Lecture 13: High throughput analyses of gene expression

A. RNA expression pattern

Northern

qRT-PCR

FISH and RNA in situ

Reporter

B. Analyses of transcriptome

I. cDNA microarrays

II. Oligonucleotide arrays

C. Protein expression pattern

Immunohistochemistry

Western blot

Mass spectrometry

Functional classification of expressed genes

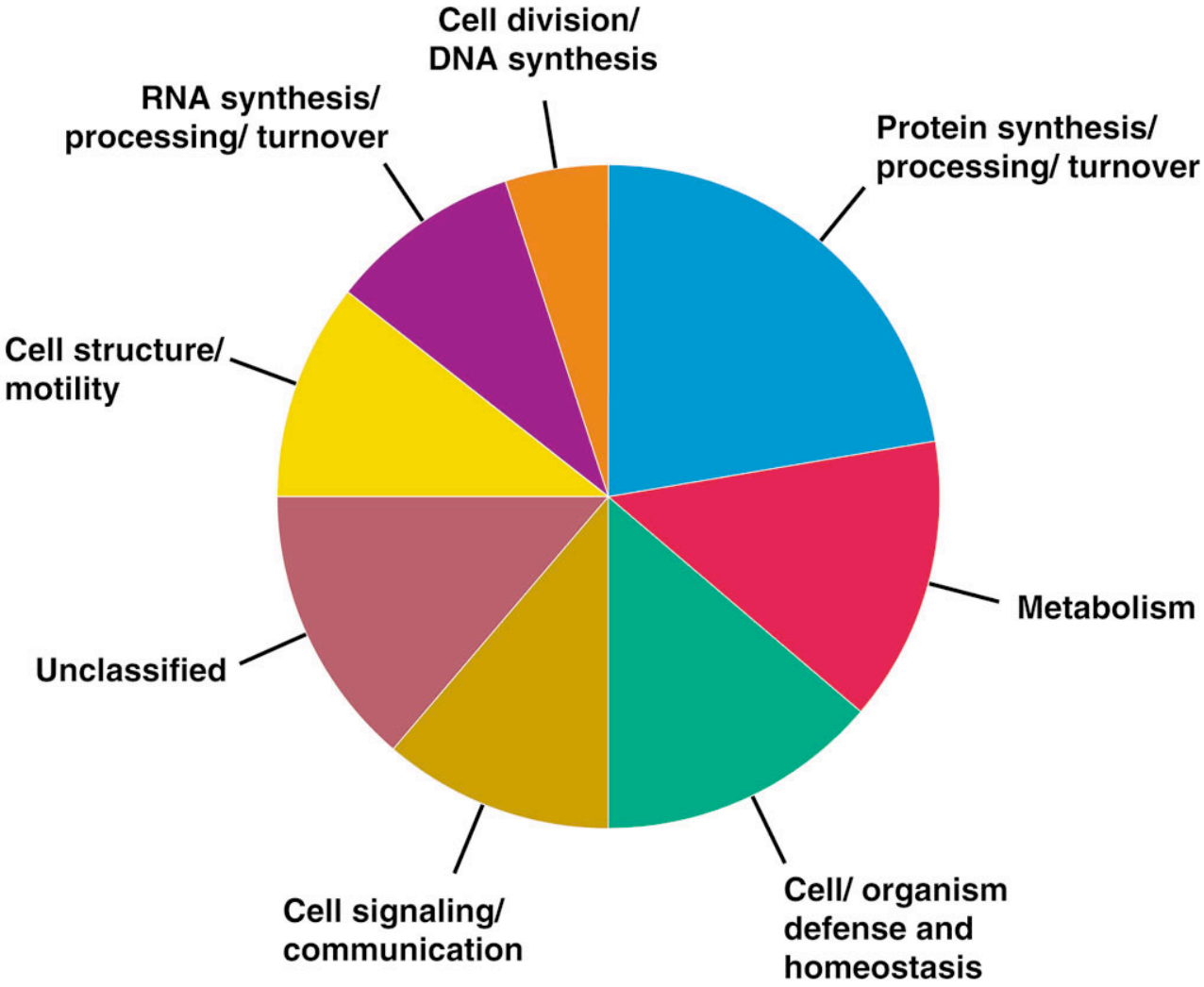
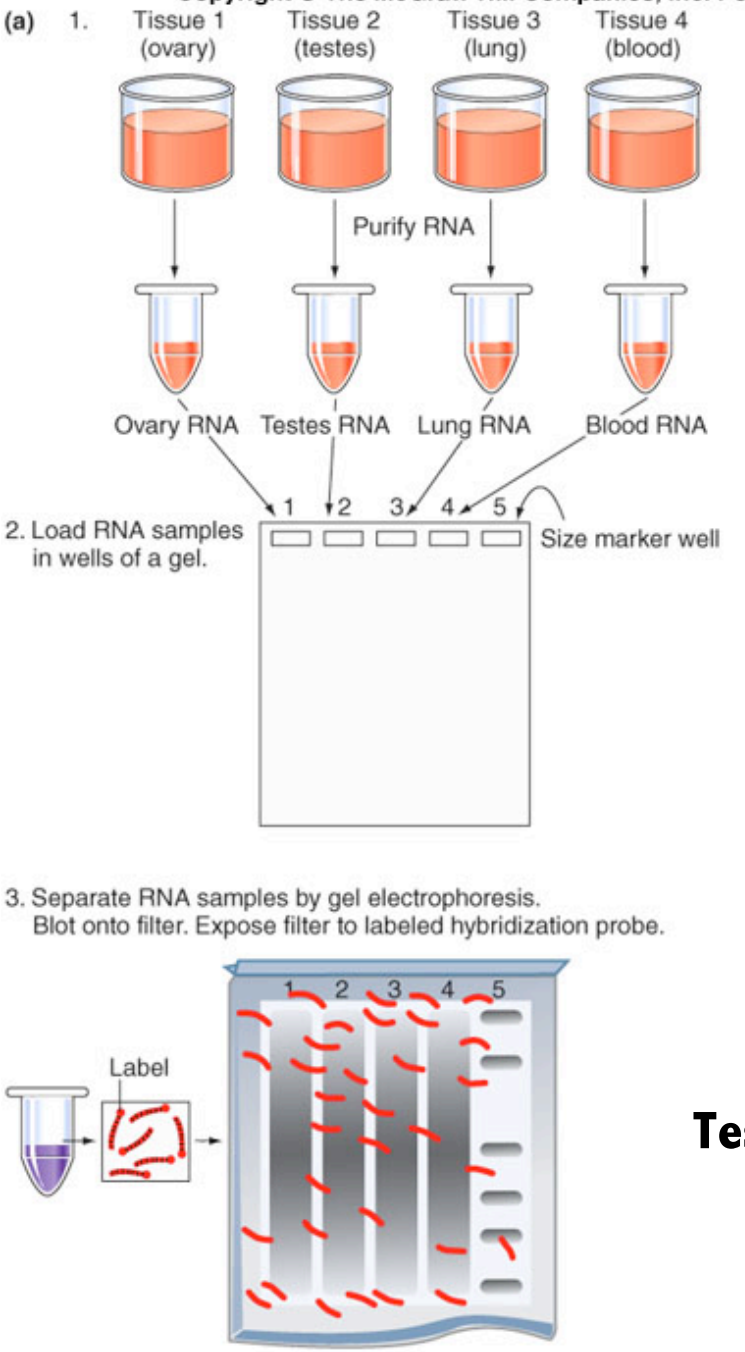
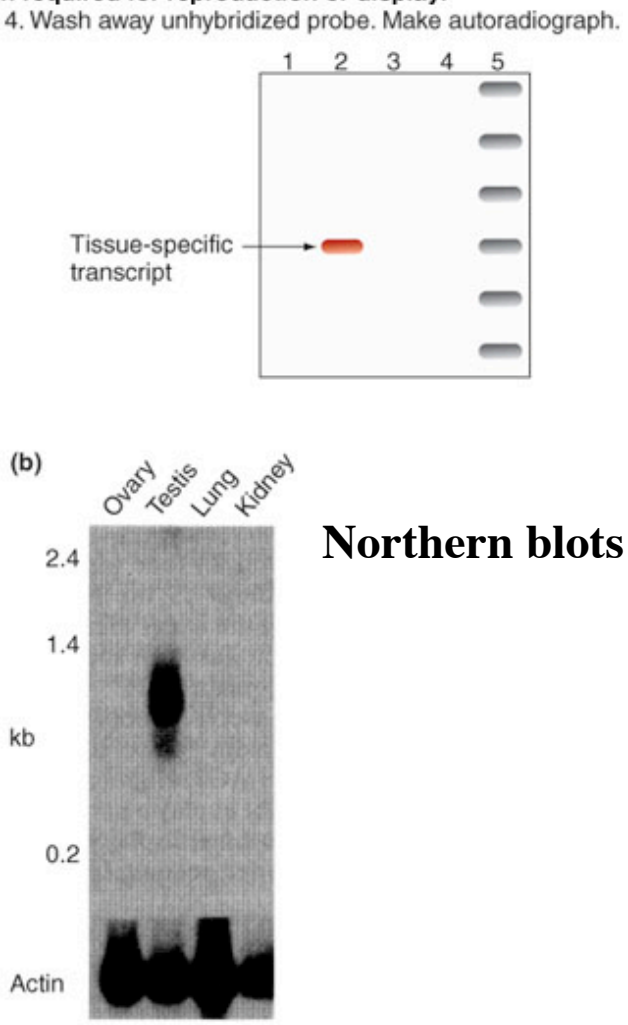


