Question 1a.

What is a proteome and how is the link between an organism’s genome and a proteome.

The answer should discuss the following topics: What is a genome, type of genomes (nuclear, mitochondrial, chloroplast). That one gene can give rise to many proteins due to alternative splicing of mRNA and that there is posttranslational modification of many proteins. Many genes do not encode proteins, tRNAs, rRNAs, micro RNAs and others. The proteome is the combined output of all protein encoding genes, all peptides/proteins, processed and modified proteins (ex. diversity of antibodies). There are thousands of protein coding genes, but hundreds of thousands – millions possible proteins in eukaryotes. The correlation between the genome and the proteome is complex, and largely dependent on the transcriptome. The proteome between various cell types varies depending on which genes are expressed, the stability of the proteins and how they are processed.

Question 1b.

Describe the principles behind 1-D and 2-D gel electrophoresis of proteins.

The answer should describe: SDS-PAGE and isoelectric focusing (IEF). That SDS-PAGE is a denaturing separating method that gives information of protein weight/size, that SDS provides negative charge to the protein (plus denaturing activity). DTT/mercaptoethanol and high temperature is used to denature the sample prior to gel electrophoresis (break disulphide bonds under reducing environment). Use of protein markers (proteins with known molecular weight) is used as a reference to estimate the size of the proteins. The visualization of proteins should also be mentioned (ex: coomassie or silver staining). IEF separates the proteins based on their isoelectric point (pl) (pH gradient gel electrophoresis), why proteins have different charges at various pH should be mentioned. 1D gel electrophoresis separates proteins based on one property ex. size/weight (one dimension) or pl (IEF). In 2D gel electrophoresis the proteins are first separated based on charge (IEF) and then on size (SDS-PAGE). Protein modifications such as phosphorylations that change the charge of a protein can be identified by 2D-gel electrophoresis. By labeling protein samples with fluorescent dyes (ex. Cy3 and Cy5) two protein samples can be analyzed in one run (difference gel electrophoresis DIGE) and the relative abundance of the protein can be assessed, ex. 2D DIGE.

Question 1c.

Describe briefly the process from you picks a (protein) spot from a 2-D gel electrophoresis and end up with a mass specter that can identify the peptide.

The answer should include: How you isolate, clean up the protein from the gel, the protein is treated with proteolytic enzymes (ex. trypsin). One technique such as MALDI-TOF should be described. How the protein digest (smaller fragments of the protein) is incorporated into a matrix. The use of A UV-laser to ionize the peptid fragments from this matrix, which is accelerated to a high velocity by an electric field in a vacuum, (all the released ions have same kinetic energy). Electrospray ionization
(ESI) is another method that can be used to produce ions from a solution. How the relationship between mass and charge (m/z ratio) together with “time of flight” (TOF) can be used to give a very precise molecular weight of the peptide fragments (their flight time depend on the mass-to-charge ratio (m/z) of the particle (heavier particles reach lower speeds). How this information can be used to search a database for peptides matching this weight.

Question 2a.

Describe briefly how polyclonal antibodies are produced and explain the term polyclonal antibody ("polyclonal antibodies").

The answer should briefly describe how antibodies are produced by B-cells in an animal after exposure to an antigen (ex. a purified protein). That the animal is injected with the antigen together with an adjuvant (oil or cells of certain killed bacteria, which enhance the immune response to the antigen), multiple injections are needed. Polyclonal antibodies can be isolated from the blood serum or ascites fluid of the immunized animal (ex. using a column coupled with protein A/G for isolating IgG). Polyclonal antibodies (derived from many different B-cells) recognize multiple epitopes, including some not specific for the antigen injected into the animal. The answer should also explain the importance of testing out the specificity of the polyclonal antibody (finding the right concentration giving low background / minimum of cross reactivity).

Question 2b.

Show the principle behind two of the methods ELISA, immunocytochemical localization, immunoaffinity chromatography, Western blot.

ELISA: The meaning of primary and secondary antibody should be explained. The difference between the various types of ELISA should be mentioned: Direct ELISA, indirect ELISA, Sandwich ELISA, competitive ELISA. Substrates and the enzymes cross-linked to the antibodies should be mentioned, as well as a brief description how the assays are performed in liquid media, ex. in 96 well plates.

