Direct Detection of Bacterial DNA and Viral RNA at Subfemtomolar Concentrations Using Single Molecule Arrays

Agenda

- Introduction of single molecule arrays (Simoa)
- Assay process for Simoa DNA/RNA detection
  - Fragmentation of target DNA/RNA
  - Improved efficiency of capture and enzyme labeling of hybridized complexes on beads
  - Capture target DNA/RNA on paramagnetic beads
  - Hybridize biotinylated detection probes to captured targets
  - Label hybridized complexes with an enzyme
- Analytical sensitivity with purified DNA/RNA
- Detection of S. aureus in whole blood and environmental water

Counting Single Molecules Using Simoa

Rissin et al., Nat. Biotechnol. 2010, 28, 595-599
Kan et al., Lab Chip 2012, 22, 577-585
Simoa - extending dynamic range above digital range

- Determine average intensity produced by the beads on an array (I_{bead})
- Determine average intensity produced by a single enzyme (I_{single})
- Analog AEB (Average enzymes per bead) is ratio of these two numbers

Rissin et al., Anal. Chem. 2011, 83, 2279-2285

Schematic of the process for DNA detection

Step A: Fragmentation via sonication or enzyme digestion
Step B: Add detection probes to the fragmented DNA
Step C: Alix with capture beads to form hybridized complex, label with enzyme
Step D: Lane DNA sequencing

Simoa detection

Fragmentation of target genomic DNA/RNA

Simoa detection of genomic DNA of S. aureus fragmented using sonication and restriction enzymes (DraI and AluI) based on the target gene, GeneBank: v01281.1, encoding nuclease from S. aureus that is 966 bp long.
The samples were either fragmented at high concentrations and then diluted before testing in Simoa ("fragment-then-dilute", red squares), or diluted to the concentrations to be tested and fragmented ("dilute-then-fragment", blue circles).

The fragmented DNA was captured by mixtures of multiple capture beads, each subpopulation of beads presenting a specific capture probe complementary to the different sites along the ~1,000 bp target gene. The number of beads was kept constant at 500,000 beads per 100 mL sample.

- Single biotin vs. multiple biotins on detection probes
  - square: (B)iotin-aaagaaagaggtgttagttatgac
  - triangle: (B)iotin-aaagaaagaggttagttatgac
  - circle: (B)iotin-aaagaaagaggttagttatgac; (B)taagtgctggcatatgtatggc; (B)agggatggctatcagtaatgtt
**Labeling of hybridized complexes**
- use of optimized enzyme concentration

The highest S/B ratio (16.3) was observed at [SBG] = 200 pM.

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**Dose-response curve**
- detection of purified genomic DNA from S. aureus

LOD (3xSD+ background): 0.04fM, equivalent to 1200 DNA molecules/50 µl sample volume, for this run.

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**Dose-response curve**
- detection of purified genomic RNA from Sendai virus

LOD (3xSD+ background): 0.06fM, equivalent to 1800 RNA molecules/50 µl sample volume, for this run.
Analytical sensitivity of Simoa to genomic DNA/RNA

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>DnaFm from S. aureus</th>
<th>DnaFm from purified viral</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>0.00002</td>
<td>0.00006</td>
<td>1,800</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.00004</td>
<td>0.00008</td>
<td>1,800</td>
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<tr>
<td>S. aureus</td>
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<td>1,800</td>
</tr>
<tr>
<td>S. aureus</td>
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<td>0.00012</td>
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</tr>
<tr>
<td>S. aureus</td>
<td>0.00010</td>
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<td>0.00018</td>
<td>1,800</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.00016</td>
<td>0.00020</td>
<td>1,800</td>
</tr>
</tbody>
</table>

Average: 0.00010 ± 0.00005

*Compared to real-time PCR

Detection of S. aureus in whole blood and river water

- LOD: 0.026 fm (396 DNA molecules per 25 µL of whole blood sample)
- LOD: 0.042 fm (1271 DNA molecules per 50 µL of river water sample)

Conclusion

- Simoa shows promise for the direct and sensitive detection of genomic DNA/RNA, not only in purified, but also in complex samples.
- Using multiple capture and detection probes for each target greatly improved the efficiency of capture and enzyme labeling, hence enabled a highly sensitive Simoa assay.
- Simoa provides an alternative approach to PCR based assays that require target amplification.
- Simoa has the benefit of assay simplicity and automation.