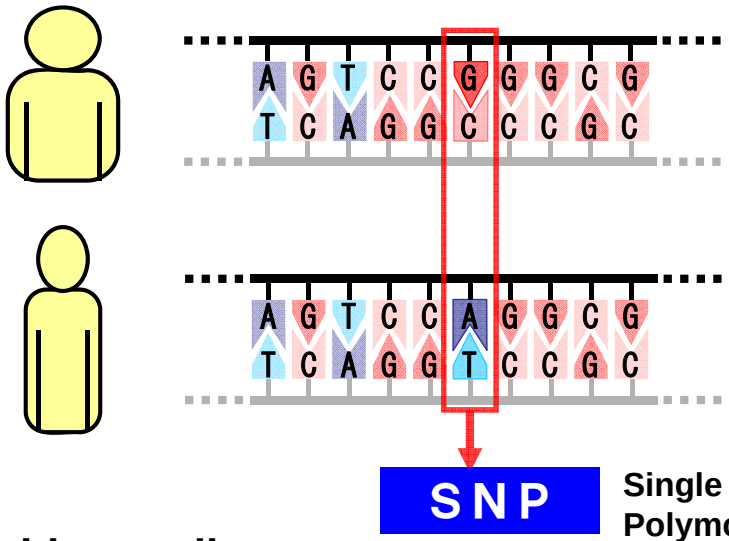
A Lab-on-Chip System for direct SNP sensing from human blood

Ichiro Yamashita[1,3], Paolo Fiorini[2]

[1]Advanced Technology Research Laboratory, Panasonic corp.
3-4 Hikaridai, Seika-cho, Soraku-gun, Kyoto 619-0237, Japan
[2]Imec, Kapeldleef 75, B-3001, Leuven, Belgium
[3]Nara Institute of Science and Technology, 8916-5 Takayama-cho Ikoma

What is SNP

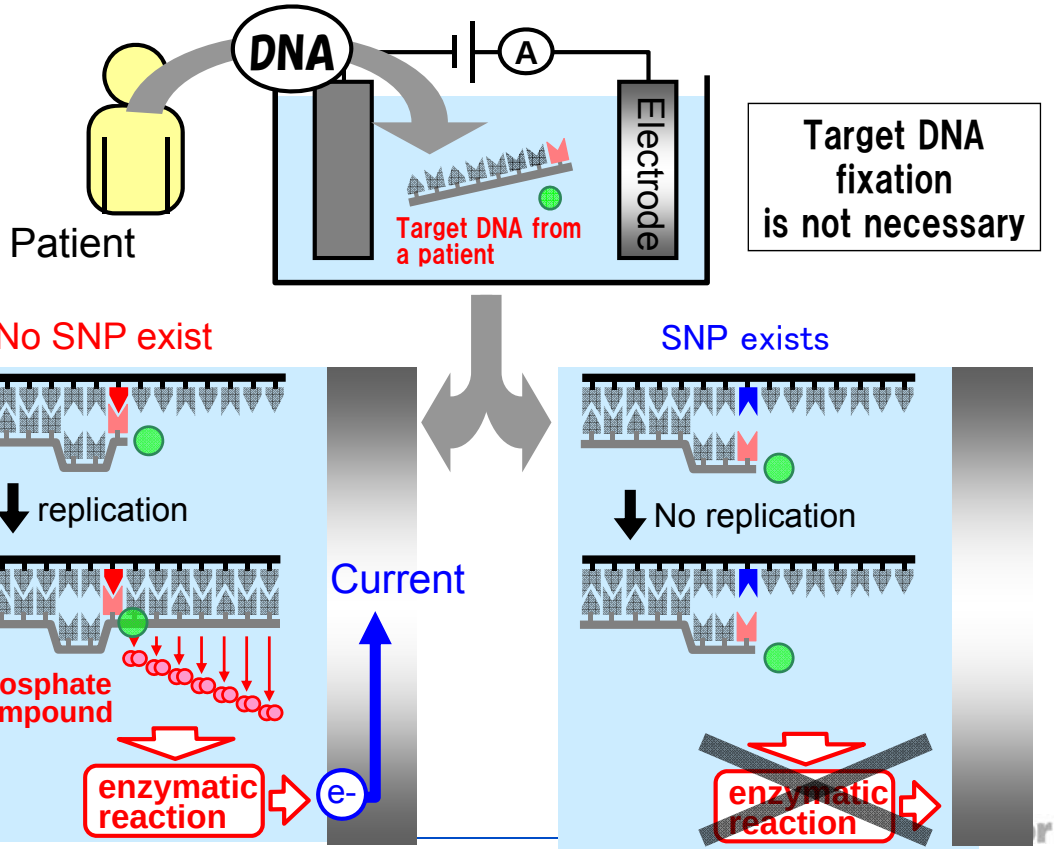
One DNA alternation can affect people's sensibility against drugs



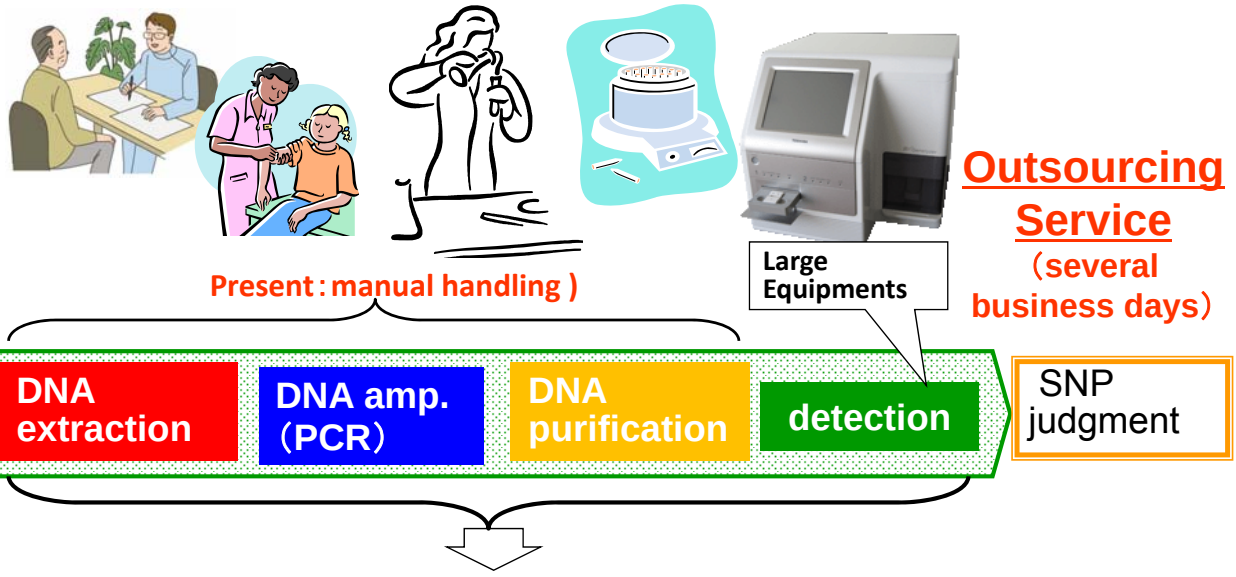
Single Nucleotide Polymorphism

predisposition to diseases, difference in reaction on drugs (ie. adverse side effects), etc

SNP sensor by Panasonic

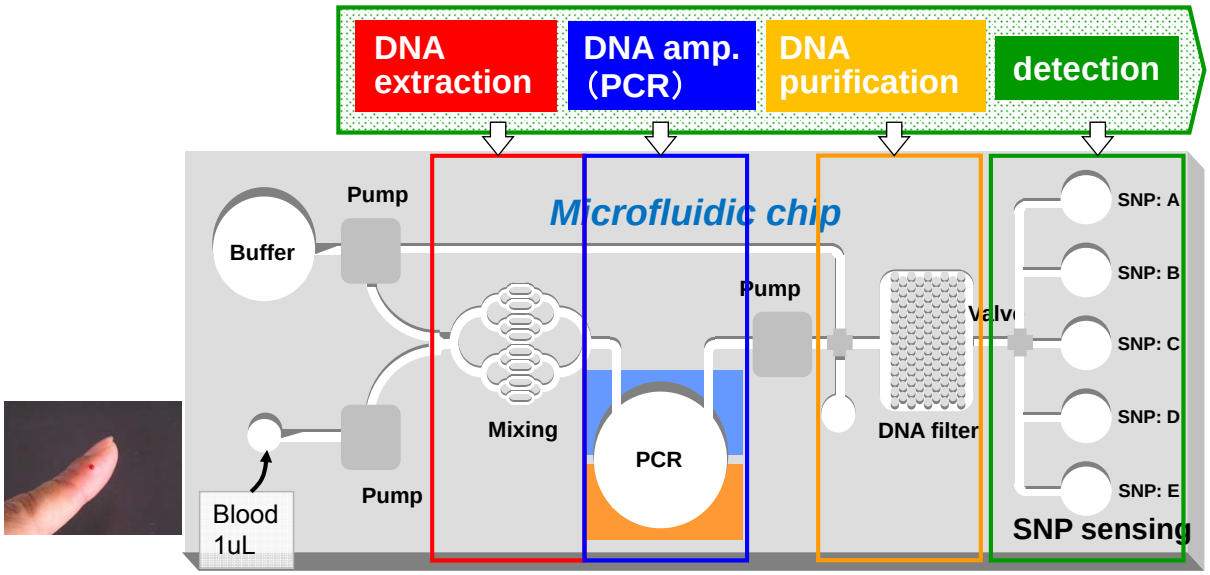


Easy and fast detection of SNP



Our target is SNP-detection from blood less than 1 hour with small one-chip.

