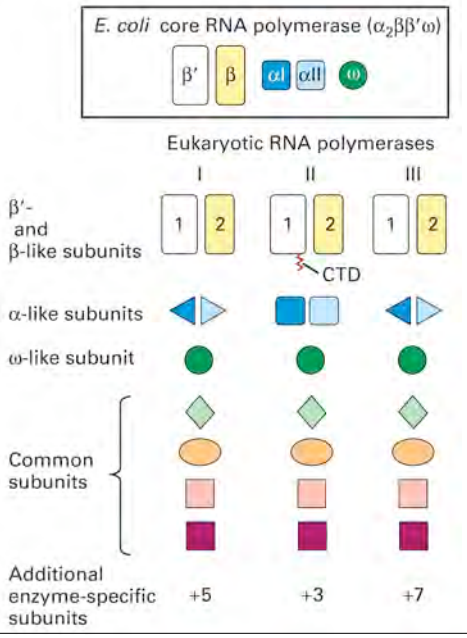
Three types of RNA polymerase in eukaryotic nuclei

Type	Location	RNA synthesized	Effect of α-amanitin
I	Nucleolus	Pre-rRNA for 18, 5.8 and 28S rRNAs	Insensitive
II	Nucleoplasm	Pre-mRNA, some snRNAs	Sensitive to 1 μg/ml
III	Nucleoplasm	Pre-tRNAs, 5S rRNA, some snRNAs	Sensitive to 10 μg/ml

- **α-amanitin from *Amanita Phalloides* binds tightly to RNA Pol II and blocks transcriptional elongation.**
- **RNA Pol I transcribe 1 gene at ~200 copies. The gene for the 45S pre-rRNA is present in tandem array.**
- **RNA Pol II transcribe ~25,000 genes;**
- **RNA Pol III transcribe 30-50 genes at variable copy numbers.**

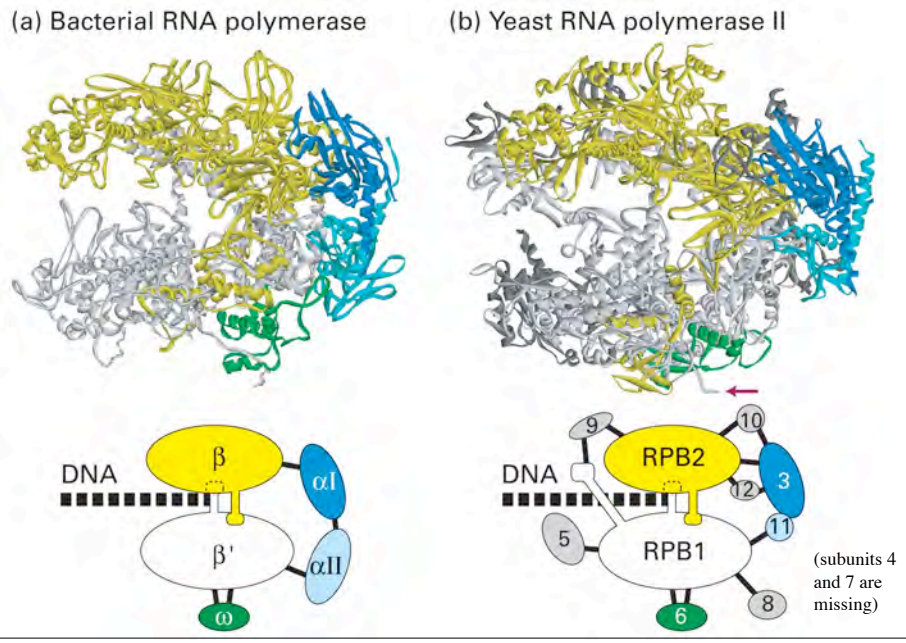
(Also- Organelle RNAPs in Mitochondria and Chloroplasts. Encoded by organelle genomes. Similar to bacterial RNAPs.)

