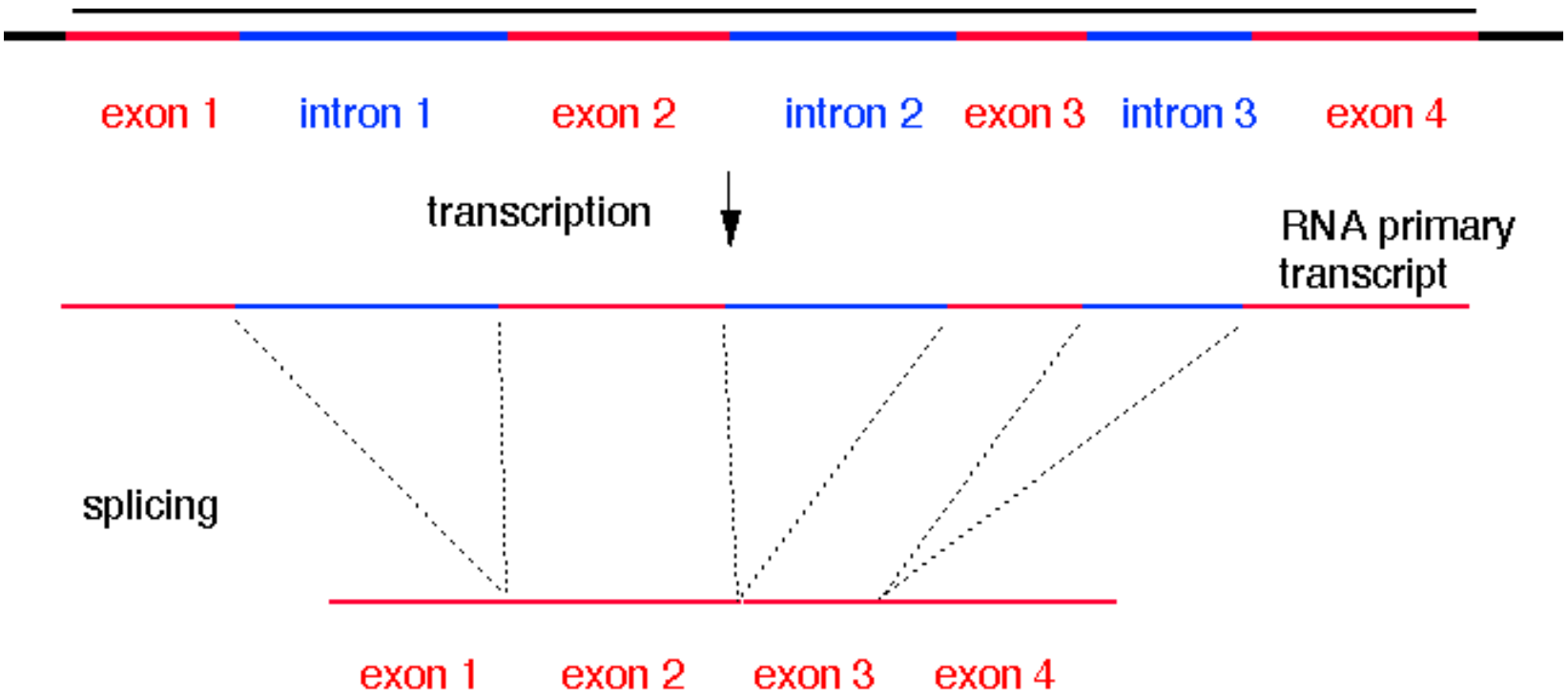
Recombinant DNA Technology

cDNA libraries

- **Library**
  - Contains >=1 copy of all sequences
    - Chromosome library
    - Genomic library
    - cDNA library

- **Step 1: Obtain DNA to be cloned**
  - **Genomic DNA cut into small pieces**
  - **cDNA prepared from mRNA with reverse transcriptase**
- Step 2: Insert DNA fragment into vector
- Step 3: Insert vector into host
- Step 4: Allow host to replicate to high population #
- Step 5: Extract DNA

Cloning euykaryotic genes in prkaryotes require special "tricks" because eukaryotic genes have introns which are removed in the nucleus of eukaryotic cells prior to translation