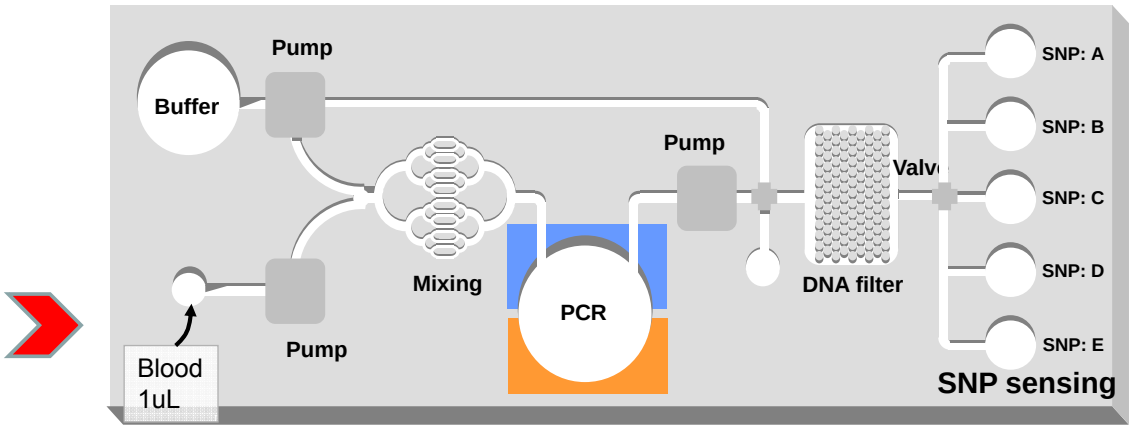
SNP Detection chip

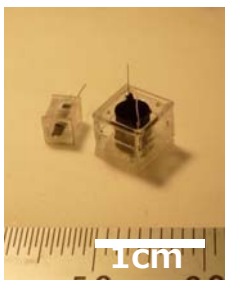


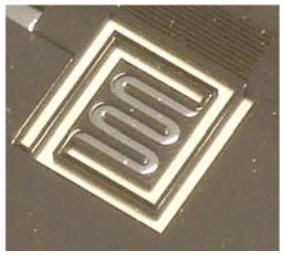
Rapid genotyping diagnostic on chip system

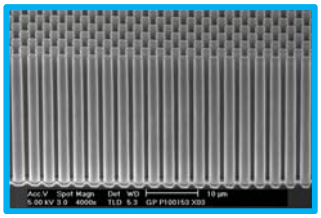
- Automated DNA extraction from blood
- Output diagnostic results within 1 hour

