Microbial Detection Array for Product and Health Safety

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IABS Adventitious Agents, May 19-20, 2011

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Lawrence Livermore National Laboratory (LLNL)

- Founded in 1952 by Nobel Laureate E.O. Lawrence
- Located in Livermore, California
- Managed by the Department of Energy
- Annual budget about $1.5B
- 1.2 square mile main site
- 6,600 employees

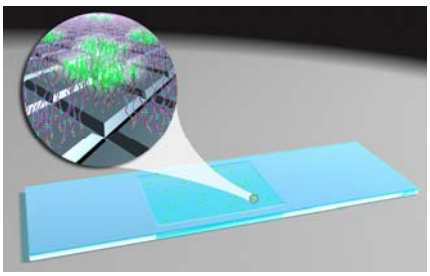
http://www.llnl.gov

We have a large multi-disciplinary bioinformatics team, focused on developing and delivering advanced detection assays

15 of ~20 current team members

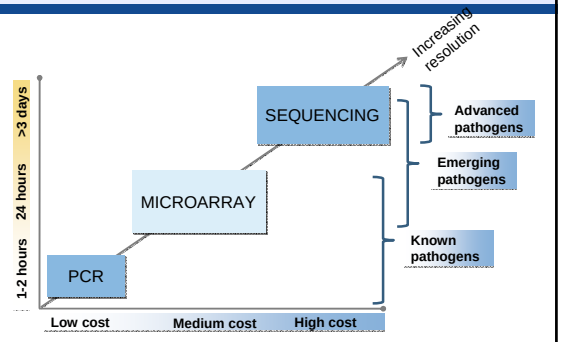
Biologists, computer scientists, statisticians, software engineer, mathematicians

Microarrays allow precise detection of large numbers of short DNA sequences



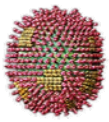
Microarrays are highly multiplexed to analyze thousands-to-millions of sequences simultaneously.

Microarrays fill some gaps between Polymerase Chain Reaction (PCR) and DNA sequencing



Lawrence Livermore Microbial Detection Array (LLMDA) has probes to detect all known viral and bacterial organisms

- 38,000 viral sequences
  - 2,200+ viral species
- 3,500 bacterial sequences
  - 900+ bacterial species



387,156 total probes

LLMDA won the US Federal Laboratory Consortium (FLC) Excellence in Technology Transfer award

A Microbial Detection Array (MDA) for Viral and Bacterial Detection. Gardner SN, Jaing CJ, McLoughlin KS, Slezak TR. *BMC Genomics*. **11**:668doi:10.1186/1471-2164-11-668, 2010.

LLMDA probes were designed on unique regions from all available viral and bacterial sequences

DNA virus: 200K bases

Bacteria: 1-5 M bases

RNA virus: 7-30K bases, very high mutation rate

Regions of apparent uniqueness

Design probe candidates 50-65 bases long with good thermodynamics

Select probes so all targets have >15-50 probes

Hundreds of thousands of cluster CPU-hours used for probe design.

Array design should be updated annually.

Lawrence Livermore Microbial Detection Array has a wide range of applications

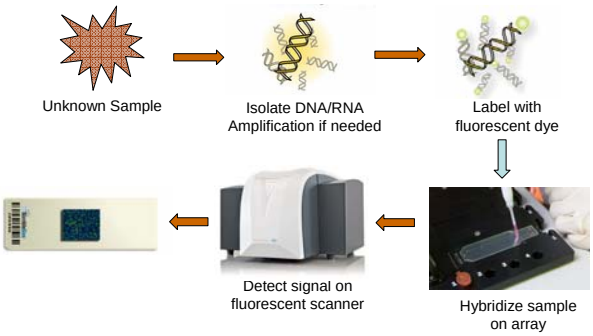
- Public health
- Product safety
- Food safety
- Biodefense
- Forensics
- Animal health
- Environmental monitoring

The LLMDA has many collaborations

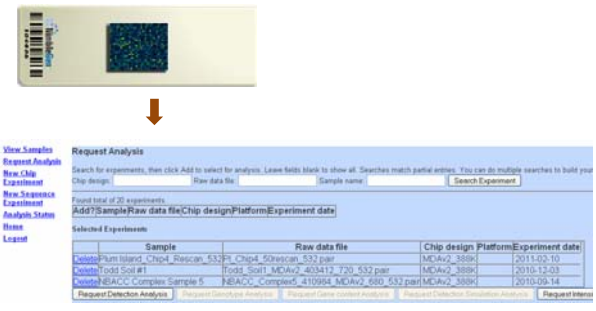
- University of California, San Francisco
- SF Blood Systems Research Institute
- University of Texas Medical Branch, Galveston
- Statens Serum Institut at Denmark
- National Institute for Public Health and the Environment, Netherlands
- Food Drug Administration
- Marine Mammal Center, Sausalito
- Boehringer Ingelheim
- Many others…

Tested on 300+ samples to date

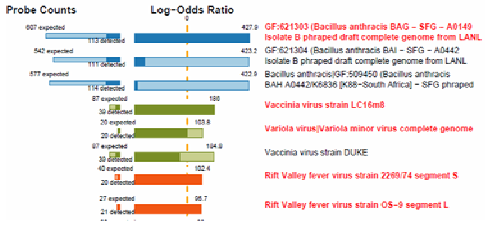
LLMDA can analyze complex clinical and environmental samples



