Quanterix

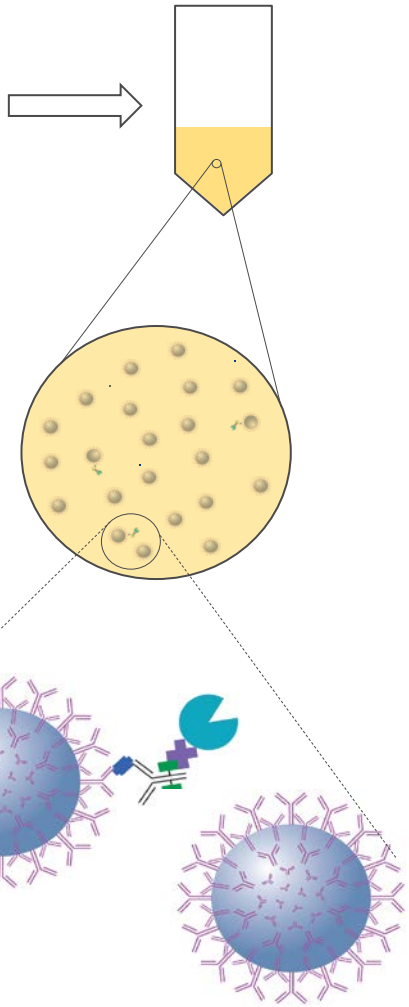
Direct Detection of Bacterial DNA and Viral RNA at Subfemtomolar Concentrations Using Single Molecule Arrays

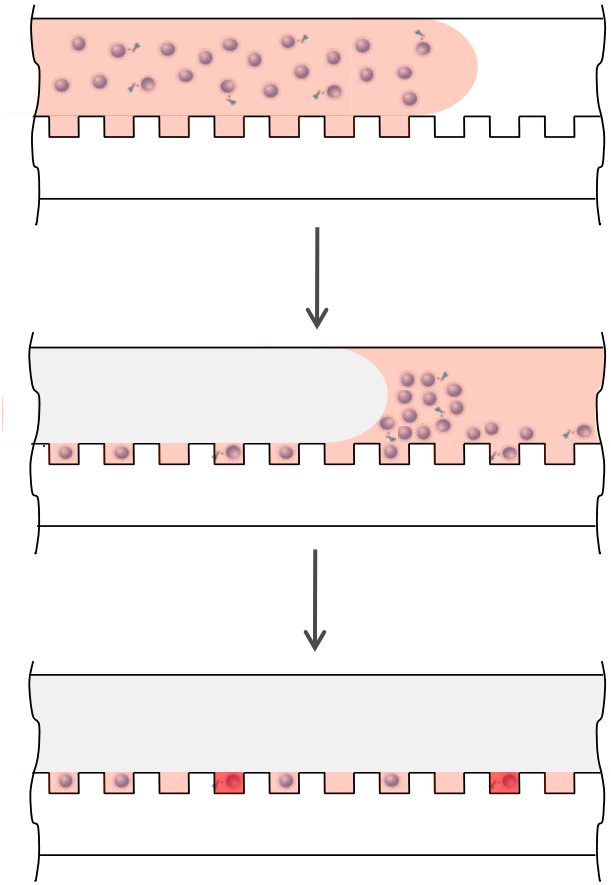
Oak Ridge Conference
April 18, 2013

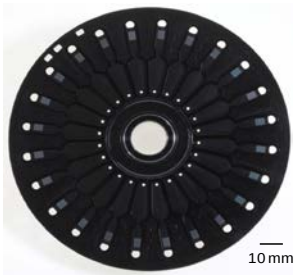
Agenda

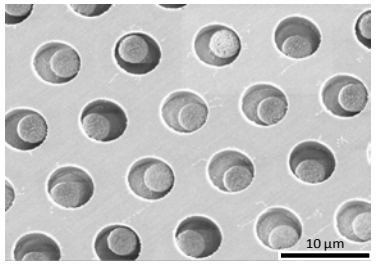
- Introduction of single molecule arrays (Simoa)
- Assay process for Simoa DNA/RNA detection
  - Fragmentation of target DNA/RNA
  - Improved efficiency of capture and enzyme labeling of hybridized complexes on beads
    - Capture target DNA/RNA on paramagnetic beads
    - Hybridize biotinylated detection 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

