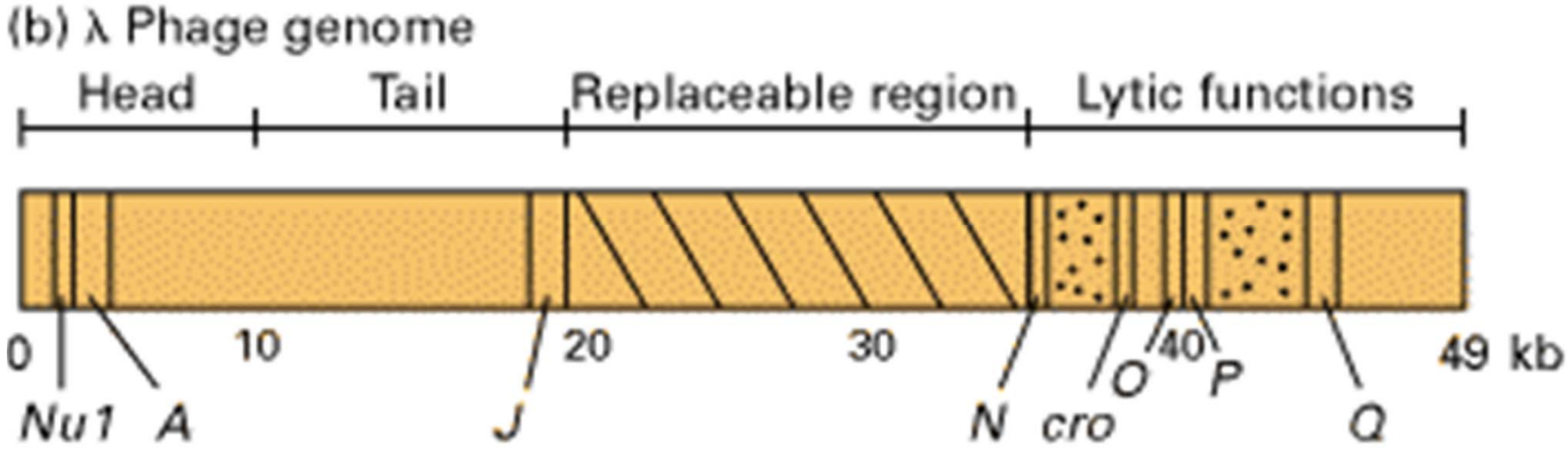
How Do You Identify and Clone a Gene of Interest?

Limitations of Plasmid Vectors

- Relatively low efficiency of *E. coli* transformation
- The small number (only a few hundred) of individual transformed colonies that can be grown on a typical culture plate