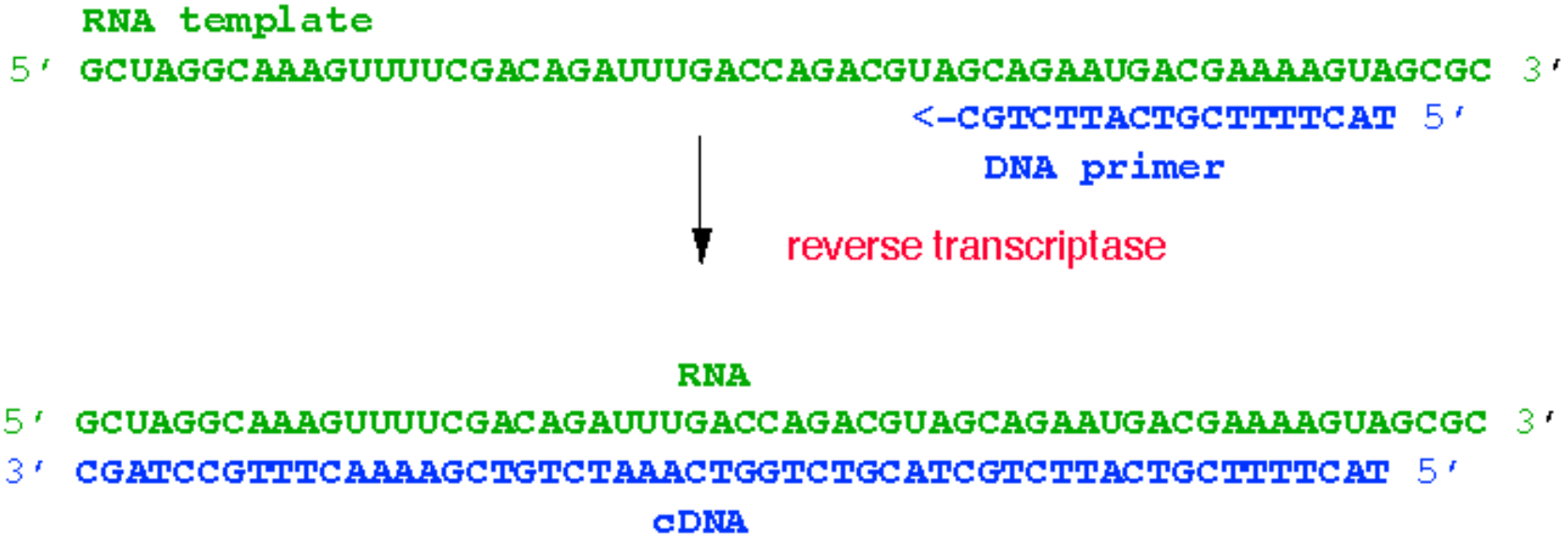
Introns can account for more than 90% of the length of a eukaryotic gene. It is hard to clone very long DNA segments. In addition, intron-containing eukaryotic genes cannot be expressed in a bacterial host because prokaryotes lack splicing apparatus.

To overcome these problems, instead of directly cloning a gene, one can clone cDNA, a DNA copy of gene mRNA.

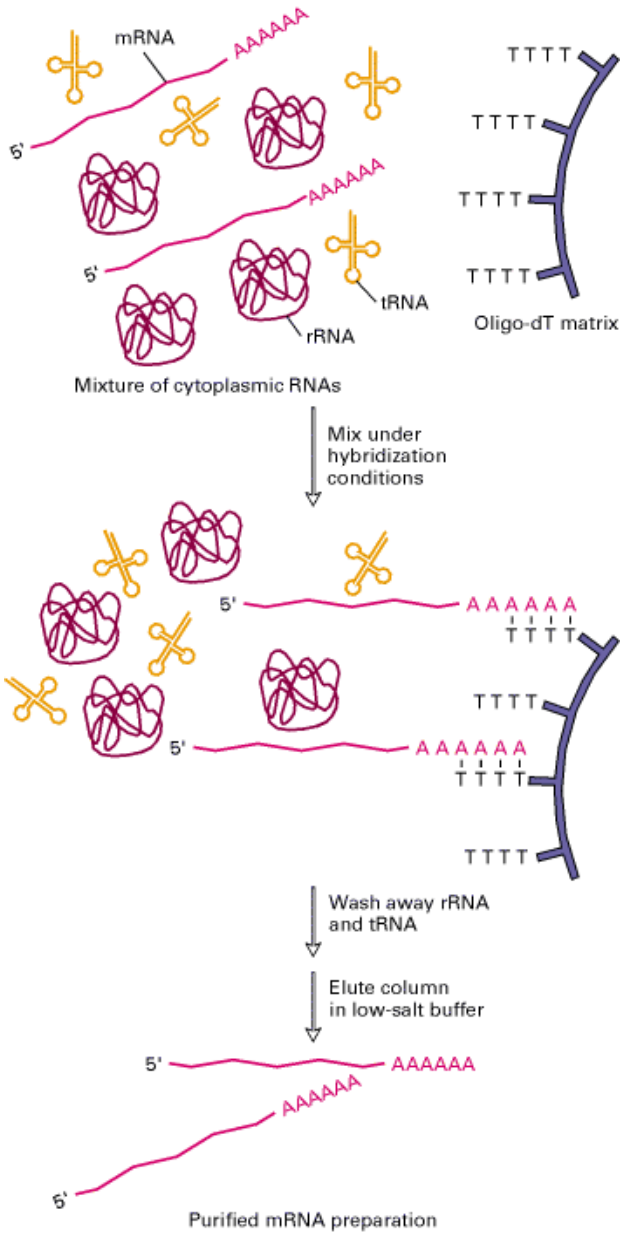
An enzyme, reverse transcriptase, is used to produce cDNA

Reverse transcriptase is an RNA-dependent DNA polymerase: it synthesizes a complementary DNA strand on the RNA template.

Similar to other DNA-polymerases, reverse transcriptase needs a PRIMER to initiate DNA synthesis.



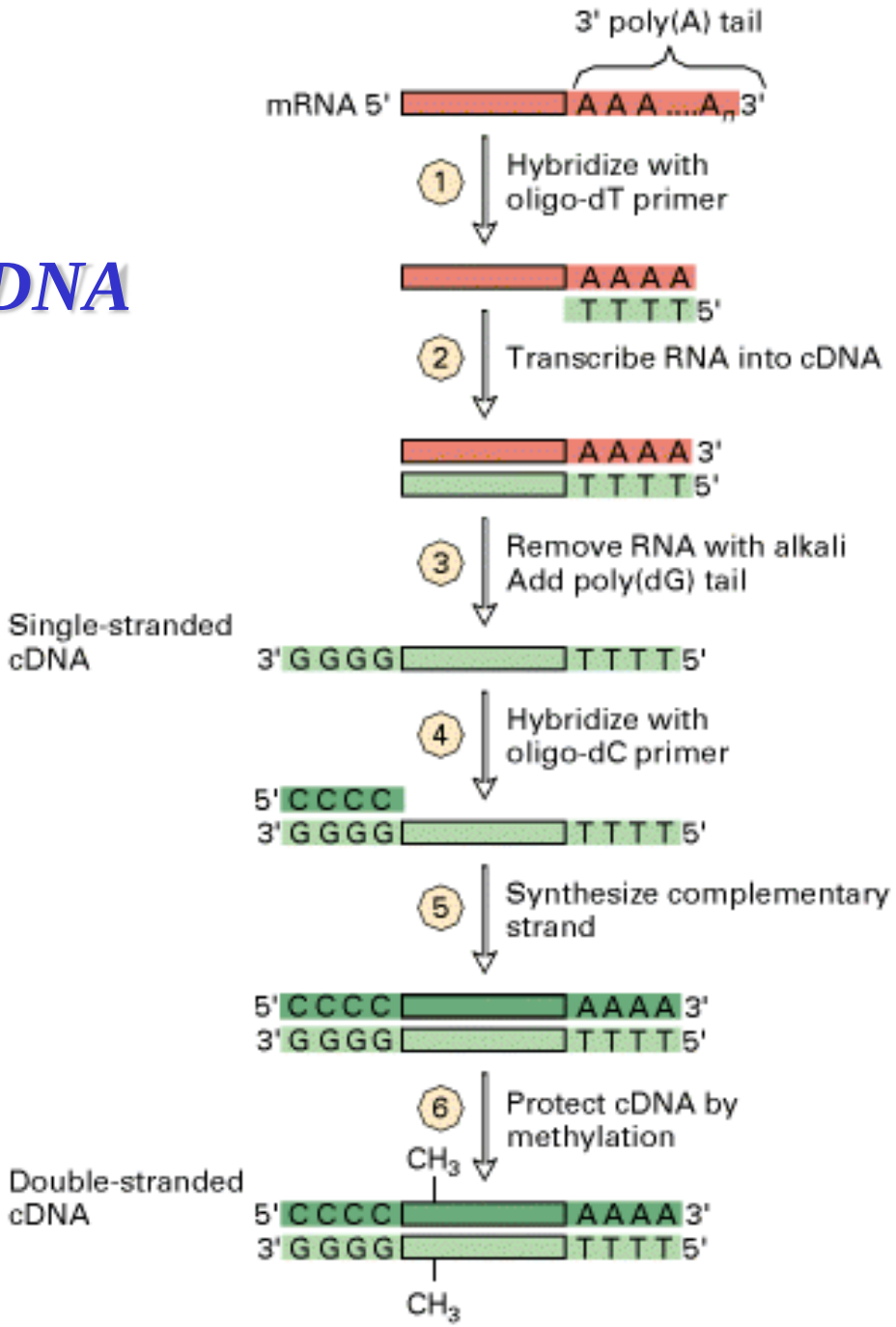
DNA strand synthesized on the RNA template is called **cDNA** (for complementary DNA)



