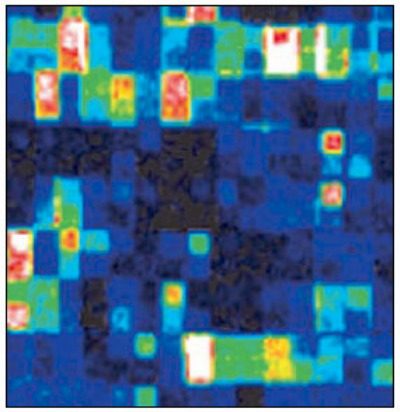
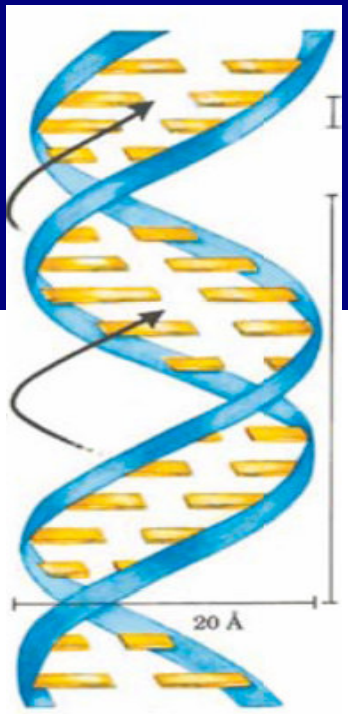
DNA Hybridization Detection

- ***Electrochemiluminescence based DNA biosensor***
- ***DNA microarray***





Minjeong So

Chem 395

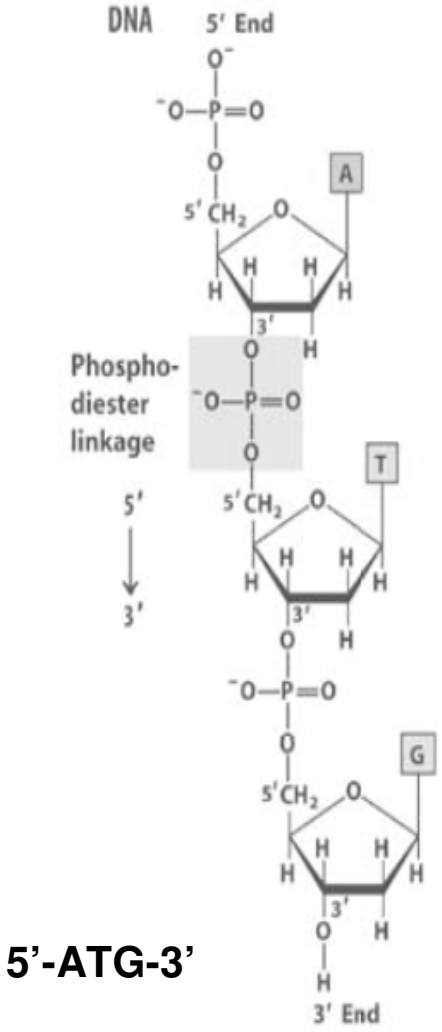
Outline

- Basic DNA and Hybridization
- Electrochemiluminescence(ECL) based DNA biosensor
  - DNA hybridization detection at high amplification with $Ru(bpy)_3^{2+}$ (*Anal.Chem, 2004, 76, 5379*)
- DNA Microarray

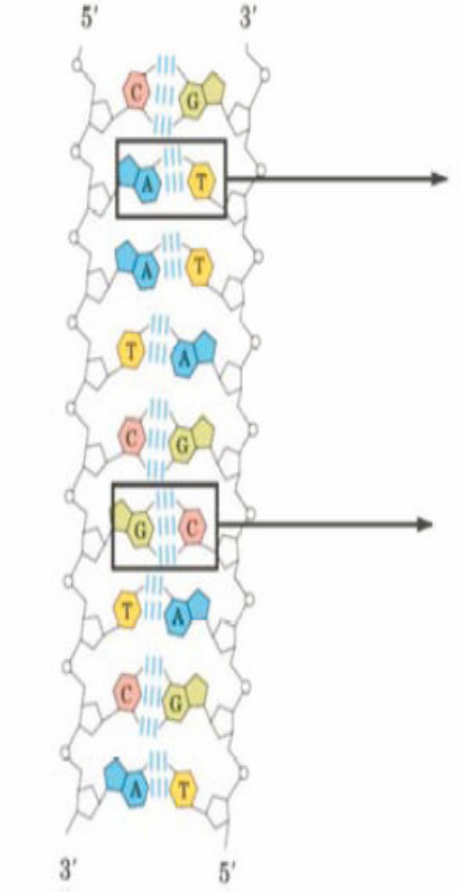
DNA Hybridization

- Diagnostic test for mutations
- Monitoring gene expression (sequence)
- Screening for targets known to play a role in disease
- Assessment of medical treatment
- Environmental investigations
- Biological warfare agent detection

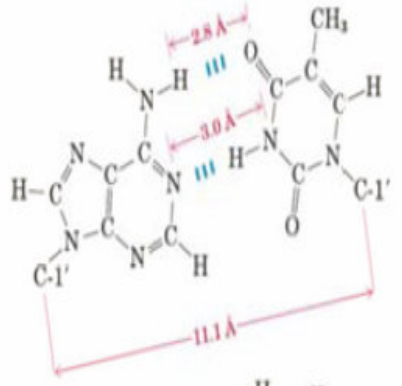
Basic DNA

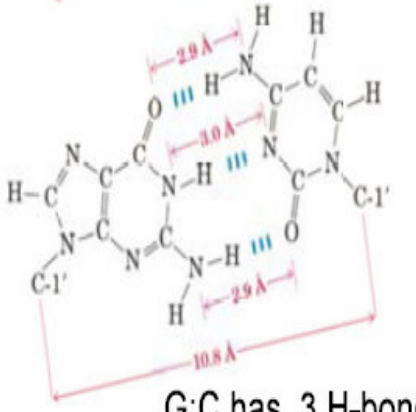


5’-ATG-3’



