

# Molecular Techniques: Tools for Success

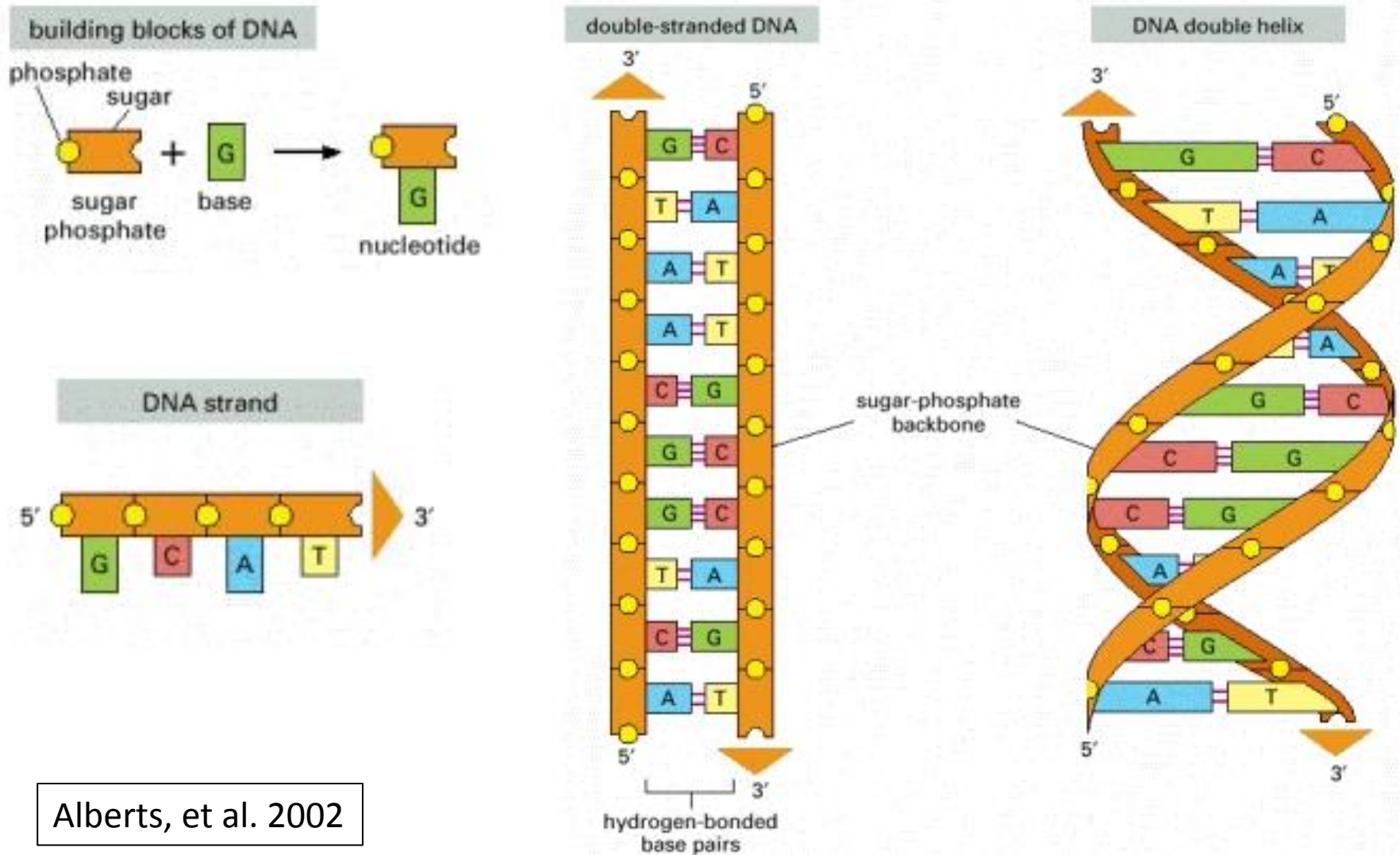
Jason Rosenzweig, PhD

# Outline

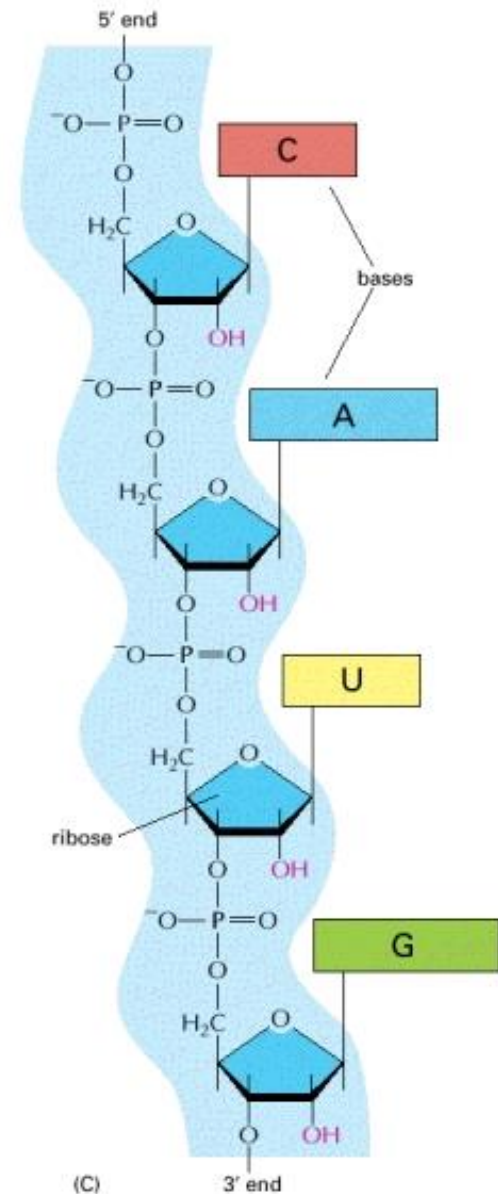
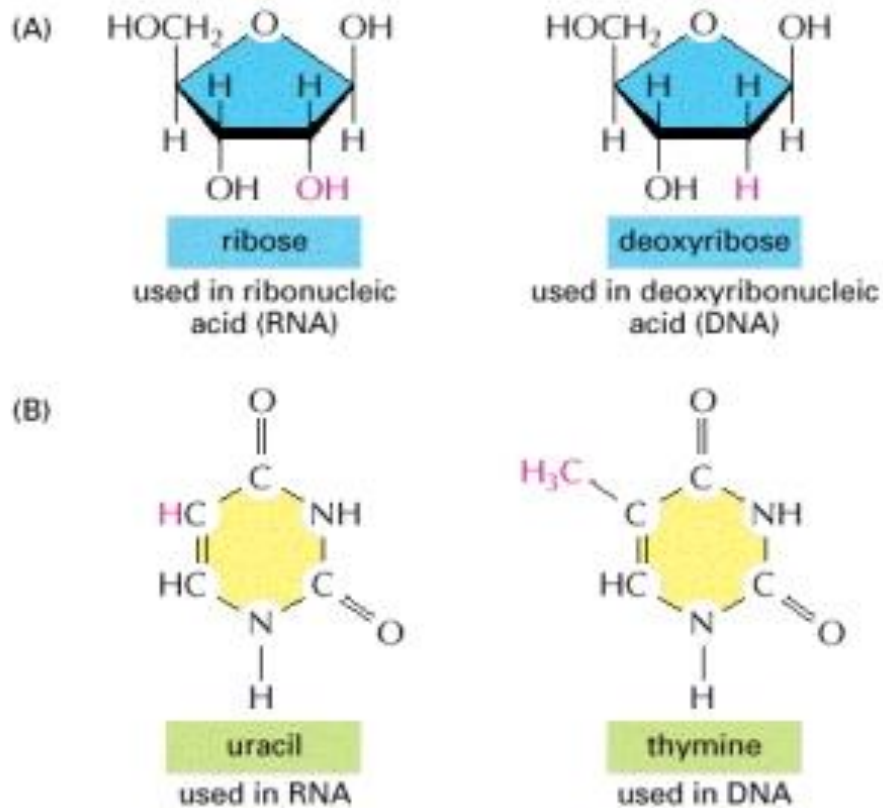
- Introduction
- DNA Cloning
- Polymerase Chain Reaction (PCR)
- Electrophoresis
- Hybridization
- Array-based expression profiling

