DATE: 27-03-22

FUNCTIONAL GENOMICS

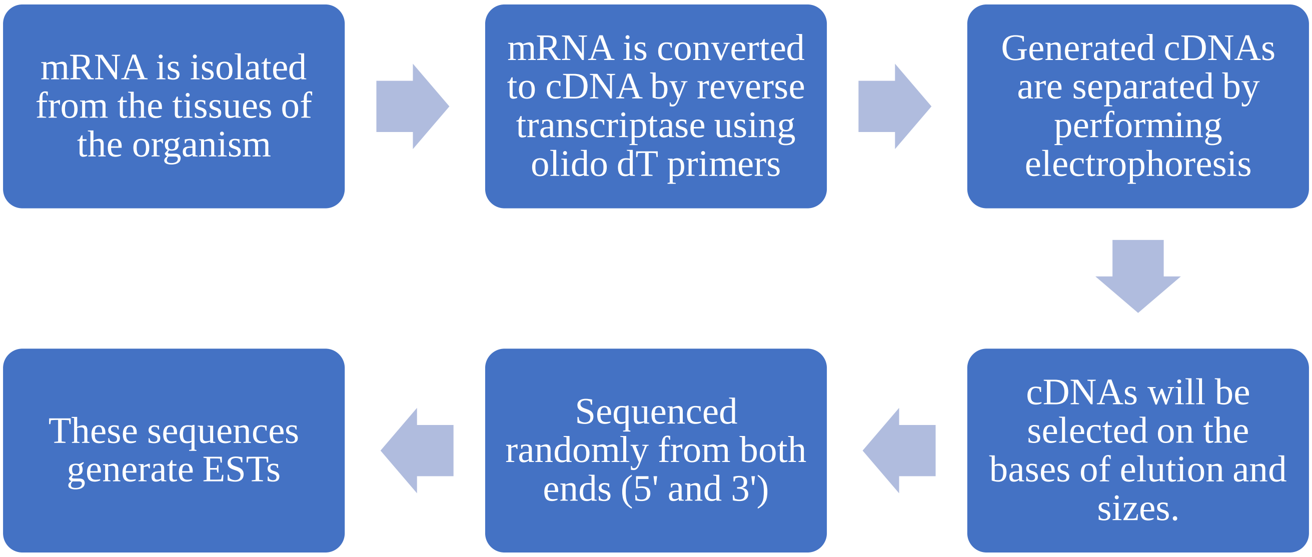
Q1) The GSS division contains (but is not limited to) the following types of data:

1. **Random “single-pass read” genome survey sequences:**
   - **Random "single pass read" genome survey sequences** are GSSs that **generated** along single pass **read by random selection**.
   - **Single-pass** sequencing with **lower fidelity** can be used on the **rapid accumulation** of genomic data but with a **lower accuracy**.
   - It includes **RAPD** (Random amplification of polymorphic DNA), **RFLP**(Restriction fragment length polymorphism), **AFLP**(Amplified fragment length polymorphism) and so on.
2. **Cosmid/BAC/YAC end sequences:**
   - **Cosmid/BAC/YAC** end sequences use “**Cosmid**” or “**Bacterial artificial chromosome**” or “**Yeast artificial chromosome**” to **sequence** the genome from the **end side**.
   - These **sequences act like** very **low copy plasmids**. Sometimes there is only **one copy per cell.**
   - **Cosmid/BAC/YAC** can also be used to get **bigger clone of DNA fragment** than vectors like **plasmid and phagemid**.
3. **Exon trapped genomic sequences:**
   - **Exon trapped sequence** is used to identify genes in **cloned DNA**, and this is achieved by **recognizing** and **trapping** carrier containing **exon sequence** of DNA.
   - **Exon trapping** has **two main features**: First, it is **independent of availability of the RNA** expressing target DNA. Second, **isolated sequences can be derived directly from clone** without knowing tissues expressing the gene which needs to be identified.
   - Since **fragment of DNA** can be **inserted into sequences**, if an **exon is inserted into intron**, the transcript will be **longer than usual** and this transcript can be **trapped by analysis**.
4. **Alu PCR sequences:**
   - **Alu repetitive element** is member of **Short Interspersed Elements** (SINE) in **mammalian genome.**
   - There are about **300 to 500 thousand copies** of Alu repetitive element in **human genome**, which means one Alu element exists in **4 to 6 kb averagely.**
   - **Alu PCR** is a "**DNA fingerprinting**" technique. This approach is **rapid and easy** to use. It is obtained from **analysis of many genomic loci** flanked by Alu repetitive elements, which are **non-autonomous retrotransposons** present in **high number** of copies in **primate genomes.**
5. **Transposon-tagged sequences:**
   - **Transposon** can be used as **tag for a DNA** with a **known sequence**.
   - Transposon can **appear at other locus** through **transcription** or **reverse transcription** by the **effect of nuclease**.

- This **appearance of transposon** proved that **genome** is **not statistical**, but always **changing the structure of itself**.
- There are **two advantages** by using transposon tagging:
    i. Transposon is **inserted into a gene sequence**; this insertion is **single and intact.**
    ii. **Many transposons** can be **found and eliminated** from tagged gene sequence when **transposase is analyzed**.

Q2) What are ESTs? Explain the protocol for EST generation

- **Expressed sequence tags** (ESTs) are short sequence reads, **typically within the range of 300–700 bp**, obtained from **randomly selected cDNA clones**.
- ESTs are **often generated** by **single-pass sequencing** of cDNA clones from one or both ends, **usually covering only a part of the transcript sequence**, and are relatively **prone to error.**
- They provide an **alternative to full-length cDNA sequencing.**
- Also, ESTs are an **inexpensive means of gene discovery.**
- The **randomly sequenced** ESTs allow us to make **gene discoveries** as they give **information of the transcribed regions of the gene**.
- **Protocol for EST Generation:**



mRNA is isolated from the tissues of the organism

mRNA is converted to cDNA by reverse transcriptase using olido dT primers

Generated cDNAs are separated by performing electrophoresis

cDNAs will be selected on the bases of elution and sizes.

Sequenced randomly from both ends (5' and 3')

These sequences generate ESTs

Q3) Write a short note:

1. STS:

- **Sequence-Tagges site (STS)** is a **relatively short**, **easily PCR-amplifed** sequence (200 to 500bp) which can be **specifically amplified** by PCR and **detected** in the **presence of all other genomic sequences** and whose **location** in the genome **is mapped**.
- The **STS concept** was introduced **by Olson** et al (1989).

- In **assessing the likely impact** of the **Polymerase Chain Reaction** (PCR) on **human genome research**, they recognized that **single-copy DNA sequences** of known map location could **serve as markers** for genetic and physical **mapping of genes** along the chromosome.
- The **advantage of STSs** over other **mapping landmarks** is that the means of **testing for the presence** of a particular STS can be completely **described as information in a database.**
- STS-based PCR produces a **simple and reproducible pattern** on agarose or polyacrylamide gel. In most cases STS markers are **co-dominant**, i.e., allow **heterorozygotes to be distinguished** from the **two homozygotes**.
- The **DNA sequence** of an STS **may contain repetitive elements**, sequences that **appear elsewhere** in the genome, but as long as the sequences at **both ends of the site are unique** and conserved, researches can **uniquely identify this portion of genome** using tools usually present in any laboratory.
- For example, some STSs can be used in **screening by PCR** to **detect microdeletions in Azoospermia** (AZF) genes in infertile men. Identification of **genes in elephants** could provide additional information for evolutionary studies and for **evaluating genetic diversity** in existing elephant populations.

2. SAGE:

- **Serial Analysis of Gene Expression** (SAGE) is a transcriptomic technique used by molecular biologists to produce a **snapshot of the messenger RNA** population in a **sample of interest** in the form of **small tags** that **correspond** to fragments of those transcripts.
- **Several variants** have been developed since, most notably a more robust version, **LongSAGE**, **RL-SAGE** and the most recent **SuperSAGE**. Many of these have **improved the technique** with the capture of **longer tags**, enabling **more confident identification** of a source gene.
- The **output** of SAGE is a list of **short sequence tags** and the **number of times** it is observed. **Using sequence databases**, a researcher can usually determine, with some confidence, from **which original mRNA** and therefore **which gene the tag was extracted.**
- **Statistical methods** can be applied to **tag and count lists** from different samples in order to **determine which genes are more highly expressed**. For example, a **normal tissue** sample can be compared **against a corresponding tumour** to determine which genes tend to be more active.
- **Once analysed**, SAGE data **provide** both a **qualitative** and **quantitative assessment** of potentially **every transcript** present in a particular cell or tissue type. Following the protocols, investigators should be able to **generate unique SAGE libraries**, which can be directly compared with our **reference library**.
- It works by **isolating short fragments** of genetic information from the **expressed genes** that are present in the **cell being studied**.