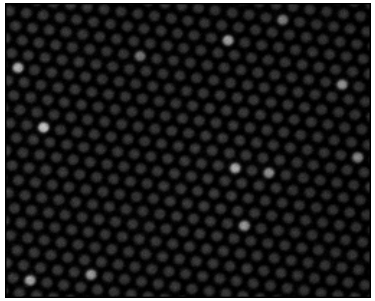
Counting Single Molecules Using Simoa





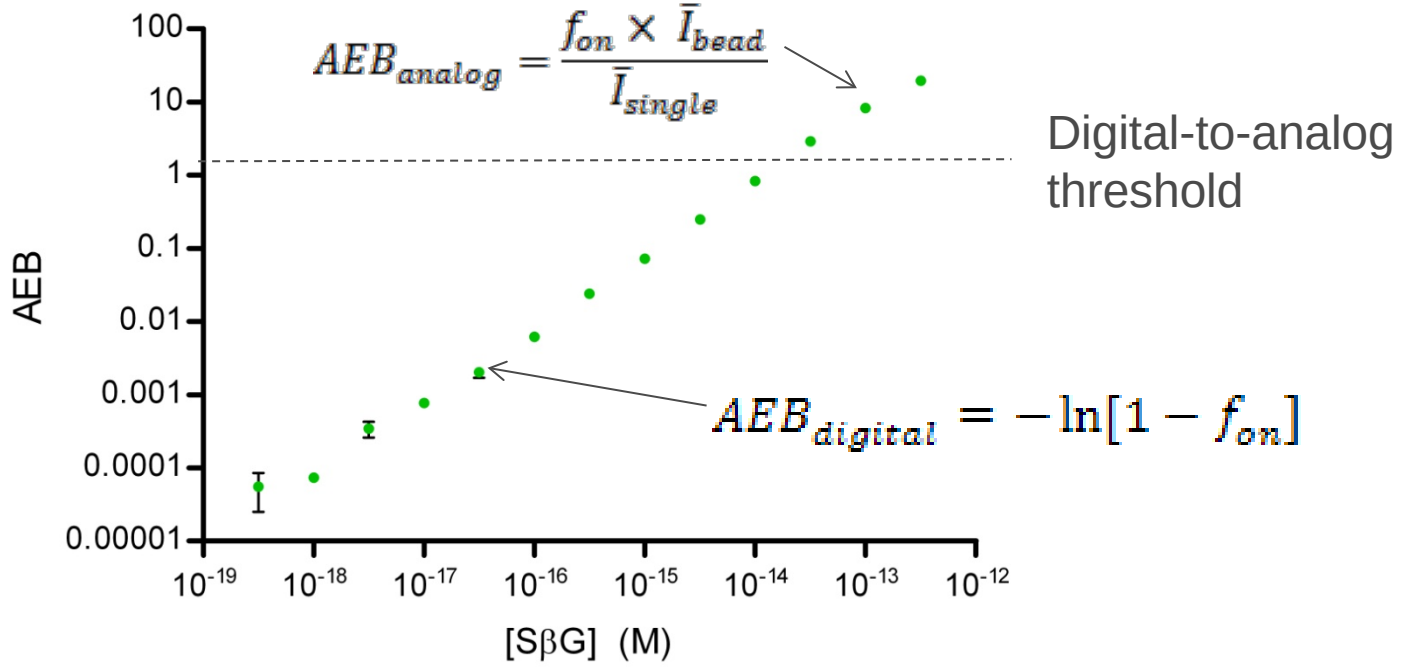






Rissin *et al.*, *Nat. Biotechnol.* **2010**, *28*, 595-599
Kan *et al.*, *Lab Chip* **2012,** *12*, 977-985

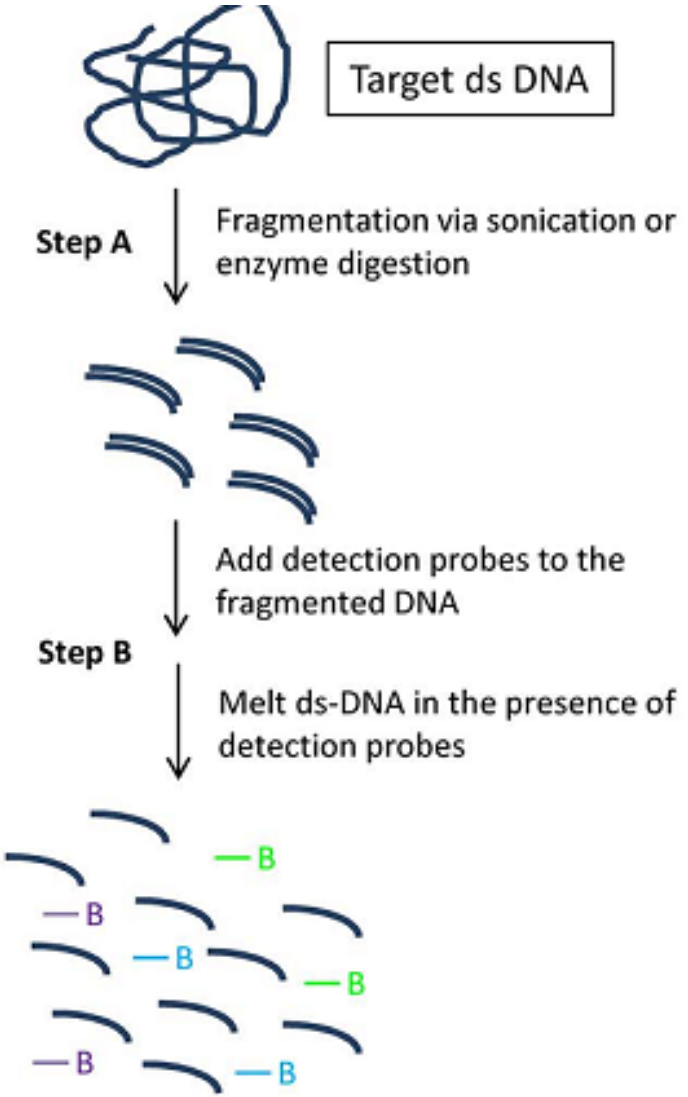
Simoa - extending dynamic range above digital range