Bacteriophage λ as Cloning Vector

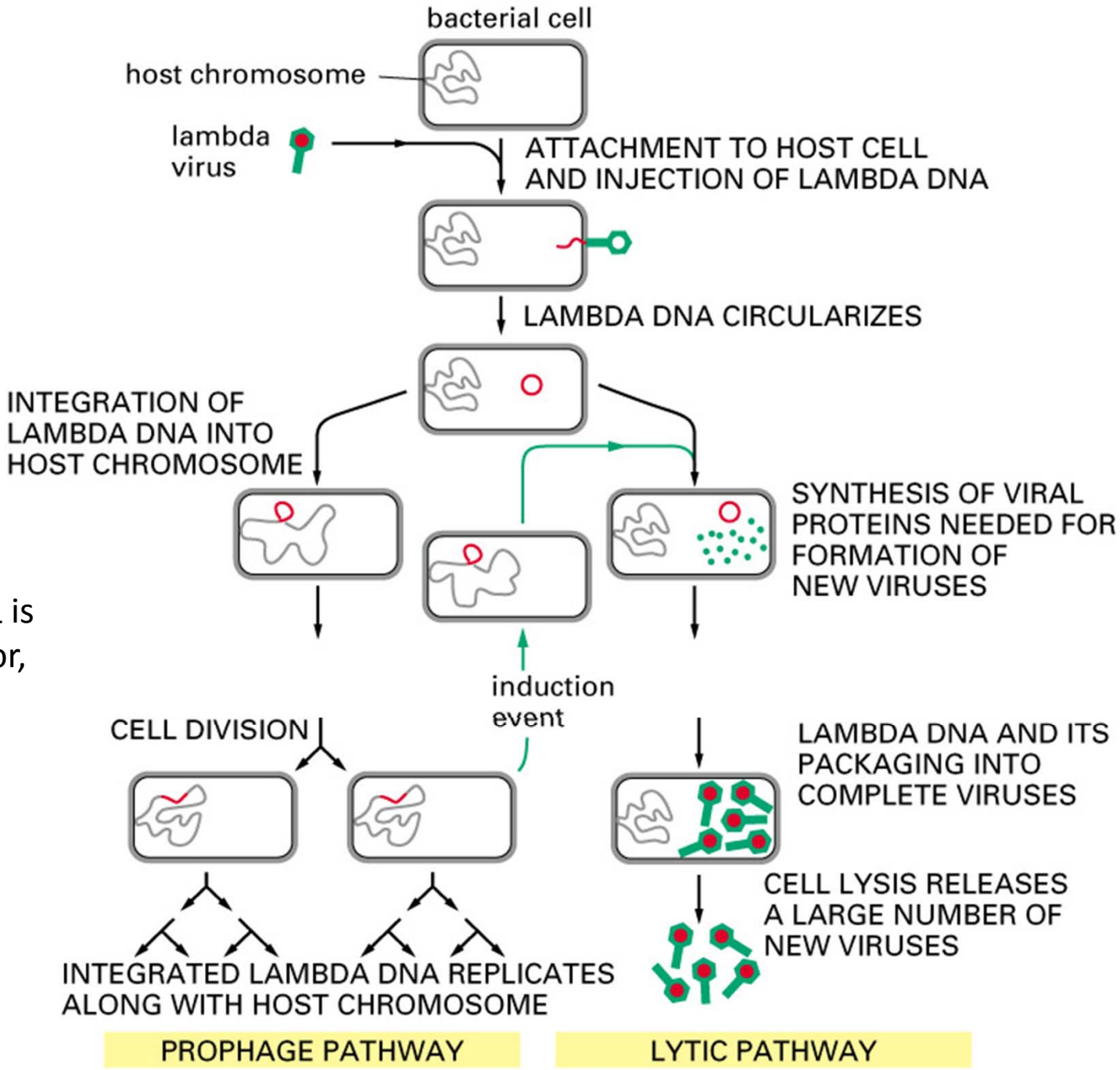
Map of the λ phage genome



Insertion of up to ≈25 kb of exogenous DNA is possible in the region between J and N

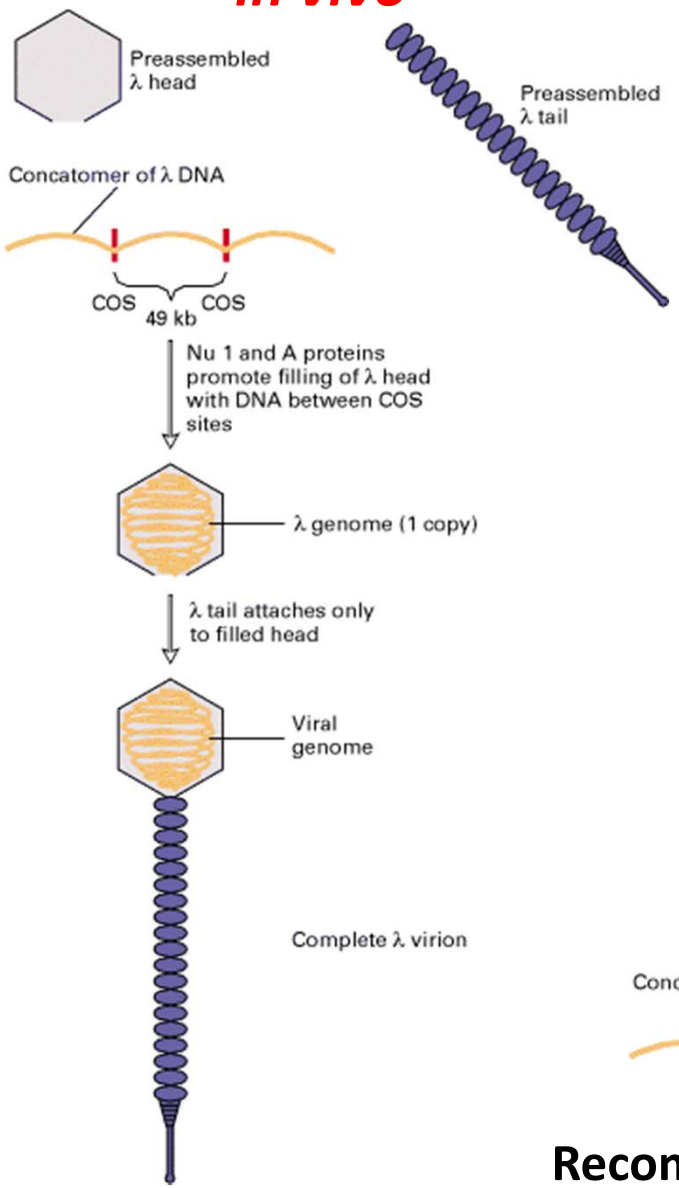
Lysogenic and Lytic growth pathways

When bacteriophage λ is used as a cloning vector, it must be capable of lytic growth



