

Assignment 1**Part 1 “A phosphosite? Are you sure?”**

Phosphorylation is a post-translational protein modification that can serve to switch the activity of the target protein on and off. There have been systematic efforts to reveal target proteins for the enzymes (kinases) that carry out phosphorylation. One strategy is to express thousands of proteins and array them on the surface of a ‘chip’ (Ptacek et al. 2005). The protein array can be exposed to a particular kinase of interest in the presence of radiolabeled (gamma-³³P) ATP.

Target proteins can be identified by virtue of becoming radiolabeled by the kinase. Ptacek *et al.* arrayed 4,200 *S. cerevisiae* proteins and identified substrates for 87 protein kinases. This study revealed, for example, 9 target proteins of the *S. cerevisiae* kinase Cmk2.

Kinases have protein sequence preferences. For example, the Cmk2 kinase reportedly phosphorylates proteins matching the pattern R-x(2)-[ST]-x-[ST] (Ptacek et al. 2005). (In English, that pattern can be read as “Arginine, anything, anything, Serine or Threonine, anything, Serine or Threonine”.) Out of the 9 proteins identified as Cmk2 targets by Ptacek *et al.*, 7 contained a single site matching the R-x(2)-[ST]-x-[ST] pattern, and 2 had no matches to that pattern. Amongst all 4,200 proteins on the array, 1,325 matched the pattern.

1. Imagine you’re looking at the sequence of your favourite protein (aptly named Yfp1), which was not measured on the Ptacek *et al.* array. You see that Yfp1 does have a match to the R-x(2)-[ST]-x-[ST]. The Yfp1 sequence is only 150 amino acids long, which is shorter than the median *S. cerevisiae* protein which is ~375 aa long (Zhang 2000). Using the table of amino acid frequencies provided below, calculate the probability that a random sequence of length 150 would match the pattern R-x(2)-[ST]-x-[ST] at least once just by chance. For the sake of simplicity, you can assume independence between positions and potential sites within the protein.
2. Assuming that the Ptacek *et al.* results are completely accurate and that the proteins measured by Ptacek *et al.* are representative of the other proteins not present on the array, what is the posterior probability that Yfp1 is phosphorylated by Cmk2, given that it matches the R-x(2)-[ST]-x-[ST] pattern?

Observed Frequency of Amino Acids in *S. cerevisiae* according to (Huntley and Golding 2000)

| Amino Acid | Frequency |
|------------|-----------|
| Ala | 5.3% |
| Arg | 4.5% |
| Asn | 6.2% |
| Asp | 5.8% |
| Cys | 1.3% |
| Gln | 3.9% |
| Glu | 6.6% |
| Gly | 4.8% |
| His | 2.2% |
| Ile | 6.6% |
| Leu | 9.8% |
| Lys | 7.4% |
| Met | 2.1% |
| Phe | 4.6% |
| Pro | 4.3% |
| Ser | 9.0% |
| Thr | 5.8% |
| Trp | 1.0% |
| Tyr | 3.3% |
| Val | 5.5% |

Part 2 “At the proteome scale”

Now that you have completed Part 1, it is time to dive deeper into the yeast proteome. In this part, you will examine the proteome of the yeast strain [BY4741](#), a widely used laboratory strain. The BY4741 is one of the parental strains included in the Yeast Knockout (YKO) collection, the first complete deletion collection of any organism (Winzeler et al. 1999; Giaever et al. 2002).

We'll use the FASTA file (“orf_trans.fasta”), which contains the reference sequences of the [BY4741 proteome](#), in the assignment folder:

1. Let's empirically count the fraction of proteins that match the pattern R-x(2)-[ST]-x-[ST], the same kinase binding motif investigated in Part 1.

Tip: There are multiple methods for pattern matching, and you are welcome to choose the method with which you're most comfortable. For example, you can take a brute-force approach by generating all possible amino acid fragments that satisfy the pattern and scanning the proteome with each one of the fragments. Alternatively, you might convert the pattern into a regular expression which can be used to quickly scan the proteome. Should you choose the regular expression approach, you might find this resource (<https://regex101.com/>) helpful to develop and debug your regular expression.