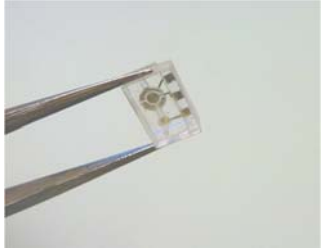
SNP Detection chip



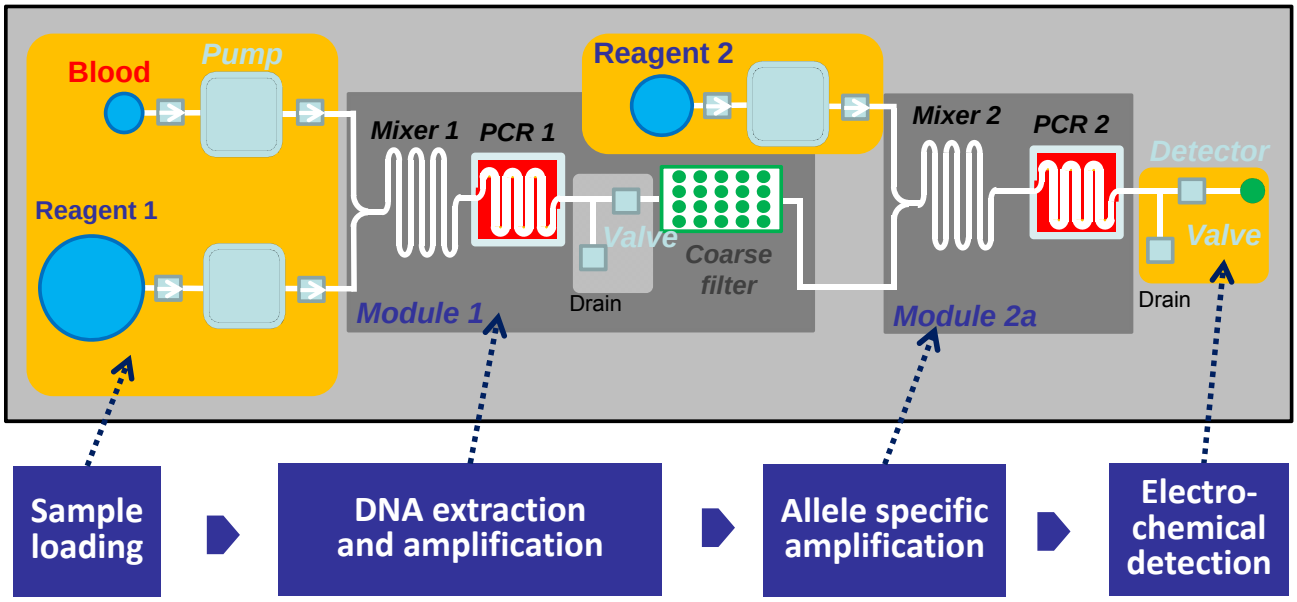




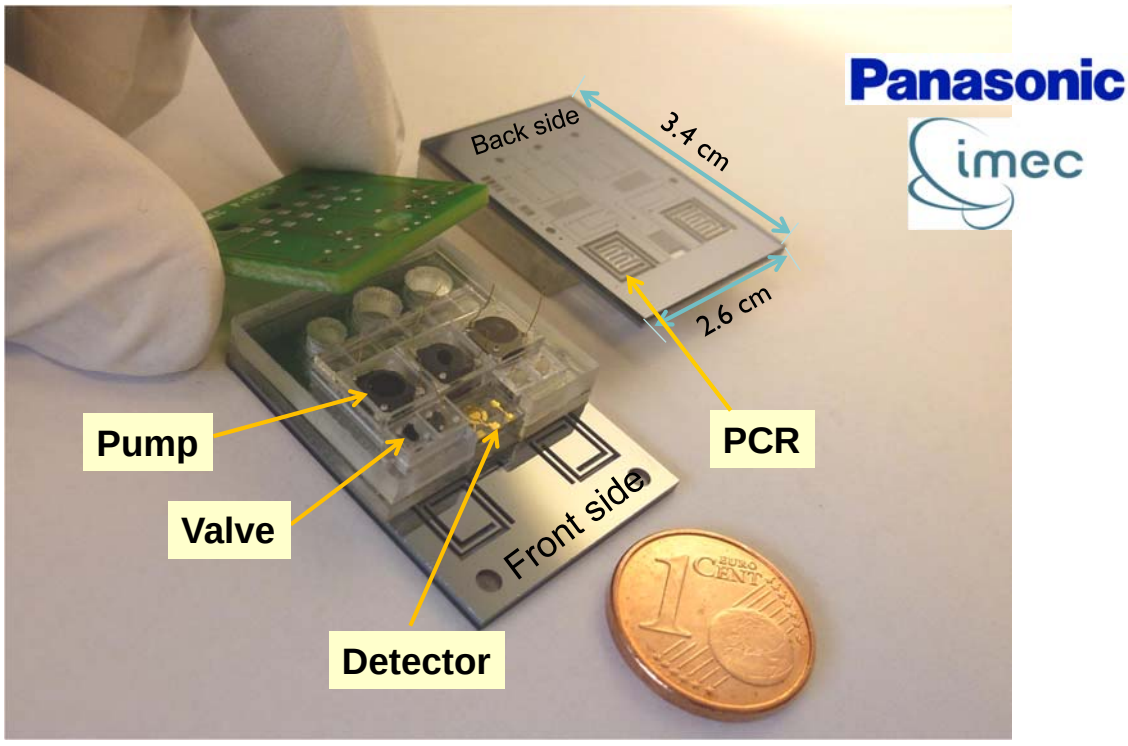




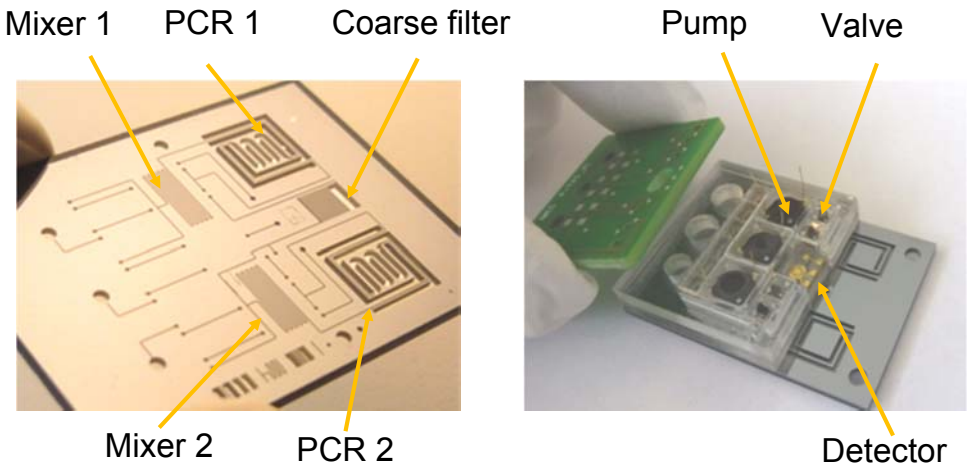
Single SNP Detection chip details

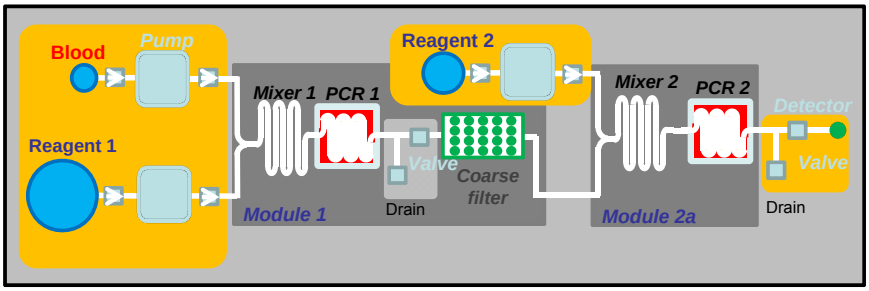


Single SNP Detection Chip

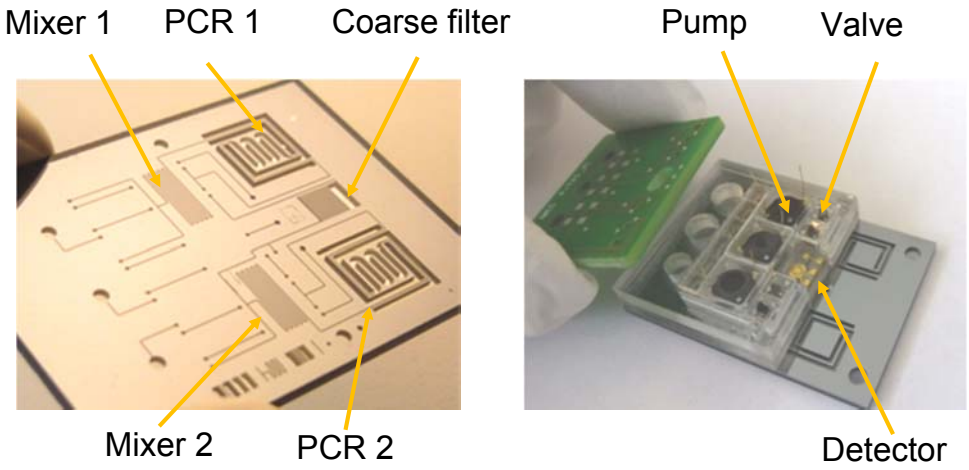


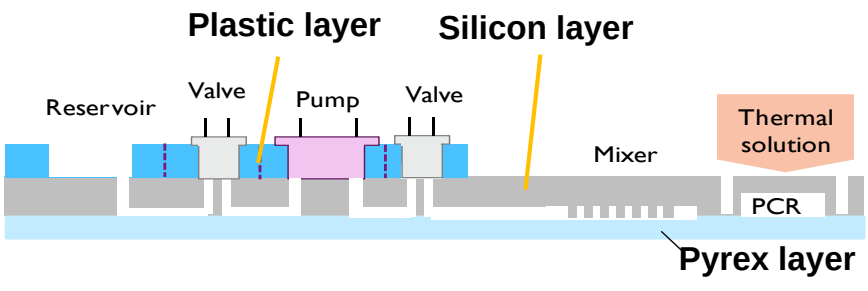
Single SNP Detection device



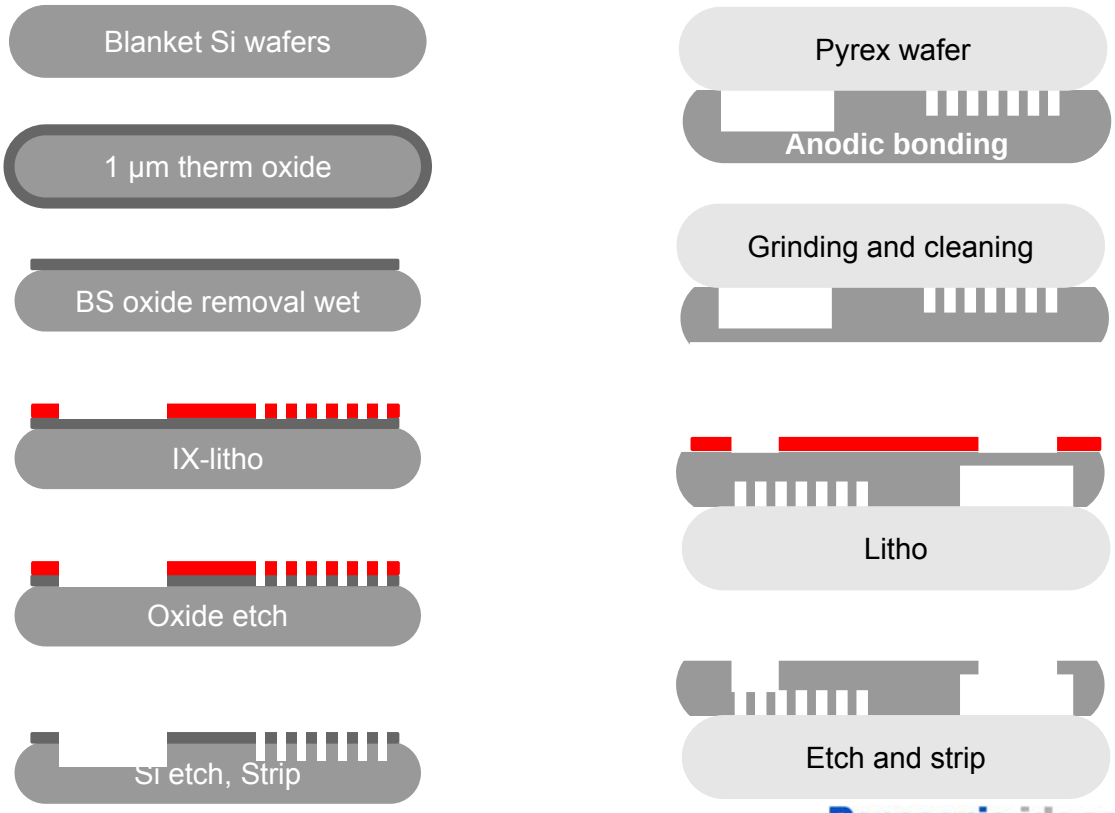


Single SNP Detection device

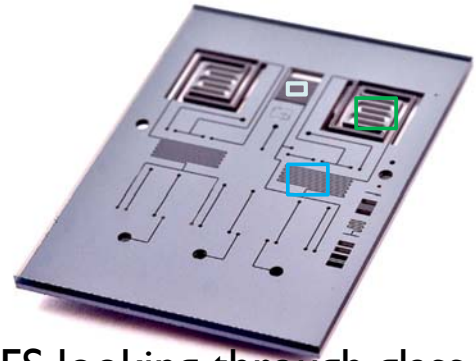




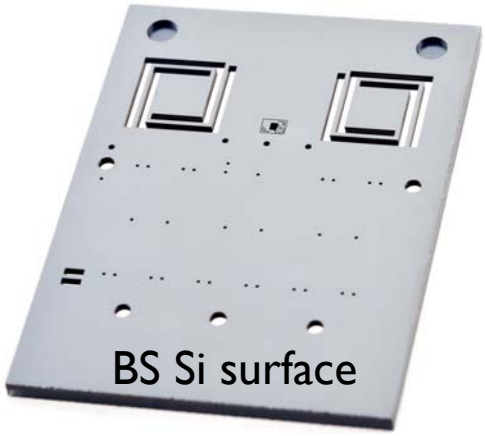
Fabrication SSD (Process flow)

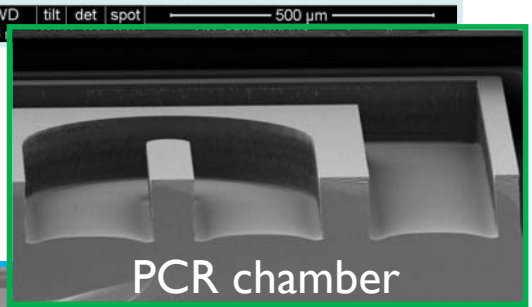


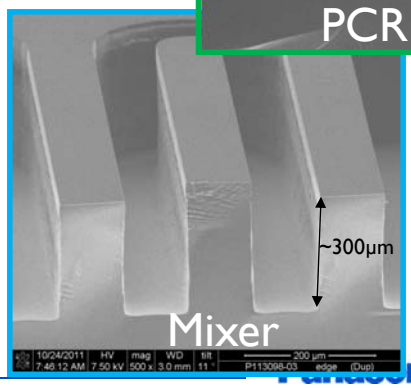
Fabrication SSD (Results)



