SEMESTER IV

17BTU413 GENOMICS AND PROTEOMICS PRACTICAL 4H - 2C

Total hours/week: L:0 T:0 P: 4 Marks: Internal: 40 External: 60 Total: 100

Practical

1. Use of SNP databases at NCBI and other sites
2. Use of OMIM database
3. Detection of Open Reading Frames using ORF Finder
4. Proteomics 2D PAGE database
5. Software for Protein localization.
6. Software for protein secondary sequencing prediction
7. Hydropathy plots
8. Native PAGE
9. SDS-PAGE

References

1. Glick, B.R., Pasternak, J.J., & Patten, C.L. (2010). *Molecular Biotechnology: Principles and Applications of Recombinant DNA* (4th ed.). American Society for Microbiology.
2. Primrose, S.B., & Twyman, R.M. (2006). *Principles of Gene Manipulation and Genomics* (7th ed.). Oxford: UK, Blackwell Publishing.
3. Pevsner, J. (2009). *Bioinformatics and Functional Genomics* (2nd ed.).John Wiley & Sons.
4. Sambrook & Russell (3rd ed.). (1989). *Molecular Cloning: A Laboratory Manual* (Vols. 1 to 3). Cold Spring Harbor Laboratory Press.

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University Established Under Section 3 of UGC Act 1956)
Coimbatore – 641 021.

LECTURE PLAN
DEPARTMENT OF BIOTECHNOLOGY

STAFF NAME: Dr Anil Kumar PK, Dr. T Sivaraman, Dr. J Anburaj, Dr. AR Sumayya
SUBJECT NAME: Genomics & Proteomics practicals
SUB.CODE:17BTU413
SEMESTER: IV CLASS: II B.Sc (BT)

S.No	Lecture Duration Period	List of Practical's
1	4	Use of SNP databases in NCBI and other sites
2	4	Use of OMIM databases
3	4	Detection of Open Reading Frame using ORF finder
4	4	Proteomics 2D PAGE database
5	4	Software for protein localization
6	4	Software for protein secondary structure prediction
7	4	Hydropathy plots
8	4	Native PAGE
9	4	SDS PAGE

CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413

1. Exploring the applications of SNP databases at NCBI and other sites

Aim: To explore unique features of SNP databases available in the public network.

Procedure

The National Center for Biotechnology Information has a pool of DNA databases along with many other databases. The protein of interest can be searched for and information regarding the same can be collected from this website. It works in collaboration with European Molecular Biology Laboratory and the DNA Data Bank of Japan.

- The amino acid sequence of proteins, nucleic acids and SNP would be downloaded in the FASTA format from the website **https://www.ncbi.nlm.nih.gov/**
- Once the FASTA file is downloaded, it can be opened in a word pad file and stored in txt format.

Sequence variations exist at defined positions within genomes and are responsible for individual phenotypic characteristics, including a person's propensity toward complex disorders such as heart disease and cancer. As tools for understanding human variation and molecular genetics, sequence variations can be used for gene mapping, definition of population structure, and performance of functional studies. The Single Nucleotide Polymorphism database (dbSNP) is a public-domain archive for a broad collection of simple genetic polymorphisms. This collection of polymorphisms includes single-base nucleotide substitutions (also known as single nucleotide polymorphisms or SNPs), small-scale multi-base deletions or insertions (also called deletion insertion polymorphisms or DIPs), and retroposable element insertions and microsatellite repeat variations (also called short tandem repeats or STRs).

The SNP database can be queried from the dbSNP homepage by using Entrez SNP, or by using the links to the six basic dbSNP search options located just below the text box at the top of the dbSNP homepage. Each of these six search options is described below.

SNP Submission Information Queries

Use this module to construct a query that will select SNPs based on submission records by laboratory (submitter), new data (called ―new batches‖ — this query limitation is more recent than a user-specified date), the methods used to assay for variation populations of interest and publication information.

dbSNP Batch Query

Use sets of variation IDs (including RefSNP (rs) IDs, Submitted SNP (ss) IDs, and Local SNP IDs) collected from other queries to generate a variety of SNP reports.

Locus Information Query

This search was originally accomplished by LocusLink, which has now been replaced by Entrez Gene. Entrez Gene is the successor to LocusLink and has two major differences that differentiate it from Locus Link: Entrez Gene is greater in scope (more of the genomes represented by NCBI Reference Sequences or RefSeqs) and Entrez Gene has been integrated for indexing and query in NCBI's Entrez system.

Between-Markers Positional Query

Use this query approach if you are interested in retrieving variations that have been mapped to a specific region of the genome bounded by two STS markers. Other map-based queries are available through the NCBI Map Viewer tool.

ADA Section 508-compliant Link

All links located on the left sidebar of the dbSNP homepage are also provided in text format at the bottom of the page to support browsing by text-based Web browsers. Suggestions for improving database access by disabled persons should be sent to the dbSNP development group at snp-admin@ncbi.nlm.nih.gov.

In addition to the NCBI, SCAN, yet another database for SNP, will also be explored. SCAN is a large-scale database of genetics and genomics data associated to a web-interface and a set of methods and algorithms that can be used for mining the data in it. The database contains two categories of single nucleotide polymorphism (SNP) annotations:

1. Physical-based annotation where SNPs are categorized according to their position relative to genes (intronic, inter-genic, etc.) and according to linkage disequilibrium (LD) patterns (an inter-genic SNP can be annotated to a gene if it is in LD with variation in the gene).
2. Functional annotation where SNPs are classified according to their effects on expression levels, i.e. whether they are expression quantitative trait loci (eQTLs) for that gene.

