Course: M.Sc. Biotechnology

Paper: BIOT4009: Genetic Engineering and Gene Therapy

UNIT – IV
Gene library

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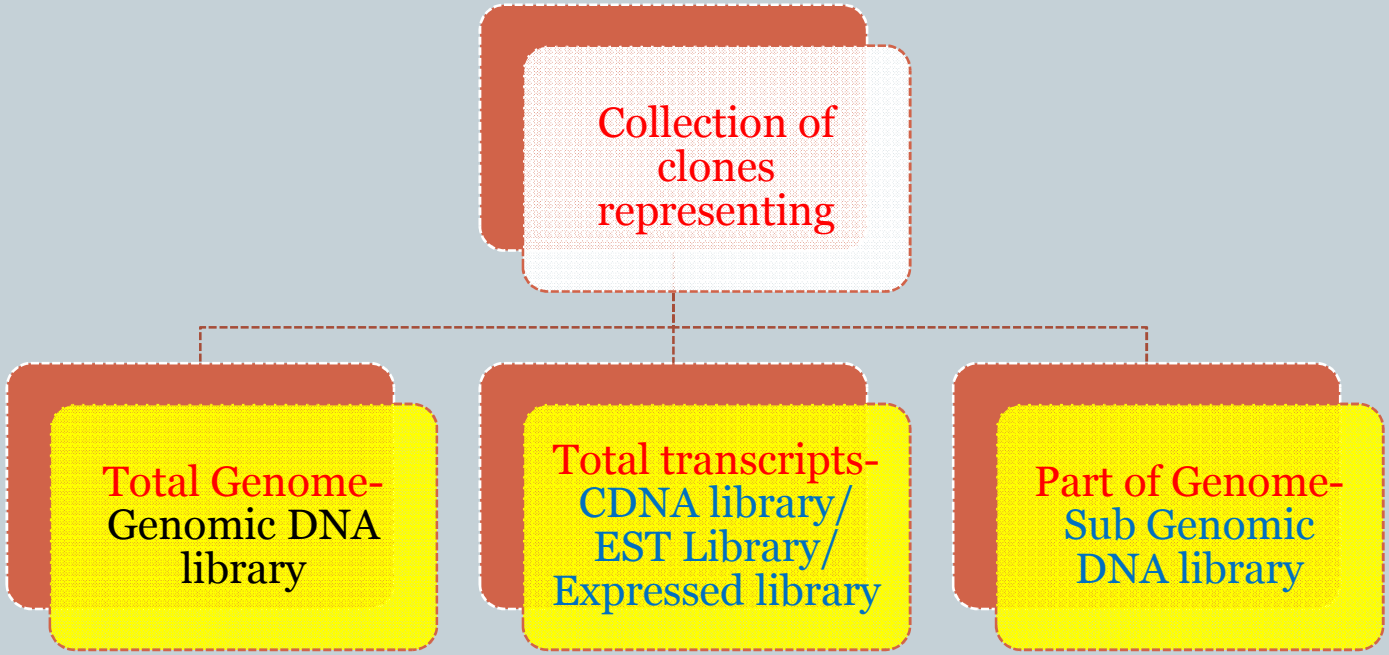
Gene library

