

Nucleic Acids-Based Methods with Enhanced Sensitivity and High Sample Throughput

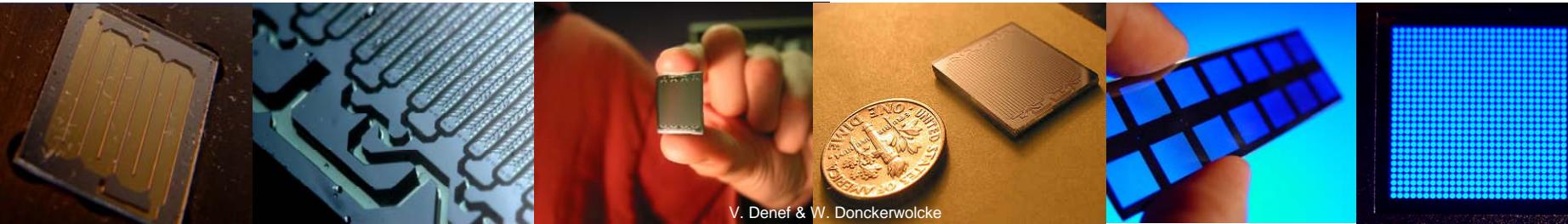
Homeland Security Annual University Network Summit on Research and Education

May 16, 2007

Syed A. Hashsham

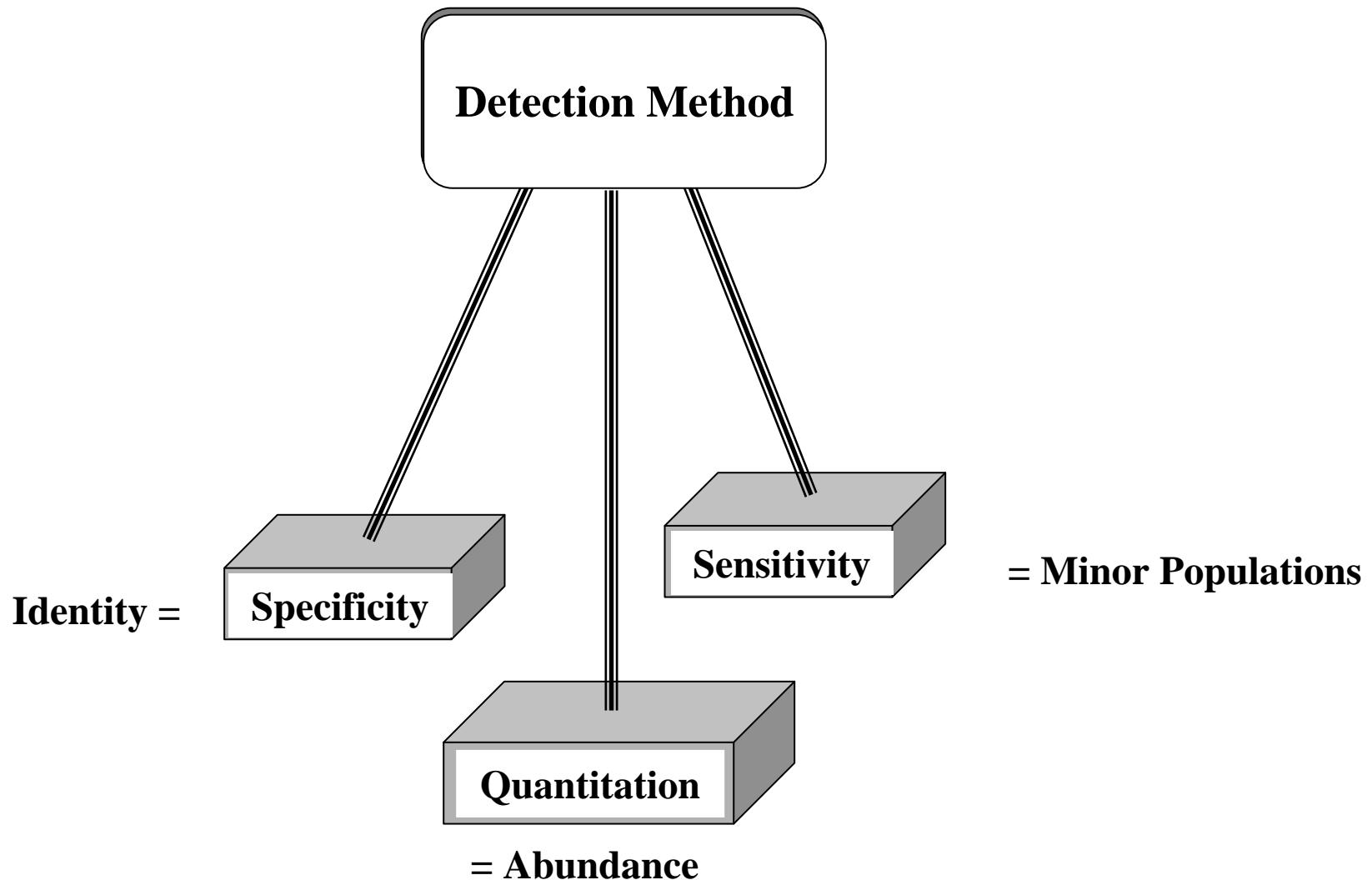
Associate Professor

Department of Civil and Environmental Engineering and
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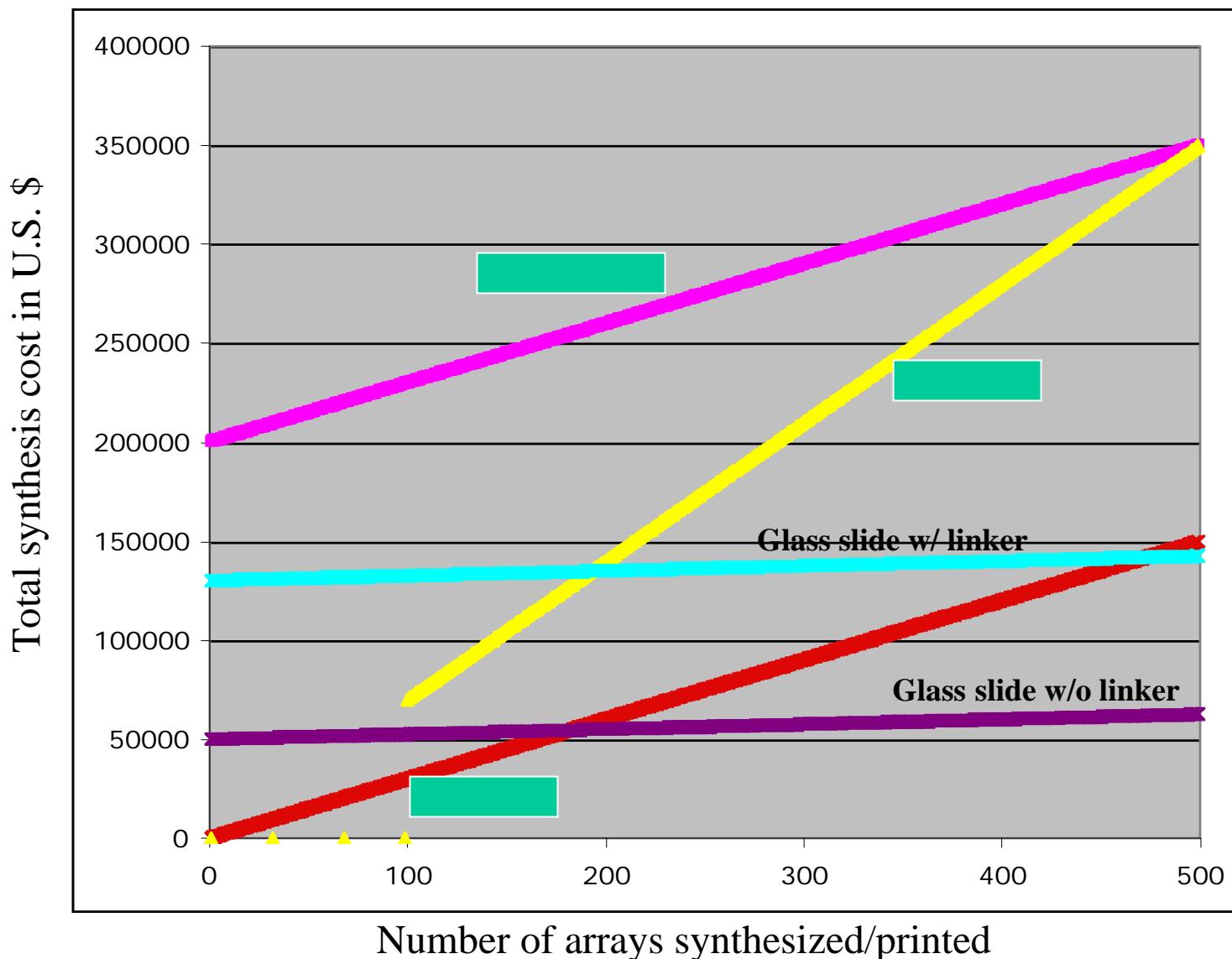