Result:

CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413

2. Exploring the use of OMIM database

Aim: To explore various unique features of OMIM database.

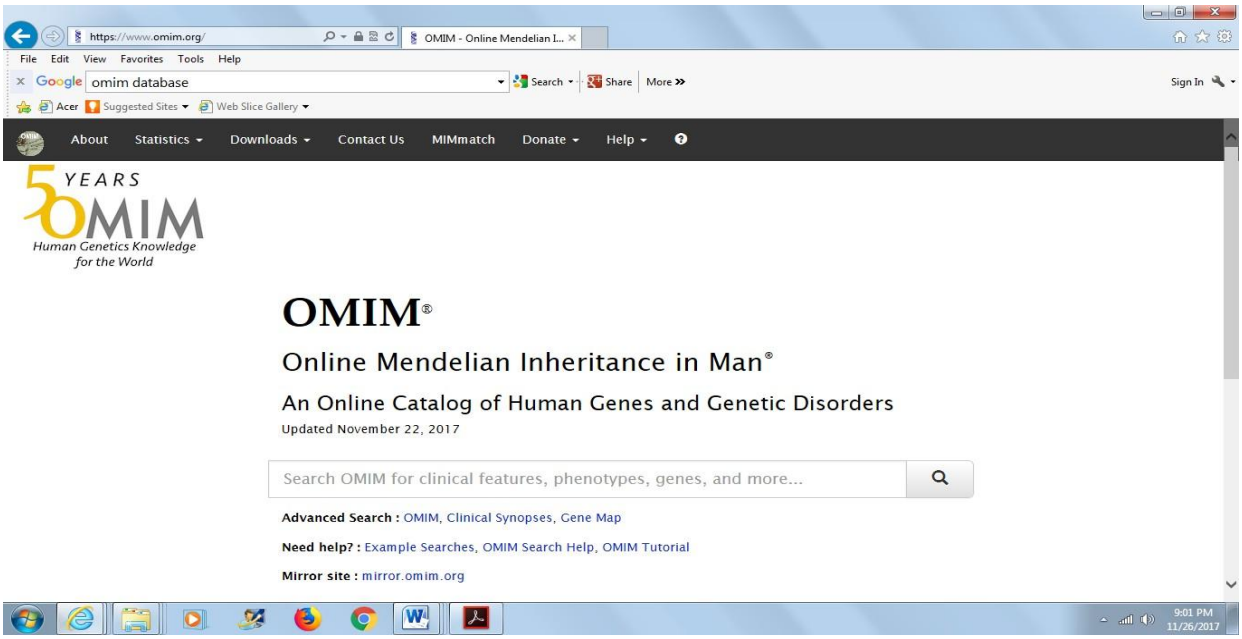
Procedure

Online Mendelian Inheritance in Man (https://www.omim.org/)

OMIM is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily. The full-text, referenced overviews in OMIM contain information on all known mendelian disorders and over 15,000 genes. OMIM focuses on the relationship between phenotype and genotype. It is updated daily, and the entries contain copious links to other genetics resources.

This database was initiated in the early 1960s by Dr. Victor A. McKusick as a catalog of mendelian traits and disorders, entitled Mendelian Inheritance in Man (MIM). Twelve book editions of MIM were published between 1966 and 1998. The online version, OMIM, was created in 1985 by a collaboration between the National Library of Medicine and the William H. Welch Medical Library at Johns Hopkins. It was made generally available on the internet starting in 1987. In 1995, OMIM was developed for the World Wide Web by NCBI, the National Center for Biotechnology Information. OMIM is authored and edited at the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, under the direction of Dr. Ada Hamosh.

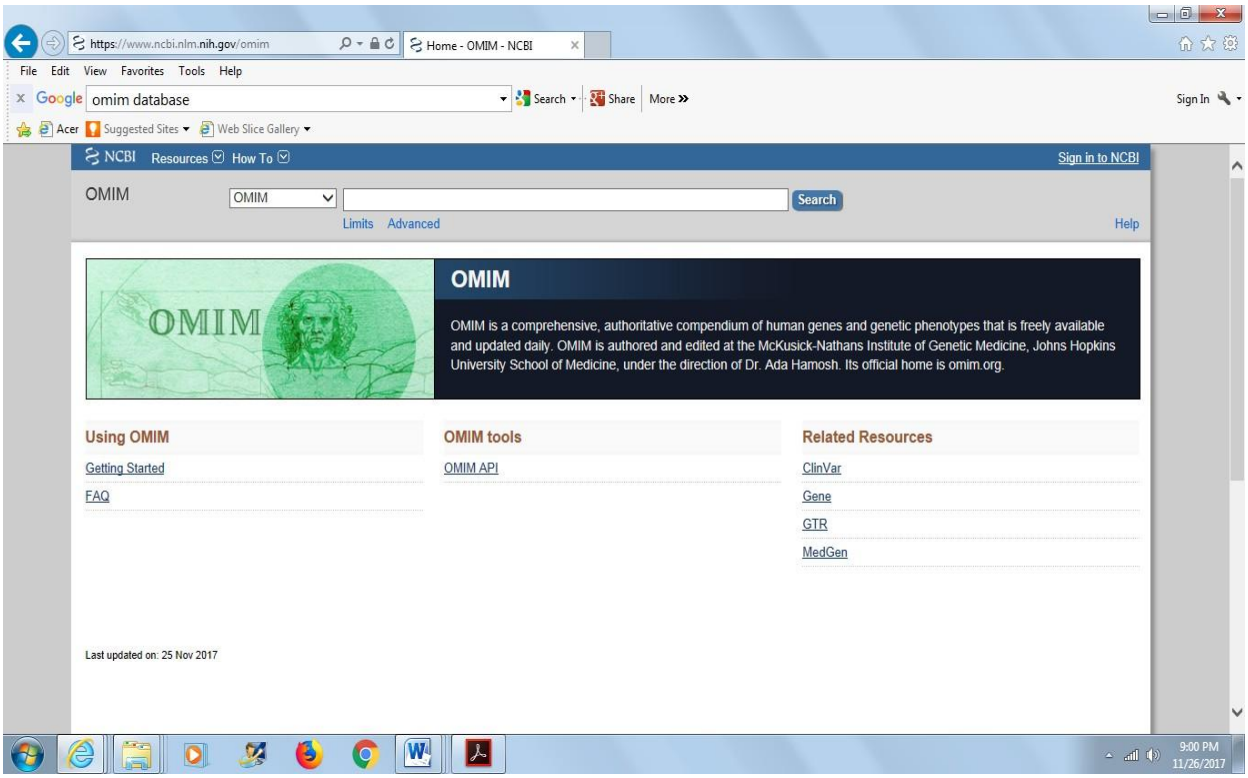
CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413