Fig. 12.17





Northern blots

Testes-determining factor (TDF)

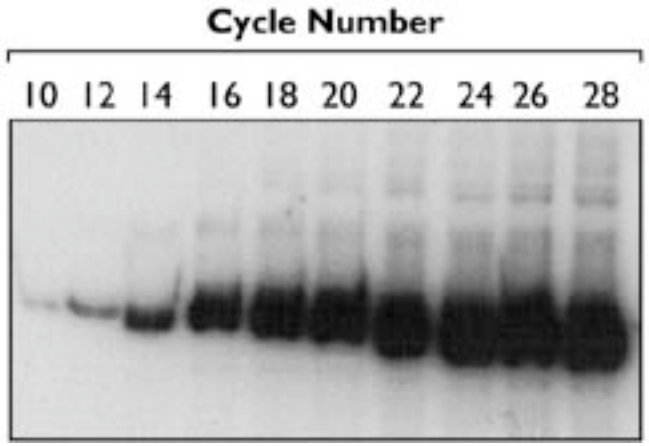
Fig. 11.20

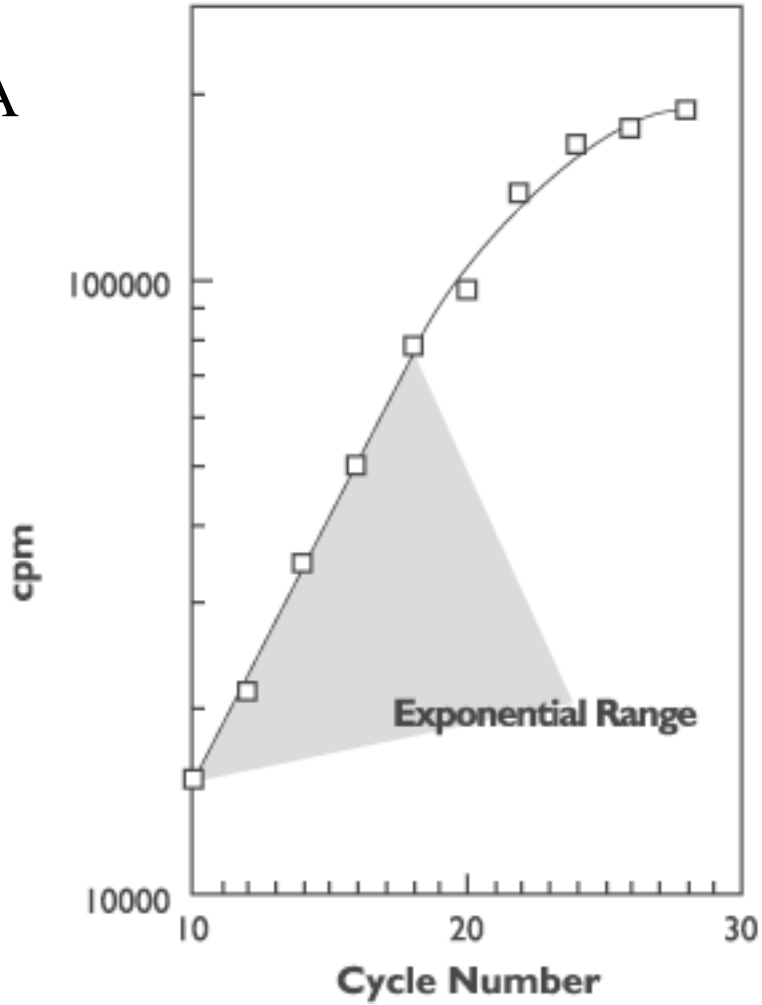
RT-PCR: measuring mRNA level

Reverse transcriptase

mRNA → cDNA

PCR





Quantitative Real Time PCR (or QRT-PCR)

