multiplex technologies.
  • Bead arrays (2016)
  • Real time PCR w/ or w/o hybridization probes (2016)
  • Digital PCR (2016-2020)

• Big challenges are in system integration and work flow.
  • Incorporating reflexive testing?
  • Consumables handling (inside and outside the instrument)
  • System monitoring and PM processes
  • Meeting laboratory quality system requirements