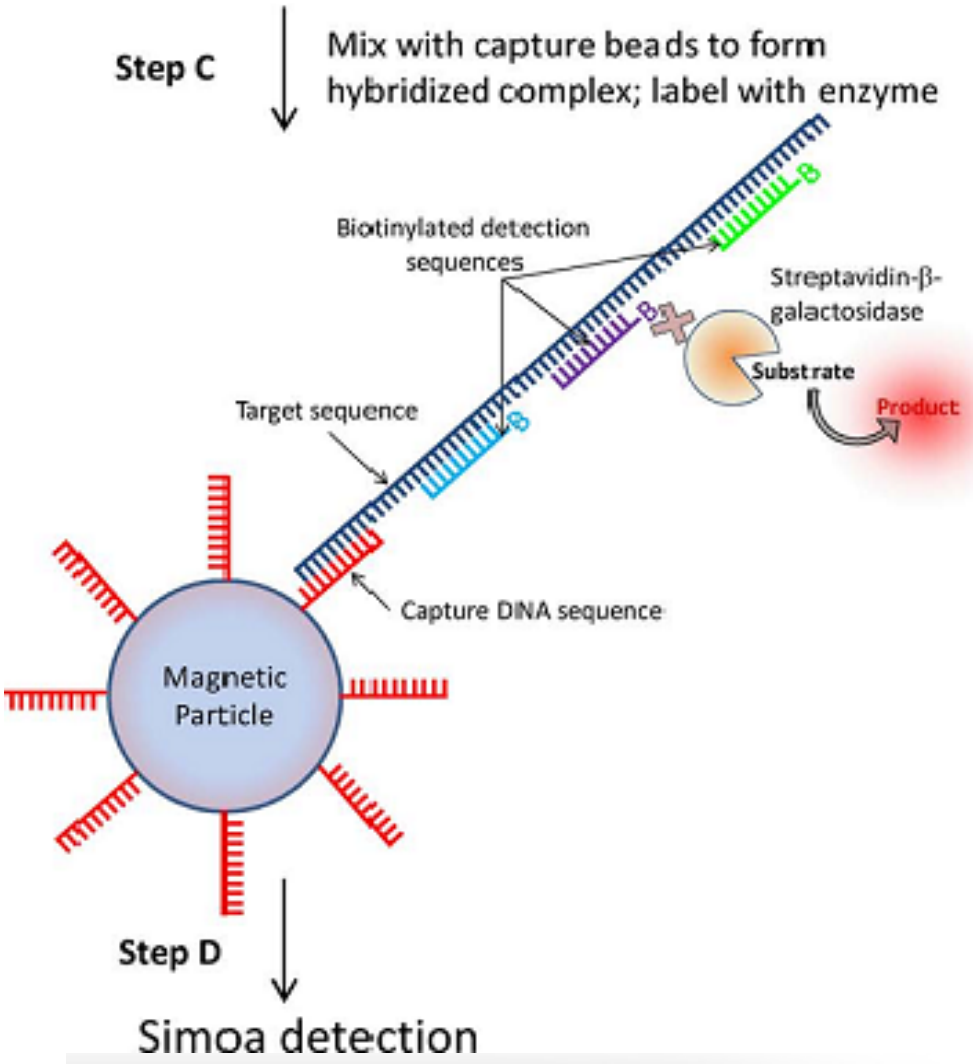


- Determine average intensity produced by the beads on an array ($\bar{I}_{bead}$)
- Determine average intensity produced by a single enzyme ($\bar{I}_{single}$)
- Analog AEB (Average enzymes per bead) is ratio of these two numbers

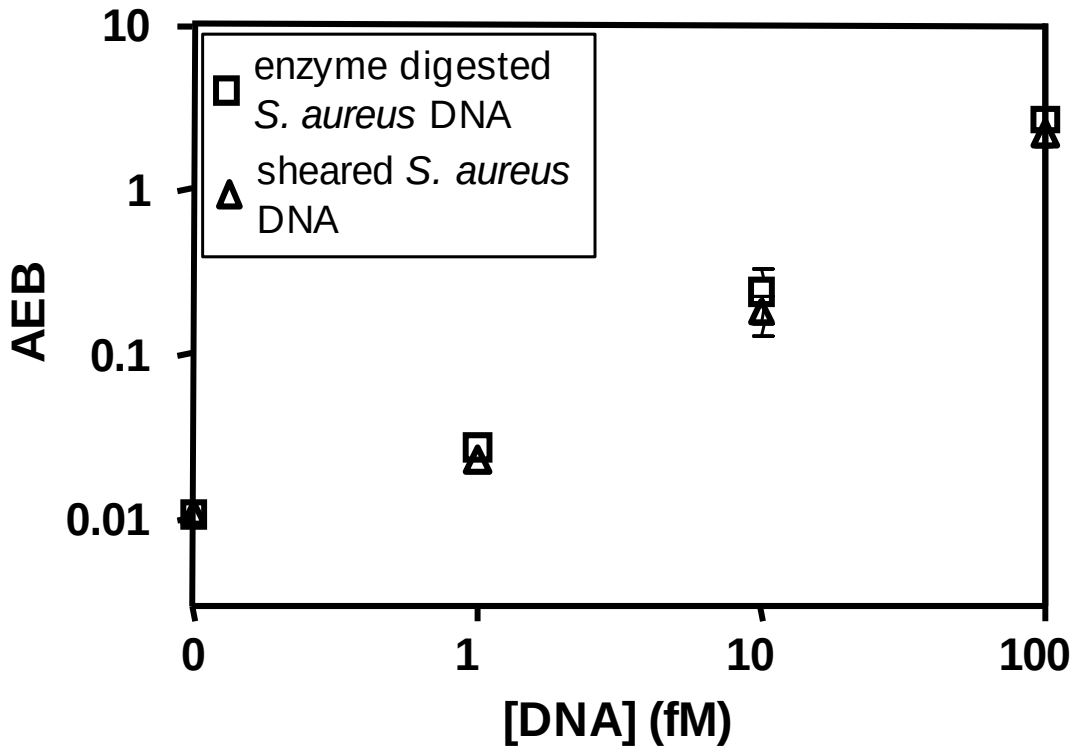
Rissin *et al.*, *Analyt.Chem.* **2011**, *83*, 2279-2285