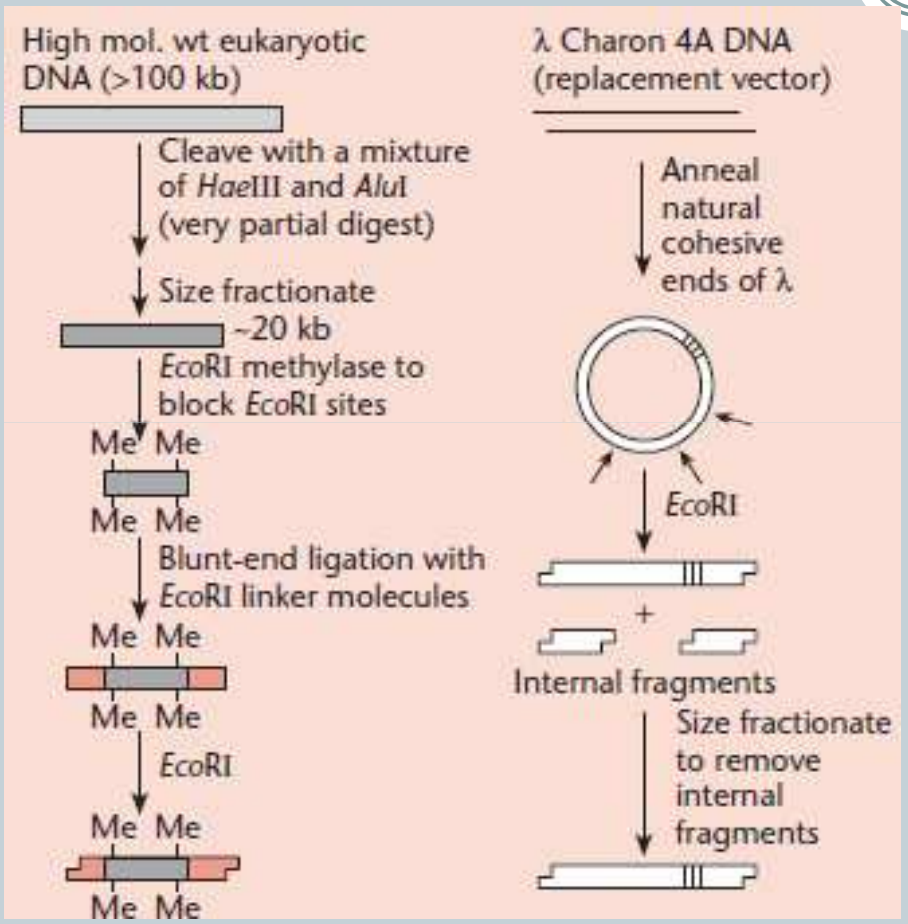
Library is collection of clones.....



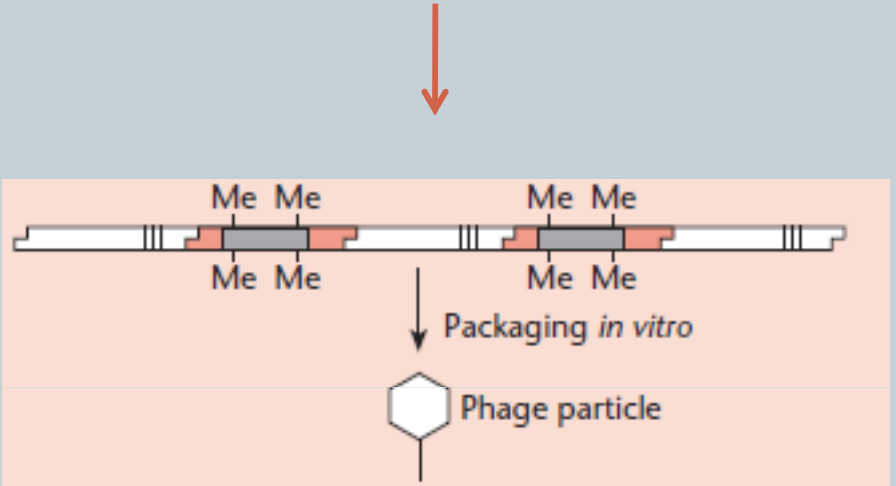
Genomic DNA Library

- **Collection of clones representing total genome**
- **Present in population of identical vectors**
- **Vectors contain clonable fragments of genomic DNA**
- **Vectors are self-replicating**
- **Vectors containing insert DNA are maintained in host cells like *E. coli* and *S. cerevisiae***

Genomic DNA Library construction method



Joining the vector and insert DNA fragments using ligase



Spread on lawn of host bacteria and count the titre

Store, distribute and use

Library may also be constructed in high capacity vectors like BAC/ YAC/ PAC

Genomic DNA Library contd.

