Genomic DNA isolation

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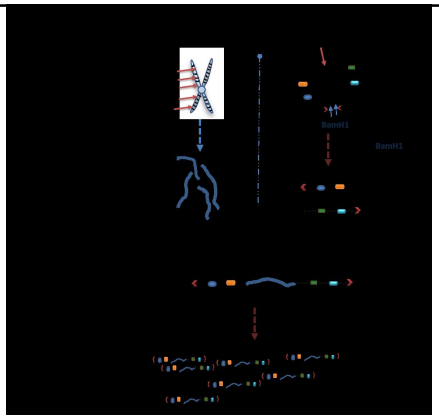
Introduction

- Gene sequence are arranged in genome in a randon fashion and selecting or isolating a gene is a big task especially when the genomic sequences are not known. A small portion of genome is transcribed to give mRNA where as a major portion remained untranscribed. Hence, there are two ways to represent a genomic sequence information into the multiple small fragments in the form of a library: (1) Genomic library (2) cDNA library.

- Preparation of Genomic Library-A genomic library represents complete genome in multiple clones containing small DNA fragments. Depending upon organism and size of genome, this library is either prepared in a bacterial vector (discussed later in future lectures) or in yeast artificial chromosome (YAC). An outline of the construction of genomic library is given in Figure 10.1. it has following steps:

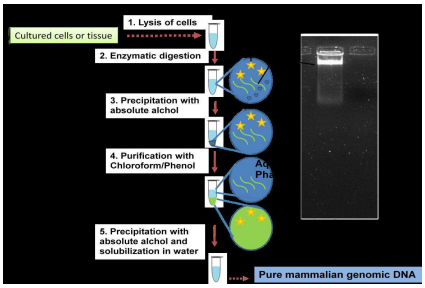
- 1. Isolation of genomic DNA
- 2. Generation of suitable size DNA fragments
- 3. Cloning in suitable vector system (depending on size)
- 4. Transformation in suitable host



- 1. Isolation of genomic DNA- Isolation of genomic DNA has following steps: 1. Lysis of cells with detergent containing lysis buffer. 2. Incubation of cells with digestion buffer containing protease-K, SDS to release genomic DNA from DNA-protein complex. 3. Isolation of genomic DNA by absolute alchol precipitation. 4. Purification of genomic DNA with phenol:chloroform mixture. Chloroform:phenol mixture has two phases, aquous phase and organic phase. In this step, phenol denatures the remaining proteins and keep the protein in the organic phase. 5. Genomic DNA present in aqueous phase is again precipitated with absolute alchol. 6. Genomic DNA is analyzed on 0.8% agarose gel and a good prepration of genomic DNA give an intact band with no visible smear (Figure 10.2).

Figure 10.2: Genomic DNA isolation. (A) Different steps in genomic DNA isolation.

(B) Agarose gel analysis of isolated genomic DNA.



- **2. Generation of suitable size fragments- Next step generation of genomic DNA into suitable small size fragments. Restriction digestion: Genomic DNA can be digested with a frequent DNA cutting enzyme such as EcoR-I, BamH-I or sau3a to generate the random sizes of DNA fragments. The criteria to choose the restriction enzyme or pair of enzymes in such a way so that a reasonable size DNA fragment will be generated. As fragments are randomly generated and are relatively big enough, it is likely that each and every genomic sequence is presented in the pool. As size of the DNA fragment is large, complete genome will be presented in very few number of clones. In addition, genomic DNA can be fragmented using a mechanical shearing. If a organism has a genome size of 2x107 kb and an average size of the fragment is 20kb, then no. of fragment, n= 106. In reality, this is the minimum number to represent a given fragment in the library where as the actual number is much larger.**

- The probability (P) of finding a particular genomic sequence in a random library of N independent clone is as follows: N=ln (1-P)/ln (1-1/n)…….**(Eq 10.1)**
  Where, N=number of clones, P=probability, n= size of average fragment size

- **3. Cloning into the suitable vector-The suitable vector to prepare the genomic library can be selected based on size of the fragment of genomic DNA and carrying capacity of the vector (Table 10.1). Size of average fragment can be calculated from the Eq 10.1 and accordingly a suitable vector can be choosen. In the case of fragment generated by restriction enzyme, vector can be digested with the same enzyme and put for ligation to get clone. In the case of mechanical shearing mediated fragment generation, putting these fragment needs additional effort. In one of the approachs, a adopter molecule can be used to generate sticky ends, alternatively a endonuclease can be used to generate sticky ends.**

- **4. Transformation to get colonies- Post ligation, clones are transformed in a suitable host to get colonies. A suitable host can be a bacterial strain or yeast. Different methods of delivering clone into the host cell is discussed in future lectures.**

S.NO	Vector	Insert Size (MB)
1	Plasmids	15
2	Phage lambda	25
3	Cosmids	45
4	Bacteriophage	70-100
5	(BAC)	120-300
6	(YAC)	250-2000

Contruction of cDNA library-

- **A cDNA library represents mRNA population present at a particular stage in a organism into multiple clones containing small DNA fragments. An outline of the construction of cDNA library is given in Figure 11.1. it has following steps: 1. Isolation of mRNA 2. Preparation of complementary DNA fragments- 3. cloning in suitable vector system 4. Transformation in suitable host .**