Automated Multichannel System for Broadband Detection and Identification of Pathogens

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How GSS Works

Complex sample → Genomic DNA tagged

Genomic DNA linearized & detected

Trace measured

Compared against database

E. coli
B. anthracis strain X
B. anthracis strain Y
S. aureus

Pathogen Confirmed

GSS = Genome Sequence Scanning
Advantages of Detection by GSS

- One reagent set detects wide range of bacteria
- Single test can detect many different bacteria
- Can analyze both isolates and complex mixtures
- No *a priori* guess about sample content is required
- Can detect “unknowns” and genetic variants
- GSS typing is reproducible and operator-independent
Sample Preparation Procedure

- Injection
- Lysis, purification
- RE digestion
- PNA tagging
- Elution
- GSS

~130 min
~20 min
~30 min
~3 min

- Sample prepared in automated reactor
- Universal protocol with universal reagents
- Entire sample preparation is ~3 hours

At different stages samples can be eluted and used for PCR and PFGE.
Single Molecule Mapping

Stretching and Excitation

Emission Output

Restriction Fragment of *E. coli*

Velocity $V = \frac{Z}{t}$
Molecule length $L = V \times T$
Data Flow for GSS Analysis

Molecule data → GeneEngineer → Raw data binary file

DNA Tracer

GSS Measurement

and / or Sorting Hat

Theoretical templates

Experimental templates

Molecule Classifier

Average Log-likelihood

Total Log-Likelihood Species

Fraction [%]
Wealth of Information for ID

*S. aureus*

- Examples of GSS signatures for molecules traveling “head-first” and inverted “tail-first”
- Experimental signatures are compared with theoretical signatures, calculated using genomic sequence
**Information Content**

**Staphylococcus aureus strain**

- Related bacteria share common fragments
- Extent of commonality correlates with phylogenetic relatedness
- Different strains exhibit clear differences
Prototype System

Sample Preparation Module

- The prototype system consists of 2 independently operating modules
- The robotic Sample Preparation Module automatically prepares 4 samples in parallel

Detector Module

- The Detector Module implements multicolor single-molecule detection and microfluidics for DNA stretching
GSS Identifies Bacterium Present at 0.1%

<table>
<thead>
<tr>
<th>EC : YE</th>
<th>Measure. time [min]</th>
<th>Molecules per min.</th>
<th>Molecules classified as EC : YE</th>
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<tbody>
<tr>
<td>1 : 1</td>
<td>32</td>
<td>527</td>
<td>2994 : 2598</td>
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<tr>
<td>10 : 1</td>
<td>32</td>
<td>668</td>
<td>6399 : 808</td>
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<td>580</td>
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<td>349</td>
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<tr>
<td>1 : 1000</td>
<td>68</td>
<td>486</td>
<td>9 : 5176</td>
</tr>
</tbody>
</table>

- Analysis of Binary Mixtures of *E. coli* (EC) and *Y. enterocolitica* (YE)
- The ratio of detected molecules (last column) approximately reflects the ratio of the cells in the input of the system
Escherichia coli Strain Typing

GSS fragment similarity identifies phylogenetic relationships between strains

Pathogenic Groups

- **EHEC/STEC**
- **ETEC**
- **EIIEC**
- **EPEC**
- **EAEC**

- The set includes commensal (black) and pathogenic (color-coded) types
- Data calculated using the genomic sequence are underlined
• A strain of *Salmonella enterica*, serotype *typhimurium* is detected in this sample (blind test)

• This strain is not in the database
Summary

• GSS demonstrated the ability to speciate and type non-sequenced bacteria with high resolution, to discriminate between strains with different pathogenicity mechanisms
• GSS uses one set of reagents for broad spectrum of microbes
• GSS is applicable to analysis of isolates or single colonies as well as to mixtures of bacteria
• Currently our company possesses 3 four-channel automated GSS prototype systems
• Systems suitable for beta-test placement in outside labs will be available in Q4, 2013