

DNA유전자칩을 이용한 인체오염 바이오마커 탐색기법

Development of Biomarker for toxic Chemicals using cDNA Microarray

박건구

파마코제네칩스

2001년 2월 사람의 genome sequence가 해독되어 발표된 것은 독성학적으로도 역사적인 사건이다. 약 29.1억 base pair로 이루어진 사람의 전체 유전자 염기서열 중 단지 1.1%에 해당하는 부분만이 exon에 해당하는 염기서열 이었고, 24%가 intron에 해당하는 부분이었으며, 나머지 75%는 유전자의 사이에 위치하는 부분이었다. 특이한 결과는 단백질을 coding하는 유전자가 26,000개 정도에 불과하다는 것과 single-nucleotide polymorphism (SNP)가 1250 bp마다 1개씩 존재한다는 사실이다. 이러한 사실은 SNP와 유전자의 발현 패턴이 독성 현상의 발현에 매우 중요하다는 것을 암시한다. 개개인마다 같은 독성 물질에 대한 감수성이 다른 것은 SNP 때문일 것이고, 한 사람에게 노출된 같은 독성 물질이라도 장기마다 독성이 다른 것은 각 장기마다 유전자의 발현 패턴이 다르기 때문일 것이다. 여러 가지 독성 물질이 같은 사람 같은 장기에 독성이 다르게 나타나는 것은 각 독성물질에 의하여 유전자의 발현 패턴이 다르게 나타나기 때문이라고 유추 할 수 있다.

사람의 전체 유전자 수가 생각보다 훨씬 적은 것은 어떤 독성 물질에 1가지 유전자만 관여하여 독성이 나타나지 않을 것이란 추측을 하게 한다. 이제는 한가지 독성물질에 의하여 변화되는 유전자가 수백개이든 수천개이든 High-Throughput Screening (HTS)로 검색하는 방법이 일반화되고 있다. 기존의 방법으로 수백 년 걸리던 일이 단 며칠만에 분석이 가능하게 되었다. 이 방법 중에 한가지가 microarray이다. microarray는 크게 oligonucleotide microarray와 cDNA microarray로 대별 할 수 있다. 전자는 주로 염기서열의 변화를 측정할 수 있고, 후자는 유전자의 발현을 측정한다. 독성물질에 의한 돌연변이나, SNP차이에 의한 독성물질에 대한 감수성의 차이는 oligonucleotide microarray를 사용하고, 유전자 발현의 패턴 변화는 cDNA microarray를 주로 사용한다. 이론적으로 모든 독성물질에 대한 각 조직에서의 모든 유전자 발현 패턴을 분석해서 Data Base를 구축하는 것이 가능하다. 이렇게 되면 유전자 발현 패턴의 변화 자체가 마커가 될 수 있다. 이러한 다수의 유전자를 조합하여 독성을 평가함으로써 보다 정확한 마커를 기대 할 수 있다.

독성물질에 대한 마커로 유전자 발현패턴의 변화를 이용하려면 cDNA microarray를 만들어야 한다. cDNA microarray를 제작하는데는 우선 각각의 유전자를 PCR하고 정제하여 특수하게 coating된 slide glass에 pin방식이나 ink-jet방식으로 고정화 한다. 지름이 약 150 um의 크기로 각각의 유전자를 22mm x 22mm 영역에 1만개 정도까지 배열 할 수 있다. 이중 독성물질의 노출에 의하여 변화되는 유전자를 측정하려면 대조군과 독성물질을 처리한 측정군에서 mRNA를 정제하고 대조군을 Cy3 그리고 측정군을 Cy5로 labelling한다. 이 두가지 probe를 혼합하여 hybridization하고 각각의 유전자에 대한 두가지 형광의 양을 측정하고 상대적인 비율을 산출하여 독성물질에 의한 발현의 증감을 측정하고, 다수의 유전자를 동시에 분석하여 다음의 발현 패턴을 도출 할 수 있다. 1. signal은 있지만 독성물질에 의하여 전혀 발현에 영향이 없는 유전자 2. 발현이 감소되는 유전자 (이 유전자의 기능이 없어져서 독성이 발현됨) 3. 발현이 증가되는 유전자 (이 유전자의 기능이 증강됨으로써 독성이 발현됨) 4. signal이 없는 전혀 발현이 되지 않는 유전자. 즉 위의 4가지 경우의 수를 1000개의 유전자를 동시에 분석해 조합하면 4^{1000} 가지의 경우의 수가 나온다. 이론적으로 모든 독성물질에 대하여 서로 다른 발현 패턴을 대응 시킬 수 있다.