OMIM @ NCBI (https://www.ncbi.nlm.nih.gov/omim)

OMIM is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily. OMIM is authored and edited at the McKusick-Nathans Institute of Genetic Medicine, John Hopkins University, School of medicine under the direction of Dr. Ada Hamosh. Its official home is omim.org.

CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413



Result:

CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413

3. Detection of Open Reading Frames using ORF finder and other computational tools

Aim: To detect the gene structure using various gene prediction tools.

Procedure

Fetch the nucleotide sequence with accession ID from EMBL database and save the file in FASTA format.

The following gene – finding programs would be employed and the results would be comprehensively analyzed.

GENSCAN

Web server: http://genes.mit.edu/GENSCAN.html

GeneID

Web server: http://genome.crg.es/geneid.html

GRAIL

Web server: http://pbil.univ-lyon1.fr/members/duret/cours/insa2004/exercise4/pgrail.html

GrailEXP is a software package that predicts exons, genes, promoters, polyas, CpG islands, EST similarities, and repetitive elements within DNA sequence. GrailEXP is used by the Computational Biosciences Section at Oak Ridge National Laboratory to annotate the entire known portion of the human genome (including both finished and draft data).

GeneMark

Web server: http://exon.gatech.edu/Genemark/genemarks.cgi

ORF Finder

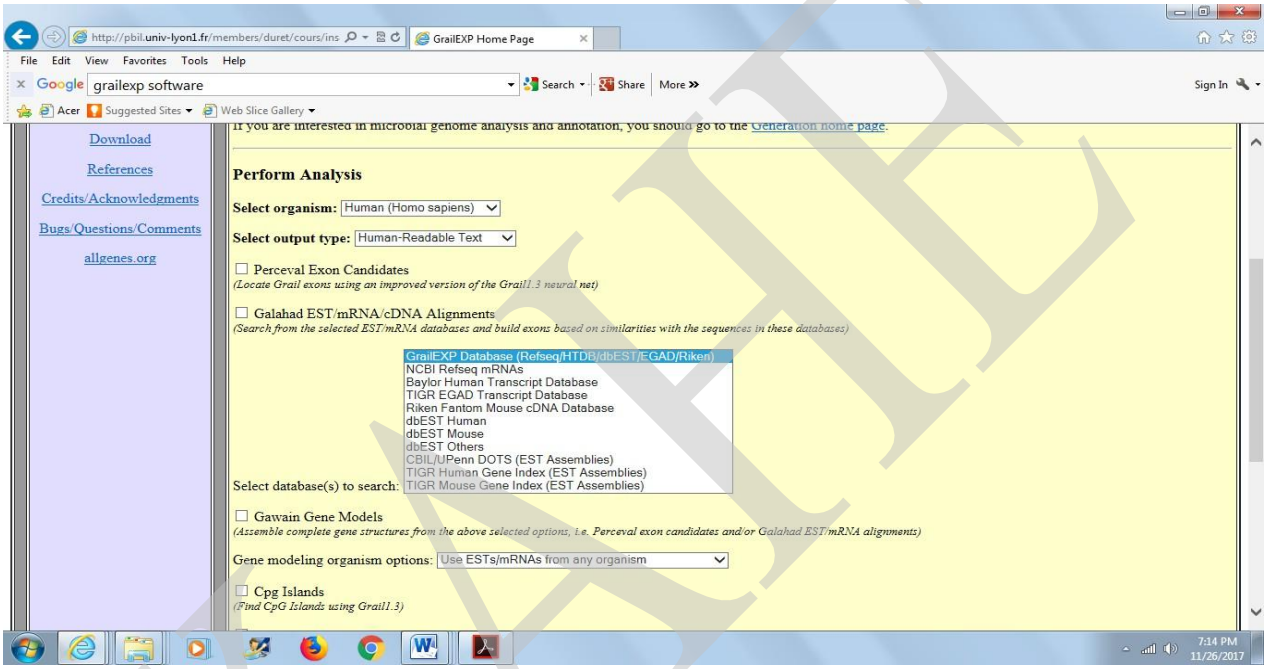
Web server: http://www.bioinformatics.org/sms2/orf_find.html