Output: A frequency.

2. Now let's double-check the amino acid frequency table we used in Part 1, by empirically measuring the marginal frequency of each of the 20 amino acids in the yeast proteome.

Output: 20 frequencies each labelled with the corresponding amino acid.

3. Compare the empirically measured amino acid frequency from Step 2 with the amino acid frequency table in Part 1. Do they agree with each other? If they disagree, think of one possible source of difference from the previous table.

Tip: When answering the questions, please make sure to explain your rationale.

Output: A paragraph describing the comparison and answering the questions.

4. Using your marginal amino acid frequencies from Step 2, calculate the expected frequencies of di-amino acid 'words', e.g., Alanine followed by Glycine forms the di-amino acid word AG. Here you should assume independence between amino acids.

Output: A 20-by-20 frequency matrix. Column names should represent the first amino acid of di-amino acid words, and row names represent the second amino acid. For example, the frequency of di-amino acid word AG should be stored in column “A” and row “G”.

5. Empirically measure di-amino acid frequencies in the BY4741 proteome.

Output: A 20-by-20 frequency matrix. Column names should represent the first amino acid of di-amino acid words, and row names represent the second amino acid. For example, the frequency of di-amino acid word AG should be stored in column “A” and row “G”.

6. Using your empirically measured di-amino acid frequencies, calculate conditional amino acid

frequencies where, for each vector, the conditioning is on a different one of the 20 possible preceding amino acids. Specifically,

- a. If the preceding amino acid is Isoleucine (I), what is the frequency of seeing each of the 20 amino acids in the next position?
- b. If the preceding amino acid is Glutamine (Q), what is the frequency of seeing each of the 20 amino acids in the next position?

Output: 2 * 20 frequencies each labelled with the corresponding amino acid.

7. Compare the conditional amino acid frequencies from Step 6 to the empirical marginal amino acid frequency from Step 2. Do they agree with each other? How did you determine that? Please offer a potential explanation for the largest differences you see between marginal and conditional frequencies.

Tip: When answering the questions, please make sure to explain your rationale.

Output: A paragraph describing the comparison and answering the questions.

Notes

1. You can use any libraries to parse the FASTA file and to perform pattern matching.
2. You are NOT allowed to use functions, libraries or packages that generate single- or di-amino acid frequencies directly from protein sequences. The purpose of this part of the assignment is to provide you with an opportunity to understand how these frequencies are generated.

Submission

Please submit one zip file (first_lastname.zip) with

1. Source code (in .R or .py format) and a README file on how to run your program.
2. Your program's output (in .txt format).
3. A document (in .doc or .pdf format) with answers to questions in Part I and II.

References

- Giaever, Guri, Angela M. Chu, Li Ni, Carla Connelly, Linda Riles, Steeve Véronneau, Sally Dow, et al. 2002. "Functional Profiling of the *Saccharomyces Cerevisiae* Genome." *Nature* 418 (6896): 387–91.
- Huntley, M., and G. B. Golding. 2000. "Evolution of Simple Sequence in Proteins." *Journal of Molecular Evolution* 51 (2): 131–40.
- Ptacek, Jason, Geeta Devgan, Gregory Michaud, Heng Zhu, Xiaowei Zhu, Joseph Fasolo, Hong Guo, et al. 2005. "Global Analysis of Protein Phosphorylation in Yeast." *Nature* 438 (7068): 679–84.
- Winzeler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson, B. Andre, R. Bangham, et al. 1999. "Functional Characterization of the *S. Cerevisiae* Genome by Gene Deletion and Parallel Analysis." *Science* 285 (5429): 901–6.
- Zhang, J. 2000. "Protein-Length Distributions for the Three Domains of Life." *Trends in Genetics: TIG* 16 (3): 107–9.