V. Denef & W. Donckerwolcke

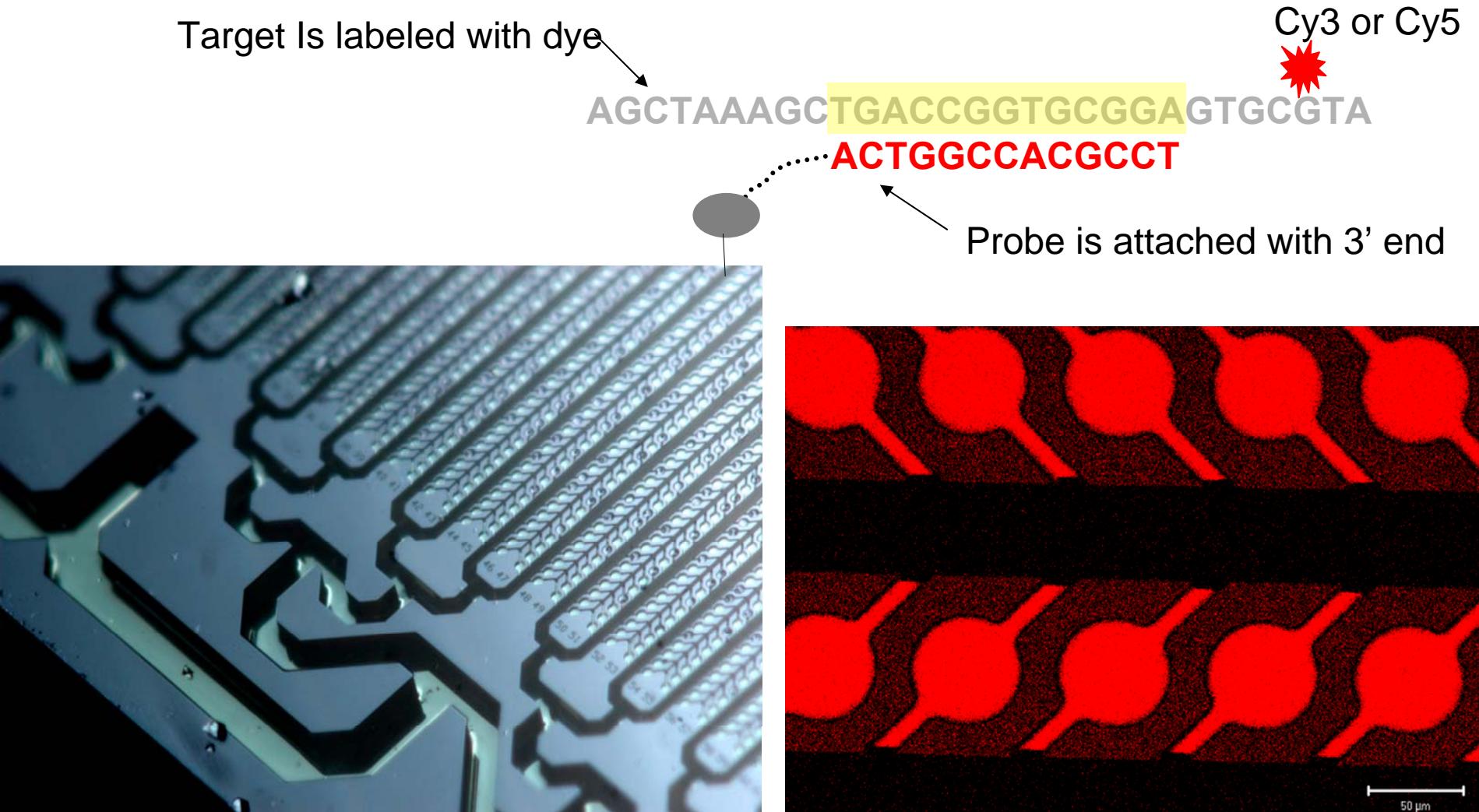
Technical Challenges



Cost effectiveness is key for community analysis



In situ Synthesized Microfluidic Chip

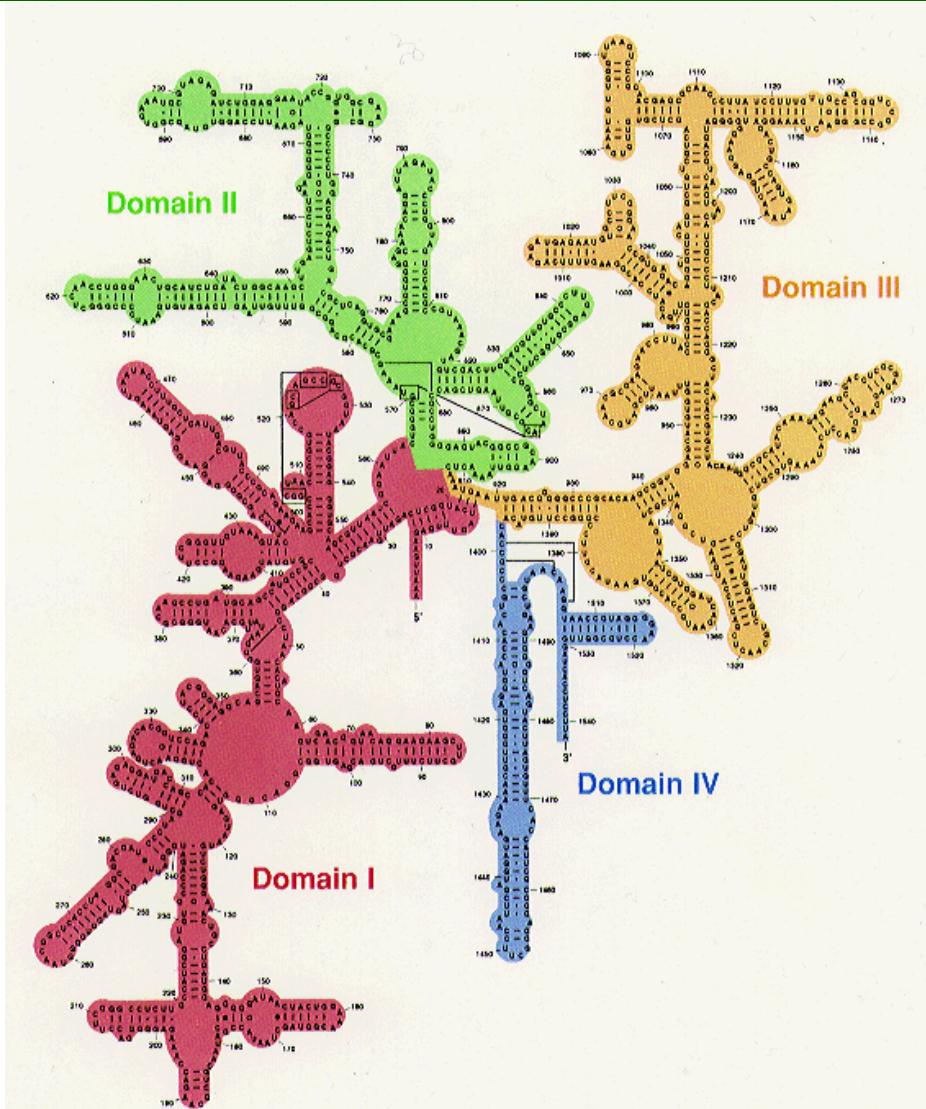


Microfluidic chip with recirculation capabilities

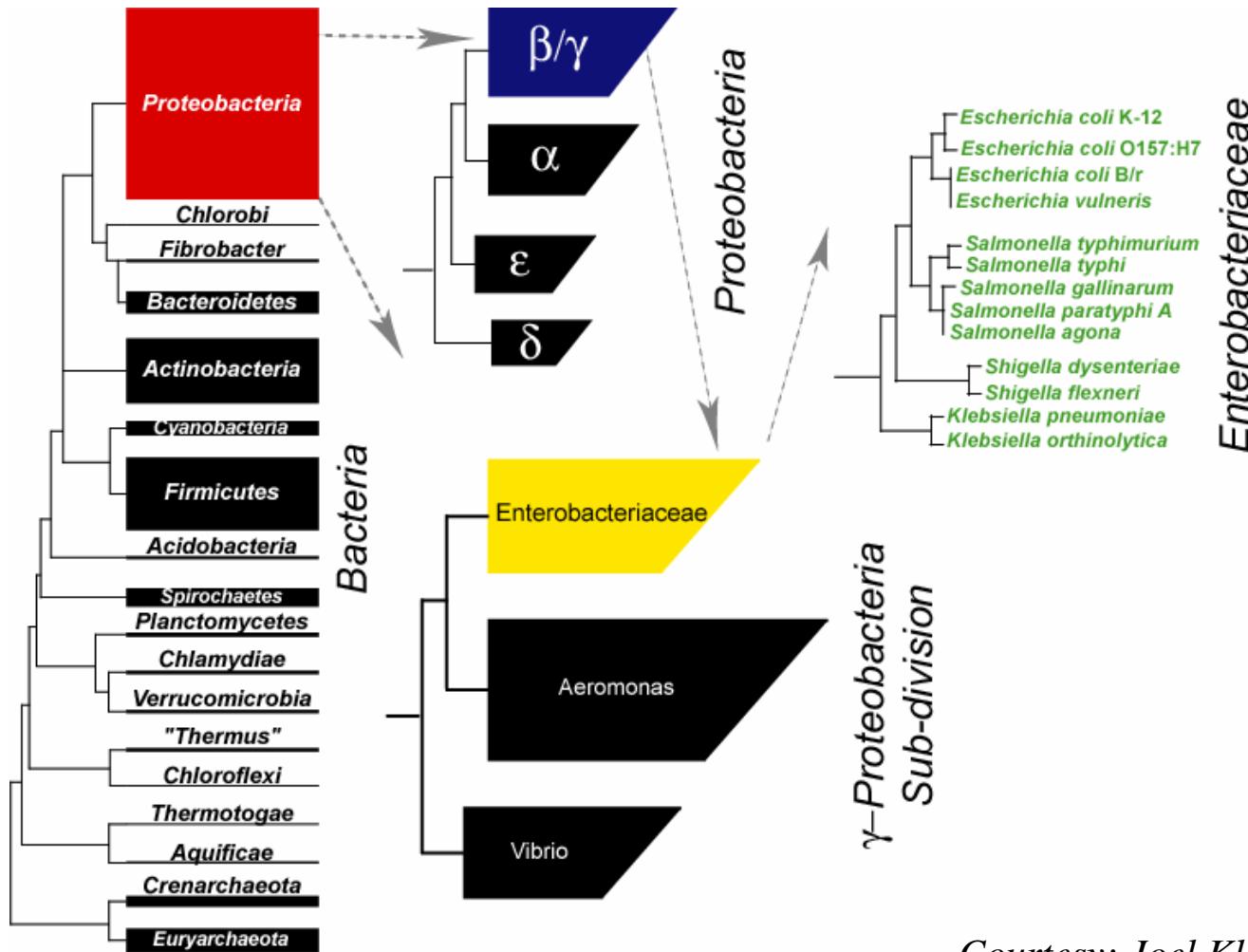
List of 20 Waterborne Pathogens

1. *Aeromonas hydrophila*
2. *Burkholderia pseudomallei, mallei*
3. *Campylobacter jejuni*
4. *Clostridium perfringens*
5. *Enterococcus faecalis, faecium*
6. *Escherichia coli, Shigella*
7. *Helicobacter pylori*
8. *Klebsiella pneumoniae*
9. *Legionella pneumophila*
10. *Leptospira interrogans*
11. *Listeria monocytogenes*
12. *Mycobacterium avium, paratuberculosis, tuberculosis, leprae*
13. *Pseudomonas aeruginosa*
14. *Salmonella typhimurium DT104*
15. *Staphylococcus aureus*
16. *Vibrio cholerae, mimicus, vulnificus*
17. *Vibrio parahaemolyticus*
18. *Yersinia enterocolitica, pestis, pseudotuberculosis*
19. *Cryptosporidium parvum, hominis*
20. *Giardia lamblia, intestinalis*