<책임연구자>

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연락처: 전화 (033-258-6777), 팩스 (033-258-6776), E-mail (kkpark@pharmacogenechips.com)

DNA 유전자칩을 이용한 인체오염 바이오마커 탐색기법

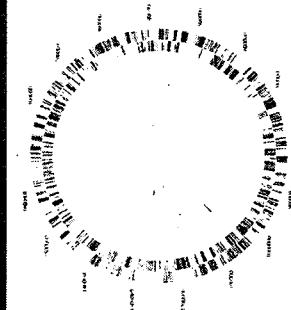
박건구
파미코제네틱스

A Pioneer in Drug Development using Functional Genomics
in DNA Chip Services
in Gene Expression Analysis

포진체 연구기자회견 주요 쟁루 (2001. 10)

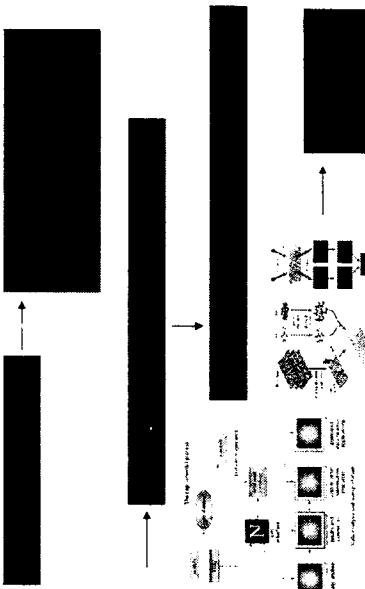
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3.5.2	2001.2.1.3.15
3.5.	1998.1.3.15
A.1.2	2001.1.3.15, 4.15
미생물	<선행기사>
	3. Ga. <i>Saccharomyces cerevisiae</i> <선행기사> 2(Bacillus subtilis)
	원세포 양孢류 (Cochliobolus pulvri) 대상 2(Enterococcus faecalis)
	3. 3(Mycobacterium tuberculosis) Haemophilus influenzae Mycoplasma genitalium Mycoplasma pneumoniae Staphylococcus aureus <선행기사> Methanobacterium thermophilic Pyrococcus horikoshii <i>Thermotoga maritima</i> <선행기사> 진한성 미생물 (Synechocystis SP.)

H. pylori Genome Sequence



A circular representation of the *H. pylori* chromosome illustrating the location of each predicted coding region. Coding regions in the outer circle are transcribed on the + strand while coding regions in the next inner circle are transcribed on the - strand.

화학기기 등 표로 쟁루



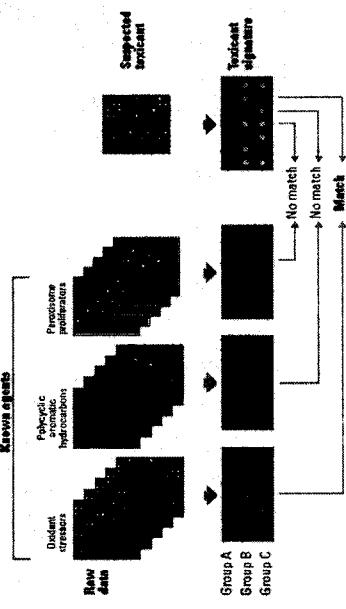
줄기 유전자와의 비교과 응용

CACCTGGCTCACTCGAACCAAGTGGACGGGACTGACATCTGGGGTGTCCTGTC
 CCTCTTACG: 30 학개의 유전자 평가서yle, 약 5%인 16개의 유전자 (UTYAGCAGACC
 CGGCCAGCTCTGAACCTGGTTCTCATCTGGCTCTCTTCATGCCCTCTCTACGGC
 오전: 간특성, 신장특성, 폐특성, GATTCAGATGCTTAAGGGCTGGTCCTGGGG
 GAACTGGCATGAACTAAAGCTGTCATCTGGTCGGTCGGTCGGCTGGTCCTGGGG
 GATGGCTGGAGAGAGAGAGACATGAGGGAGGAGAGGCTGGTCGGTCGGGG
 GGCCATTAGATCTA주의 특성과 관련된 유전자 빌려(로드)ACCCGGAGG
 CCGGGGGCTGCT
 CTTAGGGAGGGGGAGACAGTACGGGGAGAGAGGGAGGGGGAGGGGGAGGGGGAGGG
 CTGGGGAGGGGGAGGGGGAGGGGGAGGGGGAGGGGGAGGGGGAGGGGGAGGGGG
 GGCGTGTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG
 AGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG
 AGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG
 CACCACTTAATCTCACCTCTTCAGCTGGTCACTGGGGCTGGGGGGCTGGGGGGCTGGGG
 CAGCAGCTA 01 유전자를 조절하는 다른 특성을 찾는다.
 AAATGGGGCTGG
 CACCTGG

