

Question 1

1A. The most likely gene product would be a transcription factor including a sigma factor **or** sequence specific activator.

Gene specific transcription factors are the most prevalent regulatory proteins that could simultaneously control many (i.e. 30) genes. The targeted 30 genes/operons would need to have the binding site for the factor within or near their promoters.

1B. Gene expression microarray which would easily identify the battery of genes unregulated in strain C but not strain L. ChIP on chip would also work if they specify having antibody to the regulatory factor.

1C. ChIP on chip **or** ChIP combined with PCR of the 30 genes

Explanation of method

crosslink protein to DNA inside the cell

isolate QSR using a **specific antibody**

reverse crosslinks

perform chip analysis with DNA sample **or** sequence DNA directly

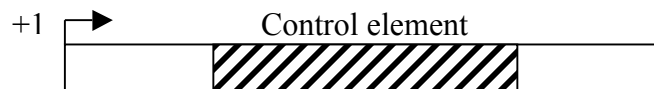
1D. Find and sequence the QSR gene in strain L **or** see if strain L will rescue when supplied with wt QSR

1E. Mutant QSR is not able to bind DNA

Mutant QSR is not able to bind RNA pol or mutation to QSR activation domain

Question 2

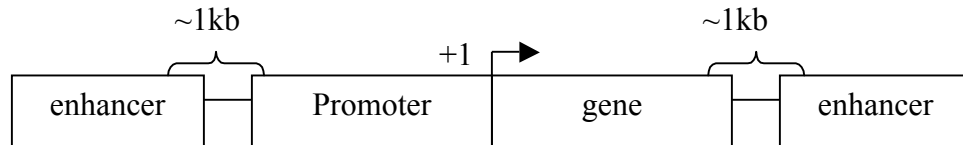
A. The promoter for RNA pol III transcripts is within the coding region of the gene – a gene internal promoter. Drawing should show a gene with control elements within the gene



B. Acceptable answers include “Deletion analysis” and/or “Linker Scanning.” “Sequence alignment” is fine to mention, but earns only 1 point in the absence of one of the other two techniques. Description must include the idea that a plasmid is used containing a reporter gene and a region of DNA to be probed by the assay that potentially contains control elements as shown below.

- a. Deletion analysis: Different plasmids with different fragments removed from the DNA in question are analyzed. Those fragments removed that show large increases (or decreases) in transcription of the reporter gene contain potential control elements.

- b. Linker scanning: Overlapping regions of mutated sequences are introduced from one end of a suspected control region to the other and again, increases (or decreases) in transcription of the reporter are assayed.
 - c. The assays can be conducted *in-vitro* or *in-vivo* so both are correct.
- C. RNA pol II genes were found to contain a core promoter proximal to the gene, and both upstream and downstream enhancers. Drawing need only show a core promoter directly upstream from a gene and a regulatory element “far away” (upstream or downstream) from the core promoter.



- D. “DNase hypersensitivity assays” or “Deletion analysis” are acceptable answers. “Linker scanning” receives partial credit since it requires prior knowledge of a defined region of DNA.
- E. Acceptable answers include “DNA affinity chromatography,” “DNase footprinting,” “Electrophoretic mobility shift assay or gel shift assay” and “Chromatin immunoprecipitation or ChIP” if they also specify that an antibody would first need to be obtained.
- F. Yes. Gene expression microarrays or ChIP-Chip work well with eukaryotes provided the entire genome sequence is available and/or on a chip.
- G. Ribosomal RNA or rRNA. RNA Polymerase in prokaryotes and RNA Polymerase I in eukaryotes

Question 3

3A. Bacterial system is **attenuation**

Description: The attenuator region, composed of sequences found within the transcribed RNA, is involved in controlling transcription from the operon after RNA polymerase has initiated synthesis. The attenuator of sequences of the RNA are found near the 5' end of the RNA termed the leader region of the RNA. The leader sequences are located prior to the start of the coding region for the first gene of the operon (the *trpE* gene). The attenuator region contains codons for a small leader polypeptide, that contains tandem tryptophan codons. This region of the RNA is also capable of forming several different stable stem-loop structures.

Depending on the level of tryptophan in the cell and hence the level of charged *trp*-tRNAs, the position of ribosomes on the leader polypeptide and the rate at which they are translating allows different stem-loops to form. If tryptophan is abundant, the ribosome prevents stem-loop 1-2 from forming and thereby favors stem-loop 3-4. The latter acts as the transcriptional terminator loop. Consequently, RNA polymerase is dislodged from the template.

Genes regulated are trp biosynthesis genes (or any genes involved in amino acid biosynthesis)

The ribosome regulates RNA polymerase elongation

3B. Insulin signaling pathway

Description: In the presence of insulin a signaling cascade activates the protein kinase Akt. To control transcription, Akt phosphorylates the FOXO transcription factor to sequester it in the cytoplasm. Akt activation also leads to phosphorylation of 4E-BP, which allows for cap dependent translation.

In the presence of low insulin, FOXO activation leads to the inhibition of cellular growth. 4E-BP activation leads to the inhibition of cap-dependent translation.

The pathway is controlled by a **metabolic feedback mechanism**. FOXO also transcribes the insulin receptor (INR) gene and 4E-BP. INR mRNA is translated even in the presence of 4E-BP due to an IRES. This increases the sensitivity of the cell to the presence of insulin.

Gene targeted - INR gene

Two important features – IRES; TF FOXO controls 4E-BP expression

3C. Coupled system is RNA processing

2 steps regulated can be 5` end capping **or** 3` end processing **or** splicing **or** transport

3D. Alternative splicing and posttranslational modification

Recombination and/or transposition will work for _ credit

Question 4

- A. Small molecules – synthetic or natural product drugs, taken in pill form, not destroyed by digestive tract etc.
Biologics – vaccines, insulin, antibodies, DNA or RNA. These larger molecules are destroyed in the digestive tract, and need to be injected.
- B. RNAi (siRNA, miRNA also accepted). dsRNA complementary to the target gene is first recognized by Dicer which cuts the dsRNA into defined lengths of ~23 bp's. These dsRNA's are then recognized by the RISC complex which uses helicase activity to unwind the dsDNA and then uses the ssRNA that remains bound to recognize the target mRNA. The formation of protein product is prevented either by degradation of the target mRNA in the case of a perfect match between the ssRNA and target, or blocking translation in the case of an imperfect match between the ssRNA and the target (either answer accepted). Drosha and Pasha/DGCR8 are also ok to mention.
- C. A mouse knockout. Answer must include these general points:
 - a. Engineer a targeting vector with a selection strategy
 - b. Transfect mouse embryonic stem cells with the targeting vector

- c. Inject cells into blastocyst
 - d. Obtain chimeric offspring that must go through a series of crosses in order to obtain a homozygous knockout
- D. Too much = weaker bones: Engineer a transgenic mouse containing/overexpressing RK1 and assay bone density.
Too little = stronger bones: Use your RK1 knockdown/knockout mouse and assay bone density.
- E. Small molecule – develop an assay for an inhibitor of RK1 and conduct a small molecule screen on a large/diverse ($\sim 10^6$ compounds) chemical library.
Biologics – Design an antibody to RK1. Antibody design was not covered in class, so an answer that contains this concept will get full credit. Also acceptable are siRNA and triplex helix formation.
- F. Human clinical trials.