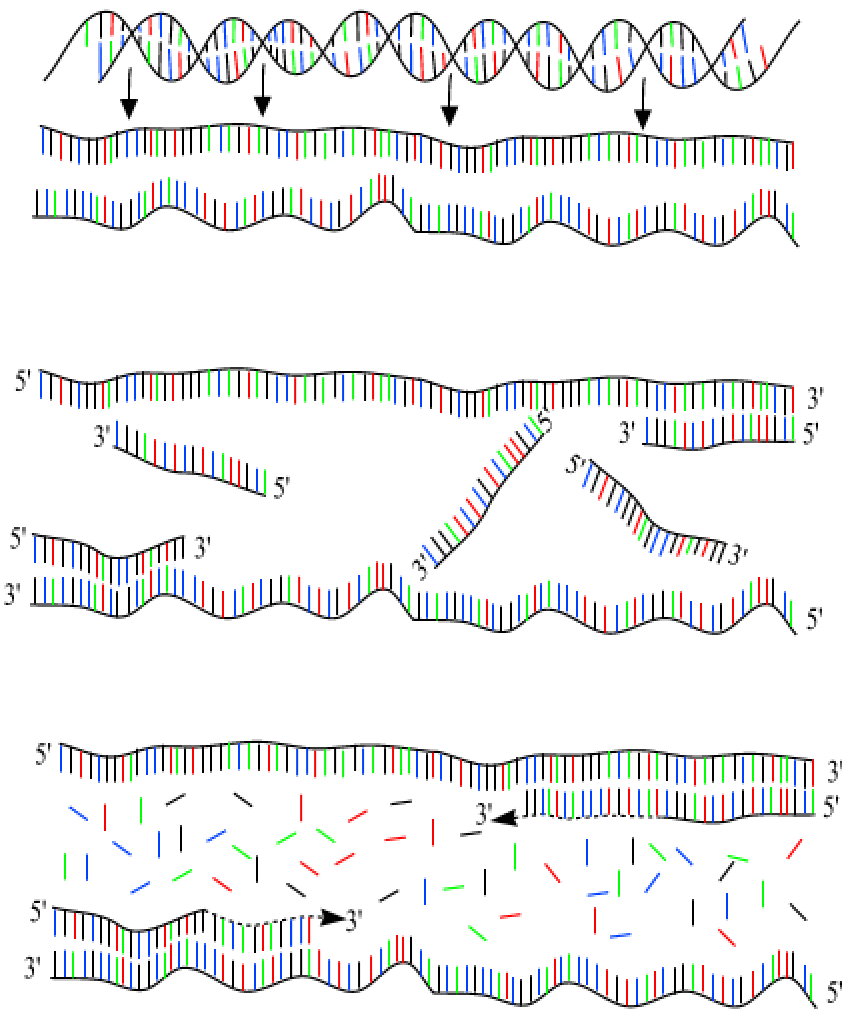
Second letter

First letter	U		C		A		G		Third letter
U	UUU UUC UUA UUG	Phe Leu	UCU UCC UCA UCG	Ser	UAU UAC UAA UAG	Tyr Stop Stop	UGU UGC UGA UGG	Cys Stop Trp	U C A G
C	CUU CUC CUA CUG	Leu	CCU CCC CCA CCG	Pro	CAU CAC CAA CAG	His Gln	CGU CGC CGA CGG	Arg	U C A G
A	AUU AUC AUA AUG	Ile Met	ACU ACC ACA ACG	Thr	AAU AAC AAA AAG	Asn Lys	AGU AGC AGA AGG	Ser Arg	U C A G
G	GUU GUC GUA GUG	Val	GCU GCC GCA GCG	Ala	GAU GAC GAA GAG	Asp Glu	GGU GGC GGA GGG	Gly	U C A G

PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

1 minut 94 °C

Step 2 : annealing

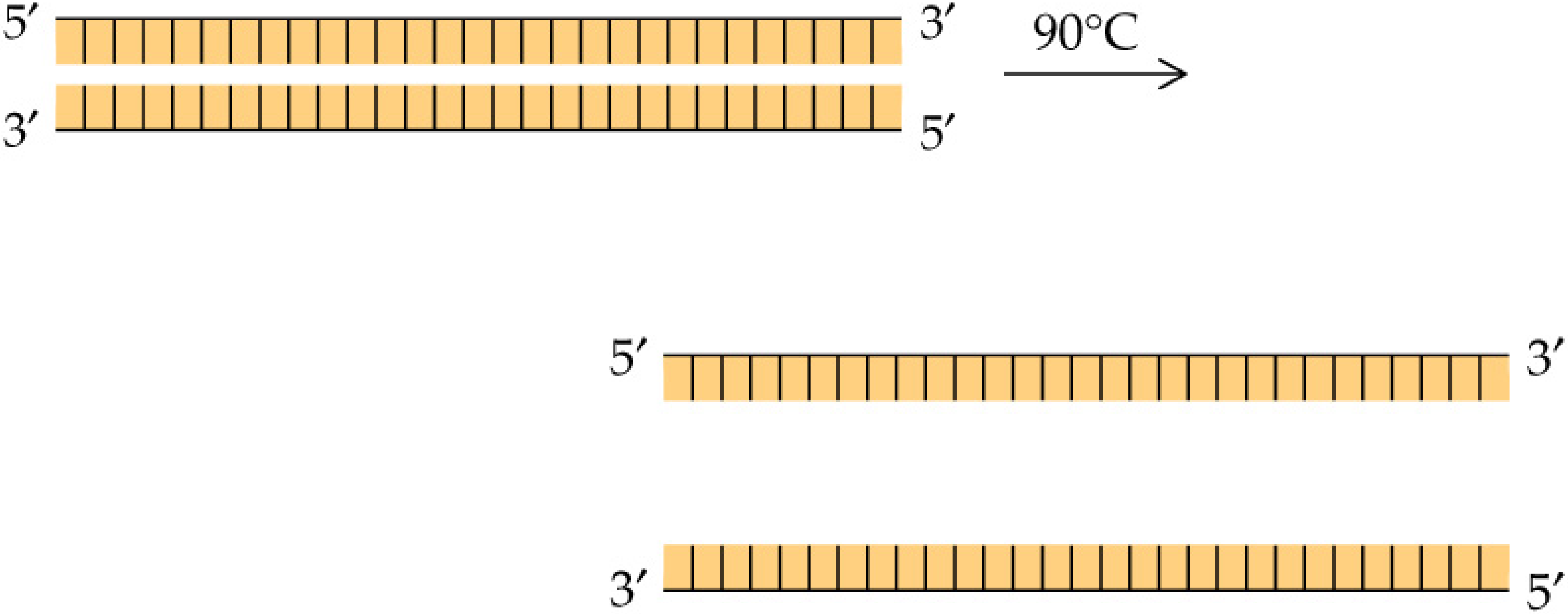
45 seconds 54 °C

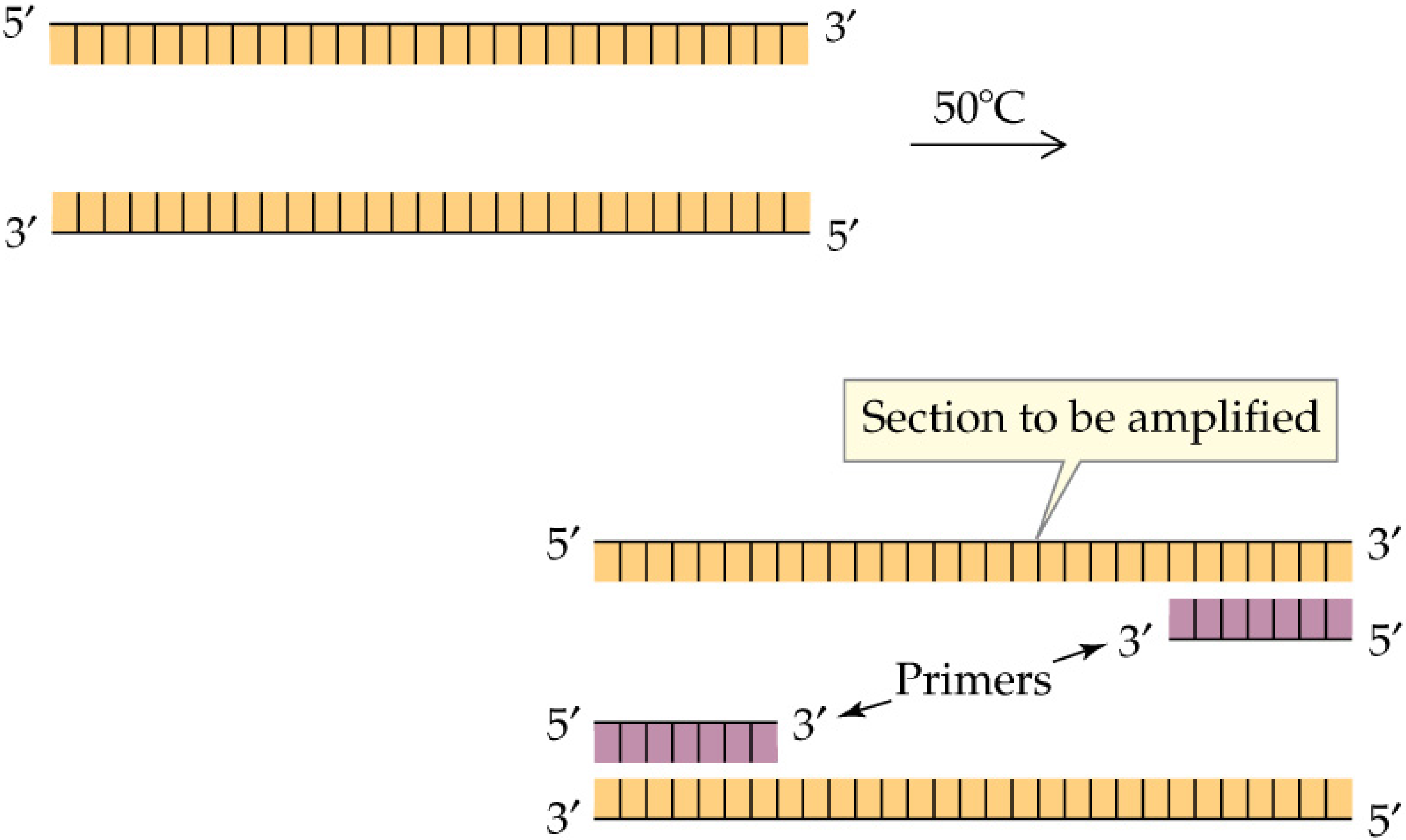
forward and reverse primers !!!

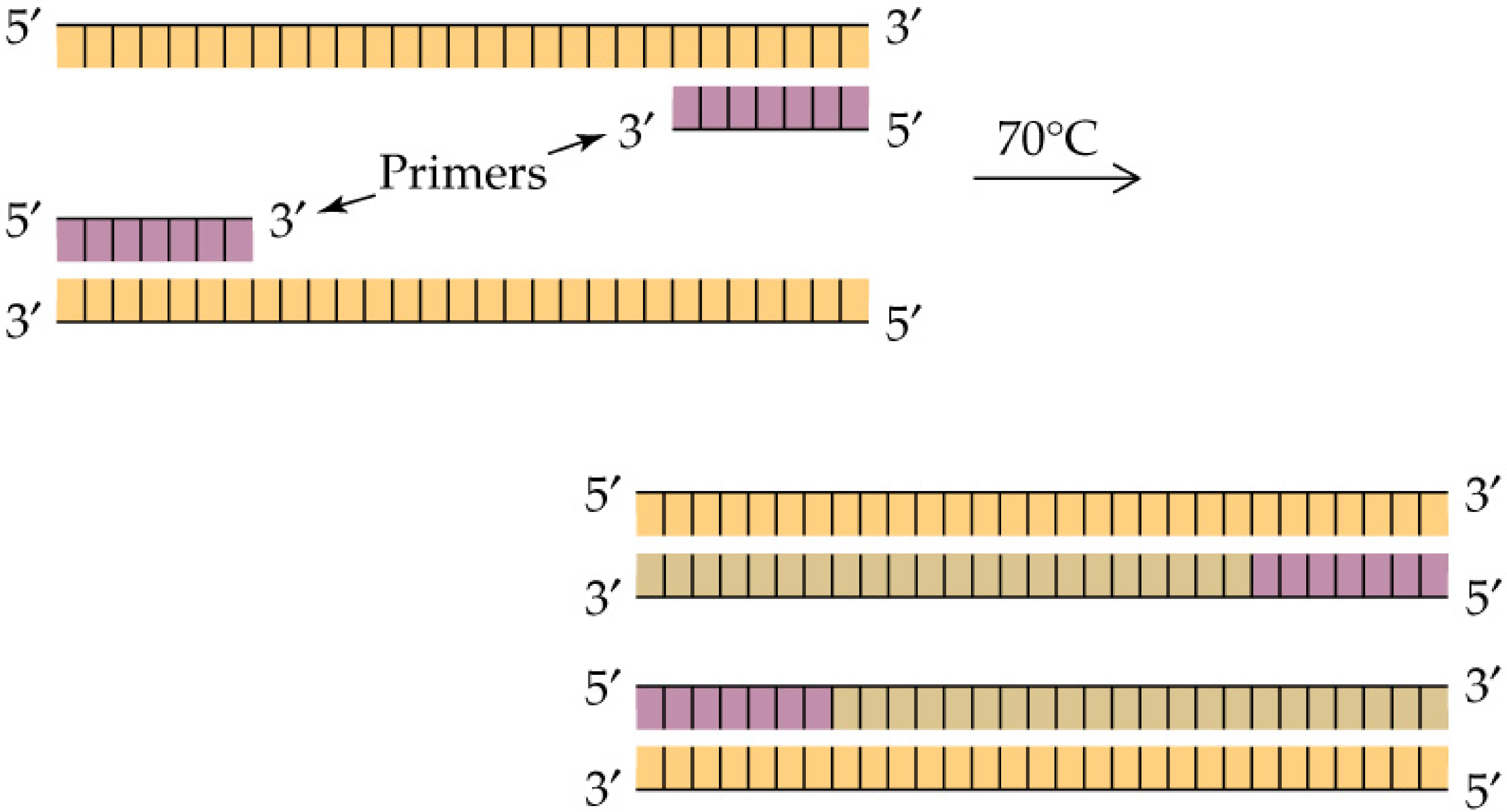
Step 3 : extension

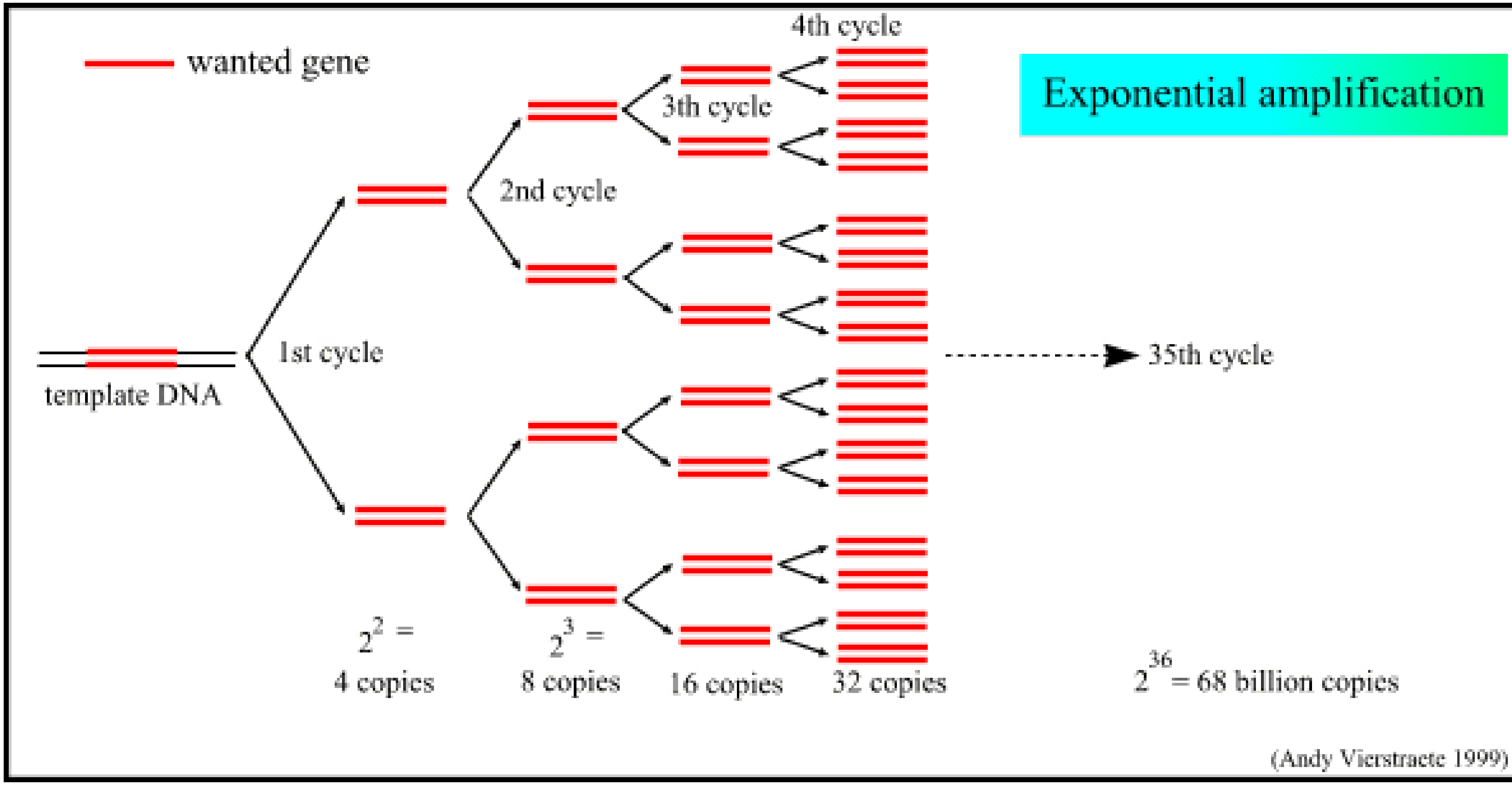
2 minutes 72 °C
only dNTP's

(Andy Vierstraete 1999)