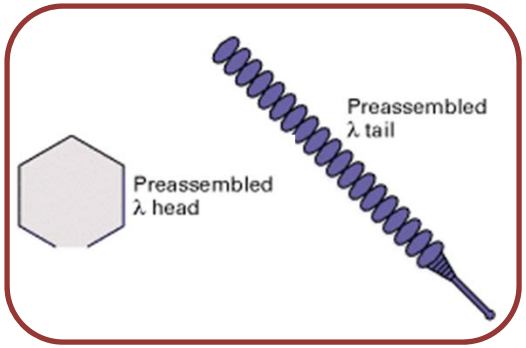
Assembly of Bacteriophage λ Virions

in vivo



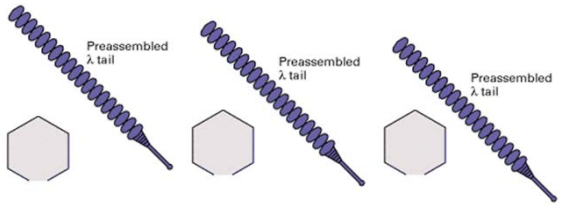
in vitro

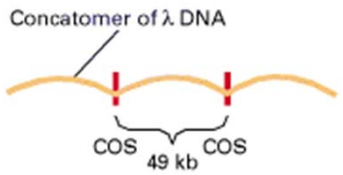
***E. coli* cells are infected with a λ mutant defective in A protein**



***E. coli* cells with preassembled λ heads and tails**

Cell lysis



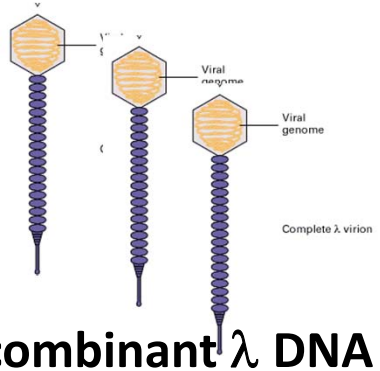


Recombinant λ DNA

+

&

Isolated A protein



Recombinant λ DNA carrying virions

Creating DNA Libraries

- Collections of cloned DNA fragments from a particular organism contained within bacteria or viruses as the host
- Screened to pick out different genes of interest
- Libraries can be used when the partial sequence of a gene (e.g., from the sequence of a homologous gene) is known and one wants to determine its entire sequence.

