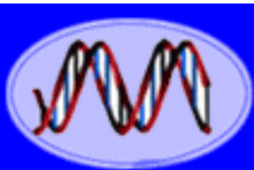
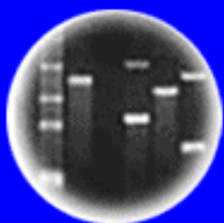


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

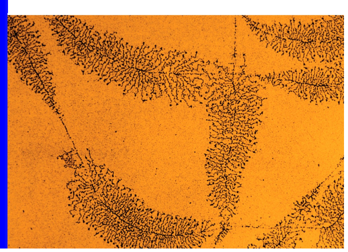
Cloning: Ethical Issues
and Future Consequences

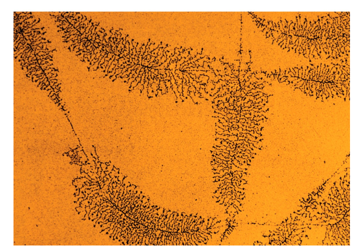
Plants of Tomorrow

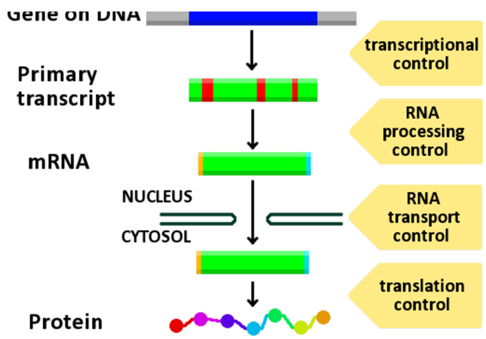
HC70AL Spring 2011
Gene Discovery Laboratory

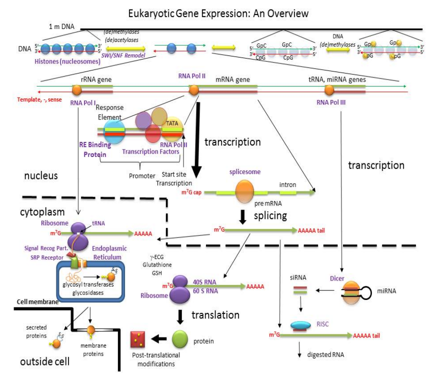
RNA and Tools For Studying Differential Gene Expression During Seed Development

4/18/11



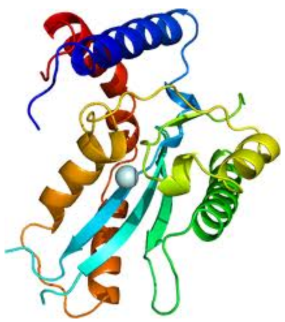


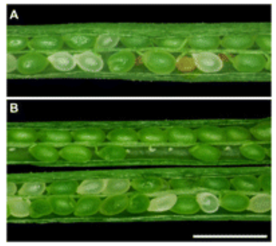




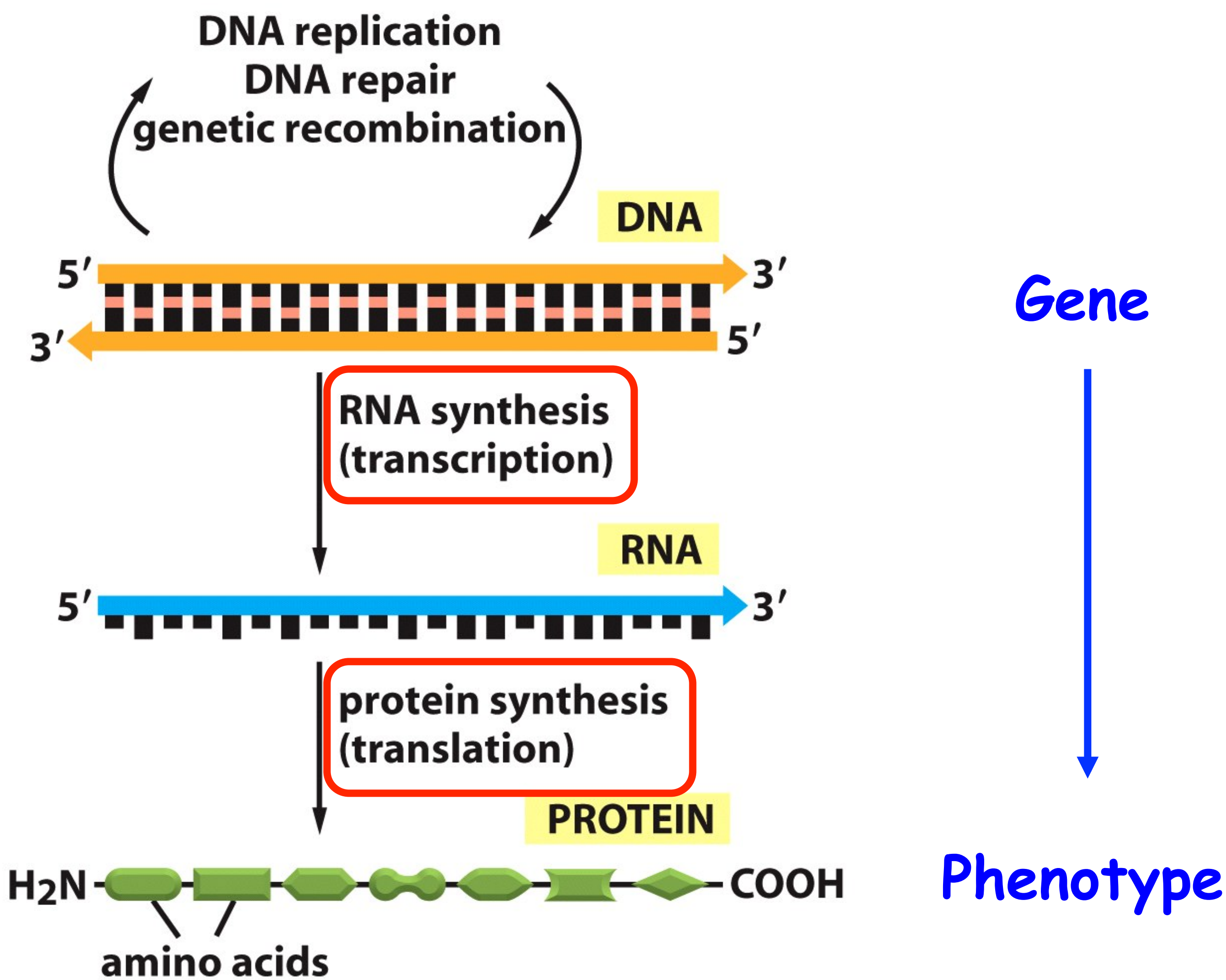
Controlling Gene Activity

From Gene to Functional Protein & Phenotype

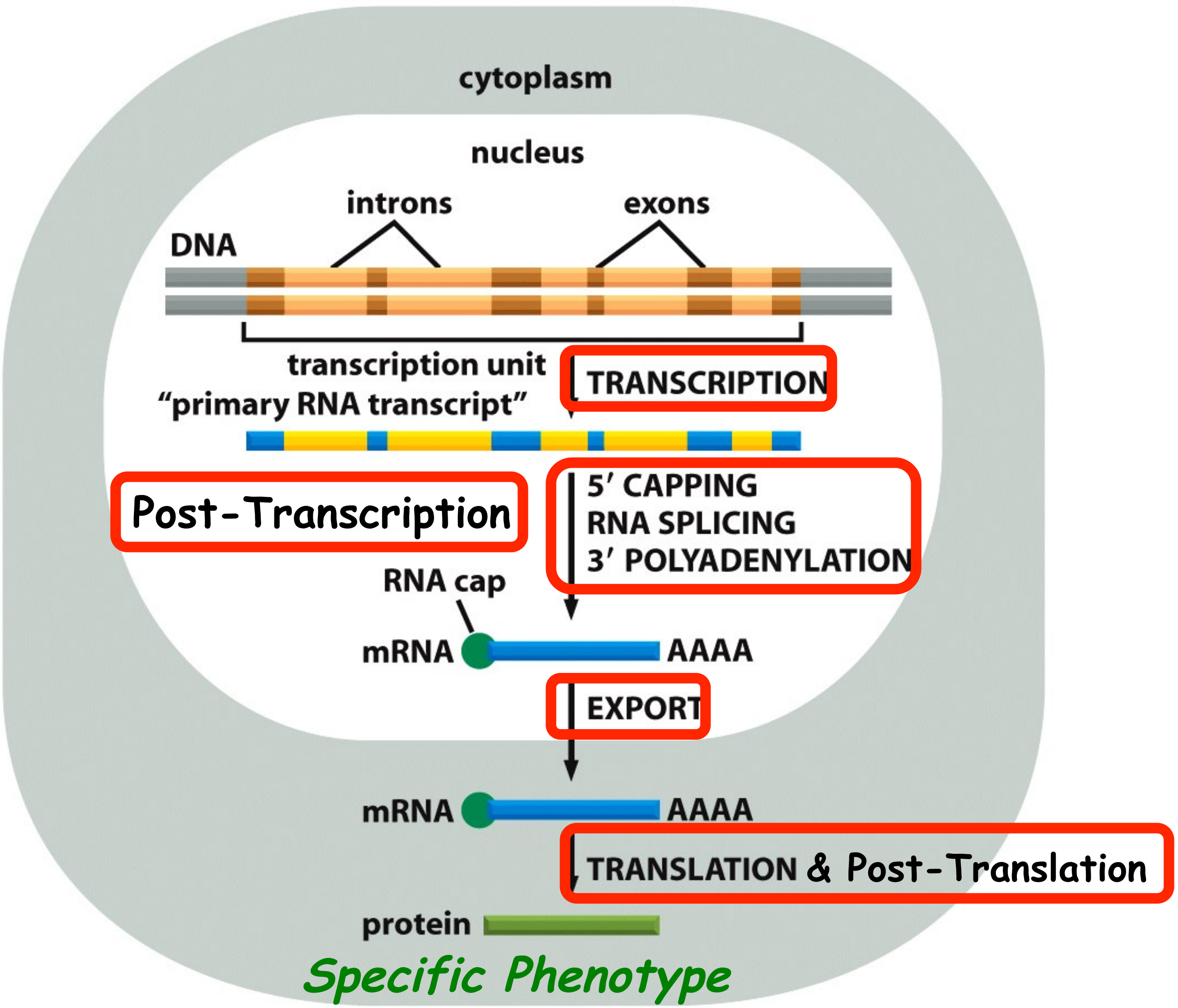




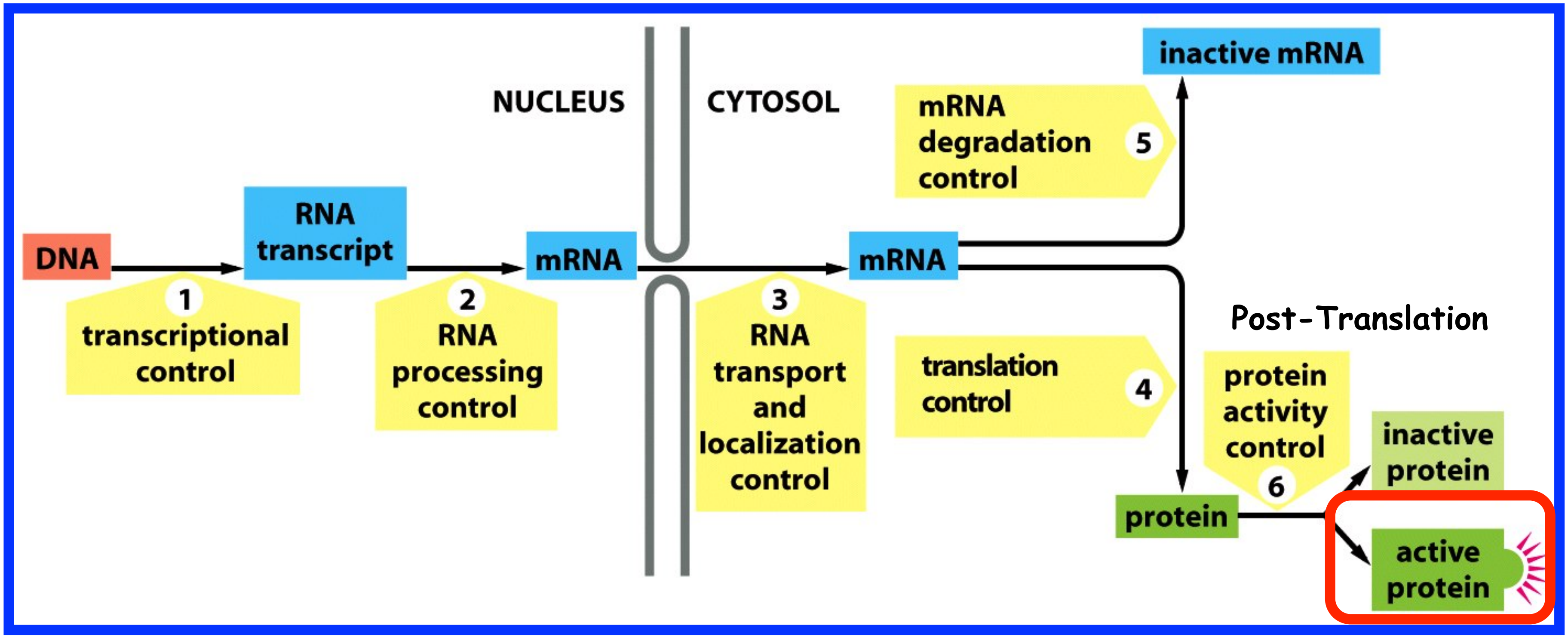
Production of the Phenotype: DNA → RNA → *Functional* Protein



Eukaryotic Gene Activity: Genes to Functional Proteins



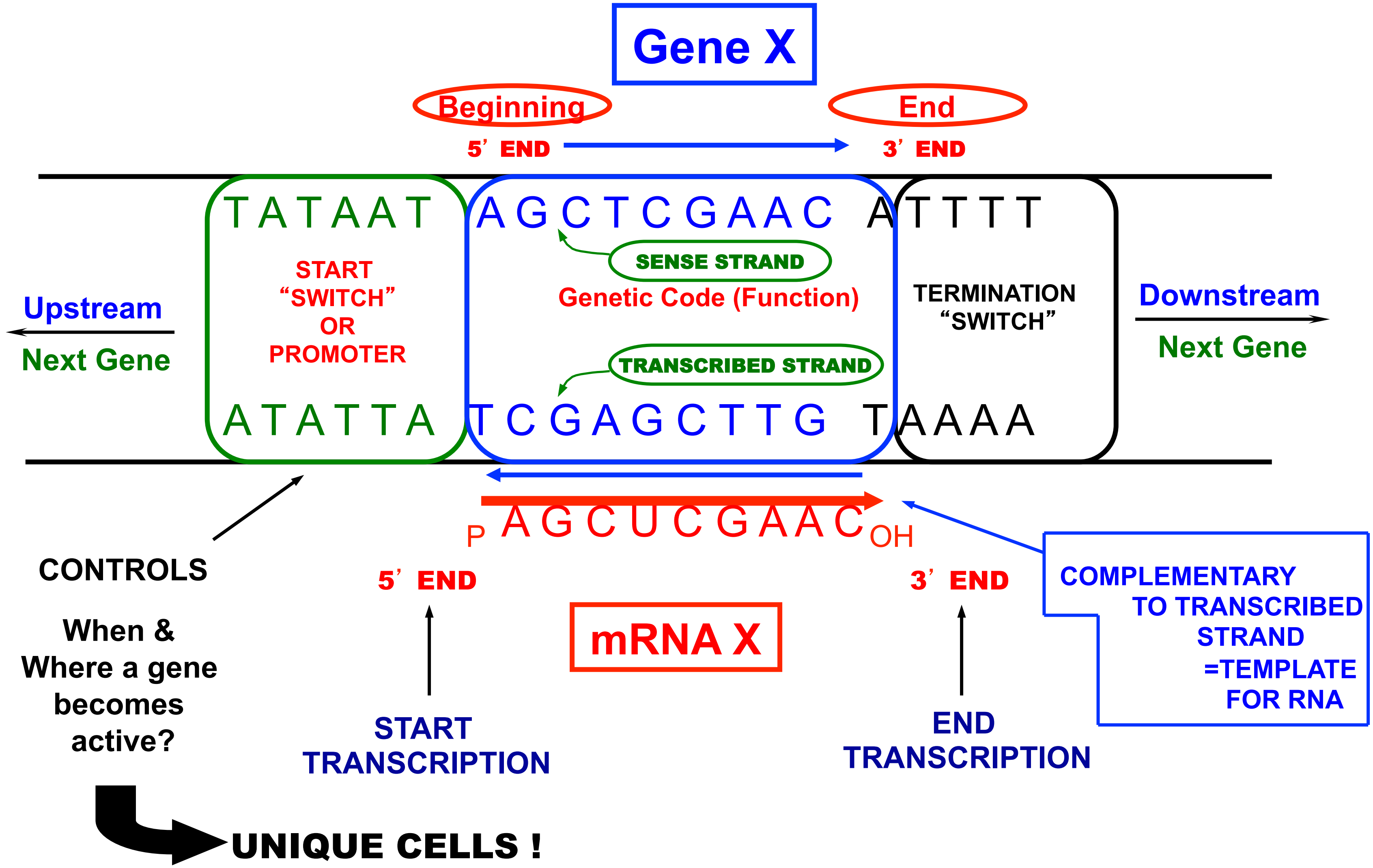
Regulating Eukaryotic Gene Activity: Major Control Points



