Surface Chemistry for Biochip and Bioanalysis

2007.05.11

박 준 원

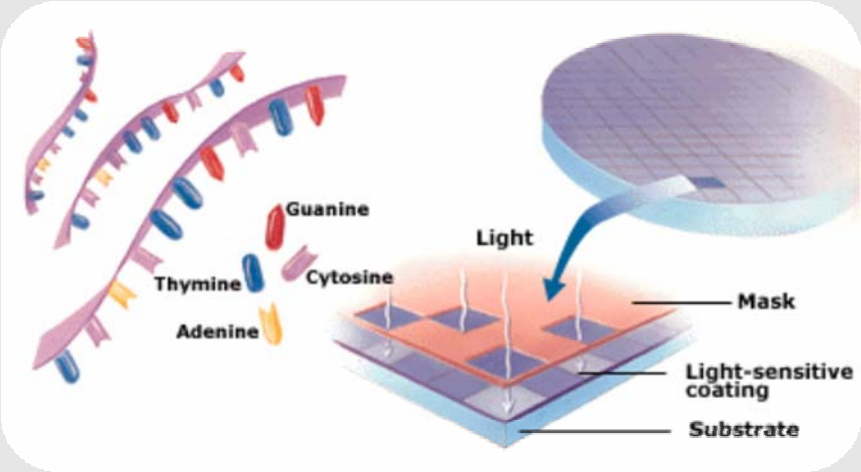
포항공과대학교

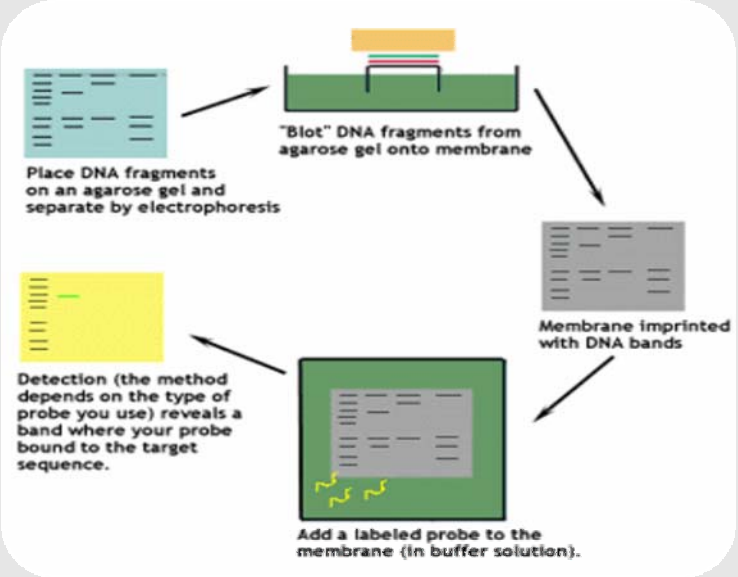
Biochip의 탄생

Light-directed, spatially addressable parallel chemical synthesis

Fodor, S. P. *et al.* Science 251(4995), 767-73, 1991

US Pat.#: 5445934, 5744305, 5677195





Conventional method – southern blotting

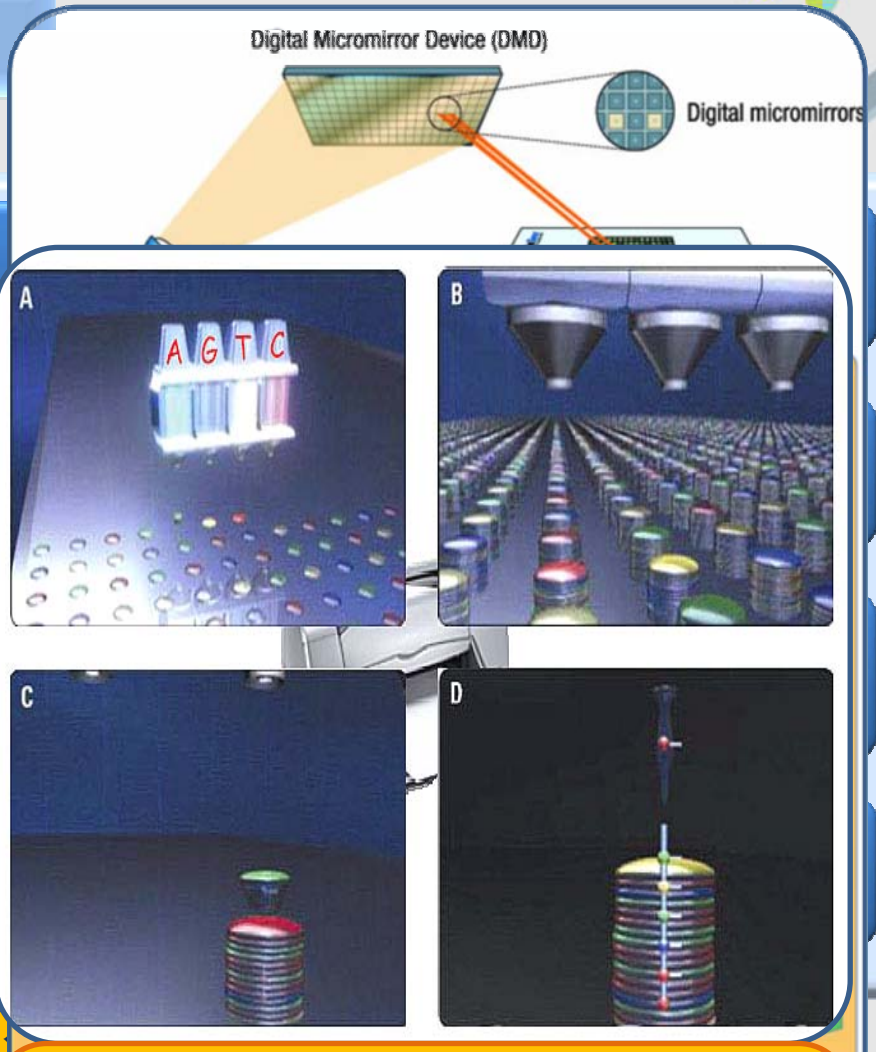
주요 회사의 기술

Affymetrix
Market leader, Mask를 이용한 GeneChip Sys

Agilent
잉크젯 프린터를 이용한 마이크로 어레이

Illumina
Bead-based technology 보유, 3μm크기

Nimblegen
Maskless Array Synthesizer (MAS) tech



(자료) Agilent Inkjet기술 도해

(자료) Nimblegen MAS기술 도해

DNA칩 시장

- DNA칩 시장은 높은 성장세를 기록 중 (단위: $ Millions)

구분	2003	2004	2005	2006	2007	CAGR
미국	548.2	712.7	904.9	1,130.50	1,388.80	29.3%
캐나다	59.6	82.9	133.1	152.3	203.3	36.1%
일본	95.4	126.5	163.3	207.2	260.1	30.7%
유럽	274.3	390	534.3	722.9	963.3	39.6%
기타	30	42.5	59.1	79.9	106.3	36.7%
합계	1,007.50	1,354.60	1,774.70	2,292.80	2,921.80	32.9%

* Global Industry Analysis Inc. "Biochips" 2004년

KFDA 바이오칩 승인현황

BAC칩, 마크로젠

태아 대상 유전자 검사, 기존

해 검사 결과를 신속하게 도출

HPV칩, 바이오메드랩, 마이진

자궁경부암, 곤지름 등을 유발

인체유두종)진단

(자료) 마이진 HPV Chip Kit

CYP 450칩(Roche, AmpliChip TM)

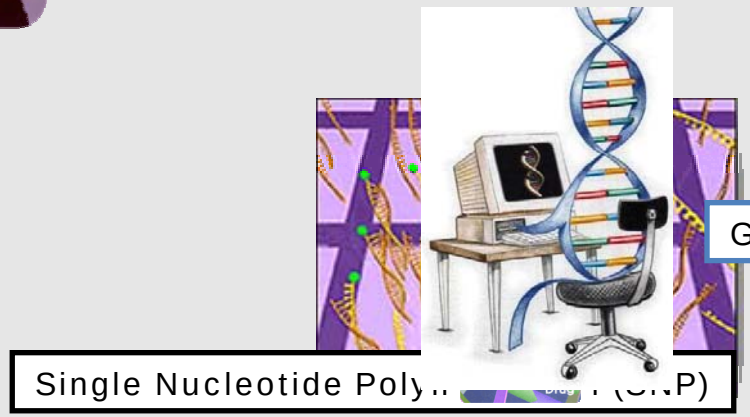
1998년, **미국에서만** 100,000**명 이상이** ADR(Adverse Drug Reaction)**을 원인으로 사망**

CYP 450 chip**으로 약물대사관련 유전자변이 진단** → UM(Ultrarapid metabolizer) PM(Poor metabolizeer) **판별** → **진통제, 항우울제, 항히스타민제, 심혈관질환 치료제의 특수처방**

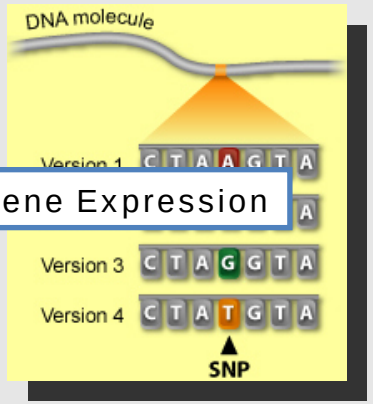
DNA Chip의 응용

"We now believe that the only disease not having some genetic component is trauma."

M.D. Collins Francis
(Director of the US National Human Genome Research Institute, Bethesda, MD, USA)



Single Nucleotide Poly.. .. (SNP)



Gene Expression

Gene Diagnostics

Gene Diagnostics

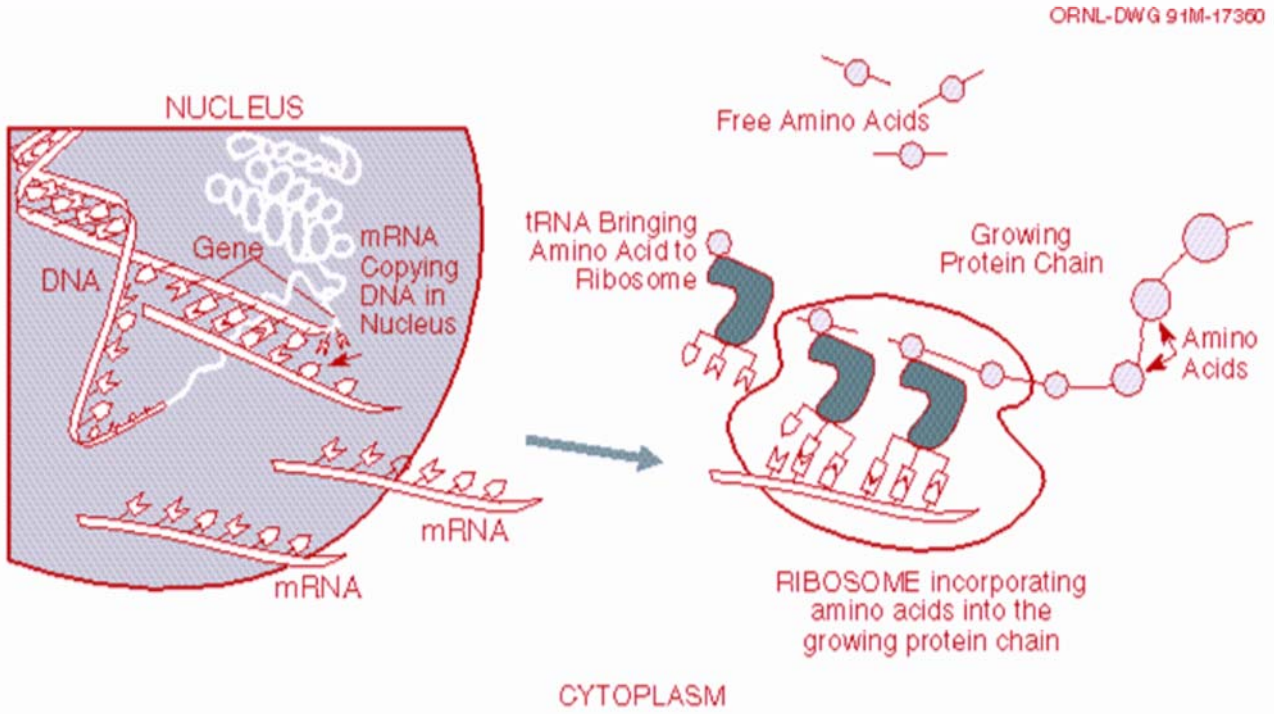
DNA Chip**은 여러 가지 병의 진단에 이용될 수 있음**

Heart disease, Cancer, HIV/AIDS, Tuberculosis, Cytomegalovirus,

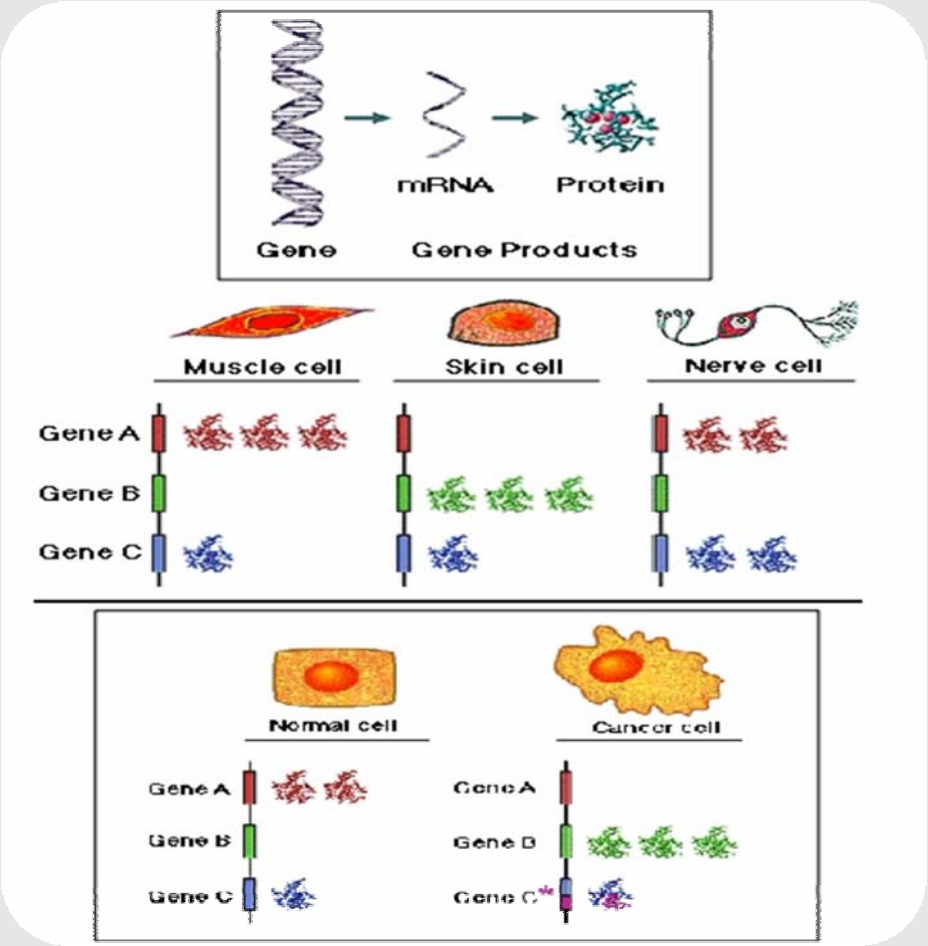
Hypertension, Osteoporosis, Infertility, Alzheimer's disease **등등**

Gene Expression

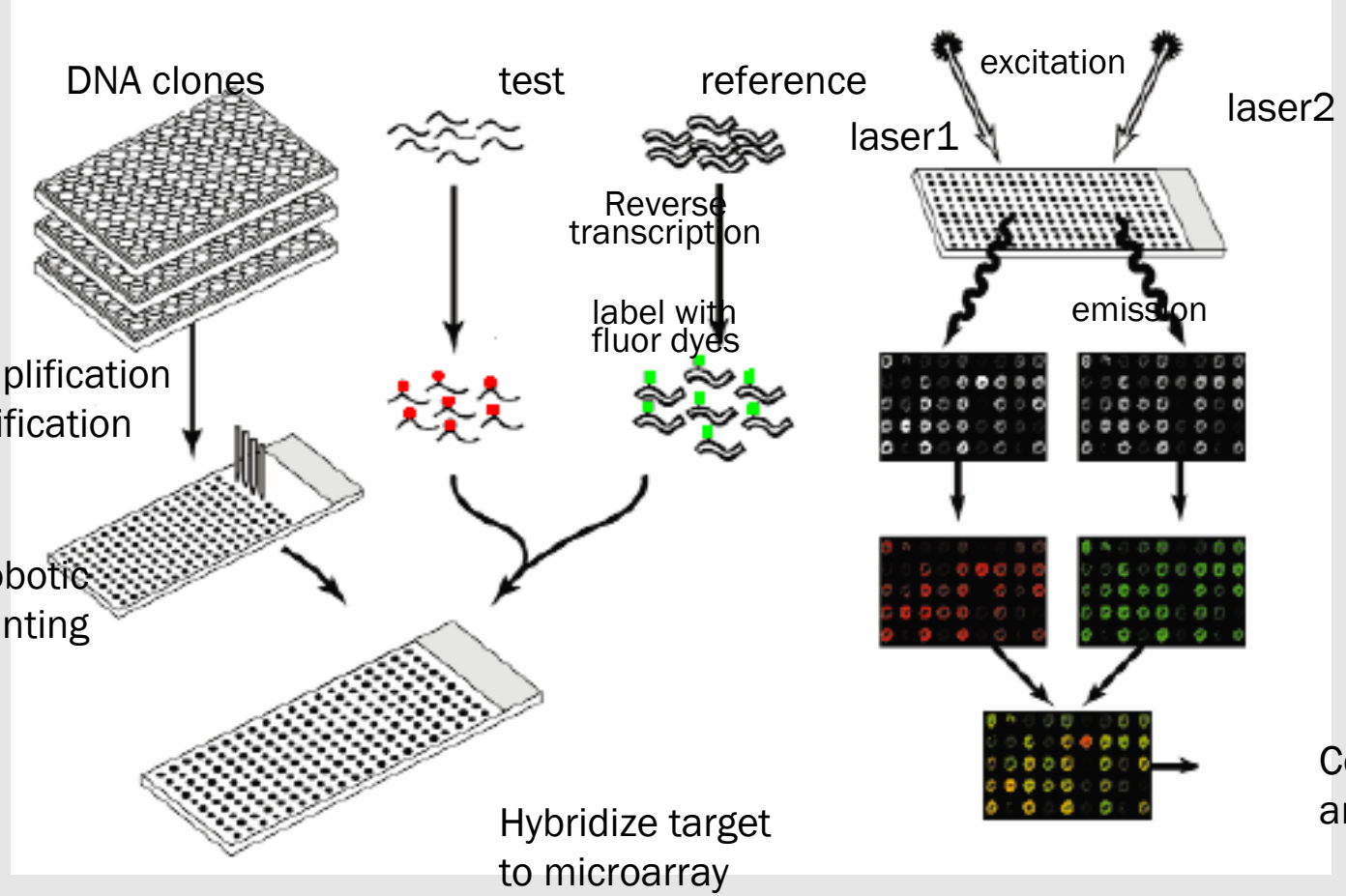
Gene의 발현상태를 밝혀냄



Gene Expression의 다양성



C-DNA Chip for Gene Expression



Single Nucleotide Polymorphism (SNP)

Nucleotide Polymorphism: Difference in DNA sequence among individuals

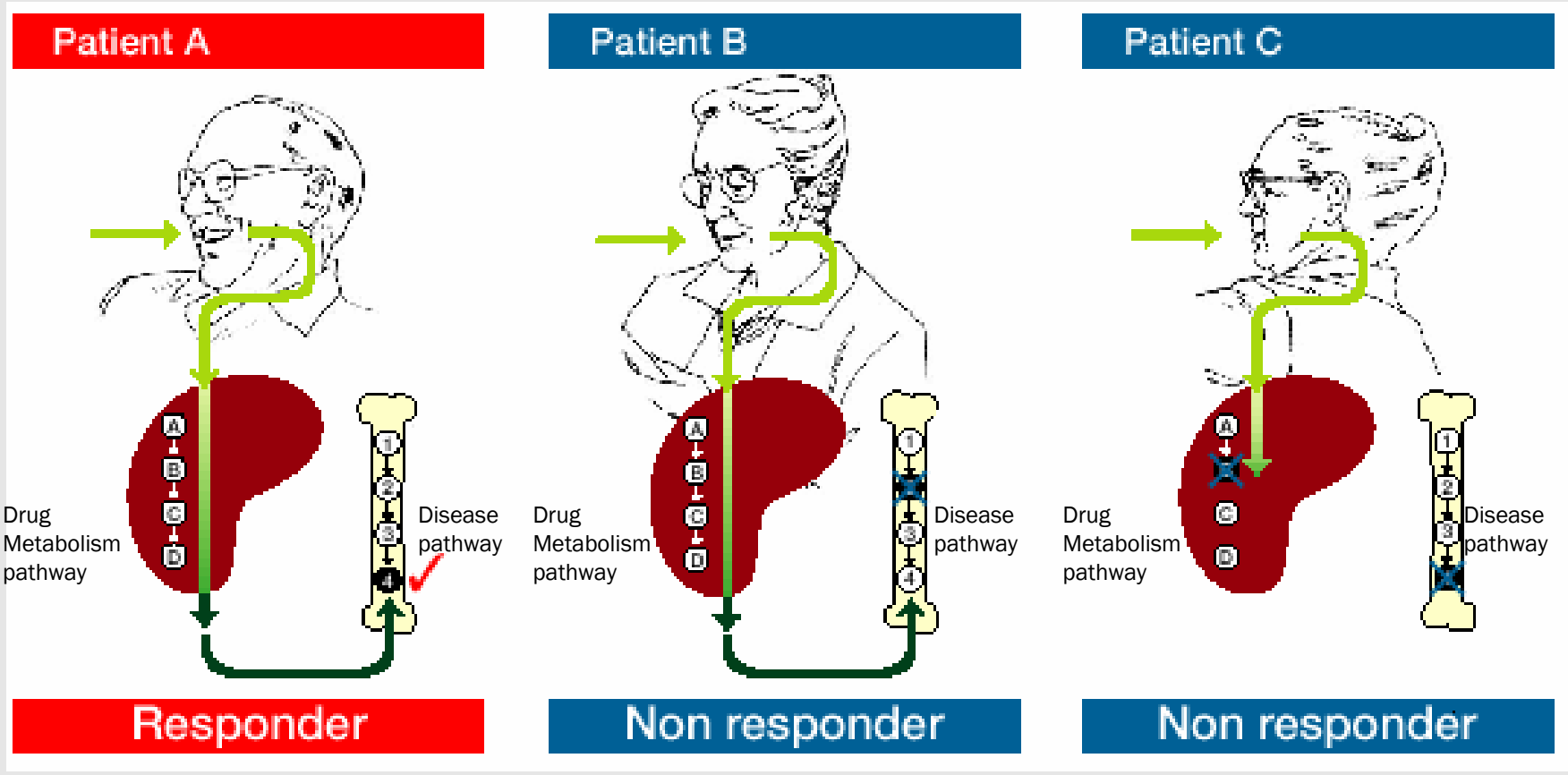
개개인의 Genome **중** 99.9 %**의 염기 서열은 서로 동일**,

단지 0.1 % **만이 개인마다 다름**

이 0.1 %**의 차이로 인해 사람마다 서로 다르게 특정 약** (drug)**에 대해 반응한다. 아울러 각 개인의 유전자 특성을 포괄적으로 확인하는 것이 경제적으로 가능하다.**

Pharmacogenetics

Pharmacogentics의 예



Personalized Medicine 시대의 도래

몇 방울의 피와 같은 소량의 샘플로 걸리기 쉬운 병과 진행 중인 병을 동시에 알아낼 수 있음

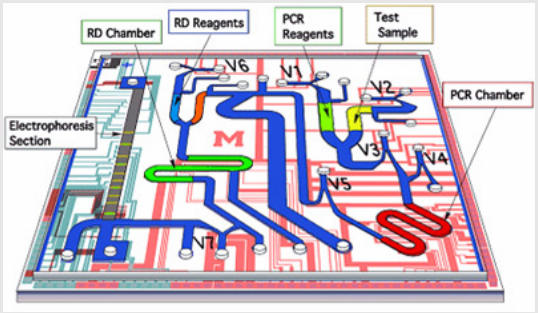
Personalized medicine 시대를 가능케 함

21세기 초반에는 실현가능

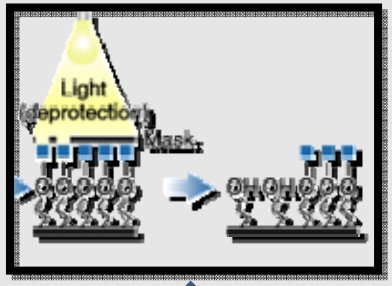
인간의 평균 수명이 100 세 정도까지 연장될 것이다.

The Core Technique of Bio Chip

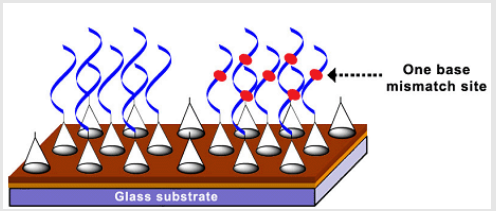
Integration



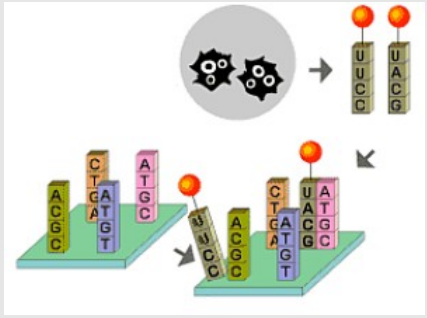
Photochemistry



Surface chemistry

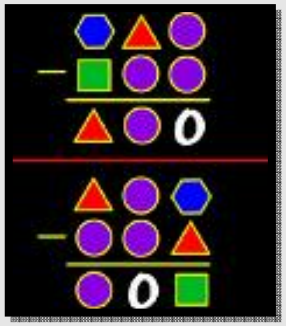


Data mining



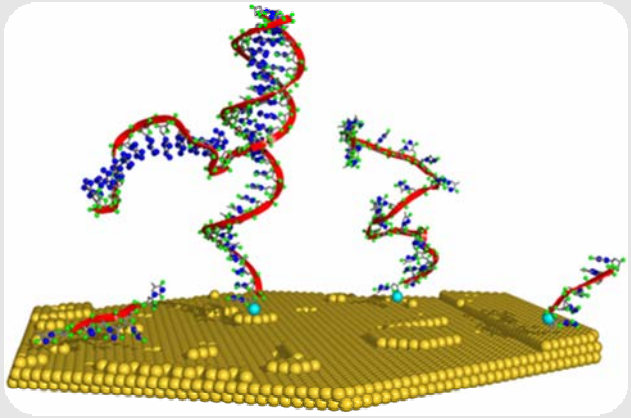
Bio Chip

Data processing

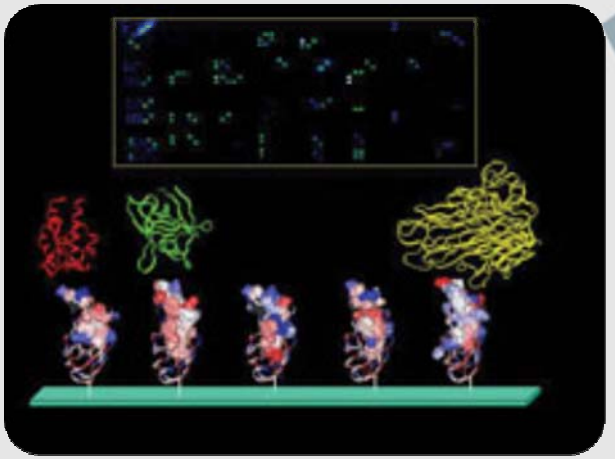


Analysis
- Fluorescence, electrochemistry

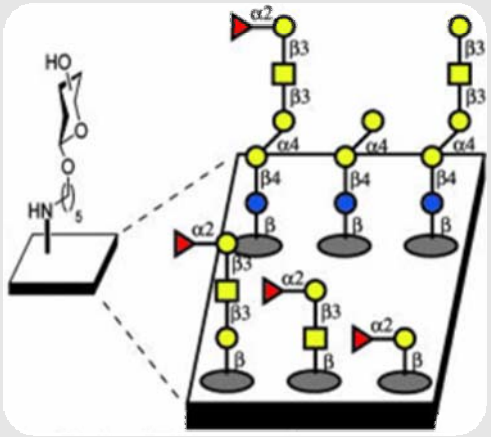
The Various Biochips



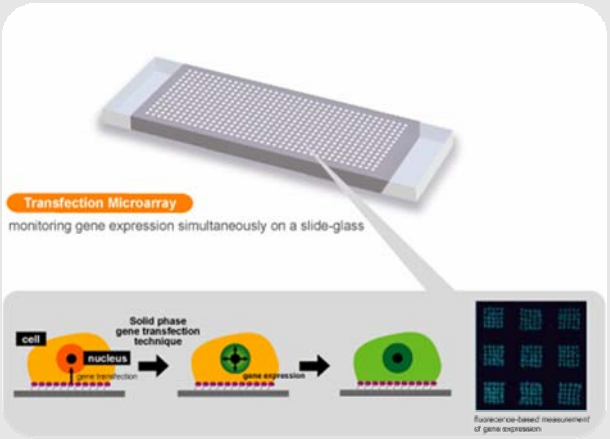
DNA chip – oligo, c-DNA, aptamer



Protein chip



Carbohydrate chip

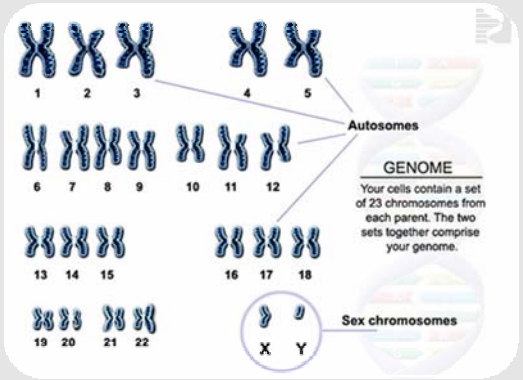


Cell chip

바이오칩 기술의 전망

- Whole genome analysis
  - Affymetrix, Agilent **등 고집적화 기술을 보유한 회사에 유리**
  - **새로운 기술이 떠 오르기 전까지 안정적**
- Human diagnosis
  - **신뢰도**, sensitivity, detection limit, **낮은** variation **등이 중요**
  - personalized medicine **시대의 도래**
- Point-of-care product
  - **의료**, **식품**, **농수산**, **애완동물**
  - **짧은 분석시간이 관건**

Blood glucose meter



범용화를 위한 과제

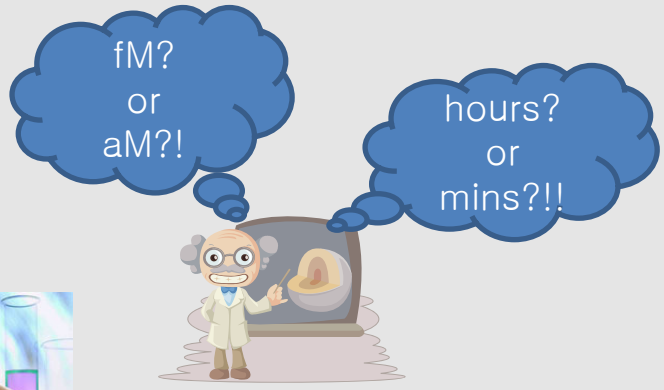
- Price of each chip, detector
- Detection limit

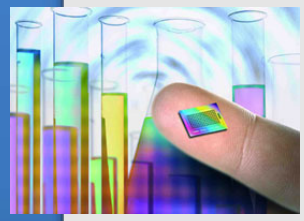
-PCR **과정 없이 소량의 샘플만으로도 분석이 가능**?

- **전체 분석에 걸리는 시간**
- **편의성**

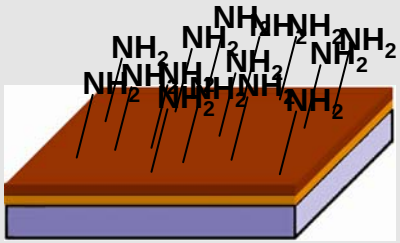
- lab-on-a-chip**의 성공**

- FDA **인증**
- **의사, 소비자들의 신뢰확보**

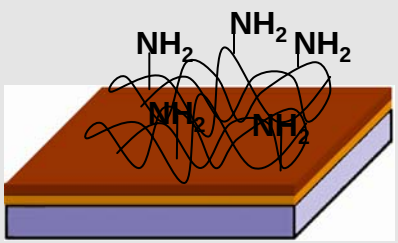




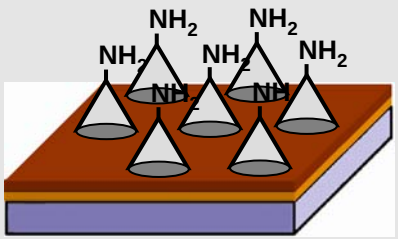
NanoCone Technology



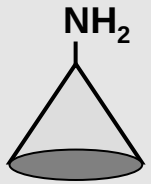
Aminosilane
Surface (2D)



Gel-like
Surface (3D)



NSB Amine Surface

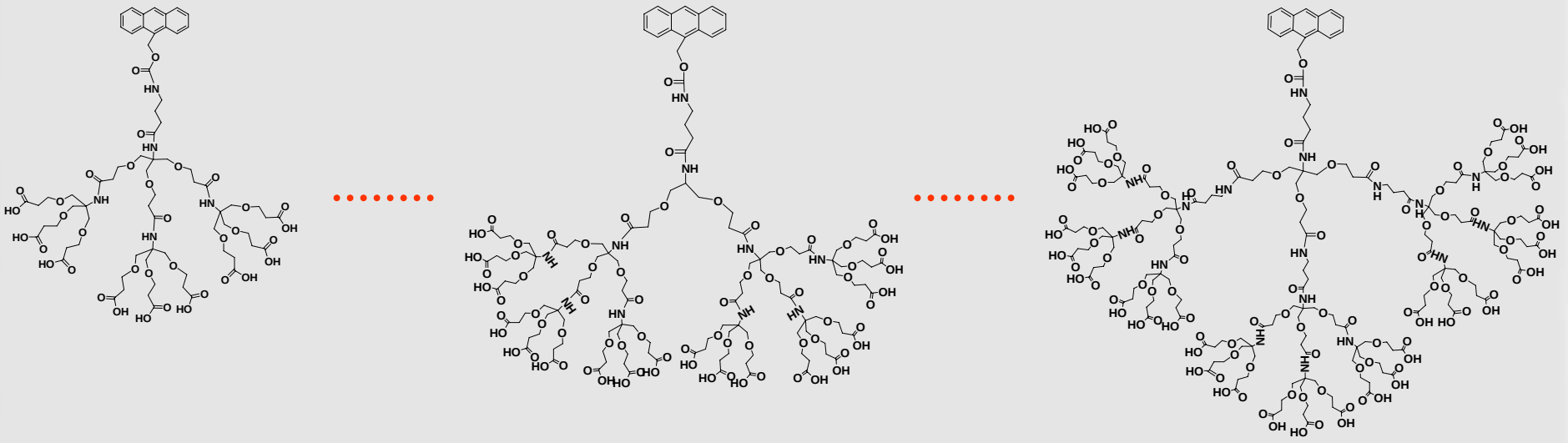


NanoCone

- **Competing technologies do not achieve homogeneity of surface-immobilized biomolecules.**
- **Severe steric hindrance and high non-specific binding result in low accuracy and low reproducibility eventually.**

- **Control of regular spacing between surface-immobilized biomolecules provides homogeneity and results in high accuracy and reproducibility.**
- **Minimized steric hindrance and low non-specific binding allow biomolecules to mimic solution-phase behavior.**

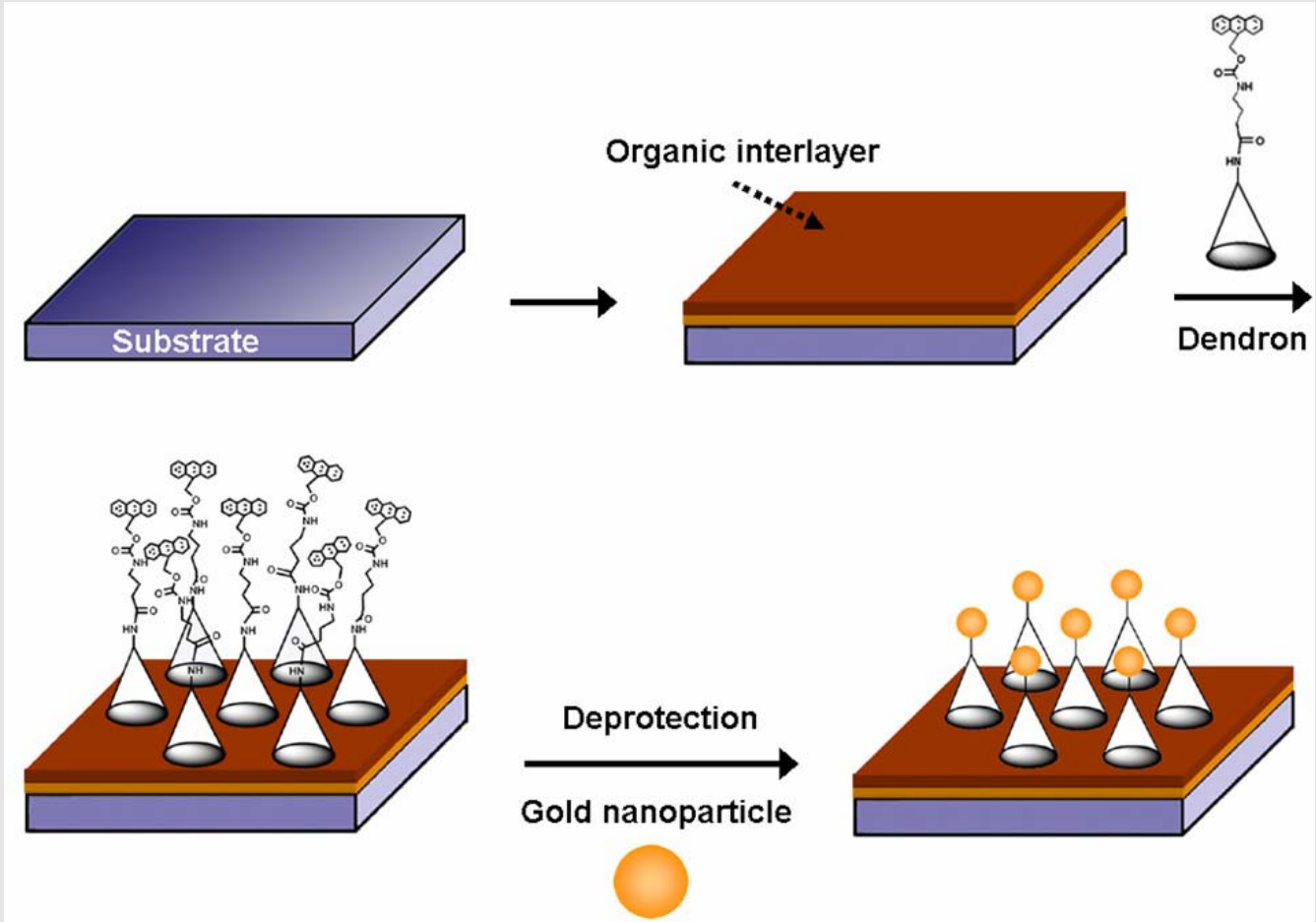
Controlled Spacing up to 10 nm



[9]-acid **[18]-acid** **[27]-acid, [81]-acid**

~ 3 nm **Spacing** **~ 10 nm**

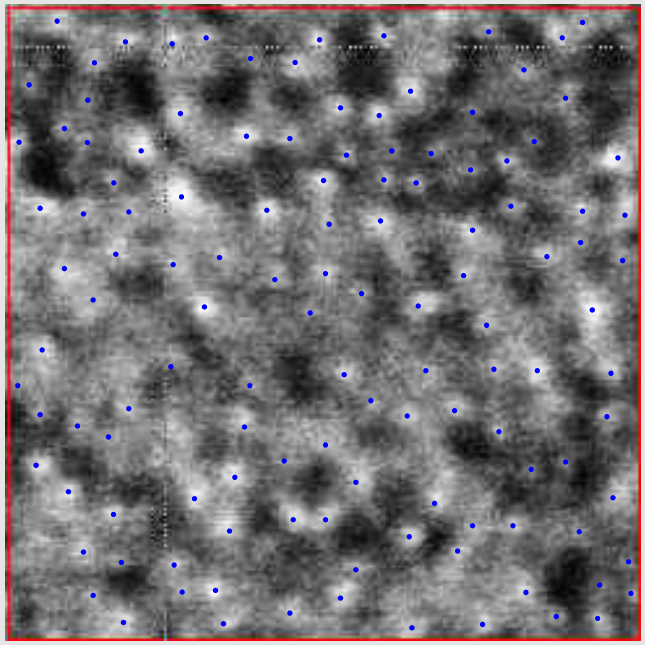
Immobilization of Gold Nanoparticles at the Dendron Surface



Langmuir, *21*, 4257 (**2005**).

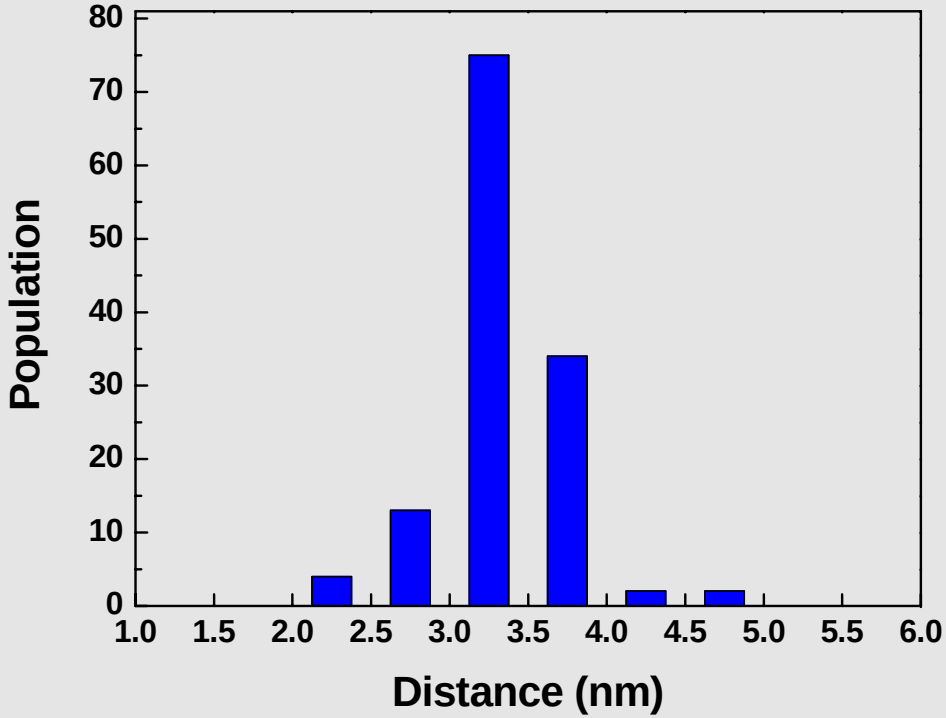
Spacing between Dendron Molecules on Surface

SEM Image



- Particle number / area
  : 130 ea / 50 x 50 nm^2
- Density: 0.05 – 0.06 ea/nm^2

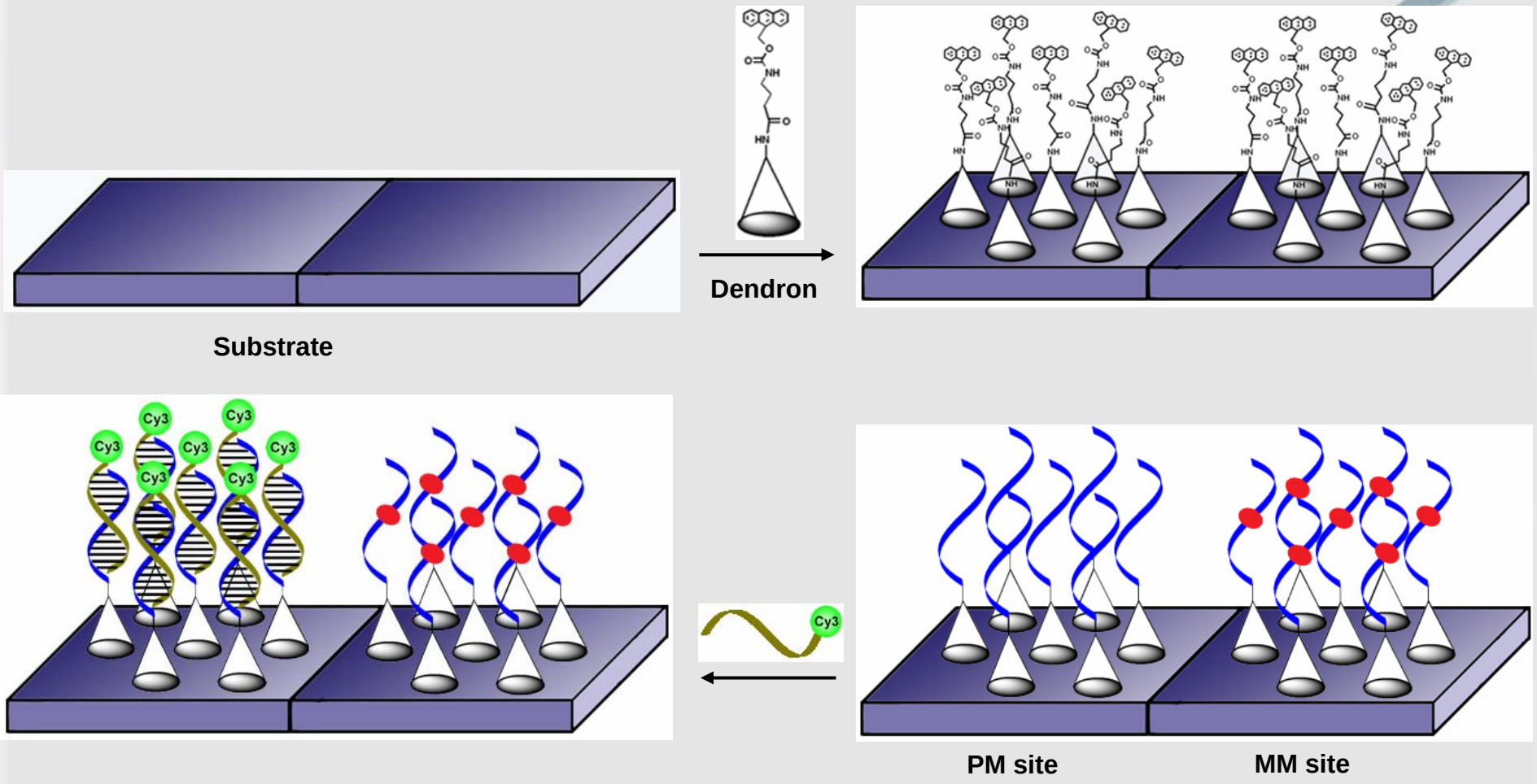
Distance between Dendron Molecules



- Average distance: 3.2 nm
- Standard deviation: 0.4 nm

DNA Microarray on the Dendron Surface Improves Significantly Detection of Single Nucleotide Variations

Fabrication of DNA Microarray on Dendron-Modified Surface



Nucleic Acids Research, *33*(12), e106 (**2005**).

Comparison of Discrimination Efficiency (I)

NSB Slide

Competitor Slide

Slide	PM	Deletion			Inser	2nd mismatch			Internal mismatch		
		EndA	2nd C	Int A		GG	GT	GA	TT	TG	TC
NSB	100	10	0.4	0.1	0.4	0.4	0.3	0.4	0	0.5	0
Competitor	100	30	15	4	9	22	21	22	6	22	0.3

Probe oligonucleotide

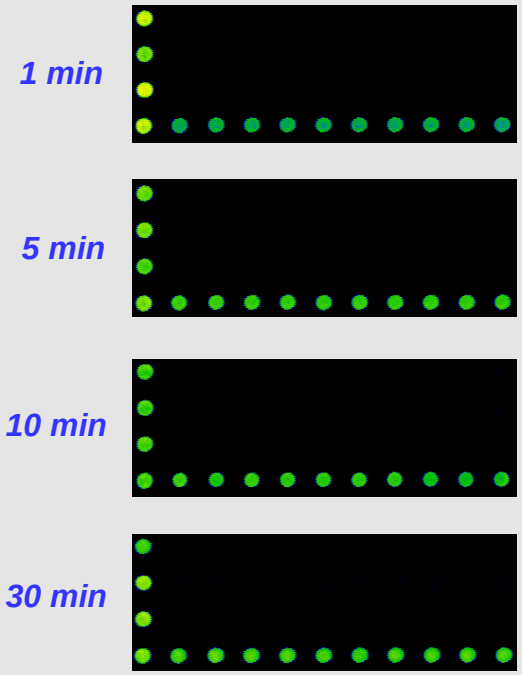
IMM: 5´-NH2-C6-CAT TCC GXG TGT CCA-3´
2nd MM: 5´-NH2-C6-CAT TCC GAG TGT CYA-3´
Insertion: 5´-NH2-C6-CAT TCC GAAG TGT CCA-3´

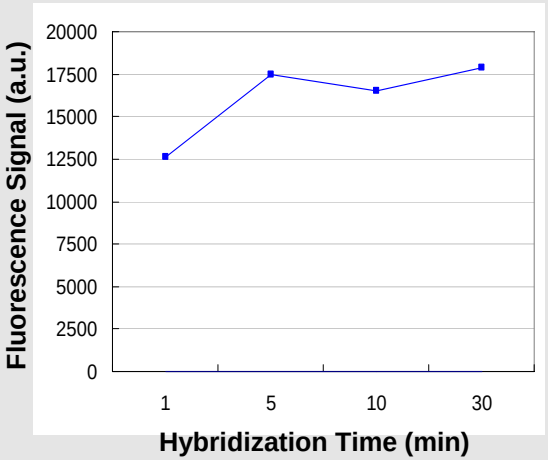
Deletion Int: 5´-NH2-C6-CAT TCC G_G TGT CCA-3´
Deletion 2nd: 5´-NH2-C6-CAT TCC GAG TGT C_A-3´
Deletion End: 5´-NH2-C6-CAT TCC GAG TGT CC_-3´

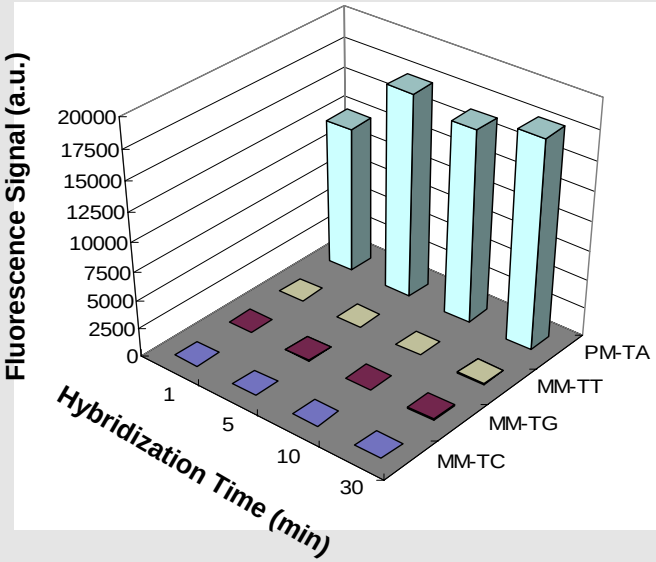
Target: 5´-Cy3-TGG ACA CTC GGA ATG-3´

Hybridization: 1 nM target at 45 °C for 1 h, 1 min washing at 50 °C

Fast & Highly Specific Hybridization



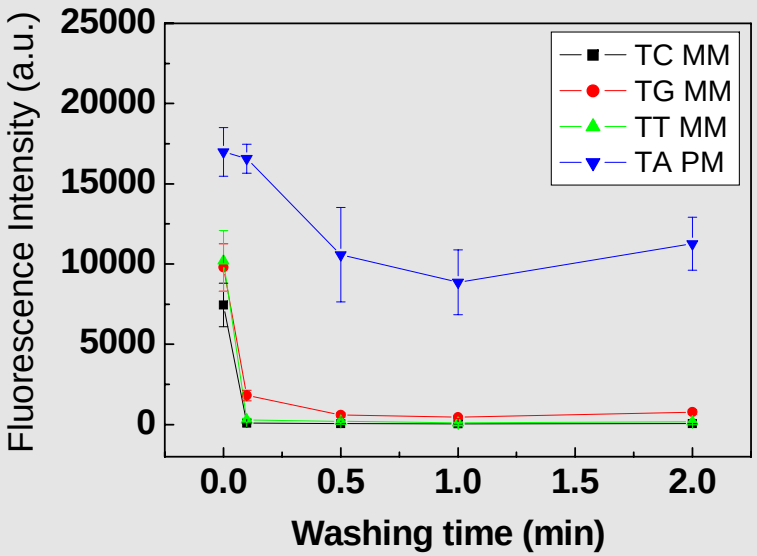




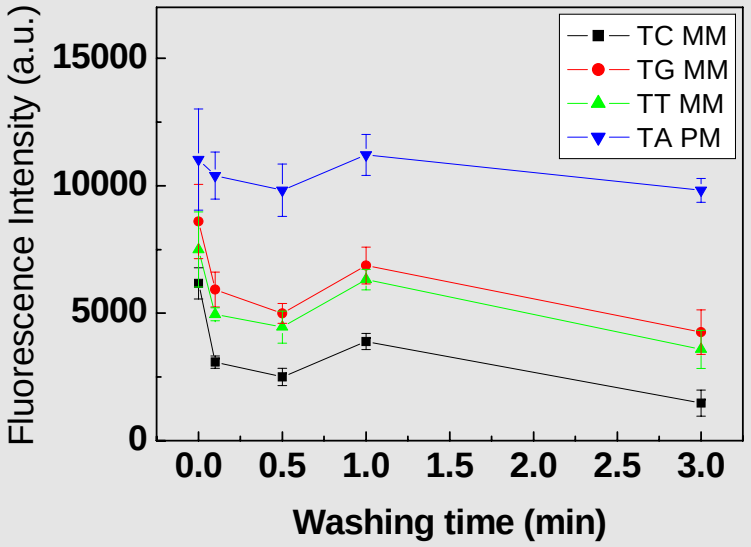
➢ Detection of single nucleotide variation in model system depending on hybridization time. All probe spots are repeated 10 times horizontally and four spots in the first column from the left are position markers which indicate each position of spotted probes.

Short Washing Time

Dendron Modified Surface



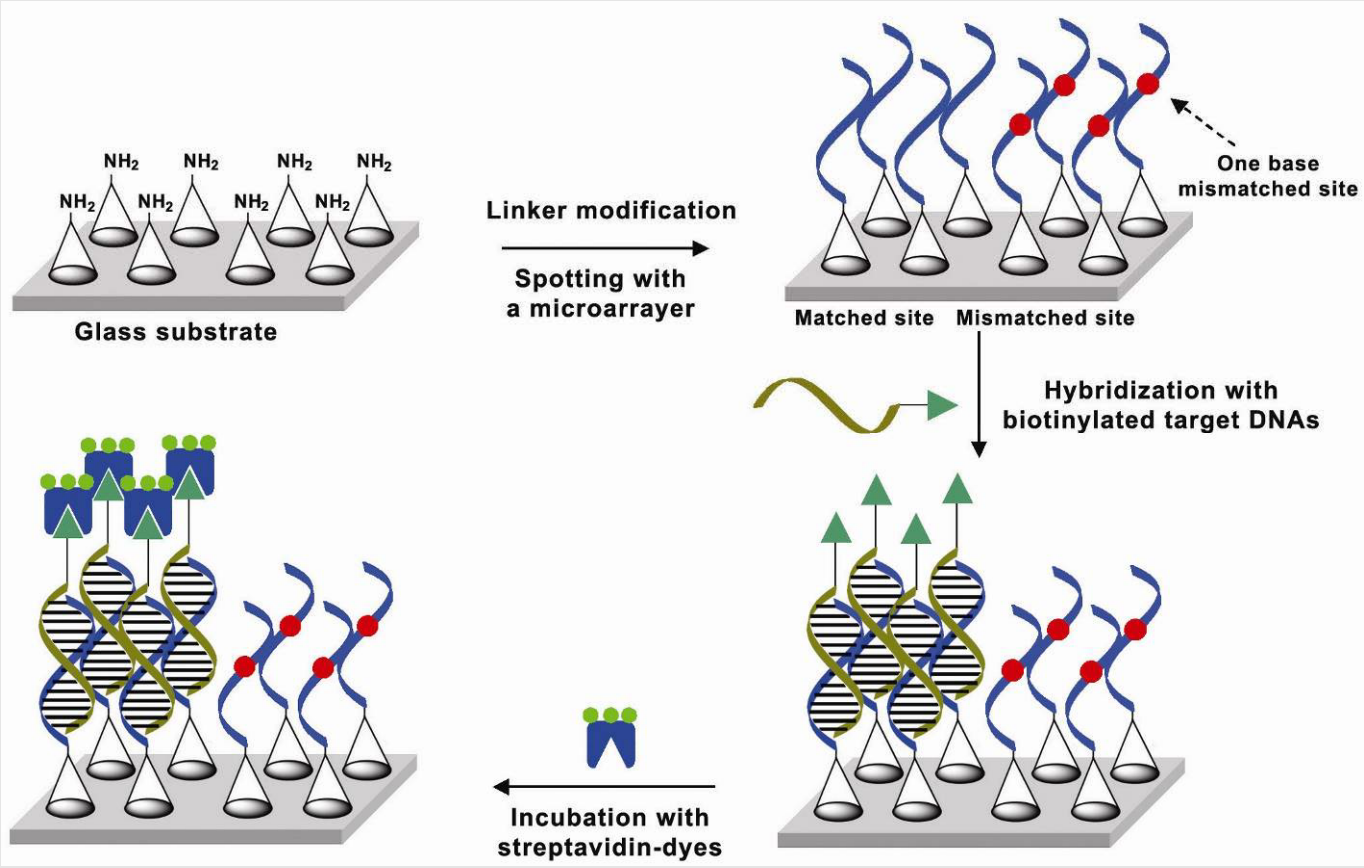
Generic Amine Surface



Hybridization: at 45 °C for 1 h, washing at 37 °C

Nucleic Acids Research, *33*(12), e106 (**2005**).

Application of Streptavidin-Dyes Conjugate

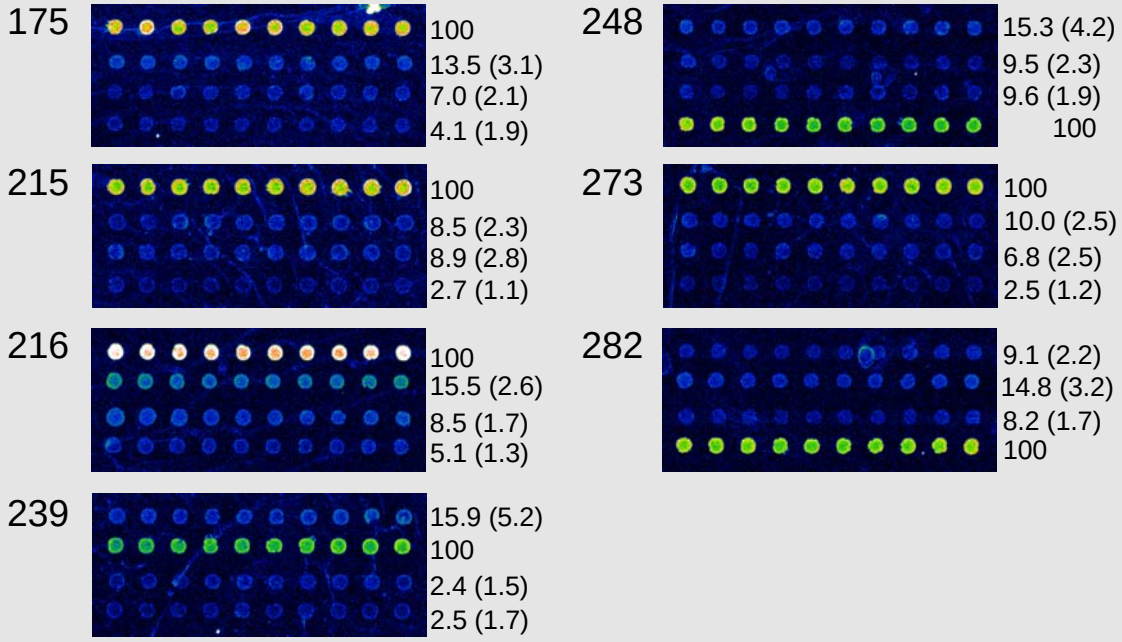


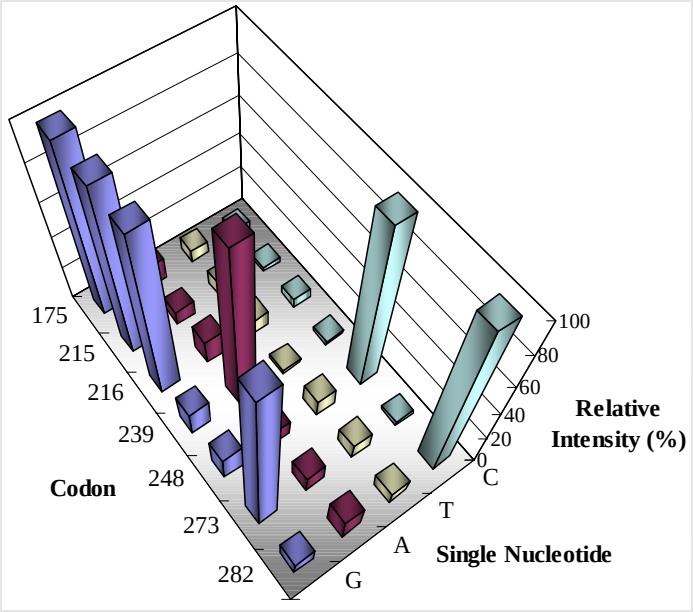
Detection limit: 50 fM

Biosensors and Bioelectronics, *22*(7), 1532 (**2007**).

DNA Microarray on the Dendron Surface Improves Significantly Detection of Single Nucleotide Variations in p53 Gene

Simultaneous Detection of 7 Hotspots of p53 Gene





Intensity less than 16% was observed for the all mutations.

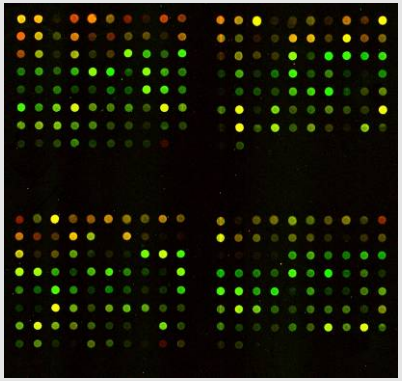
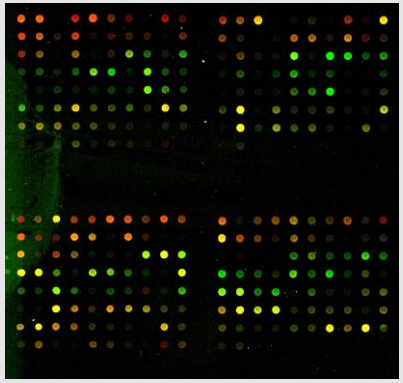
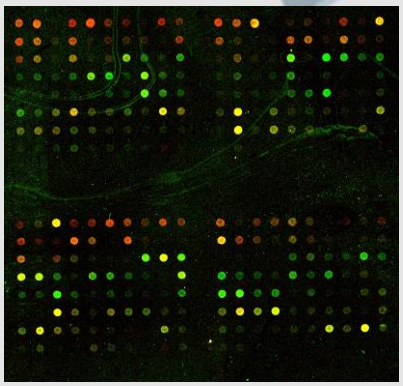
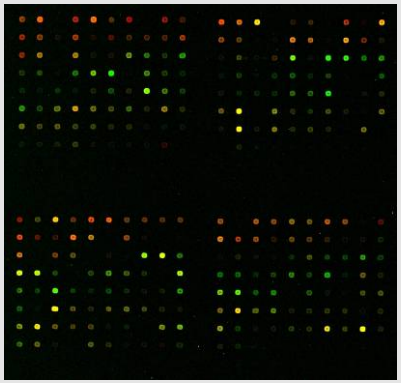
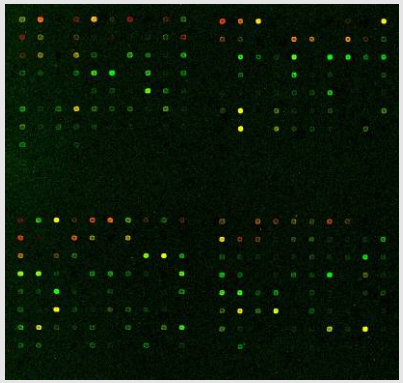
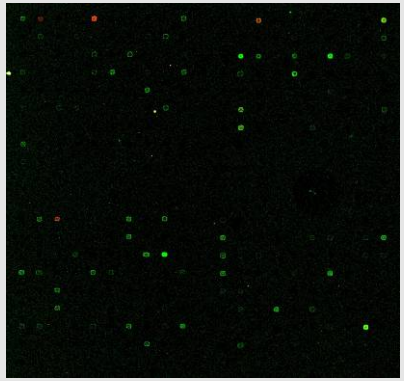
Nucleic Acids Research, *33*(10), e90 (**2005**).

NanoCone Surface Validation for Gene Expression DNA Microarray

NSB9 Amine Slides Vs Corning Slides (UltraGAPS™)

*** The following data were acquired in-house and through a contract research organization**

Extremely High Sensitivity

	50 µg total RNA	10 µg total RNA	1 µg total RNA
NSB9 Amine Slide			
Corning UltraGaps slide			

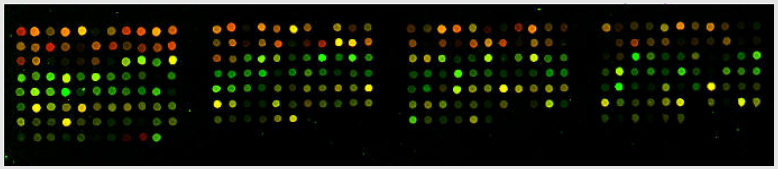
293 RNA = Cy3, HeLa RNA = Cy5

Strong background signal

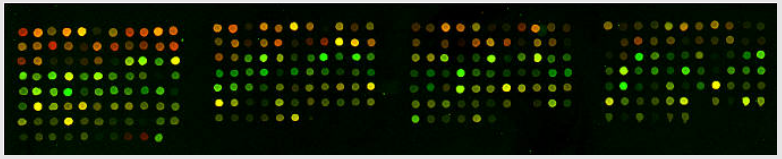
Lots of missing data

No Blocking Process

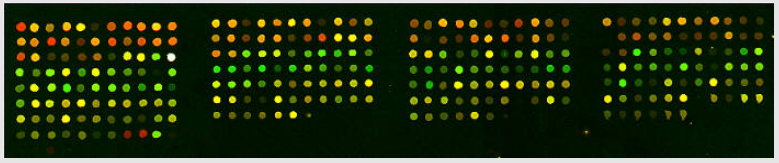
No blocking, 42°C with formamide



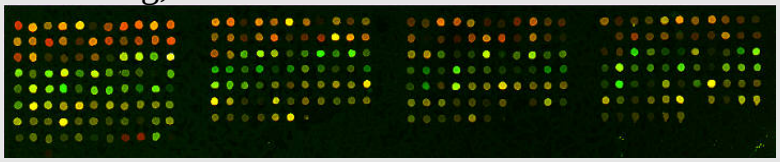
Blocking, 42°C with formamide



No blocking, 65°C



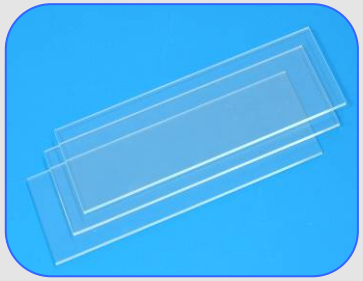
Blocking, 65°C



- POSTECH slide; No blocking, 65°C
  → High quality image

- For gene expression analysis with NanoCones slides, blocking agent was not needed, while use of BSA was required for Corning slides.

PRODUCTS



Surface functional group	Lateral spacing between surface functional groups	
	3 - 4 nm	6 - 7 nm
Amine	**NSB9 Amine Slide**	**NSB27 Amine Slide**
Epoxy	NSB9 Epoxy Slide	NSB27 Epoxy Slide
Aldehyde	NSB9 Aldehyde Slide	NSB27 Aldehyde Slide

NSB9 Amine Slide

SPECIFICATION

- **Ideal surface for oligonucleotide microarrays for SNP genotyping or gene expression profiling**
- **The outer surface is functionalized with amino group**
- **Lateral spacing between amino groups: *3 - 4 nm***
- **Density of amino groups: *0.05 - 0.06 ea/nm^2***
- **Size: 1" x 3“**

FEATURES

- **Optimized spacing for DNA hybridization**
- **Minimized steric hindrance & electrostatic repulsion**
- **High specificity & sensitivity**
- **Fast DNA hybridization**
- **High hybridization efficiency**
- **Low background signal**
- **Small amount of total RNA needed**

NSB27 Amine Slide

SPECIFICATION

- **Applicable to microarrays with cDNA, protein, peptide, aptamer, etc.**
- **The outer surface is functionalized with amino group**
- **Lateral spacing between amino groups: *6 - 7 nm***
- **Density of amino groups: *~ 0.01 ea/nm^2***
- **Size: 1" x 3"**

FEATURES

- **Larger spacing for big biomolecules**
- **Minimized steric hindrance & electrostatic repulsion**
- **High specificity & sensitivity**
- **Fast bimolecular interaction**
- **High binding efficiency**
- **Low background signal**