GENE CLONING STRATEGIES

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GENE CLONING STRATEGY

A set of techniques adopted for gene cloning for a particular purpose is said to be a gene cloning strategy.

There are several strategies of gene cloning depending on whether we need to construct genomic libraries or cDNA libraries and whether we want to clone all pieces of a DNA or a particular piece of the DNA.

Separate cloning strategies have been followed for-

- **Genomic DNA library**
- **cDNA library**
- **Chromosome walking**
- **Chromosome jumping**

Shotgun cloning method is a cloning strategy which has been done for constructing **genomic DNA library**.

A collection of clones containing all DNA segments of the genome of an organism is called **genomic DNA library**. The genomic library is created by inserting all fragments of a genomic DNA into host cells using vectors.

SHOT GUN CLONING TECHNIQUE:

Large, mammalian genomes are particularly difficult to clone, sequence and assemble because of their size and structural complexity. As a result clone-by-clone sequencing, although reliable and methodical, takes a very long time. With the emergence of cheaper sequencing and more sophisticated computer programs, researchers have therefore relied on whole genome shotgun sequencing to tackle larger, more complex genomes.

Two approaches are made on shotgun sequencing-

1. Whole genome shotgun sequencing
2. Hierarchical Shotgun sequencing

Shotgun cloning method is widely adopted to create genomic DNA libraries. cloning all DNA fragments of a genome into cleaved vectors at a time by a single cloning experiment is called **shotgun cloning**. The following steps are used to create a **genomic DNA library**.

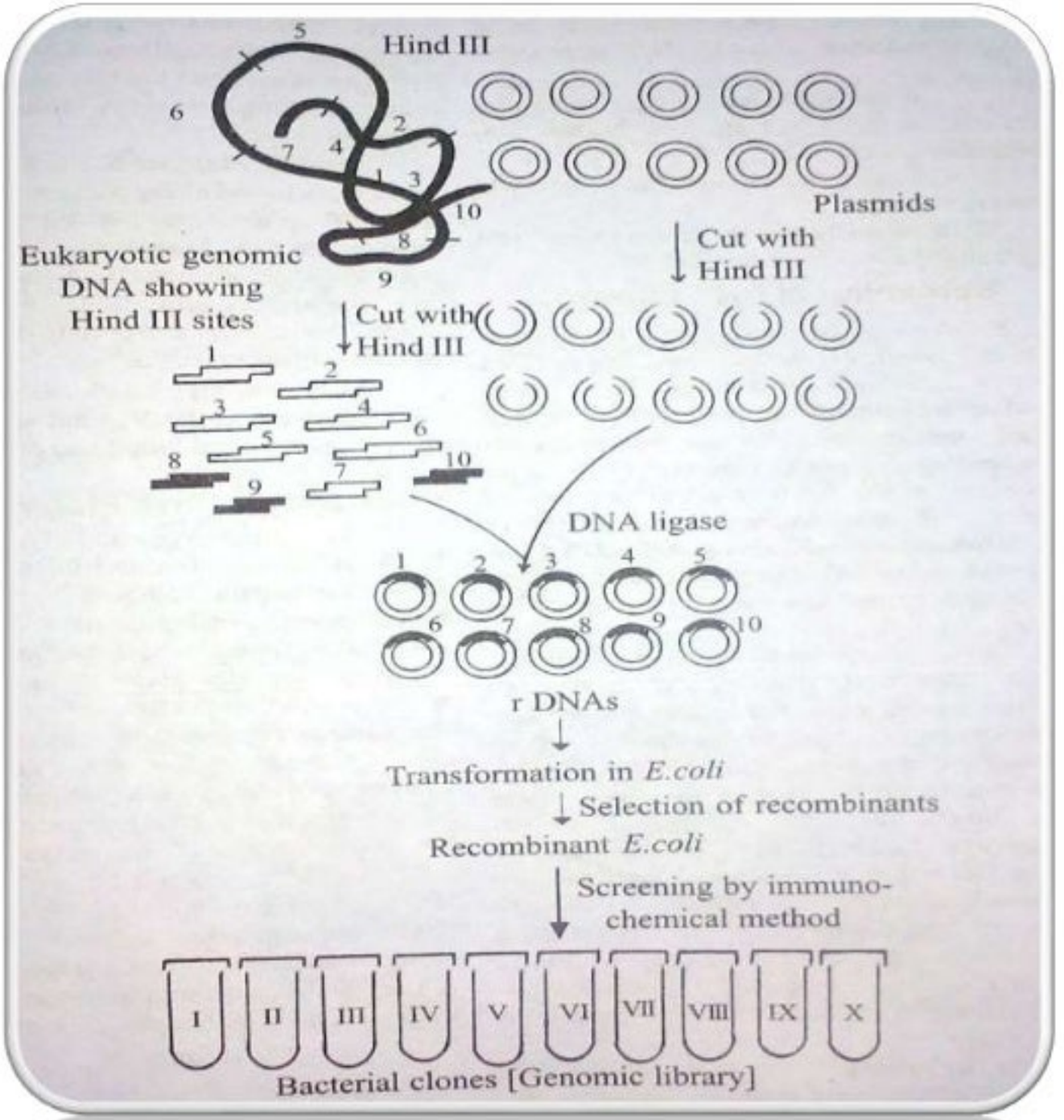
- **The genomic DNA is isolated from the source organism using a standard procedure.**
- **The isolated genomic DNA is cut with a restriction enzyme to generate small DNA fragments.**
- **The restriction digest is electrophoresed on a polyacrylamide gel in the presence of molecular weight markers in one lane.**
- **DNA fragments of clonable size are isolated from the gel and the smaller as well as larger fragments are discarded. For example, if the DNA fragments have to be cloned in plasmids, size of the fragments should be between 5 and 15 kb; so fragments lesser than 5kb and larger than 15kb are discarded.**