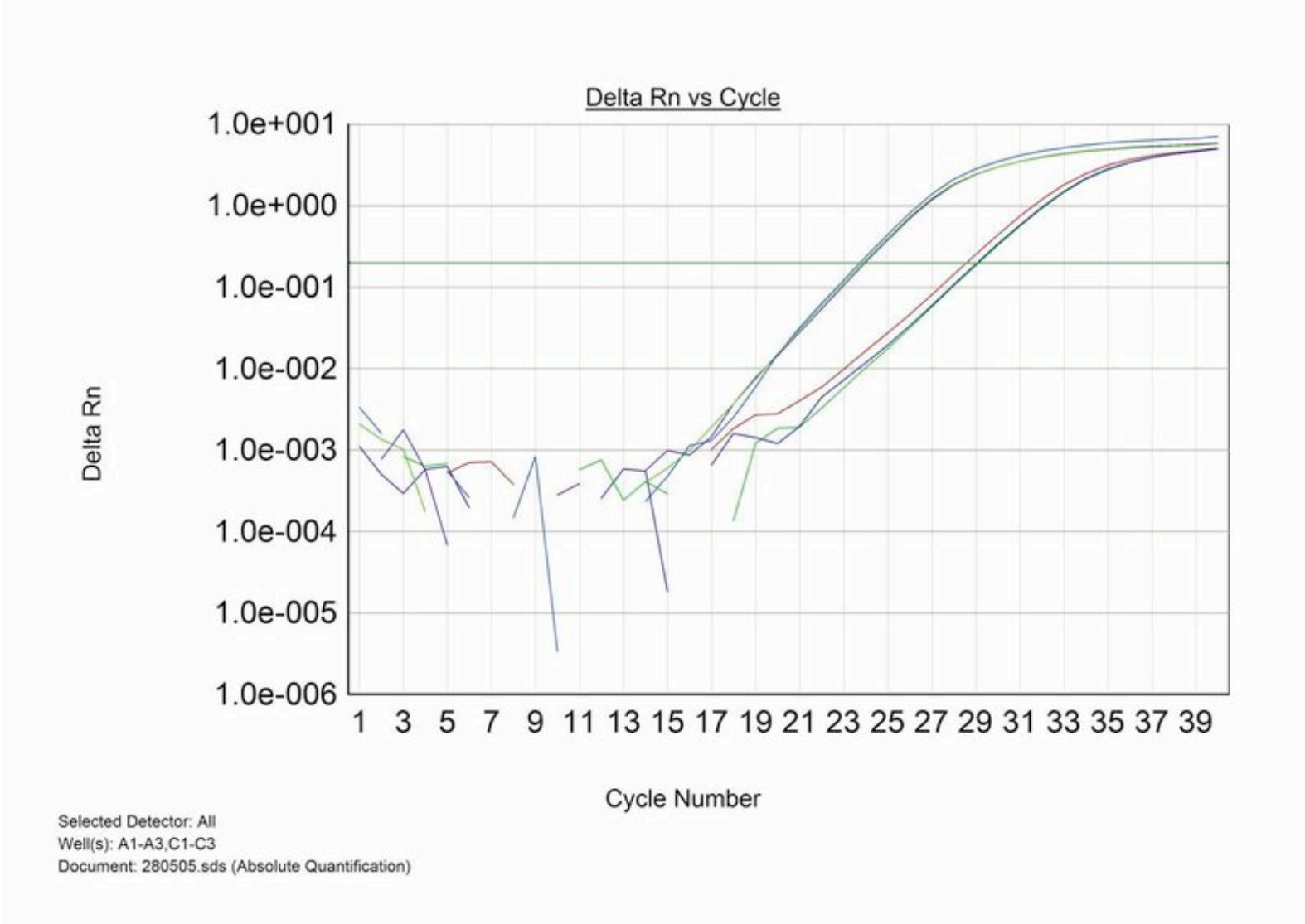
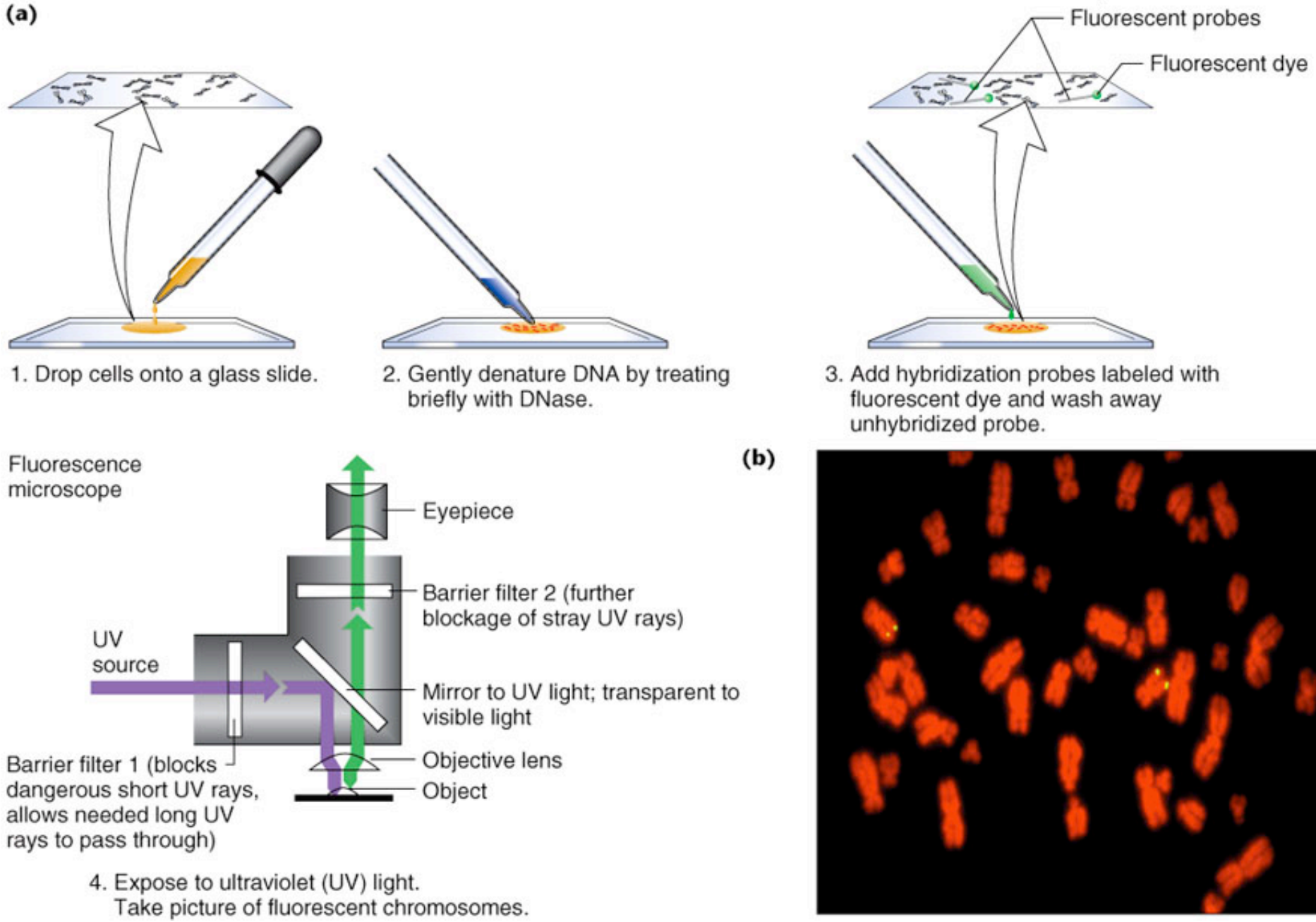
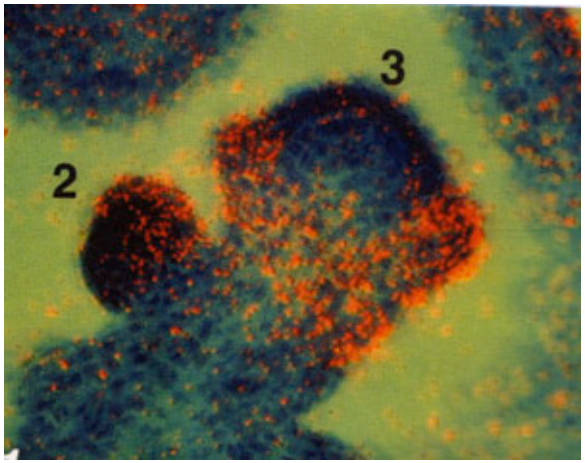


Fig. 10.6 (7.21) FISH: Fluorescent In Situ Hybridization

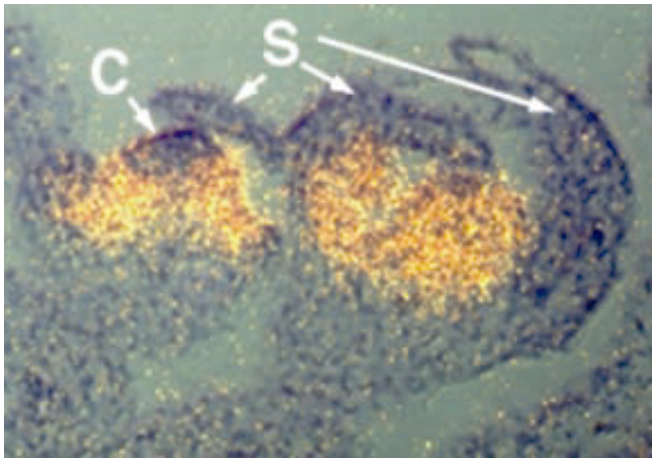


RNA in situ hybridization

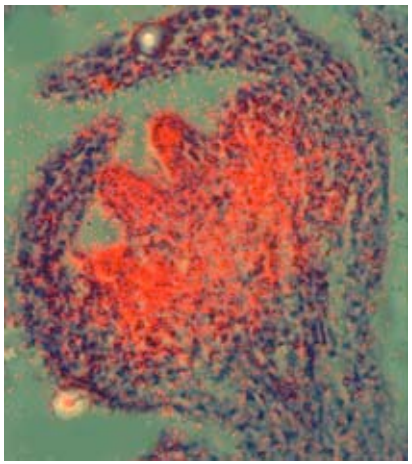
A (AP1)



B *(AP3)*

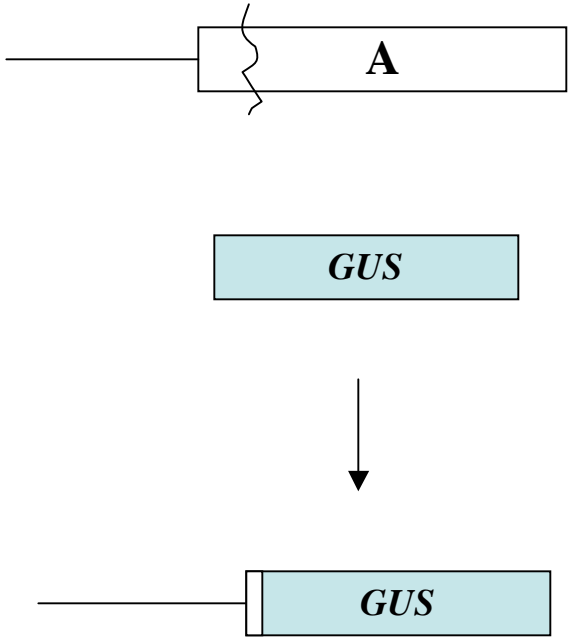


C (AG)