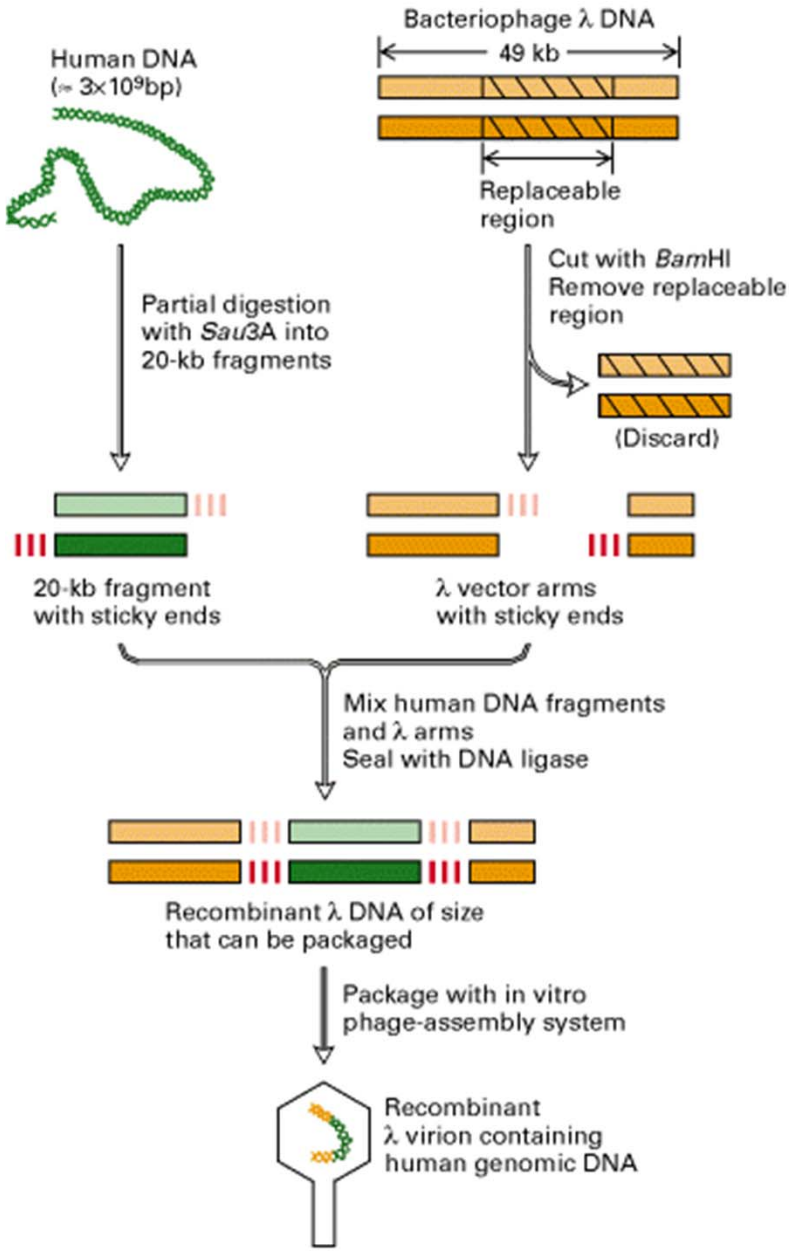
- Two Types of Libraries
  - Genomic DNA libraries
  - Complementary DNA libraries (cDNA libraries)

Genomic Libraries

- contains DNA fragments representing the entire genome of an organism
- Chromosomal DNA from the tissue of interest is isolated and digested with restriction enzyme
- Vector is digested with same enzyme and DNA ligase is used to ligate genomic DNA fragments and vector DNA
- Recombinant vectors are used to transform bacteria

Construction of a genomic library of human DNA in a plasmid vector