ORF Finder

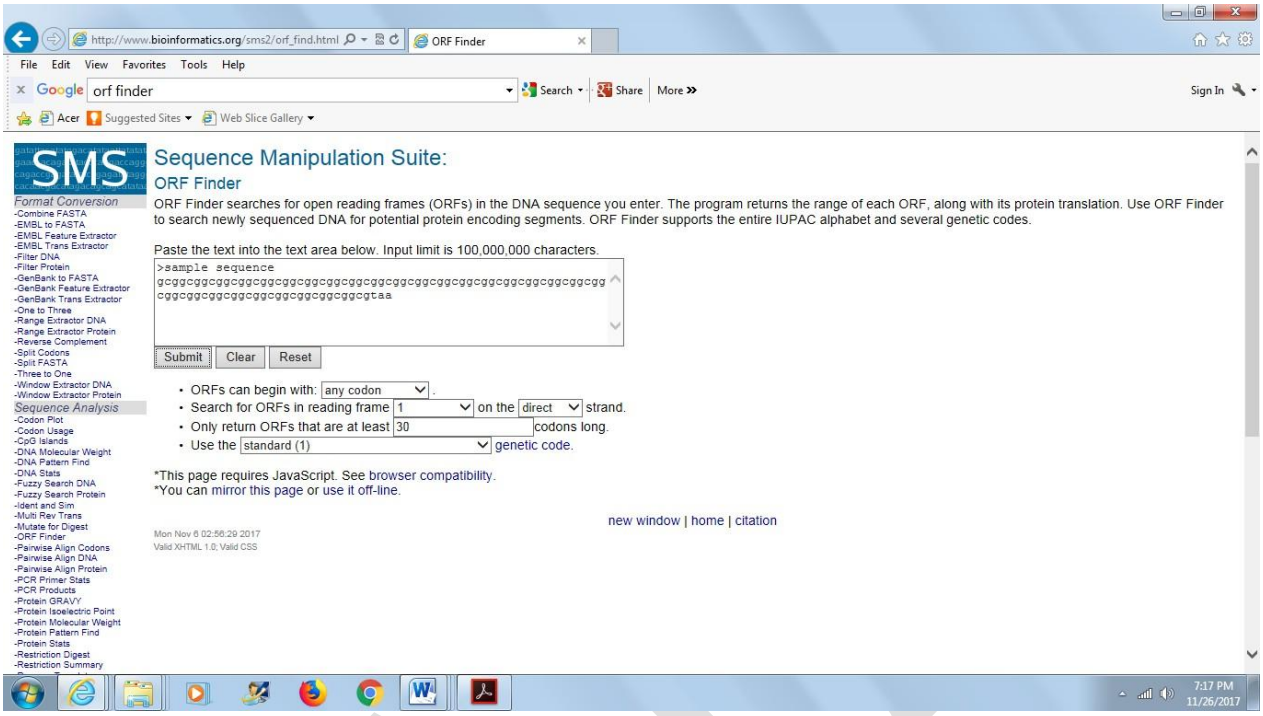
ORF Finder searches for open reading frames (ORFs) in the DNA sequence you enter. The program returns the range of each ORF, along with its protein translation. Use ORF Finder to search newly sequenced DNA for potential protein encoding segments. ORF Finder supports

the entire IUPAC alphabet and several genetic codes.

The homepage of a few gene prediction tools mentioned above are given for reference over here.



CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413



Result:

CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413

4. Estimation of hydropathy values of proteins and drawing hydropathy plots using various algorithms

Aim: To calculate residue-specific hydropathy values for the primary structures of given protein sequences (amino acid sequences).

Procedure

Retrieve protein sequences from NCBI database (https://www.ncbi.nlm.nih.gov/) and ExPASy database (http://web.expasy.org/protscale/).

Save he retrieved sequences in FASTA format and Accession IDs for all the sequences as well.

The Hydropathy values of the proteins can be calculated using various algorithms as shown below herein.

- Hphob. OMH / Sweet et al.
- Hphob. / Hopp & Woods
- Hphob. / Kyte & Doolittle
- Hphob. / Manavalan et al.
- Hphob. / Abraham & Leo
- Hphob. / Black
- Hphob. / Bull & Breese
- Hphob. / Fauchere et al.
- Hphob. / Guy
- Hphob. / Janin
- Hphob. / Miyazawa et al.
- Hphob. / Rao & Argos
- Hphob. / Roseman

- Hphob. / Tanford
- Hphob. / Wolfenden et al.
- Hphob. / Welling & al
- Hphob. HPLC / Wilson & al
- Hphob. HPLC / Parker & al
- Hphob. HPLC pH3.4 / Cowan
- Hphob. HPLC pH7.5 / Cowan

The parameter setting can be manipulated as per the following features.

ProtScale allows you to compute and represent the profile produced by any amino acid scale on a selected protein.An amino acid scale is defined by a numerical value assigned to each type of amino acid. The most frequently used scales are the hydrophobicity or hydrophilicity scales and the secondary structure conformational parameters scales, but many other scales exist which are based on different chemical and physical properties of the amino acids. This program provides 50 predefined scales entered from the literature. You can set several parameters that control the computation of a scale profile, such as the window size, the weight variation model, the window edge relative weight value, and scale normalization.

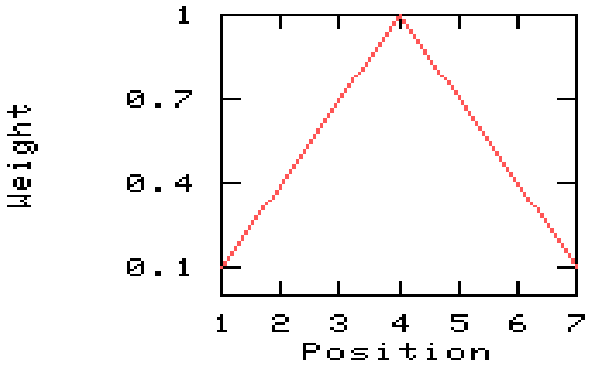
Window size

The window size is the length of the interval to use for the profile computation. When computing the score for a given residue i, the amino acids in an interval of the chosen length, centered around residue i, are considered. In other words, for a window size n, we use the i - (n-1)/2 neighboring residues on each side of residue i to compute the score for residue i. The score for residue i is the sum of the scale values for these amino acids, optionally weighted according to their position in the window.