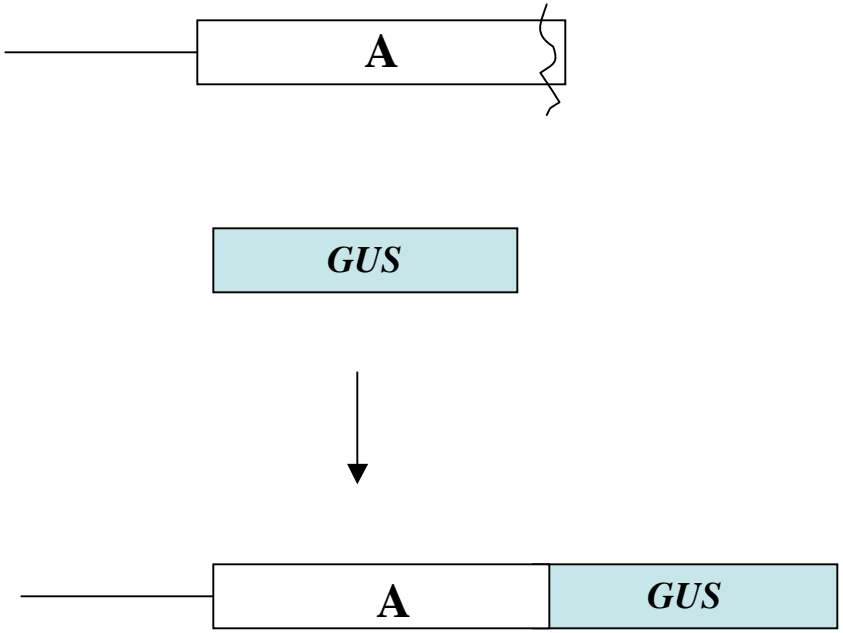
Reporter genes reports gene expression level and patterns

Promoter::GUS (β-glucuronidase)



Reporter genes reports gene expression level and patterns

Promoter::gene A-GUS chimeric protein



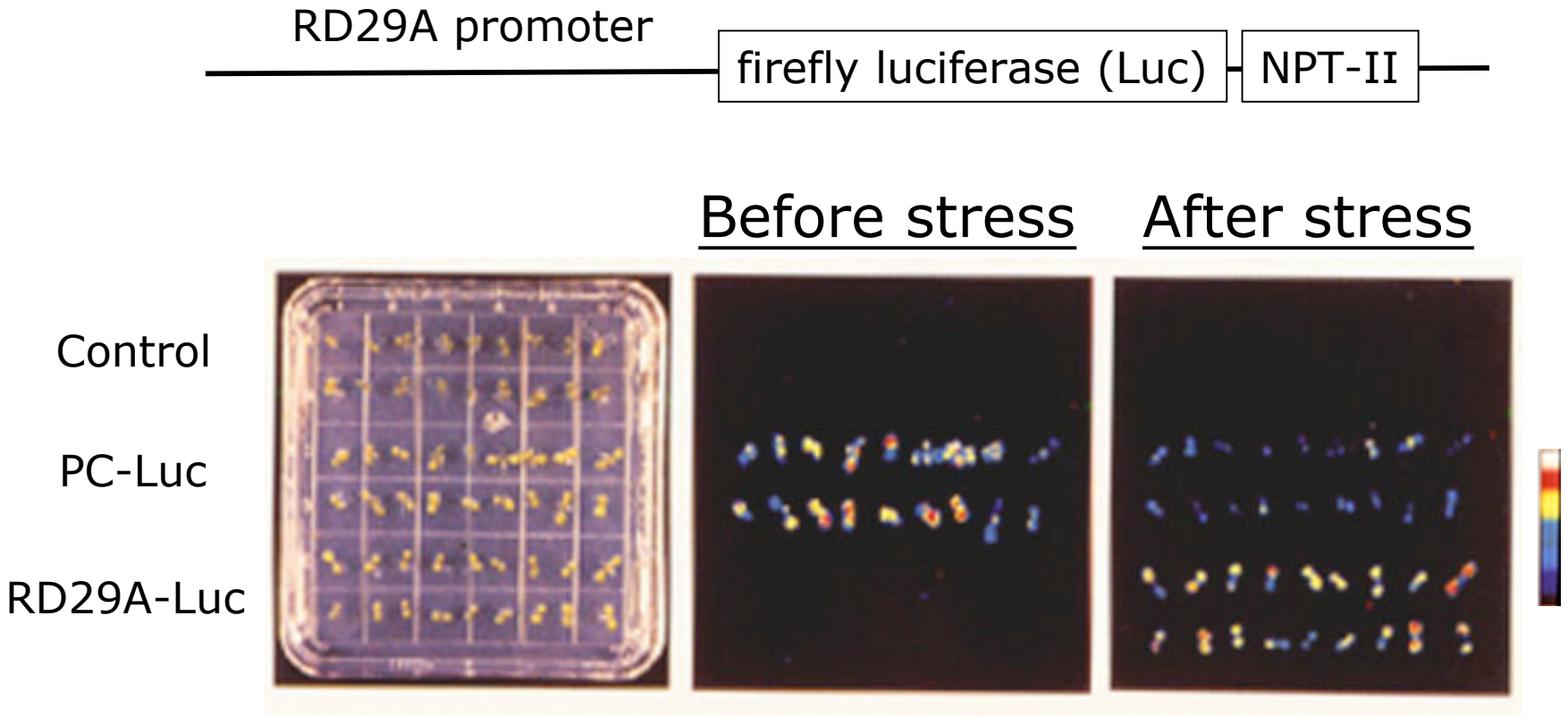
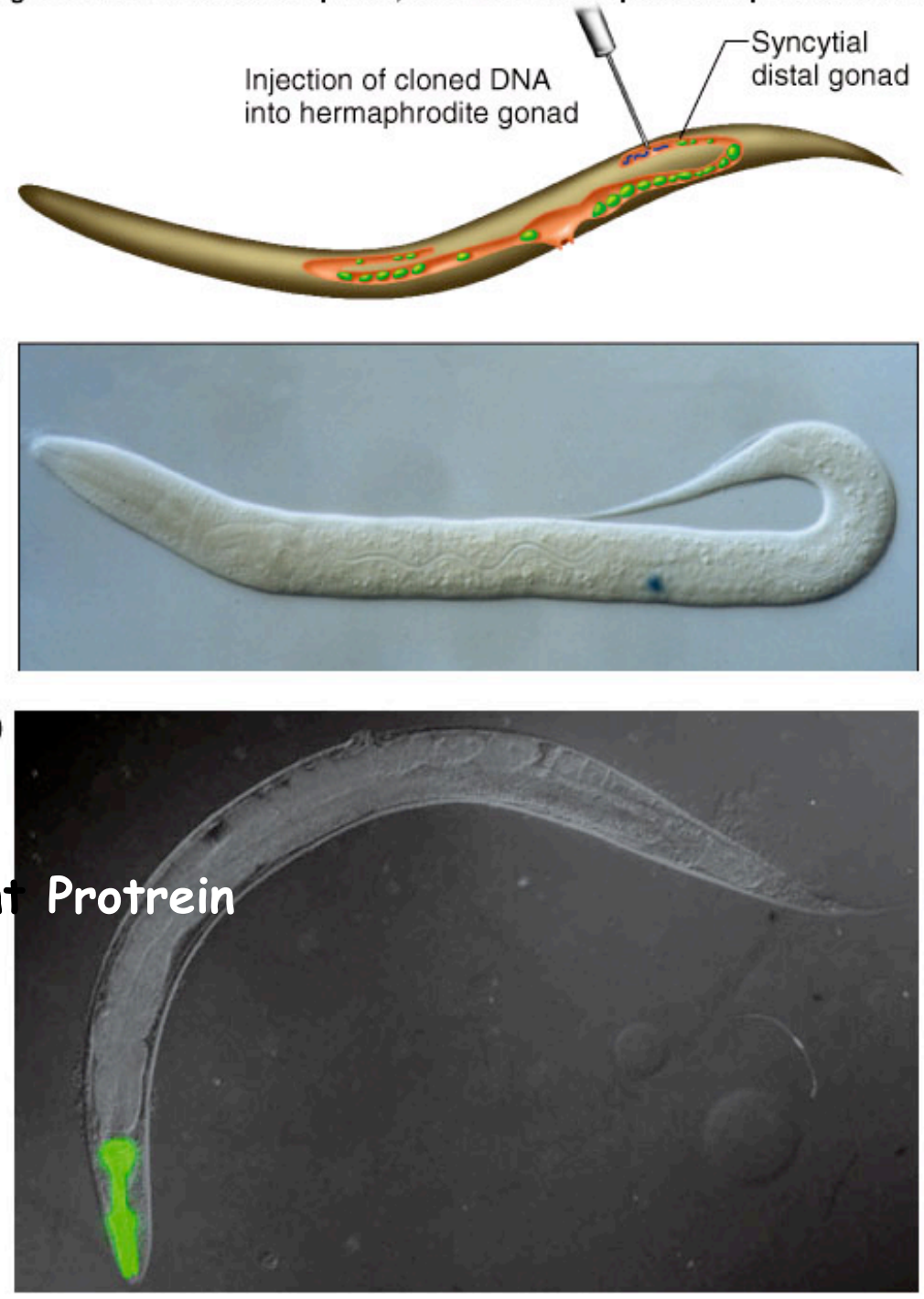