A:T has 2 H-bonds

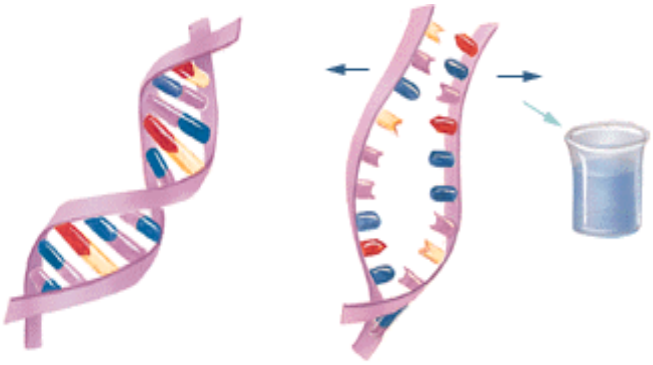




G:C has 3 H-bonds

A:T and G:C distance is (almost) the same

How Genetic Sequencing Works in DNA biosensor

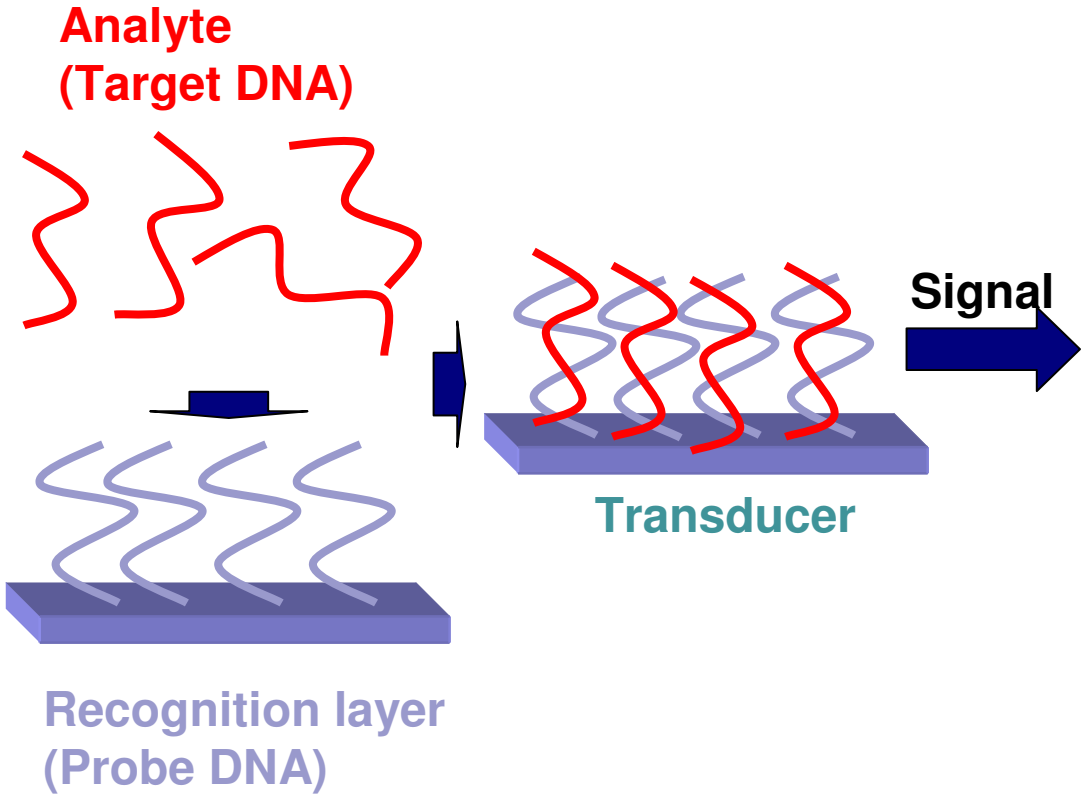


Separate ds-DNA (Probe DNA).
DNA is denatured by heat or chemical denaturant and placed in solution or on a solid substrate, forming a reference segment

Introduce unknown ss-DNA (Target DNA)
Unknown DNA sample is introduced to the reference segment. The complement of the reference segment will hybridize to it.

http://www.devicelink.com/ivdt/archive/98/09/009.html

How Hybridization is Identified



Electrochemical devices
Current signal of a redox indicator

Optical devices
Emission signal of fluorescent or chemiluminescent lables
Surface optical properties
Nanoparticle based colorimetric detection