# Introduction

# DNA



# RNA



Alberts, et al. 2002

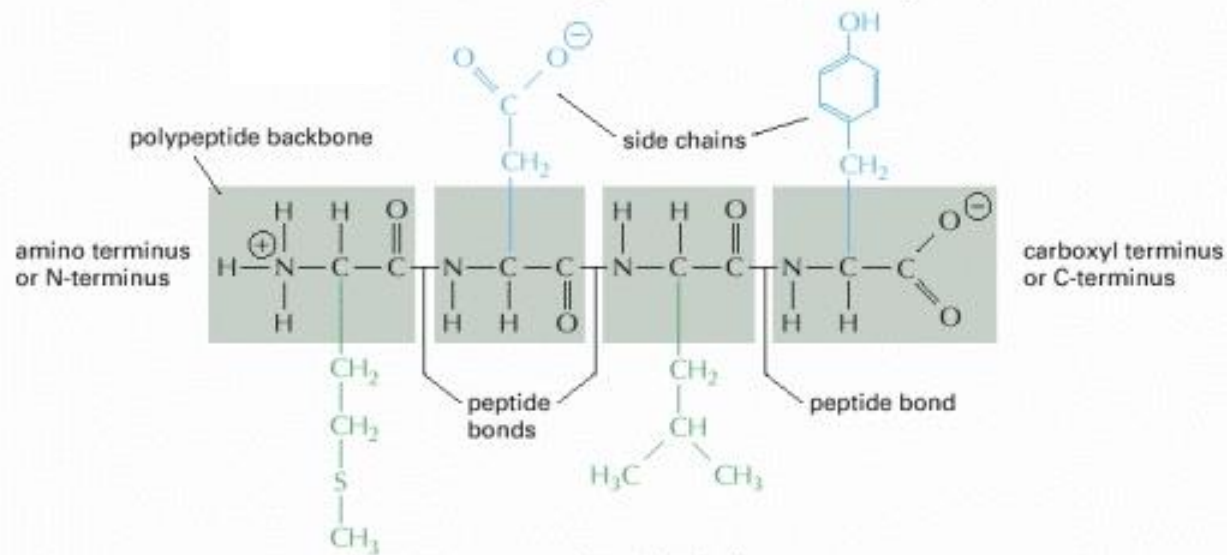
# Proteins

AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

## POLAR AMINO ACIDS

AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

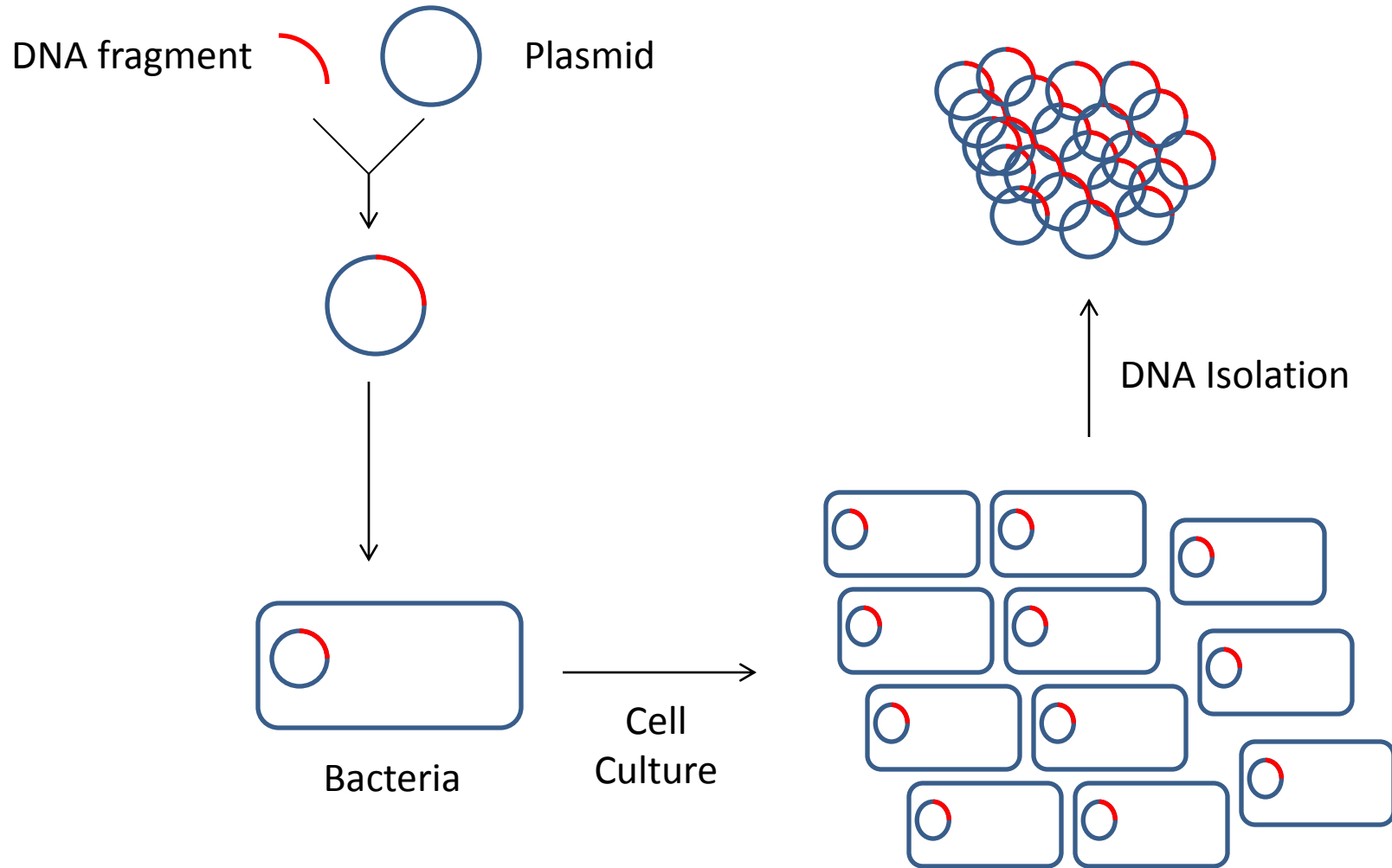
## NONPOLAR AMINO ACIDS



Alberts, et al. 2002

# DNA Cloning

# DNA Cloning

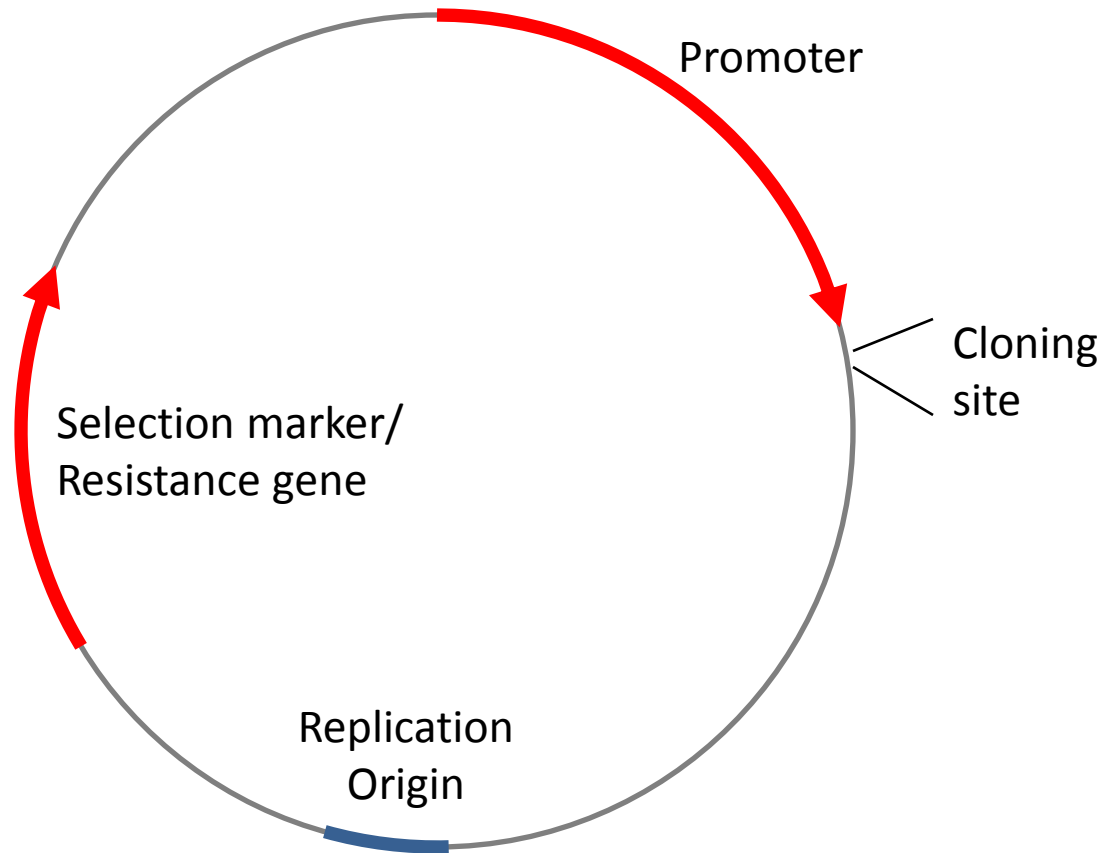




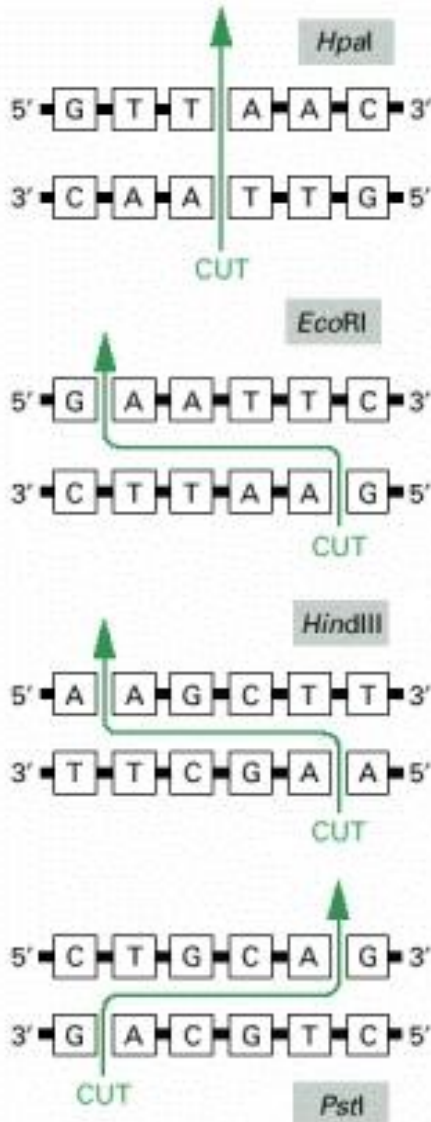