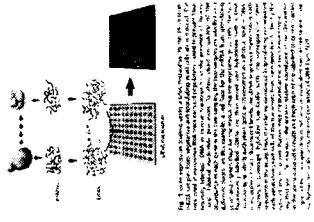
Microarray 응용

1. Treat mice with chemical. Harvest tissue.
2. Make cDNA libraries
3. Identify and count clones by hybridization with probes from most common cDNAs. Remove clones using robotics.
4. Begin high throughput sequencing of unidentified clones.
5. Determine EST frequency by counting clones identified by sequencing and hybridization.
6. Make cDNA microarrays using unique clones.
7. Search for gene expression patterns. Test models of toxicity.
8. Apply custom microarrays to additional chemical exposures.

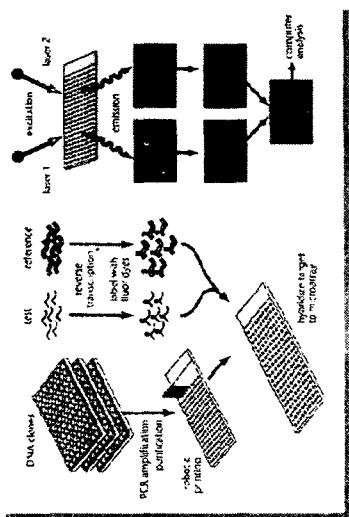
Toxicant and Gene Expression Pattern



Gene expression analysis using cDNA microarray

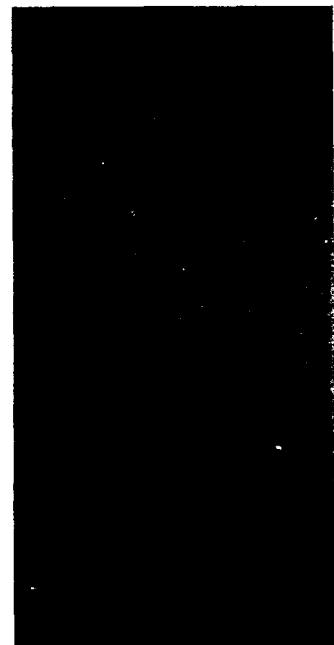


유전자 표지기 기술



DNA chip 제작 기술	특징	chip 종류	DNA chip 제작 회사
pin microarray	Pin을 이용한 micro drooping (surface contact)	cDNA & oligonucleotide	Ilyseq Incyte Pharmaceuticals
Inkjet	inkjet을 이용한 micro drooping	oligonucleotide	Affymetrix
Photolithography	Photoresist oligonucleotide 직접 헤칭	oligonucleotide	clinical Micro Sensors Nanogen
Electronic array	전기로 이용한 oligonucleotide addressing	oligonucleotide	

유전자 표지기 기술



Microarray Robot (Pin 복제)

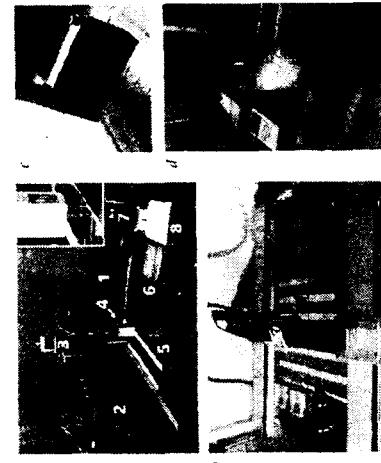
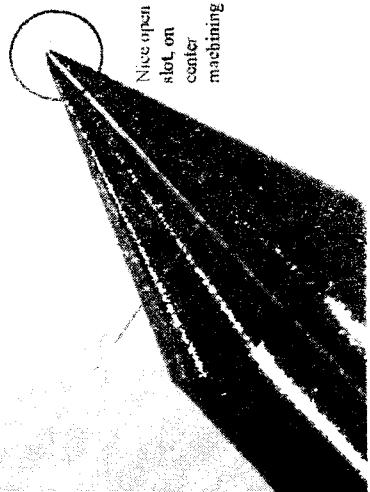
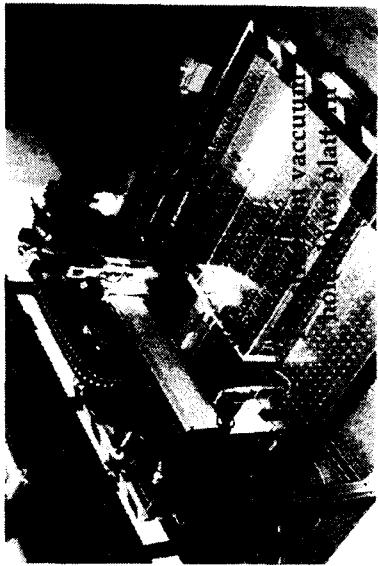
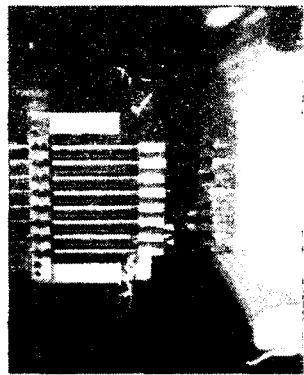