Relative weight of the window edges

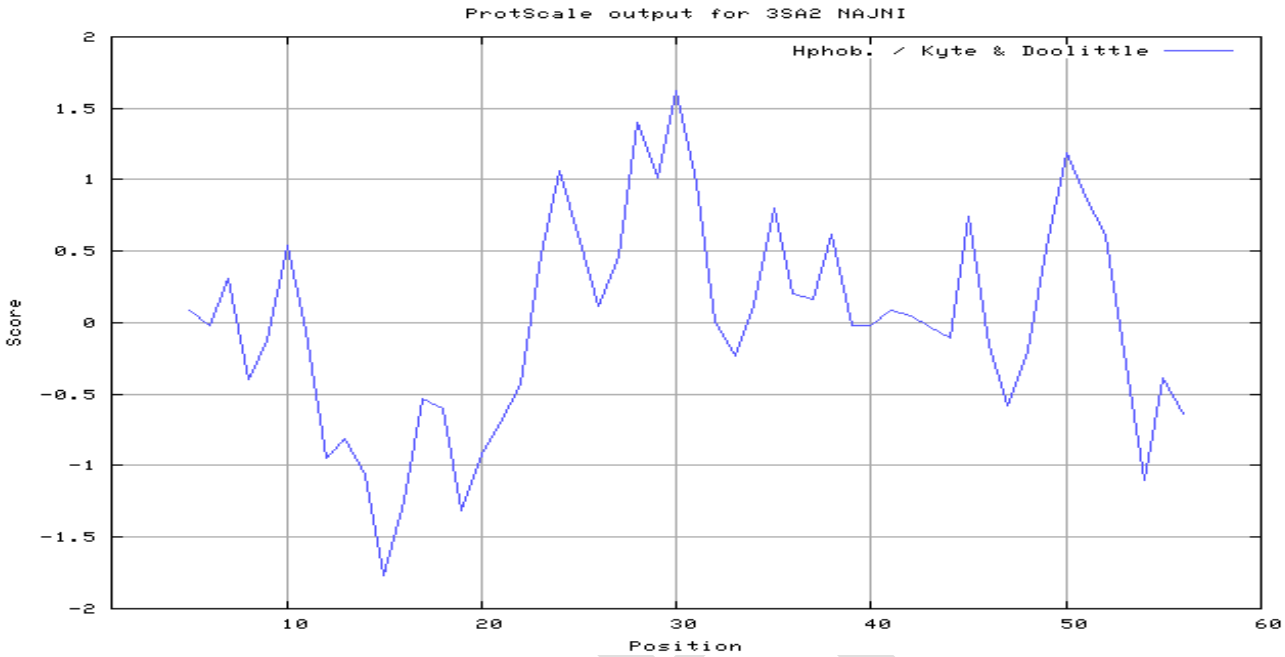
The central amino acid of the window always has a weight of 100%. By default, the amino acids at the remaining window positions have the same weight, but you can make the residue at the center of the window have a larger weight than the others by setting the weight value for the residues at the beginning and end of the interval to a value between 0 and 100%. The decrease in weight between the weight of the center and that of the edges will either be linear or exponential depending on the setting of the weight variation model option.

Weight variation modelIn the following example, the window size is 7, and the window edge relative weight value is 10%. Linear weight variation model - This option divides the weight into equally spaced intervals between 100% and the window edge relative weight (here: 10%).



Typical output for a give protein sequence is as shown below.

CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413



Results:

CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413

5. Prediction and analyses of secondary structures of proteins

Aim: To predict various secondary structural elements of proteins on the basis of their amino acid sequences.

Procedure

Retrieve protein sequences from NCBI database (https://www.ncbi.nlm.nih.gov/), ExPASy database (http://web.expasy.org/protscale/) and as well from PDB database (https://www.rcsb.org/pdb/home/home.do), if the 3D structures of the proteins are available.

Save he retrieved sequences in FASTA format and Accession IDs for all the sequences as well.

The 3D structures should be stored in .txt format using Notepad.

The secondary structures of the proteins would be estimated by means of following computational tools.

- AGADIR - An algorithm to predict the helical content of peptides
- APSSP - Advanced Protein Secondary Structure Prediction Server
- CFSSP - Chou & Fasman Secondary Structure Prediction Server
- GOR - Garnier et al, 1996
- HNN - Hierarchical Neural Network method (Guermeur, 1997)
- HTMSRAP - Helical TransMembrane Segment Rotational Angle Prediction
- Jpred - A consensus method for protein secondary structure prediction at University of Dundee

Some of them are statistical-based tools and others are knowledge-based tools. Moreover, a few of them use more than one prediction methods and deliver consensus data. In addition, the tool such as AGADIR is a prediction algorithm based on the helix/coil transition theory. The Agadir predicts the helical behaviour of monomeric peptides. It only considers short range interactions. Conditions such as pH, temperature and ionic strength are used in the calculation. Modifications of the termini are also allowed. In other words, the Agadir is not a program to predict secondary structure of proteins.