Schematic of the process for DNA detection



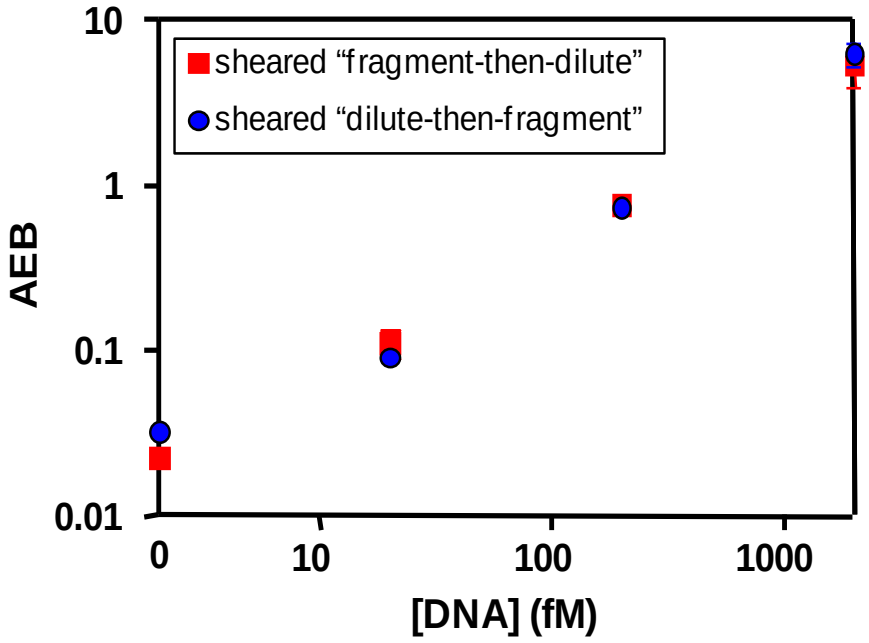


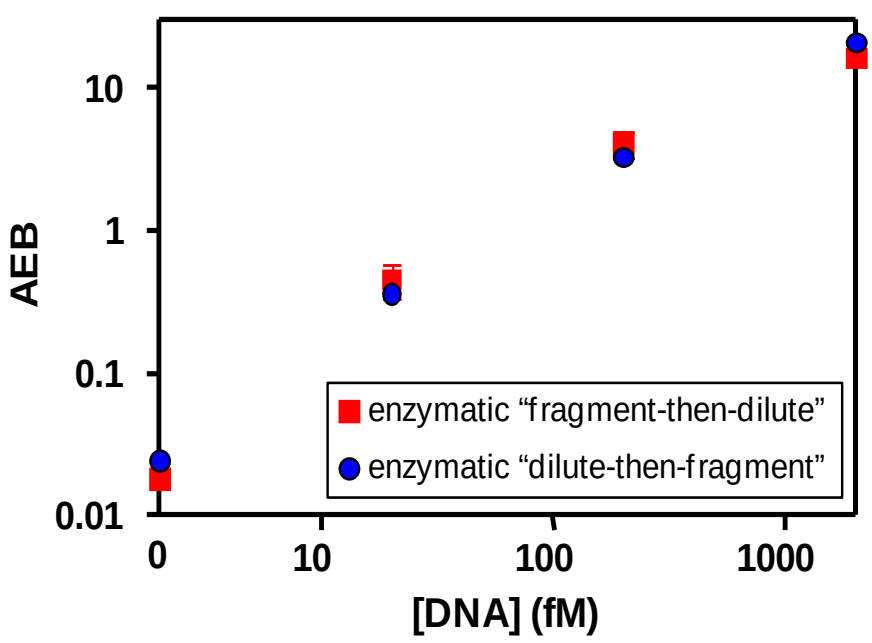
Fragmentation of target genomic DNA/RNA



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.