Fig. 14 Microarray robot. The X, Y, Z axes are selected 1, 2, and 3 respectively in the robot control software. After the sample spotting is completed in the spotting station, the slides are placed on the slide station (3). Samples are measured and stored in the storage station (4). Slides are cleaned between sample acquisitions at the washing (7) and drying (8) stations. A PCR machine (5) is used to amplify the samples. The robot can hold up to 16 slides at one time. Two wash tanks and the dryer (6) are also included in the system. When the robot is not in use, while the optical stage, power supplies for laser and Peltier cooling, the liquid stage, and most of the software are off, the robot is still active. The robotic arm, robotic hand, and other parts are stored in the metal van (9).

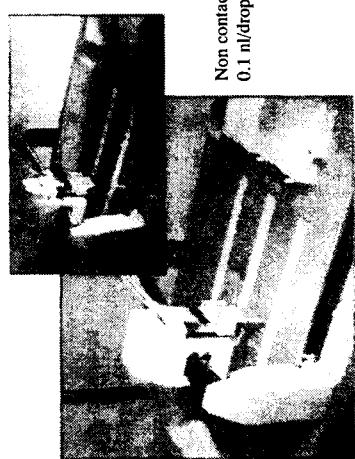
유전자 표지기 기술



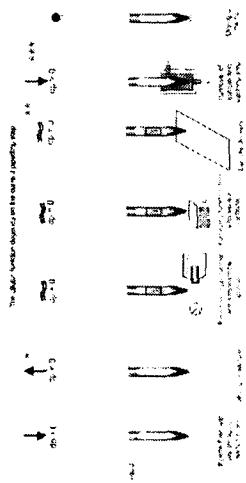
Sample aspiration
from a 384 well PCB plate



Piezoelectric



Operating Principles of the Dielectrostatic Microneedles



The nozzles of the Dielectrostatic Microneedles



Light directed oligonucleotide synthesis



Fig. 4 Light-directed oligonucleotide synthesis on solid support is demonstrated with a coumarin linker terminated with a bioreactive protecting group. Light is directed through a mask to deposit and etchate selected areas and protect nucleotides en route to the activated site. The process is repeated, activating different sets of thio and coupling until the final sequence is synthesized. This mask can be introduced at various sites during synthesis of the array.

Scanning Sensitivity

Sensitivity serial dilution detecting better than <0.1 fluorescent molecules μm^{-2} .
The faintest spot represents 0.01 fluorescent molecules μm^{-2} .



cDNA microarray Data

Data Analysis

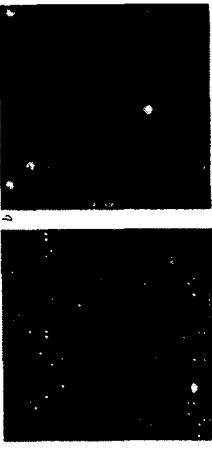
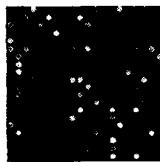
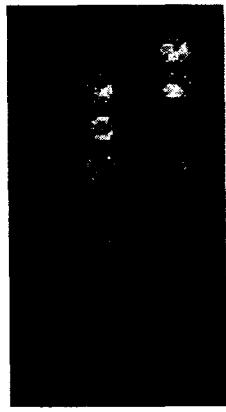


Fig. 3. a. A hybridized microarray printed by the ACOM Microarray printer was scanned by a Bio-Rad ImageStationTM system. b. A hybridized microarray printed by the ACOM Microarray printer was scanned by a Bio-Rad ImageStationTM system. The image shows a single spot of the four spot printing test. A 10 μl volume of each of the four spot printing test samples were applied to the array.