# DNA Cloning - Vector Construction

## Expression Systems:

- Bacterial
- Yeast
- Baculovirus (Insect)
- Plant
- Mammalian
- Cell-free



# DNA Cloning - Restriction Nucleases

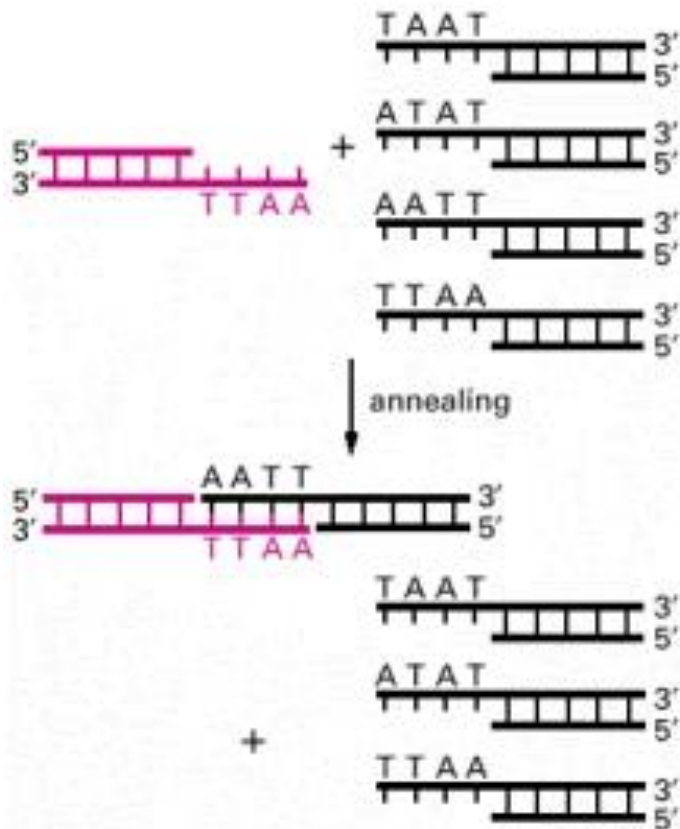


Type II Restriction nucleases:

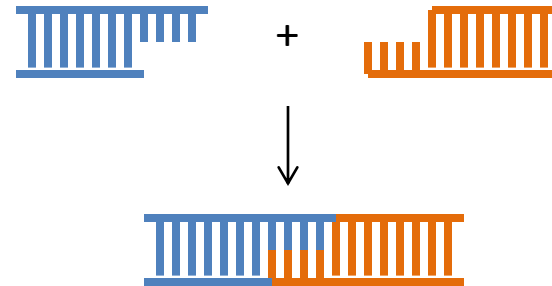
- Recognize specific DNA sequences
- Usually four to eight bases and palindromic
- Cut the DNA – can be blunt or “sticky”

Alberts, et al. 2002

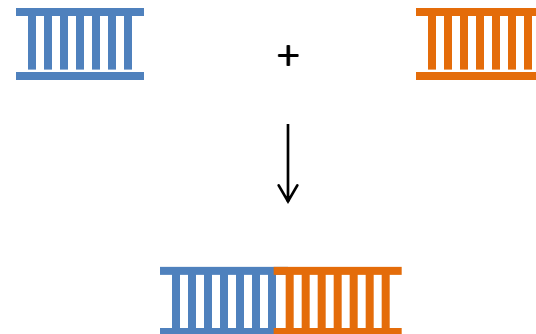
# DNA Cloning - Ligation



Sticky Ends



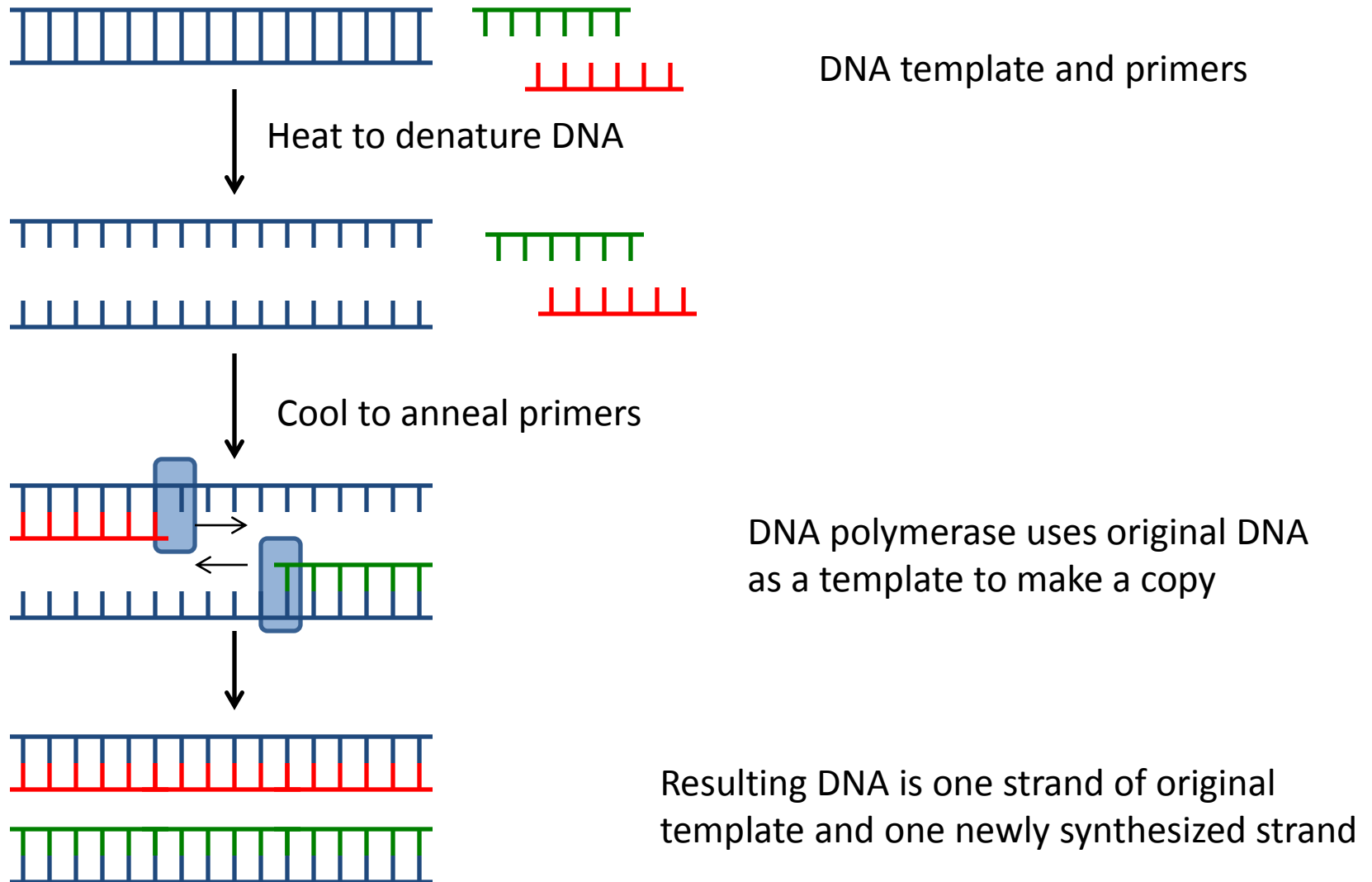
Blunt Ends



Alberts, et al. 2002

# Polymerase Chain Reaction

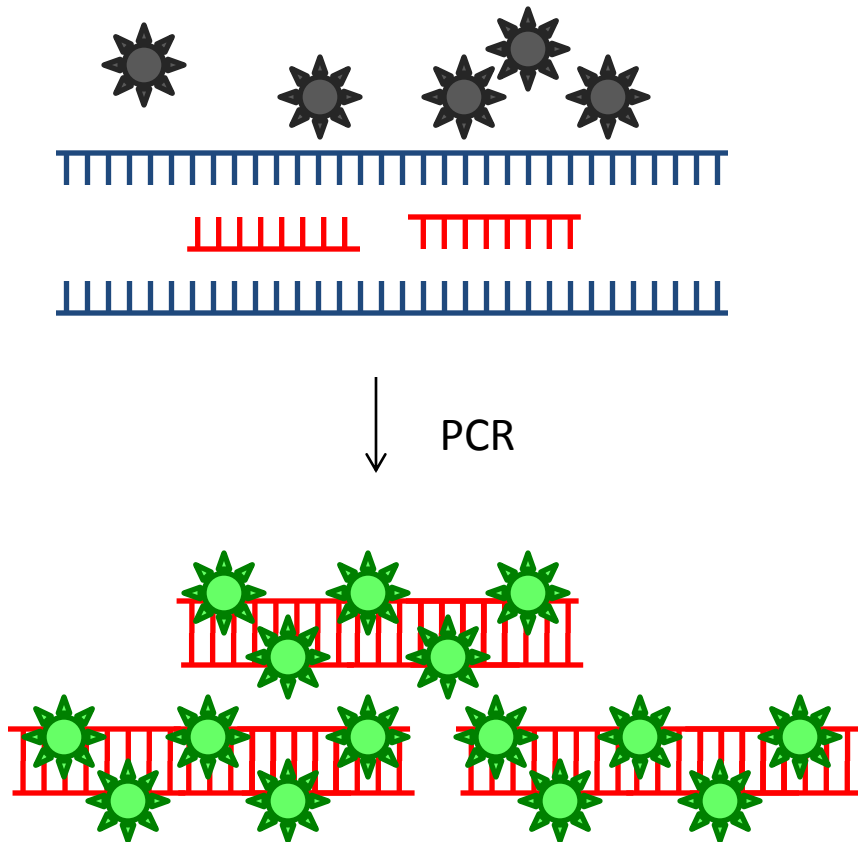
# Polymerase Chain Reaction



# Quantitative PCR (qPCR)

## Dye-based (SYBR)

DNA-binding dye fluoresces when bound to double stranded DNA

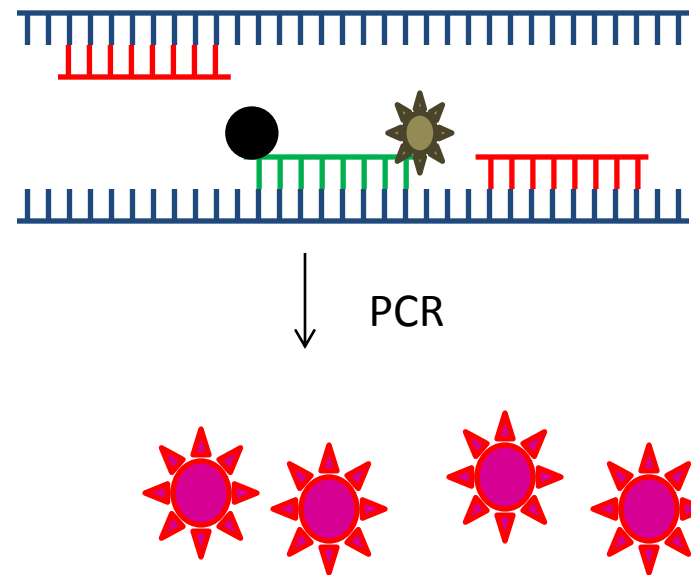


## Probe-based (TaqMan)

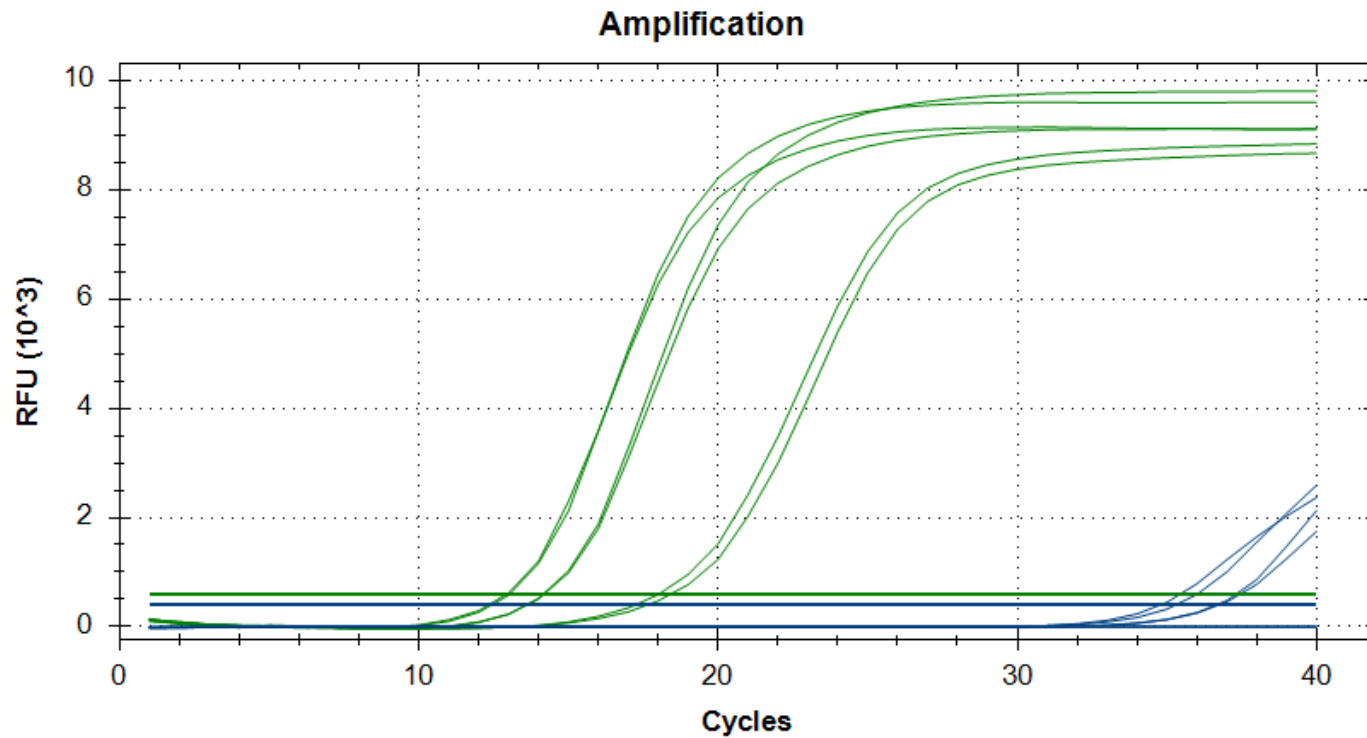
Probe labeled with a fluorophore and a quencher.

Probe binds a specific sequence between the primers

Probe is digested during DNA synthesis, releasing fluorophore from quencher.

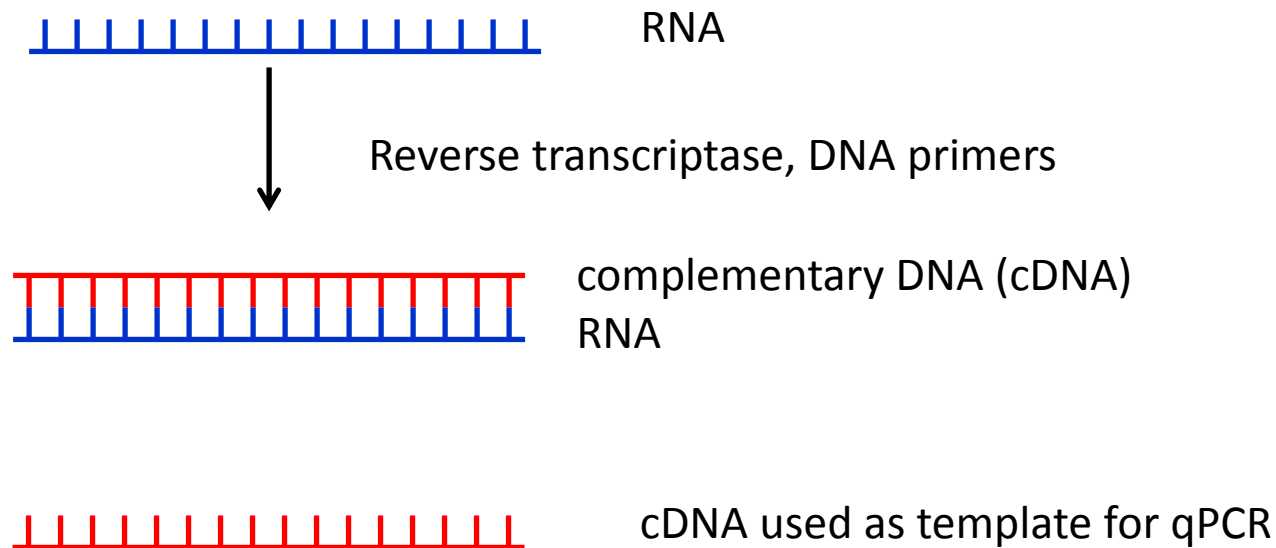


# qPCR



# Reverse Transcriptase PCR (RT-PCR)

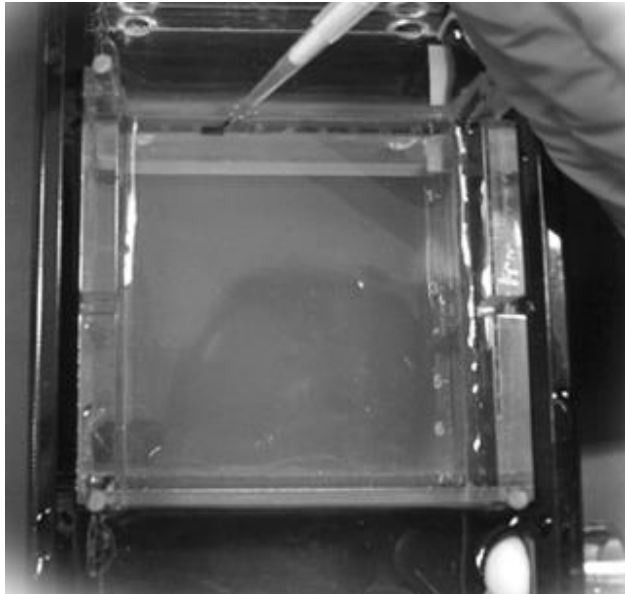
Quantifying gene expression



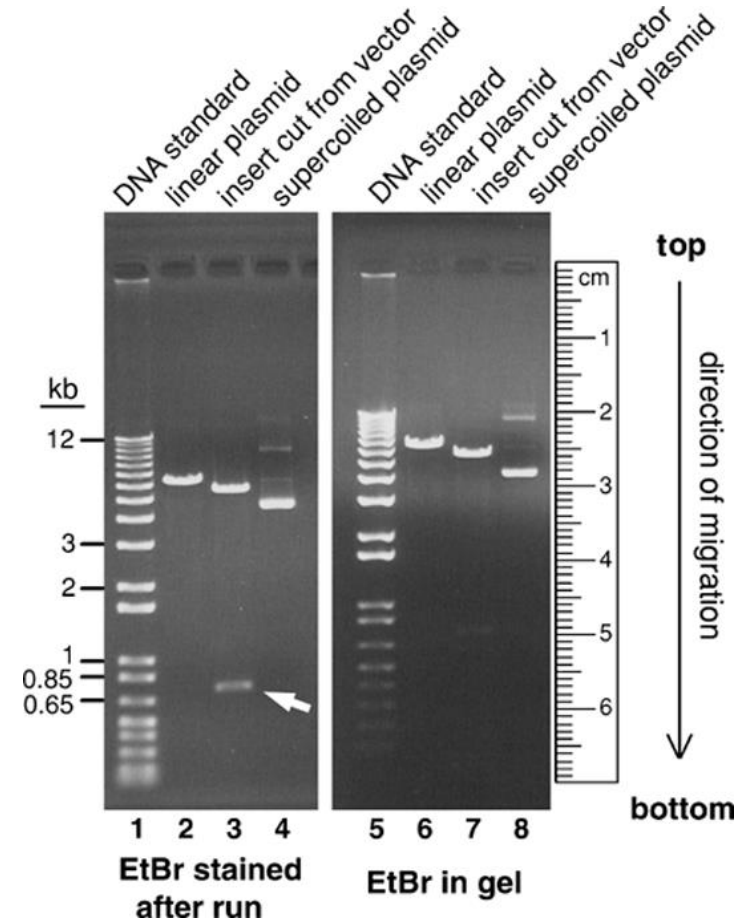


# Gel Electrophoresis

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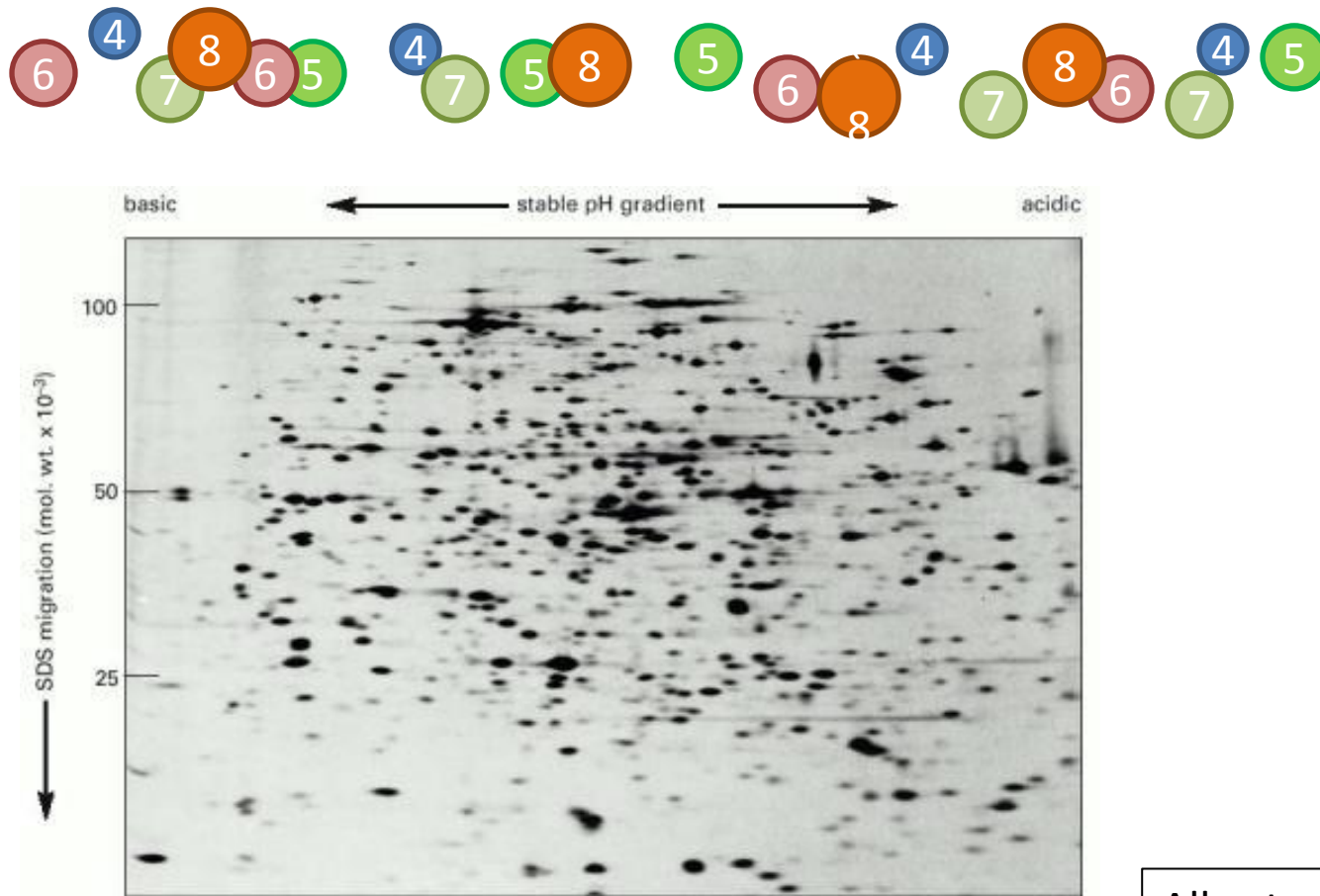


Electricity + Time



# Gel Electrophoresis – Two Dimensions

## Isoelectric Focusing



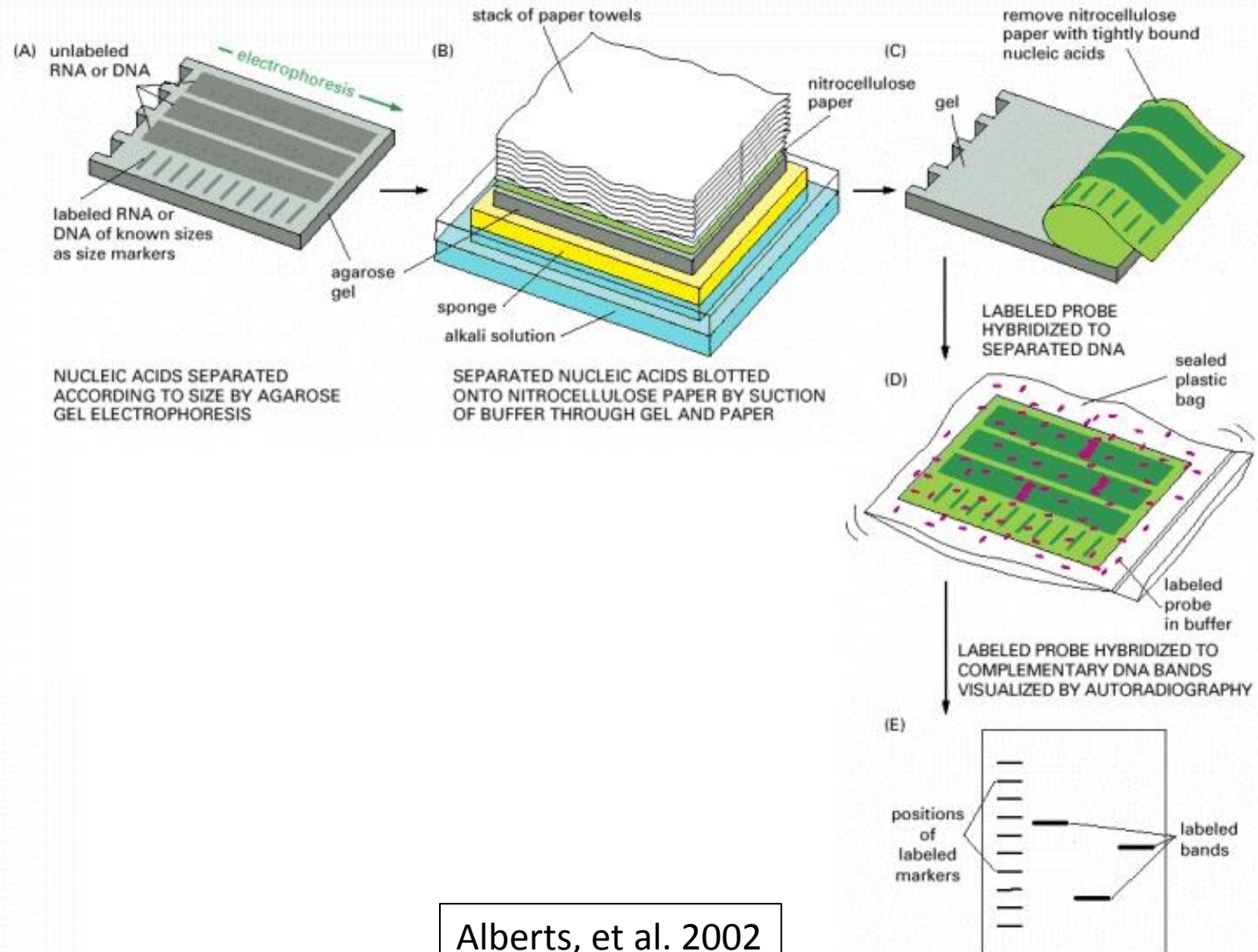
Alberts, et al. 2002

# Hybridization Techniques

# Hybridization Techniques

- Southern Blot (DNA) – labeled, single-stranded nucleic acid probes to detect DNA
- Northern Blot (RNA) – labeled, single-stranded nucleic acid probes to detect RNA
- Western Blot (Protein) – labeled probes to detect specific protein (primary and secondary antibodies)
- Enzyme-Linked Immunosorbent Assay (ELISA) -

