A study material for M.Sc. Biochemistry (Semester: III) Students
on the topic (CC-13; Unit I)

c- DNA Library

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Introduction

- cDNA libraries are prepared by isolating mRNAs from tissues, which are actively synthesizing proteins, like roots and leaves in plants ovaries or reticulocytes in mammals etc.

- The mRNA is used for copying it into cDNA through the use of **Reverse Transcriptase**

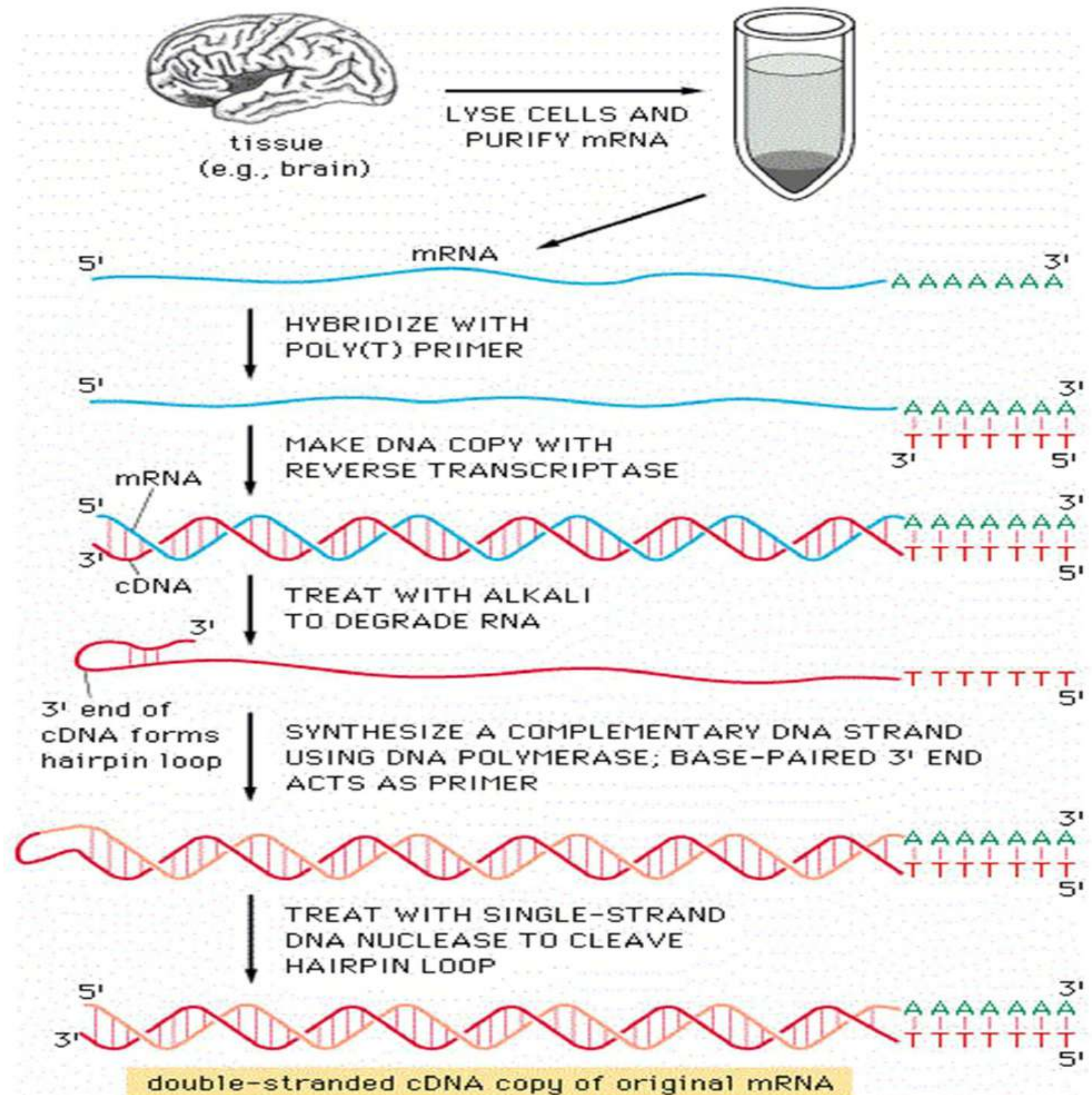
- Cloned eukaryotic cDNA have their own special uses, which derived from the fact that they lack intron sequences