Mass-sensitive devices
Frequency signal of oscillating crystal with DNA probe

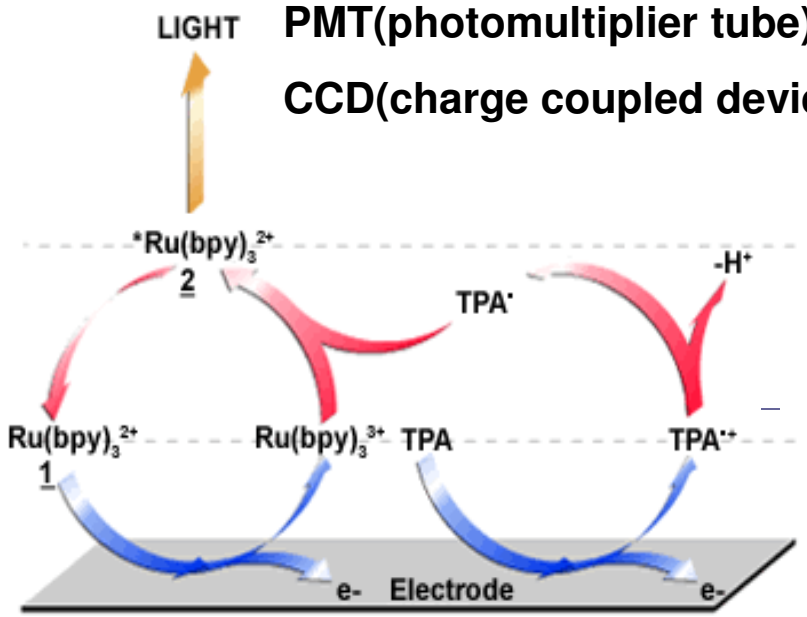
DNA Hybridizaion biosensor

- Immobilization of ss-DNA probe onto the transducer surface

- **Tranducing (association of an appropriate hybridization indicator)**

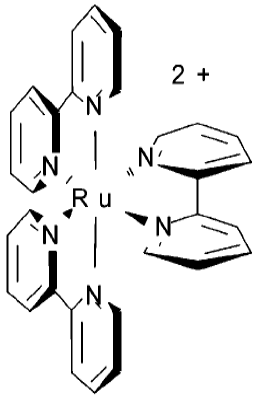
Joseph Wang, Nucleic Acids Research, 2000, 28(16), 3011

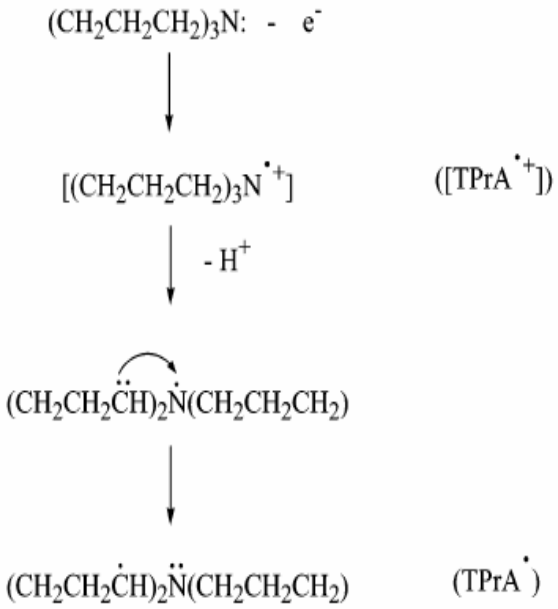
Electrochemiluminescence Process



PMT(photomultiplier tube)

CCD(charge coupled device)





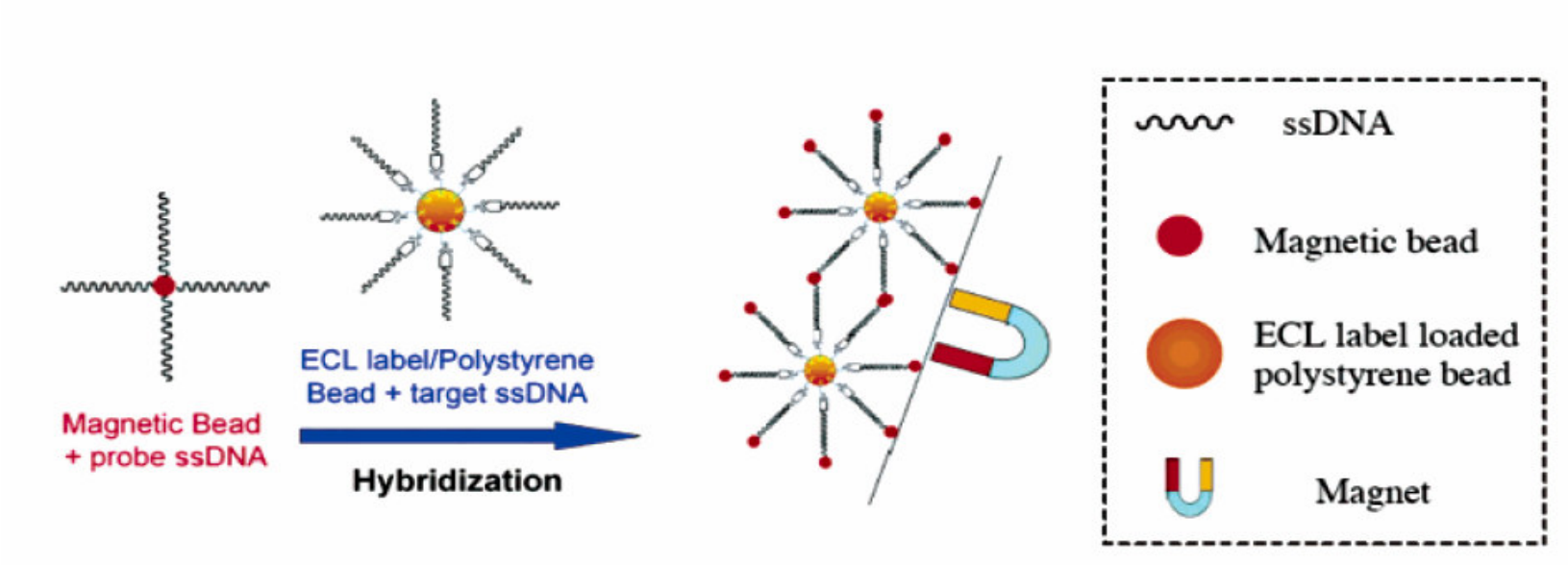
Proposed mechanism for $Ru(bpy)_3^{2+}$ /TPrA ECL system