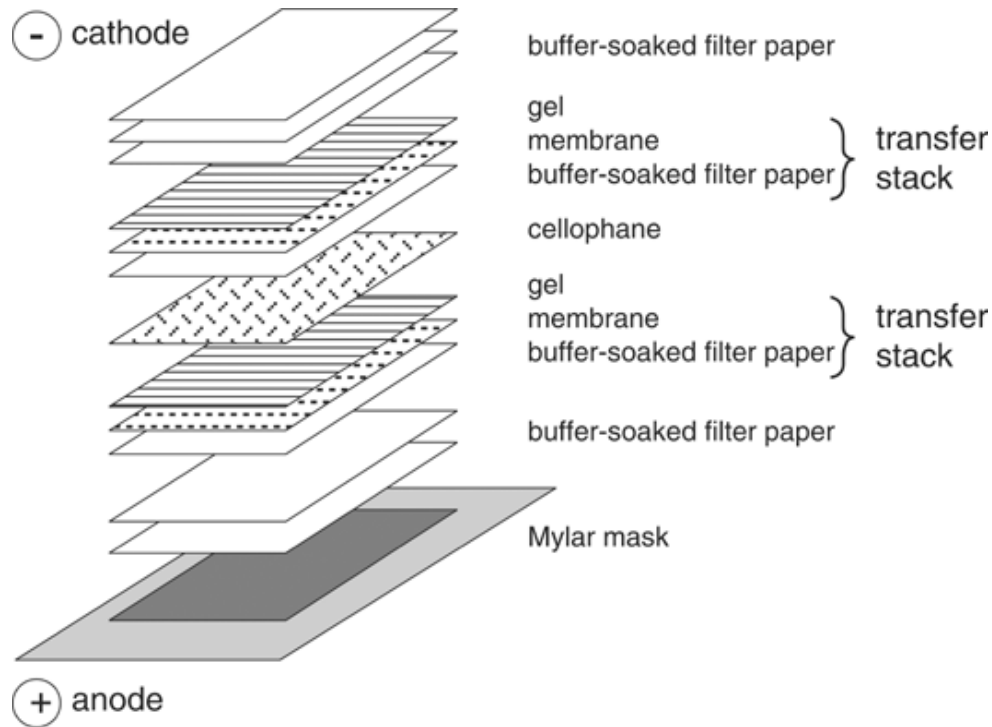
# Hybridization – Southern and Northern



Alberts, et al. 2002

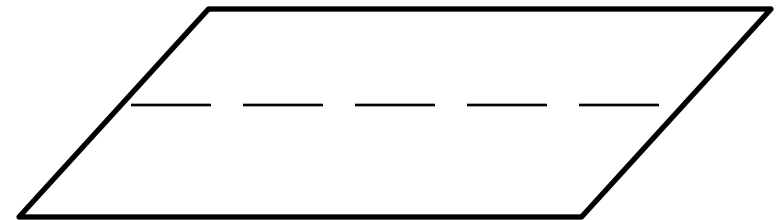
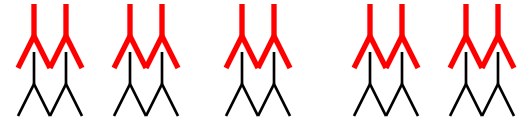
# Hybridization – Western Blot