cont.

- The isolated DNA fragments are treated with alkaline phosphatase to remove 5′ phosphate groups from the ends of the DNAs.
- The vector DNA is cleaved with the same restriction enzyme which is used to digest the genomic DNA.
- The dephosphorylated DNA fragments are mixed with the linearized vector DNA to construct rDNAs.
- The rDNAs thus constructed are introduced into *E.coli* or yeast cells.
- The recombinant cells are separated from one another using dilution plating and subsequent culture.

- **The microbial cultures are screened to detect the contigs.**

- **Based on the contigs analysis, the clones are arranged in the genomic library.**

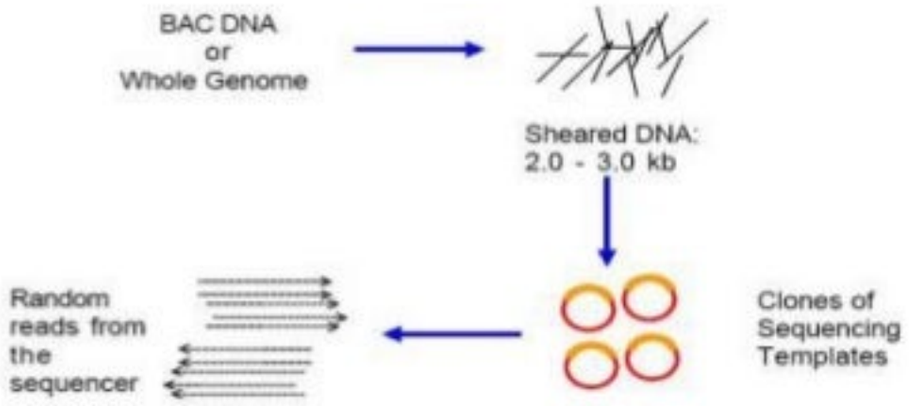


Screening of DNA libraries

Analysis of various clones to arrange them into contigs is called **screening of DNA library**. the clones are arranged into contigs based on the principle of overlapping between the adjacent clones. The continuous clones representing the entire genomic DNA of an organismin the correct order are called **contigs**.

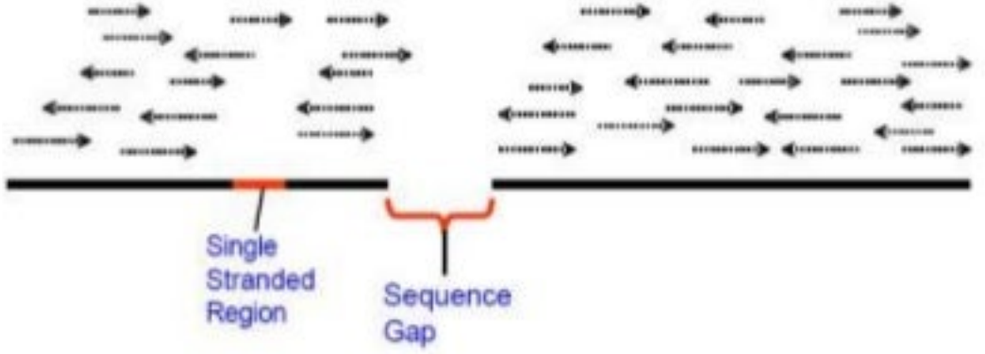
Restriction fragment fingerprinting, chromosome walking, repetitive DNA fingerprinting and STS(Sequence Tagged Sites) mapping are used to arrange the clones into contigs.

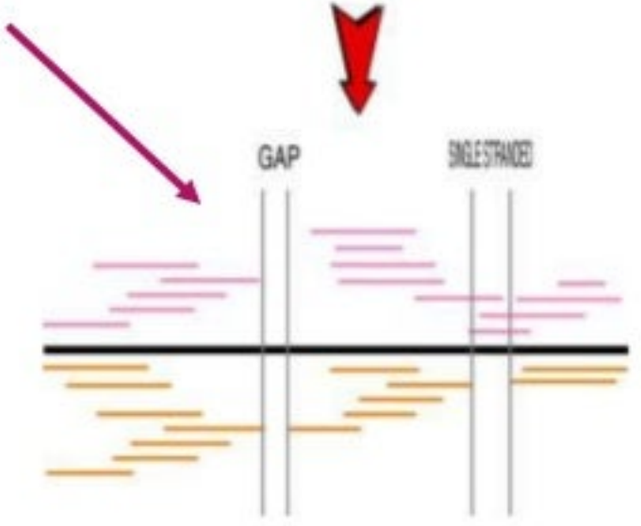
The example of Shotgun Sequencing
I :RANDOM PHASE



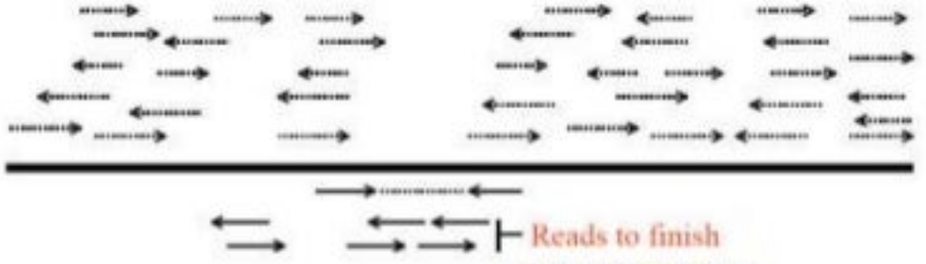
Shotgun Sequencing II:ASSEMBLY

Sequence overlap between individual reads is used to assemble a contiguous set of reads "the contig".





Shotgun sequencing III: FINISHING



•PCR or other methods are used to isolate and sequence the "unfinished regions".

Advantages-

- Much more economical and faster.
- Labor saving because the sequencing reaction is virtually fully automated and the sequences being assembled by computer programs.

Disadvantages-

- Puts a large requirement on the ability to align the overlapped fragments and reconstruct the chromosome.
- Complex process.

cDNA cloning

Cloning of cDNAs are done for the construction of cDNA libraries.

A collection of clones each of which carries a cDNA of an organism is called **cDNA library**.