FS looking through glass



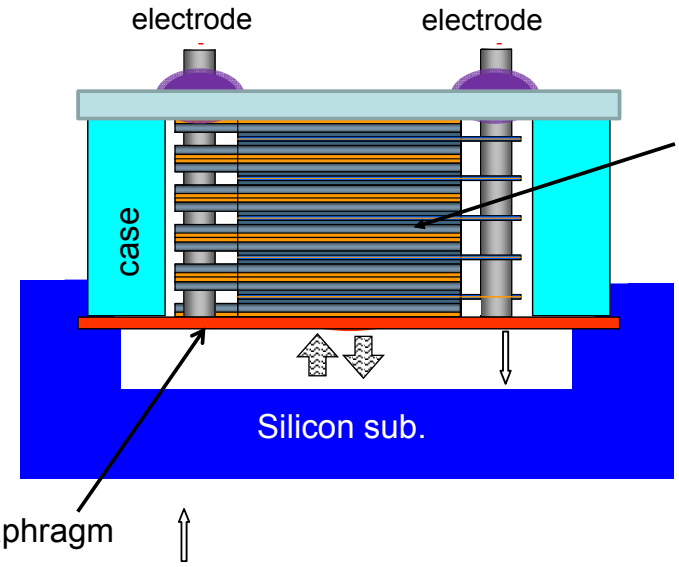


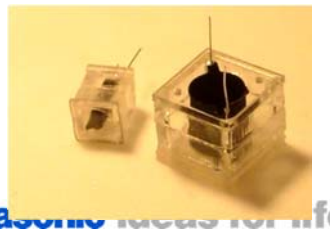


The newly developed pump unit

- Operation pressure **3MPa**
- **1.5V** battery drive
- Maximum generation pressure **30MPa**