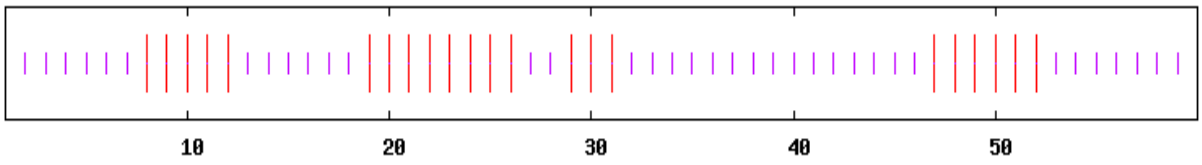
Outputs of each of the tools mentioned above are unique and reliability of the tools are also different from each other. The output of GOR tool is shown below herein as reference.

GOR4 result

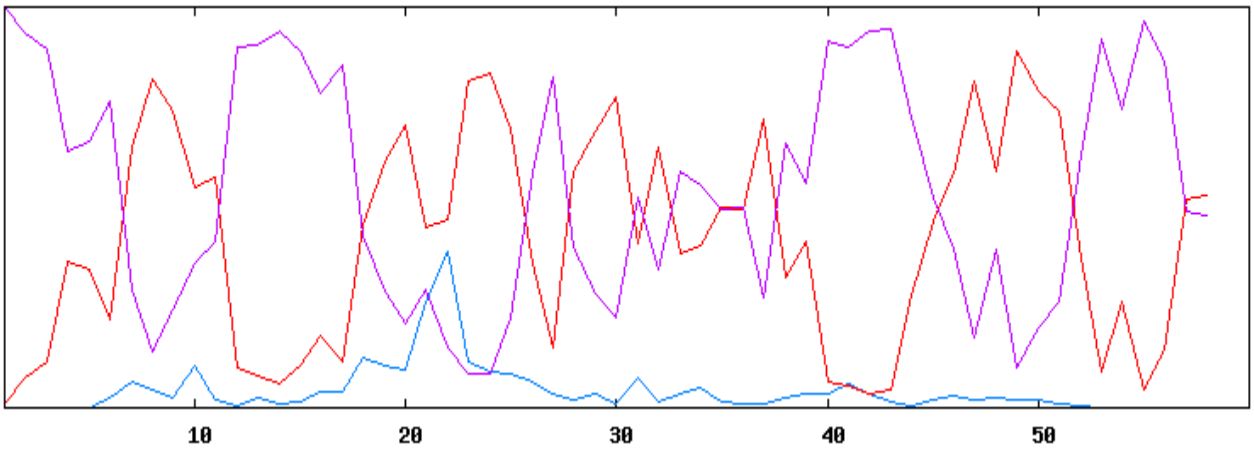
Abstract GOR secondary structure prediction method version IV, J. Garnier, J.-F. Gibrat, B. Robson, Methods in Enzymology,R.F. Doolittle Ed., vol 266, 540-553, (1996)

View GOR4 in: [AnTheProt (PC) , Download...] [HELP]

```
         10         20         30         40         50         60
          |          |          |          |          |          |
LKCNKLVPLFYKTCPAGKNLCYKMFMVSNLTVPVKRGCIDVCPKNSALVKYVCCNTDRCN
ccccccceeeeeccccccceeeeeeeecceeecccccccccccccccceeeeeccccccc
Sequence length :    60
GOR4 :
   Alpha helix      (Hh) :      0 is    0.00%
   3₁₀ helix        (Gg) :      0 is    0.00%
   Pi helix         (Ii) :      0 is    0.00%
   Beta bridge      (Bb) :      0 is    0.00%
   Extended strand  (Ee) :     22 is   36.67%
   Beta turn        (Tt) :      0 is    0.00%
   Bend region      (Ss) :      0 is    0.00%
   Random coil      (Cc) :     38 is   63.33%
   Ambiguous states (?)  :      0 is    0.00%
   Other states          :      0 is    0.00%
```



CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413



Results:

CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413

6. Exploring proteomic 2D-PAGE databases

Aim: Exploring various databases that are meant for viewing and analyzing SDS-PAGE and as well 2D PAGE data.

Procedure

A few selected 2D PAGE databases will be downloaded and their unique features towards the data analyzes would be systematically explored. For instance, the _Make2D-DB II‘ and its applications are explained below herein.

Make2D-DB II is an environment to create, convert, publish, interconnect and keep up-to-date two-dimensional gel electrophoresis (2D-PAGE) databases. It converts data from various formats into a relational format. The tool offers the possibility to automatically update data related to numerous external data resources in a highly consistent manner. It is also possible with this tool to dynamically interconnect several remote databases or projects to form a virtual global database accessible from one single entry point. Make2D-DB II can also be used without any local data, as a personal and a configurable Web portal, to link simultaneously to several remote 2D resources. Finally, this tool can be easily used to build up a 2-DE repository, accessible by any one from the Web.

Make2D-DB II is an open source software (cf. license) to create, convert, publish, interconnect and keep up-to-date 2D-PAGE databases. With this tool, one can easily convert any personal federated 2-DE database, or any 2D-PAGE database following the SWISS-2DPAGE conventions, into a more reliable format. The tool also handles XML exports from Melanie, common spreadsheets reports (e.g. Excel CSV exports), as well as simple text lists. It is also possible to create new relational databases from scratch. The tool runs on most UNIX-based operating systems (Linux, Solaris/SunOS, IRIX). Being continuously maintained, the tool is evolving in concert with the current Proteomics Standards Initiative of the Human Proteome Organization (HUPO).