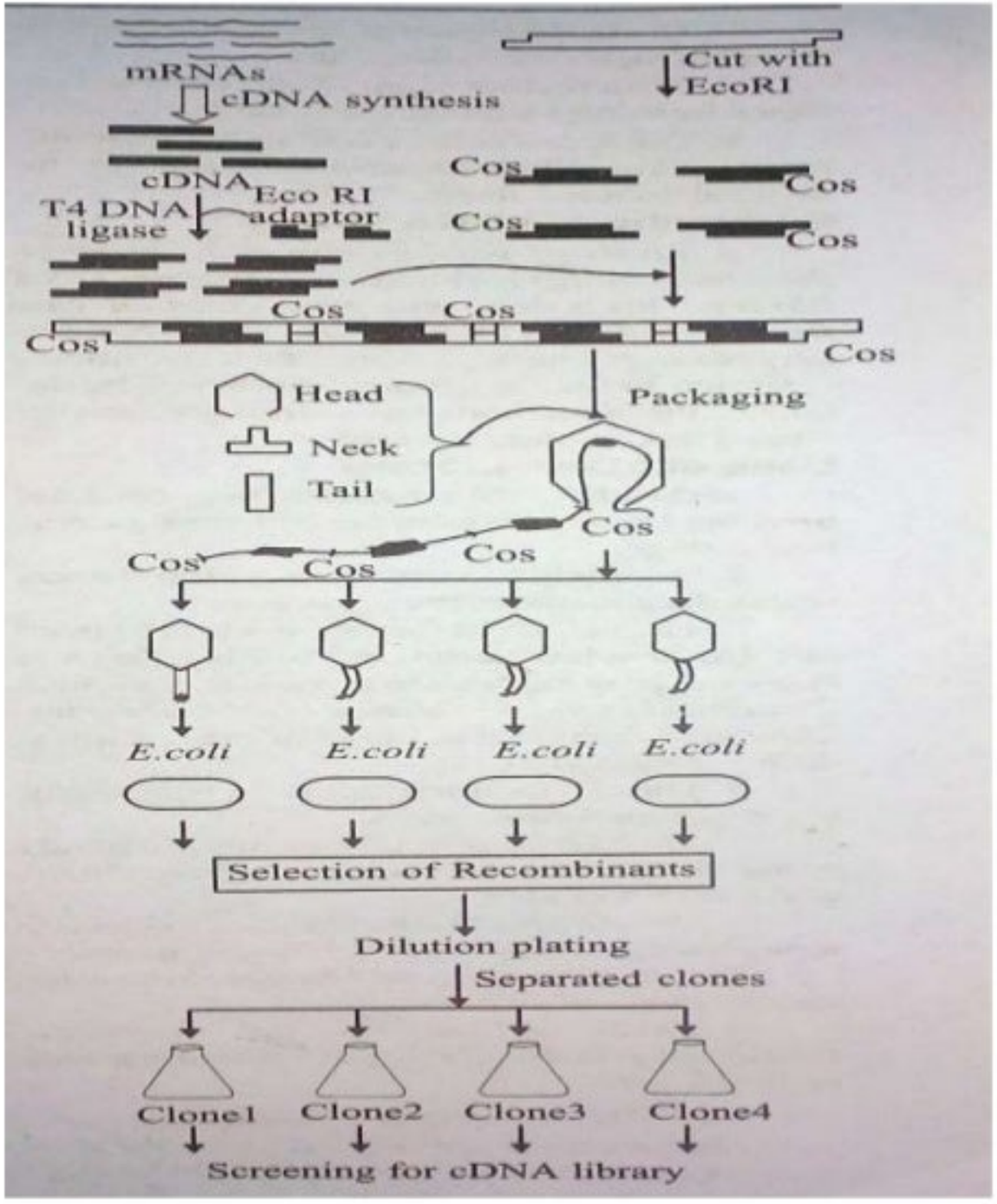
Since cDNAs have no intron, their size is always less than 10kb. Therefore, it is not necessary to use vectors that can accommodate larger DNAs. Generally plasmids, phagemids are used to construct cDNA libraries.

