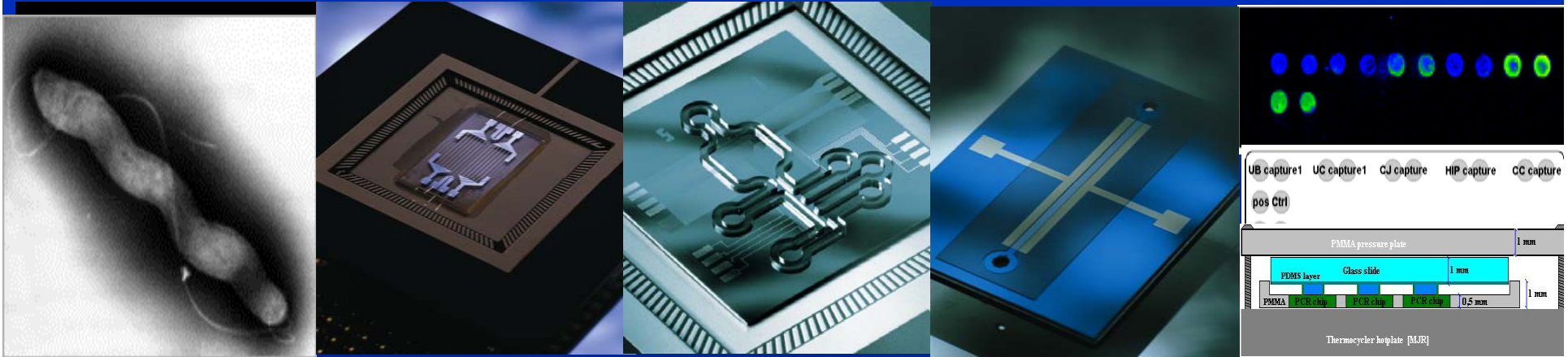


Campylobacter detection

Lab on a chip in Veterinary Research



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Outline

Introduction

- *Campylobacter*
- Campylobacteriosis

Campylobacteriosis - a global problem

A trip from a tube to a microchip

- Development of a multiplex PCR
- Development of DNA microarray
- Development of PCR chip
- Optolabcard

Future outlook

Acknowledgements



Introduction

1.1. *Campylobacter*

- * Gram negative, curved, flagellated bacteria with typical twisting motility
- * Size 0.7 μ m length 0.3 μ m width
- * Slow growth
- * Require specific atmosphere and nutrients
- * Limited biochemical activity
 - *Difficult to isolate and to identify*
 - *Many animals are symptomless carriers*
- * Among 15 known *Campylobacter* species

C. jejuni is the cause of 98% of human *Campylobacteriosis* cases



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1.2. Campylobacteriosis

Causes

Handling or consumption of Camp. contaminated food
Poultry is an important source of infection

Symptoms

Headache, fever, vomiting, abdominal pain,
Diarrhoea, faecal blood and leukocytes
Self limiting after 7-10 days

Complications

Urinal tract infection
Meningitis, arthritis
Guilain-Barre Syndrome (GBS)
* Acute, paralysis
* 1/1,000 cases