Knock-Out Mutations Can Affect Each Control Point As Well As Coding Sequences

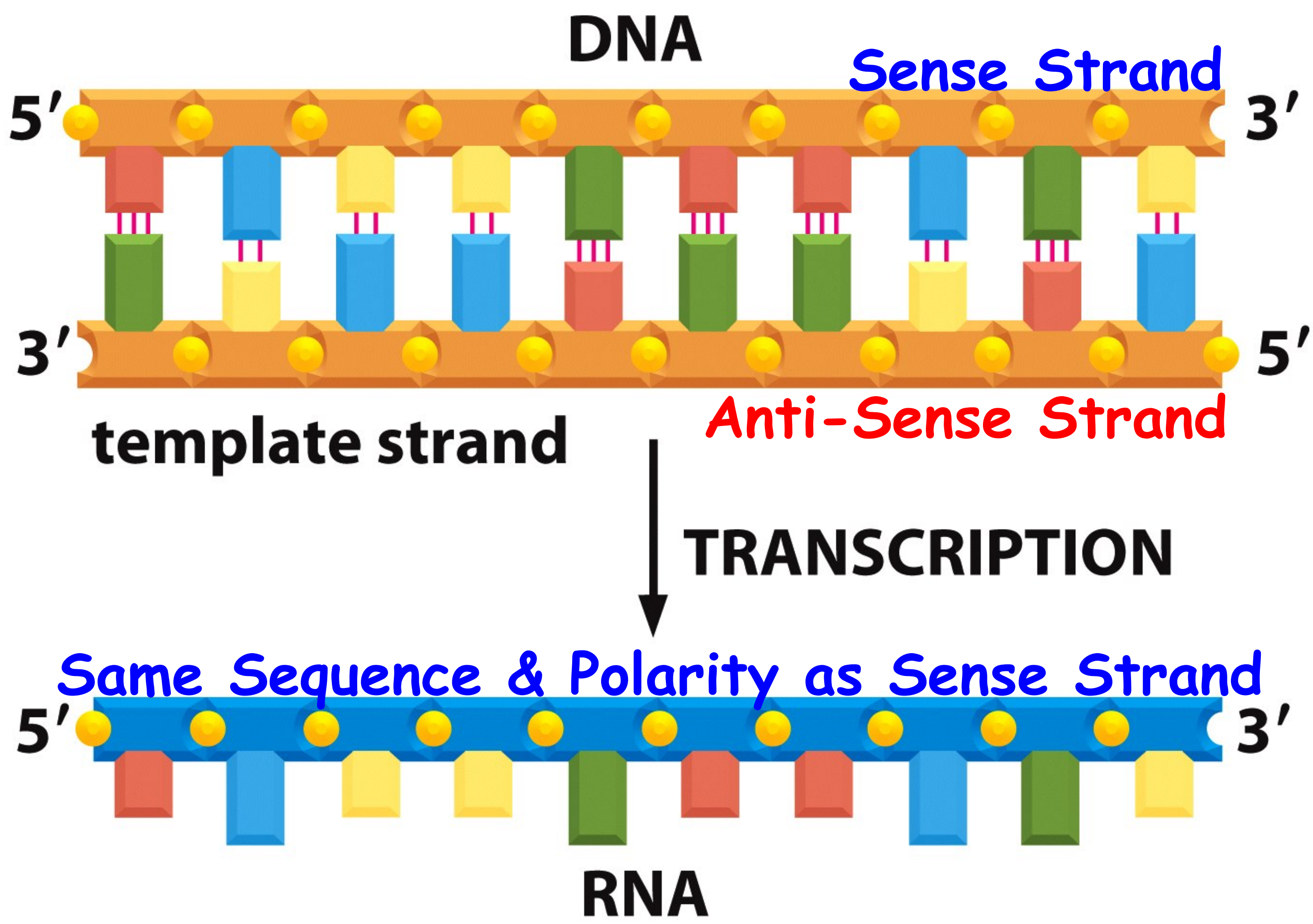
RNA Structure & Transcription

Gene Anatomy-A Review

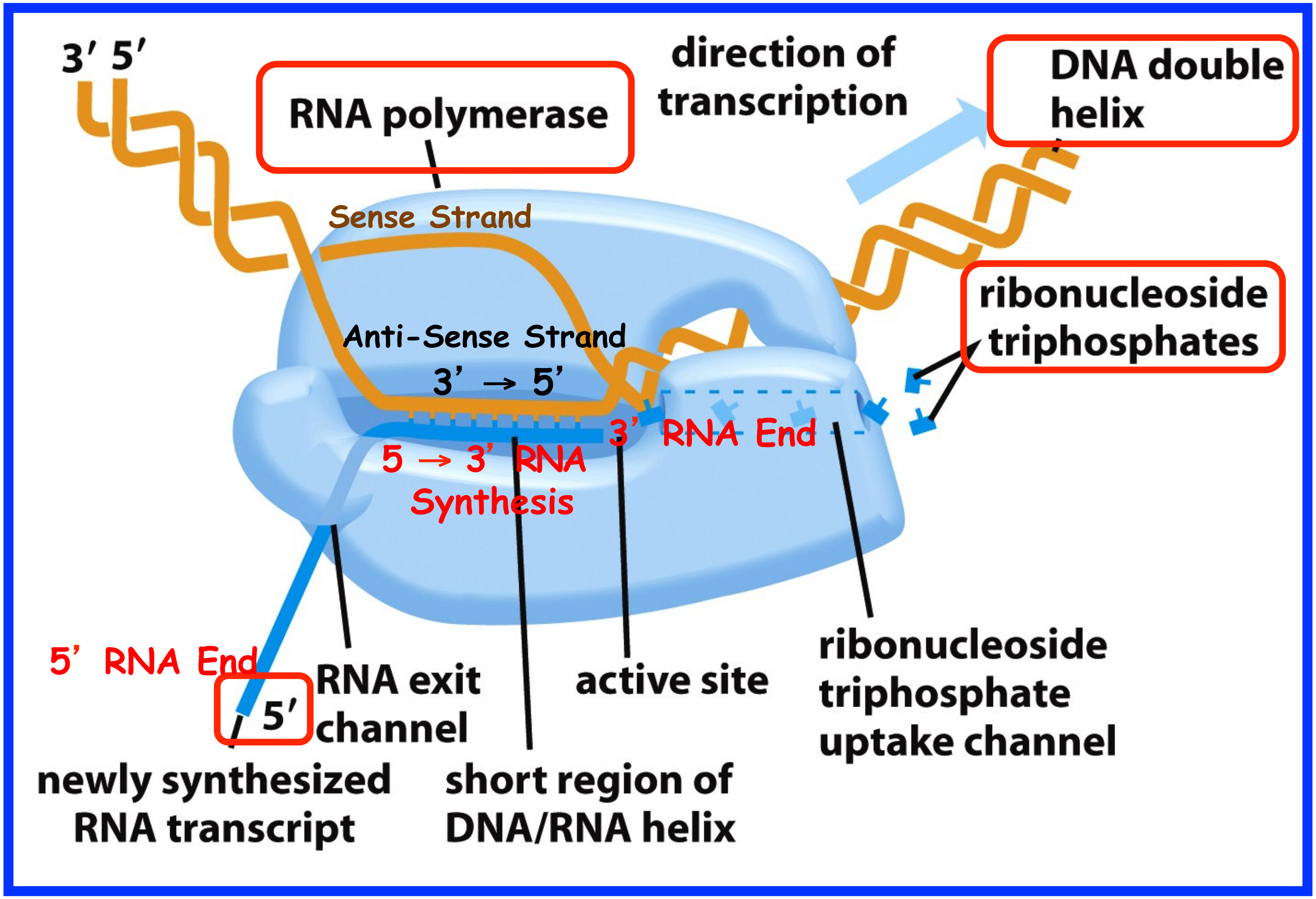


<u>Note</u>: mRNA Sequence = Sense Strand Sequence

Transcription: An Overview

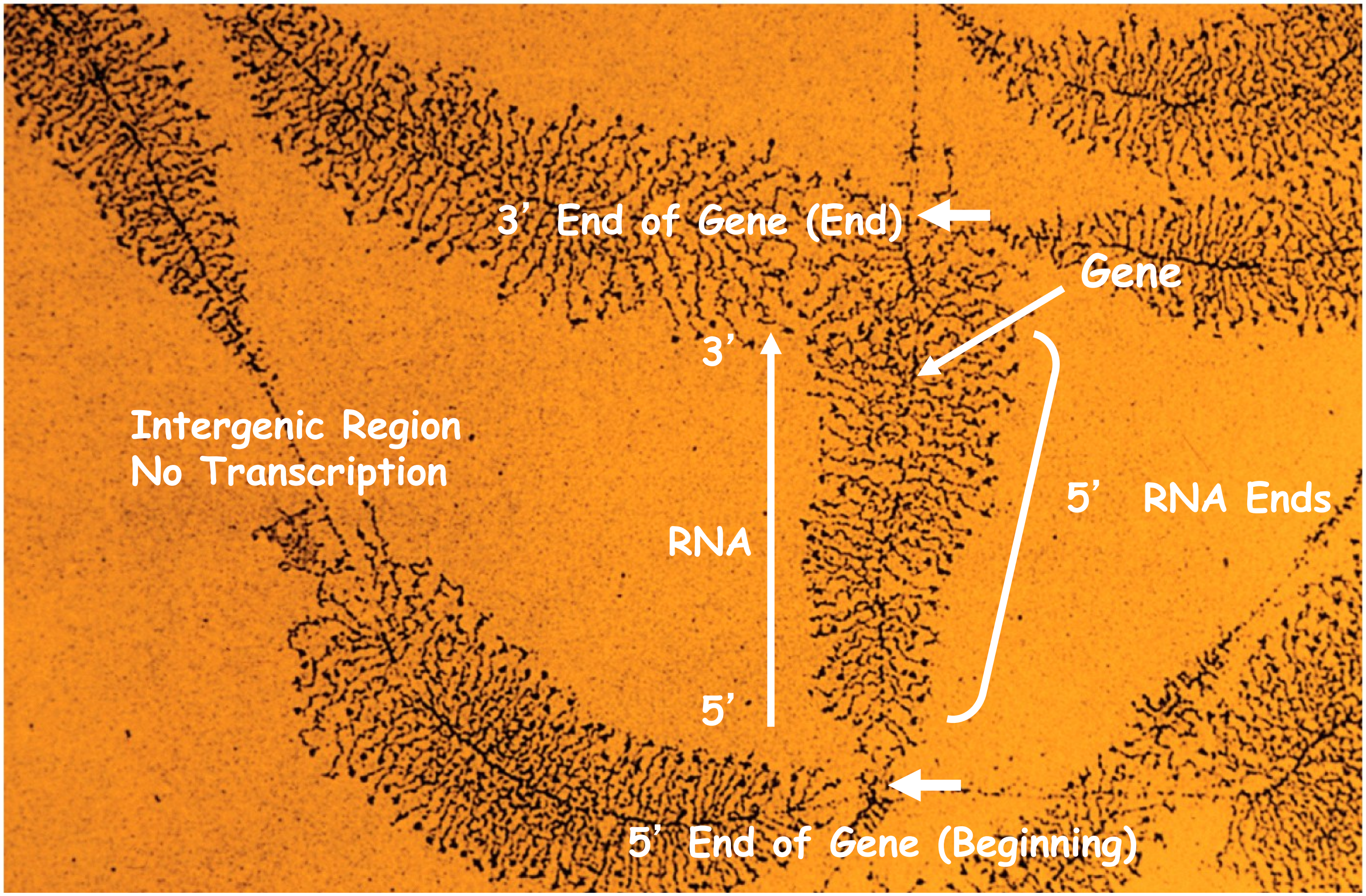


Transcription: A "Ground Level" View

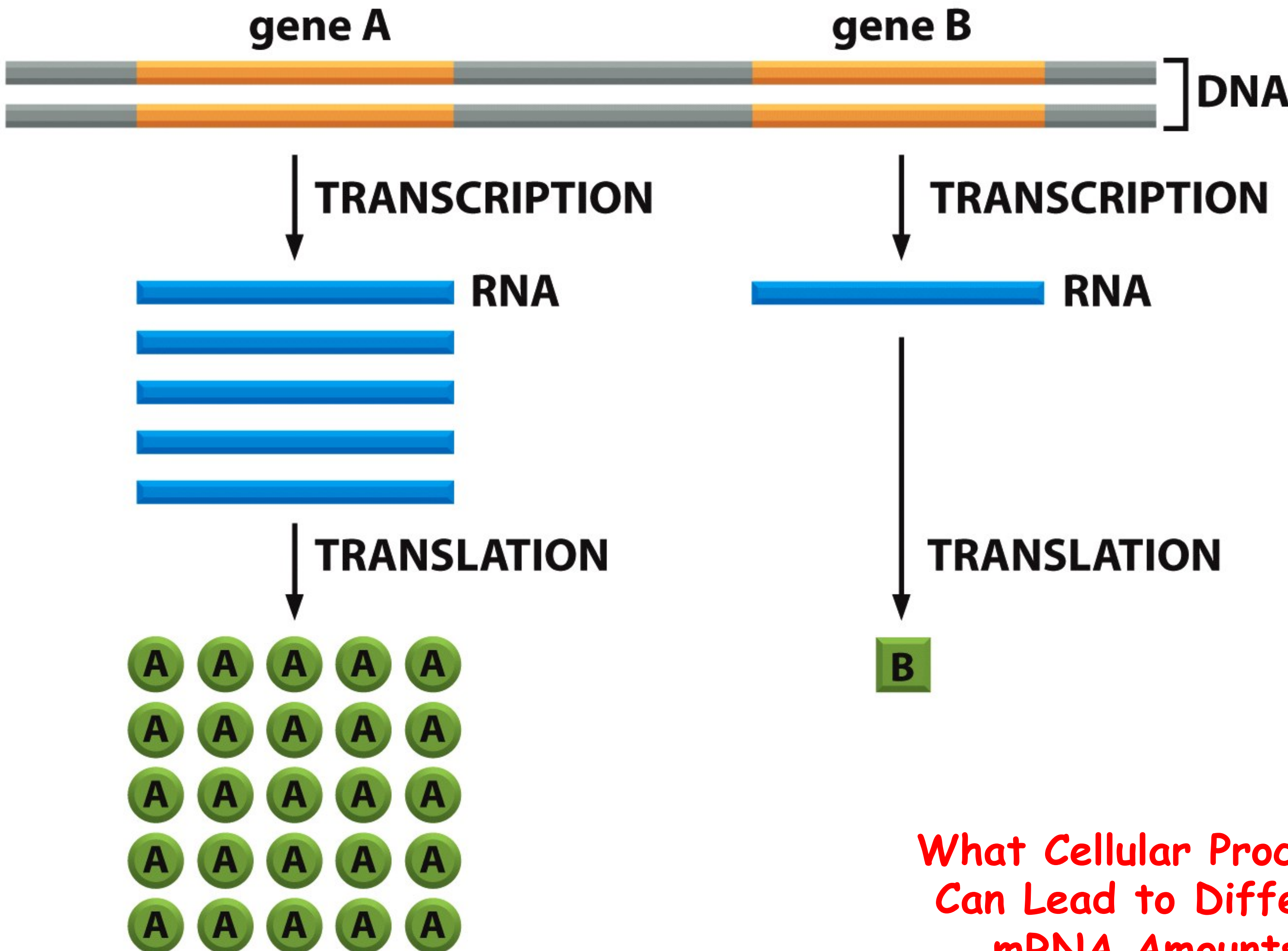


Requires: DNA Template, RNA Polymerase, and Ribonucleotides (Note: No Primer)

Visualizing Transcription in the Electron Microscope

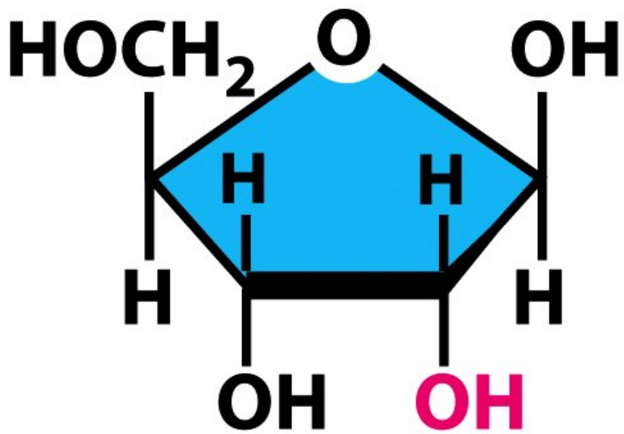


Genes Can Have Different "Expression" Levels That Are Reflected in Differing Amounts of mRNAs Accumulating in the Cytoplasm



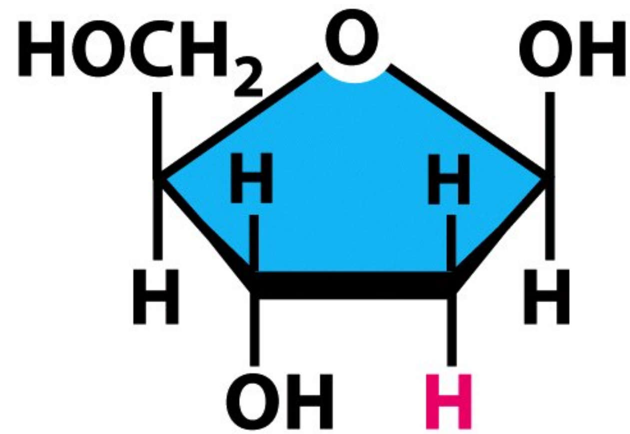
What Cellular Processes Can Lead to Different mRNA Amounts?

RNA Has Ribose Sugar in Nucleotide



ribose

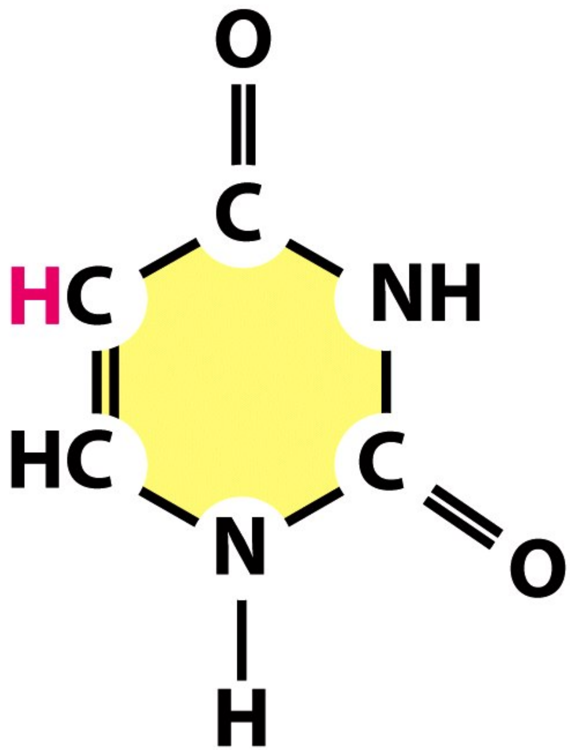
used in ribonucleic acid (RNA)



deoxyribose

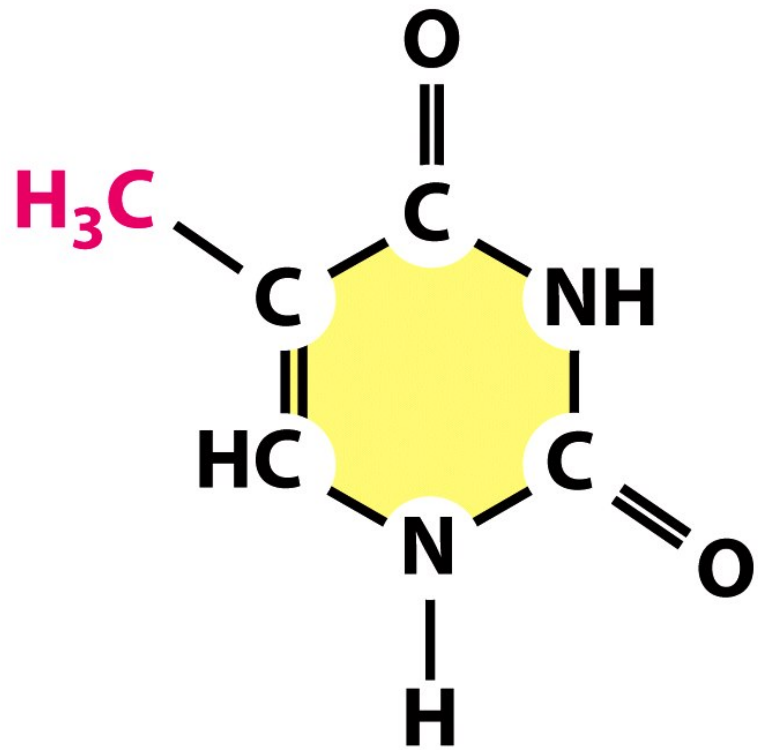
used in deoxyribonucleic acid (DNA)

RNA Has a Uracil Instead of Thymine



uracil

used in RNA

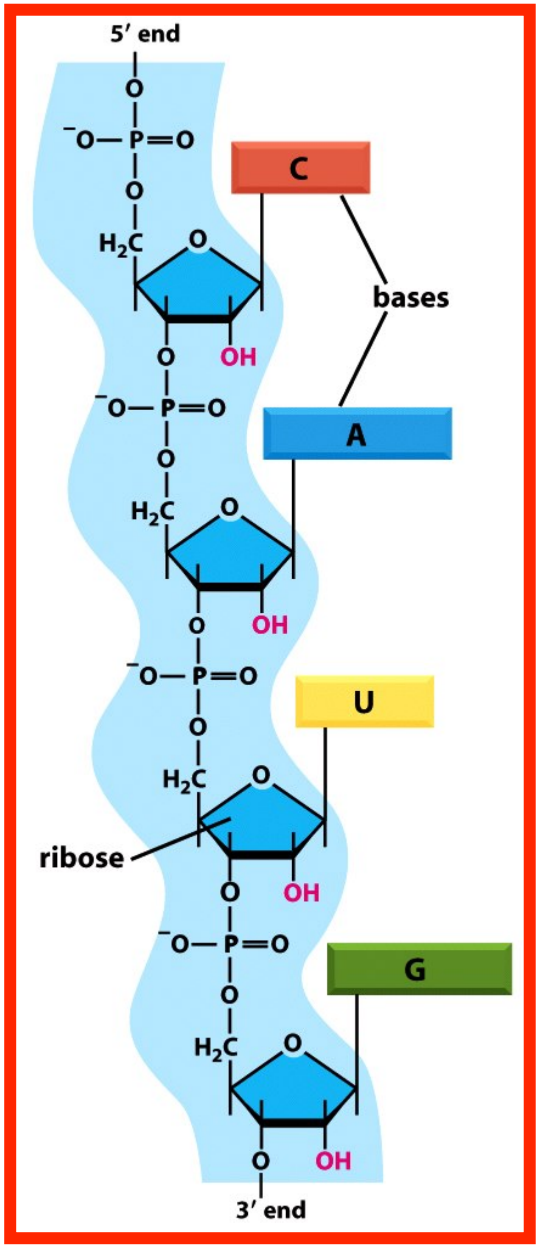


thymine

used in DNA

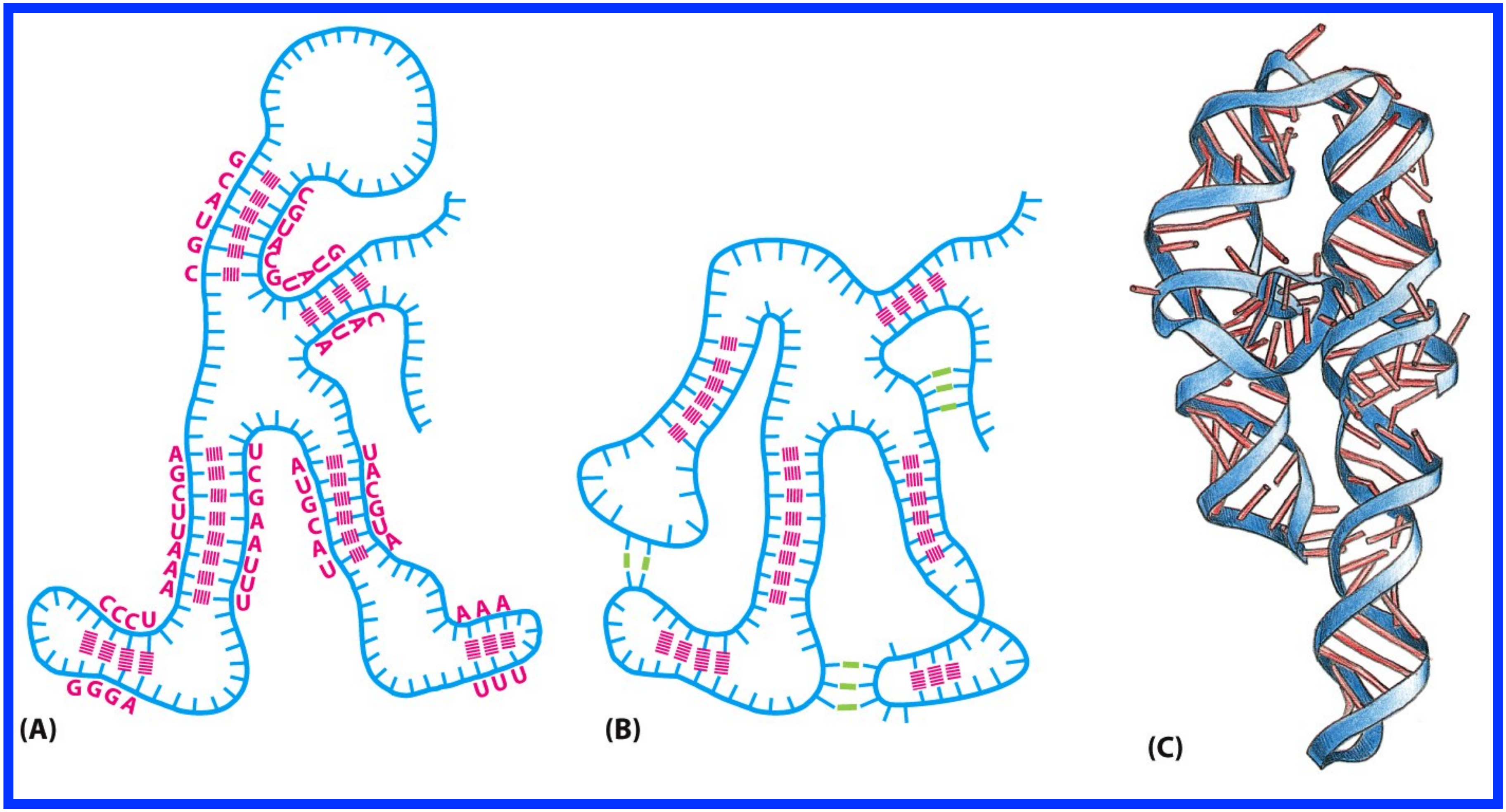
Eukaryotic RNAs Are Single-Stranded Polynucleotides

Nucleotides Joined By Phosphodiester Bonds Like All Nucleic Acids



Order 5’ → 3’ Leads to Function: Co-Linear With Gene Sequence

RNA Has Intra-Strand Double Helices or Secondary Structure



RNA Intra-Strand Secondary Structure Formed By Intra-Chain Complementary Base Pairing

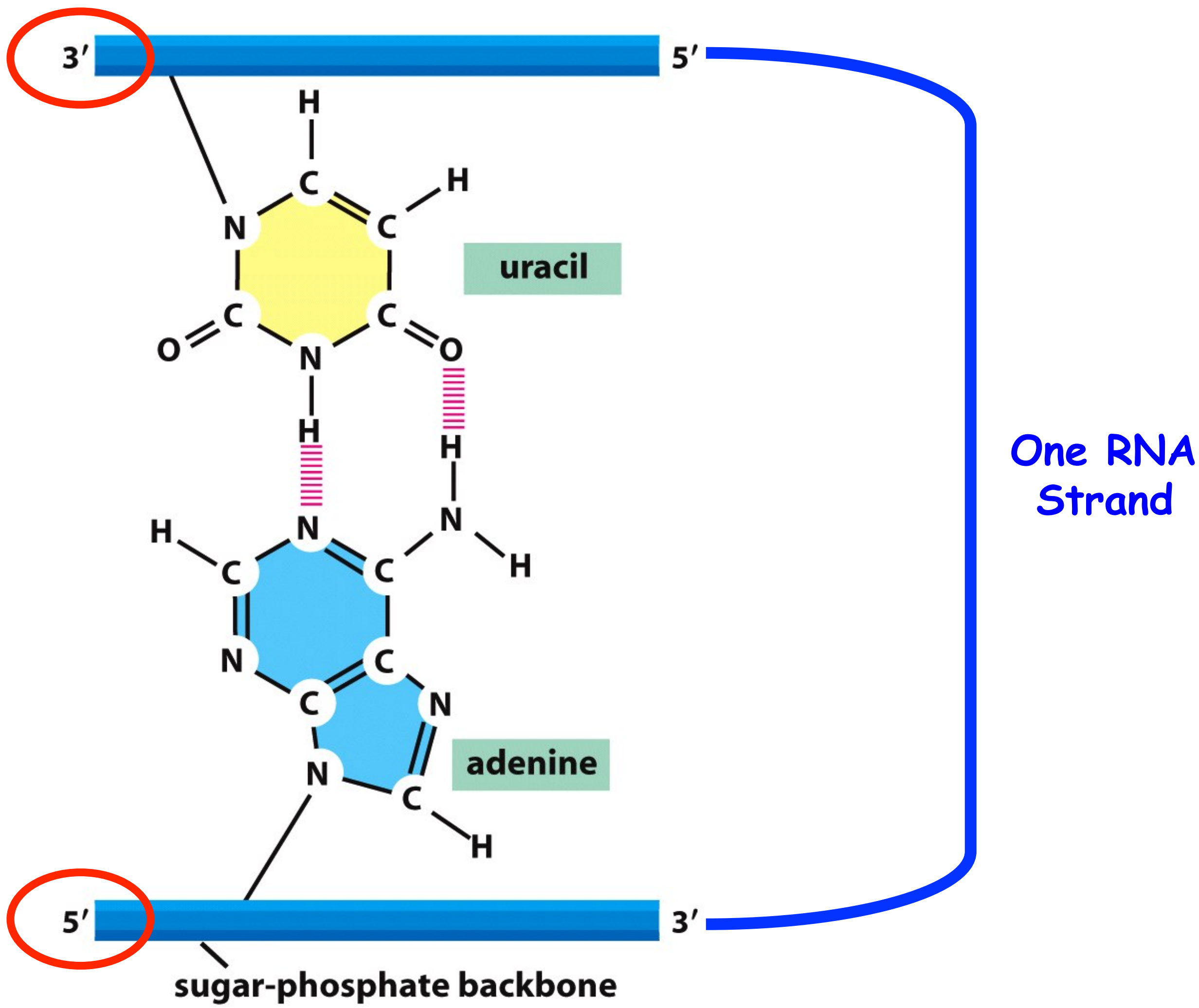


Figure 6-5 *Molecular Biology of the Cell* (© Garland Science 2008)

A Comparison of RNA and DNA Structures

Table 13.1 The structures of DNA and RNA compared

Characteristic	DNA	RNA
Composed of nucleotides	Yes	Yes
Type of sugar	Deoxyribose	Ribose
Presence of 2′-OH group	No	Yes
Bases	A, G, C, T	A, G, C, U
Nucleotides joined by phosphodiester bonds	Yes	Yes
Double or single stranded	Usually double	Usually single
Secondary structure	Double helix	Many types
Stability	Stable	Easily degraded

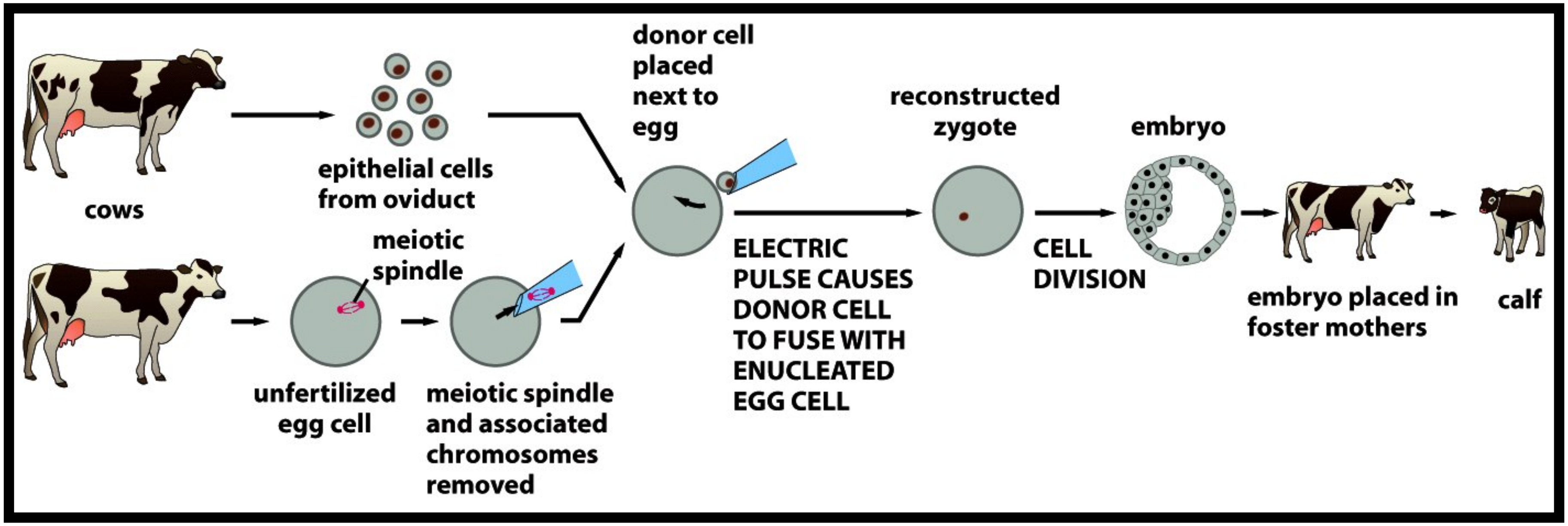
There Are Many Different Types of RNA

Table 13.2 Location and functions of different classes of RNA molecules

Class of RNA	Cell Type	Location of Function in Eukaryotic Cells*	Function
Ribosomal RNA (rRNA)	Bacterial and eukaryotic	Cytoplasm	Structural and functional components of the ribosome
Messenger RNA (mRNA)	Bacterial and eukaryotic	Nucleus and cytoplasm	Carries genetic code for proteins
Transfer RNA (tRNA)	Bacterial and eukaryotic	Cytoplasm	Helps incorporate amino acids into polypeptide chain
Small nuclear RNA (snRNA)	Eukaryotic	Nucleus	Processing of pre-mRNA
Small nucleolar RNA (snoRNA)	Eukaryotic	Nucleus	Processing and assembly of rRNA
Small cytoplasmic RNA (scRNA)	Eukaryotic	Cytoplasm	Variable
MicroRNA (miRNA)	Eukaryotic	Cytoplasm	Inhibits translation of mRNA
Small interfering RNA	Eukaryotic	Cytoplasm	Triggers (siRNA) degradation of other RNA molecules
Piwi-interacting RNA (piRNA)	Eukaryotic	Cytoplasm	Thought to regulate gametogenesis, but function poorly defined

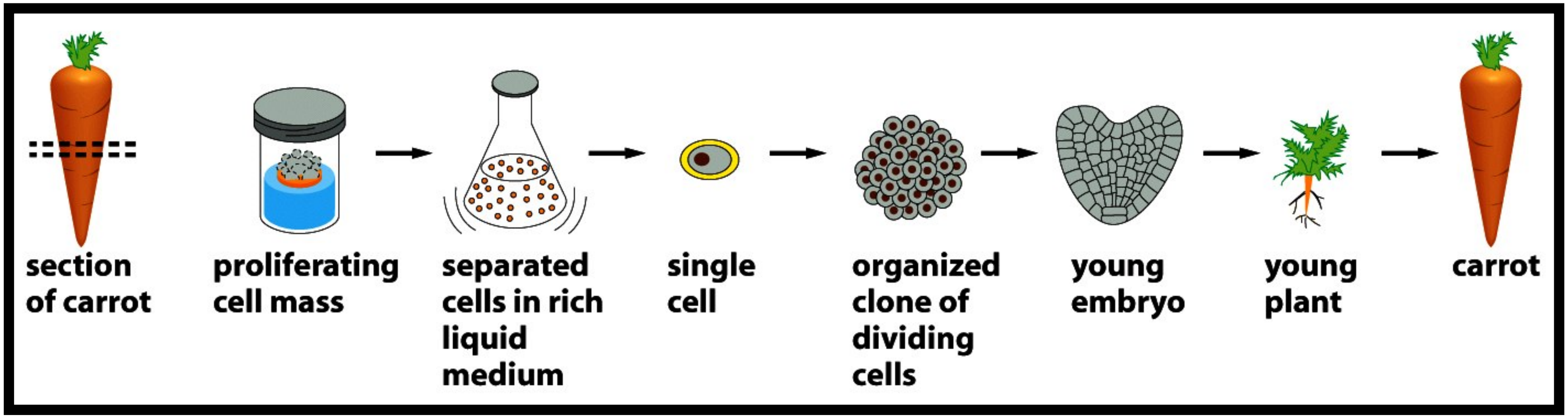
Differential Gene Activity Programs Development

Animal Cloning Demonstrates That the Genome of a Differentiated Cell Contains All of the Genes Required To Program the Entire Life Cycle



<u>Corollary</u>: Differentiation Must Be Programmed By Differential Gene Expression

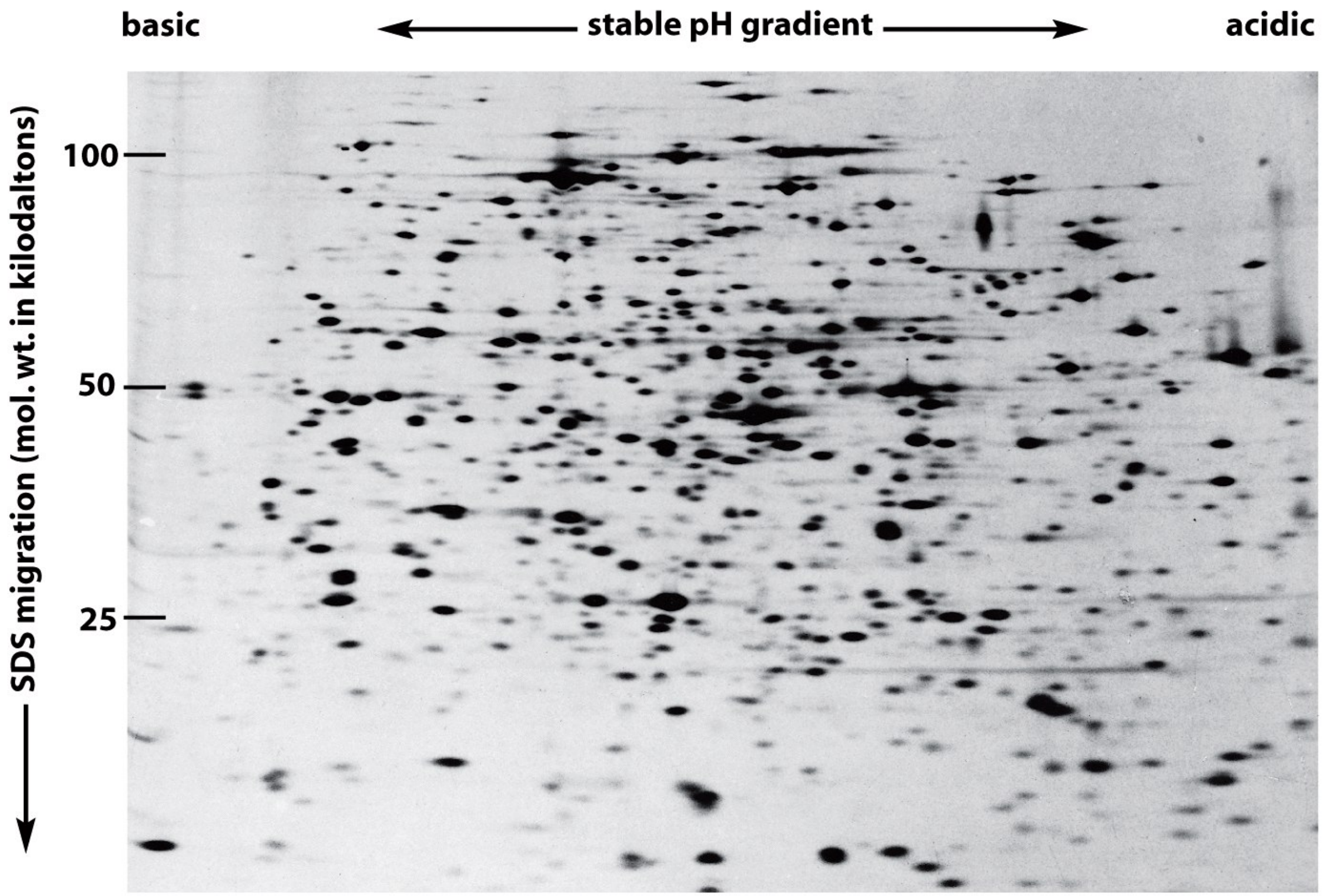
Plant Cell Cloning Demonstrates That the Genome of a Differentiated Plant Cell Contains All of the Genes Required To Program the Entire Life Cycle