RD29A promoter
firefly luciferase (Luc)
NPT-II
Before stress
After stress
Control
PC-Luc
RD29A-Luc

Fig. C.8



Reporter

LacZ
(b-galactosidase)

GFP
Green fluorescent Protrein

Summary of reporter genes

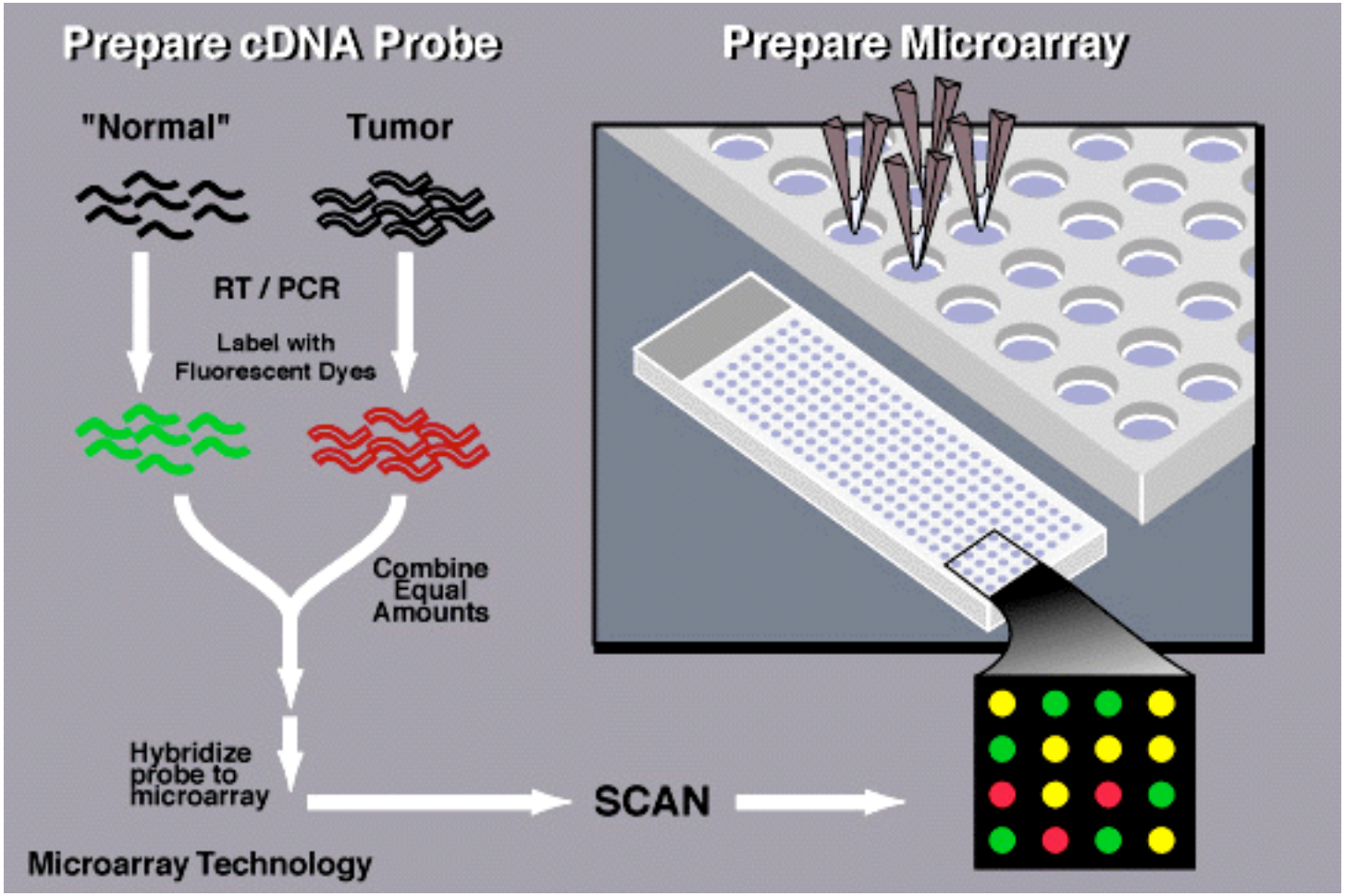
Name	Source	Substrate	Visual
GUS (β-glucuronidase)	E.coli	5-bromo-4-chloro 3-indoyl-1-glucuronide (X-gluc) →	Blue
LacZ (β-galactosidase)	E. coli	X-gal → breakdown	Blue
LUC (Luciferase)	Firefly	luciferin & ATP	emitting light
GFP (Green Flourescent Protein)	Jelly fish	none	Green

High throughput analyses of the transcriptome

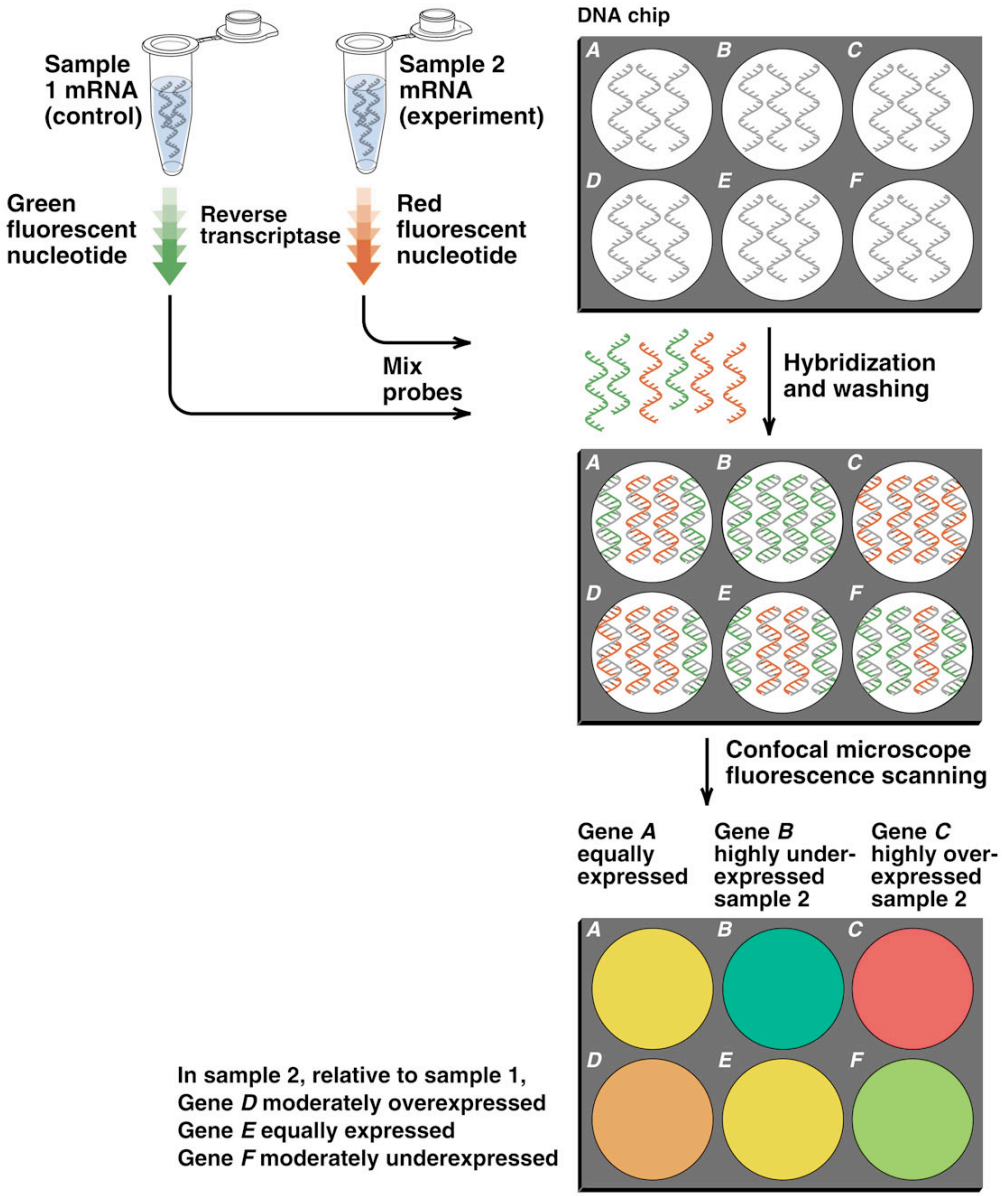
Documenting gene expression on a genome wide scale