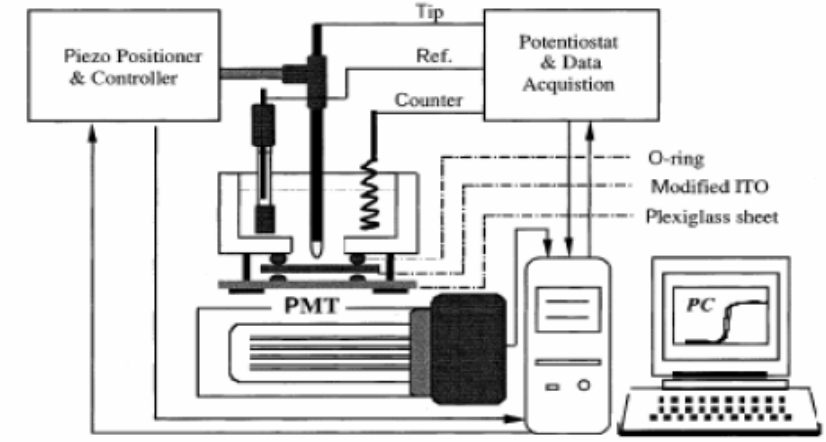
Proposed tri-n-propylamine oxidation sequence

$Ru(bpy)_3^{2+}$ /TPrA ECL system forms the basis of commercial system for immunoassay and DNA analysis.

http://www.bioveris.com/technology.htm, Richter, Chem. Rev.; 2004; 104(6); 3003-3036

DNA hybridization detection at high amplification with $Ru(bpy)_3^{2+}$





Miao, W.; Bard, A. J Anal. Chem. ; 2004; 76(18); 5379-5386.

DNA hybridization detection at high amplification with $Ru(bpy)_3^{2+}$ (continued)

1. Probe DNA–MB conjugate

- **Probe DNA** : 5'-[biotin-TEG]-AACGA TAGCT CCTAC ATTTG GAG-3'
  MB : streptavidin-coated superparamagnetic polystyrene beads

2. Target DNA-Ru/PSB/Avidin conjugate

1) Target DNA

-complementary, 5'-[biotin-TEG]-CTCCA AATGT AGGAG CTATC GTT-3' ***(t-ssDNA)***

- noncomplementary, 5'-[biotin-TEG]-TTAAC ACCTT AGCGA CGGCT AGT-3' ***(nc-ssDNA)***

- two base pair mismatched oligomer sequence,
  5'-[biotin-TEG]-CTCCA AA***C***GT AGGAG ***T***TATC GTT-3' ***(2-bp-m-ssDNA)***

2) ECL Label

: Tris(2,2'-bipyridyl)ruthenium(II) tetrakis(pentafluorophenyl)borate ($Ru(bpy)_3[B(C_6F_5)_4]_2$)

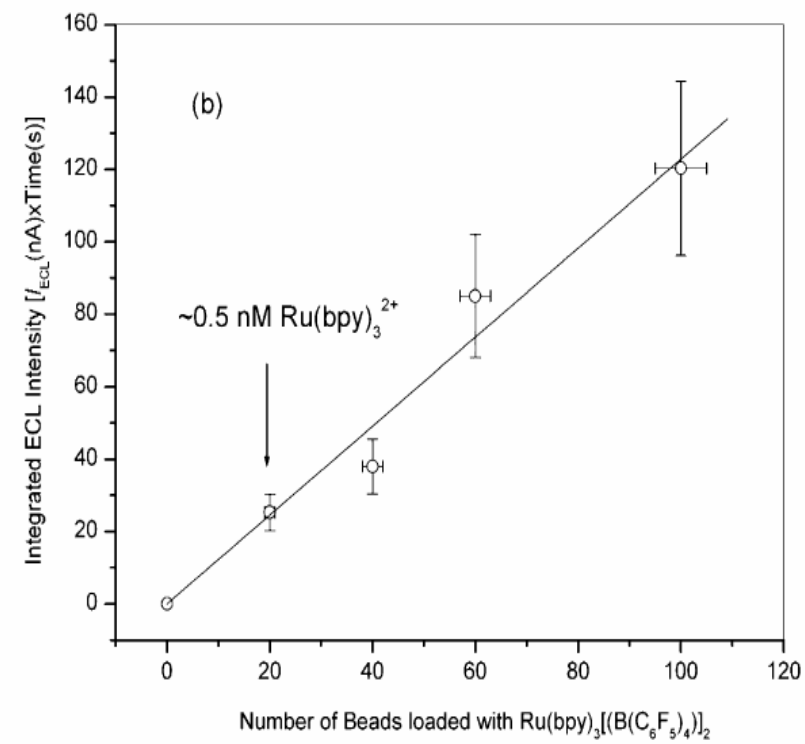
3) PSB :Carboxylate polystyrene microspheres

4) Immobilization Avidine on the surface Ru/PSB

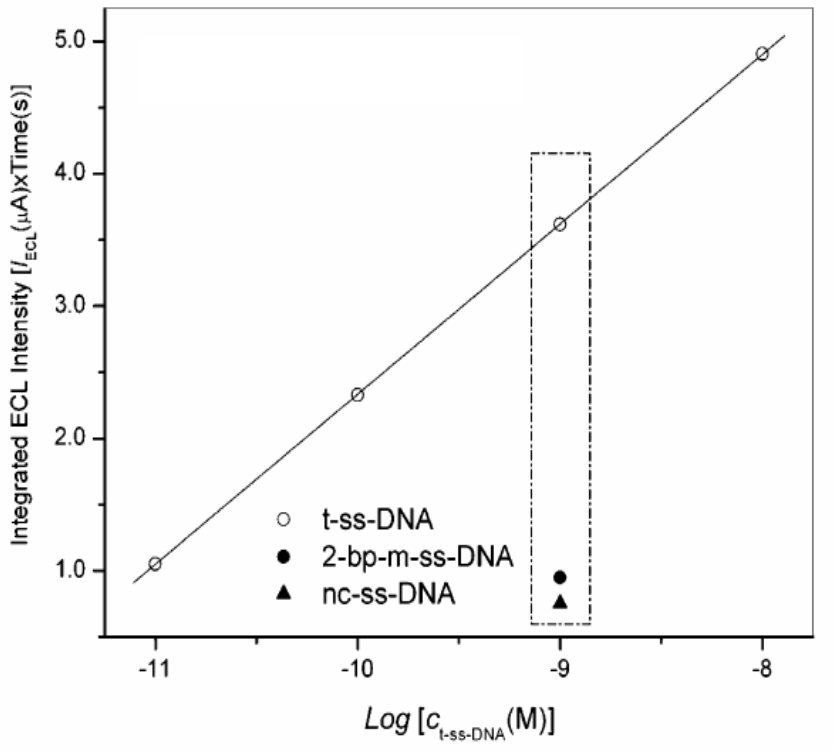
3. DNA Hybridization

- in the hybridization buffer
- Probe DNA conguage –Target DNA conguate aggregates were magnetically separated from the mixture containing free unbound Targe DNA conguate

DNA hybridization detection at high amplification with $Ru(bpy)_3^{2+}$ (continued)

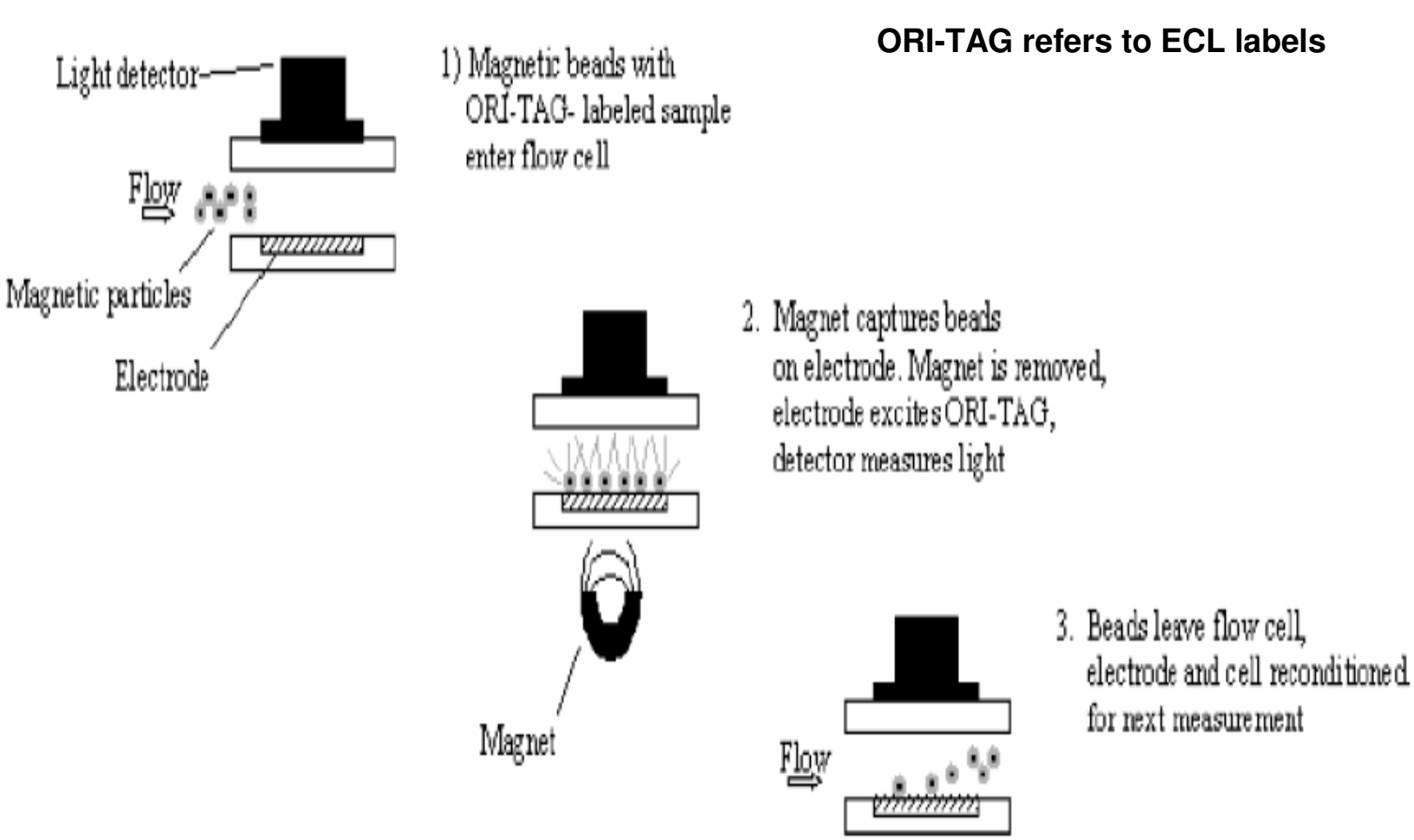


ECL intensity as a function of the number of 10- m diameter polystyrene beads loaded with $Ru(bpy)_3[B(C_6F_5)_4]_2$. The experiments were carried out in 0.50 mL of 0.10 M TPrA-0.055 M TFAA-0.10 $(TBA)BF_4$ MeCN-1% H_2O at a 2.2-mm diameter Pt electrode by applying CV potential sweeps between 0 and 3.0 V vs Ag/Ag+ at a scan rate of 50 mV/s.



ECL detection of DNA hybridization between probe DNA-MB and target DNA-Ru(II) PSB/avidin

Magnetic bead/flow cell ECL process (BioVeris Corp.)



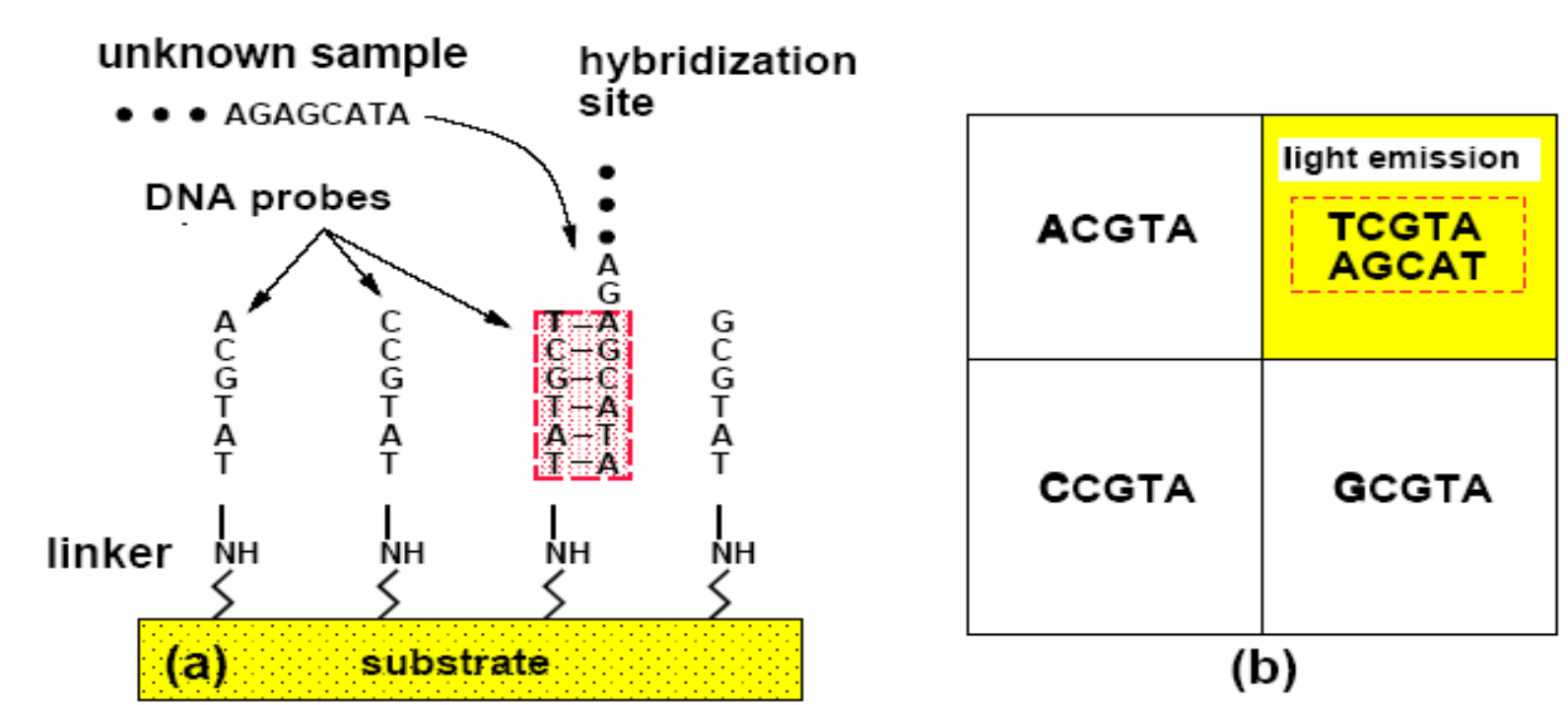
Richter, Chem. Rev.; 2004; 104(6); 3003-3036

High density DNA Microarray

- DNA microarrays, Oligonucleotide arrays, GeneChip arrays, DNA chips are all similar terms
- Revolution in the analysis of genetic information
- Hybridization is a highly parallel search by each molecule for matching partner on an affinity matrix.
- Specificity and affinity of complementary base pairing.
- Use of glass as a substrate, fluorescence for detection and the development of new technologies for synthesizing or depositing DNA have allowed the miniaturization of DNA arrays with increases in information content.

David J.Lockhart; Elizabeth A. Winzeler Nature, 2000, 405, 827

Simple Example of DNA Microarrays



(a) Example immobilized DNA probes showing hybridization of unknown(target) to specific probe.

(b) Probes are arranged a planar arrays. The hybridized regions can be detected by the fluorescence of the duplex.

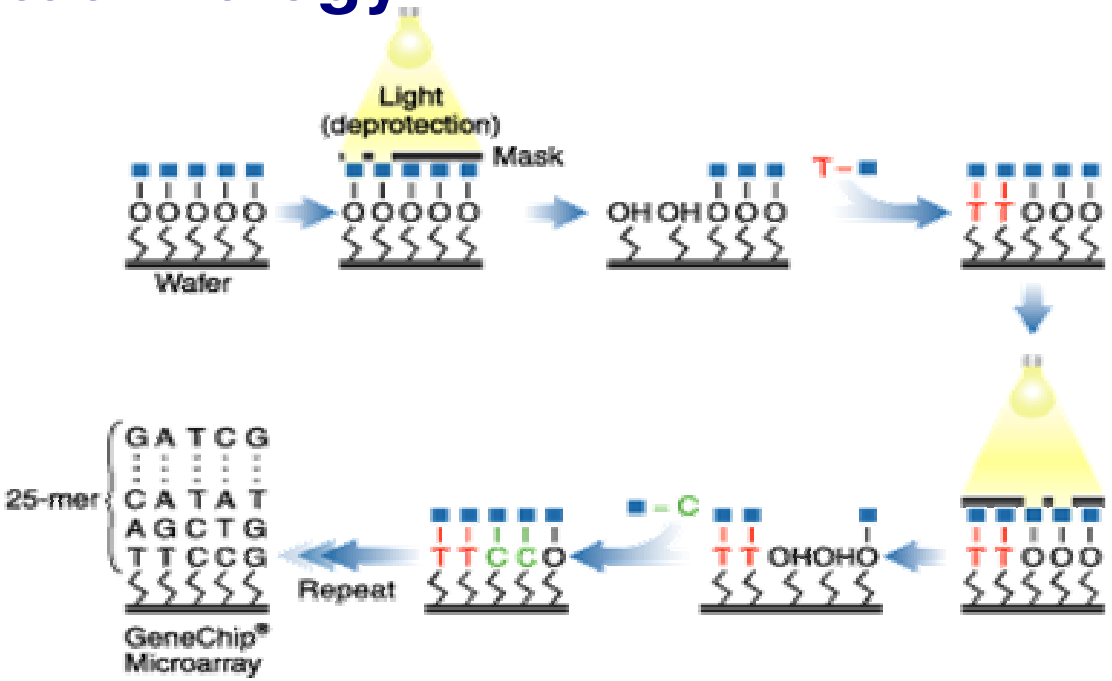
C Mastrangelo ,Adv. Sci. Technol, 1999

DNA Microarray Fabrication

- Robotic pin spotted microarray
- In situ microarray(Photolithographic method)
- Inkjet printing microarray
- Polymer photodeposition of microarray probe positions
- High density fiber optic microsphere-based microarray

Epstein J.R; Biran I; Walt D.R. Anal. Chimica. Acta 2002, 469, 3.

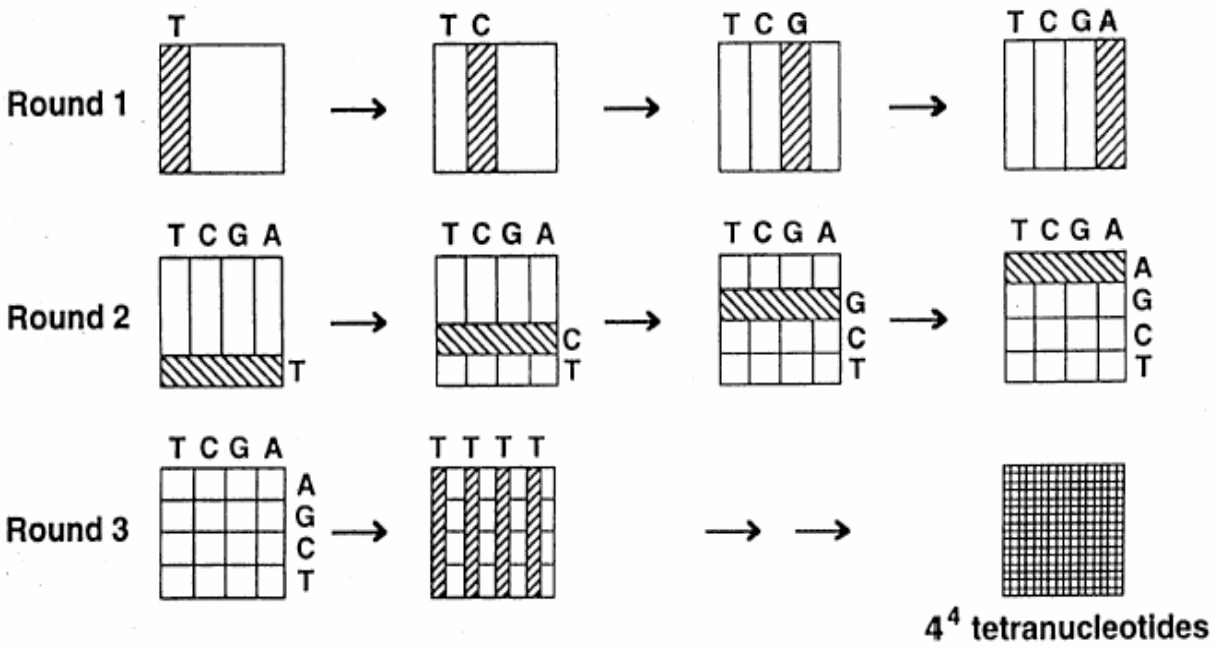
Affymetrix GeneChip array by photolithographic technology

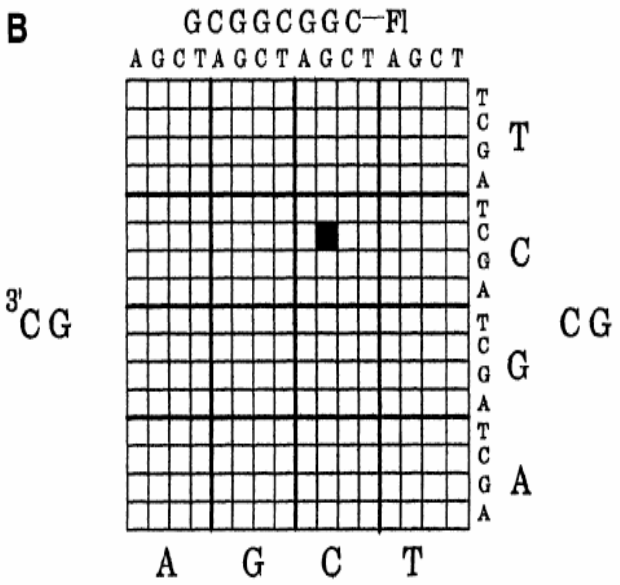


Light-directed synthesis of oligonucleotides

- A surface bearing photoprotected hydroxyls groups is illuminated through a photolithographic mask, generating free hydroxyl groups in the photodeprotected regions.
- The hydroxyl group are then coupled to a deoxynucloside phosphoramidite.
- A new mask pattern is applied, and a second photoprotected phosphoramidite is coupled.
- Rounds of illumination and coupling are repeated until the desired set of products is obtained.

http://www.affymetrix.com





Combinatorial synthesis of 4^4 tetranuclotides.

-In Round 1, one fourth of the synthesis area is activated by illumination through mask1 for coupling of the first nucleoside.

-In cycle 2 of round 1, mask 2 activates a different one-quarter section of the synthesis area and a different nucleoside is coupled.

-Further lithographic subdivisions of the array and chemical couplings generate the complete set of 256 tetranucleotides.

Hybridization of the target DNA : 5'-GCGGCGGC- fluorescein to this array
Complementary probe: 3'-CGCCGCCG (2698 counts)
Mismitches probes : 3'-CGCAGCCG(554 counts); 3'-CGCCGACG(317 counts)

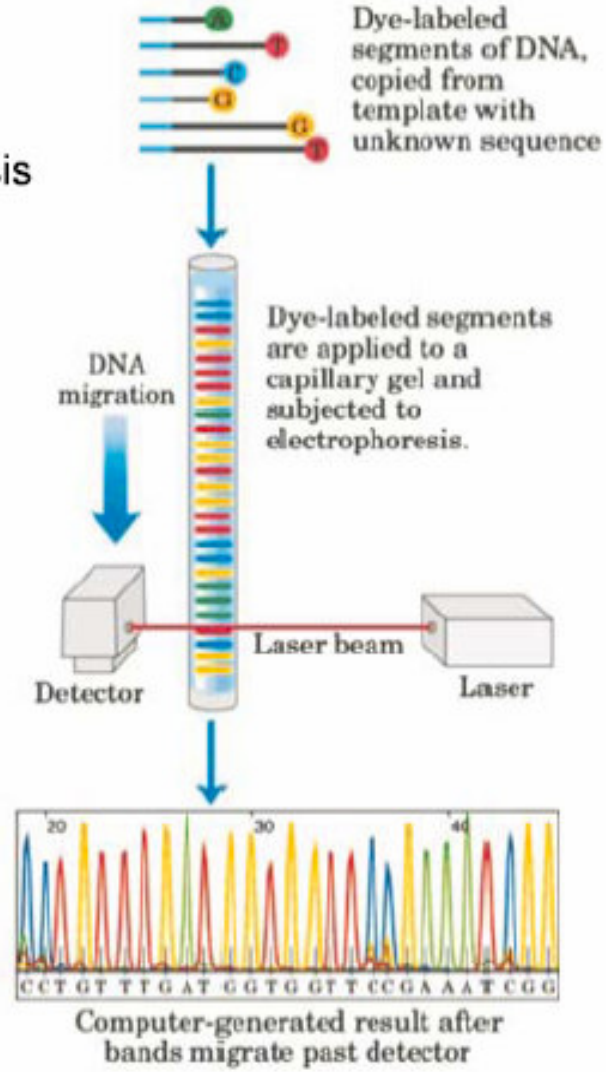
Proc.Natl.Acad.Sci, 1994, 91, 5022

Automated Sequencing: Fluorescent ddNTPs

One pot reaction

Slab or capillary gel electrophoresis

> 10^3 bases/day



Conclusion and Future(DNA Microarray)

- Data can generated in a high throughput, parallel fashion.
- Systematic examination and classification of biological processes
- DNA microarray can detect primary DNA sequences, gene expression and physiological responses.
- From specific single-base mismatch identification to global expression analysis
- The trend toward miniature probe

- high throughput design generating more information simultaneously
- low volume sampling and faster target diffusion rates
- full genomic screening and analysis capabilities in a single assay