16S rRNA Gene Based Probes



16S, 18S, and 23S rRNA Gene based Probes



FunGene Pipeline

- Harvests Functional Genes from GenBank using **Hidden Markov Model (HMM)**
- Training sequences chosen by experts is input
- Matching sequences are output

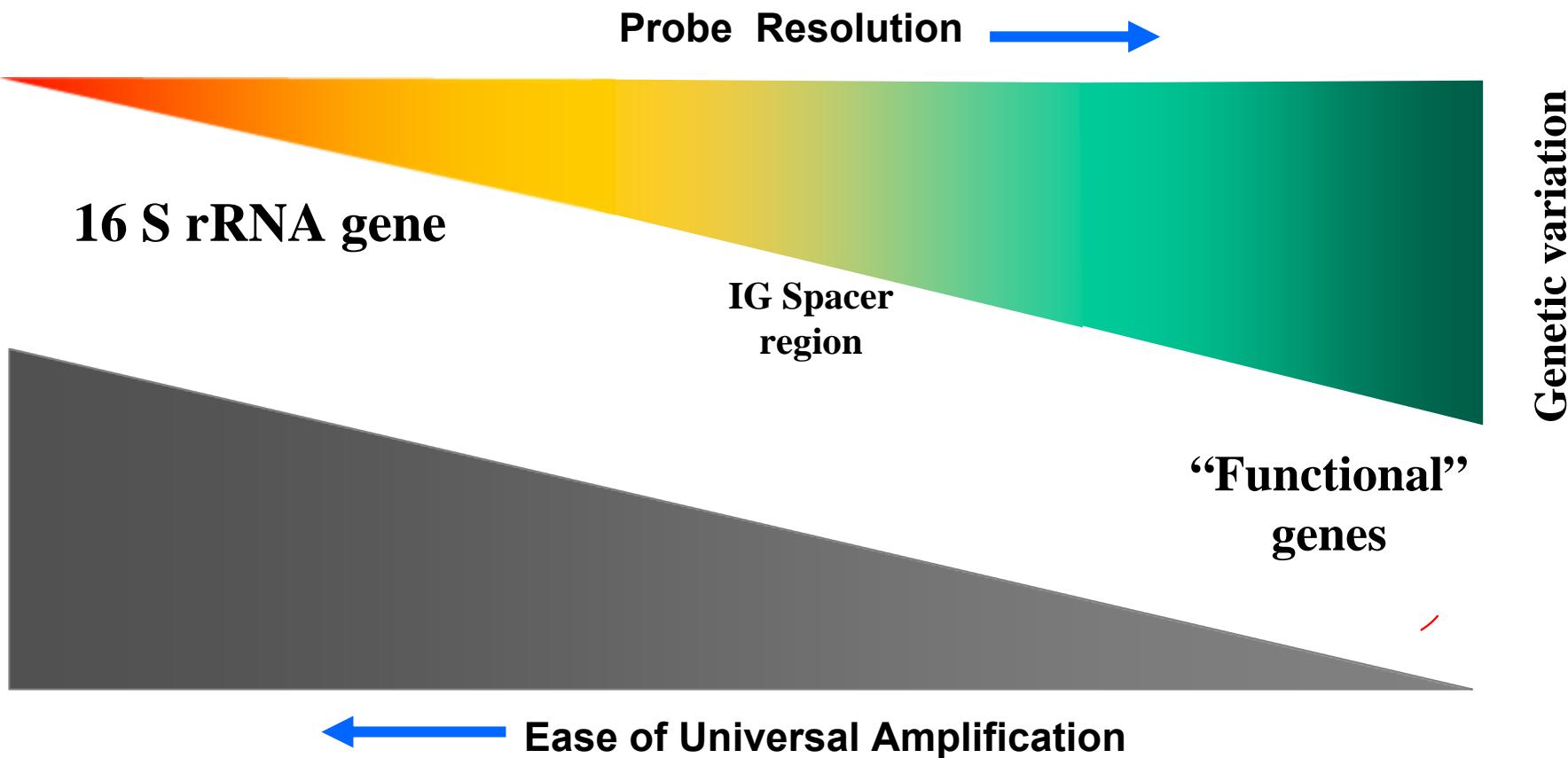


Select	Score	New Hit	PID	NID	Definition	Accession	Length	Start	End	Organism	Author
<input type="checkbox"/>	1075.8	★	AAF14359	AF121135	glycosyltransferase [Neisseria gonorrhoeae]	Neisseria gonorrhoeae	1	100	346	Harvey,H.A.	
<input type="checkbox"/>	1074.7	★	AAA92074	U15992	glycosyltransferase	Neisseria gonorrhoeae	1	100	362	Danaher,R.J.	
<input type="checkbox"/>	1073.0	★	AAA68009	U14554	glycosyl transferase	Neisseria gonorrhoeae	1	100	348	Gotschlich,E.C.	
<input type="checkbox"/>	1070.6	★	CAB883816	AL162753	lacto-N-neotetraose biosynthesis glycosyl transferase [Neisseria meningitidis Z2491]	Neisseria meningitidis Z2491	1	100	346	Parkhill,J.	
<input type="checkbox"/>	1070.4	★	AAK70338	AF313394	glycosyl transferase LgtA [Neisseria gonorrhoeae]	Neisseria gonorrhoeae	1	100	346	Tong,Y.	
<input type="checkbox"/>	1044.3	★	AAM33861	AF470659	LgtA [Neisseria meningitidis]	Neisseria meningitidis	1	100	362	Zhu,P.	
<input type="checkbox"/>	1043.4	★	AAM33875	AF470665	LgtA [Neisseria meningitidis]	Neisseria meningitidis	1	100	362	Zhu,P.	
<input type="checkbox"/>	1033.2	★	AAM33869	AF470662	LgtA [Neisseria meningitidis]	Neisseria meningitidis	1	96	348	Zhu,P.	
<input type="checkbox"/>	1031.5	★	AAM33855	AF470657	LgtA [Neisseria meningitidis]	Neisseria meningitidis	1	96	348	Zhu,P.	
<input type="checkbox"/>	1027.8	★	AAN08510	AY134876	LgtA [Neisseria lactamica]	Neisseria lactamica	1	96	348	Zhu,P.	

beginning with letters F-K	beginning with letters L-Q	beginning with letters R-T
feoA	lam	racR
feoB	lasA	rtxD
FGLN	lasB	scl
fhaB	lbpA	scpB
fhaC	lbpB	seA
filA	lef	seC
fimA	lepA	serA
fimD	lepB	sfaA
flaA	lgtA	sipB
flaB	lgtB	sipC
flhA	lic2A	ska