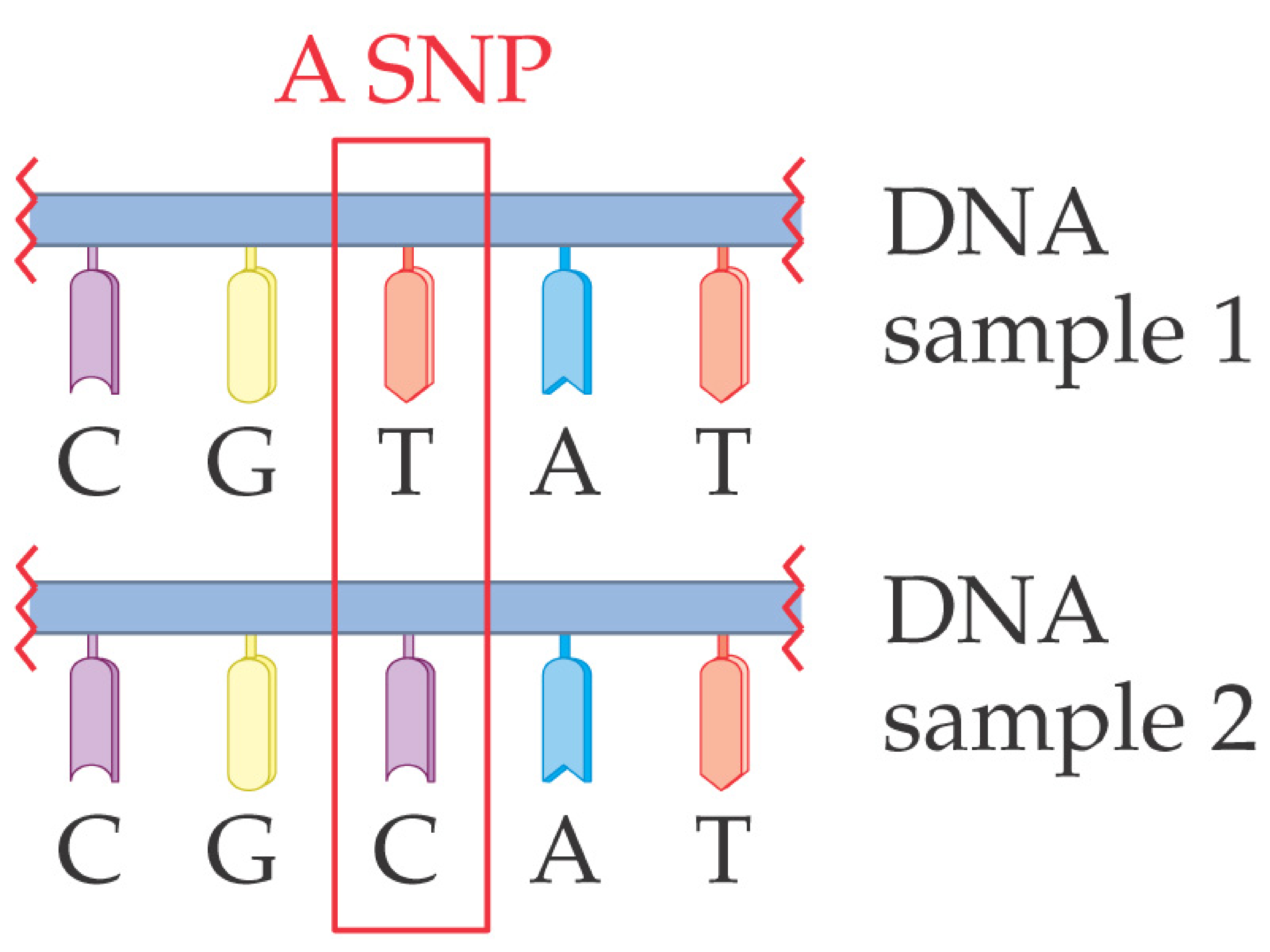

5′
3′
3′
5′
90°C
5′
3′
3′
5′


5′
3′
50°C
3′
5′
Section to be amplified
5′
3′
3′
5′
Primers
5′
3′
3′
5′

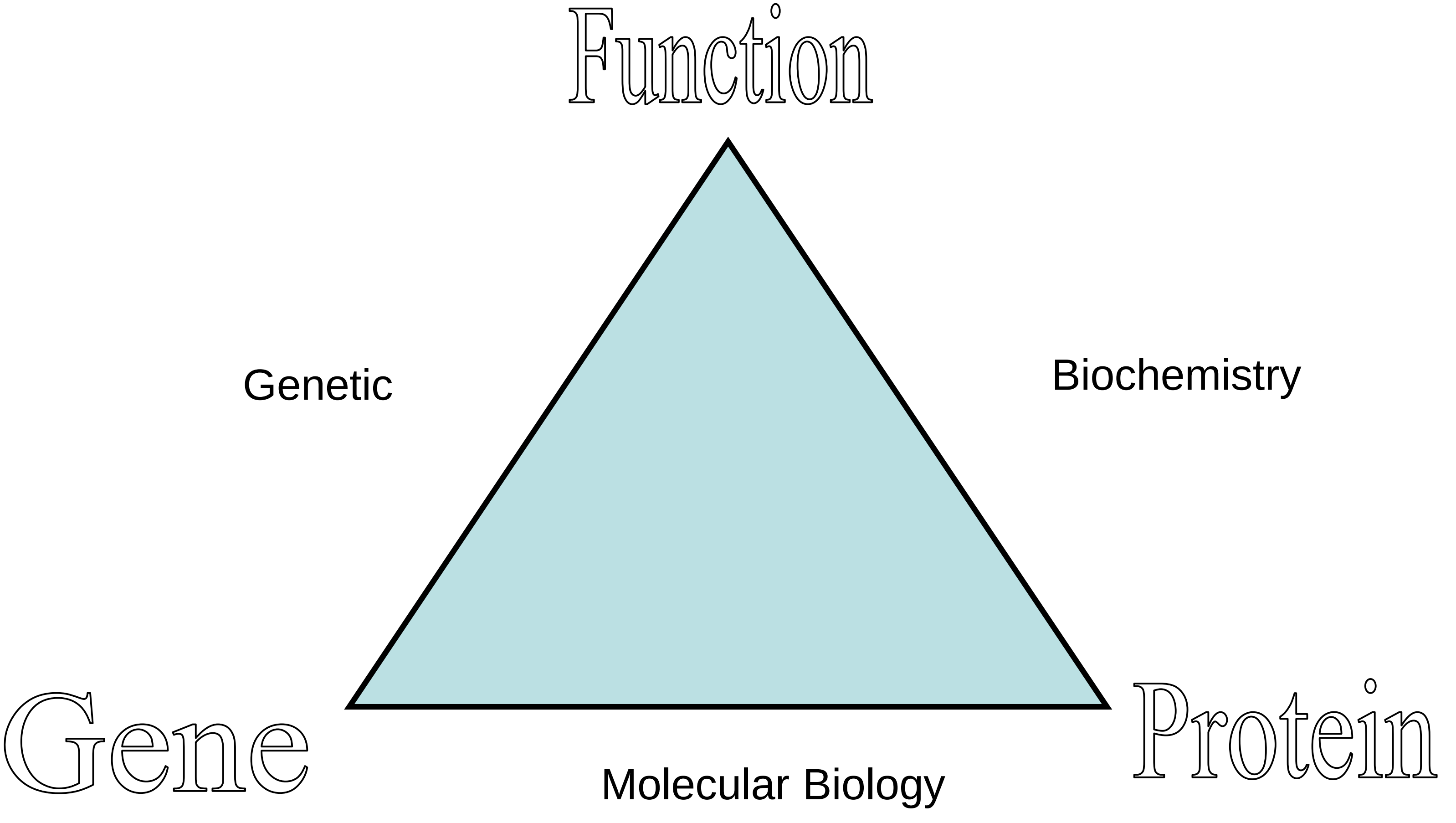

5′
3′
3′
5′
Primers
70°C
5′
3′
3′
5′
5′
3′
3′
5′
5′
3′
3′
5′

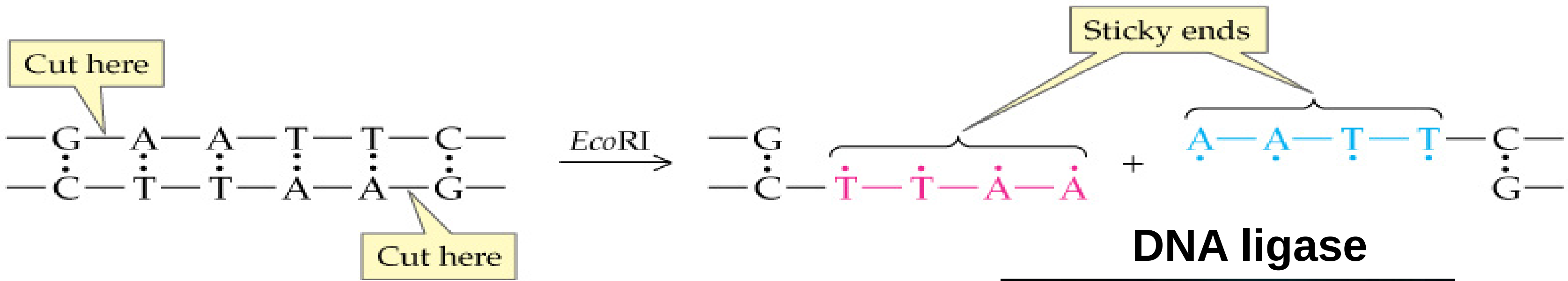


wanted gene
4th cycle
Exponential amplification
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)

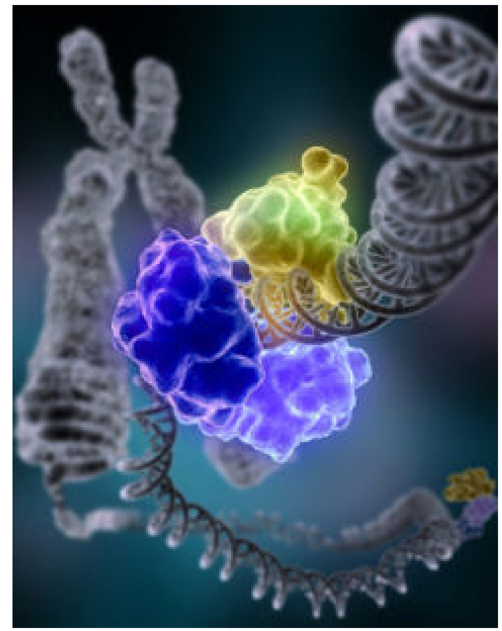

A SNP
DNA sample 1
C G T A T
DNA sample 2
C G C A T

Recombinant DNA



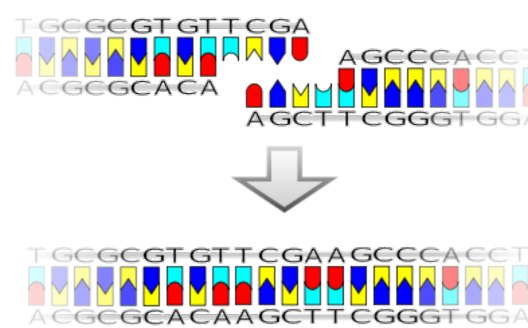


DNA ligase



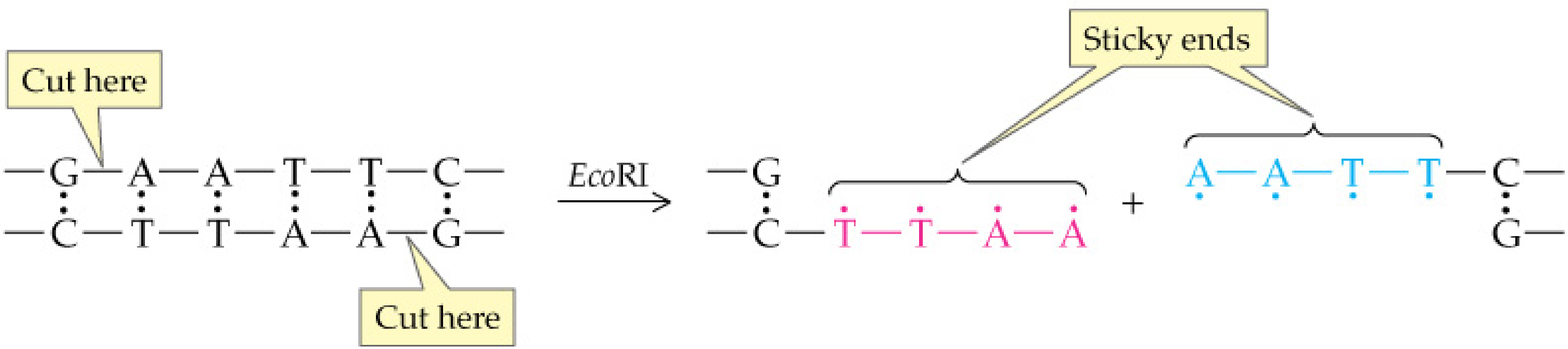
Restriction Enzyme

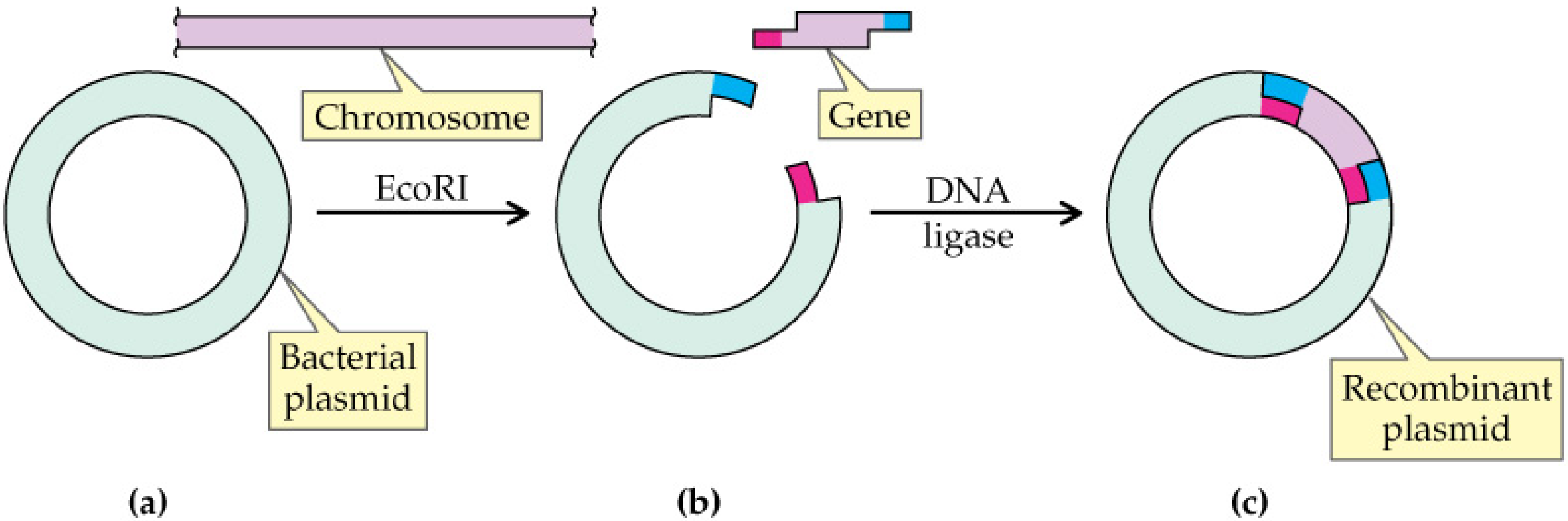
AluI	5' ...A G C T... 3' 3' ...T C G A... 5'
HaeIII	5' ...G G C C... 3' 3' ...C C G G... 5'
BamHI	5' ...G G A T C C... 3' 3' ...C C T A G G... 5'
HindIII	5' ...A A G C T T... 3' 3' ...T T C G A A... 5'
EcoRI	5' ...G A A T T C... 3' 3' ...C T T A A G... 5'



AluI and HaeIII produce blunt ends

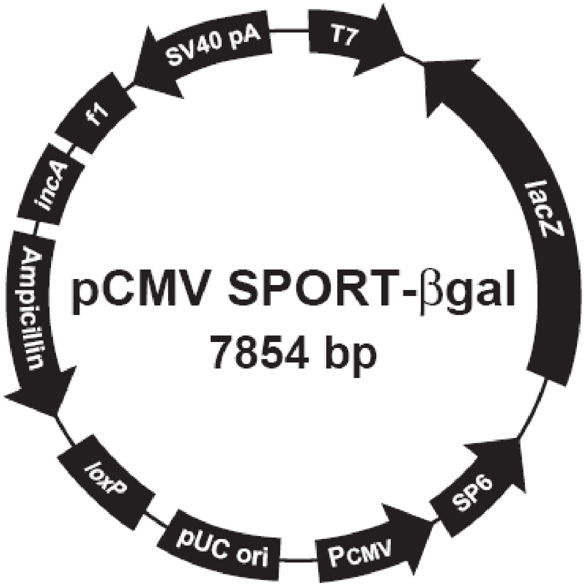
BamHI HindIII and EcoRI produce "sticky" ends

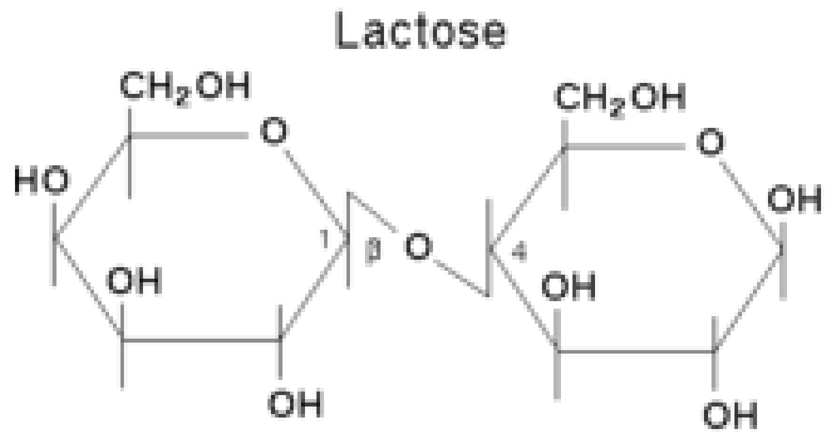

Cut here
Cut here
EcoRI
Sticky ends
G—A—A—T—T—C
C—T—T—A—A—G
G
C—T—T—A—A
+
A—A—T—T—C
G


Chromosome
Gene
EcoRI
DNA
ligase
Bacterial
plasmid
Recombinant
plasmid
(a)
(b)
(c)

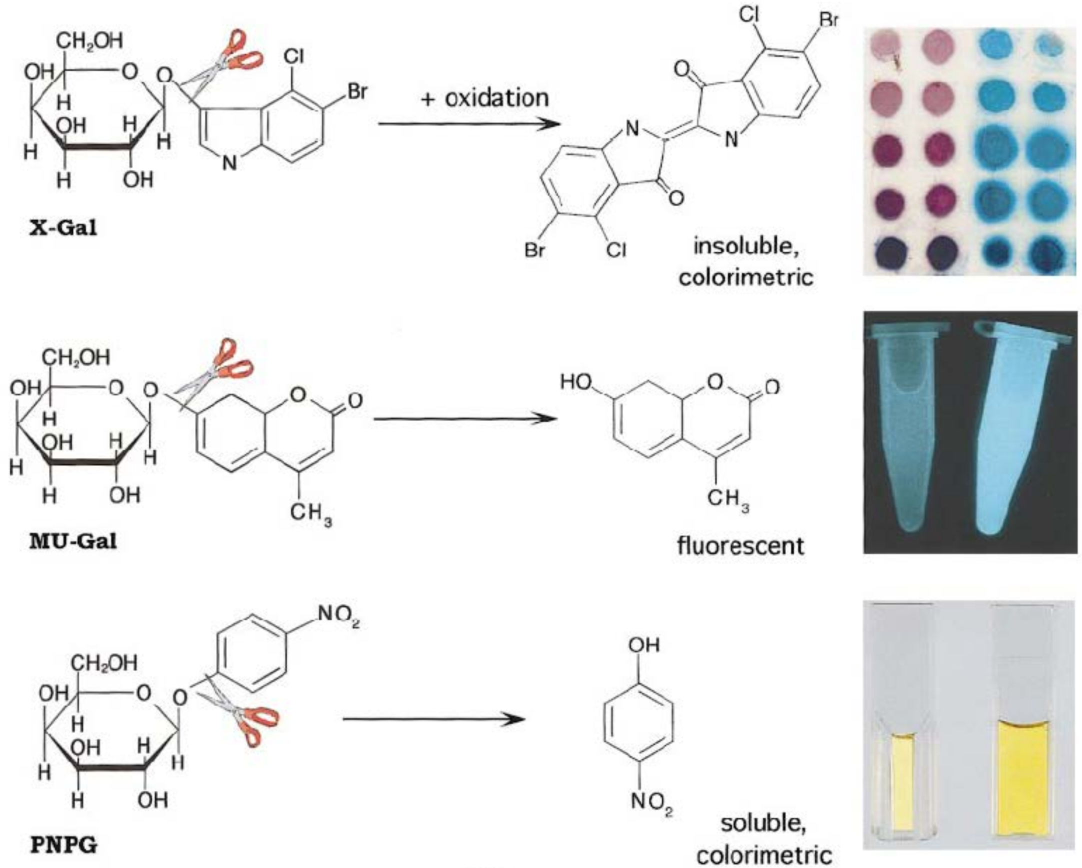
β-Galactosidase

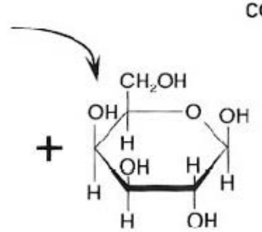
The enzyme that splits lactose into glucose and galactose. Coded by a gene (lacZ) in the lac operon of Escherichia coli.