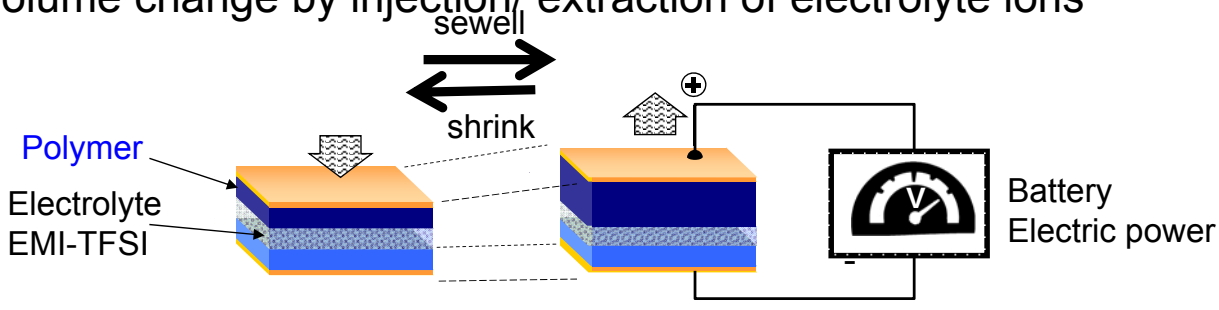
Pumping action

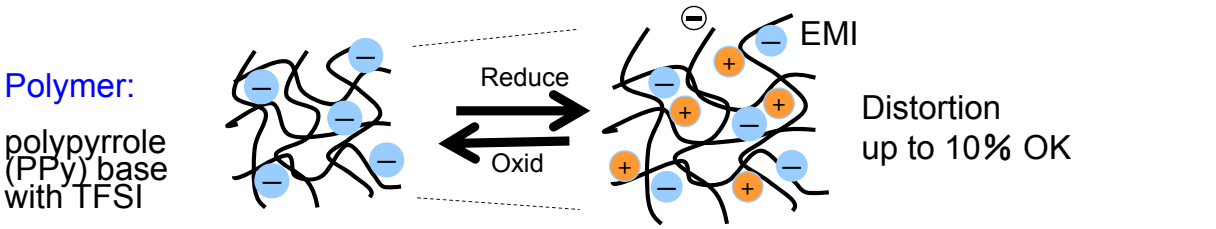


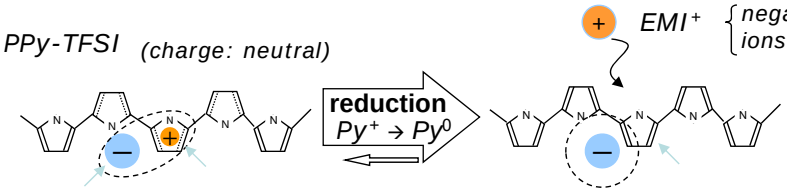


Conductive Polymer Actuator

Operating principle:
volume change by injection/ extraction of electrolyte ions



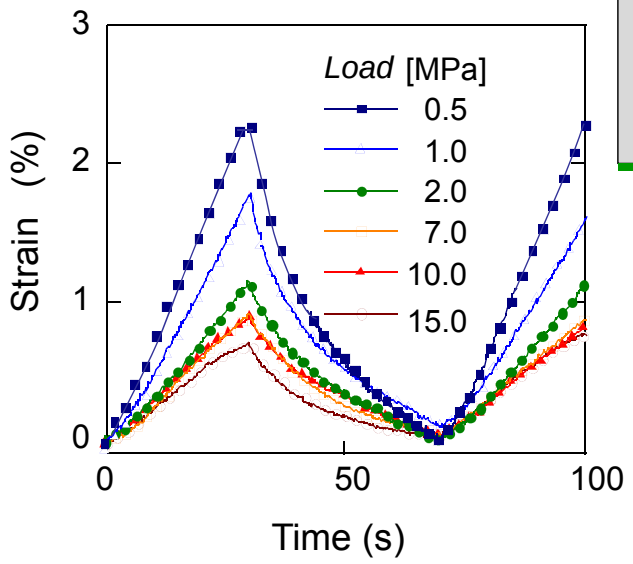


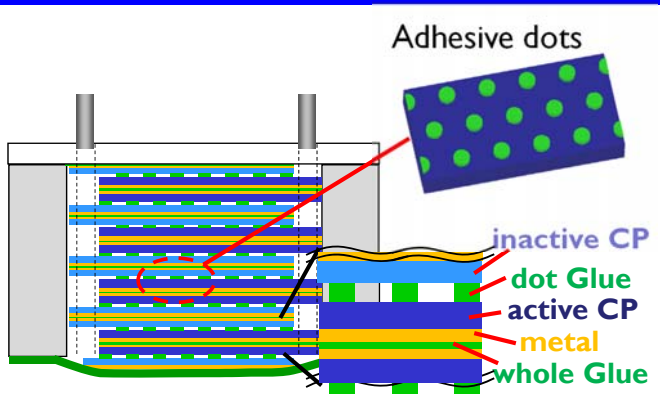


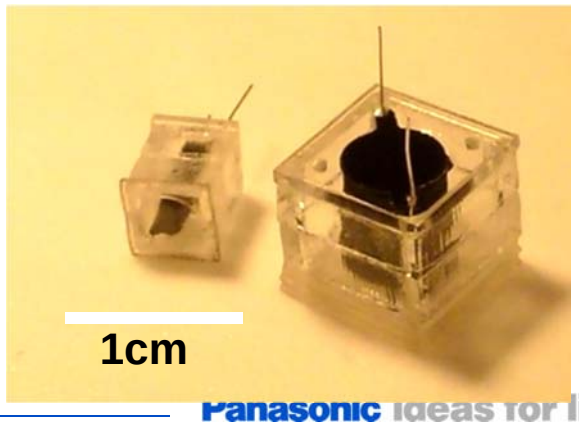
D: diffusion constant

$D_{EMI+} > D_{TFSI-}$

Polymer pump characterization

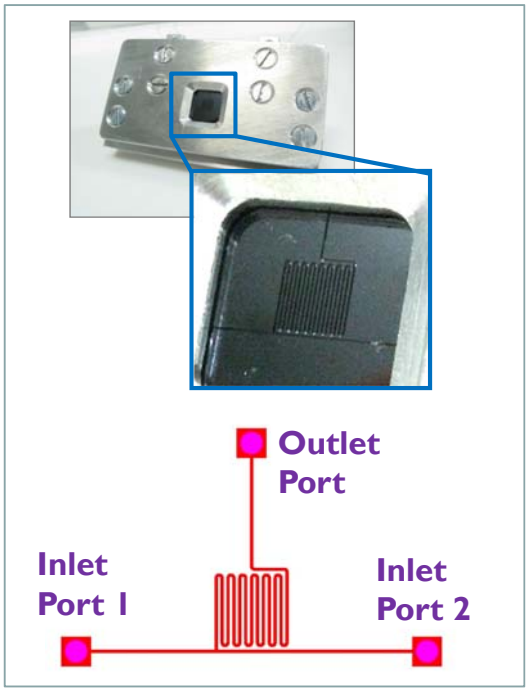


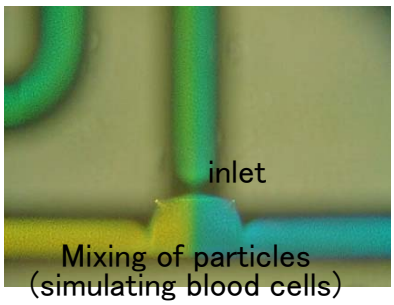




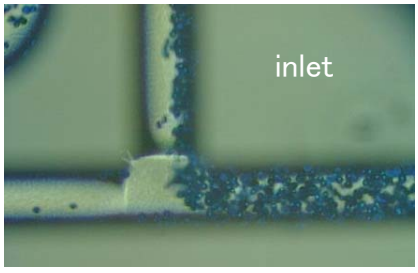
Mixers : characterization

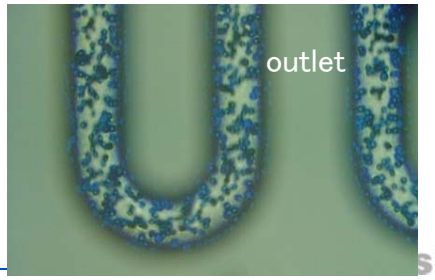
Mixing of two fluids



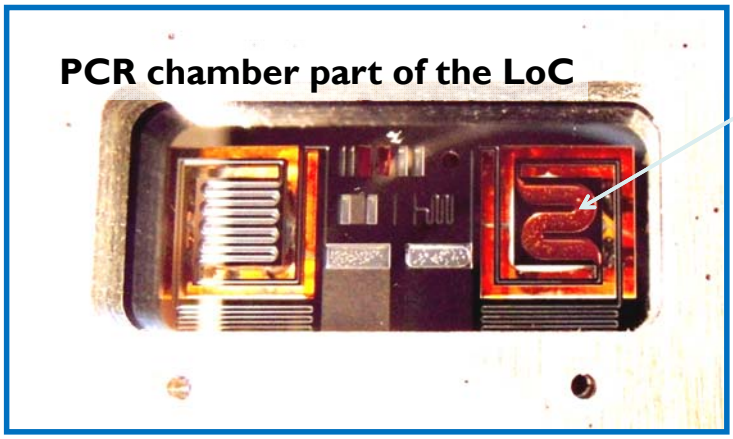


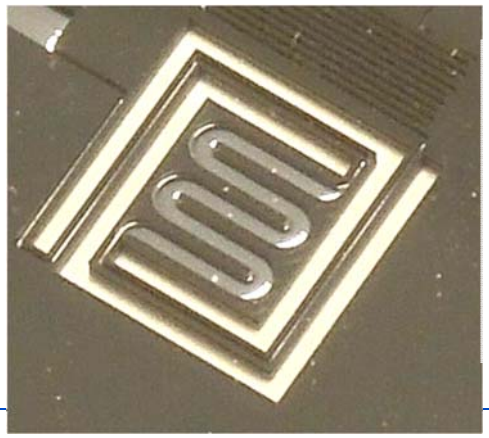
Mixing of particles (simulating blood cells)





PCR demonstration & characterization





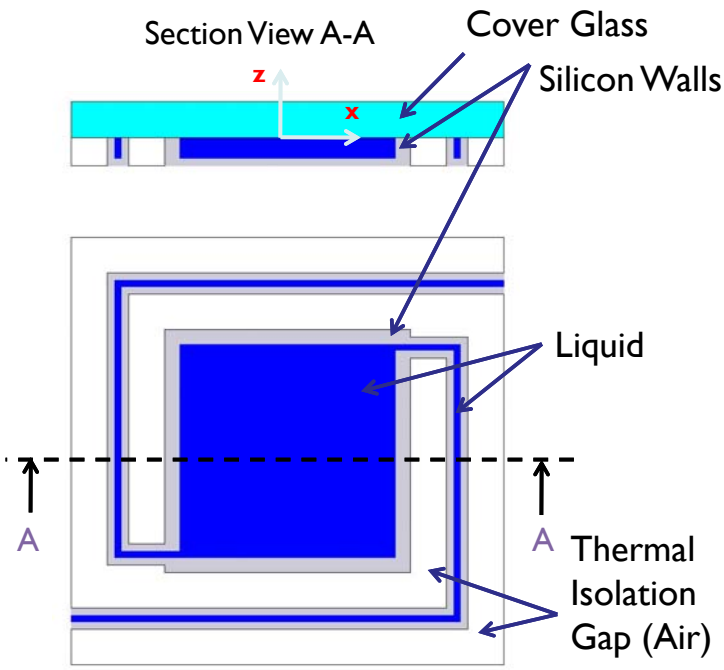
Thermal isolation is the key

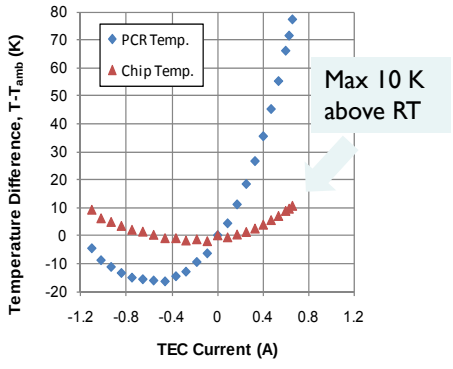
Least heat current should be realized between substrate and PCR chamber during PCR cycle

The chamber is isolated and connected only with the tiny micro-channel

On chip PCR: fabrication and thermal tests

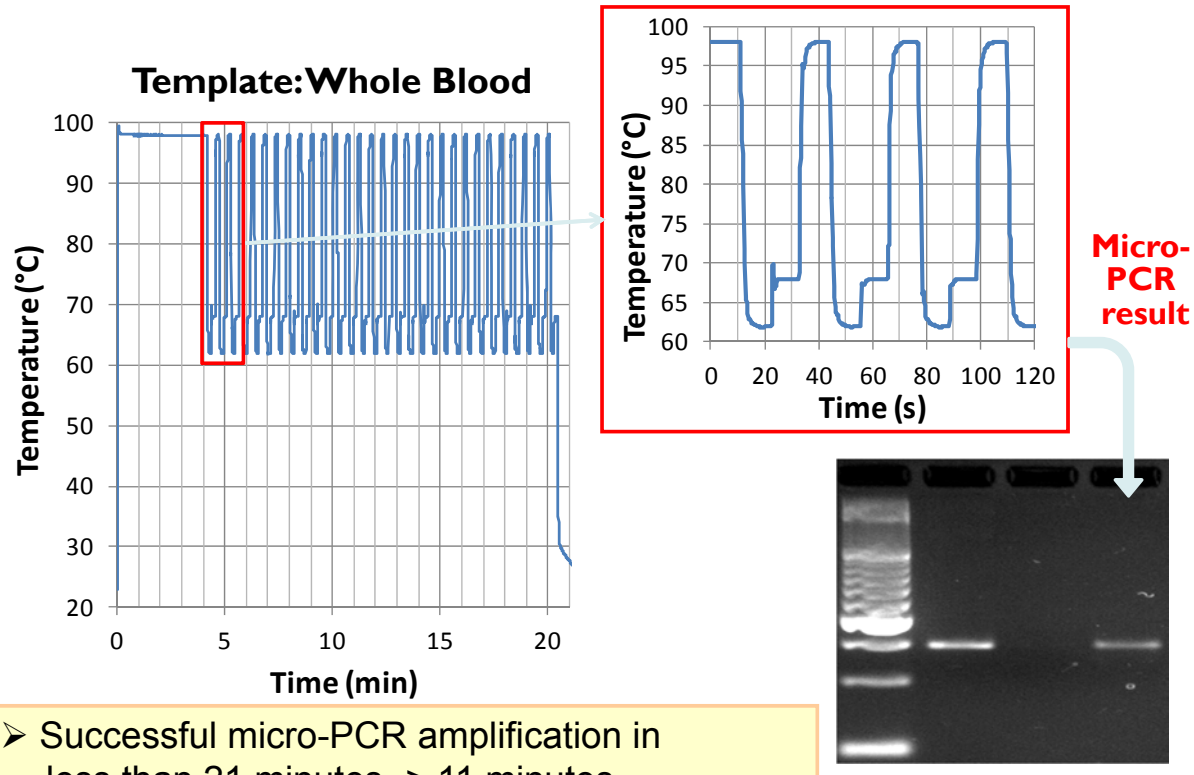
Thermal insulation is further improved by winding in/out channels around the cavity.





Steady-state temperature of the PCR chamber and bulk silicon substrate.

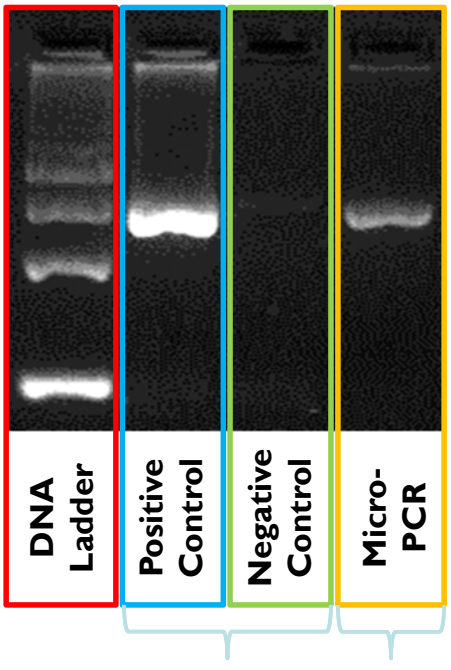
Fast Micro-PCR Demonstration



- Successful micro-PCR amplification in less than 21 minutes -> 11 minutes.