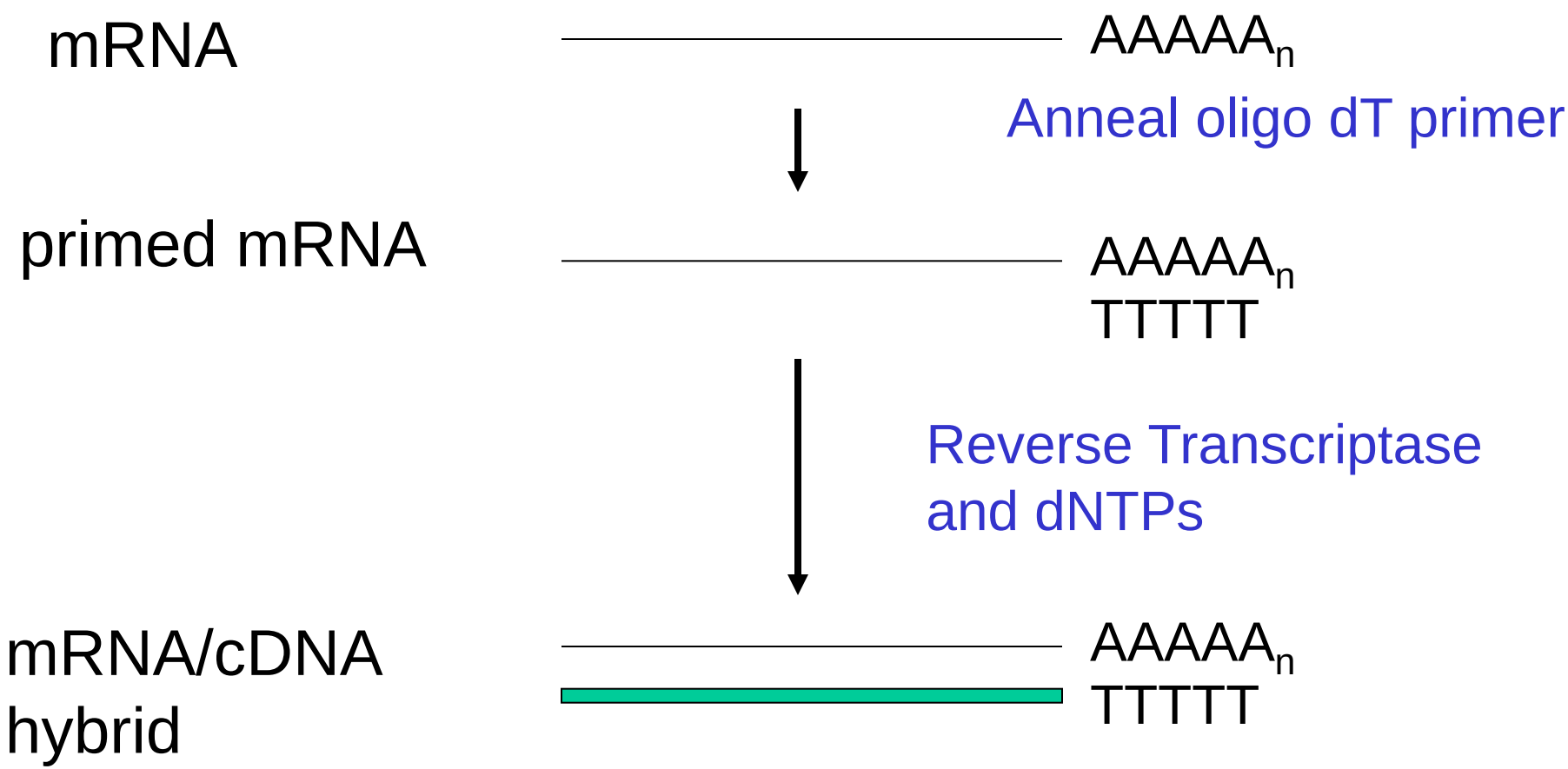
Isolation of mRNA by oligo-dT affinity chromatography.