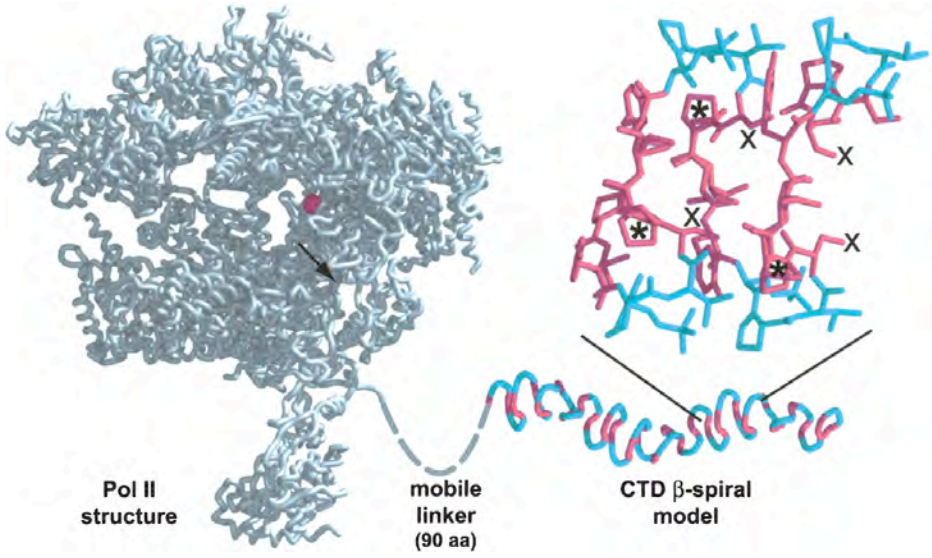
Subunit composition of eukaryotic RNA polymerases



- All three yeast polymerases have five core subunits that exhibit some homology with the β, β′, α and ω subunits in *E. coli* RNA polymerase.
- RNA polymerases I and III contain the same two non-identical α-like subunits, whereas polymerase II has two copies of a different α-like subunit.
- All three polymerases share four other common subunits. In addition, each RNA polymerase contains three to seven unique smaller subunits.
- The largest subunit (1) of RNA polymerase II also contains an essential C-terminal domain (CTD). 27 (yeast) to 52 (human) copies of (YSPTSPS).
- Phosphorylation of CTD is important for transcription and RNA processing.

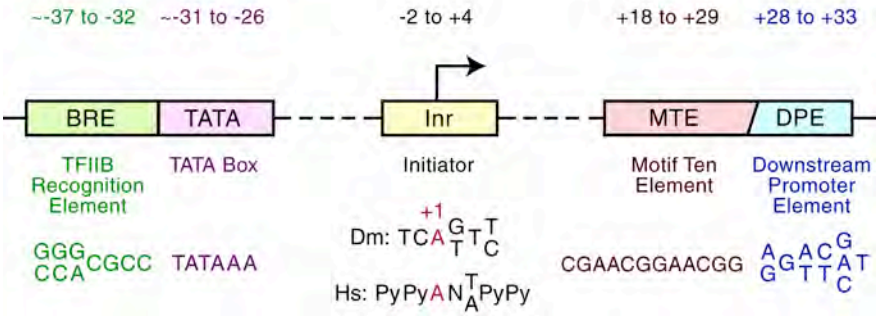
Comparison of 3-D structures of bacterial and eukaryotic RNA polymerases





Cyan: alternating β-turns; Pink: extended regions.

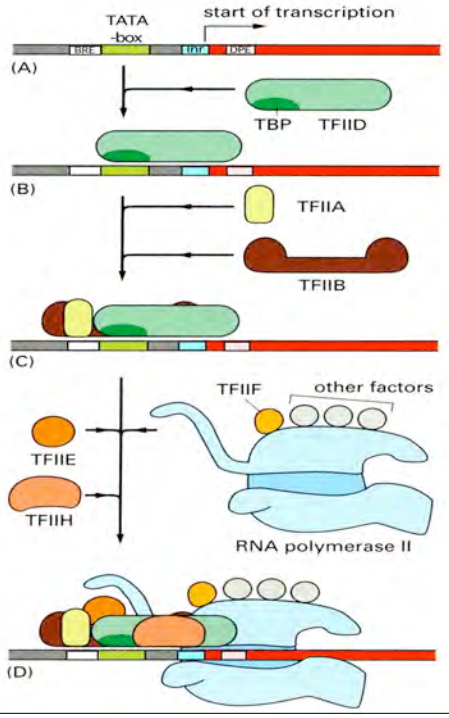
Core Promoter Elements



Many genes, which are transcribed at low rates (e.g. genes encoding the enzymes required for basic metabolic processes required in all cells, often called "housekeeping genes") do not contain a TATA box or an initiator. Most genes of this type contain a CG-rich region, or *CpG* island, of 20-50 nucleotides within ~100 base pairs upstream from the start site. Transcription of these genes can begin at any one of multiple possible sites over an extended region.

RNA Pol II is recruited to core promoter with the help from multiple general transcription factors.

In vitro stepwise assembly of the RNA Pol II pre-initiation complex (PIC) at core promoter for basal transcription



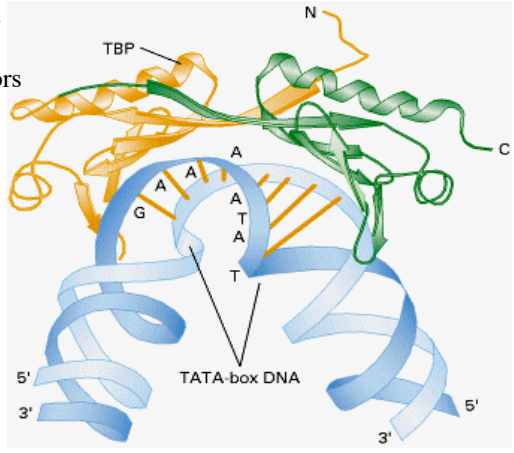
Basal ('General') Transcription Factors for RNA Polymerase II

- TFIID – consists of TBP (TATA-box binding protein) + TAFs (TBP-associated factors). Binds to core promoter motifs. TAFs interact with activator proteins. The first step in basal transcription is probably binding of TFIID to the core promoter.
- TFIIA – three (or two) small subunits. Increases affinity of TBP for DNA in vitro. Not needed for transcription in vitro. Could be an anti-inhibitor.
- TFIIB – one subunit of 35 kDa. Binds to TBP and the BRE.
- RNA Polymerase II – consists of two large subunits (IIa and IIb) as well as about eight smaller subunits. Unique feature of largest (IIa) subunit is the C-terminal domain (CTD), which is an imperfectly-repeated heptapeptide motif, YSPTSPS.
- TFIIF – also known as RAP30/74. Binds to RNA polymerase II. Two subunits of 30 and 74 kDa. Functions in transcription initiation and elongation.
- TFIIE – two polypeptides of 34 and 56 kDa. Required for assembly of TFIIH into the transcription preinitiation complex (PIC).
- TFIIH – nine polypeptides. Core TFIIH has six subunits, which include 5'–>3' and 3'–>5' DNA helicases, and is also involved in nucleotide excision repair. Also has a three subunit Cdk7/MO15 + Cyclin H + MAT1 kinase complex that phosphorylates Ser5 of the CTD during transcription initiation.

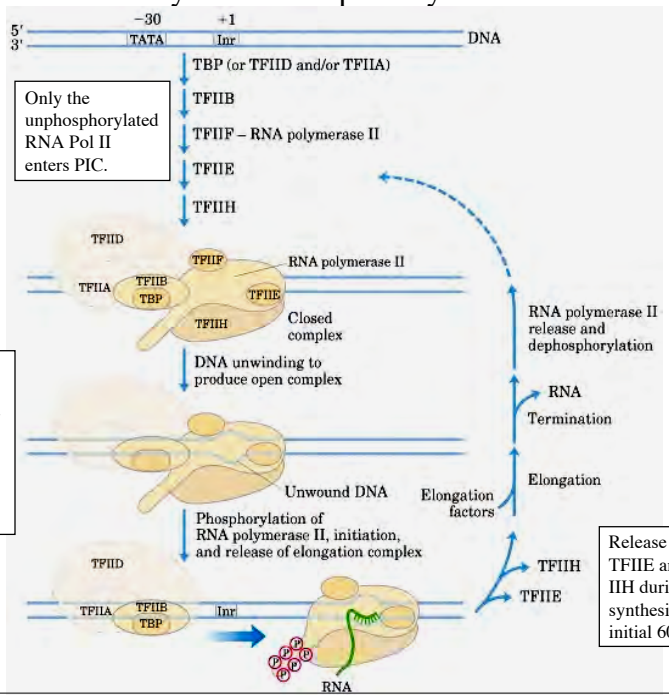
Total: 43-44 polypeptides and over 2 million daltons.

TBP (TATA-box binding protein)

- Conserved C-terminal domain of 180 amino acids.
- A monomer with a saddle-shaped structure; the two halves show an overall dyad symmetry but are not identical.
- Binds multiple transcription factors (TAFs, TFIIB and TFIIA).
- Binds in the minor groove and significantly bends DNA.



Eukaryotic transcription cycle

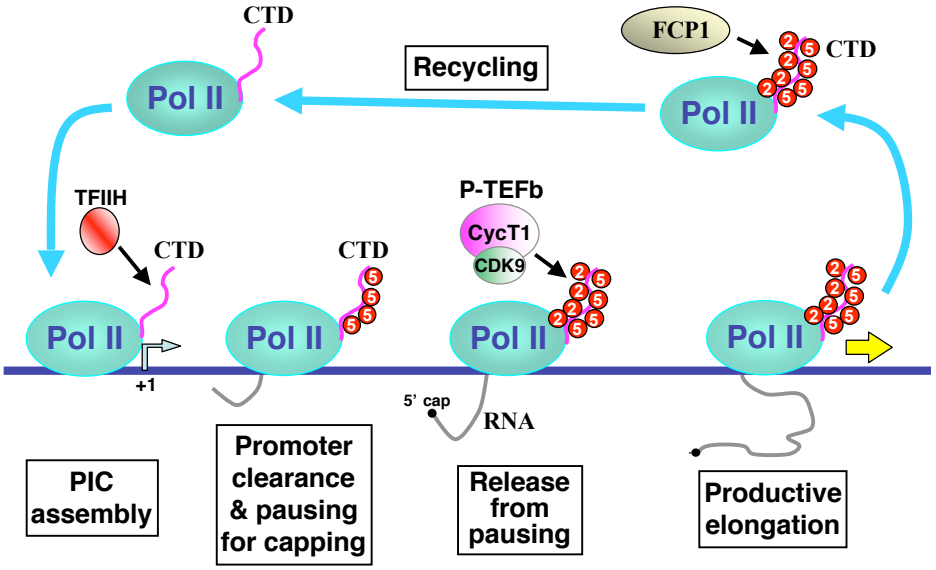


Only the unphosphorylated RNA Pol II enters PIC.

The TFIIH complex has both helicase and kinase activities that can unwind DNA and phosphorylate the CTD tail of RNA Pol II.

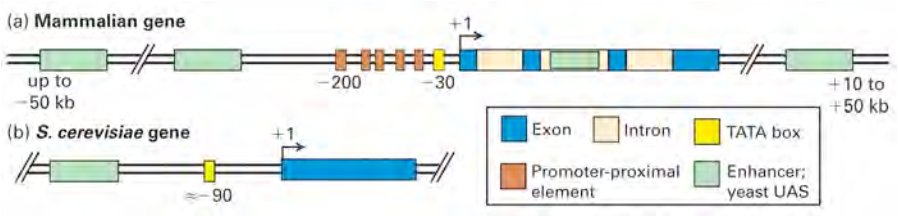
Release of TFIIE and then IIH during the synthesis of the initial 60-70nt.