PUC is a family of plasmids that have an ampicillin resistance gene and more importantly a *lacZ* gene. A functional lacZ gene will produce the protein β - galactosidase. Bacterial colonies in which β - galactosidase is produced, will form blue colonies in the presence of the substrate 5 - bromo - 4 - chloro - 3 - indolyl - b - D - galactoside or as it is more commonly referred to, X-gal.

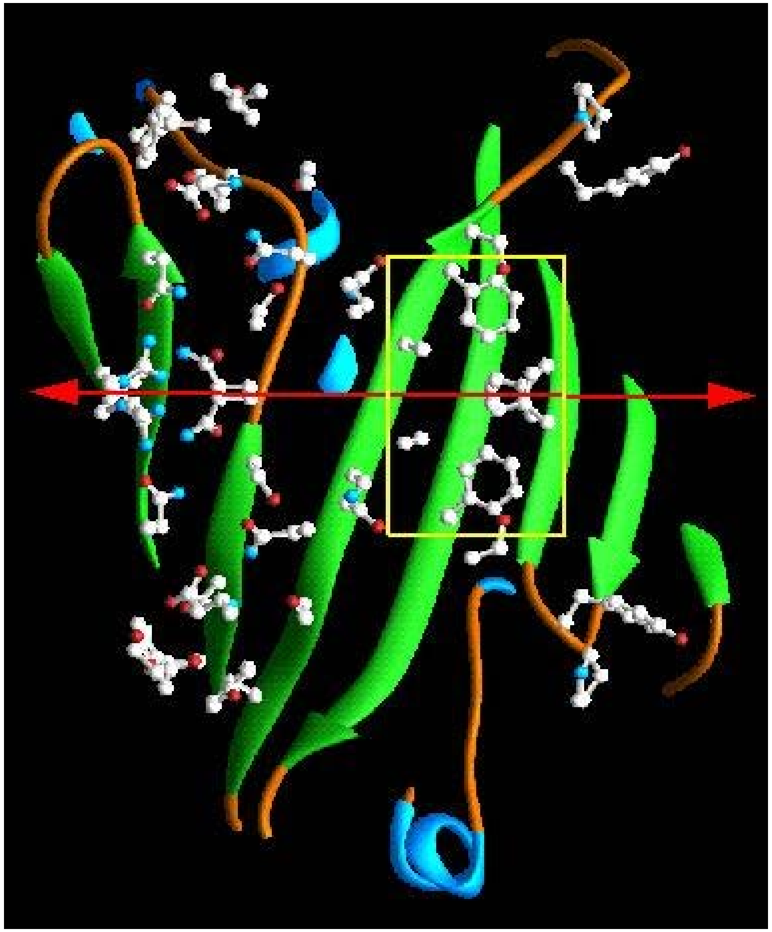




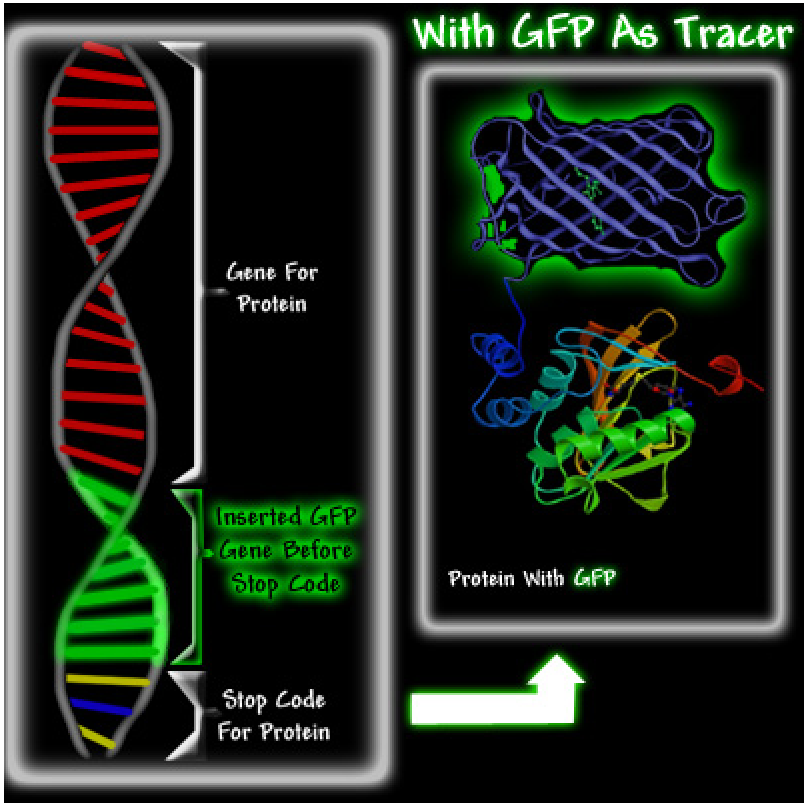
Analytical Biochemistry **285**, 1–15 (2000)

FIG. 1. Enzymatic function of β-galactosidase in cleaving indicator substrates. β-gal cleaves β-D-galactoside containing substrates with a diverse range of aglycone groups, targeting between the glycosyl oxygen and anomeric carbon as indicated (scissors). Substrates shown indicate commonly used indicators for assays on β-gal function on plates (X-Gal) or for liquid assay by measure of fluorescence (MU-Gal or MUG) or color (ONPG). Top left, X-Gal is 5-bromo-4-chloro-3-indolyl-β-D-galactoside, and when cleaved and oxidized produces the insoluble dye 5-bromo-4-chloro-indigo, as described previously (22). Right panel, top, yeast colonies expressing β-gal and exposed to X-Gal (right half) or the closely related compound Magenta-Gal (left half, see Biosynth, Inc., or Diagnostic Chemicals Limited). Middle left, MUG is methylumbelliferyl-β-D-galactoside, and when cleaved by β-gal produces the fluorescent product methylumbelliferone (first described in (102)). Right panel, middle, shows yeast lysates expressing β-gal exposed to MUG, under long-wave UV. Bottom left, PNPG and ONPG are closely related nitrophenol-β-D-galactosides with similar assay properties, e.g., (103), whose cleavage releases the yellow product nitrophenol (right panel, bottom); PNPG is shown.

Green Fluorescent Protein (GFP)

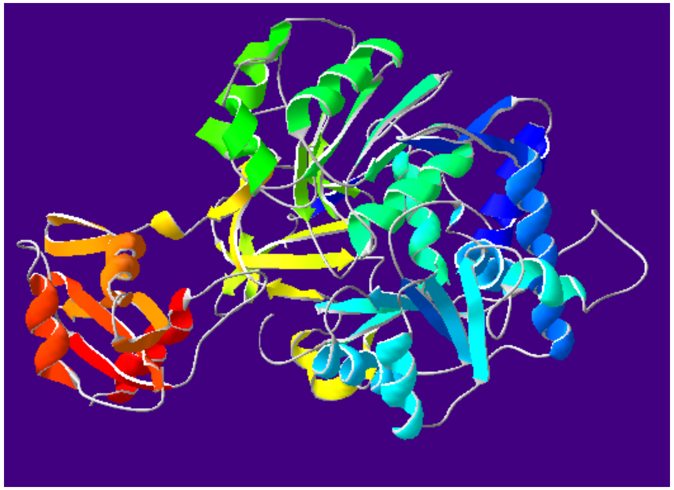


The **green fluorescent protein** (**GFP**) is a protein from the jellyfish *Aequorea victoria* that fluoresces green when exposed to blue light.



GFP Rats

Luciferase

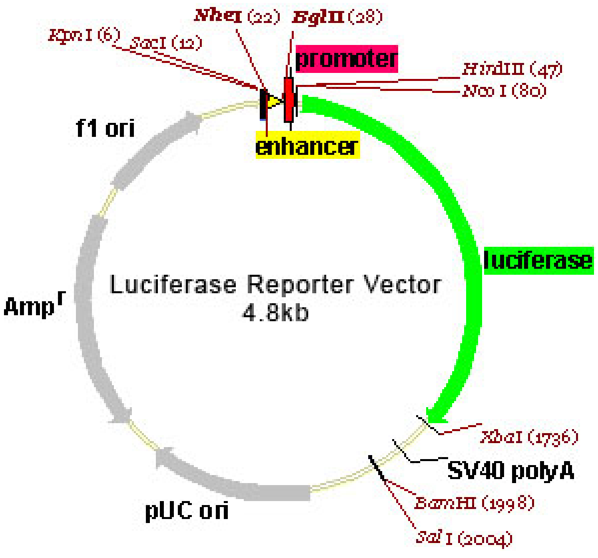


Luciferase is a generic name for enzymes commonly used in nature for bioluminescence. The name itself is derived from *Lucifer*, which means *light-bearer*. The most famous one is firefly luciferase from the firefly *Photinus pyralis*. In luminescent reactions, light is produced by the oxidation of a luciferin (a pigment), sometimes involving Adenosine triphosphate (ATP). The rates of this reaction between luciferin and oxygen are extremely slow until they are catalyzed by luciferase, often mediated by the presence of calcium ions (an analog of muscle contraction). The reaction takes place in two steps:

luciferin + ATP → luciferyl adenylate + PPi

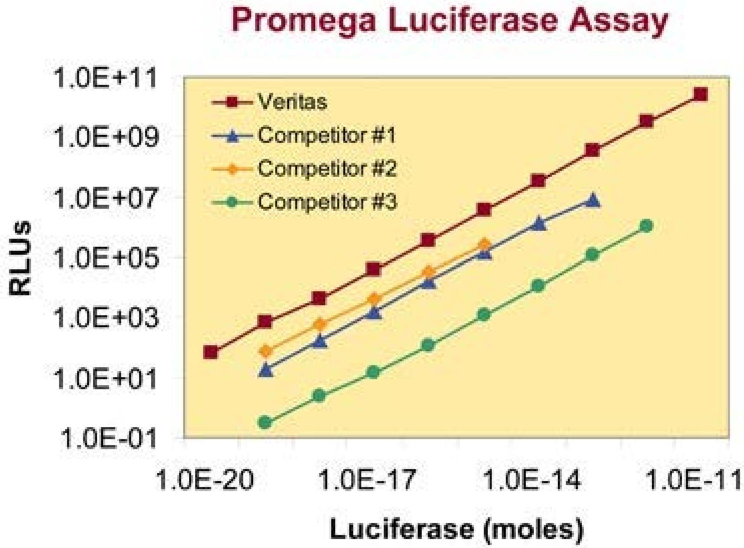
luciferyl adenylate + O2 → oxyluciferin + AMP + light

Luciferase



Dim Light

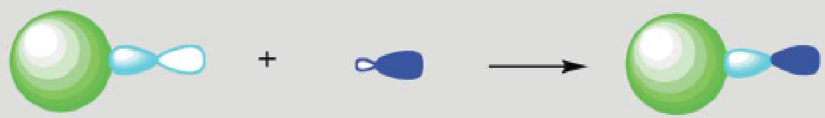
Bright Light



Fluorescent labeling techniques in biomolecules: a flashback

Harekrushna Sahoo*

Received 3rd March 2012, Accepted 18th April 2012
DOI: 10.1039/c2ra20389h



Scheme 1. Fluorescent labeling scheme: a schematic diagram showing the labeling between a biomolecule (green) linked to a tag (light blue) and a fluorophore (navy blue).

A general list of most commonly used fluorescent methods:

Fluorescent Methods		
Chemical	Enzymatic	Tagging
Maleimide-Thiol	Transglutaminase	Tetracysteine
NHS ester-Amine	Sortase	Histidine
Isothiocyanate-Amine	Cutinase	Aspartate
Alkyl Halide-Hydroxy	Intein	Lanthanide
Isocyanate-Hydroxy	Lipoic Acid ligase	CLIP
Tetrazine-Alkene/Alkyne	Biotin Ligase	Halo
	Myristoyl Transferase	SNAP
		Fluorescent proteins (Genetically Tagged)

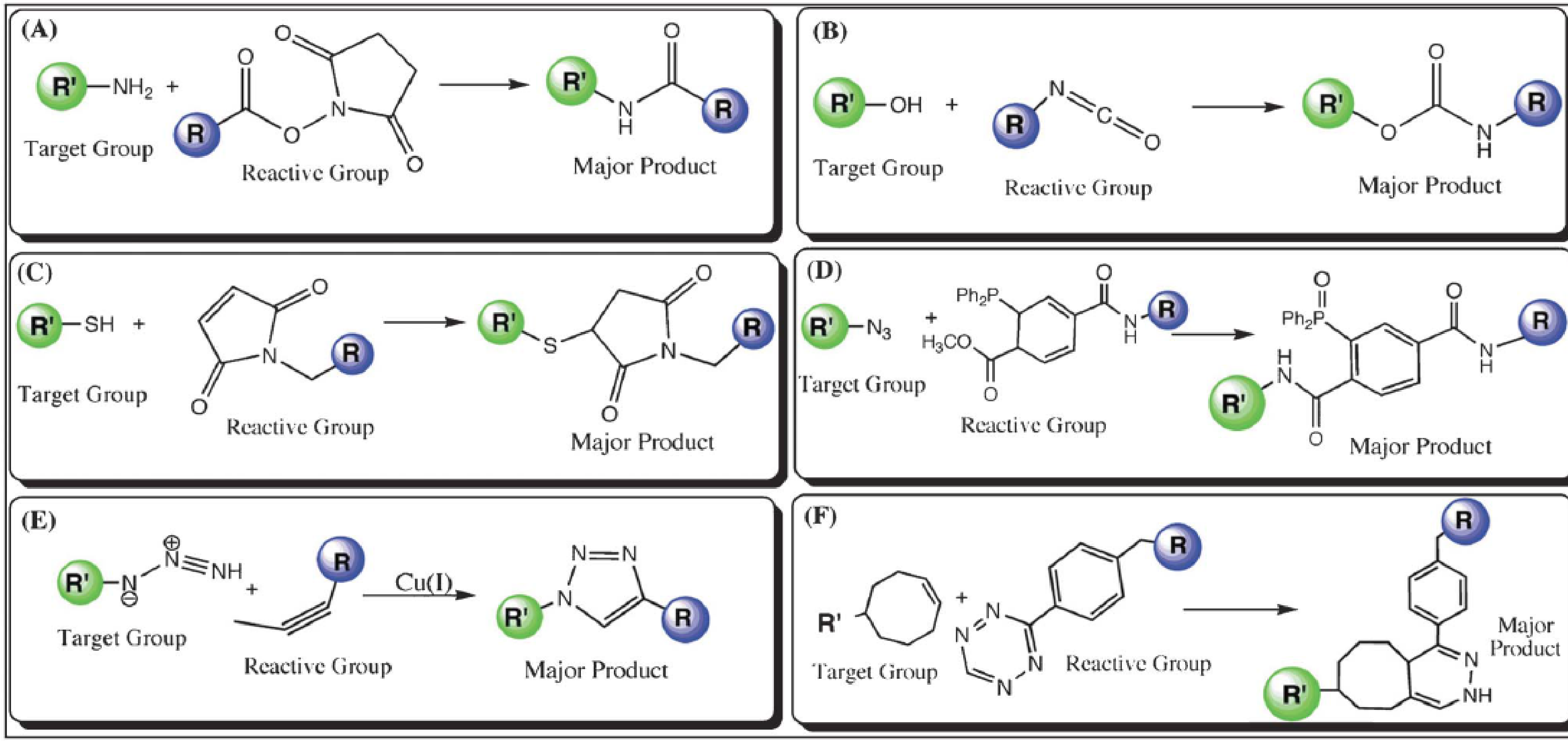


Fig. 1 Schematic diagram of chemical labeling techniques (R′ in green represents the biomolecule to be labeled with a target moiety, while R is the fluorophore linked with a reactive group); (A) Amine labeling: an NHS (*N*-hydroxysuccinimide)-ester group couples to the –NH_2 moiety, (B) Hydroxy labeling: a fluorophore is modified with an isocyanate group, which couples to the –OH moiety, (C) Thiol labeling: coupling between a maleimide modified fluorophore and the –SH moiety, (D) Azide labeling: Staudinger ligation of azide moiety with phosphine group, (E) Azide labeling: copper(I)-catalyzed cycloaddition of a fluorophore with the azide group linked to a biomolecule, and (F) Tetrazine labeling: A biomolecule labeled with tetrazine undergoes Diels–Alder cycloaddition with a dienophile linked to a fluorophore.