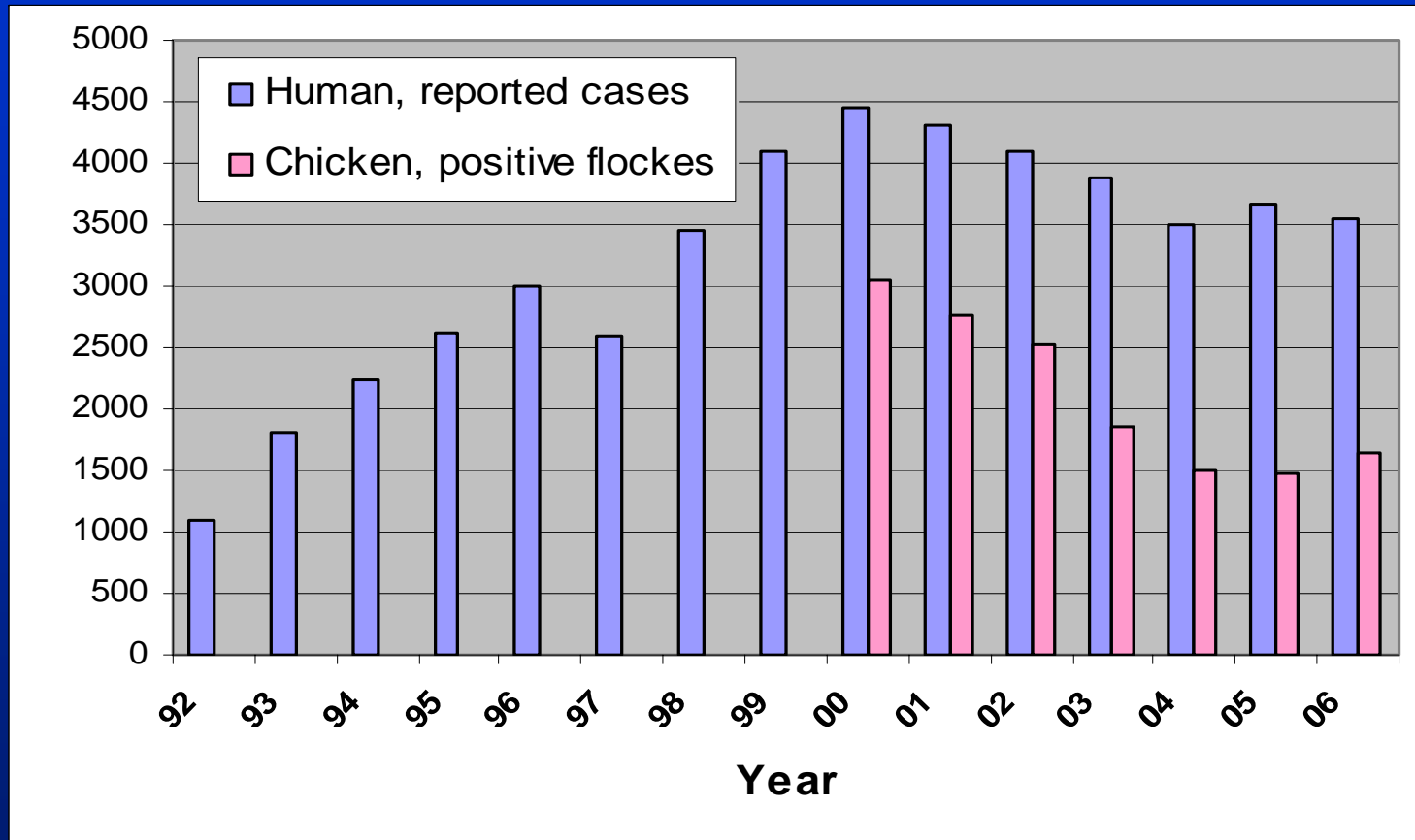
Campylobacteriosis a global problem



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Human Campylobacteriosis cases (1992 – 2006) and Campylobacter in Danish Broiler flocks



In 2005: 3672 reported cases (68 cases/100.000 inhabitants)



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Number of reported human cases of nine zoonotic agents in 14 EU member states and Norway in 2004

Pathogens & Diseases	No. of human reported cases in the EU in 2004
Campylobacteriosis	150.000
Salmonellosis	145.231
Yersiniosis	10.147
<i>E. coli</i> (VTEC)	2.664
Brucellosis	2.386
Listeriosis	860
Echinococcosis (<i>Echinococcus granulosus</i>)	266
Tuberculosis (caused by <i>M. bovis</i>)	49
Trichinellosis	48

* Source: *Trends and sources of zoonotic agents in animals, feedingstuffs, foods and man in the European Union and Norway in 2004.*

Food safety: production and distribution chain D2- Biological risks. SANCO729/2004.
www.dfvf.dk



Campylobacteriosis cases in USA

- In the United States 2 - 4 mil cases / year
- In 2006
 - 150,000 clinical visits
 - 13,174 hospitalized
 - 124 deaths

(<http://www.cfsan.fda.gov> may 2007)



Medial costs associated with Campylobacteriosis

- In the US
 - Medical cost associated with GBS
\$ 257- 425 millions / year
 - Total cost including day lost productivity
\$ 1,8 billions / year
- . In the UK (1999)
£ 150 millions.



Campylobacter infection is a global problem:

Why ?



Causes of the increase in food poisonings

- . Animal mass production
- . Globalisation of food supply
- . Increase in travelling and immigration
- . Change in human lifestyle



Total number of animals slaughtered in Denmark in 2004

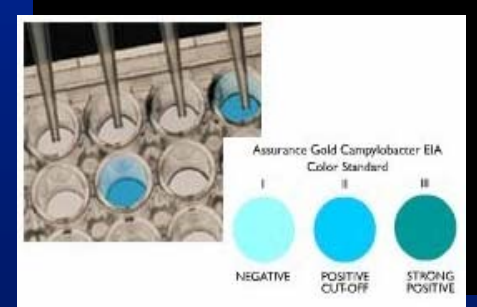
Animals	Number of Animal Slaughter
Broilers	130.4 millions
Pigs	22.5 millions
Turkeys	560,120
Cattle	574,426
Sheep, lambs and goats	75,870
Horses	2,675

Source: DFVA web.www.dfvf.dk



Conventional detection methods

- . **Bacterial culture**
(*slow growth*).
- . **Microscopy**
(*visual morphological analysis*).
- . **Biochemical testing**
(*verification of enzymatic activity*).
- . **Specific antibodies**
(*ELISA plate test or agglutination*)
 - * **Time consuming (3-5 days)**
 - **Laborious**
 - **Requires special equipments / trained staff**
 - * **Low sensitivity**



“In order to comply the demands of consumers for safe food, free of pathogens, there is an urgent need to develop a rapid method suitable for detection of *Campylobacter* spp...”



From a tube to a microchip

Development of a multiplex PCR

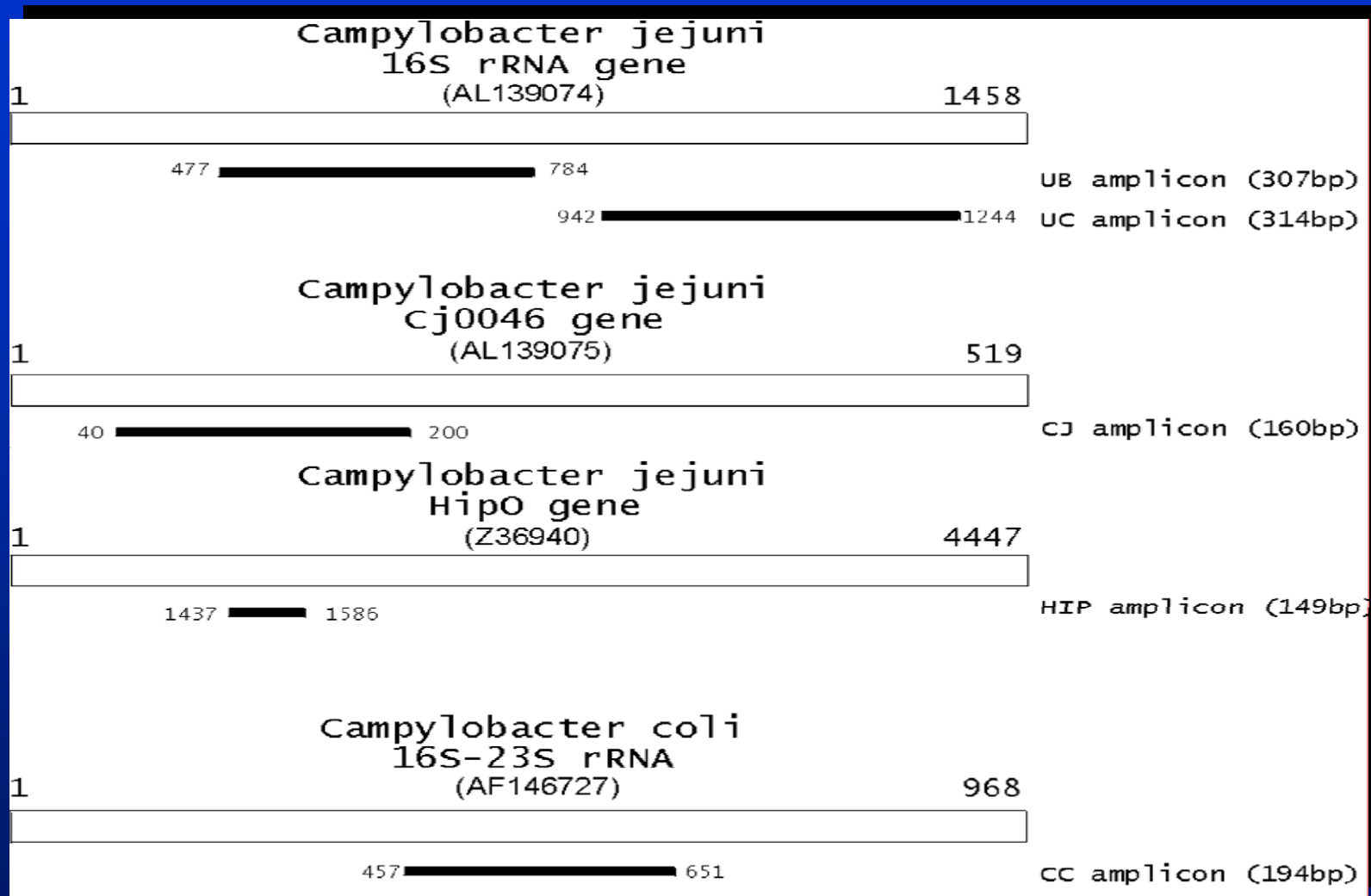
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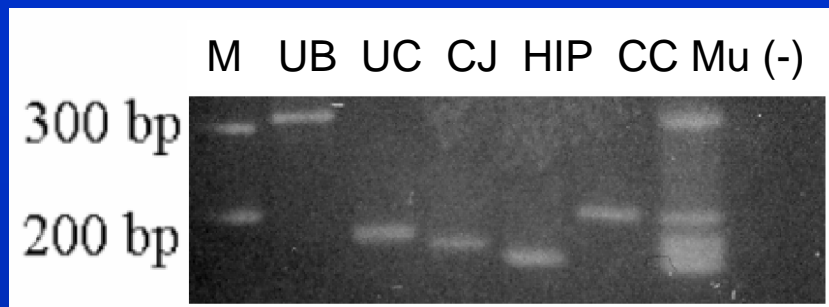
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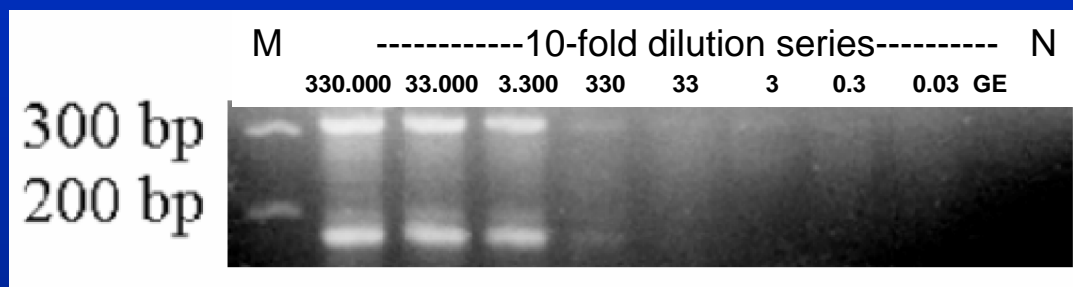
PCR primer designs



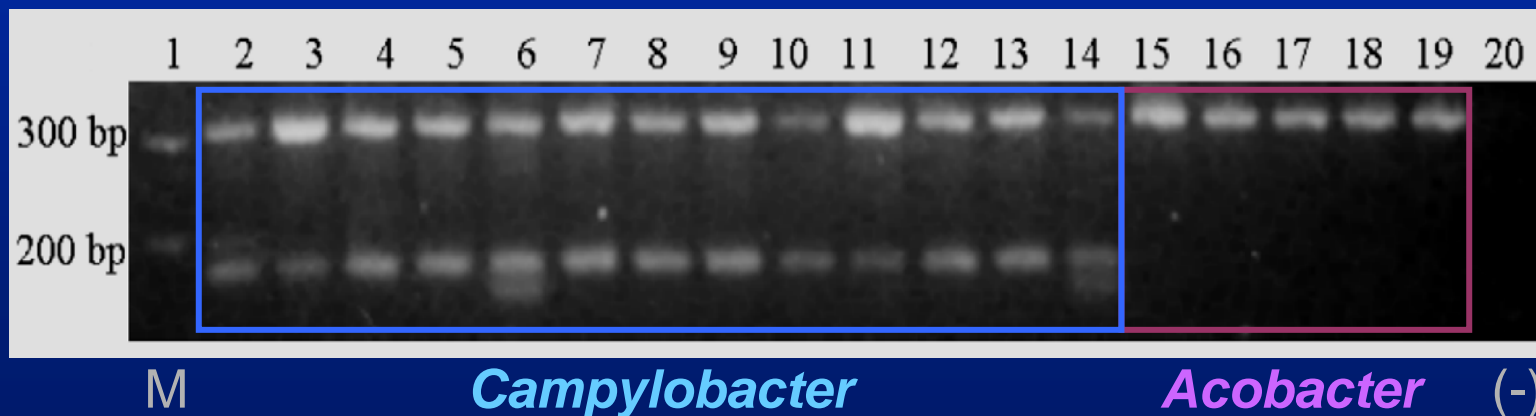
Specificity and sensitivity of the PCR



- 5 specific products:
- *Bacterial DNA*
 - *Campylobacter* spp.
 - *C. jejuni* genes (2x)
 - *C. coli* gene



Detection of 30-300
Campylobacter genome
equivalents (GE)
by electrophoresis.



Source; D. D. Bang, et al., *J. Rapid Methods Autom. Microbiol.*,9, 97-113 (2001)



Detection of *Campylobacter* directly from 65 chicken faecal pool samples by culture and multiplex PCR

Primers	No. of samples positive by	
	Culture	PCR
Universal Bacteria	NA*	65 (100%)
Universal <i>Campylobacter</i>	39 (60%)	62 (95%)
<i>Campylobacter jejuni</i>	35 (54%)	46 (71%)
<i>C. jejuni</i> (Hippuricase gene)		40 (62%)
<i>Campylobacter coli</i>	4 (6%)	6 (9%)
Negative	26 (40%)	3 (5%)
Undetermined <i>Camp.</i> spp	0	12 (19%)
Mix primers in Muktiplek PCR		62 (95%)

Source: Keramas et al. 2004 J.C.M.42: 3985-3991



Sample preparation: Isolate DNA using Kingfish system

- A 10 swabs
3ml of sterile water
Stand for 2 - 10 minutes bacterial release
- B 1ml bacterial/feces suspension
Centrifugation at 13K rpm / 1 min
Pellet 200 μ l lysis buffer
- C 100 μ l beads suspension to isolate DNA (90 mg wet weight beads)
- D Isolation DNA by kingfish system using magnetic beads
Elute DNA in 100 μ l sterile water
3 μ l/PCR

A

B

C

D



24 samples / 30 min



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Development of a DNA micro array for detection of campylobacter

Consist two steps:

- Multiplex PCR with Cy5 labeled primers
- Hybridization of Cy5 labeled PCR amplicons to capture probes

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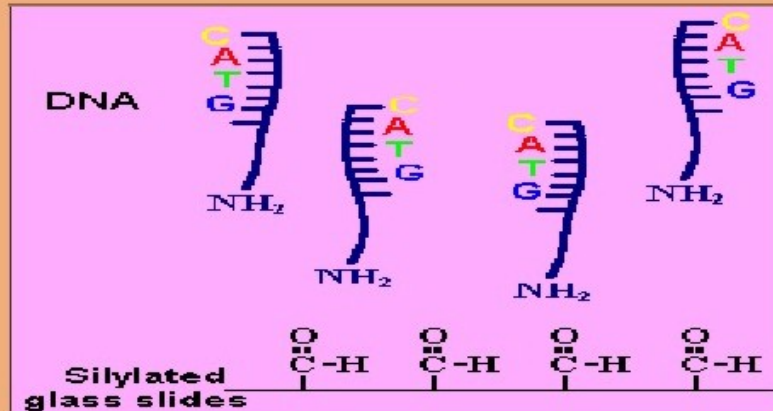
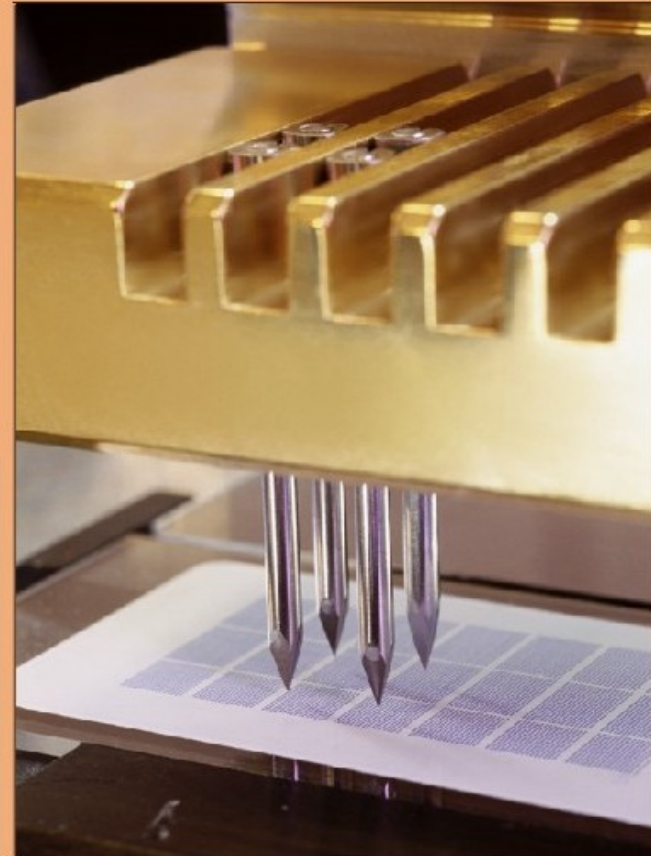
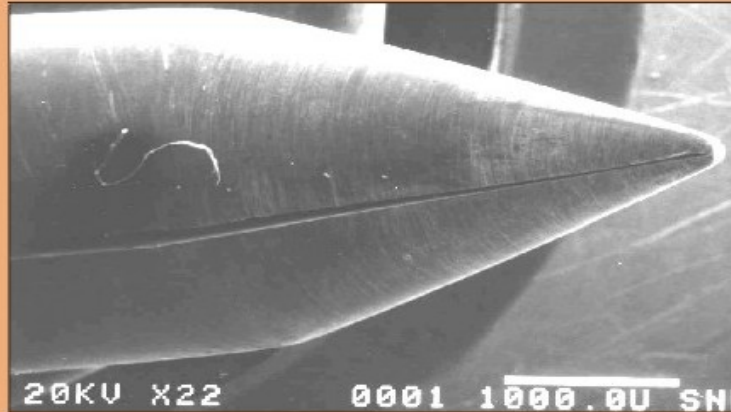


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Micro-spotting

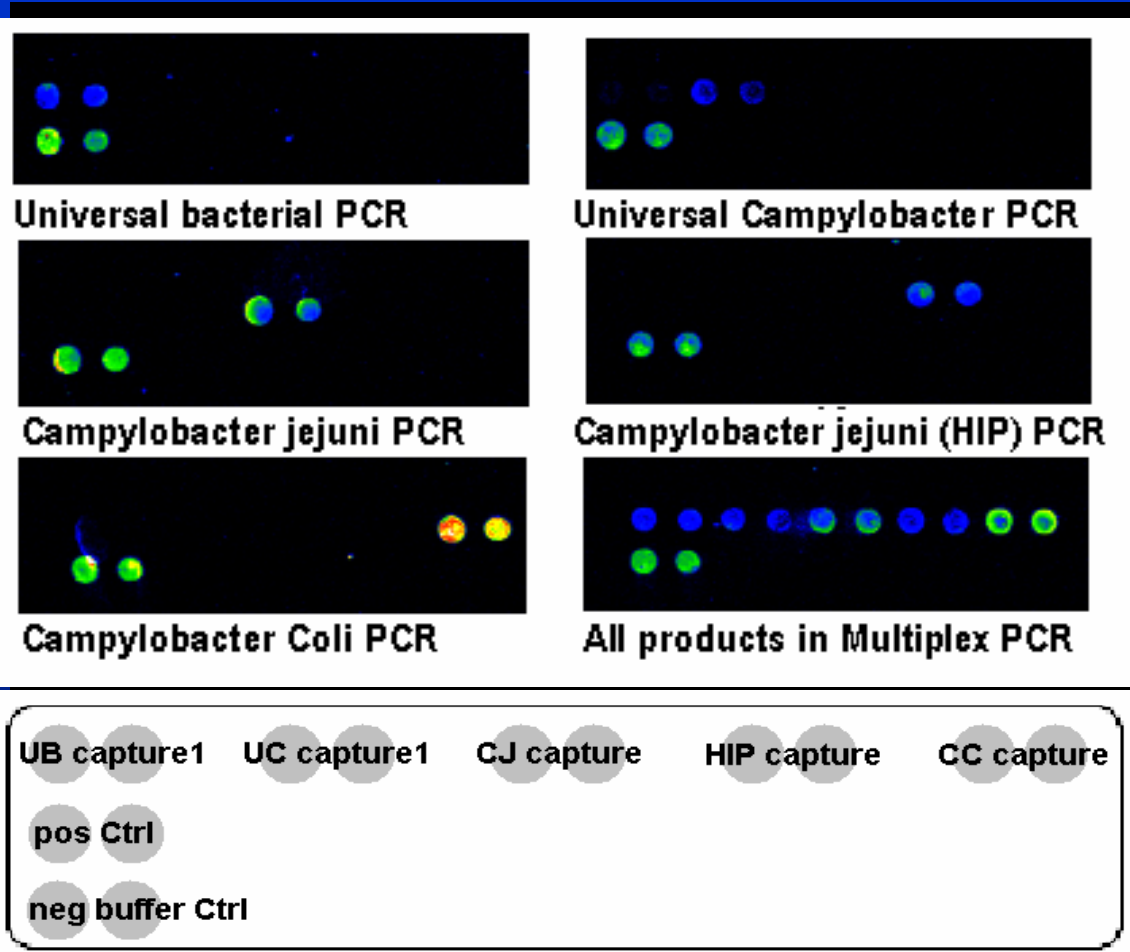
Fabrication of custom-made DNA-chips



Specificity of hybridisation to microarrays

Equal amounts of
singleplex PCR
were hybridised to
microarrays.

The individual
products hybridise
only to their
corresponding
capture probes.

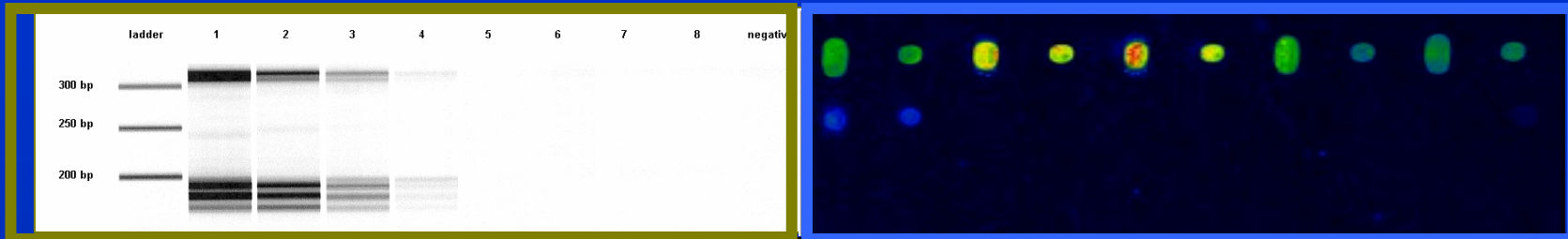


G. Keramas et al., Molecular and Cellular Probes 17: 187-196 (2003).

G. Keramas et al., J. Clin. Microbiol. 42: 3985-3991 (2004)



Sensitivity of hybridisation to microarrays



Capture probe	sample number	1	2	3	4	5	6	7	8	negative Ctrl
		6×10^{-1} ng/ μ l 330.000 GE	6×10^{-2} ng/ μ l 33.000 GE	6×10^{-3} ng/ μ l 3.300 GE	6×10^{-4} ng/ μ l 330 GE	6×10^{-5} ng/ μ l 33 GE	6×10^{-6} ng/ μ l 3 GE	6×10^{-7} ng/ μ l 0.3 GE	6×10^{-8} ng/ μ l 0.03 GE	
<i>Universal Bacterial</i>	on gel	+	+	+	+	-	-	-	-	-
	on array	+	+	+	+	+	+	-	-	-
<i>Universal Campylobacter</i>	on gel	+	+	+	+	-	-	-	-	-
	on array	+	+	+	+	+	+	-	-	-
<i>Campylobacter jejuni</i>	on gel	+	+	+	+	-	-	-	-	-
	on array	+	+	+	+	+	+	-	-	-
<i>Campylobacter Jejuni-Hippuricase</i>	on gel	+	+	+	+	-	-	-	-	-
	on array	+	+	+	+	+	+	-	-	-
<i>Campylobacter coli</i>	on gel	+	+	+	+	-	-	-	-	-
	on array	+	+	+	+	+	+	-	-	-

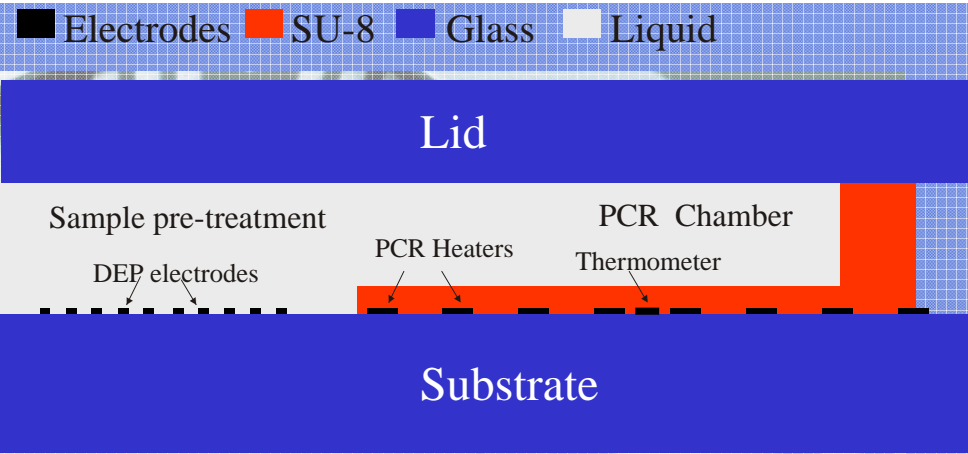
Using microarray detection a signal could be recorded even below the detection limit of DNA electrophoresis (10^{-6} ng/ μ L ~ 3 GE).

G. Keramas et al., *Molecular and Cellular Probes* 17, 187-196 (2003).

G. Kerama et al., *J. Clin. Microbiol.* 42: 3985-3991 (2004)



PCR chip



PCR chamber

Fluidic system

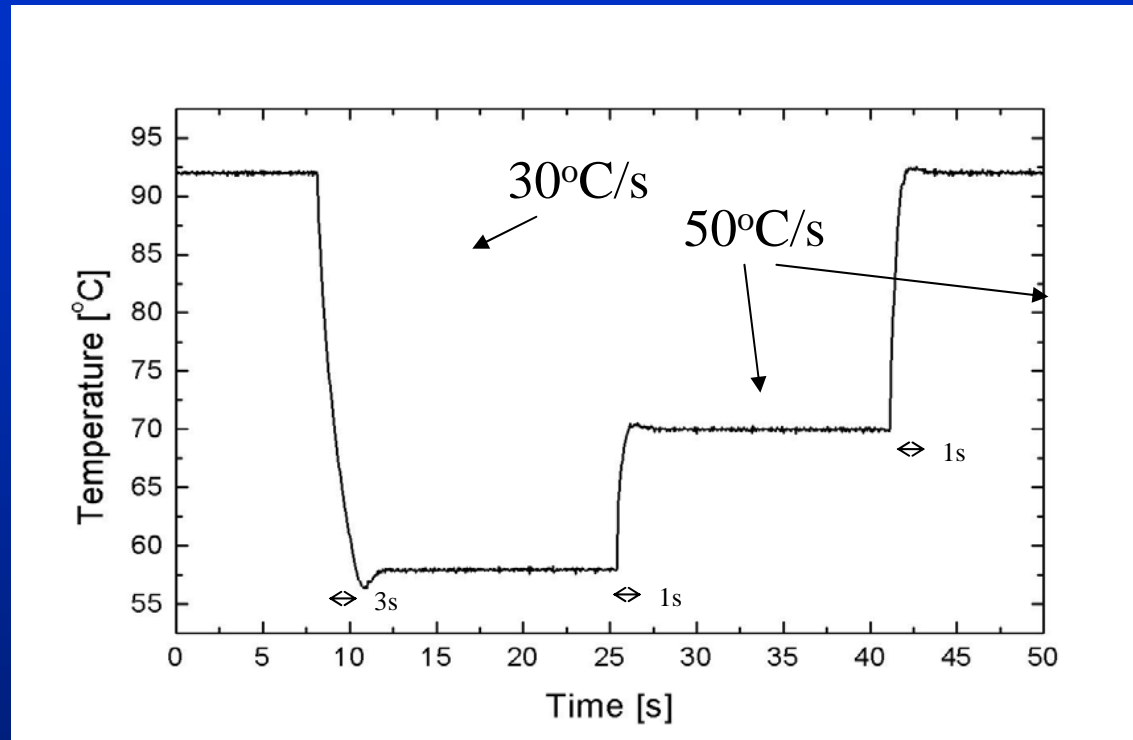
DEP electrodes

5 mm



PCR thermocycling on chip

Three temperatures: Denaturation (94°C), annealing (55°C), elongation (72°C)



Total thermal transition times during thermocycling is app. 5s

Conventional PCR thermocyclers:

Heating and cooling rate: $\sim 2^\circ\text{C/s}$

Transition time: up to 1 min.

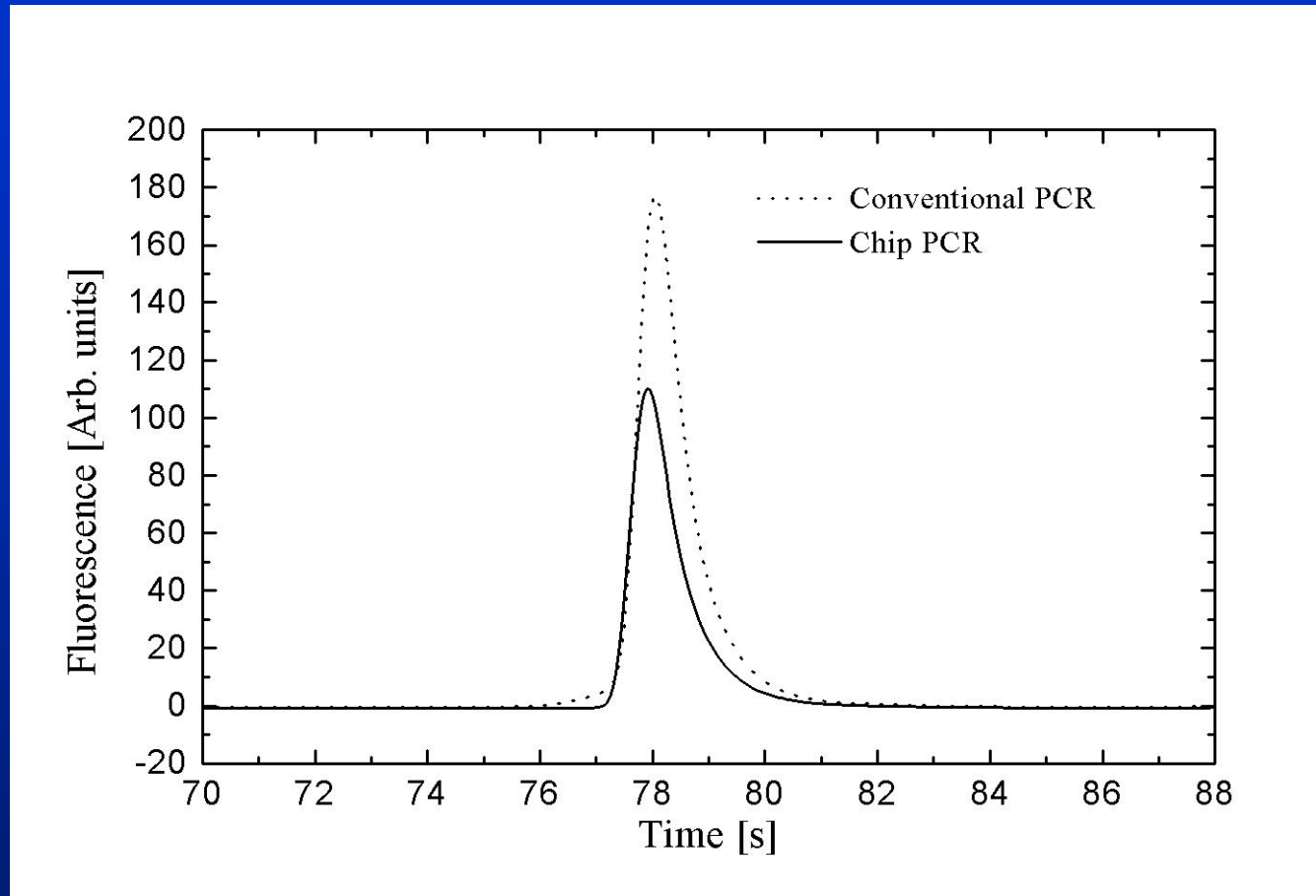
J. El-Ali et al., Sensors and Actuators A 110, 3–10 (2004).



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On chip PCR detection of *Camp. jejuni cadF* gene



Yield of PCR chip is ~2/3 of yield in conventional PCR tubes



J. El-Ali et al., Sensors and Actuators A 110, 3–10 (2004).

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PCR amplification of *C. jejuni* DNA isolated from different sources.

Samples	Chip nM/l	Tube nM/l	Ratio Chip/tube
CCUG-11284	41.5	72.2	57%
DVI-HM 679	33.5	80.4	42%
DVI-HM 682	62.0	90.4	69%
DVI-SC 455	21.8	22.2	99%

Samples

- Reference strain (CCUG 11284),
- Two human samples (DVI-HM 679 & DVI-HM 682)
- A chicken samples (DVI-SC 445)

(Claus R.P et al *J. Rapid Methods Autom. Microbiol.*,13, 111-126 (2005).



On chip PCR using *Camp. jejuni* whole cells as template

Experiment no.	Chip nM/l	Tube nM/l	Ratio Chip/tube
1	20.1	30.4	66%
2	18.0	30.4	59%
3	14.9	27.5	54%

- No DNA isolation
- Directly from whole cells – thermolysis
- Integrated sample pre-treatment



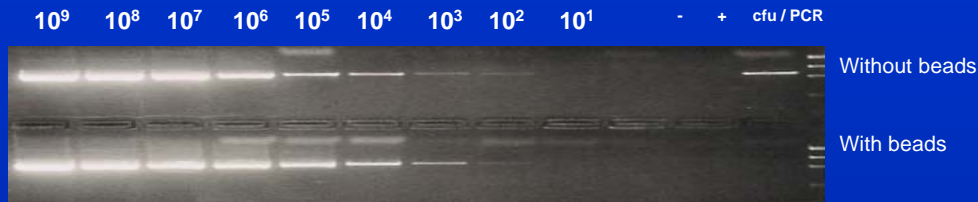
(Claus R.P et al *J. Rapid Methods Autom. Microbiol.*,13, 111-126 (2005).

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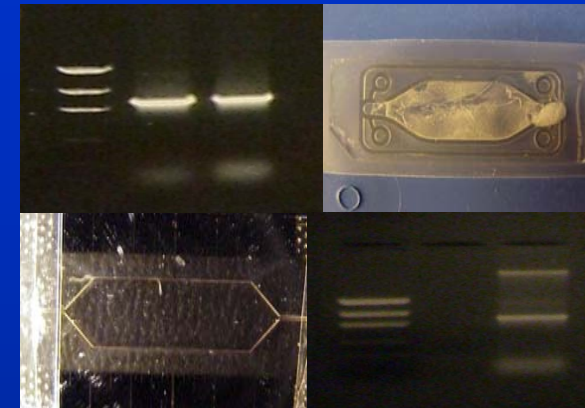


From a tube to a chip process development of a lab-card

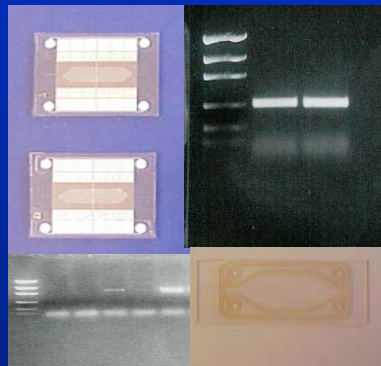
Sample preparation using magnetic beads



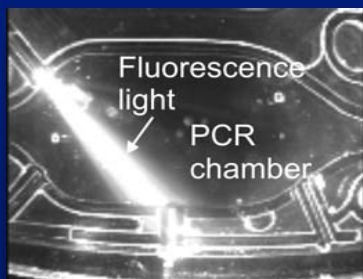
Freeze-dried PCR mixture on chip



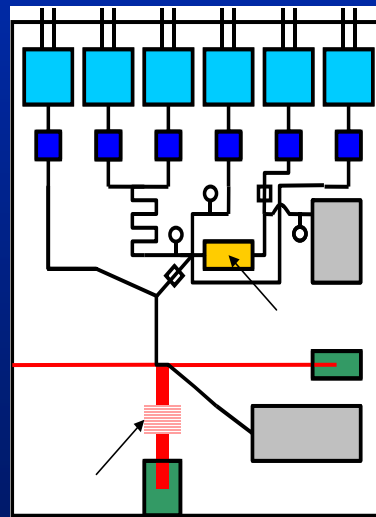
PCR on chip



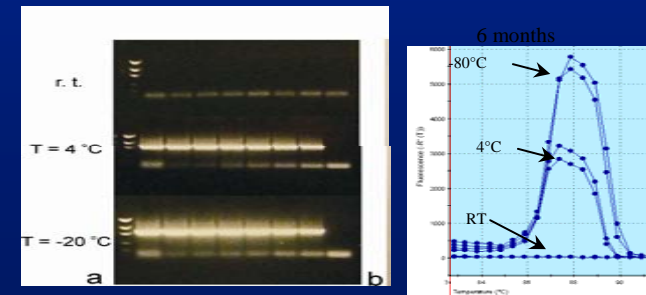
On chip optical detection



OPTOLABCARD

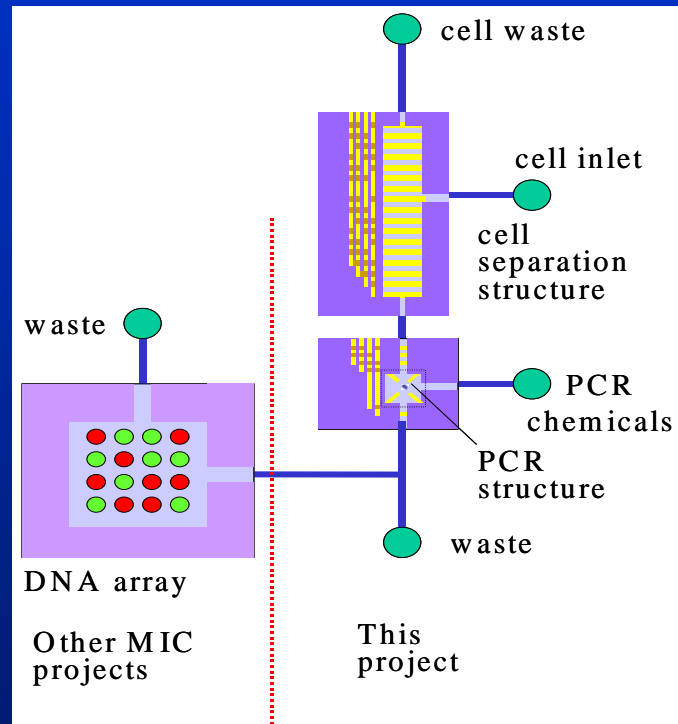


Storage the freeze-dried PCR mixture

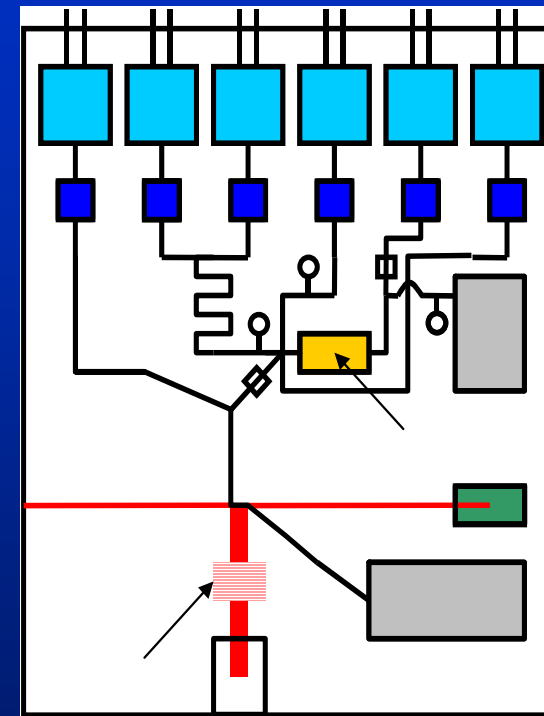


Future outlook

Total integrated Lab on a chip system
(sample preparation, PCR and DNA analysis)



Lab on a chip



Integrated Lab-card



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Thank you for your attention



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