3. cDNA:

- **Complementary DNA** (cDNA) is a DNA **copy of a messenger RNA** (mRNA) molecule **produced by reverse transcriptase**, a DNA polymerase that can **use** either **DNA or RNA as a template**.

- cDNA is **not genomic DNA**, as the transcript of genomic RNA **lacks promoters and introns**.
- **Synthesis of cDNA from mRNA** poly A tail is used as **priming site**, a **short tag** of **oligo dT with a free 3'OH group** will bind and which will be **extended by reverse transcriptase** to create cDNA.
- **mRNA is then removed** which is achieved by treating it with **RNase enzyme** resulting in the **single stranded cDNA**.
- The **single stranded cDNA** needs to be converted to **double stranded cDNA** which is achieved with the help of **DNA polymerase**.
- The **free 3'OH group** for polymerase extension is provided by the **single stranded cDNA** itself by forming a **hairpin loop like structure**, which can later be **cleaved using nuclease.**
- **Restriction endonucleases** and **DNA ligase** are then used to **clone** the sequences into **bacterial plasmids**. The **cloned bacteria** are then selected, **commonly through** the use of **antibiotic selection**. Once selected, **stocks of the bacteria** are created which can **later be grown** and sequenced to **compile the cDNA library**.

4. DNA Microarray:

- The **DNA microarray** is a tool used to **determine** whether the **DNA** from a **particular individual** contains a **mutation in genes** like **BRCA1** and **BRCA2**.
- The **chip consists** of a **small glass plate encased in plastic**. Some companies manufacture microarrays using methods similar to those used to make **computer microchips**.
- **Each chip contains** thousands of **short**, **synthetic**, **single-stranded DNA sequences**, which together add up to the **normal gene in question**, and to **variants** (mutations) of **that gene** that have been found in the **human population.**
- **DNA microarrays** were used first only as a **research tool** but scientists continue today to **conduct large-scale population studies** for example, to determine **how often individuals** with a particular mutation actually **develop breast cancer**, or to identify the **changes in gene sequences** that are most often **associated with particular diseases**.
- Also, microarrays can also be used to **study the extent** to which certain genes are **turned on or off in cells and tissues**. In this case, instead of isolating DNA from the samples, **RNA is isolated and measured**.
- Basically, it determines whether an **individual possesses a mutation for a particular disease**, a scientist first obtains a **sample of DNA** from the patient's blood as well as a **control sample** along with the one that does not contain a mutation in the **gene of interest**.
- Then the **DNA is denatured** in the samples this process will **separate the two complementary strands** of DNA into **single-stranded molecules**. The next step is to **cut the long strands of DNA into smaller**, more **manageable fragments** and then to **label each fragment** by attaching a **fluorescent dye**. The individual's **DNA is labelled with green dye** and the **control** - or normal - **DNA** is **labelled with red dye**. **Both sets** of labelled DNAs are then **inserted**

into the chip and allowed **to hybridize** - or bind – to the **synthetic DNA on the chip.**

- If the **individual does not have a mutation** for the gene, **both** the red and green **samples will bind to the sequences on the chip** that represent the **sequence without the mutation**.

5. Gene index (Gene Indices):

- Several efforts are under way to **condense single-read expressed sequence tags** (ESTs) and **full-length transcript data** on a large scale by means of **clustering or assembly**.
- One **goal of these projects** is the **construction of gene indices** where **transcripts are partitioned into index classes** (or clusters) such that they are **put into the same index class** if and only if they **represent the same gene**.
- **Accurate gene indexing** facilitates **gene expression studies** and inexpensive and **early partial gene sequence** discovery through the **assembly of ESTs** that are **derived from genes** that have **yet to be positionally cloned** or obtained directly through **genomic sequencing**.
- The **three major gene indices** use **different EST clustering methods**:
  i. **TIGR Gene Index** uses a **stringent and supervised clustering method**, which generate **shorter consensus sequences** and **separate splice variants.**
  ii. **STACK** uses a **loose** and **unsupervised clustering method**, producing **longer consensus sequences** and including **splice variants in the same index.**
  iii. A **combination** of **supervised and unsupervised methods** with **variable levels of stringency** is used in **UniGene**. **No consensus sequences** are produced.