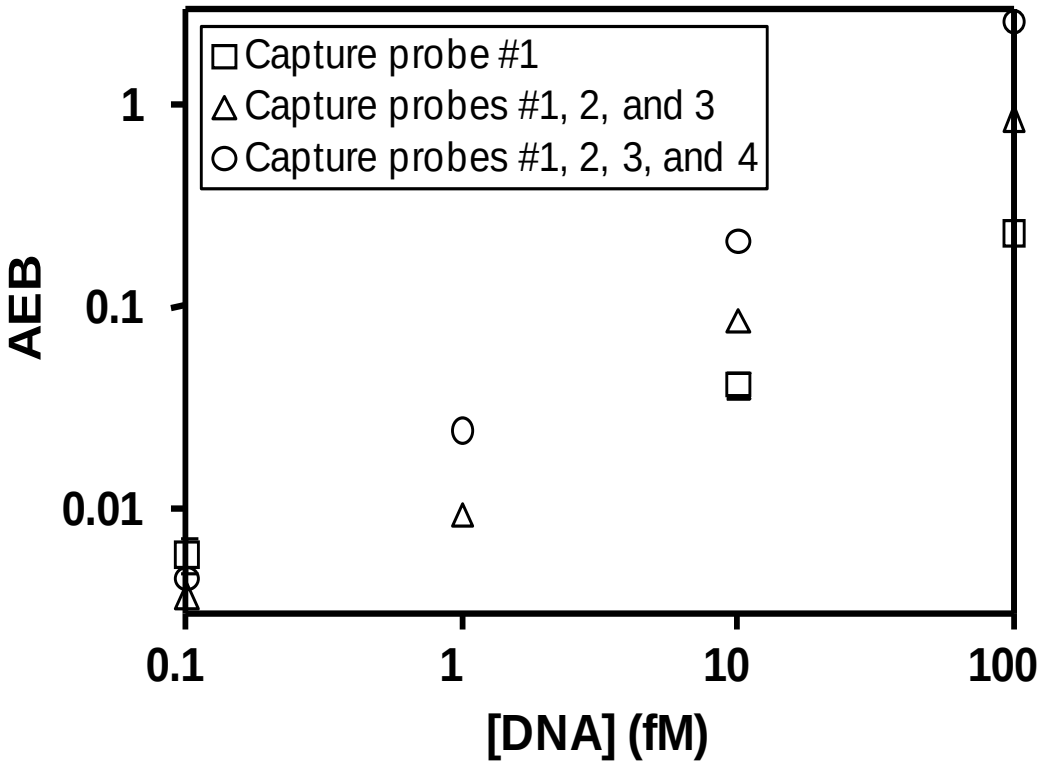
Fragmentation of target genomic DNA/RNA





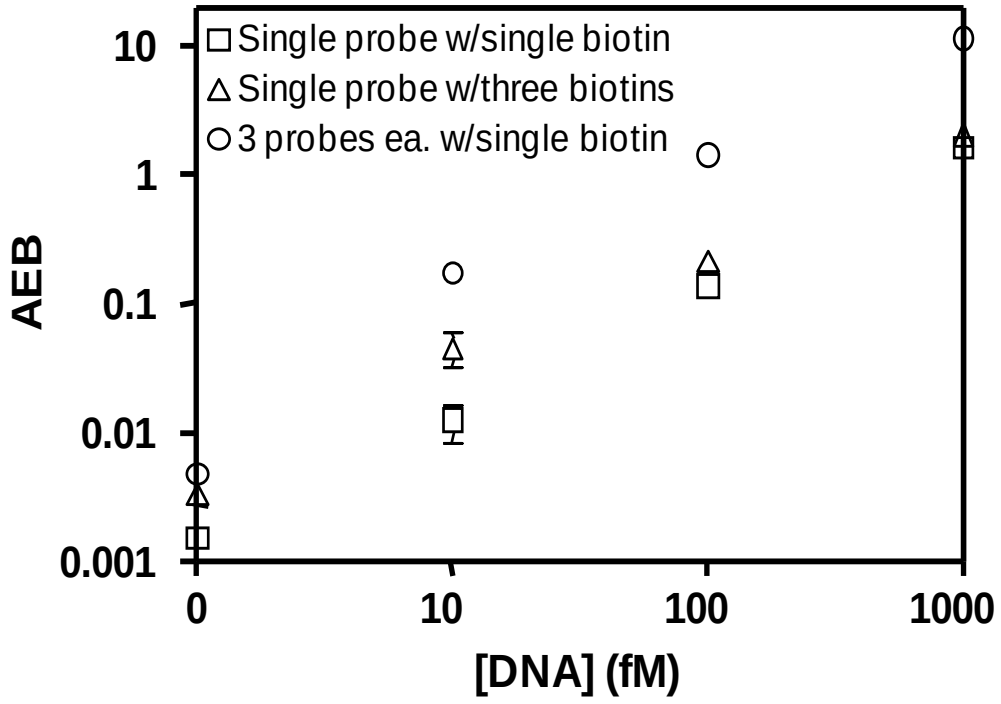
The samples were either fragmented at high concentrations and then diluted before testing in Simoa ("**fragment-then-dilute**", red squares), or diluted to the concentrations to be tested and fragmented ("**dilute-then-fragment**", blue circles).

Capture of fragmented DNA/RNA on beads - use of multiple capture probes



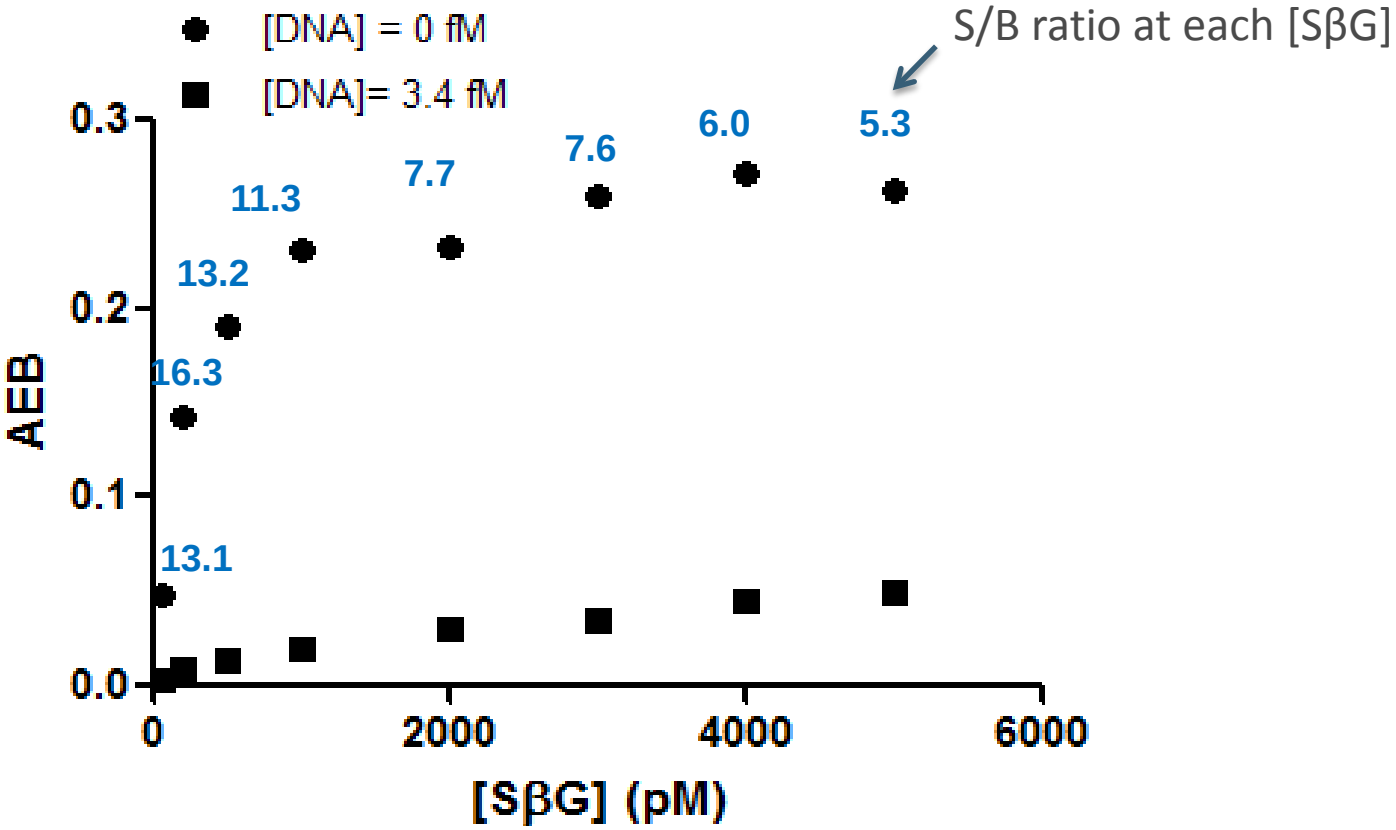
The fragmented DNA was captured by mixtures of multiple capture beads, each subpopulation of beads presenting a specific capture probe complementary to the different sites along the ~1,000 bp target gene. The number of beads was kept constant at 500,000 beads per 100 mL sample.

Labeling of hybridized complexes
- use of multiple detection probes



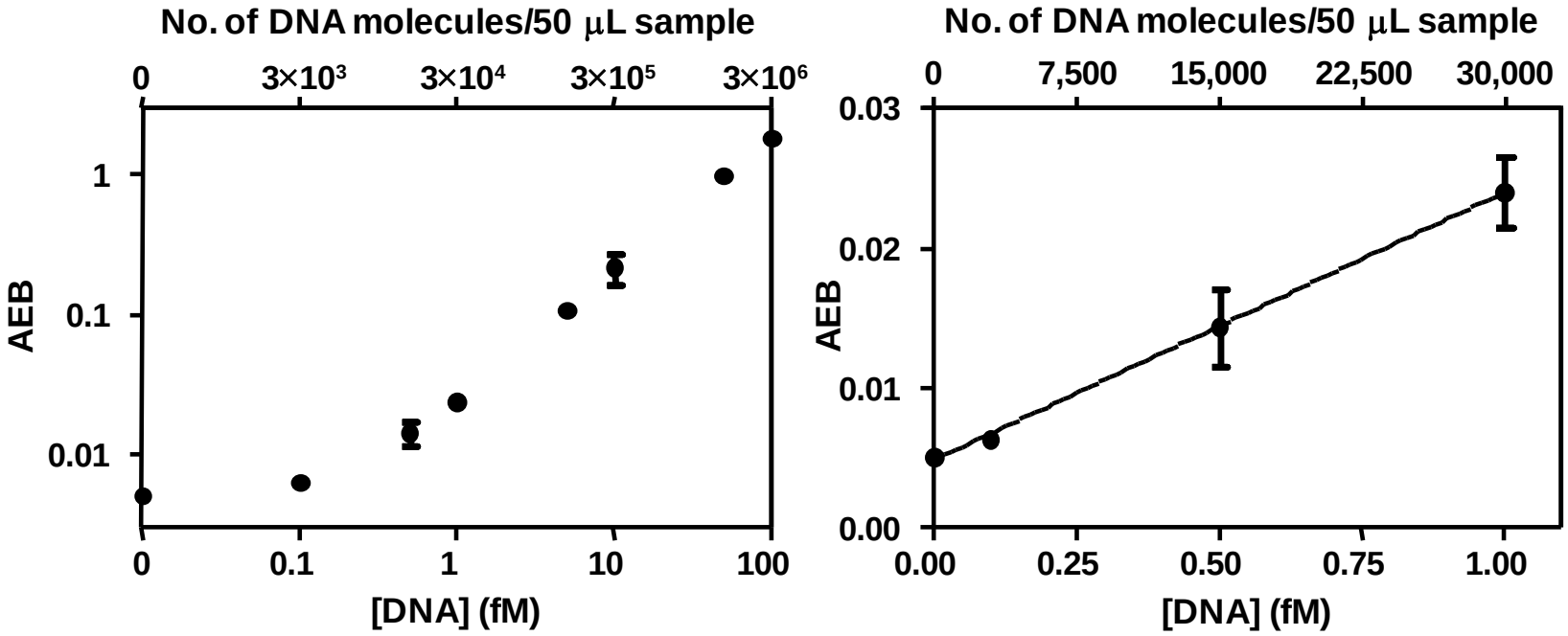
- Single biotin vs. multiple biotins on detection probes
  square: (**B**)iotin-aaagaaagaggtgttagttatgac
  triangle: (**B**)iotin-aaagaaagaggt(**B**)gttagtt(**B**)atgac
  circle: (**B**)iotin-aaagaaagaggtgttagttatgac; (**B**)-taagtgctggcatatgtatggc;
  (**B**)-agggatggctatcagtaatgtt

Labeling of hybridized complexes
- use of optimized enzyme concentration



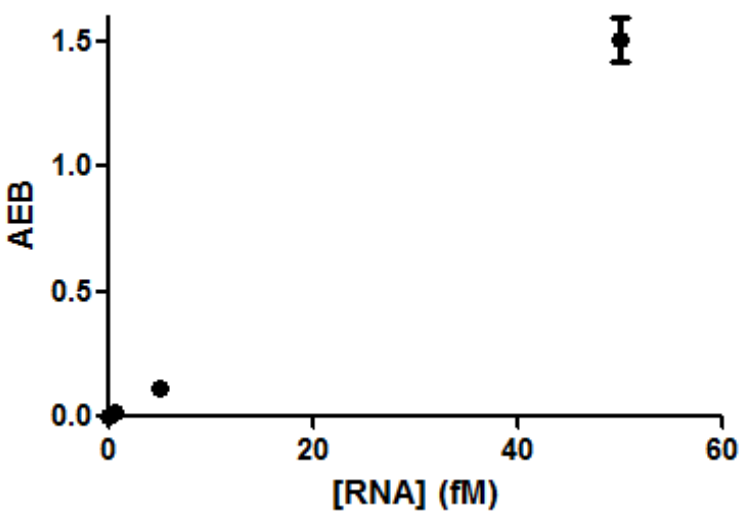
The highest S/B ratio (16.3) was observed at [SβG] = 200 pM.

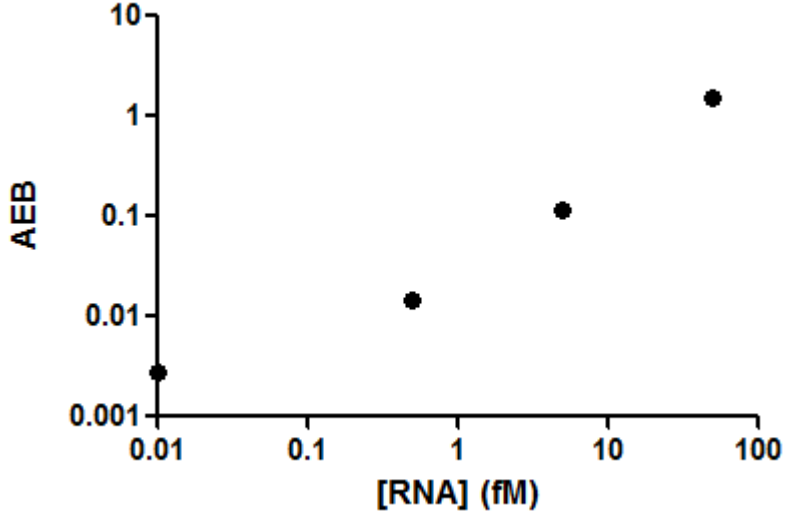
Dose-response curve
- detection of purified genomic DNA from *S. aureus*