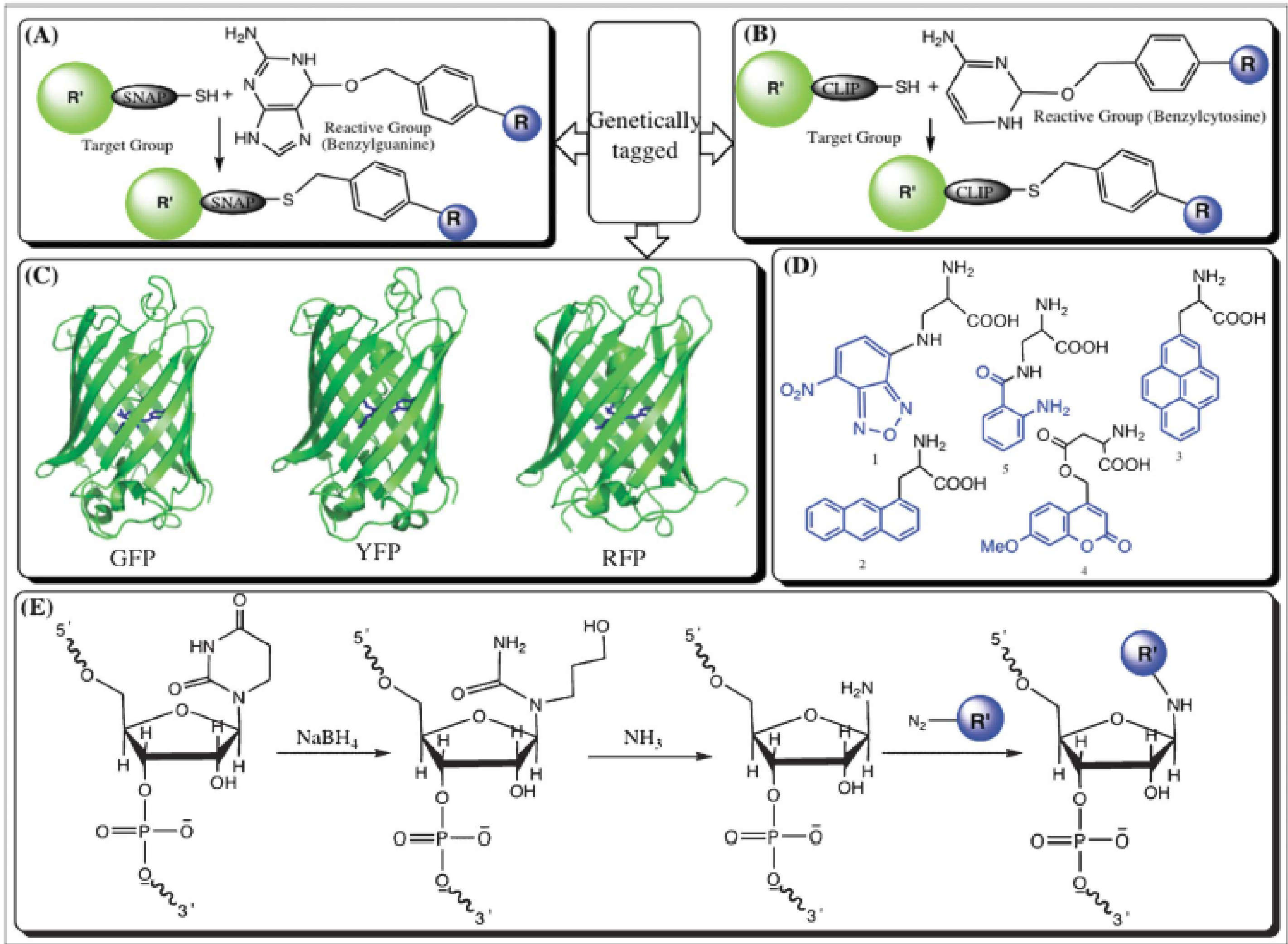
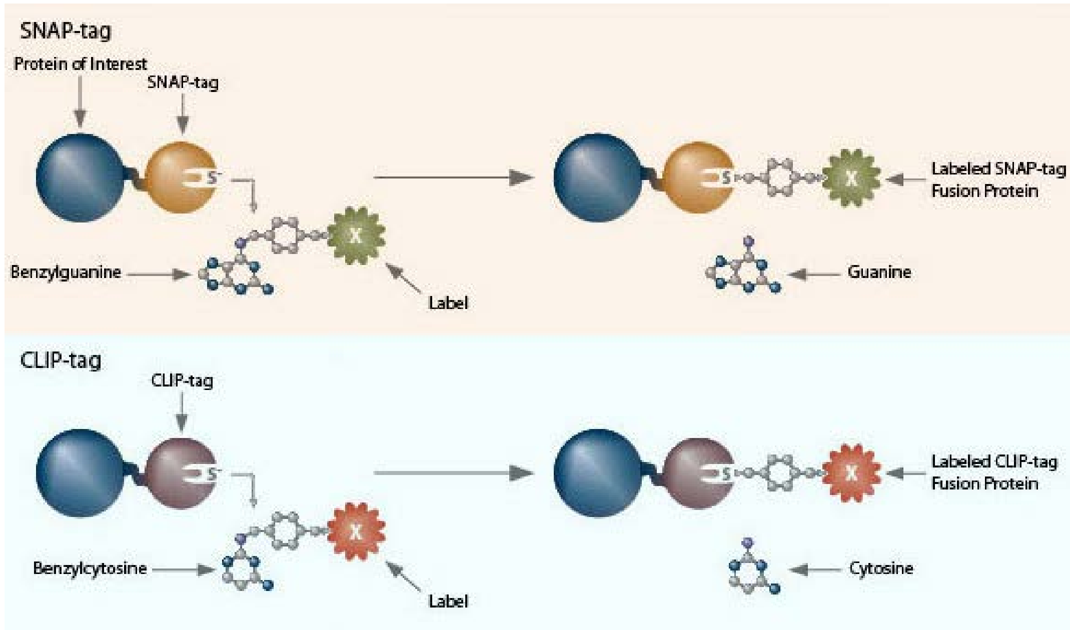
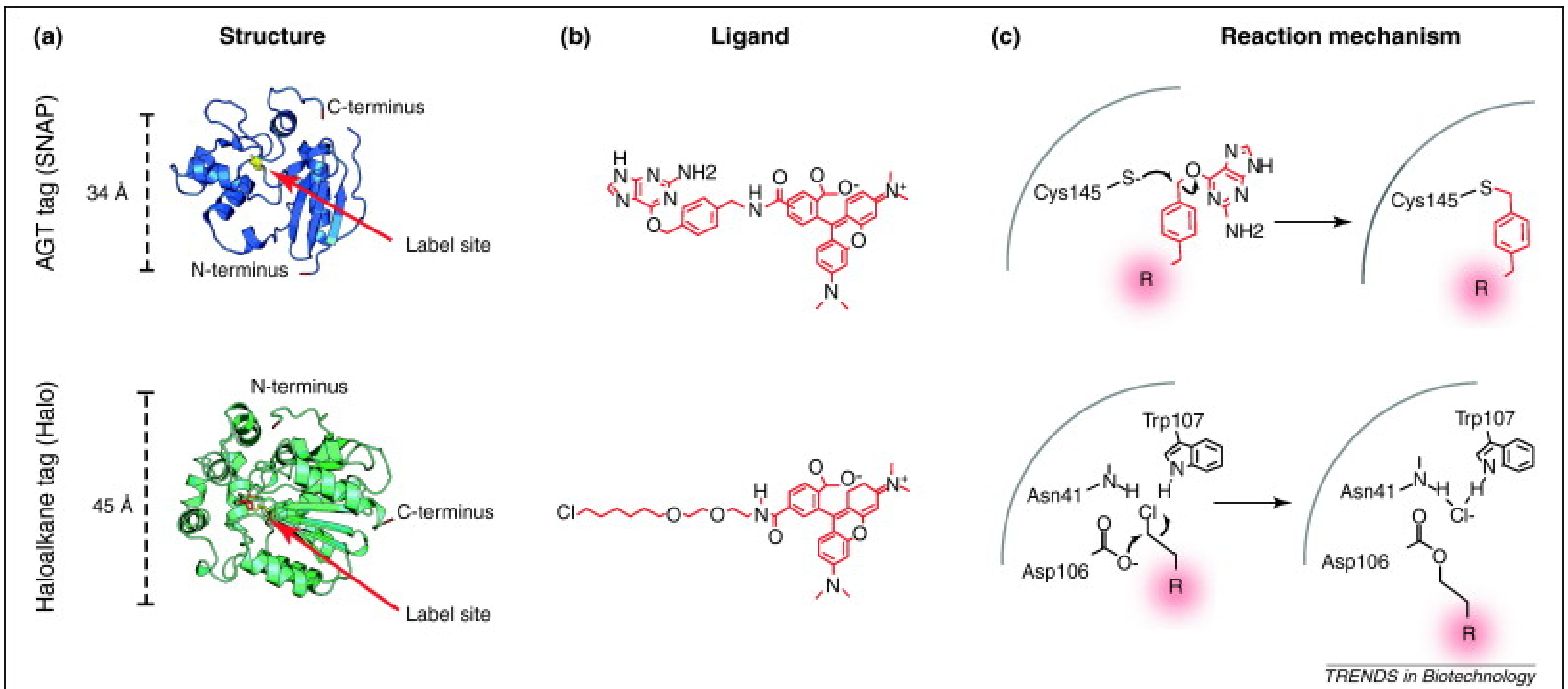


Fig. 6 Schematic representation of labeling techniques using genetic modifications: (A) SNAP-tag: attachment of benzylguanidine conjugated to a fluorophore (R, blue) to the SNAP tag fused to the target protein (R′, green); (B) CLIP-tag: attachment of benzylcytosine conjugated to a fluorophore (R, blue) to the SNAP tag fused to the target protein (R′, green); (C) Fluorescent protein: Green fluorescent proteins and a few examples of its variants, such as GFP (excitation and emission: 488 and 508 nm), YFP (excitation and emission: 514 and 530 nm) and RFP (excitation and emission: 554 and 585 nm). The blue color represents the chromophoric compound in the protein; (D) Unnatural amino acids: a few examples of widely used fluorescent non-natural amino acids, such as microenvironment sensitive (3-(4-nitrobenzo[*c*][1,2,5] oxadiazol-7-ylamino)-2-aminopropanoic acid (1), amino acids carrying 7-methoxycoumarine (4) and b-anthraniloyl (5)), 2-amino-3-(anthracen-5-yl)propanoic acid (2), which is less sensitive towards polar and nonpolar solvents and a hydrophobic labeled dye (2-amino-3-(pyren-2-yl)propanoic acid (3)). Moieties in blue indicate the fluorescent groups with a longer excitation wavelength than 320 nm, which thus remove the interference from the intrinsic fluorophores in the protein, and (E) tRNA: Reductive cleavage of dihydrouridine in the presence of $NaBH_4$ and subsequent amination to form an amine group at the target site. In the second step, a fluorophore (R in blue) modified with a dihydrazide moiety is coupled to the primary amine group of the target site.


SNAP-tag
Protein of Interest
SNAP-tag
Labeled SNAP-tag Fusion Protein
Benzylguanine
Label
Guanine
CLIP-tag
CLIP-tag
Labeled CLIP-tag Fusion Protein
Benzylcytosine
Label
Cytosine


(a) Structure
(b) Ligand
(c) Reaction mechanism
AGT tag (SNAP)
34 Å
C-terminus
N-terminus
Label site
Cys145
Asn41
Trp107
Asp106
Haloalkane tag (Halo)
45 Å
N-terminus
C-terminus
Label site
TRENDS in Biotechnology

J|A|C|S COMMUNICATIONS

Published on Web 12/15/2006

Mammalian Cell Surface Imaging with Nitrile-Functionalized Nanoprobes: Biophysical Characterization of Aggregation and Polarization Anisotropy in SERS Imaging

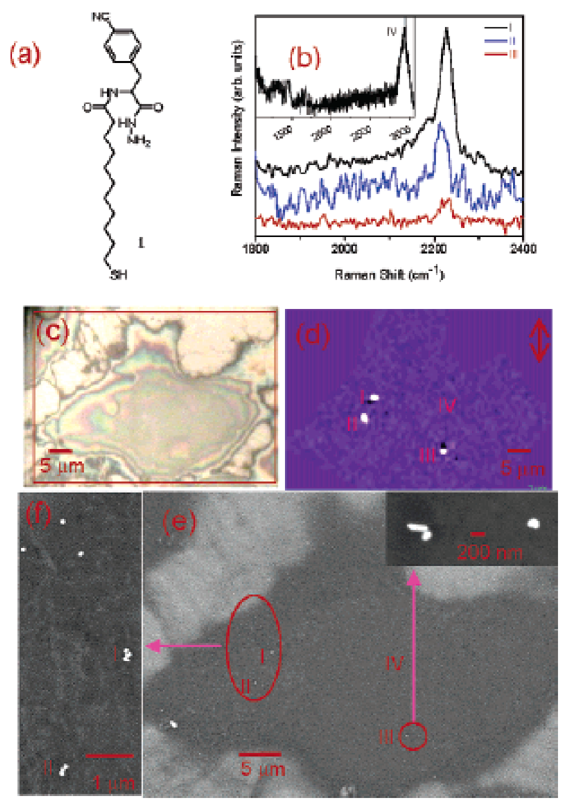


Figure 1. (a) The chemical structure of Raman reporter **1**; (b) Raman spectra of the CN vibration mode extracted from positions I, II, and III of the cell shown in the optical image (c). Inset of (b) is a cellular Raman spectrum taken from spot IV of the same cell. (d) Raman intensity map of the C≡N band of the same cell, and (e) the corresponding SEM image. Inset in (e) showed the NPs in the lower right circle. (f) The group of NPs as shown in the large oval of (e).

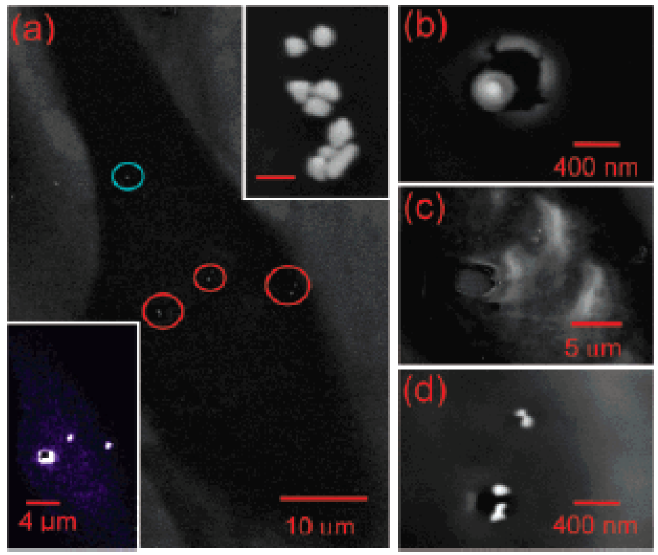
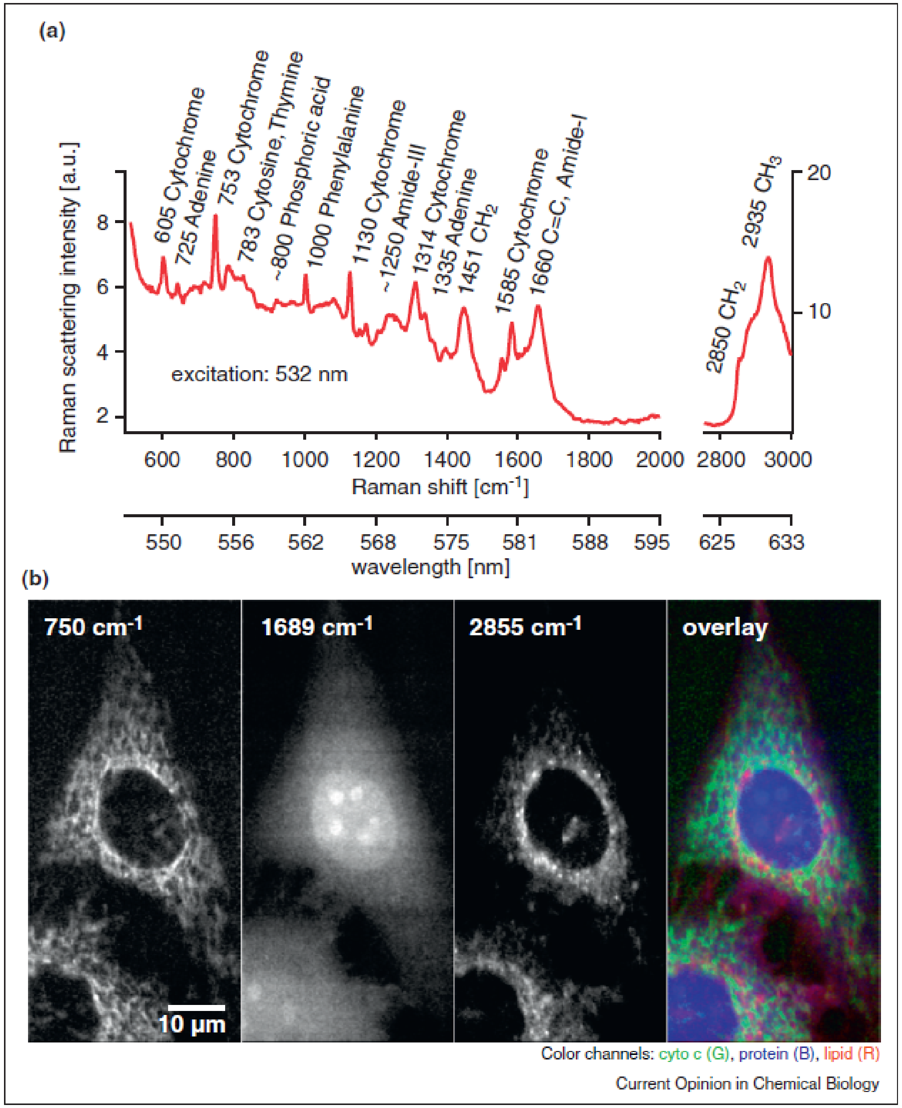


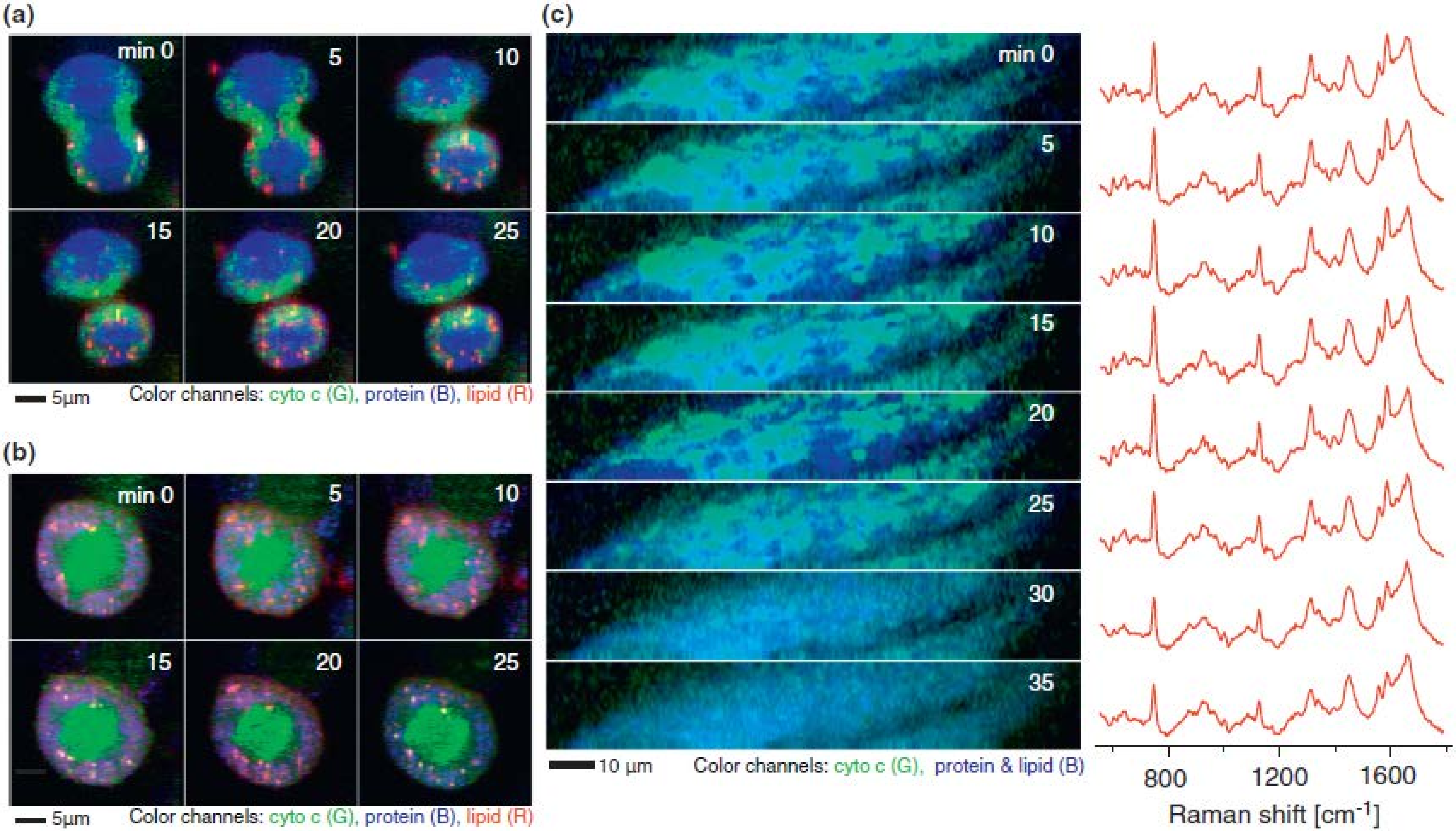
Figure 2. (a) SEM image of a cell. Upper right inset: magnification of a group of aggregated NPs. The scale bar is 200 nm. Lower left inset: the corresponding Raman intensity image of the same cell obtained with a power density of 10^5 W/cm^2. Laser-induced damage to the cell is shown in (b) the monomer (blue circle in a), (c) the aggregates, and (d) a pair of dimers.

Molecular imaging of live cells by Raman microscopy

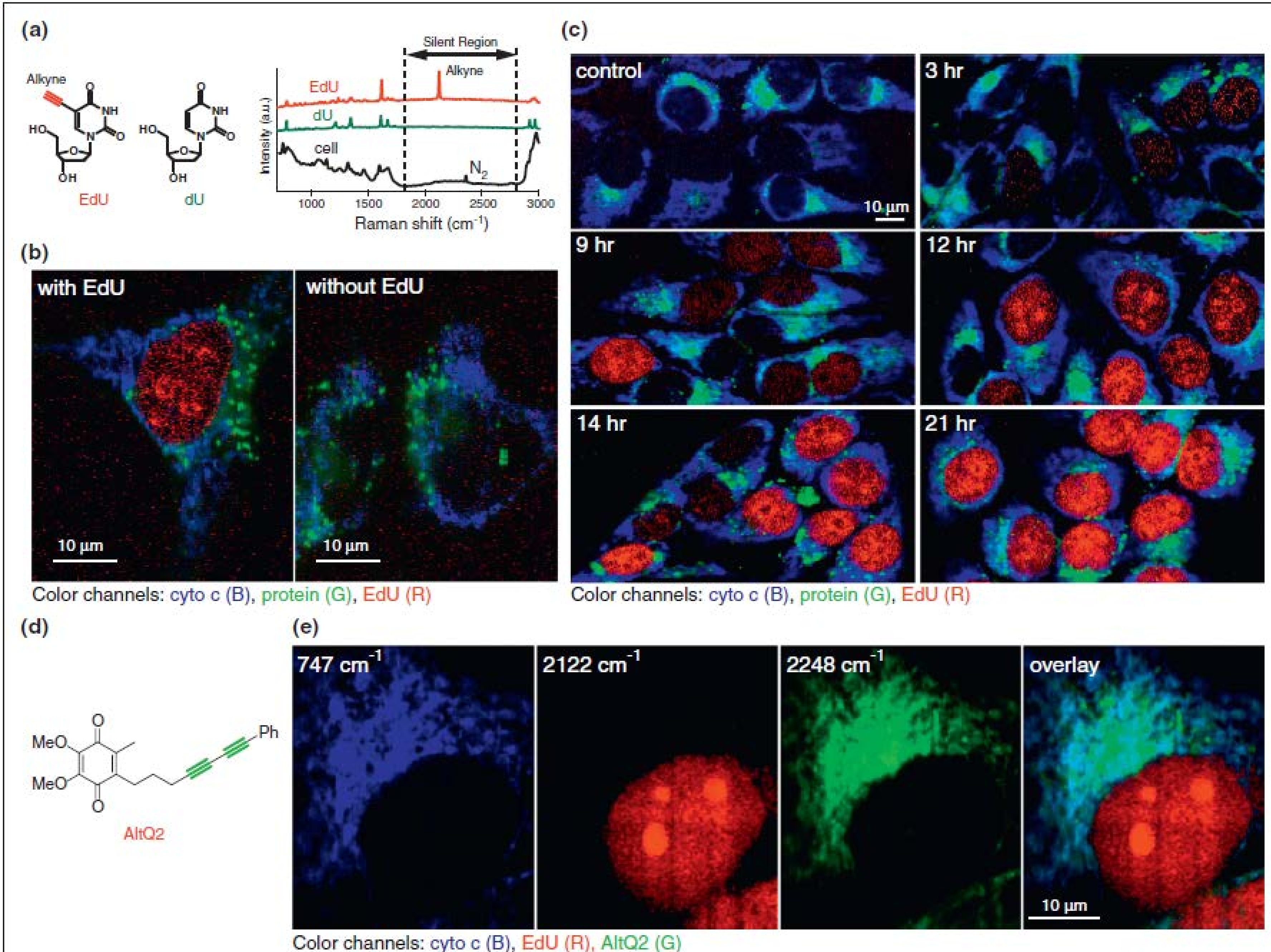
Almar F Palonpon[1,2], Mikiko Sodeoka[2,3] and Katsumasa Fujita[1,2]

Current Opinion in Chemical Biology 2013, **17**:708–715

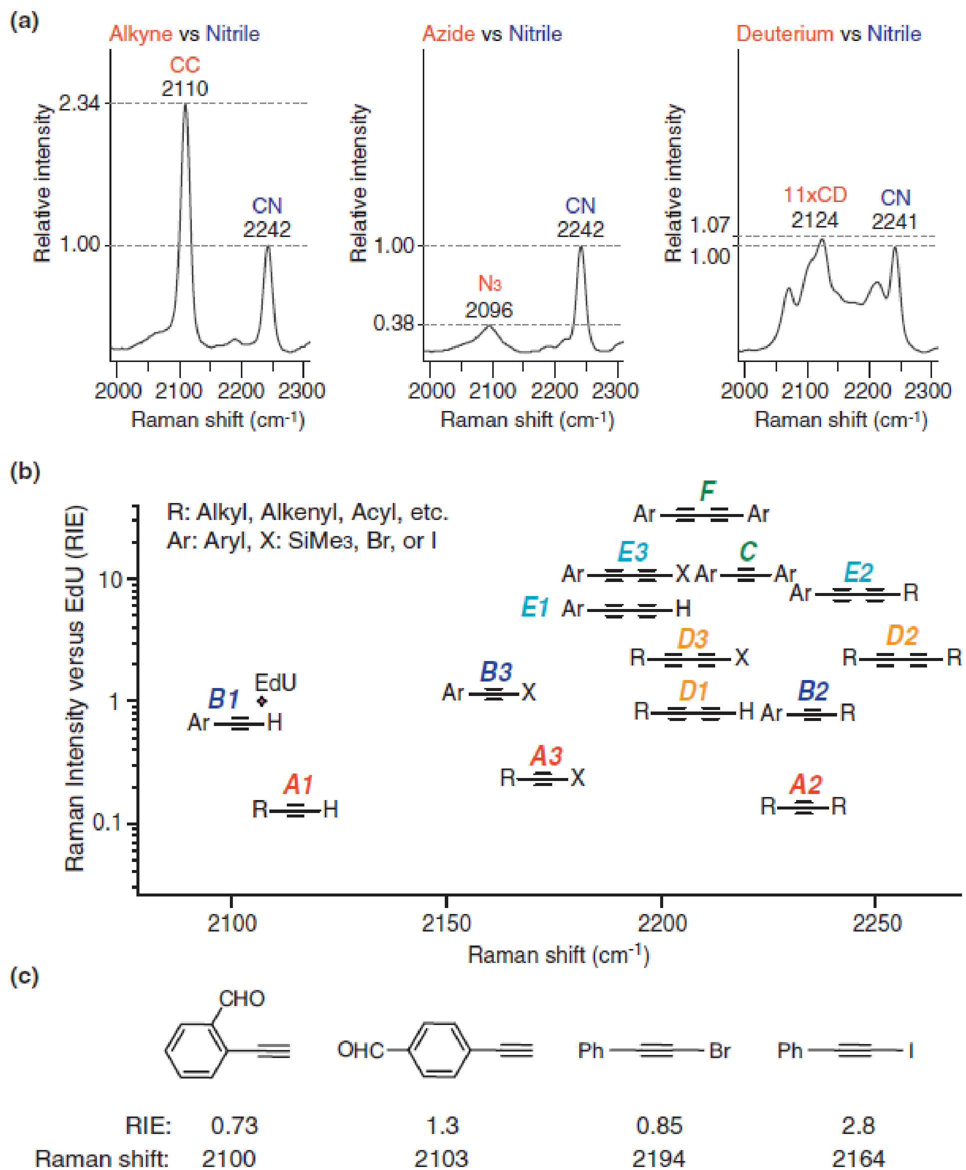




Current Opinion in Chemical Biology



Current Opinion in Chemical Biology



Current Opinion in Chemical Biology

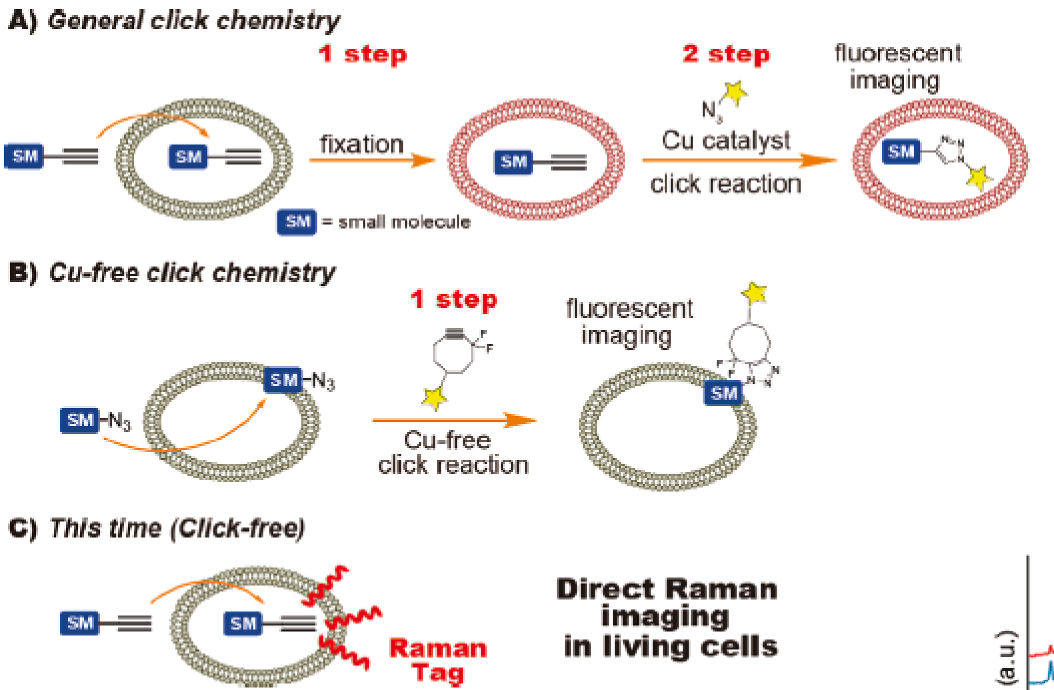
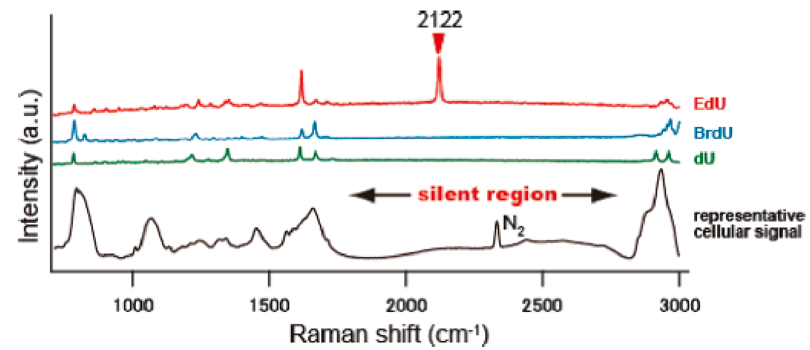


Figure 1. Concept of click-free imaging.



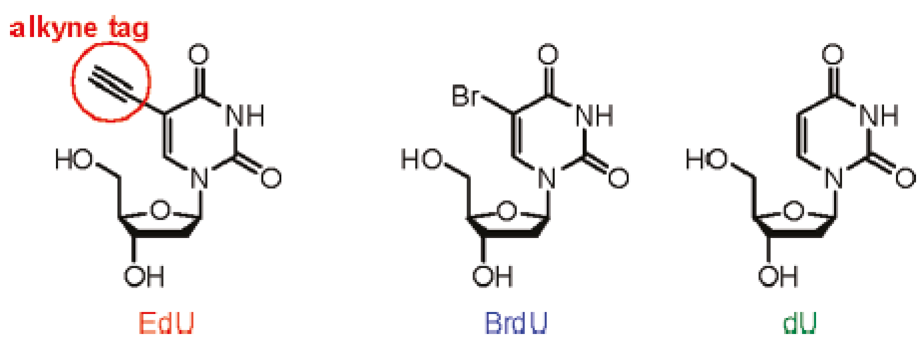
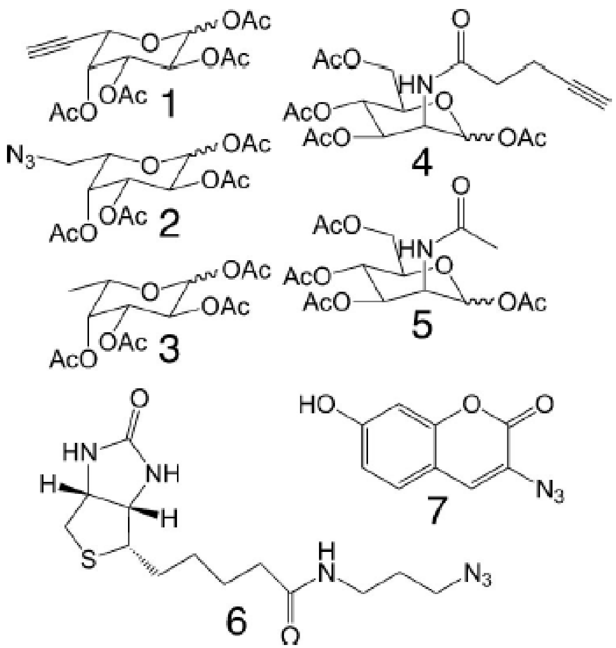
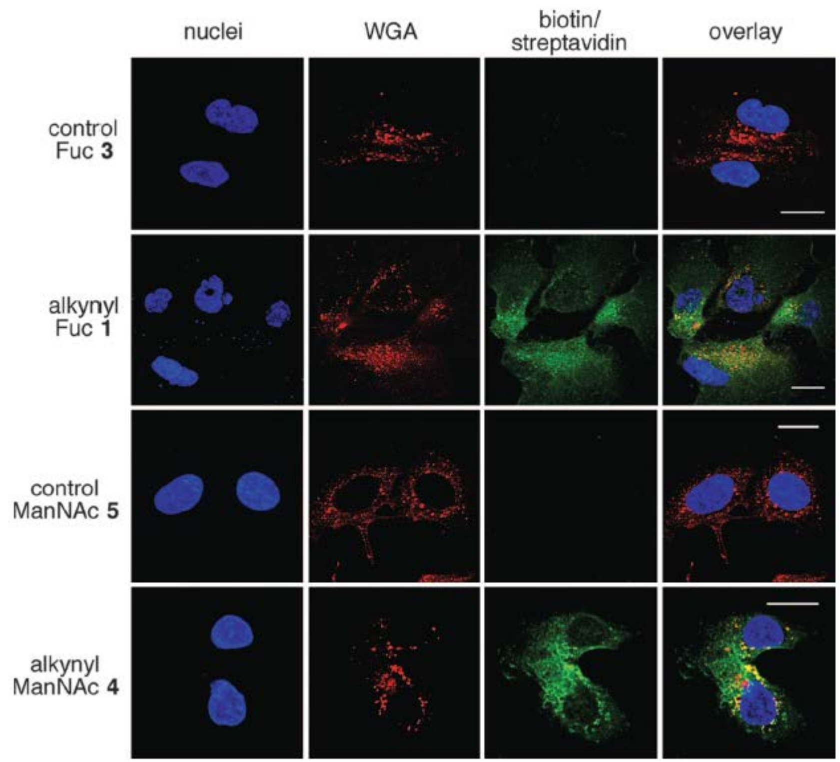


Figure 2. Structures of thymidine analogues.

Alkynyl sugar analogs for the labeling and visualization of glycoconjugates in cells



Scheme 1. Modified sugar analogs and probes used in this study.



2614–2619 | PNAS | February 20, 2007 | vol. 104 | no. 8

Cell-permeable probe for identification and imaging of sialidases

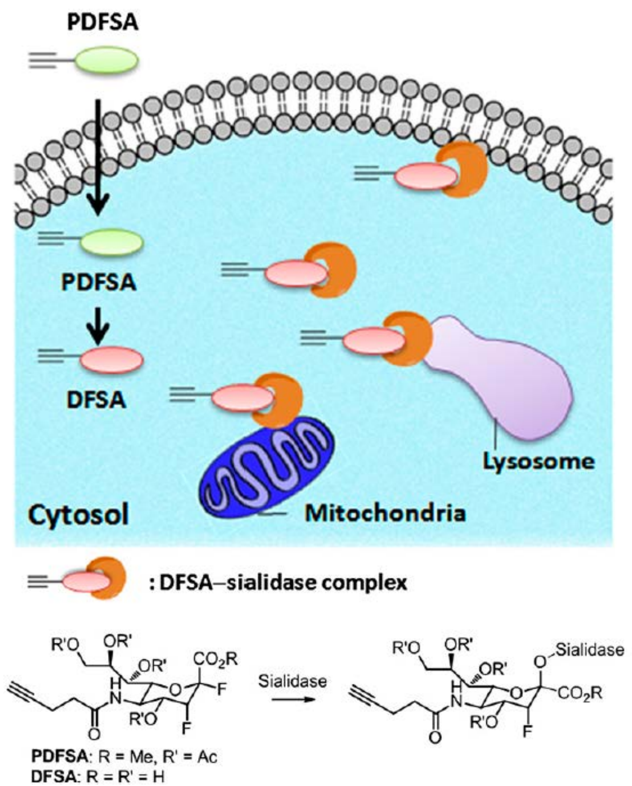
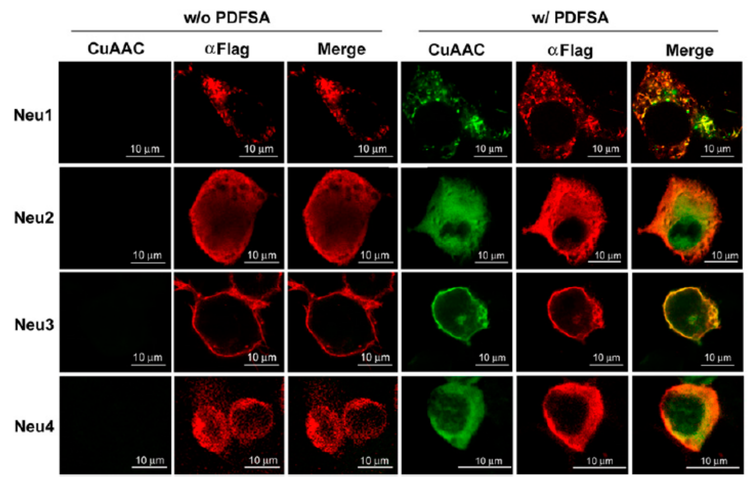
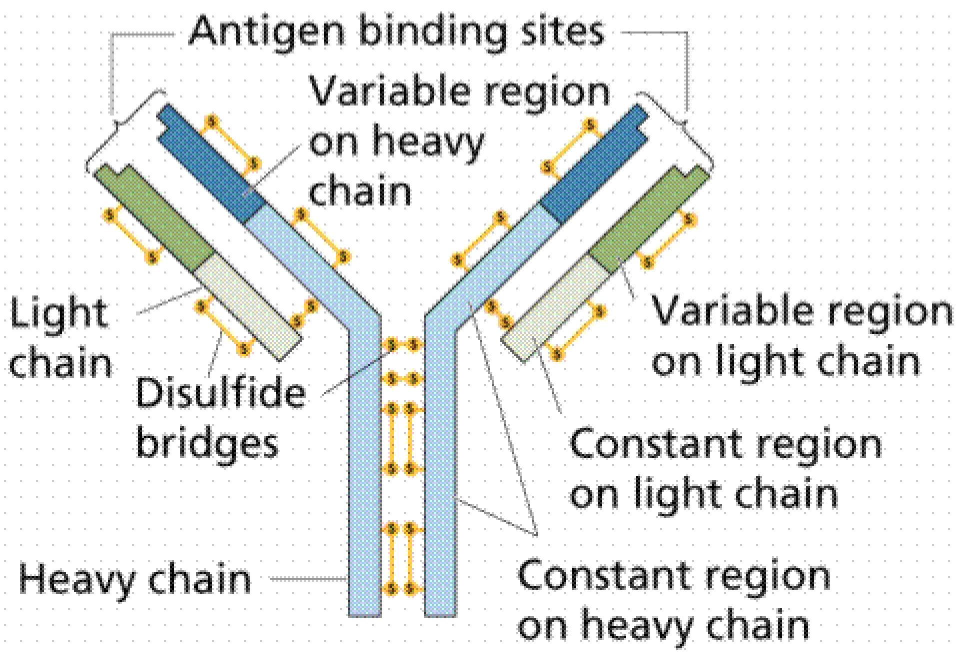


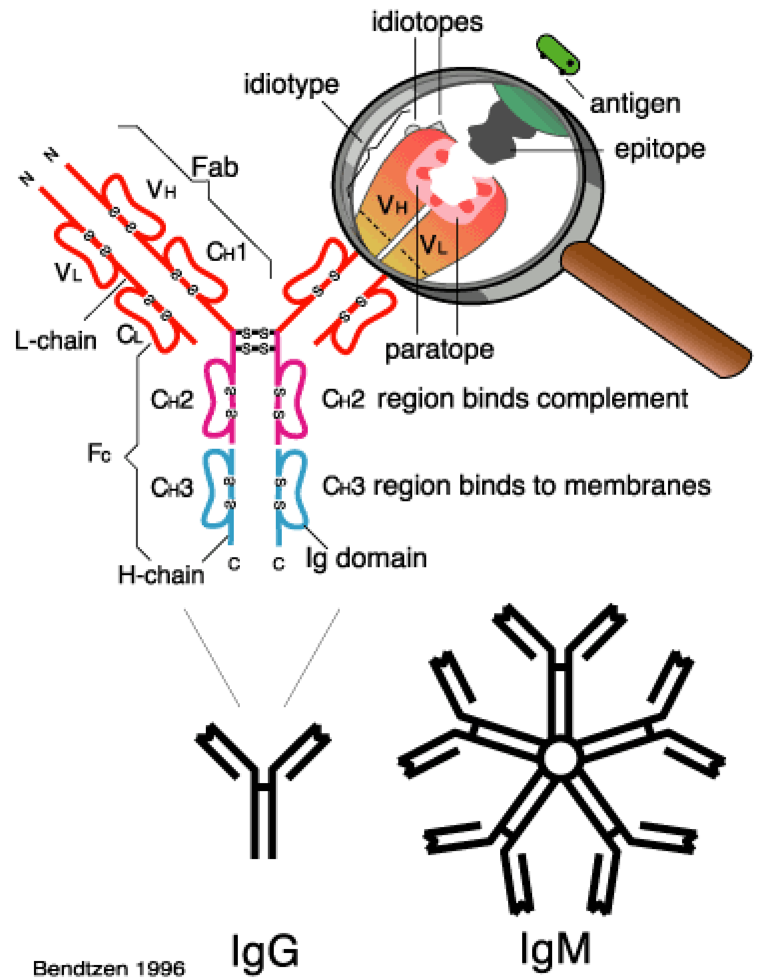
Fig. 1. Identification and imaging of sialidase with activity changes using these activity-based sialidase probes.



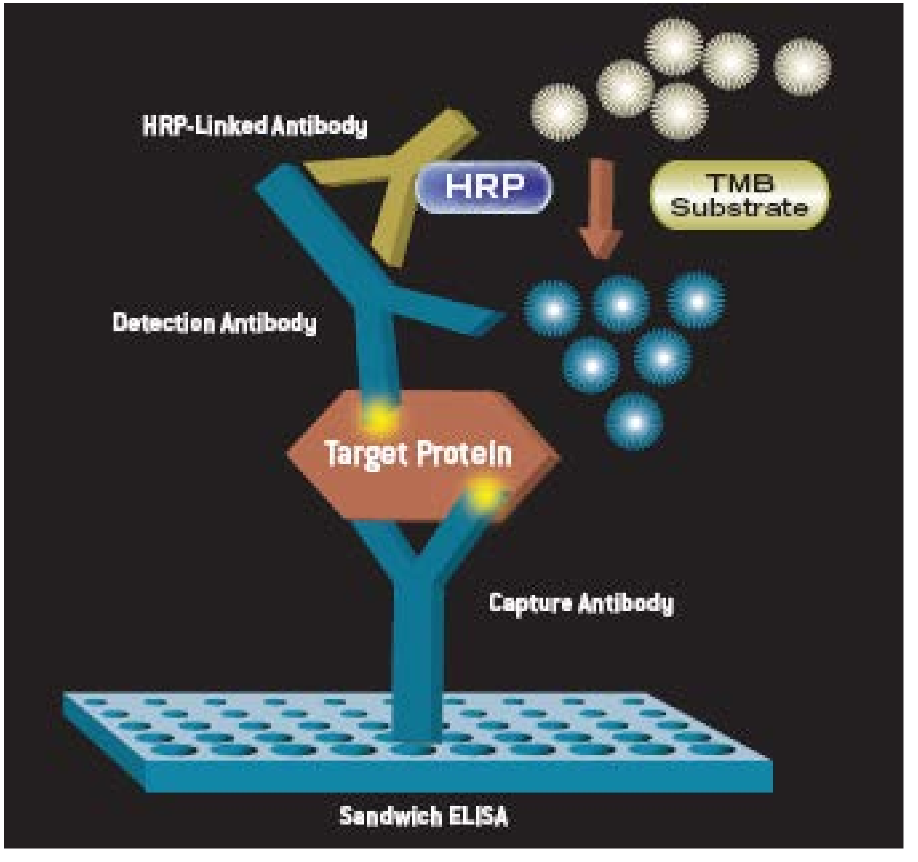
2466–2471 | PNAS | February 12, 2013 | vol. 110 | no. 7

Antibody and Antigen



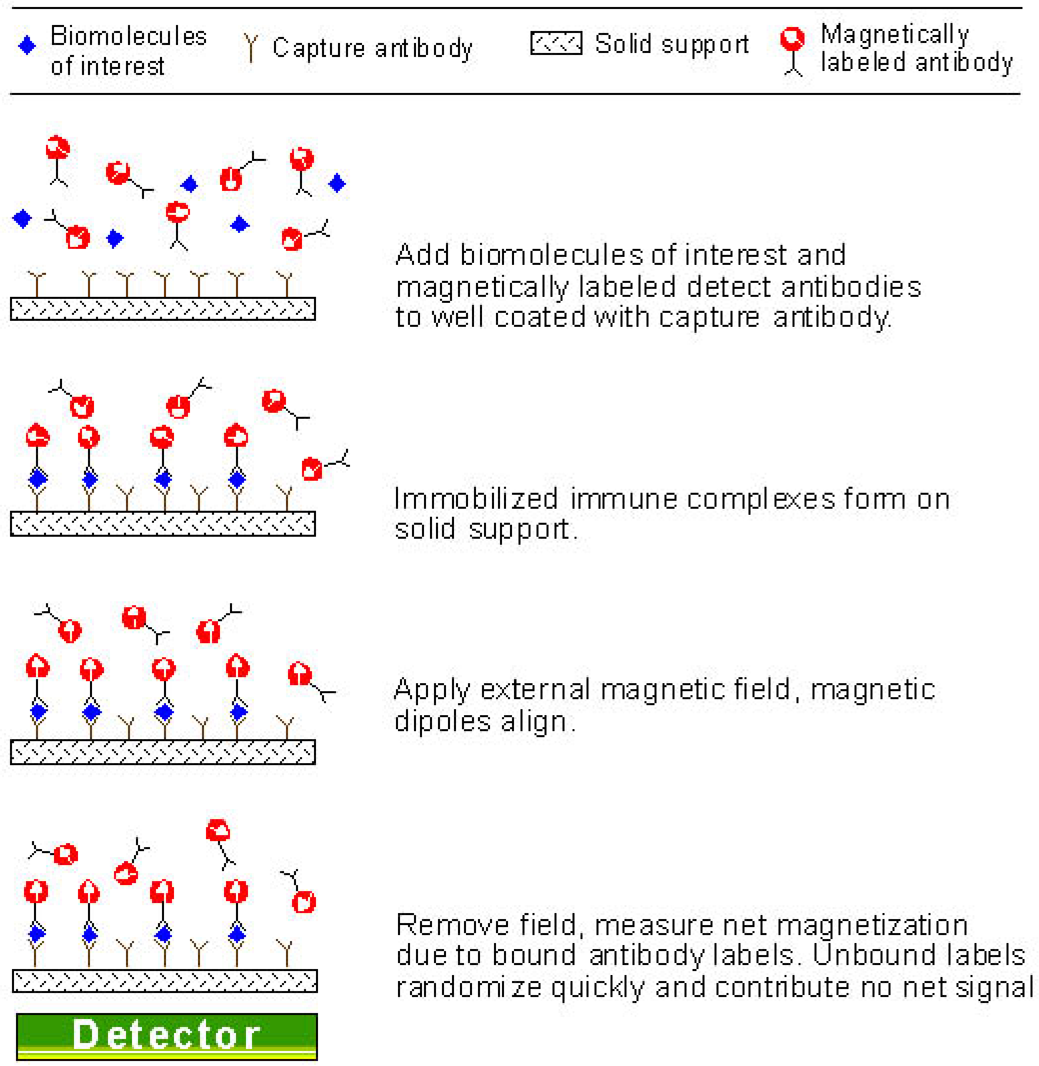


Enzyme-Linked ImmunoSorbent Assay (ELISA)

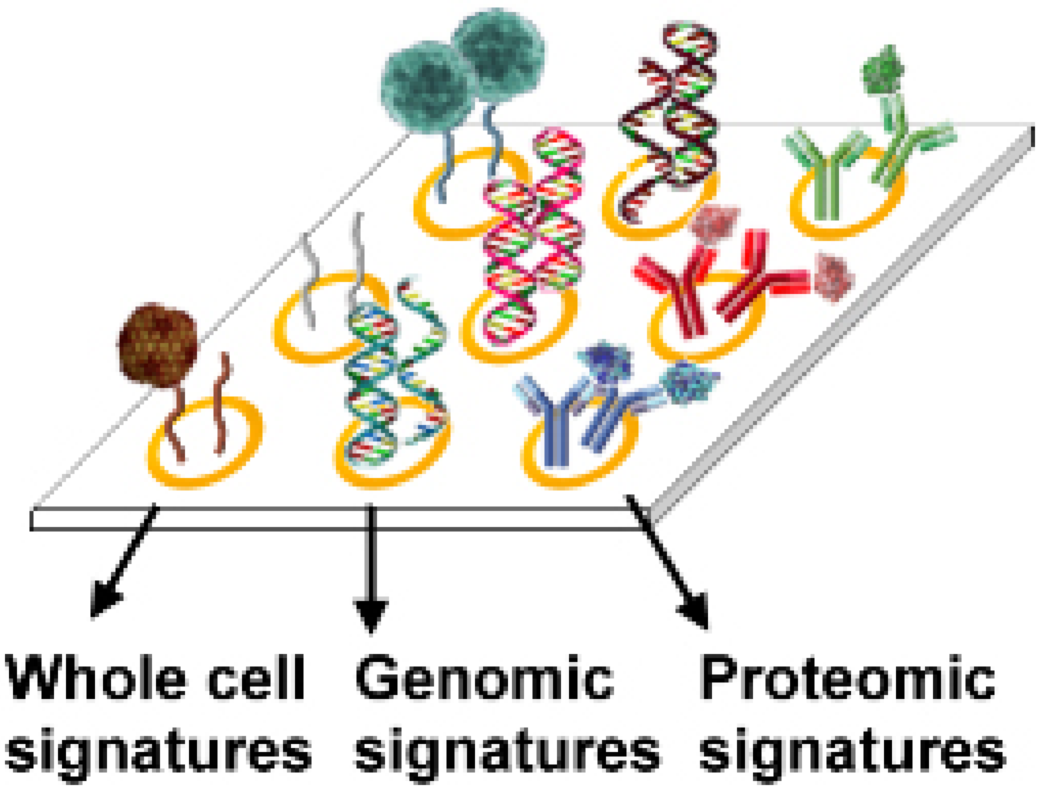


Labeling
BSA/PEG

Microarray


Biomolecules of interest
Capture antibody
Solid support
Magnetically labeled antibody
Add biomolecules of interest and magnetically labeled detect antibodies to well coated with capture antibody.
Immobilized immune complexes form on solid support.
Apply external magnetic field, magnetic dipoles align.
Remove field, measure net magnetization due to bound antibody labels. Unbound labels randomize quickly and contribute no net signal.
Detector

Microarray


Whole cell signatures
Genomic signatures
Proteomic signatures

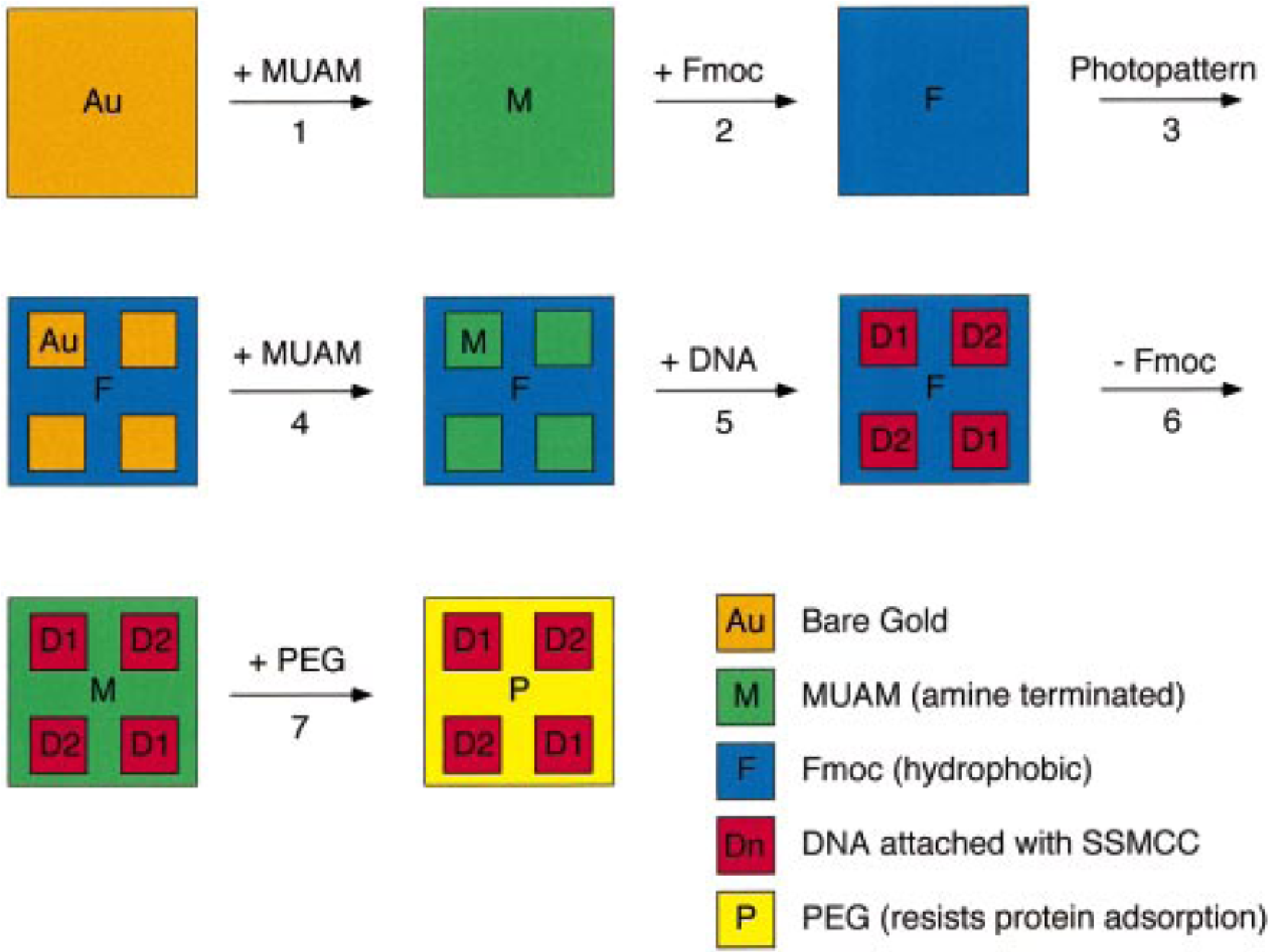


Figure 1. Fabrication scheme for the construction of multi-element DNA arrays. A clean gold surface is reacted with the amine-terminated alkanethiol MUAM, and subsequently reacted with Fmoc-NHS to create a hydrophobic surface. This surface is then exposed to UV radiation through a quartz mask and rinsed with solvent to remove the MUAM+Fmoc from specific areas of the surface, leaving bare gold pads. These bare gold areas on the sample surface are filled in with MUAM, resulting in an array of MUAM pads surrounded by a hydrophobic Fmoc background. Solutions of DNA are then delivered by pipet onto the specific array locations and are covalently bound to the surface via the bifunctional linker SSMCC. In the final two steps, the Fmoc-terminal groups on the array background are removed and replaced by PEG groups which prohibit the nonspecific binding of analyte proteins to the background.