Some 2-DE databases constructed with Make2D-DB II:

- SWISS-2DPAGE - The world famous reference database -- Swiss Institute of Bioinformatics
- WORLD-2DPAGE Repository A public standards-compliant repository for gel-based Proteomics data linked to protein identification published in the literature -- Swiss Institute of Bioinformatics
- WORLD-2DPAGE Portal - A dynamic portal to query simultaneously world-wide gel-based Proteomics databases -- Swiss Institute of Bioinformatics
- UCD-2DPAGE - 2D-PAGE Database of Escherichia coli -- Conway Institute Proteome Research Centre (CIPRC) University College Dublin, Ireland
- SIENA-2DPAGE - Multi-species 2D-PAGE Database -- Department of Molecular Biology, University of Siena, Italy
- KAIKO-2DDB - The Silkworm Genome Research Program -- National Institute of Agrobiological Sciences, Japan.
- COMPLUYEAST 2D-PAGE DATABASE - The COMPLUYEAST 2D-PAGE DATABASE -- Department Microbiology II, Universidad Complutense Madrid, Spain
- REPRODUCTION-2DPAGE - 2D-PAGE database (Human ovary, Mouse testis) -- Lab of Reproductive Medicine, Nanjing Medical University, PR. China.
- 2DBase - 2D-PAGE Database of Escherichia coli -- Fermentation Engineering Group, University of Bielefeld, Germany

Result:

CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413

7. Localizations of Proteins by computational methods

Aim: To use computational applications for finding out localizations of proteins.

Procedure

Retrieve protein sequences from NCBI database (https://www.ncbi.nlm.nih.gov/), ExPASy database (http://web.expasy.org/protscale/) and as well from PDB database (https://www.rcsb.org/pdb/home/home.do), if the 3D structures of the proteins are available.

Save he retrieved sequences in FASTA format and Accession IDs for all the sequences as well. The 3D structures should be stored in .txt format using Notepad.

A few or all of the following web-server and tools would be explored in order to predict the localization of proteins under interest.

HMMTOP

Categories: proteomics, (protein modifications) - Software type(s): website - tool

Prediction of transmembranes helices and topology of proteins.

MITOPROT [CBS]

Categories: proteomics, (protein modifications) - Software type(s): website - tool

MitoProt calculates the N-terminal protein region that can support a mitochondrial targeting sequence and the cleavage site.

PredictProtein

Categories: proteomics, (function analysis, sequence sites, features and motifs, protein modifications, protein interactions) - Software type(s): website - tool

PredictProtein integrates feature prediction for secondary structure, solvent accessibility, transmembrane helices, globular regions, coiled-coil regions, structural switch regions, B-values, disorder regions, intra-residue contacts, protein-protein and protein-DNA binding sites, sub-cellular localization, domain boundaries, beta-barrels, cysteine bonds, metal binding sites and disulphide bridges.

PSORT

Categories: proteomics, (function analysis) - Software type(s): website - tool

PSORT family of programs for subcellular localization prediction

SecretomeP [CBS]

Categories: proteomics, (protein modifications) - Software type(s): website - tool

The SecretomeP 2.0 server produces ab initio predictions of non-classical i.e. not signal peptide triggered protein secretion. The method queries a large number of other feature prediction servers to obtain information on various post-translational and localizational aspects of the protein, which are integrated into the final secretion prediction.

SignalP [CBS]

Categories: proteomics, (protein modifications) - Software type(s): website, CLI - tool

SignalP predicts the presence and location of signal peptide cleavage sites in amino acid sequences from different organisms: Gram-positive prokaryotes, Gram-negative prokaryotes, and eukaryotes. The method incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks.

TargetP [CBS]

Categories: proteomics, (function analysis, protein modifications) - Software type(s): website - tool

TargetP 1.1 predicts the subcellular location of eukaryotic proteins. The location assignment is based on the predicted presence of any of the N-terminal presequences: chloroplast transit peptide (cTP), mitochondrial targeting peptide (mTP) or secretory pathway signal peptide (SP).

TMPred [SIB: Vital-IT group]

Categories: proteomics, (protein structure) - Software type(s): website, CLI - tool

The TMpred program makes a prediction of membrane-spanning regions and their orientation. The algorithm is based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins

Result:

CLASS : II B. Sc., BT
COURSE NAME : GENOMICS AND PROTEOMICS PRACTICAL
BATCH : 2017 – 2020
COURSE CODE : 17BTU413

8. Native Polyacrylamide Gel Electrophoresis

Aim: To separate and identify various proteins from a given mixtures by using Native – PAGE method.

Principle: Polyacrylamide gel electrophoresis (PAGE), describes a technique widely used in biochemistry, forensics, genetics, molecular biology and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility. Mobility is a function of the length, conformation and charge of the molecule. As with all forms of gel electrophoresis, molecules may be run in their native state, preserving the molecules' higher-order structure. This method is called native-PAGE.

Procedure:

Separating gel buffer

Sodium dodecyl sulphate 1 gm

Tris 45.40 gm

Dissolved in 500 ml double distilled water, pH 8.9

Stacking gel buffer

Tris 6.06 gm

Dissolved in 190ml of double distilled water, pH was adjusted to 6.8 with 1N HCl, then made up to 200 ml.

Tank Buffer

Glycine 8.64 gm

Tris 1.8gm

Dissolved in 600ml double distilled water, pH was adjusted to 8.3 with 1N HCl

Sample Buffer (5X)

60mm Tris HCl, pH 6.8

25% Glycerol

0.1% Bromophenol blue and made up to 10 ml

Stock acrylamide for separating gel

Acrylamide 30.0 gm

Bisacrylamide 0.4 gm

The above materials were dissolved in 50 ml of double distilled water and was made up to 100 ml. Solution was filtered through a Whatman No.1 filter paper.

Stock acrylamide for stacking gel

Acrylamide 15 g

bis-acrylamide 0.4 g

The materials were dissolved in 30 ml of distilled water and made up to 50 ml. The solution was filtered through Whatman No.1 filter paper and stored at 4°C in a dark brown bottle.