Phosphorylation states of Pol II CTD during transcription cycle



CTD heptapeptide repeats: 27-52 x (YS^2PTS^5PS)

Cis-acting control elements



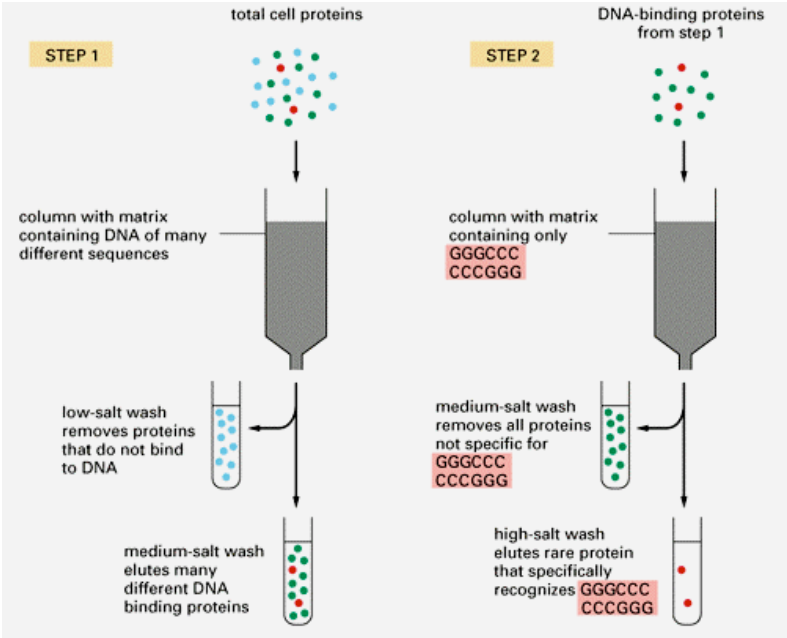
(a) Genes of multicellular organisms contain both promoter-proximal elements and enhancers (collectively referred to as cic-acting control elements) in addition to core promoter element(s).

(b) Enhancers function in a distance, position and orientation-independent manner. Long distance interactions are achieved by forming looped DNA.

(c) Most yeast genes contain only one regulatory region, called an upstream activating sequence (UAS), and a TATA box, which is ≈90 base pairs upstream from the start site. (Also note: many yeast genes do not contain introns).

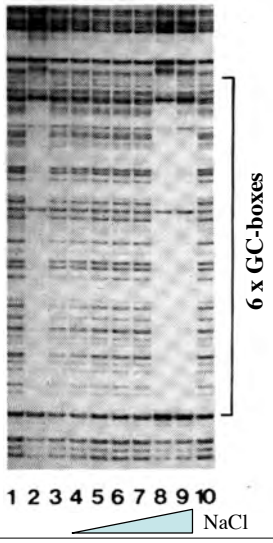
(d) In multicellular organisms, one standard promoter-proximal element often located in the CpG island is a GC-box (GGGCGGGC) recognized by the ubiquitous transcriptional activator Sp1.

DNA affinity chromatography for purification of Sp1

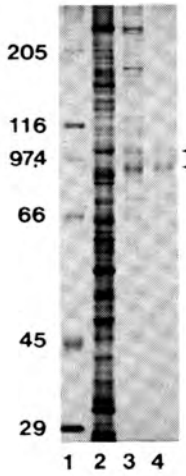


Analyses of affinity-purified Sp1 protein

DNase I footprint on SV40 promoter

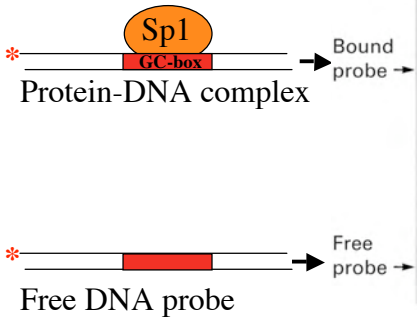


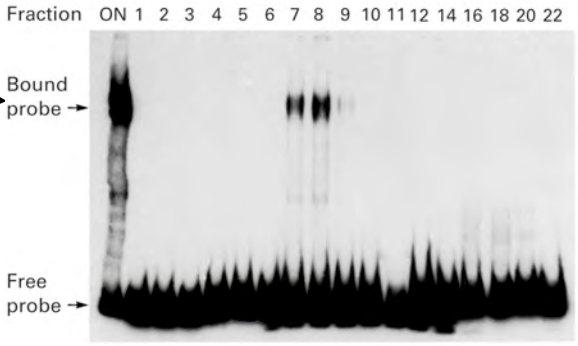
SDS-PAGE/ silverstain



Lane 2 contains total cell proteins prior to affinity purification; Lanes 3&4 contain purified Sp1 protein washed off the affinity column.