Strategy to synthetize double strand cDNA

A)



B) cDNA Synthesis

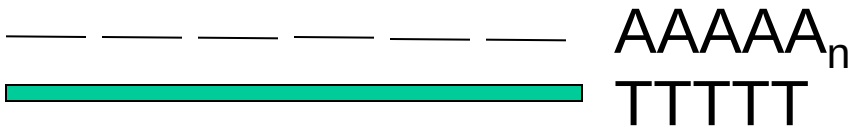


Gubler Hoffman cDNA Synthesis

mRNA/cDNA hybrid

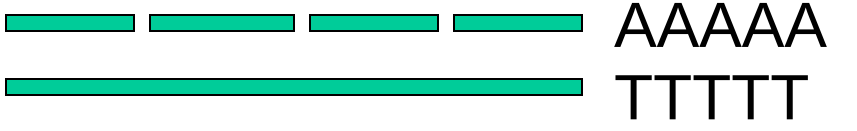
RNase H

nicked RNA



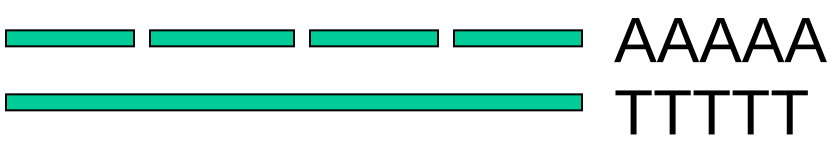
DNA Pol I

nicked RNA used as primers by Pol



Gubler Hoffman cDNA Synthesis

2nd strand cDNA in pieces



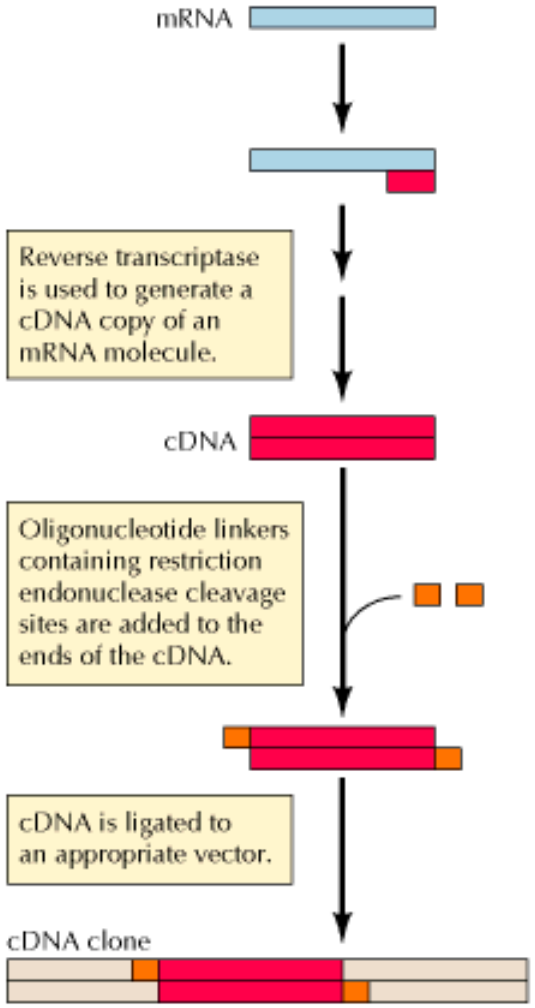
E. coli DNA Ligase

ds cDNA

Clone into vector

cDNA Library

Introduction of cDNA into a cloning vector



Insert Capacity of Vectors

Vector	max. insert size (kb)
plasmid	**up to 10**
phage λ	**25**
cosmids	**35 - 45**
phage P1	**80 - 100**
BAC	**50 - 300**
YAC	**300 - >1500**