Mutation detection. Shown are scanned images of oligonucleotide microarrays printed with TeleChem's ChipMaker 3™ Micro-spotting device

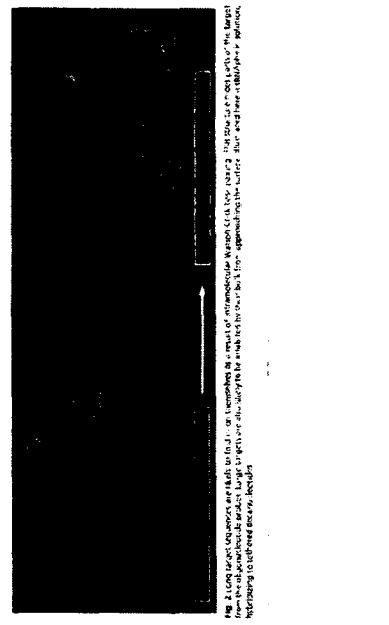


Short target sequences



-42-

Long target sequences



Short target sequences

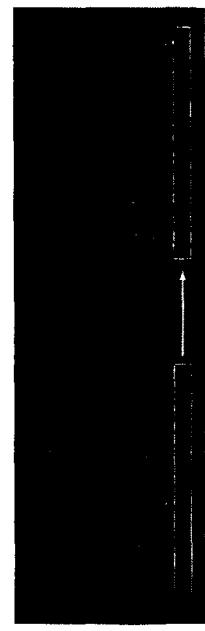


Fig. 3 Short target sequences are added to a sequencing gel. After sequencing, the bands from lanes L3 and L4 are cut out and dissolved in water. This solution is then applied to a sequencing gel. The gel is run at the same speed as the original gel. The bands from lanes L3 and L4 are visible on the sequencing gel.

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Gene Expression Monitoring with oligonucleotide arrays

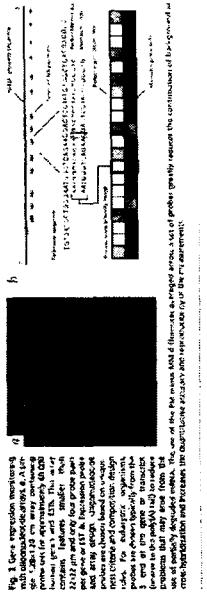


Fig. 4 Gene expression monitoring with oligonucleotide arrays. A sequencing gel with four lanes is shown. Lane L1 shows a single band at the top. Lane L2 shows a band at the top and a faint band below it. Lane L3 shows a band at the top and a strong band below it. Lane L4 shows a band at the top and a very strong band below it. A bracket on the right indicates the position of the bands.

Fig. 4 Gene expression monitoring with oligonucleotide arrays. A sequencing gel with four lanes is shown. Lane L1 shows a single band at the top. Lane L2 shows a band at the top and a faint band below it. Lane L3 shows a band at the top and a strong band below it. Lane L4 shows a band at the top and a very strong band below it. A bracket on the right indicates the position of the bands.

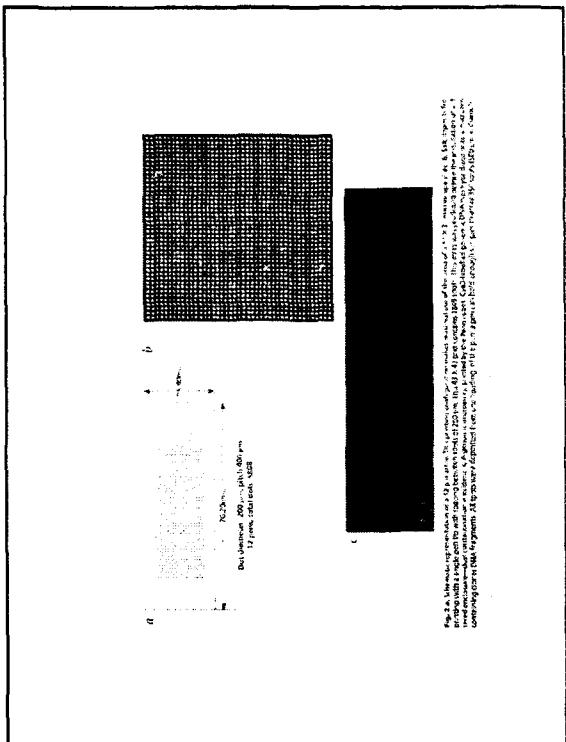


Fig. 1. A fluorescence micrograph of a 10×10 pin $40 \times$ objective $40 \times$ $\text{NA} = 1.2$ pin \times pin \times pitch $= 100 \text{ nm} \times 100 \text{ nm} \times 100 \text{ nm}$. The figure shows a standard 12 pin slide with a 100 nm pitch. The left panel shows a full array of spots. The middle panel shows a single spot at higher magnification. The right panel shows a dark control area. The slide was imaged with a 40x objective.