SSD Amplification Tests (Human Genomic DNA)

Template: Human Genomic DNA (From Commercial Source)



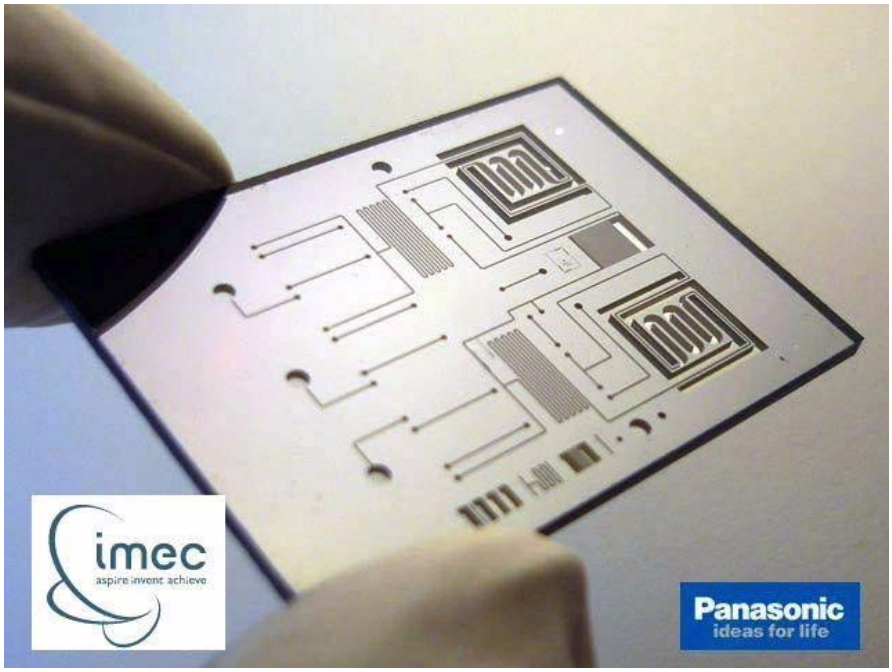
Protocol

2X buffer KOD fx	5 µL
2 mM dNTP's	1 µL
10 µM Fw primer	1 µL
10 µM Rv primer	1 µL
KOD polymerase	0.4 µL
Water	1.1 µL
Template	0.5 µL
Total	***10 µL (2 µL amplified in micro-PCR)***

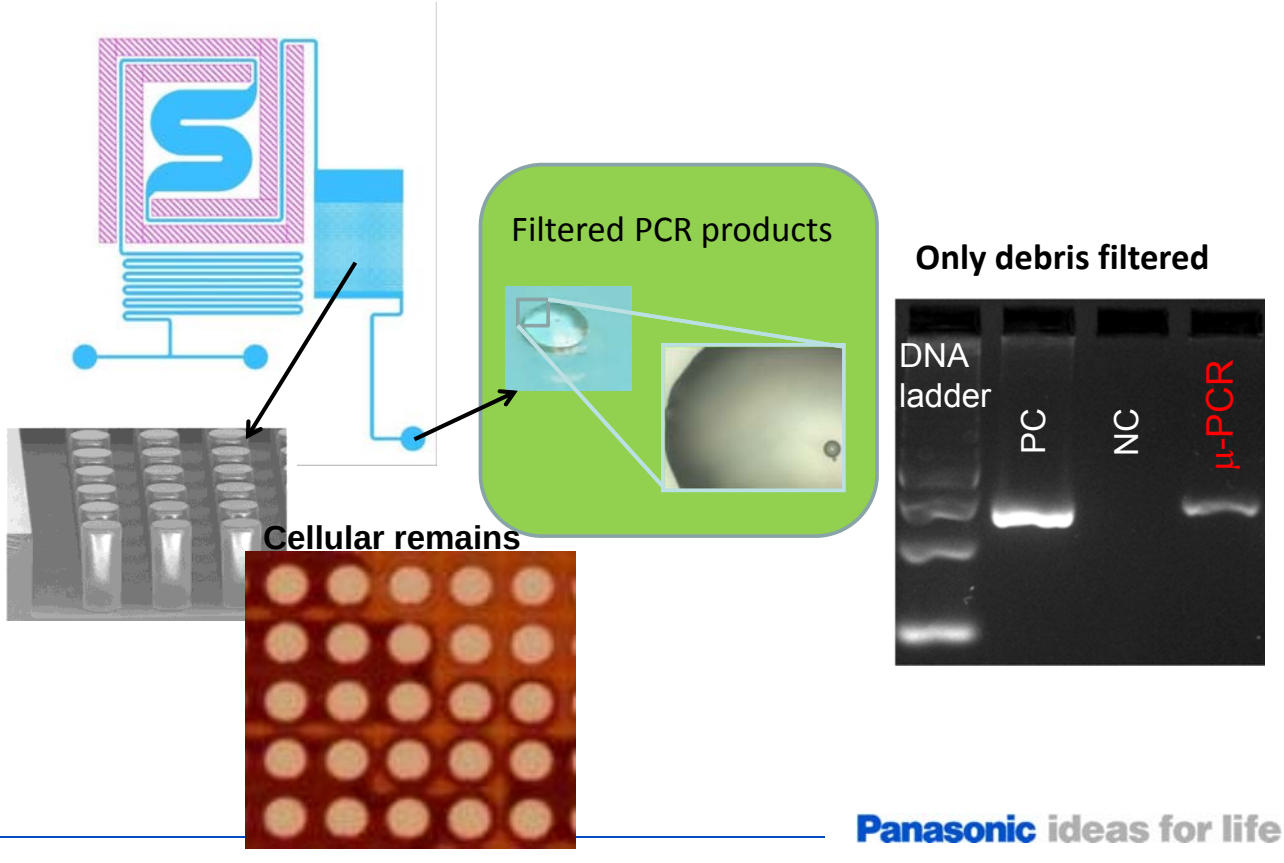
T anneal = 60° C
Cycles = 30

- Successful amplification of target fragment in micro-PCR
- Reproducibility can be improved