Transfer proteins from gel to membrane

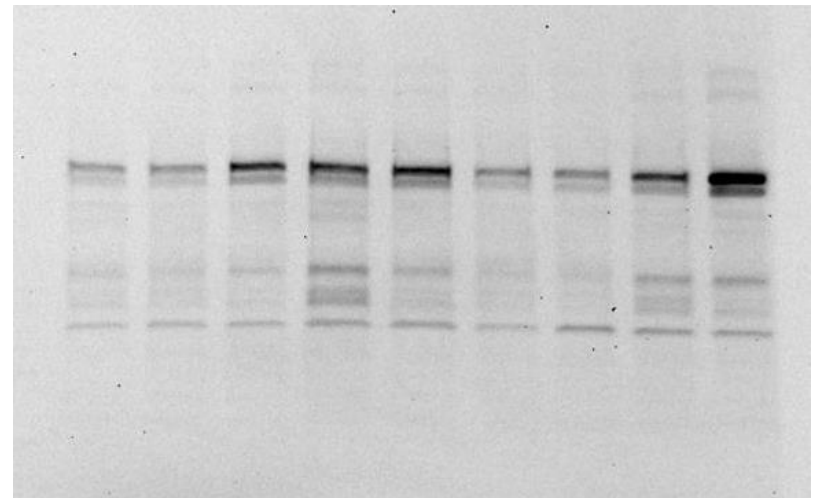


Winston et al. 2008

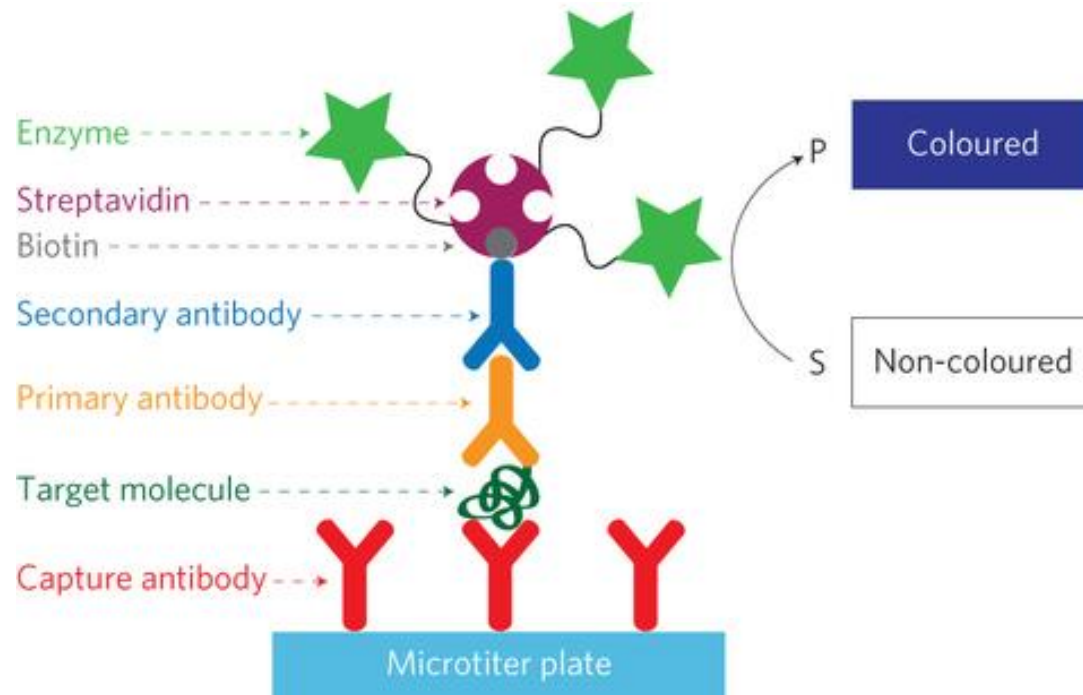
Hybridization



Visualization



# Hybridization - ELISA



- Sensitive and specific
- Quantification of picogram levels of protein

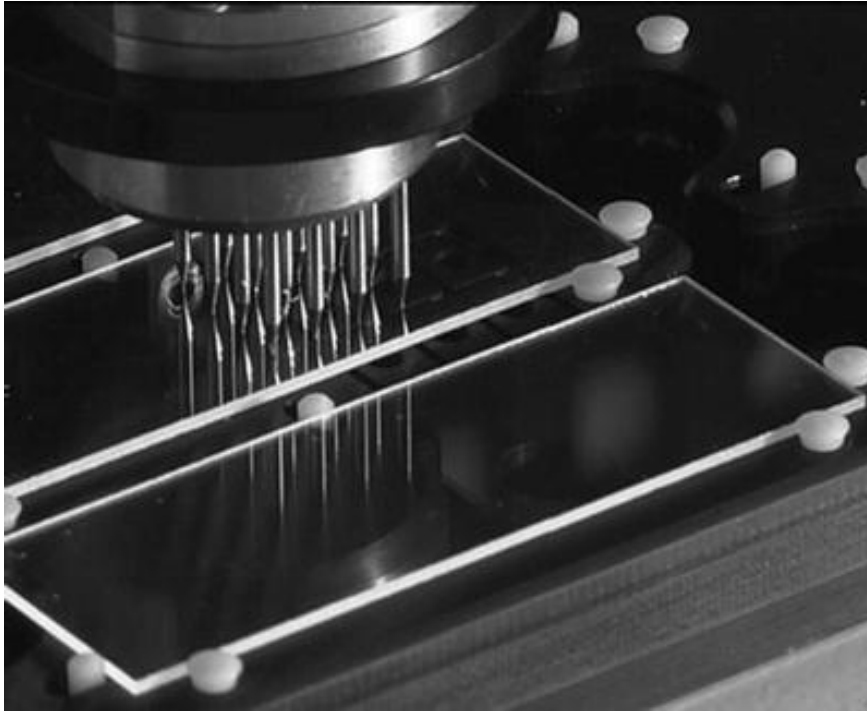
de la Rica and Stevens, 2012



# Array-based assays

# Array-based Expression Profiling

Hybridization targets are  
microprinted on to glass slides



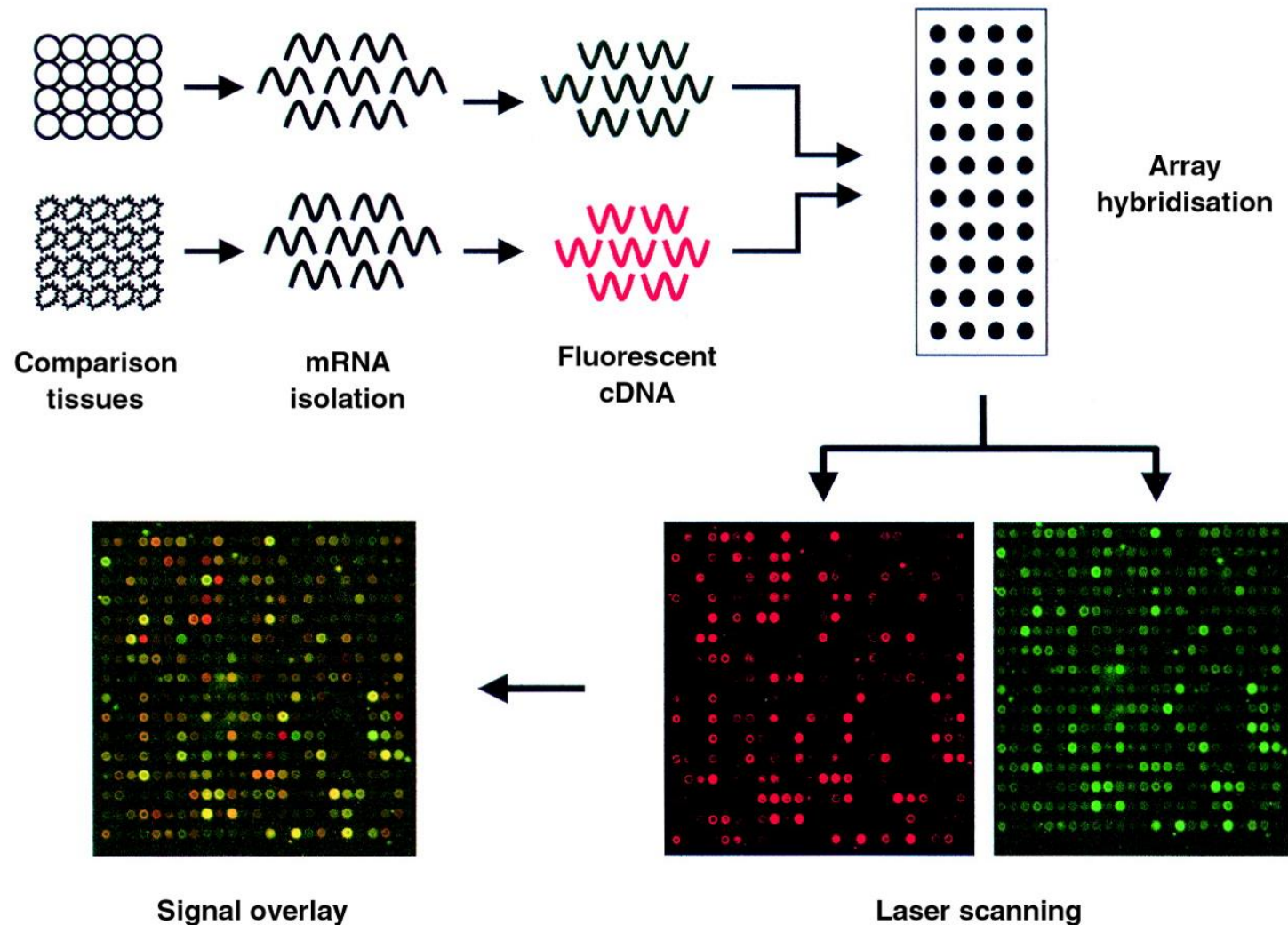
or

chip-based arrays



Blackburn and Shoko, 2011.

# Array-based Expression Profiling

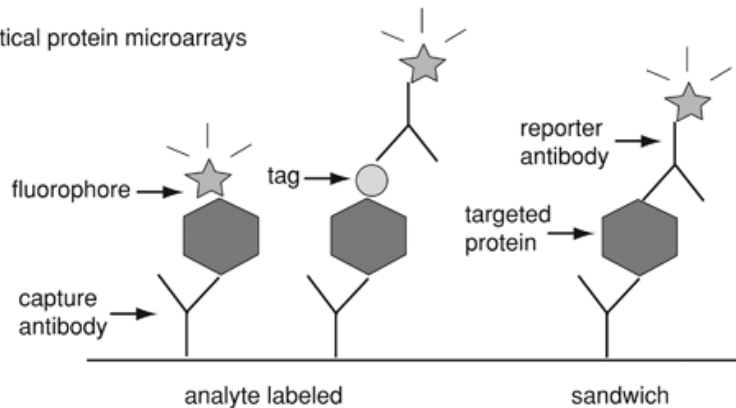


Robinson et al., 2000

# Protein Arrays

A

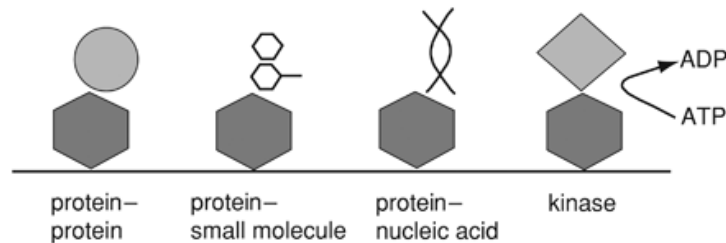
analytical protein microarrays



Analytical: Antibody-based arrays for protein detection

B

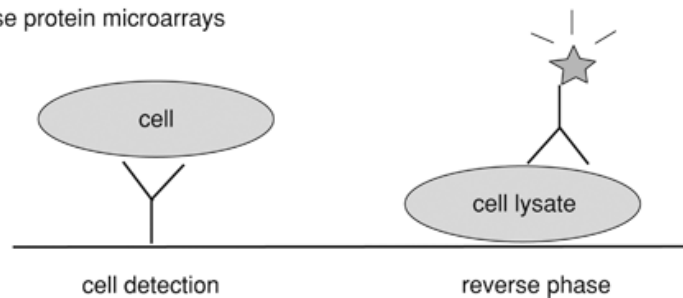
functional protein microarrays



Functional: Protein-based arrays for detection of functional interactions

C

reverse-phase protein microarrays



Reverse Phase: Analyze many samples at once.

Sutandy et al. 2013



# References

- Alberts B, Johnson A, Lewis J, et al. *Molecular Biology of the Cell*. 4th edition. New York: Garland Science; 2002.
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- Blackburn, J.M. and Shoko, A. *Protein Microarrays: Methods and Protocols. Methods in Molecular Biology*. **785**:305-330.
- Sutandy, F. R., Qian, J., Chen, C.-S. and Zhu, H. 2013. *Overview of Protein Microarrays. Current Protocols in Protein Science*. 72:27.1:27.1.1–27.1.16.