- Since size of genome of organism varies widely
- Number of clones required in library to represent total genome varies
- It depends upon
  - Type of and frequency of restriction endonuclease
  - Average size of fragments
  - Total size of genome

e.g.

Human genome size=2.8 x 10^6 Kb

Average fragment / clone size= 20 kb

Number of fragments required to represent total genome =1.4 x 10^5

The number of independent recombinants required in the library must be greater than *n,*
because sampling variation will lead to the inclusion of some sequences several times and the exclusion of other sequences in a library of just *n recombinants.*

Clarke and Carbon (1976)
P=probability of including any DNA sequence in a random library of *N* independent recombinants:

$$N = \frac{\ln(1-P)}{\ln\left(1-\frac{1}{n}\right)}$$

To achieve a 95% probability (*P = 0.95) of* including any particular sequence in a random human genomic DNA library of 20 kb fragment size
Number of clones required would be

$$N = \frac{\ln(1-0.95)}{\ln\left(1-\frac{1}{1.4\times10^5}\right)} = 4.2\times10^5$$

From: Principles of gene manipulation by Primrose et al 6th ed.

Genomic DNA library contd.

Applications

- Gene pool storage and retrieval of desired sequence
- Analysis of
- gene sequences and copy number
- gene structure
- exonic and intronic junctions
- coding and non coding sequences
- regulatory sequences
- repeats and extent etc.

Complementary DNA (cDNA) library

- **Prepared from transcripts (messenger RNA)**
- **Does not represent full genome**
- **Exhibit spatiotemporal variability**
- **It depends on cell type, age, time, stage, location etc.**
- **Represent only those gene sequences which are expressed as RNA**
- **Contains mainly ORF and UTRs**

cDNA Library contd.

- Starting material is mRNA
- mRNA is reverse transcribed to complementary DNA
- Ds complementary DNA is cloned in vectors like Plasmid, Lambda derived vectors
- Lambda vectors are preferred due to ease of
  - preparation
  - stability
  - storage and
  - transportation etc.

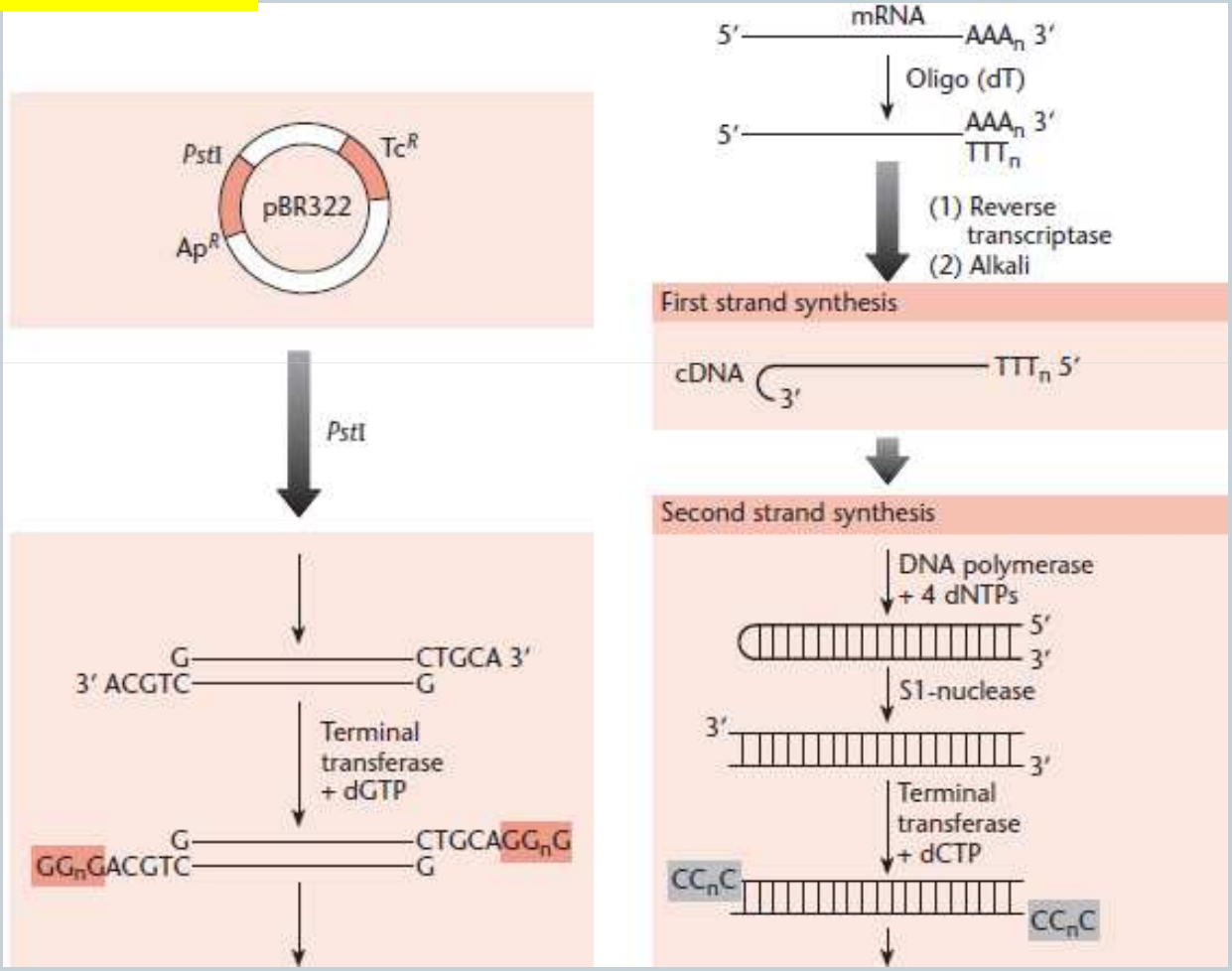
cDNA Library construction method

λZAP vector is preferred for cDNA library construction

- **High cloning capacity – upto 10 kb of foreign DNA can be cloned, most cDNAs fall in this range**
- **Presence of a polylinker with six URS makes cloning versatile**
- **Polylinkers also allow directional cloning**
- **T3 and T7 RNA polymerase sites flanking the polylinker allow transcription of**
  - **sense and**
  - **antisense RNA from cDNA cloned**

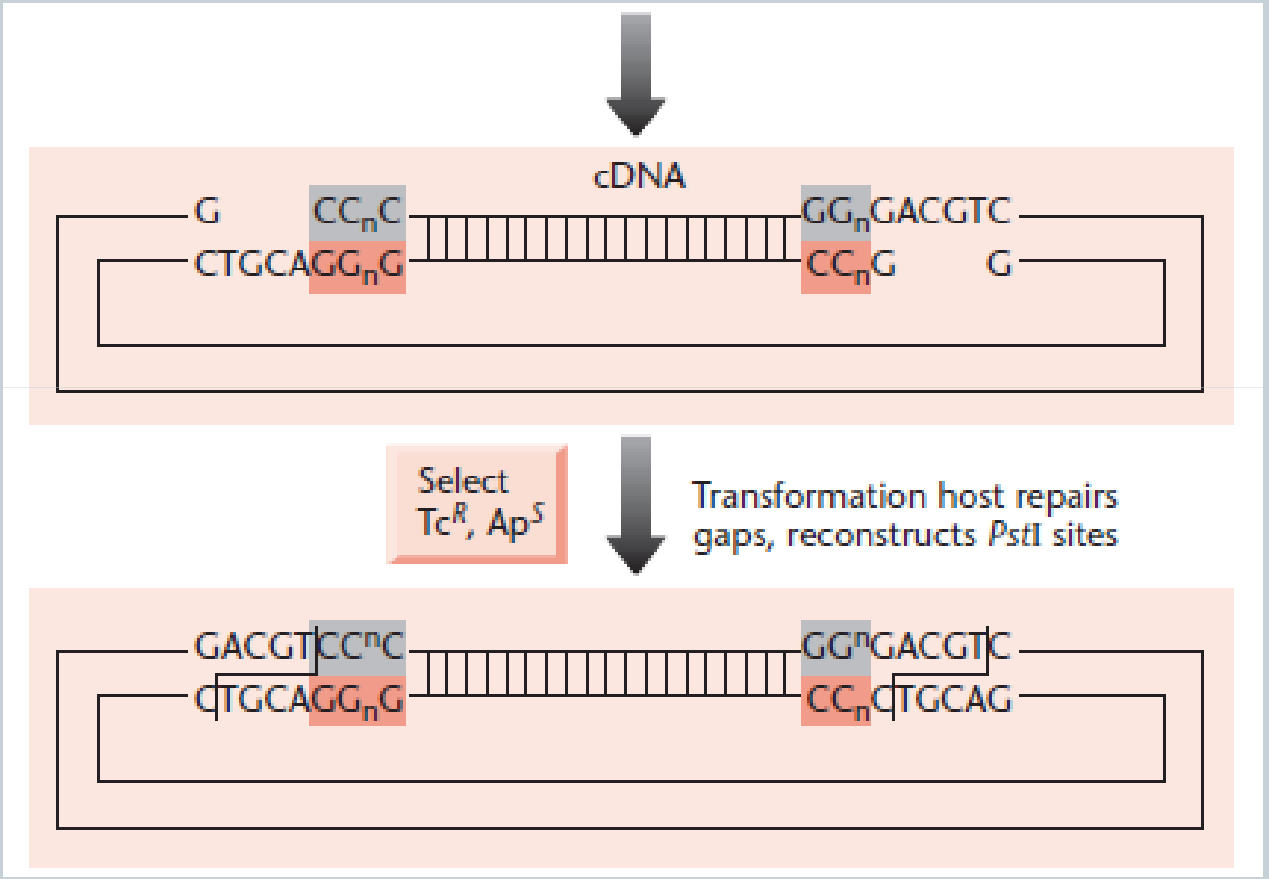
cDNA Library construction method contd.

In plasmid vector



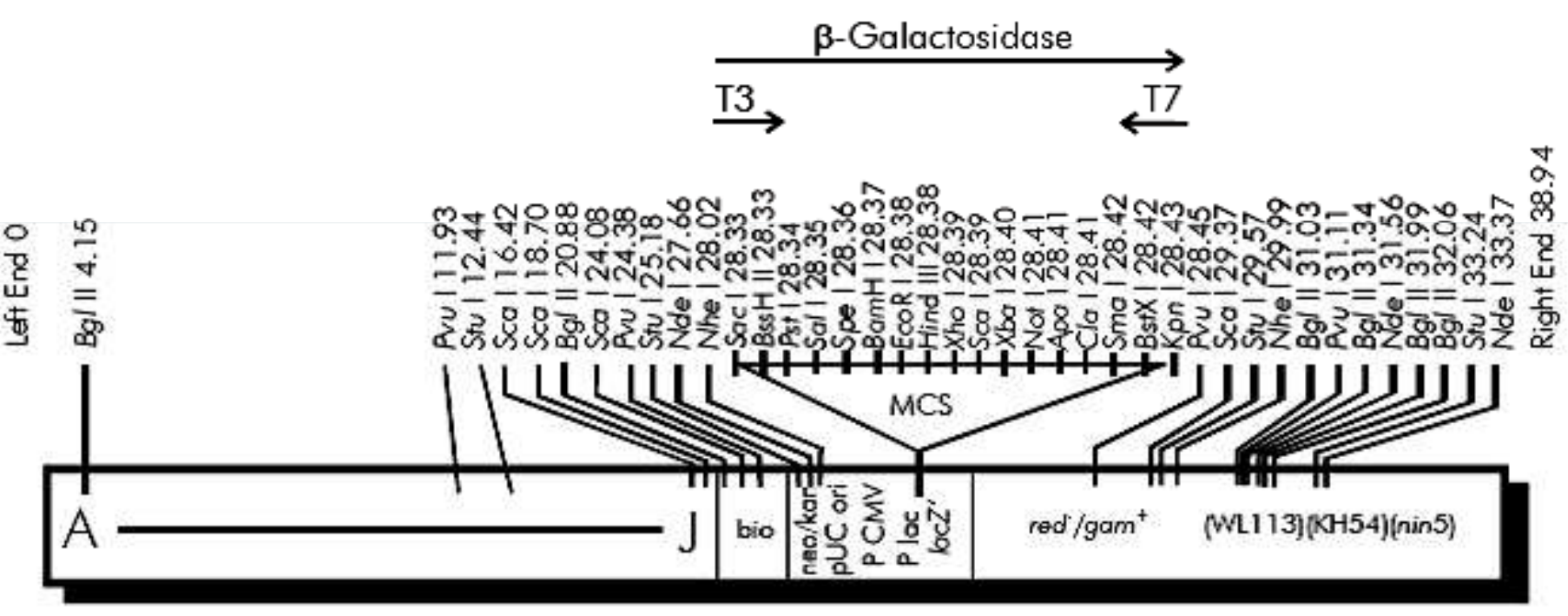
Annealing /Ligation

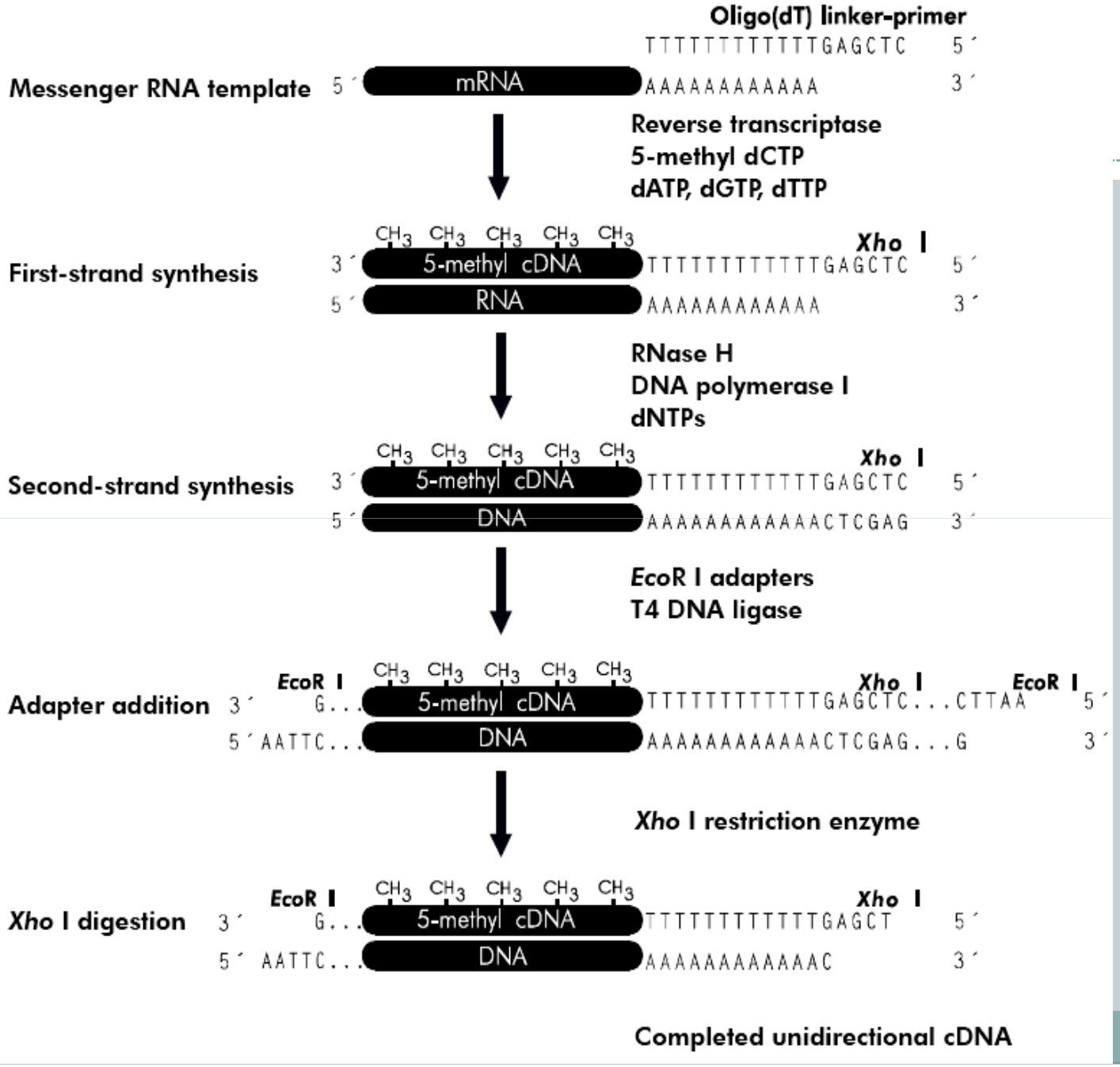
cDNA Library construction method contd.



