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Examination paper for MOL3007
Functional Genomics

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Question 1 - (25p)

a) Next Generation Sequencing (NGS) represents a set of technologies that will have a large impact on future functional genomics and systems biology.

Describe two different categories of large scale molecular characterization that is currently being studied by NGS (different classes of molecules or different classes of modification(s) of molecules).

For each category, give an example of a biological and/or biomedical question that can be answered by using this line of investigation and explain the added value from high throughput sequencing. (8p)

b) A research group has generated a knowledge resource for breast cancer with a functionality like the one shown in the figure below

Explain and discuss biomedical and bioinformatic strategies that this research group could have used in order to obtain the indicated network understanding of breast cancer. (7p)

Discuss to which extent the knowledge obtained through this knowledge resource could be used for breast cancer patients in general, to subgoups of breast cancer patients or to individual patients. (5p)

What are the implications for the health care system for each of the scenarios mentioned in the above paragraph? (5p)

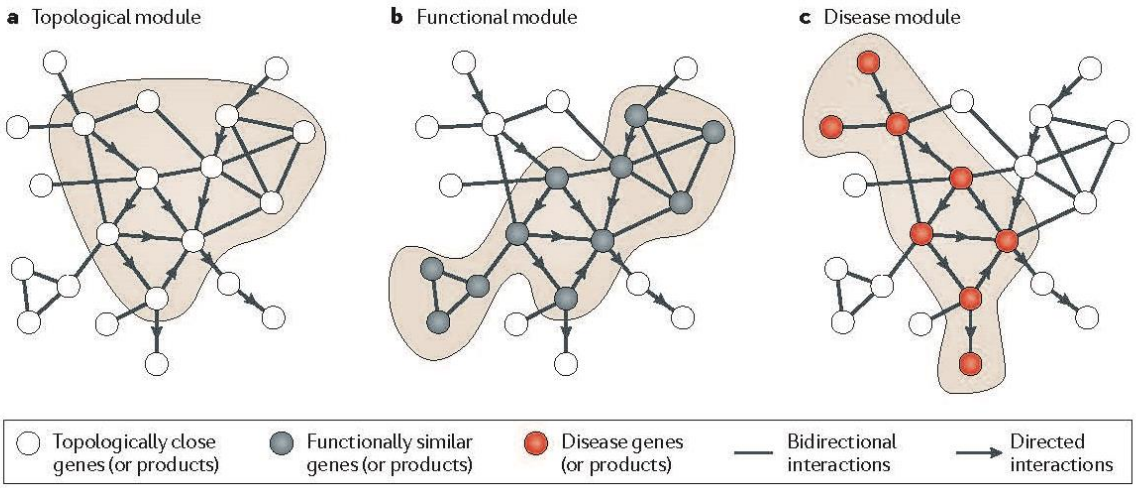

Figure 2 from "Network Medicine: a network-based approach", Barabasi and Loscalzo, NatRevGen, 2011

Question 2 - (10p)

You have been assigned a task to identify and obtain as broad sequence coverage as possible of a 60 kDa protein from a cell extract as quickly as possible. In the lab you have the tools listed below available. Outline a proper strategy to accomplish this task and briefly explain your choice of tools

- Gel electrophoresis equipment (1D and 2D)
- MALDI MS and MS/MS mass spectrometer
- Electrospray MS and MS/MS mass spectrometer
- Gel filtration column and affinity column
- HPLC system and reverse-phase (C-18) column
- Online protein database search engines
- Proteolytic enzymes (including trypsin)

Question 3 - (10p)

a) A tryptic peptide VKEGMNIVEAMER (Monoisotopic molecular mass = 1504.7) was analysed by MALDI MS. What do you expect the mass spectrum to look like? Draw the mass spectrum and indicate the m/z values and the approximated isotope distribution for the peptide ion species.

b) The same peptide VKEGMNIVEAMER was analysed by ESI MS. Draw the mass spectrum in the m/z range 400 to 1700 and indicate the m/z values and isotope distribution for the ion signals that you expect to see in the spectrum.

c) The doubly charged (i.e. doubly protonated) peptide VKEGMNIVEAMER was sequenced by ESI MS/MS. Draw the mass spectrum as you expect it to appear. Indicate the types of fragment ion series that you expect to see. Explain the appearance of the MS/MS spectrum, including assignments of peaks. A Table of amino acid residue masses is provided below.

Amino Acid Residues

Amino acid	Three (one)-letter code	Monoisotopic mass	Average mass
Glycine	Gly (G)	57.021	57.052
Alanine	Ala (A)	71.037	71.079
Serine	Ser (S)	87.032	87.078
Proline	Pro (P)	97.053	97.117
Valine	Vat (V)	99.068	99.133
Threonine	Thr (T)	101.048	101.105
Cysteine	Cys (C)	103.009	103.145
Isoleucine	Ile (I)	113.084	113.160
Leucine	Leu (L)	113.084	113.160
Asparagine	Asn (N)	114.043	114.104
Aspartic acid	Asp (D)	115.027	115.089
Glutamine	Gln (Q)	128.059	128.131
Lysine	Lys (K)	128.095	128.174
Glutamic acid	Glu (E)	129.043	129.116
Methionine	Met (M)	131.040	131.199
Histidine	His (H)	137.059	137.142
Phenylalanine	Phe (F)	147.068	147.177
Arginine	Arg (R)	156.101	156.188
Tyrosine	Tyr (Y)	163.063	163.176
Tryptophan	Trp (W)	186.079	186.213
Homoserine lactone	—	83.037	83.090
Homoserine	—	101.048	101.105
Pyroglutamic acid	—	111.032	111.100
Carbamidomethylcysteine	—	160.031	160.197
Carboxymethylcysteine	—	161.147	161.181
Pyridylethylcysteine	—	208.067	208.284

Question 4 - (15p)

In the ethical debate on genomics and research on biological materials of human origin, informed consent is a contested topic.

At least three different models for consent to this type of research is discussed on an international arena: explicit/specific, broad and dynamic consent.

Describe the pro et cons of each of them.

Which one of them, is superior to the others, in your opinion - and why?

Question 5 - (15p)

a) Explain briefly what we mean by a Gene Regulatory Network (GRN).

b) In order to build and analyze a complete GRN we need to identify all functional and active TFs. Give at least one reason why that can be challenging.

Question 6 - (15p)

a) Methods for studying gene expression in specific cell types have been developed for *Arabidopsis thaliana*. One of these methods uses the green fluorescent protein (GFP). Describe how the GFP protein can be used to isolate specific cell types for gene expression analysis, and explain what additional information you can get by performing transcriptional analyses of individual cell types.

b) What type of gene expression technology platform would you choose (using RNA from the isolated plant cell types)? Explain your choice.

c) Briefly explain what we mean by gene co-expression analysis, and explain why this method is often used when analyzing data from DNA microarray or RNAseq transcription experiments in *Arabidopsis thaliana*.

Question 7 - (10p)

GFP technology revolutionized live cell imaging and even made it possible to image subcellular events in live animals

a) You are imaging a cell line expressing a fluorescent protein

What are the advantages of using a confocal laser scanning microscope? Name at least three advantages.

How does the size of the pinhole influence the thickness of the optical slice?

Which technique can you use to investigate protein-protein interaction with the confocal microscope?

Discuss which excitation and emission properties this combination of fluorescent proteins should have and give an example of a pair for this technique

b) In the next experiment, you want to image tissues and individual cells in live animals

Which fluorescence methods can you use for this intravital imaging?

What are the penetration depths of the various techniques?

Which type of labels do you use and can you image certain structures without the need for labeling?