Transcriptome: complete set of transcripts and
their relative expression levels in a particular cell
or tissue under defined conditions

I. cDNA microarrays

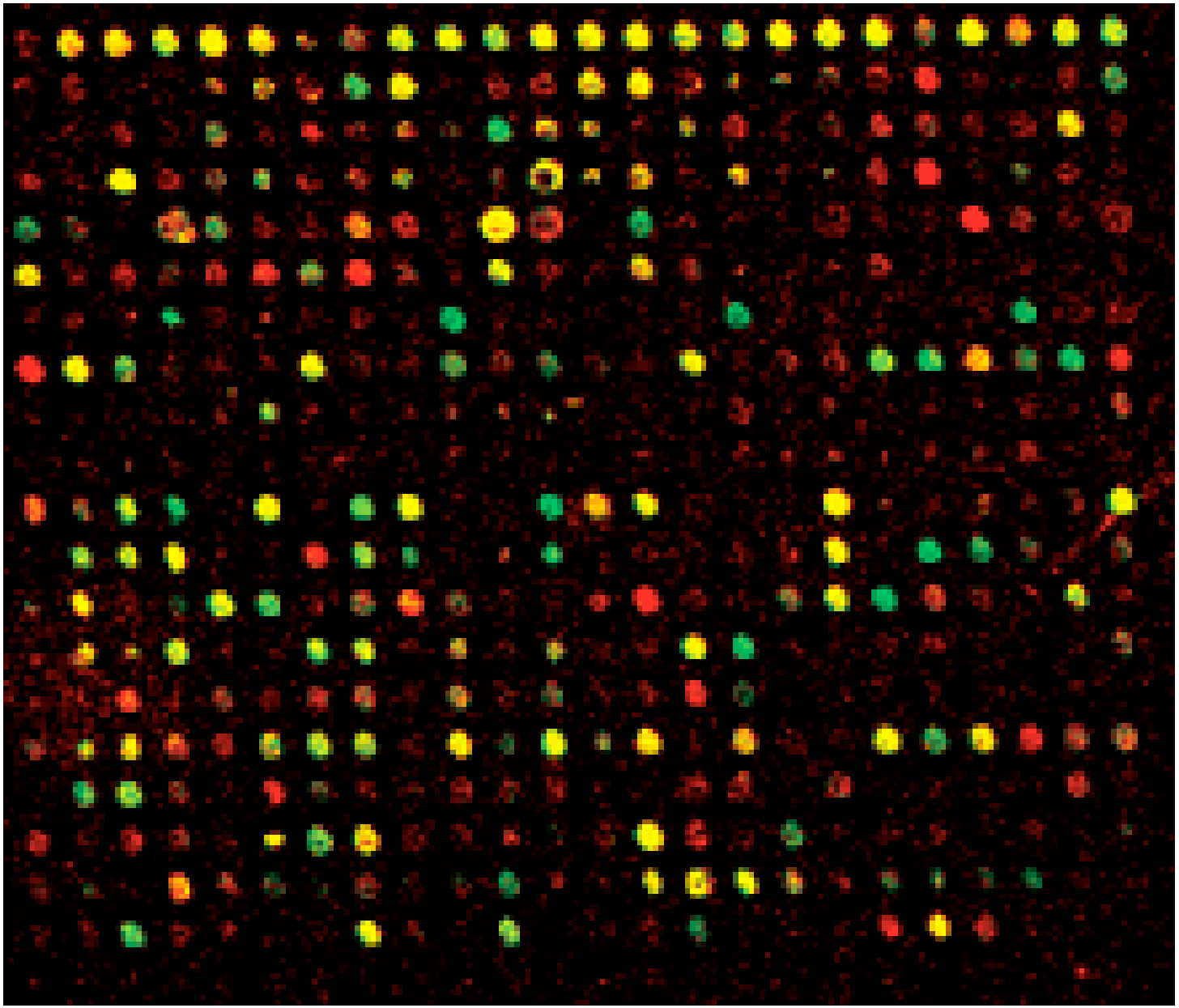


Use of DNA microarrays (chips)



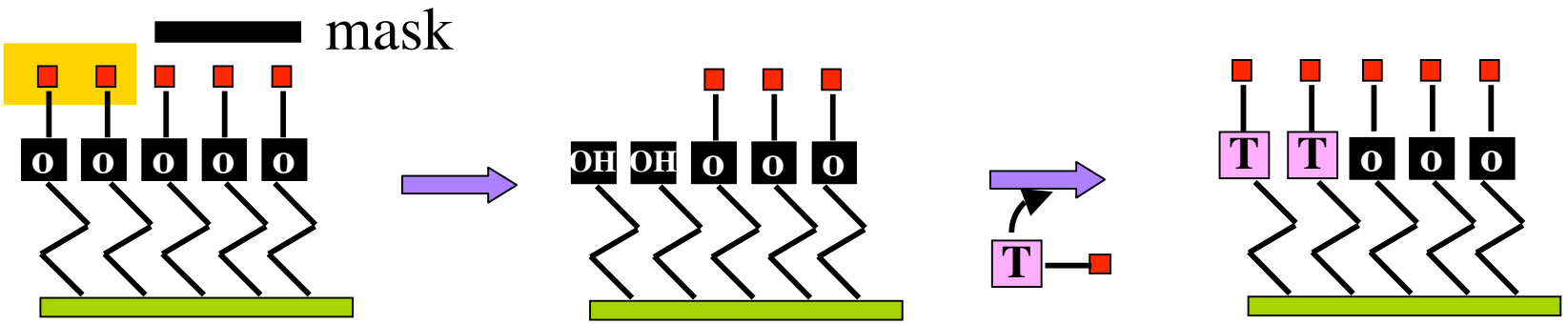
Fluorescently tagged cDNA probes are hybridized to DNA spots in the microarray for studying differential expression of thousands of genes at a time in two mRNA samples