cDNA Library construction method contd.

In λZAP Express vector




Oligo(dT) linker-primer
TTTTTTTTTTTTGAGCTC 5´
Messenger RNA template
5´ mRNA
AAAAAAAAAAAA 3´
Reverse transcriptase
5-methyl dCTP
dATP, dGTP, dTTP
First-strand synthesis
CH3 CH3 CH3 CH3 CH3
Xho I
3´ 5-methyl cDNA TTTTTTTTTTTTGAGCTC 5´
5´ RNA AAAAAAAAAAAA 3´
RNase H
DNA polymerase I
dNTPs
Second-strand synthesis
CH3 CH3 CH3 CH3 CH3
Xho I
3´ 5-methyl cDNA TTTTTTTTTTTTGAGCTC 5´
5´ DNA AAAAAAAAAAAACTCGAG 3´
EcoR I adapters
T4 DNA ligase
Adapter addition
EcoR I
CH3 CH3 CH3 CH3 CH3
Xho I
EcoR I
3´ G... 5-methyl cDNA TTTTTTTTTTTTGAGCTC...CTTAA 5´
5´AATTC... DNA AAAAAAAAAAAACTCGAG...G 3´
Xho I restriction enzyme
Xho I digestion
EcoR I
CH3 CH3 CH3 CH3 CH3
Xho I
3´ G... 5-methyl cDNA TTTTTTTTTTTTGAGCT 5´
5´ AATTC... DNA AAAAAAAAAAAAC 3´
Completed unidirectional cDNA

cDNA library construction contd.

- Vector digested with Eco RI and XhoI is dephosphorylated
- Left arm and right arm are taken and ligated to double stranded cDNA
- Ligation product is in vitro packaged in phage capsid components
- Infectious virions are synthesized
- Host cells are infected and plaques are allowed to develop on Agar plate having top agarose
- Plaques are counted and *pfu*/ titre count is calculated
- Library is amplified, stored in aliquotes, transported and analyzed.

Titre count/ *pfu* count

***pfu* in primary or amplified (secondary) library are important indicators**

$$\text{Plaque forming units } (pfu) = \frac{\text{Number of plaques } (pfu) \times \text{Dilution factor}}{\text{Volume plated } (\mu l)} \times 1000 \mu l/ml$$

cDNA Library applications

- Gene coding region analysis
- Regulatory UTRs
- Coded polypeptide analysis
- Microarray
- Comparative gene expression
- Isolation of gene for heterologous expression
- Mutagenesis
- Analysis of functional boundary of gene
- Probe construction etc.

References

- Primerose, Twyman and Old. Principles of gene manipulations (6th edition), Blackwell science.
- Maniates et al., Molecular Cloning vol 1-3
- Instruction manual, Stratagene Lamdba ZAP Express II cDNA Library construction kit

Thanks

PLEASE CONSULT MOLECULAR CLONING BY SAMBROOK ET AL.,

AND SUPPLEMENTARY STUDY MATERIAL PROVIDED FOR MORE DETAILS