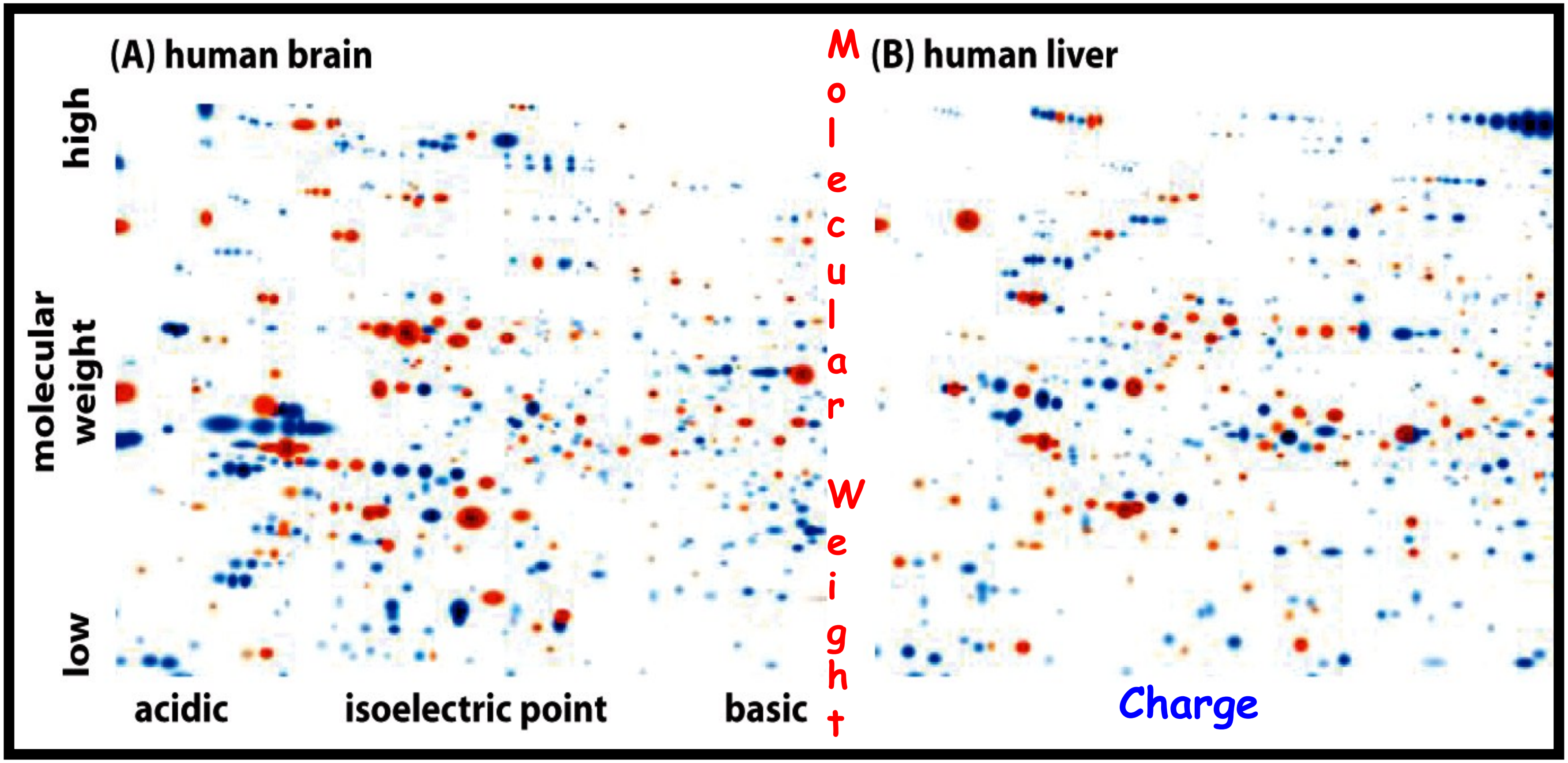
Corollary: Plant Differentiation Must Be Programmed By Differential Gene Expression

Tools For Investigating Differential Gene Activity

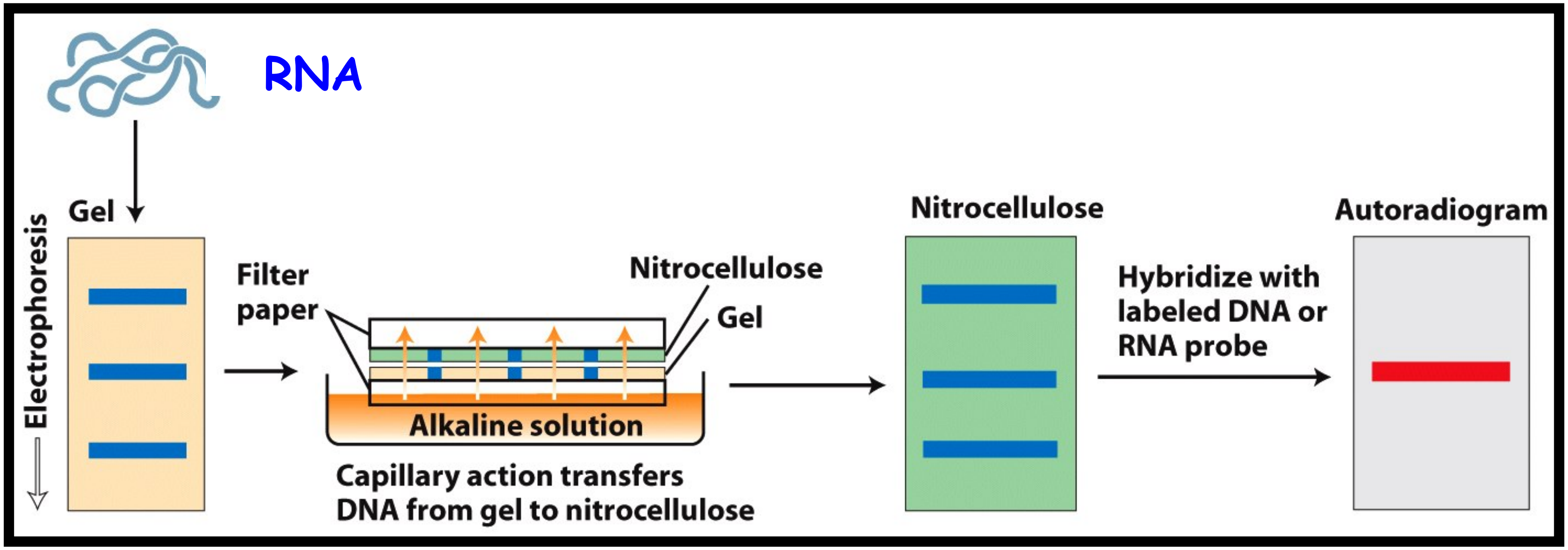
Two-Dimensional Protein Gel Electrophoresis



2-D Protein Gel Electrophoresis Demonstrates Differential Gene Activity in Animal Organs

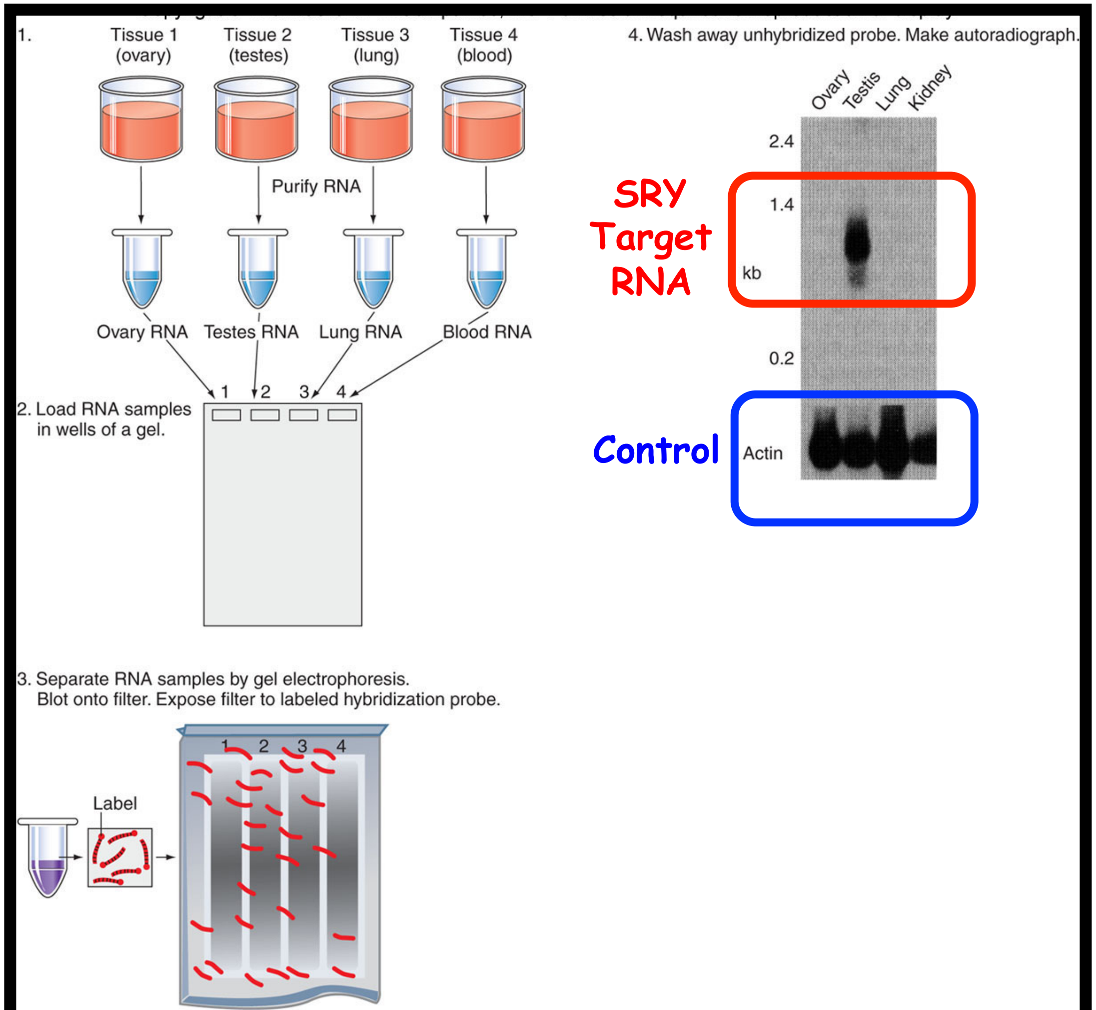


RNA Blots Detect Specific RNAs in an RNA Population



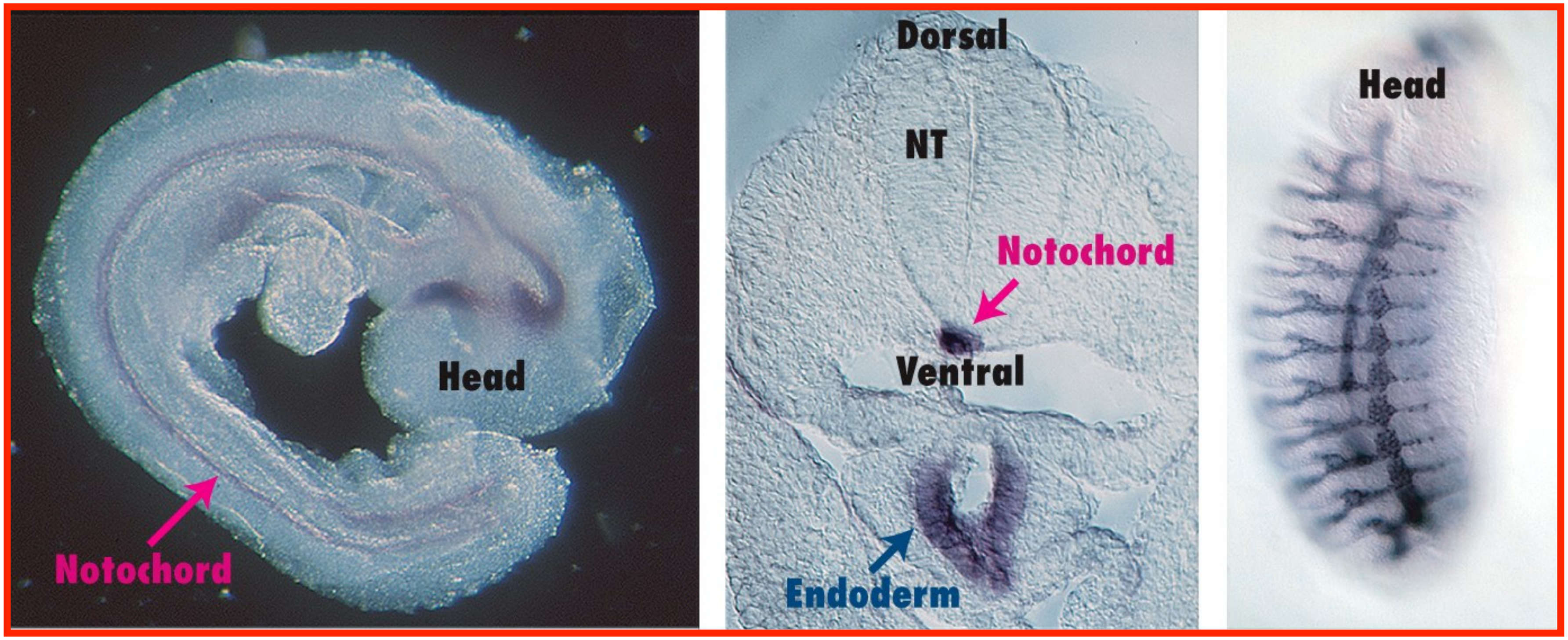
Need Specific Probe to Detect RNA

RNA Blot Demonstrates Differential Gene Activity



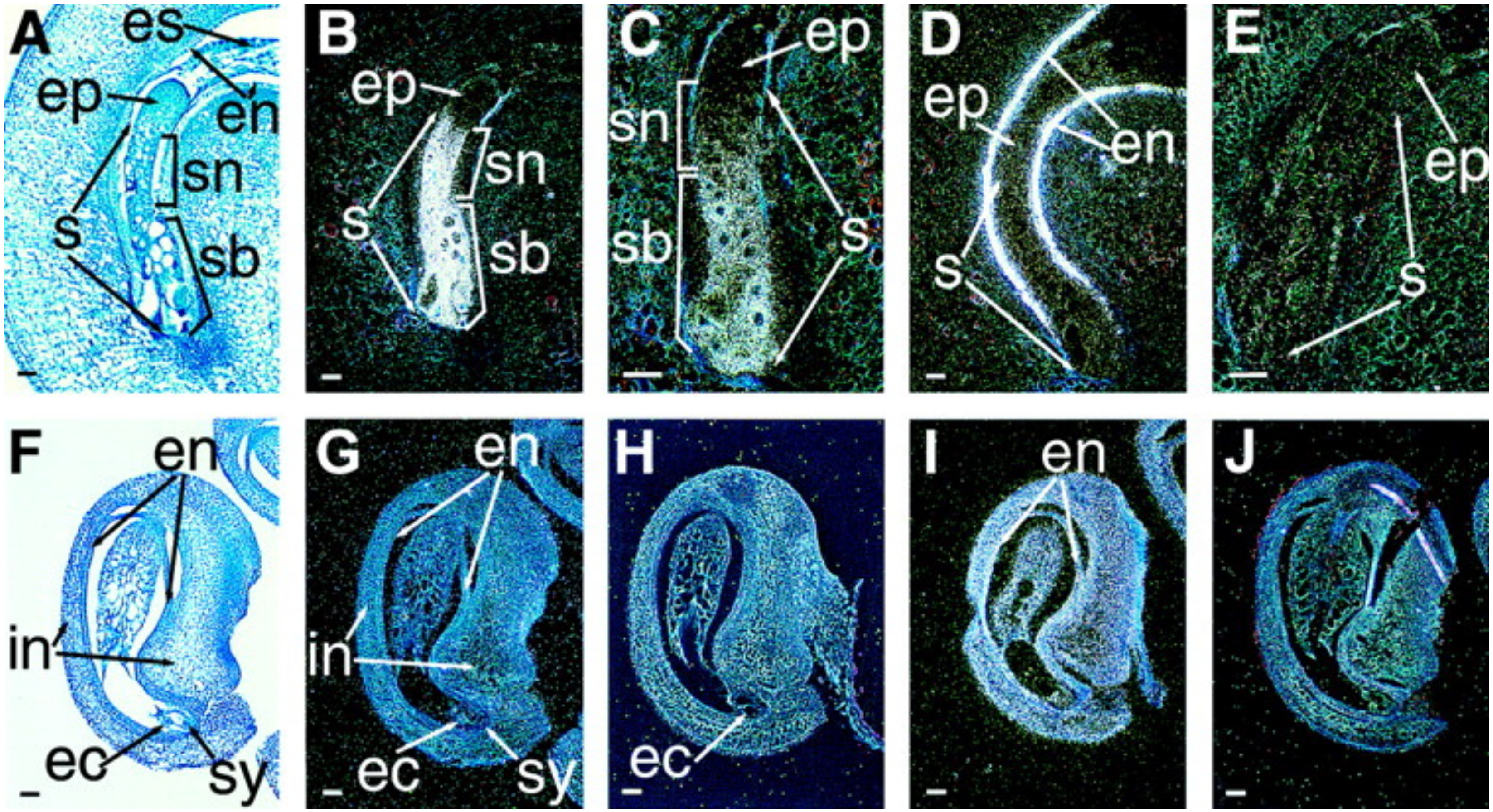
(4): Reprinted with permission from *Nature* 1990 Jul 19; 346(6281):216-7, Sinclair et al. © 1990 Macmillian Magazines Limited

In Situ Hybridization Demonstrates Differential Gene Activity



Hybridization of Specific Probe to Tissue
In Situ **(i.e., In Place)**

Localizing Gene Activity in Plant Embryo Regions



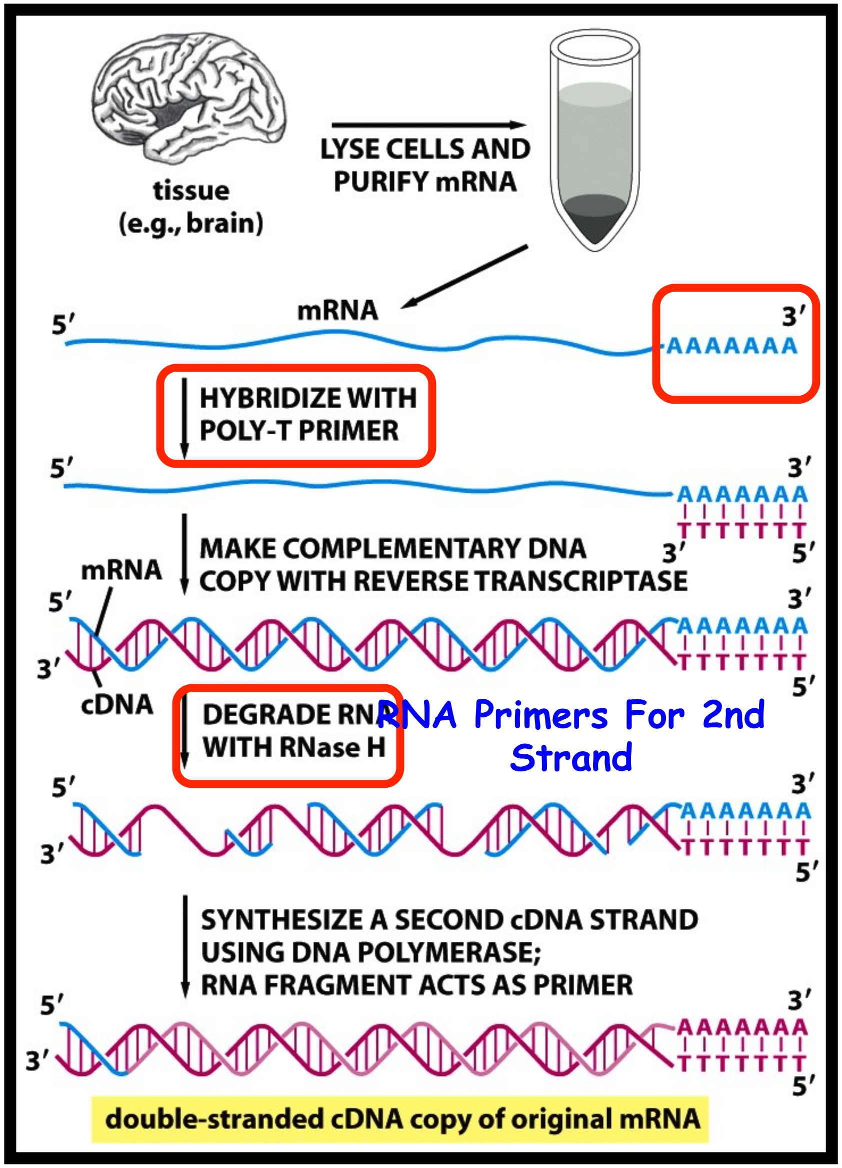
Weterings et al. Plant Cell (2001) 13,2409-2425

Using Reverse Transcriptase to Synthesize cDNA Copies of mRNAs (Note: cDNA=copy DNA)

Requirements

1. RNA Template
2. dXTPs
3. Reverse Transcriptase
4. RNase H or S-1 Nuclease
5. Oligo dT Primer

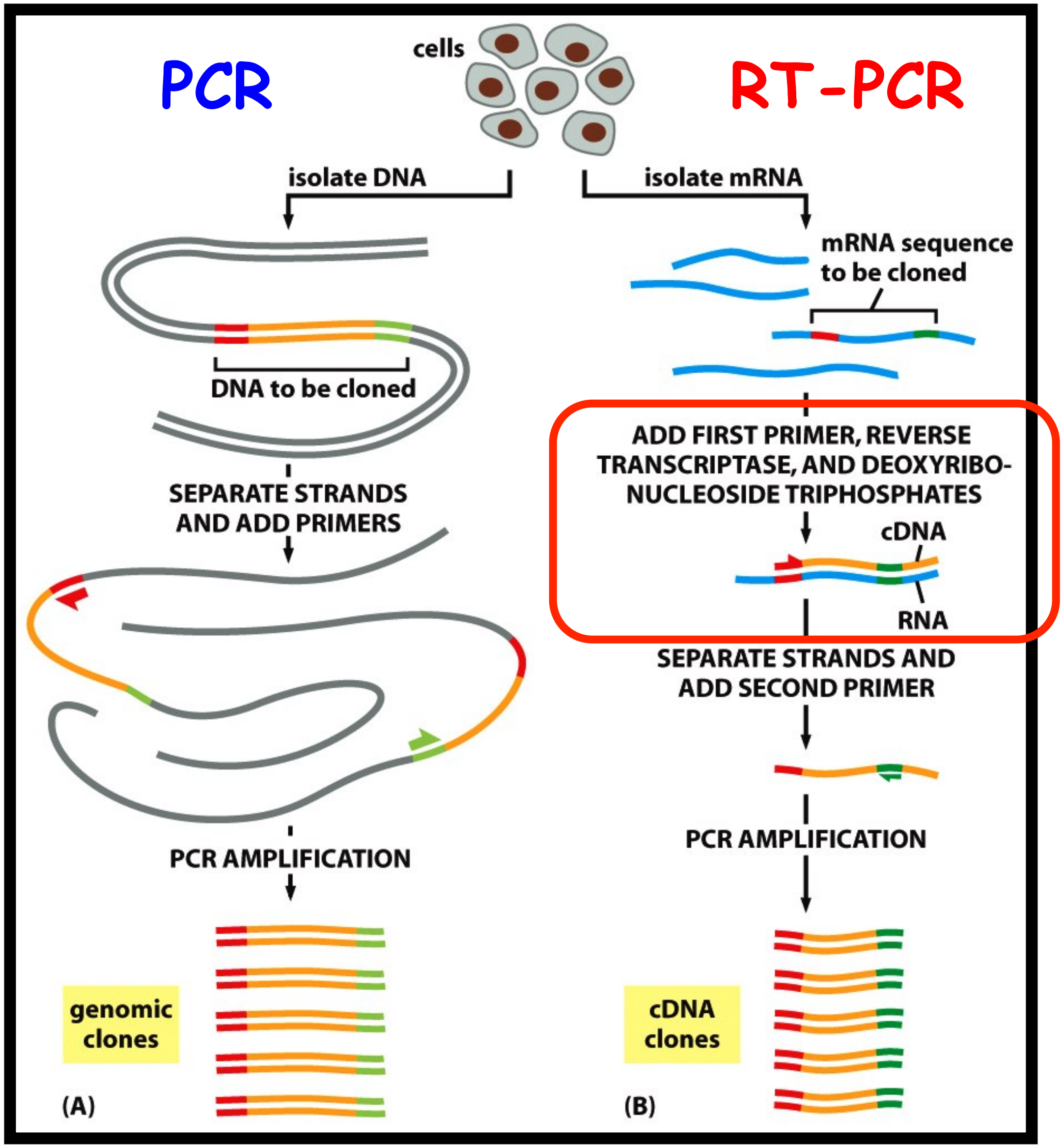
Note: Reverse Transcriptase is a DNN Polymerase



PolyA Tail

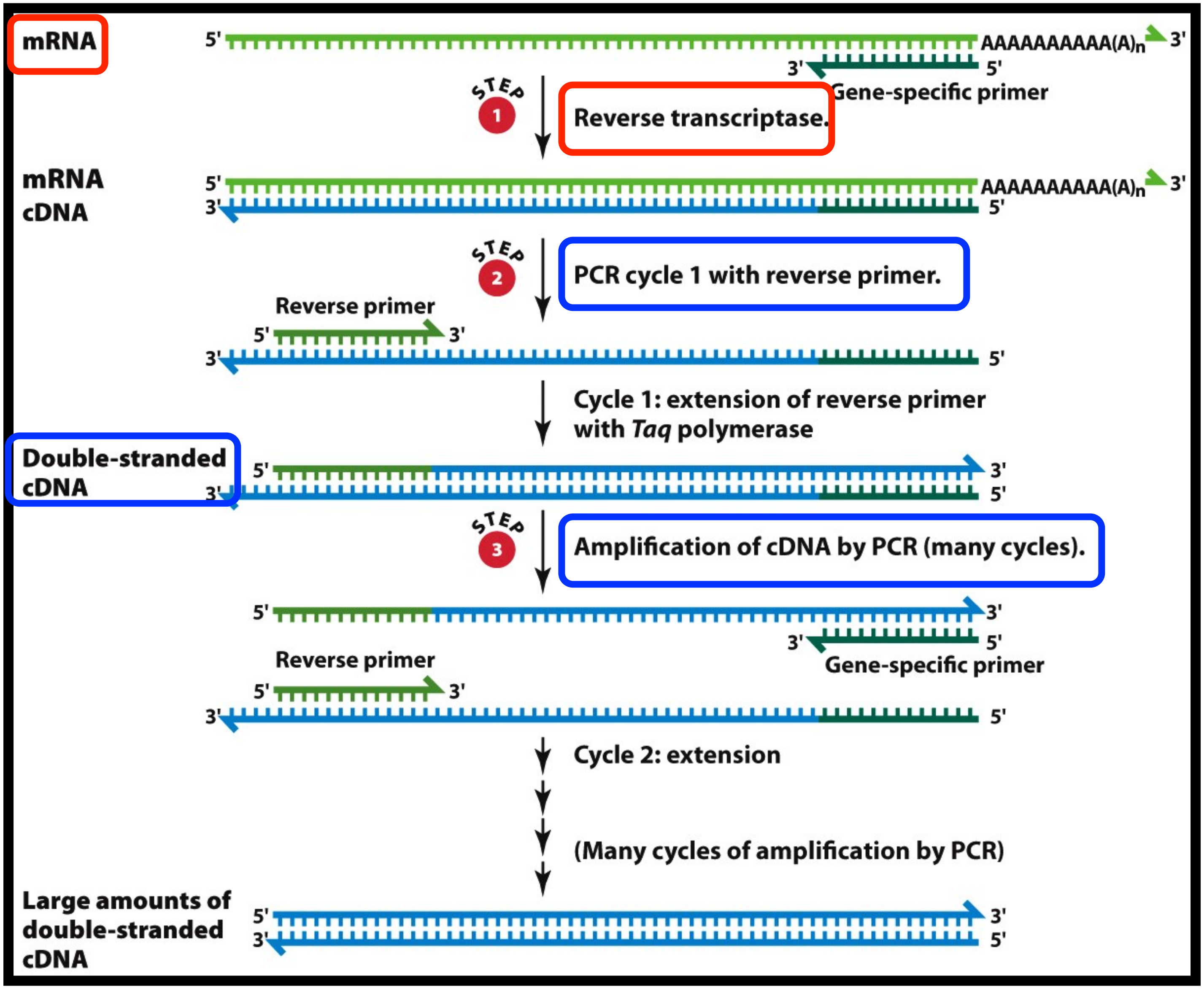
RNA Primers For 2nd Strand

A Comparison of PCR and RT-PCR

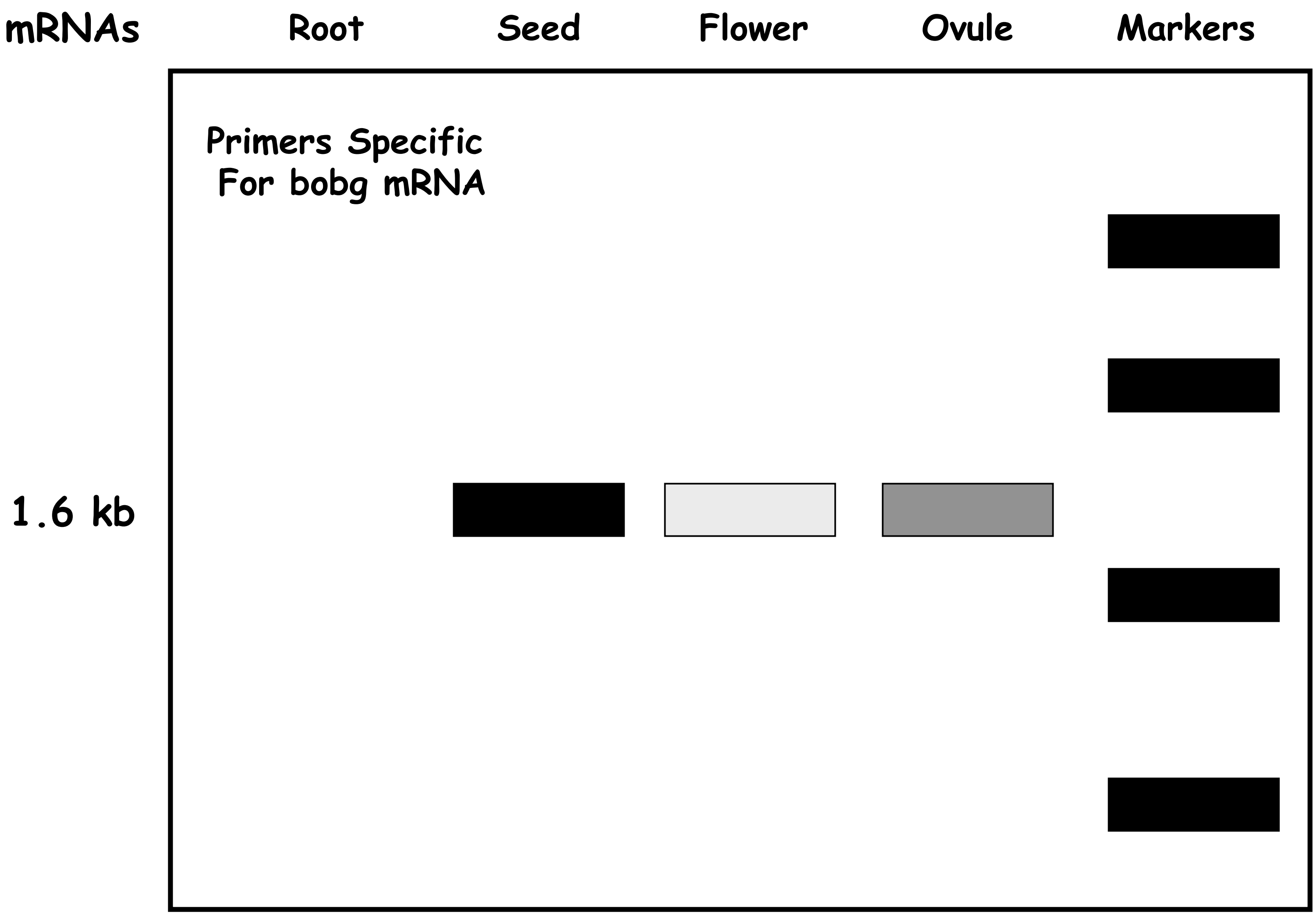


Need To Synthesize cDNA Before PCR

Generating Double-Stranded cDNA Copies of Specific mRNAs Using Reverse Transcription PCR

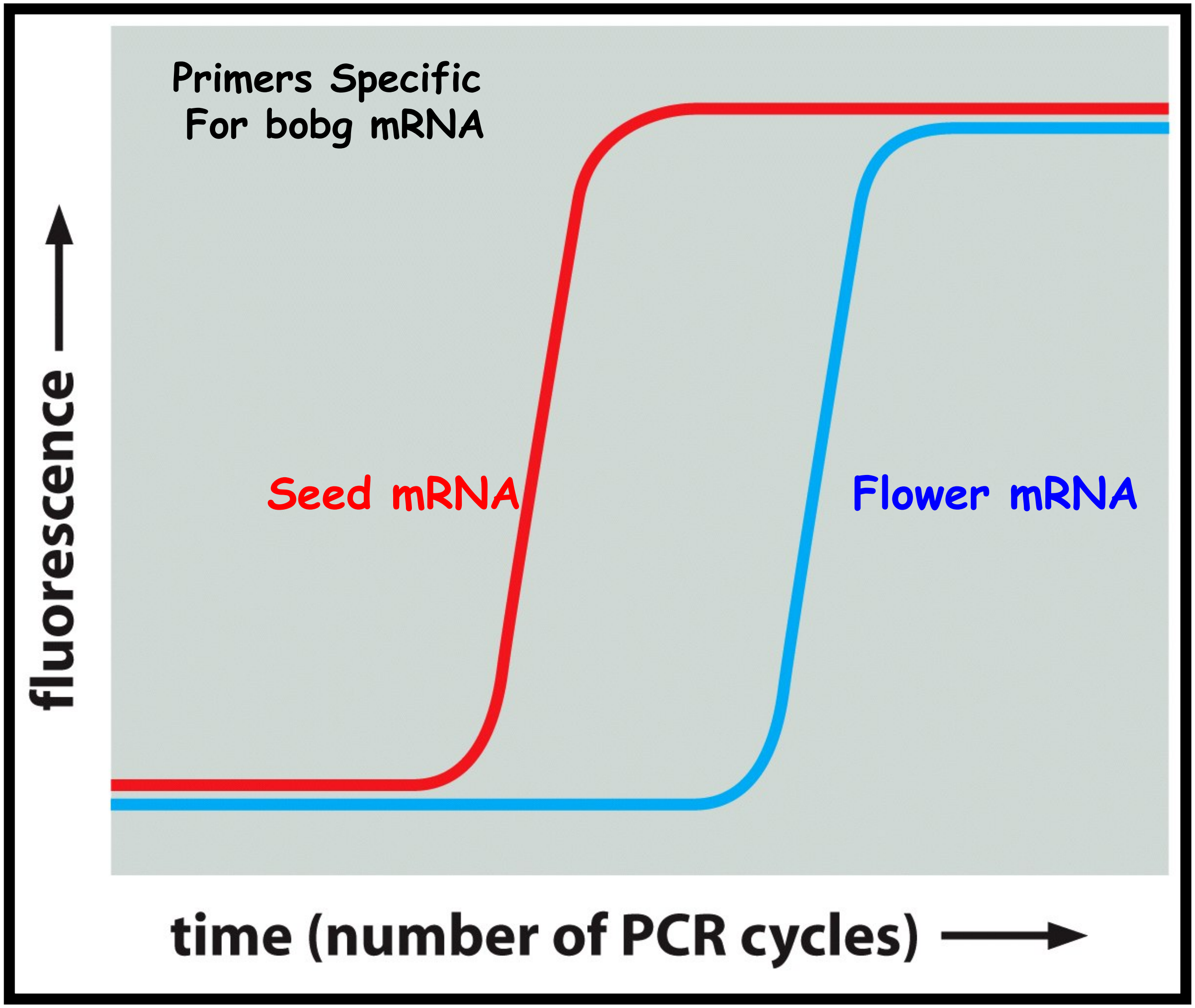


Using RT-PCR to Investigate Seed Gene Activity



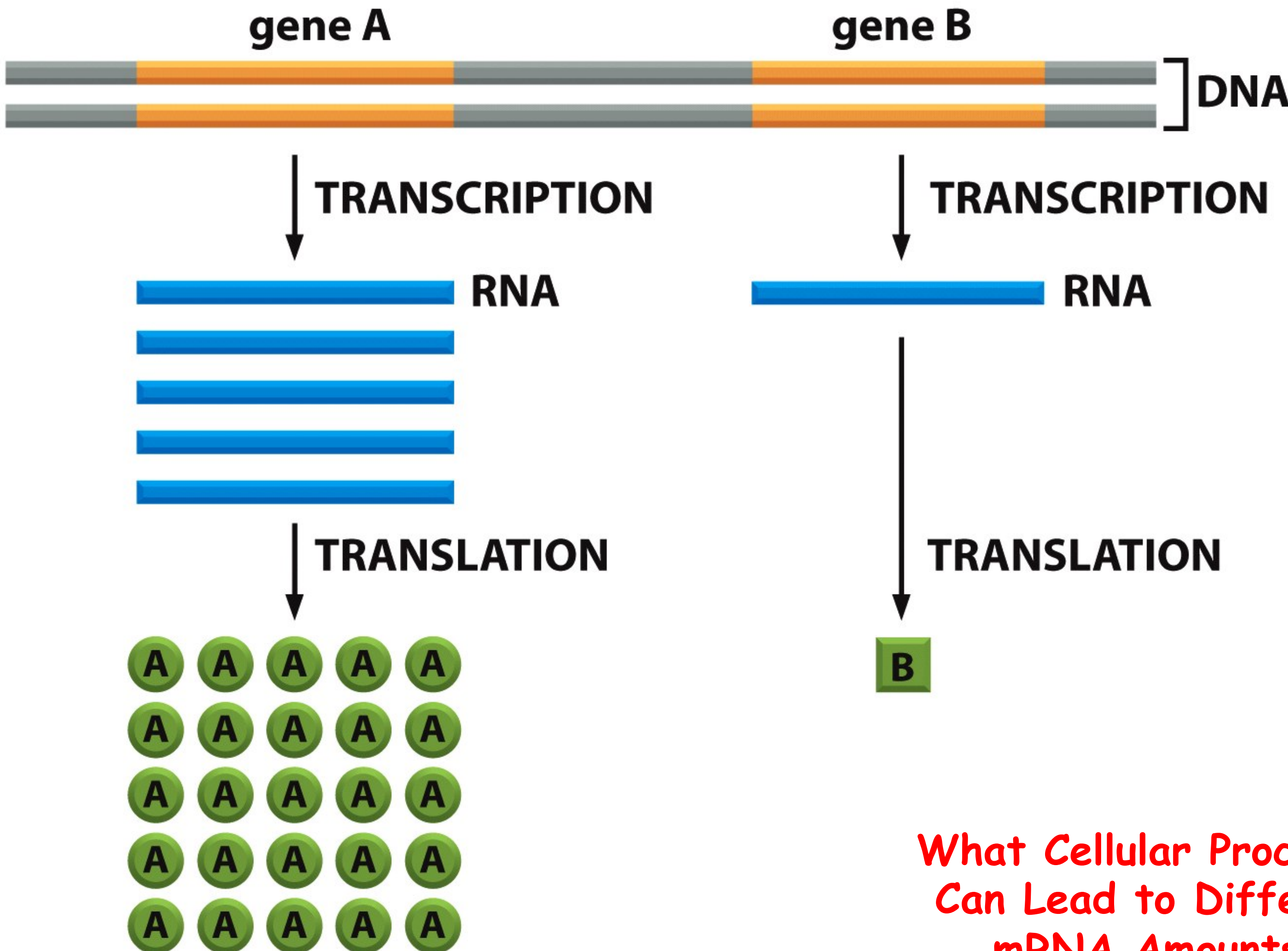
Which "Tissue" Has the Most bobg mRNA?

Using Real-Time Quantitative RT-PCR To Measure Specific mRNA Accumulation Levels



Curves Visualize the Replication Process Over Time
That is, the Amount of DNA Synthesized at Each PCR Cycle

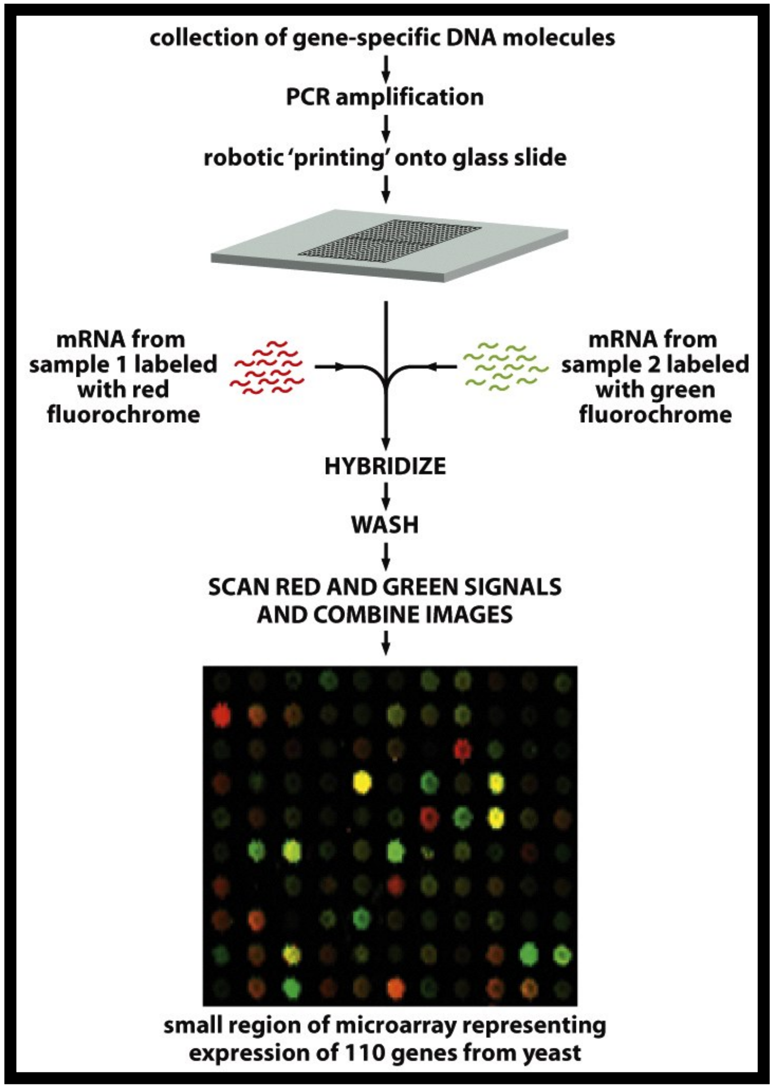
Genes Can Have Different "Expression" Levels That Are Reflected in Differing Amounts of mRNAs Accumulating in the Cytoplasm



What Cellular Processes Can Lead to Different mRNA Amounts?

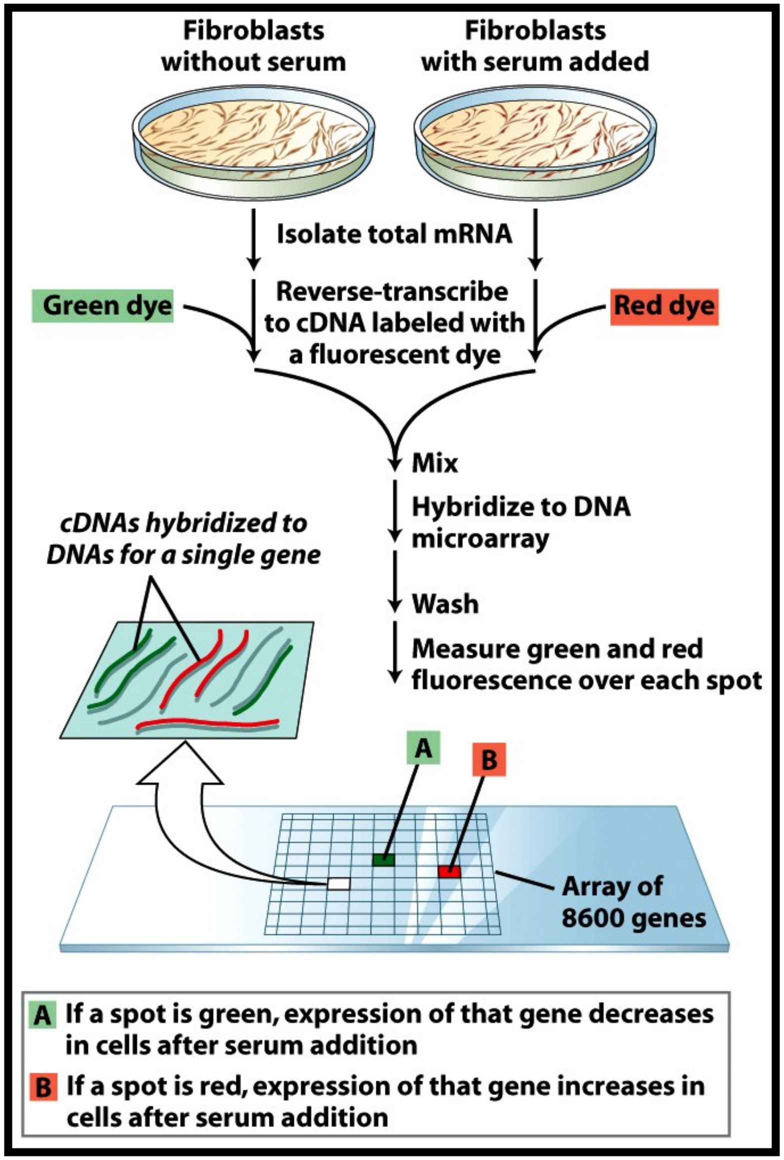
Using Microarrays to Investigate the "Expression' of Thousands of Genes at a Time: Part One

cDNAs Are Synthesized From Each mRNA With a Different Fluorescent Nucleotide



Each Spot Represents A Different Gene

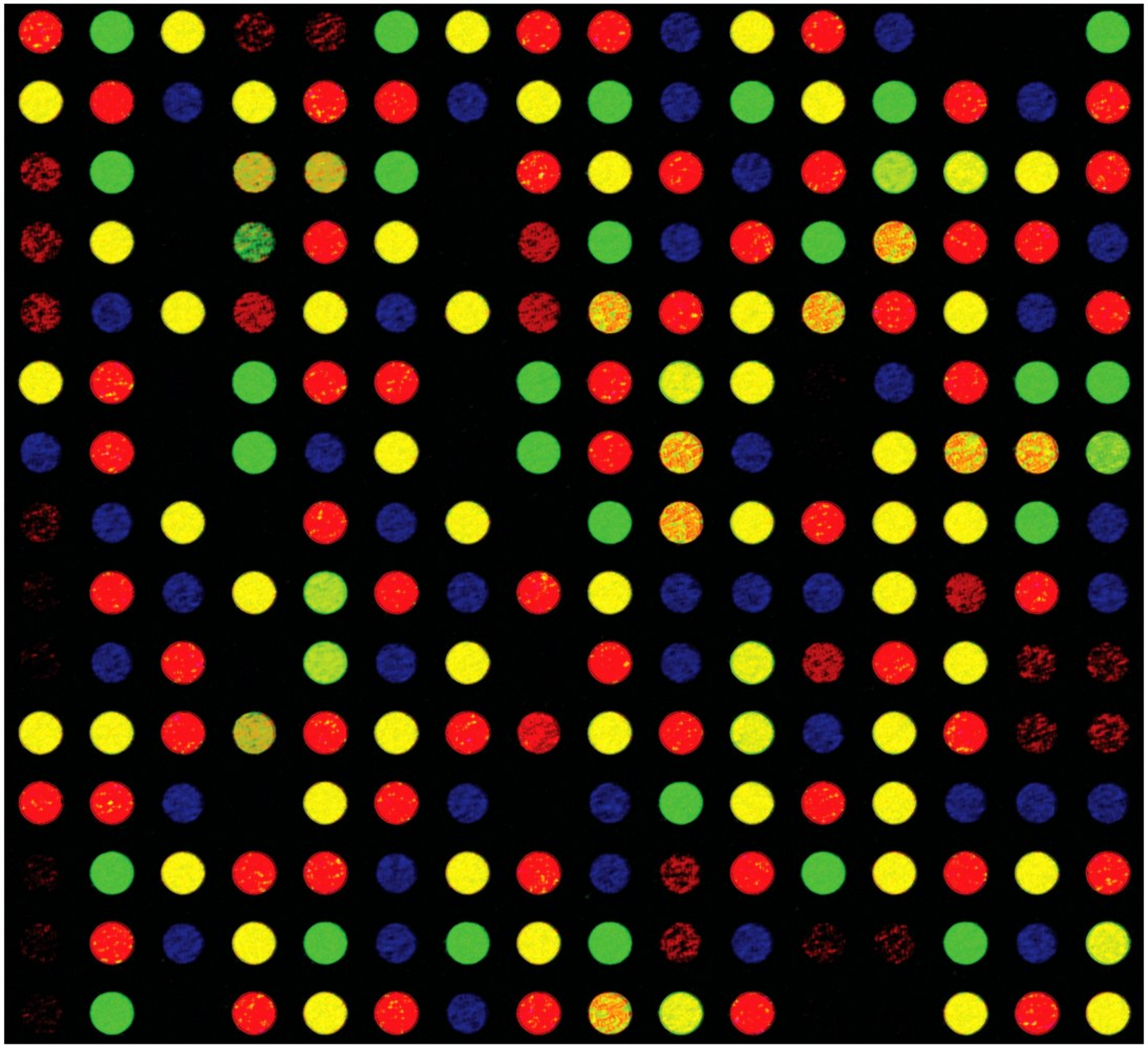
Using Microarrays to Investigate the "Expression' of <u>Thousands</u> of Genes at a Time: Part Two



Using Microarrays to Investigate the "Expression' of Thousands of Seed Genes

Red=Seed

Green=Leaf



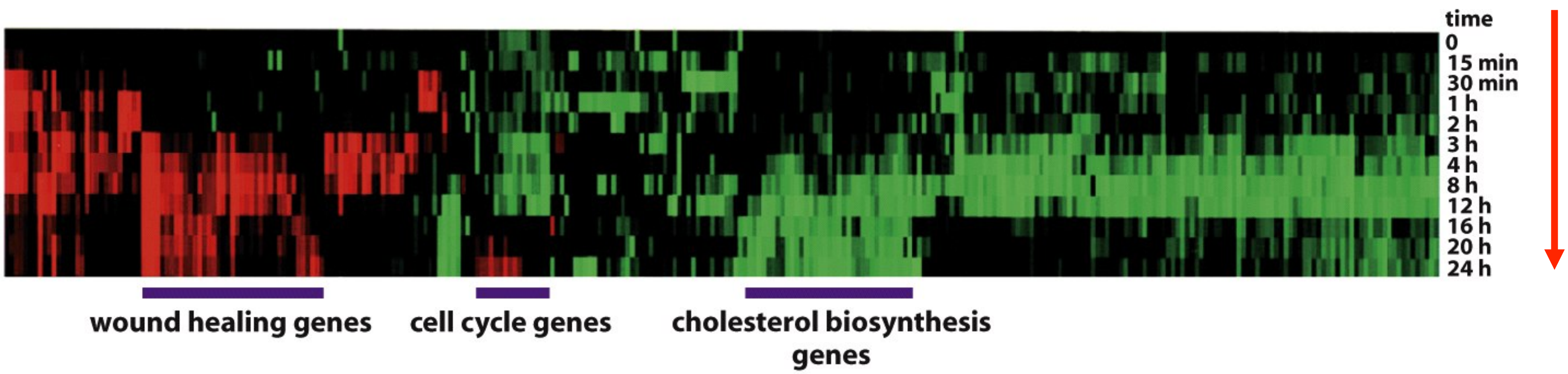
Which Genes Are

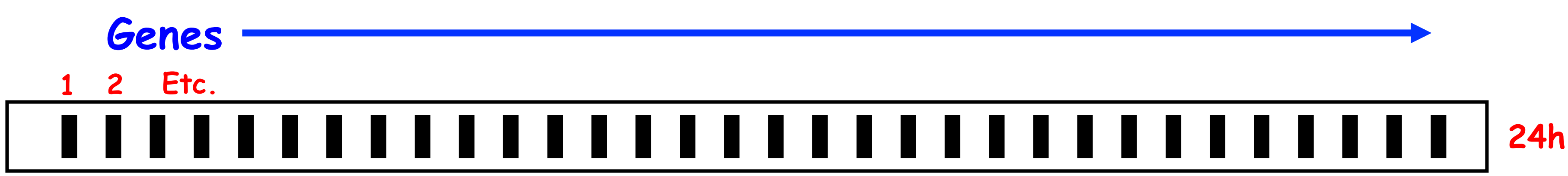
1. Seed Specific?

2. Leaf Specific?

3. Active in Both "Tissues?"

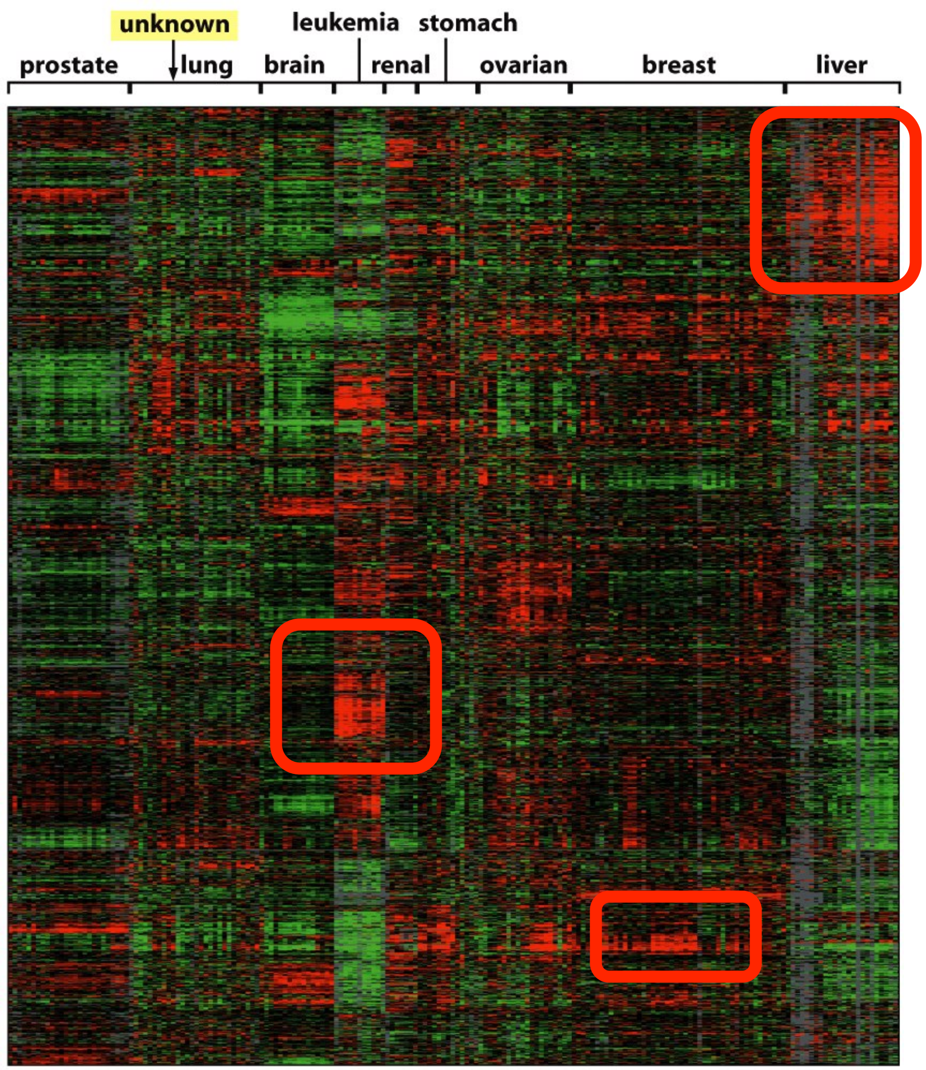
Using Hierarchical Clustering to Reveal Co-Regulated Gene (mRNA) Sets





Clustering Algorithms Find Similar Patterns

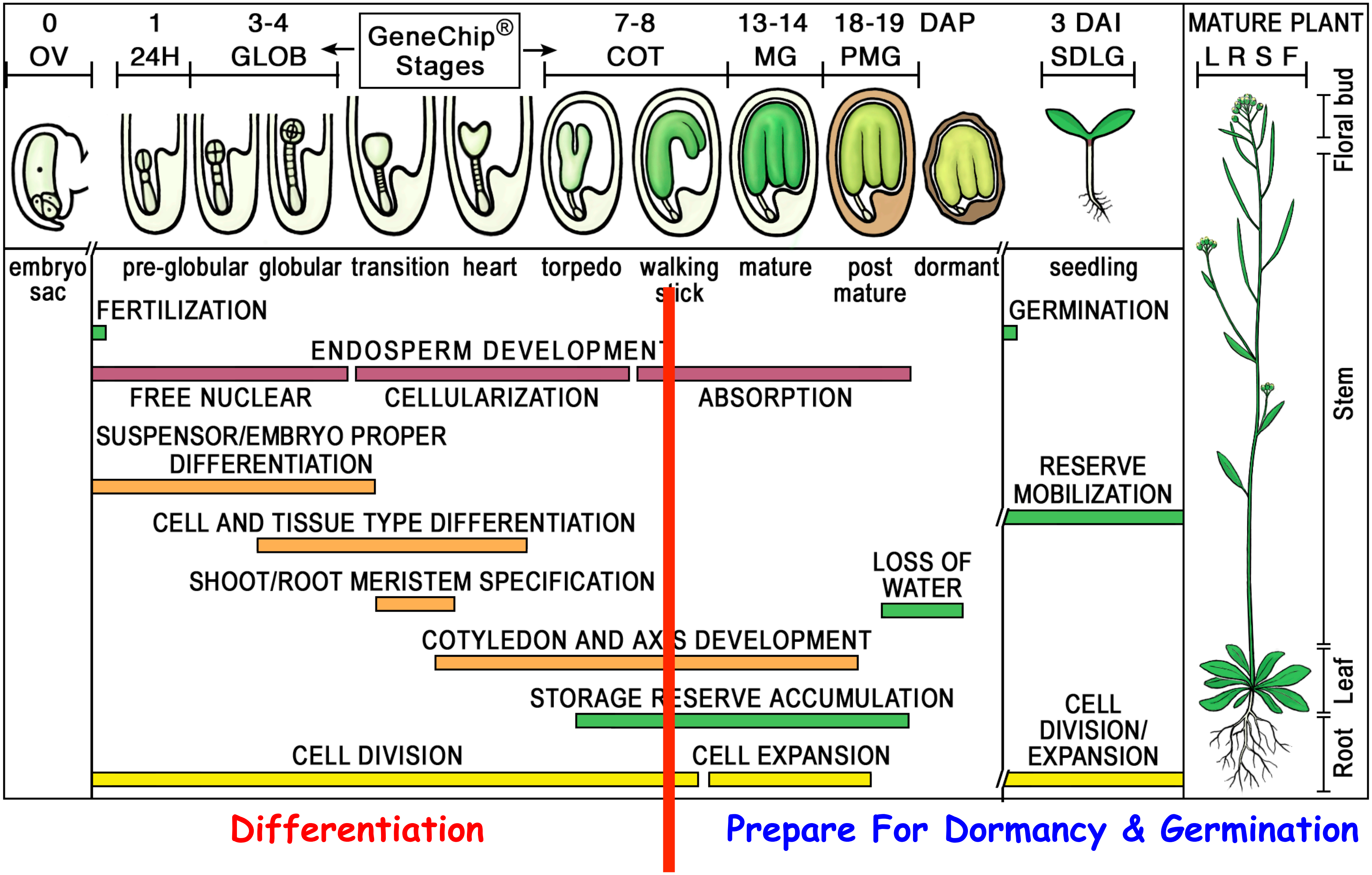
Hierarchical Clustering of Up-Regulated mRNAs in Different Cancer Tissues



Using Microarrays To Investigate Gene Activity in *Arabidopsis* Seeds

1. Whole Seeds
2. Specific Seed Compartments

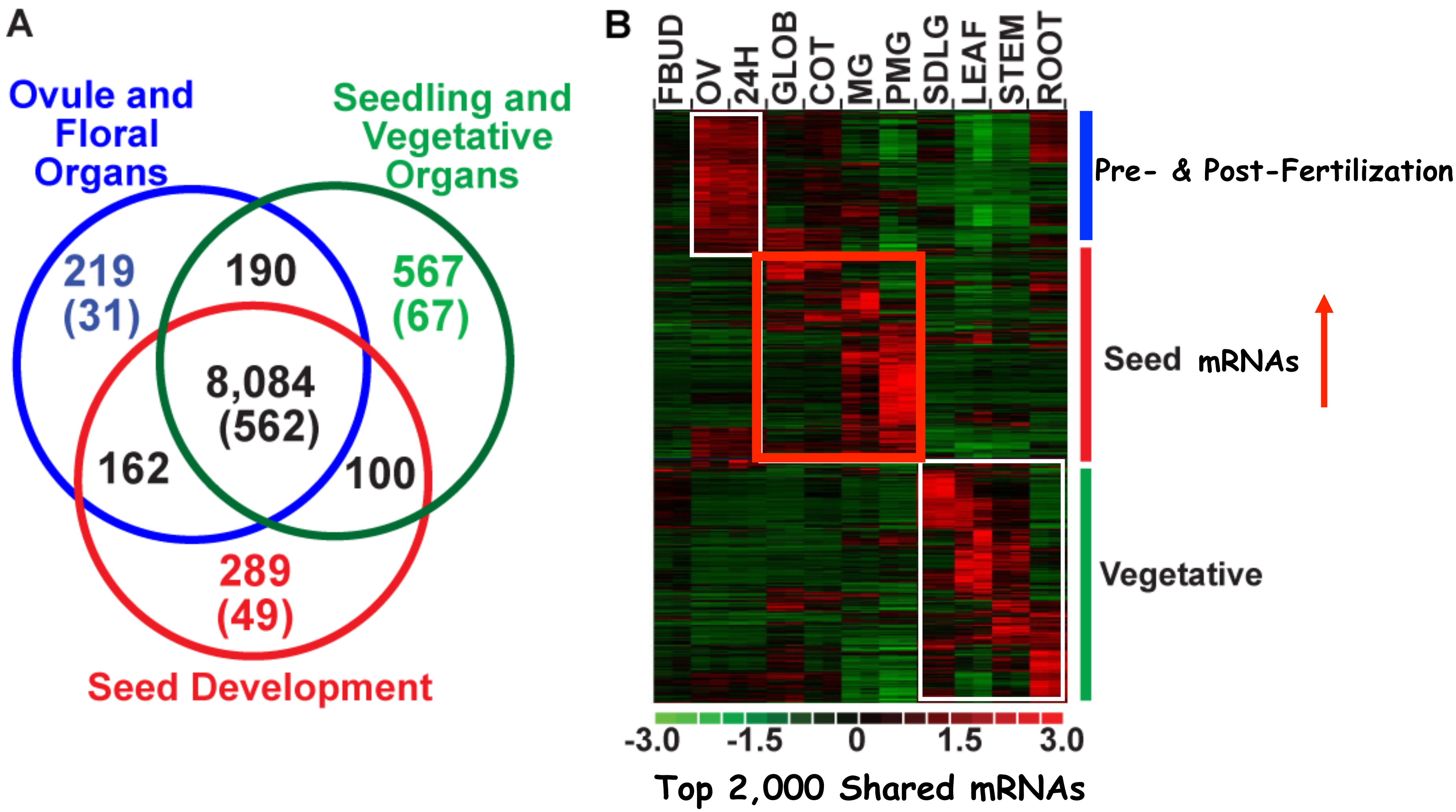
Genome-Wide Profiling of mRNAs During Arabidopsis Seed Development & Plant Life Cycle



Differentiation

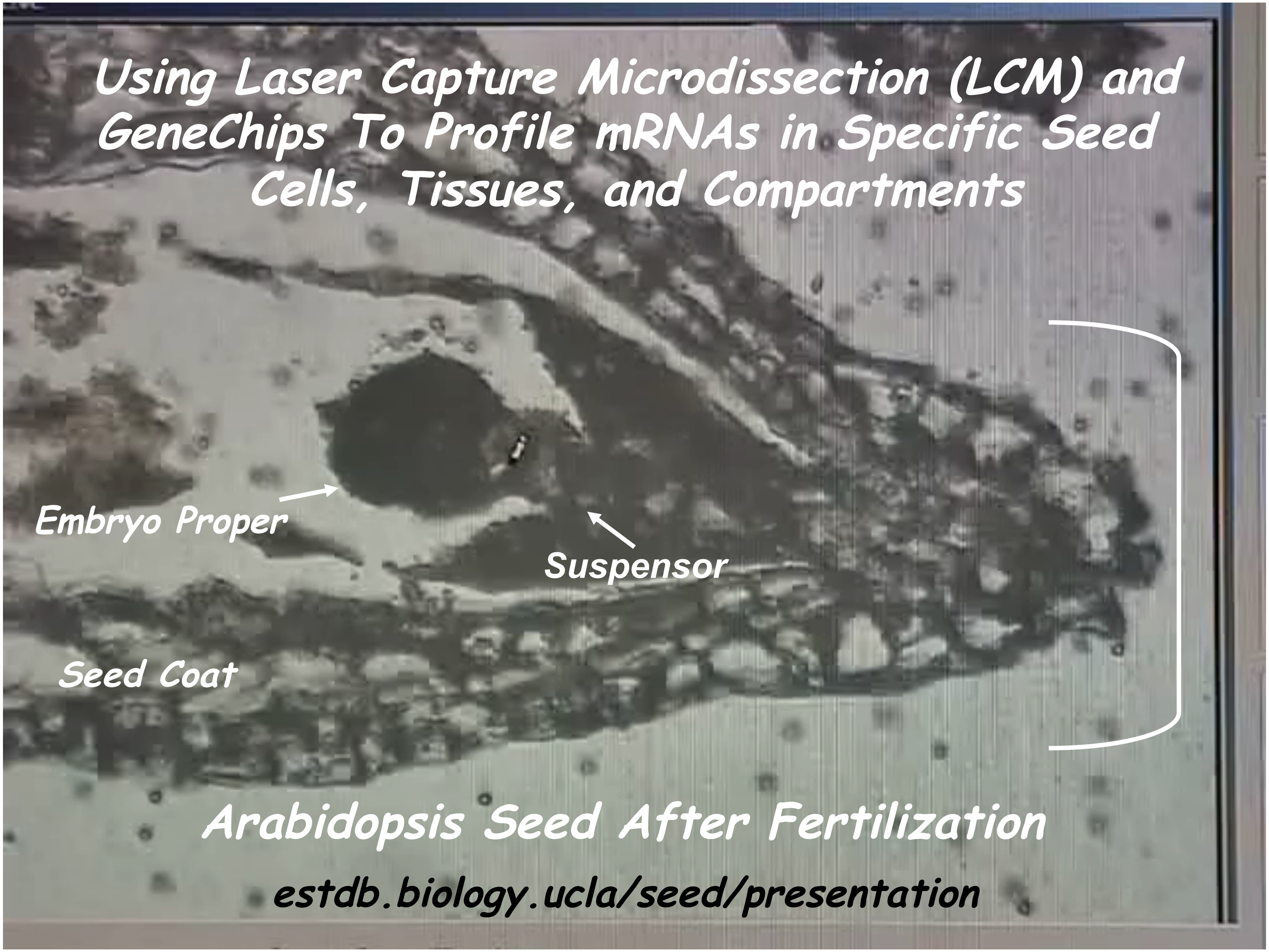
Prepare For Dormancy & Germination

Identification of Seed-Specific mRNAs in the *Arabidopsis* Life Cycle Using Whole Seeds

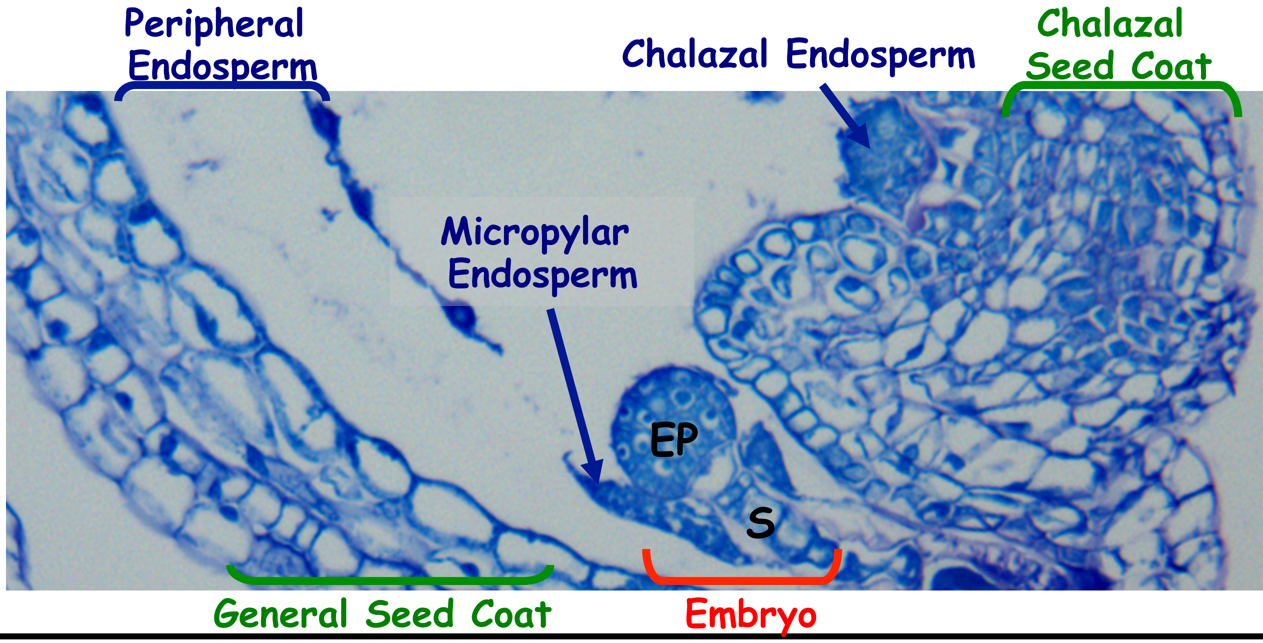


( ) Indicates number of transcription factor mRNAs

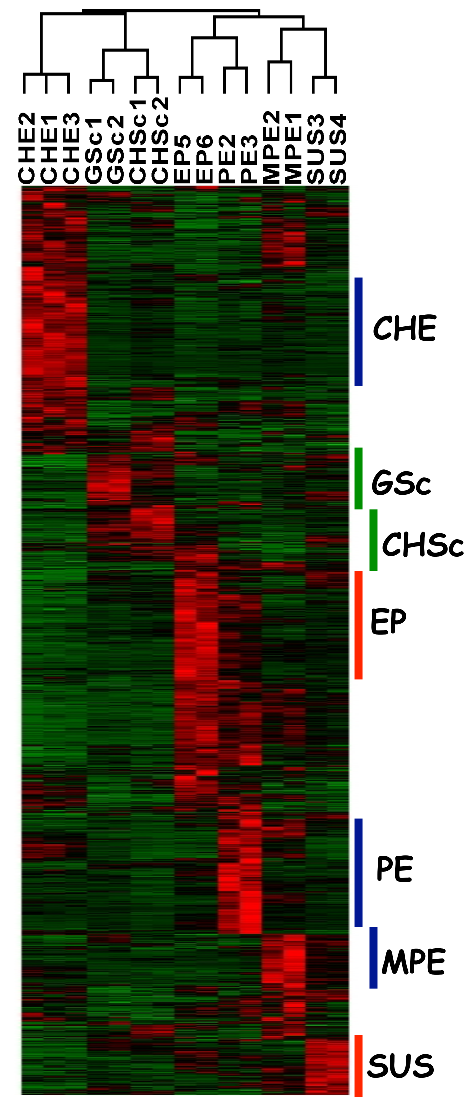
Le et al. PNAS (2010) 107, 8063-8070


Using Laser Capture Microdissection (LCM) and GeneChips To Profile mRNAs in Specific Seed Cells, Tissues, and Compartments
Embryo Proper
Suspensor
Seed Coat
Arabidopsis Seed After Fertilization
estdb.biology.ucla/seed/presentation

Gene Activity in Globular-Stage *Arabidopsis* Seed Compartments

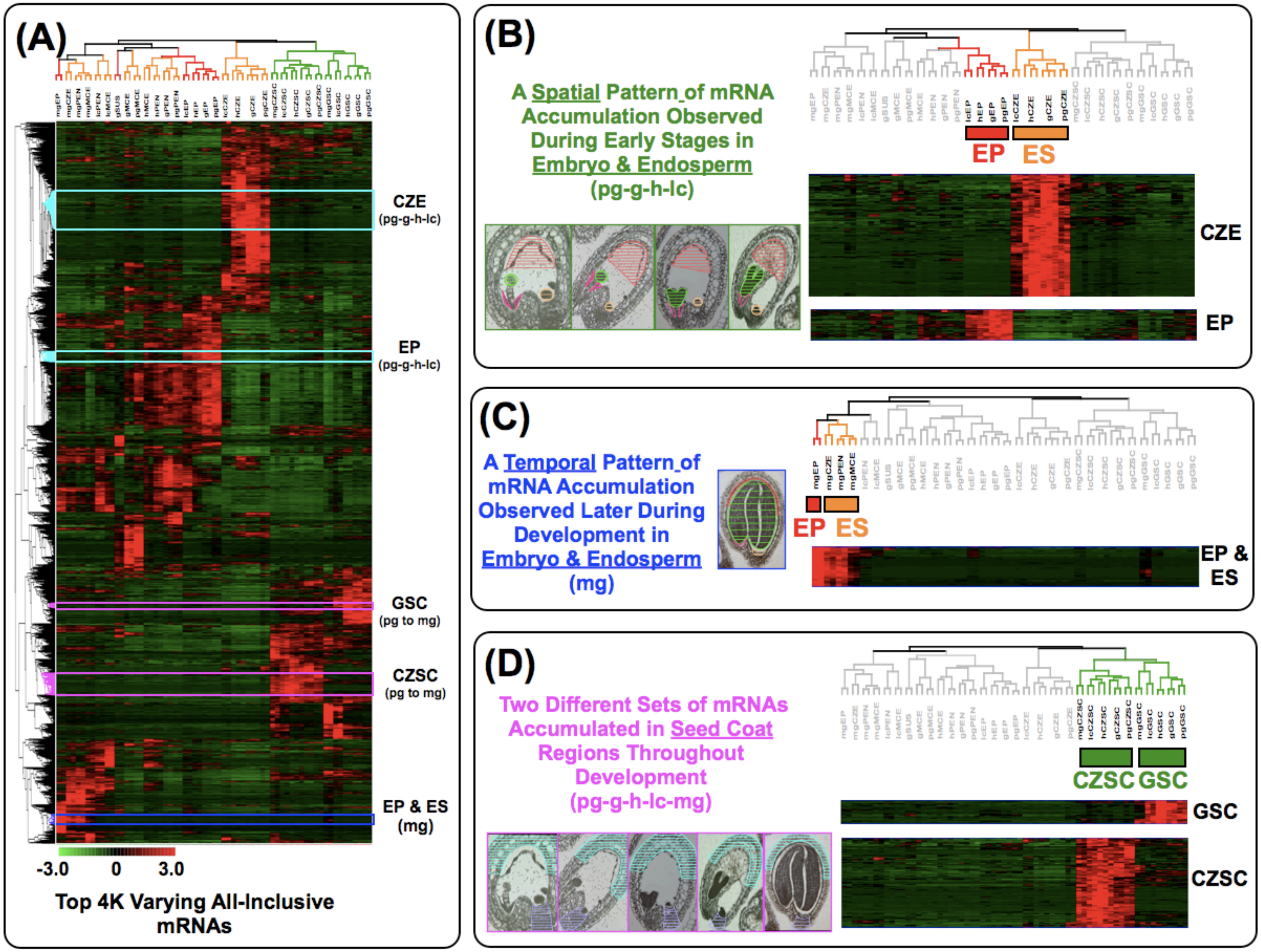


	GSC	PE	MPE	EP	SUS	CHE	CHSC
mRNA BR1	11,402	11,445	7,844	10,532	11,273	7,709	12,440
mRNA BR2	12,316	9,972	10,658	10,674	12,965	7,002	11,818
mRNA BR3	-					9,793	-
Total mRNAs	10,513	9,136	7,216	9,130	10,692	7,776	10,794
Unique mRNA	108	54	14	56	95	133	152
Unique TFs	16	3	0	17	8	10	26
Shared	4,028 →						



Top 2000 Shared mRNAs

Arabidopsis Genes You Are Investigating This Quarter Are Active in Specific Seed Compartments



Recall………….Scientific American Article on cDNA/EST Sequencing From Last Quarter

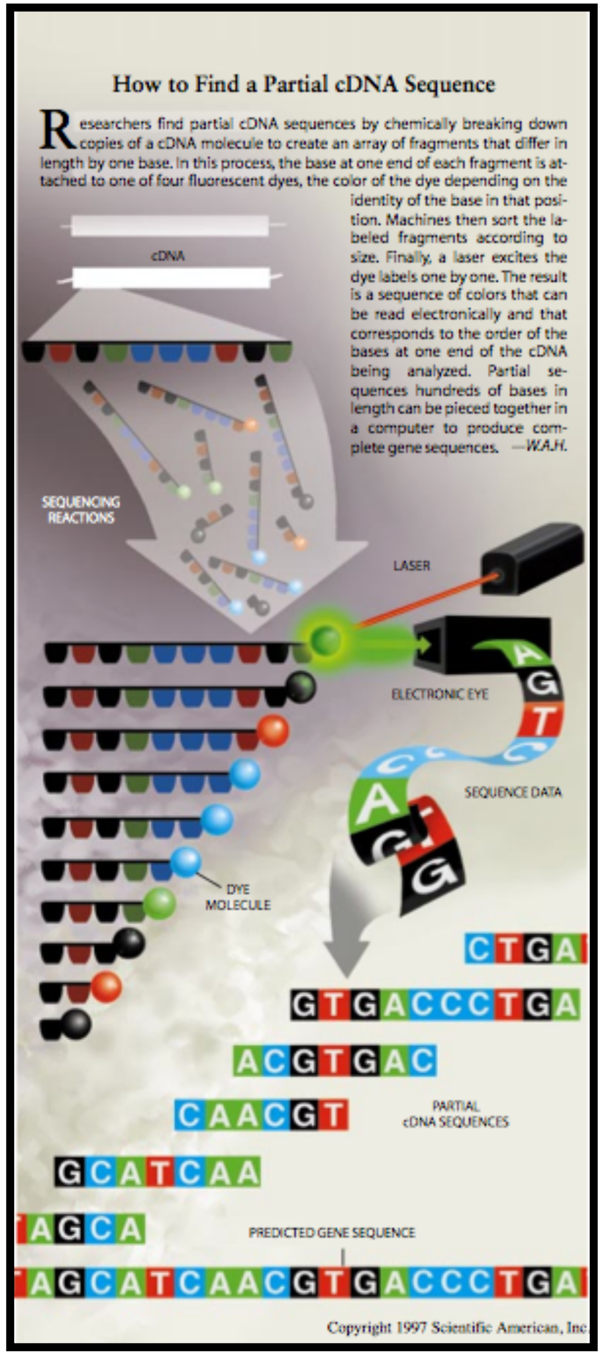
Discovering Genes for New Medicines

By identifying human genes involved in disease, researchers can create potentially therapeutic proteins and speed the development of powerful drugs

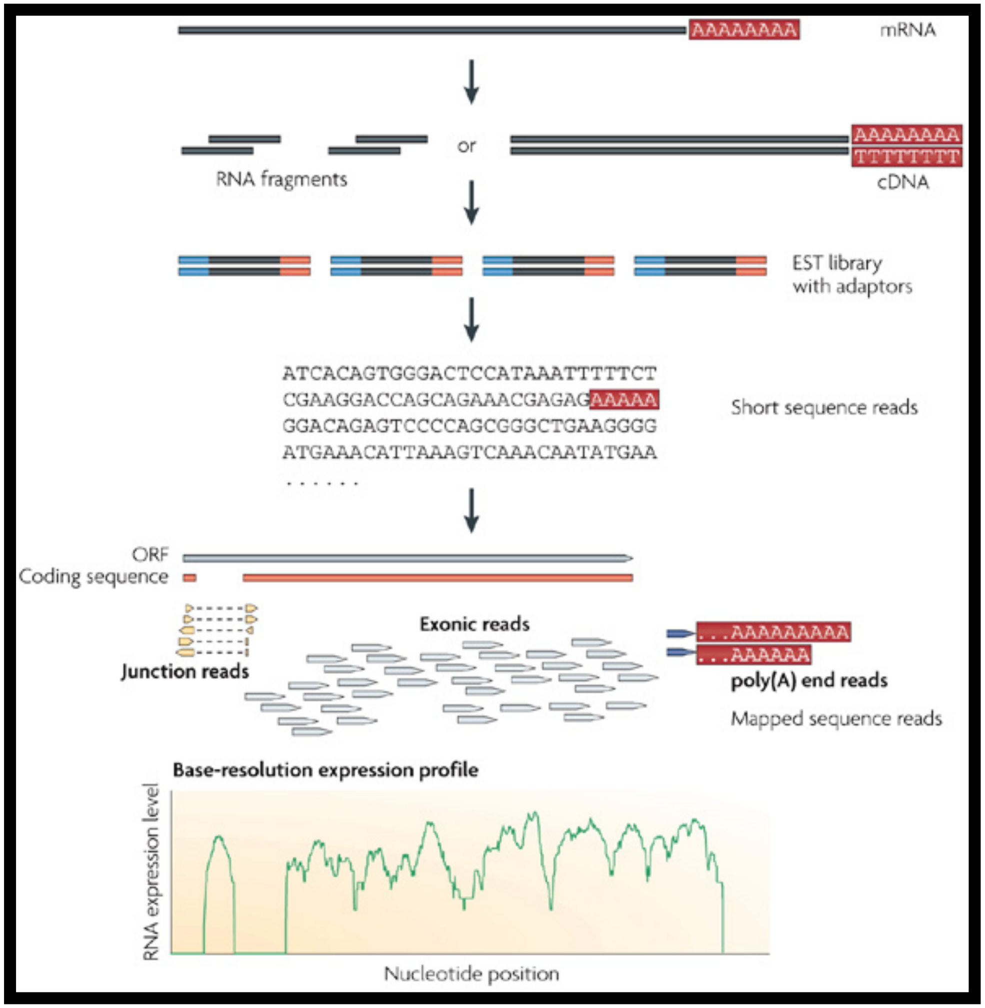
by William A. Haseltine

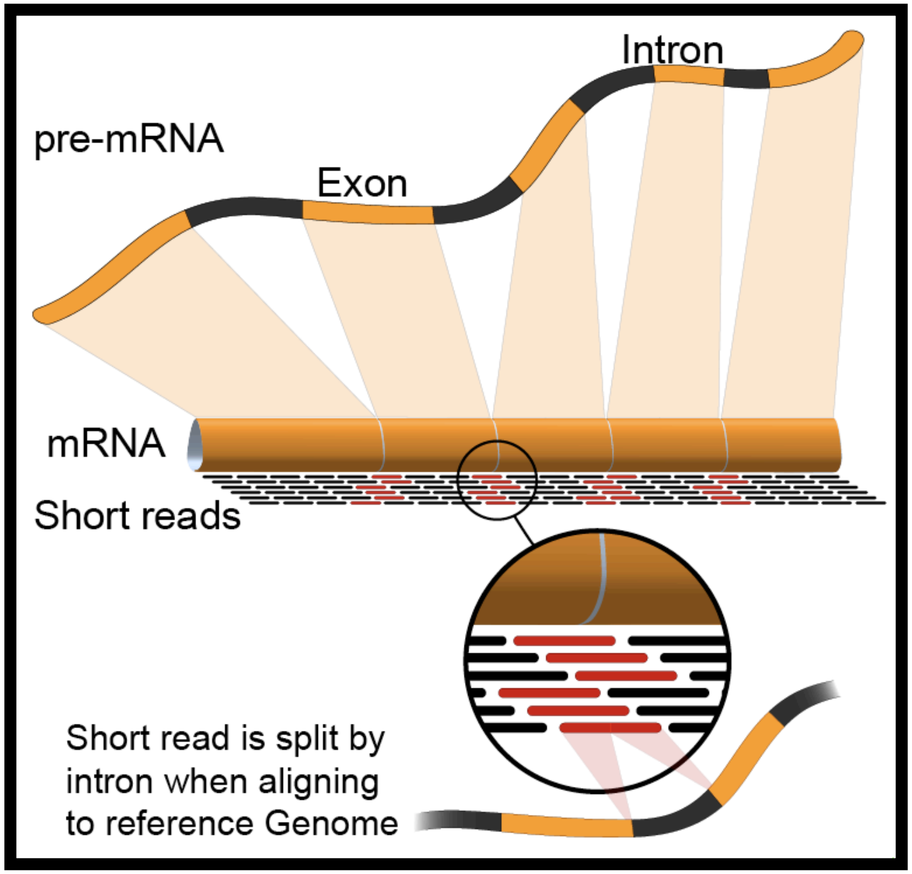
Scientific American, March 1997

Sanger Sequencing cDNAs to Discover New Genes & Drug Targets



Using RNASeq To Investigate Gene Activity



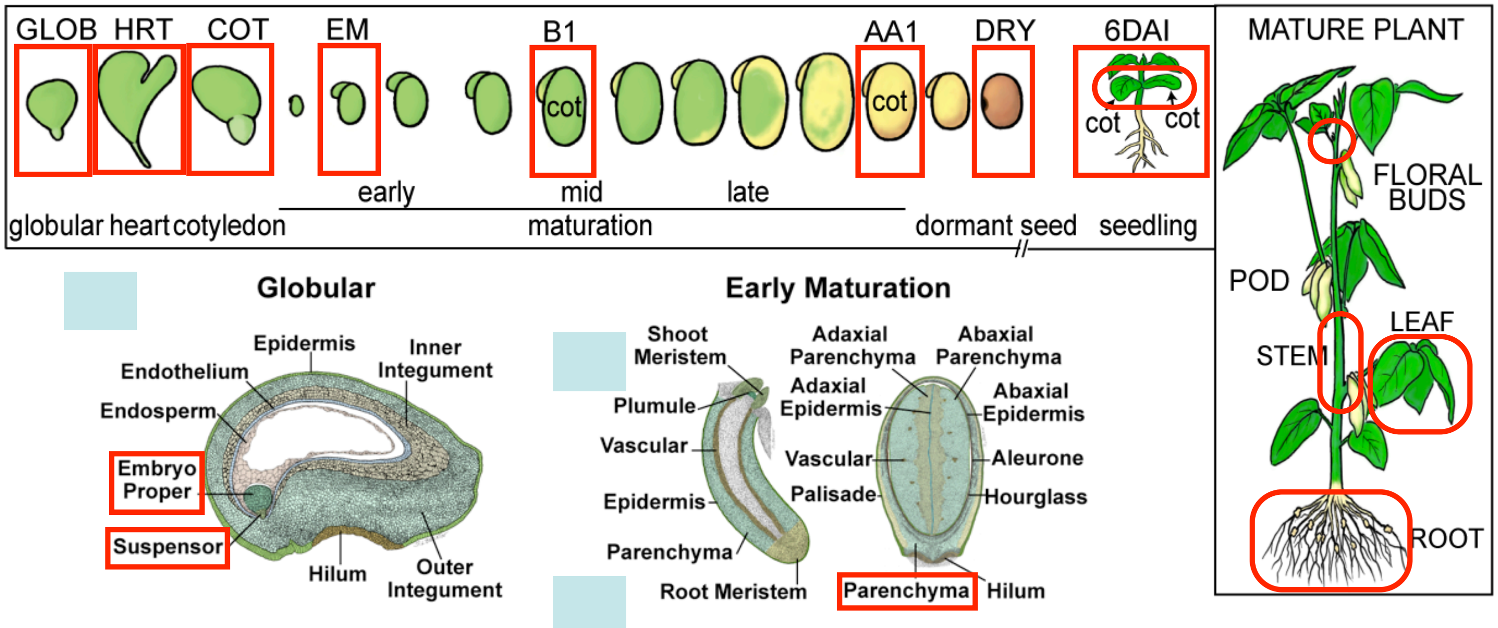


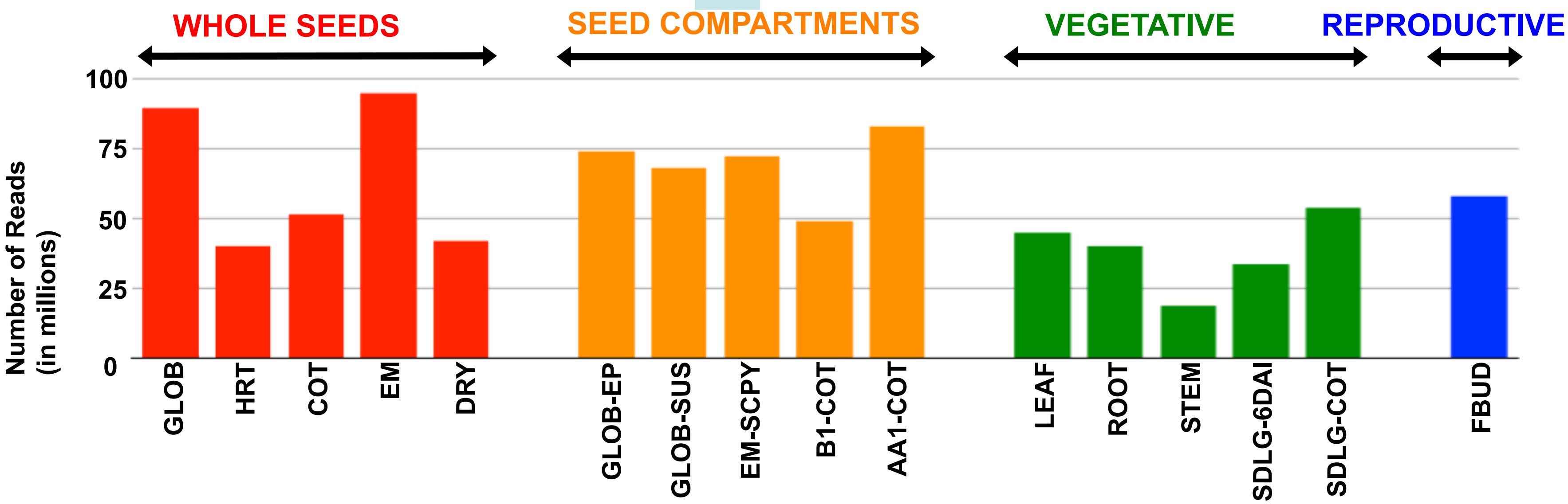
Combines EST/cDNA Sequencing & NextGen High Throughput Technology

Advantages of RNASeq To Investigate Gene Activity

- High Throughput Sequencing
- Relatively "Simple" Procedure
- Study the Activity of Entire Genome
- Very Sensitive (can identify rare mRNAs)
- Quantitative
- Useful For Genome Annotation
- Can Identify Differentially Processed mRNAs
- Can Identify SNPs in mRNAs (i.e., alleles)

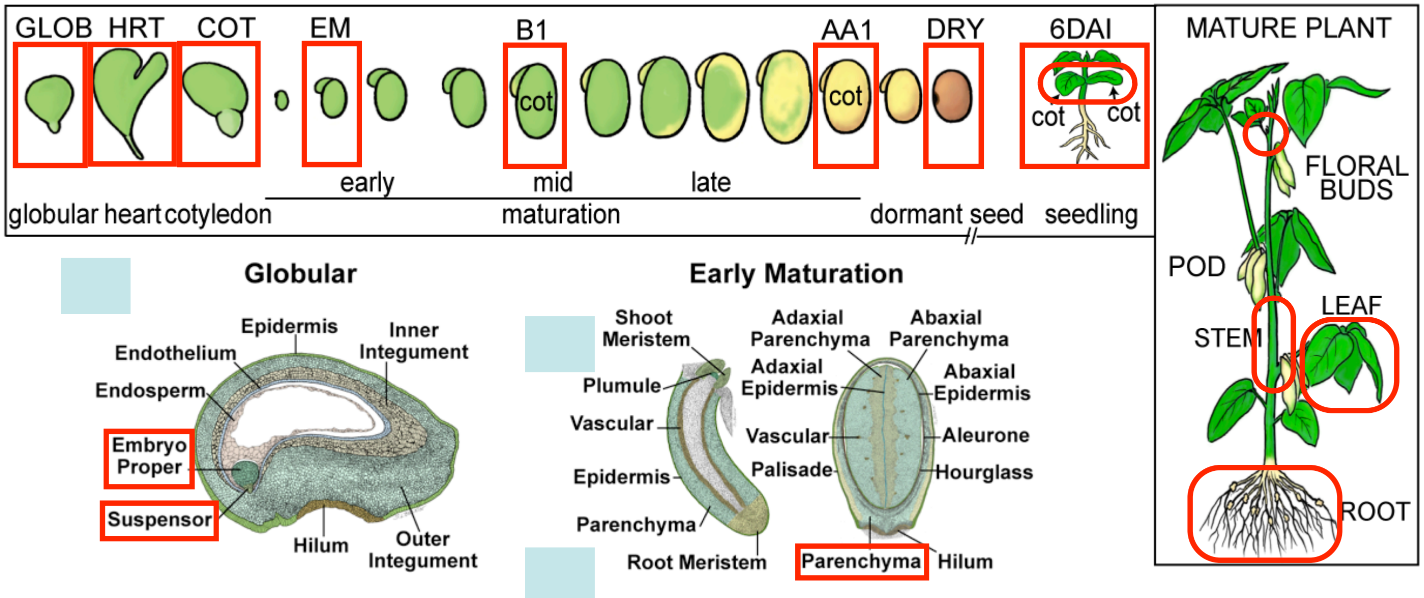
Using RNASeq to Study Gene Activity in Soybean Seed Development

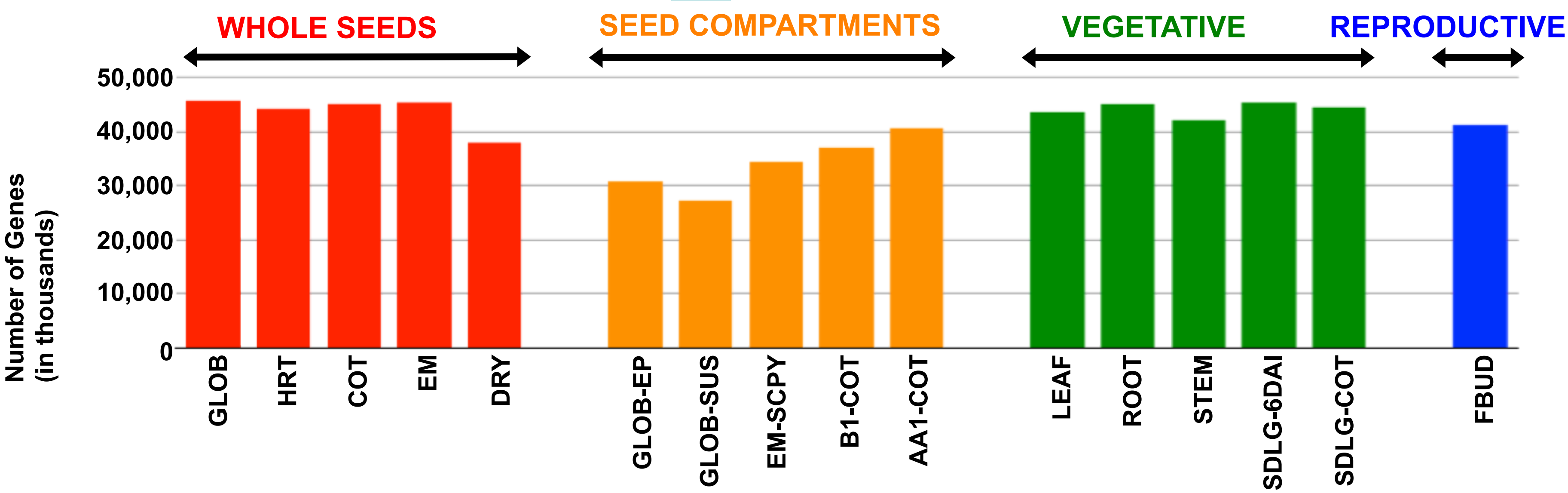




We've generated >943 million reads (~71 Gb)!!!!

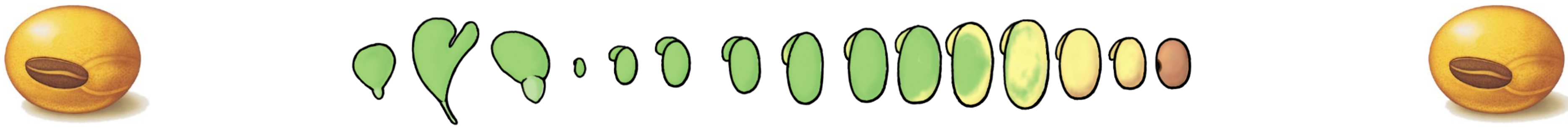
How Many Genes Are Active Throughout the Soybean Life Cycle?



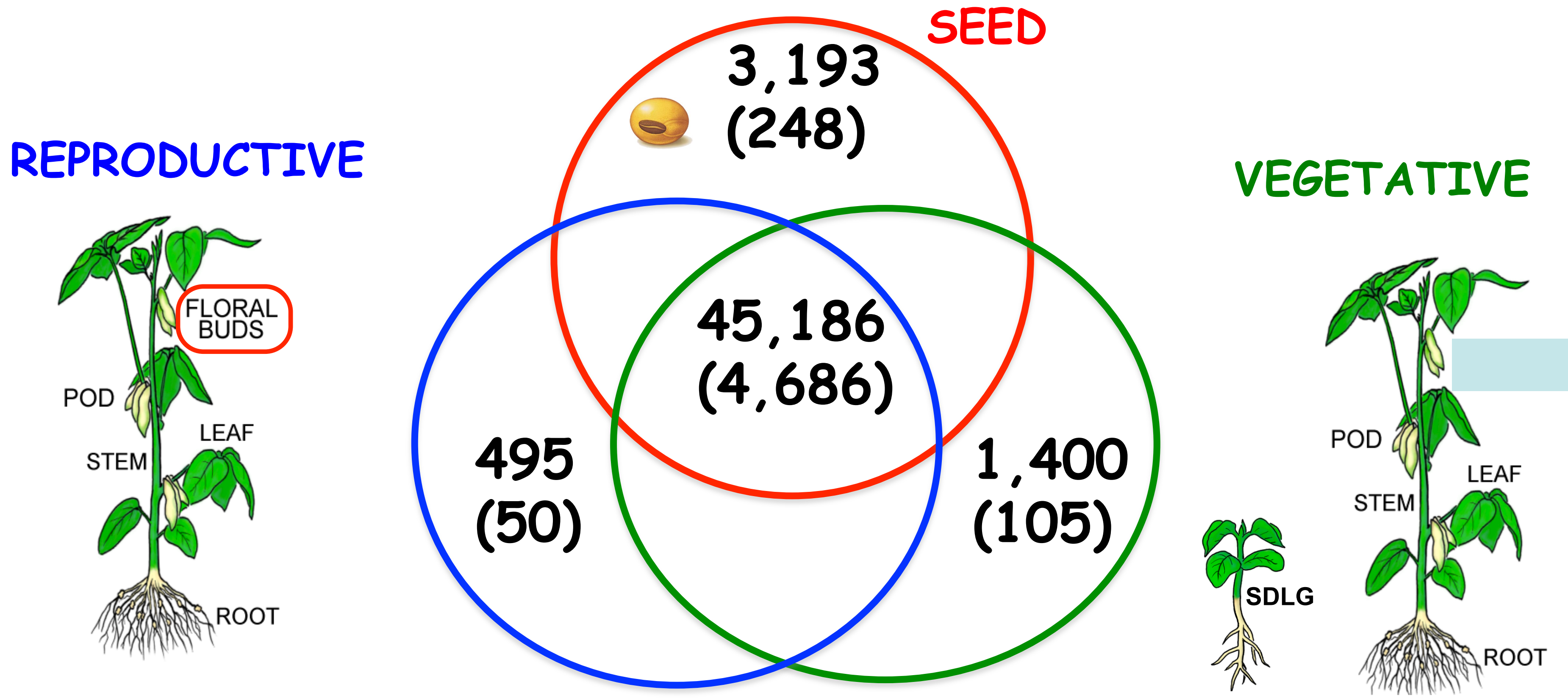


There are 55,343 Genes Active Throughout The Soybean Life Cycle!

Are There mRNAs Specific to Seed Development?



*Union of Whole Seed & Seed Compartment RNA-Seq Sets = 52,685 mRNAs (5,294 TFs)



*Union of all LCM & WM (glob-dry)