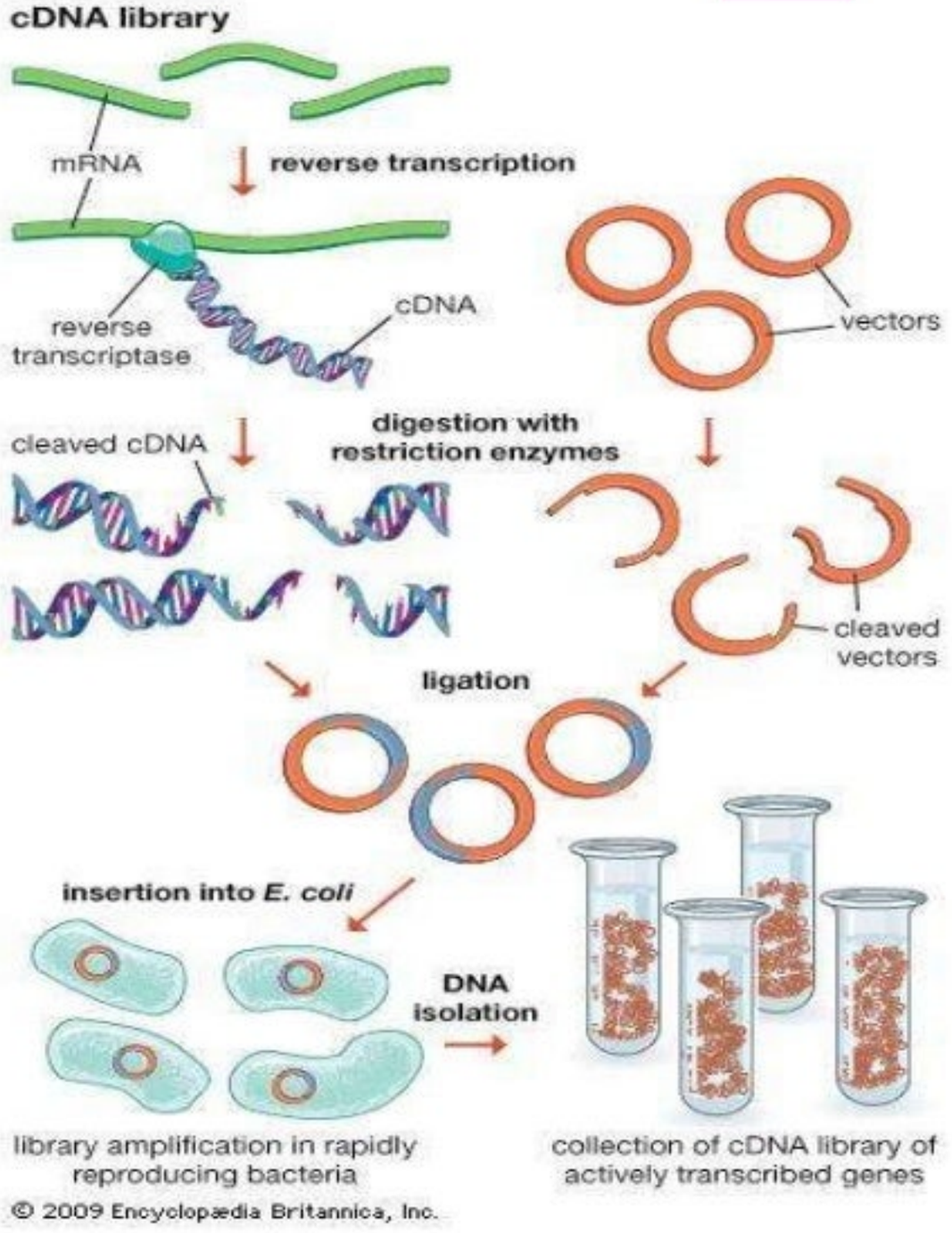
The creation of cDNA library needs a different strategy. It involves construction of cDNAs, cloning of cDNAs and screening of cDNA clones.

The cDNA is synthesized from mRNA by an enzyme called reverse transcriptase. The cDNA is used as a template to synthesize the second strand to form the duplex DNA. The double stranded DNA formed from cDNA is called cDNA clone. The cDNA clone is used for gene cloning.

cDNA cloning process:

- The cDNAs synthesized are joined with suitable adapters using DNA ligase.
- The cDNAs are then treated with alkaline phosphatase enzyme to remove 5′ phosphate group from the DNAs.
- The vector DNA is cut with a restriction enzyme and all the cDNAs are mixed together with them with treatment of DNA ligase.
- The recombinants are then infected in a bacterial culture e.g. *E.coli*
- Then recombinant *E.coli* cells are selected by using a suitable method.
- The *E.coli* culture containing recombinants is diluted and subcultured to separate individual bacterial clones.


mRNAs
cDNA synthesis
Cut with EcoRI
cDNA
Eco RI adaptor
T4 DNA ligase
Cos
Head
Neck
Tail
Packaging
E.coli
Selection of Recombinants
Dilution plating
Separated clones
Clone1
Clone2
Clone3
Clone4
Screening for cDNA library


cDNA library
mRNA
reverse transcription
reverse transcriptase
cDNA
vectors
cleaved cDNA
digestion with restriction enzymes
cleaved vectors
ligation
insertion into E. coli
DNA isolation
library amplification in rapidly reproducing bacteria
collection of cDNA library of actively transcribed genes
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