- cDNA clones application:

- 1. *Bacterial expression of eukaryotic gene*
- 2. *Study of the eukaryotic protein in <u>E.coli</u>*

cDNA library construction steps

1. mRNA isolation
2. mRNA conversion into cDNA
3. Cloning of cDNA



http://www.uic.edu/classes/phar/phar331/lecture7/

Homopolymer tailing and cDNA

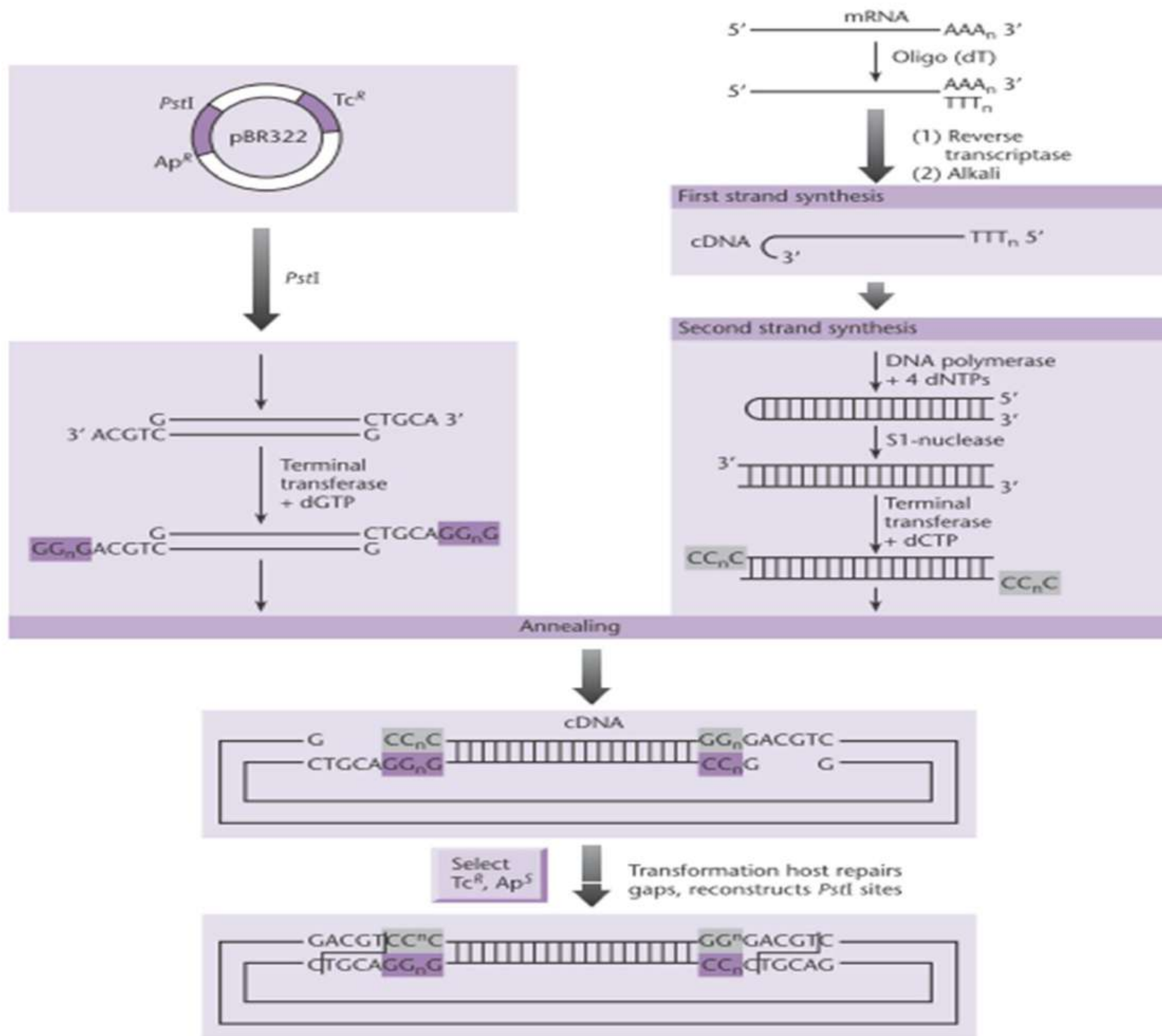
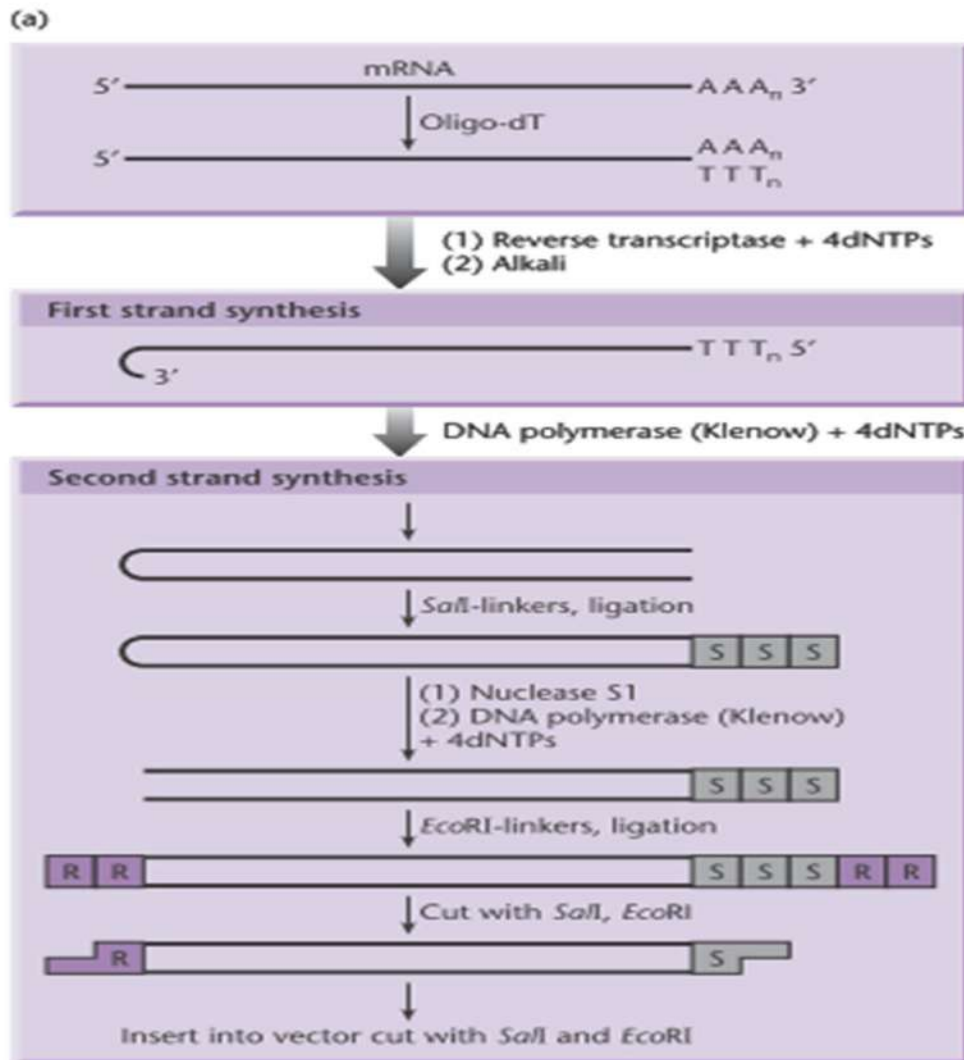


Fig. 6.5 An early cDNA cloning strategy, involving hairpin-primed second-strand DNA synthesis and homopolymer tailing to insert the cDNA into the vector.

Other strategy to cDNA cloning



Self priming method for cDNA cloning

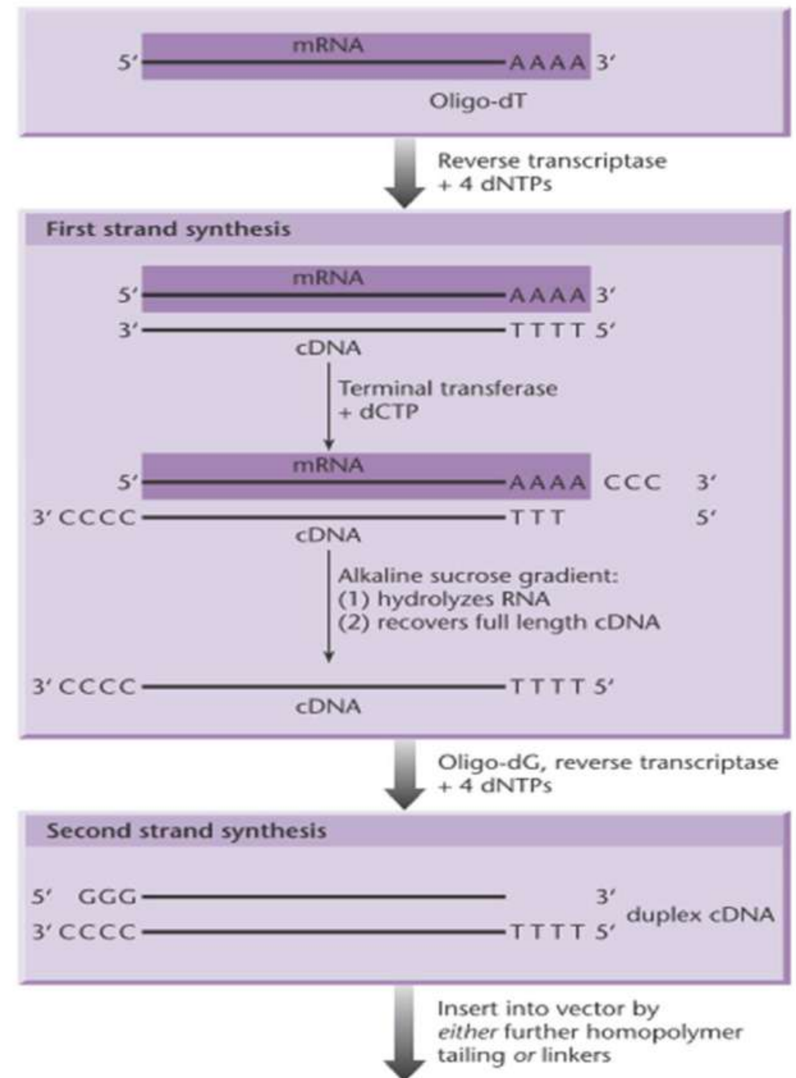
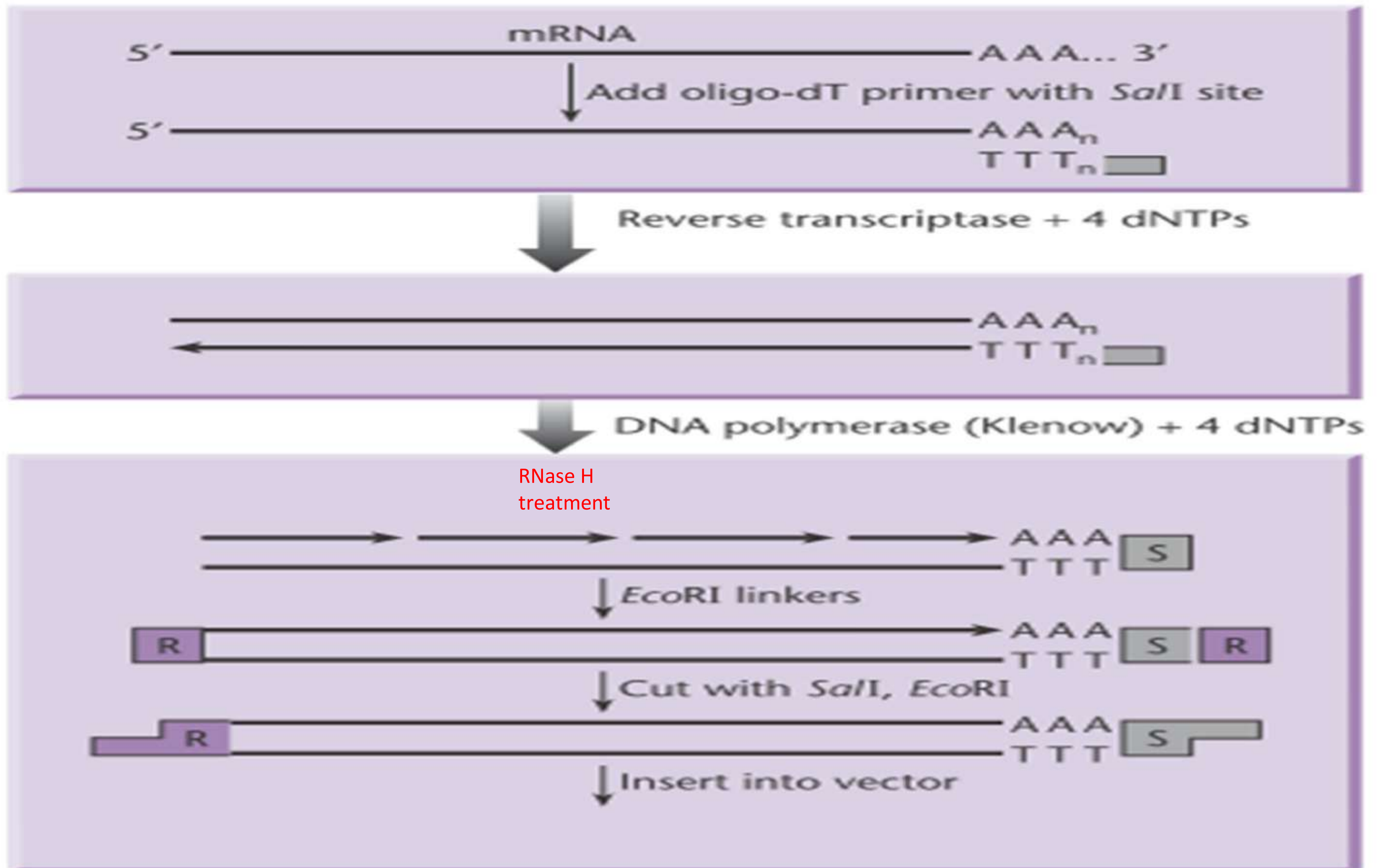