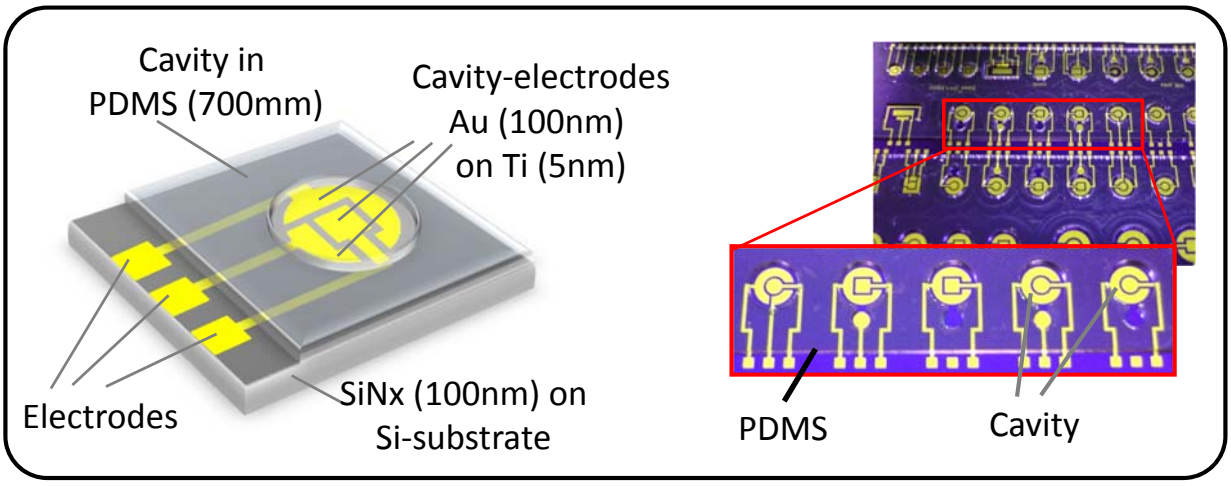
PCR demonstration & characterization



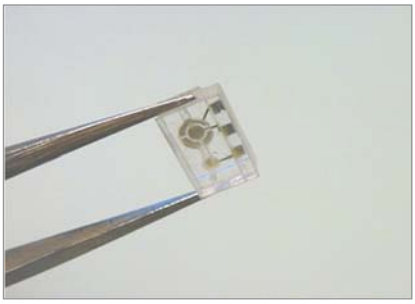
Coarse filter for debris filtration



The newly developed SNP sensor



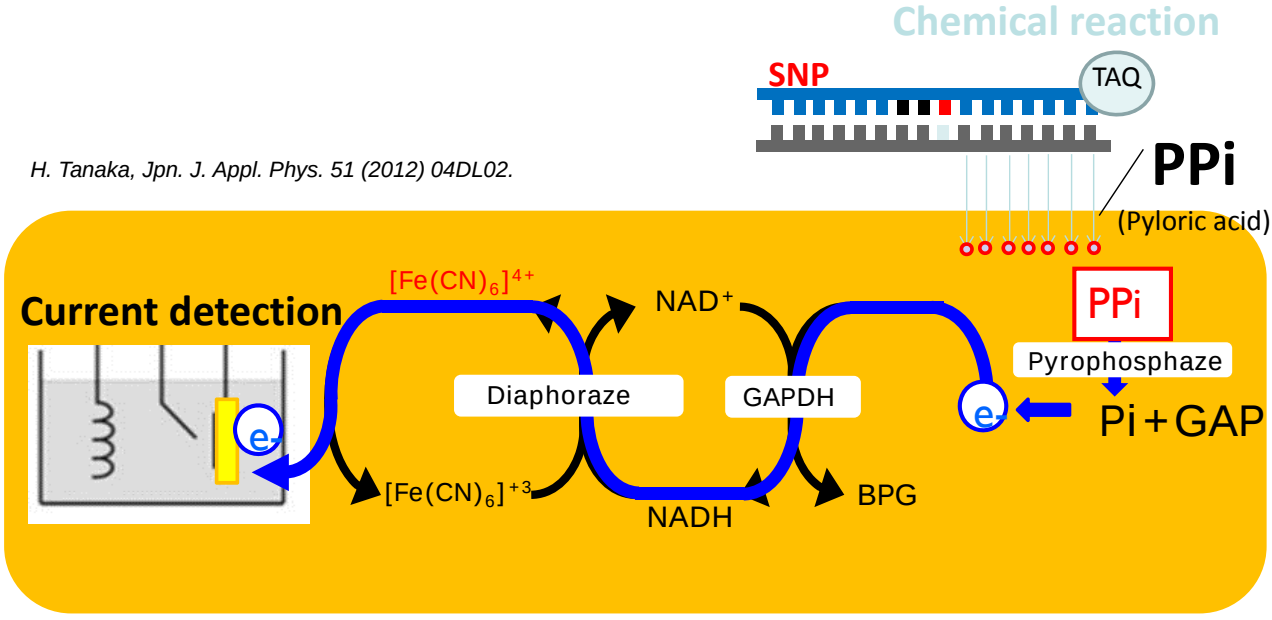
- Photolithographic process for electrode
- Small and well-defined cavities realized by PDMS molding.
- Cavity volume: down to 0.5 µL



Electrochemical SNP sensing

Electrochemical sensing of allele specific PCR products

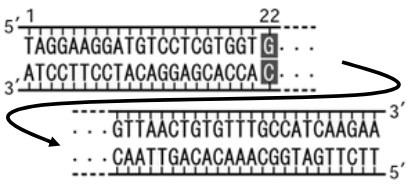
H. Tanaka, Jpn. J. Appl. Phys. 51 (2012) 04DL02.



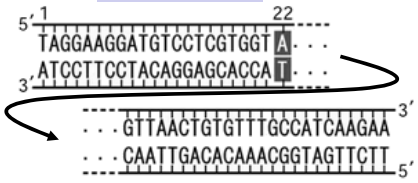
ABO gene SNP detection

Sequences of exon 6 inhuman AB,O gene

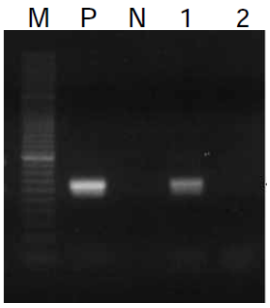
AB allele



O allele



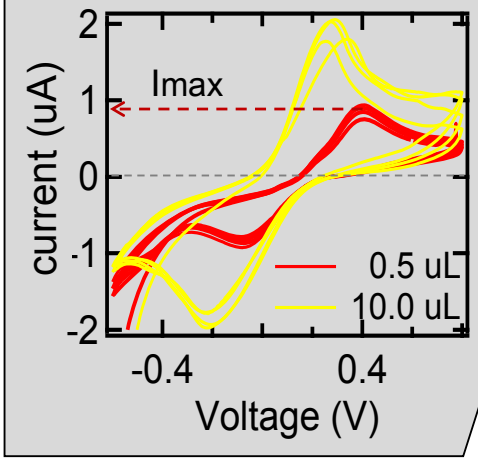
Newly designed allele-specific primer

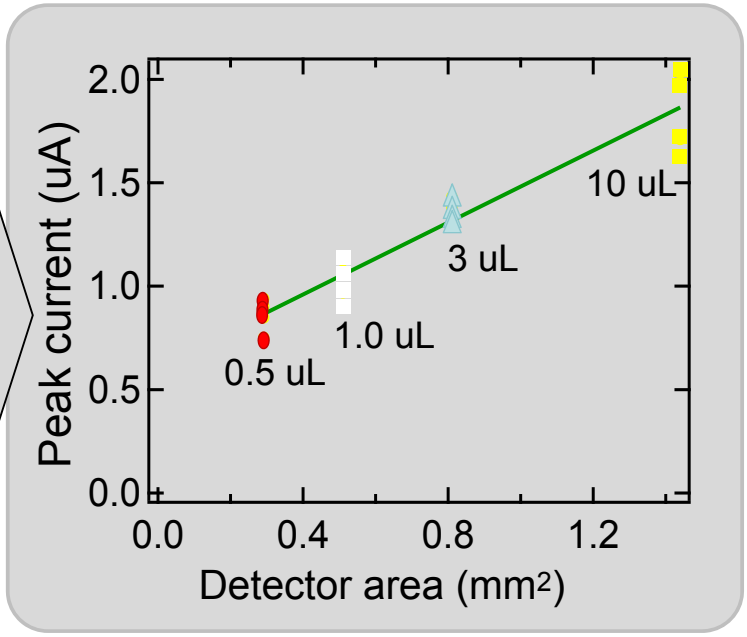


The newly developed SNP sensor

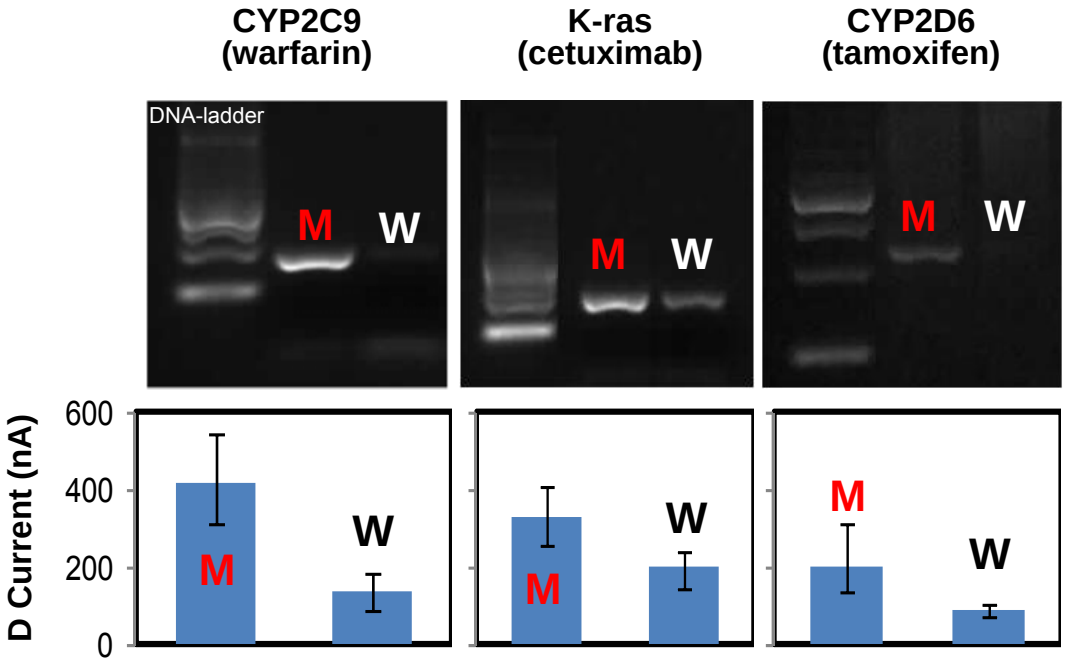
Detector is tested by using a ferricyanide solution.
Molarity is similar to the one which will be obtained in real case later.