Western blot: Transfer of proteins from a gel to a membrane (nylon or nitrocellulose) using an electric field (electroblotting), semi-dry blot or wet blot. Detection and visualization of the proteins should be discussed (ex: the importance of blocking and washing of the membrane prior to applying the primary antibody, as well as substrates and the enzymes cross-linked to the antibodies). The difference of detecting native or denatured proteins with an antibody should be mentioned (is the epitope present in the denatured protein, advantage of polyclonal / monoclonal antibodies).

Immmunoaffinity chromatography: A method used for isolating an antigen (ex. a protein) from a solution. An antibody is conjugated to a resin in a column and used to purify a specific protein with affinity for that antibody. Can also be used for affinity purification of antibodies from blood serum (ex. Using a column coated with protein A - protein G to isolate IgG antibodies).

Immunocytochemical localization: The answer should mention how a sample is fixated, embedded and sectioned prior to the Ab detection step. Should discuss the use of primary / secondary
antibodies and how protein can be detected / observed by microscopy (ex: fluorescence microscopy). Enzymes cross-linked to the antibodies and their substrates should be mentioned. High resolution detection can be achieved by the immunogold technique and electron microscopy.

**Question 2c.**

**Explain what monocl and polyclonal antibodies are used for?**

The answer should briefly describe how monoclonal antibodies are made (the fusing of myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen, production of a hybridoma cell).

Antibodies can be used for: ex. detection and quantification of a protein or molecule, localization of a protein (immunocytochemistry, immunogold electron microscopy), identification of a protein, isolation and purification of a protein. Polyclonal antibodies gives higher sensitivity (recognizes multiple epitopes), but can give unwanted cross reactivity (lower specificity, false positive detections). Monoclonal antibodies have high specificity (recognize one epitope), but may have lower sensitivity. Antibodies can be used to detect low concentrations of a substance / protein in samples (ex. human chorionic gonadotropin for pregnancy testing). Other examples: the use of monoclonal antibodies in immunocytochemistry, or therapeutic monoclonal antibodies.

**Question 3a.**

**mRNA in eukaryotes must often be processed to be functional. Describe the most common mRNA modifications and explain what function they have.**

The answer should mention. Splicing of pre-mRNA (removal of introns, what is the function of the spliceosome), capping of the 5’ end, and polyadenylation of the 3’-end.

Polyadenylation: Components that can be discussed, the AAUAAA recognition signal, the GU-rich region, the cleavage and polyadenylation specificity factor (CPSF) with endonuclease activity, the polyA polymerase, polyA-binding protein). The alternative splicing of mRNAs, self-splicing introns can also be mentioned.

Capping: a GTP residue via an unusual 5′ to 5′ triphosphate linkage added to the 5’ end. Methylation at the 7 position of the GTP residue. Provide mRNA stability, binding to the ribosome.

mRNA splicing, the splicing machinery: the small nuclear ribonucleoproteins (snRNP), five snRNPs (U1, U2, U4, US and U6) which assemble at the splice sites at intron-exon boundaries. The snRNA of the snurps recognize three sites: the 5’ splice site, the 3’ splice site and the branch site, (the branch site contain a specific adenine which is crucial during mRNA splicing). Unspliced mRNA are normally retained in the nucleus.
Additional modifications: mRNA editing (ex. use of guide RNAs), other RNA processing: processing of tRNAs (modification of bases, producing ex. pseudouridine and inosine), processing of microRNAs (miRNAs).

Question 3b.

In eukaryote cells double stranded RNA (dsRNA) is recognized as foreign and potential dangerous for the cell. How can dsRNA be formed in a cell and what is the cellular response to this?

The answer should describe how dsRNA can be produced / introduced into a cell: such as anti-sense RNA binding a mRNA → dsRNA, transcription of a miRNA genes, which folds up into a structure containing dsRNA, activation of a RNA virus (has normally stages when RNA is double stranded).

Briefly explain RNA interference (RNAi). Describe the difference between miRNAs and siRNA and how are they produced. Describe how dsRNA is detected and bound to DICER, which binds and cleaves dsRNAs into small dsRNA fragments (~ 21-23 bp), and how the RISC complex (containing the argonaute protein) process this dsRNA into ssRNA and use this as a guide RNA to “scan” mRNAs for complementary sites. When the match is 100%, (ex. siRNA) the target mRNA is cleaved by endonucleases in RISC and degraded. In case of miRNA (guide RNA have often imperfect match to target mRNA), the RISC complex induces translational repression (the mRNA will not be degraded).

How RNAi can be enhanced and spread through a mechanism that involves RNA dependent RNA polymerases (RdRP) could also be mentioned and how this can lead to gene silencing (ex. the RITS-complex).

Question 4.

You have isolated total RNA from several parts of a plant, root, leaves, stem and flower, and you want to find out more of the function of a given protein. Both protein and gene sequences are known.

Question 4a.

Describe a method where you use the isolated total RNA to find out where the gene encoding the protein is expressed and whether the mRNA expression varies between the different tissue types.

The answer should describe one of the techniques: Quantitative PCR (qPCR), northern blotting, DNA microarrays or RNAseq. (NB! DNA microarrays and RNAseq are expensive techniques and are only used if you want to get information of many transcripts in your RNA sample a transcriptome).

qPCR: Describe how mRNA can be transcribed to cDNA (use of reverse transcriptase) and how this cDNA can be used to run qPCR (real-time PCR). PCR products can be detected / quantified by: Gel electrophoresis, with Sybr Green or TaqMan probes in a qPCR machine (optical detection). How PCR products from the cDNAs are detected during the PCR run. The use of reference genes (control
genes, housekeeping genes) used for normalization and the use of Ct values should be mentioned. Statistical analysis of gene expression can also be mentioned.

DNA microarrays: Brief description of how DNA microarrays are made, how cDNA or cRNA is labeled and how this is hybridized with the probes on the DNA microarray chip, including laser scanning of the DNA chip and detection of the signals from the “spots”.

RNAseq: Short description of the Illumina or Roch 454 techniques. Describe how a cDNA library is made and how the sequencing reactions are performed. Mapping of reads to cDNAs (counting hits to gene models) and using this information to estimate how many transcripts are present in the samples.

In situ hybridization with a fluorescent DNA/RNA probe is also an option, but it requires that you have prepared sections of plant tissue. This is a method relying of microscopy and is not particular suited to quantitative analyses.

**Question 4b.**

**By using this total RNA, how would you proceed to express this protein in a bacterium?**

The answer should briefly describe: How to produce cDNA from the RNA sample. How the coding region of the gene encoding the protein is PCR amplified from the cDNA sample (ex. using primers with restriction enzymes included in the primers) and how this PCR product is isolated and cloned into a bacterial expression vector (brief description of the use of restriction enzymes). Alternatively, that you make a cDNA library which is screened using a DNA probe (non-radioactive or radioactive) from the gene encoding the protein (ending up with a positive cDNA clone containing the gene). This is followed by the verification and cloning of the coding part of the DNA into a bacterial protein expression vector.

The bacterial plasmid vectors should be discussed (ex. transformation, antibiotic resistance markers, isolation of positive bacterial clones and induction of protein expression. Adding amino acids / peptide “tags” to the protein that can be used in protein purification could also be mentioned.

**Question 4c.**

**You suspect that the protein is part of a protein complex in the plant. Describe a method that can give you information whether other proteins bind to and interact with this protein.**

Describe one of the methods. Yeast two-hybrid or protein complex immunoprecipitation.

The yeast two hybrid system relies on the use of the GAL4 the transcription factor, which can be split into two functional fragments, called the binding domain (BD) and activating domain (AD). The activating and binding domains are modular and can function when they are close to each other (does not require direct binding) and control the expression of a reporter gene. Plasmids are
engineered to produce a protein product in which the DNA-binding domain fragment is fused onto a protein (bait) while another plasmid is engineered to produce a protein product in which the activation domain fragment is fused onto another protein (pray). The plasmids are transformed into two yeast strains with different mating types (a and α). After mating yeast cells are plated onto selective medium (depending on the reporter gene used in assay), positive yeast colonies indicate that the two proteins interact.

Protein complex immunoprecipitation: Immunoprecipitation of intact protein complexes (i.e. antigen along with any proteins or ligands that are bound to it). Briefly describe how an antibody that targets a known protein (that is believed to be a member of a larger complex of proteins) is able to pull the entire protein complex out of solution and how this strategy can be used to identify unknown members of the complex.

A protein microarray (or protein chip), a high-throughput method used to track protein-protein interactions, is an alternative method which can be used for large scale studies.

Bimolecular fluorescence complementation (BiFC) is a technology that also can be used to validate protein interactions.