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

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What is SNP

One DNA alternation can affect people’s sensibility against drugs

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

SNP Single Nucleotide Polymorphism
SNP sensor by Panasonic

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

Easy and fast detection of SNP

Present: manual handling

Outsourcing Service (several business days)

DNA extraction

DNA amp. (PCR)

DNA purification
detection

SNP judgment

Large Equipments

Panasonic ideas for life
Rapid genotyping diagnostic on chip system
• Automated DNA extraction from blood
• Output diagnostic results within 1 hour

Panasonic ideas for life
Single SNP Detection chip details

Sample loading → DNA extraction and amplification → Allele specific amplification → Electro-chemical detection

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Single SNP Detection Chip

Panasonic ideas for life
Fabrication SSD (Process flow)

- Blanket Si wafers
- 1 µm therm oxide
- BS oxide removal wet
- IX-litho
- Oxide etch
- Si etch, Strip
- Pyrex wafer
- Anodic bonding
- Grinding and cleaning
- Litho
- Etch and strip

Fabrication SSD (Results)

- Coarse filter
- FS looking through glass
- BS Si surface
- PCR chamber
- Mixer

Panasonic ideas for life
The newly developed pump unit

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

Conductive Polymer Actuator

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

Polymer: polypyrrole (PPy) base with TFSI

$\text{PPy-TFSI} \quad (\text{charge: neutral})$

$\text{Py}^+ \rightarrow \text{Py}^0$

$D_{\text{EMI}^+} > D_{\text{TFSI}^-}$

Distortion up to 10% OK
Polymer pump characterization

Mixers: characterization

Mixing of two fluids

Mixing of particles (simulating blood cells)
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)

<table>
<thead>
<tr>
<th>Protocol</th>
<th>10 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X buffer KOD fx</td>
<td>5 µL</td>
</tr>
<tr>
<td>2 mM dNTP’s</td>
<td>1 µL</td>
</tr>
<tr>
<td>10 µM Fw primer</td>
<td>1 µL</td>
</tr>
<tr>
<td>10 µM Rv primer</td>
<td>1 µL</td>
</tr>
<tr>
<td>KOD polymerase</td>
<td>0.4 µL</td>
</tr>
<tr>
<td>Water</td>
<td>1.1 µL</td>
</tr>
<tr>
<td>Template</td>
<td>0.5 µL</td>
</tr>
</tbody>
</table>

Total 10 µL
(2 µL amplified in micro-PCR)

\[ T \text{ anneal} = 60^\circ \ C \]
\[ \# \text{ Cycles} = 30 \]

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

Coarse filter for debris filtration

Panasonic ideas for life
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

Chemical reaction

ABO gene SNP detection

Sequences of exon 6 in human AB, O gene

AB allele

O allele

Newly designed allele-specific primer

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

The newly developed SNP sensor

Cyclic voltammetric measurements for various detector volumes

Detector area (mm²) vs. Peak current (uA)

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SNP detection results

CYP2C9 (warfarin)  K-ras (cetuximab)  CYP2D6 (tamoxifen)

DNA-ladder

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

Panasonic ideas for life
The newly developed DNA filter

The highly precise structure for high resolution DNA filter

- DNA separation length reaches less than 50 base pair.
- The separation can be achieved 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.
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.

The newly developed DNA filter

Full automatic SNP detection LoC

Outsourcing Service (several business days)

Present: manual handling

Large Equipments

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