Fig. 6.6 Improved method for cDNA cloning. The first strand is tailed with oligo(dC) allowing the second strand to be initiated using an oligo(dG) primer.



Directional Cloning of cDNA using random priming mediated second strand synthesis

Non-Directional Cloning of cDNA

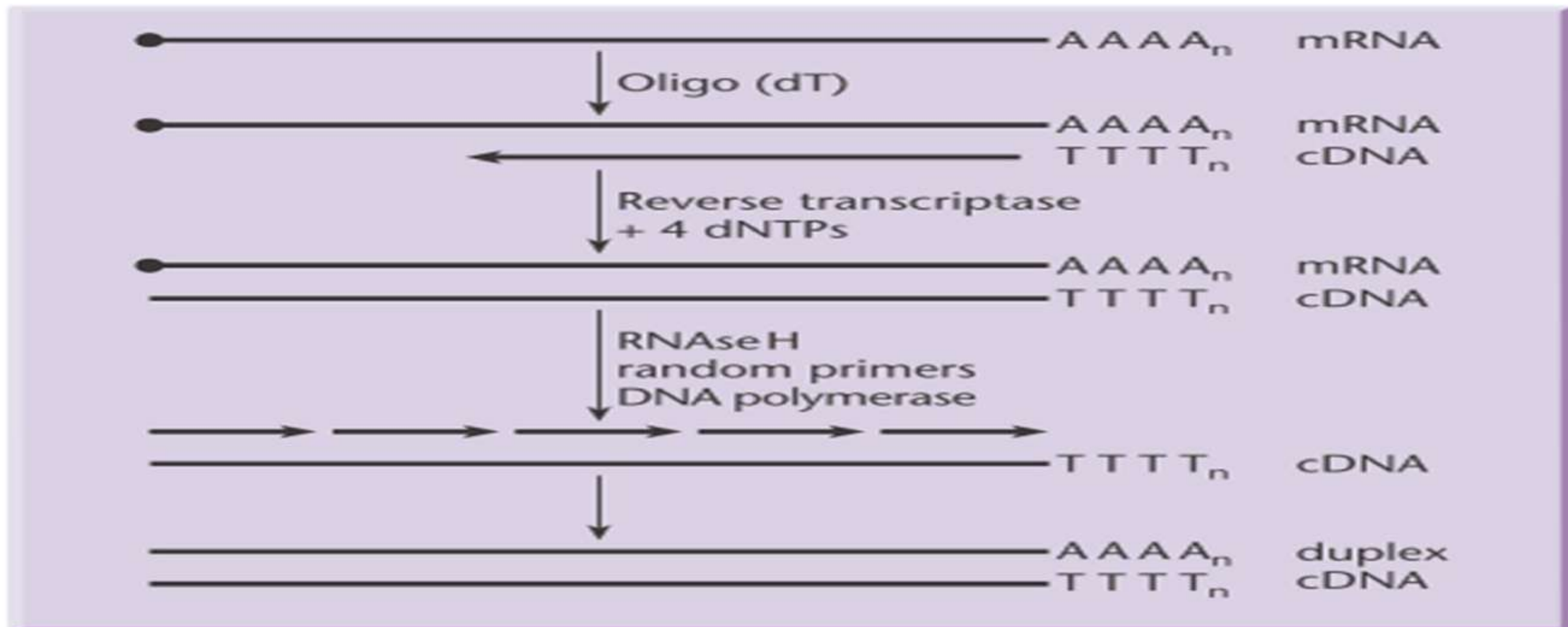


Fig. 6.8 The Gubbler–Hoffman method, a simple and general method for non-directional cDNA cloning. First-strand synthesis is primed using an oligo(dT) primer. When the first strand is complete, the RNA is removed with RNase H and the second strand is random-primed and synthesized with DNA polymerase I. T4 DNA polymerase is used to ensure that the molecule is blunt-ended prior to insertion into the vector.

Full length cDNA cloning

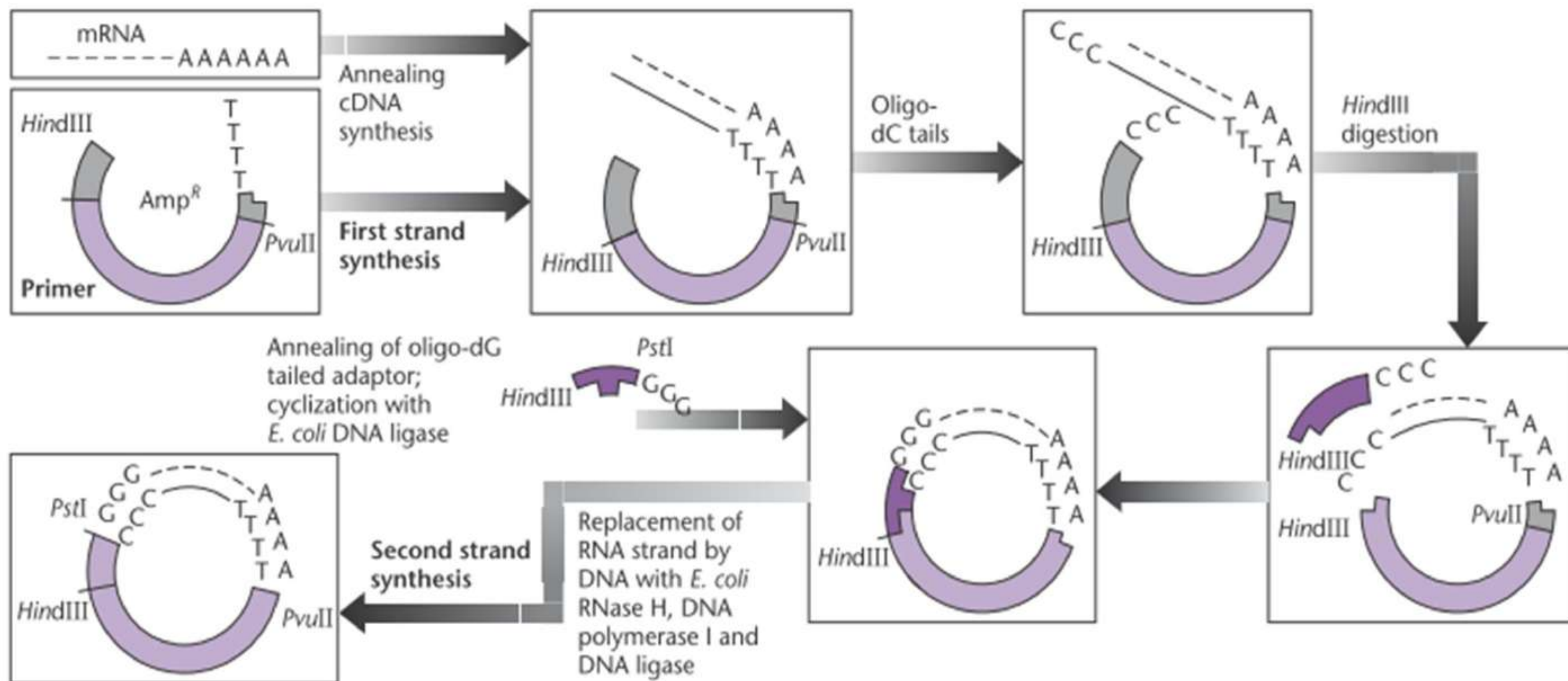
- *Aspergillus* nuclease S1 is an endonuclease enzyme derived from *Aspergillus oryzae* that splits single-stranded DNA (ssDNA) and RNA into oligo- or mononucleotides

- S1 nuclease degrade hair pin loop of duplex DNA but it also degrades the some sequences of duplex DNA, so we don’t get the full length cDNA clone.

- Disadvantage of S1 nuclease:
- Degrade some sequence of cDNA

- To get full length cDNA cloning **Okayama and Berg** and **Messing and Heidicker** method is used

Okayama and Berg method of full length cDNA cloning



This strategy was devised by Okayama & Berg (1982) and has two further notable characteristics. First, full-length cDNAs are preferentially obtained because an RNA–DNA hybrid molecule, the result of first-strand synthesis, is the substrate for a terminal transferase reaction. A cDNA that does not extend to the end of the mRNA will present a shielded 3-hydroxyl group, which is a poor substrate for tailing. Second, the second-strand synthesis step is primed by nicking the RNA at multiple sites with RNase H. Second-strand synthesis therefore occurs by a nick-translation type of reaction, which is highly efficient.

Messing and Hedicker method of full length cDNA cloning

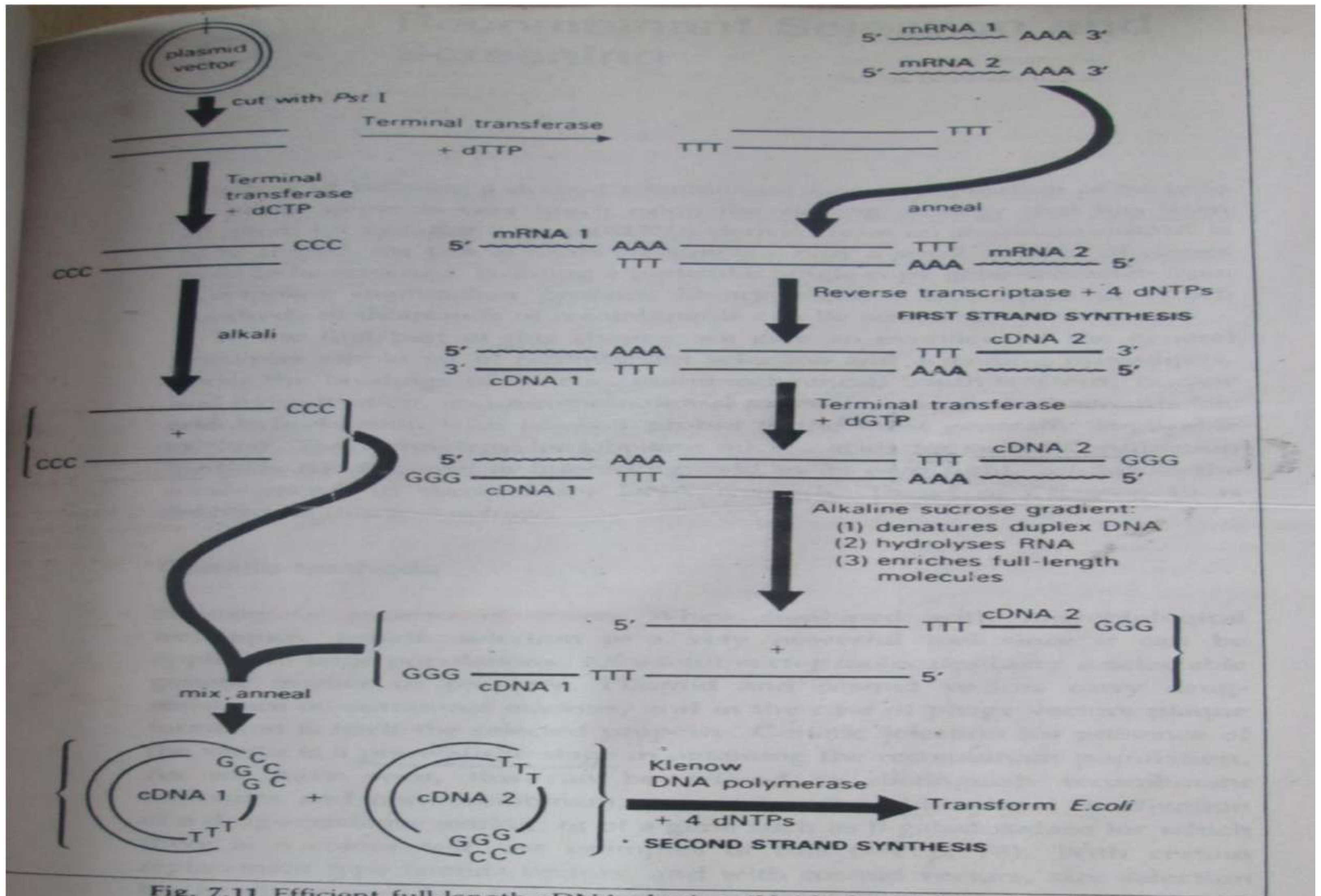


Fig. 7.11 Efficient full-length cDNA cloning (Heidecker & Messing 1983). The mRNA is annealed to linearized and oligo-dT tailed plasmid DNA, which then primes synthesis of the first cDNA strand. Oligo-dG tails are added to the cDNA-plasmid molecules, which are then centrifuged through an alkaline sucrose gradient. This step removes small molecules, hydrolyses the mRNA and separates the two cDNAs which were formerly attached to the same duplex plasmid. Denatured, oligo-dC tailed plasmid DNA is added (in excess) and conditions adjusted to favour circularization by the complementary homopolymer tails. The excess oligo-dC tailed plasmid may simply renature, but cannot circularize. The circular molecules have a free 3-hydroxyl on the oligo-dC tail which primes second-strand synthesis of the cDNA to create duplex recombinant plasmids which transform *E. coli*. Clones can be obtained with the cDNA inserted in both orientations.

Similarity between Okayama and Berg method and Messing and Heidecker method

- Both used for full length cDNA cloning
- Both methods don't require S1 nuclease
- Both method promote full length cDNA cloning with high efficiency
- Dissimilarity:
- RNAse H is used in Okyama and Berg method
- Nick translation for 2nd strand synthesis is used in Okyama and Berg method

Okyama and Berg method	Messing and Heidicker
RNase H is used	Not used
Nick translation for 2nd strand synthesis	No nick translation is used to synthesize the 2nd strand of cDNA
Direction cloning of the cDNA	Non-directional

Acknowledgement and Suggested Readings:

1. Gene Cloning and DNA Analysis: An Introduction; Sixth Edition ; T. A. Brown; Wiley – Blackwell Publications
2. Principles of Gene Manipulation; Sixth Edition; Sandy B Primrose, Richard M Twyman and Robert W. Old; Wiley – Blackwell Publications
3. Biotechnology: Applying the Genetic Revolution; David P. Clark and Nanette J. Pazdernik; Academic Press (Elsevier)

Thanks