"Gel shift": electrophoretic mobility shift assay (EMSA) for studying the interaction of Sp1 with GC-box





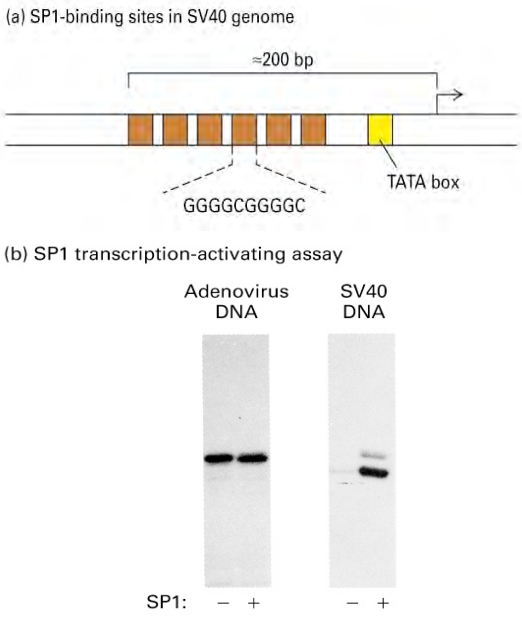
1. Prepare labeled DNA probe
2. Bind protein
3. Native gel electrophoresis

Advantage: sensitive

ON: protein mixture loaded onto an ion-exchange column.
Fraction 1-22: fractions eluted from the column with increasing salt concentrations.

Disadvantage: requires stable complex; little information about where protein is binding on DNA

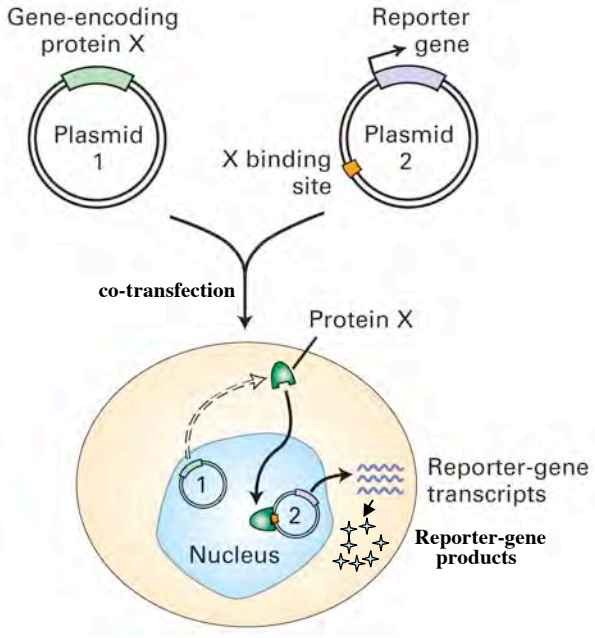
In vitro transcription assay to measure Sp1 activity



•The adenovirus DNA template used here does not contain any Sp1-binding sites (GC-box) and is therefore used as a negative control.

•In vitro transcription reactions contain template DNA, labeled ribonucleoside triphosphates, and purified general transcription factors and RNA Pol II. Purified Sp1 is added to the reactions indicated with "+".

In vivo assay for transcription factor activity



• Host cells should lack the gene encoding protein X and the reporter protein.

• The production of reporter-gene RNA transcripts or the activity of the encoded protein can be assayed.

• If reporter-gene transcription is greater in the presence of the X-encoding plasmid, then X is an activator; if transcription is less, then X is a repressor.

Identification of promoter-proximal cis-acting control elements upstream of a eukaryotic gene (part 1)

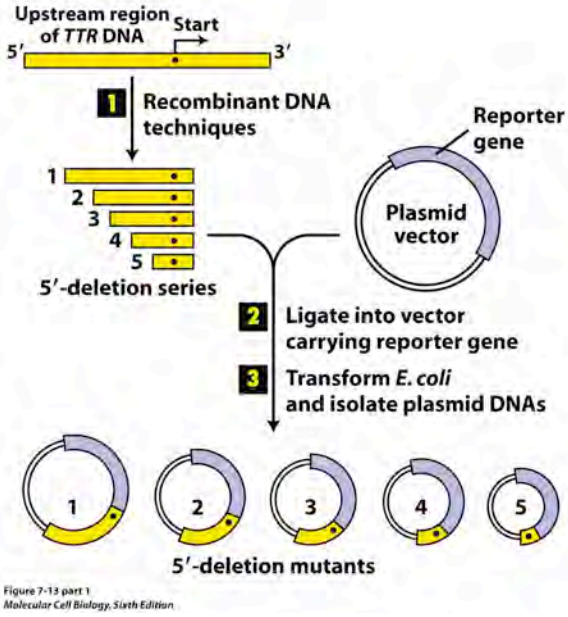
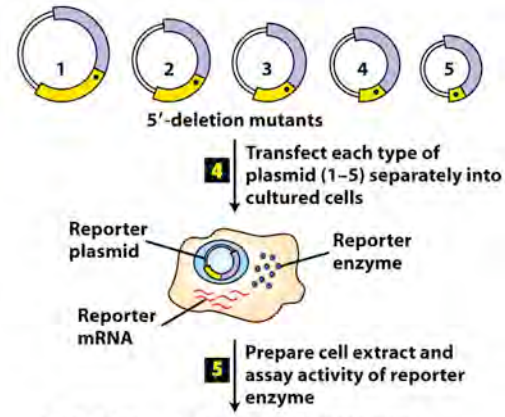


Figure 7-13 part 1
Molecular Cell Biology, Sixth Edition

Identification of promoter-proximal cis-acting control elements upstream of a eukaryotic gene (part 2)



Plasmid no.	Reporter-gene expression
1	+ + +
2	+ + +
3	+
4	+
5	–

Figure 7-13 part 2
Molecular Cell Biology, Sixth Edition

Computer-assisted search for enhancers

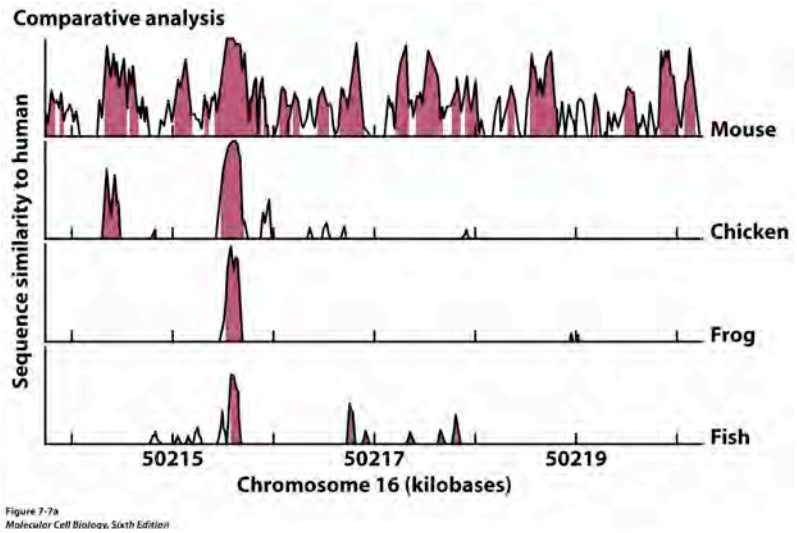


Figure 7-7a
Molecular Cell Biology, Sixth Edition

Graphic representation of the conservation of DNA sequences within a corresponding region in five different genomes reveals a region of ~500 bp of non-coding sequence that is conserved from fish to human.

The Production of DNA Microarrays and Their Use in Monitoring Global Gene Transcription