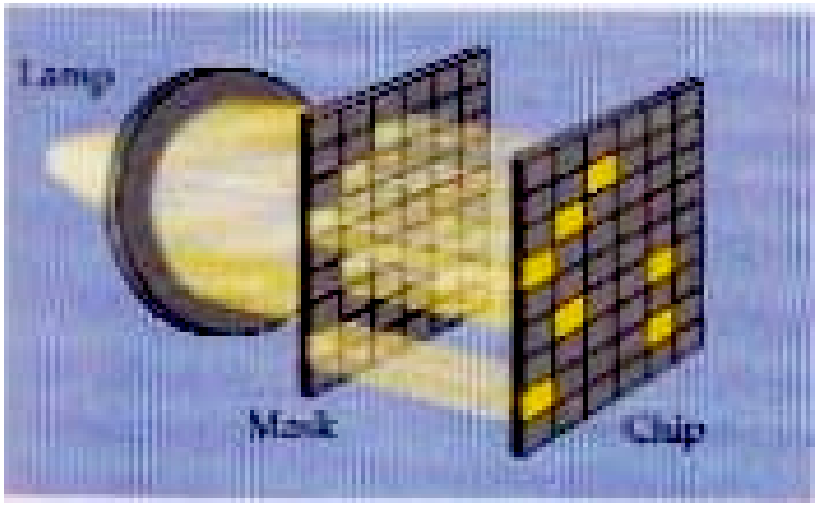
Fig. 12-21



II. Oligonucleotide microarrays (Affymetrix GeneChip)

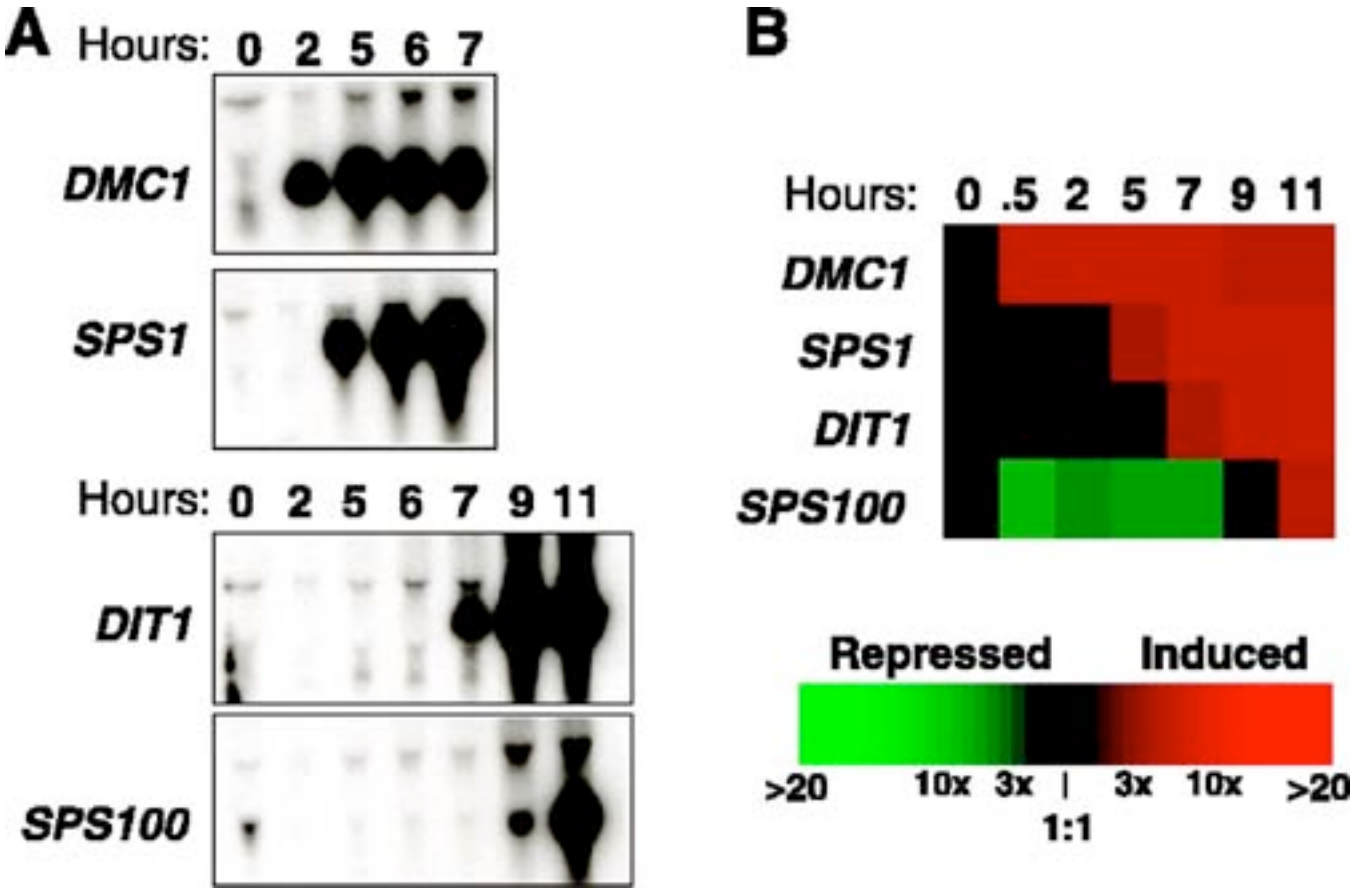
Light deprotection



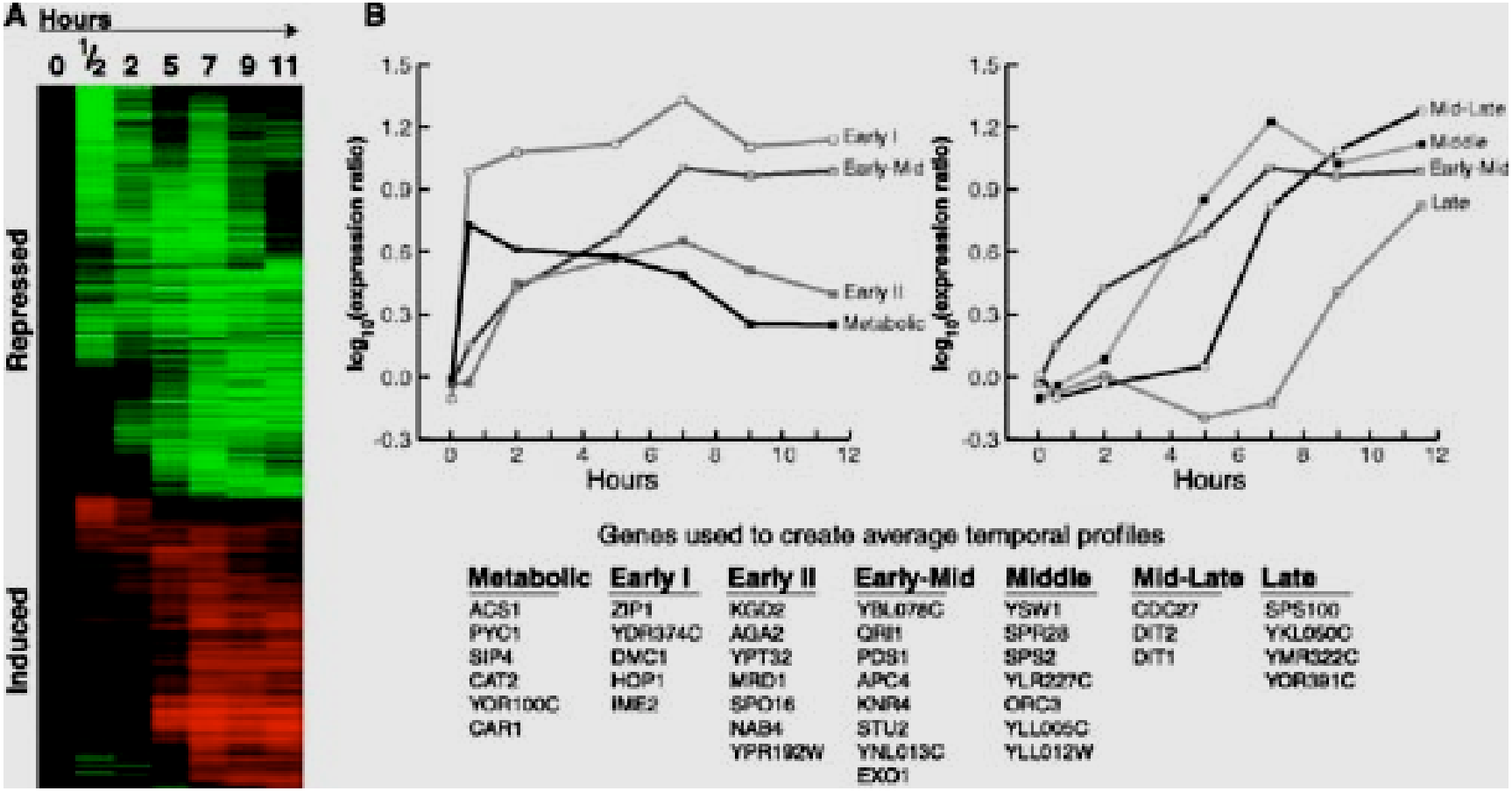


Sporulation gene expression profile in budding yeast

Chu et al., (1998) Science 282, 699-705



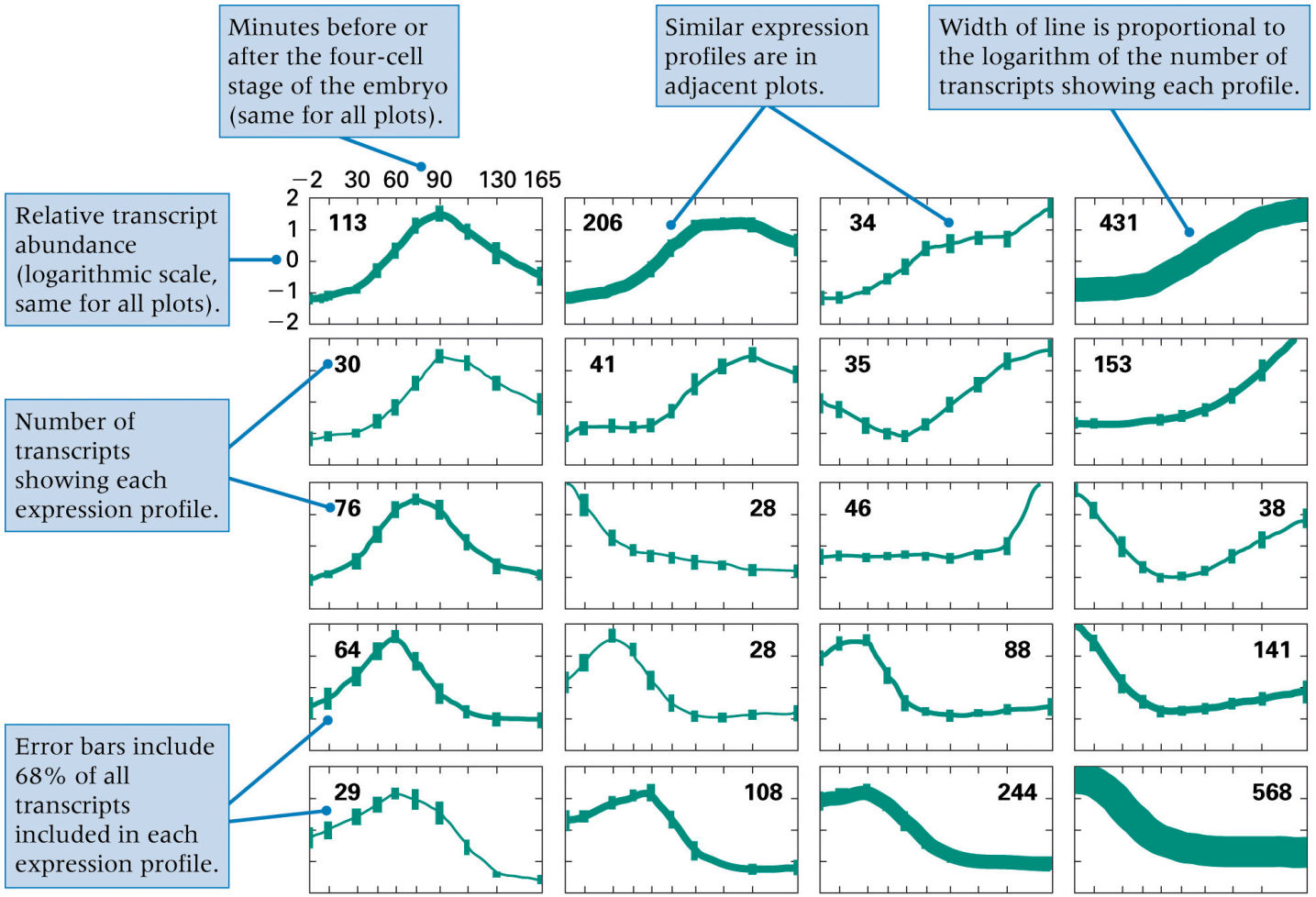
Several classes of sporulation gene expression after transfer to sporulation media