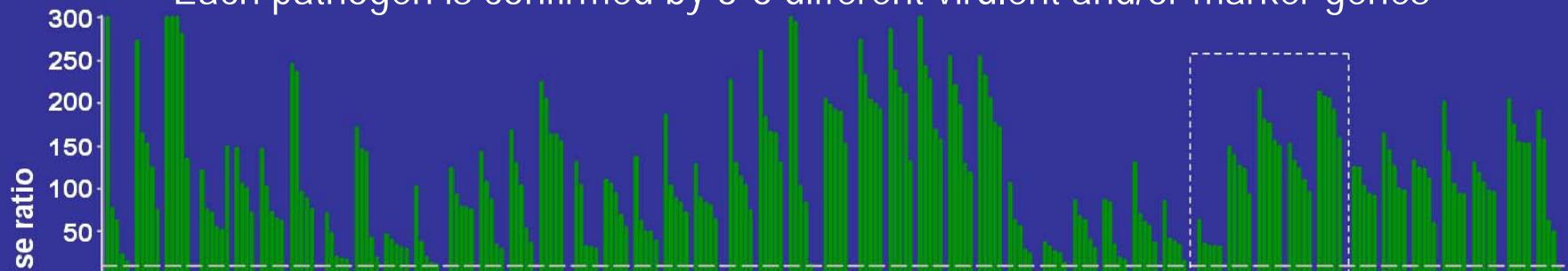
Developed by James R. Cole at MSU

Probe Resolution Hierarchy

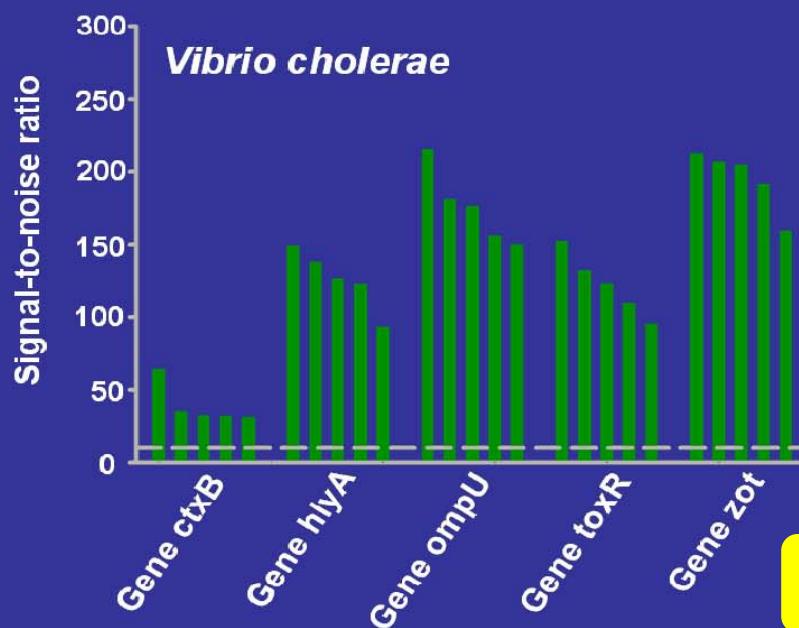


What Multiple Probes Do?

Each pathogen is confirmed by 3-6 different virulent and/or marker genes

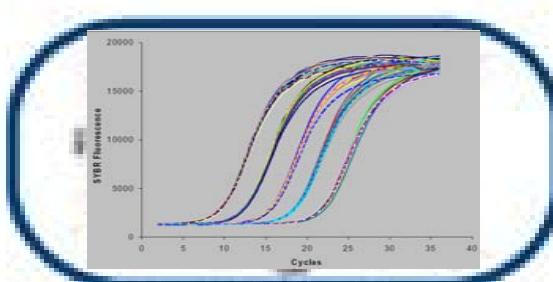


Each gene is confirmed by 5-20 probes

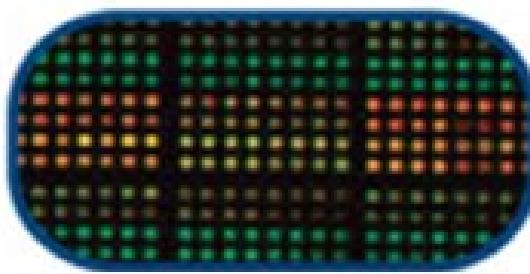


No false positive!