Automated microarray data analysis with web entry, easy to upload



LLNL custom-developed maximum likelihood analysis software detects viruses and bacteria in a sample in an automated fashion



- **Results in ~5 minutes, web interface, easy to upload data**
- **Linux based, requires at least 64 GB memory**

Application of LLMDA in vaccine safety

- **Test live attenuated viral vaccines for any potential adventitious viruses**
- **In collaboration with Dr. Eric Delwart, SF Blood Systems Research Institute**
- **Massively parallel sequencing, microarray and PCR assays**

Meruvax	**Rotarix**
Attenuvax	**Rotateq**
YF-Vax	**Oral Polio**
MMR-II	**Varivax**

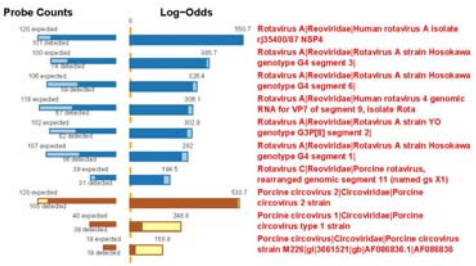
Victoria et al. 2010.

LLMDA detected non-vaccine viral sequences

Vaccine	Cell line	Non-vaccine viruses	
		454 sequencing	Microarray
Meruvax	WI-38		HEV
Attenuvax	CEF	ALV	ALV, AEV
YF-Vax	CEF		AEV
MMR-II	WI-38 and CEF		HEV, AEV
Varivax	MRC-5		HEV
Rotateq	Vero	SRV	BRV
Rotarix	Vero E6	PCV	PCV

ALV: avian leukosis virus
AEV: avian endogenous retrovirus
HEV: human endogenous retrovirus
SRV: simian endogenous retrovirus
BRV: baboon endogenous retrovirus
PCV: porcine circovirus

LLMDA array detected Rotavirus A and porcine circovirus from Rotarix vaccine



Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus. Victoria JG, Wang C, Jones MS, Jaing C, McLoughlin K, Gardner S and Delwart EL. *J. Virol*. 84: 6033-40, http://jvi.asm.org/cgi/reprint/84/12/6033, 2010.

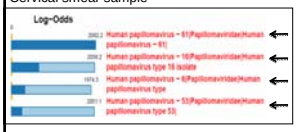
LLMDA can potentially be applied to ensure the safety of biologics

- **Successfully identified porcine circovirus from Rotarix vaccine**
- **Other potential applications:**
  - **QC raw materials**
  - **QC vaccine cell substrates**
  - **Characterize virus seeds**
  - **Detect adventitious viral, bacterial or fungal agents from biologics**
- **Cost-effective and comprehensive**

Application of LLMDA in clinical diagnostics

Swab sample

Cervical smear sample

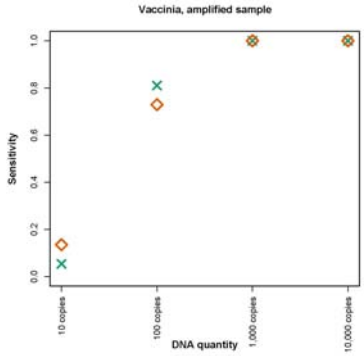


Serum sample

- In collaboration with Dr. Lena Erlandsson from Statens Serum Institut, Denmark

[The Microbial Detection Array Combined with Random Phi29 Amplification used as a Diagnostic Tool for Unbiased Virus Detection in Clinical Samples. Erlandsson et al., Submitted to PLoS ONE.]

LLMDA is highly sensitive to detect low copies of viral and bacterial DNA



- **Vaccinia virus was detected at 10 to 100 copies of DNA when coupled with random amplification.**

McLoughlin et al., manuscript in preparation

Flexible microarray platforms are cost-effective to screen large numbers of samples

388K spots

4x72K spots

2.1M spots

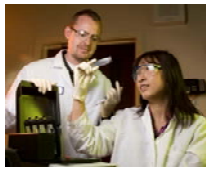
12x135K spots

4.2M spots, more multiplexing

- **High-throughput**
- **Fast**
- **High resolution**
- **Accurate**
- **Comprehensive**
- **Detection of known and unknown microorganisms**

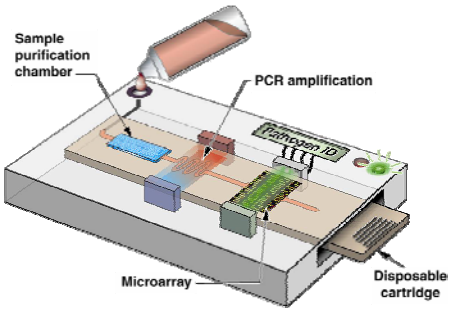
Microarrays are comprehensive and high-throughput tools for product and health safety

- **QC biologics to detect adventitious agents**
- **Pharmaceutical and clinical applications**
- **Broad spectrum, detect any of the sequenced virus or bacteria**
- **Custom array can be developed to focus on a subset of bacteria or viruses of interest**
- **Fast, results within 24 hrs**
- **Sensitive and specific**



We welcome discussions on licensing and collaborations

Future research to develop a fully integrated system from sample prep to pathogen ID



Challenges of using new technologies to screen for adventitious agents

- **How do you set a threshold of concern?**
- **How do you know that you interrogated the sample "deeply enough" to find adventitious viruses if present?**
- **How much of a virus, at what similarity threshold to previously-sequenced viruses, must be found in order to flag a potential problem?**