Construction of a genomic library of human DNA in a bacteriophage λ vector



Genomic Libraries

– Disadvantages
  - Non-protein coding pieces of DNA (introns) are cloned in addition to exons; majority of genomic DNA is introns in eukaryotes so majority of the library will contain non-coding pieces of DNA

  - Many organisms have very large genome, so searching for gene of interest is difficult at best

cDNA Libraries

- contains only complementary DNA molecules synthesized from mRNA molecules in a cell.

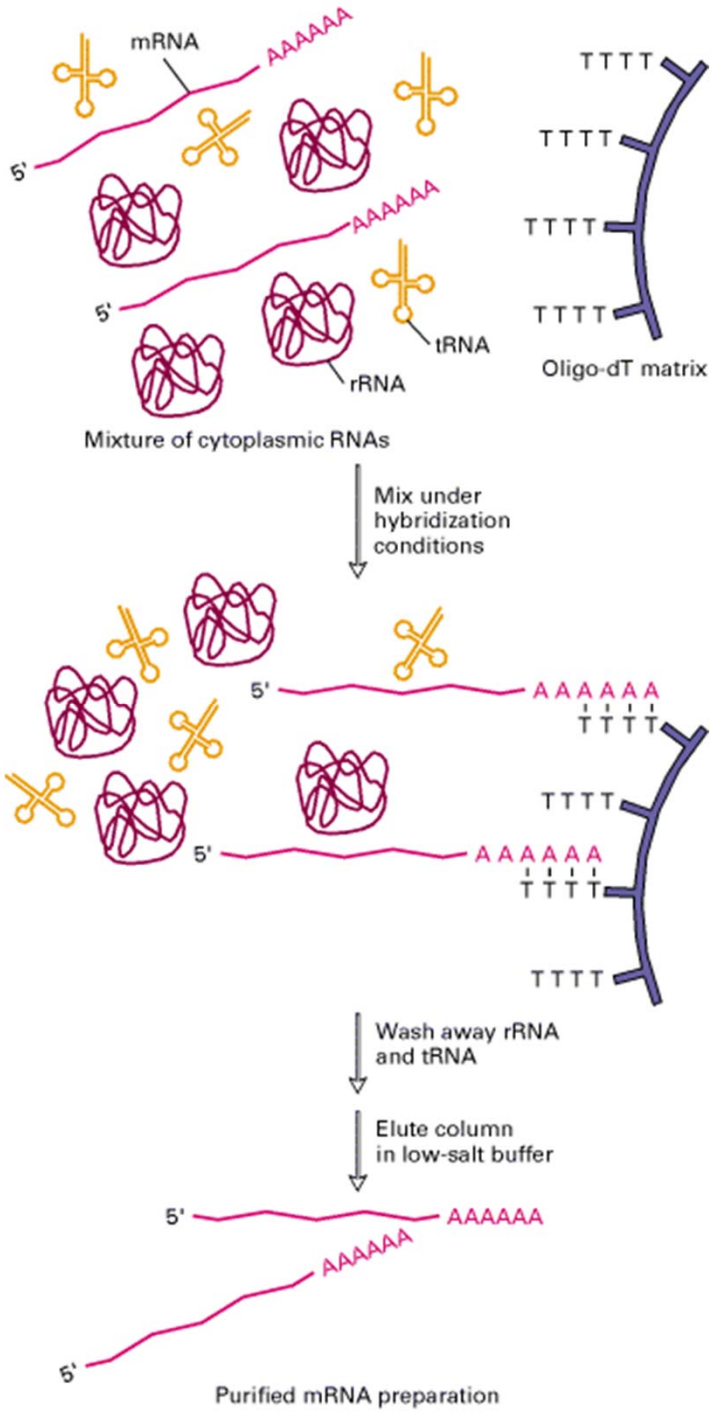
- mRNA from tissue of interest is isolated

