Bioinformatics : Written Assignment 2

Q1. (i) For a random DNA of sufficiently long length, all n-mers will occur with same frequency, for fixed values of n. Both BamH1 & Bal2 have the same length of recognition site, ie: for both cases the recognition sites are 6-mers. Hence, both are expected to occur with same frequency. Thus, the number of fragments is expected to be equal for either RE.

(ii) Both BamH1 & Bal2 produce the same overhanging "sticky" end (cohesive end) sequence. This means that a DNA strand cut with Bam H1 can easily combined with another DNA strand which had been cut using Bal 2. This is extremely useful in cases where the DNA strand of interest has recognition sites for only one of the 2 REs and the vector has recognition for only the other.

Q2. Advantages of PCR over cloning:

* PCR requires very small amounts of DNA sample for amplification
* PCR requires much less a priori information about the strand to be amplified
* PCR process is much faster than cloning and create billions of copies in very little time. (in scale of hours).
* Much simpler process. Doesn't involve steps like isolation of vector, cutting with R.E., ligation of sample DNA within vector or transformation. The entire process is in-vitro
* The replicated DNA segment in cloning is within the cells of the culture and further within the vector. Thus extraction of DNA is much more complicated. On the other hand, output of PCR is the copies of the required DNA molecules making the process of extraction much simpler.

Advantages of Cloning over PCR:

* PCR process is very prone to errors, especially during the annealing stage. Contamination by foriegn genetic material is also more likely in PCR. On the other hand, cloning error is much lower.
* Cloning allows for further manipulation and propegation of the genetic material. This includes obtaining the protien product expressed by the strand.

Q3. (i) **Role of primers:**

* Primers essentially act a as starting point for the replication process
* DNA polymerase cannot start replication with just the single stranded DNA of interest. The primers attatch to the DNA strand in a complementary manner and provide an initial strand for the polymerase to start elongation.
* Primers determines the locus of replication and the region of the DNA to be replicated.

(ii) **Role of Taq polymerase:**

* Taq polymerases are the DNA polymerases responsible for copying the DNA strand of interest by extension of the primer strand, during the elongation stage.
* Unlike other DNA polymerases, Taq polymerase can withstand high temperatures. This makes it very important for the PCR process, since the temperatures are high to allow unravelling of the DNA helix.

(iii) **Role of ddNTP:**

* ddNTPs are used for early termination of the replication process.
* 3'-OH is missing in ddNTPs and hence they bring an abrupt stop to the polymerisation of DNA.

* ddNTPs are responsible for creating the short target sequences required in Sanger sequencing technique.

Q6. Primers corresponding to the vector sequence can be used in such a case. Since the vector is completely known, we can use a primers corresponding to the region near the insert sequence, such that the insert sequence is flanked by the primers on both ends. This will result in amplification of the unknown DNA effectively.

Q5. Advantages of NGS over cDNA sequencing include:

* NGS is significantly quicker and inexpensive in comparison to other sequencing techniques.
* Requires very little amounts of genetic sample.
* Requires no a priori knowledge of the genetic material
* High throughput with single-nucleotide resolution, with high reproducibility
* Sequences both coding and non-coding RNA. cDNA sequencing, on the other hand, only sequences the coding RNA (mRNA).