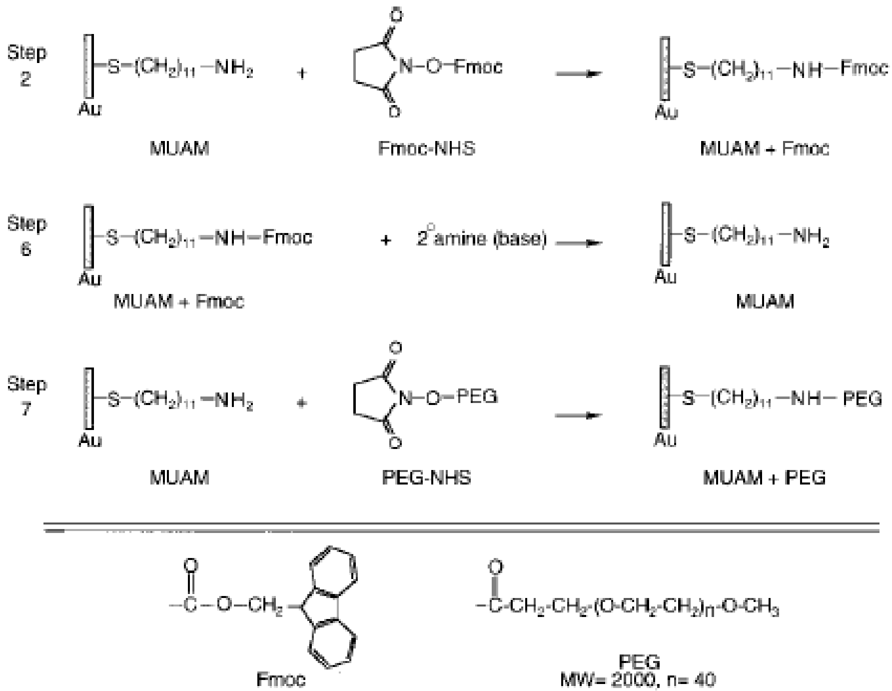


Figure 2. Surface reaction scheme showing the steps involved in the reversible modification of the array background. (Step 2) The starting amine-terminated alkanethiol surface (MUAM) is reacted with the Fmoc-NHS protecting group to form a carbamate linkage thus creating a hydrophobic Fmoc-terminated surface. (Step 6) After DNA immobilization (see Figure 3), the hydrophobic Fmoc group is removed from the surface with a basic secondary amine, resulting in the return of the original MUAM surface. (Step 7) In the final array fabrication step, the deprotected MUAM is reacted with PEG-NHS to form an amide bond that covalently attaches PEG to the array surface.

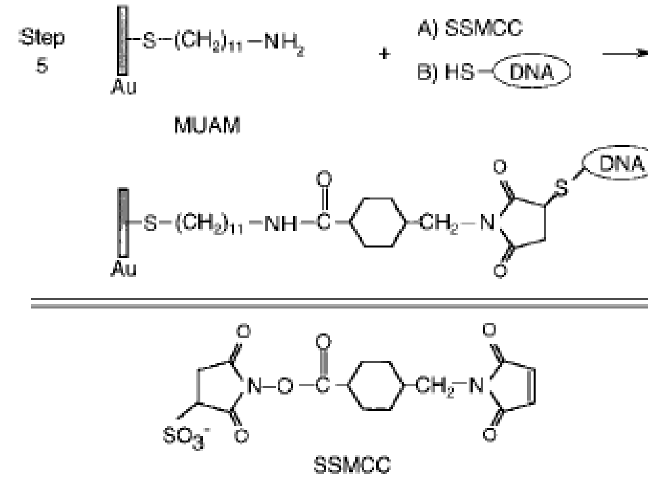


Figure 3. Surface reaction scheme showing the immobilization of thiol-terminated DNA to the array surface. In Step 5 of the DNA array fabrication, the heterobifunctional linker SSMCC is used to attach 5′-thiol modified oligonucleotide sequences to reactive pads of MUAM. This linker contains an NHSS ester functionality (reactive toward amines) and a maleimide functionality (reactive toward thiols). The surface is first exposed to a solution of the linker, whereby the NHSS ester end of the molecule reacts with the MUAM surface. Excess linker is rinsed away and the array surface is then spotted with 5′-thiol-modified DNA that reacts with the maleimide groups forming a covalent bond to the surface monolayer.

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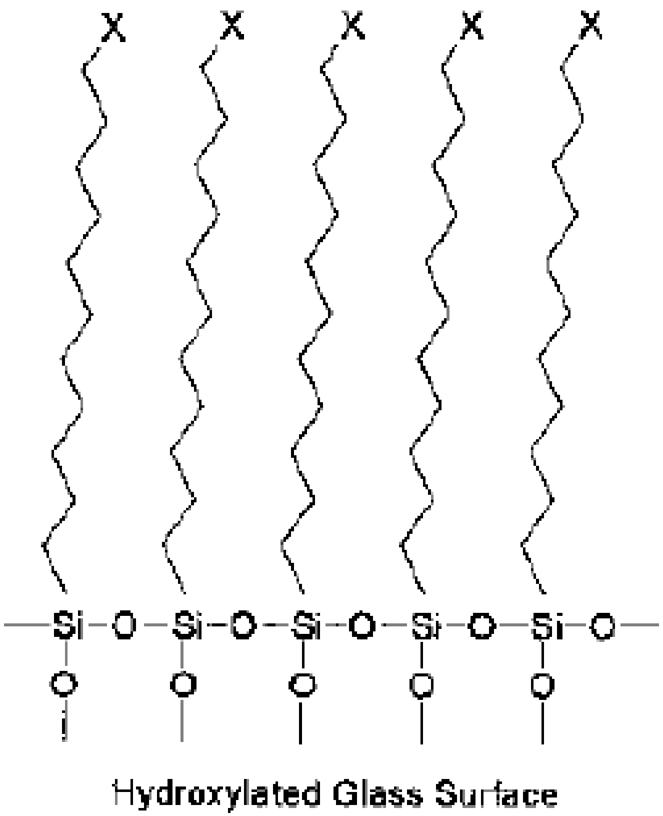
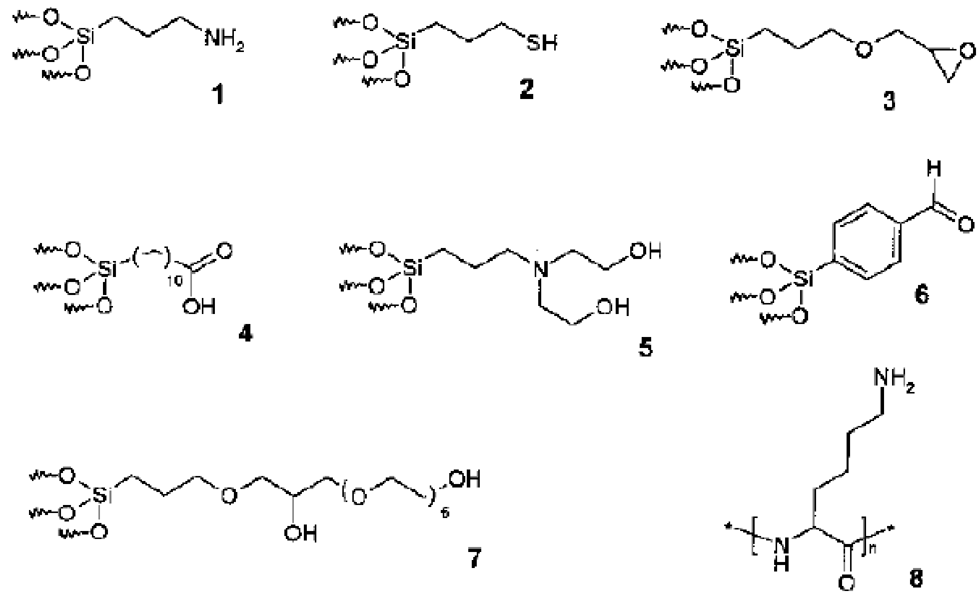
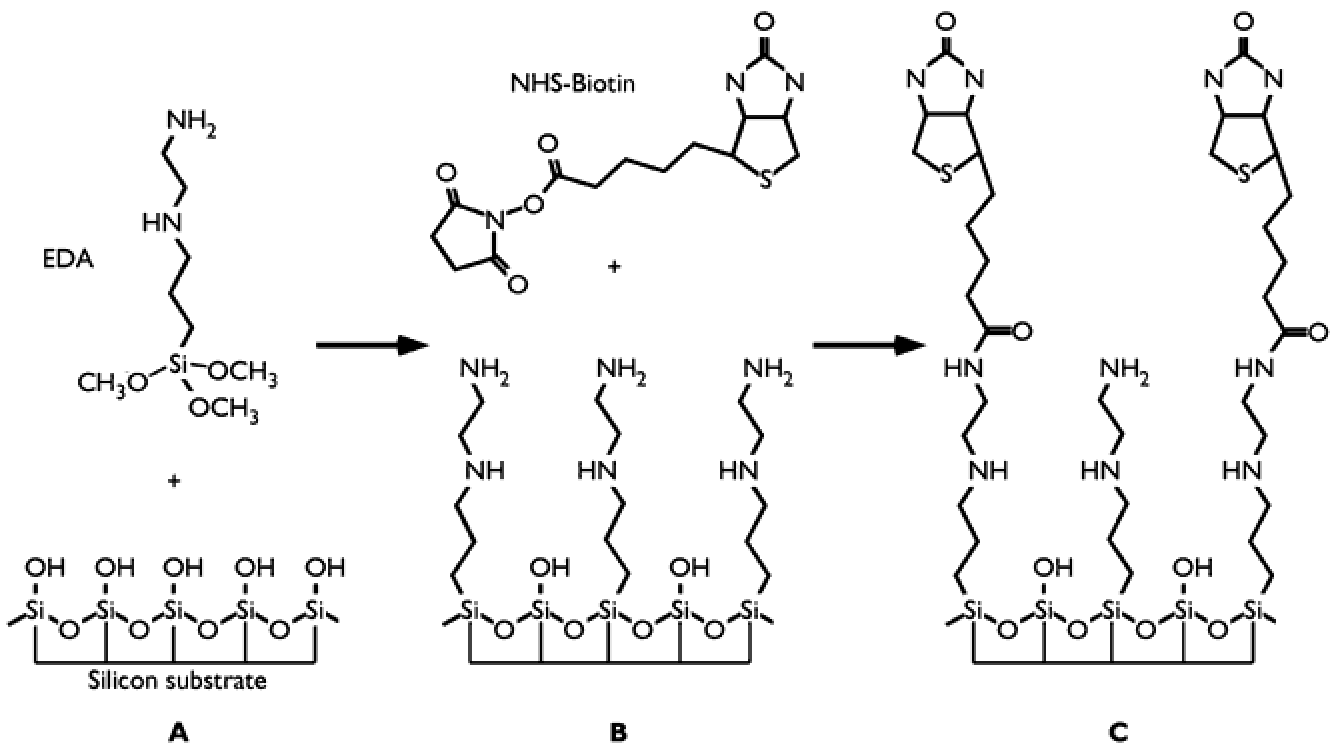
Glass Surface Modification





Scheme 2.2 Reagents for derivatization of glass surfaces. **1** APTES = aminopropyltriethoxysilane; **2** MPTS = 3-mercaptopropyltrimethoxysilane; **3** GPTS = glycidoxypropyltrimethoxysilane; **4** TETU = triethoxysilane undecanoic acid; **5** HE-APTS = bis(hydroxyethyl)aminopropyltriethoxysilane); **6** 4-trimethoxysilylbenzaldehyde; **7** GPTS/HEG = glycidoxypropyltrimethoxysilane-hexaethylene glycol; **8** poly(lysine).

Scheme 2.1 2D schematic description of a polysiloxane monolayer on a glass surface (X = terminal functional



EDA
NHS-Biotin
Silicon substrate
A
B
C

Biotin-Streptavidin

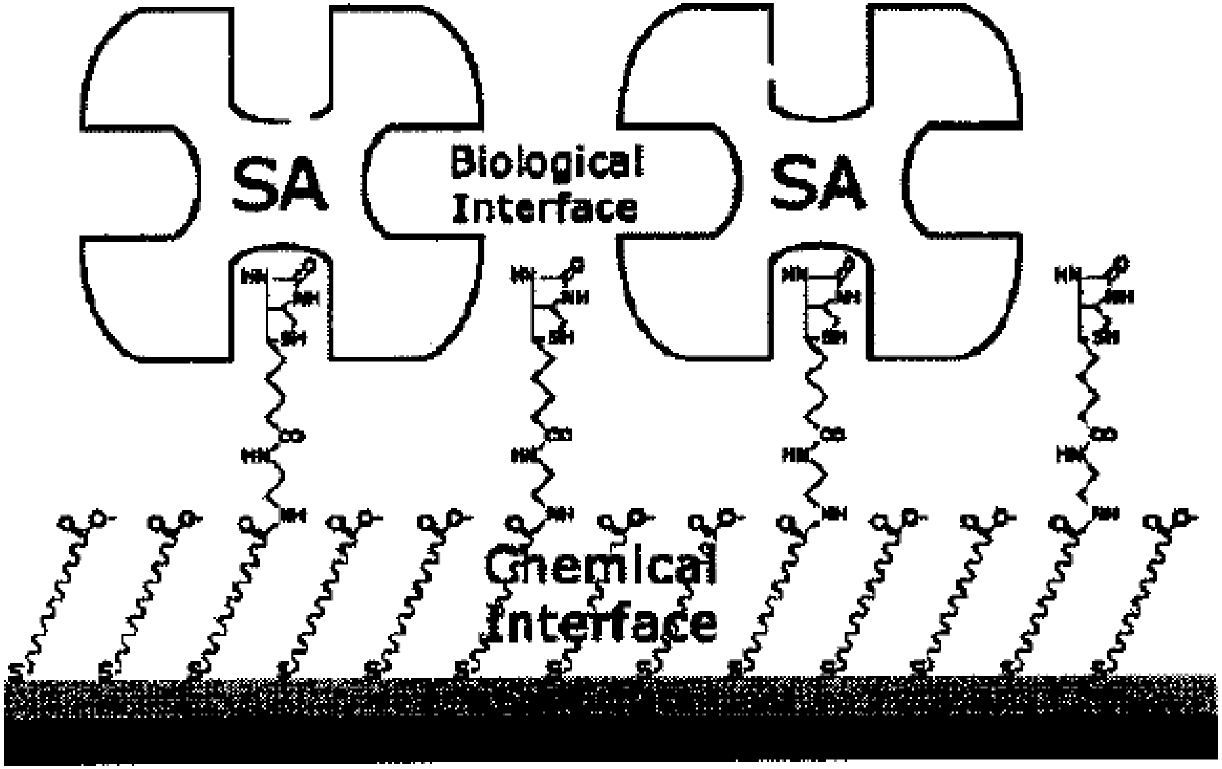
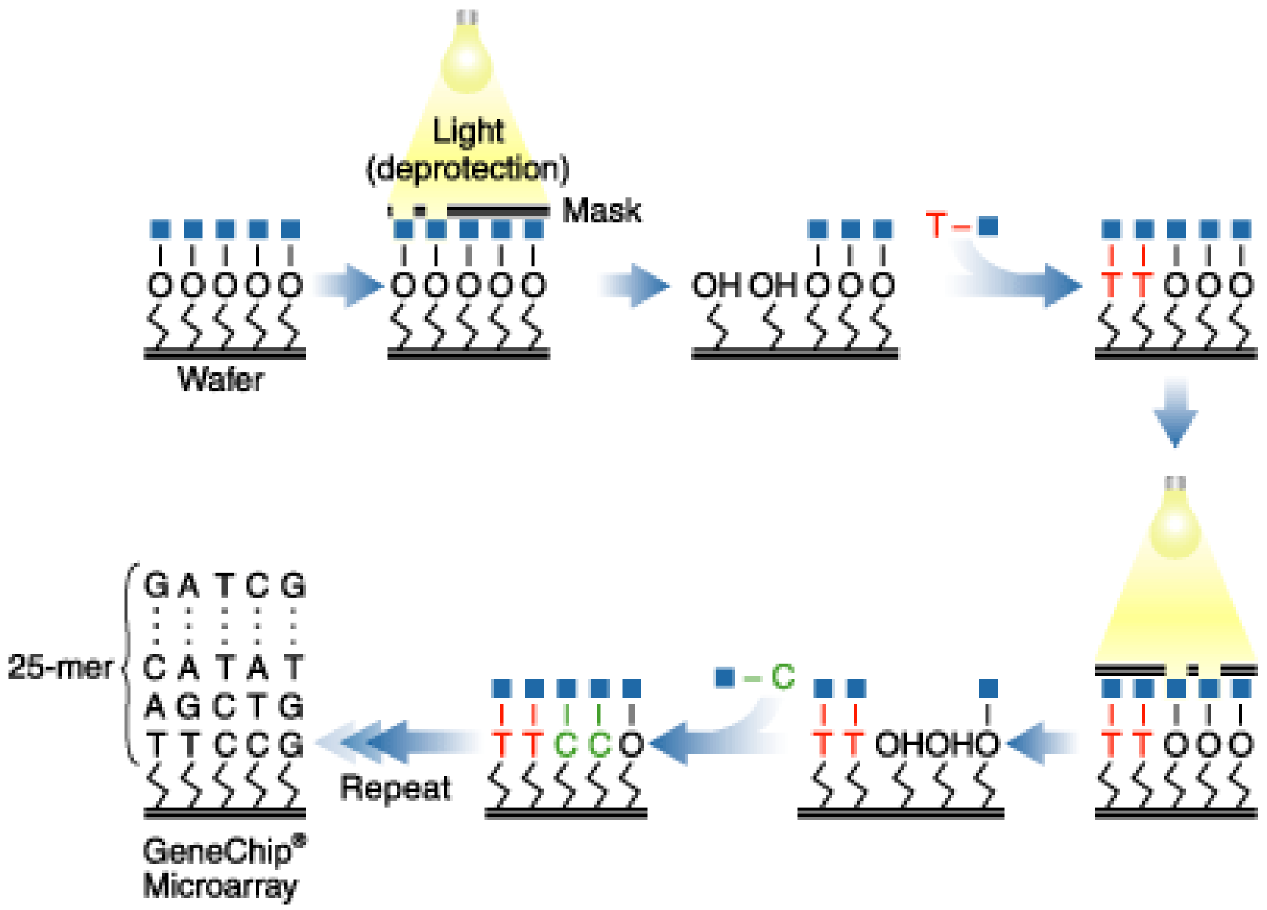