6. Functional Genomics:

- **Functional genomics** is a branch that **integrates molecular biology** and **cell biology studies**, and deals with the **whole structure**, **function** and **regulation** of a gene **in contrast** to the **gene-by-gene** approach of **classical molecular biology technique**.
- Functional genomics **focuses** on the **dynamic aspects** such as **gene transcription**, **translation**, **regulation** of gene expression and **protein–protein interactions**, **as opposed** to the **static aspects** of the **genomic information** such as **DNA sequence or structures**.
- Functional genomics is a **study** of how **genes and intergenic regions** of the genome **contribute to different biological processes**. A researcher in this field **typically studies genes or regions** on a genome-wide scale, with the hope of tightening them down to a **list of candidate genes** or regions to **analyse in more detail.**
- The **main objective** of functional genomics is to **resolve how the individual segment** of an organism work together to **produce a particular phenotype**.
- It **relies on the dynamic expression** of gene products in a **definite background** such as **during a disease** or at a **specific developmental stage**. Thus, functional genomics **involved in the development** of a model link **between genotype to phenotype.**

- There are **several specific functional genomics approaches** depending on what we are **focused on DNA level**, **RNA level**, **Protein level**, **Metabolite level**.
- These **fundamental methods** commonly **rely on genome-based sequence** datasets using **automated algorithms running in silico** for example, the **function and functional interactions** of unknown open reading frames (ORFs) can be **predicted** by using the **principle of conserved operons**.
- Functional genomics data are **predominantly stored** in one of two public databases such as **Array Express at EMBL-EBI**.
- Because of the **large quantity of data** produced by these techniques and the desire to find **biologically meaningful patterns**, bioinformatics is crucial to **analysis of functional genomics data**.
- Examples of **techniques in this class** are **data clustering** or **principal component analysis** for unsupervised machine learning as well as **artificial neural networks** or **support vector machines** for supervised machine learning.

7. Importance of the Human Genome Project:

- The project was **hugely significant** to biology and has **influenced biological research** ever since.
- The **main tasks** of the Human Genome Project were to **read and record** the **genetic instructions** contained within the human genome and **provide** that **information to researchers worldwide freely** and without restriction.
- The **sequenced human genome** is now a **crucial reference** for all of human biological research. It is a **template** against which **all human genomes are compared**. Since the **full human genome sequence became available** to the scientific community, **progress** of research into human health and disease has **accelerated dramatically**.
- The large-**scale genome research** has driven the technology advancement in **genetic testing**, **drug design**, **gene therapy**, and other **genetic related areas** such as pharmacogenetics.
- The **detailed genetic**, **physical**, and **sequence maps** developed by the Human Genome Project also will be **critical to understanding** the biological basis of **complex disorders** resulting from the **interplay of multiple genetic** and **environmental influences**, such as **diabetes**; **heart disease**; **cancer**; and **psychiatric illnesses**, including **alcoholism.**
- Human genome project **helps to understand** and **treat disease processes** at the **DNA level** are becoming the basis for a **new molecular medicine**. The discovery of **disease-associated genes** provides scientists with the **foundation for understanding** the course of **disease**, **treating disorders** with synthetic DNA or **gene products**, and assessing the **risk for future disease**.
- **Clinical tests** that detect **disease-causing mutations** in DNA are the most **immediate commercial application** of gene discovery. These tests may **positively identify** the genetic origin of an **active disease**, **foreshadow** the development of a **disease later in life**, or **identify healthy carriers** of recessive diseases **such as cystic fibrosis**.
- **Gene discovery** also provides **opportunities for developing** gene-based **treatment for hereditary and acquired diseases**.

- Although the project **reveals potential benefits**, it **raises ethical**, **legal**, and **social issues**. The outcomes of **individuals genetic information disclosure** may **lead to confidentiality and genetic discrimination issues**. In addition, **clinical relevance** of genetic testing and **psychological effect** from the results are debatable.