Survey of 1116 genes during sporulation in budding yeast

Chu et al., (1998) Science 282, 699-705

Patterns of transcriptional regulation of about 2500 genes



These 20 plots include about 80% of 3157 genes showing significant changes in transcript abundance in the first 165 minutes after the four-cell stage in development.

Fig. 12.22

Western blot

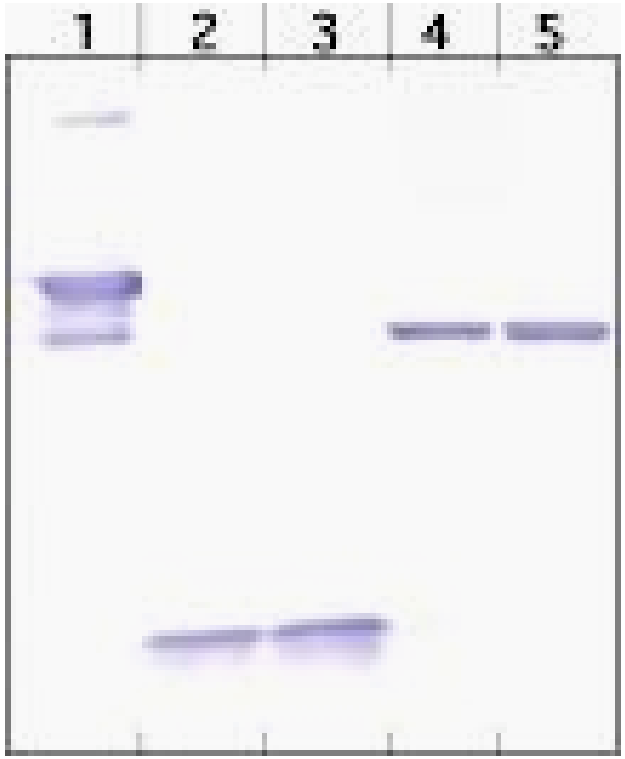


Fig. 19.27

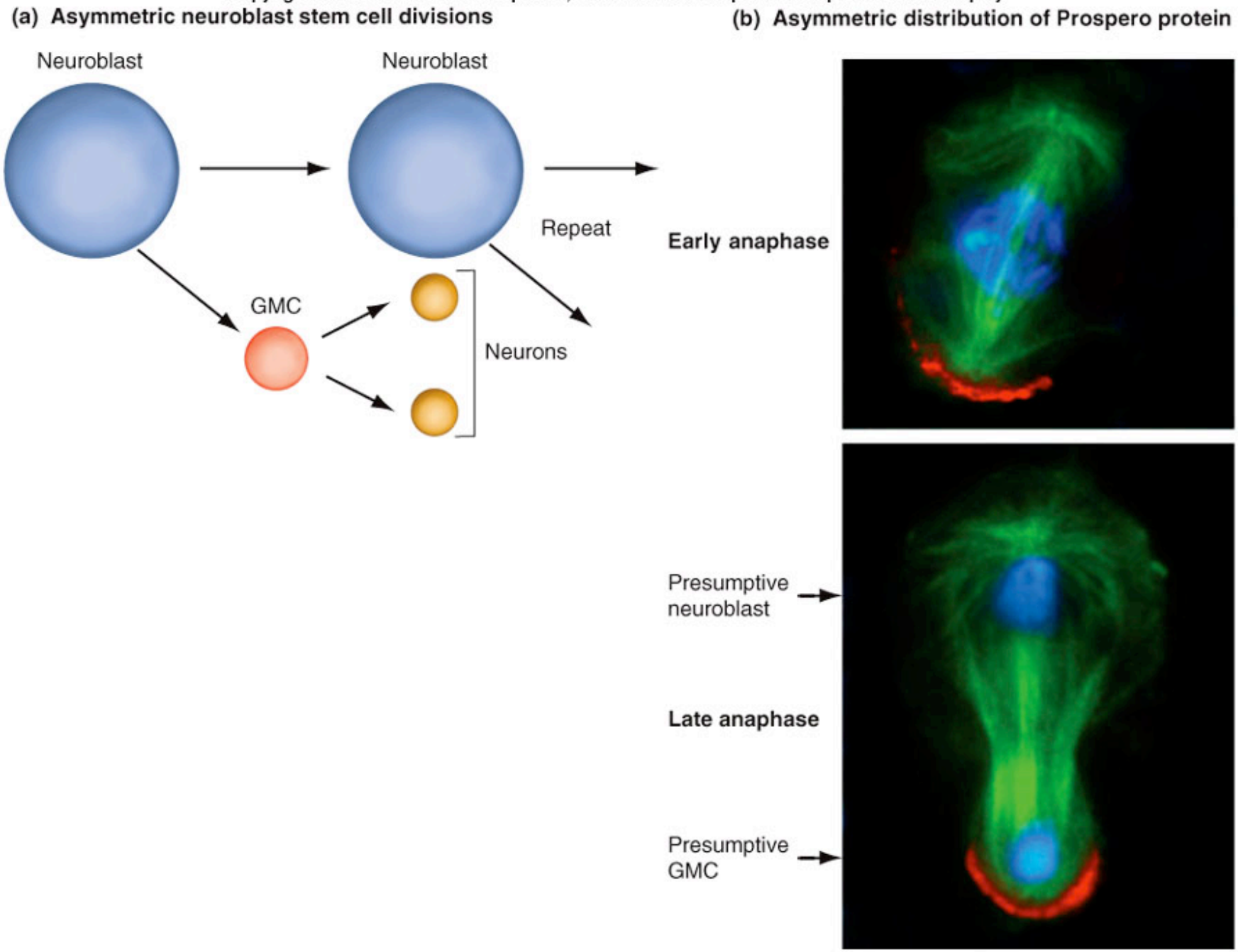


Fig. 10.29