- Converted to a double-stranded DNA by using the enzyme **reverse transcriptase**
  - Called complementary DNA (cDNA) because it is an exact copy of the mRNA
  - DNA copies of mRNAs are called complementary DNAs(cDNAs); clones of such DNA copies of mRNAs are called *cDNA clones.*

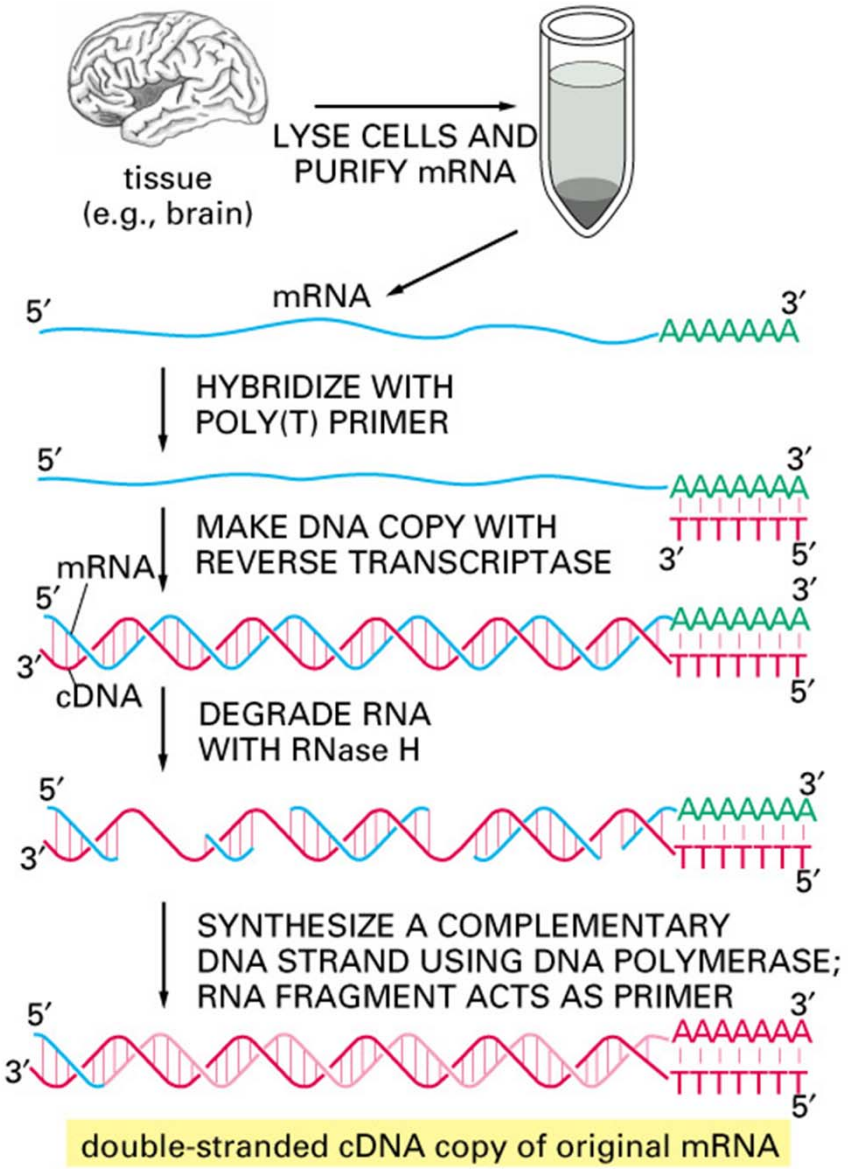
cDNA Libraries

- mRNA is degraded
- DNA polymerase used to create the second strand of DNA
- Short linker sequences are added to the end of the cDNA
  - Contain restriction enzyme recognition sites
- Cut with restriction enzyme, cut vector with same enzyme, ligate fragments to create recombinant vectors
- Vectors used to transform bacteria

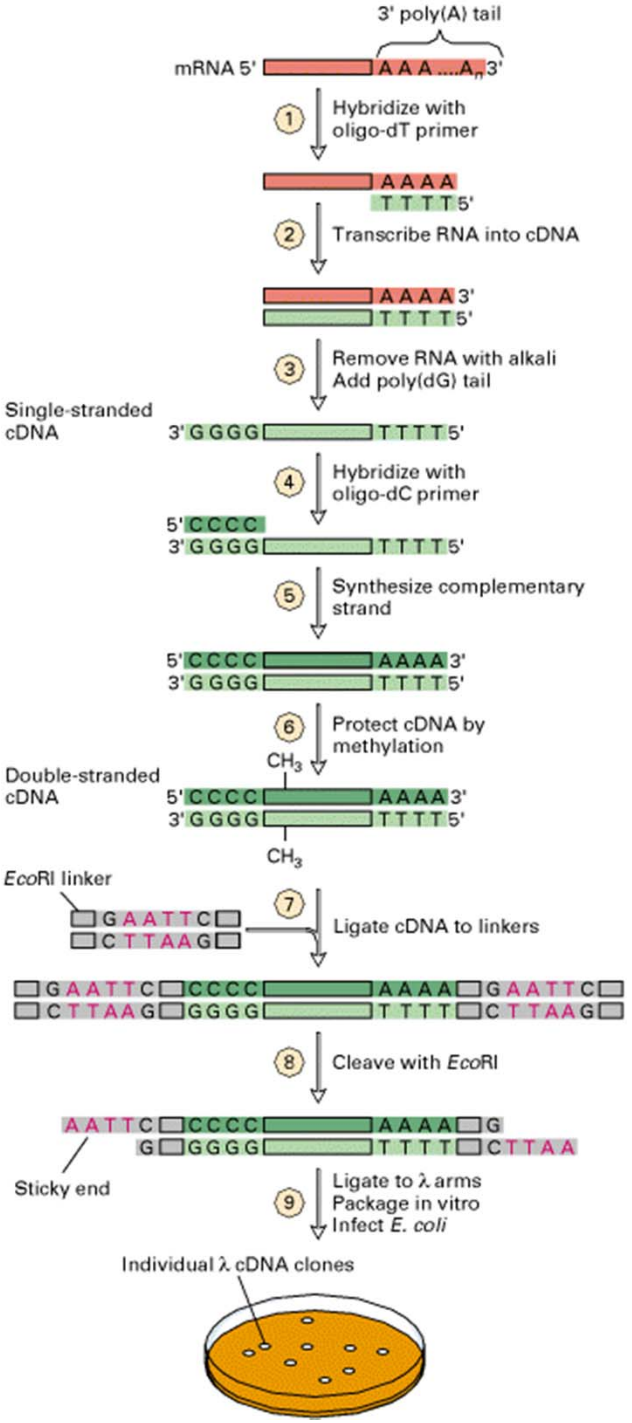
Isolation of eukaryotic mRNA by oligo-dT column affinity chromatography



cDNA Library



Preparation of a bacteriophage λ cDNA library



cDNA Libraries

- Advantages
  - Collection of actively expressed genes in the cells or tissues from which the mRNA was isolated
  - Introns are NOT cloned
  - Can be created and screened to isolate genes that are primarily expressed only under certain conditions in a tissue
- Disadvantages
  - Can be difficult to make the cDNA library if a source tissue with an abundant amount of mRNA for the gene is not available