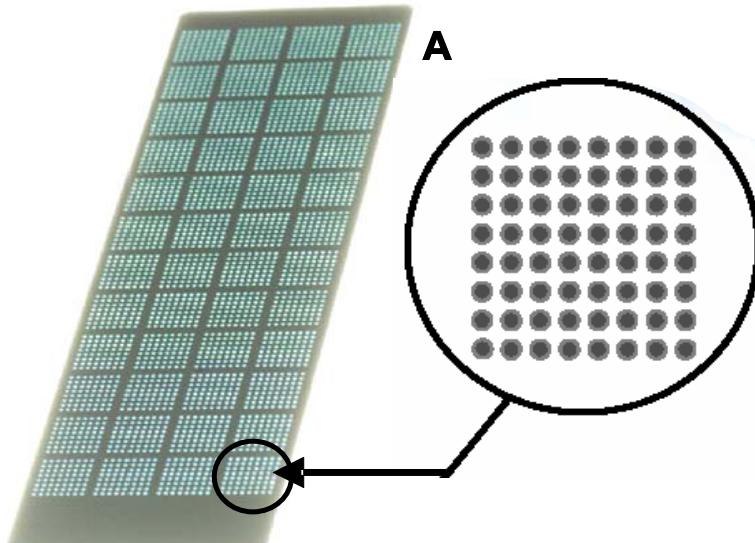
Biotrove OpenArray™



Sensitivity and
Specificity of QPCR



Throughput of
Microarray



OpenArray™

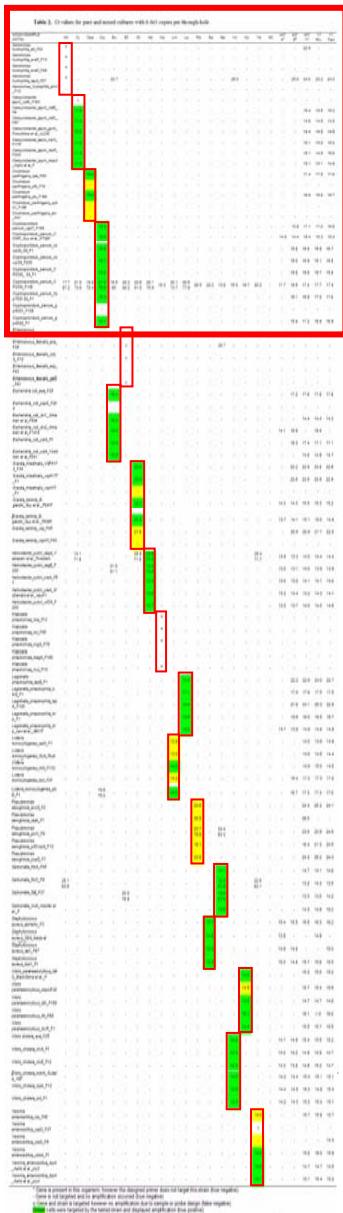


B



NT cycler and computer with
Analysis Software

Abundance of 47 virulence and marker genes in 120 samples by RT-PCR



ASSAY/SAMPLE (Ct/Tm)	Ah	Cj	Cpe	Cry	Ec	Ef	Gi	Hp	Kp	Lm	Lp	Pa	Sa	Se	Vc	Vp	Ye	NC	MT 4 ^a	MT 8 ^b	MT 17	17 Riv.	17 Tert	
<i>Aeromonas</i> <i>hydrophila</i> _alt_F33	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22.5	-	-		
<i>Aeromonas</i> <i>hydrophila</i> _exeF_F13	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Aeromonas</i> <i>hydrophila</i> _exeF_F88	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Aeromonas</i> <i>hydrophila</i> _tapA_F27	*	-	-	-	22.7	-	-	-	-	-	-	-	-	-	26.0	-	-	-	-	25.0	24.0	23.2	24.0	
<i>Aeromonas</i> <i>hydrophila</i> _arcV _F10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Campylobacter</i> <i>jejuni</i> _cdtA_F163	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Campylobacter</i> <i>jejuni</i> _cdtB_F6	-	11.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.4	15.5	15.3	-	
<i>Campylobacter</i> <i>jejuni</i> _cdtC_F57	-	11.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14.8	14.5	14.8	-	
<i>Campylobacter</i> <i>jejuni</i> _gyrA_Fukushima et al_JL238	-	10.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14.4	14.5	14.6	-	
<i>Campylobacter</i> <i>jejuni</i> _hipO_F179	-	11.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.1	15.0	15.0	-	
<i>Campylobacter</i> <i>jejuni</i> _racR_F200	-	11.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.1	14.9	15.0	-	
<i>Campylobacter</i> <i>jejuni</i> _mapA_Inglis et al_F	-	11.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.1	15.1	14.9	-	
<i>Clostridium</i> <i>perfringens</i> _cpe_F84	-	-	16.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17.4	17.5	17.6	-	
<i>Clostridium</i> <i>perfringens</i> _pfo_F14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Clostridium</i> <i>perfringens</i> _pce_F196	-	-	16.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.9	16.8	16.7	-	
<i>Clostridium</i> <i>perfringens</i> _cpb_a1_F199	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Clostridium</i> <i>perfringens</i> _ctx_F41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Cryptosporidium</i> <i>parvum</i> _cgd7_F188	-	-	-	15.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.9	17.1	17.0	16.9	
<i>Cryptosporidium</i> <i>parvum</i> _C_OWP_Guv et al_P702F	-	-	-	15.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14.5	14.4	15.4	15.3	15.3
<i>Cryptosporidium</i> <i>parvum</i> _co_wpGII_GII_F1	-	-	-	15.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.8	16.8	16.9	16.7	-
<i>Cryptosporidium</i> <i>parvum</i> _co_wpGII_F200	-	-	-	16.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.0	16.9	16.1	16.8	-
<i>Cryptosporidium</i> <i>parvum</i> _C_P23GII_GII_F1	-	-	-	15.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.8	16.8	16.7	16.8	-
<i>Cryptosporidium</i> <i>parvum</i> _C_F23GII_F152	17.7	21.0	18.8	21.4	18.5	20.2	22.6	20.1	18.3	20.1	20.6	20.5	22.2	13.8	16.3	19.7	20.2	-	17.7	18.6	17.2	17.7	17.4	
<i>Cryptosporidium</i> <i>parvum</i> _hs_p70GII_GII_F1	87.2	73.8	72.4	79.6	85	80.2	81.5	75.9	73.7	77.9	20.5	-	-	-	-	-	-	-	-	16.1	16.9	17.0	17.0	-
<i>Cryptosporidium</i> <i>parvum</i> _g_p40G1_F139	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Cryptosporidium</i> <i>parvum</i> _g_p40G2_F1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.8	17.2	16.9	16.9	16.9
<i>Enterococcus</i> <i>faecalis</i> cfaA_F99	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

^a Sample is the original source. For samples from 120 different sources of large fish, this table includes the first 100 samples.

^b Sample is a mixture of 10% original sample and 90% *C. jejuni* ATCC 43452.

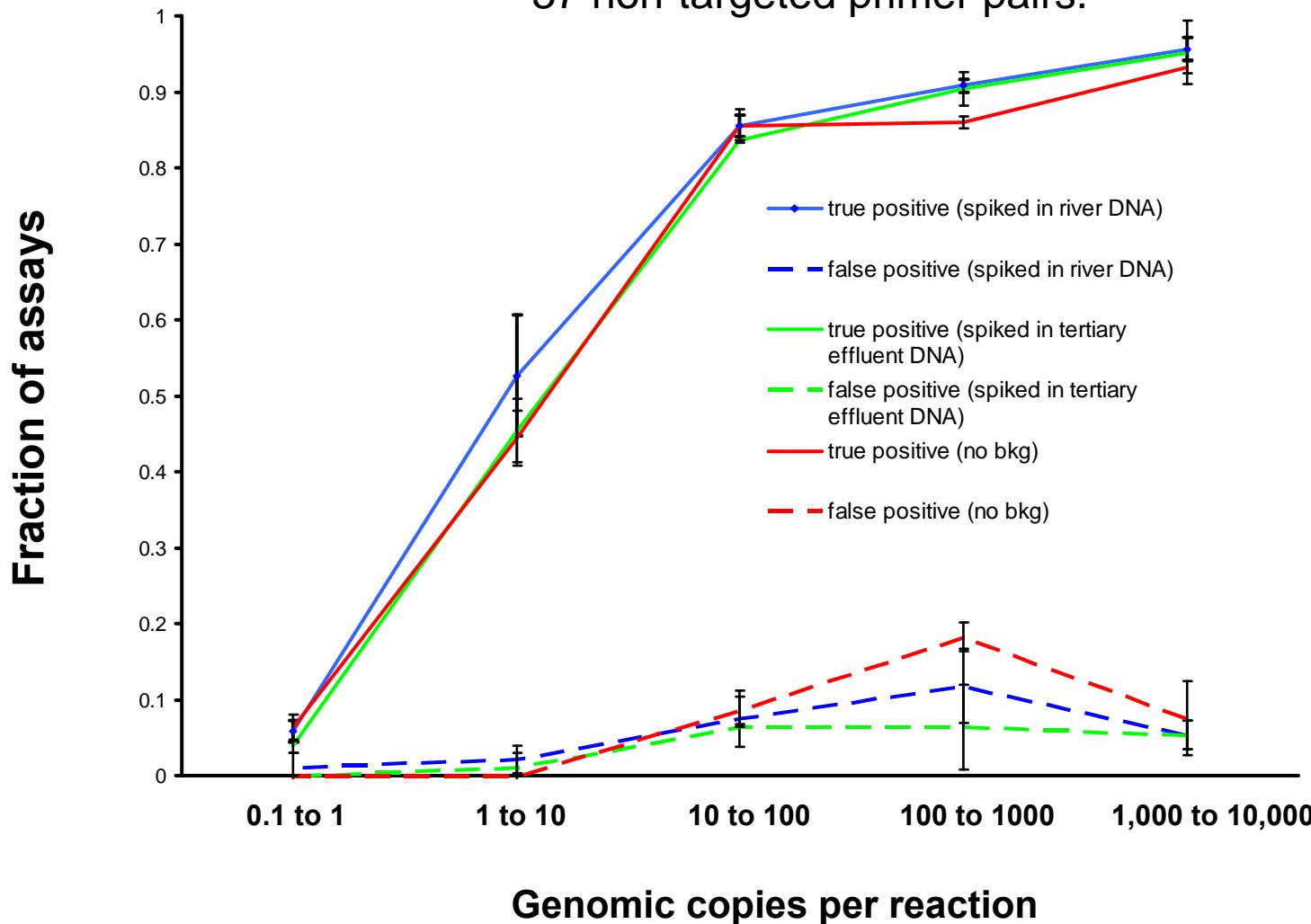
* Sample is a mixture of 10% original sample and 90% *C. jejuni* ATCC 43452.

^c Sample is a mixture of 10% original sample and 90% *C. jejuni* ATCC 43452.

^d Sample is a mixture of 10% original sample and 90% *C. jejuni* ATCC 43452.

BioTrove Performance

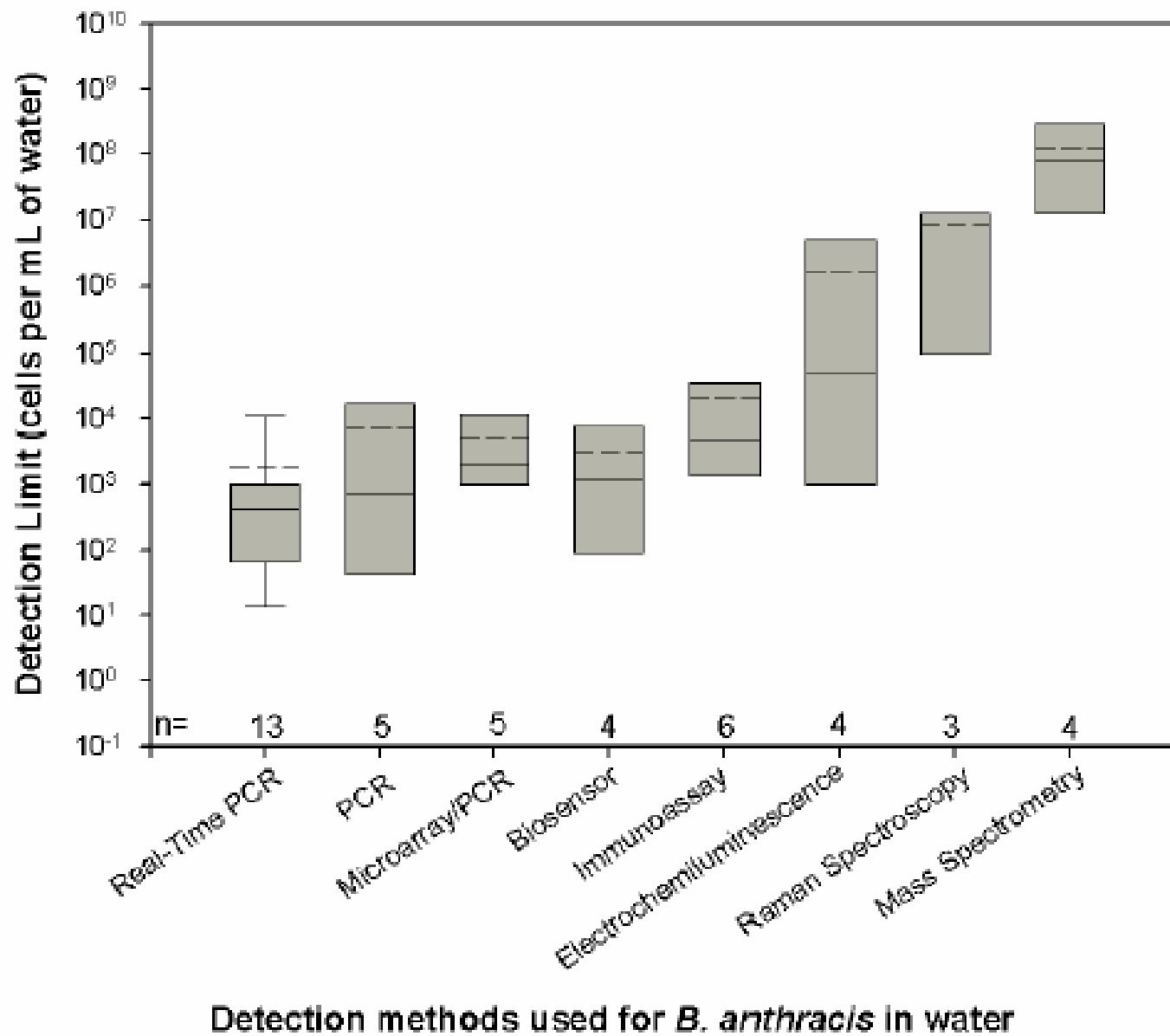
73 targeted primer pairs
37 non-targeted primer pairs.



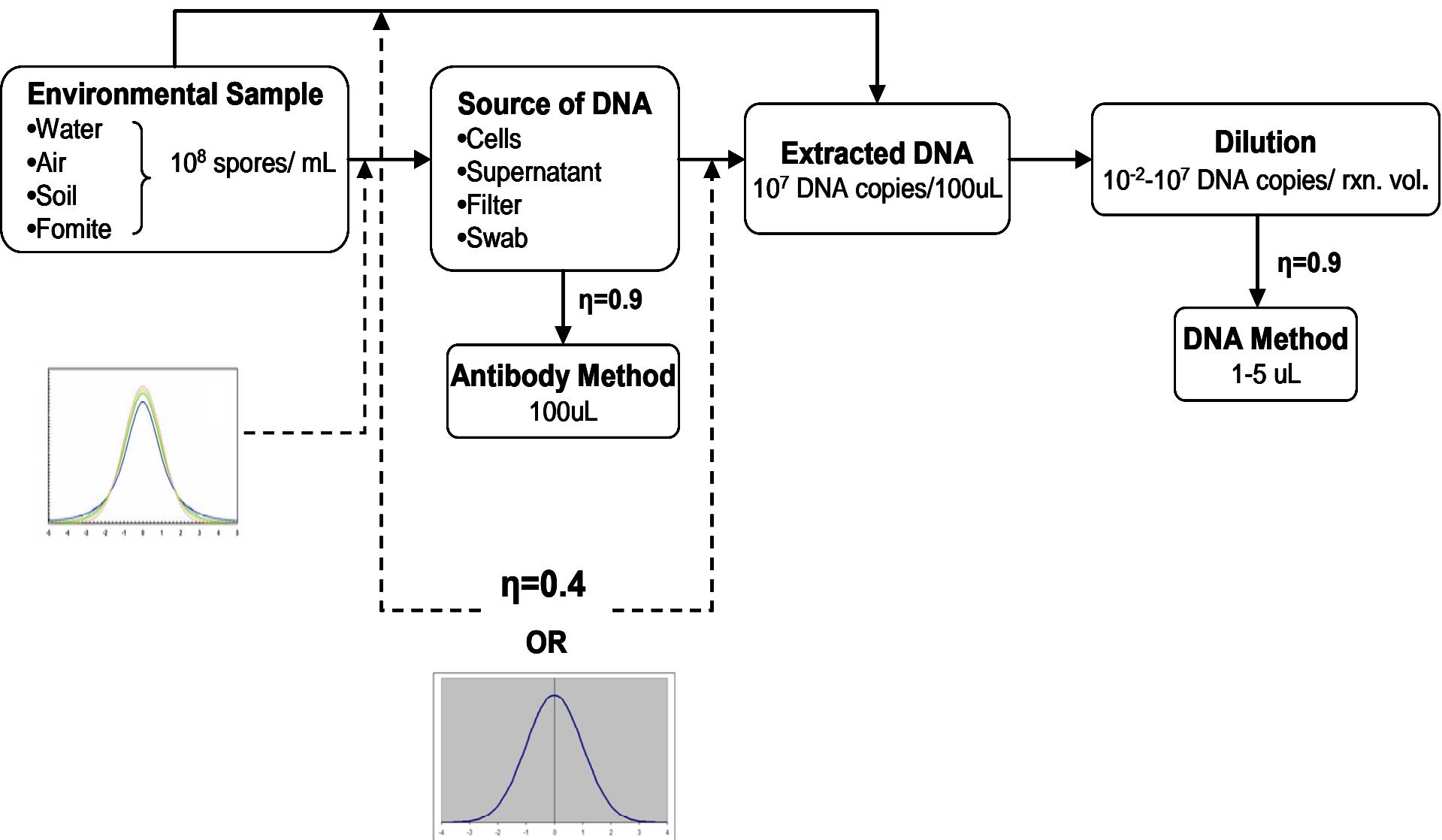
Summary

1. Number of pathogens	> 20
2. Number of genes per pathogen	3-6
3. Number of probes per gene	5-20
4. Detection limit	At least 0.001%; better with clean samples
5. Reliability	99.999% or better
6. Robustness	High
7. Speed of analysis	30 minutes detection time; 2-5 hours total
8. Ease of use	Medium
9. Cost	Per organism: < \$10, Per chip \$150-\$200

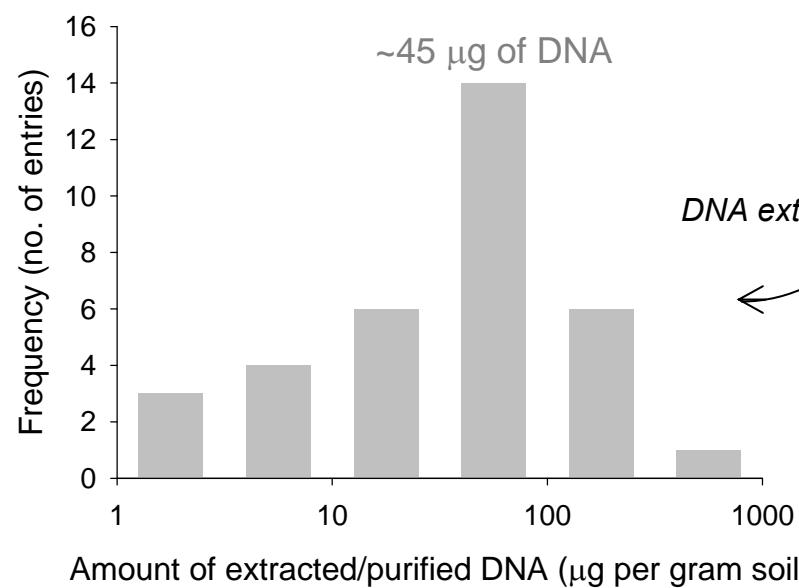
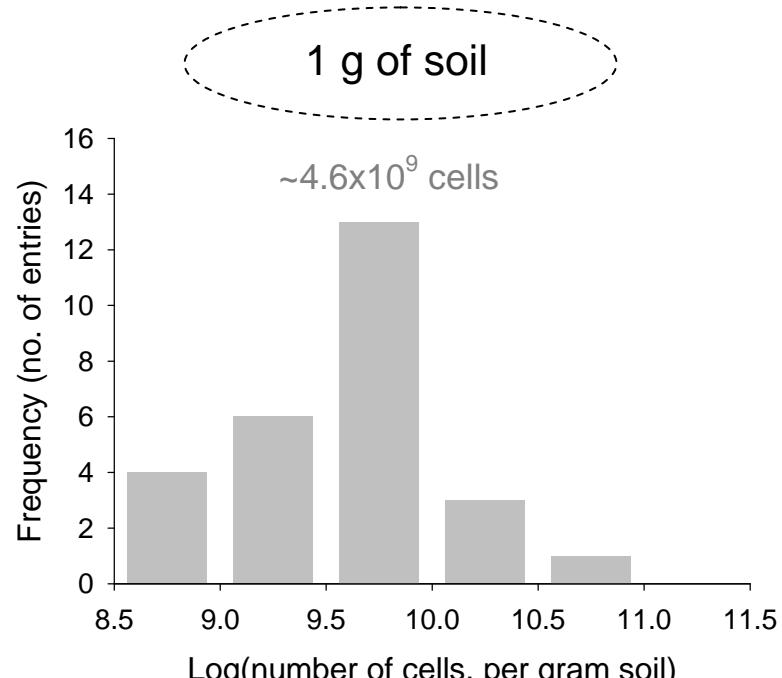
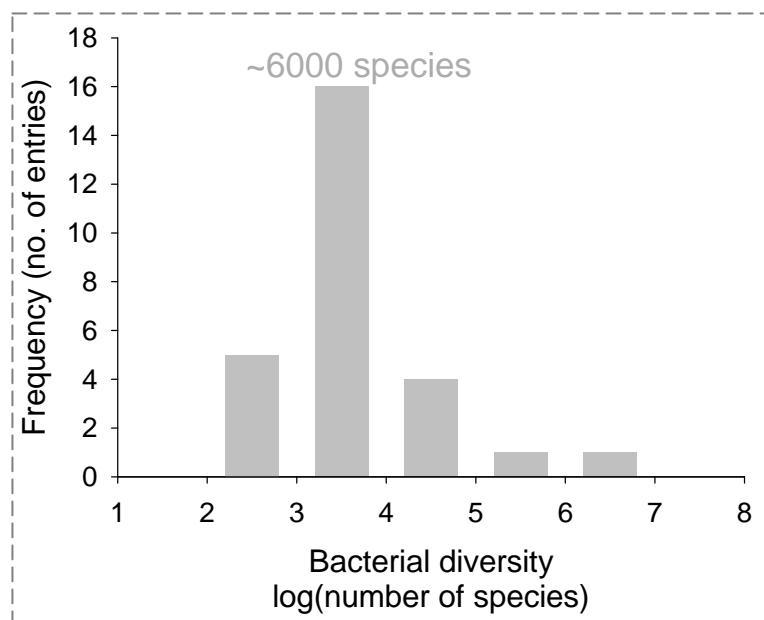
Bacillus anthracis Detection Limits



End-to-End Probability of Detection



Frequency Distribution of DNA Extraction Efficiency



DNA extraction and purification, efficiency (η)



Funding

National Institutes of Health-NCRR
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