Cyclic voltammetric measurements for various detector volumes





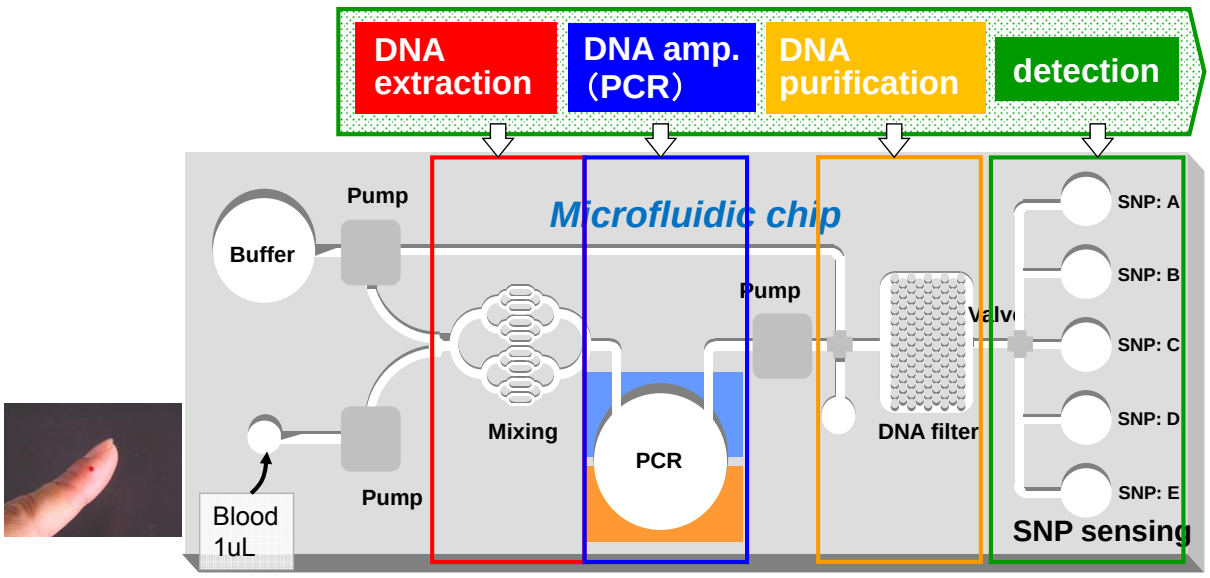
SNP detection results



M: mutant = SNP exist

W: wild = no SNP

SNP Detection chip

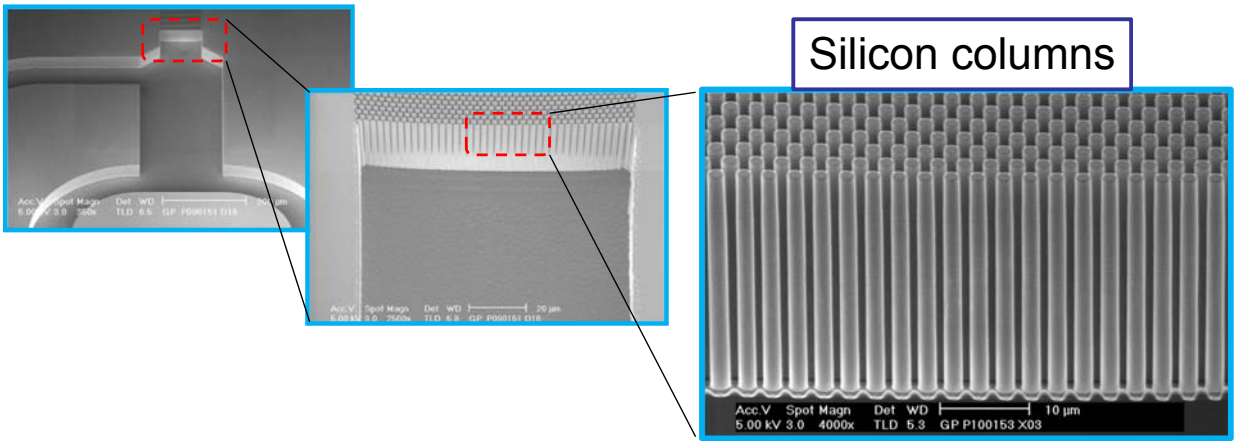


Rapid genotyping diagnostic on chip system

- Automated DNA extraction from blood
- Output diagnostic results within 2 hours

The newly developed DNA filter

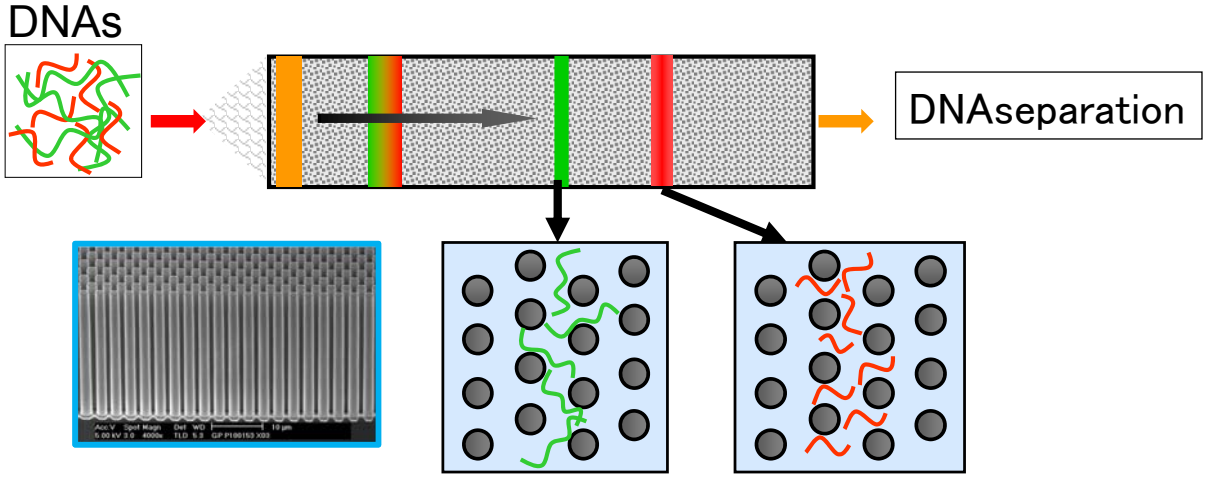
The highly precise structure for high resolution DNA filter
→
- DNA separation length reaches less than 50base pair.
- The separation can be achieve in several seconds.



DNA separation by IP-RP HLC
(ion-pair reverse phase high performance liquid chromatography)

The newly developed DNA filter

DNAs are filtered through the Si pillar forest



Silicon Pillar Array

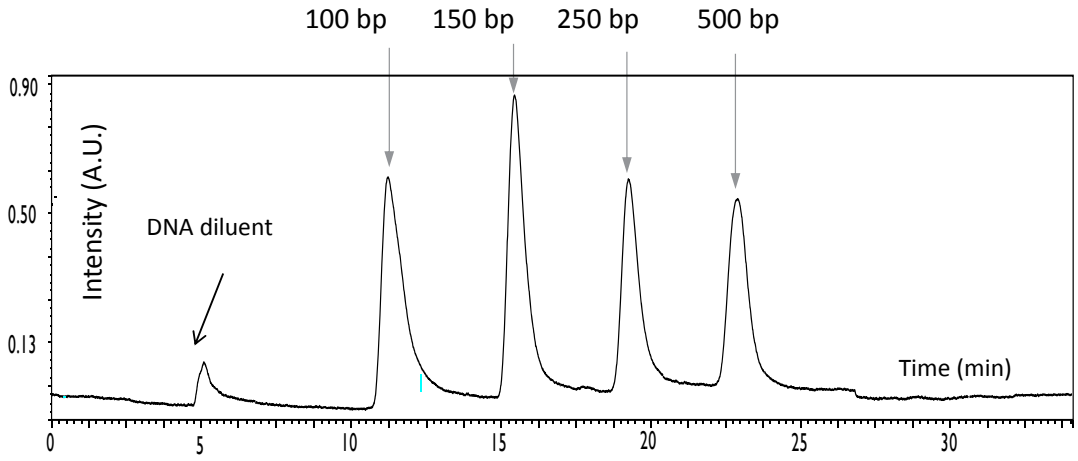
Φ1μm Height 30μm
Interval 1μm

The shorter, the faster DNAs go through the filter.

The newly developed DNA filter

DNA separation on chip based on Ion-pair reversed phase chromatography

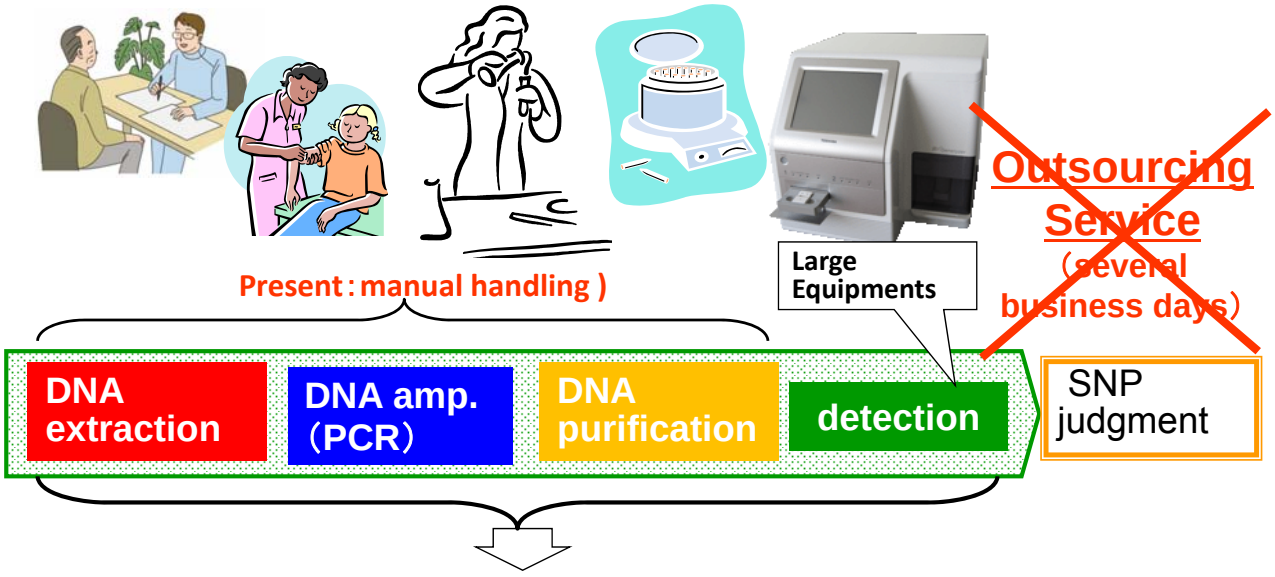
→ With gradient mobile phase (concentration is changing during filtering):



Clear separation and results are reproducible.

50 bp ladder an 10 bp ladder are separated on chip.

Full automatic SNP detection LoC



Our target is SNP-detection from blood around 1 hour with small one-chip.