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

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

# of run	purified *DNA* from S. aureus		purified *RNA* from Sendai virus	
	LOD*, fM	LOD, DNA molecules/ 50ul	LOD*, fM	LOD, RNA molecules/ 50ul
1	0.02	600	0.06	1800
2	0.094	2820	0.06	1800
3	0.094	2820	0.087	2610
4	0.015	450	0.098	2940
5	0.08	2400	0.061	1830
6	0.025	750	0.059	1770
7	0.08	2400	0.076	2280
8	0.09	2700	0.01	300
9	0.036	1080	0.008	240
10	0.086	2580	0.004	120
average	**0.062**	**1860**	**0.052**	**1569**

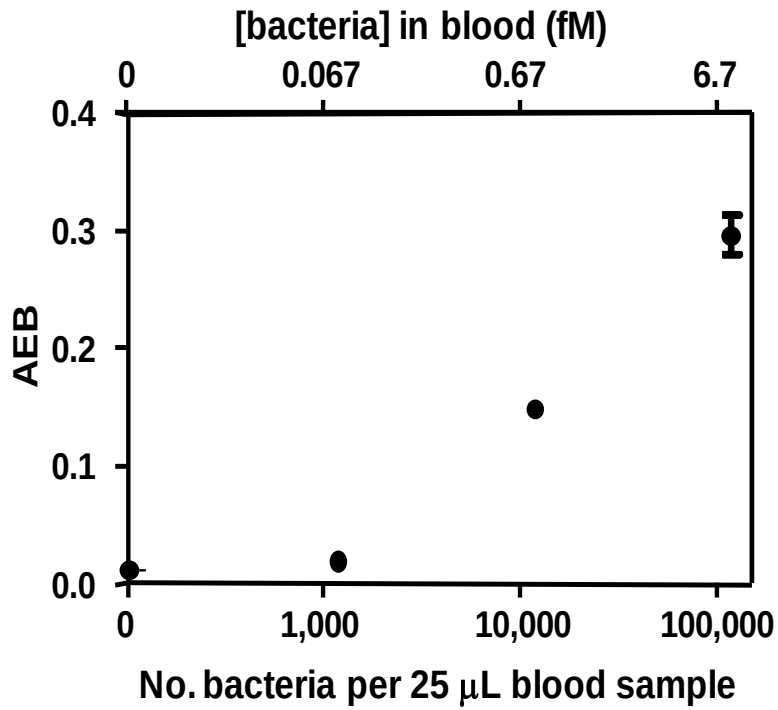
*3xSD above background

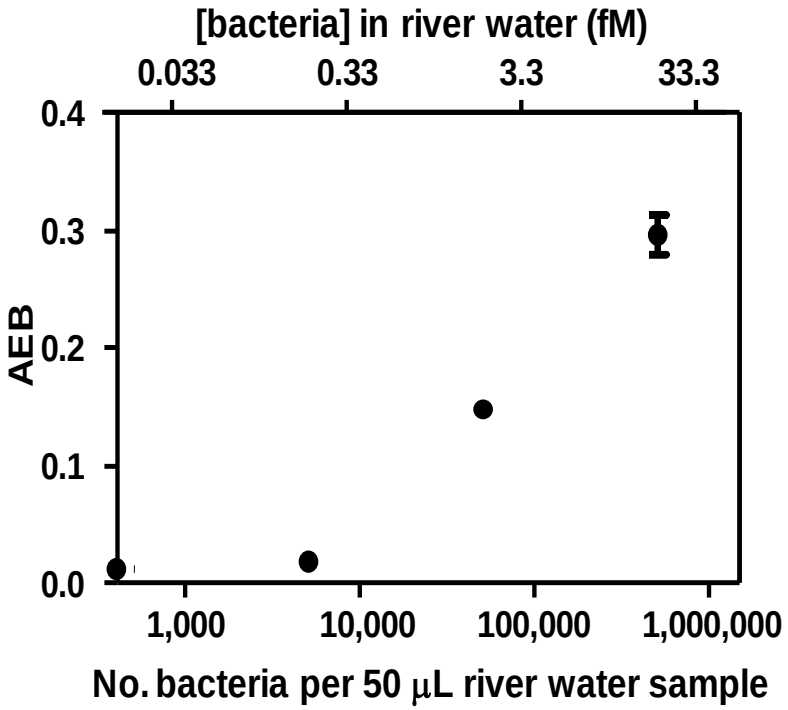
- Comparable sensitivity to real-time PCR

Technology	Strain of bacteria	Target gene	LOD, fM	LOD, number of molecules	Sample volume, µl
Simoa	*S. au* 25923	*nuc*	**0.062**	**1860**	**50**
Heminested RT-PCR[a]	*S. au* 25923	*nuc*	**0.083**	**50**	**1**
RT-PCR[b]	*S. au* 25923	*nuc*	**0.5-1**	**1200-2400**	**4**

[a]: Banada, P. P., *etc*. PLoS One 2012, 7 (2), e31126. [b]: results from internal tests using 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 sample of river water

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