Microarray 분석

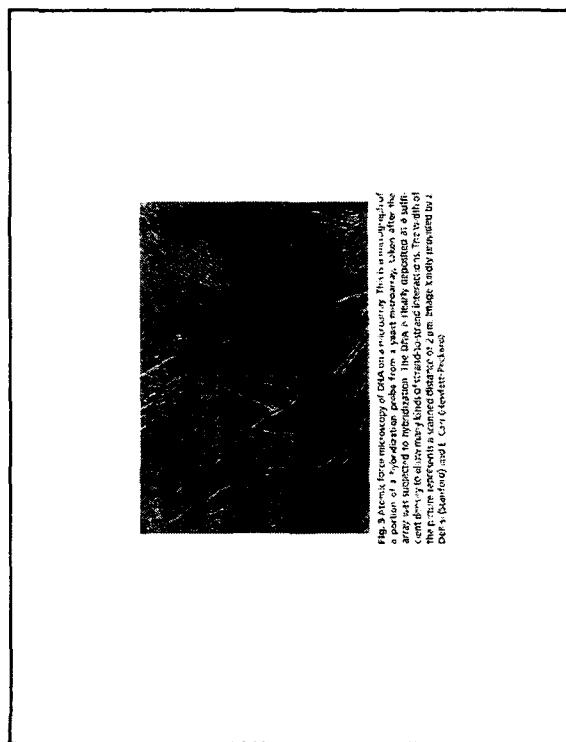
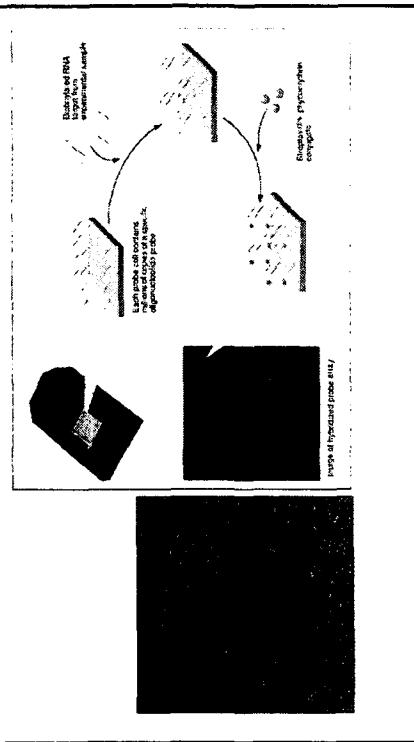
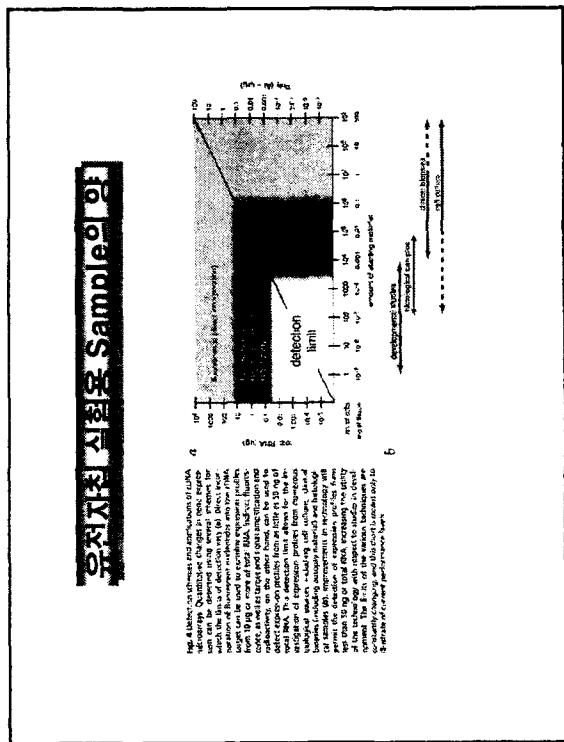


Fig. 2. A fluorescence micrograph of DNA on a microarray. This is a sample of a pin of a 10×10 pin $40 \times$ objective $40 \times$ $\text{NA} = 1.2$ pin \times pin \times pitch $= 100 \text{ nm} \times 100 \text{ nm} \times 100 \text{ nm}$, taken after the array was subjected to hydration. The DNA is clearly dehydrated as is visible. Compared to the array in Figure 1, the intensity of individual features is reduced. The figure represents a standard 12 pin $40 \times$ objective $40 \times$ $\text{NA} = 1.2$ pin \times pin \times pitch $= 100 \text{ nm} \times 100 \text{ nm} \times 100 \text{ nm}$.

유전자 조작 실험ology



The figure illustrates the methodology of cDNA microarray experiments. In the first step, total cellular RNA is isolated from the cells or tissue specimen to be analyzed. This RNA is then converted into complementary DNA (cDNA) using reverse transcriptase. From this cDNA, a probe is synthesized and labeled with a radioactive isotope. This labeled probe is then used to detect complementary probes from target cells. The target cells are usually obtained from patients with various diseases. The labeled probe is then applied to a microarray, which contains thousands of complementary probes from normal cells. After hybridization, the microarray is scanned to determine the intensity of each probe. This information is then used to calculate the ratio of expression levels between the target and normal cells. This ratio is then compared to a database of known gene expression profiles to determine if a particular gene is overexpressed or underexpressed. The final step is to analyze the results and draw conclusions about the disease process.

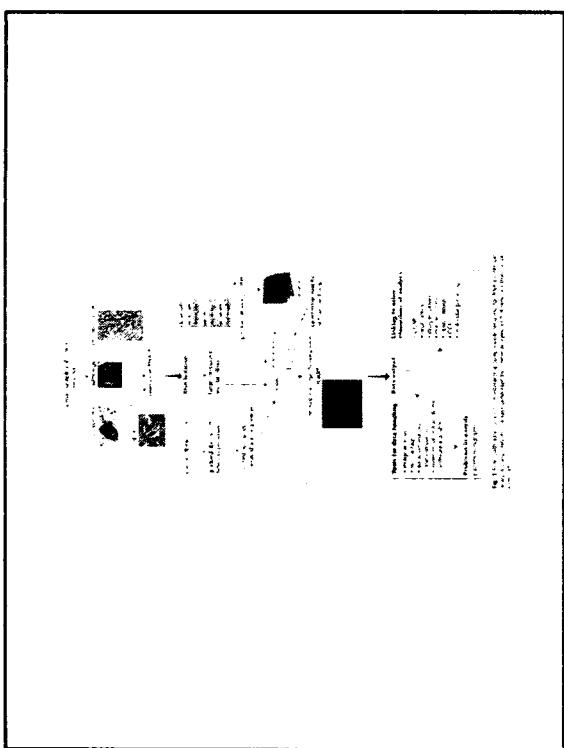


Fig. 4 Genotyping by array. **a**, A 96-well plate with over 10,000 probes designed to determine the genotype of a sample at over 3,000 loci. **b**, The agarose gel electrophoresis patterns of the samples in panel **a**. The reference lane contains a mixture of all four possible genotypes. **c**, A photograph of the printed array. **d**, Gel electrophoresis patterns of the samples in panel **a** on the array. **e**, Gel electrophoresis patterns of the samples in panel **a** on a sequencing gel. **f**, Gel electrophoresis patterns of the samples in panel **a** on a sequencing gel. **g**, Gel electrophoresis patterns of the samples in panel **a** on a sequencing gel.

Genotyping Arrays

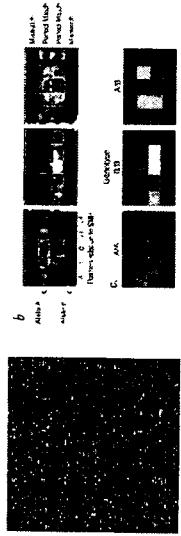
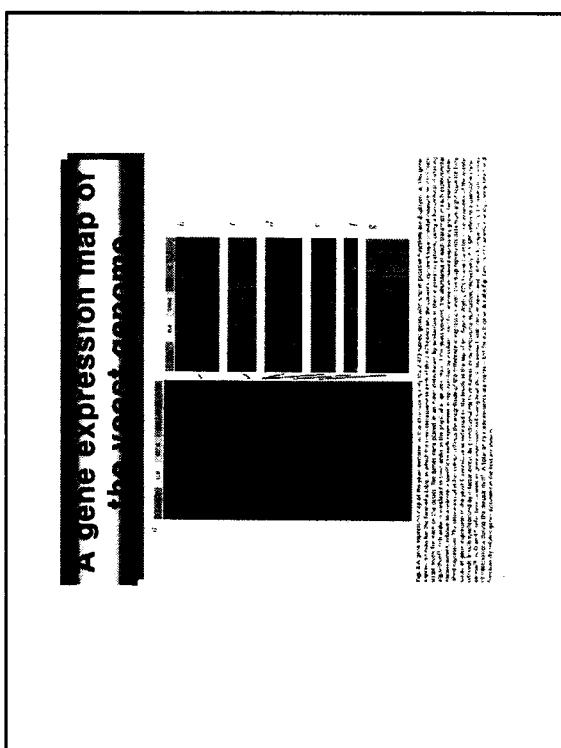


Fig. 5 Gene expression map of the yeast genome. **a**, A 96-well plate with over 10,000 probes designed to determine the expression of a sample at over 3,000 loci. **b**, The agarose gel electrophoresis patterns of the samples in panel **a**. The reference lane contains a mixture of all four possible genotypes. **c**, A photograph of the printed array. **d**, Gel electrophoresis patterns of the samples in panel **a** on the array. **e**, Gel electrophoresis patterns of the samples in panel **a** on a sequencing gel. **f**, Gel electrophoresis patterns of the samples in panel **a** on a sequencing gel. **g**, Gel electrophoresis patterns of the samples in panel **a** on a sequencing gel.



A gene expression map of the yeast genome

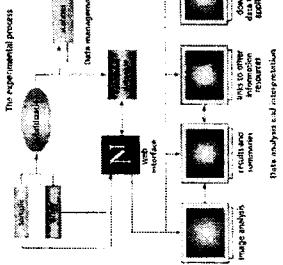
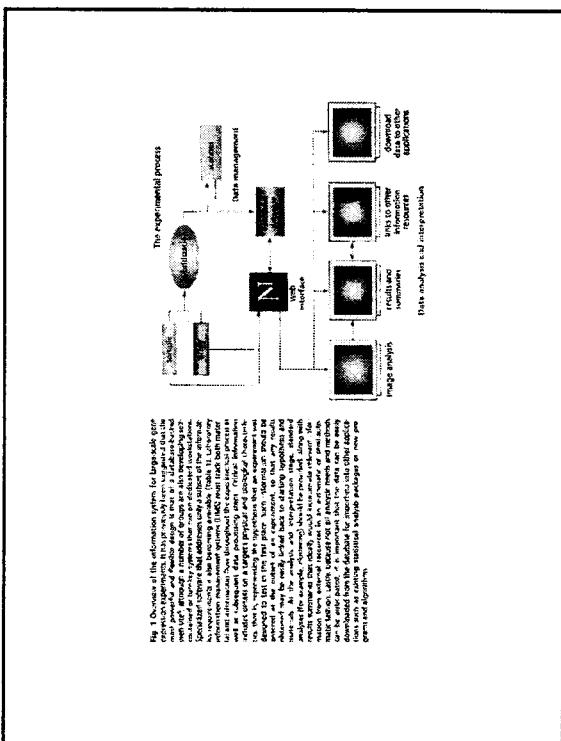


Fig. 6 Overview of the workflow system for large-scale gene expression mapping. In this figure, we have outlined the general workflow for the analysis of gene expression data. The first step is to design the experiments. This is followed by sample preparation, labeling, hybridization, and data management. The next step is nucleic acid sequencing. Finally, the data is analyzed and interpreted. The results can then be used to compare with other platforms for comparative analysis.



The Molecular Cell Biology Database

- ☆ Major sequence Repositories
 - GenBank
<http://www.ncbi.nlm.nih.gov/WebGenbank>
 - All Known nucleotide and protein sequences: International Nucleotide Sequence Database Collaboration
- ☆ Comparative Genomics
 - Clusters of Orthologous Groups(COG)
<http://www.ncbi.nlm.nih.gov/COG>
 - Phylogenetic classification of proteins from 21 complete genomes
- ☆ Gene Expression
 - Gene Expression Database(GXD)
<http://www.informatics.jax.org/gx/index.html>
 - Mouse gene expression and genomics
- ☆ Kidney Development Database
 - Kidney Development Database
<http://www.ana.ed.ac.uk/atomv/database/kidhome.html>
 - Kidney development and gene expression
- ☆ Gene identification and Structure
 - TRRD
<http://www.mbs.shinyei.nsc.tnigd.dhaisi/trrd/>
 - Regulatory regions of eukaryotic genes
- ☆ Genetic Maps
 - GenAtlas
<http://www.cit2.fr/GENATLAS/>
 - Human genes, markers and phenotypes

cDNA chip 활용 분야

DNA chip 활용 분야

☆ cDNA chip 활용 분야 <ul style="list-style-type: none"> 인체 유전자 기능 분석 연구 실험 유전자 조절 통로를 찾기 위해 유전자의 조절 단위 내적 결합 단위 찾기 유전자의 유전자의 조절 단위 찾기 유전자 치료 동물 유전자 조절 단위 찾기 환경 변화에 따른 생태학적 연구 식품 안전성 검사 신약 개발 	☆ oligonucleotide chip 활용 분야 <ul style="list-style-type: none"> 임관련 유전자 풀 엔코딩 유전자의 풀 엔코딩 유전병 관련 유전자 풀 엔코딩 DNA 암기서 열 분석 유전자 변이 기계 도구 정기 이식 가능 조직 검사 병원성 미생물을 통한 병의학적 확인(예: 확인, 천자 확인 등) DNA 고고학
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