Ammonium persulfate (APS)

Ammonium persulfate (100 mg) was dissolved in 1ml of distilled water. This solution was prepared fresh every time.

Preparation of separating gel (30 ml)

Following solutions were mixed together: Separating gel buffer: 15 ml, acrylamide stock for separating gel: 12 ml, Ammonium persulfate: 50 µl, TEMED: 50 µl were poured between two clean glass plates and layered with 5ml of n-butanol and allowed to polymerize for 30 min. After polymerization n-butanol was removed and the gel surface was rinsed with water. The comb was inserted carefully into gel sandwich until bottom of teeth reached top of front plate.

Preparation of stacking gel (10 ml)

Following solutions were mixed together: Stacking gel buffer: 1.25ml, stock acrylamide for stacking gel: 0.75ml, Ammonium persulfate: 50 µl, TEMED: 50 µl, distilled water 6 ml. The solution was poured over the separating gel and allowed to polymerize for 30 min.

Sample preparation

To the sample (200µl of the E. coli total cell extract or any other sample) sample buffer was added (to get 1X sample buffer in a mixture), vortexed thoroughly and boiled for 1 min,

cooled and spun. 50 µl of sample was loaded (depending on the protein concentration) into each well.

Electrophoresis conditions

The gel was run at 30mA constant current until the tracking Bromophenol blue dye reached the end of the gel (about 3 hrs).

Staining and destaining of the gel

The gels were stained in 0.05% (w/v) Coomassie brilliant blue R-250 in acetic acid/methanol/water (10:25:65%v/v), for 0.5 –18 h and destained repeatedly in the same solution without dye (methanol can be replaced with ethanol).

Result:

9. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

Aim: To separate and identify various proteins from a given mixtures by using SDS – PAGE method.

Principle: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a method of separating molecules based on the difference of their molecular weight. At the pH at which gel electrophoresis is carried out the SDS molecules are negatively charged and bind to proteins in a set ratio, approximately one molecule of SDS for every 2 amino acids. In this way, the detergent provides all proteins with a uniform charge-to-mass ratio. By binding to the proteins the detergent destroys their secondary, tertiary and/or quaternary structure denaturing them and turning them into negatively charged linear poly peptide chains. When subjected to an electric field in PAGE, the negatively charged poly peptide chains travel toward the anode with different mobility. Their mobility, or the distance traveled by molecules, is inversely proportional to the logarithm of their molecular weight. By comparing the relative ratio of the distance traveled by each protein to the length of the gel (Rf) one can make conclusions about the relative molecular weight of the proteins, where the length of the gel is determined by the distance traveled by a small molecule like a tracking dye.

Procedure:

Separating gel buffer

Sodium dodecyl sulphate 1 gm

Tris 45.40 gm

Dissolved in 500 ml double distilled water, pH 8.9

Stacking gel buffer

Sodium dodecyl sulphate 0.40 gm

Tris 6.06 gm

Dissolved in 190ml of double distilled water, pH was adjusted to 6.8 with 1N HCl, then made up to 200 ml.

Tank Buffer

Glycine 8.64 gm

Tris 1.8gm

Sodium dodecyl sulphate 0.6gm

Dissolved in 600ml double distilled water, pH was adjusted to 8.3 with 1N HCl

Sample Buffer (5X)

60mm tris HCl, pH 6.8

25% Glycerol

2% sodium dodecyl sulphate

14.4mm 2-Mercaptoethanol

0.1% Bromophenol blue and made up to 10 ml

Stock acrylamide for separating gel

Acrylamide 30.0 gm

Bisacrylamide 0.4 gm

The above materials were dissolved in 50 ml of double distilled water and was made up to 100 ml. Solution was filtered through a Whatman No.1 filter paper.

Stock acrylamide for stacking gel

Acrylamide 15 g

bis-acrylamide 0.4 g

The materials were dissolved in 30 ml of distilled water and made up to 50 ml. The solution was filtered through Whatman No.1 filter paper and stored at 4°C in a dark brown bottle.

Ammonium persulfate (APS)

Ammonium persulfate (100 mg) was dissolved in 1ml of distilled water. This solution was prepared fresh every time.

Preparation of separating gel (30 ml)

Following solutions were prepared for the experiment: Separating gel buffer: 15 ml, acrylamide stock for separating gel: 12 ml, Ammonium persulfate: 50 µl, TEMED: 50 µl were poured between two clean glass plates and layered with 5ml of n-butanol and allowed to

polymerize for 30 min. After polymerization n-butanol was removed and the gel surface was rinsed with water. The comb was inserted carefully into gel sandwich until bottom of teeth reached top of front plate.

Preparation of stacking gel (10 ml)

Following solutions were mixed together: Stacking gel buffer: 1.25ml, stock acrylamide for stacking gel: 0.75ml, Ammonium persulfate: 50 µl, TEMED: 50 µl, distilled water 6 ml. The solution was poured over the separating gel and allowed to polymerize for 30 min.

Sample preparation

To the sample (200µl of the *E. coli* total cell extract or any other sample) sample buffer was added (to get 1X sample buffer in a mixture), vortexed thoroughly and boiled for 1 min, cooled and spun. 50 µl of sample was loaded (depending on the protein concentration) into each well.

Electrophoresis conditions

The gel was run at 30 mA constant current until the tracking Bromophenol blue dye reached the end of the gel (about 3 hrs).

Staining and destaining of the gel

The gels were stained in 0.05% (w/v) Coomassie brilliant blue R-250 in acetic acid/methanol/water (10:25:65%v/v), for 0.5 –18 h and destained repeatedly in the same solution without dye (methanol can be replaced with ethanol).

Result: