Microfluidic Systems for Microbial Detection

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ENGINEERING

OUTLINE

A **Microbial Pathogens of Interest**

(Waterborne Pathogens, Antibiotic Resistant Bacteria.)

B **Application-Specific Issues**

(Multiplexing, Field deployability, Matrix, Volume, Cost)

C **Microfluidic Systems**

(Databases, Molecular Tools, Microfluidics)

Water and Foodborne Pathogens

	EPA's Contaminant Candidate List (CCL) 4	FDA, FIGHT BAC!
VIRUSES	Adenovirus Caliciviruses (includes Norovirus) Enterovirus (polioviruses, coxsackieviruses and echoviruses) Hepatitis A virus	Norovirus
BACTERIA	*Campylobacter jejuni* *Escherichia coli* (0157) *Helicobacter pylori* *Legionella pneumophila* *Mycobacterium avium* *Salmonella enterica* *Shigella sonnei*	Campylobacter *Clostridium botulinum* *E. coli* O157:H7 *Listeria monocytogenes* Salmonella *Staphylococcus aureus* Shigella *Vibrio vulnificus*
PROTOZOA	*Naegleria fowleri*	Cyclospora *Toxoplasma gondii*

Microbial Systems: Phylogeny vs. Function

Phylogeny: Who is there?

Function: What are they doing (or capable of doing)?

16S rRNA gene

Genes related to specific functions

Diverse Set of Sample Matrices

Blood
Swab
Tissue
Saliva
Urine
Feces
CSF

Drinking Water

Surface Water

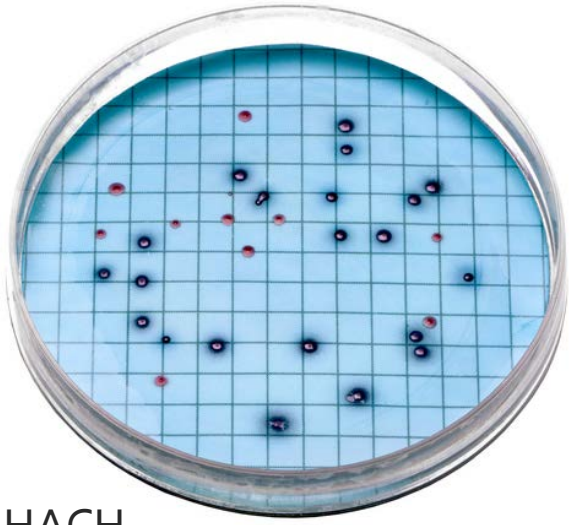
Produce

Sediment

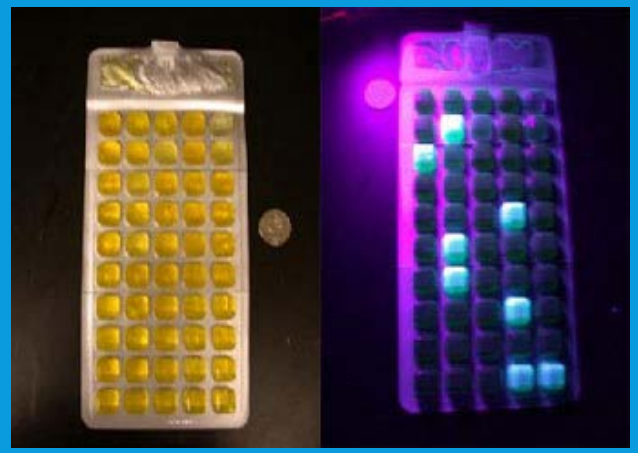
Wastewater

Manure

Waterborne Pathogens: Limit of Detection, Live vs. Dead, Time



HACH



Coliform | *E. coli*

https://www.fs.fed.us/t-d/programs/im/coliform/Ecoli_06.shtml

Issues

- Very low concentrations ~ 1 CFU/100 ml
- Live vs. Dead differentiation
- Rapid detection (<1 hr)

Virulence and Marker Genes

Linked to Regulatory Definitions!

Waterborne Pathogens

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2008, p. 2200–2209 Vol. 74, No. 7
0099-2240/08/$08.00+0 doi:10.1128/AEM.01962-07

In Situ-Synthesized Virulence and Marker Gene Biochip for Detection of Bacterial Pathogens in Water▽†

Sarah M. Miller,[1]‡ Dieter M. Tourlousse,[1]‡ Robert D. Stedtfeld,[1] Samuel W. Baushke,[1] Amanda B. Herzog,[1] Lukas M. Wick,[3] Jean Marie Rouillard,[4] Erdogan Gulari,[4] James M. Tiedje,[2] and Syed A. Hashsham[1,2]*

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Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Most probable number - loop mediated isothermal amplification (MPN-LAMP) for quantifying waterborne pathogens in <25 min

Farhan Ahmad [a], Robert D. Stedtfeld [a], Hassan Waseem [a], Maggie R. Williams [a], Alison M. Cupples [a], James M. Tiedje [b,c], Syed A. Hashsham [a,b,*]

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Appl Microbiol Biotechnol (2015) 99:7711–7722
DOI 10.1007/s00253-015-6774-z

METHODS AND PROTOCOLS

Thirty-minute screening of antibiotic resistance genes in bacterial isolates with minimal sample preparation in static self-dispensing 64 and 384 assay cards

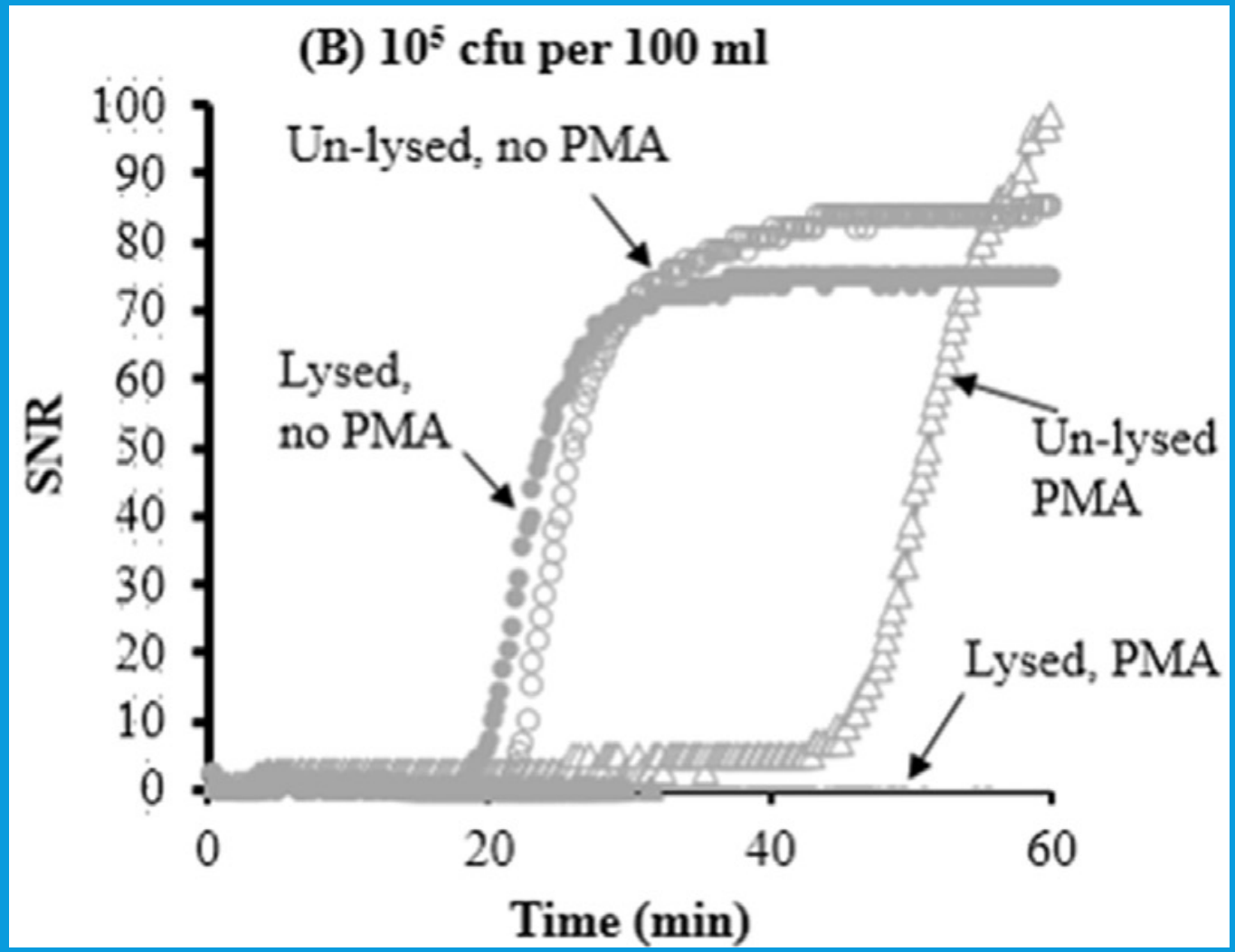
Tanja Kostić[1] · Michael Ellis[2,5] · Maggie R. Williams[2] · Tiffany M. Stedtfeld[2] · John B. Kaneene[3] · Robert D. Stedtfeld[2] · Syed A. Hashsham[2,4]

Multiplex
Shorten the time
Live/Dead
LOD
Filed deployability

LOD, Live vs. Dead, 1-hr: *Legionella pneumophila*

10 mm

1. Filter 100 ml- 1 liter of water
2. Add Propidium Monoazide
3. Crosslink by ~400 nm light
4. On-filter direct amplification
5. Reading by Gene-Z



Samhan et al. | Water Research, 2017

Filed Deployability: Direct Amplification

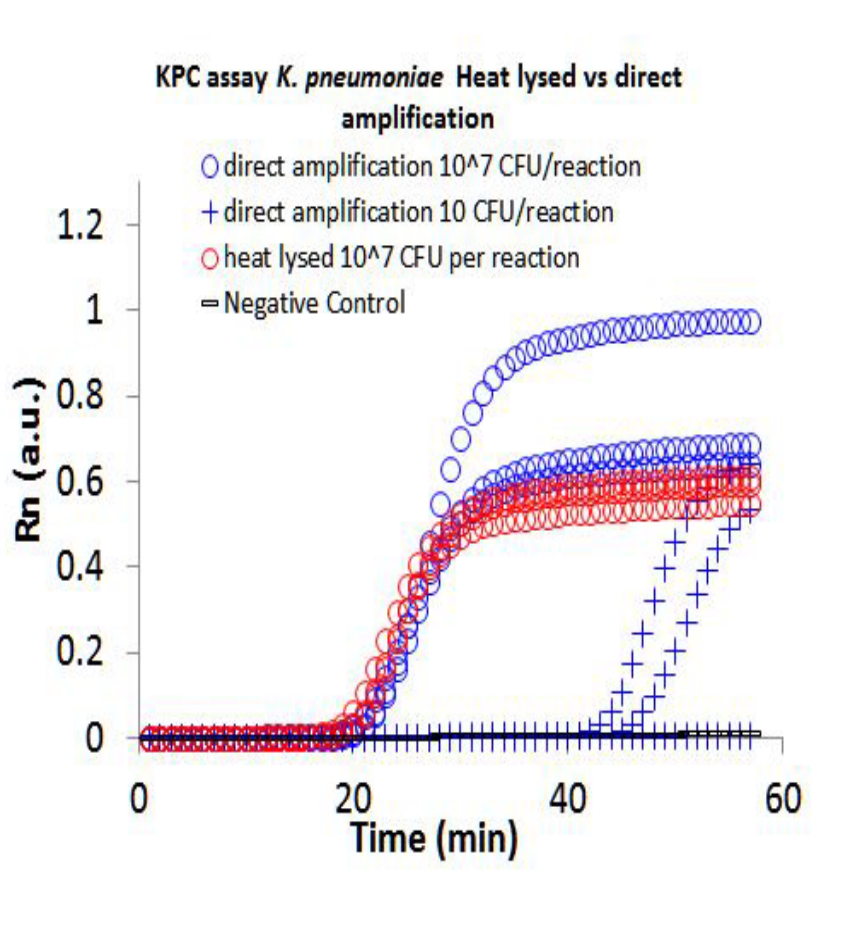
A. Traditional approach when DNA extraction is needed is complex; not suitable for POCs

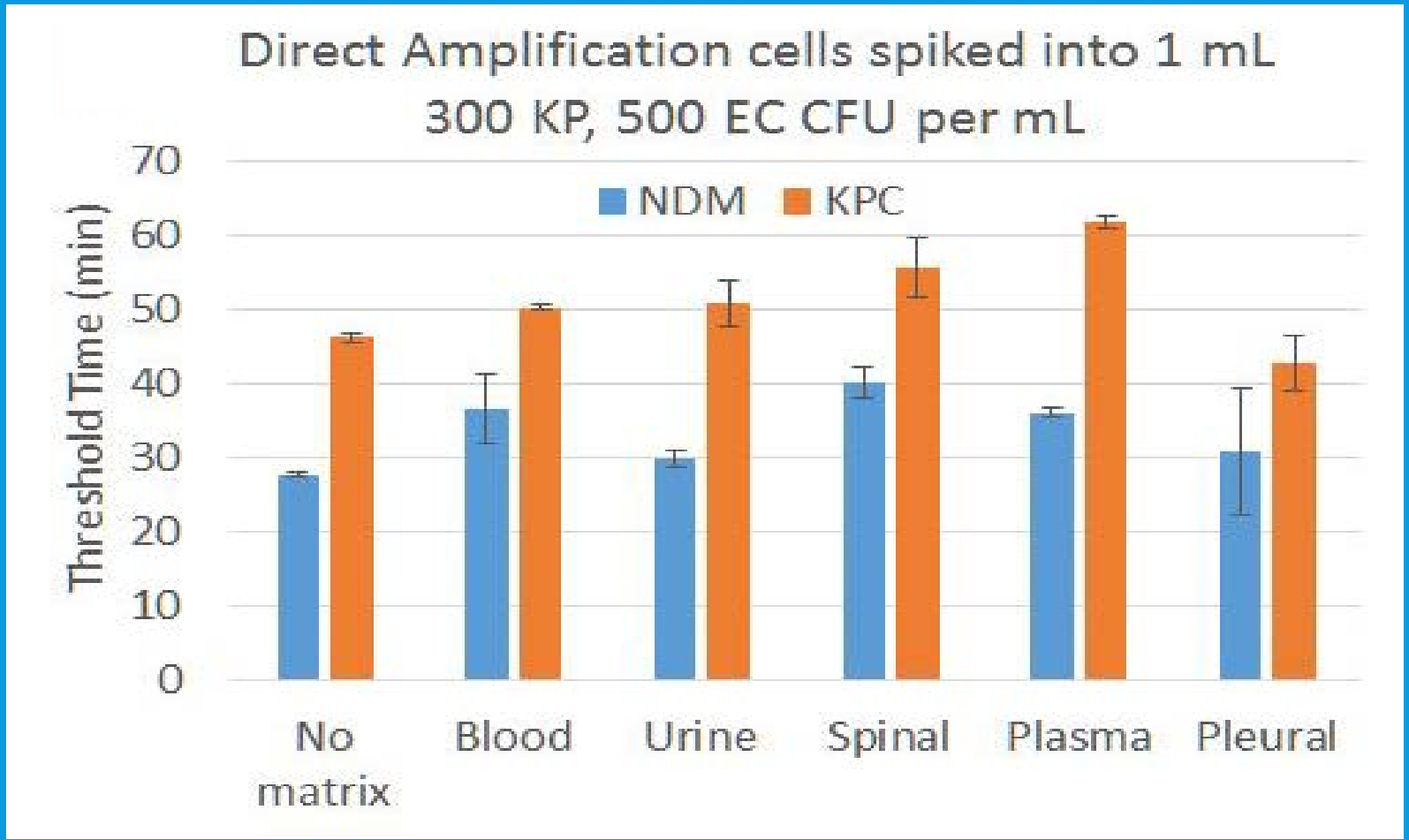
Hrs to days; in Lab

B. Approach when DNA extraction is not needed is simpler and faster

Total < 1 Hr; On site

Direct Amplification of Carbapenem Resistant Enterobacteriaceae (CRE) in body fluids





NDM-1 = New Delhi Metallo beta-lactamase 1, KPC = *Klebsiella pneumoniae* carbapenem

Direct amplification vs. Extraction and purification

Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

CRITICAL REVIEW

View Article Online
View Journal

CrossMark click for updates

Cite this: DOI: 10.1039/c6ay03405e

Implications of direct amplification for measuring antimicrobial resistance using point-of-care devices

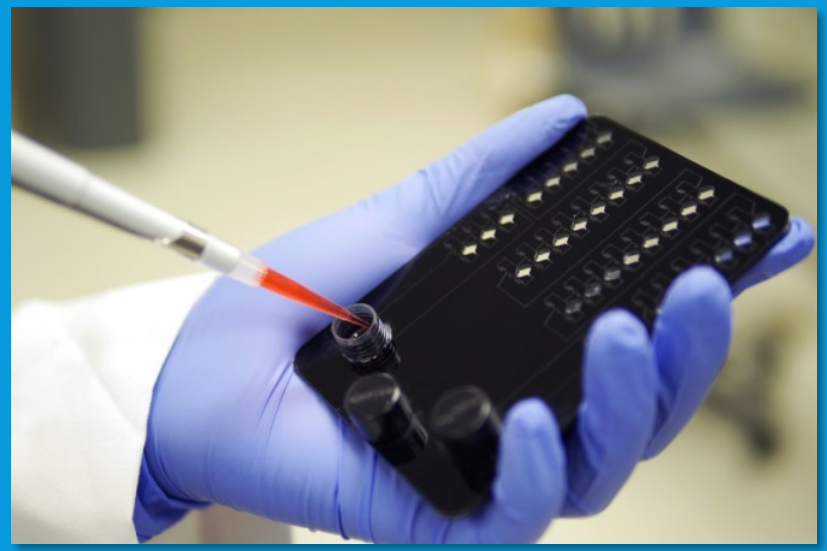
M. R. Williams,[a] R. D. Stedtfeld,[a] H. Waseem,[a] T. Stedtfeld,[a] B. Upham,[b] W. Khalife,[c] B. Etchebarne,[d] M. Hughes,[d] J. M. Tiedje[ef] and S. A. Hashsham*[aef]

DNA extraction is not always the necessary first step!

Field Deployable Real Time Amplification Readers

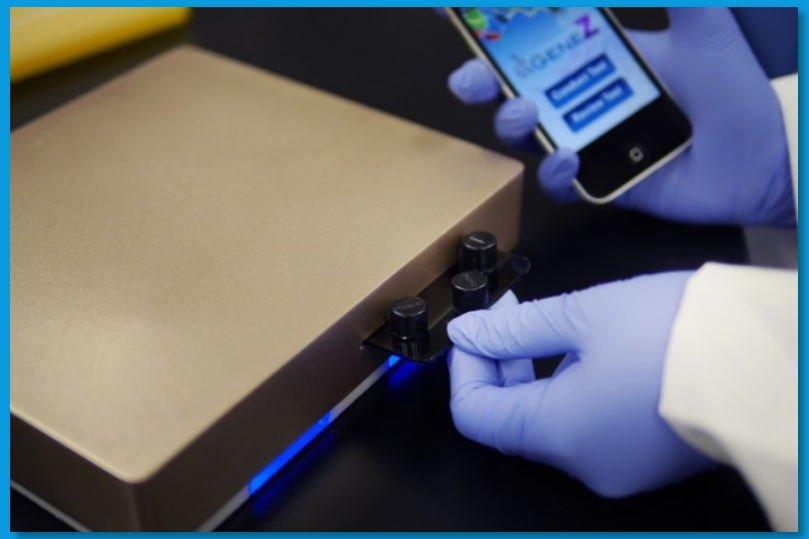
Fewer steps – good for limited resource settings

1. Load sample into the chip



A. Assay + Chips

2. Insert chip into the Gene-Z device



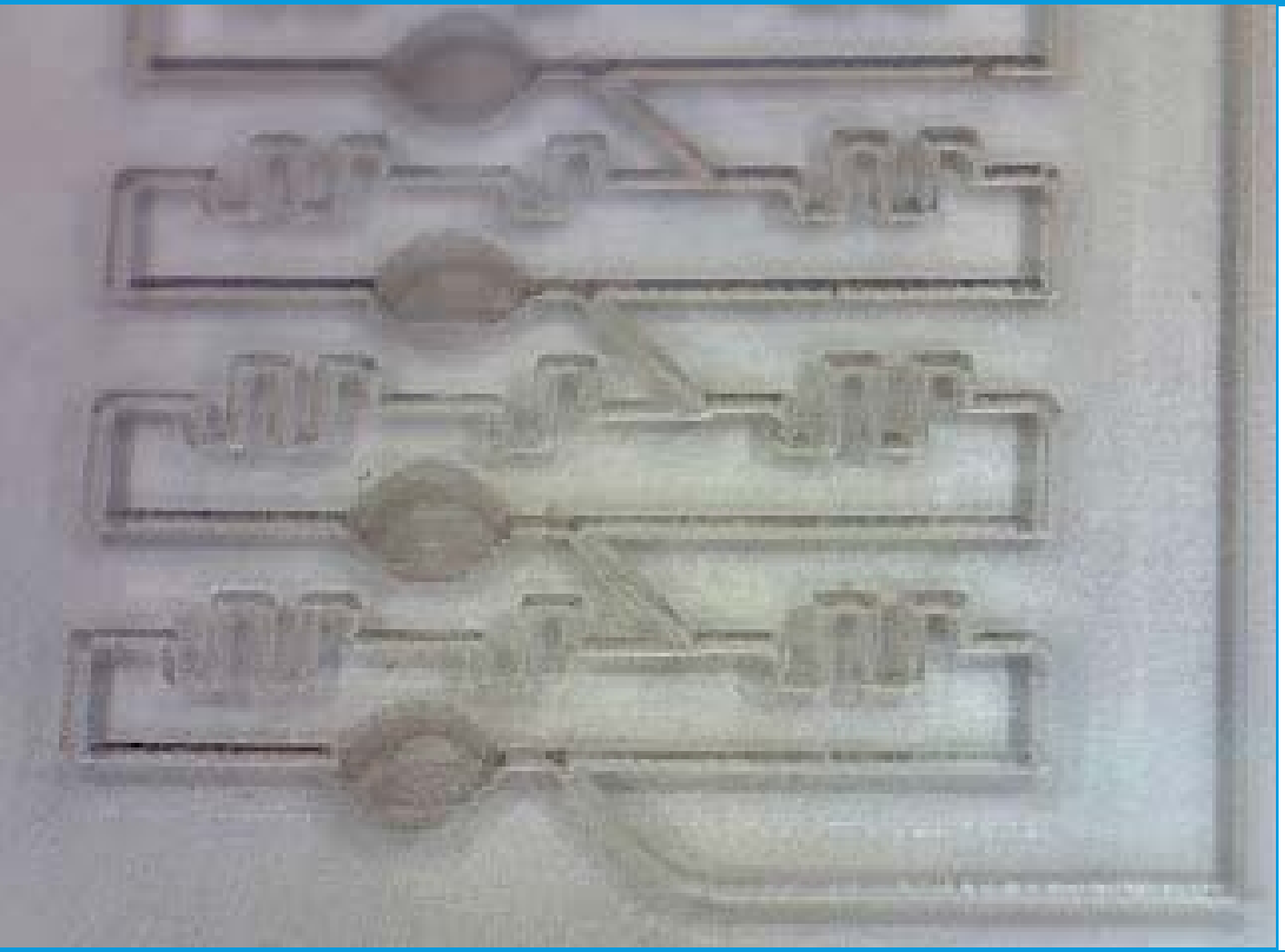
B. Device

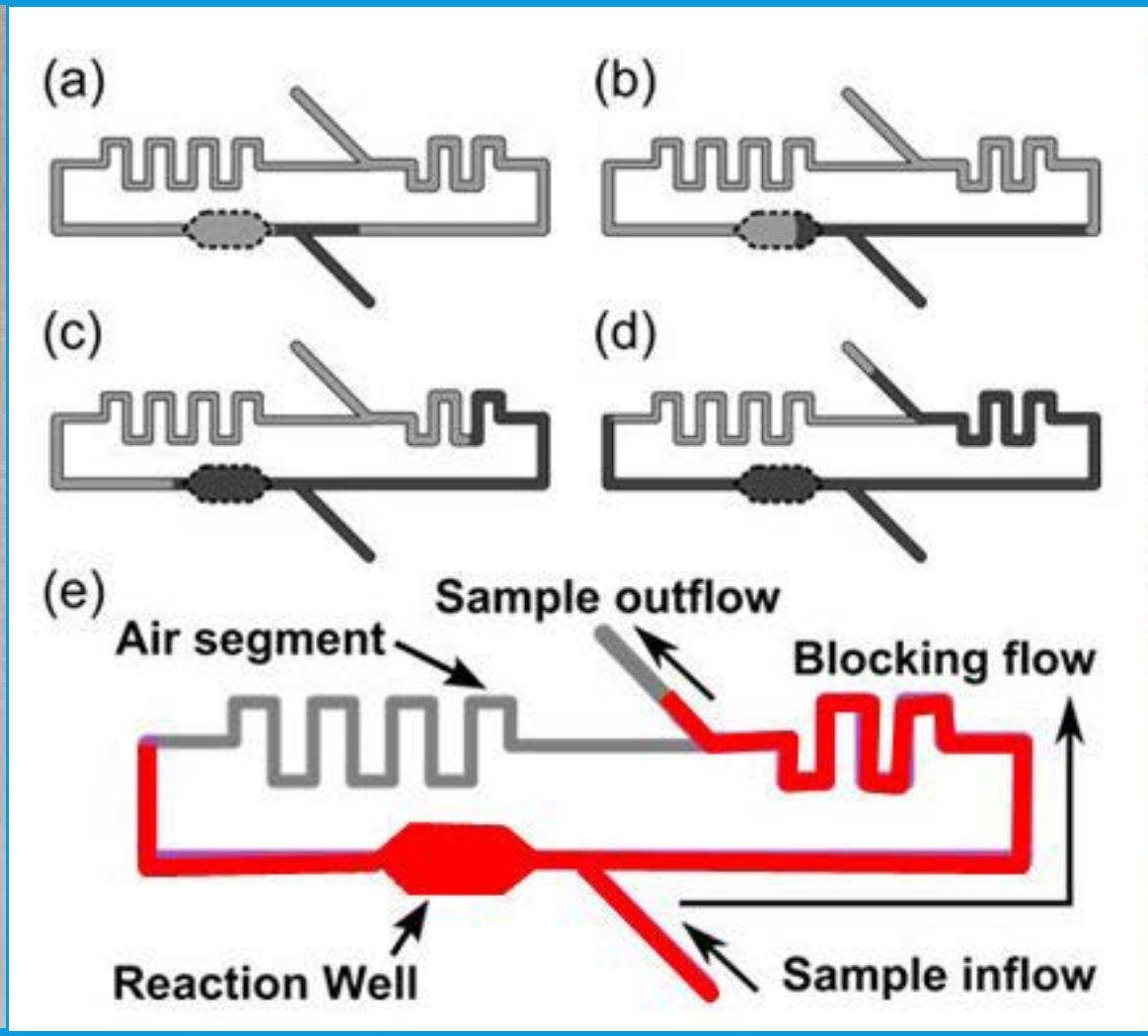
3. Press the Conduct Test button

C. OS/Database

Stedtfeld et al., Lab Chip, 2012

Field-deployable Multiplexed Microfluidic Chips





ARG 2.0 Panel

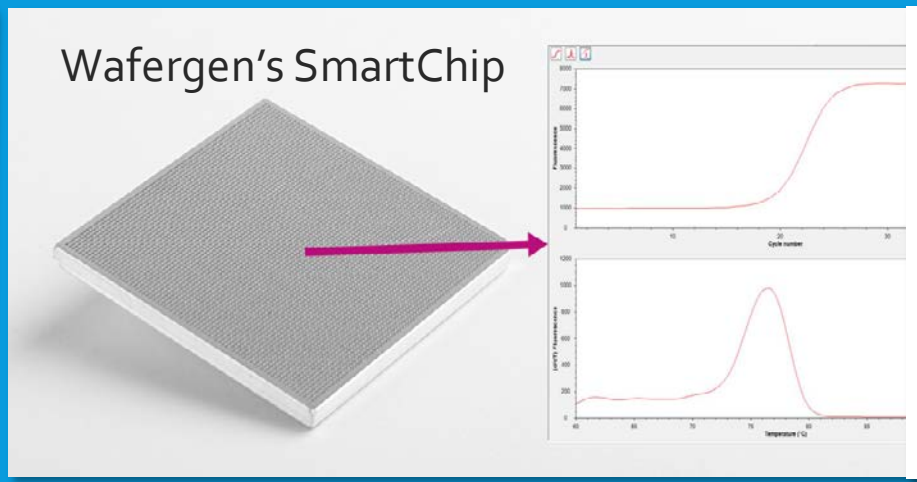
RESEARCH ARTICLE

Primer set 2.0 for highly parallel qPCR array targeting antibiotic resistance genes and mobile genetic elements

FEMS Microbiology Ecology, 94, 2018, fiy130

Robert D. Stedtfeld[1,†], Xueping Guo[2,3,4,†], Tiffany M. Stedtfeld[1], Hongjie Sheng[3,4,6], Maggie R. Williams[1], Kristin Hauschild[4], Santosh Gunturu[4], Leo Tift[4], Fang Wang[3,4,6], Adina Howe[5], Benli Chai[4], Daqiang Yin[2], James R. Cole[3,4], James M. Tiedje[3,4] and Syed A. Hashsham[1,3,4,*]

Wafergen's SmartChip



Assays	Samples	Assays	Samples
12	384	96	54
24	216	120	42
(Assay x Sample ≅ 5,184)			
54	96	248	20
72	72	296	16
80	64	384	12

ARGs in Michigan's Lakes

20+ Studies using this or an earlier version of the ARG chip

~300 Lakes Sampled
~30 Shown here!

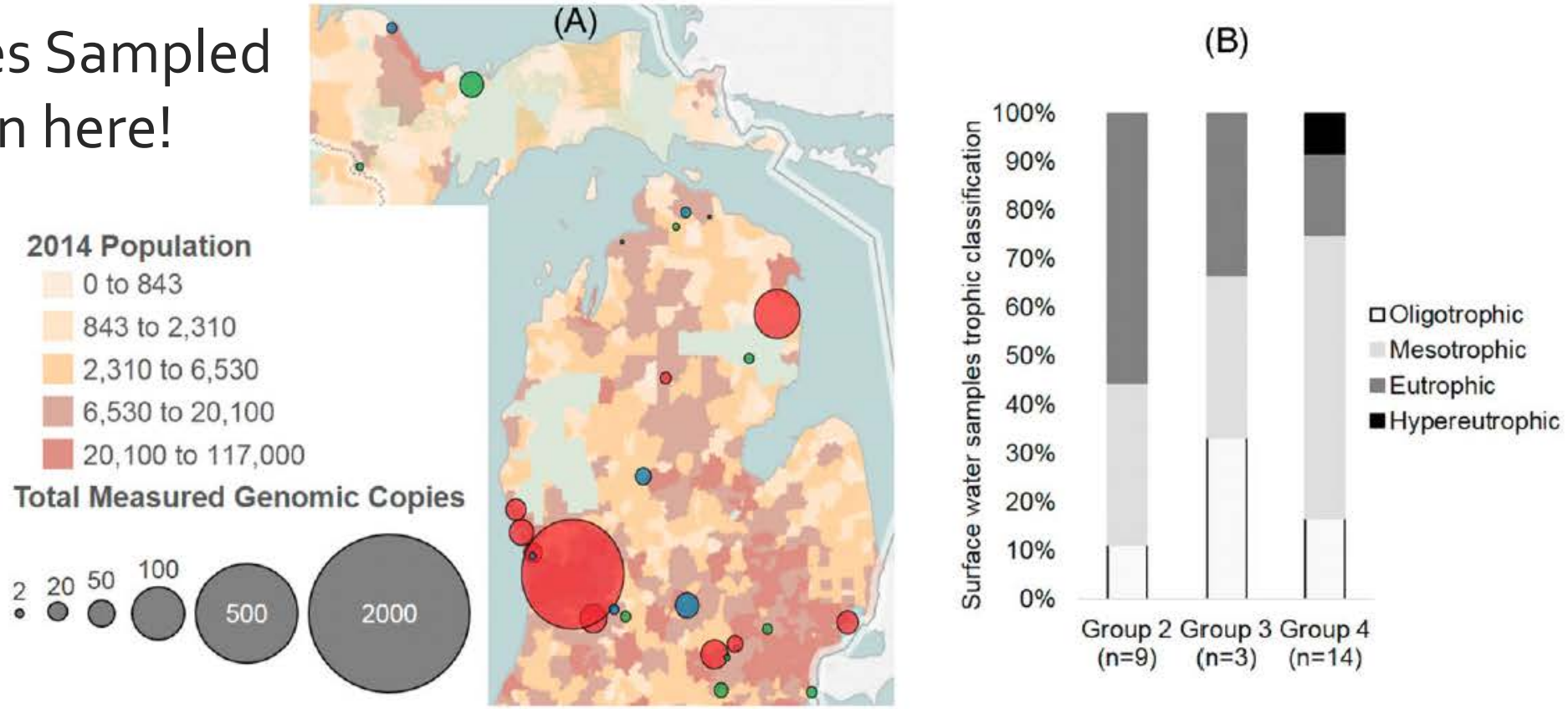
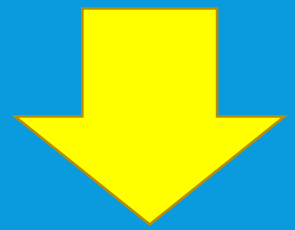


Figure 2. Mapping ARG database to identify hotspots and examine occurrence, co-occurrence and persistence of ARGs. (A) Total ARG copies versus population density for all 30 surface water samples, size of dot relates to total number of detected ARG copies, red, blue and green dots indicate samples from group 2, 3 and 4, respectively. Green portions of the map indicate no population information was available. (B) Trophic state of environmental surface waters, based on sample grouping via RDA (Fig. S2, Supporting Information).

FEMS Microbiology Ecology: 2016, Vol. 92, No. 3. doi: 10.1093/femsec/fiw020

Amplicon Recovery, Reusability

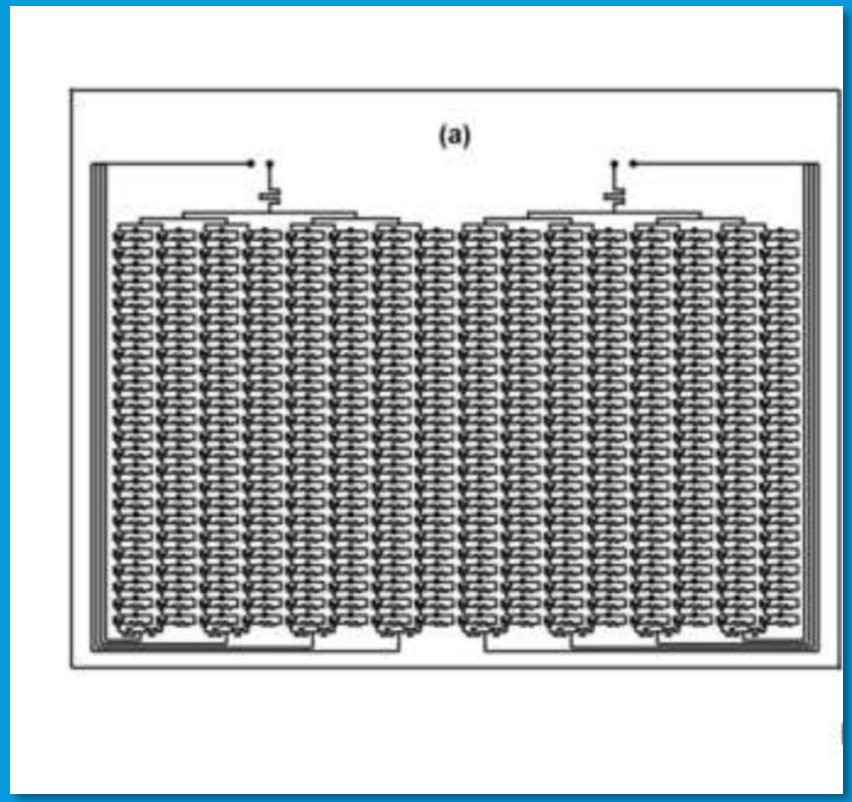
Bait-based Target Enrichment
Amplification-based Enrichment



Allelic Diversity, Expression

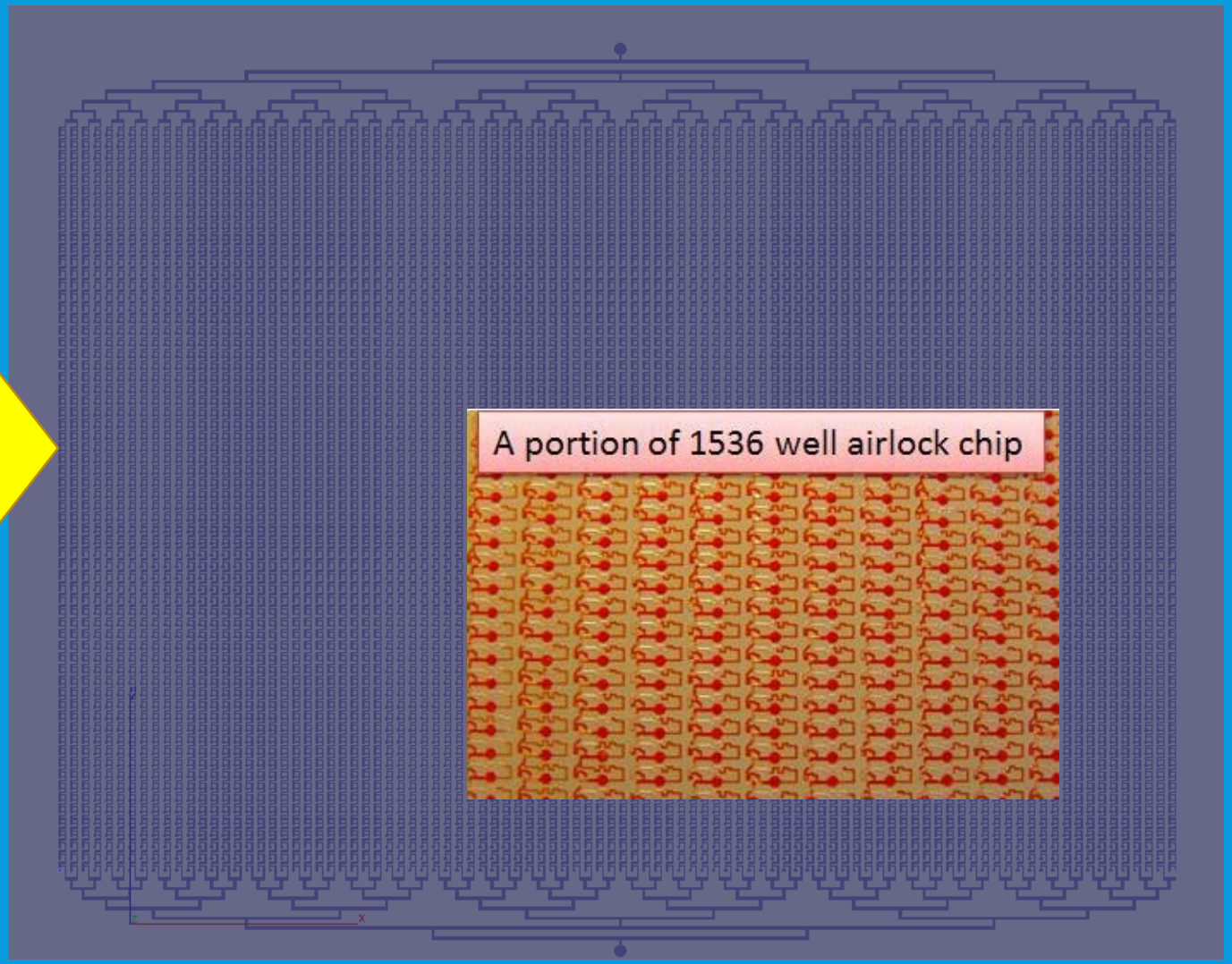
1. Moderate multiplexing (~200 primers)
2. Larger volumes (1-10 µl) to allow reasonable amount of amplicons
3. Ability to recover the amplicons after amplification
4. Reusability

Multiplexing: 64 wells → 1536 wells



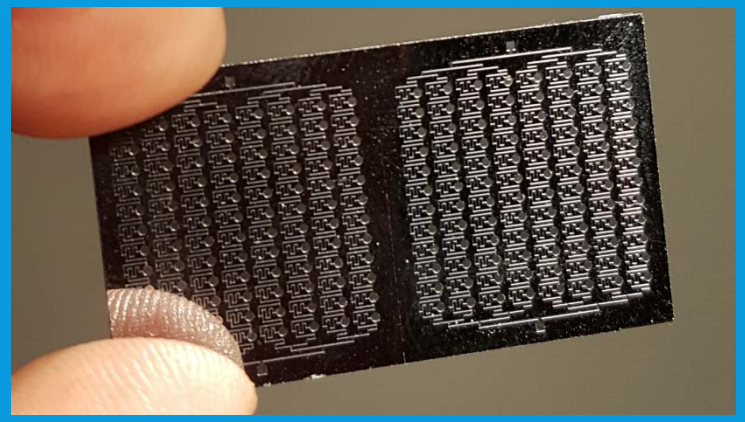
384 well chip Kostic et al., Appl Microbiol Biotechnol (2015) 99:7711–7722

1536 well chip

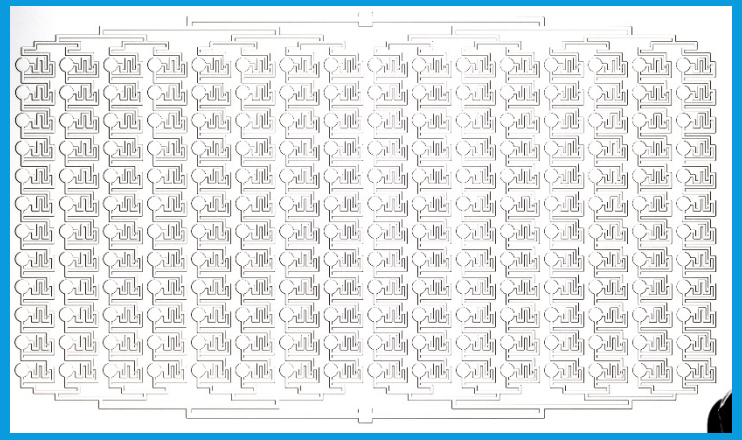


1536-wells: One entry-exit means one sample

Reusable after Amplicon Recovery?



96 well on silicon wafer



384 well on glass

Summary

1. Direct Amplification
2. Simple Microfluidic Chips and Low Cost POC Platforms
3. Application-specific needs and development

ACKNOWLEDGMENTS

Team and Collaborators

Maggie Williams
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