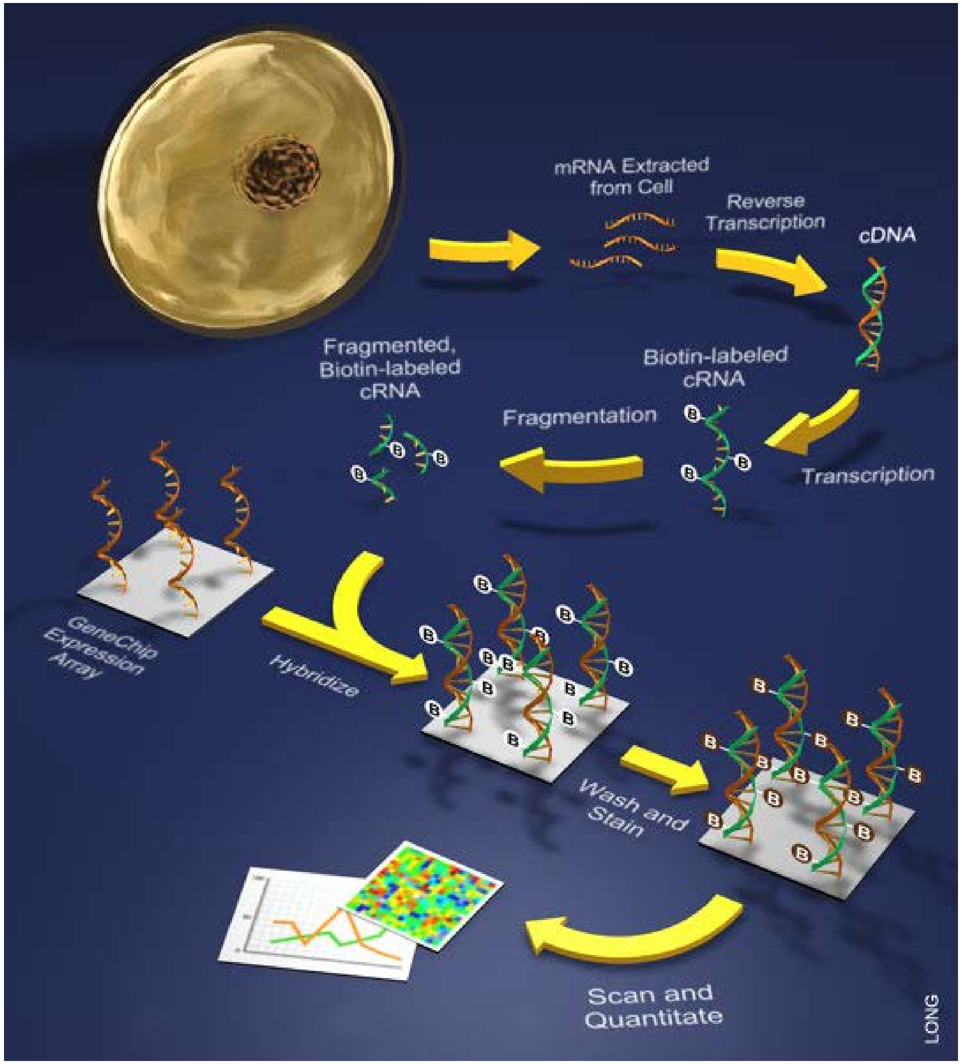
Figure 2.3 Schematic respresentation of a steptavidin sensor surface assembled on a reaction-controlled biotinylated SAM [28].

GeneChip



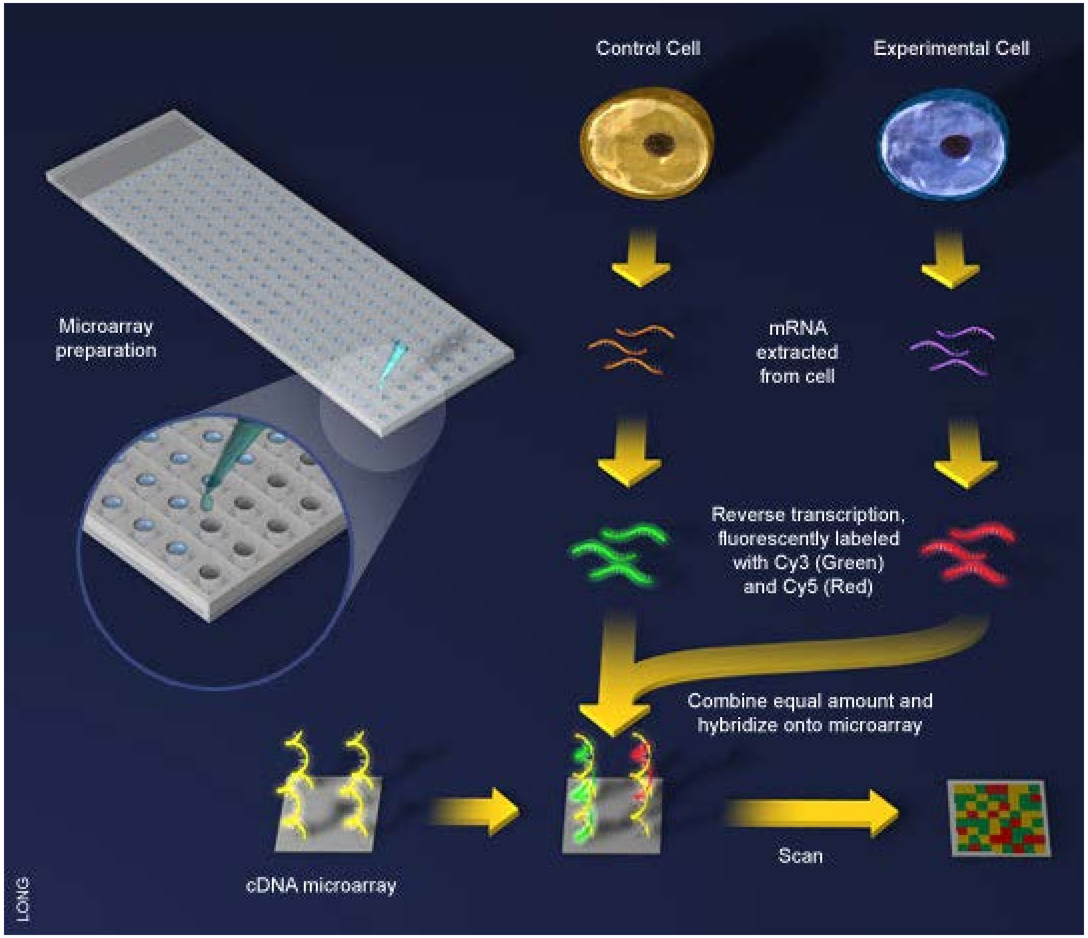
Light
(deprotection)
Mask
Wafer
T – ■
OH OH O O O
T T O O O
■ – C
T T OHOHO
T T C C O
Repeat
G A T C G
25-mer
C A T A T
A G C T G
T T C C G
GeneChip®
Microarray

Scheme

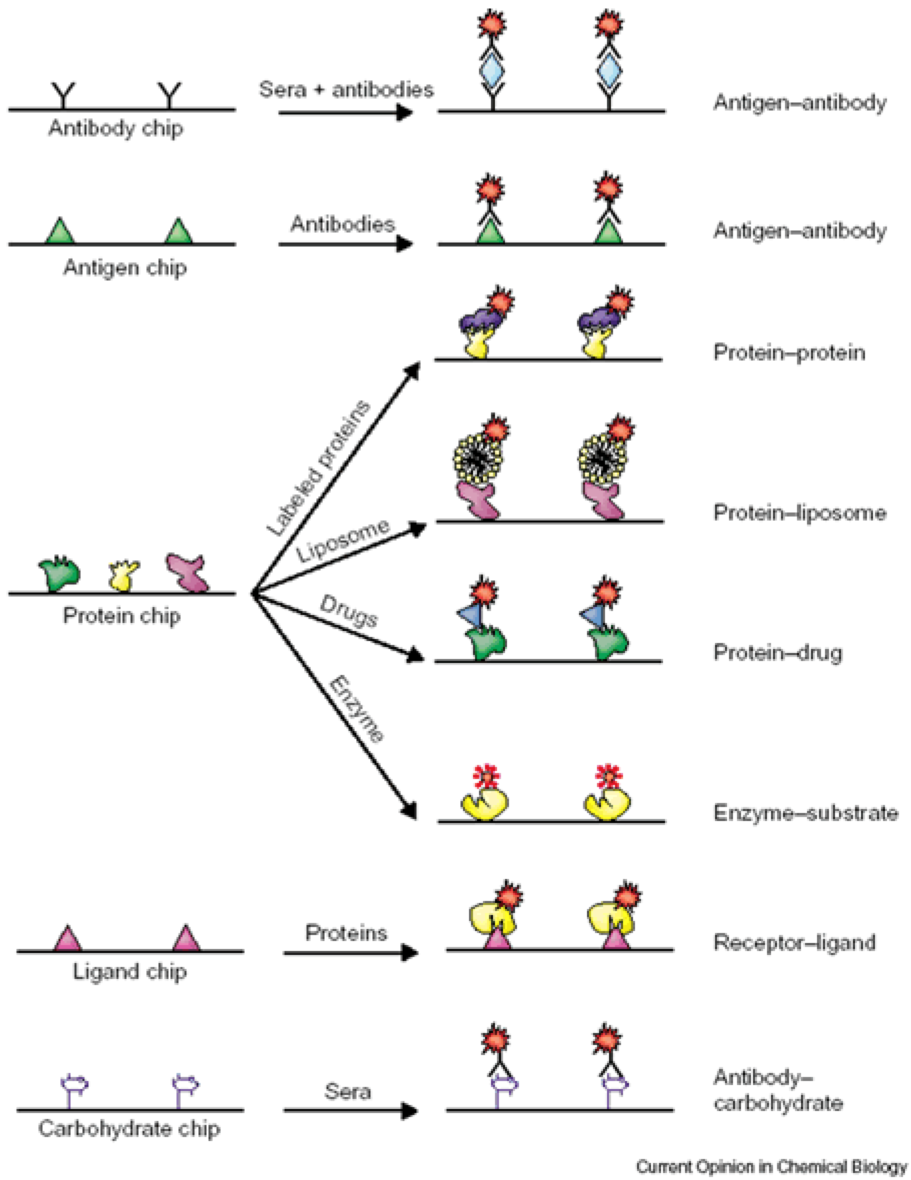


mRNA Extracted from Cell
Reverse Transcription
cDNA
Biotin-labeled cRNA
Transcription
Fragmentation
Fragmented, Biotin-labeled cRNA
GeneChip Expression Array
Hybridize
Wash and Stain
Scan and Quantitate
LONG

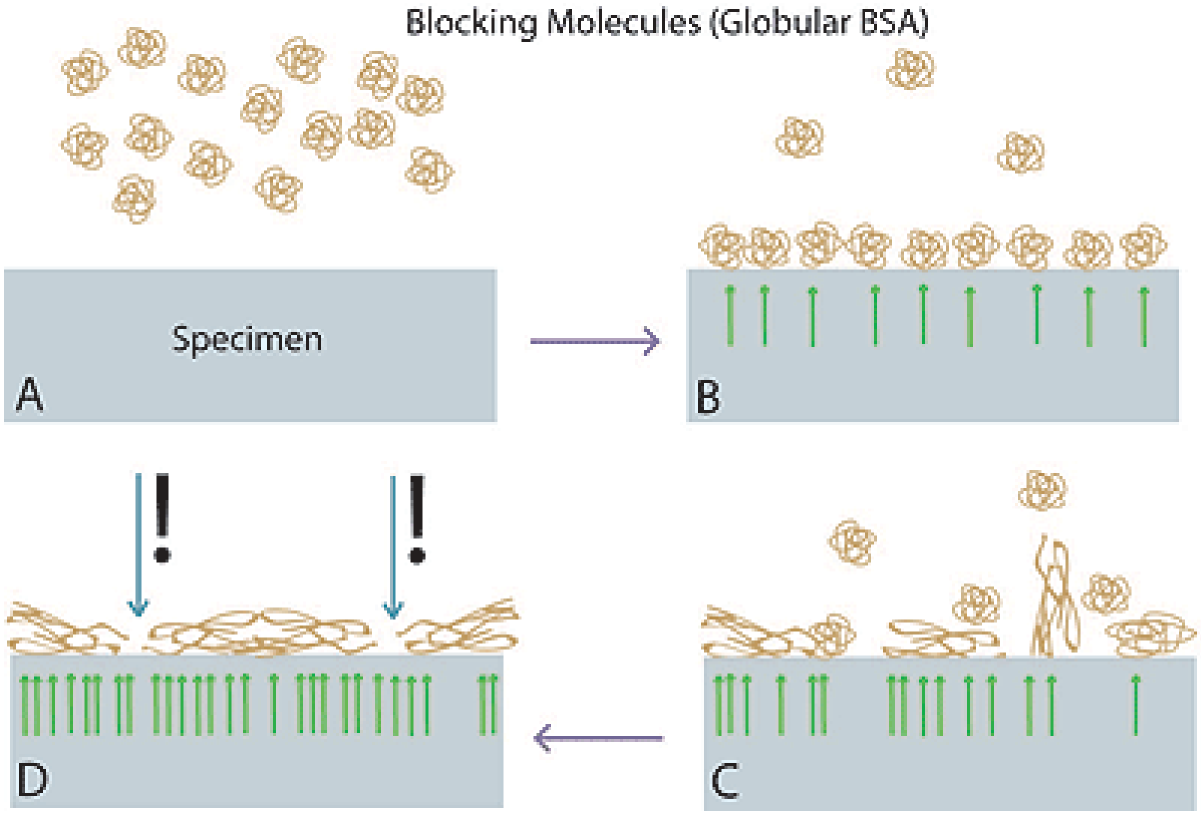
cDNA Microarray



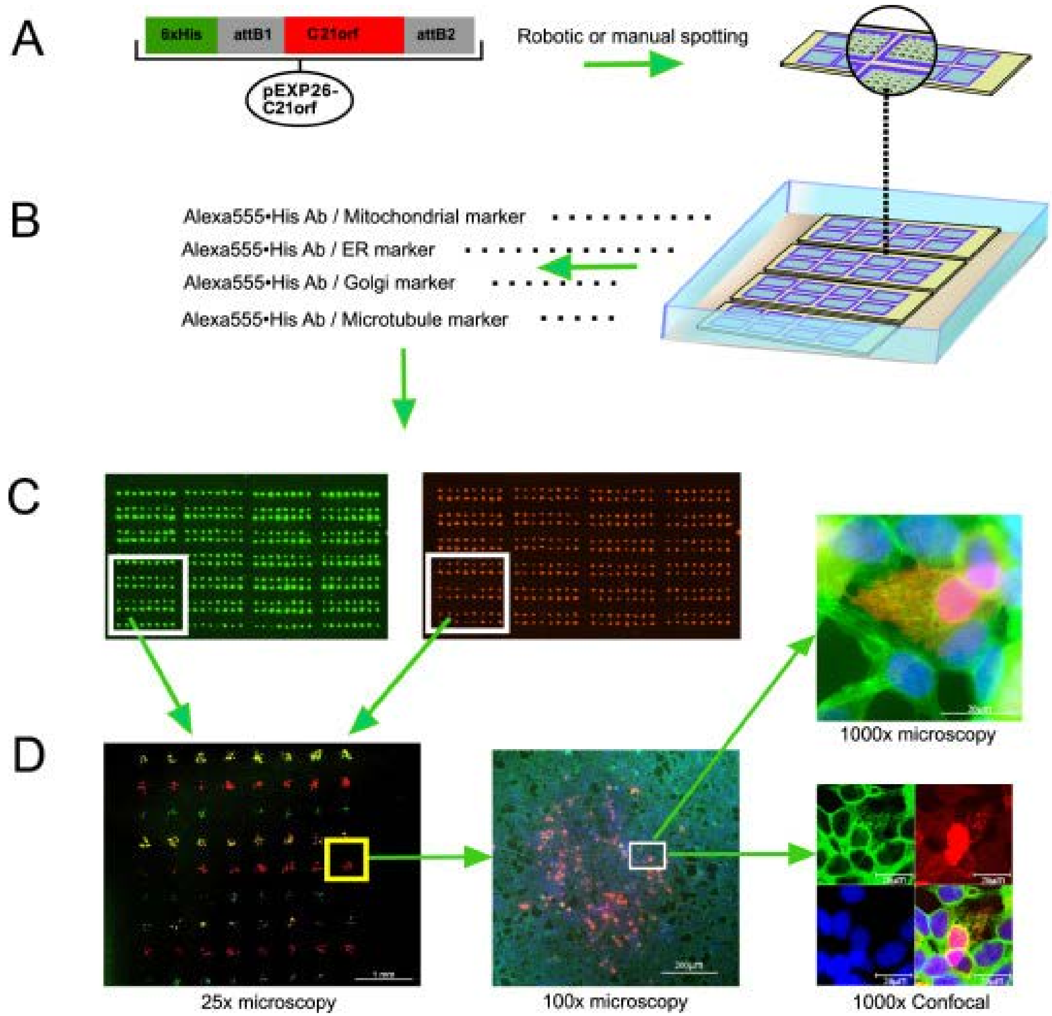
Protein Array



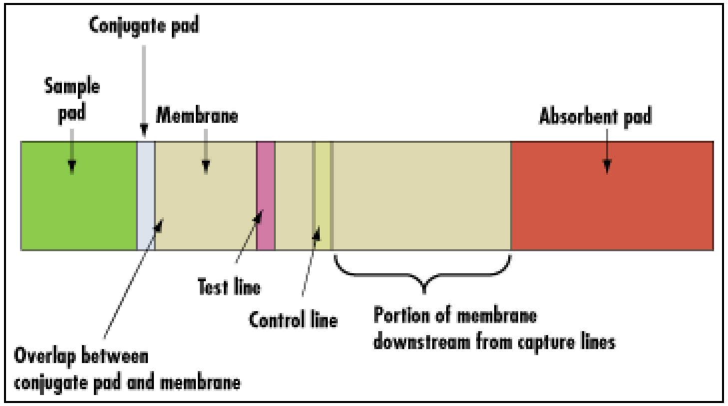
BSA Blocking



Cell Array


A
6xHis
attB1
C21orf
attB2
pEXP26-C21orf
Robotic or manual spotting
B
Alexa555•His Ab / Mitochondrial marker
Alexa555•His Ab / ER marker
Alexa555•His Ab / Golgi marker
Alexa555•His Ab / Microtubule marker
C
D
1000x microscopy
25x microscopy
100x microscopy
1000x Confocal

hCG immunoassay



human chorionic gonadotropin (hCG)

Nucleotide Sensor